IDENTIFYING TRAITS AND MOLECULAR MARKERS FOR IMPROVEMENT OF EAR FERTILITY IN WHEAT

BY

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Abstract

Wheat (*Triticum aestivum* L.) grain yield potential is generally sink-limited under favourable conditions with grain growth limited mainly by the number of grains formed per unit area. In the CIMMYT program in NW Mexico, novel large-ear phenotype traits (e.g. high assimilate partitioning to ear, high fertile florets ear^{-1}) have been developed and may offer scope for increasing grain number per unit area (Gaju *et al*., 2009). In this study, ear fertility traits and their genetic regulation were investigated in backcross lines derived from novel large-ear doubled-haploid lines (UK Rialto x novel large-ear CIMMYT L14 line cross) backcrossed into either UK spring wheat Ashby or UK winter wheat Humber. Forty four BC_3 near isogenic lines (NILs) (7 spring and 37 winter wheat NILs, currently BC_3S_5 and BC_3S_6) and 34 BC_1S_7 lines (18 spring and 16 winter wheat NILs, currently BC_1S_7) and their recurrent parents (Ashby or Humber, respectively) were chosen for physiological analysis in field experiments at University of Nottingham, UK and KWS, Thriplow, Hertfordshire, UK for evaluating novel variation for ear fertility traits and grain yield and specific introgressed target QTL on ear fertility traits and grain yield in 2012/13 and 2013/14.

In highest/lowest performing BC_3 spring NILs, above ground dry matter (AGDM) per main shoot increased by 97%, grain dry weight (GDW) ear^{-1} by 95% and grain number (GN) ear^{-1} by 68%, but grain yield was decreased by 71% associated with fewer ears m^{-2} (-87%) compared to the Ashby recurrent parent. For BC_3 winter NILs, results showed that there was variation above Humber, for grain yield 100% DM (+22%), grains m^{-2} (+16%), harvest index (HI) (+6%), spikelets ear^{-1} (+14%), fertile spikelets ear^{-1} (+15%), rachis length (cm) (+24%) and thousand grain weight (TGW) (g) (+21%) ($P<0.10$).

In the experiment at KWS Thriplow, the effects of the *Tin1A* gene in pairs of BC_1S_7 NILs contrasting in tiller number were investigated in the Ashby and Humber

backgrounds. Amongst five pairs of spring NILs contrasting for the tiller inhibition *Tin1A* locus, the *Tin1A* lines were reduced in tillers m^{-2} (-16%), grain yield (-9%) and ears m^{-2} (-19%) but increased in spikelets ear^{-1} (+12%). In two pairs of winter NILs contrasting for the *Tin1A* locus, the *Tin1A* lines reduced tillers m^{-2} (-18%), grain yield (-6%), ears m^{-2} (-16%) but increased GN ear^{-1} (+20%), spikelets ear^{-1} (+11%) and GN $spikelets^{-1}$ (+6%). The extent of tiller reduction was overall too severe in the spring NILs with Ashby background to lead to increased yield in the lines carrying the L14 allele.

In winter NILs, grain yield and biomass were increased with high canopy green area and better maintenance of green area during grain filling. This suggested grain growth may have been partly limited by source supply of assimilate during grain filling possibly during the latter stages of grain filling. A strong relationship between individual grain weight and rate of grain filling was consistent in BC_3 spring NILs. Grain weight was increased in BC_3 winter NILs with increasing rate of grain filling but duration of grain filling was decreased.

DNA samples from each BC_3S_5 line were screened using 8 SSR markers for the introgressed ear fertility QTLs identified in the Rialto x CIMMYT L14 DH population. The genotyping results showed the CIMMYT L14 allele for a novel QTL on chromosome 3A was associated with increases in ear fertility traits (grain number ear^{-1}, spikelets ear^{-1}, and grain DM $shoot^{-1}$) amongst the $BC_3S_{5\text{-}6}$ lines when compared to the Humber recurrent parent and confirmed the presence of *Tin1A* gene in the Ashby background determining ear fertility and grain yield effects.

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All the data collected in the field experiments was my own work.

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List of Abbreviations

ABA	Abscisic acid
AD	Anthesis date
AGDM	Above ground dry matter
AGE	Agarose gel electrophoresis
ANOVA	Analysis of variance
BBSRC	Biotechnology and Biological Sciences Research Council
BC	Backcross
BC_1	Backcross 1
BC_3	Backcross 3
°Cd	Degree day
CE	Control environment
CGR	Crop growth rate
Chl	Chlorophyll
Chr.	Chromosome
CHO	Carbohydrate
CIMMYT	International Maize and Wheat Improvement Center
d	Day
DAS	Days after sowing
DF	Degrees of freedom
DH	Double haploid
DM	Dry matter
DNA	Deoxyribonucleic acid
DW	Dry weight
EDM	Ear dry matter
EPI	Ear partitioning index
FE	Fruiting efficiency
FS	Fertile shoots
FLS	Flag-leaf score
GA	Gibberellic acid
GAI	Green area index
GDW	Grain dry weight
GN	Grain number
GS	Growth stages
HGCA	Home-Grown Cereals Authority
HI	Harvest index
i.e.	id est
ILs	Introgression lines
K	Potassium
LAI	Leaf area index
LI	Light interception
LOD	Logarithm of the odds
LSD	Least significant difference
Mg	Magnesium
MS	Main shoots
N	Nitrogen
NABIM	National Association of British and Irish Flour Millers
NDVI	Normalized difference vegetative index
NILs	Near-isogenic lines
NUE	Nitrogen-use efficiency
NW	Northwest
P	Phosphorus

PAGE	Polyacrylamide gel electrophoresis
PAR	Photosynthetically active radiation
PCR	Polymerase chain reaction
PGR	Plant growth regulator
PGW	Potential grain weight
PH	Plant height
QTLs	Quantitative trait loci
r	Correlation coefficient
R:FR	Red:far red ratio
R^2	Coefficient of determination
RILs	Recombinant inbred lines
RL	Rachis length
RS	NDVI senescence rate
RUE	Radiation-use efficiency
SED	Standard error of the difference of means
SEM	Standard error of means
SSR	Simple sequence repeat
Stg	NDVI stay green
T.	*Triticum*
t ha^{-1}	Tonnes per hectare
Tin1A	Tiller inhibition gene
TGW	1000 grain weight
UK	United Kingdom
USA	United States of America
WSC	Water soluble carbohydrates

CHAPTER 1 INTRODUCTION

1.1 Wheat production and world demand

Wheat (*Triticum aestivum* L.) is a staple food for more than half of the world's population. The average annual total production is approximately 600 million metric tonnes and the total cultivated land area is around 200 million hectares (FAO, 2010). World average yield is around 2.7 t ha^{-1} with high variability in both regions and countries (Rajaram and Braun, 2008). A rising global population approaching 9 billion by 2050 will result in increased food demand and require increased food production (FAO, 2010). Grain yields may also be affected by climate change (Nelson *et al.*, 2010), which may lead to a decline in the rate of genetic progress in grain yield in wheat. Therefore, there is an urgent requirement to enhance yields and to improve quality in wheat through plant breeding.

1.2 Breeding and wheat germplasm improvement

The common hexaploid bread wheat (2n = 6, x = 42) was derived from three different genomes (A, B and D). It originated from natural hybridzation, the first occurring between *Triticum urartu* which contributed genome A and *Aegilops speltoides* which contributed the genome B. The resulting tetraploid wheat, *T. turgidum* L. (AABB), then hybridised with *T. tauschii* which contributed the genome D to generate the hexaploid wheat *T. aestivum* L. (AABBDD) (Fig. 1.1) (Huang *et al.*, 2004, Shewry, 2009). The hexaploid cultivars are categorized according to (i) the duration of growing season, e.g. spring wheat (with no vernalization requirement) and winter wheat (with vernalization requirement), (ii) grain colour (red and white) and (iii) end-use quality such as high and low gluten content, hard and/or soft grain texture.

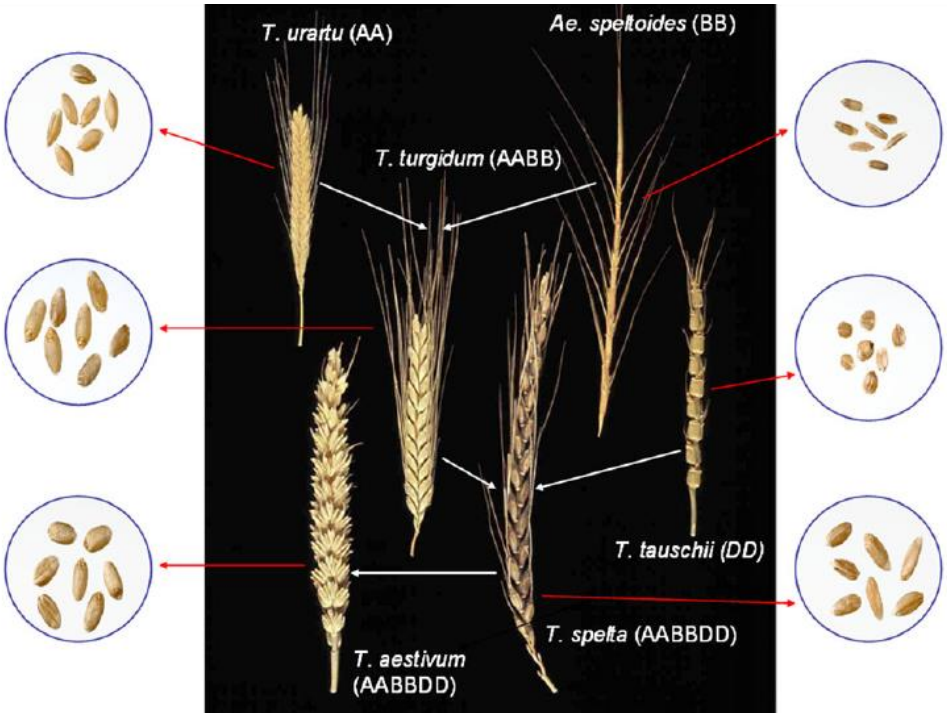

Fig. 1.1 Evolution of hexaploid wheats and relationships between cultivated bread and durum wheats (Source: Shewry (2009))

The International Maize and Wheat Improvement Center (CIMMYT) is an important worldwide organization for improving bread and durum wheat yields in developing countries through the development of cultivars with higher yield potential, resistant and/or tolerant to biotic and a biotic stresses with high adaptability to specific environments. The main purpose in the CIMMYT wheat breeding program is to fill the gap between the production and the demand in developing countries (Singh and Trethowan, 2007). Over the past century, plant breeding has resulted in productivity enhancement and new wheat varieties. Plant breeding will continue to play a major role in increasing food production. The approach of the Green Revolution in the mid-to-late 20th century, with the introduction of high-yielding varieties of wheat as well as management techniques in fertilizer and pesticides, resulted in rising cereal yields. The introduction of semi-dwarf genes such as *semi-dwarf* (*Rht-B1b* and *Rht-D1b*) genes from the Japanese source of Norin 10 not only reduced stem height and reduced lodging but also led to a greater proportion of assimilate partitioned into the

grain, resulting in greater spike growth, more grains per unit area and higher harvest index (HI) (Fischer and Stockman, 1986, Hedden, 2003). It has been reported that genetic diversity of common wheat is comparatively narrower than its two donor species due to an evolutionary bottleneck, most of the genetic variation observed in tetraploid wheat and *Ae. Tauschii* is present in the hexaploid germplasm (Li *et al*., 2014). Therefore, introgressing new genetic variation into elite wheat genetic resources to produce better genotypes is an important goal for wheat breeding (Rajaram, 2002).

1.3 Using the backcross strategy for crop improvement

Backcross breeding has been widely used by plant breeders to improve agronomic traits in the development of crop cultivars. It is a powerful approach for the introgression of several genes determining target traits from a donor parent into the genetic background of a recurrent parent (Hospital, 2002; Ye *et al.*, 2009). A backcross population can be utilized for investigating transgressive segregation and performing QTL analysis and fine mapping to identify molecular markers for crop improvement (Baohua and Peng, 2010).

The advanced backcross quantitative trait locus strategy was proposed by Tanksley and Nelson (1996) to determine and introduce beneficial QTLs from wild species to elite cultivars to enhance genetic diversity in breeding programs. Introgression lines (ILs) derived from backcross populations have also been used for QTL fine mapping (Faino *et al.*, 2012) and genome-wide metabolic profiling to understand the mechanism of cellular networks (Schauer *et al.*, 2006). The method can integrate the process of QTL analysis and genetic diversity for crop improvement and has been effectively used to map disease resistance and yield loci in several crop species such as rice (Thomson *et al.*, 2003), tomato (Fulton *et al.*, 2000), bean (Blair *et al.*, 2006) and barley (Pillen *et al.*, 2003).

In wheat, Huang *et al.* (2003) successfully identified eleven QTLs for yield, sixteen for yield components, eight for ear emergence time and five for plant height on several chromosomes from the synthetic wheat lines (*T. tauschii*, W-7984) in a German winter wheat background variety Prinz by using BC_2F_2 lines. Huang *et al.* (2004) discovered two QTLs for thousand-grain weight from the synthetic wheat (XX86) which increased thousand grain weight (TGW) by 5.2-8.0% and five QTLs linked to grain weight per ear conferring an increase 5.2%-8.1% in a German winter wheat background variety Flair by using BC_2F_1 families. BC_3F_5 progenies carrying genes for high grain protein content (*Gpc-B1*) were developed from crosses between 10 bread wheat genotypes as recipient parents and a hexaploid wheat containing *Gpc-B1* and had significantly higher GPC (14.83-17.85%) than their recurrent parental lines with no loss in yield (Kumar *et al*., 2011).

1.4 Applying physiological strategies in wheat breeding

To date improvement of yield potential has been mainly empirically based on yield *per se* (Evans, 1993). However, yield has a relatively low heritability and this approach may not be the most efficient as there is a high genotype x environment interaction (Jackson *et al.*, 1996). Therefore understanding of traits at the physiological level may help to identify indirect selection criteria that could be applied to accelerate breeding progress. Such an integrated approach may complement empirical breeding and hasten progress (Araus *et al.*, 2002, Reynolds and Borlaug, 2006).

Improving the understanding of the physiological basis of genetic progress in yield has become more important in the last decades for several reasons; for example: 1) the rate of genetic gain in yield potential resulting from plant breeding has apparently slowed down recently in some countries; and 2) the improvement in canopy sensors used in high-throughput field phenotyping of physiological traits means they are now more reliable and accurate (Fischer, 2007, Reynolds *et al*.,

2007). Examples of important physiological tools which could be used as selection criteria are canopy temperature depression (difference between ambient temperature and canopy temperature; CTD) using an infra-red thermometer indicative of photosynthetic capacity and spectral reflectance indices using spectral radiometers indicative of canopy size and biomass (Reynolds *et al.*, 1999, Babar *et al*., 2006b). Also, It was concluded that using canopy reflectance information such as: NDVI, is a valuable way to estimate the RUE and HI (Li *et al.*, 2011).

The objectives of this study are to:

1. Identify the physiological mechanisms determining novel genetic variation in ear fertility traits related to tiller production, spikelet primordia production and floret fertility as determinants of grains per m^2 and grain yield utilizing the near-isogenic lines (NILs) (currently BC_3S_5) developed from backcrossing between L14 x Rialto DH lines and winter (Humber) and spring (Ashby) wheat
2. Improve physiological understanding of the effects of the tiller inhibition (*Tin1A*) gene on tiller production and yield potential traits in the UK environment associated with large ear phenotype
3. To quantify the effects of the *Tin1A* gene in spring wheat and winter wheat in BC_1 and BC_3 genetic backgrounds
4. To identify and validate the putative QTLs associated with ear fertility traits in BC_3 population
5. To validate the novel QTL responsible for enhanced ear fertility and grain number per ear in BC_3 NILs in the spring wheat Ashby and winter wheat Humber backgrounds
6. To develop plant materials for future study for genetic regulation and mapping based cloning of target genes

CHAPTER 2 LITERATURE REVIEW

2.1 Definitions of yield potential

In wheat, genetic variation in yield potential is highly associated with the grain number produced per unit land area (Fischer, 1975, Fischer, 1985, Waddington *et al.*, 1986, Perry and D'Antuono, 1989, Sayre *et al*., 1997, Abbate *et al*., 1998, Shearman *et al*., 2005). Moreover, the final grain yield can be considered as the balance between the grain source size and sink size. The source (photosynthetic capacity of the crop to fill the grains) is the ability of the crop to supply assimilate by fixing carbon dioxide in photosynthesis whereas sink (grain storage capacity) is the capacity of grain to store assimilate. The source is comprised of the current photosynthesis during grain filling and the stem carbohydrate reserves at anthesis. Borrás *et al.* (2004) reviewed a number of 18 data sets of responses to source-sink manipulation treatments for wheat genotypes and concluded that grain yield of wheat under optimal conditions is limited by grain sink size rather than grain source size under favourable conditions. Yield potential is the final output of a cultivar when grown under optimal environments with non-limiting water and nutrients and well-controlled in biotic and abiotic stresses (Evans and Fischer, 1999, Foulkes *et al*., 2011). However, a cultivar could be grown under unfavourable growing conditions such as water stress, heat stress, diseases and pathogens and this could result in grain yield below yield potential. The divergence between the potential and actual yield or average on-farm yield is determined as the exploitable yield gap (Fischer and Edmeades, 2010). In addition, attainable yield is defined as the yield achieved by the best growers (usually about 10% above actual yield or 20-30% below potential yield). The progress in actual yield is a consequence of an increase in potential yield and/or a decrease in the exploitable yield gap (Fischer *et al*., 2014). The annual current potential yield progress of wheat in most countries is about 1.0% year^{-1} (Sayre *et al.*, 1997, Brancourt-Hulmel *et al.*, 2003, Shearman *et al.*, 2005) while

average worldwide genetic progress is 0.5% year^{-1} with a yield gap of 40% (Fischer *et al.*, 2009).

2.2 Yield component analysis

Previous studies have shown that genetic progress in wheat yield potential is highly correlated with ear fertility traits such as grains per ear and ear DM partitioning in wheat under favourable conditions (Fischer, 1985, Slafer and Savin, 1994, Reynolds *et al*., 2005, Shearman *et al*., 2005). In wheat, genetic progress in grain yield improvement has been highly associated with grain number per unit land area (GN) (Brancourt-Hulmel *et al*., 2003, Shearman *et al*., 2005, Acreche *et al*., 2008, Fischer, 2008, Foulkes *et al*., 2011) and grain number remains a critical yield-limiting factor (Gaju *et al.*, 2009). Clarke *et al.* (2012) reported that genetic variation in grain yield can be analysed using different approaches such as: (i) physiological components (above-ground biomass and harvest index) and (ii) numerical components (ears per m^2, grains per ear and individual grain weight). In terms of its numerical components, wheat grain yield is the product of plants per unit land area, ears per plant, grains per ear, spikelets per ear, grains per spikelet and individual grain weight (Slafer, 2003, Fig. 2.1).

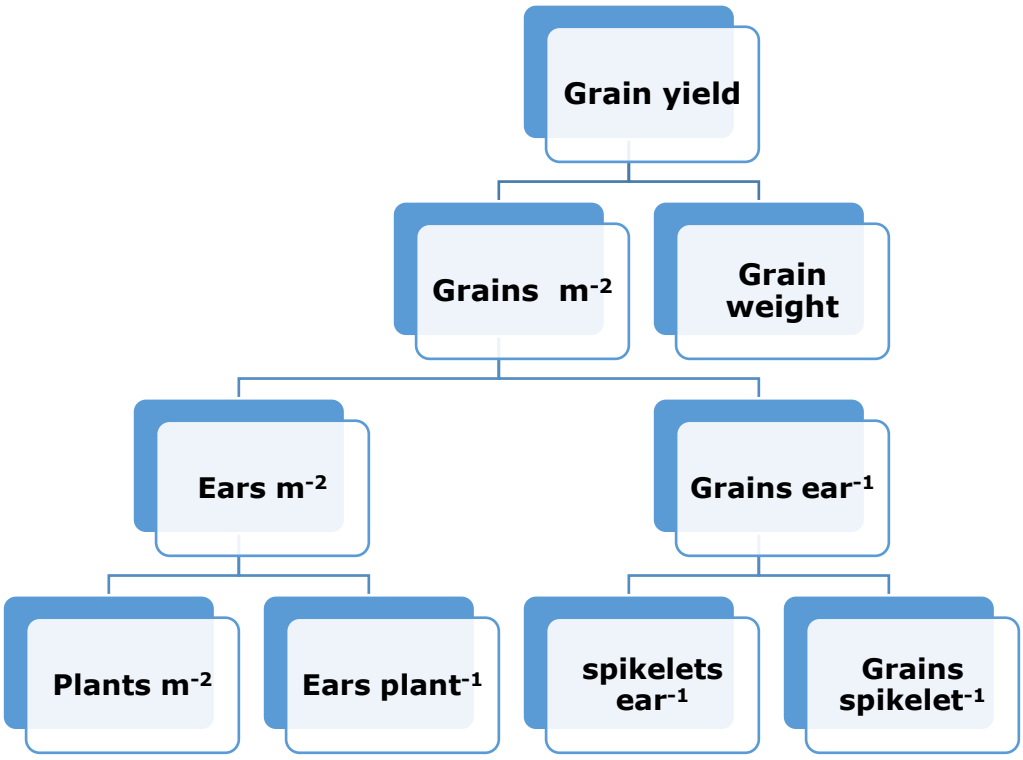

Fig. 2.1 Diagram of grain yield components (Adapted from Slafer, 2003)

In terms of the physiological components of yield, Reynolds *et al.* (2009) proposed that grain yield potential is expressed as the product of light interception (LI), radiation-use efficiency (RUE) and the partitioning of above-ground biomass to grain: (harvest index, HI) (Eqn. 2.1). Historically most grain yield potential genetic progress has been associated with increased Harvest index (Austin *et al.*, 1980, Perry and D'Antuono, 1989, Siddique *et al*., 1989a, Sayre *et al*., 1997, Shearman *et al.*, 2005, Acreche *et al*., 2008) although there is recent evidence for biomass increases e.g. Aisawi *et al.* (2015).

$$\mathbf{YP = LI \times RUE \times HI} \qquad \text{(Eqn. 2.1)}$$

Where YP = Yield potential; LI = light interception; RUE = radiation-use efficiency and HI = harvest index.

Fischer (1984) suggested that determination of grains m^2 in the pre-anthesis period may be considered as the product of ear dry weight per m^2 at anthesis (EDWa) and the fruiting efficiency (FE), i.e. number of grains per gram ear DM at anthesis (Eqn. 2.2). Moreover, during the pre-anthesis period, EDWa could be considered as the product of duration of stem-elongation phase (SEP) from GS31 to GS61 (days) (Zadoks *et al*., 1974), crop growth rate (above-ground DM per m^2 per day; CGR) during stem elongation and ear partitioning index (EPI) (Eqn. 2.3).

$$\mathbf{GN = EDWa \times FE} \qquad \text{(Eqn. 2.2)}$$

$$\mathbf{EDWa = SEP \times CGR \times EPI} \qquad \text{(Eqn. 2.3)}$$

Where EDWa = ear dry weight per m^2 at anthesis; FE (grains g^{-1} ear DM at anthesis) = fruiting efficiency; SEP = duration of stem-elongation phase and CGR = above-ground DM per m^2 per day

According to equations 2.2 and 2.3, grains per m^2 could be increased by increasing the number of fertile florets (grains) per unit ear weight at anthesis to increase FE and/or increasing the ear dry weight at anthesis via a longer duration of stem elongation as a proportion of the thermal time to anthesis (Miralles *et al*., 2000, González *et al*., 2003b, González *et al.*, 2005b). There is strong evidence that a longer stem-elongation period (from initiation of terminal spikelet to anthesis) through manipulating photoperiod responses not only reduces the rate of floret development but also increases the number of fertile florets as well as ear dry weight at anthesis (Miralles *et al.*, 2000, González *et al.*, 2003b). Furthermore, the ear DM at anthesis will be strongly influenced by the number of tillers per plant (Duggan *et al*., 2005b). The following review of physiological processes determining variation of grain number per unit area will broadly address these physiological components.

Currently, fruiting efficiency is considered as an alternative component to enhance wheat yield potential and improve grain number (Ferrante *et al.*, 2015, Slafer *et al.*, 2015). This trait might be associated with a larger proportion of floret survival at anthesis and decrease grain abortion during grain filling. Increasing the assimilate supply to the developing florets during ear growth before flowering and reducing the demand of florets for maintaining their development are the two physiological pathways to improve fruiting efficiency (Slafer *et al*., 2015). However, high fruiting efficiency may have a negative effect on individual grain weight. Ferrante *et al*. (2015) suggested that increasing fruiting efficiency could lead to an increase in the proportion of grains of smaller potential size rather than reducing grain weight constitutively.

Since the HI of wheat in modern cultivars in many countries and regions is already approaching the theoretical maximum value of ca. 64% (Austin *et al.*, 1980, Foulkes *et al.*, 2011), future breeding to improve grain yield may need to focus increasingly on increased biomass. Furthermore, grain yield and grain per m^2 could be improved through an optimal number of fertile tillers per plant to minimize the biomass in infertile tillers, together with increasing the number of fertile florets per ear, and high potential grain weight.

2.3 Crop development

Wheat can be classified as spring or winter wheat based on development responses to cold temperature. Winter wheat needs up to ca. 8-12 weeks of cold temperature (4–10°C) before being able to initiate spikelets, whereas vernalization is not required for spring wheat (Snape *et al.*, 2001, Distelfeld *et al*., 2009, Kamran *et al.*, 2014, Zikhali *et al.*, 2014). Distelfeld *et al.* (2009) reported that the vernalization (*Vrn)* genes controls the transition from the vegetative to the reproductive phase in response to temperature. In the UK, winter wheat is typically planted in the autumn, generally between September and November. It is harvested by July-August. Fig. 2.2

illustrates the different developmental stages determining grain yield and yield components in wheat.

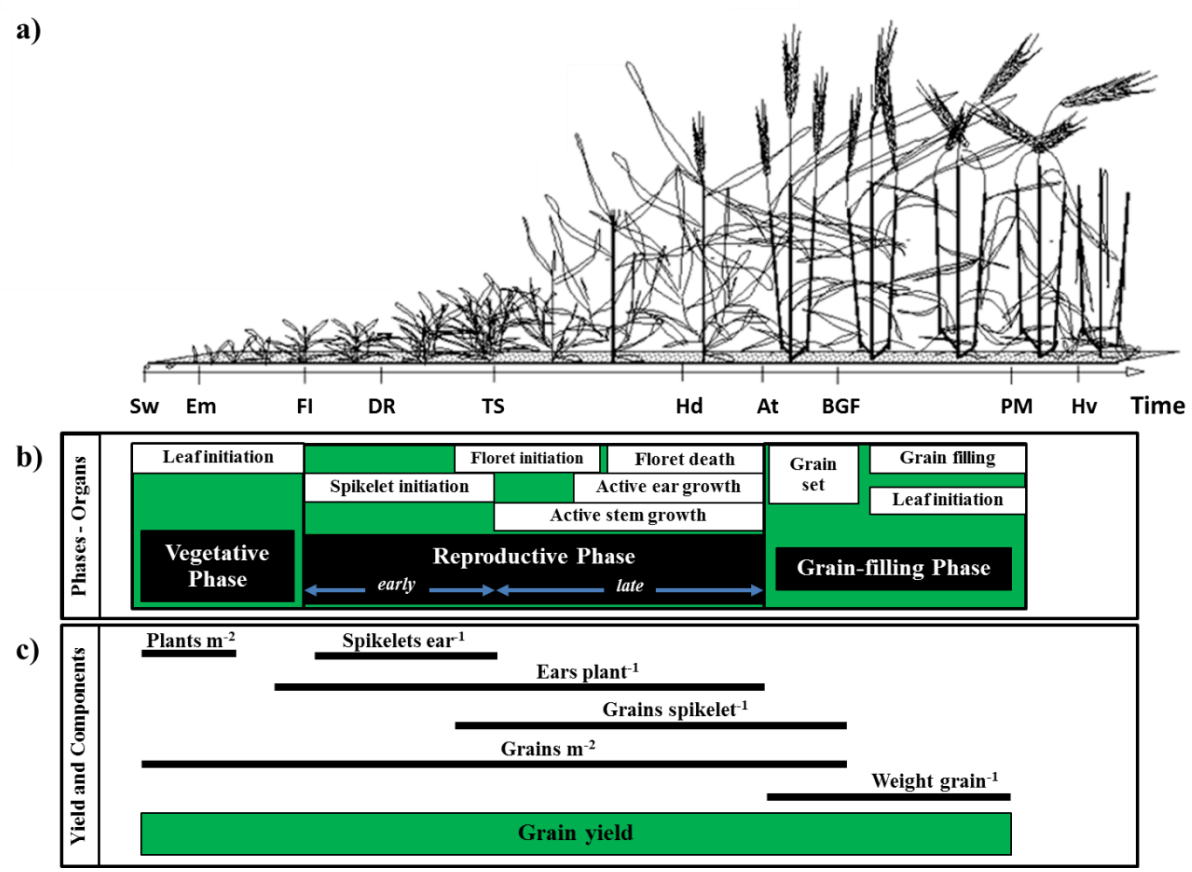

Fig. 2.2 a) Schematic diagram of wheat growth and development showing the stage of sowing (Sw), seedling emergence (Em), initation of double ridge (DR), initiation of terminal spikelet (TS), heading time (Hd), anthesis (At), beginning of grain filling period (BGF), physiological maturity (PM) and harvest (Hv), b) major phases of wheat development and c) scheme representation of grain yield components at different developmental stage. (Adapted from (Slafer and Rawson, 1994) and (Sreenivasulu and Schnurbusch, 2012))

In wheat, grain number per unit area is mainly related to the number of fertile florets that survive to anthesis; growth during the late stem-elongation phase is essential to influence the number of surviving florets and grains (Fischer, 1975, Fischer, 1985). Generally, a rapid growth rate at this phase leads to high competition for limited resources between ears and stems and results in the death of florets (Kirby, 1988). The stem-elongation phase starts at GS31 and generally coincides with the terminal spikelet stage, and extending the duration of this phase from TS to anthesis could

result in high fertile florets per ear. Miralles *et al.* (2000) and González *et al.* (2003b) found that there was a strong correlation between the number of fertile florets and ear dry weight at anthesis. Increasing ear DM partitioning at anthesis during a longer stem elongation phase without altering the flowering time could reduce assimilate competition and improve wheat yield by increased floret fertility (Miralles *et al.*, 2000, González *et al.*, 2003b).

Vegetative and stem elongation periods in wheat and barley can be modified by photoperiod sensitivity (Slafer and Rawson, 1996). Whitechurch *et al.* (2007) reported that variation in the duration of the GS31-GS61 phase was associated with changed sensitivity to photoperiod while variation in the duration of the previous phase from sowing to GS31 was related to both photoperiod and vernalization sensitivity. Miralles *et al.* (2000), who investigated the duration of the stem-elongation phase determining floret fertility in wheat and barley, found that shorter photoperiods decreased the rate of floret development while increasing the time to reach the fertile floret stage, consequently improving floret fertility by extending the duration of the late reproductive phase. Slafer *et al.* (2001) suggested that an increased stem-elongation period not only reduced assimilate competition but also increased above-ground DM growth to enhance yield potential in wheat.

Flowering time is one of the most primary traits in crop development in order to determine yield and yield components. The flowering time is mainly controlled by three groups of genes; the vernalization (*Vrn*) genes, photoperiod response (*Ppd*) genes and earliness per se (*Eps*) genes (Snape *et al.*, 2001, Distelfeld *et al.*, 2009, Kamran *et al.*, 2014, Langer *et al.*, 2014). The vernalization determines growth habit of wheat (winter and spring) while the photoperiod response classifies the crops into photoperiod-sensitive and photoperiod-insensitive classes (Distelfeld *et al.*, 2009). The UK wheat varieties are mostly winter and photoperiod sensitive wheats (Griffiths *et al.*, 2009) and the major genes regulating the response of vernalization (*VRN-1*) and photoperiod (*Ppd-1*) are fixed (Zikhali *et al.*, 2014). In addition, earliness *per se*

(*Eps*) genes have been found to influence flowering time of wheat in many studies. The *Eps* genes affect flowering time independently of vernalization and photoperiod response (Kamran *et al.*, 2014, Langer *et al.*, 2014, Zikhali *et al.*, 2014) and are involved in the fine-tuning of ear emergence time within mega-environments (Griffiths *et al.*, 2009)., i.e. agroecological zones based on their climatic characteristics.

2.4 Tiller production and survival

Tiller production and survival is one of the most important physiological processes in wheat crops because final tillers per plant determines ears per plant which is closely related to grain number determination. It is under strong genotypic regulation but is affected by the environment (Spielmeyer and Richards, 2004, Duggan *et al.*, 2005b). Optimal tiller number per plant is very important to determine grain yield. Extreme high tillers per plant could result in yield decrease due to high tiller abortion and poor grain setting before reaching physiological maturity (Davidson and Chevalier, 1990, Kebrom *et al.*, 2012) whereas small tiller numbers can lead to yield loss because of reduction in radiation interception per plant.

Tillers are lateral shoots or branches that grow from the axils of leaves at the base of the main stem. Tiller numbers increase rapidly during the first few weeks after seedling emergence and tillers can be produced until the start of stem elongation. Some smaller and later-formed tillers tend to die first between the start of stem extension and flowering. Consequently, most surviving tillers at flowering form ears and will bear grain. Kemp and Whingwiri (1980), determining the effects of tiller removal at early reproductive phase (between DR to TS) under irrigated field conditions, concluded that the competition for assimilate supply amongst tillers during floret development limited grain yield on the main shoot. Berry *et al.* (2003) reported that the economy of tillering (the ratio of grain-bearing shoots to total shoot number) can be determined by the pattern of development. For example, cultivars

with high vernalization and photoperiod requirements tend to produce high tiller number (Kirby, 1994). In addition, cultivars with more erect flag leaf attitude are likely have greater tiller survival (Foulkes *et al*., 1998).

The appearance and survival of tillers is strongly affected by the density of plants through the response to light quality (Evers *et al*., 2006). Simon and Lemaire (1987) reported that tillering appeared to cease earlier at the high plant density because the photosynthetically active radiation (PAR) density inside the canopy is decreased. Moreover, increase in plant population density is associated with a lower red light (R) to far-red light (FR) ratio which reduced the phytochrome photoequilibrium (Pr/Pfr) and led to tiller reduction (Holmes and Smith, 1977, Smith, 2000, Evers *et al.*, 2006). Sparkes *et al.* (2006) suggested that the initiation of tiller death is closely related to a critical red/far red light quality ratio (R/FR). Furthermore, Kebrom *et al.* (2012) proposed that plants respond to this light competition signal by suppressing axillary bud outgrowth, extending the stem and flowering earlier to avoid the effect of shade.

2.5 Floret fertility

Grain number per ear is one of the most important physiological traits to determine wheat yield. As modern cultivars have high grain set with more than 90% of fertile florets at anthesis set grains (Siddique *et al*., 1989a, González *et al*., 2003a, González *et al*., 2005a), the grain number is mainly determined by floret fertility at anthesis. According to González *et al.* (2005a), grain number per m^2 depends on the number of fertile florets produced per m^2. Around 6-11 floret primordia are initiated in each spikelet depending on its position on the ear (González *et al.*, 2005a). However, most of these primordia degenerate or die and about 3-5 surviving florets may develop completely at the flowering stage (Kirby, 1974, Fischer, 1984, González *et al.*, 2005a). Moreover, González *et al.* (2005a) suggested that the survival of floret primordia is mostly controlled by the assimilation supply to the ear from GS41 to

GS61. Bingham (1969) suggested that increased partitioning of photoassimilates to ear growth will increase the rate of floret survival.

2.6 Radiation interception and radiation-use efficiency

2.6.1. Radiation interception

Crop growth is influenced by the ability of the canopy to intercept incident radiation, which is the function of leaf area index and canopy architecture, and convert it into accumulated biomass (Gifford *et al*., 1984). Thus, biomass can be considered as the product of the photosynthetically active radiation intercepted by the crop accumulated during the period analysed (IPAR) and the efficiency with which the canopy converts that radiation into above-ground biomass (radiation-use efficiency, RUE) (Acreche *et al.*, 2009).

Final grain yield derives mainly from post-anthesis accumulation and translocation of carbon and nitrogen assimilates to the grains (Przulj and Momcilovic, 2001a, Przulj and Momcilovic, 2001b). Kipp *et al.* (2014) report that grain yield and grain protein concentration (GPC) are strongly affected by photosynthetic activity as determined by green leaf area and the duration of the post-anthesis phase. Therefore, further increasing, the duration of leaf greenness and maintaining the supply of assimilates may help to increase crop biomass and yield potential.

Senescence is viewed as a genetically programmed and environmentally influenced process leading to chlorophyll destruction and mineral nutrient remobilization to younger or reproductive parts of plants (Vijayalakshmi *et al*., 2010). In wheat, the onset of flag-leaf senescence is initiated by a remobilization of phloem-mobile nutrients from the vegetative tissues to developing sinks e.g. grains (Distelfeld *et al*., 2014). Zhenlin *et al.* (1998), studying the effects of source-sink manipulation on post-anthesis photosynthesis and grain set in winter wheat, reported that source reduction accelerated senescence and decreased the net photosynthetic rate (P_N) of

winter wheat after anthesis, whereas sink reduction delayed the senescence and increased the P_N.

The stay-green trait refers to delayed senescence during grain filling period (Thomas and Howarth, 2000). Crops with this phenotype maintain green leaf area longer after flowering than the senescent genotypes. Thomas and Howarth (2000) reported that delaying the start of senescence by two days led to an 11% of increase in carbon fixation by the plant in sorghum.

The stay-green trait can be further classified into two groups, functional stay green and non-functional stay green (cosmetic stay green), (Thomas and Howarth, 2000). The functional stay-green plant has an ability to photosynthesize for a longer grain filling period and the non-functional one has an ability to stay green withholding chlorophyll but lacking photosynthetic competence. The functional stay-green phenotype showed a positive correlation with grain yield in wheat in many studies under abiotic stress (Verma *et al*., 2004, Christopher *et al.*, 2008, Bogard *et al.*, 2011, Lopes and Reynolds, 2012). A negative relationship with yield has also been observed (Jiang *et al*., 2004, Kichey *et al*., 2007, Derkx *et al.*, 2012).

2.6.2. Radiation-use efficiency

Crop biomass production is determined by the amount of photosynthetically active radiation intercepted by the canopy and the efficiency with which radiant energy is used to produce biomass (Monteith and Moss, 1977). Radiation-use efficiency (RUE) is defined as the amount of above-ground biomass produced per unit intercepted radiation (IR) by the canopy (g MJ^{-1}) (Stockle and Kemanian, 2009). RUE in wheat under favourable conditions ranged from 1.09 to 1.30 g DM MJ^{-1} based on solar radiation interception in three tall and semi-dwarf cultivars (Siddique *et al*., 1989a) and 1.52 to 2.40 g DM MJ^{-1} based on PAR interception in a range of old and modern wheat cultivars (Gregory *et al.*, 1992, Yunusa *et al*., 1993). Modern wheat cultivars had higher RUE than older ones (Siddique *et al*., 1989a, Yunusa *et al*., 1993).

However, Calderini *et al.* (1997) found in Argentina that old wheat varieties converted intercepted radiation to biomass more efficiently than modern cultivars pre-anthesis. Miralles and Slafer (1997) reported that decreased internode length in modern cultivars was related to lower pre-anthesis RUE associated with poorer canopy architecture and radiation distribution.

Radiation-use efficiency can be affected by traits at the level of the canopy, single leaf and Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco). Canopy architecture plays an important role in the photosynthesis activity processes. Vertical leaves enable more light to penetrate to lower leaf levels and more light to be reflected to the canopy than to the atmosphere, resulting in optimal canopy photosynthesis (Duncan, 1971). The decrease in post-anthesis RUE results from decrease in photosynthetic activity as the leaves decline with leaf aging (Gallagher and Biscoe, 1978). Senescence also has a negative effect on the net assimilation rate. Green (1989) suggested that since the carbon assimilates are needed for the growing grains, senescence of slower leaves is increased to maintain the photosynthetic activity. Senescence was often slower in erect-leaved cultivars, a phenomenon which may depend on illumination of the lower leaves (Monteith and Elston, 1983).

Rubisco is the major enzyme related to the photosynthetic activity. It has been suggested that modification of the CO_2 fixation enzyme Rubisco by increasing its carboxylase activity and decreasing its oxygenase activity could result in RUE improvement (Austin, 1999). Several approaches to enhance Rubisco activity have been proposed by Parry *et al*. (2011), such as increasing the amount of Rubisco protein in the chloroplast, identifying a Rubisco with a higher catalytic rate, maintaining Rubisco activity, faster RuBP (ribulose bisphosphate) regeneration, increasing CO_2 at the Rubisco catalytic site and reducing the photorespiration or increasing the probability of recapturing CO_2 released in the photorespiratory pathway.

2.7 Agronomic improvement of ear fertility and yield

Wheat yield increased during the second half of the twentieth century due to improved genotypes with higher yield potential and lodging resistance and high application in N fertilizer (Erisman *et al*., 2008). Results from several studies showed that grain yield can be affected by N application depending on time of application. For example, with applications at the end of tillering improving tiller survival and ears ((Sarandón and Gianibelli, 1992) and application at flowering increasing individual grain weight and grain protein content (Strong, 1982). Demotes-Mainard *et al.* (1999) investigating ear DM and N accumulation before anthesis under various N nutrition conditions, found a strong positive correlation was detected between ear N content and ear DM at anthesis, resulted in high grains ear^{-1}. Peltonen (1992) suggested that nitrogen fertilizer application at the double-ridges stage can increase the number of grains per ear and spraying nitrogen at pollination also increased grain weight and bread-making quality in spring wheat. Sinclair and Jamieson (2008) suggested that grain number can be determined as a consequence of N and C accumulation during the pre-anthesis phase, and not solely assimilate supply to the ear. Currently, grain yield stability has become an important target trait with climate change increasing the frequency of stress environments and due to N fertilizer becoming more expensive; there are associated environmental concerns of the impacts of N of global warming through the release of N_2O by soil bacteria, so improving N-use efficiency (NUE; grains DM yield/ N available from soil and fertilizer) will be important for reducing excessive input of fertilizers whilst maintaining an acceptable yield. Raising NUE, defined as grain DM yield divided by nitrogen supply from the soil and fertilizer, relates to gains in yields and/or reductions in nitrogen uptake (Gaju *et al.*, 2011). For future genetic increase in biomass, improved uptake of nitrogen will likely be required, e.g. via deeper rooting.

In addition, increased spikelets per ear could be related to environment factors e.g. temperature and light intensity. The number of spikelets and fertile spikelets can be increased by low plant density in the period between double ridges formation and ear emergence (Puckridge, 1968). In addition, the number of spikelets is influenced by day length (Wislocka, 1959), nitrogen supply (Single, 1964), temperature and light intensity (Friend, 1965).

2.8 Genetic improvement of yield potential

2.8.1 Trends in wheat yield potential

Gains in wheat yield potential were reported worldwide in both spring and winter wheat. In spring wheat rates of yield gain from historic experiments have been observed over the period from 1950 to 2009. In NW Mexico, genetic progress for seven cultivars released from 1950 to 1982 was 59 kg ha^{-1} yr^{-1} (1.1% yr^{-1}) (Waddington *et al*., 1986) while for eight short cultivars released between 1962 and 1988, it was 67 kg ha^{-1} yr^{-1} (0.88% yr^{-1}) (Sayre *et al*., 1997). Perry and D'Antuono (1989) reported the annual genetic gain for 28 cultivars released in Australia from 1884 to 1982 was 5.8 kg ha^{-1} yr^{-1} (0.57% yr^{-1}). In Western Australia, Siddique *et al*. (1989a) examining ten cultivars released between 1960s and 1986 reported that yield of the most recent cultivar (released in 1986) increased by 63% compared to the oldest ones (released in 1860s). In North Dakota, USA, genetic progress of 30.4 kg ha^{-1} yr^{-1} of grain yield (1.3% yr^{-1}) was observed in a linear regression of 33 cultivars released from 1968 to 2006 (Underdahl *et al.*, 2008). In NW Mexico, recent increases in yield potential in CIMMYT cultivars of 0.6% $year^{-1}$ released between 1966 and 2009 have been associated with increases in above ground dry matter (AGDM), potential grain weight (PGW) and final grain weight (Aisawi *et al.*, 2015).

Similarly, rates of yield genetic progress from historic studies have been carried out worldwide from 1830 to 2010 in winter wheat. Grain yield progress of 0.44% yr^{-1} in the USA was observed in 14 genotypes released in 1873 from 1990s (Donmez *et al.*,

2001). Between 1874 and 1987, grain yield of 35 hard red winter wheats in Kansas, USA was increased by 16.2 kg ha^{-1} yr^{-1} (Cox *et al*., 1988). The genetic progress in winter wheat in France was 49 kg ha^{-1} yr^{-1} in 14 cultivars released from 1946 to 1992 (Brancourt-Hulmel *et al*., 2003) while genetic gain in yield of 15 cultivars released from 1940 to 2000 in Iran was 66.8 kg ha^{-1} y^{-1} (0.86% yr^{-1}) (Miri, 2009). In China, Zhou *et al.* (2007) found the range of genetic gain in grain yield for 47 cultivars in different areas released between 1960 and 2000 was 32.1 to 72.1 kg ha^{-1} yr^{-1} (0.48 to 1.23%) with a mean of 64.27 kg ha^{-1} yr^{-1} (1.23% yr^{-1}), while Wu *et al.* (2014) calculating data from 1945 to 2010, reported that genetic gain in wheat yield across different zones in China ranged from 20 to 103 kg ha^{-1} $year^{-1}$ with a mean of 0.33 to 1.42% yr^{-1}. In the UK, Austin *et al.* (1989) comparing 13 winter wheat varieties introduced from 1830 to 1986, found that the most current cultivar 'Slejpner' gave 80% more grain yield with a 56% increase in HI compared to the oldest cultivar 'Squarshead Master'. Shearman *et al.* (2005) reported the genetic progress in grain yield for eight cultivars released in the UK from 1972 to 1995 was 120 kg ha^{-1} yr^{-1} (1.2% yr^{-1}).

2.8.2 Effects of the Green Revolution and introduction of semi-dwarf genes

The Green Revolution in the mid-to-late 20th century associated with the introduction of high-yielding semi-dwarf varieties of wheat as well as management techniques in fertilizer and pesticides, has resulted in raising wheat yields. The introduction of semi-dwarf genes such as *Rht-B1b* and *Rht-D1b* in the 1960s and 1970s not only reduced stem height and reduced lodging but also led to a greater proportion of assimilate partitioned into the ear, resulting in greater ear growth, more grains per unit land area and higher HI (Fischer and Stockman, 1986, Hedden, 2003).

The introduction of *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) semi-dwarfing genes which confer insensitivity to gibberellin results in a less-extensible cell wall (Keyes *et al.*, 1990) and a less elongated cell unchanged in cell number (Keyes *et al*., 1989, Hoogendoorn *et al.*, 1990) compared to standard height (*Rht-B1a* and *Rht-D1a*, *rht*) genotypes. The reduction of cell size contributes to a decrease in the length of coleoptile and seedling vigour (Keyes *et al*., 1989, Hoogendoorn *et al*., 1990, Botwright *et al*., 2005, Rebetzke *et al*., 2011). Moreover, alternative semi-dwarfing genes known as gibberellic acid (GA) sensitive (*Rht8*, *Rht9*, *Rht13*) genes have been identified conferring shorter plant height and greater harvest index without decreasing coleoptile length (Ellis *et al*., 2004, Rebetzke *et al*., 2011). Rebetzke and Richards (2000) suggested that stem height reduction with greater coleoptile length and more seedling vigour is promising for wheat improvement in dry Australian environments.

Many studies demonstrated genetic gains in wheat yield associated with *Rht-B1b* and *Rht-D1b* gibberellin-insensitive dwarfing genes which have been used widely in breeding programs (Flintham *et al*., 1997, Hedden, 2003, Butler *et al.*, 2005). The presence of *Rht* (gibberellin insensitive) alleles resulted in a 20% reduction in plant height, decrease in biomass, but greater HI, and yield increases (Flintham *et al.*, 1997). Semi-dwarf recombinant inbred lines (RILs) with *Rht1* gene increased grain yield, tiller number, and grains per ear compared to the tall lines (*rht*) but tall lines had heavier grain weight and higher grain protein than semi-dwarf lines (McClung *et al*., 1986). Wheat cultivars with combinations of semi-dwarf genes showed different reductions in plant height: short (*Rht1* and *Rht2*), semi-dwarf (*Rht1* or *Rht2*) and tall (*rht*), with greater HI values (41.4, 38.0 and 31.6, respectively) (Allan, 1983). Borrell *et al.* (1991) observed that near-isogenic spring wheat lines possessing the *Rht1* gene had more grains ear^{-1} whilst lines with *Rht2* gene were associated with higher ears plant^{-1}, both lines showed increased assimilate supply to the developing ear at the stem elongation phase compared to standard height (*rht*). Some studies

showed that recombinant inbred spring wheat lines carrying the *Rht1* gene had higher yield than those with the *Rht2* gene under the fully irrigated conditions e.g. Butler *et al.* (2005). However, Mathews *et al.* (2006) argued that although grain yield in semi-dwarf near-isogenic lines for *Rht1* and *Rht2* was higher under less stressed conditions, both semi-dwarf and standard near-isogenic lines showed similar adaptive performance under drought environments.

Overall, introduction of the semi-dwarfing genes was largely impacted through the increase in HI. The theoretical maximum HI for wheat was estimated as 0.62 (Austin, 1980) and as 0.64 (Foulkes *et al.*, 2011). Foulkes *et al.* (2007) demonstrated that above-ground dry matter (AGDM) at harvest was strongly associated with genetic yield progress since ca. 1990 in UK winter wheat. Strategies with regard to plant components (roots, leaves, stems and infertile tillers) to enhance assimilate partitioning to grains were predicted to increase HI to the theoretical maximum (Fischer, 1985, Kirby, 1988, Foulkes *et al.*, 2011).

2.8.3 Physiological traits associated yield progress in wheat

Cultivars released in the last decades have been compared for yield and yield components and physiological traits associated with genetic improvement to better understand yield-limiting factors. Several studies suggest that increases in HI and grains per unit land area are mainly related to genetic gain in spring wheat. For example, amongst the spring wheat cultivars released from 1940 to the 1970s in the Mediterranean area of Spain, grain yield was positively correlated with improved HI and grains m^{-2} but not correlated with grain weight and biomass production (Acreche *et al*., 2008). Waddington *et al.* (1986), studying the yield potential of spring wheat cultivars released in NW Mexico from 1950 to 1982, found gains in yield potential associated with grains m^{-2} and higher C assimilation during a longer duration of the stem-elongation phase. Perry and D'Antuono (1989) reported that yield progress in Australian wheat from 1860s to 1982 was positively correlated with HI, grains ear^{-1}

and grains m^{-2}. Siddique *et al.* (1989a) detected genetic yield progress and HI increased systematically from old to modern wheat cultivars released from 1960s to 1986 in Western Australia. Yield potential progress in semi-dwarf bread wheat in NW Mexico (1962-1988) was associated with HI and grains m^{-2} but not correlated with AGDM, grain weight and ear m^{-2} and grains ear^{-1} (Sayre *et al.*, 1997). In the same experiments, Fischer *et al.* (1998) reported that yield potential in semi-dwarf spring cultivars released in NW Mexico between 1962 and 1988 was associated with increased flag-leaf stomatal conductance, flag-leaf photosynthetic rate and canopy temperature depression (CTD), but not with flag-leaf area and specific leaf weight. Moreover, Abbate *et al.* (1998), for six Argentine semi-dwarf awned spring wheat cultivars released from 1980s until early 1990s, reported that enhancement in fruiting efficiency led to increase in grain number m^{-2} rather than an increase in ear dry matter partitioning. In the findings of Lopes and Reynolds (2012), stay-green calculated as an estimation of NDVI (normalized difference vegetation index) at physiological maturity and the rate of senescence had positive and negative correlations with yield, respectively, under heat and heat combined with drought environments. The genetic improvement of yield potential in CIMMYT cultivars released between 1966 and 2009 was associated with increased potential grain weight and final grain weight (Aisawi *et al*., 2015).

In winter wheat, results from many studies on sets of historic cultivars showed that genetic progress in yield was associated with increment in HI, above-ground biomass production, grain number per unit area and decreased plant height. Modern cultivars showed large increases in HI due to the presence of the semi-dwarf genes. For example, Austin *et al.* (1980), who evaluated the increase in winter wheat yield in the UK from 1908 to 1978, reported grain yield was strongly related to HI. Austin *et al.* (1989) compared 13 winter wheat varieties representing very old, old, intermediate and modern groups introduced from 1830 to 1986 and found that increased grain yield of modern varieties was associated with a trend for higher

above-ground biomass and reduced plant height. Grain yield progress of winter wheat in the Great Plains of the USA was associated with increased grains ear^{-1} and ears m^{-2} but not grain weight (Donmez *et al*., 2001). Decrease in plant height was correlated with genetic gains in winter wheat cultivars in France during the second half of the 20th century (1946-1992) and improved HI and more grains m^{-2} but there was no modification of grain weight (Brancourt-Hulmel *et al.*, 2003). In Iran, yield progress in winter wheat cultivars released from 1940 to 2000 was associated with HI, grains ear^{-1}, flag-leaf transpiration rate, flag-leaf stomatal conductance and reduced plant height (Miri, 2009). Acuña *et al.* (2011) suggested that increased grains ear^{-1} was the main factor to improve grain yield in the high-rainfall zone (HRZ) of southern Australia (1989-2009). Furthermore, Pedro *et al.* (2011) suggested that yield improvement was more closely correlated to above-ground biomass than HI and increased ear DM partitioning at anthesis resulted in more grains m^{-2}. In UK breeding, the genetic gains in winter wheat grain yield resulted from the improvement of growth rate during the pre-anthesis period leading to an increase in grain number per m^2 (Austin *et al*., 1980). In addition, genetic progress in yield of UK winter wheat was associated with not only increasing HI values and accumulated above-ground biomass at harvest, but also a larger source for grain filling via improving radiation-use efficiency (RUE) and consequently greater water soluble carbohydrate reserves in the stem (Shearman *et al*., 2005). Overall, increases in HI have started to level off since the 1990s, and there is evidence that most of the recent gains have come from enhanced biomass. There is some evidence that pre-anthesis RUE may be increasing the grains sink strength and reducing the extent of sink limitation in modern cultivars via source/sink manipulation treatments applied at anthesis (e.g. Reynolds *et al.* (2005) and Acreche and Slafer (2009)).

In China, improvement in HI and above-ground biomass, as well as a decrease in stem height, resulted in increased grain yield of cultivars released since 1945 (Wu *et al.*, 2014). Amongst CIMMYT spring wheat cultivars released from 1966 to 2009,

genetic gain in yield was related to increase in above-ground dry matter (AGDM) at harvest and high grain weight associated with longer rachis length per spike (Aisawi *et al.*, 2015). Therefore, enhancing biomass accumulation whilst maintaining the HI is an essential approach to increase yield potential in the future (Foulkes *et al*., 2007).

Most studies on historic sets of bread wheat cultivars showed no significant association between individual grain weight and grain yield. Khanna-Chopra and Viswanathan (1999) suggested that grain weight is more related to grain yield stability than grain number per m^2. *Cox et al.* (1988) reported that the genetic gain in yield of hard red winter wheat cultivars in Kansas released from 1874 to 1987 was associated with an increase in thousand grain weight (TGW) and a decrease in days to heading and plant height. Yield improvement in hard red spring wheat (HRSW) cultivars released by North Dakota State University (NDSU) since 1968 was also associated with enhance in grain weight and lodging resistance (Underdahl *et al*., 2008). Sadras and Lawson (2011) reported that grain yield of South Australian wheat varieties was positively related to a 2-fold increase in grain size (since 1982) in HI over the year of release (1958-2007) and ear DM (since 1980) with grain number increasing until 1980. Their results also suggested that grain number was related to crop growth rate in the pre-anthesis phase while grain size was correlated with crop growth rate per grain. Across different zones in China, a significant positive correlation was found between grain weight and wheat yield with year of release from 1949 to 2010 in spring and winter wheat (Wu *et al.*, 2014). In order to improve understanding about raising yield potential, Bustos *et al.* (2013), who observed spring DH lines derived from across between contrasting for high grain number m^{-2} and high grain weight, found that yield was strongly related to above-ground biomass and grains m^{-2} but not to grain weight. Aisawi *et al.* (2015) investigating the physiological basis of genetic progress in grain yield in CIMMYT spring wheat, found a positive association between grain yield and increased AGDM at harvest and heavier

grain weight and a linear increase in pre-anthesis flag-leaf stomatal conductance with year of release from 1966 to 2009. The presence of dwarfing genes and the 1B/1R translocation in the Northern China Winter Wheat Region from 1960 to 2000 led to improved grain yield with increased HI and grain weight ear^{-1} but reduced plant height (Zhou *et al*., 2007). According to Slafer (2003), improved understanding of physiological traits determining yield potential could provide a better rationale for breeders to use candidate physiological traits as selection criteria.

2.8.3.1 DM partitioning

Dry matter partitioning is the allocation of assimilate produced by source organs via a transport path to the sink organs (Marcelis, 1996). Many studies have reported that the proportion of assimilates partitioned to the ear at anthesis was positively associated with an increase in grains m^2. In the investigation of Slafer *et al.* (1990), ear dry weight at anthesis and ear-dry-weight: ear partitioning index (EPI) was closely related to HI and grains m^{-2}. Increase in HI with less stem assimilate partitioning has been reported, e.g. Austin *et al.* (1980) reported that the increase of HI in UK cultivars released between 1908 to 1978 mainly resulted from a decrease in stem dry weight and these authors argued that a great increase in HI could be achieved by decreasing stem and leaf sheath partitioning further at harvest while maintaining the above-ground biomass. On the other hand, Abbate *et al.* (1998) reported that in the six Argentine semi-dwarf awned spring wheat cultivars from 1980s until early 1990s, yield improvement was associated with the increase of fruiting efficiency rather than the ear dry partitioning index at anthesis. González *et al.* (2011) reported that onset of floret death was associated with the starting time of ear growth at the maximum rate during stem-elongation and the rate of floret death was negatively related to ear weight at anthesis. However, assimilate supply to the ear during the rapid ear growth phase at pre-anthesis may also be increased in increasing ear DM partitioning at anthesis (Foulkes *et al*., 2011).

Increase in grain number m^{-2} in Argentina was associated with increased partitioning to ear before anthesis (Slafer and Andrade, 1993, González *et al*., 2003b), and in Spain was associated with ear dry weight at anthesis and the fruiting efficiency (Acreche *et al.*, 2008). In the UK, the increase in grain number was associated with ear dry weight at anthesis (Shearman *et al*., 2005). In other studies, the fruiting efficiency was not improved during the improvement of ear partitioning (Calderini *et al*., 1999b, Slafer *et al.*, 2015). However, FE is an important target for future breeding.

2.8.3.2 Potential grain weight

The potential grain weight is determined by the total number of endosperm cells which are formed at the end of the cellularization phase (Brocklehurst, 1977, Foulkes *et al.*, 2011). In addition, at the end of the cellularization phase or early in grain filling, grain weight appears to be closely associated with maximum grain water content in wheat (Millet and Pinthus, 1984, Saini and Westgate, 1999) and this grain volume showed a strong correlation with the final grain weight (Lizana *et al*., 2010). Lopes and Larkins (1993) reported that the endosperm is mainly divided into cells, and the seed coat and pericarp form the outer layers of the developing grain during early grain development. Many authors suggested that the determination of grain weight was influenced by the maternally derived ovary from which the pericarp develops, which limits grain expansion and as a result limits grain weight (Calderini *et al*., 1999a, Ugarte *et al*., 2007, Lizana *et al.*, 2010). The duration between booting and anthesis is an important period for determining grain weight, as the floret carpels are widened quickly (Calderini *et al.*, 1999a, Ugarte *et al*., 2007, Foulkes *et al*., 2011). High temperature during pre-anthesis can affect the carpel size and lead to reduced grain weight (Calderini *et al*., 1999a, Savin *et al.*, 1999, Calderini *et al*., 2001). Improving understanding of the factors e.g. temperature affecting grain

weight, grain number and the maternal tissues is required to increase potential grain weight (Foulkes *et al.*, 2011).

2.9 Genetic regulation and QTL analysis

Grain yield and yield components are complex traits, generally controlled by a number of quantitative trait loci (QTLs) and affected by multiple genes. Gene by environment interactions, make it difficult to control and improve these traits in breeding programs (Kato *et al*., 2000). However, the advance of molecular markers has potential to detect the QTLs controlling yield-related traits and the relationship between grain yield and its components. It is possible to estimate the number of loci controlling genetic variation in a segregating population of lines and to identify these loci with regard to map position, gene action, phenotypic effects, pleiotropic effects and epistatic interactions with other QTLs (Xiao *et al.*, 1996).

2.9.1 Genetic regulation of tiller production

The tiller inhibition (*tin*) gene is a single recessive gene on chromosome 1A and is linked to the hairy glumes trait, the presence of the *tin* gene results in decreased tiller number as well as a larger ear and thick leaves and stems (Richards, 1988). Donald (1968) and Duggan *et al.* (2005b) suggested that a decrease in infertile tillers may enhance grain number per ear and per m^2 associated with reduced internal competition between the developing ears and younger tillers for assimilate.

The *tiller inhibition* (*tin*) gene strongly limited tiller number in near-isogenic lines across environmental conditions and treatments in Australia (Richards, 1988, Spielmeyer and Richards, 2004). The growth of *tin* lines was found to be stunted at low temperature and after long photoperiods (Duggan *et al.*, 2002). Marshall and Boyd (1985) and Richards (1988) reported that lines containing the *tin* gene produced a few leaves with wider and longer leaves, thicker stems, and larger ears

with more grains per ear than the commercial lines. Motzo *et al.* (2004) found that the presence of the *tin* gene in bread wheat lines and high grains per ear was associated with greater spikelet fertility. Spring wheat lines possessing the *tin* gene showed a large ear phenotype and produced a high grain number per ear with an increase in grains per spikelet, spikelets per ear, rachis length and ear partitioning index at anthesis compared to the check cultivar Bacanora in NW Mexico (Gaju *et al.*, 2009). Moreover, Gaju *et al.* (2014b) found that the presence of the *tin* gene in lines of the DH population (LSP2 (*tin*) x Rialto (non-*tin*)) increased ear partitioning index (EPI) (+6.3%) and grains per ear (+13.9%), but decreased ears per m^2 (-5.7%).

The effects of *tin* on grain yield vary with different genetic backgrounds and environmental conditions. Mitchell *et al.* (2012), who determined the effect of the *tin* gene on grain yield screening in multiple genetic backgrounds, found that grain yield was decreased in Silverstar *tin* lines but unchanged in *tin* lines in other variety backgrounds (Brookton, Chara and Wyalkatchem) in field experiments in southern Australian environments. Duggan *et al.* (2005b) reported that near-isogenic lines with the *tin* gene produced fewer ears at maturity, but this was balanced by more grains per ear with greater individual grain weight than the standard lines; HI was also greater for the *tin* lines and biomass was largely unaffected. According to van Herwaarden *et al.* (1998b), in a field study in Australia *tin* lines produce more yield than check lines under terminal drought condition in part associated with an increase in water-soluble carbohydrate (WSC) in the stem. In the study of Mitchell *et al.* (2013) greater grain weight of *tin* Silvester NILs under stress conditions in Australia was related to an increase in water-soluble carbohydrate (WSC) in the stem and more biomass accumulation at anthesis. Furthermore, *tin* lines tended to be more stunted in some genetic backgrounds under long photoperiod and at low temperature limiting their application in breeding (Duggan *et al.*, 2002, Kebrom and Richards, 2013). The spikelet number per ear also influences grains ear^{-1} and spikelets per ear was increased in tiller inhibition (*Tin1A*) gene lines; increased spikelet number in

Tin1A lines may be associated with a longer thermal duration for spikelet primordia production (Gaju *et al.*, 2009).

In wheat, although a single gene responsible for tiller inhibition was mapped on chromosome arm 1AS (Richards, 1988, Spielmeyer and Richards, 2004), the underlying variation for tillering is controlled mostly by a number of quantitative trait loci (QTL) (Li and Gill, 2005). Kato *et al.* (2000) showed the presence of minor QTLs for tillering associated with the vernalization gene (*Vrn-A*) on wheat chromosome 5A. QTL located on chromosome 6AS and 1DS showed a strong effect on increasing tiller number per plant in the spring wheat cv. Opata allele (Li *et al*., 2002). The *tiller inhibition* (*tin3*) mutant producing monoculm plants contrasting with wild type plants was located on the long arm of chromosome $3A^m$ (Kuraparthy *et al*., 2007). Naruoka *et al.* (2011) suggested that high tiller number was controlled by QTL on chromosome 6B in spring wheat. Moreover, a fertile tiller inhibition gene (*ftin*) was mapped on chromosome 1AS which differed from *tin1A* and showed a decrease in tiller growth rather than tiller inhibition (Zhang *et al.*, 2013). Peng *et al.* (1998) reported another tiller inhibition gene (*Tin2*) which is a dominance gene and located on chromosome 2A.

2.9.2 Genetic regulation of ear fertility traits and grain yield

Quantitative trait loci (QTLs) for ear length, spikelet number, and compactness in wheat were mapped in a doubled-haploid (DH) population, 4 to 6 QTLs were identified on chromosome 1A, 2A, 2B and 2D with individual effects ranging from 6.9% to 21.8% of total phenotypic variation (Sourdille *et al.*, 2000) while Narasimhamoorthy *et al.* (2006) reported that seven of ten putative QTLs were located on chromosomes 2 and 3 and three QTLs were derived from a synthetic parent for grain hardness, grains ear^{-1}, and tiller number. Jantasuriyarat *et al.* (2004) found four QTLs for ear length, number of spikelets and ear compactness on chromosomes 1B (18% and 7% of the variation in ear length and ear compactness,

respectively), 4A (11%, 14% and 12% of the variation in ear length, ear compactness and spikelet number, respectively), 6A (27% of the phenotypic variance for ear compactness), and 7A (18% of the variation in spikelet number). In the study of Maccaferri *et al.* (2008), QTLs specific for plant height were identified on chromosomes 1BS, 3AL, and 7AS in a durum wheat population (RILs) in 16 environments (10 rainfed and 6 irrigated) and a further three major QTL for heading date on chromosomes 2AS, 2BL, and 7BS showed linked effects on grain yield. Zhang *et al.* (2010b) found that 12 of 55 meta-QTLs for yield and yield related traits were located on wheat chromosomes 1A, 1B, 2A, 2D, 3B, 4A, 4B, 4D and 5A, and the major genes such as *Rht* and *Vrn* affected the genetic control of yield and its components. Twelve QTLs for grain size and yield traits were detected on chromosome 2D using a mutant line induced by ethyl methane sulfonate (EMS) in wheat (Zhang *et al.*, 2015). Li *et al.* (2015) identified a number of QTLs for plant height (3 QTLs), 1000-grain weight (10 QTLs), grains ear^{-1} (8 QTLs), ears m^{-2} (7 QTLs), and grain yield (9 QTLs) under water-limited and full irrigation environments using 4 recombinant inbred lines (RILs).

The genetic analysis of grain yield and yield components on chromosome 3A was established in Nebraska in the 1980s (Campbell *et al.*, 2003). Reciprocal sets of chromosome substitution lines were developed from two hard red winter wheat cultivars, Cheyenne (CNN) and Wichita (WI); the results showed that chromosome 3A from WI in the CNN background increased grain yield by 12-15% (Berke *et al.*, 1992a, 1992b). Subsequently, a set of recombinant inbred chromosome lines for chromosome 3A (RICL-3A) was generated from a cross between CNN and CNN (WI3A) and QTLs for ears m^{-2}, GN ear^{-1}, 1000 grain weight and plant height were identified (Shah *et al.*, 1999a, 1999b). To increase QTL detection precision, Campbell *et al.* (2003) evaluated CNN(RICLs-3A) for grain yield and yield-related traits across seven locations and a number of QTLs were detected for grain yield, grain weight, TGW, grains ear^{-1}, grains m^{-2}, ears m^{-2} and plant height and located on three regions

of chromosome 3A. The major QTL for grain yield described 28% of the variation (Campbell *et al.*, 2003). To accurately identify the QTLs, a linkage map was constructed using 223 CNN(RICLs-3A) and 32 microsatellite markers; 19 QTLs for grain yield, grain weight, TGW, grains ear^{-1}, ears m^{-2}, plant height and anthesis date explained 4.6 to 16.8% of the phenotypic variation (Ali *et al*., 2011). Twelve QTLs for grain yield, grain weight, TGW, grains ear^{-1}, grains m^{-2}, plant height and anthesis date were detected describing the phenotypic variation ranging from 12% for ears m^{-2} to 53% for grain weight, using the mirror population WI(RICLs-3A) (Mengistu *et al*., 2012) and this study confirmed the QTL mapping on chromosome 3A for grain yield and yield components reported by Ali *et al.* (2011) (Mengistu *et al.*, 2012). Three novel QTLs for shoot biomass, total biomass and grains per ear were identified using a high-density composite map of wheat chromosome 3A (Rustgi *et al*., 2013). Major QTLs on chromosome 3A identified for grain yield, grains per ear, grain weight and ears per m^{-2} explained 23.2%, 24.2%, 20.5% and 20.2% of the phenotypic variation, respectively (Rustgi *et al.*, 2013).

The specific hypotheses of this study are to:

1. Novel genetic materials from large ear phenotype CIMMYT source can increase ear fertility traits, grains per unit area and grain yield per unit area
2. There is a positive relationship between genetic increase of grain yield in spring wheat and grains ear^{-1} with reduction in ears plant^{-1}
3. There is a positive relationship between genetic increase of grain yield in winter wheat and grains ear^{-1} with increase in ear fertility
4. It is possible to identify genes for increased grains per ear without a matching reduction in ears m^{-2}
5. NILs in the Ashby and Humber backgrounds (BC_3S_5 and BC_3S_6) produce higher grain yield than their respective recurrent parental lines.

6. There are significant increases amongst BC_3 lines in grains per unit area and grains ear^{-1} and associated traits compared to the recurrent parental lines.
7. Genetic variation in grain yield amongst NILs is positively associated with grains m^{-2} and above-ground biomass.
8. There is a positive correlation between fertile spikelets ear^{-1} and rachis length and grains ear^{-1} amongst the NILs and significant differences in BC_3 lines compared to the recurrent parental lines.
9. BC_3 NILs have higher ear DM partitioning index and therefore greater ear DM and more fertile florets per unit area at anthesis than their recurrent parents.
10. There is a positive correlation between grains m^{-2} and fruiting efficiency and grains per ear amongst the BC_3 NILs.
11. NILs can be identified with more spikelets ear^{-1} and more fertile florets $spikelets^{-1}$ in NILs hence more fertile florets ear^{-1} compared to their recurrent parents.
12. There is a positive correlation between fertile florets ear^{-1} at anthesis and grains per ear at harvest amongst the BC_3 NILs.
13. There is a positive correlation between EPI and FE at anthesis amongst the BC_3 NILs.
14. There is a positive correlation between grain weight and grain yield amongst the BC_3 NILs.
15. There is a positive correlation between grain weight and duration and rate of grain filling amongst the BC_3 NILs.
16. Duration and rate of grain filling is associated with post-anthesis source-type traits including NDVI, flag-leaf senescence duration and rate and flag-leaf chlorophyll content (SPAD) amongst the BC_3 NILs.
17. Grain yield is source-limited during the latter stages of grain filling so that there is a correlation amongst BC_3 lines between source-type traits under favourable post-anthesis conditions and grain weight and grain yield.

18. There is higher expression of source-type traits in BC_3 NILs than the recurrent parents Ashby and Humber.
19. The +*Tin1A* BC_3 NILs have reduced ears m^{-2} at harvest but increased GN ear^{-1}, spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length leading to higher grains m^{-2} compared to the –*Tin1A* BC_3 NILs and the recurrent parent(s) in the UK rainfed environment.
20. The +*Tin1A* BC_1S_7 NILs in the Ashby and Humber backgrounds produce fewer ears m^{-2}, but higher grains ear^{-1}, grains m^{-2} and grain yield than contrasting –*Tin1A* BC_1S_7 lines.
21. The BC_3 NILs with the allele from the CIMMYT L14 for a QTL on chromosome 3A have increased spikelets ear^{-1}, grains m^{-2} and grain yield compared to BC_3 NILs with the allele from Humber and the Humber recurrent parent in the UK rainfed environment.
22. The presence of an allele from Rialto for a QTL on chromosome 7B (WMC696) in the BC_3 NILs increases grain number per unit area compared to BC_3 NILs with the Humber and also the combined Humber recurrent parents.

CHAPTER 3 MATERIALS AND METHODS

This chapter describes the materials and methods used in the field experiments carried out at University of Nottingham farm, Sutton Bonington Campus, Leicestershire and KWS UK Ltd, Thriplow, Hertfordshire in 2012/13 and 2013/14.

Two field experiments were conducted in 2012/13 and 2013/14 at University of Nottingham farm, Sutton Bonington Campus, Leicestershire and another in 2013/14 at KWS, Thriplow, Hertfordshire. The plant materials for these experiments were 44 selected BC_3 near-isogenic lines (NILs) in spring wheat Ashby and winter wheat Humber backgrounds and the recurrent parents (Ashby and Humber). In the experiment at Thriplow, a subset of 15 BC_3 winter NILs and 34 selected BC_1S_7 NILs the in Ashby and Humber backgrounds contrasting for the presence/absence of the *Tin1A* allele and their recurrent parents together with check lines were used. In each experiment, the genotypes were assessed for yield and yield components and associated physiological traits.

3.1 Plant materials

138 doubled-haploid (DH) lines were developed jointly by Nottingham University and the International Maize and Wheat Improvement Centre (CIMMYT) from a cross between the CIMMYT large-ear phenotype spring wheat advanced line L14 and UK winter wheat Rialto using the maize-pollination technique. In collaboration with KWS UK ltd, NILs (BC_3S_5 in 2013 and BC_3S_6 in 2014, respectively) were generated from back-crosses between selected DH lines as donors and each of a UK spring elite cultivar (Ashby) and a UK winter wheat elite cultivar (Humber) as a recurrent parent and then self-fertilization for 3 generations (Fig. 3.1). In the present study, 44 promising BC_3 lines (7 spring and 37 winter BC_3 lines) and their recurrent parents (Ashby and Humber) were selected (based on preliminary phenotyping data in the

BC_3S_4 population) and used for determining yield and yield components at harvest and detailed physiological trait assessment (Table 3.1). In the BC_3 lines, backcross lines were selected by phenotyping for ear fertility traits and genotyping for *Ppd-D1b* (photoperiod sensitivity) and *Rht-B1a* (semi-dwarf plant stature) for UK backgrounds which similar to the parental genotypes for these major adaptive genes.

At KWS, Thriplow, the following genotypes were examined for yield, yield components and physiological traits:

- a subset of 15 BC_3S_6 Humber (winter) NILs
- nine pairs of BC_1S_7 NILs in the Ashby (spring) background differing in tiller number
- eight pairs of BC_1S_7 NILs in the Humber (winter) background differing in tiller number
- the check winter wheat cultivars Santiago, Belugo, Rialto and spring wheat cultivar doubled haploid (DH) line (+*Tin1A*) were examined for yield, yield components and physiological traits
- the Ashby and Humber parents

Each pair of BC_1 NILs was developed by selecting ears from contrasting plants in a single ear row established from a common BC_1S_4 ear selected by phenotype focusing on *Tin1A* characteristics including tiller number per plant and spikelets per ear. The pairs of NILs were selected to show different degrees of contrast in tiller number per plant. The degree of contrast was divided into three categories: i) high tillers per plant in the –*Tin1A* line versus low tillers per plant in the +*Tin1A* line; ii) moderate tillers per plant in the –*Tin1A* line versus low tillers per plant in the +*Tin1A* line; and iii) low to moderate tillers per plant in the –*Tin1A* line versus low tillers per plant in the +*Tin1A* line.

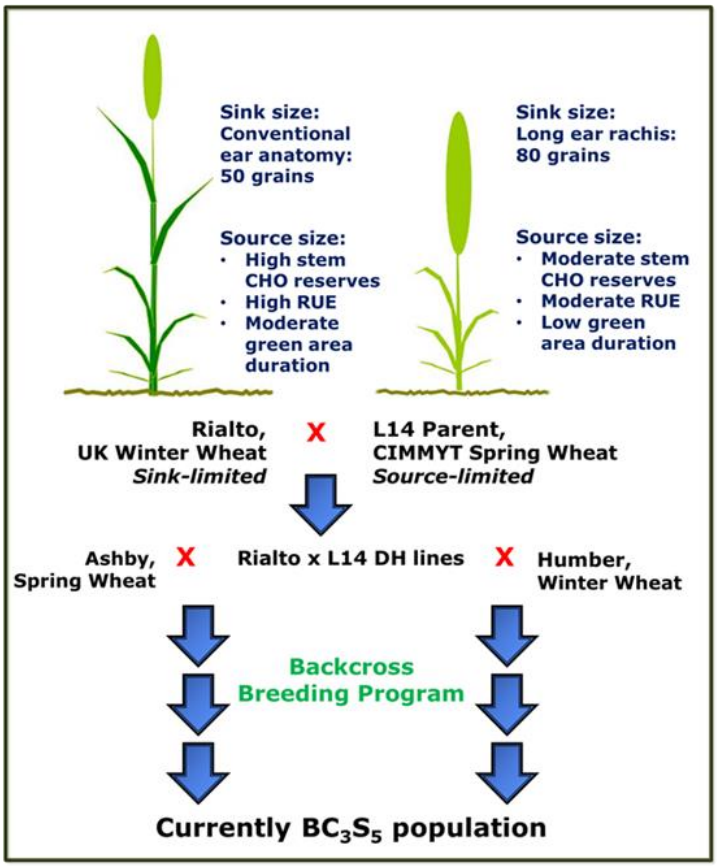

Fig. 3.1 Schematic figure of how the BC plant materials were developed.

The CIMMYT spring bread wheat advanced line L14 was developed during the 1990s from a wide crossing program including *Agropyron elongatum* L., *Triticum polonicum* L. and *Triticum aestivum* L. var. Morocco wheat (Rajaram and Reynolds, 2001). The UK winter wheat cultivar Rialto was released in 1995 and shows high expression of grains per ear, radiation-use efficiency (RUE) and stem carbohydrate reserves traits amongst UK winter wheat cultivars (Shearman *et al.*, 2005). The L14 genotype has a longer rachis, more spikelets ear^{-1} and more grains ear^{-1} than a check CIMMYT spring wheat cultivar Bacanora and has the tiller inhibition (*Tin1A*) gene on chromosome 1A (Gaju *et al*., 2014b). The L14 advanced line has a dominant allele *Vrn-A1* on chr 5A controlling vernalization response, whereas the winter wheat Rialto contains the recessive allele (*Vrn-a1*). Similarly, the L14 advanced line contains the dominant allele (*Ppd-D1a*) for photoperiod insensitivity on chromosome 2D, whereas Rialto has

the recessive allele (*Ppd-D1b*) for photoperiod sensitivity. Thus, the L14 x Rialto DH progeny segregates for spring/winter vernalization and photoperiod sensitivity/insensitivity feature (Gaju *et al.*, 2014b). For plant height, the DH population and the parents were genotyped for the two major semi-dwarfing genes (*Rht-B1b*, *Rht1* and *Rht-D1b*, *Rht2*) using perfect markers developed by (Ellis *et al*., 2002); results showed that the L14 advanced line carries *Rht-B1b* (dwarf), and the Rialto contains the *Rht-B1a* (wild type/tall) gene. Both genotypes possessed the *Rht-D1b* gene.

Ashby is a high yielding National Association of British and Irish Flour Millers (NABIM) Group 2 spring wheat which has some breadmaking potential (bred by KWS UK Ltd). It has large and robust grain, good yellow rust resistance and performance in both late autumn and spring sown dates; it was released in 2001 (HGCA, 2014). Humber is a hard wheat NABIM Group 4 winter wheat, it was released in 2007 (HGCA, 2009). This variety has high yield potential and is suited to early sowing.

In the present study, 44 NILs (BC_3S_5 in 2012/13 and BC_3S_6 in 2013/14) were derived from 7 doubled-haploid lines (UK Rialto x novel large-ear CIMMYT L14 line cross; line 12, 25, 28, 48, 78, 124 and 136). These lines were backcrossed into UK spring wheat Ashby and winter wheat Humber. Lines were selected phenotypically, except that molecular markers were used to select for the semi-dwarf *Rht-D1b/rht-D1b* and photoperiod sensitive *Ppd-D1b* backgrounds. The perfect markers for *Rht-B1* and *Ppd-D1* was used for selection (Beales *et al*., 2007, Ellis *et al.*, 2002)

Nine pairs of BC_1S_7 NILs in the Ashby background and eight pairs of BC_1S_7 NILs in the Humber background differing in tiller number were developed as described above. The lines were selected phenotypically to contrast for ± tiller inhibition phenotypes.

3.2 Field experiment sites

Two field experiments, one in each of in 2012/13 and 2013/14, were conducted at University of Nottingham farm, Sutton Bonington Campus, Leicestershire, UK (52° 49′ 59′′ N, 1° 14′ 50′′ W). A complete randomized block design was used for the field experiments with 44 BC_3 lines (7 spring and 37 winter BC_3 lines) and the two parental lines with two replicates in each year (Table A1-2 for 2012/13 and Table A1-3 for 2013/14). Duplicate plots of the parental lines were included in each replicate. The plot size was 1 x 1.2 m^2. Each plot consisted of six 1 m rows 20 cm apart: the two outer rows were discards (Ashby was used as the discard rows for the spring BC lines and Humber as the discard rows for winter BC lines) and the four inner rows were comprised of BC_3 lines (Fig. 3.2). In each year, the seed was used was that from six 'high ear fertility' ears selected visually at harvest from the preceding field cycle. The seed from two largest selected ears (based on grains per ear) in the previous harvest was sown in the 2^{nd} and 3^{rd} row, respectively, and the bulked seed from the other four ears were sown in the 4^{th} and 5^{th} rows with the same seed rate (25 seed per 1 m row). The soil type was a medium stony loam (Dunington & Heath Series) with pH 7.5, containing 67.8 mg l^{-1} P, 510.0 mg l^{-1} K, 241.0 mg l^{-1} Mg available in the top 30 cm, and 78.2 kg ha^{-1} N available in the top 90 cm.

Fig. 3.2 The field trials at Sutton Bonington Campus, The University of Nottingham for BC_3S_5 population in 2012/13 and BC_3S_6 population in 2013/14.

Fig. 3.3 The field trial at KWS, Thriplow, Hertfordshire for BC_1S_7 population in 2013/14.

The field experiment at KWS-UK Ltd in 2013/14 was carried out at field site near Thriplow, Hertfordshire, UK (52° 05′ 59′′ N, 0° 06′ 16.8′′ E), 30 m above sea level. A complete randomized block design with two replicates was used for the field experiment. The soil type was a sandy clay loam. The plot size was 1 x 7 m^2 with 8 rows 12 cm apart per plot with the one seed rate (250 seeds m^{-2}).

Table 3.1 Forty-four BC_3 lines used in the experiments.

Line No.	Ref.	Line ID	Line No.	Ref.	Line ID
1	1	Ashby*[12]	28	131	B_3S_5 29914-10-207-4[2]
2	1	Ashby*[12]	29	150	B_3S_5 29915-3-219-1*[12]
3	5	B_3S_5 29907-4-37-3*[1]	30	151	B_3S_5 29915-3-219-2*
4	6	B_3S_5 29907-4-37-4[1]	31	152	B_3S_5 29915-3-219-3*
5	19	B_3S_5 29907-12-58-2*	32	167	B_3S_5 29915-7-225-3*
6	20	B_3S_5 29907-12-58-3*	33	170	B_3S_5 29917-5-273-1*[12]
7	21	B_3S_5 29907-12-58-4*	34	171	B_3S_5 29917-5-273-2*[12]
8	25	B_3S_5 29907-13-60-2*[1]	35	172	B_3S_5 29917-5-273-3*[2]
9	32	B_3S_5 29908-1-67-3[1]	36	173	B_3S_5 29917-5-273-4*[2]
10	33	B_3S_5 29908-6-79-4*[1]	37	174	B_3S_5 29917-5-273-5*[2]
11	36	B_3S_5 29908-9-85-1*[1]	38	175	B_3S_5 29917-5-273-6*[2]
12	53	Humber*[12]	39	176	B_3S_5 29917-5-273-7*[2]
13	53	Humber*[12]	40	177	B_3S_5 29917-5-273-8*[2]
14	67	B_3S_5 29911-3-159-3*	41	178	B_3S_5 29917-5-274-1*
15	68	B_3S_5 29911-3-159-4*[1]	42	179	B_3S_5 29917-5-274-2*
16	72	B_3S_5 29911-14-171-1*	43	180	B_3S_5 29917-5-274-3*
17	73	B_3S_5 29911-14-171-2*[12]	44	181	B_3S_5 29917-5-274-4*
18	74	B_3S_5 29911-14-171-3*	45	184	B_3S_5 29917-8-282-1*
19	75	B_3S_5 29911-14-171-4*	46	185	B_3S_5 29917-8-282-2*
20	76	B_3S_5 29911-14-172-1*[1]	47	191	B_3S_5 29917-8-283-1*
21	77	B_3S_5 29911-14-172-2*[12]	48	194	B_3S_5 29917-8-283-5*
22	78	B_3S_5 29911-14-172-3*	49	195	B_3S_5 29917-8-283-6*[2]
23	79	B_3S_5 29911-14-172-5*	50	196	B_3S_5 29917-8-283-7*[1]
24	80	B_3S_5 29911-14-172-6*	51	223	B_3S_5 29923-12-406-4*
25	81	B_3S_5 29911-14-172-7*	52	229	B_3S_5 29923-14-411-1*[2]
26	82	B_3S_5 29911-14-172-8*	53	238	B_3S_5 29923-14-412-2[1]
27	83	B_3S_5 29914-5-196-1[2]			

* 44 lines including Ashby and Humber were selected to determine yield and yield components at harvest and physiological processes including crop growth analysis at anthesis, floret fertility traits (2014) and senescence traits

[1] a subset of 17 lines including 7 Ashby and 10 Humber backgrounds were selected to determine floret fertility traits in 2013.

[2] a subset of 15 lines including Ashby and Humber were selected to determine yield and yield components at harvest at KWS

3.3 Plot management in field experiments

3.3.1 Sutton Bonington

3.3.1.1 Previous cropping, sowing date and fertilizer and PGR application

In 2012/13, the field site was planted with winter oats in the previous season. The field was ploughed and pressed on 12 September and sowing date was 5 November 2012. Manganese (1.0 t ha^{-1}) was applied on 27 February 2013 while nitrogen fertilizer in the form of ammonium nitrate prill was applied in three doses: 40 kg N ha^{-1} (March), 80 kg N ha^{-1} (April) and 60 kg N ha^{-1} (May). Plant growth regulators were applied on 2 May 2013 as Chlormequat (1.4 l ha^{-1}) on 13 May as Moddus (0.2 l ha^{-1}) and on 3 June as Terpal (0.5 l ha^{-1}). In 2013/14, spring oilseed rape was planted in the previous season. The field was ploughed and pressed on 8 October and sown on 24 October 2013. The spring and winter wheat lines were sown on the same date in each year. In this year, nitrogen fertilizer in the form of ammonium nitrate prill was applied in three doses: 40 kg N ha^{-1} (May), 80 kg N ha^{-1} (April) and 60 kg N ha^{-1} (May). In addition, Human extra (1.0 t ha^{-1}) and Magnor (1.0 t ha^{-1}) were applied before anthesis. Plant growth regulators were applied on 2 April as Adjust (1.0 l ha^{-1}) and on 28 April as Adjust (0.8 l ha^{-1}) and Cleancrop Cutlass (0.2 l ha^{-1}).

3.3.1.2 Herbicide, fungicide and insecticide application

Crop protection chemicals were applied following standard management practices to minimize any effects of diseases, pests and weeds.

In 2012/13, herbicides were applied four times as: twice in October pre-drilling (Round-up 2.5 t ha^{-1}), and on 10 April 2013 (Hetra 1.0 l ha^{-1}, Charge 2.0 l ha^{-1}, Liberator 0.3 l ha^{-1} and Zeal 0.3 l ha^{-1}) and 3 June 2013 (Lorate 25 g ha^{-1}). Fungicides were applied three times on 2 May 13 (Kestrel 1.0 l ha^{-1}, Phoenix 1.0 l ha^{-1}, Flexity 0.14 l ha^{-1}) (GS30), 3 June (Brutus 1.25 l ha^{-1}, Amistar Opti 1.0 l ha^{-1}, Vegas 0.15 l ha^{-1}) (GS39)

and 26 June (Brutus 1.0 l ha^{-1}, Bravo 1.0 l ha^{-1} and Instinct 0.5 l ha^{-1}) (GS61). Insecticide was applied as: metaldehyde slug pellets (3.0 kg ha^{-1}) on 19 November 2012.

In 2013/14, herbicides were applied three times on 12 March (Hatra 1.2 l ha^{-1}, Headland charge 2.0 l ha^{-1} and Zeal 0.3 l ha^{-1}), 14 May (Traton SX 45 g ha^{-1}) and Kantor 150 ml ha^{-1}) and 16 May (Axial 0.39 l ha^{-1} and Adigor 0.75 l ha^{-1}). Fungicides were applied four times during GS30-GS65 on 2 April (Brutus 0.5 l ha^{-1}, Bravo 500 1.0 l ha^{-1} and Vegas 0.13 l ha^{-1}), 28 April (Kestrel 0.75 l ha^{-1}, Vegas 0.13 l ha^{-1} and Phoenix 0.6 l ha^{-1}), 21 May (Opera 1.0 l ha^{-1}, Phoenix 1.0 l ha^{-1} and Brutus 1.2 l ha^{-1}) and 16 June (Kestrel 0.75 l ha^{-1} and Kantor 0.15 l ha^{-1}). Insecticide was applied on 14 March as Minuet (0.1 l ha^{-1}).

3.3.2 KWS UK Ltd Thriplow

3.3.2.1 Previous cropping, sowing date and fertilizer application

At KWS, Thriplow the previous cropping was oilseed rape and the plants were sown on 15 October 2013. A complete randomized block design was used for the field experiments with 2 replicates and the soil type was a sandy clay loam. The plot size at KWS was 1 x 7 m^2 with 8 rows 12 cm apart per plot with the one seed rate (250 seeds m^{-2}). These plants were grown in breeders' trials under optimal conditions with full fertiliser, PGR and pest management protocols to optimise yield potential and minimize any effects of weeds, diseases and pests. Fertilizer applications were Multisulph 20 kg ha^{-1} and nitrogen fertilizer as Nitram in two doses 80 and 145 kg N ha^{-1}, respectively.

3.4 Crop measurements

3.4.1 Measurements at Sutton Bonington

3.4.1.1 Crop development

According to the decimal code developed by (Zadoks *et al.*, 1974) and refined by (Tottman, 1987), the growth stages (GS) were determined every 3-4 days to determine dates of GS31 (onset of stem extension), GS39 (flag-leaf ligule visible) and GS61 (onset of anthesis) in each plot for the 44 BC_3 lines and the recurrent parents (Ashby and Humber). These stages were taken as when more than 50% of the plants at GS31 and the shoots at GS39 and GS61 reached the stage.

3.4.1.2 Shoot number

Shoot number was recorded every two weeks from before GS31 until GS61. In each plot, five plants per plot were selected randomly and labelled in January. In addition, the number of fertile shoots (healthy green shoot before ear emergence and shoot with an ear after ear emergence) and infertile shoots (a yellow or senescent shoot before ear emergence or those without after ear emergence) were counted and recorded at GS39 and GS61. At harvest, the tagged plants were sampled for analysis of dry matter partitioning and yield components. In addition, the number of fertile shoots (those with an ear) was counted in a 50 cm row length in each plot 3-4 days prior to harvest.

3.4.1.3 Crop growth at GS61

At anthesis, in order to determine DM per shoot and DM partitioning, five fertile shoots per plot were sampled at random avoiding outer rows. Their fresh weights were recorded and the shoots were then separated into four components: ears, stems and attached leaf sheaths, green leaf lamina and dead leaf lamina. The green area of the lamina, stem and ear was recorded using a leaf area meter (LICOR 3100, NE, USA). Each component was

weighed separately after oven-drying at 85°C for 48 hours. The spikelet number, length of rachis and ear were recorded for each ear.

3.4.1.4 Floret fertility and ear DM partitioning at GS61

At anthesis (GS61), ears of BC_3 spring and winter wheat lines (3 randomly selected primary shoots and those shoots within 10 cm of the tallest shoot in the plot per plot) were dissected to determine the developmental stage of all florets and to count the total number of fertile florets per spikelet under a binocular microscope. The score of floret fertility and anther colour (green, yellow or white) was measured using the semi-quantitative scale established by (Waddington *et al.*, 1983) which ranged from W3.5 (stamen primordia present) to W10 or fertile floret (styles curved and stigmatic branches spread wide, pollen grains on well-developed stigmatic hairs) (Table A2). The assessment was done for all fertile spikelets down one side of the ear. Florets were considered as fertile when they were at W9.5 or more on the Waddington scale. Moreover, spikelet number and dry matter per shoot were also recorded. Lines selected to determine floret fertility were shown in Table 3.1.

3.4.1.5 Harvest assessments

Grain yield was evaluated based on the machine-harvested grain from each plot (after removing the discard rows). For yield components and HI assessment, the five tagged individual plants in each plot were sampled by cutting at ground level immediately prior to harvest and assessed. The number of fertile (those with an ear) and infertile shoots were counted for each plant. The fertile shoots were further separated into four components: leaf lamina, stem with attached leaf sheath, grain and chaff. Dry matter of crop components was obtained by weighing after drying for 48 h at 85°C. 200 grains from the seed counter (model Contador, Pfeuffer GmbH, Kitzingen, Germany) were

counted and weighed after drying for 48 h at 85°C. Harvest index (HI), above-ground dry matter (AGDM), grain dry weight (GDW), grain number (GN) ear^{-1}, GN spikelet^{-1}, spikelets ear^{-1}, thousand grain weight (TGW), and rachis length (RL) were recorded for the main shoot (main shoots previously tagged pre-GS31) and for all fertile shoots plant^{-1}. Ten ears per 5-plant sample were randomly selected to determine spikelet number, fertile spikelet number, and rachis length. Using these data, estimates of yield components and final AGDM were calculated as shown below:

- Grains yield (m^{-2}) = machine harvested grain yield adjusted to 100% DM (see 3.4.1.7)
- 1000 grain weight (g) = DM 200 grains x 5
- Grains m^{-2} = (grain yield g m^{-2} / (TGW g/1000)
- Grain No. ear^{-1} = Grain No. plant^{-1} / ears plant^{-1}
- Ears m^{-2} = (100,000 x grain yield)/(TGW x GN ear^{-1})
- AGDM (g m^{-2}) = Grain yield / Harvest index

3.4.1.6 Plant height

Plant height was measured in the laboratory by measuring height from the bottom of the stem to the tip of the ear using a wooden ruler on 3 randomly selected shoots from the 5-plant sample at harvest per plot.

3.4.1.7 Combine grain yield

After removing the outer discard rows, combine grain yield was obtained by machine harvest from an area of 1.2 m^2 in 2013 and 2014. Moisture content was measured from a sub-sample from the combined harvest grain per plot and grain yield expressed at 100%DM.

3.4.1.8 Flag leaf chlorophyll content

Leaf chlorophyll content was estimated approximately weekly from about two weeks after anthesis until complete senescence using a hand-held portable SPAD meter (SPAD-502, Konica-Minolta, Japan) (Fig. 3.4) on three randomly selected flag leaves as the average reading from three leaf positions (basal, middle and apical) of each leaf. The chlorophyll content was measured by the SPAD meter which measures relative chlorophyll content via light transmittance with compensation for differing leaf thicknesses. In 2013, the first post-anthesis SPAD reading was taken around 15-20 days after anthesis (11 July 2013) as well as in 2014 (2 July 2014). The data for SPAD were fitted to thermal time post-anthesis (GS61, base temp. 0°C) using a linear function and the slopes were taken as the SPAD senescence rates.

Fig. 3.4 The hand-held meter (model SPAD 502, Minolta, Spectrum Technologies Inc., Plainfield, IL).

3.4.1.9 Visual assessment of flag leaf senescence

In each experiment, the visual senescence scores for the flag leaf (for the five tagged plants) were observed visually using a standard diagnostic scale of percentage green area senesced from 0 (0% senesced) to 10 (100% senesced) (Fig. 3.5). Measurements were carried out in all plots in the field experiments in 2012/13 and 2013/14 once a week from GS61 until the flag-leaf was completely senesced. The flag-leaf senesced data

were fitted to thermal time (°Cd, base temperature 0 °C) from anthesis (GS61) using a logistic curve.

$$\mathbf{FLS = A + C/(1 + EXP(-B*(X - M)))}$$

FLS = flag leaf score; A and C = the lower and the differences between the lower and upper asymptotes, respectively; B = the senescence rate; M = the accumulated thermal time when the senescence rate is at the maximum; X = the accumulated thermal time after anthesis.

Onset of senescence was taken as thermal time when FLS = 1 and end of senescence when FLS = 9. Values of senescence parameters were estimated for each plot and subjected to ANOVA.

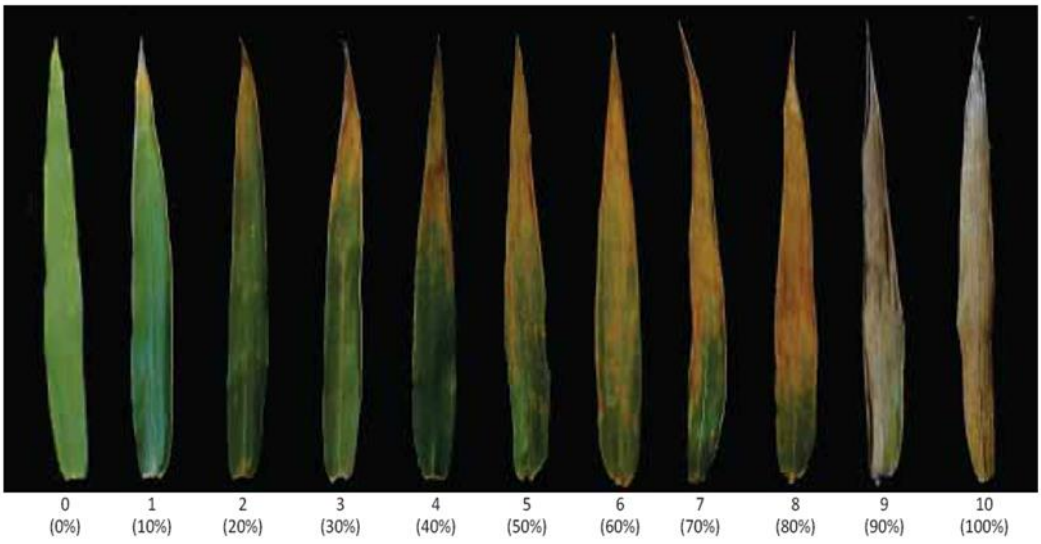

Fig. 3.5 Flag leaf senescence scale (Pask *et al*., 2012).

3.4.1.10 Flag leaf chlorophyll fluorescence

Flag leaf chlorophyll fluorescence measurements, using a fluorometer, provide an indirect estimation of the functional levels of photosynthesis such as the primary light reactions, thylakoid electron transport reactions, etc. From GS61 until harvest, chlorophyll fluorescence was measured using a hand-held fluorpen (FP-10, PSI, Brno,

Czech Republic) on three randomly selected flag leaves per plot (Fig. 3.6). Flag-leaf chlorophyll fluorescence was measured as PSII quantum yield (ϕPSII or ΔF/Fm') (Murchie and Lawson, 2013) approximately every 14d starting at approximately GS61+14d in both 2013 and 2014. The data for flag-leaf chlorophyll fluorescence were used to calculate the average of the three readings during the post-anthesis period.

Fig. 3.6 The hand-held fluorometer (model FP-10, PSI, Brno, Czech Republic).

3.4.1.11 Normalized difference vegetation index (NDVI)

The normalized difference vegetation index (NDVI) is widely used to estimate canopy green area and biomass (Babar *et al.*, 2006a, Lopes and Reynolds, 2012). At GS61 until harvest, NDVI was estimated weekly using a Green-seeker handheld crop spectrotradiometer (GreenSeeker, Trimble, OL, USA) (Fig. 3.7). NDVI is calculated from measurements of light reflectance in the red and near infrared (NIR) regions of the spectrum. A healthy green canopy will absorb most of the red light and reflect most of NIR light.

$$\mathbf{NDVI = (R_{NIR} - R_{Red}) / (R_{NIR} + R_{Red})}$$

Where R_{NIR} is the reflectance at near infrared (710-980 nm) and R_{Red} is the reflectance at red wavelength (550-670 nm). NDVI values range from -1 to +1.

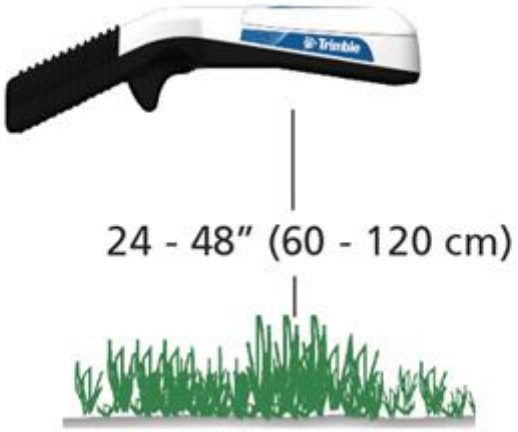

Fig. 3.7 The green seeker handheld crop spectrotradiometer (GreenSeeker, Trimble, US).

The measured data were fitted to thermal time (base temp 0°C) post anthesis (GS61) using a linear function and the slope taken as the NDVI senescence rate. Onset of senescence taken as thermal time when NDVI = 0.9 and end of senescence when NDVI = 0.1. Values of NDVI senescence parameters were estimated for each plot and subjected to ANOVA.

3.4.2. Measurements at KWS-UK Ltd

Grain yield adjusted to 100% DM was evaluated based on the machine-harvested grain harvest from the each plot. For yield components, grains ear^{-1}, 1000 grain weight, spikelets ear^{-1} and GN spikelets^{-1}, 30 ears per plot were randomly sampled at KWS, Thriplow. The number of shoots was counted in 30 x 30 cm quadrats in each plot, 3-4 days prior to harvest, two replicates. The number of spikelets per ear, grain number per ear, grain number per spikelet, 1000 grain weight, anthesis date and plant height were calculated from these data.

3.5 Molecular analysis

3.5.1. Sutton Bonington experiment

For the 44 BC_3S_5 lines and the recurrent parents (Ashby and Humber), young leaves from the five tagged individual plants per plot were sampled at GS31 in 2013. Bulks of DNA sample were extracted from leaves from 7-10 individual plants for each genotype using the DNeasy Plant Mini Kit (QIAGEN) and kept at -80°C in the freezer. DNA was also extracted from plants of the parents of the DH population, Rialto and L14 (L14 and Rialto DNA samples were from seed grown under CE conditions extracted in 2011). Bulks of DNA samples from 44 BC_3S_5 NILs and the parents (Ashby, Humber, Rialto and L14) were genotyped using 8 SSR markers linked to *Tin1A* and novel QTL for ear fertility traits, grain number, and yield on chromosome 1A, 2A, 3A, 7B and 7D identified in the DH population to validate the putative QTLs for ear fertility traits (Mayes, S. Pers. Comm.) (Table 3.2).

The PCR analysis was carried out in a final volume of 20 μl containing 10X PCR buffer with $MgCl_2$ 1.5 mM, 100 mM dNTPs, 200 μM dye-labelled M13 primer, 20 μM M13-tagged forward primer, 200 μM reverse primer, *lab Taq* DNA polymerase and 50 ng template DNA. The PCR cycle was one pre-cycle at 94°C for 3 min, followed by 40 cycles at 94°C for 1 min, at annealing temperature for 1 min (Table 3.2), and 72°C for 2 min, with a final extension for 7 min at 72°C and hold at 4°C. PCR products were checked on 2% agarose gel and the allele size compared with a 2-log ladder. The PCR products were sent to the Genomic service at School of Biosciences, University of Nottingham to run on a Beckmann CEQ 8000 machine to size accurately the microsatellite alleles. The allele size from CEQ results of BC_3 NILs was compared to the allele size of parents; Ashby, Humber, L14 and Rialto.

3.5.2. KWS experiment

For the 17 pairs of BC_1S_7 lines contrasting in tiller number plant^{-1} and the subset of 15 BC_3S_5 NILs, leaf samples were taken from 3 plants per plot for each line for DNA extraction on 12 June 2014 in the field experiment at KWS-UK Ltd. Bulks of DNA samples from both BC_1S_7 and the subset of 15 BC_3S_5 NILs were genotyped separately using 2 SSR markers linked to *Tin1A* on chromosome 1A and a novel QTL for ear fertility traits, grain number, and yield on chromosome 3A (Table 3.2). DNA was sent for analysis at KWS lab in Einbeck, Germany.

Table 3.2 Eight SSR markers linked to *Tin1A* gene and novel QTL for ear fertility traits, grain number, and yield on chromosome 1A, 2A, 3A, 7B and 7D in DH population.

Location	SSR Marker	Primers	PCR Annealing Temperature	Allele sizes (base pair)
Chr 1A (*Tin1A*)	XPSP2999 (W49)	Forward TCCCGCCATGAGTCAATC Reverse TTGGGAGACACATTGGCC	60°	123-151
	WMS136**	Forward *GACAGCACCTTGCCCTTTG Reverse CATCGGCAACATGCTCATC	60°	300-378
Chr 2A	WMS294	Forward GGATTGGAGTTAAGAGAGAACCG Reverse GCAGAGTGATCAATGCCAGA	55°	108
Chr 3A	WMC169	Forward *TACCCGAATCTGGAAAATCAAT Reverse TGGAAGCTTGCTAACTTTGGAG	55°	161-165
	WMC428**	Forward *TTAATCCTAGCCGTCCCTTTTT Reverse CGACCTTCGTTGGTTATTTGTG	55°	278-284
Chr 7B	WMC606	Forward*CCGATGAACAGACTCGACAAGG Reverse GGCTTCGGCCAGTAGTACAGGA	65°	200-207
	WMC696	Forward*ACCCGAGAGAGATTAGGGCTTG Reverse CACTCGCAGCCTCTCTTCTACC	62°	141-159
Chr 7D	WMC702	Forward *GAATCACATCGAATGGATCTCA Reverse GAGGCCTTTTTCGATATTCTGC	60°	185-214

*M13-tagged CACGACGTTGTAAAACGAC

** SSR marker analysis for samples in KWS experiment

3.6 Statistical analysis

Analysis of variance (ANOVA) and correlation and regression analysis was performed on data collected in field experiments, with year and genotype as the main factors using Genstat 16th edition. In the ANOVA genotype and years were considered as fixed effects and replicates as random effects. Unbalanced designs were used to compare between the means of recurrent parent groups (absence/presence of introgression allele) and NILs groups and between NILs groups carrying heterozygous and homozygous alleles from L14 or Rialto. The statistical analysis of spring lines was conducted separately from winter lines. All trait data were tested for normality prior to ANOVA and transformed before analysis if data were not normally distributed.

When the ANOVA indicated significant differences, the mean values of treatment were calculated and compared using the least significant difference (5%) of the means of Fisher, calculated from standard errors of the difference of the means using appropriate degrees of freedom.

Correlations among traits were calculated using the mean values of lines. Pearson's correlations were used to determine the relationship between traits in each year.

Curve fitting to calculate senescence parameters is described in Chapter 6.

CHAPTER 4 HARVEST CROP GROWTH IN BC_3 NILs IN THE ASHBY AND HUMBER BACKGROUNDS

4.1 Introduction

Yield potential is the final output of a cultivar when grown under an optimal environment with non-limiting water and nutrients and with well-controlled biotic stresses (Evans and Fischer, 1999, Foulkes *et al*., 2011). The progress in actual yield is a consequence of an increase in potential yield and/or a decrease in the exploitable yield gap (Fischer *et al*., 2014). The annual current potential yield progress of wheat in most countries is about 1.0% $year^{-1}$ (Sayre *et al.*, 1997, Brancourt-Hulmel *et al.*, 2003, Shearman *et al.*, 2005) while average worldwide yield gain is 0.5% $year^{-1}$ associated with a yield gap of approximately 40% (Fischer *et al.*, 2009). Wheat yield potential is generally sink-limited under favourable conditions with grain growth limited mainly by the number of grains formed per unit area (Foulkes *et al.*, 2011). Wheat yield improvement has been highly associated with harvest index (HI) and grain number per unit land area (GN) (Brancourt-Hulmel *et al*., 2003, Shearman *et al*., 2005, Acreche *et al*., 2008, Fischer, 2008, Foulkes *et al*., 2011). Generally, genetic progress in grain yield worldwide in the last decades was mainly associated with changes in grains per m^2 rather than in individual grain weight (Fischer and Edmeades, 2010). There is evidence that recent increases in yield potential in CIMMYT cultivars have been associated with increases in PGW and final grain weight (Aisawi *et al*., 2015).

Tiller production and survival is one of the most important physiological processes in wheat crops because it determines ears $plant^{-1}$, a key component of grains m^{-2} and grain yield. Increased partitioning of assimilates to the developing ear at anthesis has historically had the single greatest impact on improving grain m^{-2} yield potential in wheat (Foulkes *et al.*, 2011). Furthermore, the floret fertility is a key component of

grain number m^{-2} and is largely determined by floret survival during the rapid ear growth phase from GS41 to GS61 (González *et al.*, 2011).

In this study, backcross lines derived from selected high ear fertility (spikelets ear^{-1}, grains ear^{-1} and grains m^{-2}) doubled-haploid lines (UK winter wheat Rialto x large-ear-phenotype CIMMYT spring wheat advanced line L14 DH population) backcrossed three times in each of UK spring wheat Ashby and winter wheat Humber backgrounds were investigated for enhanced expression of ear fertility traits and grain yield.

The specific hypotheses tested in this chapter are:

1. NILs in the Ashby and Humber backgrounds (BC_3S_5 and BC_3S_6) produce higher grain yield than their respective recurrent parental lines.
2. There are significant increases amongst BC_3 lines in grains per unit area and grains ear^{-1} and associated traits compared to the recurrent parental lines.
3. Genetic variation in grain yield amongst NILs is positively associated with grains m^{-2} and above-ground biomass.
4. There is a positive correlation between fertile spikelets ear^{-1} and rachis length and grains ear^{-1} amongst the NILs and significant differences in BC_3 lines compared to the recurrent parental lines.

4.2 Materials and Methods

4.2.1 Plant materials

138 DH lines were developed jointly by Nottingham University and CIMMYT from a cross between the CIMMYT spring wheat large-ear phenotype L14 and UK winter wheat Rialto using the maize-pollination technique. In collaboration with KWS UK ltd, a total of 246 BC_3S_4 lines were generated from back-crosses between selected DH lines with high expression of grain m^{-2} and ear fertility traits as donors and each of a

spring elite cultivar (Ashby) and a winter elite cultivar (Humber) as a recipient. In the present study 44 promising BC_3 individuals (7 spring and 37 winter BC_3 lines) and their recurrent parents (Ashby and Humber) were selected (based on preliminary phenotyping data, Foulkes Pers. Comm.) and examined in field experiments for grain yield, yield components and associated physiological traits (for full details see Chapter 3.1 Materials and Methods).

4.2.2 Field experiments

The field experiments in 2012/13 and 2013/14 were conducted at the University of Nottingham farm, Sutton Bonington Campus, Leicestershire, UK (52° 49' 59'' N, 1° 14' 50'' W) (52.833 N, -1.247 W). Full details of the site experimental design and plot management are described in Chapter 3.3. A complete randomized block design was used for the field experiments.

4.2.3 Harvest assessments

Grain yield was evaluated based on the machine-harvested grain from the each plot (after removing the outer discard rows) adjusted to 0% moisture content. Yield components and HI assessment, were carried out on five randomly selected individual plants per plot. The grain number was counted using a seed counter (model Contador, Pfeuffer GmbH, Kitzingen, Germany).

4.2.4 Statistical analysis

ANOVA and correlation analysis using the genotype means was performed to determine the significance of effects of genotype, year and year x genotype using Genstat 16th edition. Treatment means were calculated and compared using the least significant difference (5%).

Regression analysis and Pearson correlations were calculated using the mean values across two replicates to estimate the associations between grain yield and physiological traits and between physiological traits using Genstat 16th edition (Payne *et al.*, 2009) and GraphPad Prism version 6.05 (Motulsky, 2003).

4.3 Results

4.3.1 Genetic variation in grain yield and yield components

4.3.1.1 BC_3 spring wheat NILs

The frequency distribution of the spring wheat BC_3 lines and Ashby for grain yield, yield components, harvest index, above-ground dry matter, plant height and rachis length is shown in 2013 (Figs. 4.1 and 4.2) and 2014 (Figs. 4.3 and 4.4). In 2013, positive variation above the Ashby recurrent parent was observed for HI (+13%, *P<0.10*), TGW (+24%, *P<0.10*), grain number (GN) ear^{-1} (+92%, *P<0.001*), AGDM main-shoot $(MS)^{-1}$ (+88%, *P<0.05*), grain DW MS^{-1} (+100%, *P<0.05*), MS GN ear^{-1} (+64%, *P<0.01*), MS fertile spikelets ear^{-1} (+18%, *P<0.05*), MS GN $spikelet^{-1}$ (+40%, *P<0.01*) and MS rachis length (+33%, *P<0.01*). In 2014, positive variation above Ashby was observed for HI (+5%, *P<0.05*), TGW (+32%, *P<0.05*), GN ear^{-1} (+128%, *P<0.001*), AGDM MS^{-1} (+146%, *P<0.001*), grain DW MS^{-1} (+142%, *P<0.001*), MS GN ear^{-1} (+116%, *P<0.001*), MS spikelets ear^{-1} (+19%, *P<0.01*), MS fertile spikelets ear^{-1} (+31%, *P<0.01*), MS GN $spikelet^{-1}$ (+63%, *P<0.01*) and MS rachis length (+55%, *P<0.001*).

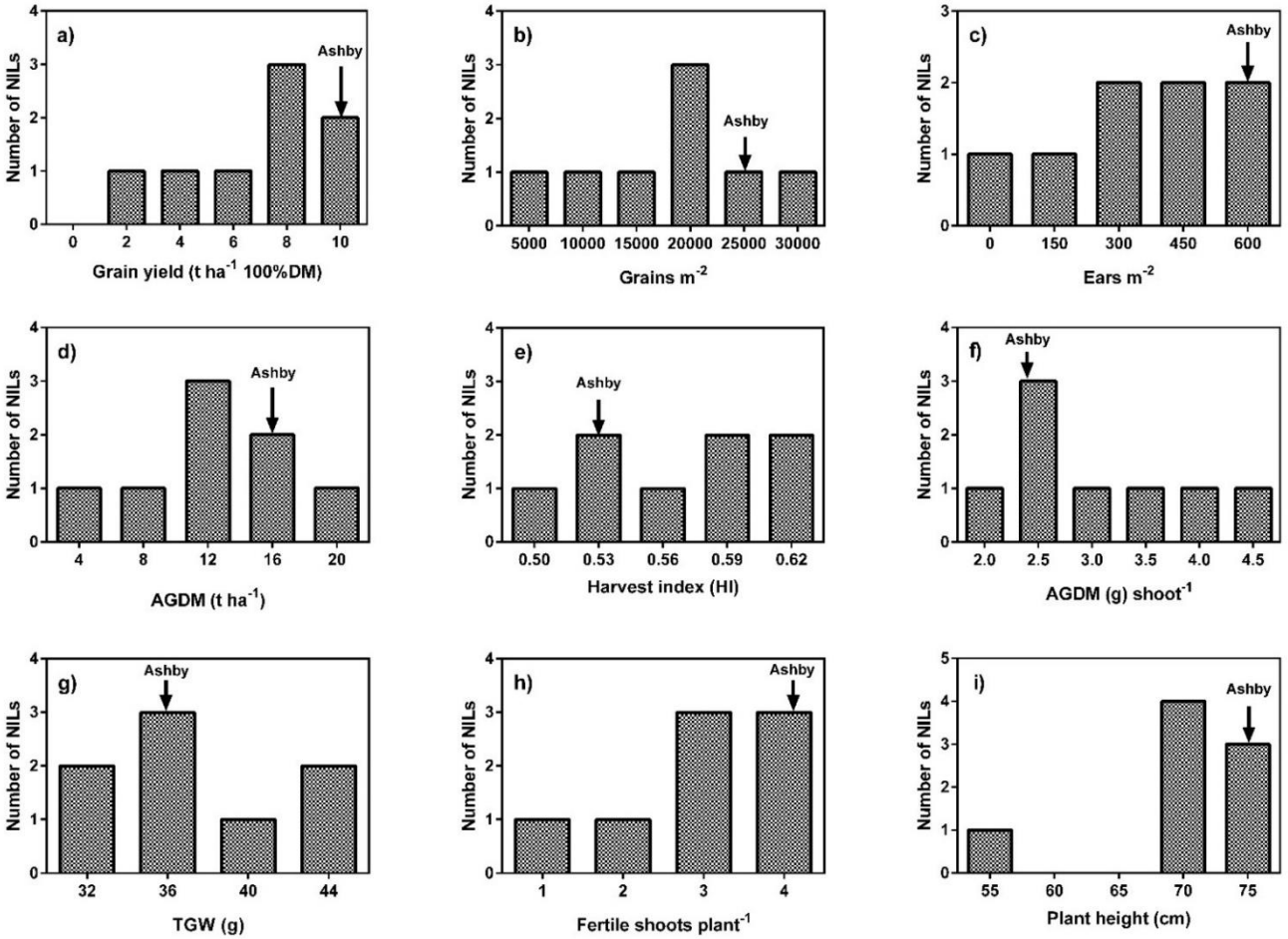

Fig. 4.1 Frequency distribution of BC_3S_5 spring wheat NILs and the Ashby recurrent parent for a) grain yield (100% DM), b) grains m^{-2}, c) ears m^{-2}, d) above-ground dry matter (AGDM) m^{-2}, e) harvest index (HI), f) AGDM $shoot^{-1}$, g) 1000 grain weight (TGW), h) fertile shoots $plant^{-1}$ and i) plant height (PH) in 2013. Mean values for the recurrent parent are indicated with arrows. The number on X axis indicated the mid-point of the range.

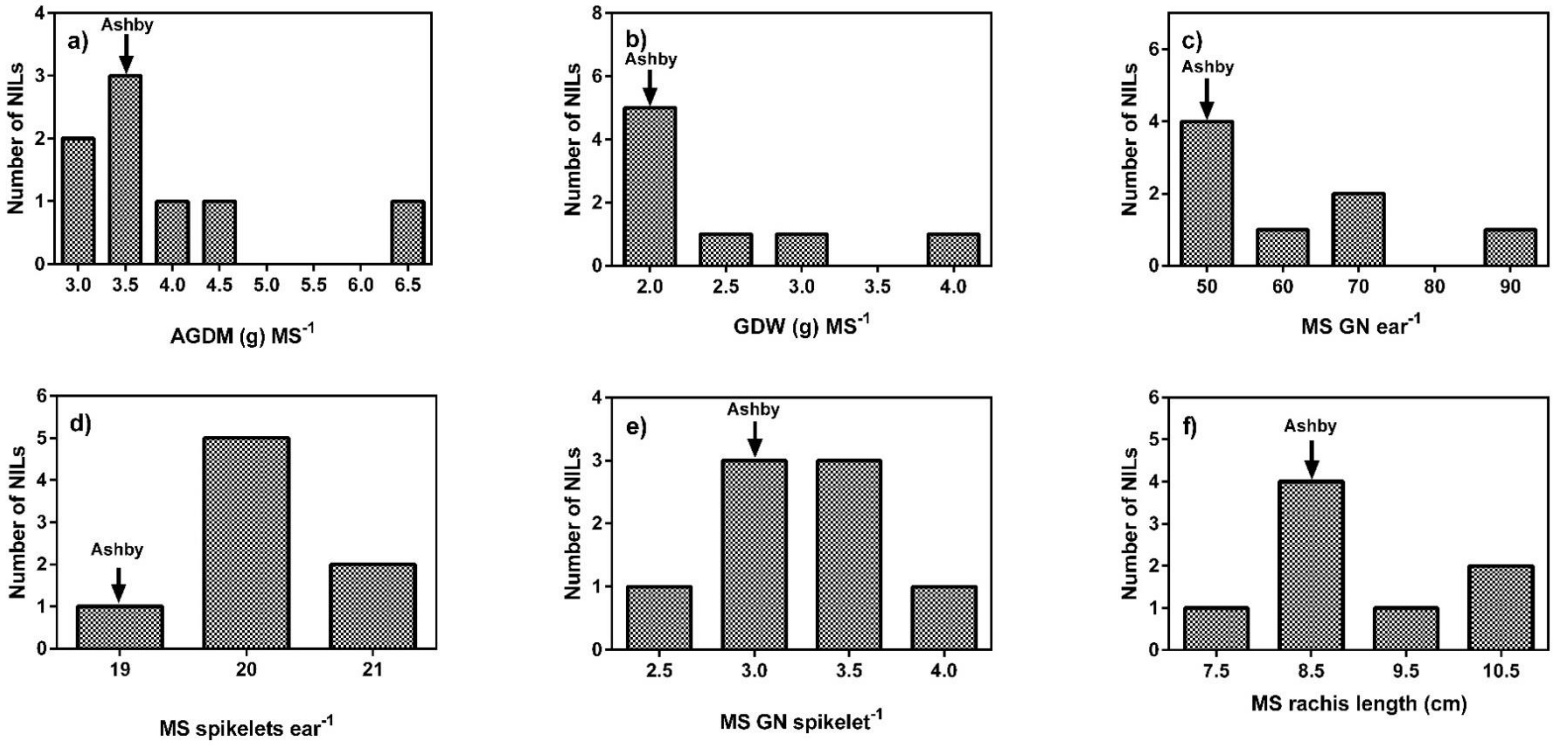

Fig. 4.2 Frequency distribution of BC_3S_5 spring wheat NILs and the Ashby recurrent parent for a) AGDM per main shoot (MS), b) grain dry weight (GDW) per MS, c) MS GN ear^{-1}, d) MS spikelets ear^{-1}, e) MS GN spikelet^{-1} and f) MS rachis length (RL) in 2013. Mean values for the recurrent parent are indicated with arrows. The number on X axis indicated the mid-point of the range.

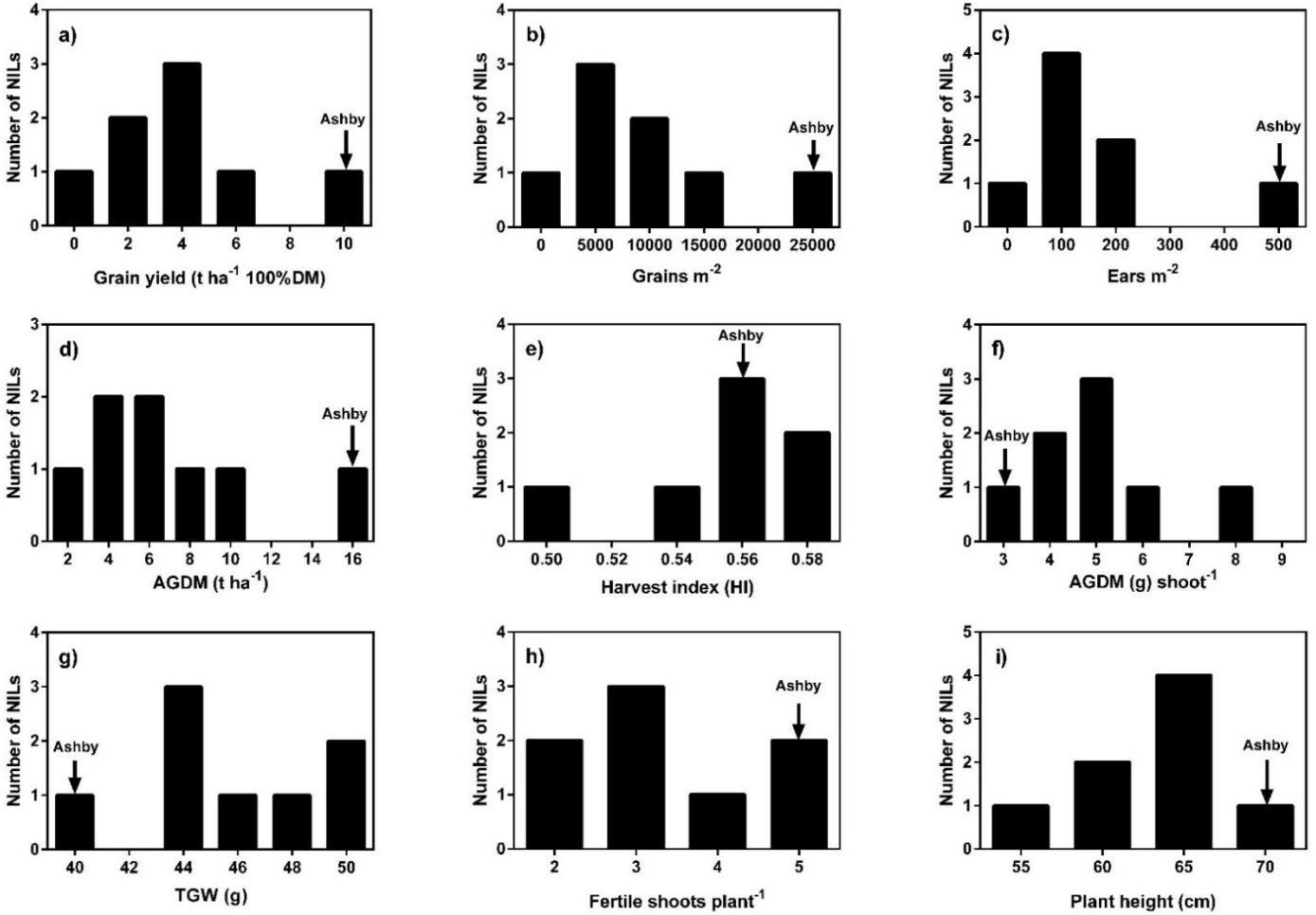

Fig. 4.3 Frequency distribution of BC_3S_6 spring wheat NILs and the Ashby recurrent parent for a) grain yield (100% DM), b) grains m^{-2}, c) ears m^{-2}, d) above-ground dry matter (AGDM) m^{-2}, e) harvest index (HI) f) AGDM $shoot^{-1}$, g) 1000 grain weight (TGW), h) fertile shoots $plant^{-1}$ and i) plant height (PH) in 2014. Mean values for the recurrent parent are indicated with arrows. The number on X axis indicated the mid-point of the range.

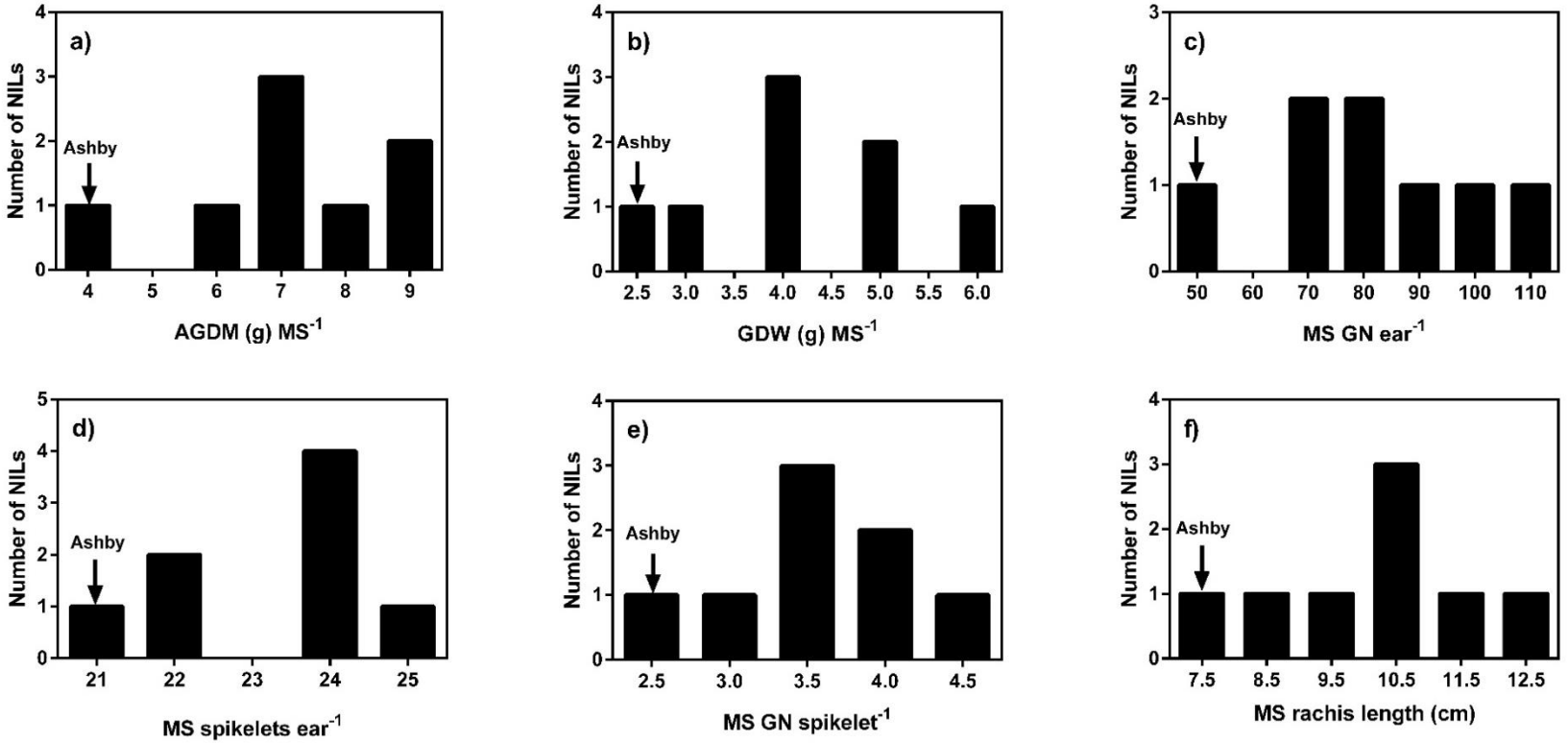

Fig. 4.4 Frequency distribution of BC_3S_6 spring wheat NILs and the Ashby recurrent parent for a) AGDM per main shoot (MS), b) grain dry weight (GDW) per MS, c) MS GN ear^{-1}, d) MS spikelets ear^{-1}, e) MS GN spikelet^{-1} and f) MS rachis length (RL) in 2014. Mean values for the recurrent parent are indicated with arrows. The number on X axis indicated the mid-point of the range.

In 2013, BC_3S_5 lines differed in GN ear^{-1} (ranging from 38.3 to 78.4) with more GN spikelet^{-1} (range 2.46 to 3.82) and a longer rachis length (range 6.87 to 11.08 cm) compared to Ashby (40.8 GN ear^{-1}; *P<0.001*, 2.46 GN spikelet^{-1}; *P<0.01* and 7.85 cm RL; *P<0.01*, respectively) (Table 4.1). However, NILs generally produced fewer fertile shoots plant^{-1} (range 0.43 to 4.00) compared to Ashby (3.65) (*P<0.10*). Generally decreased grain yield in NILs was observed compared to Ashby (9.13 t ha^{-1}) ranging from 1.93 to 9.48 t ha^{-1} (*P<0.05*). Ear m^{-2} varied from 55.0 to 591.0 in NILs compared to 655.0 m^{-2} in Ashby (*P<0.05*), while grains m^{-2} ranged from 4,967 to 28,992 compared to 26,354 in Ashby (*P<0.05*).

In 2014, no difference in fertile shoots plant^{-1} (1.80-4.50) for NILs compared to the Ashby parent (5.10) was observed (*P>0.10*). For HI, one line (range 0.49-0.59) was less and for TGW, four lines (range 43.8-51.8 g) were higher than Ashby (0.56 HI and 39.3 g TGW) (*P<0.05*). AGDM shoot^{-1} (3.71-8.22 g) and spikelets ear^{-1} (20.2-24.8) were increased in NILs compared to Ashby (3.03 g AGDM shoot^{-1}; *P<0.01* and 22.1 spikelets ear^{-1}; *P<0.05*). The NILs overall produced lower grain yield in 2014 than in 2013. The BC_3S_6 lines showed lower grain yield (range 0.97 to 5.59), ears m^{-2} (range 20.0 to 227.0) and grains m^{-2} (range 1,962 to 12,783) compared to Ashby (9.03 t ha^{-1}; *P<0.01*, 510.0 ears m^{-2}; *P<0.001* and 22,960 grains m^{-2}; *P<0.01*, respectively). There were differences amongst BC_3 NILs in HI *(P<0.05)*, AGDM shoot^{-1} (*P<0.01*), TGW *(P<0.05)*, spikelets ear^{-1} (*P<0.05*), GN ear^{-1} (*P<0.001*), GN spikelet^{-1} (*P<0.01*) and rachis length (*P<0.001*), but not in GDW plant^{-1} and GN plant^{-1} (*P>0.10*).

Averaging across years, grain yield amongst the 7 spring BC_3 lines ranged from 2.36 to 6.68 t ha^{-1} and ears m^{-2} from 61.0 to 371.0 compared to 9.08 t ha^{-1} and 583.0 m^{-2}, respectively, for the Ashby recurrent parent (*P<0.001*). Grains m^{-2} ranged from 5,269 to 18,473 and fertile shoots plant^{-1} from 1.93 to 3.79 in BC_3 lines compared to 24,657 m^{-2} and 4.38 fertile shoots plant^{-1} in Ashby, respectively (*P<0.001* and *P<0.01*, respectively). There were differences amongst BC_3 NILs in AGDM shoot^{-1}

(*P<0.01*), TGW (*P<0.01*), spikelets ear^{-1} (*P<0.01*), fertile spikelets ear^{-1} (*P<0.001*), GN ear^{-1} (*P<0.001*), GN spikelet^{-1} (*P<0.001*) and rachis length (*P<0.001*). There was a year effect on grain yield (*P<0.05*), ears m^{-2} (*P<0.05*), grains m^{-2} (*P<0.01*), TGW (*P<0.05*), spikelets ear^{-1} (*P<0.10*), fertile spikelets ear^{-1} (*P<0.10*), GN ear^{-1} (*P<0.05*) and rachis length (*P<0.10*); positive segregation above the Ashby recurrent parent was found for AGDM shoot^{-1}, TGW, spikelets ear^{-1}, fertile spikelets ear^{-1}, GN ear^{-1}, GN spikelet^{-1} and rachis length.

Year x genotype interactions were observed for grain yield, grains m^{-2}, fertile shoots plant^{-1}, GN plant^{-1}, TGW, spikelets ear^{-1}, fertile spikelets ear^{-1} (*P<0.05*), HI (*P<0.01*), AGDM shoot^{-1} (*P<0.01*) and rachis length (*P<0.001*).

Table 4.1 Minimum, maximum and mean values for grain yield (100% DM), ears m^{-2}, grains m^{-2}, harvest index (HI), fertile shoots (FS) $plant^{-1}$, above-ground dry matter (AGDM) $shoot^{-1}$, grain dry weight (GDW) $plant^{-1}$, grain number (GN) $plant^{-1}$, 1000 grain weight (TGW), spikelets ear^{-1}, fertile spikelets ear^{-1}, GN ear^{-1}, GN $spikelet^{-1}$ and rachis length (RL) (average for all fertile shoots per plant) for 7 BC_3 spring wheat NILs and the Ashby recurrent parent in 2013, 2014 and SEDs for BC_3 line, year and interaction.

	Grain yield (t ha^{-1})	Ears m^{-2}	Grains m^{-2}	HI	FS $plant^{-1}$	AGDM (g) $shoot^{-1}$	GDW (g) $plant^{-1}$	GN $plant^{-1}$	TGW (g)	Spikelets ear^{-1}	Fertile spikelets ear^{-1}	GN ear^{-1}	GN $spikelet^{-1}$	RL (cm)
2013 BC_3S_5 lines														
Minimum	1.93	55.0	4,967.0	0.49	0.43	2.22	3.52	94.0	32.9	17.8	14.0	38.3	2.46	6.87
Maximum	9.48	591.0	28,992.0	0.61	4.00	5.14	8.24	221.0	43.0	21.7	21.5	78.4	3.82	11.08
Mean	6.62	347.1	17,537.0	0.57	2.78	3.35	6.27	161.1	37.9	19.5	17.9	55.5	3.00	8.85
Parental line (Ashby)	9.13	655.0	26,354.0	0.54	3.65	2.40	5.31	151.0	34.8	18.8	16.2	40.8	2.46	7.85
SED between BC_3 and parental line (DF=8)	1.70*	126.8*	5,142.00*	0.03†	0.89†	1.08	2.16	46.8	2.72†	1.14	1.70†	6.78***	0.24**	0.57**
SED within BC_3 lines (DF=8)	1.97*	146.4*	5,938.00*	0.04†	1.03†	1.24	2.50	54.0	3.15†	1.31	1.97†	7.83***	0.28**	0.66**
2014 BC_3S_6 lines														
Minimum	0.97	20.0	1,962.0	0.49	1.80	3.71	4.01	73.0	43.8	20.2	20.0	56.3	2.61	8.30
Maximum	5.59	227.0	12,783.0	0.59	4.50	8.22	14.8	328.0	51.8	24.8	24.1	102.6	4.14	12.5
Mean	3.39	111.3	7,267.0	0.56	3.21	5.15	9.31	200.4	47.2	22.8	22.6	72.0	3.14	10.5
Parental line (Ashby)	9.03	510.0	22,960.0	0.56	5.10	3.03	9.17	232.0	39.3	22.1	20.0	45.0	2.25	8.22
SED between BC_3 and parental line (DF=7)	1.34**	52.7***	3,049.00**	0.014*	1.37	0.77**	3.36	72.6	2.52*	0.87*	0.98*	5.45***	0.27**	0.39***
SED within BC_3 lines (DF=7)	1.55**	60.9***	3,521.00**	0.016*	1.58	0.89**	3.88	83.9	2.91*	1.01*	1.13*	6.29***	0.31**	0.45***
Cross-year ANOVA BC_3 lines														
Minimum	2.36	61.0	5,269.0	0.54	1.93	2.96	5.62	135.0	39.9	19.8	18.3	47.3	2.58	7.59
Maximum	6.68	371.0	18,473.0	0.59	3.79	5.41	10.2	234.0	45.9	22.3	22.1	90.5	3.98	11.2
Mean	4.94	226.7	12,286.0	0.56	2.66	4.24	7.73	180.0	42.5	21.1	20.2	63.7	3.07	9.64
Parental line (Ashby)	9.08	583.0	24,657.0	0.55	4.38	2.71	7.24	192.0	37.1	20.4	18.2	42.9	2.35	8.04
SED between BC_3 and parental line (DF=7)	1.17***	64.2***	3,082.6***	0.017†	0.59**	0.64**	1.88	40.90	1.89**	0.58**	0.83***	4.35***	0.18***	0.34***
SED within BC_3 lines (DF=7)	1.35***	74.2***	3,559.5***	0.020†	0.68**	0.74**	2.17	47.20	2.18**	0.67**	0.96***	5.03***	0.21***	0.40***
SED Year (DF=2)	0.31*	46.90*	730.20**	0.005	0.58	0.63	1.82	41.60	1.37*	0.99†	1.33†	1.95*	0.04	0.38†
SED Year*BC_3 and parental line (DF=15)	1.56*	96.10†	4,089.3*	0.023**	0.96*	1.05*	3.05	67.60*	2.82*	1.25*	1.72*	6.01	0.24	0.59***
SED Year*BC_3 lines (DF=15)	1.83*	109.50†	4,801.8*	0.027**	1.07*	1.17*	3.41	75.40*	3.21*	1.33*	1.85*	6.98	0.28	0.65***

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Table 4.2 Minimum, maximum and mean values for AGDM per Main Shoot (MS), plant height (PH), GDW MS^{-1}, GN MS^{-1}, MS spikelets ear^{-1}, MS fertile spikelets ear^{-1}, MS GN $spikelet^{-1}$ and MS rachis length (RL) for 7 BC_3 spring wheat NILs and the Ashby recurrent parent in 2013, 2014 and SEDs for BC_3 line, year and interaction.

	AGDM (g) MS^{-1}	**PH (cm)**	**GDW (g) MS^{-1}**	**GN MS^{-1}**	**MS Spikelets ear^{-1}**	**MS fertile spikelets ear^{-1}**	**MS GN $spikelet^{-1}$**	**MS RL (cm)**
2013 BC_3S_5 lines								
Minimum	3.01	57.2	1.77	48.4	19.4	17.0	2.35	7.71
Maximum	6.35	76.7	3.83	86.2	21.0	20.8	4.15	11.0
Mean	4.01	69.4	2.43	61.5	20.0	18.9	3.24	9.23
Parental line (Ashby)	3.40	73.4	1.90	52.6	19.7	17.7	2.97	8.28
SED between BC_3 and parental line (DF=8)	0.59*	2.91**	0.38*	6.09**	0.50	0.90*	0.26**	0.48**
SED within BC_3 lines (DF=8)	0.69*	3.36**	0.43*	7.04**	0.58	1.03*	0.30**	0.55**
2014 BC_3S_6 lines								
Minimum	5.55	55.9	3.12	65.7	21.6	20.0	3.10	8.43
Maximum	9.13	65.5	5.80	110.7	25.2	25.2	4.39	12.4
Mean	7.44	62.7	4.36	84.7	23.6	23.3	3.63	10.8
Parental line (Ashby)	3.67	71.8	2.35	51.3	21.2	19.2	2.68	7.97
SED between BC_3 and parental line (DF=7)	0.54***	1.34***	0.39***	5.64***	0.64**	0.88**	0.24**	0.43***
SED within BC_3 lines (DF=7)	0.63***	1.55***	0.45***	6.51***	0.74**	1.02**	0.28**	0.50***
Cross-year ANOVA								
BC_3 lines								
Minimum	4.37	61.3	2.45	58.5	20.5	18.5	3.09	8.07
Maximum	7.74	69.4	4.81	98.5	23.0	23.0	4.27	11.4
Mean	5.71	66.1	3.38	73.0	21.8	21.1	3.43	9.98
Parental line (Ashby)	3.54	72.6	2.12	52.0	20.4	18.4	2.82	8.12
SED between BC_3 and parental line (DF=15)	0.41***	1.68***	0.26***	4.15***	0.42***	0.66***	0.18***	0.35***
SED within BC_3 lines (DF=15)	0.48***	1.94***	0.30***	4.79***	0.49***	0.76***	0.21***	0.41***
SED Year (DF=2)	0.26**	1.79†	0.20*	3.33*	0.34*	0.41*	0.11	0.35†
SED Year*BC_3 and parental line (DF=15)	0.60***	2.83***	0.39*	6.36**	0.65*	0.96	0.26**	0.58**
SED Year*BC_3 lines (DF=15)	0.69***	3.14***	0.45*	7.21**	0.73*	1.10	0.30**	0.65**

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Averaging across years, NILs showed significant positive genetic variation for main shoot traits: AGDM MS^{-1} (4.37-7.74 g), plant height (61.3-69.4 cm), GDW MS^{-1} (2.45-4.81 g), GN MS^{-1} (58.5-98.5), MS spikelets ear^{-1} (20.5-23.0), MS fertile spikelets ear^{-1} (18.5-23.0), MS GN $spikelet^{-1}$ (3.09-4.27) and RL (8.07-11.4 cm) compared to Ashby parent (*P<0.001*). There was a year effect on AGDM MS^{-1} (*P<0.01*), GDW MS^{-1} (*P<0.05*), GN MS^{-1} (*P<0.05*), MS spikelets ear^{-1} (*P<0.05*), MS fertile spikelets ear^{-1} (*P<0.05*), plant height (*P<0.10*) and rachis length (*P<0.10*). Year x genotype interactions were observed for AGDM MS^{-1} (*P<0.001*), plant height (*P<0.001*), GDW MS^{-1} (*P<0.05*), GN MS^{-1} (*P<0.01*), MS spikelets ear^{-1} (*P<0.05*), GN $spikelets^{-1}$ (*P<0.01*) and rachis length (*P<0.01*).

For the main-shoot traits, there was a positive relationship between GN $spikelet^{-1}$ and GN ear^{-1} (R^2 = 0.903 (*P<0.01*); 2013, R^2 = 0.893 (*P<0.01*); 2014), and between GN ear^{-1} and GDW (g) ear^{-1} (R^2 = 0.868 (*P<0.01*); 2013, R^2 = 0.962 (*P<0.001*); 2014; Fig. 4.5, a-b). The positive associations between fertile spikelets ear^{-1} and GN ear^{-1} (R^2 = 0.345 (*P>0.10*); 2013, R^2 = 0.621 (*P<0.05*); 2014) and between rachis length (cm) and GN ear^{-1} (R^2 = 0.102 (*P>0.10*); 2013, R^2 = 0.590 (*P<0.05*); 2014) were for 2014 only (Fig. 4.5, c-d).

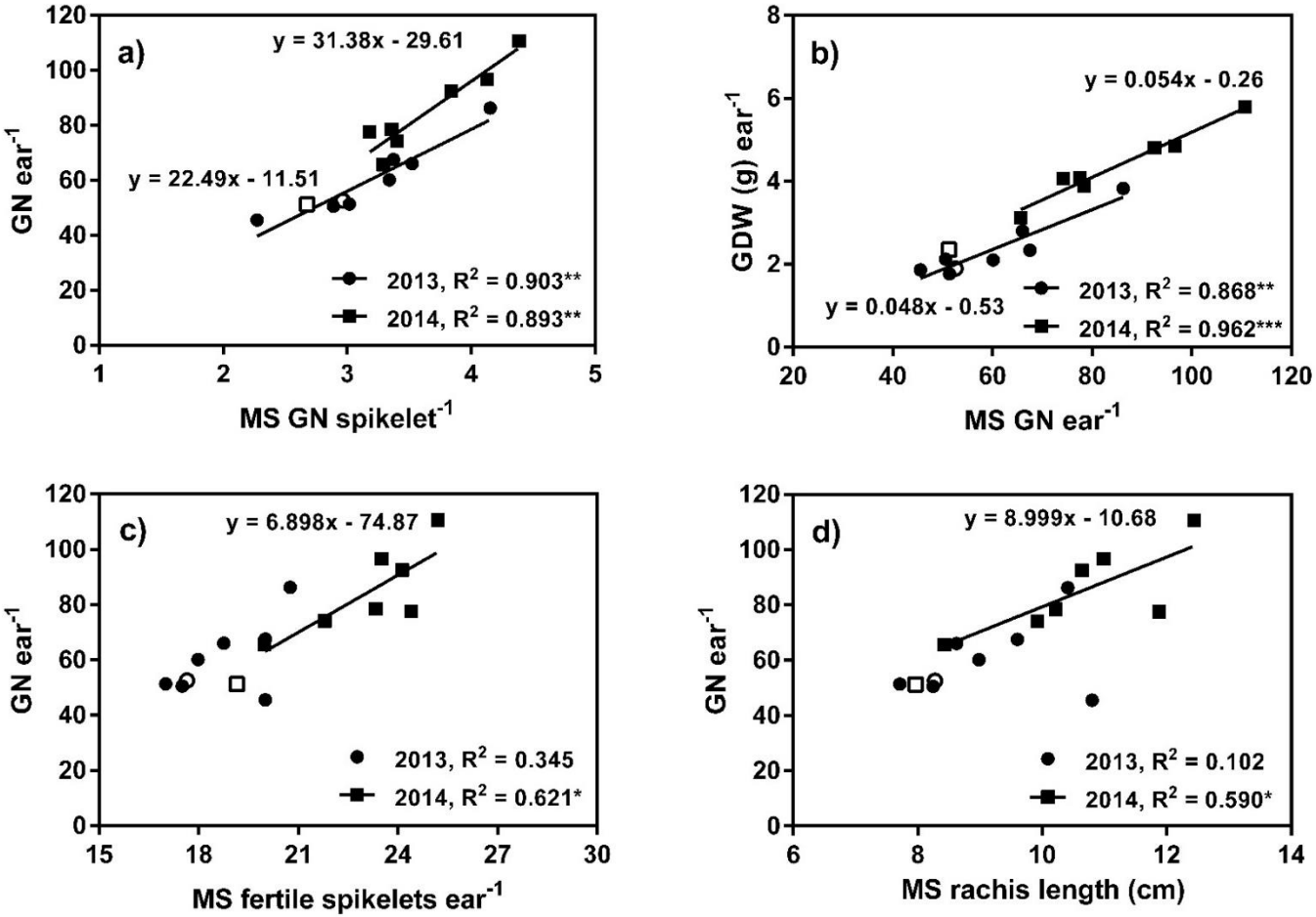

Fig. 4.5 Linear relationship between a) main-shoot (MS) GN spikelet^{-1} and GN ear^{-1}, b) MS GN ear^{-1} and GDW ear^{-1}, c) MS fertile spikelets ear^{-1} and GN ear^{-1}, and d) MS rachis length and GN ear^{-1} in BC_3 spring wheat NILs in 2013 (closed circles) and 2014 (closed squares) and the recurrent parent (Ashby) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *** *P<0.001*.

4.3.1.2 BC_3 winter wheat NILs

The frequency distribution of NILs for harvest traits is shown for winter wheat BC_3 NILs in 2013 (Figs. 4.6 and 4.7) and 2014 (Figs. 4.8 and 4.9). Significant variation above the Humber recurrent parent was observed in 2013 for grain yield (+23%, *P<0.05*), HI (+7%, *P<0.05*), TGW (+27%, *P<0.01*), MS plant height (cm) (+32%, *P<0.001*), MS spikelets ear^{-1} (+12%, *P<0.01*), MS fertile spikelets ear^{-1} (+17%, *P<0.05*) and MS RL (cm) (+28%, *P<0.001*). In 2014, variation above the Humber recurrent parent was observed for HI (+5%, *P<0.05*), AGDM (g) MS^{-1} (+35%, *P<0.05*), GN MS^{-1} (+29%, *P<0.05*), MS plant height (cm) (+23%, *P<0.001*), MS spikelets ear^{-1} (+12%, *P<0.001*), MS fertile spikelets ear^{-1} (+15%, *P<0.001*), MS GN $spikelet^{-1}$ (+23%, *P<0.05*) and MS RL (cm) (+25%, *P<0.001*). Moreover, trends for increasing grain yield (+35%) grains m^{-2} (+24%), TGW (g) (+17%), GDW (g) MS^{-1} (+35%) compared to the recurrent parent were also observed in across-year analysis (*P<0.10*) (Table 4.3).

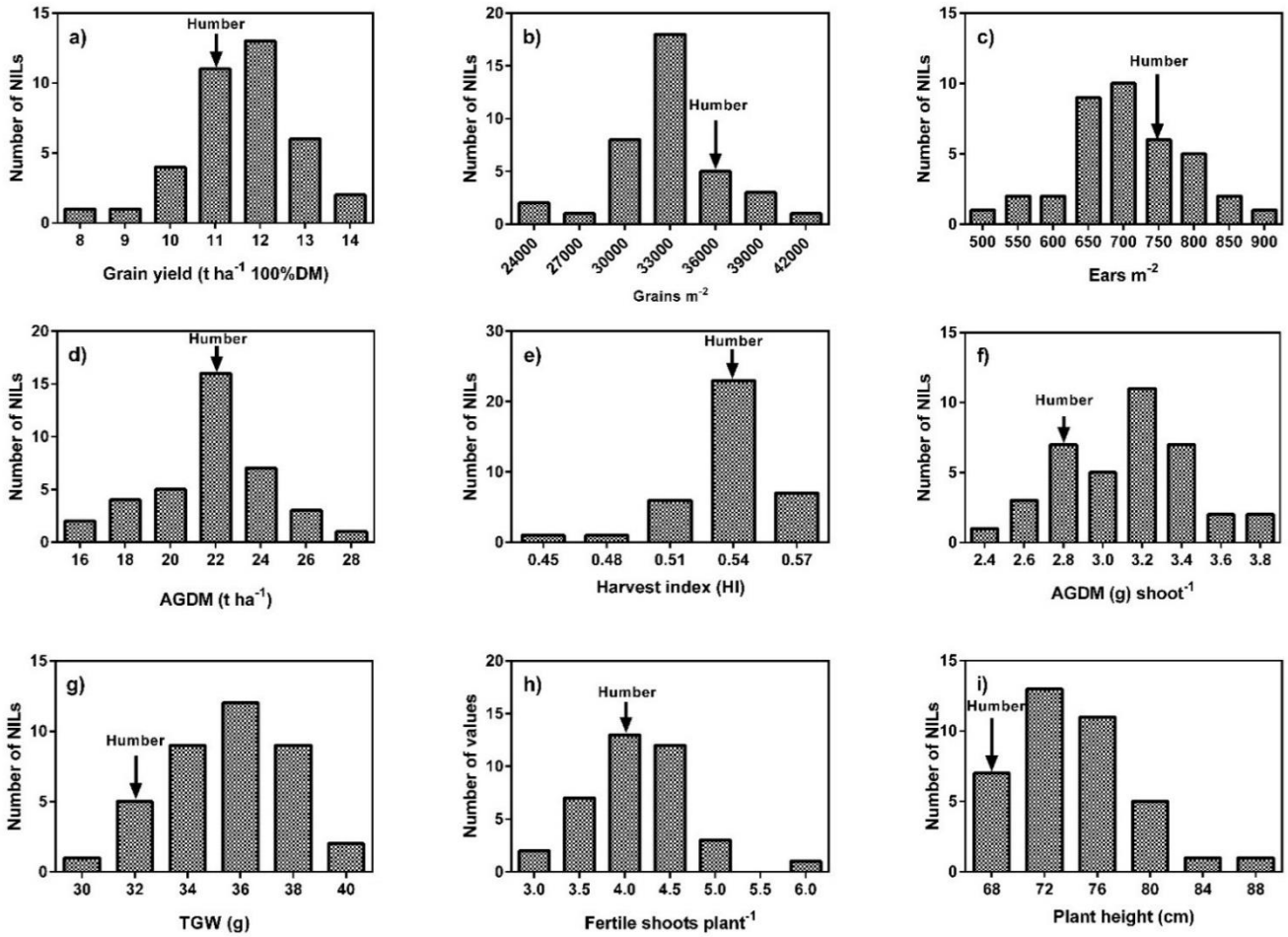

Fig. 4.6 Frequency distribution of BC_3S_5 winter wheat NILs and the Humber recurrent parent for a) grain yield (100% DM), b) grains m^{-2}, c) ears m^{-2}, d) above-ground dry matter (AGDM) m^{-2}, e) harvest index (HI), f) AGDM $shoot^{-1}$, g) 1000 grain weight (TGW), h) fertile shoots $plant^{-1}$ and i) plant height (PH) in 2013. Mean values for the recurrent parent are indicated with arrows. The number on X axis indicated the mid-point of the range.

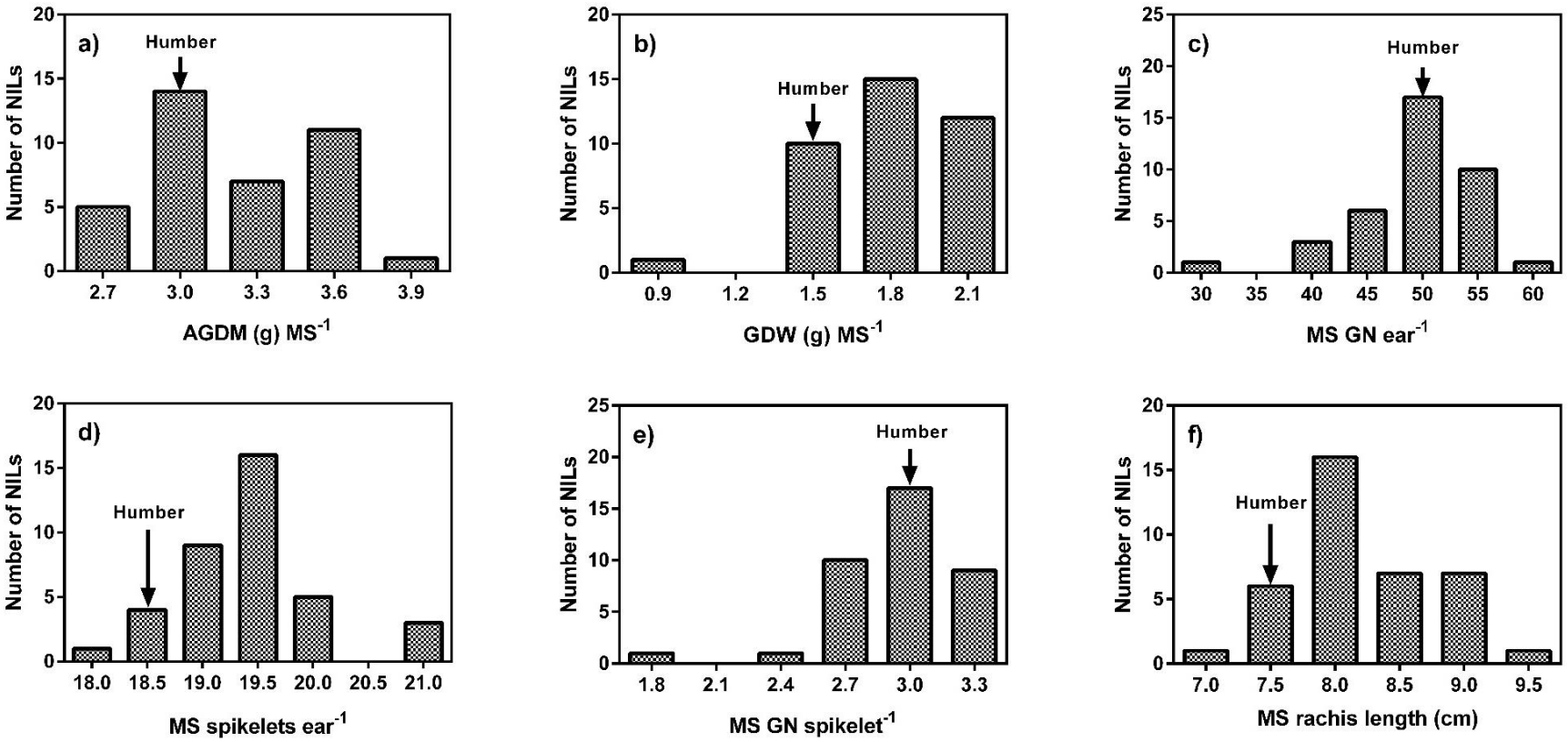

Fig. 4.7 Frequency distribution of BC_3S_5 winter wheat NILs and the Humber recurrent parent for a) AGDM per main shoot (MS), b) grain dry weight (GDW) per MS, c) MS GN ear^{-1}, d) MS spikelets ear^{-1}, e) MS GN spikelet^{-1} and f) MS rachis length (RL) in 2013. Mean values for the recurrent parent are indicated with arrows. The number on X axis indicated the mid-point of the range.

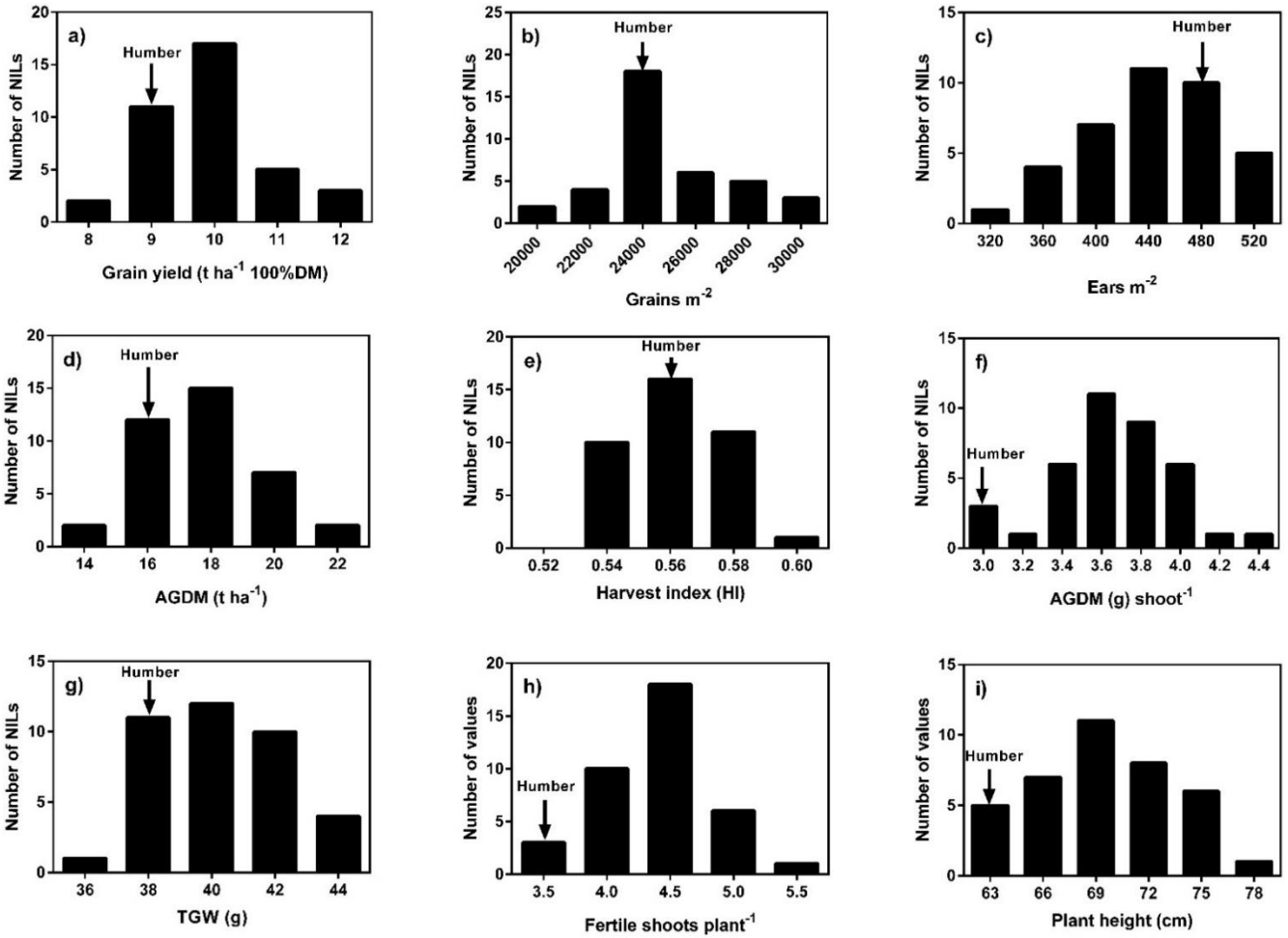

Fig. 4.8 Frequency distribution of BC_3S_6 winter wheat NILs and the Humber recurrent parent for a) grain yield (100% DM), b) grains m^{-2}, c) ears m^{-2}, d) above-ground dry matter (AGDM) m^{-2}, e) harvest index (HI), f) AGDM $shoot^{-1}$, g) 1000 grain weight (TGW), h) fertile shoots $plant^{-1}$ and i) plant height (PH) in 2014. Mean values for the recurrent parent are indicated with arrows. The number on X axis indicated the mid-point of the range.

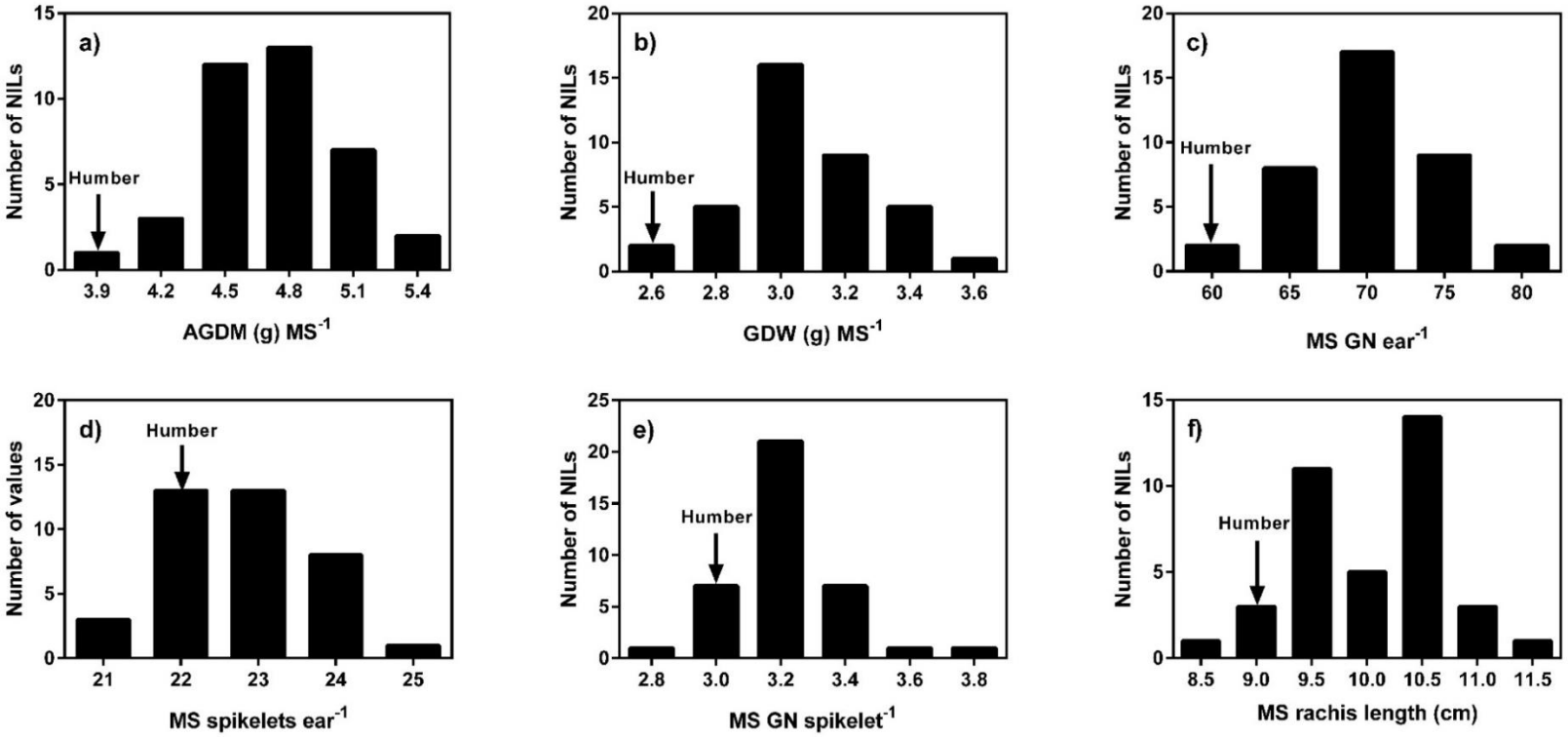

Fig. 4.9 Frequency distribution of BC_3S_6 winter wheat NILs and the Humber recurrent parent for a) AGDM per main shoot (MS), b) grain dry weight (GDW) per MS, c) MS GN ear^{-1}, d) MS spikelets ear^{-1}, e) MS GN spikelet^{-1} and f) MS rachis length (RL) in 2014. Mean values for the recurrent parent are indicated with arrows. The number on X axis indicated the mid-point of the range.

In 2013, there were no differences in ears m^{-2}, grains m^{-2}, fertile shoots $plant^{-1}$, GDW $plant^{-1}$, GN $plant^{-1}$, GN ear^{-1} and GN $spikelet^{-1}$ (*P>0.10*) amongst the 37 BC_3 lines (Table 4.3). Grain yield ranged from 8.43 to 14.07 t ha^{-1} in NILs compared to 11.5 t ha^{-1} in Humber (*P<0.05*) and HI from 0.44 to 0.58 compared to 0.54 in Humber (*P<0.05*). AGDM $shoot^{-1}$ was increased in NILs (range 2.50-3.83 g) compared to Humber (2.70 g; *P<0.05*). BC_3S_5 lines generally produced a longer rachis (range 7.46 to 9.92 cm, *P<0.001*) with higher spikelets ear^{-1} (range 19.1 to 22.3, *P<0.01*) and heavier TGW (range 30.5 to 40.3 g, *P<0.01*) compared to the Humber parent (7.88 cm RL, 19.5 spikelets ear^{-1} and 31.7 g TGW).

In 2014, there was no difference in grain yield, ears m^{-2}, grains m^{-2}, fertile shoots $plant^{-1}$, AGDM $shoot^{-1}$, TGW, GN $plant^{-1}$ and GN ear^{-1} (*P>0.10*) amongst the 37 BC_3 lines. HI varied from 0.54 to 0.59 in NILs compared to 0.56 in Humber (*P<0.05*). AGDM $shoot^{-1}$ (range 3.08 to 4.44 g) did not differ in NILs compared to Humber but GDW $plant^{-1}$ (range 6.86 to 13.27 g) tended to be heavier than Humber in NILs (2.99 g AGDM $shoot^{-1}$ and 7.13 g GDW $plant^{-1}$) (*P<0.10*). Spikelets ear^{-1} (range 20.3 to 24.2) and rachis length (range 8.10 to 11.43 cm) were generally greater in BC_3 lines than the Humber parent (21.3 spikelets ear^{-1} and RL 8.86 cm, respectively) (*P<0.001*).

Averaging across years, grain yield ranged amongst the 37 winter BC_3 lines higher than Humber from 8.42 to 12.56 t ha^{-1} and HI from 0.51 to 0.58 compared to 10.28 t ha^{-1} (*P<0.05*) and 0.55 (*P<0.01*), respectively, for the Humber parent. AGDM $shoot^{-1}$ (range 2.79 to 4.14 g) and TGW (range 34.4 to 42.2 g) in the BC_3 lines were generally greater compared to Humber (2.85 g AGDM $shoot^{-1}$; *P<0.001* and 35.0 g TGW; *P<0.001*, respectively). Moreover, NILs also differed in spikelets ear^{-1} (*P<0.001*), fertile spikelet ear^{-1} (*P<0.001*), GN $spikelet^{-1}$ (*P<0.05*) and RL (*P<0.001*). There was no difference amongst NILs in ears m^{-2}, fertile shoots $plant^{-1}$, GN $plant^{-1}$ and GN ear^{-1} (*P>0.10*), but grains m^{-2} and GDW $plant^{-1}$ tended to be increased slightly in the highest BC_3 lines (*P<0.10*) compared to the Humber parent. There was

a year effect on grain yield (*P<0.05*), ears m^{-2} (*P<0.001*), grains m^{-2} (*P<0.01*), HI (*P<0.01*), AGDM $shoot^{-1}$ (*P<0.05*), TGW (*P<0.05*), GN ear^{-1} (*P<0.01*), spikelets ear^{-1} (*P<0.05*), fertile spikelets ear^{-1} (*P<0.05*) and RL (*P<0.05*). Year x genotype interactions were significant for HI and rachis length (*P<0.05*).

Table 4.3 Minimum, maximum and mean values for grain yield (100% DM), ears m^{-2}, grains m^{-2}, harvest index (HI), fertile shoots (FS) $plant^{-1}$, above-ground dry matter (AGDM) $shoot^{-1}$, grain dry weight (GDW) $plant^{-1}$, grain number (GN) $plant^{-1}$, 1000 grain weight (TGW), spikelets ear^{-1}, fertile spikelets ear^{-1}, GN ear^{-1}, GN $spikelet^{-1}$ and rachis length (RL) (average for all fertile shoots per plant) for 37 BC_3 winter wheat NILs and the Humber recurrent parent in 2013, 2014 and SEDs for BC_3 line, year and interaction.

	Grain yield (t ha^{-1})	**Ears m^{-2}**	**Grains m^{-2}**	**HI**	**FS $plant^{-1}$**	**AGDM (g) $shoot^{-1}$**	**GDW (g) $plant^{-1}$**	**GN $plant^{-1}$**	**TGW (g)**	**Spikelets ear^{-1}**	**Fertile spikelets ear^{-1}**	**GN ear^{-1}**	**GN $spikelet^{-1}$**	**RL (cm)**
2013 BC_3S_5 lines														
Minimum	8.43	518.0	24,481	0.44	3.00	2.50	5.24	142.3	30.5	19.1	16.9	36.5	2.11	7.46
Maximum	14.07	890.0	41,630	0.58	5.80	3.83	11.02	301.4	40.3	22.3	20.9	52.7	2.91	9.92
Mean	11.6	700.8	32,750	0.54	4.18	3.14	7.01	197.2	35.5	20.5	18.7	47.3	2.58	8.63
Parental line (Humber)	11.5	783.0	36,290	0.54	4.15	2.70	6.17	195.2	31.7	19.5	18.0	46.6	2.65	7.88
SED between BC_3 and parental line (DF=39)	1.12*	106.0	3,826	0.023*	0.59	0.29*	1.47	39.0	1.93**	0.57**	0.76*	4.52	0.21	0.33***
SED within BC_3 lines (DF=39)	1.29*	122.4	4,417	0.027*	0.68	0.34*	1.70	45.0	2.23**	0.66**	0.88*	5.22	0.24	0.38***
2014 BC_3S_6 lines														
Minimum	7.69	302.0	19,863	0.54	3.60	3.08	6.86	180.7	37.0	20.3	19.0	49.3	2.31	8.10
Maximum	12.27	519.7	29,569	0.59	5.30	4.44	13.27	339.5	44.7	24.2	23.1	65.5	2.97	11.43
Mean	9.97	441.5	24,727	0.56	4.43	3.67	10.10	250.4	40.4	22.4	21.1	56.4	2.65	9.79
Parental line (Humber)	9.06	478.7	23,854	0.56	3.70	2.99	7.13	185.1	38.3	21.3	19.8	50.0	2.49	8.86
SED between BC_3 and parental line (DF=39)	1.20	60.25	3,030.8	0.013*	0.59	0.33	1.45†	37.0	2.07	0.57***	0.85**	4.39	0.15†	0.37***
SED within BC_3 lines (DF=39)	1.39	69.57	3,499.7	0.015*	0.68	0.39	1.68†	42.7	2.39	0.66***	0.98**	5.06	0.18†	0.43***
Cross-year ANOVA														
BC_3 lines														
Minimum	8.42	435.6	22,300	0.51	3.45	2.79	6.79	177.4	34.4	19.7	18.0	45.2	2.38	8.26
Maximum	12.56	663.6	34,852	0.58	5.20	4.14	11.23	289.7	42.2	23.2	21.8	56.7	2.85	10.39
Mean	10.77	571.2	28,739	0.55	4.30	3.41	8.55	223.8	38.0	21.5	19.9	51.8	2.62	9.21
Parental line (Humber)	10.28	630.7	30,072	0.55	3.93	2.85	6.65	190.1	35.0	20.4	18.9	48.3	2.57	8.37
SED between BC_3 and parental line (DF=37)	0.83*	61.23	2,445.0†	0.013**	0.41	0.22***	1.04†	26.58	1.44***	0.40***	0.57***	3.15	0.13*	0.25***
SED within BC_3 lines (DF=37)	0.96*	70.70	2,823.2†	0.015**	0.48	0.26***	1.20†	30.69	1.66***	0.46***	0.66***	3.64	0.15*	0.29***
SED Year (DF=2)	0.31*	7.16***	382.2**	0.001**	0.40	0.06*	0.87†	21.32	0.60*	0.37*	0.39*	0.45**	0.03	0.20*
SED Year*BC_3 and parental line (DF=78)	1.19	85.40	3,419.5	0.018*	0.70	0.32	1.69	42.65	2.09	0.67	0.88	4.41	0.18	0.40*
SED Year*BC_3 lines (DF=78)	1.37	98.95	3,959.6	0.021*	0.78	0.37	1.89	47.86	2.39	0.74	1.00	5.10	0.21	0.45*

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Table 4.4 Minimum, maximum and mean values for AGDM per Main Shoot (MS), plant height (PH), GDW MS^{-1}, GN MS^{-1}, MS spikelets ear^{-1}, MS fertile spikelets ear^{-1}, MS GN $spikelet^{-1}$ and MS rachis length (RL) for 37 BC_3 winter wheat NILs and the Humber recurrent parent in 2013, 2014 and SEDs for BC_3 line, year and interaction.

	AGDM (g) MS^{-1}	**PH (cm)**	**GDW (g) MS^{-1}**	**GN MS^{-1}**	**MS Spikelets ear^{-1}**	**MS Fertile spikelets ear^{-1}**	**MS GN $spikelet^{-1}$**	**MS RL (cm)**
2013 BC_3S_5 lines								
Minimum	2.63	67.9	1.05	31.7	17.9	14.9	1.94	7.19
Maximum	3.84	86.9	2.04	58.9	20.9	19.1	3.32	9.65
Mean	3.21	74.3	1.77	49.8	19.5	16.9	2.95	8.23
Parental line (Humber)	2.95	66.1	1.62	50.2	18.7	16.4	3.05	7.64
SED between BC_3 and parental line (DF=39)	0.38	2.43***	0.23	6.29	0.50**	0.81*	0.31	0.39***
SED within BC_3 lines (DF=39)	0.44	2.80***	0.26	7.26	0.58**	0.94*	0.36	0.45***
2014 BC_3S_6 lines								
Minimum	4.09	62.2	2.63	60.2	21.0	19.5	2.83	8.54
Maximum	5.41	76.9	3.53	80.2	24.6	23.8	3.72	11.44
Mean	4.75	69.4	3.08	70.2	22.8	21.8	3.22	10.06
Parental line (Humber)	4.02	62.7	2.64	62.1	21.9	20.7	3.00	9.12
SED between BC_3 and parental line (DF=39)	0.28*	1.84***	0.22	4.32*	0.51***	0.65***	0.16*	0.42***
SED within BC_3 lines (DF=39)	0.33*	2.12***	0.26	4.99*	0.59***	0.75***	0.18*	0.49***
Cross-year ANOVA								
BC_3 lines								
Minimum	3.49	65.0	2.15	51.4	19.5	17.2	2.58	8.08
Maximum	4.49	81.0	2.76	66.6	22.8	21.2	3.41	10.24
Mean	3.98	71.8	2.42	60.0	21.1	19.3	3.08	9.14
Parental line (Humber)	3.49	64.4	2.13	56.1	20.3	18.6	3.02	8.38
SED between BC_3 and parental line (DF=37)	0.24**	1.50***	0.16*	3.81*	0.35***	0.52***	0.17†	0.29***
SED within BC_3 lines (DF=37)	0.27**	1.73***	0.18*	4.40*	0.41***	0.60***	0.20†	0.33***
SED Year (DF=2)	0.12**	1.03*	0.06**	0.45***	0.20**	0.16**	0.007***	0.10**
SED Year*BC_3 and parental line (DF=78)	0.35	2.33	0.23	5.32	0.53*	0.73*	0.24	0.41*
SED Year*BC_3 lines (DF=78)	0.40	2.63	0.26	6.16	0.60*	0.85*	0.28	0.47*

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Averaging across years, BC_3 NILs differed for main-shoot traits in the following ranges: AGDM shoot^{-1} (3.49-4.49 g, *P<0.01*), plant height (65.0-81.0 cm, *P<0.001*), GDW ear^{-1} (2.15-2.76 g, *P<0.05*), GN ear^{-1} (51.4-66.6, *P<0.05*), spikelets ear^{-1} (19.5-22.8, *P<0.001*), fertile spikelets ear^{-1} (17.2-21.2, *P<0.001*) and rachis length (8.08-10.24 cm, *P<0.001*). The upper values of these ranges were generally significantly higher than the Humber recurrent parent. Year had effects on AGDM shoot^{-1} (*P<0.01*), plant height (*P<0.05*), GDW ear^{-1} (*P<0.01*), GN ear^{-1} (*P<0.001*), spikelets ear^{-1} (*P<0.01*), fertile spikelets ear^{-1} (*P<0.01*), GN spikelet^{-1} (*P<0.001*) and RL (*P<0.01*). The year x genotype interaction was significant for spikelets ear^{-1}, fertile spikelets ear^{-1} and rachis length (*P<0.05*).

In each year for main-shoot traits, a positive linear relationship was found between GN spikelet^{-1} and GN ear^{-1} (R^2 = 0.798 (*P<0.001*); 2013, R^2 = 0.328 (*P<0.001*); 2014), and between GN ear^{-1} and GDW (g) ear^{-1} (R^2 = 0.524 (*P<0.001*); 2013, R^2 = 0.626 (*P<0.001*); 2014) in winter wheat NILs (Fig. 4.10, a-b). In addition, a positive linear relationship was found between fertile spikelets ear^{-1} and GN ear^{-1} (R^2 = 0.369 (*P<0.001*); 2013, R^2 = 0.441 (*P<0.001*); 2014); and between rachis length (cm) and GN ear^{-1} (R^2 = 0.251 (*P<0.01*); 2013, R^2 = 0.558 (*P<0.001*); 2014) (Fig. 4.10, c-d).

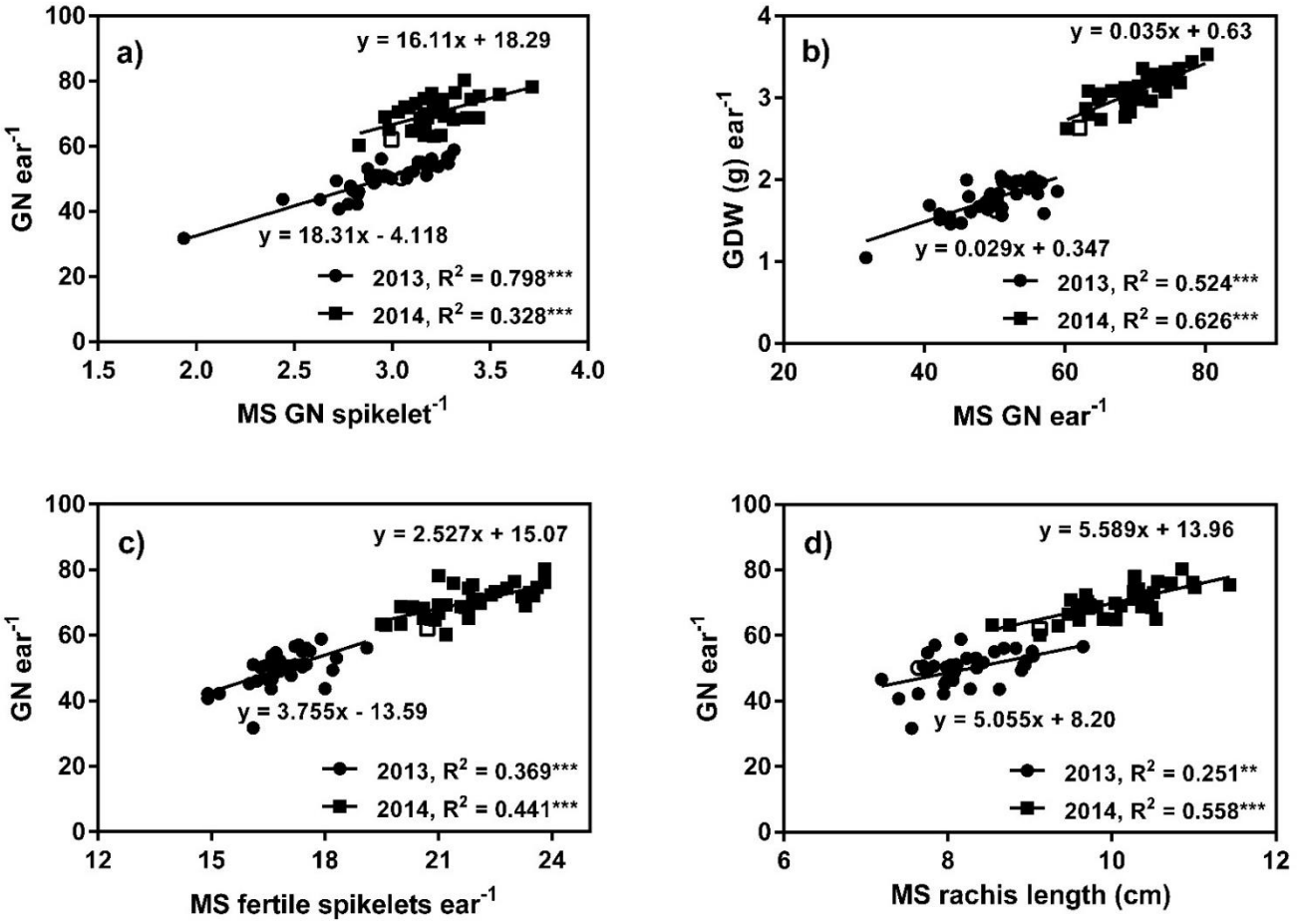

Fig. 4.10 Linear relationship between a) main-shoot (MS) GN spikelet^{-1} and GN ear^{-1}, b) MS GN ear^{-1} and GDW ear^{-1}, c) MS fertile spikelets ear^{-1} and GN ear^{-1}, and d) MS rachis length and GN ear^{-1} in BC_3 winter wheat NILs in 2013 (closed circles) and 2014 (closed squares) and the recurrent parent (Humber) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *** *P<0.001*.

4.3.2 Correlations among yield-related traits

4.3.2.1 BC_3 spring wheat NILs

In 2013, grain yield was positively correlated amongst NILs with ears m^{-2} (r=0.870, *P≤0.05*), grains m^{-2} (r=0.973, *P≤0.001*), AGDM m^{-2} (r=0.980, *P≤0.001*), plant height (r=0.889, *P≤0.01*) and fertile shoots (FS) $plant^{-1}$ (r=0.867, *P≤0.05*) (Table 4.5, full table in Appendices, Table A2-1). AGDM per unit area showed a positive correlation with ears m^{-2} (r=0.930, *P≤0.01*), grains m^{-2} (r=0.992, *P≤0.001*), plant height (PH) (r=0.879, *P≤0.01*), FS $plant^{-1}$ (r=0.812, *P≤0.05*). Grains m^{-2} was also positively associated with FS $plant^{-1}$ (r=0.813, *P≤0.05*). GN ear^{-1} was positively associated with fertile spikelets ear^{-1} (r=0.887, *P≤0.01*), GN $spikelet^{-1}$ (r=0.979, *P≤0.001*) and rachis length (r=0.822, *P≤0.05*). Rachis length was positively correlated with fertile spikelets ear^{-1} (r=0.952, *P≤0.001*) and GN $spikelet^{-1}$ (r=0.694, *P≤0.10*).

In 2014, grain yield was positively correlated with ears m^{-2} (r= 0.985, *P≤0.001*), grains m^{-2} (r=0.996, *P≤0.001*) and AGDM (r=0.996, *P≤0.001*) and negatively with RL (r=-0.950, *P≤0.01*). Grains m^{-2} was positively correlated with ears m^{-2} (r=0.987, *P≤0.001*) and negatively with RL (r=-0.933, *P≤0.01*). AGDM showed a positive correlation with ear m^{-2} (r=0.990, *P≤0.001*) and grains m^{-2} (r=0.995, *P≤0.001*). HI was positively correlated with plant height (r=0.819, *P≤0.05*). GN ear^{-1} was positively associated with GN $spikelet^{-1}$ (r=0.973, *P≤0.001*) but negatively associated with grains m^{-2} (r=-0.702, *P≤0.10*) and fertile shoots $plant^{-1}$ (r=-0.793, *P≤0.05*).

Significant correlations were found between main-shoot traits in BC_3 spring wheat lines (Table 4.6, full table in Appendices, Table A2-2). In 2013, GN MS^{-1} was positively correlated with AGDM MS^{-1} (r=0.902, *P≤0.01*), GDW MS^{-1} (r=0.932, *P≤0.01*), and GN $spikelet^{-1}$ MS^{-1} (r=0.950, *P≤0.01*) whilst GDW MS^{-1} was positively correlated with GN ear^{-1} (r=0.824, *P≤0.05*), AGDM MS^{-1} (r=0.960, *P≤0.001*) and GN $spikelet^{-1}$ MS^{-1} (r=0.854, *P≤0.05*).

In 2014, a positive correlation was found between GN MS^{-1} and AGDM MS^{-1} (r=0.914, *P≤0.01*), GDW MS^{-1} (r=0.981, *P≤0.001*), MS fertile spikelet ear^{-1} (r=0.788, *P≤0.05*), MS GN $spikelet^{-1}$ (r=0.945, *P≤0.01*) and MS RL (r=0.768, *P≤0.05*) but there was a negative correlation with grain yield (r=-0.759, *P≤0.05*) and ears m^{-2} (r=-0.794, *P≤0.05*). MS GN $spikelet^{-1}$ showed a significant positive correlation with GN ear^{-1} (r=0.941, *P≤0.01*) and GDW MS^{-1} (r=0.903, *P≤0.01*) but a negative correlation with fertile shoots $plant^{-1}$ (r=-0.762, *P≤0.05*).

Table 4.5 Pearson correlation (r) amongst BC_3 spring wheat NILs for grain yield (100% DM), ears m^{-2}, grains m^{-2}, HI, AGDM m^{-2}, plant height (PH), fertile shoots $plant^{-1}$, TGW, GN ear^{-1}, fertile spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length (RL) in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Ears m^{-2}**	**Grains m^{-2}**	**HI**	**AGDM (m^{-2})**	**PH (cm)**	**FS $plant^{-1}$**	**TGW (g)**	**GN ear^{-1}**	**Fertile spikelets ear^{-1}**	**GN $spikelet^{-1}$**	**RL ear^{-1}**
Grain yield	-	**0.985*****	**0.996*****	0.178	**0.996*****	0.303	0.582	**-0.699**†	**-0.739**†	-0.467	-0.644	**-0.950****
Ears m^{-2}	**0.870***	-	**0.987*****	0.093	**0.990*****	0.253	0.546	-0.654	**-0.747**†	-0.385	**-0.674**†	**-0.909****
Grains m^{-2}	**0.973*****	**0.916****	-	0.164	**0.995*****	0.336	0.536	**-0.730**†	**-0.702**†	-0.399	-0.618	**-0.933****
HI	-0.339	**-0.705**†	-0.510	-	0.092	**0.819***	0.148	-0.205	0.275	-0.550	0.447	-0.408
AGDM m^{-2}	**0.980*****	**0.930****	**0.992*****	-0.515	-	0.245	0.559	**-0.700**†	**-0.761***	-0.410	-0.279	**-0.924****
PH	**0.889****	**0.697**†	**0.839***	-0.249	**0.879****	-	-0.109	-0.566	0.378	-0.140	0.487	-0.415
FS $plant^{-1}$	**0.867***	**0.696**†	**0.813***	-0.136	**0.812***	0.785*	-	0.044	**-0.793***	**-0.671**†	**-0.734**†	-0.538
TGW	0.100	-0.192	-0.107	0.625	-0.03	0.368	0.249	-	0.160	0.114	0.068	**0.668**†
GN ear^{-1}	-0.393	**-0.733**†	-0.441	0.652	-0.456	-0.091	-0.292	-0.362	-	0.474	**0.973*****	0.634
Fertile spikelets ear^{-1}	-0.533	**-0.818***	-0.551	0.590	-0.577	-0.345	-0.464	0.073	**0.887****	-	0.274	0.630
GN $spikelet^{-1}$	-0.255	-0.617	-0.317	0.638	-0.326	-0.070	-0.160	0.473	**0.979*****	**0.778***	-	0.485
RL ear^{-1}	-0.660	**-0.818***	-0.617	0.398	-0.654	-0.473	-0.577	-0.131	**0.822***	**0.952*****	**0.694**†	-

† *P≤0.10,* * *P≤0.05*, ** *P≤0.01*, *** *P≤0.001*

Table 4.6 Pearson correlation (r) amongst BC_3 spring wheat NILs for grain yield (100% DM), $ears_{CAL.}$ m^{-2}, grains m^{-2}, plant height (PH), fertile shoots $plant^{-1}$, GN ear^{-1}, AGDM MS^{-1}, GDW MS^{-1}, GN MS^{-1}, MS fertile spikelets ear^{-1}, MS GN $spikelet^{-1}$, MS rachis length and $ears_{COUNT}$ m^{-2} in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Ears m^{-2} CAL.**	**Grains m^{-2}**	**PH (cm)**	**FS $plant^{-1}$**	**GN ear^{-1}**	**AGDM (g) MS^{-1}**	**GDW (g) MS^{-1}**	**GN MS^{-1}**	**MS Fertile spikelets ear^{-1}**	**MS GN $spikelet^{-1}$**	**MS RL (cm)**	**Ears m^{-2} COUNT**
Grain yield (t ha^{-1})	-	**0.985*****	**0.996*****	0.303	0.582	**-0.739**†	**-0.718**†	**-0.821***	**-0.759***	**-0.829***	-0.593	**-0.955*****	**0.921****
Ears m^{-2} CAL.	**0.870***	-	**0.987*****	0.253	0.546	**-0.747**†	**-0.780***	**-0.858***	**-0.794***	**-0.845***	-0.640	**-0.939****	**0.913****
Grains m^{-2}	**0.973*****	**0.916****	-	0.336	0.536	**-0.702**†	**-0.693**†	**-0.802***	**-0.730**†	**-0.801***	-0.570	**-0.937****	**0.933****
PH (cm)	**0.889****	**0.697**†	**0.839***	-	-0.109	0.378	0.319	0.276	0.342	-0.048	0.479	-0.232	0.444
FS $plant^{-1}$	**0.867***	**0.696**†	**0.813***	**0.785***	-	**-0.793***	-0.536	-0.636	**-0.731**†	-0.426	**-0.762***	-0.516	0.363
GN ear^{-1}	-0.393	**-0.733**†	-0.441	-0.091	-0.292	-	**0.872***	**0.955*****	**0.981*****	**0.729**†	**0.941****	**0.736**†	-0.546
AGDM MS^{-1}	-0.037	-0.287	-0.123	0.407	0.059	**0.695**†	-	**0.933****	**0.914****	**0.843***	**0.799***	**0.757***	-0.528
GDW MS^{-1}	-0.138	-0.468	-0.251	0.269	0.015	**0.824***	**0.960*****	-	**0.981*****	**0.816***	**0.903****	**0.814***	**-0.676**†
GN MS^{-1}	0.084	-0.260	0.005	0.427	0.215	**0.773***	**0.902****	**0.932****	-	**0.788***	**0.945****	**0.768***	-0.608
MS Fertile spikelets ear^{-1}	-0.549	**-0.823***	-0.567	-0.373	-0.323	**0.907****	0.426	0.617	0.588	-	0.545	**0.946****	**-0.786***
MS GN $spikelet^{-1}$	0.330	-0.020	-0.245	0.661	0.387	0.566	**0.889****	**0.854***	**0.950****	0.308	-	0.543	-0.421
MS RL	**-0.718**†	**-0.835***	-0.663	-0.569	-0.542	**0.782***	0.241	0.388	0.319	**0.915****	0.024	-	**-0.909****
Ears m^{-2} COUNT	**0.931****	**0.960*****	**0.927****	**0.821***	**0.719**†	-0.601	-0.108	-0.278	-0.085	**-0.780***	0.212	**-0.877****	-

† *P≤0.10*, * *P≤0.05*, ** *P≤0.01*, *** *P≤0.001*

4.3.2.2 BC_3 winter wheat NILs

In 2013, grain yield was positively correlated amongst NILs with ears m^{-2} (r=0.611, *P≤0.001*), grains m^{-2} (r=0.784, *P≤0.001*), AGDM m^{-2} (r=0.888, *P≤0.001*), anthesis date (AD) (r=0.503, *P≤0.01*), plant height (r=0.409, *P≤0.05*), TGW (r=0.387, *P≤0.05*) and GN $spikelet^{-1}$ (r=0.348, *P≤0.05*) (Table 4.7, full table in Appendices, Table A2-3). FS $plant^{-1}$ showed a positive correlation with AGDM $plant^{-1}$ (r=0.833, *P≤0.001*), GDW $plant^{-1}$ (r=0.783, *P≤0.001*), GN $plant^{-1}$ (r=0.883, *P≤0.001*), fertile spikelets ear^{-1} (r=0.498, *P≤0.01*) and a negative correlation with GN $spikelet^{-1}$ (r=-0.365, *P≤0.05*). Moreover, GN ear^{-1} was positively associated with fertile spikelets ear^{-1} (r=0.439, *P≤0.01*), GN $spikelet^{-1}$ (r=0.773, *P≤0.001*) and RL (r=0.525, *P≤0.001*).

In 2014, grain yield was positively correlated amongst NILs with ears m^{-2} (r=0.766, *P≤0.001*), grains m^{-2} (r=0.863, *P≤0.001*), AGDM (r=0.967, *P≤0.001*) and AD (r=0.420, *P≤0.01*). Fertile shoots $plant^{-1}$ was positively associated with AGDM $plant^{-1}$ (r=0.856, *P≤0.001*), GDW $plant^{-1}$ (r=0.848, *P≤0.001*) and GN $plant^{-1}$ (r=0.867, *P≤0.001*). GN ear^{-1} was positively associated with fertile spikelets ear^{-1} (r=0.524, *P≤0.001*), GN $spikelet^{-1}$ (r=0.685, *P≤0.001*) and rachis length (r=0.800, *P≤0.001*).

In BC_3 winter wheat lines in 2013, positive correlations were found between GN MS^{-1} and AGDM MS^{-1} (r=0.395, *P≤0.05*), GDW MS^{-1} (r=0.724, *P≤0.001*), MS fertile spikelets ear^{-1} (r=0.607, *P≤0.001*), MS GN $spikelet^{-1}$ (r=0.893, *P≤0.001*) and MS RL (r=0.501, *P≤0.01*) and negative associations with ears m^{-2} (r=-0.371, *P≤0.05*). GDW MS^{-1} was positively correlated with HI (r=0.498, *P≤0.01*), AD (r=0.325, *P≤0.05*), PH (r=0.493, *P≤0.01*), GN ear^{-1} (r=0.651, *P≤0.001*), AGDM MS^{-1} (r=0.554, *P≤0.001*), MS fertile spikelets ear^{-1} (r=0.403, *P≤0.05*), MS GN $spikelet^{-1}$ (r=0.667, *P≤0.001*) and MS RL (r=0.603, *P≤0.001*) (Table 4.8, full table in Appendices, Table A2-4). AGDM MS^{-1} was positively correlated with plant height (r=0.456, *P≤0.01*) and

negatively with HI (r=-0.365, *P≤0.05*). $Ears_{COUNT}$ m^{-2} showed a positive correlation with grains m^{-2} (r=0.394, *P≤0.05*).

In 2014, strong positive correlations were found between GN MS^{-1} and AGDM MS^{-1} (r=0.810, *P≤0.001*), GDW MS^{-1} (r=0.791, *P≤0.001*), MS fertile spikelets ear^{-1} (r=0.664, *P≤0.001*), MS GN $spikelet^{-1}$ (r=0.573, *P≤0.001*) and MS RL (r=0.747, *P≤0.01*) and negative associations with ears m^{-2} (r=-0.592, *P≤0.001*). AGDM MS^{-1} positively associated with PH (r=0.496, *P≤0.01*) and negatively with ears m^{-2} (r=-0.564, *P≤0.001*).

Table 4.7 Pearson correlation (r) amongst BC_3 winter wheat NILs for grain yield (100% DM), ears m^{-2}, grains m^{-2}, HI, AGDM $plant^{-1}$, anthesis date (AD), plant height (PH), fertile shoots $plant^{-1}$, TGW, GN ear^{-1}, fertile spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length (RL) in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Ears m^{-2}**	**Grains m^{-2}**	**HI**	**AGDM (g) $plant^{-1}$**	**AD**	**PH (cm)**	**FS $plant^{-1}$**	**TGW (g)**	**GN ear^{-1}**	**Fertile spikelets ear^{-1}**	**GN $spikelet^{-1}$**	**RL ear^{-1}**
Grain yield	-	**0.766*****	**0.863*****	-0.113	-0.068	**0.420****	0.244	-0.155	0.169	-0.041	-0.231	0.213	-0.047
Ears m^{-2}	**0.611*****	-	**0.816*****	**-0.372***	**-0.499****	**0.410***	-0.062	**-0.325***	-0.172	**-0.500****	**-0.445****	-0.137	**-0.483****
Grains m^{-2}	**0.784*****	**0.776*****	-	**-0.424****	-0.097	**0.488****	0.150	-0.117	**-0.350***	0.082	-0.160	**0.288**†	-0.029
HI	-0.078	**-0.511****	-0.261	-	0.167	**-0.540*****	**-0.390***	0.187	**0.625*****	-0.041	0.192	-0.260	0.044
AGDM $plant^{-1}$	0.067	-0.206	-0.005	-0.168	-	0.005	0.241	**0.856*****	0.071	**0.701*****	**0.490****	**0.393***	**0.731*****
AD	**0.503****	0.172	**0.275**†	-0.160	0.046	-	**0.594*****	-0.101	-0.196	0.053	**-0.403***	**0.493****	-0.019
PH	**0.409***	-0.093	0.086	-0.100	0.121	**0.738*****	-	-0.110	0.152	**0.375***	-0.109	**0.579*****	**0.356***
FS $plant^{-1}$	-0.158	-0.016	0.005	-0.179	**0.833*****	-0.239	**-0.299**†	-	-0.042	**0.342***	**0.384***	0.072	**0.444****
TGW	**0.387***	-0.208	**-0.267**†	**0.297**†	0.108	**0.351***	**0.484****	-0.242	-	-0.243	-0.119	-0.176	-0.036
GN ear^{-1}	0.041	**-0.565*****	0.069	**0.376***	**0.365***	0.103	**0.293**†	0.044	-0.035	-	**0.524*****	**0.685*****	**0.800*****
Fertile spikelets ear^{-1}	**-0.413***	**-0.492****	-0.264	0.258	**0.433****	**-0.359***	**-0.269**†	**0.498****	-0.244	**0.439****	-	-0.249	**0.673*****
GN $spikelet^{-1}$	**0.348***	-0.263	**0.265**†	**0.268**†	0.027	**0.380***	**0.510****	**-0.365***	0.146	**0.773*****	-0.216	-	**0.344***
RL ear^{-1}	0.154	**-0.307**†	-0.002	-0.078	**0.519****	**0.510****	**0.659*****	0.169	0.225	**0.525*****	**0.285**†	**0.353***	-

† *P≤0.10*, * *P≤0.05*, ** *P≤0.01*, *** *P≤0.001*

Table 4.8 Pearson correlation (r) amongst BC_3 winter wheat NILs for grain yield (100% DM), $ears_{CAL.}$ m^{-2}, grains m^{-2}, HI, anthesis date (AD), plant height (PH), GN ear^{-1}, AGDM MS^{-1}, GDW MS^{-1}, GN MS^{-1}, MS fertile spikelets ear^{-1}, MS GN $spikelet^{-1}$, MS rachis length and $ears_{COUNT}$ m^{-2} in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Ears m^{-2} CAL.**	**Grains m^{-2}**	**HI**	**AD**	**PH (cm)**	**GN ear^{-1}**	**AGDM (g) MS^{-1}**	**GDW (g) MS^{-1}**	**GN MS^{-1}**	**MS Fertile spikelets ear^{-1}**	**MS GN spikelet^{-1}**	**MS RL (cm)**	**Ears m^{-2} COUNT**
Grain yield	-	**0.766*****	**0.863*****	-0.113	**0.420****	0.244	-0.041	-0.052	-0.145	-0.248	**-0.406***	0.128	-0.182	0.181
Ears m^{-2} CAL.	**0.611*****	-	**0.816*****	**-0.372***	**0.410***	-0.062	**-0.500****	**-0.564*****	**-0.649*****	**-0.592*****	**-0.524*****	-0.191	**-0.553*****	0.224
Grains m^{-2}	**0.784*****	**0.776*****	-	**-0.424****	**0.488****	0.150	0.082	-0.153	**-0.304**†	-0.125	**-0.357***	0.236	-0.151	0.081
HI	-0.078	**0.511****	-0.261	-	**-0.540*****	**-0.390***	-0.041	0.186	**0.455****	0.176	**0.433****	-0.250	0.144	0.046
AD	**0.503****	0.172	**0.275**†	-0.160	-	**0.594*****	0.053	-0.079	-0.197	**-0.281**†	**-0.713*****	**0.423****	-0.219	0.085
PH	**0.409***	-0.093	0.086	-0.100	**0.738*****	-	**0.375***	**0.496****	**0.326***	0.131	**-0.413***	**0.624*****	0.187	-0.071
GN ear^{-1}	0.041	**-0.565*****	0.069	**0.376***	0.103	**0.293**†	-	**0.760*****	**0.677*****	**0.834*****	**0.348***	**0.703*****	**0.738*****	-0.240
AGDM MS^{-1}	0.205	-0.158	-0.013	**-0.365***	**0.295**†	**0.456****	**0.302**†	-	**0.945*****	**0.810*****	**0.388***	**0.627*****	**0.700*****	-0.256
GDW MS^{-1}	0.226	**-0.500****	-0.093	**0.498****	**0.325***	**0.493****	**0.651*****	**0.554*****	-	**0.791*****	**0.479****	**0.503****	**0.662*****	0.226
GN MS^{-1}	0.023	**-0.371***	0.114	**0.337***	0.146	0.175	**0.729*****	**0.395***	**0.724*****	-	**0.664*****	**0.573*****	**0.747*****	**-0.381***
MS fertile spikelets ear^{-1}	**-0.334***	**-0.526*****	-0.244	0.215	**-0.285**†	-0.155	**0.501****	**0.337***	**0.403***	**0.607*****	-	-0.231	**0.658*****	-0.184
MS GN spikelet^{-1}	0.214	-0.169	**0.277**†	**0.317**†	**0.335***	**0.293**†	**0.623*****	**0.271**†	**0.667*****	**0.893*****	0.188	-	0.250	**-0.309**†
MS RL	0.155	**-0.301**†	0.001	-0.023	**0.465****	**0.598*****	**0.510****	**0.554*****	**0.603*****	**0.501****	**0.437****	**0.372***	-	-0.056
Ears m^{-2} COUNT	**0.321**†	**0.378***	**0.394***	-0.110	0.253	0.091	-0.111	-0.198	-0.164	-0.136	**-0.282**†	-0.020	-0.131	-

† *P≤0.10,* * *P≤0.05,* ** *P≤0.01,* *** *P≤0.001*

A positive linear relationship was found in both years between grain m^{-2} and grain yield (R^2 = 0.614 (*P<0.001*); 2013, R^2 = 0.744 (*P<0.001*); 2014), and between AGDM and grain yield (R^2 = 0.788 (*P<0.001*); 2013, R^2 = 0.935 (*P<0.001*); 2014) in BC_3 winter wheat NILs (Fig. 4.11). In addition, a positive linear relationship was found between ears m^{-2} and grain yield (R^2 = 0.373 (*P<0.001*); 2013, R^2 = 0.586 (*P<0.001*); 2014) and between anthesis date and grain yield (R^2 = 0.268 (*P<0.01*); 2013, R^2 = 0.190 (*P<0.01*); 2014). There was a positive linear association between plant height and above-ground biomass (R^2 = 0.208 (*P<0.01*); 2013, R^2 = 0.246 (*P<0.01*); 2014) and between anthesis date and plant height (R^2 = 0.500 (*P<0.001*); 2013, R^2 = 0.370 (*P<0.001*); 2014) (Fig. 4.12). Positive relationships between plant height and GN spikelet^{-1} (R^2 = 0.260 (*P<0.01*); 2013, R^2 = 0.335 (*P<0.01*); 2014) and TGW (R^2 = 0.234 (*P<0.01*); 2013) were also observed in BC_3 winter NILs.

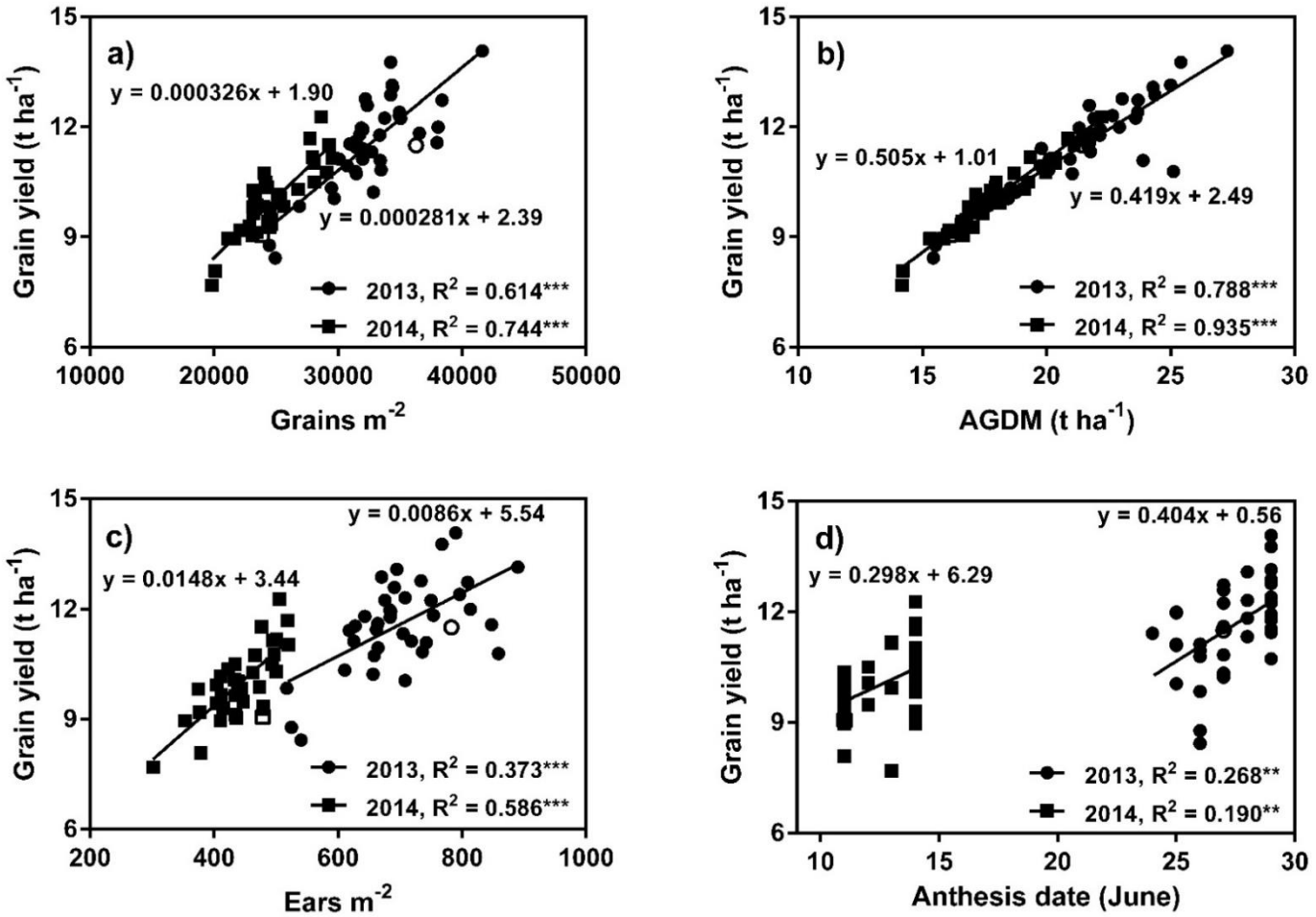

Fig. 4.11 Linear relationship between grain yield and traits associated with improved grain yield in BC_3 NILs in a) grains m^{-2}, b) AGDM m^{-2}, c) ears m^{-2} and d) anthesis date in 2013 (closed circles) and 2014 (closed squares) and the recurrent parent (Humber) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *** *P<0.001*.

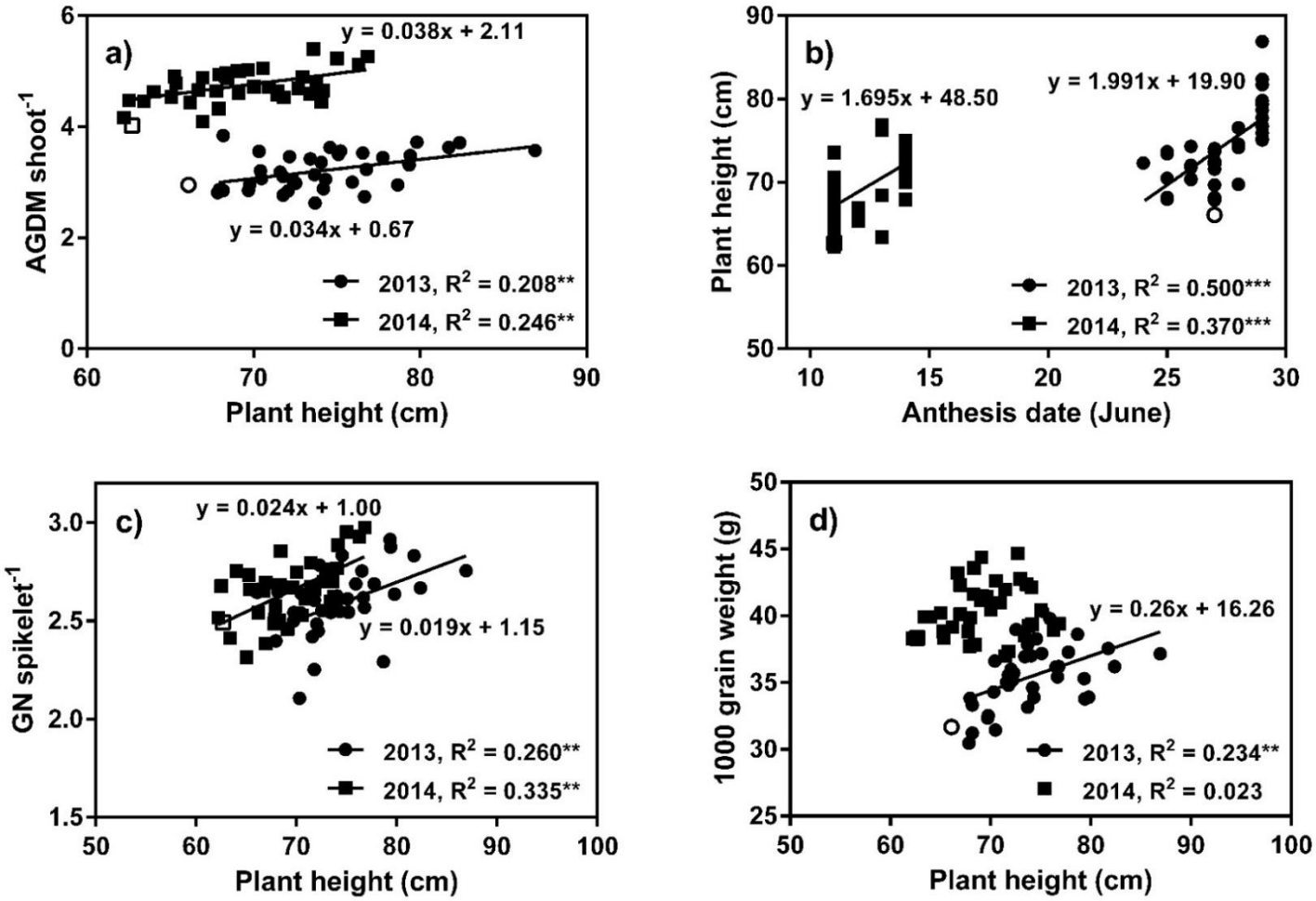

Fig. 4.12 Linear relationship between a) plant height and AGDM shoot^{-1}, b) anthesis date and plant height, c) plant height and GN spikelets^{-1}, d) plant height and TGW in Humber BC_3 NILs in in 2013 (closed circles) and 2014 (closed squares) and the recurrent parent (Humber) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *** *P<0.001*.

4.4 Discussion

Studying the physiological basis of transgressive segregation in BC_3 lines is important in order to improve physiological understanding of the effects of chromosome fragments introgression on yield potential traits in the UK environment. The discussion will focus on the physiological basis of significant differences in BC_3 lines taking account of the effects of anthesis date and plant height. The implications for breeders will also be discussed.

4.4.1 Physiological basis of grain yield and grains m^{-2} in BC_3 lines

In the present study, the grain yield of BC_3 spring wheat NILs was lower than the Ashby recurrent parent. It is possible that this was associated with the tiller inhibition (+*Tin1A*) gene in some of the BC_3 lines since ears m^{-2} and tiller survival were largely reduced but grains ear^{-1} was higher at harvest compared to Ashby.

On the other hand, for BC_3 winter wheat NILs, grain yield was generally similar to Humber, and higher than the Humber parent for two NILs (lines 174 and 177). In the spring wheat background, HI was increased in some lines compared to the recurrent parent, but ears m^{-2} and grains m^{-2} were lower than Ashby. In winter wheat, there was a slight increase in HI in BC_3 lines compared to the recurrent parent. There were no differences in ears m^{-2} and grains m^{-2} in winter wheat NILs compared to the Humber parent. Overall, grains m^{-2}, AGDM, ears m^{-2}, TGW, anthesis date and plant height were the main traits associated with higher yield in Humber BC_3 lines. Increase in grains m^{-2} might be associated with increased assimilate supply to the developing ear (see chapter 5 for further discussion on anthesis partitioning traits).

Present results suggested that grain yield was increased in the winter wheat NILs partly through biomass, in turn, suggesting efficiency of photosynthesis processes (RUE and LI) were improved while maintaining HI. Above-ground biomass contributed to yields above the Humber recurrent parent. Also, AGDM could be

improved by increasing plant height. Aisawi *et al.* (2015) reported findings for increased biomass and yield potential in taller semi-dwarf spring wheat CIMMYT cultivars introduced from 1966 to 2009. Reynolds *et al.* (2005) reported that the biomass increase in 7DL.7Ag introgression lines not only related to increased carbon assimilation rate during ear growth phase but also increased photosynthetic rate during grain filling. Biomass increases could also be related to senescence traits; these traits and underlying mechanisms will be investigated further in Chapter 6. The genetic gains in grain yield related to crop growth rate and DM production and partitioning pre-anthesis affecting grains m^{-2} will also be examined in more detail in Chapter 5.

The reduction in fertile tillers per plant and fertile tillers per unit area in BC_3 spring wheat lines could be due to tiller inhibition gene (*Tin1A*) as mentioned above, since the L14 CIMMYT parent possessed the *Tin1A* gene (see Chapters 5 and 7). In addition, GN MS^{-1} and GDW MS^{-1} were significantly improved in BC_3 spring wheat lines with fewer shoots m^{-2} than the Ashby parent and more fertile spikelets ear^{-1}, higher GN $spikelet^{-1}$ and longer rachis length compared to the Ashby recurrent parent. Poorer plant establishment could also partly explain the reduced shoots m^{-2} in some of the spring NILs as plant establishment was lower in 2014 in some BC_3 spring wheat NILs compared to Ashby (see Chapter 5). In Australia, Duggan *et al.* (2005b), who investigated the effects of *Tin1A* gene in NILs on yield and yield components under irrigated conditions where 50 kg ha^{-1} nitrogen was applied, reported that the grain yield was 3.81 t ha^{-1} in +*Tin1A* lines compared to 4.27 t ha^{-1} in freely tillering cultivars. No difference in fertile shoots per plant and the effect of plant establishment were observed in the winter NILs.

For winter wheat, grain yield, AGDM $shoot^{-1}$, TGW, spikelets ear^{-1}, fertile spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length were significantly increased in NILs compared to Humber. Since the grain number can be determined by floret fertility at anthesis, the effects on florets ear^{-1} and per unit area and underlying mechanisms will

investigated further in Chapter 5. An increase in rachis length per spikelets may provide an increase in potential grain weight (PGW) via raising ear photosynthesis per grain after anthesis duration (Savin *et al*., 1999, Ugarte *et al.*, 2007). This may partly explain the increased TGW in some NILs compared to Humber in the present study.

4.4.2 Effects of anthesis date and plant height on yield and yield components

In the present study, anthesis date was positively associated with grain yield and grains m^{-2} in winter NILs in 2013 and 2014, and plant height was positively correlated with grain yield and grains m^{-2} in spring and winter NILs in 2013. The height of BC_3 spring wheat lines was up to 16% shorter than Ashby while BC_3 winter wheat lines were up to 26% taller than Humber. An optimum crop height in the range 70-100 cm has been found in the modern wheat cultivars with greater yield and yield stability (Perry and D'Antuono, 1989). Similarly, highest grain yield, biomass, canopy light interception and leaf gas exchange was observed in winter bread wheat NILs with plant height ranging between 80 and 90 cm (Gent, 1995). The reduction in plant height with the introduction of semi-dwarf genes such as semi-dwarf (*Rht-B1b*, *Rht1* and *Rht-D1b*, *Rht2*) genes from the Japanese wheat variety (Norin 10) not only reduced stem height and lodging but also led to a greater proportion of assimilate partitioned to the ear, resulting in greater ear growth, more grains per unit land area and higher HI (Fischer and Stockman, 1986, Hedden, 2003). Evans (1998) reported that 70% of the commercial wheat cultivars carry either *Rht1* or *Rht2.* The yield gain of these two dwarfing alleles tend to be greatest in well managed, irrigated environments and high fertilizer inputs (Pearman *et al.*, 1978). Height differences in present BC_3 lines were, however, likely not associated with *Rht1* or *Rht2* since in the development of the BC lines, DH lines were selected to be *Rht2* semi-dwarfs, using a perfect marker for *Rht2* (see Chapter 3), so other plant

height genes must be determining the plant height differences. The effects of height and anthesis date on physiological traits will be examined in further detail in Chapter 5. The genetic control of markers controlling plant height in the BC_3 lines will be discussed further in Chapter 7.

4.4.3 Implications for breeders

There was genetic variability in ear fertility traits in winter NILs in the present results. Several of these traits were highly correlated with grain yield. Hence, there is much scope for using BC_3 winter lines in increase yield potential in the future UK breeding programs.

In spring wheat, the tiller inhibition gene likely contributed to less scope for using the BC_3 spring lines in increase yield potential in the future. However, increased ear partitioning index, could contribute to higher harvest index and grain weight. It has been also suggested that NILs with *+Tin1A* gene may have a greater water and nutrient uptake, cooler canopy temperature and better maintenance of green leaf area during grain filling (Hendriks *et al.*, 2016). However, since there appears to be a significant influence of genetic backgrounds and environment, careful consideration of these effects should be given to selection of genetic background for the tiller inhibition gene in UK wheat breeding.

The competition for assimilate supply between stem and ear during floret development results in grain yield limitation (Austin *et al.*, 1980, Kemp and Whingwiri, 1980, Slafer *et al*., 1990, Abbate *et al.*, 1998). In the current study, positive relationships between rachis length, spikelets ear^{-1}, GN ear^{-1} and grain dry weight per ear and negative relationships between each of grain yield, and grains m^{-2} and ears m^{-2} were observed in spring NILs. Gaju *et al.* (2009) suggested that large-ear phenotype with longer rachis length per spikelet can maintain potential grain weight better with increasing grain number. Higher grain numbers per ear and

heavier grain weight were associated with reduced tiller numbers per plant in spring NILs in the present study.

In winter wheat, grain yield, HI, TGW and ear fertility traits at harvest showed variation above the Humber recurrent parent. The increases in biomass appeared to contribute to grain yield and to be associated with plant height. Increase in plant height may favour biomass accumulation and heavier grain weight as taller lines are better adapted to capture radiation during vegetative growth and also greater stem sink-strength may upregulate photosynthesis pre-anthesis.

In the UK, genetic gain in grain yield was associated with increased growth rate during pre-anthesis and stem soluble carbohydrate reserves (WSC) providing assimilate during grain filling (Shearman *et al.*, 2005). In the present study, grain yield was associated with increased above-ground biomass, late flowering and taller plants, and these traits may be related to increase stem WSC in winter NILs. It has been reported that WSC contributed 10-20% of grain yield under favourable conditions and 50% or more under stress conditions where photosynthesis is reduced (Blum, 1998, van Herwaarden *et al*., 1998a, van Herwaarden *et al*., 1998b, Gebbing *et al.*, 1999, McIntyre *et al.*, 2012, Dreccer *et al.*, 2013). Since stem WSC has high heritability and positive relationship with grain yield (Shearman *et al.*, 2005, Ruuska *et al.*, 2006, Dreccer *et al*., 2009), the winter BC_3 lines may represent useful germplasm for increasing stem WSC in UK plant breeding programs.

Fruiting efficiency (FE) (grains per unit ear dry weight at anthesis) is an alternative component to enhance wheat yield potential and improve grain number (Ferrante *et al.*, 2015, Slafer *et al.*, 2015). Higher FE may be associated with a larger proportion of florets surviving at anthesis and/or a decrease in grain abortion during grain filling. However, a negative relationship between EPI and FE in wheat was reported in some previous studies (Gaju *et al*., 2009, González *et al*., 2011). In the present results (in Chapter 5), a negative relationship between EPI and FE was found in

spring NILs in 2014 and a positive relationship between EPI and FE in winter NILs in 2013 (Table 5.5). It will be important to improve understanding of any trade-offs between these traits when introgressing novel variation for EPI and FE into adapted UK wheat germplasm.

4.4.4 Summary

Four hypotheses were tested in this chapter:

Hypothesis 1*: NILs in the Ashby and Humber backgrounds (BC_3S_5 and BC_3S_6) produce higher grain yield than their respective recurrent parental lines.*

Results showed that the grain yield of BC_3 spring wheat lines was not significantly higher than the Ashby parent mainly due to limited fertile shoots plant^{-1} and poor plant establishment, so this hypothesis was not confirmed for spring wheat. For the winter wheat, grain yield of BC_3 winter wheat lines was significantly higher than the Humber parent for 2 lines (+22%), so the hypothesis was confirmed.

Hypothesis 2*: There are significant increases amongst BC_3 lines in grains per unit area and grains ear^{-1} and associated traits compared to the recurrent parental lines.*
Spring NILs had significantly higher grains ear^{-1} (+111%) but grains m^{-2} was decreased (-79%) compared to Ashby, so this hypothesis was confirmed for grains ear^{-1} but not for grains m^{-2}. In winter NILs, there was a trend for increased grains m^{-2} for 1 line (+16%) but there were no significant increases amongst winter NILs in grains ear^{-1} compared to Humber, so this hypothesis was confirmed for grains m^{-2} but not for grains ear^{-1}.

***Hypothesis 3**: Genetic variation in grain yield amongst NILs is positively associated with grains m^{-2} and above-ground biomass.*

Genetic variation in grain yield amongst spring and winter NILs is positively associated with grains m^{-2} and above-ground biomass (AGDM) m^{-2}, so this hypothesis was confirmed.

***Hypothesis 4:** There is a positive correlation between fertile spikelets ear^{-1} and rachis length and grains ear^{-1} amongst the NILs and significant differences in BC_3 lines compared to the recurrent parental lines.*

A positive correlation between fertile spikelets ear^{-1} and rachis length and grains ear^{-1} was observed amongst the winter NILs and significant increases in BC_3 winter lines compared to the Humber recurrent parent, so this hypothesis was confirmed in winter NILs. In spring NILs, MS fertile spikelets ear^{-1} and rachis length were associated with MS grains ear^{-1} in 1 year out of 2 so this hypothesis was partially supported but not fully confirmed.

CHAPTER 5 PHYSIOLOGICAL TRAITS ASSOCIATED WITH GRAIN YIELD AND YIELD COMPONENTS IN BC_3 NILs

5.1 Introduction

Previous studies have shown that yield potential of wheat is highly correlated with ear fertility traits under favourable conditions (Fischer, 1985, Fischer, 2008, Slafer and Savin, 1994, Reynolds *et al*., 2005, Shearman *et al*., 2005). Grain yield can be analysed using: (i) physiological components (above-ground biomass x harvest index) or (ii) numerical components (ears per m^2 x grains per ear x individual grain weight). In wheat, genetic progress in grain yield improvement has been highly associated with grain number per unit land area (GN) (Brancourt-Hulmel *et al.*, 2003, Shearman *et al.*, 2005, Acreche *et al.*, 2008, Fischer, 2008, Foulkes *et al.*, 2011, Clarke *et al.*, 2012) and grain number per m^2 remains a critical yield-limiting factor (Gaju *et al*., 2009, 2014b). Fischer (1984) suggested that determination of grain number per m^2 may be considered as the product of ear dry weight per m^2 at anthesis (EDWa) and the fruiting efficiency (FE), i.e. grains g^{-1} ear DM at anthesis (Eqn. 5.1). Moreover, during the pre-anthesis period, ear dry weight (EDWa) can be considered as the product of duration of stem-elongation phase (SEP) from GS31 to GS61 (days) (Zadoks *et al.*, 1974), crop growth rate (above-ground DM per m^2 per day; CGR) during stem elongation and ear dry matter partitioning index (EPI) (Eqn. 5.2).

$$\mathbf{GN = EDWa \times FE} \qquad \text{(Eqn. 5.1)}$$

$$\mathbf{EDWa = SEP \times CGR \times EPI} \qquad \text{(Eqn. 5.2)}$$

According to Eqn. 5.1 and 5.2, grains per m^2 could be increased by increasing the number of fertile florets (grains) per unit ear weight at anthesis and/or increasing the ear dry weight at anthesis via a longer duration of stem elongation as a proportion of

the thermal time to anthesis (Slafer and Rawson, 1996). There is strong evidence that a longer stem-elongation period (from initiation of terminal spikelet to anthesis) not only reduces the rate of floret development but also increases the number of fertile florets as well as ear dry weight at anthesis (Miralles *et al*., 2000, González *et al*., 2003a). Furthermore, the ear DM at anthesis will be strongly influenced by the number of tillers per plant (Duggan *et al.*, 2005a). Spikelet number per ear and spikelet fertility also influence grain number per ear. The grains per m^2 is strongly affected by the number of florets which survive to form fertile florets at anthesis determined by the assimilate supply during the rapid ear-growth phase from early booting to anthesis (GS61) (González *et al.*, 2011).

Specific hypotheses tested in this chapter

1. BC_3 NILs have higher ear DM partitioning index and therefore greater ear DM and more fertile florets per unit area at anthesis than their recurrent parents.
2. There is a positive correlation between grains m^{-2} and fruiting efficiency and grains per ear amongst the BC_3 NILs.
3. NILs can be identified with more spikelets ear^{-1} and more fertile florets $spikelets^{-1}$ in NILs hence more fertile florets ear^{-1} compared to their recurrent parents.
4. There is a positive correlation between fertile florets ear^{-1} at anthesis and grains per ear at harvest amongst the BC_3 NILs.
5. There is a positive correlation between EPI and FE at anthesis amongst the BC_3 NILs.

5.2. Materials and Methods

5.2.1 Field experiments

The field experiments in 2012/13 and 2013/14 were conducted at University of Nottingham farm, Sutton Bonington Campus, Leicestershire, UK (52° 49' 59'' N, 1° 14' 50'' W) (52.833 N, -1.247 W). A complete randomized block design was used for the field trials. The 44 BC_3S_{5-6} lines were planted in two replications in each of 2012/13 and 2013/14. Each line was grown in 1 x 1.2 m^2 plots. Full details are given in Chapter 3.

5.2.2 Crop measurements

5.2.2.1 Crop development, shoot number and DM partitioning

In each plot, shoot counts on five tagged plants were recorded at GS31 and GS61. The number of fertile shoots (healthy green shoot) and infertile shoots (a yellow shoot pre GS39 or after GS39 a shoot with no evidence of booting) were counted and recorded.

At anthesis (GS61), in order to determine dry matter partitioning, five fertile shoots per plot were randomly sampled. Their fresh weights were recorded and the shoots were then separated into four components: ears, stems and attached leaf sheaths, green leaf lamina and dead leaf lamina. The green area of the lamina, stem and ear was recorded using a leaf area meter (LICOR 3100, NE, USA). Each component was weighed separately after oven-drying at 85°C for 48 hours. The spikelet number and length of rachis were recorded for each ear.

5.2.2.2 Floret fertility and ear DM partitioning

At anthesis (GS61), ears of BC_3 spring and winter wheat lines, 3 randomly selected primary shoots (those shoots within 10 cm of tallest shoot in plot) per plot were dissected to determine the developmental stage of all florets and to count the total number of fertile florets per spikelet under a binocular microscope. Full details are given in Chapter 3.

In 2013, 15 selected lines including 6 BC_3 spring and 9 BC_3 winter wheat lines and their parents (Ashby and Humber) were selected from the preliminary results on BC_3S_5 lines (6 spring lines, and 9 winter lines were assessed) with high expression for fertile florets, spikelet number ear^{-1}, and grain number ear^{-1} and used to determine floret fertility traits, these lines included lines containing *Tin1A* gene in the Ashby background and a chromosome 3A fragment in the Humber background (see Chapter 7). In 2014, all the 44 selected lines were assessed.

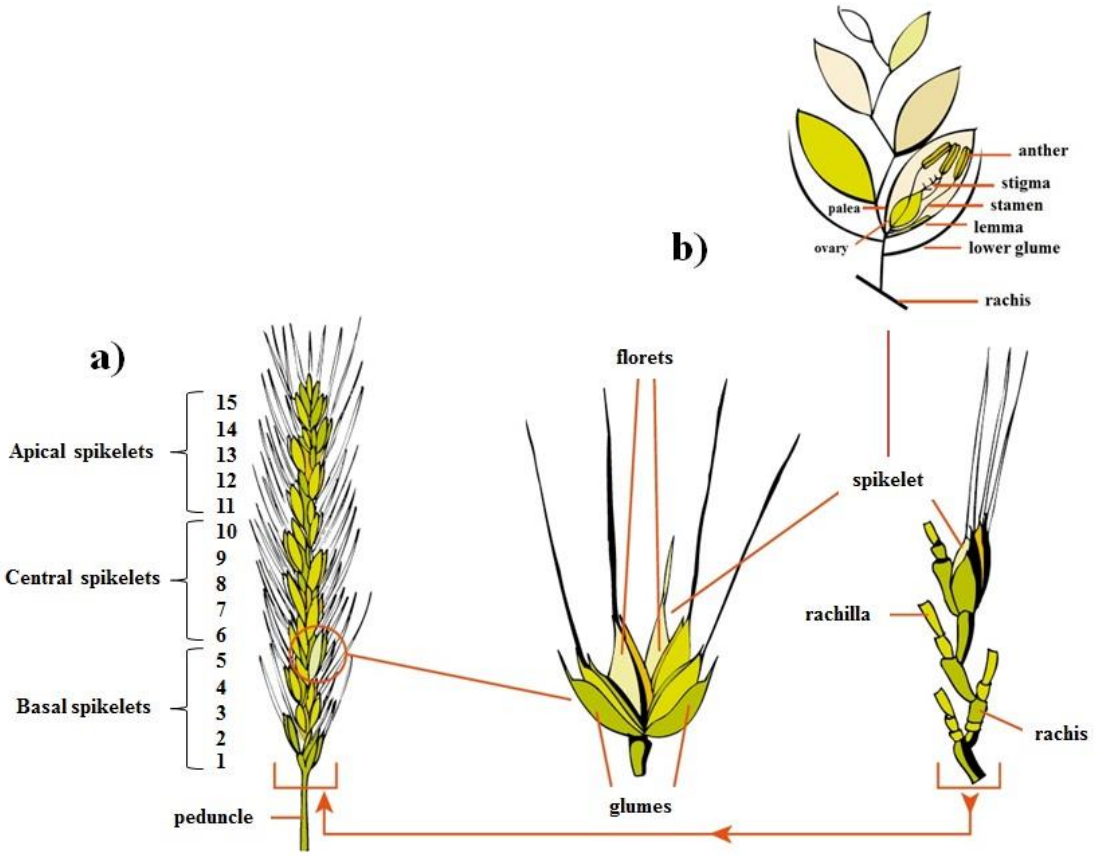

Fig. 5.1 Wheat ear showing (a) different spikelet position, (b) spikelet and florets (Adapted from (Bowden *et al*., 2008))

5.2.3 Statistical analysis

Analysis of variance (ANOVA) and correlation and regression analysis was performed on data, with year and genotype as fixed factors and replicates as random factors using Genstat 16th edition. When the ANOVA indicated significant differences, the mean values of treatments were calculated and compared using the least significant difference (5%) of the means of Fisher, calculated from standard errors of the difference of the means using appropriate degrees of freedom. Correlations among traits and between traits and grain yield were calculated using the mean values of genotypes.

5.3 Results

5.3.1 Crop development and tillering

In 2013, only small variation was found in date of GS31 from 14-19 May and in GS61 from 23-25 June amongst BC_3 spring wheat lines (Fig. 5.2, a-c). In 2014, the BC_3 spring wheat lines again reached GS31 in a narrow range from 25-26 April and similarly at GS61 from 11-12 June (Fig. 5.2, d-f). In both years, most NILs reached GS31, GS39 and GS61 on the same day or within one day of the parental line (Ashby) (Table 5.1).

In winter wheat BC_3 lines, slightly larger variations were found in dates of GS31, GS39 and GS61 in both years (Table 5.1; Fig. 5.3). At GS31, there was a range of 2 days amongst the BC_3 lines in both years. In 2013, the NILs reached GS61 from 24-29 June (5 days) and in 2014 from 11-14 June (3 days). In 2013 and 2014, some NILs reached GS61 later than the Humber recurrent parent by 2 and 3 days ($P<0.001$).

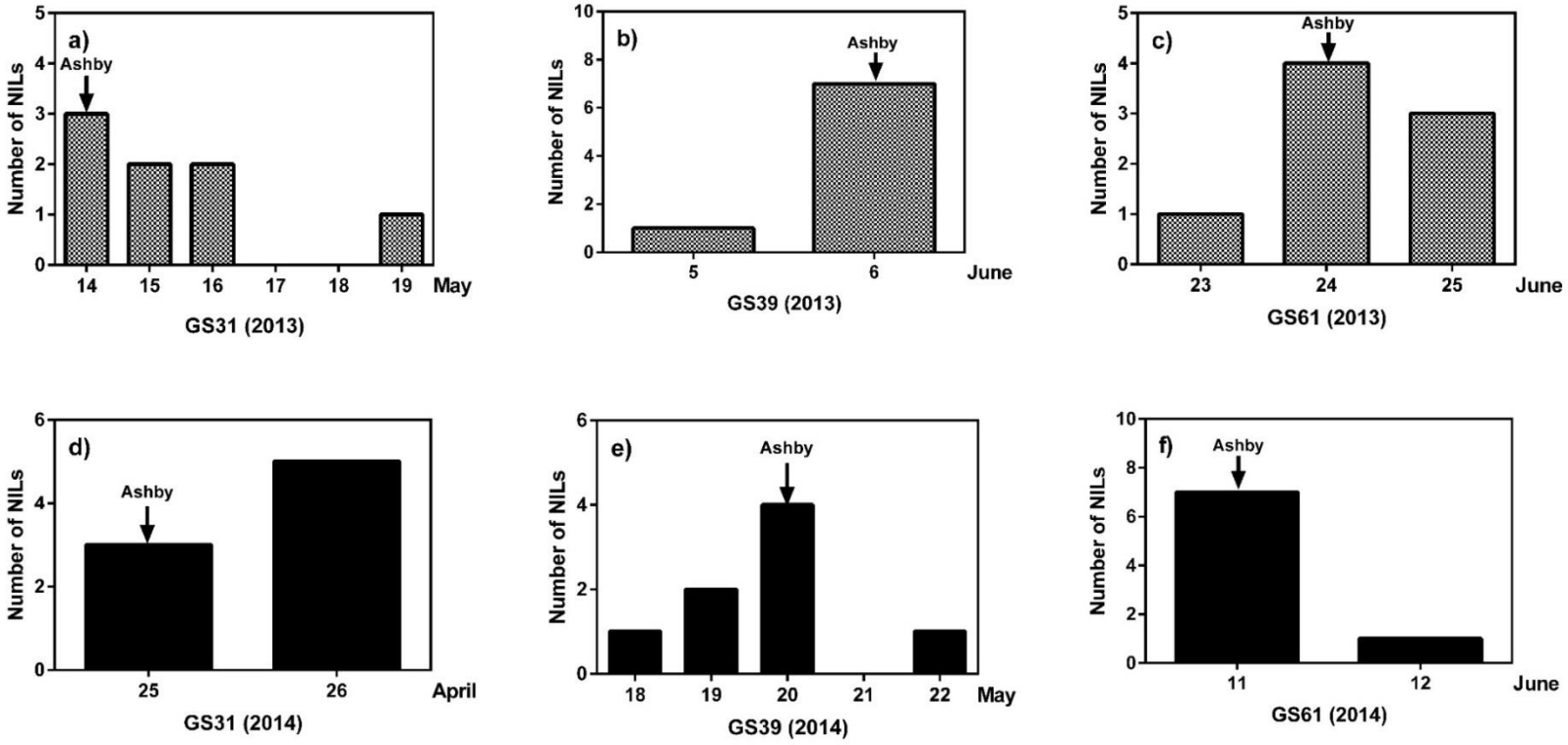

Fig. 5.2 Frequency distribution for 7 BC_3 spring wheat NILs and their Ashby recurrent parent for dates of GS31, GS39 and GS61 in 2013 (a-c) and 2014 (d-f), respectively. Mean values for the recurrent parent are indicated with arrows.

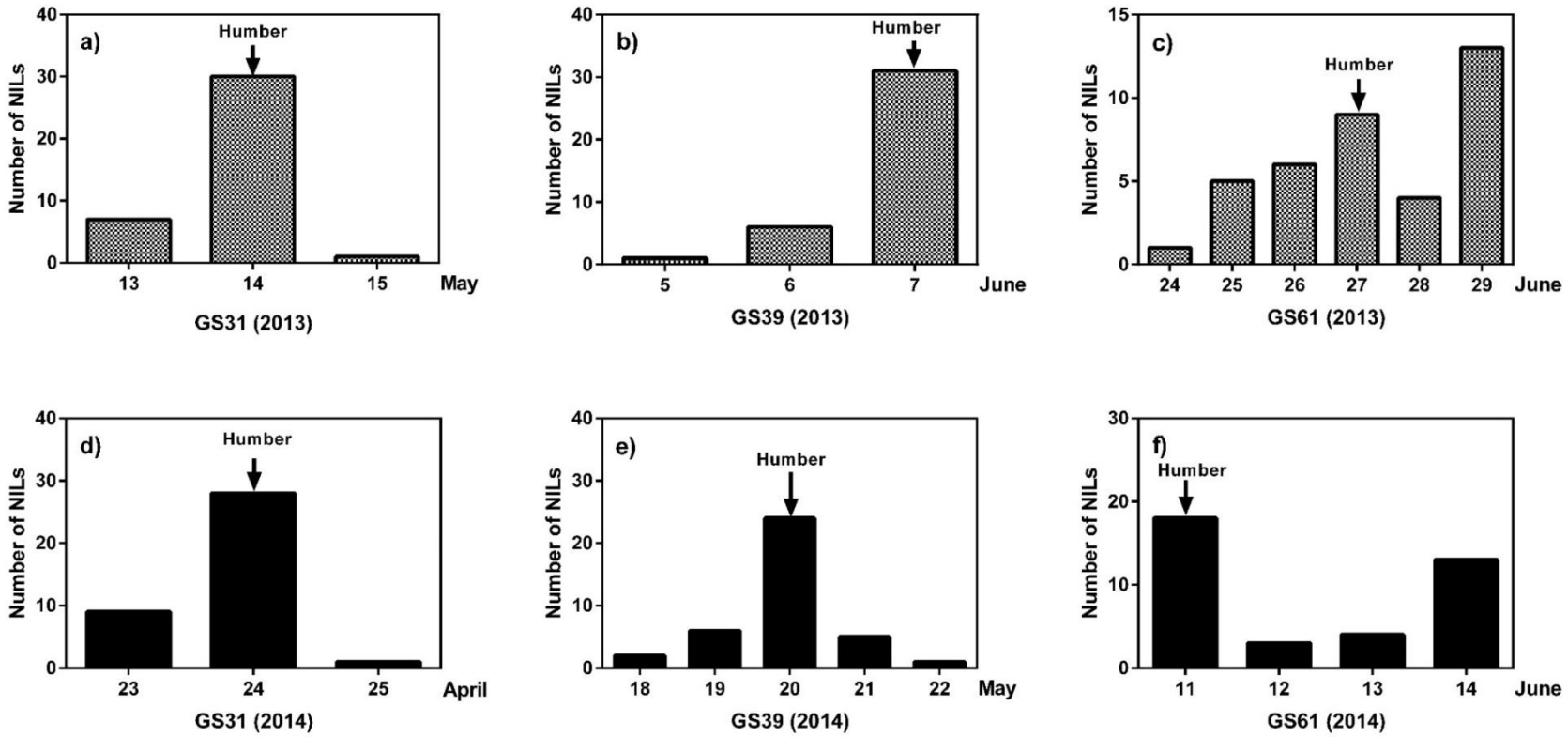

Fig. 5.3 Frequency distribution for 37 BC_3 winter wheat NILs and their Humber recurrent parent for dates of GS31, GS39 and GS61 in 2013 (a-c) and 2014 (d-f), respectively. Mean values for the recurrent parent are indicated with arrows.

Table 5.1 Date of developmental stages (GS31=onset of stem extension, GS39 = flag-leaf ligule is visible, GS61 = anthesis) among 7 BC_3 spring and 37 BC_3 winter wheat NILs and the recurrent parent (Ashby or Humber) in 2013, 2014 and SEDs for BC_3 NILs.

	Spring wheat NILs				Winter wheat NILs			
	GS31	**GS39**	**GS61**	**Days sowing to anthesis**	**GS31**	**GS39**	**GS61**	**Days sowing to anthesis**
2013 BC_3S_5 lines								
Minimum	14 May	5 Jun	23 Jun	230.0	13 May	5 Jun	24 Jun	231.0
Maximum	18 May	6 Jun	25 Jun	232.5	15 May	7 Jun	29 Jun	236.5
Mean	15 May	5 Jun	24 Jun	231.7	13 May	6 Jun	27 Jun	234.4
Parental line (Ashby or Humber)	14 May	6 Jun	24 Jun	231.0	14 May	7 Jun	27 Jun	234.0
SED BC_3 vs parental line (DF =8 or 39)	0.65**	0.77	0.92	0.92	0.32	0.44*	0.85***	0.85***
SED within BC_3 lines (DF = 8 or 39)	0.75**	0.89	1.06	1.06	0.36	0.51*	0.99***	0.99***
2014 BC_3S_6 lines								
Minimum	25 Apr	18 May	11 Jun	230.0	23 Apr	18 May	11 Jun	230.0
Maximum	26 Apr	22 May	12 Jun	231.0	25 Apr	22 May	14 Jun	233.0
Mean	25 Apr	19 May	11 Jun	230.4	24 Apr	19 May	12 Jun	231.5
Parental line (Ashby or Humber)	25 Apr	20 May	11 Jun	230.5	24 Apr	20 May	11 Jun	230.5
SED BC_3 vs parental line (DF =8 or 39)	0.45	1.30	0.65	0.65	0.64	0.88	0.50***	0.50***
SED within BC_3 lines (DF =8 or 39)	0.52	1.50	0.75	0.75	0.74	1.01	0.58***	0.58***
Cross-year ANOVA								
SED BC_3 vs parental line (DF=7 or 37)	0.38***	0.67	0.55	0.56	0.37	0.49*	0.49***	0.49***
SED within BC_3 lines (DF=7 or 37)	0.44***	0.77	0.64	0.64	0.42	0.57*	0.57***	0.57***
SED Year (DF=2)	0.18***	1.03***	0.76***	0.75	0.19***	0.27***	0.60***	0.60*
SED Year*BC_3 lines and parental line (DF=17 or 78)	0.52***	1.34	1.05	1.04	0.54	0.73	0.91	0.91
SED Year*BC_3 lines (DF=17 or 78)	0.61***	1.45	1.14	1.14	0.62	0.83	1.00	1.00

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

5.3.1.1 Tiller production and survival in spring wheat NILs

In 2013, spring BC_3S_5 lines at GS31 ranged from 6.9 to 8.5 fertile shoots plant^{-1} compared to Ashby with 7.7 shoots plant^{-1} (*P>0.10*) (Fig. 5.4; Table 5.2). At GS61, fertile shoots plant^{-1} decreased and varied from 0.4 to 4.0 in BC_3 lines compared to 3.7 in Ashby (*P<0.10*). Ears m^{-2} for NILs estimated from a direct count before harvest (range 68.0-485.0) differed in some NILs from Ashby (572.0) (*P<0.001*) but NILs did not differ in tiller survival (*P>0.10*).

In 2014, BC_3S_6 lines at GS31 ranged from 7.0 to 8.9 fertile shoots plant^{-1} while Ashby produced 7.1 shoots plant^{-1} (*P>0.10*). At GS61, fertile shoots plant^{-1} were decreased and varied from 1.8 to 4.5 in NILs compared to 5.1 in Ashby (*P>0.10*). Ears m^{-2} for NILs from direct counts were lower (range 135.0-363.0) than Ashby (554.0) (*P<0.01*), but there were no differences in tiller survival (*P>0.10*).

Averaging across years, there was no differences in fertile shoots plant^{-1} at GS31 but fertile shoots differed at GS61 and at harvest in NILs (range 1.93-3.79) compared to Ashby (4.38; *P<0.01*) (Table 4.1). Some spring wheat NILs showed trends for decreases in percentage tiller survival (26.2%-51.7%) compared to the recurrent parent Ashby (60.7%) (*P<0.10*). NILs overall produced fewer ears m^{-2} (range 153.0-414.0) than Ashby (563.0; *P<0.001*). A year effect and year x BC_3 line interaction were observed on % tiller survival and ears m^{-2} at harvest (*P<0.05*). The genetic control of tiller production and survival of these lines is investigated further in Chapter 7.

There was a positive linear relationship amongst NILs between ears m^{-2} and each of grain yield (R^2 = 0.860; 2013, R^2 = 0.865; 2014) and grains m^{-2} (R^2 = 0.860; 2013, R^2 = 0.871; 2014) in both years and between fertile shoots plant^{-1} and grain yield in 2013 (R^2 = 0.751; 2013, R^2 = 0.339; 2014) (Fig. 5.5). There was a negative linear relationship between fertile shoots plant^{-1} and GN ear^{-1} (R^2 = 0.085; 2013, R^2 = 0.629; 2014) in 2014 (Fig. 5.5).

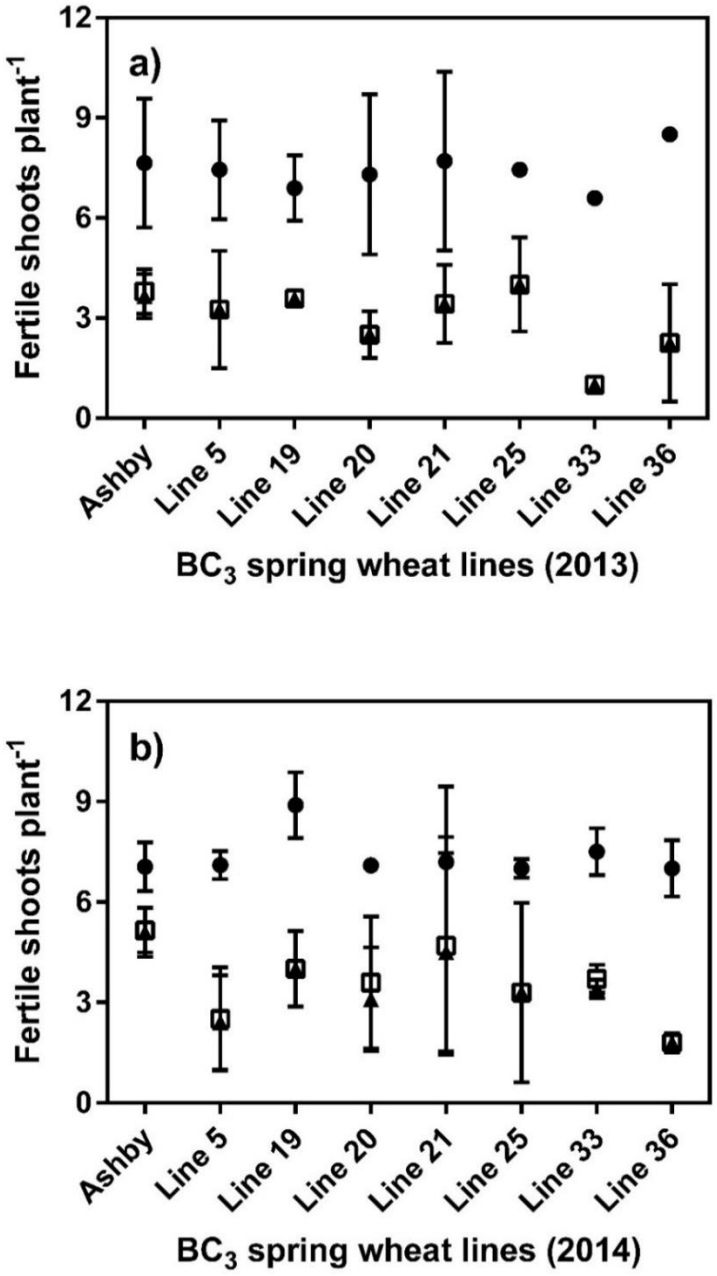

Fig. 5.4 Fertile shoots per plant in spring wheat NILs and Ashby recurrent parent measured at GS31 (closed circles), GS61 (open squares) and harvest (closed triangles), respectively in (a) 2013 and (b) 2014. Each point is the mean of two replicates, and the standard error of mean (SEM) is indicated with bars.

Table 5.2 Fertile shoots production among 7 BC_3 spring wheat and 37 BC_3 winter NILs and the recurrent parent (Ashby or Humber) in 2013, 2014 and SEDs for BC_3 NILs.

	Spring wheat NILs (DF=8)			Winter wheat NILs (DF=39)		
	% Shoot survival[1]	**Ears m^{-2} COUNT**	**Plants m^{-2} COUNT**	**% Shoot survival[1]**	**Ears m^{-2} COUNT**	**Plants m^{-2} COUNT**
2013 BC_3S_5 lines						
Minimum	14.3	68.0		40.9	415.0	
Maximum	53.8	485.0		62.6	700.0	
Mean	38.3	324.7		51.9	523.8	
Parental line (Ashby or Humber)	48.8	572.0		51.5	635.0	
SED between BC_3 and parental line	11.02	54.50***		5.26†	61.01*	
SED within BC_3 lines	12.72	63.00***		6.07†	70.45*	
2014 BC_3S_6 lines						
Minimum	25.7	135.0	65.0	60.3	460.0	95.0
Maximum	58.9	363.0	95.0	83.0	592.5	135.0
Mean	43.2	220.1	74.3	72.2	518.5	112.3
Parental line (Ashby or Humber)	72.6	554.0	110.0	68.4	508.7	121.3
SED between BC_3 and parental line	17.76	65.10**	14.99	5.84	36.87	11.88
SED within BC_3 lines	20.51	75.20**	17.31	6.74	42.58	13.72
Cross-year ANOVA						
BC_3 lines						
Minimum	26.2	153.0		55.8	447.5	
Maximum	51.7	414.0		69.0	603.8	
Mean	40.8	272.6		62.0	521.1	
Parental line (Ashby or Humber)	60.7	563.0		59.9	571.9	
SED between BC_3 and parental line	10.58†	42.2***		3.93	35.64†	
SED within BC_3 lines	12.22†	48.7***		4.54	41.16†	
SED Year (DF=2)	1.67*	30.8		3.32*	11.6	
SED between Year*BC_3 and parental line (DF=17 or 37)	13.92	63.1**		6.39*	50.88*	
SED within Year*BC_3 line (DF=17 or 37)	16.38	71.9**		7.15*	58.61*	

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

1 % tiller survival based on shoots numbers at GS31 and GS61

Shoot survival = percentage of fertile shoot of GS31 on ears at harvest.

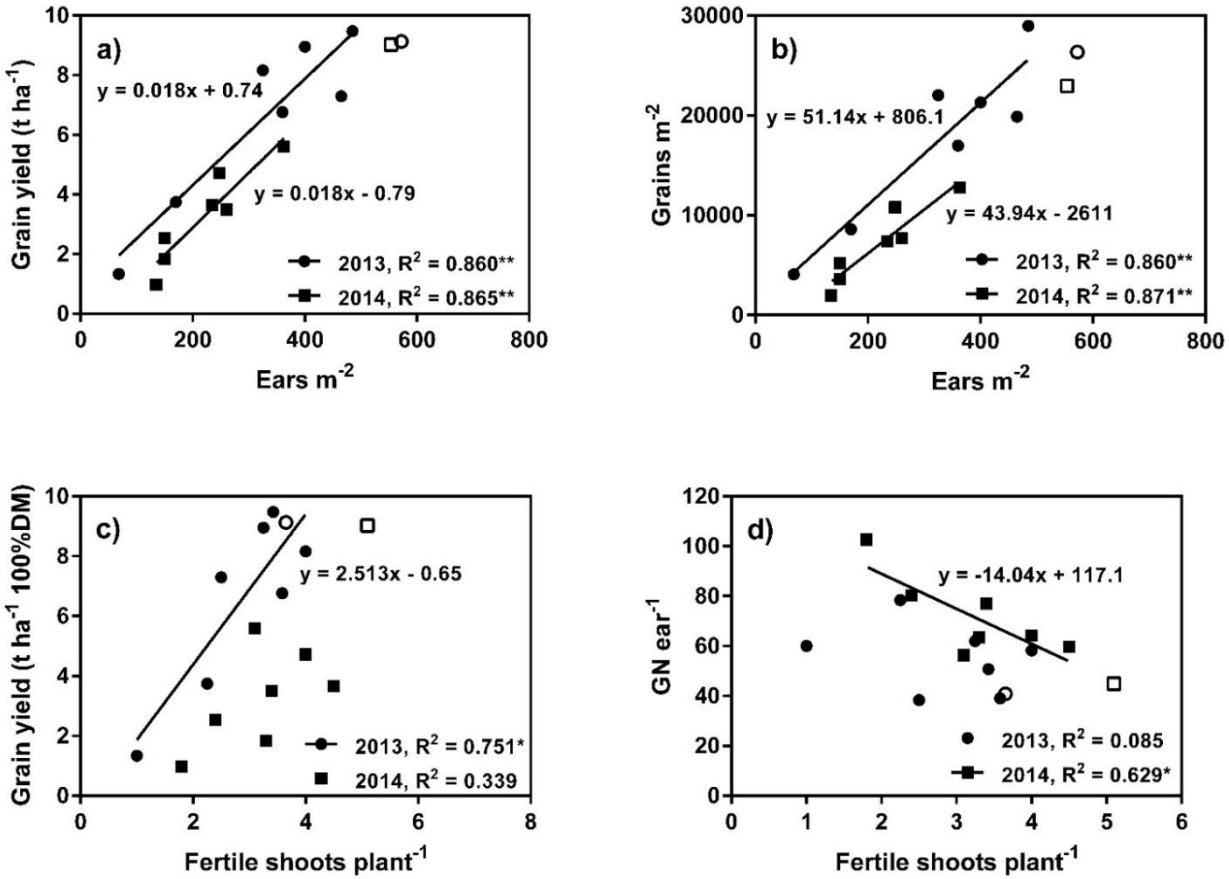

Fig. 5.5 (a) Linear relationship between ears m^{-2} and grain yield, (b) ears m^{-2} and grain m^{-2}, (c) fertile shoots $plant^{-1}$ at harvest and grain yield and (d) fertile shoots $plant^{-1}$ and GN ear^{-1} in 7 BC_3 spring wheat NILs in 2013 (closed circles) and 2014 (closed squares) and parental line (Ashby) in 2013 (open circle) and 2014 (open square). Significance level * *P<0.05*, ** *P<0.01*.

5.3.1.2 Tiller production and survival in winter wheat NILs

In winter wheat, at GS31 BC_3S_5 ranged from 6.0 to 10.2 shoots plant^{-1} compared to Humber with 8.0 shoots plant^{-1} (Fig. 5.6; Table 5.2; *P>0.10*). At anthesis, fertile shoots plant^{-1} in BC_3 lines ranged from 3.0 to 6.4 compared to 4.2 in Humber, but differences compared to the recurrent parent were not statistically significant (*P>0.10*).

In 2014, at GS31 BC_3S_6 lines ranged from 5.2 to 7.1 fertile shoots plant^{-1} compared to Humber at 5.5 (*P>0.10*). At GS61, the number of fertile shoots plant^{-1} varied from 3.7 to 5.2 in BC_3 lines compared to 3.7 in Humber. However, there were no statistically significant differences in fertile shoots plant^{-1} at GS31 or GS61 or in percent tiller survival and ears m^{-2} (*P>0.10*).

Averaging across years, tiller survival (%) and fertile shoots plant^{-1} at harvest did not differ amongst the NILs (range 3.45-5.20 fertile shoots plant^{-1} (Table 4.3) and 55.8-69.0% tiller survival (Table 5.2)). Ears m^{-2} from direct count differed amongst the 37 winter BC_3 lines ranging from 447.5 to 603.8 ears m^{-2} compared to Humber (571.9) (*P<0.10*). Year and year x genotype interaction were significant in %tiller survival (P<0.05) and %tiller survival and ears m^{-2} (*P<0.05*), respectively.

A positive linear relationship amongst NILs was found in 2013 between ears m^{-2} and grains m^{-2} (R^2 = 0.155; 2013), and in 2014 between tiller survival (%) and GN plant^{-1} (R^2 = 0.210; 2014), and in both years between fertile shoots plant^{-1} and GN plant^{-1} (R^2 = 0.779; 2013, R^2 = 0.751; 2014) and between fertile shoots plant^{-1} and AGDM plant^{-1} (R^2 = 0.693; 2013, R^2 = 0.733; 2014) (Fig. 5.7).

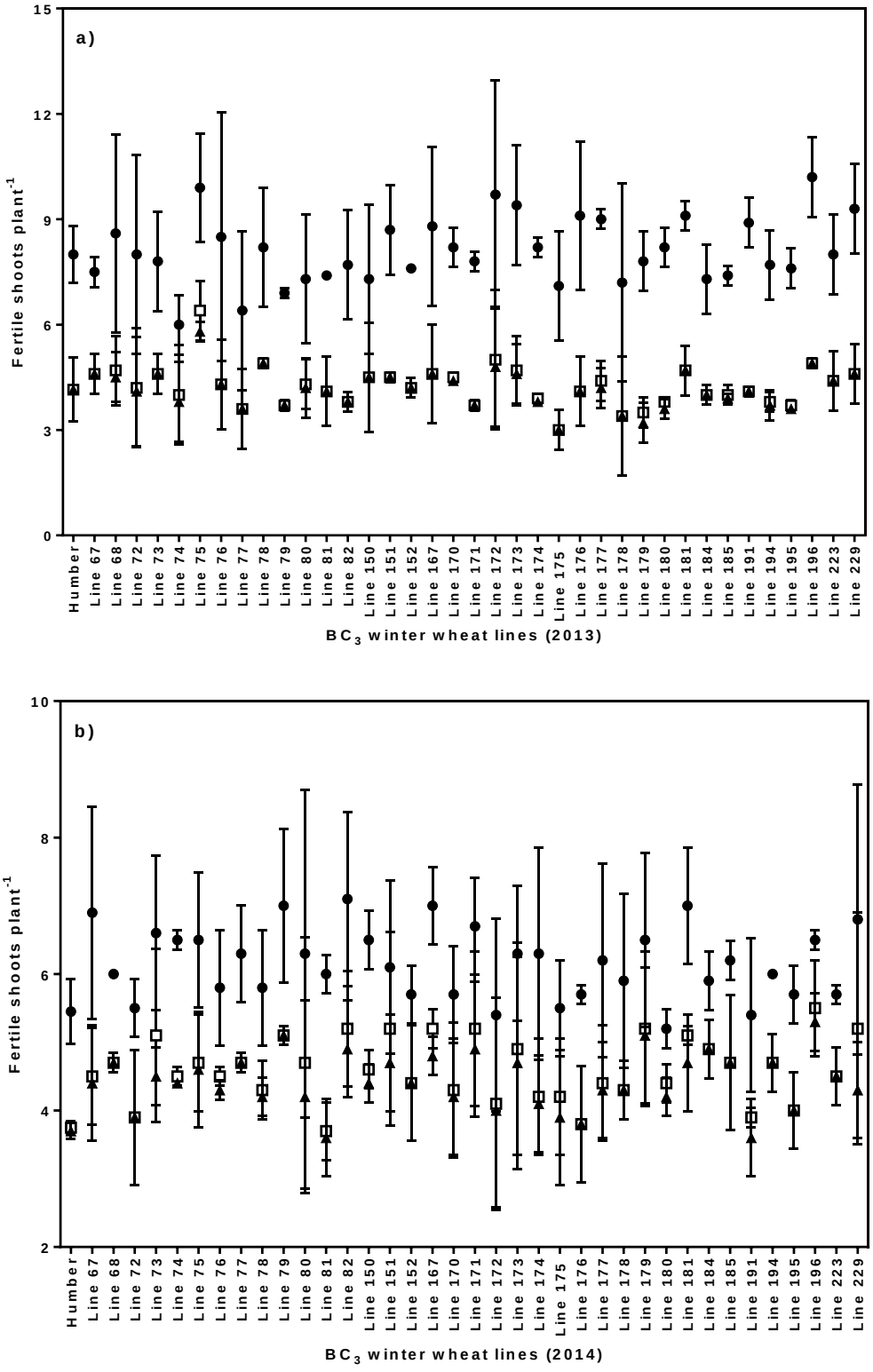

Fig. 5.6 Fertile shoots per plant in winter wheat NILs and Humber recurrent parent measured at GS31 (closed circles), GS61 (open squares) and harvest (closed triangles), respectively in (a) 2013 and (b) 2014. Each point is the mean of two replicates, and the standard error of mean (SEM) is indicated with bars.

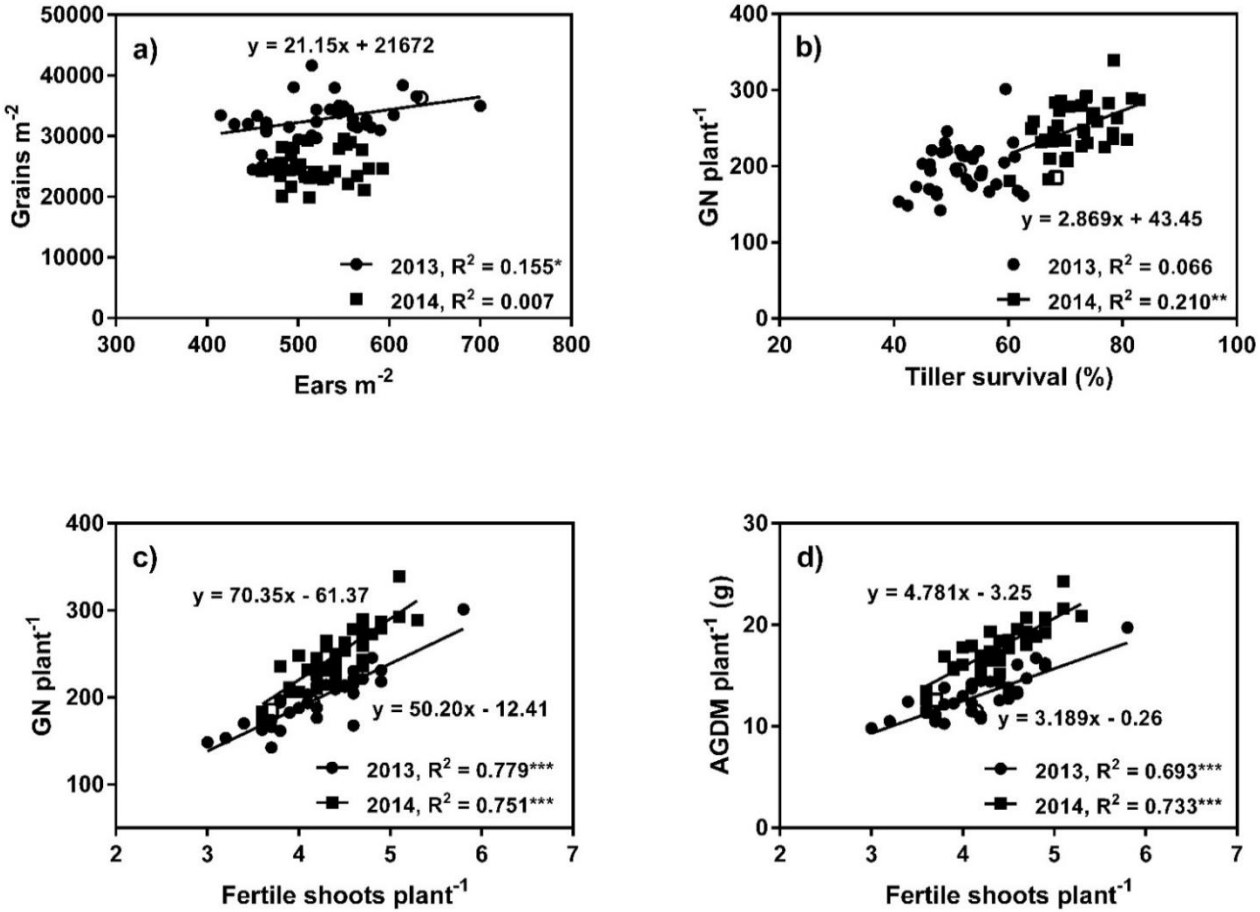

Fig. 5.7 (a) Linear relationship between ears m^{-2} and grain m^{-2}, (b) tiller survival (%) and GN $plant^{-1}$, (c) fertile shoots $plant^{-1}$ and GN $plant^{-1}$ and (d) Fertile shoots $plant^{-1}$ and AGDM $plant^{-1}$ in 37 BC_3 winter wheat NILs in 2013 (closed circles) and 2014 (closed squares) and parental line (Humber) in 2013 (open circle) and 2014 (open square). Significance level * *P<0.05*, ** *P<0.01*.

5.3.2 DM production and ear partitioning at anthesis

5.3.2.1 Spring wheat BC_3 NILs

In 2013, above-ground DM shoot^{-1} at anthesis from the five shoot samples per plot varied from 3.10 to 4.22 g shoot^{-1} in NILs compared to 2.95 for Ashby (*P<0.05*), and ear DM shoot^{-1} from 0.57 to 0.98 g compared to 0.57 g for Ashby at anthesis (*P<0.05*) (Table 5.3). Ear partitioning index ranged from 0.18 to 0.23 in NILs compared to 0.19 in Ashby (*P<0.10*), and FE (grains g^{-1}) from 20.2 to 57.7 grains g^{-1} in BC_3S_5 compared to Ashby (24.1 grains g^{-1}) (*P<0.01*).

In 2014, the above-ground DM shoot^{-1} ranged from 3.97 to 8.49 g shoot^{-1} in the BC_3 NILs compared to 2.35 g shoot^{-1} for Ashby (*P<0.01*). DM per ear amongst BC_3S_6 lines ranged from 0.99 to 2.18 g compared to 0.47 g for Ashby (*P<0.01*) and EPI from 0.22 to 0.29 compared to 0.20 for Ashby (*P<0.01*). Plant height in the NILs (range 42.6-52.5 cm) was decreased in several NILs compared to the recurrent parent (59.5 cm) (*P<0.01*).

Averaging across years, there were differences in green area shoot^{-1} (*P<0.001*), AGDM shoot^{-1} (*P<0.001*), ear partitioning index (*P<0.001*) and fruiting efficiency (*P<0.001*) compared to Ashby. Green area shoot^{-1} was increased in NILs (range 133.6-220.3 cm^2) compared to Ashby (119.2 cm^2; *P<0.001*). EPI varied from 0.22 to 0.26 compared to 0.20 for Ashby (*P<0.001*) and FE from 19.2-37.3 grains g^{-1} compared to 22.6 grains g^{-1}, respectively (*P<0.001*). There were effects of year for green area shoot^{-1} (*P<0.05*), AGDM shoot^{-1} (*P<0.01*), EPI (*P<0.05*) and FE (*P<0.001*) and year x genotype interactions were significant for green area shoot^{-1}, above-ground DM shoot^{-1}, EPI and FE (*P<0.001*).

5.3.2.2 Winter wheat BC_3 NILs

In 2013, there was little difference in AGDM shoot^{-1} ranged from 2.24 to 2.91 g shoot^{-1} in NILs compared to 2.43 g for Humber (*P>0.10*) (Table 5.4), EPI from 0.17 to 0.22 compared to 0.19 (*P<0.05*) and FE from 17.2 to 30.4 compared to 25.8, respectively (*P>0.10*).

In 2014, AGDM shoot^{-1} ranged from 2.71 to 3.40 g in NILs compared to 2.46 g for Humber (*P<0.001*); ear DM shoot^{-1} (0.57-0.76 g) and stem DM shoot^{-1} (1.62-2.09 g) were higher in NILs than Humber (0.50 g ear DM shoot^{-1}; *P<0.01* and 1.49 g stem DM shoot^{-1}; *P<0.001*). EPI ranged from 0.20 to 0.24 compared to 0.20 for Humber (*P<0.01*); and plant height was slightly increased (range 65.3-78.6 cm) in NILs compared to the recurrent parent (64.8 cm) (*P<0.001*).

Averaging across years, anthesis date (230.8-234.8 DAS, *P<0.001*), green area shoot^{-1} (115.6-145.5 cm^2, *P< 0.001*), EPI (0.19 to 0.22, *P<0.001*), and FE (17.4 to 25.1 grains g^{-1}, *P<0.05*) differed for NILs compared to Humber (232.3, 121.2 cm^2, 0.20, 22.6 grains g^{-1}, respectively). The year effect was significant for AGDM shoot^{-1} (*P<0.01*) and EPI (*P<0.05*) and the year x genotype interaction was significant for stem DM shoot^{-1} (*P<0.05*), ear DM shoot^{-1} (*P<0.01*) and EPI (*P<0.05*).

Table 5.3 Days sowing to anthesis, green area per shoot, AGDM shoot^{-1}, ear partitioning index (EPI), fruiting efficiency (FE) and plant height at anthesis for 7 BC_3 spring wheat NILs and the Ashby recurrent parent in 2013, 2014 and SEDs for BC_3 NILs.

	Days sowing to anthesis	Green area shoot^{-1} (cm^2)	AGDM shoot^{-1} (g)	DM shoot^{-1} (g)			EPI	FE Grains g^{-1}	Plant height (cm)
				Green lamina	Stem	Ear			
2013 BC_3S_5 lines									
Minimum	230.0	131.4	3.10	0.47	2.03	0.57	0.18	20.2	
Maximum	232.5	154.2	4.22	0.65	2.59	0.98	0.23	57.7	
Mean	231.7	139.8	3.48	0.52	2.23	0.73	0.21	29.1	
Parental line (Ashby)	231.0	123.7	2.95	0.41	1.96	0.57	0.19	24.1	
SED between BC_3 and parental line (DF=8)	0.92	7.86†	0.24*	0.04*	0.16†	0.08*	0.011†	5.00**	
SED within BC_3 lines (DF=8)	1.06	9.07†	0.28*	0.05*	0.19†	0.09*	0.012†	5.77**	
2014 BC_3S_6 lines									
Minimum	230.0	135.8	3.97	0.72	2.25	0.99	0.22	16.6	42.6
Maximum	231.0	303.0	8.49	1.59	4.72	2.18	0.29	18.9	52.5
Mean	230.4	239.7	6.96	1.33	3.85	1.78	0.26	17.9	48.3
Parental line (Ashby)	230.5	114.7	2.35	0.42	1.46	0.47	0.20	21.1	59.5
SED between BC_3 and parental line (DF=7)	0.65	24.68**	0.88**	0.18**	0.49**	0.23**	0.012**	1.13†	2.31**
SED within BC_3 lines (DF=7)	0.75	28.50**	1.02**	0.21**	0.57**	0.27**	0.013**	1.30†	2.67**
Cross-year ANOVA									
BC_3 lines									
Minimum	230.0	133.6	3.77	0.60	2.28	0.88	0.22	19.2	
Maximum	231.5	220.3	6.10	1.06	3.45	1.65	0.26	37.3	
Mean	231.1	192.6	5.33	0.94	3.09	1.30	0.23	23.5	
Parental line (Ashby)	230.8	119.2	2.65	0.42	1.71	0.52	0.20	22.6	
SED between BC_3 and parental line (DF=7)	0.56	12.68***	0.44***	0.09***	0.25***	0.12***	0.008***	2.65***	
SED within BC_3 lines (DF=7)	0.64	14.64***	0.51***	0.10***	0.29***	0.14***	0.009***	3.06***	
SED Year (DF=2)	0.75	14.85*	0.22**	0.06**	0.11*	0.06**	0.005*	0.28***	
SED Year*BC_3 and parental line (DF=15)	1.04	22.23***	0.62***	0.13***	0.35***	0.17***	0.011***	3.47***	
SED Year*BC_3 line (DF=15)	1.14	24.53***	0.72***	0.15***	0.40***	0.19***	0.013***	4.08***	

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001* # Assessment based on 5-shoot samples at GS61.

Table 5.4 Days sowing to anthesis, green area per shoot, AGDM shoot^{-1}, ear partitioning index (EPI), fruiting efficiency (FE) and plant height at anthesis for 37 BC_3 winter wheat NILs and the Humber recurrent parent in 2013, 2014 and SEDs for BC_3 NILs.

	Days sowing to anthesis	Green area shoot^{-1} (cm^2)	AGDM shoot^{-1} (g)	DM shoot^{-1} (g)			EPI	FE Grains g^{-1}	Plant height (cm)
				Green lamina	Stem	Ear			
2013 BC_3S_5 lines									
Minimum	231.0	103.8	2.24	0.34	1.45	0.40	0.17	17.2	
Maximum	236.5	138.5	2.91	0.47	1.90	0.60	0.22	30.4	
Mean	234.4	121.2	2.52	0.41	1.64	0.48	0.19	24.3	
Parental line (Humber)	234.0	120.0	2.43	0.45	1.53	0.45	0.19	25.8	
SED between BC_3 and parental line (DF=39)	0.85***	8.16*	0.16	0.037	0.10*	0.046†	0.010*	2.36	
SED within BC_3 lines (DF=39)	0.99***	9.42*	0.18	0.042	0.12*	0.053†	0.012*	2.73	
2014 BC_3S_6 lines									
Minimum	230.0	117.6	2.71	0.42	1.62	0.57	0.20	17.2	65.3
Maximum	233.0	152.5	3.40	0.60	2.09	0.76	0.24	19.9	78.6
Mean	231.5	132.3	3.05	0.52	1.86	0.67	0.22	18.7	71.8
Parental line (Humber)	230.5	122.4	2.46	0.47	1.49	0.50	0.20	19.4	64.8
SED between BC_3 and parental line (DF=39)	0.50***	7.59**	0.17***	0.035**	0.10***	0.050**	0.007**	0.79	1.69***
SED within BC_3 lines (DF=39)	0.58***	8.77**	0.20***	0.040**	0.12***	0.057**	0.009**	0.91	1.95***
Cross-year ANOVA									
BC_3 lines									
Minimum	230.8	115.6	2.49	0.41	1.54	0.50	0.19	17.4	
Maximum	234.8	145.5	3.07	0.51	1.96	0.63	0.22	25.1	
Mean	233.0	126.8	2.79	0.46	1.75	0.57	0.20	21.5	
Parental line (Humber)	232.3	121.2	2.45	0.46	1.51	0.48	0.20	22.6	
SED between BC_3 and parental line (DF=37)	0.49***	5.50***	0.12***	0.025*	0.07***	0.034**	0.006***	1.24*	
SED within BC_3 lines (DF=37)	0.57***	6.35***	0.13***	0.029*	0.08***	0.039**	0.007***	1.44*	
SED Year (DF=2)	0.60*	2.81†	0.03**	0.019*	0.03*	0.010**	0.005*	0.30**	
SED Year*BC_3 and parental line (DF=78)	0.91	8.14	0.16*	0.039*	0.10*	0.048**	0.010*	1.75	
SED Year*BC_3 line (DF=78)	1.00	9.30	0.19*	0.044*	0.12*	0.055**	0.011*	2.03	

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001* # Assessment based on 5-shoot samples at GS61.

5.3.2.3 Correlation among physiological traits at anthesis

5.3.2.3.1 BC_3 spring wheat NILs

In 2013, although lower grains m^{-2} was produced in spring BC_3S_5 lines than Ashby, grains ear^{-1} was positively correlated amongst NILs with above-ground biomass (r=0.820, *P≤0.05*), ear DM $shoot^{-1}$ (r=0.816, *P≤0.05*) and EPI (r=0.737, *P≤0.10*) (Table 5.5). Increased AGDM $shoot^{-1}$ was related to increased ear DM $shoot^{-1}$ (r=0.991, *P≤0.001*) and EPI (r=0.915, *P≤0.05*).

In 2014, grains m^{-2} was positively associated with FE (r=0.736, *P≤0.05*). GN ear^{-1} was correlated with fertile spikelets per ear (r=0.877, *P≤0.01*). AGDM $shoot^{-1}$ was strongly positively related to ear DM $shoot^{-1}$ (r=0.963, *P≤0.001*), but negatively with FE (r=-0.712, *P≤0.05*). A negative correlation between EPI and FE was observed (r=-0.711, *P≤0.05*). Date of GS31 was positively associated with GN ear^{-1} (r=0.880, *P≤0.05*), spikelets ear^{-1} (r=0.950, *P≤0.001*) and fertile spikelets ear^{-1} (r=0.897, *P≤0.01*).

5.3.2.3.2 BC_3 winter wheat NILs

In 2013, grains m^{-2} showed a trend for a positive correlation with anthesis date (r=0.275, *P≤0.10*) and days GS31 to anthesis (r=0.315, *P≤0.10*), but was negatively associated with spikelets ear^{-1} (r=-0.400, *P≤0.05*). GN ear^{-1} was positively related with fertile spikelets ear^{-1} (r=0.607, *P≤0.001*) and FE (r=0.508, *P≤0.01*). Strong positive correlations were observed between spikelets ear^{-1} and fertile spikelets ear^{-1} (r=0.854, *P≤0.001*), between AGDM $shoot^{-1}$ and ear DM $shoot^{-1}$ (r=0.809, *P≤0.001*) and between ear DM $shoot^{-1}$ and EPI (r=0.800, *P≤0.001*). In addition, a positive correlation was found between EPI and FE (r=0.423, *P≤0.01*).

In 2014, grains m^{-2} was positively related to ear DM $shoot^{-1}$ (r=0.419, *P≤0.01*), EPI (r=0.302, *P≤0.10*) and dates of GS31 to GS61 (r=0.584, *P≤0.001*) but negatively

with fertile spikelets ear^{-1} (r=-0.357, *P≤0.05*). GN ear^{-1} was strongly positively related to fertile spikelets ear^{-1} (r=0.664, *P≤0.001*) and FE (r=0.312, *P≤0.10*). There was no correlation between EPI and FE in this year.

In spring NILs, in each year a positive linear relationship was found between spikelets ear^{-1} and GN ear^{-1} (2013; R^2=0.398, 2014; R^2=0.730), between AGDM shoot^{-1} and ear DM shoot^{-1} (2013; R^2=0.982, 2014; R^2=0.927), between ear DM shoot^{-1} and EPI (2013; R^2=0.922, 2014; R^2=0.571) and a negative relationship between EPI and FE (2013; R^2=0.003, 2014; R^2=0.506) (Fig. 5.8).

Similarly, in winter NILs, in each year a positive linear relationship was found between ear DM shoot^{-1} and grains m^{-2} (2013; R^2=0.078, 2014; R^2=0.176), FE and GN ear^{-1} (2013; R^2=0.258, 2014; R^2=0.098). In 2013, there was a positive relationship between EPI and FE (R^2=0.179).

Table 5.5 Pearson correlation (r) amongst BC_3 spring and winter wheat NILs for grains m^{-2} and physiological traits related to DM production and partitioning at anthesis in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grains m^{-2}	MS GN ear^{-1}	MS spikelets ear^{-1}	MS fertile spikelets ear^{-1}	AGDM $shoot^{-1}$ (g)	Ear DM $shoot^{-1}$ (g)	EPI	FE	PH	Days sowing to anthesis	Days sowing to GS31	Days GS31 to anthesis
BC_3 spring wheat NILs												
Grains m^{-2}	-	**-0.841****	**-0.804***	**-0.879****	-0.490	-0.492	-0.535	**0.736***	**0.692†**	0.119	**-0.700†**	0.507
GN ear^{-1}	0.005	-	**0.854****	**0.877****	0.508	0.488	0.399	-0.497	-0.583	-0.407	**0.880***	**-0.784***
MS spikelets ear^{-1}	-0.269	0.631	-	**0.977*****	0.284	0.261	0.279	-0.491	-0.419	-0.368	**0.953*****	**-0.808***
MS fertile spikelets ear^{-1}	-0.567	0.588	**0.837***	-	0.366	0.380	0.443	**-0.626†**	-0.578	-0.322	**0.897****	**-0.747***
Above-ground DM $shoot^{-1}$	**-0.893***	**0.820***	0.698	**0.787†**	-	**0.963*****	0.566	**-0.712***	**-0.673†**	0.024	0.244	-0.140
Ear DM $shoot^{-1}$ (g)	**-0.889***	**0.816***	0.705	**0.806†**	**0.991*****	-	**0.756***	**-0.756***	**-0.819***	-0.085	0.182	-0.163
Ear partitioning index (EPI)	**-0.837***	**0.737†**	0.695	**0.791†**	**0.915***	**0.960****	-	**-0.711***	**-0.960*****	-0.341	0.099	-0.256
Fruiting efficiency (FE)	0.075	-0.281	0.399	0.244	-0.233	-0.184	-0.054	-	**0.756***	-0.148	-0.284	0.094
Plant height (PH)	**-**	-	-	-	-	-	-	-	-	0.282	-0.281	0.337
Days sowing to anthesis	0.192	-0.275	-0.128	-0.534	-0.118	-0.204	-0.327	-0.087	-	-	-0.394	**0.817***
Days sowing to GS31	**-0.909****	-0.168	0.252	0.587	**0.766†**	**0.769†**	**0.747†**	0.080	-	-0.303	-	**-0.852****
Days GS31 to anthesis	**0.805***	0.022	-0.253	**-0.686†**	-0.609	-0.666	-0.729	-0.099	-	0.648	**-0.923****	-
BC_3 winter wheat NILs												
Grains m^{-2}	-	-0.125	-0.202	**-0.357***	0.259	**0.419****	**0.302†**	0.254	0.253	**0.488****	**-0.368***	**0.584*****
GN ear^{-1}	0.114	-	**0.551*****	**0.664*****	-0.002	-0.088	-0.121	**0.312†**	0.061	**-0.281†**	0.129	**-0.311†**
MS spikelets ear^{-1}	**-0.400***	**0.457****	-	**0.937*****	**-0.417***	**-0.445****	-0.109	0.240	**-0.295†**	**-0.646*****	0.080	**-0.649*****
MS fertile spikelets ear^{-1}	-0.244	**0.607*****	**0.854*****	-	**-0.443****	**-0.449****	-0.074	**0.289†**	**-0.416†**	**-0.713*****	0.124	**-0.728*****
Above-ground DM $shoot^{-1}$	**-0.311†**	0.077	0.161	0.127	-	**0.825*****	-0.054	-0.262	**0.787*****	**0.619*****	-0.165	**0.650*****
Ear DM $shoot^{-1}$ (g)	**-0.279†**	-0.058	0.037	0.107	**0.809*****	-	**0.514****	-0.230	**0.499****	**0.719*****	0.053	**0.680*****
Ear partitioning index (EPI)	-0.119	-0.152	-0.105	0.047	**0.296†**	**0.800*****	-	-0.037	**-0.299†**	**0.298†**	**0.339***	0.185
Fruiting efficiency (FE)	0.219	**0.508****	0.126	0.235	-0.086	0.194	**0.423****	-	**-0.312†**	-0.183	-0.218	-0.111
Plant height (PH)	-	-	-	-	-	-	-	-	-	**0.571*****	-0.179	**0.607*****
Days sowing to anthesis	**0.275†**	0.146	**-0.289†**	**-0.285†**	-0.260	**-0.514****	**-0.560*****	-0.201	-	-	0.050	**0.953*****
Days sowing to GS31	-0.122	-0.062	0.010	-0.024	**-0.505****	**-0.477****	-0.257	-0.066	-	0.262	-	-0.257
Days GS31 to anthesis	**0.315†**	0.147	**-0.292†**	**-0.285†**	-0.176	**-0.436****	**-0.520*****	-0.196	-	**0.980*****	0.089	-

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

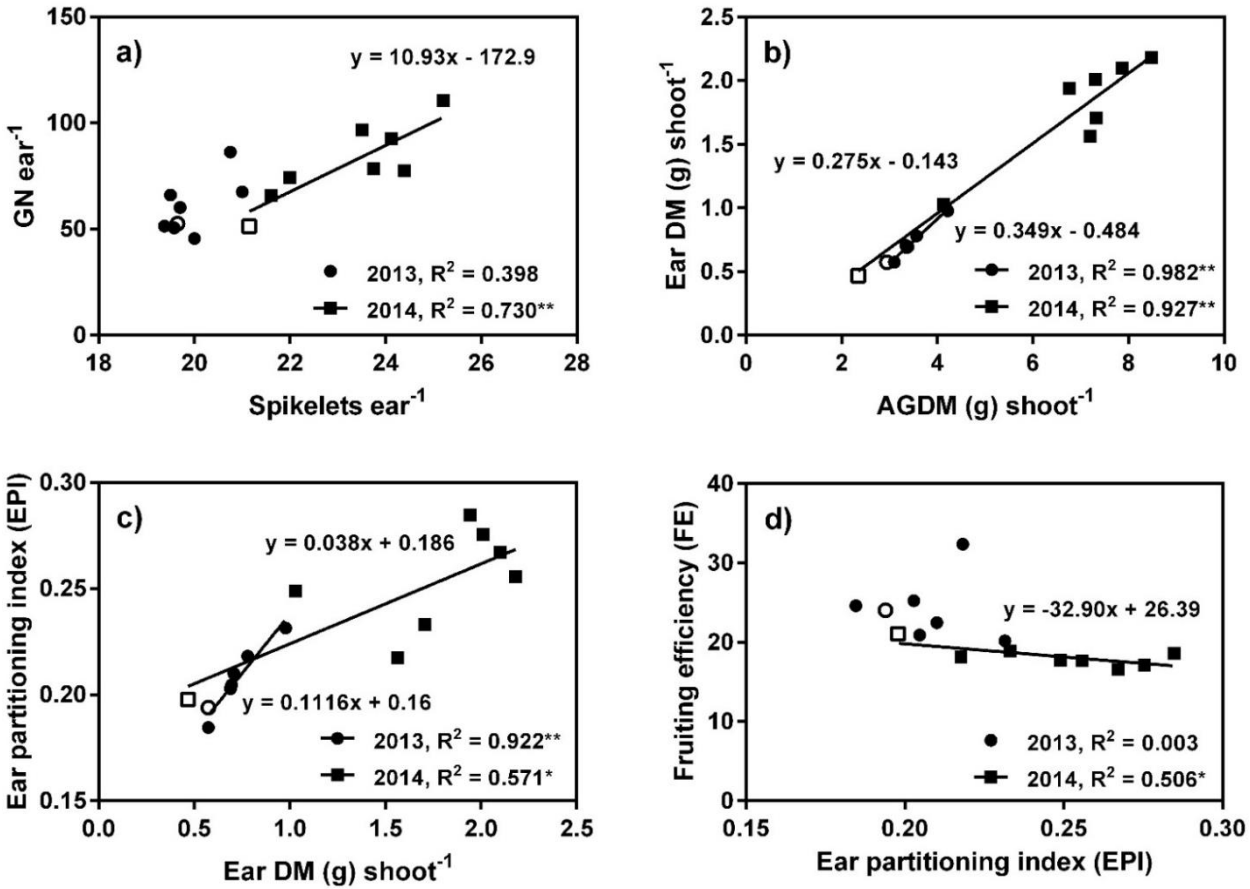

Fig. 5.8 (a) Linear relationship between total spikelets ear^{-1} and grains ear^{-1}, (b) AGDM shoot^{-1} and ear DM shoot^{-1} at GS61, (c) ear DM shoot^{-1} and EPI and (d) EPI and FE in BC_3 spring wheat NILs in 2013 (closed circles) and 2014 (closed squares) and parental line (Ashby) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *P<0.001*.

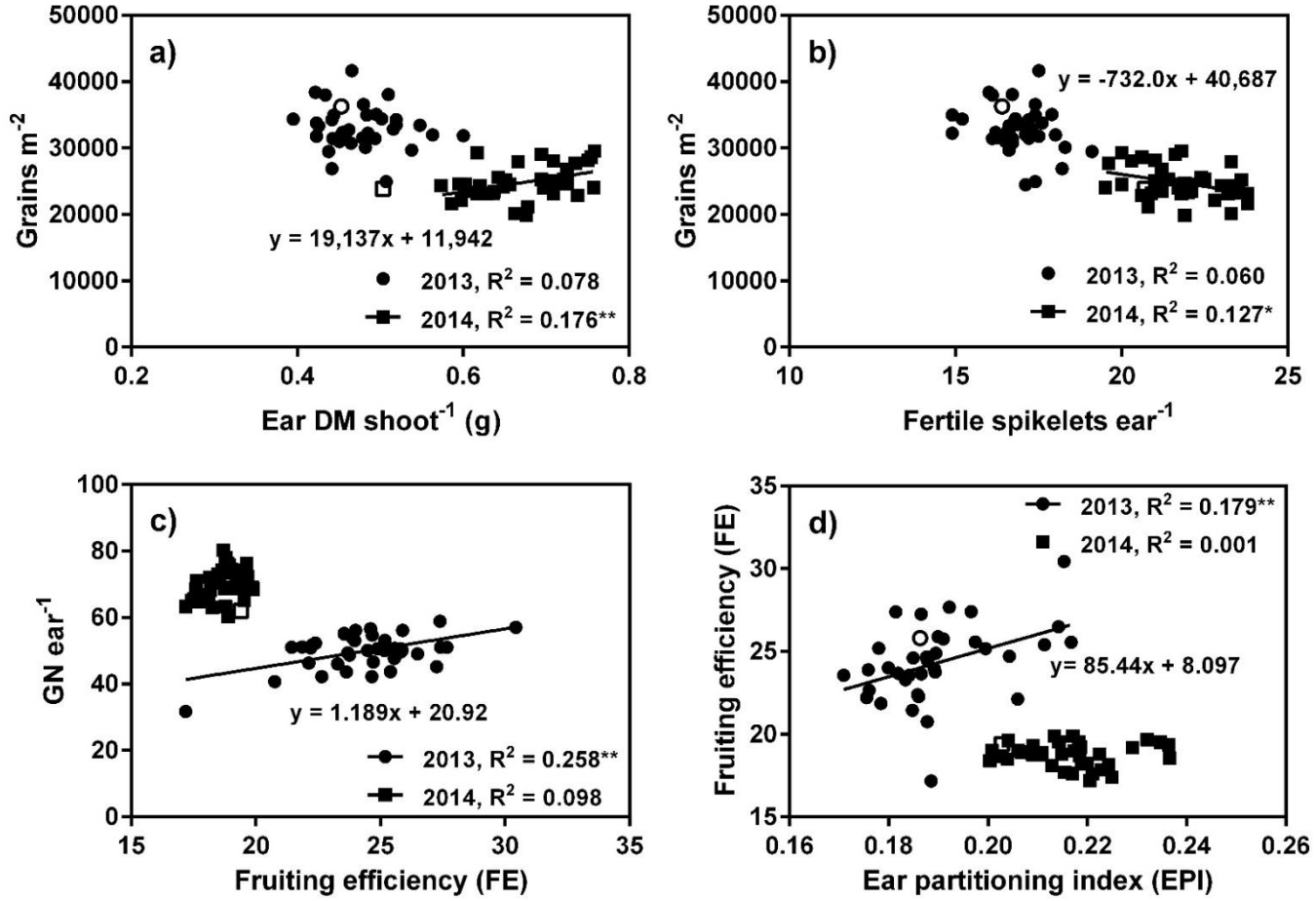

Fig. 5.9 (a) Linear relationship between ear DM shoot^{-1} at anthesis and grains m^{-2}, (b) fertile spikelets ear^{-1} and grains m^{-2}, (c) FE and GN ear^{-1} and (d) EPI and FE in BC_3 winter wheat NILs in 2013 (closed circles) and 2014 (closed squares) and parental line (Humber) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *P<0.001*.

5.3.3 Floret fertility at anthesis

In 2013, at GS61, ears of 3 main shoots per genotype from 15 selected BC_3S_5 lines (6 spring and 9 winter NILs) and the recurrent parents were dissected to determine the developmental stage of all florets, fertile florets per spikelet and fertile florets per ear (Table A1; Fig. 5.10).

Fig. 5.10 Developmental florets with Waddington score (8.5-10) and dehydrated florets (D-dehydration, LD-late dehydration) at anthesis under a binocular microscope (the white line represents 0.1 mm).

5.3.3.1 Floret fertility in spring wheat NILs

In 2013, fertile florets ear^{-1} ranged from 65.0 to 137.3 compared to 57.7 for Ashby (*P<0.001*) (Table 5.6). The number of fertile florets ear^{-1} was increased in 4 BC_3S_5 lines compared to the parental line (*P<0.001*). The increase was 138% for line 33 which had 6.91 fertile florets per central $spikelet^{-1}$ compared to Ashby (3.79 fertile florets central $spikelet^{-1}$). Overall, more fertile florets $spikelet^{-1}$ were produced in the central spikelets than the basal and apical spikelets of the ear. Fertile florets $spikelet^{-1}$ in line 33 was also higher than Ashby (2-5 fertile florets) in the basal and apical

spikelets. The increased fertile florets ear^{-1} was therefore associated with more fertile florets spikelet^{-1} in basal, central and apical spikelets (Fig. 5.11; a-d for 2013 and e-h for 2014).

In 2014, fertile florets ear^{-1} increased from 64.2 in Ashby to 125.5 in BC_3 line 33 which producing fewer tillers plant^{-1} (95.5%). Overall, higher fertile florets ear^{-1} in BC_3 spring wheat lines tended to be associated with more fertile florets spikelet^{-1}, with higher spikelets ear^{-1} and longer rachis length.

Averaging across years, for the 4 common BC_3 spring wheat lines (lines 5, 25, 33 and 36), the BC_3 NILs had significant variation above Ashby in all floret fertility traits: spikelets ear^{-1}, fertile spikelets ear^{-1}, rachis length ear^{-1} and fertile florets ear^{-1}. Fertile florets ear^{-1} was highest for NILs with lower tiller number at anthesis (e.g. 131.5 for line 33 and 106.5 for line 36) compared to Ashby (61.0). A year effect was significant for spikelets ear^{-1} ($P<0.01$) and fertile spikelets ear^{-1} ($P<0.05$). The year x genotype interaction was significant for fertile florets spikelet^{-1} ($P<0.05$).

Table 5.6 Minimum, maximum and mean values for spikelets ear^{-1}, fertile spikelets ear^{-1}, rachis length (RL), fertile florets ear^{-1}, fertile florets spikelet^{-1} (basal, central and apical), ear partitioning index (EPI), ear dry matter (EDM) and FE at anthesis for BC_3 spring wheat NILs and the Ashby parent in 2013, 2014 and SEDs from cross-year ANOVA for BC_3 lines, year and interaction.

	Spikelets ear^{-1}	Fertile spikelets ear^{-1}	Rachis length (cm)	Fertile florets ear^{-1}	Fertile florets spikelet^{-1}			EDM (g) ear^{-1}	EPI	FE
					Basal	Central	Apical			
2013 BC_3S_5 lines										
Minimum	20.7	18.7	9.68	65.0	1.61	3.79	2.87	0.52	0.17	20.2
Maximum	23.5	23.4	12.6	137.3	6.84	6.91	4.13	1.04	0.23	32.4
Mean	21.6	20.9	11.1	93.7	3.87	5.12	3.51	0.81	0.20	25.5
Parental line (Ashby)	19.4	17.4	8.84	57.7	1.86	3.79	2.91	0.44	0.19	24.1
SED between BC_3 and parental line (DF=7)	0.45**	0.38***	0.21***	5.04***	0.21***	0.22***	0.30*	0.05***	0.010**	5.23
SED within BC_3 lines (DF=7)	0.52**	0.44***	0.24***	5.82***	0.24***	0.26***	0.35*	0.06***	0.011**	6.04
2014 BC_3S_6 lines										
Minimum	23.2	22.5	10.6	94.3	3.50	4.76	3.28	0.81	0.22	16.6
Maximum	25.7	25.7	12.5	125.5	4.94	5.71	4.13	1.21	0.29	18.9
Mean	24.0	23.8	11.5	106.1	4.18	5.12	3.62	1.04	0.26	17.9
Parental line (Ashby)	21.8	20.6	9.59	64.2	2.10	3.73	2.81	0.47	0.20	21.1
SED between BC_3 and parental line (DF=9)	0.61**	0.59***	0.59*	13.8*	0.79†	0.58	0.42	0.16*	0.012**	1.13†
SED within BC_3 lines (DF=9)	0.70**	0.69***	0.69*	15.9*	0.91†	0.67	0.48	0.19*	0.013**	1.30†
Cross-year ANOVA										
BC_3 lines										
Minimum	22.2	22.2	10.8	96.7	3.84	5.05	3.32	0.88	0.22	19.2
Maximum	24.5	24.5	12.2	131.5	5.93	6.21	3.92	1.11	0.27	25.3
Mean	23.2	23.2	11.6	109.8	4.66	5.50	3.63	1.01	0.24	22.1
Parental line (Ashby)	20.6	19.0	9.2	61.0	1.98	3.76	2.86	0.45	0.20	22.6
SED between BC_3 and parental line (DF=4)	0.37***	0.37***	0.34***	6.08***	0.37***	0.21***	0.19***	0.07***	0.008***	3.04
SED within BC_3 lines (DF=4)	0.43***	0.43***	0.40***	7.02***	0.42***	0.25***	0.22***	0.08***	0.009***	3.51
SED Year (DF=2)	0.17**	0.28*	0.11	2.47	0.11	0.12	0.03	0.03†	0.008	0.87*
SED Year*BC_3 and parental line (DF=11)	0.49	0.54	0.44	7.98	0.47†	0.29*	0.24*	0.09†	0.013**	3.89
SED Year*BC_3 lines (DF=11)	0.58	0.62	0.52	9.40	0.55†	0.34*	0.28*	0.10†	0.014**	4.61

† *P<0.1*, * *P<0.05*, ** *P<0.01*, *** *P<0.001* # Assessment based on 3-shoot samples at GS61.

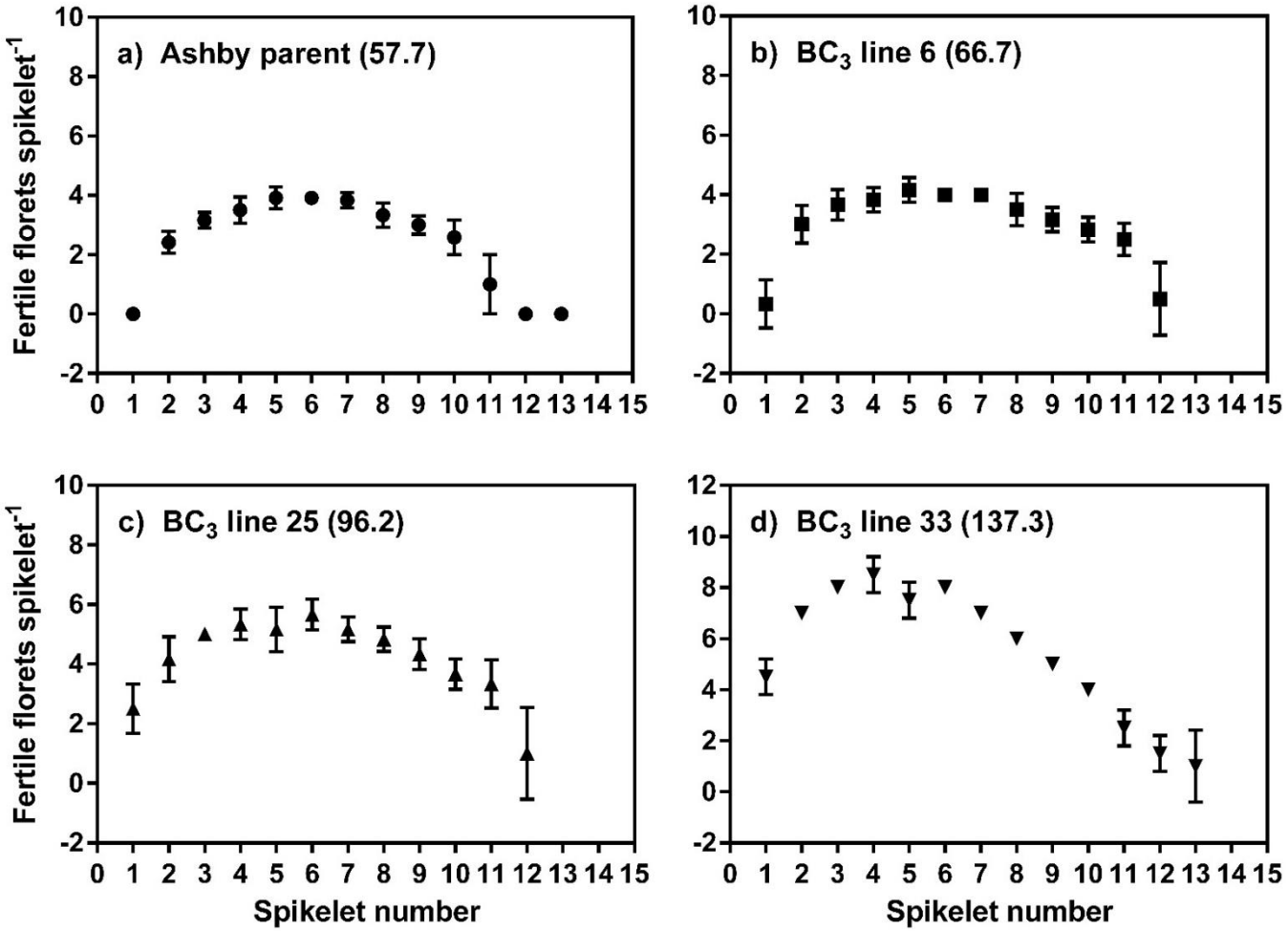

Fig. 5.11 Fertile florets spikelet^{-1} from basal to apical spikelet in (a) Ashby parent and 3 BC_3S_5 spring wheat NILs representative of (b) minimum, (c) mean and (d) maximum values for fertile florets ear^{-1} in 2013 (e) Ashby parent and 3 BC_3S_6 spring wheat NILs representative of (f) minimum, (g) mean and (h) maximum values for fertile florets ear^{-1} in 2014. Assessment based on three replicates in each line. Error bars represent standard error of the mean (SEM). The fertile florets ear^{-1} are indicated in brackets.

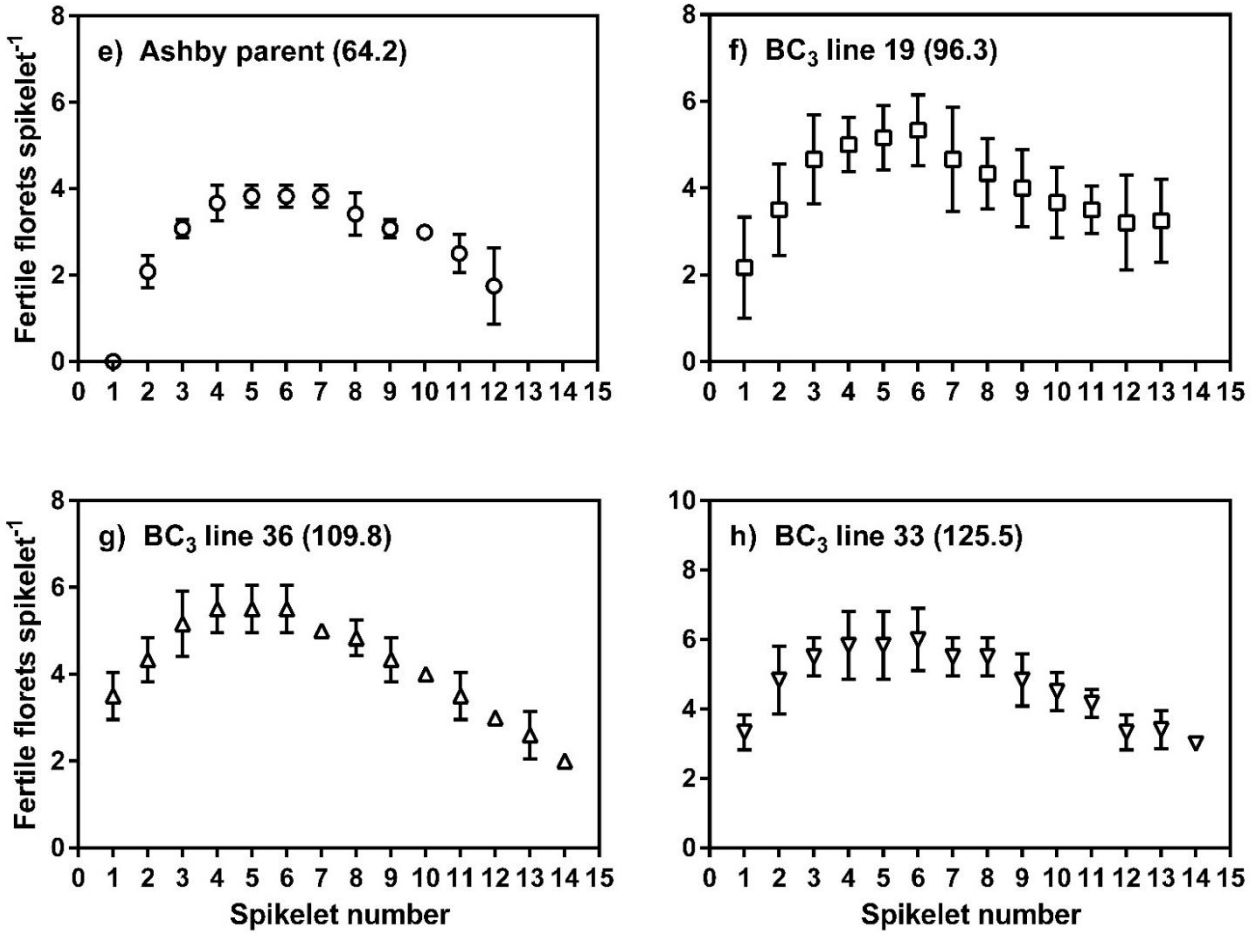

Fig. 5.11 Fertile florets spikelet^{-1} from basal to apical spikelet in (a) Ashby parent and 3 BC_3S_5 spring wheat NILs representative of (b) minimum, (c) mean and (d) maximum values for fertile florets ear^{-1} in 2013 (e) Ashby parent and 3 BC_3S_6 spring wheat NILs representative of (f) minimum, (g) mean and (h) maximum values for fertile florets ear^{-1} in 2014. Assessment based on three replicates in each line. Error bars represent standard error of the mean (SEM). The fertile florets ear^{-1} are indicated in brackets.

Pearson correlation among floret fertility traits in spring NILs in both years is shown in Table 5.7. In 2013, increase in fertile florets ear^{-1} was related to increase in spikelets ear^{-1} (r=0.938, *P≤0.01*), fertile spikelets ear^{-1} (r=0.971, *P≤0.001*), rachis length (r=0.921, *P≤0.01*), ear DM ear^{-1} (r=0.911, *P≤0.01*), AGDM shoot^{-1} (r=0.865, *P≤0.05*) and EPI (r=0.864, *P≤0.05*). In 2014, fertile florets ear^{-1} was positively correlated with spikelets ear^{-1} (r=0.823, *P≤0.05*), fertile spikelets ear^{-1} (r=0.903, *P≤0.01*), fertile florets spikelet^{-1} (r=0.975, *P≤0.001*) and ear DM (r=0.945, *P≤0.001*). The number of fertile florets ear^{-1} was positively related to GN MS^{-1} at harvest (r=0.790, *P≤0.05*) but negatively with plant height (r=-0.732, *P≤0.05*) and days from GS31 to anthesis (r=-0.657, *P≤0.10*).

In each year for floret fertility traits in spring NILs, a positive linear relationship was found between fertile spikelets ear^{-1} and fertile florets ear^{-1} (2013; R^2=0.958, 2014; R^2=0.586), between fertile florets spikelet^{-1} and fertile florets ear^{-1} (2013; R^2=0.999, 2014; R^2=0.857). In 2013 only a positive linear relationship was found between EPI and fertile florets ear^{-1} (2013; R^2=0.895) and between fertile florets ear^{-1} at anthesis and MS GN ear^{-1} at harvest (2013; R^2=0.881) in BC_3 lines (Fig. 5.12).

Table 5.7 Pearson correlation (r) amongst BC_3 spring wheat NILs for floret fertility traits at anthesis in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Spikelets ear^{-1}	Fertile spikelets ear^{-1}	RL	Fertile florets ear^{-1}	Fertile florets spikelet^{-1}				EDM (g) ear^{-1}	Above-ground DM shoot^{-1}	EPI	FE	PH	Days GS31 to anthesis	GN MS^{-1}
					Overall	Basal	Central	Apical							
Spikelets ear^{-1}	-	**0.979*** **	**0.747***	**0.823***	**0.680†**	**0.800***	**0.628†**	0.341	**0.680†**	0.425	0.372	**-0.641†**	-0.403	-0.609	**0.696†**
Fertile spikelets ear^{-1}	**0.928** **	-	**0.732***	**0.903** **	**0.789***	**0.892** **	**0.750***	0.476	**0.785***	0.516	0.430	**-0.634†**	-0.484	**-0.655†**	**0.751***
Rachis length (cm)	**0.952*** **	**0.937** **	-	**0.630†**	0.553	**0.641†**	0.521	0.284	0.559	0.501	0.325	**-0.781***	-0.462	-0.236	**0.710***
Fertile florets ear^{-1}	**0.938** **	**0.971*** **	**0.921** **	-	**0.975*** **	**0.989*** **	**0.956*** **	**0.789***	**0.945*** **	**0.660†**	**0.674†**	**-0.627†**	**-0.732***	**-0.657†**	**0.790***
Fertile florets spikelet^{-1}	**0.928** **	**0.961*** **	**0.914** **	**0.999*** **	-	**0.967*** **	**0.995*** **	**0.894** **	**0.961*** **	**0.703†**	**0.750***	-0.599	**-0.813***	-0.590	**0.754***
Basal	**0.900** **	**0.948** **	**0.855***	**0.989*** **	**0.990*** **	-	**0.950*** **	**0.755***	**0.945*** **	**0.663†**	0.613	-0.585	**-0.707***	**-0.709***	**0.856** **
Central	**0.909** **	**0.968*** **	**0.893** **	**0.996*** **	**0.996*** **	**0.993*** **	-	**0.917** **	**0.946*** **	**0.686†**	**0.756***	-0.574	**-0.820***	**-0.564***	**0.725***
Apical	0.110	0.311	0.377	0.138	0.127	0.043	0.141	-	**0.838** **	**0.670†**	**0.828***	-0.490	**-0.834***	-0.278	0.436
EDM (g) ear^{-1}	**0.824***	**0.956*** **	**0.914** **	**0.911** **	**0.905** **	**0.874***	**0.917** **	0.503	-	**0.852** **	**0.714***	**-0.668†**	**-0.799***	-0.550	**0.753***
AGDM shoot^{-1}	0.713	**0.814***	**0.808†**	**0.865***	**0.876***	**0.831***	**0.843***	**0.790†**	**0.899***	-	0.566	**-0.712***	**-0.673†**	-0.140	0.508
Ear part. index (EPI)	0.508	0.798	0.602	**0.864***	**0.878***	**0.918***	**0.905***	0.662	**0.879***	**0.872***	-	**-0.711***	**-0.960*** **	-0.256	0.399
Fruiting efficiency (FE)	0.3674	0.308	0.229	0.279	0.247	0.284	0.306	-0.219	0.202	-0.237	-0.098	-	**0.756***	0.094	-0.497
Plant height (PH)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.337	-0.583
Days GS31 to anthesis	-0.871	-0.787	-0.783	**-0.913***	**-0.928***	**-0.943***	**-0.907***	0.281	-0.630	-0.649	-0.656	-0.051	-	-	**-0.784***
GN ear^{-1}	-0.106	0.088	0.167	-0.106	-0.116	-0.204	-0.120	**0.944***	0.365	**0.991** **	**0.972***	-0.459	-	0.312	-

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

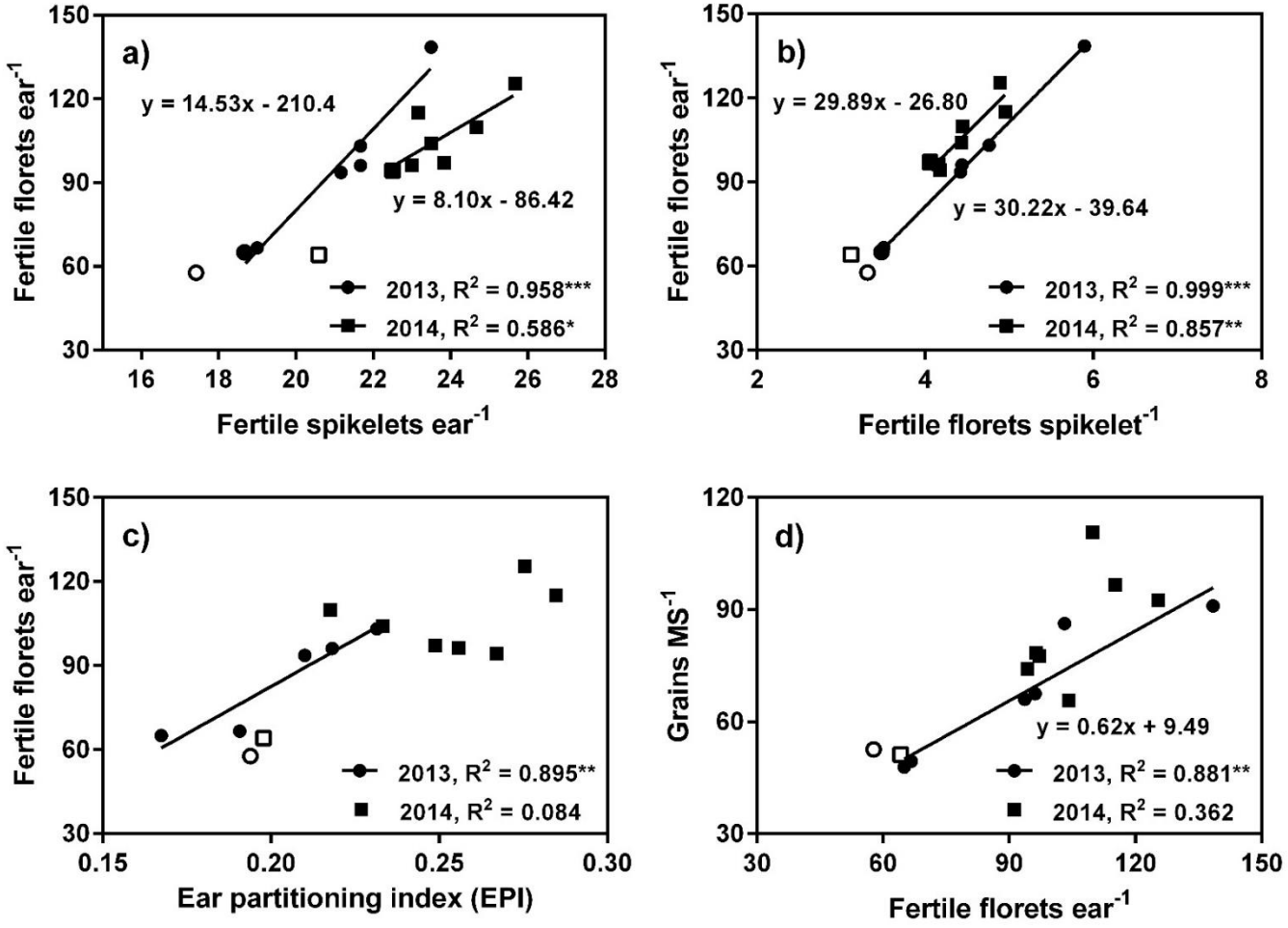

Fig. 5.12 (a) Linear relationship between fertile spikelets ear^{-1} and fertile florets ear^{-1}, (b) fertile florets spikelet^{-1} and fertile florets ear^{-1}, (c) EPI and fertile florets ear^{-1} and (d) fertile florets ear^{-1} and MS GN ear^{-1} in BC_3 spring wheat NILs in 2013 (closed circles) and 2014 (closed squares) and parental line (Ashby) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *P<0.001*.

5.3.3.2 Floret fertility in winter wheat NILs

In 2013, ears from 9 selected BC_3 winter wheat lines and the Humber recurrent parent were dissected to determine the developmental stage of all florets and fertile florets per spikelet. Fertile spikelets ear^{-1} ranged from 17.7 to 21.3 compared to 17.9 in Humber (*P<0.01*) (Table 5.8) and fertile florets ear^{-1} from 61.3 to 82.7 compared to 65.1 for Humber (*P<0.05*). Fertile florets central $spikelet^{-1}$ was increased in BC_3S_5 lines compared to Humber by 27% in line 73 with 4.71 fertile florets central $spikelet^{-1}$ compared to Humber (4.19 fertile florets central $spikelet^{-1}$). A higher number of fertile florets $spikelet^{-1}$ in NILs were produced in central (4.00-4.71) than apical (2.79-3.63) spikelets, which in turn produced more than basal spikelets (1.50-3.06) (Fig. 5.12).

In 2014, 37 BC_3 winter wheat lines and the Humber parents were assessed. Fertile spikelets ear^{-1} ranged from 21.2 to 24.0 in NILs compared to 20.8 for Humber (*P<0.001*). In NILs, fertile florets ear^{-1} ranged from 77.2 to 95.5 compared to 68.3 for Humber (*P<0.05*). Fertile florets ear^{-1} was increased by 40% in line 175 compared to Humber (*P<0.05*). Central spikelets had more fertile florets per spikelet than apical and basal spikelets.

Averaging across years for the 8 common BC_3 winter wheat lines (lines 68, 73, 76, 77, 150, 170, 171 and 196), the BC_3 NILs had significant variation above Humber in all floret fertility traits: spikelets ear^{-1} (*P<0.001*), fertile spikelets ear^{-1} (*P<0.001*), rachis length ear^{-1} (*P<0.001*), fertile florets ear^{-1} (*P<0.001*) and ear DM (*P<0.001*). Fertile florets ear^{-1} in NILs (75.2-86.3) was increased above Humber (66.7; *P<0.001*) with higher fertile florets $spikelets^{-1}$ for central (*P<0.001*), apical (*P<0.01*) and basal spikelets (*P<0.01*). The year effect was significant for spikelets ear^{-1} (*P<0.05*), fertile spikelets ear^{-1} (*P<0.01*), rachis length (*P<0.05*), fertile florets ear^{-1} (*P<0.001*) and EDM ear^{-1} (*P<0.05*). Year x genotype interaction was significant for fertile florets ear^{-1} (*P<0.001*), fertile florets $spikelet^{-1}$ (*P<0.01*) and EPI (*P<0.05*). Amongst the 8 common BC_3 winter wheat lines and the Humber parent, all 8 lines had more fertile florets ear^{-1} (75.2-86.3) compared to Humber (66.7) (*P<0.001*).

Table 5.8 Minimum, maximum and mean values for spikelets ear^{-1}, fertile spikelets ear^{-1}, rachis length (RL), fertile florets ear^{-1}, fertile florets spikelet^{-1} (basal, central and apical), ear partitioning index (EPI), ear dry matter (EDM) and FE at anthesis for BC_3 winter wheat NILs and the Humber parent in 2013, 2014 and SEDs from cross-year ANOVA for BC_3 lines, year and interaction.

	Spikelets ear^{-1}	Fertile spikelets ear^{-1}	Rachis length (cm)	Fertile florets ear^{-1}	Fertile florets spikelet^{-1}			EDM (g) ear^{-1}	EPI	FE
					Basal	Central	Apical			
2013 BC_3S_5 lines										
Minimum	20.2	17.7	9.45	61.3	1.50	4.00	2.79	0.52	0.19	21.4
Maximum	23.0	21.3	10.9	82.7	3.06	4.71	3.63	0.69	0.21	26.5
Mean	21.1	19.3	10.1	72.7	2.27	4.39	3.38	0.58	0.20	24.1
Parental line (Humber)	19.5	17.9	8.75	65.1	2.31	4.19	3.28	0.48	0.19	25.8
SED between BC_3 and parental line (DF=11)	0.49***	0.61**	0.34**	4.22*	0.32*	0.15**	0.13**	0.05†	0.009†	1.12*
SED within BC_3 lines (DF=11)	0.56***	0.71**	0.39**	4.87*	0.37*	0.17**	0.16**	0.06†	0.011†	1.29*
2014 BC_3S_6 lines										
Minimum	21.5	21.2	9.88	77.2	2.54	4.18	2.89	0.57	0.20	17.2
Maximum	24.7	24.0	12.5	95.5	3.79	5.09	3.79	0.76	0.24	19.9
Mean	22.9	22.4	11.2	86.9	3.21	4.61	3.44	0.67	0.22	18.7
Parental line (Humber)	21.8	20.8	9.59	68.3	2.30	3.92	3.06	0.50	0.20	19.4
SED between BC_3 and parental line (DF=39)	0.51***	0.57***	0.41***	5.13*	0.37†	0.26*	0.16***	0.05**	0.007**	0.79
SED within BC_3 lines (DF=39)	0.59***	0.66***	0.48***	5.92*	0.43†	0.31*	0.19***	0.06**	0.009**	0.91
Cross-year ANOVA										
BC_3 lines										
Minimum	21.5	19.8	10.2	75.2	2.29	4.40	3.26	0.58	0.20	19.8
Maximum	23.1	22.3	11.2	86.3	3.26	4.75	3.67	0.68	0.22	22.9
Mean	22.0	20.9	10.8	80.7	2.76	4.59	3.42	0.63	0.21	21.4
Parental line (Humber)	20.6	19.4	9.17	66.7	2.30	4.05	3.17	0.49	0.20	22.6
SED between BC_3 and parental line (DF=8)	0.34***	0.41***	0.25***	2.54***	0.19**	0.14***	0.09**	0.03***	0.006*	0.66**
SED within BC_3 lines (DF=8)	0.40***	0.48***	0.29***	2.94***	0.22**	0.16***	0.10**	0.04***	0.007*	0.76**
SED Year (DF=2)	0.24*	0.17**	0.18*	0.18***	0.15*	0.05†	0.07	0.02*	0.008	0.30**
SED Year*BC_3 and parental line (DF=20)	0.51	0.57	0.38	3.35***	0.29***	0.19**	0.13**	0.045†	0.011*	0.92†
SED Year*BC_3 lines (DF=20)	0.58	0.66	0.43	4.00***	0.33***	0.22**	0.15**	0.052†	0.012*	1.07†

† *P<0.1*, * *P<0.05*, ** *P<0.01*, *** *P<0.001* #Assessment based on 3-shoot samples at GS61.

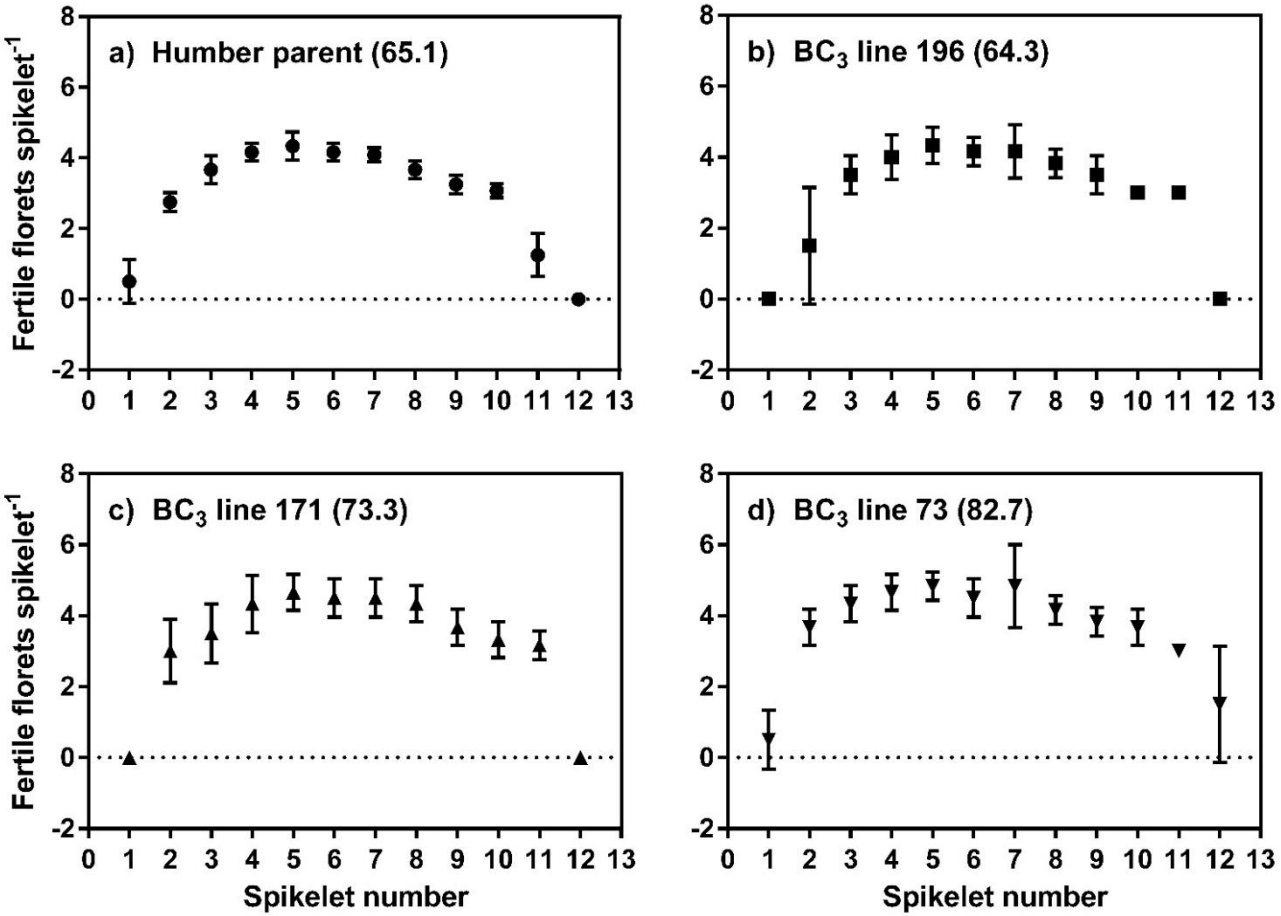

Fig. 5.13 Fertile florets spikelet^{-1} from basal to apical spikelet in (a) Humber parent and 3 BC_3S_5 winter wheat NILs representative of (b) minimum, (c) mean and (d) maximum values for fertile florets ear^{-1} in 2013 and (e) Humber parent and 3 BC_3S_6 winter wheat NILs representative of (f) minimum, (g) mean and (h) maximum values for fertile florets ear^{-1} in 2014. Assessment based on three replicates in each line. Error bars represent standard error of the mean (SEM). The fertile florets ear^{-1} are indicated in brackets.

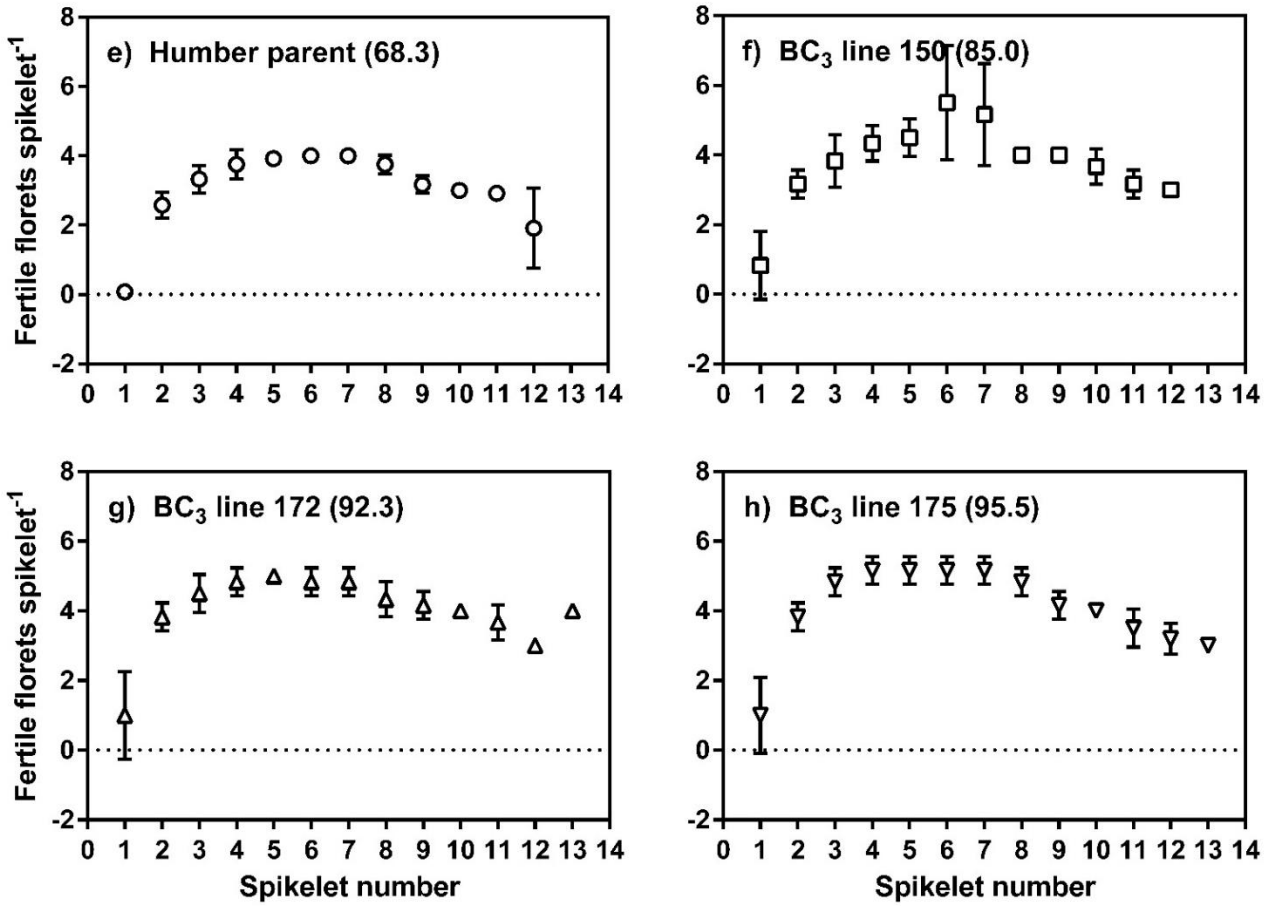

Fig. 5.13 Fertile florets spikelet^{-1} from basal to apical spikelet in (a) Humber parent and 3 BC_3S_5 winter wheat NILs representative of (b) minimum, (c) mean and (d) maximum values for fertile florets ear^{-1} in 2013 and (e) Humber parent and 3 BC_3S_6 winter wheat NILs representative of (f) minimum, (g) mean and (h) maximum values for fertile florets ear^{-1} in 2014. Assessment based on three replicates in each line. Error bars represent standard error of the mean (SEM). The fertile florets ear^{-1} are indicated in brackets.

In 2013, increased fertile florets ear^{-1} in winter NILs was positively correlated with increase in spikelets ear^{-1} (r=0.665, *P≤0.05*), fertile spikelets ear^{-1} (r=0.788, *P≤0.01*), fertile florets spikelet^{-1} (r=0.760, *P≤0.05*), and ear DM (r=0.694, *P≤0.05*) (Table 5.9). Increased fertile florets ear^{-1} resulted from increased fertile florets spikelet^{-1} in basal (r=0.856, *P≤0.01*) and central (r=0.774, *P≤0.01*) rather than basal spikelets. A positive correlation between fertile spikelets ear^{-1} and EPI (r=0.696, *P≤0.05*) was observed.

In 2014, fertile florets ear^{-1} was positively related to fertile spikelets ear^{-1} (r=0.340, *P≤0.05*), rachis length (r=0.658, *P≤0.001*), fertile florets spikelets^{-1} (r=0.849, *P≤0.001*), ear DM (r=0.867, *P≤0.001*), AGDM shoot^{-1} (r=0.779, *P≤0.001*) and EPI (r=0.446, *P≤0.01*).

There were positive relationships amongst NILs, between fertile florets spikelet^{-1} and fertile florets ear^{-1} (2013; R^2=0.567, 2014; R^2=0.668), between DM per ear and fertile florets ear^{-1} (2013; R^2=0.405, 2014; R^2=0.692) as well as EPI and fertile florets ear^{-1} (2013; R^2=0.230, 2014; R^2=0.159). However, there was no relationship between fertile florets ear^{-1} at anthesis and GN ear^{-1} at harvest. A longer rachis was associated with more fertile spikelets ear^{-1} and fertile florets spikelet^{-1} and fertile florets ear^{-1}.

Table 5.9 Pearson correlation (r) amongst BC_3 winter wheat NILs for floret fertility traits at anthesis in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Spikelets ear^{-1}	Fertile spikelets ear^{-1}	RL	Fertile florets ear^{-1}	Fertile florets spikelet^{-1}				EDM (g) ear^{-1}	Above-ground DM shoot^{-1}	EPI	FE	PH	Days GS31 to anthesis	GN MS^{-1}
					Overall	Basal	Central	Apical							
Spikelets ear^{-1}	-	**0.940*** **	**0.443** **	0.170	**-0.348***	-0.100	-0.267	**-0.625*** **	-0.092	-0.083	0.032	0.013	-0.081	**-0.367***	**0.443** **
Fertile spikelets ear^{-1}	**0.888*** **	-	**0.492** **	**0.340***	-0.207	0.153	-0.143	**-0.545*** **	0.076	0.036	0.163	-0.039	-0.067	**-0.278†**	**0.367***
Rachis length (cm)	0.451	0.242	-	**0.658*** **	**0.410***	**0.347***	**0.459** **	0.210	**0.478** **	**0.541*** **	0.097	**-0.342***	**0.581*** **	**0.304†**	**0.351***
Fertile florets ear^{-1}	**0.665***	**0.788** **	0.447	-	**0.849*** **	**0.837*** **	**0.794*** **	**0.539*** **	**0.867*** **	**0.779*** **	**0.446** **	**-0.302†**	**0.588*** **	**0.566*** **	0.162
Fertile florets spikelet^{-1}	0.124	0.200	0.437	**0.760***	-	**0.776*** **	**0.911*** **	**0.869*** **	**0.860*** **	**0.794*** **	**0.369***	**-0.300†**	**0.660*** **	**0.751*** **	-0.041
Basal	0.539	**0.814** **	0.104	**0.856** **	0.501	-	**0.608*** **	**0.476** **	**0.801*** **	**0.618*** **	**0.566*** **	-0.200	**0.323***	**0.450** **	-0.004
Central	0.163	0.276	0.304	**0.774** **	**0.942*** **	0.528	-	**0.795*** **	**0.740*** **	**0.747*** **	0.222	**-0.338***	**0.674*** **	**0.670*** **	-0.013
Apical	-0.348	-0.284	0.276	0.334	**0.829** **	0.047	**0.778** **	-	**0.636*** **	**0.647*** **	0.136	-0.177	**0.604*** **	**0.722*** **	-0.158
EDM (g) ear^{-1}	0.523	0.501	**0.866** **	**0.694***	**0.556†**	0.520	0.459	0.274	-	**0.858*** **	**0.548*** **	**-0.272†**	**0.565*** **	**0.668*** **	0.047
AGDM shoot^{-1}	0.146	-0.042	0.298	-0.117	-0.183	-0.291	-0.336	-0.017	0.123	-	0.047	**-0.300†**	**0.810*** **	**0.645*** **	0.116
Ear part. index (EPI)	0.243	**0.696***	0.087	0.530	0.293	**0.656†**	0.334	0.290	0.367	0.129	-	-0.070	-0.206	0.212	-0.051
Fruiting efficiency (FE)	-0.089	0.302	-0.155	0.293	0.240	**0.596†**	0.293	0.063	0.182	**-0.786***	0.345	-	**-0.342***	-0.132	0.253
Plant height (PH)	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.617*** **	0.144
Days GS31 to anthesis	-0.488	**-0.689***	0.206	**-0.664†**	-0.568	**-0.667***	**-0.684***	-0.411	0.011	0.167	-0.387	-0.140	-	-	-0.254
GN ear^{-1}	-0.100	-0.221	-0.422	-0.375	-0.484	-0.336	-0.185	-0.562	-0.479	-0.219	-0.377	-0.168	-	-0.100	-

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

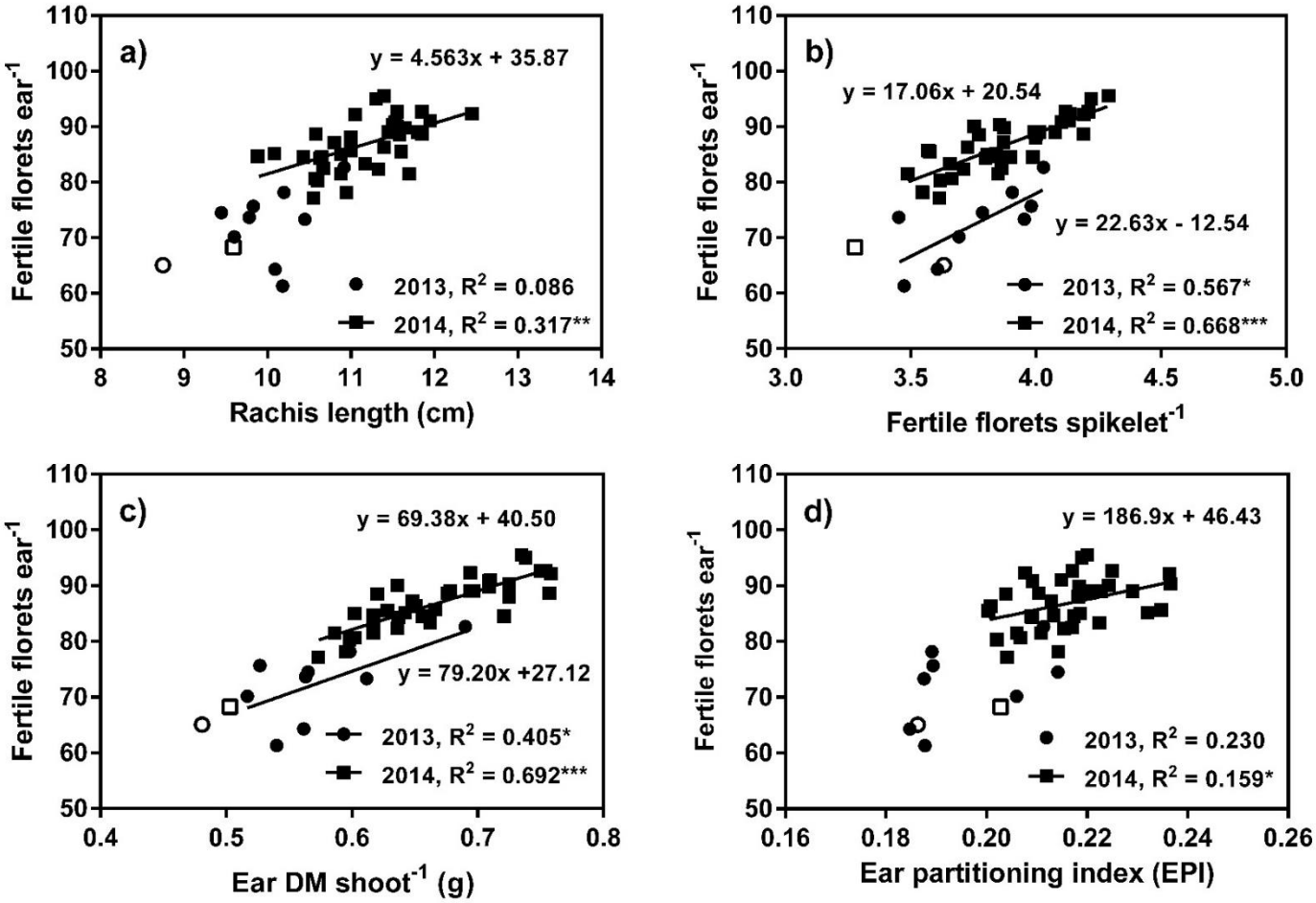

Fig. 5.14 (a) Linear relationship between rachis length and fertile florets ear^{-1} and, (b) fertile florets spikelet^{-1} and fertile florets ear^{-1}, (c) fertile florets ear^{-1} and ear DM shoot^{-1} and (d) EPI and fertile florets ear^{-1} in BC_3 winter wheat NILs in 2013 (closed circles) and 2014 (closed squares) and parental line (Humber) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *P<0.001*.

5.4 Discussion

Results showed that increased grain yield in BC_3 NILs was associated with increased grains m^{-2} and ears m^{-2} compared to the recurrent parents In addition, grain yield showed positive correlations with anthesis date, plant height, thousand grain weight and ear fertility traits in BC_3 winter NILs. The effects of the genotypes on the physiological components of grain number per m^2 described in the following equations are discussed in relation to the grain number model below, as outlined in Chapter 2:

$$\mathbf{GN = EDWa \times FE} \qquad \text{(Eqn. 5.1)}$$

$$\mathbf{EDWa = SEP \times CGR \times EPI} \qquad \text{(Eqn. 5.2)}$$

5.4.1 Determinants of ear DM per m^2

Flowering time is a major trait affecting plant adaptation, yield potential and yield stability (Snape *et al*., 2001, Langer *et al*., 2014). The genetic control of flowering time is complex and is mainly controlled by three groups of genes; the vernalization (*Vrn*) genes, photoperiod (*Ppd*) genes and earliness *per se* (*Eps*) genes responses (Snape *et al.*, 2001, Distelfeld *et al.*, 2009, Kamran *et al.*, 2014, Langer *et al.*, 2014). The period of stem elongation (from GS31 to GS61) is a key determinant of grains m^{-2} and yield determination. In the present study, there was no significant difference in anthesis date in BC_3 spring wheat lines, but there were small differences in date of GS31 associated with spikelets ear^{-1} in 2014. GN ear^{-1} in spring NILs was strongly related to spikelets and fertile spikelets per ear. Increased spikelets ear^{-1} in spring BC_3 lines could be due to a longer duration of spikelet primordia production (Gaju *et al*., 2009). It has been suggested that extending the duration from floral initiation to terminal spikelet formation might increase the number of spikelets ear^{-1} (Rawson, 1970). The earliness *per se* or intrinsic earliness can affect the rate of spikelet primordia production and the duration of early developmental phases (Jamieson *et al.*, 1998, Lewis *et al.*, 2008). The longer duration of the ear

development and more spikelets per ear were related to the presence of the *Eps-A^{m}1* allele from cultivated diploid wheat in NILs (Lewis *et al.*, 2008). There was a small difference of 3 days in anthesis date in BC_3 winter wheat NILs and days from GS31 to GS61 varied by 5 days. A positive correlation between grain yield and later anthesis date and days from GS31 to GS61 was found in the present study. Fischer (1985) reported that the late reproductive phase associated with rapid ear growth starts about 20 d before anthesis and this phase played an important role to determining wheat yield. The duration of this phase was positively related to AGDM shoot^{-1} and grains per m^2 in winter wheat lines in the present study. It has been suggested by many studies that extending the stem-elongation phase (from initiation of terminal spikelet to anthesis) will be an avenue to increase grains per m^2 and yield (Slafer *et al*., 2001, González *et al*., 2003b, Foulkes *et al*., 2011). Flowering was dependent on vernalization and photoperiod response in the winter NILs while it was independent of vernalization in the spring wheat background. A strong correlation between spikelet number and flowering time was observed by Shaw *et al.* (2013) with later flowering allowing more spikelet primordia formation and an expanded period of transition from the vegetative to reproductive stage. Although spikelet number was increased in winter NILs compared to Humber in the present study, there was no difference in date of GS31 as an indicator of the critical time in determination of spikelet number.

In the present study, ear partitioning index was positively associated with grains ear^{-1} in 2013 in spring wheat NILs, and with grains m^{-2} in winter wheat NILs in 2014 (Table 5.5). BC_3 spring and winter wheat lines had up to 217% and 31% higher ear DM per m^2 associated with increases of 30% and 10% in EPI compared to Ashby and Humber, respectively. There were no statistically significant differences in fertile shoots plant^{-1} in winter wheat NILs but a trend for decreased ears m^{-2} in some lines compared to the recurrent parent. Some BC_3 spring wheat lines showed evidence of tiller inhibition (associated with lower shoot survival) and produced larger ears with

more fertile florets ear^{-1} at anthesis and GN ear^{-1} at harvest compared to Ashby parent. The number of fertile florets per ear was increased largely in spring NILs as a results of low tillers plant^{-1}. In spring wheat, the restricted tillering was associated with ear DM shoot^{-1} and EPI, but these traits showed negative correlation with grains m^{-2}. The extent of tiller reduction was likely too severe in spring NILs with Ashby background. Gaju *et al.* (2009) and (2014b) reported effects of lower yields and reduced ears m^{-2} associated with the *Tin1A* gene in CIMMYT advanced lines compared to a check CIMMYT wheat cultivar and also amongst lines of the L14 x Rialto DH population.

In the winter wheat background, ear DM shoot^{-1} was increased up to 31% and had a positive association with plant height and days from GS31 to GS61 compared to the Humber recurrent parent. However, there were no differences in grains m^{-2} at harvest. These effects were associated with a small increase in spikelets ear^{-1} and fertile spikelets ear^{-1} in BC_3 winter wheat NILs. According to Calderini *et al.* (1999b), the greatest breeding impact on improving wheat yield potential has been through increasing partitioning of assimilates to the developing ear and grain. The yield potential is reported to be sink limited rather than source limited under optimal conditions (Borrás *et al*., 2004) and sink strength during grain filling is therefore the main factor limiting wheat yield potential (Reynolds *et al.*, 2009). Slafer *et al.* (1990) reported that improvement of ear DM partitioning was positively related to genetic progress in Argentinian wheat yield released between 1912 and 1980. Moreover, the increase of HI in the UK cultivars released between 1908 and 1978 was mainly due to increased ear dry weight at anthesis and decreased stem dry weight (Austin *et al.*, 1980). Recent genetic increases in grain yield in the UK was associated with pre-anthesis growth rate and stem water soluble carbohydrates (WSC) at anthesis (Shearman *et al*., 2005). However, a recent investigation showed that EPI had decreased with breeding amongst CIMMYT spring wheat semi-dwarf cultivars released from 1966 to 2009 (Aisawi *et al.*, 2015).

Reduced plant height was observed in spring NILs and reduction in plant height increased ear DM shoot^{-1}. Brooking and Kirby (1981) reported that among two F_4 lines with similar numbers of spikelets and floret primordia, the lines possessing the semi-dwarfing gene (*Rht-D1b* formerly *Rht2*) partitioned more DM to the developing ear and less to the stem resulting in more fertile florets and grains spikelet^{-1}. Moreover, the presence of semi-dwarfing genes (*Rht-B1b* and *Rht-D1b*) induced more distal fertile florets spikelet^{-1} (Miralles *et al.*, 1998). The competition for assimilates between the stem and growing ear at stem-elongation duration has been reported by many authors (Austin *et al*., 1980, Slafer *et al*., 1990, Abbate *et al*., 1998, Reynolds *et al*., 2009). Those authors suggested that the higher DM partitioning to the ear during stem-elongation phase, the greater the ear DM partitioning at anthesis, consequently, more grains ear^{-1} were produced. However, a negative correlation between EPI and grain yield at harvest was observed in spring wheat. The low yield in spring NILs with high EPI could be attributed mainly to their reduced tiller number and fewer ears m^{-2}.

In the study of Dreccer *et al.* (2014), more fertile florets ear^{-1} was related to higher concentration in water soluble carbohydrates (WSC). Stem WSC is often negatively associated with tiller number (Dreccer *et al*., 2013). Grain number per ear can be determined by floret survival within each spikelet, and fertilization at anthesis. In the experiments, the number of fertile florets per ear at anthesis in BC_3 spring wheat lines was positively related to grains ear^{-1} at harvest in 2013. In 2014, grain m^{-2} in BC_3 winter wheat lines was positively related to ear DM shoot^{-1} and anthesis date, and especially a longer duration from GS31 to anthesis (Table 5.5) but there was no correlation between fertile florets per ear at anthesis and grains ear^{-1} at harvest in BC_3 winter wheat lines (Table 5.9). This could be due to later flowering in BC_3 winter wheat lines resulting in a shorter grain filling period. Also these could be effects of grain set post-anthesis in the Humber background. There are several factors that can affect grain set, e.g. pollen production or high stem WSC.

5.4.2 Fruiting efficiency and floret fertility

In the present study, fruiting efficiency was decreased in spring NILs in 2014, whereas FE did not differ in winter NILs in 2013 and 2014 compared to the recurrent parent. A trade-off between EPI and FE was found in spring wheat NILs in 2014, but there was no trade-off between EPI and FE in BC_3 winter wheat lines in either year. A positive relationship between EPI and FE was observed in winter wheat lines in 2013 ($r = 0.423$; $P< 0.01$) (Table 5.5). Slafer *et al.* (2015) suggested that the FE can be improved by two different strategies; (i) increase in ear DM partitioning to developing florets pre-anthesis and (ii) decreased assimilate demand of florets to maintain their normal development. Abbate *et al.* (1998) observed that the genetic improvement of Argentinian wheat grain yield was associated with FE rather than EPI. However, a negative relationship between EPI and FE was observed by Gaju *et al.* (2009). Foulkes *et al.* (2011) suggested that a greater allocation of assimilate within the developing ear to developing florets rather than the other parts of the ear (rachis, glumes and paleas) as a physiological approach to break the negative relationship between EPI and FE.

Results indicated that basal spikelets were most responsive to increasing fertile florets spikelet^{-1} to change in assimilate supply in terms of increasing ear DM. Although the stem WSC was not measured in the present study, the WSC in the stem might be another factor contributing to high above-ground DM and high fertile florets per ear at anthesis in winter NILs. Wheat yield is limited by the amount of assimilates allocated to the ear the during pre-anthesis phase (Fischer, 1984) and high rate of ear growth was associated with decreased stem soluble carbohydrates and decreased fertile florets in wheat (*Triticum aestivum*) cultivar Buck Manantial (Ghiglione *et al*., 2008). A positive relationship between fertile florets per ear and ear DM at anthesis was observed in NILs, and it is possible that high stem WSC was associated with these effects. High WSC in wheat was associated with slower rate of floret development and increase in floret development score during stem elongation (Dreccer *et al.*, 2014). The mechanism associated with increase in stem WSC and

floret fertility could be: (i) increased ear DM due to high partitioning to the ear or high carbon fixation in high WSC lines, (ii) high sucrose production at booting leading to high amounts of available sucrose transport to the ear, or (iii) high biomass and sucrose in the ear resulting in higher floret survival in the ear at anthesis. There is also evidence that faster rate of ear development is associated with lower grain set by the grain lacking the minimum size or inactive metabolic capacity to transit fertile florets into grains (Dreccer *et al*., 2014). The lower shoots plant^{-1} in high WSC lines was associated with differences in stem C/N metabolism (McIntyre *et al.*, 2011) and a small total area of sheaths per plant but larger internode cross-section and wall area was also associated with high stem WSC (Dreccer *et al.*, 2013).

Ear DM per fertile shoot was associated with fertile floret number per ear in the present study supporting previous associations between assimilate supply and floret fertility. A positive relationship between the number of fertile florets at anthesis (or grains at maturity) and ear dry weight was observed in many investigations (Kirby, 1988, Siddique *et al.*, 1989b, González *et al*., 2003a, González *et al.*, 2005a). Present results showed increased rachis length was positively related to spikelets and fertile spikelets ear^{-1}. This could in part be associated with increased ear photosynthesis increasing assimilate supply to spikelets. More fertile florets were produced in central than in basal spikelets which in turn produced more than apical spikelets, in spring NILs. In winter NILs, again most fertile florets were produced in central spikelets but apical spikelets produced more florets than basal spikelets. The decrease in fertile florets in basal spikelets in winter NILs compared to Humber could be partly due to more infertile spikelets at the base of ear. Floret development is initiated in the middle of the ear, then continued to the apical and finally basal spikelets of the ear (Kirby, 1974, Whingwiri and Stern, 1982, Stockman *et al*., 1983). In the findings of Mishra and Mohapatra (1987), slow-growing and sterile spikelets were mostly observed in the basal spikelets of the ear with higher concentration of soluble carbohydrates than the apical and middle spikelets, respectively. In addition, the number of fertile florets per basal spikelet initiated was reduced when there was

a deficit of assimilates before anthesis (Stockman *et al*., 1983, Sibony and Pinthus, 1988, González *et al*., 2011). There was a large increase in fertile florets per basal spikelet in spring NILs compared to Ashby, and the increase in basal spikelets was greater than in central or apical spikelets.

In spring NILs, the large increase in fertile florets ear^{-1} was likely due to fewer tillers competing with the main shoot for assimilate supplies. Guo and Schnurbusch (2015) examining the effects of detillering on floret fertility found that tiller removal allowed florets to delay degradation in most cases and detillering was highly associated with enhanced maximum floret primordia, fertile florets and final grains per spikelet. Their study also reported that the number of fertile florets at flowering may rely on the alternation of two components; (i) the increase of apical floret primordia and (ii) the increase in basal florets per spikelet. González-Navarro *et al.* (2015) reported that the final number of fertile florets is related to survival of floret primordia and extended duration of floret development can maximize floret survival.

In the present study, ear DM per fertile shoot was increased in the Humber NILs. Guo and Schnurbusch (2015) suggested that the maximum number of floret primordia could be improved by increasing main shoot DM. Moreover, plant hormones play an important role in the determination of floret fertility. Wang *et al.* (2001) gave examples of hormones that could influence fertile floret number in rice, e.g. zeatin (cytokinin) and abscisic acid (ABA). Zeatin not only encouraged the floret development and raised the number of fertile florets and grain set but also increased the sugar concentration in developing ears at anthesis. In contrast, several hormones e.g. IAA, GA_3 and ABA inhibited floret development and decreased the number of fertile florets and grain set (Wang *et al*., 2001). Lejeune *et al.* (1998) found that applied cytokinins stimulated sucrose transport into cultured ears of wheat and this resulted in more grain set. More recently, a gene for a quantitative trait loci (QTL) was identified that increased grain number by approximately 21%; this was identified

as *Gn1a* in rice. This gene codes for expression of a cytokinin oxidase/dehydrogenase enzyme (OsCKX2) that degrades cytokinin (Ashikari *et al*., 2005).

5.4.3 Summary

Five hypotheses were tested in this chapter:

Hypothesis 1*: BC_3 NILs have higher ear DM partitioning index and therefore greater ear DM and more fertile florets per unit area at anthesis than their recurrent parents.*

This hypothesis was confirmed in both spring and winter NILs. BC_3 spring and winter NILs had up to 217% and 31% higher ear DM shoot^{-1} than Ashby and Humber respectively, associated with increases in EPI (30% and 10% in spring and winter NILs, respectively) and fertile florets ear^{-1} (116% and 29% in spring and winter NILs, respectively).

Hypothesis 2*: There is a positive correlation between grains m^{-2} and fruiting efficiency and grains per ear amongst the BC_3 NILs.*

A positive correlation between grains m^{-2} and fruiting efficiency was observed in spring BC_3 NILs in 2014 and a positive correlation between grains per ear and fruiting efficiency was observed in winter BC_3 NILs in 2013 and 2014 (Table 5.5), so this hypothesis was only confirmed for spring NILs in 2014.

***Hypothesis 3**: NILs can be identified with more spikelets ear^{-1} and more fertile florets spikelets^{-1} in NILs hence more fertile florets ear^{-1} compared to their recurrent parents.*

More spikelets ear^{-1} and more fertile florets spikelets^{-1} hence more fertile florets ear^{-1} were observed in spring NILs (Table 5.7) and winter NILs (Table 5.9) compared to their recurrent parent (Ashby and Humber, respectively), so this hypothesis was confirmed in both spring and winter NILs.

***Hypothesis 4**: There is a positive correlation between fertile florets ear^{-1} at anthesis and grains per ear at harvest amongst the BC$_3$ NILs.*

In this study, there was a positive correlation between fertile florets ear^{-1} at anthesis and grains ear^{-1} at harvest amongst the BC$_3$ spring NILs but not in the BC$_3$ winter NILs, so this hypothesis was confirmed in spring NILs but not winter NILs.

***Hypothesis 5**: There is a positive correlation between EPI and FE at anthesis amongst the BC$_3$ NILs.*

There was a positive correlation between EPI and FE at anthesis amongst the BC$_3$ winter NILs in 2013 but no correlation in 2014. A negative relationship between EPI and FE at anthesis was observed in the BC$_3$ spring NILs in 2014, so this hypothesis was confirmed only in winter NILs in 2013.

CHAPTER 6 PHYSIOLOGICAL TRAITS ASSOCIATED WITH GRAIN YIELD AND YIELD COMPONENTS IN BC_3 NILs DURING THE POST-ANTHESIS PERIOD

6.1 Introduction

Sources are defined as photosynthetically active tissues which export the fixed carbon dioxide as sucrose to inactive plant parts which import the photoassimilate as sinks (Gaju, 2007). The supply of carbon assimilation for grain growth is contributed from the current photosynthesis organs, e.g. leaves and ears (source size) and remobilized from reserve carbohydrates in stem to grains (Schnyder, 1993, Blum *et al.*, 1994). The contribution of carbon assimilation to grain yield depends on photoassimilate transport to various organs and the source and sink relationship. The capacity of the sources to fill the sinks is a necessary component for yield potential improvement (Blum, 1996). Source limitation during grain filling can lead to poorly filled grains which have smaller endosperm cells and less starch than corresponding cells in normal grains (Brocklehurst *et al.*, 1978). Stem reserve carbohydrates mainly as water soluble carbohydrates (WSC) accumulate in wheat stems during growth and make an important contribution to grain filling, particularly under conditions when assimilation is limited (Ruuska *et al*., 2006) or when the post-anthesis photoassimilates do not meet the requirements of grain growth (Bonnett and Incoll, 1993, Schnyder, 1993). Thus, if both post-anthesis photoassimilates and stem reserves are limited, grain growth may be restricted by assimilate supply (Cook and Evans, 1978). Gifford and Evans (1981) showed that the store of soluble carbohydrates in stem tissue may increase until 2-3 weeks after anthesis because of high assimilate production with limited grain demand. Shearman *et al.* (2005) found a positive association between grain yield potential and WSC content in stem and leaf sheaths shortly after anthesis in the UK winter wheat and that stem WSC increased from 1972 to 1995 by 4.6 g m^{-2} yr^{-1}. Dreccer *et al.* (2009) reported that high WSC

lines produced similar or higher yields with lower grains m^{-2} but heavier grain weight compared to low WSC lines and suggested that lines with high amount of stem WSC may compensate for low grains m^{-2} by increasing the grain weight. In the UK, the recent increase in above-ground biomass in the UK winter wheat cultivars with year of release was partly associated with an increase in the stem WSC (Foulkes *et al.*, 2007).

The final grain weight is determined by the rate and the duration of grain filling. Generally, high temperature increases the rate of grain filling while duration of grain filling is shortened (Calderini *et al*., 1999a). Wardlaw *et al.* (1980) found that increasing temperatures from 21/16 °C to 30/25 °C during the post-anthesis period significantly reduced grain dry weight in wheat; the increase in grain filling rate did not compensate for the shorter period of grain filling. Reduction in grain weight due to increasing temperature was reported by several studies. For instance, Ugarte *et al.* (2007), determining the effect of pre-anthesis temperature on the Argentina grain weight in field experiments during three seasons, reported that a 5.5 °C increase above air temperature decreased grain weight up to 23%. Yin *et al.* (2009) studying the source-sink relationships during grain filling in six winter wheat genotypes grown in climate-controlled greenhouses under two temperature regimes (day/night: 20/15 and 25/20 °C) found that a 5 °C increase in temperature above 20 °C increased the grain filling rate from 1.32 to 1.67 mg $grain^{-1}$ d^{-1} (20%) but decreased the grain filling period by 12 days (>30%).

With regard to genetic variation in grain weight, high grain filling rate with longer duration of grain filling may be an important trait for increasing grain weight. Austin (1982) reported that genotypes with a longer duration of grain filling were more productive than those with a shorter grain filling phase under favourable conditions associated with a slower decrease in their photosynthetic capacity and lower respiratory losses of assimilated carbon. However, Bruckner and Frohberg (1987) found that, under heat and/or water stress, genotypes with heavier grain weight had

high rates of grain filling but shorter duration of grain filling. Motzo *et al.* (2010) suggested that the genetic variation in the individual grain weight was primarily associated with differences in the rate of grain filling. According to a weak relationship between grain filling rate and duration of grain filling, it was possible to select for high grain weight through increasing the rate of grain filling rate without shortening the duration of grain filling, or vice versa (Gebeyehou *et al*., 1982, Bruckner and Frohberg, 1987).

Most literature suggests that grain yield in wheat is generally sink limited under favorable conditions (Slafer and Rawson, 1994, Borrás *et al*., 2004, Zhang *et al*., 2010a), but modern cultivars may be moving closer towards source limitation (Shearman *et al.*, 2005, Aisawi *et al*., 2015). Therefore it is important to quantify relationships between source-type traits and grain yield in the post-anthesis period in the present experiments as it is possible that post-anthesis photosynthetic capacity may be limiting grain growth in some genotypes. This chapter therefore investigates the genotypic variation in the physiological processes from anthesis to physiological maturity and their association with grain yield and yield components in the BC_3 spring and winter NILs. The relationships between grain yield and physiological processes post-anthesis including senescence-related traits and the duration and rate of grain filling will be discussed.

Specific hypotheses tested in this chapter

1. There is a positive correlation between grain weight and grain yield amongst the BC_3 NILs.
2. There is a positive correlation between grain weight and duration and rate of grain filling amongst the BC_3 NILs.
3. Duration and rate of grain filling is associated with post-anthesis source-type traits including NDVI, flag-leaf senescence duration and rate and flag-leaf chlorophyll content (SPAD) amongst the BC_3 NILs.
4. Grain yield is source-limited during the latter stages of grain filling so that there is a correlation amongst BC_3 NILs between source-type traits under favourable post-anthesis conditions and grain weight and grain yield.
5. There is higher expression of source-type traits in BC_3 NILs than the recurrent parents Ashby and Humber.

6.2. Material and Methods

Measurements were carried out to quantify the post-anthesis physiological processes associated with grain yield and yield components in BC_3 NILs and their recurrent parents in field experiments at University of Nottingham Farm in 2012/13 and 2013/14 as described in Chapter 3.2. Leaf and canopy traits including flag-leaf senescence duration and rate, and flag-leaf chlorophyll content and the crop normalized difference vegetation index (NDVI) were measured approximately weekly in both 2013 and 2014, as described in Chapter 3.4.1.8-3.4.1.11. Measurements for flag-leaf visual score started at GS61 and for NDVI and SPAD at approximately 15-20 days after GS61. Flag-leaf chlorophyll fluorescence was measured as PSII quantum yield (ϕPSII or ΔF/Fm') (Murchie and Lawson, 2013) approximately every 14d starting at approximately GS61+14d in both 2013 and 2014, as described in Chapter 3.4.1.8-3.4.1.11.

Grain growth duration and grain growth rate were estimated using final grain weight and assuming grain growth occurred from GS61 to physiological maturity (taken as x=650 °Cd, (Shearman *et al*., 2005)) and were calculated based on the thermal time using a base temperature of 0 °C.

The flag-leaf visual senescence score data were fitted to thermal time (°Cd, base temperature 0 °C) from anthesis (GS61) using a logistic curve. For flag-leaf senescence score (FLS), onset of senescence was taken as the thermal time when FLS=1 and the end of senescence as FLS=9. The data for NDVI and flag-leaf chlorophyll content (SPAD) were fitted to thermal time post-anthesis using a linear function and the slopes were taken as the NDVI and SPAD senescence rates. The data for flag-leaf chlorophyll fluorescence were used to calculate the average of the three readings during post-anthesis as an estimate of post-anthesis chlorophyll fluorescence. Values of senescence parameters were estimated for each plot and subjected to ANOVA. For SPAD and NDVI, the value at physiological maturity was taken as an indicator of stay-green. The date of physiological maturity was estimated as the date when complete flag-leaf senescence occurred for 50% of the fertile shoots.

6.3 Results

6.3.1 Senescence traits during grain filling

6.3.1.1 Normalized difference vegetative index (NDVI)

Normalized difference vegetative index (NDVI) was measured five times in 2013 and four times in 2014 from 19-43 days after anthesis. In spring wheat lines, NDVI at the initial assessment at approximately GS61+20d in BC_3 spring NILs varied from 0.62 to 0.76 in 2013 and 0.49 to 0.69 in 2014 compared to Ashby (0.75; *P<0.01* and 0.72; *P<0.10*, respectively; Table 6.1). NDVI at this stage averaging across years ranged amongst BC_3 lines from 0.60 to 0.73 compared to 0.74 for Ashby (*P<0.01*). NDVI at

the final assessment at approximately GS61+35d amongst spring BC_3 lines varied from 0.22 to 0.26 compared to 0.20 for Ashby (*P<0.10*). The year and genotype interaction was significant for NDVI at GS61+20d (*P<0.10*) but there was no year effect.

The data from all readings were fitted to thermal time post-anthesis in each year using a linear function; the slope is the NDVI senescence rate and NDVI at physiological maturity (taken as x=650 °Cd) was taken as an indicator of stay-green (Lopes and Reynolds, 2012). Onset of NDVI senescence was estimated as the thermal time when NDVI = 1 and the end of canopy senescence when NDVI = 0. The NDVI rate of senescence (RS) (-0.00109 to -0.00071 $°Cd^{-1}$) and NDVI stay green at physiological maturity (Stg) (0.36-0.95) differed in BC_3 spring NILs compared to Ashby in 2013 (-0.00110 $°Cd^{-1}$; *P<0.001* and 0.38; *P<0.05*, respectively). In 2014, BC_3 lines also differed in NDVI rate of senescence (RS) (-0.00125 to -0.00070 $°Cd^{-1}$) compared to Ashby (-0.00139 $°Cd^{-1}$; *P>0.05*), but did not differ in NDVI stay green (Stg) (0.37-0.71) compared to Ashby (0.53; *P>0.10*). Averaging across years, the NDVI rate of senescence (RS) (-0.00116 to -0.00079 $°Cd^{-1}$) was generally slower and NDVI stay green (Stg) (0.36-0.83) higher in BC_3 spring NILs compared to Ashby (-0.00124 $°Cd^{-1}$; *P<0.001* and 0.45; *P<0.01*, respectively).

In winter wheat, BC_3 NILs differed in NDVI at the initial assessment in early to mid grain filling at approximately GS61+15d (13-19d after anthesis) (*P<0.01*) and during late grain filling at approximately GS61+35d (33-39 days after anthesis) (*P<0.001*) in both years (Table 6.2). In 2013, NDVI at GS61+15d in BC_3 winter NILs varied from 0.72 to 0.80 and NDVI at GS61+35d from 0.23 to 0.54 compared to Humber (0.78; *P<0.01* and 0.38; *P<0.001*, respectively). The NDVI senescence rate (-0.00116 to -0.00100 $°Cd^{-1}$) differed in BC_3 NILs compared to Humber (-0.00110 $°Cd^{-1}$; P<*0.001*) but the NILs did not differ in Stg (0.30-0.77) compared to Humber (0.44; *P>0.10*). In 2014, NDVI at GS61+15d in BC_3 winter NILs varied from 0.70 to 0.74 and at GS61+35d from 0.21 to 0.38 compared to Humber (0.73; *P<0.10* and

0.30; *P<0.001*, respectively). In 2014, BC_3 NILs differed in the NDVI senescence rate (-0.00138 to -0.00102 °Cd^{-1}) and Stg (0.39-1.04) compared to Humber (-0.00125 °Cd^{-1}; *P<0.01* and 0.49; *P<0.01*, respectively).

Averaging across years, the NDVI rate of senescence (RS) (-0.00124 to -0.00103 °Cd^{-1}) and stay green (Stg) (0.39-0.89) differed in BC_3 winter NILs (8 and 5 lines were significantly slower and higher than Humber, respectively) compared to Humber (-0.00118 °Cd^{-1}; *P<0.001* and 0.46; *P<0.01*, respectively). The effect of year was significant for NDVI stay green (*P<0.01*) and NDVI rate of senescence (*P<0.01*) and there was significant year x genotype interaction for NDVI rate of senescence (*P<0.05*).

Table 6.1 Minimum, maximum and mean values for normalized difference vegetation index (NDVI), flag-leaf senescence score (FLS), flag-leaf chlorophyll content (SPAD) and chlorophyll fluorescence (fluorpen) during the grain filling period among 7 BC_3 spring wheat NILs and the Ashby parent in 2013, 2014 and SED for BC_3 line, year and interaction.

	NDVI				FLS			SPAD			Fluorpen
	Stay green (Stg)	**Rate of senescence (RS) (°Cd^{-1})**	**NDVI GS61+20d**	**NDVI GS61+35d**	**Thermal time (°Cd) at the start of senescence**	**Thermal time (°Cd) at the end of senescence**	**Rate of senescence (°Cd^{-1})**	**SPAD rate (°Cd^{-1})**	**SPAD at 650 °Cd**	**SPAD at GS61+14d**	**Chlorophyll fluorescence Post-anthesis (ϕPSII)**
2013 BC_3S_5 lines											
Minimum	0.36	-0.00109	0.62	0.27	366.7	797.1	0.0053	-0.097	26.0	58.8	0.56
Maximum	0.95	-0.00071	0.76	0.37	459.5	886.9	0.0103	-0.059	42.3	61.1	0.69
Mean	0.55	-0.00099	0.72	0.30	409.4	841.5	0.0072	-0.080	34.0	59.8	0.62
Parental line (Ashby)	0.38	-0.00110	0.75	0.22	382.9	769.4	0.0087	-0.095	26.9	57.4	0.63
SED between BC_3 and parental line (DF=8)	0.13*	0.000040***	0.016**	0.039	23.21	19.21**	0.0010*	0.012	5.09	2.10	0.033
SED within BC_3 lines (DF=8)	0.15*	0.000046***	0.019**	0.046	26.80	22.19**	0.0012*	0.014	5.88	2.43	0.038
2014 BC_3S_6 lines											
Minimum	0.37	-0.00125	0.49	0.25	389.7	821.0	0.0052	-0.162	23.6	62.9	0.57
Maximum	0.71	-0.00070	0.69	0.31	437.1	865.0	0.0084	-0.115	40.0	81.3	0.66
Mean	0.56	-0.00097	0.62	0.27	408.6	841.6	0.0069	-0.141	30.6	69.3	0.63
Parental line (Ashby)	0.53	-0.00139	0.72	0.24	420.5	796.0	0.0101	-0.148	17.5	59.0	0.60
SED between BC_3 and parental line (DF=9)	0.15	0.000152*	0.065†	0.028	21.22	41.20	0.0021	0.011*	4.81*	4.61*	0.047
SED within BC_3 lines (DF=9)	0.17	0.000176*	0.075†	0.033	24.50	47.60	0.0024	0.013*	5.56*	5.32*	0.055
Cross-year ANOVA											
BC_3 lines											
Minimum	0.36	-0.00116	0.60	0.22	389.6	815.5	0.0056	-0.130	26.7	61.6	0.60
Maximum	0.83	-0.00079	0.73	0.26	436.7	876.1	0.0093	-0.099	39.7	70.7	0.66
Mean	0.56	-0.00098	0.67	0.24	409.0	841.6	0.0070	-0.111	32.3	64.6	0.63
Parental line (Ashby)	0.45	-0.00124	0.74	0.20	401.7	782.7	0.0094	-0.121	22.2	58.2	0.62
SED between BC_3 and parental line (DF=7)	0.10**	0.000081***	0.035**	0.016†	15.68	23.15*	0.0012*	0.008*	3.50**	2.58**	0.029
SED within BC_3 lines (DF=7)	0.11**	0.000093***	0.040**	0.018†	18.11	26.73*	0.0014*	0.009*	4.04**	2.98**	0.034
SED Year (DF=2)	0.07	0.000095	0.042	0.030	14.01	5.28	0.0010	0.014†	3.39	1.03*	0.014
SED Year*BC_3 and parental lines (DF=17)	0.15	0.000142*	0.062†	0.036	24.81	30.67	0.0019	0.017*	5.68	3.52*	0.041
SED Year*BC_3 line (DF=17)	0.17	0.000156*	0.068†	0.039	27.92	36.03	0.0021	0.018*	6.36	4.10*	0.047

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Table 6.2 Minimum, maximum and mean values for normalized difference vegetation index (NDVI), flag leaf senescence score (FLS), flag-leaf chlorophyll content (SPAD) and chlorophyll fluorescence (fluorpen) during the grain filling period among 37 BC_3 winter wheat NILs and the Humber parent in 2013, 2014 and SED for BC_3 line, year and interaction.

	NDVI				FLS			SPAD			Fluorpen
	Stay green (Stg)	**Rate of senescence (RS) (°Cd^{-1})**	**NDVI GS61+15d**	**NDVI GS61 +35d**	**Thermal time (°Cd) at the start of senescence**	**Thermal time (°Cd) at the end of senescence**	**Rate of senescence (°Cd^{-1})**	**SPAD rate (°Cd^{-1})**	**SPAD at 650 °Cd**	**SPAD at GS61+14d**	**Chlorophyll fluorescence Post-anthesis (ϕPSII)**
2013 BC_3S_5 lines											
Minimum	0.30	-0.00116	0.72	0.23	308.6	722.4	0.0070	-0.089	20.7	50.6	0.54
Maximum	0.77	-0.00100	0.80	0.54	471.4	862.8	0.0151	-0.047	37.4	55.6	0.67
Mean	0.45	-0.00106	0.78	0.37	398.4	777.2	0.0103	-0.070	29.2	53.3	0.63
Parental line (Humber)	0.44	-0.00110	0.78	0.38	397.0	778.7	0.0106	-0.081	26.5	54.4	0.65
SED between BC_3 and parental line (DF=39)	0.14	0.000023***	0.013**	0.055***	22.39**	22.36***	0.0018*	0.0069***	2.09***	1.48	0.027*
SED within BC_3 lines (DF=39)	0.16	0.000027***	0.016**	0.063***	25.86**	25.81***	0.0021*	0.0080***	2.42***	1.70	0.031*
2014 BC_3S_6 lines											
Minimum	0.39	-0.00138	0.70	0.21	386.7	785.4	0.0051	-0.139	16.2	50.1	0.58
Maximum	1.04	-0.00102	0.74	0.38	469.6	936.6	0.0130	-0.085	30.1	60.3	0.71
Mean	0.55	-0.00123	0.72	0.29	429.9	844.2	0.0088	-0.112	22.0	54.4	0.64
Parental line (Humber)	0.49	-0.00125	0.73	0.30	458.2	864.4	0.0087	-0.129	20.7	54.3	0.69
SED between BC_3 and parental line (DF=39)	0.13**	0.000080**	0.010†	0.031***	18.37*	28.75***	0.0018*	0.0103**	2.97*	1.85†	0.035*
SED within BC_3 lines (DF=39)	0.15**	0.000092**	0.012†	0.036***	21.21*	33.20***	0.0021*	0.0119**	3.43*	2.14†	0.040*
Cross-year ANOVA											
BC_3 lines											
Minimum	0.39	-0.00124	0.71	0.20	389.1	758.6	0.007	-0.112	18.9	51.6	0.58
Maximum	0.89	-0.00103	0.76	0.37	444.1	879.8	0.013	-0.066	32.3	56.8	0.68
Mean	0.50	-0.00115	0.75	0.27	414.2	810.7	0.010	-0.091	25.6	53.9	0.63
Parental line (Humber)	0.46	-0.00118	0.75	0.26	427.6	821.6	0.010	-0.105	23.6	54.3	0.67
SED between BC_3 and parental line (DF=37)	0.10**	0.000042***	0.009***	0.025***	14.48*	18.21***	0.0013**	0.006***	1.82***	1.18*	0.022***
SED within BC_3 lines (DF=37)	0.11**	0.000048***	0.010***	0.029***	16.72*	21.03***	0.0015**	0.007***	2.10***	1.37*	0.026***
SED Year (DF=2)	0.01**	0.000005**	0.013*	0.024	11.38	9.02*	0.0005	0.002**	0.44**	0.58	0.010
SED Year*BC_3 and parental line (DF=78)	0.13	0.000058*	0.018	0.043	23.12***	26.87	0.0019*	0.009	2.56	1.74	0.032
SED Year*BC_3 line (DF=78)	0.15	0.000067*	0.019	0.047	25.97***	30.71	0.0021*	0.010	2.96	1.99	0.037

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

6.3.1.2 Flag-leaf chlorophyll content (SPAD)

The first SPAD reading at approximately GS61+14d in BC_3 spring NILs varied from 58.8 to 61.1 in 2013 and 62.9 to 81.3 in 2014 compared to Ashby (57.4; *P>0.10* and 59.0; *P<0.05*, respectively). There were no differences amongst spring NILs in SPAD senescence rate (-0.097 to -0.059 °Cd^{-1}) and SPAD at 650°Cd (26.0 to 42.3) in 2013 (*P>0.10*). In 2014, SPAD senescence rate varied from -0.162 to -0.115 °Cd^{-1} compared to -0.148 °Cd^{-1} in Ashby (*P<0.05*) while SPAD at 650 °Cd was higher in NILs (23.6-40.0) compared to Ashby (17.5; *P<0.05*).

Averaging across years, there were differences in SPAD senescence rate with a slower senescence rate in 2 NILs (-0.130 to -0.099 °Cd^{-1}) compared to Ashby (-0.121; *P<0.05*). Year and year x genotype effects were significant for SPAD rate (*P<0.10* and *P<0.05*, respectively) and SPAD at GS61+14d (*P<0.05*).

In winter wheat, there were differences in SPAD senescence rate and SPAD at 650 °Cd in both years. In 2013, NILs differed in SPAD senescence rate (-0.089 to -0.047 °Cd^{-1}) and SPAD at 650 °Cd (20.7-37.4) compared to Humber (-0.081 °Cd^{-1}; *P<0.001* and 26.5; *P<0.001*, respectively). In 2014, NILs differed in SPAD rate (-0.139 to -0.085 °Cd^{-1}) and SPAD at 650 °Cd (16.2-30.1) compared to Humber (-0.129 °Cd^{-1}; *P<0.01* and 20.7; *P<0.05*, respectively).

Averaging across years, SPAD at GS61+14d (51.6-56.8) in 1 BC_3 line was higher and in 3 BC_3 lines were lower than Humber (54.3; *P<0.05*). SPAD at 650 °Cd in 11 NILs (18.9-32.3) was also higher in NILs compared to Humber (23.6; *P<0.001*) with slower SPAD rate observed in 17 NILs (-0.112 to -0.066) compared to Humber (-0.105; *P<0.001*). The year effect was significant for SPAD rate (*P<0.01*) and SPAD at 650 °Cd (*P<0.01*) but not for SPAD at GS61+14d (*P>0.10*). There was no year x genotype interaction for SPAD at GS61+14d, SPAD at 650 °Cd and SPAD rate (*P>0.10*).

6.3.1.3 Flag-leaf visual senescence score

In 2013, the end of senescence (797.1-886.9 °Cd) and rate of senescence (0.0053-0.0103 °Cd^{-1}) differed amongst BC_3 spring wheat lines compared to Ashby (769.4 °Cd^{-1}; *P<0.01* and 0.0087; *P<0.05*, respectively). There were no differences in 2014. Averaging across years, there were no differences in the start of senescence (*P>0.10*) but the end of senescence was delayed in four NILs (815.5-876.1 °Cd) compared to Ashby (782.7 °Cd; *P<0.05*) while rate of senescence was significantly slower in 3 NILs (0.0056-0.0093 °Cd^{-1}) than Ashby (0.0094 °Cd^{-1}; *P<0.05*). There was no year and year x genotype interaction for thermal time at the start and end of senescence and rate of senescence (*P>0.10*).

In winter wheat, the start (308.6-471.4 °Cd) and the end of senescence (722.4-862.8 °Cd) differed in NILs compared to Humber (397.0 °Cd; *P<0.01* and 778.7 °Cd; *P<0.001*, respectively) in 2013, while rate of senescence ranged from 0.0070 to 0.0151 °Cd^{-1} compared to Humber (0.0106 °Cd; *P<0.05*). In 2014, the start of senescence was generally earlier in NILs (386.7-469.6 °Cd) compared to Humber (458.2 °Cd; *P<0.05*), while the end of senescence was later in several NILs (785.4-936.6 °Cd) compared to Humber (864.4 °Cd; *P<0.001*). The rate of senescence ranged from 0.0051 to 0.0130 °Cd^{-1} in NILs compared to Humber (0.0087 °Cd^{-1}; *P<0.05*). Averaging across years, the start of senescence was earlier in 8 NILs (389.1-444.1 °Cd) compared to Humber (427.6 °Cd; *P<0.05*), and the end of senescence was delayed in 5 NILs (758.6-879.8 °Cd) compared to Humber (821.6 °Cd; *P<0.001*). The rate of senescence differed in NILs (0.0066-0.0132) compared to Humber (0.0096; *P<0.01*). The year effect was significant for the end of senescence (*P<0.05*), and year x genotype interaction was significant for the start of senescence (*P<0.001*) and rate of senescence (*P<0.05*).

6.3.1.4 Flag-leaf chlorophyll fluorescence

Averaging across assessment dates, there were no differences in post-anthesis chlorophyll fluorescence in spring NILs in both years. Averaging across years and assessment dates, chlorophyll fluorescence (ϕPSII or ΔF/Fm') ranged from 0.60 to 0.66 in NILs compared to 0.62 in Ashby (*P>0.10*). There was no year effect or year x genotype interaction (*P>0.10*).

In winter wheat NILs, in 2013 post-anthesis chlorophyll fluorescence (ϕPSII or ΔF/Fm') averaging across assessment dates was generally lower (0.54-0.67) compared to Humber (0.65; *P<0.05*). In 2014, chlorophyll fluorescence was again generally lower in NILs ranging from 0.58 to 0.71 compared to Humber (0.69; *P<0.05*). Averaging across years and assessment dates, 17 NILs showed decreased chlorophyll fluorescence in the range 0.58-0.68 compared to Humber (0.67; P<*0.001*). There was no year effect or year x genotype interaction (*P>0.10*).

6.3.2 Grain weight and duration and rate of grain growth

In spring wheat in 2013, grain weight varied from 25.6 to 42.4 mg in BC_3 NILs compared to 34.3 mg in Ashby (*P<0.05*) (Table 6.3). Rate of grain filling differed in BC_3 lines (0.027-0.048 mg °Cd^{-1}) compared to Ashby (0.042 mg °Cd^{-1}) (*P<0.01*). BC_3 lines also differed in duration of grain filling (804.3-959.1 °Cd) compared to Ashby (824.8 °Cd; *P<0.05*). There were no differences in individual grain weight, rate of grain filling and grain filling duration in 2014. Averaging across years, grain weight (34.2-43.0 mg) was generally higher in BC_3 lines than Ashby (35.9 mg; *P<0.05*) and the rate of grain filling (0.038-0.049 mg °Cd^{-1}) was generally increased compared to Ashby (0.043 mg °Cd^{-1}; *P<0.05*). Grain filling duration (823.7-904.9 °Cd^{-1}) was also generally increased in NILs compared to the recurrent parent (833.9 °Cd; *P<0.01*). The year effect was significant for rate of grain filling (*P<0.05*) and

the year x genotype interaction was significant for grain weight (*P<0.05*), rate of grain filling (*P<0.01*) and duration of grain filling (*P<0.05*).

In winter wheat NILs in 2013, grain weight (30.4-40.6 mg) was generally increased in BC_3 lines compared to Humber (31.6 mg; *P<0.05*) with higher rate of grain filling (0.035-0.047 mg °Cd^{-1}) in BC_3 lines compared to Humber (0.035 mg °Cd^{-1}; *P<0.01*). However, thermal duration of grain filling (796.2-899.5 °Cd) generally decreased in BC_3 lines compared to Humber (899.5 °Cd; *P<0.05*). In 2014, there were trends for grain weight (34.3-43.4 mg) and rate of grain filling (0.043-0.054 mg °Cd^{-1}) to be increased in several BC_3 lines compared to Humber (36.2 mg; *P<0.10* and 0.043 mg °Cd^{-1}; *P<0.10,* respectively). Grain filling duration (799.4-850.6 °Cd) was generally decreased in BC_3 lines compared to Humber (843.0 °Cd; *P<0.001*). Averaging across years, grain weight (33.5-41.0 mg) was increased in 22 BC_3 lines compared to Humber (33.9 mg; *P<0.01*), and the rate of grain filling (0.040-0.050 mg °Cd^{-1}) was higher in 32 BC_3 lines than Humber (0.039 mg °Cd^{-1}; *P<0.001*). However, the duration of grain filling (817.3-875.1 °Cd) was decreased in 25 BC_3 lines compared to Humber (871.2 °Cd; *P<0.05*). The year effect was significant for individual grain weight (*P<0.05*), rate of grain filling (*P<0.05*), thermal duration of grain filling (*P<0.05*) and duration of grain filling in days (*P<0.10*), and the year x genotype interaction was significant for thermal duration of grain filling (*P<0.001*) and duration of grain filling in days (*P<0.001*).

A positive linear relationship was found between rate of grain filling and individual grain weight in both BC_3 spring and winter wheat NILs in both years (Fig. 6.1). In winter wheat in 2013, duration of grain filling was associated with rate of grain filling (R^2 = 0.131; *P<0.05*). In 2014, there was a trend for a positive association between duration of grain filling and grain weight (R^2 = 0.087; *P<0.10*) in BC_3 winter wheat NILs. No statistically significant relationships between the duration of grain filling and grain weight and rate of grain filling were observed in spring wheat lines.

Table 6.3 Minimum, maximum and mean values for individual grain weight, rate of grain filling, thermal duration of grain filling and duration of grain filling in days among 7 BC_3 spring wheat NILs and 37 BC_3 winter wheat NILs and the recurrent parent (Ashby or Humber) in 2013, 2014 and SEDs from cross-year ANOVA for BC_3 line, year and interaction.

	Spring wheat NILs				Winter wheat NILs			
	Individual GW (mg)	**Rate of grain filling (mg °Cd^{-1})**	**Thermal duration grain filling (°Cd)**	**Duration of grain filling (days)**	**Individual GW (mg)**	**Rate of grain filling (mg °Cd^{-1})**	**Thermal duration grain filling (°Cd)**	**Duration of grain filling (days)**
2013 BC_3S_5 lines								
Minimum	25.6	0.027	804.3	34.5	30.4	0.035	796.2	44.0
Maximum	42.4	0.048	959.1	44.0	40.6	0.047	899.5	50.0
Mean	36.9	0.042	884.3	39.6	35.6	0.042	847.8	47.0
Parental line (Ashby or Humber)	34.3	0.042	824.8	36.0	31.6	0.035	899.5	50.0
SED between BC_3 and parental line (DF=8 or 39)	3.27*	0.0032**	33.55*	2.03*	2.11*	0.0024**	29.17*	1.75*
SED within BC_3 lines (DF=8 or 39)	3.78*	0.0037**	38.74*	2.35*	2.43*	0.0028**	33.69*	2.02*
2014 BC_3S_6 lines								
Minimum	38.9	0.045	834.5	39.0	34.3	0.043	799.4	37.0
Maximum	44.5	0.052	850.6	40.0	43.4	0.054	850.6	40.0
Mean	41.8	0.049	844.0	39.6	39.0	0.047	824.9	38.5
Parental line (Ashby or Humber)	37.4	0.044	843.0	39.5	36.2	0.043	843.0	39.5
SED between BC_3 and parental line (DF=8 or 39)	3.14	0.0037	9.86	0.63	2.26†	0.0027†	8.20***	0.49***
SED within BC_3 lines (DF=8 or 39)	3.63	0.0043	11.39	0.73	2.60†	0.0031†	9.46***	0.57***
Cross-year ANOVA								
BC_3 lines								
Minimum	34.2	0.038	823.7	47.0	33.5	0.040	817.3	46.5
Maximum	43.0	0.049	904.9	52.0	41.0	0.050	875.1	50.0
Mean	39.4	0.046	864.1	49.6	37.3	0.045	836.3	47.7
Parental line (Humber)	35.9	0.043	833.9	47.8	33.9	0.039	871.2	49.8
SED between BC_3 lines and parental line (DF=8 or 39)	2.27*	0.0024*	17.05**	1.04**	1.54**	0.0018***	15.15*	0.91*
SED within BC_3 lines (DF=8 or 39)	2.62*	0.0028*	19.68**	1.20**	1.78**	0.0021***	17.50*	1.05*
SED Year (DF=2)	1.70	0.0010*	18.70	1.01	0.73*	0.0010*	4.64*	0.34†
SED Year*BC_3 lines and parental line (DF=17 or 78)	3.41*	0.0033**	29.08*	1.69*	2.27	0.0027	21.56***	1.31***
SED Year*BC_3 lines (DF=17 or 78)	3.89*	0.0039**	32.24*	1.89*	2.59	0.0031	24.86***	1.50***

† *P<0.1*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

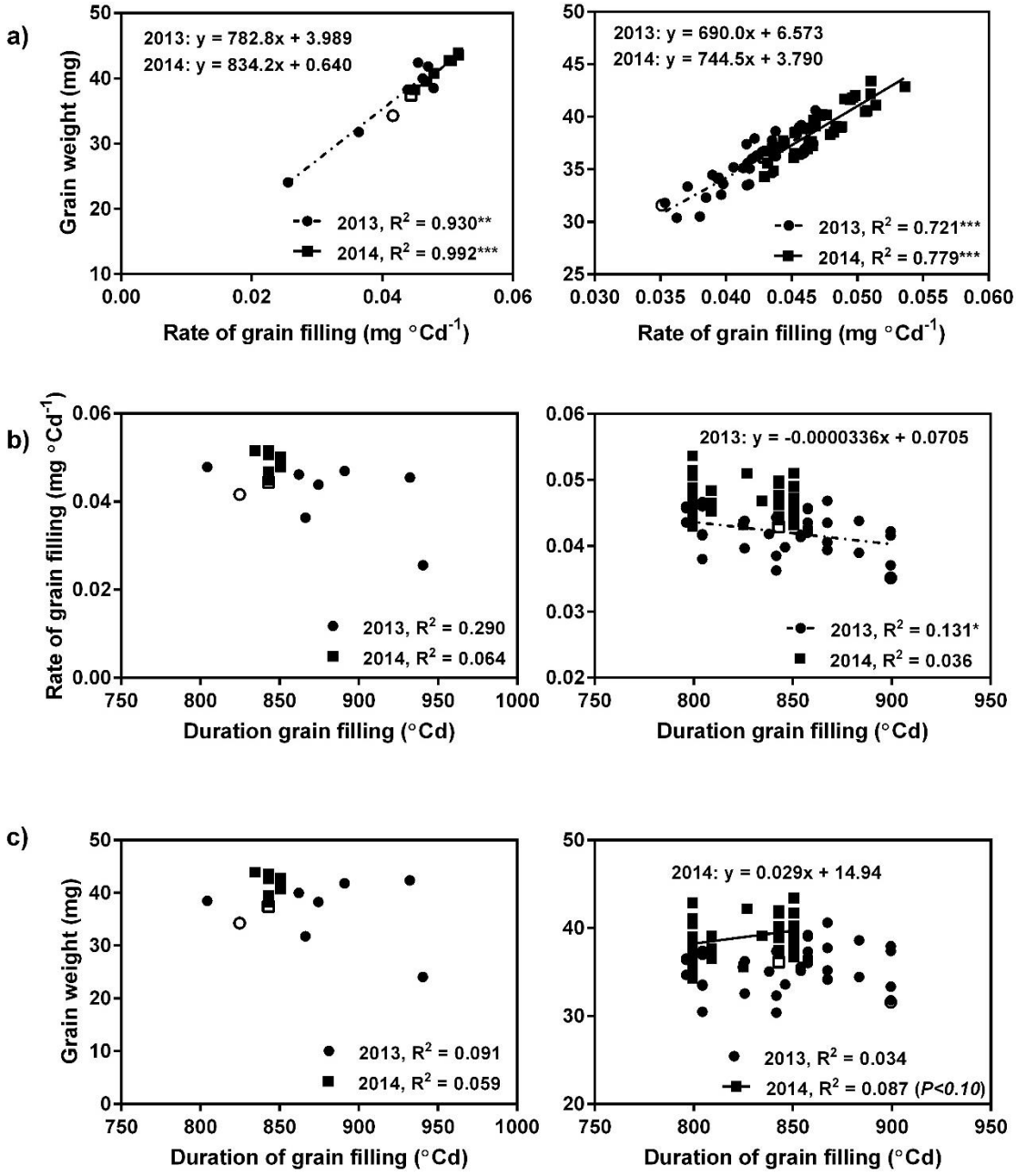

Fig. 6.1 Linear relationship between a) rate of grain filling (mg °Cd^{-1}) and individual grain weight (mg), b) duration of grain filling and rate of grain filling (mg °Cd^{-1}) and c) duration of grain filling (°Cd) and individual grain weight (mg) in BC_3 spring (left hand panels) and winter NILs (right hand panels), respectively in 2013 (closed circles) and 2014 (closed squares) and the recurrent parental lines (Ashby and Humber) in 2013 (open circle) and 2014 (open square). Statistical significance; * *P<0.05*, ** *P<0.01*, *** *P<0.001*.

6.3.3 Trait correlations

6.3.3.1 Correlations between post-anthesis traits in spring wheat NILs

In spring wheat NILs, there were only 7 lines so correlations should be interpreted with caution. In 2013, grain yield was positively correlated with NDVI at GS61+15d (r=0.886, *P≤0.01*). High grain yield was associated with slower NDVI senescence rate (r=-0.948, *P≤0.05*) but high FLS rate (r=0.849, *P≤0.05*) (Table 6.4). Longer stay green (NDVI at 650 °Cd) was associated with higher chlorophyll content at 650 Cd° (r=0.882, *P≤0.01*), and higher post-anthesis chlorophyll fluorescence (r=0.774, *P≤0.05*). Moreover, slower FLS rate was associated with higher NDVI Stg (r=-0.674, *P≤0.10*) and higher NDVI senescence rate (r=-0.752, *P≤0.10*).

In 2014, high grain yield was associated with high NDVI at GS61+15d (r=0.730, *P≤0.10*) and with slower NDVI rate of senescence (r=-0.836, *P≤0.05*), whereas TGW was positively correlated with NDVI Stg (r=0.856, *P≤0.05*). Higher FLS rate was associated with higher fertile shoots plant^{-1} (r=0.884, *P≤0.01*) and higher NDVI at GS61+15d (r=0.901, *P≤0.01*), earlier FLS start of senescence (r=0.800, *P≤0.05*) and correlated with earlier FLS end of senescence (r=-0.750, *P≤0.10*) and NDVI slower senescence rate (r=-0.736, *P≤0.10*).

Table 6.4 Phenotypic correlation (r) amongst BC_3 spring wheat NILs for senescence traits in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**HI**	**AGDM (g) shoot^{-1}**	**TGW (g)**	**Fertile shoots plant^{-1}**	**NDVI at GS61 +15d**	**NDVI at 650 °Cd (Stg)**	**NDVI rate (RS) (°Cd^{-1})**	**Start of FLS °Cd**	**End of FLS °Cd**	**FLS rate °Cd^{-1}**	**SPAD at GS61 +14d**	**SPAD at 650 °Cd**	**SPAD rate**	**Chl. fluorescence**
Grain yield 100%DM (t ha^{-1})	-	0.178	**- 0.793***	**-0.699†**	**-0.739†**	**0.730†**	-0.609	**-0.836***	0.322	-0.637	0.500	-0.254	-0.349	-0.074	-0.072
HI	**-0.756***	-	0.068	-0.205	0.148	-0.123	0.155	0.100	0.200	0.137	0.023	0.269	0.348	0.092	-0.163
AGDM (g) shoot^{-1}	-0.219	**-0.704†**	-	0.248	**-0.818***	**-0.901****	0.272	**0.897****	**-0.682†**	0.551	**-0.683†**	**0.769***	0.583	-0.234	0.129
TGW (g)	0.354	-0.257	-0.459	-	0.044	-0.050	**0.856***	0.264	0.303	0.085	0.230	-0.170	-0.117	0.174	0.064
Fertile shoots plant^{-1}	**0.877****	**-0.703†**	-0.380	0.582	-	**0.859***	-0.056	**-0.695†**	**0.911****	-0.470	**0.884****	-0.599	-0.584	0.128	-0.002
NDVI GS61+15d (°Cd)	**0.886****	**-0.883****	-0.582	0.598	**0.853***	-	-0.122	**-0.944****	**0.689†**	**-0.807***	**0.901****	-0.581	**-0.730†**	-0.033	-0.084
NDVI at 650 °Cd (Stg)	**-0.674†**	0.621	0.230	0.045	-0.431	-0.613	-	0.216	0.178	0.076	0.108	-0.106	-0.076	0.101	-0.308
NDVI rate (RS) °Cd^{-1}	**-0.948***	**0.836***	0.485	-0.519	**-0.886****	**-0.966*****	**0.699†**	-	-0.470	**0.840***	**-0.736†**	0.528	**0.701†**	0.108	0.248
Start FLS °Cd	0.448	-0.043	0.485	0.104	0.164	0.265	-0.062	-0.249	-	-0.259	**0.800***	-0.627	-0.326	0.452	0.172
End FLS °Cd	-0.469	**0.796***	0.668	0.016	-0.567	-0.549	0.564	0.565	0.475	-	**-0.750†**	0.089	0.589	0.364	0.064
FLS rate °Cd^{-1}	**0.849***	**-0.832***	-0.203	-0.048	**0.690†**	**0.732†**	**-0.674†**	**-0.752†**	0.340	-0.657	-	-0.385	-0.619	-0.049	0.085
SPAD at GS61+14d	-0.078	0.658	**0.676†**	0.252	0.049	-0.303	0.412	0.215	0.323	0.639	-0.391	-	0.526	-0.463	0.265
SPAD at 650 °Cd	-0.461	0.617	0.324	0.233	-0.352	-0.443	**0.882****	0.502	0.280	**0.793***	-0.652	0.564	-	0.477	0.535
SPAD rate	-0.396	0.349	-0.004	0.330	-0.224	-0.263	**0.885****	0.374	0.155	0.547	-0.526	0.302	**0.930****	-	0.424
Chl. fluorescence	-0.498	0.555	0.064	0.522	-0.128	-0.329	**0.774***	0.420	-0.162	0.490	**-0.712†**	0.595	**0.720†**	**0.692†**	-

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

6.3.3.2 Correlations between post-anthesis traits in winter wheat NILs

In winter NILs 2013, grain yield was positively correlated with NDVI at GS61+20d (r=0.593, *P≤0.001*), FLS end of senescence (r=0.419, *P≤0.01*), flag-leaf SPAD at GS61+14d (r=0.500, *P≤0.01*), flag-leaf SPAD at 650 °Cd (r=0.534, *P≤0.001*), flag-leaf SPAD rate (r=0.482, *P≤0.01*) and flag-leaf chlorophyll fluorescence (r=0.547, *P≤0.001*) (Table 6.5). TGW was also positively associated with SPAD at GS61+14d (r=0.327, *P≤0.05*), flag-leaf SPAD at 650 °Cd (r=0.253, *P≤0.05*) and flag-leaf SPAD rate (r=0.276, *P≤0.10*). NDVI at GS61+20d was positively correlated with anthesis date (r=0.552, *P≤0.001*), plant height (r=0.347, *P≤0.05*), FLS start of senescence (r=0.476, *P≤0.01*), FLS end of senescence (r=0.359, *P≤0.05*), flag-leaf SPAD at GS61+14d (r=0.343, *P≤0.05*) and chlorophyll fluorescence (r=0.499, *P≤0.05*). Faster NDVI senescence rate was associated with slower FLS rate (r=-0.488, *P≤0.01*) and higher SPAD rate (r=0.483, *P≤0.01*). In addition, slower FLS rate was correlated with higher AGDM shoot^{-1} (r=-0.346, *P≤0.05*), increased plant height (r=-0.440, *P≤0.05*) and later FLS end of senescence (r=-0.580, *P≤0.05*).

In 2014, higher grain yield was correlated with higher NDVI at early at GS61+20d (r=0.530, *P≤0.001*), higher NDVI Stg (r=0.307, *P≤0.10*), higher NDVI senescence rate (r=0.318, *P≤0.10*), later FLS end of senescence (r=0.345, *P≤0.05*), higher SPAD at 650 °Cd (r=0.284, *P≤0.10*) and faster SPAD rate (r=0.509, *P≤0.01*). Earlier FLS start of senescence was correlated with lower SPAD at GS61+14d (r=-0.463, *P≤0.01*), lower SPAD at 650 °Cd (r=-0.420, *P≤0.01*) and lower SPAD rate (r=-0.472, *P≤0.01*).

Table 6.5 Phenotypic correlation (r) amongst BC_3 winter wheat NILs for senescence traits in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield	HI	AGDM (g) shoot^{-1}	TGW (g)	Anthesis date	Plant height	NDVI at GS61 +20d	NDVI at 650 °Cd (Stg)	NDVI rate (RS) (°Cd^{-1})	Start FLS (°Cd)	End FLS (°Cd)	FLS rate (°Cd^{-1})	SPAD at GS61+14 d	SPAD at 650 ° Cd	SPAD rate	Chl. fluorescence
Grain yield	-	-0.113	0.153	0.169	**0.420****	0.244	**0.530*****	**0.307†**	**0.318†**	-0.183	**0.345***	-0.199	-0.094	**0.284†**	**0.509****	0.214
HI	-0.078	-	0.032	**0.625*****	**-0.540*****	**-0.390***	**-0.404***	-0.122	-0.254	**0.476****	-0.212	**0.332***	-0.197	-0.243	**-0.443****	**-0.342***
AGDM (g) shoot^{-1}	**0.341***	0.086	-	0.240	0.144	**0.583*****	-0.005	0.169	0.182	0.008	0.266	-0.108	0.235	**0.367***	0.266	0.086
TGW (g)	**0.387***	**0.297†**	**0.580*****	-	-0.196	0.152	-0.206	-0.112	-0.073	0.242	-0.047	0.179	-0.056	-0.052	-0.176	-0.182
Anthesis date	**0.503****	-0.160	**0.455****	**0.351***	-	**0.594*****	**0.422****	0.233	**0.575*****	**-0.781*****	**0.299†**	**-0.471****	**0.552*****	**0.572*****	**0.754*****	**0.463****
Plant height	**0.409***	-0.100	**0.624*****	**0.484****	**0.738*****	-	0.230	0.265	**0.434****	**-0.439****	**0.477****	**-0.464****	**0.329***	**0.455****	**0.520*****	**0.360***
NDVI at GS61 +20d	**0.593*****	-0.102	0.160	0.212	**0.552*****	**0.347***	-	**0.281†**	0.254	-0.118	**0.411***	-0.259	0.034	**0.360***	**0.499****	**0.505****
NDVI at 650 °Cd (Stg)	0.242	-0.019	0.083	0.127	0.126	0.095	0.183	-	**0.616*****	-0.111	**0.536*****	**-0.362***	-0.054	**0.312†**	**0.355***	0.040
NDVI rate (RS) (°Cd^{-1})	0.054	**-0.289†**	**0.349***	-0.037	**0.418***	**0.497****	-0.262	0.129	-	**-0.336***	**0.654*****	**-0.583*****	0.260	**0.730*****	**0.704*****	0.398
Start FLS (°Cd)	0.216	0.166	-0.010	-0.037	0.074	-0.094	**0.476****	-0.033	-0.193	-	-0.272	**0.667*****	**-0.463****	**-0.420****	**0.472****	-0.257
End FLS (°Cd)	**0.419****	-0.118	**0.536*****	0.218	**0.577*****	**0.655*****	**0.359***	**0.290†**	**0.481****	-0.006	-	**-0.845*****	0.044	**0.643*****	**0.617*****	**0.468****
FLS rate (°Cd^{-1})	-0.032	0.209	**-0.346***	-0.137	-0.240	**-0.440****	0.216	-0.059	**-0.488****	**0.726*****	**-0.580*****	-	-0.226	**-0.625*****	**-0.640*****	**-0.453****
SPAD at GS61+14d	**0.500****	-0.057	0.149	**0.327***	**0.475****	**0.342***	**0.343***	-0.127	0.152	0.213	0.186	-0.039	-	**0.520*****	**0.284†**	**0.327***
SPAD at 650 °Cd	**0.534*****	-0.186	**0.514****	**0.253***	**0.770*****	**0.703*****	**0.584*****	**0.310†**	**0.454****	0.262	**0.820*****	**-0.292†**	**-0.371***	-	**0.868*****	**0.636*****
SPAD rate	**0.482****	-0.108	**0.555*****	**0.276†**	**0.822*****	**0.733*****	**0.532*****	**0.312†**	**0.483****	0.186	**0.811*****	**-0.306†**	0.184	**0.943*****	-	**0.569*****
Chl. fluorescence	**0.547*****	0.071	0.222	0.103	**0.523*****	0.206	**0.499****	-0.047	0.076	**0.419****	**0.303†**	0.195	**0.403***	**0.472****	**0.479****	-

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

6.4 Discussion

6.4.1 Relationships between grain yield and physiological processes post-anthesis

Correlations between each of grain yield and AGDM and post-anthesis source-type traits were observed in both spring and winter wheat NILs. In spring wheat lines in 2014, high grain yield was associated with high fertile shoots plant^{-1} and high NDVI at GS61+15d, but a high FLS rate. Higher yield associated with increased flag-leaf senescence rate could be due to later onset of senescence, correlating with a high senescence rate. In contrast, BC_3 spring lines with low fertile shoot plant^{-1} were associated with stay-green traits (high NDVI at 650 °Cd and high NDVI rate of senescence) and lower FLS rate. Duration of post-anthesis photosynthesis is closely associated with leaf N status (Thomas and Smart, 1993). Some of the BC_3 spring wheat lines had fewer tillers per plant with increased leaf thickness (see Chapter 5) at anthesis compared to the recurrent parent; these leaf effects might be assoiated with increased N source at anthesis for grain growth and delayed senescence during N remobilization. In the study of Borrell and Hammer (2000), higher leaf thickness increased the demand of leaves for N and this in turn contributed to increased photosynthesis rate per unit leaf area. Hendriks *et al.* (2016), studying the effects of the tiller inhibition gene on root-shoot carbon partitioning in rainfed environments, reported that the NILs with fewer tillers plant^{-1} (+*Tin*) reduced total leaf area and biomass (60%) but increased root length (120%) and root biomass (145%) and led to high availability of water, cooler canopy temperatures, more stomatal conductance and high maintenance of green leaf area during the grain filling phase. In spring NILs, grain yield and grain weight was not generally well correlated with post-anthesis senescence traits in the present study, so present results suggest that grain growth was likely limited by sink size.

In winter wheat lines, grain yield and biomass were increased with high canopy green area (NDVI measured around 2 weeks after anthesis) and better maintenance

of green area during grain filling (higher NDVI at 650 °Cd and slower NDVI rate of senescence). Grain yield was also positively correlated with flag-leaf chlorophyll content at GS61+14d and at 650 °Cd and later end of flag-leaf senescence (FLS). According to Thomas and Howarth (2000), the stay green traits can be classified into functional stay green and non-functional stay green (photosynthetic function not maintained). Flag-leaf chlorophyll content in several BC_3 spring and winter lines decreased later and the decrease was slower compared to the recurrent parent, indicating that they potentially had an ability to photosynthesize for a longer period during grain filling period (functional stay green). It has been suggested that crops may maintain a long duration of active photosynthesis in two ways; i) conserving more chlorophyll content during senescence and ii) conserving higher photosynthetic activity during the loss of chlorophyll (Zhang *et al*., 2012). Due to positive correlations between photosynthetic rate per unit area and chlorophyll content per leaf area (Evans, 1983) and between SPAD relative chlorophyll content and maximum net photosynthetic rate in wheat (Gutiérrez-Rodŕiguez *et al.*, 2000), it is possible that the low SPAD senescence rate might be associated with high flag-leaf photosynthetic rate in the BC_3 lines during grain filling duration.

The correlations between SPAD senescence rate, SPAD at 650 °Cd and grain yield could indicate source limitation of grain growth in winter NILs. There is recent evidence for co-limitation of yield by source and sink in modern wheat cultivars under favourable conditions (Shearman *et al*., 2005, Acreche and Slafer, 2009, Aisawi *et al*., 2015). Possible mechanisms that could explain differences in stay green in this study might be deeper roots and higher N accumulation at anthesis and slower post-anthesis N remobilization (Foulkes *et al.*, 2009). Previous studies reported that delayed onset of senescence is associated with increased nitrogen uptake at anthesis and/or reduced post-anthesis N remobilization at post-anthesis period (Mi *et al.*, 2000, Borrell *et al.*, 2001, Gaju *et al.*, 2011). In the current study, SPAD was an indicator of leaf N content, but associations between SPAD at GS61+14d and senescence traits were inconsistent across years, e.g. there was a positive

association with SPAD at 650 °Cd in 2014 and negative association in 2013 in the winter NILs. Stay-green differences could also be due to differences in post-anthesis N remobilization efficiency. Borrell and Hammer (2000) proposed that stay-green genotypes uptake more N during grain filling, which may in turn be asociated with a more extensive root system, leading to extented root growth and hence more N assimilation by roots (O'Toole and Bland, 1987, Ta and Weiland, 1992)

Although bread wheat has been frequently characterised as sink-limited during grain filling, evidence for co-limitation of grain yield by source and sink in modern cultivars was reported in several studies, e.g. Acreche and Slafer (2009) reported that modern Spanish and Italian wheat cultivars have increased grains per unit land area but reduced the source capacity due to reduction in leaf net photosynthetic rate during grain filling.

In the present study in the winter wheat NILs, later anthesis date was correlated with both higher grain yield and stay-green traits (e.g. later end of FLS senescence and higher flag-leaf SPAD and NDVI at physiological maturity) in both years, so some caution is needed in interpeting the correlations between yield and senescence traits since they could be autocorrelations with anthesis date. Bogard *et al.* (2011) found genetic variation in leaf senescence was mostly explained by flowering time in a winter wheat population segregating for flowering time QTLs. Wingler *et al.* (2010) who studied the relationship between flowering time and leaf senescence during grain filling in *Arabidopsis* hypothesised that flowering could result in increased sugar content in the leaves activating senescence. Overall, in winter NILs, there was evidence for source limitation of grain yield or co-limitation of grain yield by source and sink in high yielding lines. Delayed leaf senescence might also increase maintenance of radiation-use efficiency and allow more carbon and nitrogen to be assimilated during grain filling.

6.4.2 Duration and rate of grain filling

A strong relationship between individual grain weight and rate of grain filling was consistent in BC_3 spring NILs. In winter wheat, individual grain weight was increased in BC_3 lines with increasing rate of grain filling, whereas duration of grain filling was decreased. These effects could partly be associated with anthesis date, as later flowering in BC_3 winter NILs was associated with a shorter grain filling period and plants might counteract this with a faster grain filling rate. However, duration of grain filling is not only determined by stay-green effects, but also by genetic factors influencing grain growth development (Gallagher *et al*., 1976, Metzger *et al*., 1984, Bruckner and Frohberg, 1987). Genetic differences for the rate and duration of grain filling have been reported in spring wheat (Przulj and Mladenov, 1999, Talbert *et al.*, 2001, Kamaluddin *et al*., 2007) and winter wheat (Mou and Kronstad, 1994).

In the current study, the results showed that greater grain weight was associated with higher rate of grain filling in both spring and winter wheat NILs. In spring wheat, grain weight was strongly associated with grain filling rate but no trade-off was observed with duration of grain filling. Associations between duration of grain filling and grain weight were inconsistent in BC_3 winter wheat lines in the two seasons. The duration of grain filling was negatively associated with rate of grain filling in 2013 while the duration of grain filling was positively related to grain weight in 2014 (Fig. 6.1). The correlation between genetic variation in grain filling duration and grain weight has been reported by many authors. The increase in final grain weight amongst five spring wheat genotypes (*Triticum aestivum* L.) differing in time to maturity was associated with higher grain filling rate and shorter grain filling period (Duguid and Brule-Babel, 1994). Although the rate of grain filling was important to determine grain yield, no association was found between the duration of grain filling and grain yield in ten spring wheat cultivars grown in Canada (Nass and Reiser, 1975). Due to a weak relationship between grain filling rate and duration of grain filling, it was possible to select for high grain weight through increasing the rate of

grain filling rate without shortening the duration of grain filling, or vice versa in North America (Gebeyehou *et al*., 1982, Bruckner and Frohberg, 1987).

Potential grain weight is determined by the carpel size and endosperm cell division and expansion at around anthesis (Fischer, 2011). Calderini *et al.* (1999a) found a negative effect of higher temperature on carpel size pre-anthesis resulting in reduced final grain weight. The carpel weight at anthesis potentially limited the grain growth after anthesis and may be important in determining grain size during grain filling (Calderini *et al*., 1999a, Calderini and Reynolds, 2000). However, the weight of carpels was not measured in the present study. The final grain weight can be determined by the number of cells in the endosperm at the end of the cellularization phase (Brocklehurst, 1977, Foulkes *et al.*, 2011). Moreover, the duration of grain filling could be increased in source-limited conditions by introduction of the stay-green genes in wheat (Reynolds *et al.*, 1999).

Accumulated starch in the endosperm composes 65% to 75% of the final grain dry weight (Housley *et al*., 1981, Dale and Housley, 1986, Hurkman *et al*., 2003) and grain filling is mainly associated with the processes of starch biosynthesis and accumulation (Yang *et al.*, 2004). It has been suggested that faster rate of grain filling is mainly associated with an increase in sink activity by regulating key enzymes involved in the sucrose-to-starch pathway e.g. sucrose synthase (SuSase), starch synthase (soluble StSase, SSS) and starch branching enzyme (SBE) in wheat grains under mild water deficit during grain filling (Yang *et al*., 2004). At high temperature, decrease in starch accumulation was correlated with reduced enzyme activities in the starch biosynthetic pathway (Hurkman *et al*., 2003). In addition, abscisic acid (ABA) plays an important role in the regulation of this process (Yang *et al.*, 2004). An increased ABA level was observed in faster grain filling in water-stressed grains (Yang *et al.*, 2001). Peng *et al.* (2003) reported that ABA can enhance the phloem cells in sink organs by inducing ATPase activation and controls the metabolism of assimilates in cells. The small vascular bundle can limit the transport pathway of photoassimilates into the endosperm during grain-filling progress (Hoshikawa, 1968);

therefore, the rate of grain growth could be increased by improving the vascular connections in the rachis and in the rachilla within the spikelets and increasing the rate of starch accumulation in the grain through an increase of enzyme activities involved in sucrose-to-starch pathway.

6.4.3 Summary

Five hypotheses were tested in this chapter:

Hypothesis 1*: There is a positive correlation between grain weight and grain yield amongst the BC_3 NILs.*

There was a positive correlation between grain weight and grain yield amongst the in BC_3 winter NILs in 2013 but no correlation in 2014, so this hypothesis was only confirmed in 2013 (Table 6.5). For spring NILs, there was no correlation between grain weight and grain yield in BC_3 spring NILs in 2013 but a negative correlation in 2014 (Table 6.4), so this hypothesis was not confirmed in BC_3 spring NILs.

Hypothesis 2*: There is a positive correlation between grain weight and duration and rate of grain filling amongst the BC_3 NILs.*

In the present study, grain weight was strongly associated with rate of grain filling in both spring and winter NILs. In spring wheat, no association was found between grain weight and duration of grain filling, whereas the association between grain weight and duration of grain filling was inconsistent in BC_3 winter wheat lines across years. So, the hypothesis that there is a positive correlation between grain weight and rate of grain filling amongst the BC_3 NILs was confirmed, but not between duration of grain filling and grain weight.

***Hypothesis 3**: Duration and rate of grain filling is associated with post-anthesis source-type traits including NDVI, flag-leaf senescence duration and rate and flag-leaf chlorophyll content (SPAD) amongst the BC_3 NILs.*

In this study, correlations between duration and rate of grain filling and the post-anthesis source traits were generally not signficant in BC_3 spring NILs, so this hypothesis was generally not confirmed. However, in winter NILs some associations between duration and rate of grain filling and source-type traits were observed, e.g. NDVI at GS61+15d, later FLS, SPAd at 650 °Cd and SPAD senescence rate , so this hypothesis was confirmed.

***Hypothesis 4**: Grain yield is source-limited during the latter stages of grain filling so that there is a correlation amongst BC_3 NILs between source-type traits under favourable post-anthesis conditions and grain weight and grain yield.*

The results in the spring wheat NILs suggesting that grain yield is sink limited rather than source limited under favourable post-anthesis conditions whereas in winter NILs, there was evidence for source limitation of grain yield or co-limitation of grain yield by source and sink i.e. positive correlations between grain yield and several source-type traits including NDVI at GS61+15d, later FLS, SPAd at 650 °Cd and SPAD senescence rate were consistent in winter NILs (Table 6.5).

***Hypothesis 5**: There is higher expression of source-type traits in BC_3 NILs than the recurrent parents Ashby and Humber.*

There was higher expression of source-type traits in BC_3 near-isogenic lines than the recurrent parents Ashby and Humber e.g. NDVI stay green, NDVI senescence rate, SPAD at 650 °Cd, SPAD senescence rate and FLS rate.

CHAPTER 7 MOLECULAR ANALYSIS FOR SSR MARKERS IN BC_3 AND BC_1 NILs

7.1 Introduction

According to the previous QTL mapping in the L14 x Rialto DH population (field tested at Nottingham and KWS Thriplow) carried out in BBSRC project BB/D008972/1 "Raising the ceiling on UK wheat yields/introgression of novel 'large-ear' CIMMYT germplasm into UK pre-breeding lines" in 2007/09 (Foulkes *et al.*, 2010), five major novel QTLs were identified for ear fertility traits and grain yield on chromosomes 1A, 1B, 2A, 3A and 7B (Table 7.1). The QTL on chromosome 1A coincided with the *Tin1A* locus on chromosome 1A (Spielmeyer and Richards, 2004). This QTL made a significant contribution to spikelets ear^{-1} and floret fertility leading to high grains ear^{-1} without an effect on either fruiting efficiency (FE) or grain weight. Furthermore, grain weight was significantly affected by a QTL on chromosome 1B while a QTL on chromosome 2A increased grains ear^{-1}, grains m^{-2}, FE and grain yield. A QTL on chromosome 3A increased spikelet number, grains ear^{-1}, grains m^{-2} and grain yield either an indicative or a significant effect across experiments in 2007/09 while spikelets ear^{-1} was modified by a QTL on chromosome 7B.

Table 7.1 Summary of QTLs identified in L14 x Rialto DH population in 2007/09.

Chr	Nearest marker	LOD	Add effect (%) of CIMMYT allele	Phenotype	Environment
1A	WMS136	6.5, 4.3, 10.3	+16, +15, +23	Spikelets/ear	K08; K09; N09
1A	WMS136	3.9, 7.0 , 3.4	+11, +22,+11	Grains/ear	N09; K08; K09
1B	CFD48	4.6, 8.5, 5.7, 3.6	-19, -21, -14, -10	1000 Grain weight	N07; N08; N09;K09
2A	WMS294	3.1, 6.1, 4.5	+6, +12, +11	Spikelets/ear, Grains/ear, Fruiting efficiency	K08*
2A	WMS294	2.9, 3.0	+6, +12	Grains m^{-2}, Grain yield	N08*
3A	WMS155	2.6, 2.9, (2.2)	+10, +10, +5	Grains m^{-2}	N08; K08; K09
3A	WMS155	4.0, 3.4, (2.3)	+14, +10, +6	Grain yield	N08; K08; K09
7B	wPt82117B	5.2, 3.6, 5.6	-13, -9, -18	Spikelets/ear	KM07; KM08; K09

MQM analysis using MapQTL5; significance threshold by permutation = 3.1; * in absence of *Tin1A* N07: full plot trial at Nottingham in 2006/07; K09: full plot trial KWS Thriplow in 2008/09; KM09: microplot (1.5 x 1 m) trial KWS Thriplow in 2008/09

The Backcross (BC) method (repeated crossing to a single genotype) is an important breeding strategy in order to transfer alleles from a donor parent to an elite cultivar (Reyes-Valdes, 2000, Hospital, 2005). This method is useful to investigate the genetic architecture of quantitative traits because it introgresses one or more genes of interest on a chromosomal segment carrying the trait of interest into a different genetic background (Hospital, 2005). This method can be used to validate the additive effect of a quantitative trait locus (QTL) or a target trait, but can also be used to fine map a previous QTL detection and to enhance the accuracy of the QTL mapping (Hospital, 2005). The backcross populations are generated by crossing the donor parent which contains the trait of interest with the recurrent parent (another genetic background general from an elite variety) to produce the F_1 hybrid. The F_1 hybrid is then backcrossed with the recurrent parent to produce the first backcross generation (BC_1) followed by repeated crossing of selected BC plants with the recurrent parent to produce BC_2, BC_3, BC_4, respectively. After the first backcross to the recurrent parent, in each generation the proportion of donor genome in the progeny is expected to be reduced by 50% and that in the recurrent parent increased

by 50% (Hospital, 2005). Hence, the proportion of recurrent genome in the BC_1, BC_2 and BC_3 progeny is expected to be 75%, 87.5% and 93.75%, respectively.

Molecular markers play a vital role in plant breeding. Molecular or DNA markers are DNA fragments revealing mutations or variants, which can be used to detect polymorphism between different genotypes (or alleles) of a specific DNA sequence in a population (Guo-Liang, 2013). Moreover, they can be used to scan the genome of individual lines to determine which carries the favourable alleles from the non-recurrent parent genome and the greatest proportion of the recurrent genome (Moose and Mumm, 2008). Microsatellite markers are PCR-based markers. They are tandem array repeats of short nucleotide motifs (2-6 base pairs). Microsatellite loci are individually amplified by PCR using pairs of oligonucleotide primers specific to the unique DNA sequences flanking the SSR sequence. In addition, these markers are characterized by their hyper-variability, reproducibility, co-dominant nature, locus-specificity, and random genome-wide distribution, in most cases (Guo-Liang, 2013). The advantages of SSR markers include that they can be readily analyzed by PCR and are easily detected by PAGE or AGE. SSR markers can be multiplexed, leading to high throughput genotyping and can be automated.

The development of near-isogenic lines (NILs) is a very important tool for genetic and physiological dissection of grain yield in wheat. In this study, the BC_3 NILs in two different genetic backgrounds, Ashby spring wheat and Humber winter wheat, were phenotyped and genotyped. Eight simple sequence repeat (SSR) markers close to the previously reported QTL locations (Table 7.1) were used to genotype the BC_3S_5 lines in both the Ashby and Humber backgrounds in experiments at University of Nottingham and two markers (WMS136 and WMC428) were used to screen the BC_1 and BC_3 lines in an experiment at KWS, Thriplow.

The specific hypotheses tested in this chapter are:

1. The +*Tin1A* BC_3 NILs have reduced ears m^{-2} at harvest but increased GN ear^{-1}, spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length leading to higher grains m^{-2} compared to the –*Tin1A* BC_3 NILs and the recurrent parent(s) in the UK rainfed environment.
2. The +*Tin1A* BC_1S_7 NILs in the Ashby and Humber backgrounds produce fewer ears m^{-2}, but higher grains ear^{-1}, grains m^{-2} and grain yield than contrasting –*Tin1A* BC_1S_7 lines.
3. The BC_3 NILs with the allele from the CIMMYT L14 for a QTL on chromosome 3A have increased spikelets ear^{-1}, grains m^{-2} and grain yield compared to BC_3 NILs with the allele from Humber and the Humber recurrent parent in the UK rainfed environment.
4. The presence of an allele from Rialto for a QTL on chromosome 7B (WMC696) in the BC_3 NILs increases grain number per unit area compared to BC_3 NILs with the Humber and also the combined Humber recurrent parents.

7.2 Materials and Methods

7.2.1 Plant materials

The plant materials for the experiment at Sutton Bonington were the NILs (BC_3) in the spring and winter wheat backgrounds as described in Chapter 3. At KWS Thriplow, 17 selected BC_3S_6 wheat NILs, nine pairs of NILs (BC_1S_7) in an Ashby background and eight pairs of NILs in a Humber background, selected to contrast for tiller number and *Tin1A* characteristics, were grown and assessed for yield and yield components. The BC_1S_7 lines were developed by Dr. Peter Werner of KWS UK Ltd as part of BBSRC project BB/D008972/1 prior to this PhD program. Each pair of BC_1 NILs was developed by phenotypic selection of the ears from contrasting plants in a single ear row derived from a common BC_1S_4 ear according to *Tin1A* characteristics including tiller number per plant and spikelets per ear. The pairs of NILs were

selected to show different degrees of contrast in tiller number per plant. The degree of contrast was divided into three categories: i) high tillers per plant in the *–Tin1A* line versus low tillers per plant in the *+Tin1A* line; ii) moderate tillers per plant in the *–Tin1A* line versus low tillers per plant in the *+Tin1A* line; and iii) low – moderate tillers per plant in the *–Tin1A* line versus low tillers per plant in the *+Tin1A* line. The parental cultivars Humber and Ashby were also included in the experiments, and the modern check winter wheat cultivars Santiago, Belugo, Rialto and Ashby.

7.2.2 Field experiments

Two field experiments were carried out at Sutton Bonington Campus, The University of Nottingham, Leicestershire in 2012/13 and 2013/14 and a third experiment at KWS-UK Ltd in Thriplow, Hertfordshire in 2013/14. The experimental design and management were described in Chapter 3. The yield and trait measurements were described in Chapter 3.

7.2.3 Molecular analysis

Young leaves from five tagged individual plants per plot in the experiments at Nottingham were sampled at GS31 (onset of stem extension). Bulks of DNA samples were extracted from 7-10 individual plants using the DNeasy Plant Mini Kit (QIAGEN) and kept at -80°C in a freezer. For 17 pairs of BC_1S_7 lines and the check cultivars in the experiment at KWS, Thriplow, leaf samples were taken from three plants per plot for DNA extraction. Bulks of DNA samples from BC_3S_5 spring and winter backgrounds were genotyped using 8 SSR primer pairs, closely linked to the *Tin1A* gene (W49 and WMS136) and the QTL on 1A, 2A, 3A, 7B and 7D. With regard to the *Tin1A* locus, one marker linked to the *Tin1A* locus (WMS136) was screened on the pairs of NILs BC_1S_7 spring and winter backgrounds and the other marker WMC428 was genotyped

in the BC_1S_7 winter wheat lines at KWS. The full details of the molecular marker methodology were given in Chapter 3.

7.2.4 Statistical analysis

ANOVA and correlation analysis were performed on the phenotypic data collected in the field experiments using Genstat 16th edition (Payne *et al.*, 2009). Treatment means were calculated and compared using the least significant difference (5%) of the means of Fisher when the ANOVA indicated significant differences. Unbalanced design ANOVA was used to compare between the means of parents and groups of lines according to the presence and absence of the *Tin1A* allele from L14 and to the presence and absence of the L14 allele for the QTL on 3A in the spring and winter wheat lines, respectively.

Regression analysis and Pearson correlations were performed on the mean values across two replicates to estimate the associations between physiological traits using Genstat 16th edition (Payne *et al.*, 2009) and GraphPad Prism version 6.05 (Motulsky, 2003).

7.3 Results

7.3.1 Validation of possession of the tiller inhibition gene (*Tin1A*) marker and of the chromosome 3A QTL (*QTL3A*) in BC_3 NILs

Bulks of DNA samples extracted from the BC_3S_5 NILs (7-10 individual plants) and their recurrent parents (Ashby, Humber, L14 and Rialto) were screened by the SSR marker (WMS136) which is tightly linked to the tiller inhibition (*Tin1A*) gene in wheat (Spielmeyer and Richards, 2004) and the presence of the L14 or non-L14 allele used as an indicator of the presence/absence of the tiller inhibition *Tin1A* locus. The size of

the generated PCR products was compared with the expected allele sizes from the four parents.

The results showed that lines homozygous for the *Tin1A* allele (corresponding to W49 and WMS136 alleles) from the CIMMYT L14 advanced line were identified only in the Ashby background lines (no homozygous lines for the *Tin1A* allele from L14 in the Humber background) (Table 7.2). Five of the BC_3S_5 spring NILs carried heterozygous alleles from the L14 and Ashby for *Tin1A,* and two were homozygous for +*Tin1A* from the L14. The *Tin1A* scores were clear and consistent in the BC_1S_7 and BC_3S_5 populations. Most of the BC_3S_5 spring NILs carried heterozygous alleles from the L14 and Ashby lines for *Tin1A* (W49 and WMS136) (lines 5, 19, 20, 21 and 25) and homozygous for the allele from Rialto line on chromosome 7B (WMC696) (lines 19, 20, 21 and 25). No allelic variation was found on chromosome 2A (WMS294), 3A (WMC428), 7B (WMC606) and 7D (WMC702) in the Ashby background for the alleles with all four markers being homozygous for the Ashby allele, implying that the QTLs for these regions were fixed and were not the L14 derived alleles.

In the winter wheat background, BC_3S_5 lines in the Humber background carried the L14 allele for the QTL on chromosome 3A as indicated by the SSR markers (WMC169 and WMC428). WMC169 (3A) was polymorphic between L14, Rialto and the Humber recurrent parent and WMC696 (7B) was also polymorphic between the L14, Rialto and the Humber recurrent parent. WMC428 (3A) was polymorphic between L14 and Humber. The alleles from Rialto were present on chromosome 3A (WMC169), 7B (WMC696) and 7D (WMC702) in BC_3S_5 winter population. No allelic variation was found on chromosome 2A (WMS294) and 7B (WMC606) with the alleles carried being derived from the L14 line.

Table 7.2 Molecular results at Nottingham after screening 44 BC_3S_5 NILs (7 spring and 37 winter wheat) with 8 SSR marker on 5 chromosome and Rialto, L14 and the recurrent parents (Ashby or Humber).

Line	1A		2A	3A		7B		7D
	W49	WMS136	WMS294	WMC428	WMC169	WMC606	WMC696	WMC702
BC_3 spring wheat NILs								
5	Ash/L14	Ash/L14	Ash	Ash	Ash/Rial	Ash	Ash	Ash
19	Ash/L14	Ash/L14	Ash	Ash	Ash	Ash	Rial	Ash
20	Ash/L14	Ash/L14	Ash	Ash	Ash	Ash	Rial	Ash
21	Ash/L14	Ash/L14	Ash	Ash	Ash	Ash	Rial	Ash
25	Ash/L14	Ash/L14	Ash	Ash	Ash	Ash	Rial	Ash
33	L14	L14	Ash	Ash	Ash	Ash	Ash	Ash
36	L14	L14	Ash	Ash	Ash/L14	Ash	Ash	Ash
BC_3 winter wheat NILs								
67	Hum	Hum	Hum	Hum/L14	Hum	Hum	Hum	Hum
68	Hum	Hum	Hum	Hum/L14	Hum	Hum	Hum	Hum
72	Hum	Hum	Hum	L14	Hum	Hum	Hum/Rial	Hum
73	Hum	Hum	Hum	L14	Hum	Hum	Hum	Hum
74	Hum	Hum	Hum	L14	Hum	Hum	Hum	Hum
75	Hum	Hum	Hum	L14	Hum	Hum	Hum/Rial	Hum
76	Hum	Hum	Hum	L14	Hum	Hum	Hum	Hum
77	Hum	Hum	Hum	L14	Hum	Hum	Hum	Hum
78	Hum	Hum	Hum	L14	Hum	Hum	Hum	Hum
79	Hum	Hum	Hum	L14	Hum	Hum	Hum	Hum
80	Hum	Hum	Hum	L14	Hum	Hum	Hum	Hum
81	Hum	Hum	Hum	Hum/L14	Hum/L14	Hum	Hum	Rial
82	Hum	Hum	Hum	L14	Hum	Hum	Hum	Hum
150	Hum	Hum	Hum	L14	Rial	Hum	Hum	Hum
151	Hum	Hum	Hum	L14	Rial	Hum	Hum	Hum
152	Hum	Hum	Hum	Hum/L14	Rial	Hum	Hum	Hum
167	Hum/L14	Hum	Hum	L14	Hum	Hum	Hum/Rial	Hum
170	Hum/L14	Hum	Hum	L14	Hum/Rial	Hum	Rial	Hum
171	Hum	Hum	Hum	L14	Hum/Rial	Hum	Rial	Hum
172	Hum	Hum	Hum	L14	Hum	Hum	Rial	Hum
173	Hum	Hum	Hum	L14	Rial	Hum	Rial	Hum
174	Hum	Hum	Hum	L14	Hum/Rial	Hum	Rial	Hum
175	Hum	Hum	Hum	L14	Hum	Hum	Rial	Hum
176	Hum	Hum	Hum	L14	Hum/Rial	Hum	Rial	Hum
177	Hum	Hum	Hum	L14	Hum	Hum	Rial	Hum
178	Hum	Hum	Hum	Hum/L14	Hum/Rial	Hum	Hum/Rial	Hum
179	Hum	Hum	Hum	L14	Hum	Hum	Rial	Hum
180	Hum	Hum	Hum	Hum/L14	Hum/Rial	Hum	Rial	Hum
181	Hum	Hum	Hum	Hum/L14	Hum/Rial	Hum	Hum	Hum
184	Hum	Hum	Hum	Hum/L14	Hum	Hum	Hum	Hum
185	Hum	Hum	Hum	Hum/L14	Hum	Hum	Hum/Rial	Hum
191	Hum	Hum	Hum	Hum/L14	Hum	Hum	Rial	Hum
194	Hum	Hum	Hum	Hum/L14	Hum	Hum	Rial	Hum
195	Hum	Hum	Hum	Hum/L14	Hum	Hum	Rial	Hum
196	Hum	Hum	Hum	Hum/L14	Hum	Hum	Rial	Hum
223	Hum	Hum	Hum	Hum/L14	Hum/L14	Hum	L14	Hum
229	Hum	Hum	Hum	L14	Hum	Hum	Hum/Rial	Hum

The molecular results confirmed that there were two groups of BC_3S_5 spring wheat lines carrying homozygous (+*Tin1A*) and heterozygous (±*Tin1A*) alleles from the L14 advanced lines. Similar to the spring wheat, the 37 DNA pools of the winter wheat BC_3S_5 NILs were screened with the SSR marker WMC428 for the 3A QTL. Then, the PCR products were compared with the allele size of parents. BC_3 winter NILs were divided into two groups ($\pm 3A_{WMC428}$ and $+3A_{WMC428}$). The presence of the heterozygous alleles could be ascribed to their segregation at earlier generations. The BC_3S_5 seed were selected from six ears per genotype in the BC_3S_4 population

lines and seed was multiplied to determine grain yield in 2013 and 2014, respectively.

7.3.2 Effects of the *Tin1A* locus on chromosome 1A in BC_3 spring wheat NILs on grain yield and yield components

7.3.2.1 Nottingham 2012/13 and 2013/14

With regard to the *Tin1A* locus, the seven BC_3 spring wheat lines were categorized into two groups; heterozygous *±Tin1A* lines (BC_3 lines 5, 19, 20, 21 and 25) and homozygous *+Tin1A* (BC_3 lines 33 and 36) based on the molecular analysis. These groups were compared with the Ashby parent.

In 2013, grain yield and grains m^{-2} were lower in *+Tin1A* lines (3.12 t ha^{-1} and 7,360 grains m^{-2}, respectively) compared to *±Tin1A* BC_3 lines (8.09 t ha^{-1} and 21,785 grains m^{-2}, respectively) and Ashby (9.09 t ha^{-1} and 26,294 grains m^{-2}, respectively) (*P<0.01*) (Fig. 7.2, Table 7.3). There was a trend for lower fertile shoots $plant^{-1}$ in *+Tin1A* lines (1.96) compared to Ashby (3.62) (*P<0.10*). In addition, ears m^{-2} and AGDM m^{-2} were decreased in *+Tin1A* lines compared to *±Tin1A* BC_3 lines and Ashby. $Ears_{COUNT}$ m^{-2} in *+Tin1A* lines (135.9) was also lower than *±Tin1A* BC_3 lines (405.7) and the Ashby recurrent parent (571.2) (*P<0.001*). There were no differences in HI (*P>0.10*), AGDM $shoot^{-1}$ (*P>0.10*) and TGW (*P>0.10*) amongst the groups. Grain number ear^{-1} and GN $spikelet^{-1}$ were greater in *+Tin1A* lines (73.0 and 3.01, respectively) compared to *±Tin1A* BC_3 lines (49.5 and 2.17, respectively) and the Ashby recurrent parent (40.7; *P<0.01* and 1.93; *P<0.05*, respectively).

In 2014, two of the heterozygote *±Tin1A* BC_3 lines (line 5 and 25) were omitted from the ANOVA analysis as these lines showed similar phenotypes to *+Tin1A* lines with low ears m^{-2} and large ears in the experiment in 2014. Fertile shoots $plant^{-1}$ at harvest was reduced to 2.62 in *+Tin1A* lines compared to 5.12 in Ashby (*P<0.10*).

Grain yield and grains m^{-2} were lower in +*Tin1A* lines (2.22 t ha^{-1} and 4,811 grains m^{-2}, respectively) compared to ±*Tin1A* BC_3 lines (4.88 t ha^{-1} and 10,951 grains m^{-2}, respectively) and Ashby (9.02 t ha^{-1} and 22,938 grains m^{-2}, respectively; *P<0.001*). TGW was increased to 47.4 g in +*Tin1A* lines compared to 39.3 g in Ashby (*P<0.01*). There were no differences in spikelets ear^{-1} (*P>0.10*) but fertile spikelets ear^{-1} and grain number ear^{-1} were higher in +*Tin1A* lines (23.4 and 89.7, respectively) and ±*Tin1A* BC_3 lines (22.2 and 60.2, respectively) compared to Ashby (20.2; *P<0.05* and 44.9; *P<0.001*, respectively). $Ears_{COUNT}$ m^{-2} in +*Tin1A* lines (195.2) was lower than ±*Tin1A* BC_3 lines (289.8) and the Ashby recurrent parent (551.4) (*P<0.001*). The +*Tin1A* lines produced a normal number of tillers at GS31 but few tillers survived as fertile tillers at later stages (Fig. 7.1)

Fig. 7.1 Tiller inhibition lines which produced a normal number of tillers at seedling stage and GS31 but fewer fertile tillers at later stages

Averaging across years, grain yield and grains m^{-2} were reduced in +*Tin1A* lines (2.73 t ha^{-1} and 6,228 grains m^{-2}, respectively) compared to ±*Tin1A* BC_3 lines (6.67 t ha^{-1} and 17,054 grains m^{-2}, respectively) and Ashby (9.30 t ha^{-1} and 25,098 grains m^{-2}, respectively) (*P<0.001*). Fertile shoots $plant^{-1}$ was lower in +*Tin1A* lines (2.06) than ±*Tin1A* BC_3 lines (3.30) and Ashby (4.37) (*P<0.01*) and ears m^{-2} was lower for +*Tin1A* lines compared to ±*Tin1A* BC_3 lines and Ashby (*P<0.001*). There was no differences in AGDM $plant^{-1}$, grain dry weight $plant^{-1}$ and grain number $plant^{-1}$ (*P>0.10*). However, GN ear^{-1} and fertile spikelets ear^{-1} were increased in +*Tin1A* lines (80.2 and 21.9, respectively) and ±*Tin1A* BC_3 lines (54.1 and 19.1, respectively) compared to the Ashby recurrent parent (42.3; *P<0.001* and 18.4; *P<0.05*, respectively). There was a trend for higher TGW and spikelets ear^{-1} in +*Tin1A* lines compared to Ashby (*P<0.10*). There was a year effect on grain yield (*P<0.001*), ears m^{-2} (*P<0.001*), grains m^{-2} (*P<0.001*), AGDM $shoot^{-1}$ (*P<0.001*), AGDM $plant^{-1}$ (*P<0.01*), GDW $plant^{-1}$ (*P<0.01*), GN $plant^{-1}$ (*P<0.05*), TGW (*P<0.001*), GN ear^{-1} (*P<0.01*) and spikelets ear^{-1} (*P<0.001*) and GN $spikelet^{-1}$ (*P<0.01*). Year x genotype interactions were observed for grain yield, grains m^{-2} and ears m^{-2} (*P<0.10*). However, year x genotype interactions were few and weak.

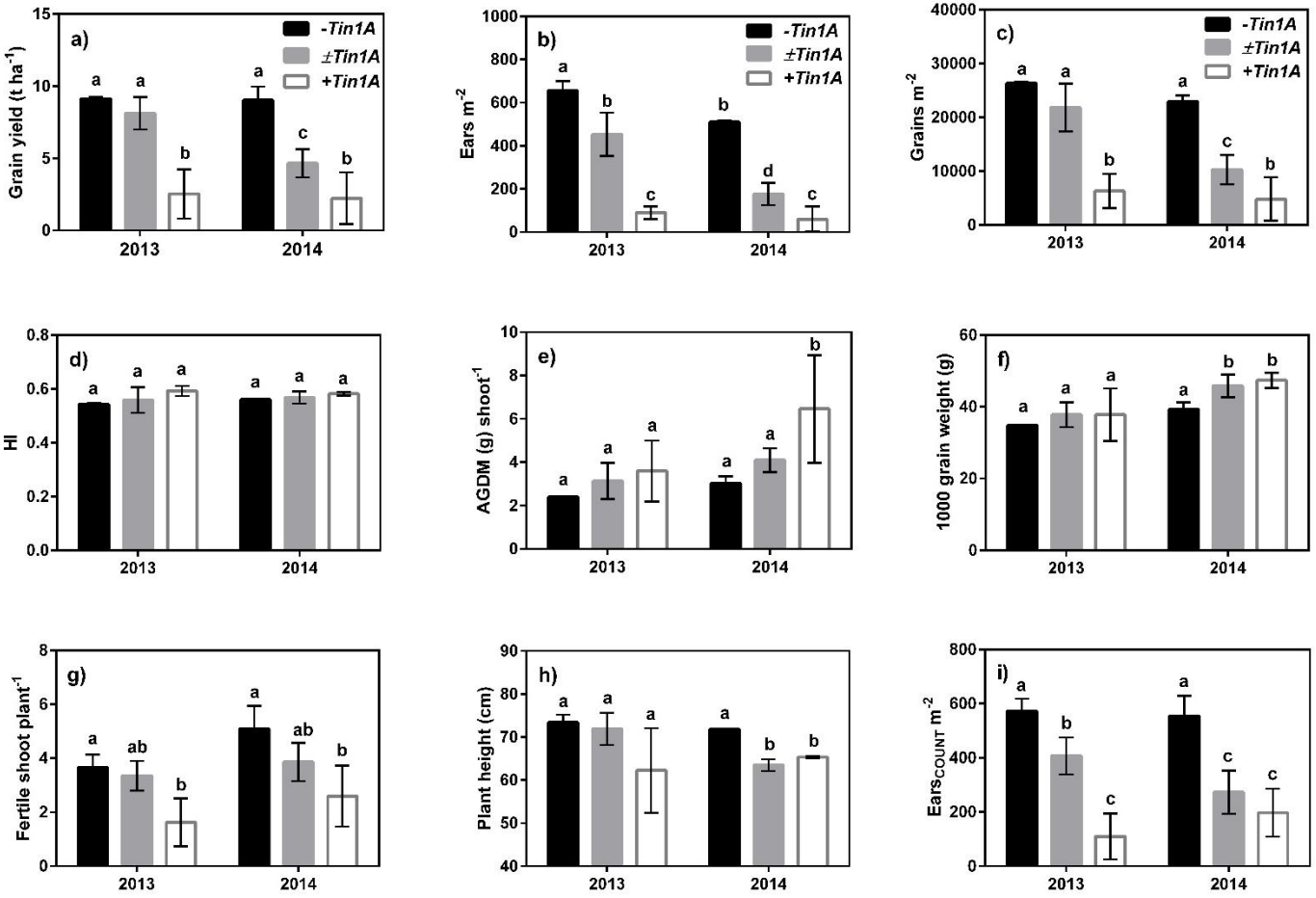

Fig. 7.2 Grain yield and yield components; a) grain yield (100% DM), b) ears m^{-2}, c) grains m^{-2}, d) HI, e) AGDM $shoot^{-1}$, f) 1000 grain weight, g) fertile shoots $plant^{-1}$, h) plant height and i) $ears_{COUNT}$ m^{-2} in spring NILs (BC_3) in 2013 and 2014. Black, grey and white bars (mean±SEM) indicate the Ashby parent (*-Tin1A*) and the BC_3 NIL groups (±*Tin1A* and *+Tin1A*), respectively. Different letters above the bars indicate significant differences between genotypes ($P<0.05$).

Table 7.3 Mean values in 2013 and 2014 and cross-year means for grain yield (100% DM), ears m^{-2}, grains m^{-2}, HI, AGDM shoot^{-1}, FS plant^{-1}, AGDM plant^{-1}, GDW plant^{-1}, GN plant^{-1}, TGW, spikelets ear^{-1}, fertile spikelets ear^{-1}, GN ear^{-1}, GN spikelet^{-1} and $ears_{COUNT}$ m^{-2} (average for all fertile shoots per plant) for the Ashby recurrent parent (-*Tin1A*) and the BC_3 NIL groups (±*Tin1A* and +*Tin1A*) and SEDs for genotype groups.

	Grain yield (t ha^{-1})	**Ears m^{-2}**	**Grains m^{-2}**	**HI**	**AGDM shoot^{-1}**	**FS plant^{-1}**	**AGDM (g) plant^{-1}**	**GDW (g) plant^{-1}**	**GN plant^{-1}**	**TGW (g)**	**Spikelets ear^{-1}**	**Fertile spikelets ear^{-1}**	**GN ear^{-1}**	**GN spikelet^{-1}**	**$ears_{COU.}$ m^{-2}**
2013															
Ashby parent (-*Tin1A*)	9.09	655.6	26,294	0.54	2.37	3.62	9.60	5.22	149.0	34.8	18.8	16.2	40.7	1.93	571.2
BC_3 NIL group (±*Tin1A*)	8.09	453.5	21,785	0.56	3.11	3.32	11.1	6.27	164.3	37.7	19.0	16.8	49.5	2.17	405.7
BC_3 NIL group (+*Tin1A*)	3.12	93.3	7,360	0.60	3.39	1.96	11.0	6.54	155.5	39.9	20.7	20.7	73.0	3.01	135.9
SED between NIL group and Ashby parent (DF=13)	1.08**	82.70***	3,473**	0.028	0.77	0.55†	2.60	1.54	34.82	2.48	0.78	1.41	6.58**	0.26*	45.03***
SED within NIL group (DF=13)	1.40**	107.37***	4,510**	0.036	1.00	0.72†	3.37	2.00	45.20	3.23	1.14	2.06	8.54**	0.33*	58.46***
2014															
Ashby parent (-*Tin1A*)	9.02	510.3	22,938	0.56	3.03	5.12	16.3	9.15	231.9	39.3	22.1	20.2	44.9	1.88	551.4
BC_3 NIL group (±*Tin1A*)	4.88	187.1	10,951	0.56	4.15	4.04	18.5	10.5	231.2	45.2	22.2	22.0	60.2	2.42	289.8
BC_3 NIL group (+*Tin1A*)	2.22	60.7	4,811	0.58	6.46	2.62	18.5	10.8	227.8	47.4	23.4	23.4	89.7	2.69	195.2
SED between NIL group and Ashby parent (DF=9)	0.90***	40.06***	2,199***	0.012	0.97*	0.83†	4.78	2.80	59.46	1.94**	0.76	0.88*	7.07***	0.50	60.15***
SED within NIL group (DF=9)	0.95***	42.08***	2,310***	0.013	1.02*	0.94†	5.02	2.95	62.46	2.04**	0.80	0.92*	7.43***	0.53	63.19***
Cross-year ANOVA															
Ashby parent (-*Tin1A*)	9.30	605.6	25,098	0.55	2.76	4.37	13.3	7.35	191.8	37.2	20.5	18.4	42.3	2.01	572.2
BC_3 NIL group (±*Tin1A*)	6.67	338.5	17,054	0.56	3.54	3.30	14.2	8.02	192.0	40.9	20.4	19.1	54.1	2.44	354.5
BC_3 NIL group (+*Tin1A*)	2.73	79.3	6,228	0.59	4.72	2.06	14.2	8.36	186.3	43.2	21.9	21.9	80.2	3.74	159.5
SED between NIL group and Ashby parent (DF=2)	0.73***	48.49***	2,153***	0.018	0.66*	0.49**	2.81	1.64	36.02	1.77†	0.61†	1.03*	5.34***	0.19***	41.03***
SED within NIL groups (DF=2)	0.91***	60.25***	2,675***	0.023	0.82*	0.61**	3.49	2.04	44.75	2.20†	0.74†	1.26*	6.63***	0.24***	50.97***
SED Year (DF=1)	0.58***	38.56***	1,712***	0.015	0.53**	0.39	2.23**	1.31**	28.64*	1.41***	0.46***	0.78***	4.24**	0.15**	32.63†
SED Year*NIL groups and Ashby parent (DF=21)	0.84†	55.94†	2,484†	0.021	0.76	0.57	3.24	1.90	41.55	2.04	0.65	1.10	6.16	0.22	47.33†
SED Year*NIL group (DF=21)	1.24†	82.43†	3,660†	0.031	1.12	0.84	4.77	2.79	61.22	3.01	1.07	1.81	9.07	0.33	69.74†

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

The effect of the +*Tin1A* locus on main-shoot traits at harvest is shown in Fig. 7.3 and Table 7.4. In 2013, AGDM per main shoot (MS), and GDW per MS were higher in +*Tin1A* lines compared to ±*Tin1A* lines and Ashby (*P<0.10* and *P<0.05,* respectively). For main shoots, spikelets ear^{-1} and GN ear^{-1} were higher in +*Tin1A* lines (20.6 and 73.9, respectively) and ±*Tin1A* lines (19.8 and 58.9, respectively) compared to Ashby (19.6; *P>0.10* and 52.3; *P<0.10,* respectively). Rachis length was also greater in +*Tin1A* and ±*Tin1A* lines compared to Ashby (*P<0.01*). There were no differences in plant height or GN spikelet^{-1} amongst the groups (*P>0.10*).

In 2014, AGDM MS^{-1} and GN MS^{-1} were higher in +*Tin1A* lines and ±*Tin1A* lines compared to Ashby (*P<0.001* and *P<0.001,* respectively). Spikelet number per ear was higher at 24.7 in +*Tin1A* lines compared to 22.6 in ±*Tin1A* lines and 21.2 in Ashby (*P<0.01*). GN spikelet^{-1} and rachis length were also higher in +*Tin1A* and ±*Tin1A* lines compared to Ashby (*P<0.001* and *P<0.01*, respectively).

Averaging across years, AGDM MS^{-1} was higher in +*Tin1A* lines compared to Ashby (*P<0.001*) and GDW MS^{-1} was higher in +*Tin1A* lines (4.14 g) compared to Ashby (2.07 g) (*P<0.001*). GN MS^{-1} also was higher in +*Tin1A* lines (85.8) compared to Ashby (51.0) (*P<0.001*) together with greater spikelets ear^{-1} in +*Tin1A* lines (22.4) than Ashby (20.2) (*P<0.001*). GN spikelet^{-1} and rachis length were higher/longer in +*Tin1A* lines (3.80 and 10.98 cm, respectively) than the recurrent parent (2.81; *P<0.01* and 8.13 cm; *P<0.001,* respectively). There was a year effect on most traits: AGDM MS^{-1} (*P<0.001*), plant height (*P<0.01*), GDW MS^{-1} (*P<0.001*), GN MS^{-1} (*P<0.01*), MS spikelets ear^{-1} (*P<0.001*), MS fertile spikelets ear^{-1} (*P<0.001*), and rachis length (*P<0.001*). Year x genotype interactions were observed for AGDM MS^{-1} (*P<0.01*), plant height (*P<0.10*), GDW MS^{-1} (*P<0.10*), GN MS^{-1} (*P<0.10*) and spikelets ear^{-1} (*P<0.05*).

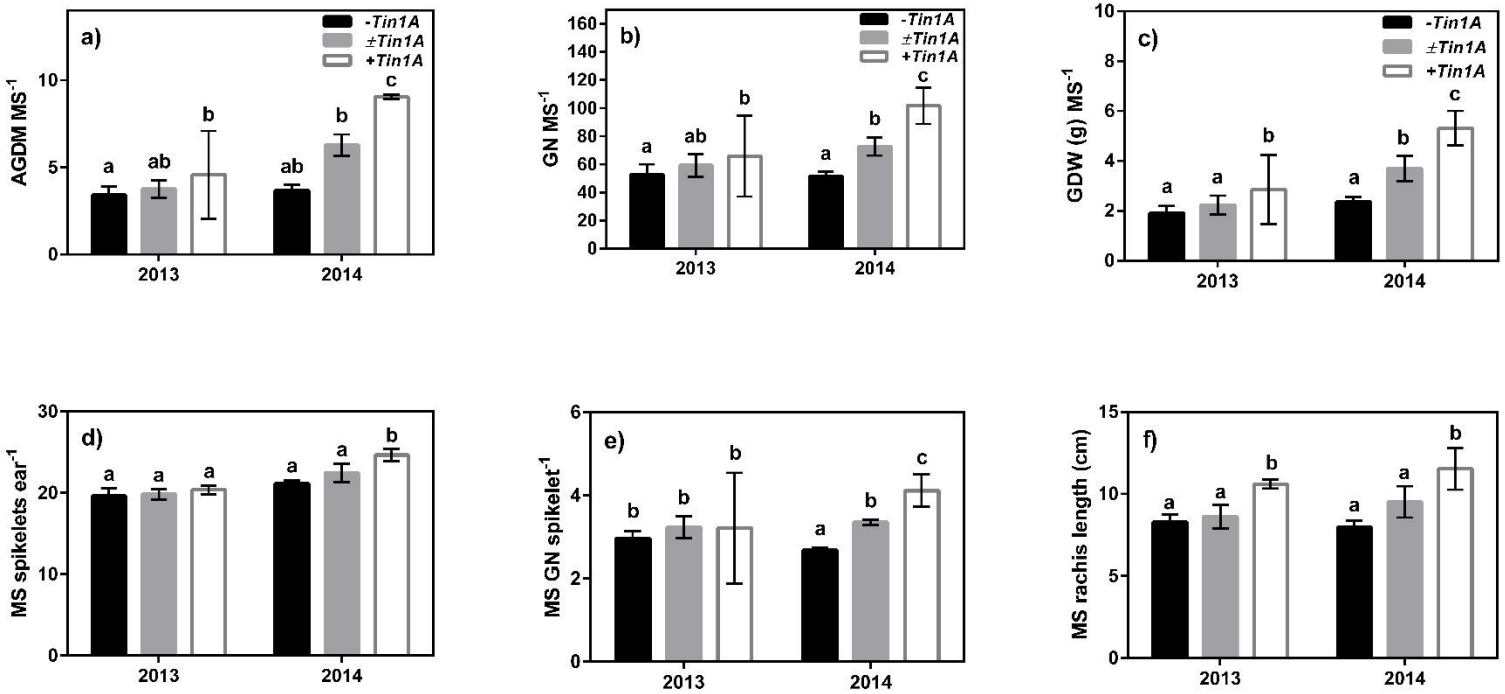

Fig. 7.3 Grain dry matter and yield components for main-shoot (MS); a) above-ground DM MS^{-1}, b) grain number MS^{-1}, c) grain DM MS^{-1}, d) MS spikelets ear^{-1}, e) MS grain number $spikelet^{-1}$ and f) MS rachis length in spring NILs (BC_3) in 2013 and 2014. Black, grey and white bars (mean±SEM) indicate the Ashby parent (*-Tin1A*) and the BC_3 NIL groups *(±Tin1A* and *+Tin1A*), respectively. Different letters above the bars indicate significant differences between genotypes (*P<0.05*).

Table 7.4 Mean values in 2013 and 2014 and cross-year means for above-ground DM per main shoot (MS)$^{-1}$, plant height, GDW MS^{-1}, GN MS^{-1}, MS spikelets ear^{-1}, MS fertile spikelets ear^{-1}, MS GN spikelet^{-1} and MS rachis length for the Ashby recurrent parent (-*Tin1A*) and the BC_3 NIL groups (±*Tin1A* and +*Tin1A*) and SEDs for genotype groups.

	AGDM (g) MS^{-1}	**PH (cm)**	**GDW (g) MS^{-1}**	**GN MS^{-1}**	**MS Spikelets ear^{-1}**	**MS Fertile spikelets ear^{-1}**	**MS GN spikelet^{-1}**	**MS RL (cm)**
2013								
Ashby parent (-*Tin1A*)	3.38	73.3	1.88	52.3	19.6	17.6	2.96	8.27
BC_3 NIL group (±*Tin1A*)	3.72	71.8	2.22	58.9	19.8	18.2	3.22	8.62
BC_3 NIL group (+*Tin1A*)	5.26	65.2	3.24	73.9	20.6	20.6	3.57	10.60
SED between NIL group and Ashby parent (DF=13)	0.59†	2.81	0.36*	6.99†	0.41	0.73*	0.30	0.43**
SED within NIL group (DF=13)	0.76†	3.65	0.47*	9.07†	0.53	0.94*	0.39	0.56**
2014								
Ashby parent (-*Tin1A*)	3.66	71.9	2.35	51.4	21.2	19.2	2.68	7.95
BC_3 NIL group (±*Tin1A*)	6.23	63.7	3.61	72.4	22.6	21.7	3.34	9.47
BC_3 NIL group (+*Tin1A*)	9.03	65.4	5.31	101.7	24.7	24.7	4.12	11.52
SED between NIL group and Ashby parent (DF=9)	0.45***	0.81***	0.40***	6.21***	0.65**	0.96**	0.18***	0.64**
SED within NIL group (DF=9)	0.48***	0.85***	0.42***	6.53***	0.68**	1.01**	0.19***	0.67**
Cross-year ANOVA								
Ashby parent (-*Tin1A*)	3.47	72.2	2.07	51.0	20.2	18.1	2.81	8.13
BC_3 NIL group (±*Tin1A*)	4.80	68.2	2.81	64.6	21.0	19.7	3.27	8.98
BC_3 NIL group (+*Tin1A*)	6.87	65.2	4.14	85.8	22.4	22.4	3.80	10.98
SED between NIL groups and Ashby parent (DF=2)	0.45***	1.89†	0.29***	5.32***	0.40***	0.65***	0.21**	0.41***
SED within NIL group (DF=2)	0.56***	2.35†	0.36***	6.61***	0.50***	0.80***	0.26**	0.51***
SED Year (DF=1)	0.36***	1.50**	0.23***	4.23**	0.32***	0.51***	0.17	0.33*
SED Year*NIL group and Ashby parent (DF=21)	0.52**	2.18†	0.33†	6.13†	0.47*	0.75	0.24	0.47
SED Year*NIL group (DF=21)	0.77**	3.22†	0.49†	9.04†	0.69*	1.10	0.36	0.70

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Averaging across years for anthesis traits, AGDM and ear DM shoot^{-1} were higher in +*Tin1A* lines and ±*Tin1A* lines compared to Ashby (*P<0.001*). EPI was increased in +*Tin1A* lines (0.24) and ±*Tin1A* lines (0.23) compared to Ashby (0.20) (*P<0.05*) but there were no differences in FE between +*Tin1A* lines, ±*Tin1A* lines and Ashby (*P>0.10*). NILs carrying the *Tin1A* locus produced more fertile florets ear^{-1} (100.5-115.9) and higher fertile florets spikelet^{-1} (4.45-4.88) than the recurrent parent (61.8 and 3.25, respectively) (*P<0.001*). Spikelets ear^{-1} was substantially higher in +*Tin1A* lines (23.8) and ±*Tin1A* lines (22.6) compared to Ashby (20.7) (*P<0.001*). All spikelets in +*Tin1A* lines were fertile. Year effects were observed for AGDM shoot^{-1} (*P<0.001*), ear DM (*P<0.001*), EPI (*P<0.001*), FE (*P<0.05*), spikelets ear^{-1} (*P<0.001*) and fertile spikelets ear^{-1} (*P<0.001*). Year x genotype interactions were observed for AGDM shoot^{-1} (*P<0.001*), ear DM (*P<0.001*) and EPI (*P<0.10*).

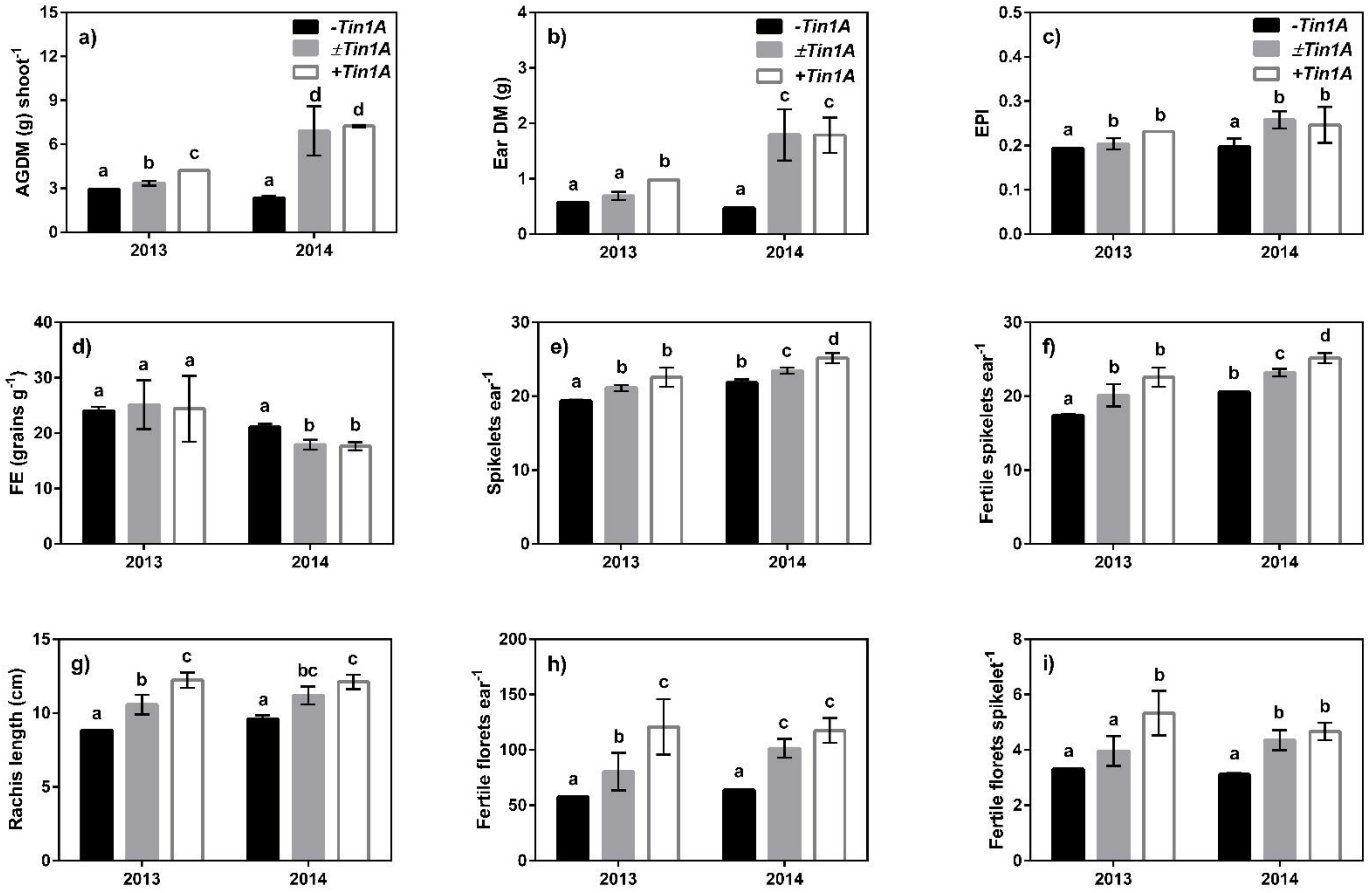

Fig. 7.4 Effects of *Tin1A* locus on anthesis traits: a) above-ground DM shoot^{-1}, b) ear DM shoot^{-1}, c) EPI, d) FE, e) spikelets ear^{-1}, f) fertile spikelets ear^{-1} g) rachis length, h) fertile florets ear^{-1} and i) fertile florets spikelet^{-1} in spring NILs (BC_3) in 2013 and 2014. Black, grey and white bars (mean±SEM) indicate the Ashby parent (*-Tin1A*) and the BC_3 NIL groups *(±Tin1A* and *+Tin1A*), respectively. Different letters above the bars indicate significant differences between genotypes (*P<0.05*).

Table 7.5 Mean values in 2013 and 2014 and cross-year means for above-ground DM per shoot, ear DM shoot^{-1}, ear partitioning index (EPI), fruiting efficiency (FE), spikelets ear^{-1}, fertile spikelets ear^{-1}, rachis length, fertile florets ear^{-1} and fertile florets spikelet^{-1} at anthesis for the Ashby recurrent parent (-*Tin1A*) and the BC_3 NIL groups (±*Tin1A* and +*Tin1A*) and SEDs for genotype groups.

	Ear DM partitioning (DF=12)				**Floret fertility (DF=11 in 2013, DF=14 in 2014)**				
	AGDM (g) shoot^{-1}	**Ear DM (g)**	**EPI**	**FE**	**Spikelets ear^{-1}**	**Fertile spikelets ear^{-1}**	**Rachis length (cm)**	**Fertile floret ear^{-1}**	**Fertile florets spikelet^{-1}**
2013									
Ashby parent (-*Tin1A*)	2.95	0.57	0.19	24.1	19.4	17.4	8.8	57.9	3.32
BC_3 NIL group (±*Tin1A*)	3.35	0.69	0.20	25.1	21.1	20.1	10.6	80.6	3.97
BC_3 NIL group (+*Tin1A*)	4.22	0.98	0.23	23.0	22.3	22.2	12.1	114.2	5.12
SED between NIL groups and Ashby recurrent parent (DF=12 or 11)	0.16***	0.06**	0.008*	3.56	0.42***	0.76**	0.34***	9.56**	0.32**
SED within NIL groups (DF=12 or 11)	0.23***	0.08**	0.012*	4.63	0.52***	0.96**	0.43***	12.00**	0.41**
2014									
Ashby parent (-*Tin1A*)	2.35	0.47	0.20	21.1	21.8	20.6	9.6	64.2	3.12
BC_3 NIL group (±*Tin1A*)	7.03	1.80	0.25	17.7	23.5	23.2	11.2	101.4	4.36
BC_3 NIL group (+*Tin1A*)	7.22	1.84	0.25	18.0	25.2	25.2	12.1	117.7	4.67
SED between NIL groups and Ashby recurrent parent (DF=12 or 14)	0.80***	0.23***	0.015**	0.73**	0.41***	0.43***	0.44***	8.80***	0.35**
SED within NIL groups (DF=12 or 14)	1.02***	0.29***	0.020**	0.93**	0.49***	0.51***	0.52***	10.52***	0.42**
Cross-year ANOVA									
Ashby parent (-*Tin1A*)	2.61	0.53	0.20	22.6	20.7	19.1	9.3	61.8	3.25
BC_3 NIL group (±*Tin1A*)	5.20	1.25	0.23	21.6	22.6	22.5	11.1	100.5	4.45
BC_3 NIL group (+*Tin1A*)	5.72	1.41	0.24	25.4	23.8	23.8	12.1	115.9	4.88
SED between NIL groups and Ashby recurrent parent	0.50***	0.15***	0.011*	3.52	0.41***	0.41***	0.31***	7.02***	0.26***
SED within NIL groups	0.58***	0.17***	0.012*	4.18	0.50***	0.50***	0.37***	8.49***	0.32***
SED Year (DF=1)	0.41***	0.12***	0.009***	2.96*	0.33***	0.33***	0.25	5.65	0.21
SED Year*NIL group and Ashby parent (DF=23 or 14)	0.45***	0.13***	0.010†	3.44	0.56	0.56	0.42	9.54	0.36
SED Year*NIL group (DF=23 or 14)	0.90***	0.26***	0.019†	6.18	0.67	0.67	0.50	11.4	0.43

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

7.3.2.2 KWS-UK Ltd, Thriplow 2014

In 2014 at Thriplow, nine pairs of BC_1S_7 spring wheat lines (Ashby background) and eight pairs of BC_1S_7 winter wheat lines (Humber background) were assessed for yield and yield components. All pairs of BC_1S_7 spring and winter wheat lines were genotyped with the SSR marker linked to the *Tin1A* gene (WMS136) (Table 7.6) and lines were assigned to two groups; +*Tin1A* lines and –*Tin1A* lines based on the molecular results (Fig. 7.5 and Table 7.7).

Table 7.6 Molecular results from genotyping 18 BC_1S_7 spring wheat NILs, 16 BC_1S_7 winter wheat NILs and their recurrent parents (Ashby and Humber) with the SSR marker (WMS136) linked to the *Tin1A* locus on chromosome 1A.

Pair	Lines	References		WMS136 (1A)
	BC_1S_7 spring NILs			
1	B1 A#109-8-2-277-2-8-2-0-0	10	tin-	Ash
	B1 A#109-8-2-277-2-4-5-0-0	10	tin+	L14
2	B1 A#109-9-3-283-1-3-1-0-0	12	tin-	Ash
	B1 A#109-9-3-283-2-8-1-0-0	12	tin+	L14
3	B1 A#124-3-6-312-1-5-6-0-0	15	tin-	L14
	B1 A#124-3-6-312-2-8-6-0-0	15	tin+	L14
4	B1 A#25-3-3-21-2-5-1-0-0	101	tin-	Ash
	B1 A#25-3-3-21-2-5-2-0-0	101	tin+	L14
5	B1 A#48-13-1-91-1-8-3-0-0	103	tin-	Ash
	B1 A#48-13-1-91-1-8-6-0-0	103	tin+	L14
6	B1 A#48-13-1-91-3-1-4-0-0	104	tin-	Ash
	B1 A#48-13-1-91-3-1-2-0-0	104	tin+	Ash
7	B1 A#109-3-1-253-1-1-4-1-0	105	tin-	L14
	B1 A#109-3-1-253-1-1-3-0-0	105	tin+	L14
8	B1 A#109-3-1-253-1-8-3-0-0	107	tin-	Ash
	B1 A#109-3-1-253-1-8-4-0-0	107	tin+	L14
9	B1 A#58-9-2-131-1-2-5-0-0	118	tin-	L14
	B1 A#58-9-2-131-1-2-2-0-0	118	tin+	L14
	BC_1S_7 winter NILs			
1	B1 H#48-5-1-553-6-8-2-0-0	33	tin-	Hum
	B1 H#48-5-1-553-6-2-6-0-0	33	tin+	Hum
2	B1 H#61-7-3-648-3-5-2-0-0	39	tin-	Hum
	B1 H#61-7-3-648-3-2-6-0-0	39	tin+	L14
3	B1 H#65-11-3-703-1-4-1-0-0	41	tin-	Hum
	B1 H#65-11-3-703-2-3-2-0-0	41	tin+	Hum
4	B1 H#78-15-2-727-3-3-6-0-0	42	tin-	Hum
	B1 H#78-15-2-727-4-8-3-0-0	42	tin+	L14
5	B1 H#25-8-1-411-3-8-5-0-0	109	tin-	Hum
	B1 H#25-8-1-411-3-8-1-0-0	109	tin+	Hum
6	B1 H#25-8-2-413-4-8-2-0-0	112	tin-	Hum
	B1 H#25-8-2-413-4-2-1-0-0	112	tin+	Hum
7	B1 H#48-22-1-599-5-4-4-0-0	114	tin-	Hum
	B1 H#48-22-1-599-5-4-2-0-0	114	tin+	Hum
8	B1 H#61-7-3-648-2-7-1-0-0	115	tin-	L14
	B1 H#61-7-3-648-2-7-6-0-0	115	tin+	L14

According to the molecular results in Table 7.6, not all the pairs of BC_1 spring NILs contrasted for presence of +*Tin1A* and –*Tin1A* alleles. Four pairs of spring NILs (pair 3, 6, 7 and 9) were both homozygous for either +*Tin1A* or –*Tin1A* alleles (i.e. did not contrast for *Tin1A*).

The pairs of lines selected to show high a contrast in tillers plant^{-1} in the –*Tin1A* versus the +*Tin1A* line (pairs 1, 2 and 3) had fewer shoots m^{-2} and ears m^{-2} in the +*Tin1A* lines compared to –*Tin1A* lines (*P<0.001*) (Fig. 7.5 and Table 7.7). In pair 1, grain yield and grains m^{-2} were lower at 8.3 t ha^{-1} and 15,581 in +*Tin1A* line compared to –*Tin1A* line (9.9 t ha^{-1} and 19,102; *P<0.001*), while GN ear^{-1} and spikelets ear^{-1} were increased to 90.3 and 28.6, respectively, in +*Tin1A* line compared to 74.2 and 25.1, respectively, compared to in –*Tin1A* line (*P<0.001*).

For the pairs of lines selected for a moderate degree of *Tin1A* contrasts (pairs 4, 6 and 9), ears m^{-2} for pair 4 was lower in the +*Tin1A* line (222.6) compared to the –*Tin1A* line (301.0) (*P<0.001*), whereas GN ear^{-1} and spikelets ear^{-1} tended to be higher in the +*Tin1A* line (41.8 and 22.0, respectively) compared to the –*Tin1A* line (33.5 and 20.6, respectively; *P>0.10*).

For the pairs of lines selected for low to moderate contrast in *Tin1A* phenotype (pairs 5, 7 and 8), no differences were found in shoots m^{-2}, ears m^{-2}, GN ear^{-1} and TGW, but for pair 8 grain yield and grains m^{-2} were lower in the +*Tin1A* line (9.1 t ha^{-1} and 16,080 grains m^{-2}, respectively) compared to –*Tin1A* line (10.7 t ha^{-1} and 19,857 grains m^{-2}, respectively) (*P<0.001*).

For the five pairs of spring NILs (pairs 1, 2, 4, 5 and 8) where the *Tin1A* phenotypes were confirmed by the genotyping averaging over the five pairs of NILs, +*Tin1A* BC_1S_7 lines produced fewer tillers per m^2 (448.5) than -*Tin1A* BC_1 lines (532.1) and the check lines e.g. Santiago (547.2), Rialto (475.4), Ashby (584.8) (*P<0.05*). Although ears m^{-2} was lower in the +*Tin1A* BC_1 lines (244.1) compared to -*Tin1A* BC_1 lines (300.0) and Ashby (362.8) (*P<0.001*), there were no differences in grains m^{-2}

and grain yield between +*Tin1A* BC_1 lines (14,708 and 8.5 t ha^{-1}, respectively) and -*Tin1A* BC_1 lines (17,165; *P>0.10* and 9.3 t ha^{-1}; *P<0.10*, respectively). There were no differences in 1,000 grain weight and grains ear^{-1} between +*Tin1A* BC_1S_7 lines, -*Tin1A* BC_1S_7 lines and the check cultivars (*P>0.10*). Spikelets ear^{-1}, however, was increased in +*Tin1A* lines (24.3) compared to –*Tin1A* lines (21.8) and Ashby (20.1) (*P< 0.01*). Plant height was also slightly decreased in +*Tin1A* lines (77.3 cm) compared to –*Tin1A* lines (81.1 cm) and Ashby (79.0 cm) (*P<0.10*).

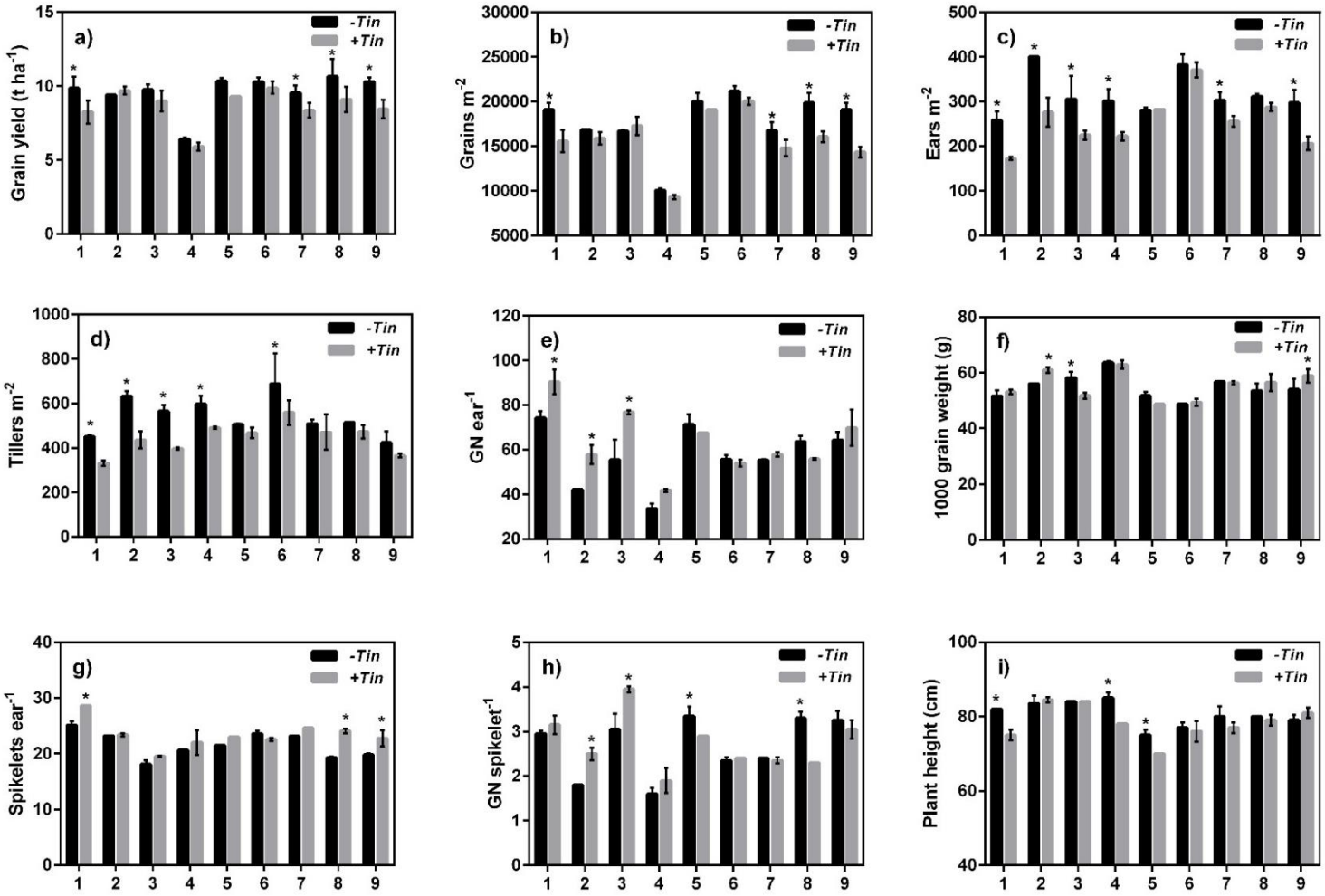

Fig. 7.5 Comparisons of grain yield and yield components; a) grain yield (100% DM), b) grains m^{-2}, c) ears m^{-2}, d) Tillers m^{-2}, e) GN ear^{-1}, f) 1000 grain weight, g) spikelets ear^{-1}, h) GN $spikelet^{-1}$ and i) plant height in BC_1 spring NILs in 2014. Black and grey bars (mean±SEM) indicate the pairs of BC_1S_7 lines contrasting in tiller inhibition gene; (*-Tin phenotype*) and (*+Tin phenotype*), respectively. * above the bars indicates significantly higher than contrasting line of the pair (*P<0.05*).

Table 7.7 Effects of tiller inhibition (*Tin1A*) locus on tillers$_{COUNT}$ m^{-2}, grain yield (100% DM), grains m^{-2}, ears m^{-2}, heading date, plant height, GN ear^{-1}, 1000 grain weight, spikelets ear^{-1} and GN spikelet^{-1} in 9 pairs of BC_1S_7 spring NILs and mean of five +*Tin1A* BC_1S_7 spring NILs compared to five –*Tin1A* BC_1S_7 spring NILs and check cultivars at KWS, Thriplow 2014.

Pair no.	BC_1S_7 lines with degree of contrast in tiller no. and ear traits	*Tin1A* allele	Tillers$_{COU.}$ per m^2	Grain yield (t ha^{-1})	Grains m^{-2}	Ears m^{-2}	Ear emergence (May)	Plant height (cm)	GN ear^{-1}	1000 grain weight (g)	Spikelets ear^{-1}	GN spikelet^{-1}
1	10 tin-	-	450.3	9.9	19,102	258.0	27.5	82.0	74.2	51.7	25.1	3.0
	10 tin+ extreme	+	331.9	8.3	15,581	172.4	23.0	75.0	90.3	53.1	28.6	3.2
2	12 tin-	-	633.3	9.4	16,846	400.8	21.5	83.5	42.0	56.0	23.2	1.8
	12 tin+ extreme	+	435.9	9.7	15,892	276.4	22.5	84.5	57.7	61.0	23.4	2.5
3	15 tin-	+	565.1	9.7	16,721	305.6	25.5	84.0	55.5	58.3	18.1	3.1
	15 tin+ extreme	+	398.3	9.0	17,277	225.1	27.0	84.0	76.7	51.8	19.5	3.9
4	101 tin-	-	597.4	6.4	10,051	301.0	29.0	85.0	33.5	63.6	20.6	1.6
	101 tin+ moderate	+	491.6	5.9	9,292	222.6	28.0	78.0	41.8	63.0	22.0	1.9
5	103 tin-	-	505.9	10.4	20,020	281.2	26.5	75.0	71.2	51.8	21.5	3.3
	103 tin+ mild	+	468.2	9.3	19,093	282.7	24.0	70.0	67.5	48.7	23.0	2.9
6	104 tin-	-	687.1	10.3	21,190	382.1	23.5	77.0	55.5	48.8	23.6	2.4
	104 tin+ moderate	-	559.7	9.9	20,036	371.0	24.0	76.0	54.0	49.3	22.6	2.4
7	105 tin-	+	508.6	9.5	16,774	303.2	22.5	80.0	55.3	56.8	23.2	2.4
	105 tin+ mild	+	470.9	8.3	14,799	256.0	22.0	77.0	57.8	56.4	24.6	2.3
8	107 tin-	-	473.6	10.7	19,857	312.1	28.5	80.0	63.6	53.6	19.3	3.3
	107 tin+ mild	+	514.9	9.1	16,080	288.1	25.5	79.0	55.8	56.5	24.1	2.3
9	118 tin-	+	423.4	10.3	19,086	297.8	28.0	79.0	64.3	54.1	19.8	3.2
	118 tin+ moderate	+	366.9	8.5	14,343	206.3	20.0	81.0	69.8	58.9	22.8	3.1
	SED (DF=18)		45.91***	0.55***	766.70***	21.88***	0.69***	1.43***	4.03***	1.86***	0.75***	0.16***
	LSD (DF=18)		96.45***	1.16***	1,625.30***	46.39***	1.44***	3.01***	8.55***	3.95***	1.58***	0.34***
Santiago		-	547.2	11.7	22,378	389.1	31.0	77.5	57.6	52.2	19.9	2.9
Beluga		-	610.0	10.9	19,144	390.4	24.0	70.0	49.1	56.8	18.6	2.6
Rialto		-	475.4	9.7	18,893	313.6	27.0	78.0	60.4	51.4	22.2	2.7
Rialto x L14 DH line		+	378.5	9.0	16,560	183.4	23.0	78.5	90.3	54.6	23.8	3.8
Ashby		-	584.8	10.6	18,038	362.8	25.5	79.0	49.8	58.7	20.1	2.5
-*Tin1A* BC_1S_7 lines (5 lines)		-	532.1	9.3	17,165	300.0	26.6	81.1	58.5	55.2	21.8	2.7
+*Tin1A* BC_1S_7 lines (5 lines)		+	448.5	8.5	14,708	244.1	24.6	77.3	62.1	57.3	24.3	2.5
SED between BC_1 NIL groups (DF=22)			31.47*	0.64†	1,507.00	19.24***	1.03*	1.85†	7.13	2.08	0.99*	0.26
SED between BC_1 NIL groups and check lines (DF=22)			54.51*	1.11†	2,610.20	33.32***	1.79*	3.20†	12.35	3.60	1.72*	0.45
SED between check lines (DF=22)			70.38*	1.43†	3,369.70	43.01***	2.31*	4.13†	15.94	4.64	2.22*	0.58

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Similarly, all pairs of BC_1 winter wheat lines were genotyped with the SSR primer linked to the *Tin1A* gene (WMS136) and lines ascribed to two groups; +*Tin1A* lines and –*Tin1A* lines based on the molecular results (Fig. 7.6 and Table 7.8).

According to the molecular results, two of eight pairs of BC_1 winter NILs contrasted for +*Tin1A* and –*Tin1A* alleles (pairs 2 and 4). Six pairs of winter NILs (pair 1, 3, 5, 6, 7 and 8) did not contrast and were homozygous for either the -*Tin* (five pairs) or +*Tin* allele (1 pair). Pairs 2 and 4 were selected to show high contrast for *Tin1A* alleles. For pair 2, grain yield and grains m^{-2} were similar between the +*Tin1A* line (10.1 t ha^{-1} and 23,149 grains m^{-2}, respectively) and –*Tin1A* line (10.4 t ha^{-1}; *P<0.10* and 22,641 grains m^{-2}; *P<0.001*, respectively). There was no difference in shoots m^{-2} (*P>0.10*) and ears m^{-2} (*P>0.10*) between +*Tin1A* and –*Tin1A* line in pair 2 but differences in GN ear^{-1} (*P<0.001*). For pair 4, grain yield and grains m^{-2} were also similar between the +*Tin1A* line (9.5 t ha^{-1} and 18,259 grains m^{-2}, respectively) and –*Tin1A* line (10.3 t ha^{-1}; *P<0.10* and 18,259 grains m^{-2}; *P<0.001*, respectively) and no differences were found in ears m^{-2} between contrasting lines (*P>0.10*). However, the +*Tin1A* line produced more grains ear^{-1} (79.8) than the -*Tin1A* line (66.3) (*P<0.001*) with higher spikelets ear^{-1} (24.4 and 21.1, respectively) (*P<0.001*). Thousand grain weight was also decreased in the +*Tin1A* line (52.2 g) compared to the -*Tin1A* line (56.0 g) (*P<0.001*).

Averaging across these two pairs of NILs, shoots m^{-2} and ears m^{-2} were decreased in +*Tin1A* BC_1S_7 lines (437.7 and 265.7, respectively) compared to -*Tin1A* BC_1S_7 lines (536.4 tillers m^{-2}; *P<0.05* and 315.3 ears m^{-2}; *P<0.01*, respectively). Grain yield was lower in the +*Tin1A* BC_1S_7 lines (9.8 t ha^{-1}) compared to -*Tin1A* BC_1S_7 lines (10.4 t ha^{-1}) and check cultivars e.g. Humber (10.5 t ha^{-1}), Rialto (9.7 t ha^{-1}) and Santiago (11.7 t ha^{-1}) (*P<0.001*). GN ear^{-1} was higher in +*Tin1A* BC_1S_7 lines (78.3) compared to -*Tin1A* BC_1S_7 lines (65.4) and Humber (60.3) (*P<0.001*). Spikelets ear^{-1} was also higher in +*Tin1A* BC_1S_7 lines (23.0) than -*Tin1A* BC_1S_7 lines (20.7) (*P<0.05*) while

GN spikelet^{-1} was greater at 3.4 in +*Tin1A* BC_1S_7 lines compared to 3.2 in -*Tin1A* BC_1S_7 lines (*P<0.001*).

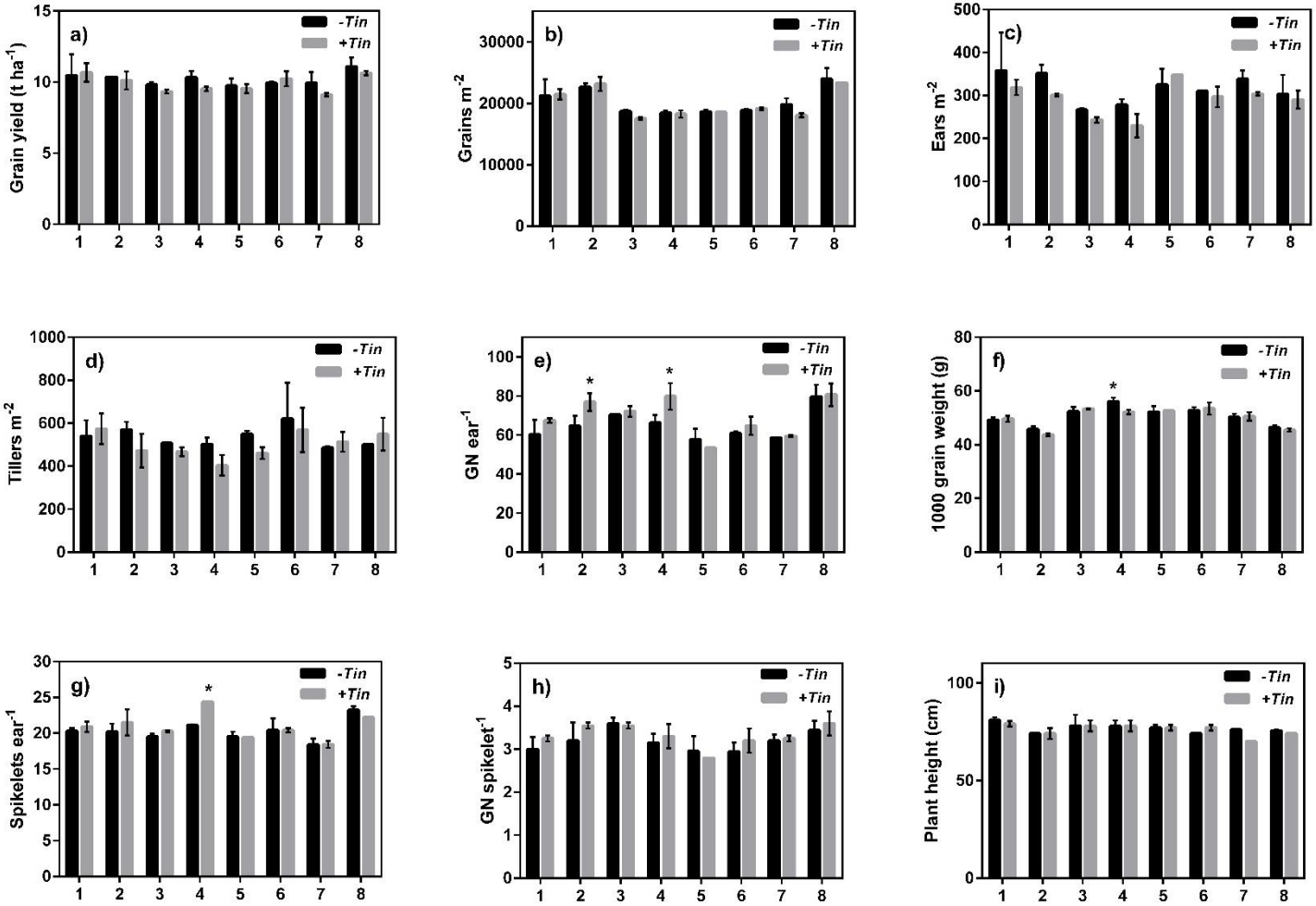

Fig. 7.6 Comparisons of grain yield and yield components; a) grain yield (100% DM), b) grains m^{-2}, c) ears m^{-2}, d) Tillers m^{-2}, e) GN ear^{-1}, f) 1000 grain weight, g) spikelets ear^{-1}, h) GN $spikelet^{-1}$ and i) plant height in BC_1 winter NILs in 2014. Black and grey bars (mean±SEM) indicate the pairs of BC_1S_7 lines contrasting in tiller inhibition gene; (*-Tin* phenotype) and (*+Tin* phenotype), respectively. * above the bars indicates significantly higher than contrasting line of the pair (*P<0.05*).

Table 7.8 Effects of tiller inhibition (*Tin1A*) locus on tillers$_{COUNT}$ m^{-2}, grain yield (100% DM), grains m^{-2}, ears m^{-2}, heading date, plant height, GN ear^{-1}, 1000 grain weight, spikelets ear^{-1} and GN spikelet^{-1} in 9 pairs of BC_1S_7 winter NILs and mean of two +*Tin1A* BC_1S_7 winter NILs compared to two –*Tin1A* BC_1S_7 winter NILs and check cultivars at KWS, Thriplow 2014.

Pair no.	BC_1S_7 lines with degree of contrast in tiller no. and ear traits	*Tin1A* allele	Tillers$_{COU.}$ per m^2	Grain yield (t ha^{-1})	Grains m^{-2}	Ears m^{-2}	Heading date (May)	Plant height (cm)	GN ear^{-1}	1000 grain weight (g)	Spikelets ear^{-1}	GN spikelet^{-1}
1	33 tin-	-	540.0	10.5	21,233	357.9	24.0	81.0	60.3	49.2	20.3	3.0
	33 tin+ mild	-	574.1	10.7	21,484	318.7	22.5	79.0	67.4	49.7	20.9	3.2
2	39 tin-	-	570.5	10.4	22,641	351.6	29.0	74.0	64.5	45.8	20.2	3.2
	39 tin+ extreme	+	471.8	10.1	23,149	301.3	28.5	74.0	76.8	43.7	21.5	3.6
3	41 tin-	-	507.7	9.8	18,708	266.3	20.5	78.0	70.2	52.5	19.5	3.6
	41 tin+ moderate	-	466.4	9.3	17,527	243.4	21.5	78.0	72.1	53.3	20.3	3.5
4	42 tin-	-	502.3	10.3	18,460	279.0	24.0	78.0	66.3	56.0	21.1	3.1
	42 tin+ extreme	+	403.6	9.5	18,259	230.1	28.5	78.0	79.8	52.2	24.4	3.3
5	109 tin-	-	549.0	9.7	18,641	325.5	29.5	77.0	57.6	52.2	19.5	3.0
	109 tin+ extreme	-	461.1	9.5	18,579	347.9	27.5	77.0	53.4	52.6	19.4	2.8
6	112 tin-	-	620.7	9.9	18,827	309.8	30.5	74.0	60.8	52.7	20.4	3.0
	112 tin+ moderate	-	568.7	10.2	19,144	296.8	28.5	77.0	64.7	53.5	20.4	3.2
7	114 tin-	-	486.2	9.9	19,783	338.2	23.5	76.0	58.5	50.2	18.4	3.2
	114 tin+ mild	-	513.1	9.1	18,042	303.6	23.5	70.0	59.4	50.6	18.4	3.2
8	115 tin-	+	500.5	11.1	23,961	302.6	29.5	75.5	79.6	46.3	23.2	3.4
	115 tin+ moderate	+	549.0	10.6	23,375	290.8	28.5	74.0	80.6	45.5	22.2	3.6
	SED (DF=16)		65.85	0.56†	988.5***	30.95*	0.64†	2.16*	4.49***	1.30***	0.83***	0.24*
	LSD (DF=16)		139.60	1.20†	2,106.9***	65.98*	1.35†	4.57*	9.56***	2.78***	1.77***	0.50*
Santiago		-	547.2	11.7	22,377.9	389.1	31.0	77.5	57.6	52.2	20.0	2.9
Beluga		-	610.0	10.9	19,143.6	390.4	24.0	70.0	49.1	56.8	18.7	2.6
Rialto		-	475.4	9.7	18,892.6	313.6	27.0	78.0	60.4	51.4	22.2	2.7
Rialto x L14 DH line		+	378.5	9.0	16,560.3	183.4	23.0	78.5	90.3	54.6	23.8	3.8
Humber		-	488.3	10.5	19,057.2	318.4	26.3	73.7	60.3	54.9	19.9	3.0
-*Tin1A* BC_1S_7 lines (2 lines)		-	536.4	10.4	20,550.6	315.3	26.5	76.0	65.4	50.9	20.7	3.2
+*Tin1A* BC_1S_7 lines (2 lines)		+	437.7	9.8	20,704.1	265.7	28.5	76.0	78.3	47.9	23.0	3.4
SED between BC_1 NIL groups (DF=10)			38.99*	0.24***	1,497.22	25.81**	1.18**	1.74†	2.12***	3.03	0.87*	0.13***
SED between BC_1 NIL groups and check lines (DF=10)			47.75*	0.29***	1,833.71	31.61**	1.44**	2.13†	2.59***	3.71	1.07*	0.16***
SED between check lines (DF=10)			55.14*	0.34***	2,117.39	36.49**	1.67**	2.46†	3.00***	4.28	1.23*	0.18***

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

7.3.3 Effect of the *3A* QTL in BC_3 winter wheat NILs on grain yield and yield components

7.3.3.1 Nottingham 2012/13 and 2013/14

In order to determine the effects of the putative 3A QTL on yield and ear fertility traits, the 37 winter NILs were assigned to two groups (14 $\pm 3A_{WMC428}$ BC_3 lines and 23 $+3A_{WMC428}$ BC_3 lines) based on the presence of the WMC428 L14 allele on chromosome 3A. These two groups were compared with the Humber recurrent parent ($-3A_{WMC428}$) (Fig. 7.7 and Table 7.9).

In 2013, there were no differences in grain yield and grains m^{-2} in $+3A_{WMC428}$ lines (11.3 t ha^{-1} and 32,271 grains m^{-2}, respectively) and $\pm 3A_{WMC428}$ BC_3 lines (12.0 t ha^{-1} and 33,537 grains m^{-2}, respectively) compared to Humber (11.5 t ha^{-1} and 36,290 grains m^{-2}, respectively) (*P>0.10*). HI was also similar between $+3A_{WMC428}$ lines (0.54) and Humber (0.54) (*P>0.10*), whereas ears m^{-2} was slightly decreased in $+3A_{WMC428}$ lines (677.8) compared to $\pm 3A_{WMC428}$ BC_3 lines (738.5) and Humber (782.8) (*P<0.05*). TGW was increased in $+3A_{WMC428}$ lines (35.2 g) and $\pm 3A_{WMC428}$ BC_3 lines (36.0 g) compared to Humber (31.7 g) (*P<0.05*). Rachis length was also increased in $+3A_{WMC428}$ lines (8.77 cm) and $\pm 3A_{WMC428}$ lines (8.41 cm) compared to Humber (7.88 cm) (*P<0.01*), as was plant height (74.9 cm, 73.3 and 66.1 cm for Humber, respectively (*P<0.01*). Spikelets ear^{-1} was higher in $+3A_{WMC428}$ lines (20.8) and $\pm 3A_{WMC428}$ lines (20.0) than Humber (19.5) (*P<0.001*). AGDM $shoot^{-1}$ tended to be increased in $+3A_{WMC428}$ lines (3.16 g) and $\pm 3A_{WMC428}$ lines (3.11 g) compared to Humber (2.70 g) (*P<0.10*).

In 2014, there was no difference in grain yield, grains m^{-2}, ears m^{-2} and HI between the $+3A_{WMC428}$ lines, the $\pm 3A_{WMC428}$ lines and Humber (*P>0.10*). AGDM $shoot^{-1}$ was increased in $+3A_{WMC428}$ lines (3.75 g) and $\pm 3A_{WMC428}$ BC_3 lines (3.55 g) compared to Humber (2.99 g) (*P<0.001*), while plant height was slightly higher in $+3A_{WMC428}$ lines (70.0 cm) and $\pm 3A_{WMC428}$ BC_3 lines (68.4 cm) than Humber (62.7 cm) (*P<0.01*). The

+$3A_{WMC428}$ and ±$3A_{WMC428}$ NILs produced more grains ear^{-1} (57.7 and 54.2, respectively) than Humber (50.0) (*P<0.001*). The spikelet number and rachis length were also higher in +$3A_{WMC428}$ lines (22.8 and 10.03 cm, respectively) and ±$3A_{WMC428}$ BC_3 lines (21.8 and 9.39 cm, respectively) than Humber (21.3 and 8.86 cm, respectively) (*P<0.01*).

Averaging across years, there was no difference in grain yield, grains m^{-2}, HI, TGW and GN spikelet^{-1} between +$3A_{WMC428}$ and Humber (*P>0.10*). Ears m^{-2} tended to decrease in +$3A_{WMC428}$ lines (556.0) compared to Humber (580.0) (*P<0.10*). Plant height was increased in +$3A_{WMC428}$ lines (72.5 cm; +14%) and ±$3A_{WMC428}$ BC_3 lines (70.8 cm; +11%) compared to Humber (63.9 cm) (*P<0.001*), while AGDM shoot^{-1} was heavier in +$3A_{WMC428}$ lines (3.46 g; +17%) and ±$3A_{WMC428}$ BC_3 lines (3.33 g; +10%) than Humber (3.02 g) (*P<0.05*). Grains (+8%; *P<0.01*), spikelets (+6%; *P<0.001*) and fertile spikelets (+6%; *P<0.001*) per ear were increased in +$3A_{WMC428}$ lines associated with a longer rachis (+12%; *P<0.001*) compared to Humber. The year effect was significant for most of the yield-related traits; grain yield, ears m^{-2}, grains m^{-2}, HI, plant height, AGDM shoot^{-1}, TGW, spikelets ear^{-1}, fertile spikelets ear^{-1}, GN ear^{-1} and rachis length (*P<0.001*). There was no year x genotype interaction for the traits (*P>0.10*).

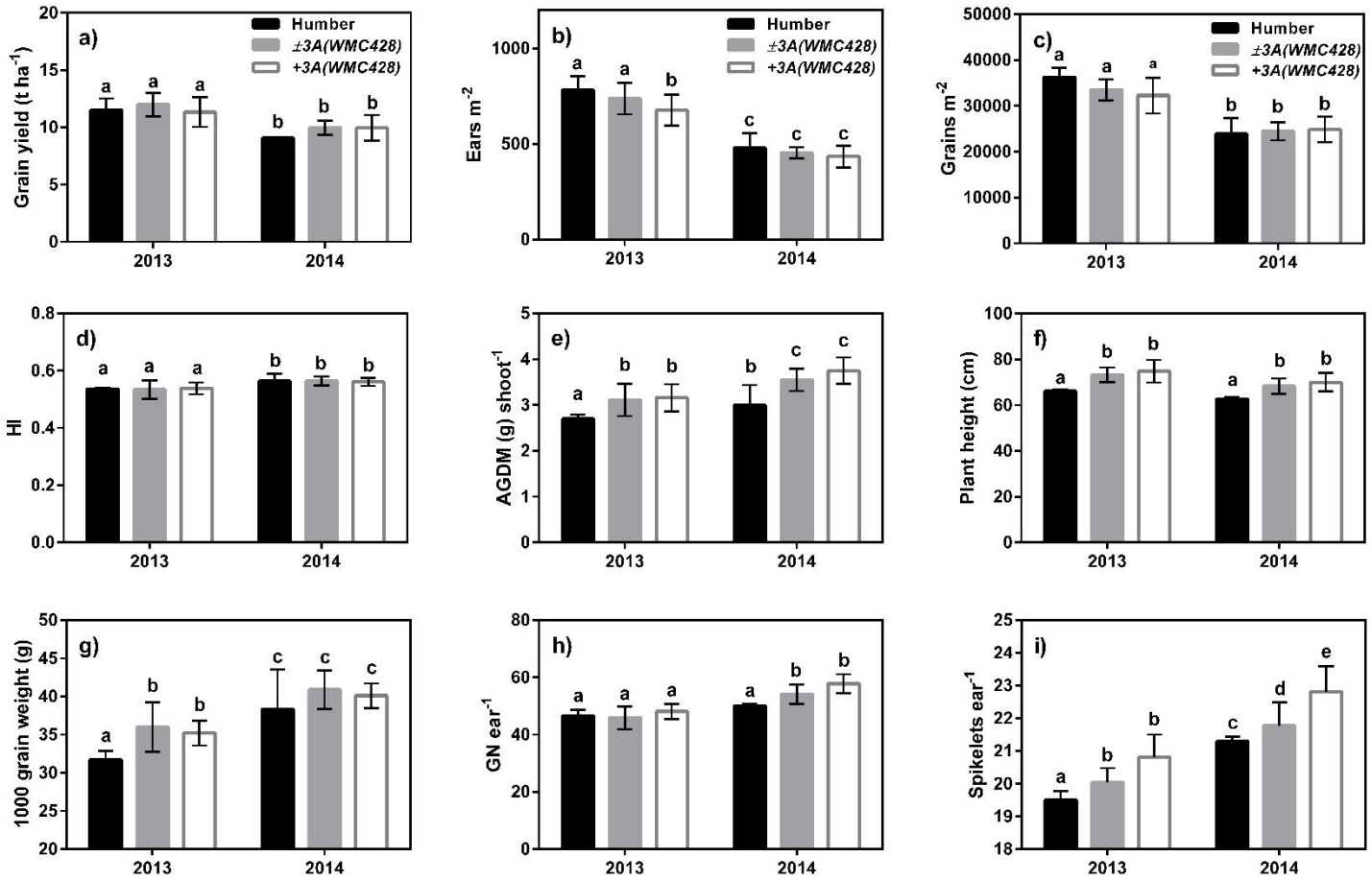

Fig. 7.7 Yield and yield components; a) grain yield (100% DM), b) ears m^{-2}, c) grains m^{-2}, d) HI, e) AGDM $shoot^{-1}$, f) plant height, g) 1000 grain weight, h) GN ear^{-1} and i) spikelets ear^{-1} in winter NILs (BC_3) in 2013 and 2014. Black, grey and white bars (mean±SEM) indicate the Humber parent (*-3A$_{WMC428}$*) and the BC_3 groups (*±3A$_{WMC428}$* and *+3A$_{WMC428}$*), respectively. Different letters above the bars indicate significant differences between genotypes (*P<0.05*).

Table 7.9 Mean values in 2013 and 2014 and cross-year means for grain yield (100% DM), ears m^{-2}, grains m^{-2}, HI, plant height, AGDM m^{-2}, AGDM $plant^{-1}$, GDW (g) $plant^{-1}$, GN $plant^{-1}$, TGW, spikelets ear^{-1}, fertile spikelets ear^{-1}, GN ear^{-1}, GN $spikelet^{-1}$ and rachis length (average for all fertile shoots per plant) for the Humber parent (*-3A*$_{WMC428}$) and the BC_3 groups (*±3A*$_{WMC428}$ and *+3A*$_{WMC428}$) and SEDs for genotype groups.

	Grain yield (t ha^{-1})	Ears m^{-2}	Grains m^{-2}	HI	PH	AGDM $shoot^{-1}$	AGDM (g) $plant^{-1}$	GDW (g) $plant^{-1}$	GN $plant^{-1}$	TGW (g)	Spikelets ear^{-1}	Fertile spikelets ear^{-1}	GN ear^{-1}	GN $spikelet^{-1}$	RL (cm)
2013															
Humber parent (-*3A*$_{WMC428}$)	11.5	782.8	36,290	0.54	66.1	2.70	11.5	6.17	195.3	31.7	19.5	18.0	46.6	2.65	7.88
BC_3 group (±*3A*$_{WMC428}$)	12.0	738.5	33,537	0.53	73.3	3.11	12.7	6.80	188.8	36.0	20.0	18.2	45.9	2.57	8.41
BC_3 group (+*3A*$_{WMC428}$)	11.3	677.8	32,271	0.54	74.9	3.16	13.3	7.14	202.4	35.2	20.8	19.0	48.1	2.59	8.77
SED between BC_3 group and Humber parent (DF=74)	0.36	28.63*	1,103	0.01	1.13**	0.09†	0.71	0.40	10.65	0.67*	0.18***	0.24**	1.18	0.06	0.14**
SED within BC_3 group (DF=74)	0.80	63.84*	2,459	0.02	2.53**	0.21†	1.59	0.89	23.74	1.50*	0.41***	0.54**	2.62	0.13	0.31**
2014															
Humber parent (-*3A*$_{WMC428}$)	9.06	478.7	23,854	0.56	62.7	2.99	12.6	7.13	185.1	38.3	21.3	19.8	50.0	2.49	8.86
BC_3 group (±*3A*$_{WMC428}$)	10.0	453.4	24,481	0.56	68.4	3.55	17.4	9.81	240.8	40.9	21.8	20.4	54.2	2.63	9.39
BC_3 group (+*3A*$_{WMC428}$)	10.0	434.2	24,876	0.56	70.1	3.75	18.3	10.27	256.3	40.1	22.8	21.6	57.7	2.66	10.03
SED between BC_3 groups and Humber parent (DF=74)	0.33	16.57	846.0	0.004	0.96**	0.09***	0.73**	0.43**	10.69**	0.62	0.21***	0.28***	1.18***	0.05	0.15***
SED within BC_3 group (DF=74)	0.73	36.94	1,887	0.009	2.14**	0.21***	1.62**	0.96**	23.85**	1.39	0.47***	0.62***	2.63***	0.11	0.33***
Cross-year ANOVA															
Humber parent (-*3A*$_{WMC428}$)	9.89	580.0	28,232	0.56	63.9	3.02	12.7	7.09	196.1	36.4	20.5	19.1	49.2	2.61	8.41
BC_3 group (±*3A*$_{WMC428}$)	11.0	596.0	29,009	0.55	70.8	3.33	15.0	8.31	214.8	38.4	20.9	19.3	50.0	2.60	8.90
BC_3 group (+*3A*$_{WMC428}$)	10.7	556.0	28,574	0.55	72.5	3.46	15.8	8.70	229.3	37.7	21.8	20.3	52.9	2.63	9.40
SED between BC_3 group and Humber parent (DF=2)	0.25	16.50†	693.0	0.004	0.75***	0.07*	0.55†	0.31	7.97	0.46	0.15***	0.19***	0.83**	0.04	0.10***
SED within BC_3 group (DF=2)	0.73	49.19†	2,066	0.013	2.24***	0.20*	1.63†	0.93	23.76	1.38	0.44***	0.57***	2.49**	0.11	0.31***
SED Year (DF=1)	0.23***	15.59***	654.8***	0.004***	0.71***	0.06***	0.52***	0.30***	7.53***	0.44***	0.14***	0.18***	0.79***	0.04	0.10***
SED Year*BC_3 group and Humber parent (DF=147)	0.30	20.30	853.0	0.005	0.93	0.08	0.67	0.38	9.80	0.57	0.18	0.24	1.03	0.05	0.13
SED Year*BC_3 group (DF=147)	1.02	68.83	2,891	0.018	3.14	0.28	2.28	1.30	33.25	1.93	0.61	0.80	3.48	0.16	0.44

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

The effect of the *+3A$_{WMC428}$* on main-shoot traits is shown in Fig. 7.8 and Table 7.10. Averaging across years 2013-14, AGDM MS^{-1} (+11%; *P<0.05*), grain DM MS^{-1} (+14%; *P<0.05*), GN MS^{-1} (+11%; *P<0.01*), MS spikelets ear^{-1} (+7%; *P<0.001*), MS fertile spikelets ear^{-1} (+7%; *P<0.001*), and MS rachis length (+12%; *P<0.001*) were increased in *+3A$_{WMC428}$* lines compared to Humber. Year was significant for AGDM MS^{-1}, GDW MS^{-1}, GN MS^{-1}, spikelets ear^{-1}, fertile spikelets ear^{-1}, GN MS^{-1}, GN spikelet^{-1}, spikelets ear^{-1}, fertile spikelets ear^{-1} and rachis length (all; *P<0.001*). Year x genotype interactions were observed for AGDM MS^{-1} (*P<0.10*).

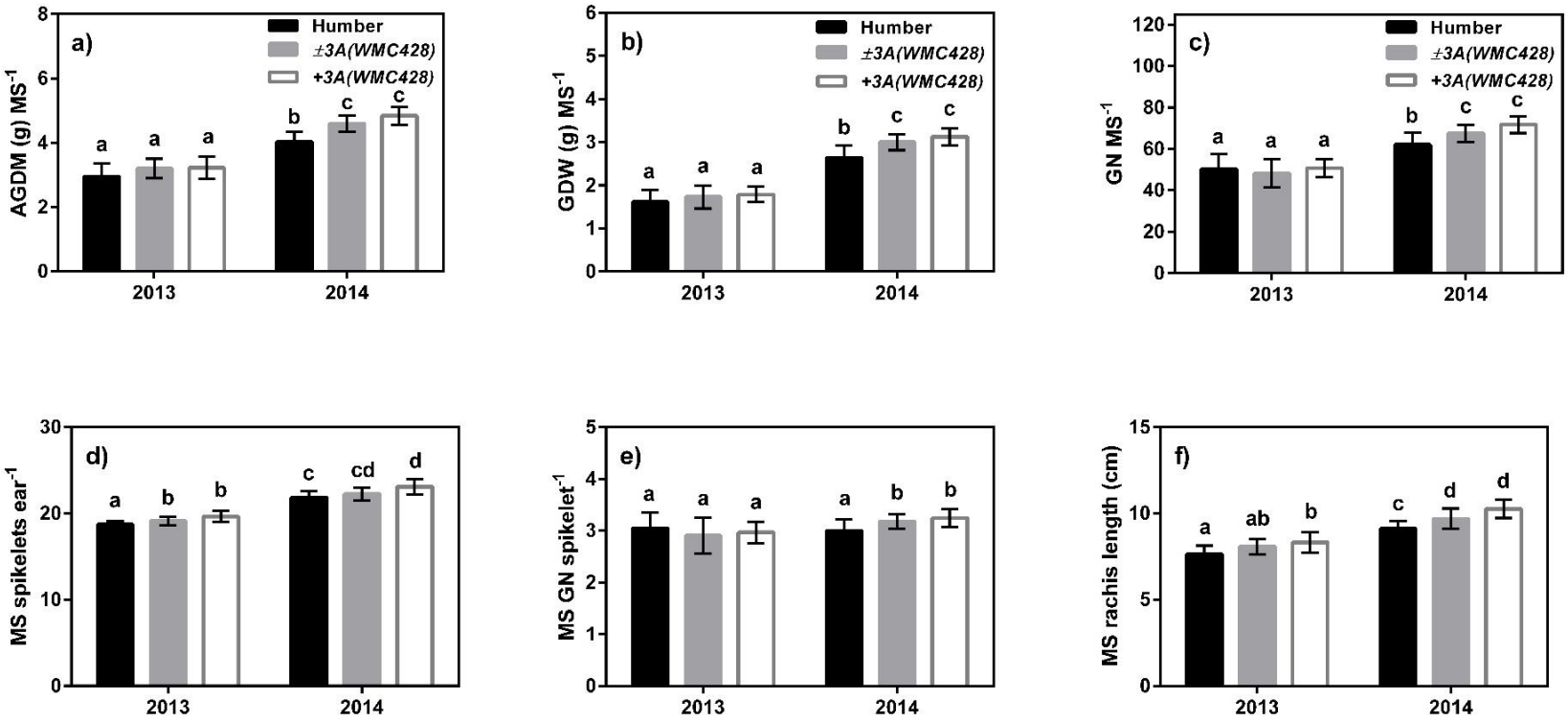

Fig. 7.8 Grain yield (100% DM) and yield components; a) above-ground DM MS^{-1}, b) GDW MS^{-1}, c) GN MS^{-1}, d) MS spikelets ear^{-1}, e) MS GN $spikelet^{-1}$ and f) MS rachis length in winter NILs (BC_3) in 2013 and 2014. Black, grey and white bars (mean±SEM) indicate the Humber parent (*-3A$_{WMC428}$*) and 2 BC_3 groups (*±3A$_{WMC428}$* and *+3A$_{WMC428}$*), respectively. Different letters above the bars indicate significant differences between genotypes (*P<0.05*).

Table 7.10 Mean values in 2013 and 2014 and cross-year means for above-ground DM per main shoot $(MS)^{-1}$, plant height, grain dry weight (GDW) MS^{-1}, grain number (GN) MS^{-1}, MS spikelets ear^{-1}, MS fertile spikelets ear^{-1}, MS GN $spikelet^{-1}$ and MS rachis length for the Humber parent (*-3A$_{WMC428}$*) and 2 BC_3 groups (*±3A$_{WMC428}$* and *+3A$_{WMC428}$*) and SEDs for genotype groups.

	AGDM (g) MS^{-1}	**GDW (g) MS^{-1}**	**GN MS^{-1}**	**MS spikelets ear^{-1}**	**MS Fertile spikelets ear^{-1}**	**MS GN $spikelet^{-1}$**	**MS RL (cm)**
2013							
Humber parent (*-3A$_{WMC428}$*)	2.95	1.62	50.2	18.7	16.4	3.05	7.64
BC_3 group (*±3A$_{WMC428}$*)	3.20	1.73	48.2	19.1	16.5	2.91	8.08
BC_3 group (*+3A$_{WMC428}$*)	3.22	1.79	50.8	19.6	17.1	2.97	8.33
SED between BC_3 group and Humber parent (DF=74)	0.11	0.07	1.78	0.17**	0.26†	0.09	0.15*
SED within BC_3 groups (DF=74)	0.25	0.15	3.96	0.38**	0.57†	0.20	0.33*
2014							
Humber parent (*-3A$_{WMC428}$*)	4.02	2.64	62.1	21.9	20.7	3.00	9.12
BC_3 group (*±3A$_{WMC428}$*)	4.60	3.00	67.5	22.3	21.2	3.18	9.70
BC_3 group (*+3A$_{WMC428}$*)	4.84	3.13	71.8	23.1	22.1	3.25	10.27
SED between BC_3 group and Humber parent (DF=74)	0.09***	0.06**	1.30***	0.22***	0.30**	0.05*	0.16***
SED within BC_3 groups (DF=74)	0.19***	0.14**	2.91***	0.49***	0.66**	0.11*	0.35***
Cross-year ANOVA							
Humber parent (*-3A$_{WMC428}$*)	3.55	2.19	55.4	20.1	18.3	3.02	8.28
BC_3 group (*±3A$_{WMC428}$*)	3.90	2.37	57.8	20.7	18.9	3.04	8.89
BC_3 group (*+3A$_{WMC428}$*)	4.03	2.46	61.3	21.4	19.6	3.11	9.30
SED between BC_3 group and Humber parent (DF=2)	0.07*	0.05*	1.11**	0.14***	0.20***	0.05	0.11***
SED within BC_3 group (DF=2)	0.21*	0.14*	3.30**	0.42***	0.59***	0.15	0.32***
SED Year (DF=1)	0.07***	0.04***	1.04***	0.13***	0.19***	0.05***	0.10***
SED Year*BC_3 group and Humber parent (DF=147)	0.09†	0.06	1.36	0.17	0.24	0.06	0.13
SED Year*BC_3 group (DF=147)	0.29†	0.20	4.61	0.59	0.82	0.21	0.45

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

At anthesis averaging across years, AGDM shoot^{-1} was increased in $+3A_{WMC428}$ lines (2.80 g) and $\pm 3A_{WMC428}$ (2.76 g) lines compared to Humber (2.39 g) (*P<0.001*) and ear DM was increased (0.58 g, 0.57 g and 0.47 g, respectively (*P<0.001*). EPI did not differ between groups, but there was a trend for FE to decrease in $\pm 3A_{WMC428}$ lines (21.0) compared to $+3A_{WMC428}$ lines (21.9) and $-3A_{WMC428}$ lines (22.0) (*P<0.10*). NILs carrying $+3A_{WMC428}$ produced more fertile florets ear^{-1} (81.1) and fertile florets spikelet^{-1} (3.9) than the recurrent parent (66.1; *P<0.001* and 3.4; *P<0.01* respectively). Spikelets ear^{-1} and rachis length were increased in $+3A_{WMC428}$ lines (22.1 and 10.8 cm, respectively) and $\pm 3A_{WMC428}$ lines (21.7 and 10.6 cm, respectively) compared to Humber (20.8; *P<0.01* and 9.3 cm; *P<0.001*, respectively). Year effects were observed for AGDM shoot^{-1} (*P<0.001*), ear DM shoot^{-1} (*P<0.001*), EPI (*P<0.001*), FE (*P<0.001*), spikelets ear^{-1} (*P<0.001*), fertile spikelets ear^{-1} (*P<0.001*), rachis length (*P<0.001*) and fertile florets ear^{-1} (*P<0.001*) but not fertile florets spikelet^{-1} (*P<0.001*). Year x genotype interactions were observed for AGDM shoot^{-1} (*P<0.01*), ear DM (*P<0.01*), fertile floret ear^{-1} (*P<0.05*) and fertile florets spikelet^{-1} (*P<0.05*).

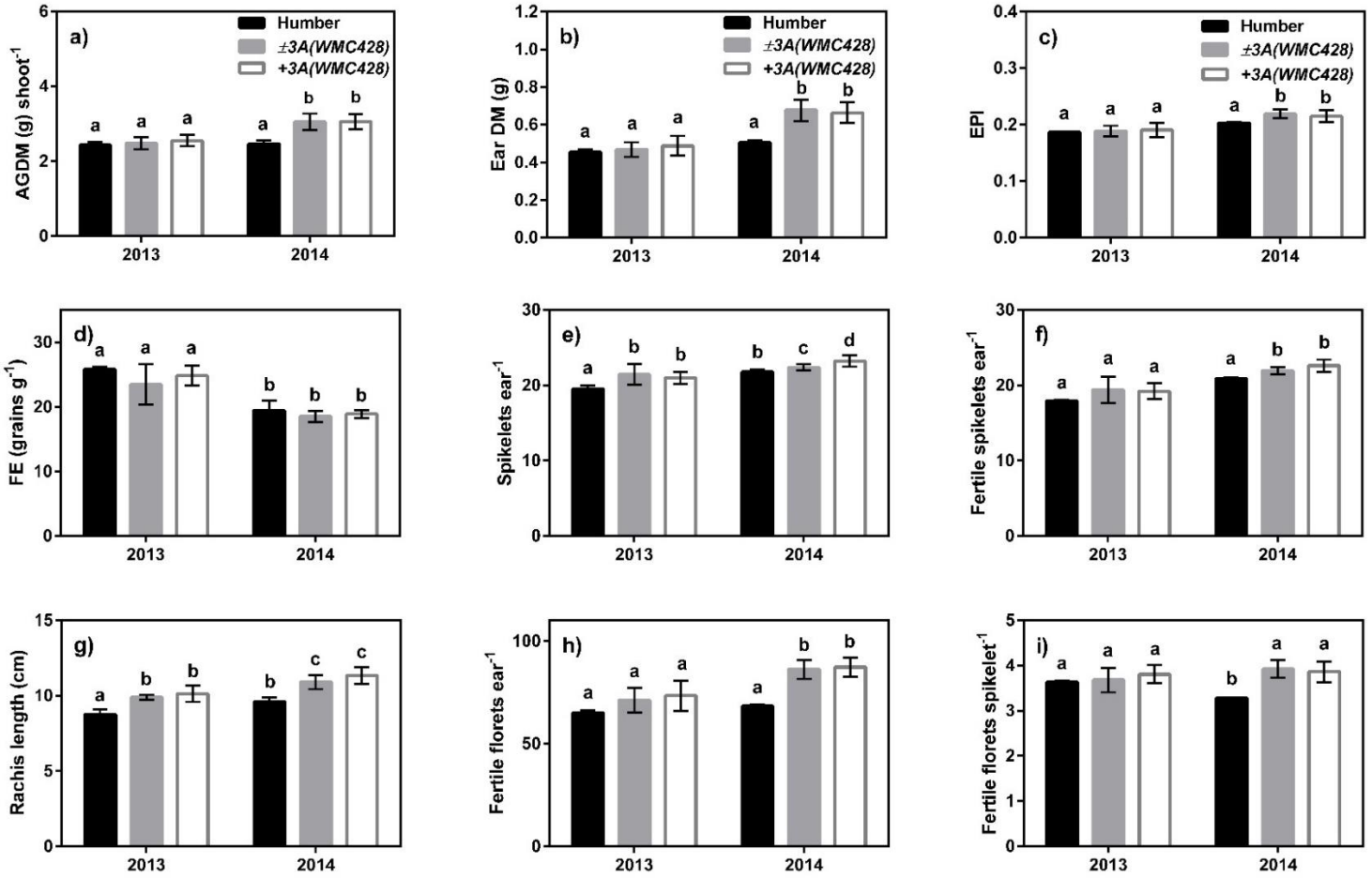

Fig. 7.9 Effects of $3A_{WMC428}$ locus on anthesis traits: a) above-ground DM shoot^{-1}, b) ear DM shoot^{-1}, c) EPI, d) FE, e) spikelets ear^{-1}, f) fertile spikelets ear^{-1} g) rachis length, h) fertile florets ear^{-1} and i) fertile florets spikelet^{-1} in winter NILs (BC_3) in 2013 and 2014. Black, grey and white bars (mean±SEM) indicate the Humber parent (*-$3A_{WMC428}$*) and the BC_3 NIL groups *(±$3A_{WMC428}$* and *+$3A_{WMC428}$*), respectively. Different letters above the bars indicate significant differences between genotypes (*$P<0.05$*).

Table 7.11 Mean values in 2013 and 2014 and cross-year means for above-ground DM per Shoot, ear DM shoot^{-1}, ear partitioning index (EPI), fruiting efficiency (FE), spikelets ear^{-1}, fertile spikelets ear^{-1}, rachis length, fertile florets ear^{-1} and fertile florets spikelet^{-1} at anthesis for the Humber parent (*-3A$_{WMC428}$*) and the BC$_3$ NIL groups (*±3A$_{WMC428}$* and *+3A$_{WMC428}$*) and SEDs for genotype groups.

	Ear DM partitioning (DF=74)				Floret fertility (DF=18 in 2013, DF=74 in 2014)				
	AGDM (g) shoot^{-1}	**Ear DM (g)**	**EPI**	**FE**	**Spikelets ear^{-1}**	**Fertile spikelets ear^{-1}**	**Rachis length (cm)**	**Fertile floret ear^{-1}**	**Fertile florets spikelet^{-1}**
2013									
Humber parent (*-3A$_{WMC428}$*)	2.43	0.45	0.186	25.8	19.5	17.9	8.75	65.1	3.63
BC$_3$ NIL group (*±3A$_{WMC428}$*)	2.48	0.47	0.189	23.5	21.4	19.4	9.90	71.2	3.68
BC$_3$ NIL group (*+3A$_{WMC428}$*)	2.55	0.49	0.191	24.8	21.0	19.2	10.13	73.4	3.80
SED between NIL groups and Humber recurrent parent	0.05	0.01	0.003	0.71†	0.50*	0.64	0.26	3.61	0.12
SED within NIL groups	0.11	0.03	0.008	1.58†	0.65*	0.82	0.33	4.66	0.15
2014									
Humber parent (*-3A$_{WMC428}$*)	2.46	0.50	0.203	19.4	21.8	20.8	9.59	68.3	3.28
BC$_3$ NIL group (*±3A$_{WMC428}$*)	3.05	0.68	0.219	18.5	22.4	21.9	10.9	86.2	3.93
BC$_3$ NIL group (*+3A$_{WMC428}$*)	3.05	0.66	0.215	18.9	23.2	22.6	11.3	87.3	3.86
SED between NIL groups and Humber recurrent parent	0.06***	0.02***	0.003*	0.22	0.18***	0.20***	0.15***	1.49***	0.06***
SED within NIL groups	0.13***	0.04***	0.006*	0.50	0.41***	0.45***	0.33***	3.31***	0.14***
Cross-year ANOVA									
Humber parent (*-3A$_{WMC428}$*)	2.38	0.47	0.195	22.0	20.8	19.4	9.33	66.1	3.42
BC$_3$ NIL group (*±3A$_{WMC428}$*)	2.76	0.57	0.204	21.0	21.7	20.4	10.6	79.3	3.88
BC$_3$ NIL group (*+3A$_{WMC428}$*)	2.80	0.58	0.203	21.9	22.1	21.0	10.8	81.1	3.85
SED between NIL groups and Humber recurrent parent	0.04**	0.01**	0.002	0.37†	0.32*	0.38**	0.20***	2.58***	0.09**
SED within NIL groups	0.12**	0.03**	0.007	1.11†	0.45*	0.54**	0.28***	3.62***	0.13**
SED Year (DF=1)	0.04***	0.01***	0.002***	0.35***	0.25***	0.30***	0.15***	2.00***	0.007
SED Year*NIL group and Humber parent (DF=147 or 31)	0.05**	0.01**	0.003	0.46	0.32	0.38	0.20	2.58*	0.09*
SED Year*NIL groups (DF=147 or 31)	0.16**	0.05**	0.009	1.55	0.59	0.72	0.37	4.80*	0.17*

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Pearson correlations amongst the 23 $+3A_{WMC428}$ BC_3 NILs for grain yield and yield components in 2013 and 2014 are shown in Table 7.12. In 2013, grain yield was positively correlated with grains m^{-2} (r=0.917, *P≤0.001*), ears m^{-2} (r=0.746, *P≤0.001*), AGDM $shoot^{-1}$ (r=0.436, *P≤0.05*), anthesis date (r=0.463, *P≤0.05*), plant height (r=0.421, *P≤0.05*) and GN $spikelet^{-1}$ (r=0.491, *P≤0.05*) but negatively correlated with spikelets ear^{-1} (r=-0.429, *P≤0.05*) (Table 7.14). Plant height was positively associated with anthesis date (r=0.772, *P≤0.001*), TGW (r=0.467, *P≤0.05*), GN ear^{-1} (r=0.493, *P≤0.05*), GN $spikelet^{-1}$ (r=0.705, *P≤0.001*) and rachis length (r=0.722, *P≤0.001*). GN ear^{-1} was positively correlated with GN $spikelet^{-1}$ (r=0.727, *P≤0.001*) and rachis length (r=0.599, *P≤0.001*). Spikelets ear^{-1} was positively associated with fertile spikelets ear^{-1} (r=0.922, *P≤0.001*) but negatively with GN $spikelet^{-1}$ (r=0.524, *P≤0.05*).

In 2014, grain yield was positively associated amongst $+3A_{WMC428}$ BC_3 lines with grains m^{-2} (r=0.926, *P≤0.001*), ears m^{-2} (r=0.852, *P≤0.001*) and anthesis date (r=0.583, *P≤0.01*) but negatively correlated with fertile spikelets ear^{-1} (r=-0.414, *P≤0.05*). HI was positively related with TGW (r=0.521, *P≤0.05*) but negatively with anthesis date (r=-0.597, *P≤0.01*) and plant height (r=-0.464, *P≤0.05*). GN ear^{-1} was positively correlated with plant height (r=0.549, *P≤0.05*), GN $spikelet^{-1}$ (r=0.712, *P≤0.001*) and rachis length (r=0.773, *P≤0.001*) while plant height was positively associated with anthesis date (r=0.605, *P≤0.01*) and GN $spikelet^{-1}$ (r=0.700, *P≤0.001*). However, a negative correlation was observed between GN $spikelet^{-1}$ and spikelets ear^{-1} (r=-0.546, *P≤0.01*) and fertile spikelets ear^{-1} (r=-0.513, *P≤0.05*).

Table 7.12 Pearson correlation (r) amongst *+3A$_{WMC428}$* BC_3 winter NILs for grain yield, grains m^{-2}, ears m^{-2}, harvest index (HI), above-ground dry matter (AGDM) $shoot^{-1}$, anthesis date (AD), plant height (PH), 1,000 grain weight (TGW), GN ear^{-1}, spikelets ear^{-1}, fertile spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length (RL) in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Grains m^{-2}**	**Ears m^{-2}**	**HI**	**AGDM $shoot^{-1}$**	**AD**	**PH (cm)**	**TGW**	**GN ear^{-1}**	**Spikelets ear^{-1}**	**Fertile spikelets ear^{-1}**	**GN $spikelet^{-1}$**	**RL (cm)**
Grain yield (t ha^{-1})	-	**0.926*** **	**0.852*** **	-0.211	0.072	**0.583** **	0.272	0.177	-0.190	-0.209	**-0.414***	0.194	-0.156
Grains m^{-2}	**0.917*** **	-	**0.899*** **	-0.406	-0.047	**0.686*** **	0.232	-0.208	-0.160	-0.316	**-0.448***	0.251	-0.206
Ears m^{-2}	**0.746*** **	**0.886*** **	-	-0.368	-0.389	**0.516***	-0.025	-0.149	**-0.566** **	-0.314	**-0.485***	-0.088	**-0.509***
HI	-0.345	**-0.429***	**-0.473***	-	-0.090	**-0.597** **	**-0.464***	**0.521***	0.003	**0.425***	0.394	-0.345	0.079
AGDM $shoot^{-1}$	**0.436***	0.148	-0.142	-0.223	-	0.269	**0.692*** **	0.322	**0.827*** **	0.163	0.142	**0.649*** **	**0.756*** **
AD	**0.463***	0.373	0.255	**-0.508***	**0.510***	-	**0.605** **	-0.288	0.168	-0.349	-0.398	**0.537** **	0.124
PH (cm)	**0.421***	0.196	-0.029	-0.333	**0.690*** **	**0.772*** **	-	0.114	**0.549** **	-0.218	-0.219	**0.700*** **	0.409
TGW	0.087	-0.316	**-0.448***	0.298	**0.659*** **	0.127	**0.467***	-	-0.054	0.303	0.122	-0.153	0.140
GN ear^{-1}	0.333	0.215	-0.245	-0.041	**0.660*** **	0.266	**0.493***	0.268	-	0.115	0.222	**0.712*** **	**0.773*** **
Spikelets ear^{-1}	**-0.429***	**-0.439***	**-0.509***	0.299	**-0.128**	**-0.451***	-0.348	0.122	0.143	-	**0.921*** **	**-0.546** **	**0.525***
Fertile spikelets ear^{-1}	-0.249	-0.207	-0.303	0.237	**-0.152**	-0.412	-0.387	-0.024	0.175	**0.922*** **	-	**-0.513***	**0.572** **
GN $spikelet^{-1}$	**0.491***	0.346	0.021	-0.178	**0.688*** **	**0.527** **	**0.705*** **	0.282	**0.727*** **	**-0.524***	**-0.538** **	-	0.300
RL (cm)	0.252	0.064	-0.209	-0.288	**0.710*** **	**0.651*** **	**0.722*** **	**0.422***	**0.599** **	0.139	0.181	0.392	-

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

7.3.3.2 KWS-UK Ltd, Thriplow 2013/14

According to the genotyping results on the DNA samples taken from the BC_3S_6 winter NILs in the Thriplow 2013/14 experiment (Table 7.13), BC_3 winter wheat lines were grouped into two groups; three *-3A*$_{WMC428}$ BC_3 lines and twelve *+3A*$_{WMC428}$ BC_3 lines based on the SSR marker (WMC428) on chromosome 3A. These two groups were compared and with the Humber parent (Fig. 7.10 and Table 7.14).

Table 7.13 Molecular results after screened 15 BC_3S_6 NILs and the Humber recurrent parent with SSR marker (WMC428) linked to the 3A QTL on chromosome 3A.

Lines	Reference no.	WMC428 (3A)
B3 29911-14-171-2-0-0	73	L14
B3 29911-14-172-2-0-0	77	L14
B3 29914-5-196-1-0-0	83	Hum
B3 29914-10-207-4-0-0	131	Hum
B3 29915-3-219-1-0-0	150	L14
B3 29917-5-273-1-0-0	170	L14
B3 29917-5-273-2-0-0	171	L14
B3 29917-5-273-3-0-0	172	L14
B3 29917-5-273-4-0-0	173	L14
B3 29917-5-273-5-0-0	174	L14
B3 29917-5-273-6-0-0	175	L14
B3 29917-5-273-7-0-0	176	L14
B3 29917-5-273-8-0-0	177	L14
B3 29917-8-283-6-0-0	195	Hum
B3 29923-14-411-1-0-0	229	L14

There was no difference in grain yield and grains m^{-2} in *+3A*$_{WMC428}$ BC_3 lines (10.5 t ha^{-1} and 18,759, respectively) compared to *-3A*$_{WMC428}$ BC_3 lines (10.2 t ha^{-1} and 17,816, respectively) compared to *-3A*$_{WMC428}$ the Humber recurrent parent (10.3 t ha^{-1} and 18,750, respectively) (*P>0.10*). Plant height in *+3A*$_{WMC428}$ BC_3 lines (80.5 cm) was higher than *-3A*$_{WMC428}$ BC_3 lines (76.7 cm) and Humber (73.9 cm) (*P<0.001*). In addition, GN ear^{-1} was increased in *+3A*$_{WMC428}$ BC_3 lines (62.7) compared to *-3A*$_{WMC428}$ BC_3 lines (57.5) and *-3A*$_{WMC428}$ Humber (61.8) (*P<0.01*). GN $spikelet^{-1}$ was also increased in *+3A*$_{WMC428}$ BC_3 lines (3.3) compared to *-3A*$_{WMC428}$ BC_3 lines (3.1) and Humber (3.1) (*P<0.01*). However, spikelets ear^{-1} was similar among the groups (*P>0.10*).

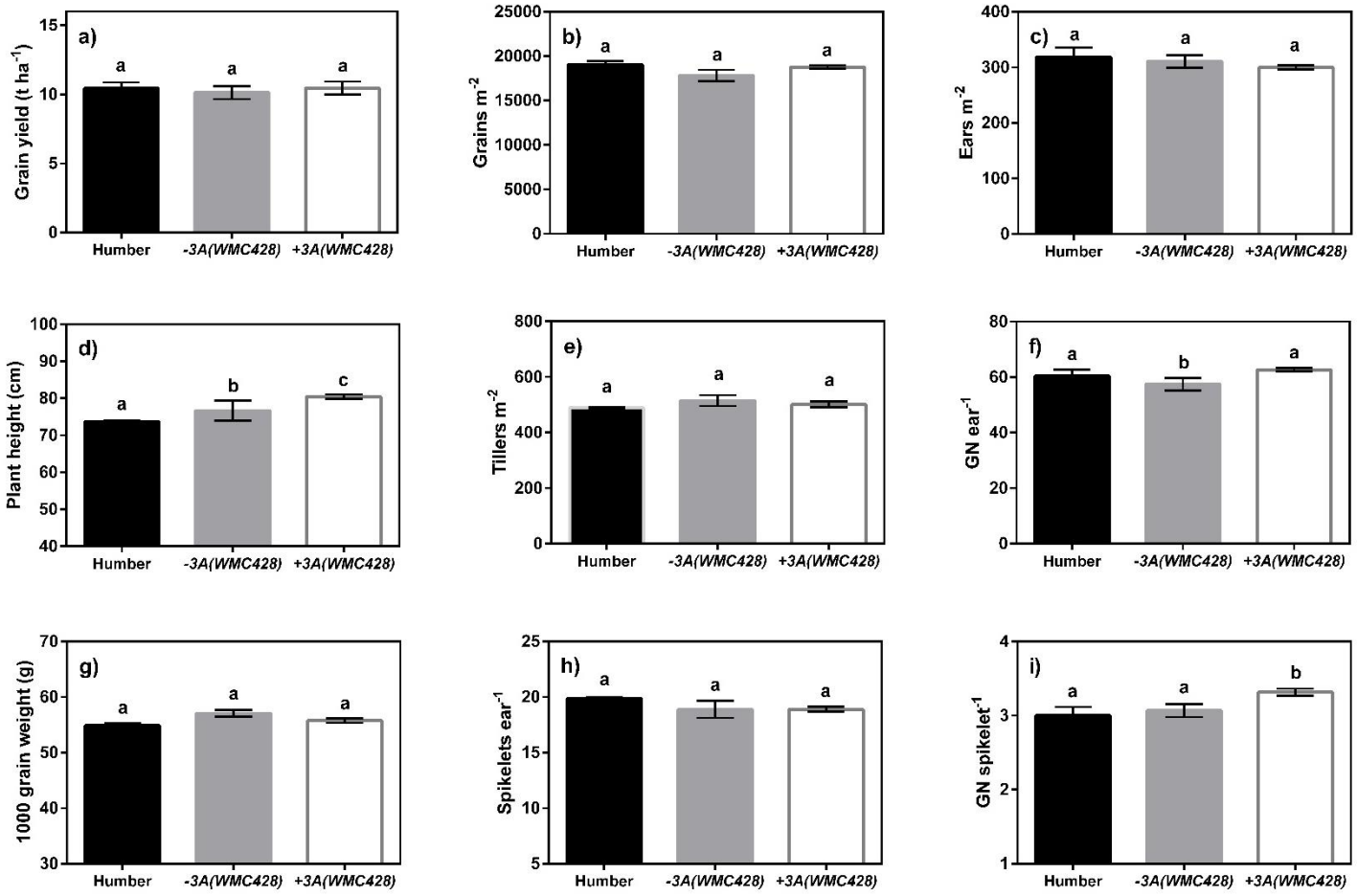

Fig. 7.10 Comparisons of yield and yield components; a) grain yield (100% DM), b) grains m^{-2}, c) ears m^{-2}, d) plant height, e) shoots m^{-2}, f) GN ear^{-1}, g) 1000 grain weight, h) spikelets ear^{-1} and i) GN $spikelet^{-1}$ in winter NILs (BC_3) in 2013-14. Black, grey and white bars (mean±SEM) indicate the Humber parent (*-3A*$_{WMC428}$) and the BC_3 groups (*-3A*$_{WMC428}$ and *+3A*$_{WMC428}$), respectively. Different letters above the bars indicate significant differences between genotypes (*P<0.05*).

Table 7.14 Mean values in 2014 for grain yield (100% DM), grains m^{-2}, ears m^{-2}, heading date, plant height, tillers m^{-2}, GN ear^{-1}, 1000 grain weight, spikelets ear^{-1} and GN $spikelet^{-1}$ for the Humber parent (*-3A$_{WMC428}$*) and 2 BC_3 groups *(-3A$_{WMC428}$* and *+3A$_{WMC428}$*) at KWS, Thriplow and SEDs for genotype groups.

	Grain yield (t ha^{-1} 100 %DM)	**Grains m^{-2}**	**Ears m^{-2}**	**Heading date (May)**	**Plant height (cm)**	**Tillers m^{-2}**	**GN ear^{-1}**	**1000 grain weight (g)**	**Spikelets ear^{-1}**	**GN $spikelet^{-1}$**
2014 BC_3S_6 lines										
Humber parent (*-3A$_{WMC428}$*)	10.3	18,750	305.8	26.3	73.9	486.1	61.8	54.8	19.8	3.12
BC_3 group (*-3A$_{WMC428}$*)	10.2	17,816	310.7	25.3	76.7	515.2	57.5	57.1	18.9	3.05
BC_3 group (*+3A$_{WMC428}$*)	10.5	18,759	299.8	26.0	80.5	501.4	62.7	55.8	18.9	3.32
SED between BC_3 group and Humber parent (DF=31)	0.34	495.5	9.84	1.16	1.27***	21.14	1.39**	0.87	0.42	0.09**
SED within BC_3 groups (DF=31)	0.47	679.0	13.49	1.59	1.74***	28.97	1.91**	1.19	0.57	0.12**

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Table 7.15 Pearson correlations (r) amongst BC_3 winter wheat lines containing *+3A$_{WMC428}$* for grain yield (100% DM), grains m^{-2}, ears m^{-2}, ear emergence date, plant height, tillers m^{-2}, GN ear^{-1}, 1000 grain weight (TGW), spikelets ear^{-1} and GN $spikelet^{-1}$ at KWS, Thriplow in 2014

	Grain yield (t ha^{-1})	**Grains m^{-2}**	**Ears m^{-2}**	**Ear emergence date (May)**	**Plant height (cm)**	**Tillers m^{-2}**	**GN ear^{-1}**	**TGW (g)**	**Spikelets ear^{-1}**	**GN $spikelet^{-1}$**
Grain yield (t ha^{-1})	-									
Grains m^{-2}	**0.819****	-								
Ears m^{-2}	**0.584***	**0.625***	-							
Heading date (May)	-0.423	-0.217	-0.233	-						
Plant height (cm)	-0.374	**-0.531†**	-0.069	0.163	-					
Tillers per m^2	0.237	-0.070	0.442	-0.476	**0.499†**	-				
GN ear^{-1}	0.123	0.272	**-0.581***	0.065	-0.475	**-0.627***	-			
1000 grain weight (g)	**0.641***	0.085	0.204	-0.433	0.066	**0.528†**	-0.178	-		
Spikelets ear^{-1}	0.172	0.061	-0.067	**-0.776****	-0.211	0.267	0.131	0.203	-	
GN $spikelet^{-1}$	-0.027	0.129	-0.350	**0.642***	-0.063	**-0.587***	**0.576***	-0.228	**-0.702***	-

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

Amongst $+3A_{WMC428}$ BC_3 winter wheat lines at KWS, Thriplow in 2014 (Table 7.15), grain yield was positively correlated with grains m^{-2} (r=0.819, *P≤0.01*), ears m^{-2} (r=0.584, *P≤0.05*) and TGW (r=0.641, *P≤0.05*). Grains m^{-2} was positively related with ears m^{-2} (r=0.625, *P≤0.05*) but ears m^{-2} was negatively associated with GN ear^{-1} (r=-0.581, *P≤0.05*). Ear emergence date was positively correlated with GN $spikelet^{-1}$ (r=0.642, *P≤0.05*) and negatively with spikelets ear^{-1} (r=-0.776, *P≤0.01*). GN $spikelet^{-1}$ was positively associated with GN ear^{-1} (r=0.576, *P≤0.05*).

7.3.4 Effects of the 7B QTL in BC_3 winter wheat NILs on grain yield and yield components

7.3.4.1 Nottingham 2012/13 and 2013/14

In order to determine the effect of the putative 7B QTL on yield and ear fertility traits, the 37 winter NILs were divided into 3 groups (16 $-7B_{WMC696}$, 16 $\pm7B_{WMC696}$ and 14 $+7B_{WMC696}$ BC_3 lines) based on the presence of alleles for the WMC696 marker from Rialto on chromosome 7B. All BC_3 groups of genotypes were compared with the Humber recurrent parent ($-7B_{WMC696}$).

Averaging across years, grain yield was increased in 2 genotype groups; $+7B_{WMC696}$ lines (11.26 t ha^{-1}) and $\pm7B_{WMC696}$ lines (10.83 t ha^{-1}) compared to Humber parent (9.89 t ha^{-1}; *P<0.01*), but HI was decreased in $+7B_{WMC696}$ lines (0.54) compared to the Humber parent (0.56; *P<0.05*). There were no differences in grains m^{-2}, ears m^{-2} and GN ear^{-1} (*P>0.10*), whereas TGW (*P<0.001*), AGDM $shoot^{-1}$ (*P<0.001*), plant height (*P<0.001*) and rachis length (*P<0.001*) were increased in all BC_3 genotype groups compared to Humber. Fertile spikelets ear^{-1} was increased in $\pm7B_{WMC696}$ lines (20.64) and $-7B_{WMC696}$ lines (20.05) compared to $+7B_{WMC696}$ lines (19.43) and Humber (19.09; *P<0.001*). Year effects was significant for most traits; grain yield, ears m^{-2}, grains m^{-2}, HI, plant height, AGDM $shoot^{-1}$, TGW, GDW ear^{-1}, GN ear^{-1}, spikelets ear^{-}

^{1}, fertile spikelets ear^{-1}, and rachis length (*P<0.001*). Year x genotype interaction was significant for TGW and rachis length (*P<0.10*).

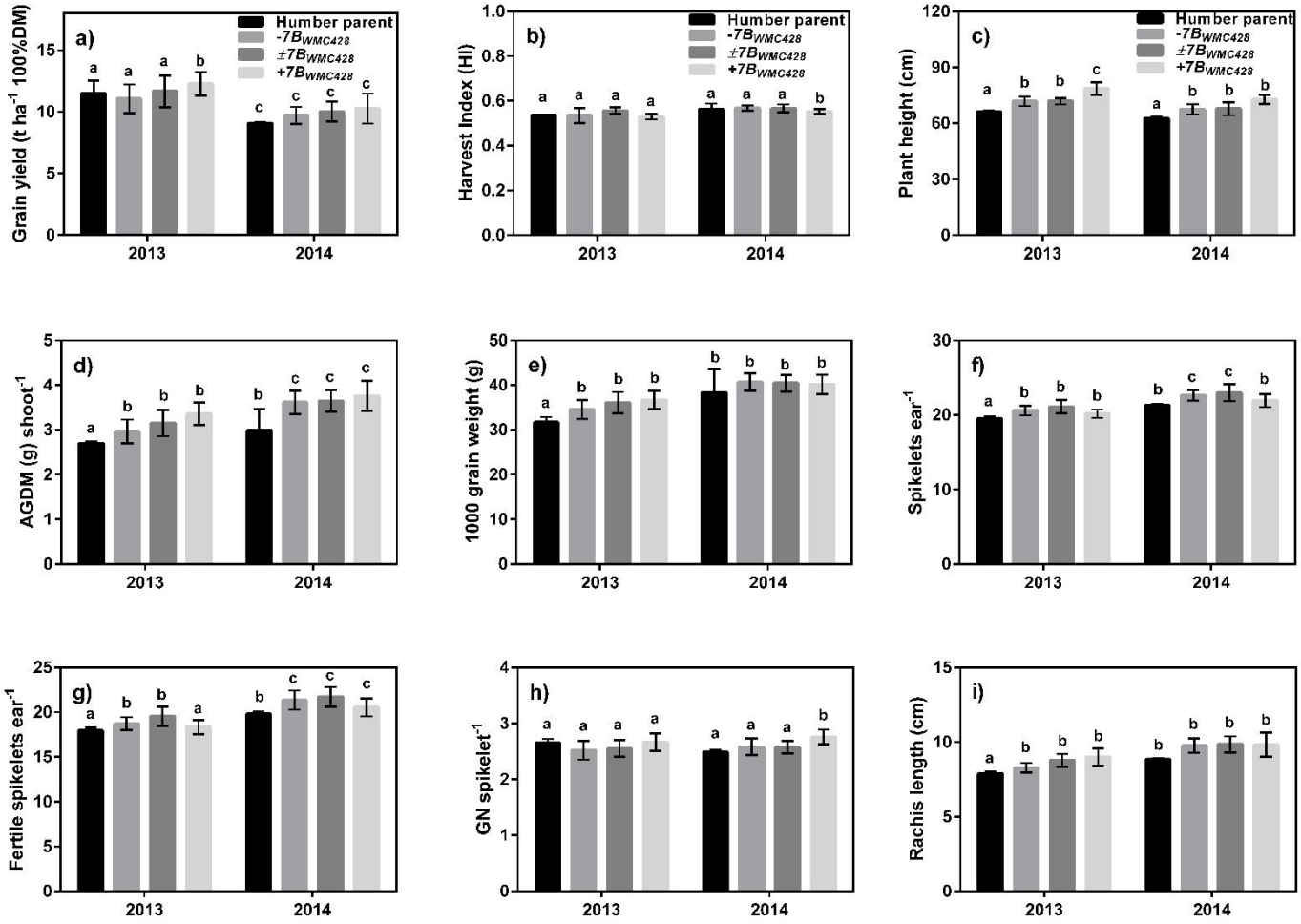

Fig. 7.11 Yield and yield components; a) grain yield (100% DM), b) HI, c) plant height, d) AGDM (g) shoot^{-1}, e) 1000 grain weight (g), f) spikelets ear^{-1}, g) fertile spikelets ear^{-1}, h) GN spikelet^{-1} and i) rachis length (cm) in winter NILs (BC_3) in 2013 and 2014. Bars (mean±SEM) indicate the Humber parent (*-7B$_{WMC696}$*) and the BC_3 groups (*-7B$_{WMC696}$*, *±7B$_{WMC696}$* and *+7B$_{WMC696}$*), respectively. Different letters above the bars indicate significant differences between genotypes (*P<0.05*).

Table 7.16 Mean values in 2013 and 2014 and cross-year means for grain yield (100% DM), ears m^{-2}, grains m^{-2}, HI, plant height (cm), AGDM (g) $shoot^{-1}$, TGW, GDW ear^{-1}, GN ear^{-1}, spikelets ear^{-1}, fertile spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length (average for all fertile shoots per plant) for the Humber parent *(-7B$_{WMC696}$)* and the BC_3 groups (*-7B$_{WMC696}$*, *±7B$_{WMC696}$* and *+7B$_{WMC696}$*) and SEDs for genotype groups.

	Grain yield (t ha^{-1})	Ears m^{-2}	Grains m^{-2}	HI	Plant height (PH) (cm)	AGDM (g) $shoot^{-1}$	TGW (g)	GDW (g) ear^{-1}	GN ear^{-1}	Spikelets ear^{-1}	Fertile spikelets ear^{-1}	GN $spikelet^{-1}$	RL (cm)
2013													
Humber parent (*-7B$_{WMC696}$*)	11.50	782.8	36,290	0.54	66.1	2.70	31.7	1.44	46.6	19.5	18.0	2.65	7.88
BC_3 group (*-7B$_{WMC696}$*)	11.04	704.1	32,181	0.54	71.8	2.97	34.6	1.59	46.2	20.6	18.7	2.52	8.29
BC_3 group (*±7B$_{WMC696}$*)	11.65	669.7	32,458	0.56	71.8	3.15	36.1	1.72	48.6	21.1	19.6	2.56	8.78
BC_3 group (*+7B$_{WMC696}$*)	12.27	713.6	33,518	0.53	78.6	3.36	36.7	1.76	47.7	20.2	18.3	2.66	8.99
SED between BC_3 group and Humber parent (DF=72)	0.37*	31.98	1,207.00	0.008	0.88***	0.09***	0.67***	0.06**	1.29	0.21**	0.26**	0.060	0.14***
SED within NILs group (DF=72)	1.23*	107.01	4,039.00	0.027	2.95***	0.31***	2.25***	0.19**	4.32	0.69**	0.87**	0.201	0.45***
2014													
Humber parent (*-7B$_{WMC696}$*)	9.06	478.7	23,854	0.56	62.7	2.99	38.3	1.65	50.0	21.3	19.8	2.49	8.86
BC_3 group (*-7B$_{WMC696}$*)	9.71	429.8	23,941	0.57	67.5	3.62	40.7	2.03	55.8	22.6	21.4	2.58	9.77
BC_3 group (*±7B$_{WMC696}$*)	10.01	445.0	24,733	0.57	67.8	3.65	40.4	2.03	56.4	23.0	21.7	2.58	9.85
BC_3 group (*+7B$_{WMC696}$*)	10.26	453.2	25,558	0.55	72.8	3.76	40.2	2.04	56.9	21.9	20.5	2.76	9.82
SED between BC_3 group and Humber parent (DF=72)	0.35	18.08	907.00	0.004*	0.79***	0.103*	0.68	0.066†	1.36	0.24**	0.32**	0.048**	0.18
SED within NILs group (DF=72)	1.18	60.52	3,034.00	0.014*	2.65***	0.345*	2.27	0.221†	4.55	0.82**	1.07**	0.161**	0.60
Cross-year ANOVA													
Humber parent (*-7B$_{WMC696}$*)	9.89	580.0	28,232	0.56	63.9	3.02	36.4	1.67	49.2	20.5	19.1	2.61	8.41
BC_3 group (*-7B$_{WMC696}$*)	10.37	566.9	28,061	0.55	69.6	3.29	37.6	1.81	51.0	21.6	20.1	2.55	9.03
BC_3 group (*±7B$_{WMC696}$*)	10.83	557.4	28,595	0.56	69.8	3.40	38.3	1.88	52.5	22.1	20.6	2.57	9.31
BC_3 group (*+7B$_{WMC696}$*)	11.26	583.4	29,538	0.54	75.7	3.56	38.4	1.90	52.3	21.1	19.4	2.71	9.41
SED between BC_3 group and Humber parent (DF=72)	0.26**	18.33	753.00	0.005*	0.60***	0.069***	0.48*	0.044	0.94	0.17***	0.21***	0.038**	0.12***
SED within NILs group (DF=72)	0.98**	69.94	2,872.00	0.017*	2.31***	0.265***	1.84*	0.167	3.58	0.64***	0.81***	0.147**	0.44***
SED Year (DF=1)	0.23***	16.04***	658.70***	0.004***	0.53***	0.061***	0.42***	0.038***	0.82***	0.15***	0.19***	0.034	0.10***
SED Year*BC_3 group and Humber parent (DF=143)	0.35	25.04	1,028.00	0.006	0.83	0.095	0.66†	0.060	1.28	0.23	0.29	0.053	0.16†
SED Year*BC_3 groups (DF=143)	1.41	100.17	4,114.00	0.025	3.30	0.379	2.63†	0.240	5.13	0.91	1.16	0.210	0.63†

† *P<0.10*, * *P<0.05*, ** *P<0.01*, *** *P<0.001*

7.4 Discussion

The discussion will focus firstly on the effects of the *Tin1A* locus on yield and yield components in BC_3 and BC_1 spring wheat near-isogenic pairs of lines. Then, the effects of introgression of the 3A and 7B QTL on ear fertility traits will be discussed, respectively.

7.4.1 Validation of the tiller inhibition gene on chromosome 1A and a QTL on chromosome 3A

The spring BC_3 lines carrying the *Tin1A* locus produced a normal shoot number at GS31 but fewer tillers survived as fertile tillers at anthesis and harvest (Fig. 7.1). The reduction of tiller numbers in NILs containing the *Tin1A* gene is ascribable to an earlier cessation of tiller bud outgrowth rather than a decreased rate of tiller bud production (Kebrom *et al.*, 2012), observations which are consistent with the findings of Duggan *et al.* (2002). Kebrom *et al.* (2012) reported that tiller inhibition in *Tin1A* wheat is related to early development of basal internodes which are more solid than the wild type so that sucrose is diverted away from the axillary buds to support internode elongation; therefore, there is restricted development of secondary tillers. In addition, the inhibition of bud outgrowth in *Tin1A* lines is related to the expression of genes responsive to several dormancy-inducing hormonal and environmental signals (*TB1-like* and *DRM1-like*) (Kebrom *et al.*, 2012). This relates to the present finding in that tiller death in later-formed tillers may be associated with early development of solid basal internodes of the main shoot and tiller 1 and 2.

Although a single gene responsible for tiller inhibition was mapped on chromosome arm 1AS (Richards, 1988, Spielmeyer and Richards, 2004), a few investigations have reported further tiller inhibition *Tin* genes. Peng *et al.* (1998) found a dominant gene on chr 2A (*Tin2*) which affected tiller number. The *tiller inhibition* (*Tin3*) gene located on the long arm of chromosome $3A^m$ was identified in mutants of diploid wheat

(*Triticum monococcum* subsp. *monococcum*) which produced one culm in contrast to wild type plants (Kuraparthy *et al*., 2007) while a fertile tiller inhibition gene (*ftin*) was mapped on chromosome 1AS which differed from *Tin1A* and showed a reduction in tiller growth rather than tiller inhibition (Zhang *et al.*, 2013). In addition, most of the underlying variation for tillering is controlled by a number of quantitative trait loci (QTL) (Li and Gill, 2005). Kato *et al.* (2000) showed the presence of minor QTLs for tillering associated with the vernalization gene (*VrnA*) on wheat chromosome 5A. QTL located on chr 6AS and 1DS showed strong effects, increasing tillers per plant from the spring wheat cv. Opata allele (Li *et al*., 2002). Naruoka *et al.* (2011) suggested that tiller number was controlled by a QTL on chromosome 6B in spring wheat. In all cases, different cultivars and populations were used, so it would be expected that different QTL or combinations of QTL may act in any one cross or cultivar.

In the winter wheat NILs, molecular results showed allelic variation on chromosome 1A (W49), 3A (WMC428 and WMC169) and 7B (WMC696) from the CIMMYT L14 line and the UK wheat Rialto. The WMC428 L14 allele on chromosome 3A was the most common version, whereas the WMC169 and WMC696 alleles were contributed by the UK Rialto winter wheat. The CIMMYT spring bread wheat advanced line L14 was developed from a wide crossing program including *Agropyron elongatum* L., *Triticum polonicum* L. and *Triticum aestivum* L. var. Morocco wheat during the 1990s (Rajaram and Reynolds, 2001). It has been suggested that the crosses between wheat relatives and conventional lines can lead to transgressive segregation of trait values in breeding programs (Reynolds *et al*., 2007). For example, Trethowan *et al*. (2005) reported that cultivars developed from the crosses between synthetic wheats and elite wheat cultivars displayed enhanced drought tolerance. The cross between exotic parents also showed significant differences in moisture adaptation under stress environments (Trethowan *et al.*, 2003). A number of recombinant inbred lines (RILs) generated from a synthetic-hexaploid (W7985) × bread wheat (Opata 85) cross had

quality values consistently greater than those of the parental lines (Nelson *et al*., 2006).

Among the several QTLs for yield and yield components identified on homoeologous groups 2, 3, 5, 7 chromosomes (Gupta *et al*., 2006), QTLs on chromosome 3A were the most reproducibly identified and most important (Baenziger *et al*., 2011). Wheat chromosome 3A has been identified as an outstanding influence on grain yield and yield component traits (Rustgi *et al*., 2013). QTLs for yield components have been identified on chromosome 3A such as anthesis date, plant height, grains ear^{-1}, 1000 grain weight and ears m^{-2} and grains m^{-2} but not for the grain yield itself (Campbell *et al*., 2003, Ali *et al*., 2011, Mengistu *et al*., 2012, Rustgi *et al*., 2013).

A few studies have investigated QTL for yield components on chromosome group 7. Quarrie *et al.* (2006), who investigated yield QTL on 7AL and 7BL from 95 bread wheat DH lines across 3 locations, reported that the yield QTL on 7AL modified biomass accumulation and flag-leaf chlorophyll content.

7.4.2 Effects of the *Tin1A* locus on grains m^{-2} and yield in NILs

In the present study, all experiments showed that ears m^{-2} was reduced in NILs possessing *Tin1A* compared to the recurrent parents (Ashby or Humber), but there was a wide range of effects on yield and yield components. In BC_3 spring wheat lines, the *Tin1A* lines reduced fertile shoots plant^{-1} by 53%, grain yield by 71%, ears m^{-2} by 87%, grains m^{-2} by 75% but maintained HI at harvest. The *Tin1A* introgression lines were also increased in main shoot traits: AGDM shoot^{-1} by 97%, GDW ear^{-1} by 100%, GN ear^{-1} by 68%, spikelets ear^{-1} by 11%, fertile spikelets ear^{-1} by 24%, GN spikelet^{-1} by 35% and rachis length by 35%. At anthesis, AGDM shoot^{-1} was raised by 119%, EPI by 20%, fertile floret ear^{-1} by 88%, fertile florets spikelet^{-1} by 50% whilst FE was unaffected in the *Tin1A* lines.

In the pairs of BC_1S_7 spring wheat lines, for the *Tin1A* lines showing the largest degree of contrast in tiller number (pair 1), grain yield was slightly decreased by 16%, grains m^{-2} by 18% and tillers m^{-2} by 26% but GN ear^{-1} increased by 22% and spikelets ear^{-1} by 26%. In the moderate degree of contrast (pair 4), tillers m^{-2} was reduced by 18% in *Tin1A* line but there was no differences in grain yield, GN ear^{-1}, spikelets ear^{-1} and GN $spikelet^{-1}$. Averaged across the five pairs of lines contrasting for the *Tin1A* locus, the *Tin1A* lines were reduced in tillers m^{-2} by 16%, grain yield by 9%, ears m^{-2} by 19% but increased in spikelets ear^{-1} by 12%. The relatively small reductions in ears m^{-2} in the BC_1 lines in the present study compared to previous investigations indicated that there may be further tillering genes which are modifying the effect of *Tin1A*. Overall, the effect of the *Tin1A* locus was too strong in BC_3 UK spring wheat Ashby background to increase grain yield. Therefore, the tiller inhibition effect needs to be modified by tiller promoting genes or the effects of increasing the seed rate needs to be investigated.

Similarly, the BC_1S_7 winter wheat pairs of contrasting *Tin1A* allele were investigated for the effect of *Tin1A* in the Humber background. The NILs showed variation in tillers $plant^{-1}$ but only two of the eight pairs contrasted for the *Tin1A* locus. Averaging across these two pairs for the contrasting *Tin1A* loci, the *Tin1A lines* reduced tillers m^{-2} by 18%, grain yield by 6%, ears m^{-2} by 16% but did not differ in grains m^{-2}. The BC_1S_7 winter wheat lines containing the *Tin1A* gene increased the GN ear^{-1} by 20%, spikelets ear^{-1} by 11% and GN $spikelet^{-1}$ by 6%.

Previously, many studies showed that tillers compete with the main shoot for resources. An increase in ear DM per unit area and grain number per unit area at anthesis in the +*Tin1A* BC_3 Ashby background could be attributed to less competition from stem growth associated with improved economy of tillering with the *Tin1A* gene which is consistent with findings of Siddique *et al.* (1989a). Reducing infertile tiller numbers might increase resources for growth for the main shoot and surviving shoots, especially to the ears at anthesis, increasing ear partitioning index and floret

fertility. In addition, more assimilate partitioning to floral development can delay floral degradation, leading to more fertile florets and final grains, respectively (Guo and Schnurbusch, 2015). In the study of Hendriks *et al.* (2016), the *Tin1A* gene had an effect on increased root-shoot carbon partitioning and improved water use efficiency in the 'Banks' genetic background under rainfed conditions. With regard to the effects of *Tin1A* on DM in infertile shoots, these effects appeared to be too small in the present experiment to compensate for reduced ears m^{-2}.

Poor plant establishment could also partly explain the reduced grain yield with *Tin1A* in the BC_3 spring wheat NILs in the present study. From the same seed rate (25 seeds = 150 seeds m^{-2} per 1 m row) at sowing, there were decrease in ears m^{-2} in 7 BC_3 spring lines, partly contributing to lower grain yield compared to the Ashby recurrent parent. Grain yield, grains m^{-2} and ears m^{-2} were largely reduced in +*Tin1A* BC_3 group (2.73 t ha^{-1}, 6,228 grains m^{-2} and 79.3 ears m^{-2}, respectively) compared to ±*Tin1A* BC_3 group (6.67 t ha^{-1}, 17,054 m^{-2} and 338.5 m^{-2}, respectively) and the Ashby parent (9.30 t ha^{-1}, 25,098 m^{-2} and 605.6 m^{-2}, respectively) (Table 7.3). In Australia, Duggan *et al.* (2005b), who investigated the effects of four near-isogenic pairs containing *Tin1A* gene on yield and yield components at 2 sowing rates (150 and 300 seed m^{-2}) under irrigated conditions, reported that there were no effects of the *Tin1A* gene on grain yield averaged over experiments and genetic backgrounds; ears m^{-2} were reduced by 11% in NILs carrying *tin* gene while grains ear^{-1} and grain weight were increased by 9% and 2%, respectively. These authors also found an interaction between high seed rate and the *tin* gene on grain yield, increased sowing density (300 seed m^{-2}) resulted in 0.2 t ha^{-1} more yield in +*Tin1A* lines than –*Tin1A* lines (Duggan *et al*., 2005b).

The present results showed contrasting the effects of the *Tin1A* gene in different genetic backgrounds. Different proportional effects of *Tin1A* are also reported in the literature. Mitchell *et al.* (2012), who determined the effects of the *Tin1A* gene on grain yield across environments in Australia in multiple genetic backgrounds, found

that grain yield was decreased by 12% in +*Tin1A* Silverstar background with 21-50% fewer ears m^{-2} than the free-tillering Silverstar lines. Gaju *et al.* (2014b) reported that the presence of the *Tin1A* gene in the DH population (CIMMYT spring wheat advanced line L14 x UK winter wheat Rialto) increased ear partitioning index (+6.3%) and grains ear^{-1} (+13.9%) but decreased ears m^{-2} (-5.7%). In the study of Moeller *et al.* (2014), investigating the effects of a different *tin* gene in two pairs of NILs differing in genetic backgrounds (B_{tin} and B; *tin* derived from the Israeli uniculm line 492 and 7770_{tin} and 7770; *tin* derived from the CSIRO line CS971) on physiological characterisation, reported that ear m^{-2} was reduced by 16% and 40% in *tin* lines (line 7770_{tin} and B_{tin}, respectively) compared to the recurrent parents (line 7770 and B, respectively). According to Duggan *et al*. (2005a), the *tin* line was limited in the ability to produce tillers that would compensate for gaps in the canopy but the genotype was able to compensate by increasing yield components such as GN ear^{-1} and grain weight. Present results indicated that the presence of the *Tin1A* gene in the Ashby background resulted in fewer ears m^{-2} and grains m^{-2}, more GN ear^{-1} and heavier grain weight. However, the increased GN ear^{-1} and grain weight did not compensate for the reductions in ears m^{-2}, so that yield was reduced. Future experiments should aim to quantify the effects of *Tin1A* in UK conditions at increased seed rates, as mentioned above.

7.4.3 Effects of the $3A_{WMC428}$ locus on chromosome 3A on grain m^{-2} and grain yield in NILs

In the present study, the presence of the 3A allele from L14 advance line had no effect on grain yield, grains m^{-2} and HI between the BC_3 groups and the recurrent parent. Ears m^{-2} tended to slightly decrease in the $+3A_{WMC428}$ BC_3 groups (-4%) compared to Humber. NILs containing the $+3A_{WMC428}$ loci increased plant height by 14%, AGDM $shoot^{-1}$ by 17% and ear fertility traits including spikelets ear^{-1} by 6%,

fertile spikelets ear^{-1} by 6%, GN ear^{-1} by 8%, and rachis length by 12% compared to the Humber recurrent parent.

At anthesis, the *+3A$_{WMC428}$* locus increased spikelets ear^{-1} by 6%, fertile spikelets ear^{-1} by 8%, rachis length by 16%, fertile florets ear^{-1} by 23% and fertile florets spikelet^{-1} by 13%. Plant height in NILs containing the *+3A$_{WMC428}$* from L14 line was closely related to biomass production, anthesis date, GN ear^{-1} and GN spikelet^{-1}. However, the semi-dwarfing gene does not contribute to the difference in plant height. In a study in Nebraska, Mengistu *et al.* (2012) reported that the WMC428 locus on 3A was related to plant height trait in a WI(RICLs3A) mapping population, whereas Ali *et al.* (2011) found this allele was associated with increased grains ear^{-1} and mapped in Region 3 on chromosome 3A of CNN(RICLs3A) population and that the effect was active across environments. In the findings of Rustgi *et al.* (2013), the WMC428 loci was associated with grain weight and ears m^{-2}.

The present results suggested that the substitution of a chromosome segment on chromosome 3A in BC_3 NILs was responsible for increased plant height resulting in increased GN ear^{-1} and GN spikelet^{-1}. It would not be expected that increased plant height would lead to an increase in EPI and GN ear^{-1}, it is possible that the 3A QTL had an effect on spikelet primordia production increasing spikelets ear^{-1}, EPI and GN ear^{-1}. In addition, the increase in plant height is likely to be contributing to increased AGDM shoot^{-1}. However, trade-offs were observed between GN spikelet^{-1} and spikelets ear^{-1} and between GN spikelet^{-1} and fertile spikelets ear^{-1}. Flintham *et al.* (1997) reported that wheat lines with height approximate ranging 70-100 cm produced more grains m^{-2} than shorter or taller lines. The allocation of biomass to the ear at anthesis was greater in BC_3 NILs than the Humber recurrent parent and it is possible that the high sink capacity (spikelets ear^{-1}, GN spikelets^{-1}, rachis length) contributed to the increased allocation of dry matter to the ear. Also, the increase in spikelets per ear could be associated with an effect of the 3A QTL on earliness *per se*. It has been reported in many studies that the *earliness per se* genes are present on

chromosome 3A (Hoogendoorn, 1985, Miura and Worland, 1994, Miura *et al.*, 1999) and that anthesis date was related to identified regions also on chromosome 3A (Ali *et al.*, 2011). However, in the present results, there was no effect of 3A on date of GS31 and anthesis date. So, further investigation is needed on any potential effects on development.

In previous QTL mapping in the L14 x Rialto DH population in 2007-09, WMS155 was the nearest marker on 3A linked to an increase in grain yield and grains m^{-2} across two environments (Foulkes *et al*., 2010). Association between grain yield and the WMS155 SSR marker on chromosome 3A was observed in a population of CNN(RICLs3A) (Ali *et al.*, 2011). Moreover, a number of markers were associated with grain weight, ears m^{-2}, plant height and anthesis date in the flanking region of WMS155 (Ali *et al.*, 2011). In the study of Sajjad *et al.* (2014), who identified QTLs for yield related traits on chromosome 3A, it was reported that grain yield $plant^{-1}$ and plant height were associated with WMS155 locus. This locus was also associated with many flanking DArT markers linked to grain yield on chromosome 3AL (Crossa *et al.*, 2007). A future study could be to fine map the 3A QTL with additional markers to validate and refine the precise location of the putative QTL on chromosome 3A.

7.4.4 Effects of the $7B_{WMC696}$ locus on chromosome 7B on grains m^{-2} and grain yield in NILs

In the present study, the presence of the $7B_{WMC696}$ locus from Rialto UK winter wheat had an effect on grain yield and yield components. Grain yield was increased in $+7B_{WMC696}$ BC_3 lines by 14% and $\pm 7B_{WMC696}$ BC_3 lines by 10% compared to the Humber recurrent parent, but there were no differences in grains m^{-2} and ears m^{-2}. HI was decreased in the $+7B_{WMC696}$ BC_3 lines by 3% compared to Humber. NILs containing $+7B_{WMC696}$ locus also increased plant height by 19%, AGDM $shoot^{-1}$ by

18%, TGW by 6% and rachis length by 12% compared to Humber but spikelets ear^{-1} was similar to Humber.

In the previous QTL mapping, ePt82111 loci on Chr 7B showed negative effects on spikelets ear^{-1} in L14 x Rialto DH population across 3 locations (Foulkes *et al.*, 2010). It is possible that chromosomal interactions may have an influence on yield and yield components. In the study of Maccaferri *et al.* (2008), QTLs specific for plant height were identified on chromosomes 1BS, 3AL, and 7AS in a durum wheat population (RILs) in 16 environments (10 rainfed and 6 irrigated) and a further three major QTL for heading date on chromosomes 2AS, 2BL, and 7BS showed limited on grain yield. These authors also found an epistasis effect between the chromosome 2BL and 3BS QTL was detected across several environments.

7.4.5 Summary

Four hypotheses were tested in this chapter:

Hypothesis 1*: The +Tin1A BC$_3$ NILs have reduced ears m^{-2} at harvest but increased GN ear^{-1}, spikelets ear^{-1}, GN spikelet^{-1} and rachis length leading to higher grains m^{-2} compared to the –Tin1A BC$_3$ NILs and the recurrent parent(s) in the UK rainfed environment.*

+Tin1A BC$_3$ NILs had reduced ears m^{-2} at harvest but increased GN ear^{-1} (+90%), spikelets ear^{-1} (+7%), fertile spikelets ear^{-1} (+19%), GN spikelet^{-1} (+86%) and rachis length (+35%) compared to the *–Tin1A* BC$_3$ NILs and the Ashby recurrent parent in the UK rainfed environment. However, this did not lead to higher grains m^{-2} due to the effect of *Tin1A* gene on spikelet number and fertile floret number was too small to compensate for ears m^{-2} and grains m^{-2} in the present experiment.

***Hypothesis 2**: The +Tin1A BC_1S_7 NILs in the Ashby and Humber backgrounds produce fewer ears m^{-2}, but higher grains ear^{-1}, grains m^{-2} and grain yield than contrasting –Tin1A BC_1S_7 lines.*

In the KWS 2014 experiment, the results showed that the *+Tin1A* BC_1S_7 NILs in the Ashby and Humber backgrounds produced fewer tillers m^{-2} and ears m^{-2} , higher spikelets ear^{-1}, GN ear^{-1} and GN $spikelet^{-1}$ but similar grains m^{-2} and grain yield compared to *–Tin1A* BC_1S_7 NILs , so overall this hypothesis was not confirmed. Results also indicated that the degree of tiller inhibition with tiller inhibition gene may be dependent on the genetic background.

***Hypothesis 3**: The BC_3 NILs with the allele from the CIMMYT L14 for a QTL on chromosome 3A have increased spikelets ear^{-1}, grains m^{-2} and grain yield compared to BC_3 NILs with the allele from Humber and the Humber recurrent parent in the UK rainfed environment.*

The BC_3 winter NILs with the allele from CIMMYT L14 for the QTL on chromosome 3A ($+3A_{WMC428}$ BC_3 groups) caused increases in spikelets ear^{-1} (+6%) but no significant effects on grain yield and grains m^{-2} compared to the BC_3 winter NILs with the allele from Humber and the Humber recurrent parent.

***Hypothesis 4**: The presence of an allele from Rialto for a QTL on chromosome 7B (WMC696) in the BC_3 NILs increases grain number per unit area compared to BC_3 NILs with the Humber and also the combined Humber recurrent parents.*

The presence of the allele from Rialto for a QTL on chromosome 7B (WMC696) in the BC_3 winter NILs had a positive effect on grain yield (+14%) but no effects on grains m^{-2} compared to BC_3 NILs with the Humber recurrent parent.

CHAPTER 8 GENERAL DISCUSSION

8.1 Physiological basis of grain yield and grains m^{-2} in BC_3 lines

In the present study, the grain yield of BC_3 spring NILs was lower than Ashby but HI was increased in some BC_3 lines. The tiller inhibition (+*Tin1A*) gene was present in two of the lines. The +*Tin1A* BC_3 spring lines increased ear partitioning by 9% which increased grains per spikelet and grains per ear (Fischer and Stockman, 1986). However, the *Tin1A* gene effect on grains per ear did not compensate for the reduction in ears m^{-2} so that grain yield was reduced in+*Tin1A* BC_3 lines. For winter wheat, the highest BC_3 NILs increased grain yield by 22%, AGDM $shoot^{-1}$ by 45%, TGW by 21%, spikelets ear^{-1} by 14%, fertile spikelets ear^{-1} by 15%, GN $spikelet^{-1}$ by 11% and rachis length by 24% compared to Humber. Grain yield increased in two NILs compared to Humber and AGDM, anthesis date and 1000 grain weight were associated with these increases in the two NILs.

Flowering time is a major trait affecting plant adaptation, yield potential and yield stability (Snape *et al*., 2001, Langer *et al*., 2014). In the present study, there was no difference in anthesis date in BC_3 spring wheat lines, but there were small differences in the date of GS31 which were positively associated with spikelets ear^{-1} in 2014. GN ear^{-1} in spring BC_3 NILs was strongly related to fertile spikelets per ear. Increased spikelets ear^{-1} in spring BC_3 lines could be due to a longer duration of spikelet primordia production (Gaju *et al*., 2009). It has been suggested that extending the duration from floral initiation to terminal spikelet formation might increase spikelets ear^{-1} (Rawson, 1970) and the earliness *per se* response can affect this duration via an effect on the rate of spikelet primordia production (Jamieson *et al.*, 1998, Lewis *et al.*, 2008).

A positive correlation was found between grain yield and anthesis date in the winter BC_3 lines. Fischer (1985) reported that the late reproductive phase associated with rapid ear growth starts about 20 d before anthesis and played an important role to

determining wheat yield. The duration of the GS31 to GS61 phase was positively related to grains m^{-2} in winter wheat lines in the present study. It has been suggested by many studies that extending the stem-elongation phase (from initiation of terminal spikelet to anthesis) will be an avenue to increase grains per m^2 and yield (Slafer *et al.*, 2001, González *et al.*, 2003b, Foulkes *et al.*, 2011).

Correlations between each of grain yield and AGDM and post-anthesis source-type traits were observed in winter wheat near-isogenic lines. In winter wheat lines, grain yield and biomass were increased with high canopy green area (as indicated by NDVI about 2 weeks after anthesis) and better maintenance of green area during grain filling (higher NDVI at 650 °Cd and slower NDVI rate of senescence). Grain yield was also positively correlated with flag-leaf chlorophyll content at GS61+14d and at 650 °Cd and end of flag-leaf senescence (FLS). According to Thomas and Howarth (2000), stay-green traits can be classified into functional stay green and non-functional stay green (photosynthetic function not maintained) types. Flag-leaf chlorophyll content in several winter BC_3 NILs started to decreased later and the rate of decrease was slower compared to the recurrent parent, indicating that they had ability to photosynthesize for a longer period during grain filling period (potentially functional stay green).

The correlations between SPAD senescence rate, SPAD at 650 °Cd and grain yield could indicate source limitation of grain growth in the winter wheat background. There is recent evidence for co-limitation of yield by source and sink in modem wheat cultivars under favourable conditions (Shearman *et al*., 2005, Acreche and Slafer, 2009, Aisawi *et al*., 2015). Possible mechanisms that could explain differences in stay green in this study might be deeper roots and higher N accumulation at anthesis and slower post-anthesis N remobilization (Foulkes *et al.*, 2009). Gaju *et al*. (2014a) reported that an increase in AGDM with later flowering was associated with larger root biomass and high N uptake capacity during ear growth from booting to anthesis. Previous studies reported that delayed onset of senescence is associated with increased nitrogen uptake at anthesis and/or reduced post-anthesis N remobilization

at post-anthesis period (Mi *et al.*, 2000, Borrell *et al.*, 2001, Gaju *et al.*, 2011). Borrell and Hammer (2000) proposed that stay-green genotypes uptake more N during grain filling, which may in turn be asociated with a more extensive root system, leading to extented root growth and hence more N assimilation by roots (O'Toole and Bland, 1987, Ta and Weiland, 1992).

Although bread wheat has been frequently characterised as sink-limited during grain filling under optimal conditions, there is recent evidence for co-limitation of grain yield by source and sink in modern cultivars in several studies. Acreche and Slafer (2009) reported that modern Spanish and Italian wheat cultivars have increased grains per unit land area but reduced the source capacity due to reduction in leaf net photosynthetic during grain filling. In the winter NILs, there was evidence for source limitation of grain yield or co-limitation of grain yield by source and sink. Delayed leaf senescence might also increase maintenance of radiation-use efficiency and allow more carbon and nitrogen to be assimilated during grain filling. Present results would support the hypothesis that grain growth of modern UK winter wheat lines is co-limited by sink size in the first part of the grain filling phase but by source size in the latter stages of grain filling.

8.2 QTLs for improved ear fertility and grain yield

8.2.1. Effects of the tiller inhibition gene (*Tin 1A*)

Tiller production and survival is one of the most important physiological processes in wheat crops because final tillers per plant determines ears per plant which is closely related to grain number and grain weight determination. In the present study, grain yield, grains m^{-2} and ears m^{-2} were reduced in BC_3 spring NILs possessing *Tin1A* by 71%, 75% and 87%, respectively but HI was maintained compared to the Ashby parent. However, the *Tin1A* BC_3 lines increased EPI by 20%, fertile florets ear^{-1} by 88% at anthesis and grains ear^{-1} by 68% at harvest. The effects of *Tin1A* locus overall were too strong in the BC_3 UK spring Ashby background to increase grain

yield. The results for the BC_1 lines were different from the BC_3 lines. In BC_1 spring NILs, grain yield and ears m^{-2} were reduced by 9% and 19%, respectively, and in BC_1 winter NILs grain yield and ears m^{-2} were also decreased by 6% and 16%, respectively; however, there was no statistically significant differences in grains m^{-2} in both spring and winter BC_1 NILs in this study. An increase in ear DM and grain number per fertile shoot at anthesis was observed in +*Tin1A* BC_3 Ashby background which could be attributed to less competition from infertile tillers associated with improved economy of tillering with the *Tin1A* gene (Siddique *et al*., 1989a). Reducing infertile tiller numbers might increase resources for growth and development for the main shoot and surviving shoots, especially to the ears at anthesis, increasing ear partitioning index and floret fertility. In addition, more biomass partitioning to floral development can delay floral degradation, leading to more fertile florets and final grains, respectively (Guo and Schnurbusch, 2015).

Results showed that the spring BC_3 NILs carrying the *Tin1A* locus produced a normal shoot number at GS31 but fewer tillers survived as fertile tillers at anthesis and harvest. Tiller death in later-formed tillers in *Tin1A* genotypes may be associated with early development of solid basal internodes of the main shoot and tiller 1 and 2 (Kebrom *et al.*, 2012). Present contrasting results in the BC_1 and BC_3 lines are consistent with the suggestion that the effects of *Tin1A* gene on tiller restriction and ear fertility and grain yield depend on the genetic background (Mitchell *et al.*, 2013, Gaju *et al*., 2014b, Hendriks *et al*., 2016).

8.2.2 Effects of a 3A QTL on grain yield and ear fertility traits

In the present study, the presence of the $3A_{WMC428}$ allele from L14 advanced line had no effect on grain yield, grains m^{-2} and HI or EPI and FE. However, this allele increased spikelets ear^{-1} by 7%, fertile spikelets ear^{-1} by 8%, rachis length by 16%, fertile florets ear^{-1} by 23% and fertile florets $spikelet^{-1}$ by 13%. It is possible that the 3A QTL is having an effect on spikelet primordia production increasing spikelets ear^{-1} and GN ear^{-1}.It is also possible that the high sink capacity (spikelets ear^{-1}, GN

spikelets^{-1}, rachis length) contributed to the increased allocation of dry matter to the ear. Also, the increase in spikelets per ear could be associated with an effect of the 3A QTL on earliness *per se*. For future application of this QTL in winter wheat breeding it may be necessary to combine the 3A QTL with QTL(s) for enhancing florets per spikelet and/or other QTLs for traits reducing the trade-off between spikelet per ear and florets/grain per spikelet.

Among the several QTLs for yield and yield components identified on homoeologous groups 2, 3, 5, 7 chromosomes (Gupta *et al*., 2006), it has been suggested that QTLs on chromosome 3A are the most reproducibly identified and most important (Baenziger *et al*., 2011). Wheat chromosome 3A has been identified as an outstanding influence of grain yield and yield component traits (Rustgi *et al*., 2013). QTLs for yield components have been identified on chromosome 3A such as anthesis date, plant height, grains ear^{-1}, 1000 grain weight and ears m^{-2} and grains m^{-2} but not for the grain yield itself (Campbell *et al*., 2003, Ali *et al*., 2011, Mengistu *et al*., 2012, Rustgi *et al*., 2013). The presence of *earliness per se* genes on chromosome 3A was reported (Hoogendoorn, 1985, Miura and Worland, 1994, Miura *et al.*, 1999). A future study could be to fine map the 3A QTL in segregating lines developed from further backcrosses and with additional markers to validate and refine the precise location of the QTL.

8.3 Developing a wheat ideotype with physiological traits to increase ear fertility and grain yield

Breeders have been very successful in the past in direct selections for higher yield potential. Those selections were mainly made directly for grain yield and with limited consideration for the physiological processes limiting grain yield potential and therefore the mechanisms responsible for yield progress could not be identified with certainty in breeding programs.

Designing strategies for future yield improvement requires understanding the physiological processes determining grain yield. Studying traits from the physiological level to the gene level may be more effective way to improve yield by combining yield-enhancing traits in new genotypes than direct empirical selection for yield (Foulkes *et al*., 2011). Furthermore, because the genetic gain in yield potential resulting from traditional breeding has slowed down recently, improving the understanding of physiology has now become more important.

Using the ideotype approach in rice breeding was an effective way to produce the second generation of the new plant type of rice in China which yielded as high as 12 t ha^{-1} in farmers' fields (Peng *et al*., 2008). Moreover, it is very important to focus on the traits which are limiting yield and combine work between physiology and molecular studies to identify markers for selection for important yield-enhancing traits and to identify synergies between traits to improve wheat yield potential. Trait-based physiological breeding has been used successfully to improve drought adaptation of wheat in Australia and by CIMMYT, including higher transpiration efficiency, greater early vigour, reduced tillering, and dehydration avoidance (Reynolds and Tuberosa, 2008).

The presently reported yield variation above the Humber recurrent parent in the BC_3 and BC_1 lines in the winter wheat background were associated with improvement in traits to increase ear fertility and photosynthetic capacity through introgression of genes from wider germplasm. These traits included:

- Increased duration of GS31 to GS61 phase as a proportion of thermal time to anthesis
- Spikelet number per ear
- Fruiting efficiency
- Post-anthesis stay-green traits (slow NDVI senescence rate and high NDVI value at physiological maturity)

This combination of traits was supported by a stepwise multiple regression analysis (all subsets) carried in in Genstat version 16th. Firstly from the trait correlation matrices in Chapter 4-6 (for traits relating to grain yield) and in Chapter 5 (for traits relating to grains m^{-2}), those traits most consistently associated with grain yield and grains m^{-2} in the correlation matrices were identified. These traits were then used in the stepwise multiple linear regression analysis. The subsets of traits initially identified were:

Grain yield: days sowing to GS31 ($Days_{31}$); Fruiting efficiency (FE); NDVI at GS61+20 d ($NDVI_{20d}$); NDVI senescence rate ($NDVI_{SR}$).

Grains m^{-2}: Fruiting efficiency (FE); NDVI at GS61+20d ($NDVI_{20d}$); Spikelets per main shoot ear ($SPNO_{MS}$).

For grain yield the multiple linear regression was based on genotypes means across 2013 and 2014, since correlations identified were consistent in both years. For grain m^{-2}, the multiple linear regression analysis was based on 2014 only since trait correlations were not consistent across years, and the strongest correlations were identified in 2013.

The results of the multiple linear regression analysis are shown in Table 8.1 and 8.2 below.

Table 8.1 Stepwise multiple linear regression for 37 Humber BC_3 lines for traits for grain yield. Values based on means of 2013 and 2014.

	Significance level				
	$NDVI_{20d}$	$NDVI_{SR}$	FE	$Days_{31}$	Adjusted R^2
1 Trait	< 0.001	-	-	-	44.6
	-	0.019	-	-	12.3
	-	-	-	0.12	4.11
	-	-	0.185	-	2.2
2 Traits	< 0.001	0.019	-	-	51.6
	< 0.001	-	0.057	-	48.8
	< 0.001	-	-	0.098	47.4
	-	0.003	-	0.017	23.8
	-	0.025	0.243	-	13.3
	-	-	0.107	0.072	8.7
3 traits	< 0.001	0.002	-	0.01	59.3
	< 0.001	0.025	0.075	-	54.8
	< 0.001	-	0.024	0.04	53.6
	-	0.004	0.112	0.01	27.4
4 traits	< 0.001	0.002	0.02	0.003	64.7

Best fitted model:

GY (t ha^{-1}) = 183.7 + 42.61 x $NDVI_{20d}$ + 4956 x $NDVI_{SR}$ - 0.159 x FE -1.052 x $Days_{31}$

Table 8.2 Stepwise multiple linear regression for 37 Humber BC_3 lines for traits for grains m^{-2}. Values based on means in 2013.

	Significance Level			
	$NDVI_{20d}$	FE	$SPNO_{MS}$	Adjusted R^2
1 Trait	-	-	0.014	
	0.034	-	-	13.6
	-	0.194	-	9.7
				2.1
2 Traits	0.067	-	0.028	19.5
	-	0.08	0.007	18.8
	0.27	0.144	-	12.8
3 Traits	0.054	0.064	0.014	25.4

Best fitted model:

Grains m^{-2} = -1929 + 91109 x $NDVI_{20d}$ + 408 x FE - 2079 x $SPNO_{MS}$

The best fitted model for grain yield included both source and sink-type traits consistent the suggestion that grain yield was co-limited by source and sink in the winter BC_3 NILs. The best model for grains m^{-2} confirmed the importance of combining high source capacity ($NDVI_{20d}$) with efficient conversion ear DM to grains (FE) for enhancing grain number per unit area.

The broad sense heritability is defined as the ratio of genetic variance to the genetic and environment variation (Meredith, 1984) and the higher ratio the more heritable the traits are. In this study, an indicator of heritability was taken as the ratio of the sums of squares for genotype to the sums of squares for (i) genotypes , (ii) year and (iii) year x genotype from the cross-year ANOVA. On this basis, there was high heritability for ears per m^2 (0.66), grains ear^{-1} (0.78) and grains $spikelet^{-1}$ (0.91) in spring NILs and high heritability for grains $spikelet^{-1}$ (0.65) but moderate heritability for ears per m^2 (0.44), rachis length (0.42) and spikelets ear^{-1} (0.38) in winter NILs. There were year x genotype effects for grain yield, grains m^{-2}, ears m^{-2}, HI, fertile shoots $plant^{-1}$, AGDM $shoot^{-1}$, TGW, spikelets ear^{-1}, fertile spikelets ear^{-1} and rachis length in BC_3 spring NILs and year x genotype effects for HI and rachis length in BC_3 winter NILs. Nevertheless, it is encouraging that the heritability for key target traits such as grains ear^{-1}, spikelets ear^{-1}, grains $spikelet^{-1}$ and rachis length were higher than grain yield in both BC_3 spring and winter NILs.

In the present study, there was generally a negative correlation between ear fertility traits and tillering traits. However, some BC_3 winter lines (line 167, 171, 177) showed a combination of high grains ear^{-1} and ears m^{-2}. EPI may be strongly linked to shoots m^{-2}, whereas FE and spikelets per ear appeared to be less associated with tillering traits, so it may be possible to combine high ears per m^2 with FE and spikletes per m^2 to increase the efficiency of breeding for greater yield potential. So there are prospects for uncoupling grains per ear and shoots per m^2 through selection for higher FE, spikelets per ear and shoot number per plant. A positive

relationship between genetic variation in tillers per plant and spikelets per ear has been reported by Kato *et al.* (2000).

In the present study, significant genetic variation in ear fertility traits including spikelets ear^{-1}, fertile spikelets ear^{-1}, grains ear^{-1} and grains spikelet^{-1} and the number of fertile shoots plant^{-1} was observed in BC_1S_7 NILs in the spring (Ashby) and winter (Humber) wheat backgrounds. In BC_1 spring and winter lines, although ears m^{-2} was fewer in +*Tin1A* BC_1 lines compared to -*Tin1A* BC_1 lines, +*Tin1A* lines produced more grains per ear than –*Tin1A* lines and resulted in similar grain yield and/or grains m^{-2} between +*Tin1A* and –*Tin1A* BC_1 NILs. In future work, crossing between selected BC_1 lines with the *Tin1A* and the Chr 3A QTLs to each Ashby or Humber background could be carried out to produce BC_2 and BC_3 NILs, with orthogonal contrasts of the *Tin1A* and the Chr 3A QTLs and associated traits for ears m^{-2}, grains ear^{-1} and spikelets ear^{-1} to test if there are additive effects of these QTLs to increase grains m^{-2} and map QTL associated with these traits.

8.4 Conclusions

- Results showed that grain yield of BC_3 spring wheat lines was lower than the Ashby parent mainly due to fewer fertile shoots plant^{-1} and lower grains m^{-2} but spring NILs had higher grains ear^{-1} compared to Ashby. On the other hand, grain yield of BC_3 winter wheat lines was higher than the Humber parent for two lines (up to 22%). Although there were no significant increases amongst winter NILs in grains m^{-2} and grains ear^{-1} compared to Humber, there were more spikelets ear^{-1} and more fertile florets spikelets^{-1} in NILs hence more fertile florets ear^{-1} compared to the recurrent parent.
- Grain yield variation amongst winter BC_3 NILs was associated with AGDM, TGW, and anthesis date, whereas in spring BC_3 NILs it was associated with ears m^{-2} and AGDM.

- The hypothesis that there is a positive correlation between grain weight and grain yield amongst the BC_3 NILs was confirmed in winter wheat lines. No correlation between grain weight and grain yield was observed in BC_3 spring wheat lines.
- The hypothesis that +*Tin1A* BC_3 NILs have reduced ears m^{-2} at harvest but increased GN ear^{-1}, spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length compared to the –*Tin1A* BC_3 NILs and the Ashby recurrent parent in the UK rainfed environment was confirmed in spring wheat NILs but this did not lead to higher grains m^{-2} since the effect of *Tin1A* gene on spikelet number and fertile floret number was too small to compensate for the reduced ears m^{-2}.
- In the present study, grain weight was strongly associated with rate of grain filling in both spring and winter NILs. No consistent association was found between grain weight and duration of grain filling in BC_3 spring or winter wheat lines.
- There is higher expression of source-type traits in BC_3 near-isogenic lines than recurrent parents Ashby and Humber e.g. NDVI stay green, NDVI senescence rate, SPAD at 650 °Cd, SPAD senescence rate and FLS rate.
- The results in in winter NILs showed there was evidence for source limitation of grain yield or co-limitation of grain yield by source and sink.
- In the KWS experiment, the +*Tin1A* BC_1S_7 NILs in the Ashby and Humber backgrounds produce fewer tillers m^{-2} and ears m^{-2} but higher or lower values for grain yield and yield components compared to the check line depending on the NIL pair indicating that degree of tiller inhibition and associated effects on yield and yield components with tiller inhibition gene may be dependent on the genetic background.
- The BC_3 NILs with the allele from CIMMYT L14 for a QTL on chromosome 3A caused increases in plant height, AGDM $shoot^{-1}$ and ear fertility traits including spikelets ear^{-1}, fertile spikelets ear^{-1}, GN ear^{-1} and rachis length but no significant effects on grain yield, grains m^{-2} and HI compared to the BC_3 NILs

compared with the allele from Humber and the Humber recurrent parent in the UK rainfed environment.

- The presence of an allele from Rialto for a QTL on chromosome 7B (WMC696) in the BC_3 NILs had a positive effects on grain yield but no differences in grains m^{-2} and ears m^{-2} compared to BC_3 NILs with the Humber recurrent parent.

8.5 Future work

1. To study the effects of tiller inhibition gene and the interaction with plant density, it will be important to increase seed rate to qualify the optimum plant densities. Also, the effects of the tiller inhibition gene on root-shoot carbon partitioning in the Ashby background under rainfed environments should be investigated
2. More detailed physiological studies to examine the physiological basis of apparent spikelet number in BC_3 with plant breeding and measurements of spikelet primordia production and rate in relation to earliness *per se* responses should be investigated.
3. Wider ranges of novel germplasm (synthetic wheats, landraces, introgression lines, diploid progenitors etc.) should be screened to identify further genetic variation in the target traits identified in this study.
4. Also the development of the near-isogenic lines (BC_3 to BC_6) through back-crossing will be crucial for precise physiological studies and fine mapping to develop molecular markers for the key traits for use in marker-assisted selection. Markers can be used for progeny selection and to identify parental crosses with synergistic combinations of traits. The use of crop simulation to model and to predict the joint optimization of traits in new ideotypes will be important in this context.

5. Future Research approaches should also combine physiological and genetic analysis to strengthen understanding of the genetic controls of ear partitioning index (EPI) and fruiting efficiency (FE). It may be useful to place some emphasis on defining the nature and impact of hormonal signalling (meristem/spikelet hormone concentration (ABA, ACC, IAA, cytokinins) in determining thresholds of assimilate requirement for floret survival in the spikelets.
6. Present results indicated associations between post-anthesis source-type traits (e.g. NDVI, leaf SPAD and leaf senescence visual score and senescence parameters) and grain yield in winter BC_3 NILs. Further work is required to develop new in-field high-throughput phenotyping tools for phenotyping canopy photosynthetic capacity, such as:

   - NDVI, (canopy size and architecture) and other spectral reflectance indices, e.g. NIR Water Index (Biomass)
   - Chlorophyll fluorescence (leaf photosynthetic efficiency)
   - Canopy Temperature Depression (canopy photosynthesis),

   including the development of aerial platforms for these canopy sensors.

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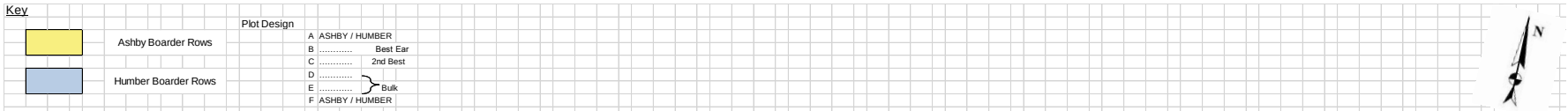
Appendices

Appendix 1. Materials and Methods

Table A1-1 Scale of ear initial and pistil morphogenesis for wheat (Waddington *et al*., 1983).

Developmental score (stage)	Description	Days after emergence
1 5	Transition apex	14
2	Early double ridge stage	20
2·5	Double ridge stage	26
3	Glume primordium present	30
(3·25)	Lemma primordium present	32
3 5	Floret primordium present	35
4	Stamen primordium present	38
(4·25)	Pistil primordium present	41
4 5	Carpel primordium present	46
5	Carpel extending round three sides of ovule	49
5·5	Stylar canal closing; ovarian cavity enclosed on all sides but still open above	53
6	Stylar canal remaining as a narrow opening; two short round style primordia present	59
6·5	Styles begin elongating [Fig. 6(a)]	64
7	Stigmatic branches just differentiating as swollen cells on styles	69
7·5	Unicellular hairs just differentiating on ovary wall; stigmatic branches elongating	72
8	Stigmatic branches and hairs on ovary wall elongating [Fig. 6(b)]	78
8·5	Stigmatic branches and hairs on ovary wall continue to elongate; stigmatic branches form a tangled mass	82
9	Styles and stigmatic branches erect; stigmatic hairs differentiating [Fig. 6(c)]	89
9·5	Styles and stigmatic branches spreading outwards. Stigmatic hairs well developed	93
10	Styles curved outwards and stigmatic branches spread wide; pollen grains on well-developed stigmatic hairs	98

Table A1-2 Field trial at Sutton Bonington Campus, The University of Nottingham in 2012/13: Experimental design.



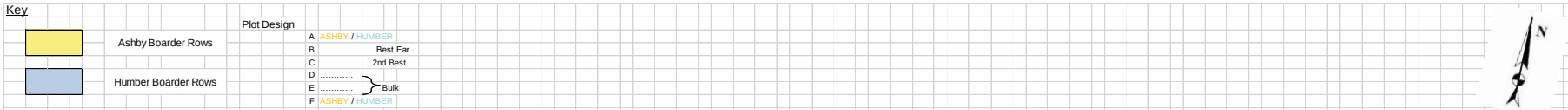
Rep 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ashby	Ashby	B3S3 29907-4-37-3	B3S3 29907-4-37-4	B3S3 29907-12-58-2	B3S3 29907-12-58-3	B3S3 29907-12-58-4	B3S3 29907-12-58-7	B3S3 29907-13-60-2	B3S3 29907-13-60-8	B3S3 29908-1-67-3	B3S3 29908-6-79-4	B3S3 29908-6-80-2	B3S3 29908-6-80-3	B3S3 29908-9-85-1	B3S3 29908-13-95-1	B3S3 29909-6-109-3	Humber	Humber	B3S3 29910-9-139-1
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
B3S3 29910-9-139-2	B3S3 29910-9-139-6	B3S3 29910-9-140-1	B3S3 29911-3-159-1	B3S3 29911-3-159-2	B3S3 29911-3-159-3	B3S3 29911-3-159-4	B3S3 29911-14-171-1	B3S3 29911-14-171-2	B3S3 29911-14-171-3	B3S3 29911-14-171-4	B3S3 29911-14-172-1	B3S3 29911-14-172-2	B3S3 29911-14-172-3	B3S3 29911-14-172-5	B3S3 29911-14-172-6	B3S3 29911-14-172-7	B3S3 29911-14-172-8	B3S3 29914-5-196-1	B3S3 29914-5-197-2
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
B3S3 29914-6-198-1	B3S3 29914-6-198-2	B3S3 29914-6-198-3	B3S3 29914-6-198-4	B3S3 29914-6-198-5	B3S3 29914-6-199-3	B3S3 29914-6-199-7	B3S3 29914-6-200-3	B3S3 29914-6-200-5	B3S3 29914-6-200-6	B3S3 29914-8-203-1	B3S3 29914-8-203-7	B3S3 29914-8-204-2	B3S3 29914-9-205-1	B3S3 29914-10-207-1	B3S3 29914-10-207-2	B3S3 29914-10-207-3	B3S3 29914-10-207-4	B3S3 29914-10-207-8	B3S3 29914-12-209-1
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
B3S3 29915-2-217-2	B3S3 29915-2-217-5	B3S3 29915-2-217-6	B3S3 29915-3-218-1	B3S3 29915-3-218-2	B3S3 29915-3-219-1	B3S3 29915-3-219-2	B3S3 29915-3-219-3	B3S3 29915-3-219-4	B3S3 29915-4-222-2	B3S3 29915-4-222-5	B3S3 29915-7-225-3	B3S3 29917-5-273-1	B3S3 29917-5-273-2	B3S3 29917-5-273-3	B3S3 29917-5-273-4	B3S3 29917-5-273-5	B3S3 29917-5-273-6	B3S3 29917-5-273-7	B3S3 29917-5-273-8
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
B3S3 29917-5-274-1	B3S3 29917-5-274-2	B3S3 29917-5-274-3	B3S3 29917-5-274-4	B3S3 29917-8-282-1	B3S3 29917-8-282-2	B3S3 29917-8-283-1	B3S3 29917-8-283-5	B3S3 29917-8-283-6	B3S3 29917-8-283-7	B3S3 29917-10-285-2	B3S3 29917-10-285-3	B3S3 29922-8-382-3	B3S3 29922-8-382-7	B3S3 29923-8-397-1	B3S3 29923-8-397-2	B3S3 29923-12-406-4	B3S3 29923-14-411-1	B3S3 29923-14-412-2	B3S3 29923-14-412-7

Rep 2

101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
Ashby	Ashby	B3S3 29908-1-67-3	B3S3 29907-12-58-2	B3S3 29909-6-109-3	B3S3 29907-13-60-2	B3S3 29908-13-95-1	B3S3 29907-4-37-4	B3S3 29908-6-79-4	B3S3 29908-6-80-3	B3S3 29907-4-37-3	B3S3 29908-9-85-1	B3S3 29907-12-58-4	B3S3 29908-6-80-2	B3S3 29907-13-60-8	B3S3 29907-12-58-3	B3S3 29907-12-58-7	Humber	Humber	B3S3 29915-2-217-2
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140
B3S3 29917-5-273-2	B3S3 29914-5-196-1	B3S3 29917-10-285-3	B3S3 29914-12-209-1	B3S3 29914-5-197-2	B3S3 29914-6-199-7	B3S3 29915-7-225-3	B3S3 29914-6-198-1	B3S3 29917-5-274-2	B3S3 29911-3-159-1	B3S3 29917-5-273-3	B3S3 29914-10-207-4	B3S3 29917-5-273-7	B3S3 29915-2-217-6	B3S3 29911-3-159-4	B3S3 29917-8-283-6	B3S3 29914-10-207-8	B3S3 29917-5-274-1	B3S3 29911-14-172-8	B3S3 29914-9-205-1
141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
B3S3 29917-5-273-8	B3S3 29911-14-171-3	B3S3 29917-5-274-3	B3S3 29910-9-139-6	B3S3 29917-8-283-7	B3S3 29911-14-172-1	B3S3 29917-5-274-4	B3S3 29911-14-171-4	B3S3 29914-10-207-1	B3S3 29915-2-217-5	B3S3 29914-6-198-4	B3S3 29914-10-207-3	B3S3 29914-6-200-3	B3S3 29911-14-171-2	B3S3 29917-8-282-2	B3S3 29914-8-203-7	B3S3 29910-9-139-2	B3S3 29914-8-204-2	B3S3 29911-14-172-2	B3S3 29915-4-222-5
161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
B3S3 29917-5-273-1	B3S3 29910-9-140-1	B3S3 29915-3-219-3	B3S3 29923-14-412-7	B3S3 29911-14-171-1	B3S3 29923-8-397-2	B3S3 29911-14-172-6	B3S3 29917-10-285-2	B3S3 29915-3-219-1	B3S3 29911-14-172-5	B3S3 29915-4-222-2	B3S3 29910-9-139-1	B3S3 29917-8-283-1	B3S3 29914-8-203-1	B3S3 29915-3-218-2	B3S3 29917-5-273-4	B3S3 29914-6-200-5	B3S3 29917-5-273-5	B3S3 29914-6-200-6	B3S3 29922-8-382-3
181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
B3S3 29923-14-412-2	B3S3 29914-10-207-2	B3S3 29914-6-198-2	B3S3 29915-3-219-4	B3S3 29914-6-199-3	B3S3 29923-12-406-4	B3S3 29917-5-273-6	B3S3 29911-3-159-3	B3S3 29917-8-282-1	B3S3 29923-8-397-1	B3S3 29914-6-198-3	B3S3 29915-3-218-1	B3S3 29915-3-219-2	B3S3 29911-14-172-7	B3S3 29917-8-283-5	B3S3 29914-6-198-5	B3S3 29911-3-159-2	B3S3 29922-8-382-7	B3S3 29923-14-411-1	B3S3 29911-14-172-3

Table A1-3 Field trial at Sutton Bonington Campus, The University of Nottingham in 2013/14: Experimental design**.**



Rep 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1-1de	1-67bc	5-15b	6-64bc	19-12bc	20-127de	21-78de	23-65de	25-6bc	27-138bc	32-129bc	33-124b	34-9de	35-18de	36-130bc	44-138de	52-10bc	53-106bc	53-147bc	54-173de
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
55-85de	59-174bc	62-51de	65-114bc	66-148bc	67-36de	68-239de	72-94de	73-98de	74-56de	75-42bc	76-211bc	77-155de	78-40bc	79-203de	80-45de	81-27bc	82-153de	83-157bc	88-162bc
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
91-31de	92-60de	93-26bc	94-168bc	95-231de	98-142bc	102-232bc	106-170de	108-101bc	109-291de	112-34de	118-223de	121-99bc	124-112bc	128-149bc	129-93bc	130-201bc	131-88bc	135-209bc	136-100bc
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
141-225de	144-113bc	145-117bc	148-90bc	149-240bc	150-56bc	151-207bc	152-263de	153-97bc	158-44bc	160-160bc	167-92de	170-284bc	171-88de	172-41de	173-33de	174-36bc	175-38de	176-109bc	177-221bc
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
178-175bc	179-87bc	180-52de	181-110bc	184-43de	185-165de	191-180de	194-50bc	195-146bc	196-55de	199-82de	200-173bc	204-34bc	207-108bc	209-296de	210-29bc	223-174de	229-104de	238-105de	243-280bc

Rep 2

101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
52-129de	35-133bc	19-16de	36-140de	23-74bc	27-138bc (2)	5-15b (2)	33-124b (2)	20-127de (2)	6-64bc (2)	32-129bc (2)	1-64de	44-14bc	25-6bc (2)	34-135bc	21-127bc	1-67de	124-102de	175-239bc	92-31bc
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140
108-277de	72-145bc	199-233bc	145-267de	151-205de	179-84de	102-219de	184-212bc	243-273de	106-296bc	172-96bc	153-150de	68-288bc	160-144de	77-273bc	178-234de	191-266bc	54-143bc	238-261bc	149-284de
141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
209-276bc	94-294de	67-219bc	121-146de	200-230de	76-278de	130-266de	144-143de	66-232de	53-106de	91-265bc	176-288de	82-230bc	204-86de	136-292de	98-214de	170-265de	118-267bc	83-221de	171-150bc
161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
141-222bc	78-103de	195-297de	81-240de	53-147de	128-270de	180-82bc	62-274bc	194-262de	93-166de	112-292bc	210-152de	75-295de	158-227de	131-289de	152-262bc	55-178bc	185-238bc	148-236bc	223-210bc
181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
167-275bc	181-220de	74-170bc	177-293de	109-269bc	174-81de	88-282de	150-142de	73-226bc	59-209de	229-90bc	129-201de	79-238bc	95-227bc	207-274de	173-166bc	65-268de	196-294bc	135-280de	80-203bc

Table A1-4 Field trial at Sutton Bonington Campus, The University of Nottingham in 2012/13: Agronomy.

Title		Ear Fertility BC3 Mini Plots	
Researcher		John Foulkes	
	Date	**Details**	**Operator**
Field		S26	
Previous crop		Winter Oats	
SNS N Index	2/3/2013	20 kg/ha, SNS Index 0	JPA / MGT
Soil Indices		P:4, K:4, Mg:4, pH:7.5	
Cultivations	12/9/2012	Plough + Press	JPA
	5/11/2012	Roll after drilling	JPA
Crop/variety		Various (see seedrates tab)	
TGW (g)		Various (see seedrates tab)	
Sowing	5/11/2012		JPA / RW
Seed rate (m-2)		250 seeds m^2	
Drill type		Haldrup mini plot drill	
Row width (m)		0.2	
Plot length (m)		1.0	
Plot width (m)		1.0	
Fertiliser	27/2/2013	Manganese 15% @1l/ha	MGT
	6/3/2013	116 kg/ha 34.5% Nitram (40kg/ha N)	JPA
	21/3/2013	Manganese 15% @ 2l/ha	MGT
	17/4/2013	232 kg/ha 34.5% Nitram (80kg/ha N)	JPA
	2/5/2013	Manganese 15% @ 2l/ha	MGT
	13/5/2013	Manganese 15% @ 1l/ha	MGT
	21/5/2013	174 kg/ha 34.5% Nitram (60kg/ha N)	JPA
	3/6/2013	Magnor @ 1.0 l/ha	JPA
Herbicide	2/10/2012	Round-up @ 2.5 l/ha (Pre-drilling)	JPA
	30/10/2012	Round-up @ 2.5 l/ha (Pre-drilling)	JPA
	10/4/2013	Hatra @ 1l/ha + Charge @ 2l/ha + Liberator @ 0.3l/ha + Zeal @ 0.3l/ha	MGT
	3/6/2013	Lorate @ 25g/ha	JPA
Fungicide	2/5/2013	Kestrel @ 1l/ha + Phoenix @ 1l/ha + Flexity @ 0.14l/ha	MGT
	3/6/2013	Brutus @ 1.25 l/ha + Amistar Opti @ 1.0 l/ha + Vegas @ 0.15 l/ha	JPA
	26/6/2013	Brutus @ 1l/ha + Bravo @ 1l/ha + Instinct @ 0.5l/ha	MGT
Insecticide	19/11/2012	Slug pellets @ 3kg/ha	MGT
PGR	2/5/2013	Chlormequat @ 1.4l/ha	MGT
	13/5/2013	Moddus @ 0.2l/ha	MGT
	3/6/2013	Terpal @ 0.5 l/ha	JPA

Table A1-5 Field trial at Sutton Bonington Campus, The University of Nottingham in 2013/14: Agronomy.

Title		Ear Fertility BC3 Mini Plots	
Researcher		John Foulkes	
	Date	**Details**	**Operator**
Field		S2	
Previous crop		Spring OSR	
SNS N Index		54 kg/ha, SNS Index 0	JPA / MGT
Soil Indices		P:5, K:2, Mg:4, pH:6.8	
Cultivations	8/10/2013	Plough + Press	JPA
		Roll after drilling	
Crop/variety		Various (see seedrates tab)	
TGW (g)		Various (see seedrates tab)	
Sowing	24/10/2013		MGT/JPA
Seed rate (m-2)		250 seeds m^2	
Drill type		Haldrup mini plot drill	
Row width (m)		0.2	
Plot length (m)		1.0	
Plot width (m)		1.0	
Fertiliser	12/3/2014	116 kg/ha 34.5% Nitram (40kg/ha N)	JPA
	14/3/2014	Human extra @ 1l/ha	MGT
	2/4/2014	Human extra @ 1l/ha	MGT
	28/4/2014	232 kg/ha 34.5% Nitram (80kg/ha N)	JPA
	28/4/2014	Human extra @ 1.1l/ha	MGT
	14/5/2014	174 kg/ha 34.5% Nitram (60kg/ha N)	JPA
	21/5/2014	Magnor @ 1l/ha	MGT
Herbicide	12/3/2014	Hatra @ 1.2l/ha + Headland charge @ 2l/ha + Zeal @ 0.3l/ha	MGT
	14/5/2014	Traton SX @ 45g/ha + Kantor @ 150ml/ha	MGT
	16/5/2014	Axial @ 0.39l/ha + Adigor @ 0.75l/ha	MGT
Fungicide	2/4/2014	Brutus @ 0.5l/ha + Bravo 500 @ 1l/ha + Vegas @ 0.13l/ha	MGT
	28/4/2014	Kestrel @ 0.75l/ha + Vegas @ 0.13l/ha + Phoenix @ 0.6l/ha	MGT
	21/5/2014	Opera @ 1l/ha + Phoenix @ 1l/ha + Brutus @ 1.2l/ha	MGT
	16/6/2014	Kestrel @ 0.75l/ha + Kantor @ 0.15l/ha	MGT
Insecticide	14/3/2014	Minuet @ 0.1l/ha	MGT
PGR	2/4/2014	Adjust @ 1l/ha	MGT
	28/4/2014	Adjust @ 0.8l/ha + Cleancrop Cutlass @ 0.2l/ha	MGT

Table A1-6 Active ingredient(s) of fertilizer, PGR, herbicide, fungicide and insecticide.

Common names	Active ingredient(s)
Fertilizer and PGR	
Chlormequat	chlormequat chloride
Moddus	trinexapac-ethyl
Terpal	mepiquat chloride + 2-chloroethyl phosphonic acid
Cleancrop Cutless	trinexapac-ethyl
Herbicide	
Round-up	glyphosate
Charge	carfentrazone-ethyl + sulfentrazone
Liberator	flufenacet + diflufenican
Zeal	metsulfuron-methyl
Lorate	metsulfuron-methyl
Headland charge	mecoprop-p
Traton SX	metsulfuron-methyl + tribenuron-methyl
Kantor	florasulam
Axial	pinoxaden + cloquintocet-mexyl
Adigor	methylated rapeseed oil + ethoxylated alcohols
Fungicide	
Kestrel	prothioconazole + tebuconazole
Phoenix	lactofen
Flexity	metrafenone
Brutus	epoxiconazole + metconazole
Amistar Opti	azoxystrobin + chlorothalonil
Vegas	cyflufenamid
Bravo	chlorothalonil
Instinct	fenpropidin
Opera	epoxiconazole + pyraclostrobin
Insecticide	
Minuet	zeta-cypermethrin
Slug pellets	metaldehyde

Table A2-1 Pearson correlation (r) amongst BC_3 spring wheat lines for grain yield, $ears_{CAL.}$ m^{-2}, grains m^{-2}, HI, AGDM m^{-2}, AGDM $plant^{-1}$, anthesis date (AD), plant height (PH), fertile shoots $plant^{-1}$, TGW, GN ear^{-1}, GDW $plant^{-1}$, GN $plant^{-1}$, fertile spikelets ear^{-1}, GN $spikelet^{-1}$ and rachis length ear^{-1} in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Ears m^{-2} $_{CAL.}$**	**Grains m^{-2}**	**HI**	**AGDM (t ha^{-1})**	**AGDM (g) $plant^{-1}$**	**AD**	**PH (cm)**	**FS $plant^{-1}$**	**TGW (g)**	**GN ear^{-1}**	**GDW (g) $plant^{-1}$**	**GN $plant^{-1}$**	**Fertile spikelets ear^{-1}**	**GN $spikelet^{-1}$**	**RL ear^{-1}**
Grain yield	-	**0.985*****	**0.996*****	0.178	**0.996*****	0.318	0.132	0.303	0.582	**-0.699**†	**-0.739**†	0.321	0.367	-0.467	-0.644	**-0.950****
Ears m^{-2} $_{CAL.}$	**0.870***	-	**0.987*****	0.093	**0.990*****	0.245	0.223	0.253	0.546	-0.654	**-0.747**†	0.241	0.287	-0.385	**-0.674**†	**-0.909****
Grains m^{-2}	**0.973*****	**0.916****	-	0.164	**0.995*****	0.355	0.098	0.336	0.536	**-0.730**†	**-0.702**†	0.356	0.401	-0.399	-0.618	**-0.933****
HI	-0.339	**-0.705**†	-0.510	-	0.092	0.402	0.093	**0.819***	0.148	-0.205	0.275	0.488	0.442	-0.550	0.447	-0.408
AGDM m^{-2}	**0.980*****	**0.930****	**0.992*****	-0.515	-	0.293	0.119	0.245	0.559	**-0.700**†	**-0.761***	0.288	0.339	-0.410	-0.279	**-0.924****
AGDM $plant^{-1}$	0.588	0.186	0.5004	0.272	0.504	-	-0.476	**0.707**†	0.045	**-0.690**†	0.187	**0.995*****	**0.995*****	-0.038	0.241	-0.262
AD	0.092	0.494	0.192	-0.653	0.200	-0.308	-	-0.190	0.507	0.484	-0.383	-0.452	-0.493	-0.398	-0.301	-0.149
PH	**0.889****	**0.697**†	**0.839***	-0.249	**0.879****	**0.761***	0.039	-	-0.109	-0.566	0.378	**0.760***	**0.734**†	-0.140	0.487	-0.415
FS $plant^{-1}$	**0.867***	**0.696**†	**0.813***	-0.136	**0.812***	**0.727**†	0.183	0.785*	-	0.044	**-0.793***	0.065	0.045	**-0.671**†	**-0.734**†	-0.538
TGW	0.100	-0.192	-0.107	0.625	-0.03	0.521	-0.230	0.368	0.249	-	0.160	**-0.672**†	**-0.741**†	0.114	0.068	**0.668**†
GN ear^{-1}	-0.393	**-0.733**†	-0.441	0.652	-0.456	0.425	-0.642	-0.091	-0.292	-0.362	-	0.206	0.165	0.474	**0.973*****	0.634
GDW $plant^{-1}$	0.493	0.053	0.381	0.434	0.383	**0.985*****	-0.398	**0.669**†	0.659	0.599	0.512	-	**0.993*****	-0.083	0.271	-0.291
GN $plant^{-1}$	0.579	0.171	0.513	0.288	0.488	**0.971*****	-0.350	**0.669**†	0.733†	0.367	0.416	**0.962*****	-	-0.092	0.236	-0.331
Fertile spikelets ear^{-1}	-0.533	**-0.818***	-0.551	0.590	-0.577	0.180	**-0.778***	-0.345	-0.464	0.073	**0.887****	0.267	0.225	-	0.274	0.630
GN $spikelet^{-1}$	-0.255	-0.617	-0.317	0.638	-0.326	0.540	-0.577	-0.070	-0.160	0.473	**0.979*****	0.620	0.510	**0.778***	-	0.485
RL ear^{-1}	-0.660	**-0.818***	-0.617	0.398	-0.654	0.016	-0.568	-0.473	-0.577	-0.131	**0.822***	0.080	0.071	**0.952*****	**0.694**†	-

† *P≤0.10*, * *P≤0.05*, ** *P≤0.01*, *** *P≤0.001*

Table A2-2 Pearson correlation (r) amongst BC_3 spring wheat lines for grain yield, $ears_{CAL.}$ m^{-2}, grains m^{-2}, HI, plant height (PH), fertile shoots $plant^{-1}$, GN ear^{-1}, fertile spikelets ear^{-1}, AGDM MS^{-1}, GDW MS^{-1}, GN MS^{-1}, MS fertile spikelets ear^{-1}, MS GN $spikelet^{-1}$, MS rachis length and $ears_{COUNT}$ m^{-2} in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Ears m^{-2} CAL.**	**Grains m^{-2}**	**HI**	**PH (cm)**	**FS $plant^{-1}$**	**GN ear^{-1}**	**Fertile spikelets ear^{-1}**	**AGDM (g) MS^{-1}**	**GDW (g) MS^{-1}**	**GN MS^{-1}**	**MS Fertile spikelets ear^{-1}**	**MS GN $spikelet^{-1}$**	**MS RL (cm)**	**Ears m^{-2} COUNT**
Grain yield (t ha^{-1})	-	**0.985*****	**0.996*****	0.178	0.303	0.582	**-0.739**†	-0.467	**-0.718**†	**-0.821***	**-0.759***	**-0.829***	-0.593	**-0.955*****	**0.921****
Ears m^{-2} CAL.	**0.870***	-	**0.987*****	0.093	0.253	0.546	**-0.747**†	-0.385	**-0.780***	**-0.858***	**-0.794***	**-0.845***	-0.640	**-0.939****	**0.913****
Grains m^{-2}	**0.973*****	**0.916****	-	0.164	0.336	0.536	**-0.702**†	-0.399	**-0.693**†	**-0.802***	**-0.730**†	**-0.801***	-0.570	**-0.937****	**0.933****
HI	-0.339	**-0.705****	-0.510	-	**0.819***	0.148	0.275	-0.550	0.300	0.315	0.282	-0.104	0.434	-0.211	0.264
PH (cm)	**0.889****	**0.697**†	**0.839***	-0.249	-	-0.109	0.378	-0.140	0.319	0.276	0.342	-0.048	0.479	-0.232	0.444
FS $plant^{-1}$	**0.867***	**0.696**†	**0.813***	-0.136	**0.785***	-	**-0.793***	**-0.671**†	-0.536	-0.636	**-0.731**†	-0.426	**-0.762***	-0.516	0.363
GN ear^{-1}	-0.393	**-0.733**†	-0.441	0.652	-0.091	-0.292	-	0.474	**0.872***	**0.955*****	**0.981*****	**0.729**†	**0.941****	**0.736**†	-0.546
Fertile spikelets ear^{-1}	-0.533	**-0.818***	-0.551	0.590	-0.345	-0.464	**0.887****	-	0.416	0.335	0.405	0.560	0.236	0.561	-0.275
AGDM MS^{-1}	-0.037	-0.287	-0.123	0.345	0.407	0.059	**0.695**†	0.366	-	**0.933****	**0.914****	**0.843***	**0.799***	**0.757***	-0.528
GDW MS^{-1}	-0.138	-0.468	-0.251	0.582	0.269	0.015	**0.824***	0.530	**0.960*****	-	**0.981*****	**0.816***	**0.903****	**0.814***	**-0.676**†
GN MS^{-1}	0.084	-0.260	0.005	0.473	0.427	0.215	**0.773***	0.411	**0.902****	**0.932****	-	**0.788***	**0.945****	**0.768***	-0.608
MS Fertile spikelets ear^{-1}	-0.549	**-0.823***	-0.567	**0.687**†	-0.373	-0.323	**0.907****	**0.875****	0.426	0.617	0.588	-	0.545	**0.946****	**-0.786***
MS GN $spikelet^{-1}$	0.330	-0.020	-0.245	0.292	0.661	0.387	0.566	0.160	**0.889****	**0.854***	**0.950****	0.308	-	0.543	-0.421
MS RL	**-0.718**†	**-0.835***	-0.663	0.421	-0.569	-0.542	**0.782***	**0.877****	0.241	0.388	0.319	**0.915****	0.024	-	**-0.909****
Ears m^{-2} COUNT	**0.931****	**0.960*****	**0.927****	-0.542	**0.821***	**0.719**†	-0.601	**-0.751**†	-0.108	-0.278	-0.085	**-0.780***	0.212	**-0.877****	-

† *P≤0.10,* * *P≤0.05,* ** *P≤0.01,* *** *P≤0.001*

Table A2-3 Pearson correlation (r) amongst BC_3 winter wheat near-isogenic lines for grain yield, ears m^{-2}, grains m^{-2}, HI, AGDM m^{-2}, AGDM plant^{-1}, anthesis date (AD), plant height (PH), fertile shoots plant^{-1}, TGW, GN ear^{-1}, GDW plant^{-1}, GN plant^{-1}, fertile spikelets ear^{-1}, GN spikelet^{-1} and rachis length ear^{-1} in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Ears m^{-2}**	**Grains m^{-2}**	**HI**	**AGDM (t ha^{-1})**	**AGDM (g) plant^{-1}**	**AD**	**PH (cm)**	**FS plant^{-1}**	**TGW (g)**	**GN ear^{-1}**	**GDW (g) plant^{-1}**	**GN plant^{-1}**	**Fertile spikelets ear^{-1}**	**GN spikelet^{-1}**	**RL ear^{-1}**
Grain yield	-	**0.766*****	**0.863*****	-0.113	**0.967*****	-0.068	**0.420****	0.244	-0.155	0.169	-0.041	-0.088	-0.131	-0.231	0.213	-0.047
Ears m^{-2}	**0.611*****	-	**0.816*****	**-0.372***	**0.812*****	**-0.499****	**0.410***	-0.062	**-0.325***	-0.172	**-0.500****	**-0.548*****	**-0.468****	**-0.445****	-0.137	**-0.483****
Grains m^{-2}	**0.784*****	**0.776*****	-	**-0.424****	**0.921*****	-0.097	**0.488****	0.150	-0.117	**-0.350***	0.082	-0.176	-0.030	-0.160	**0.288**†	-0.029
HI	-0.078	**-0.511****	-0.261	-	**-0.362***	0.167	**-0.540*****	**-0.390***	0.187	**0.625*****	-0.041	**0.353***	0.113	0.192	-0.260	0.044
AGDM m^{2}	**0.888*****	**0.751*****	**0.781*****	**-0.522*****	-	-0.103	**0.528*****	**0.327***	-0.189	-0.008	-0.022	-0.170	-0.146	-0.264	0.270	-0.051
AGDM plant^{-1}	0.067	-0.206	-0.005	-0.168	0.138	-	0.005	0.241	**0.856*****	0.071	**0.701*****	**0.981*****	**0.960*****	**0.490****	**0.393***	**0.731*****
AD	**0.503****	0.172	**0.275**†	-0.160	**0.474****	0.046	-	**0.594*****	-0.101	-0.196	0.053	-0.101	-0.021	**-0.403***	**0.493****	-0.019
PH	**0.409***	-0.093	0.086	-0.100	**0.372***	0.121	**0.738*****	-	-0.110	0.152	**0.375***	0.154	0.100	-0.109	**0.579*****	**0.356***
FS plant^{-1}	-0.158	-0.016	0.005	-0.179	-0.048	**0.833*****	-0.239	**-0.299**†	-	-0.042	**0.342***	**0.848*****	**0.867*****	**0.384***	0.072	**0.444****
TGW	**0.387***	-0.208	**-0.267**†	**0.297**†	0.205	0.108	**0.351***	**0.484****	-0.242	-	-0.243	0.188	-0.174	-0.119	-0.176	-0.036
GN ear^{-1}	0.041	**-0.565*****	0.069	**0.376***	-0.139	**0.365***	0.103	**0.293**†	0.044	-0.035	-	**0.659*****	**0.745*****	**0.524*****	**0.685*****	**0.800*****
GDW plant^{-1}	0.042	**-0.358***	-0.085	0.129	-0.021	**0.955*****	0.004	0.094	**0.783*****	0.198	**0.476****	-	**0.932*****	**0.506****	**0.320**†	**0.702*****
GN plant^{-1}	-0.099	-0.253	0.048	0.017	-0.093	**0.901*****	-0.137	-0.114	**0.883*****	-0.219	**0.488****	**0.911*****	-	**0.539*****	**0.394***	**0.713*****
Fertile spikelets ear^{-1}	**-0.413***	**-0.492****	-0.264	0.258	**-0.461****	**0.433****	**-0.359***	**-0.269**†	**0.498****	-0.244	**0.439****	**0.508****	**0.613*****	-	-0.249	**0.673*****
GN spikelet^{-1}	**0.348***	-0.263	**0.265**†	**0.268**†	0.174	0.027	**0.380***	**0.510****	**-0.365***	0.146	**0.773*****	0.107	0.044	-0.216	-	**0.344***
RL ear^{-1}	0.154	**-0.307**†	-0.002	-0.078	0.141	**0.519****	**0.510****	**0.659*****	0.169	0.225	**0.525*****	**0.507****	**0.395***	**0.285**†	**0.353***	-

† *P≤0.10*, * *P≤0.05*, ** *P≤0.01*, *** *P≤0.001*

Table A2-4 Pearson correlation (r) amongst BC_3 winter wheat near-isogenic lines for grain yield, $ears_{CAL.}$ m^{-2}, grains m^{-2}, HI, anthesis date (AD), plant height (PH), fertile shoots $plant^{-1}$, GN ear^{-1}, fertile spikelets ear^{-1}, AGDM MS^{-1}, GDW MS^{-1}, GN MS^{-1}, MS fertile spikelets ear^{-1}, MS GN $spikelet^{-1}$, MS rachis length and $ears_{COUNT}$ m^{-2} in 2013 (unshaded matrix) and 2014 (shaded matrix).

	Grain yield (t ha^{-1})	**Ears m^{-2} CAL.**	**Grains m^{-2}**	**HI**	**AD**	**PH (cm)**	**FS plant^{-1}**	**GN ear^{-1}**	**Fertile spikelets ear^{-1}**	**AGDM (g) MS^{-1}**	**GDW (g) MS^{-1}**	**GN MS^{-1}**	**MS Fertile spikelets ear^{-1}**	**MS GN spikelet^{-1}**	**MS RL (cm)**	**Ears m^{-2} COUNT**
Grain yield	-	**0.766*****	**0.863*****	-0.113	**0.420****	0.244	-0.155	-0.041	-0.231	-0.052	-0.145	-0.248	**-0.406***	0.128	-0.182	0.181
Ears m^{-2} CAL.	**0.611*****	-	**0.816*****	**-0.372***	**0.410***	-0.062	**-0.325***	**-0.500****	**-0.445****	**-0.564*****	**-0.649*****	**-0.592*****	**-0.524*****	-0.191	**-0.553*****	0.224
Grains m^{-2}	**0.784*****	**0.776*****	-	**-0.424****	**0.488****	0.150	-0.117	0.082	-0.160	-0.153	**-0.304**†	-0.125	**-0.357***	0.236	-0.151	0.081
HI	-0.078	**0.511****	-0.261	-	**-0.540*****	**-0.390***	0.187	-0.041	0.192	0.186	**0.455****	0.176	**0.433****	-0.250	0.144	0.046
AD	**0.503****	0.172	**0.275**†	-0.160	-	**0.594*****	-0.101	0.053	**-0.403***	-0.079	-0.197	**-0.281**†	**-0.713*****	**0.423****	-0.219	0.085
PH	**0.409***	-0.093	0.086	-0.100	**0.738*****	-	-0.110	**0.375***	-0.109	**0.496****	**0.326***	0.131	**-0.413***	**0.624*****	0.187	-0.071
FS plant^{-1}	-0.158	-0.016	0.005	-0.179	-0.239	**-0.299**†	-	**0.342***	**0.384***	0.203	0.236	**0.324**†	**0.310**†	0.085	**0.406***	-0.088
GN ear^{-1}	0.041	**-0.565*****	0.069	**0.376***	0.103	**0.293**†	0.044	-	**0.524*****	**0.760*****	**0.677*****	**0.834*****	**0.348***	**0.703*****	**0.738*****	-0.240
Fertile spikelets ear^{-1}	**-0.413***	**-0.492****	-0.264	0.258	**-0.359***	-0.269	**0.498****	**0.439****	-	**0.432****	**0.459****	**0.655*****	**0.799*****	-0.011	**0.637*****	**-0.353***
AGDM MS^{-1}	0.205	-0.158	-0.013	**-0.365***	**0.295**†	**0.456****	0.147	**0.302**†	-0.059	-	**0.945*****	**0.810*****	**0.388***	**0.627*****	**0.700*****	-0.256
GDW MS^{-1}	0.226	**-0.500****	-0.093	**0.498****	**0.325***	**0.493****	-0.079	**0.651*****	0.119	**0.554*****	-	**0.791*****	**0.479****	**0.503****	**0.662*****	0.226
GN MS^{-1}	0.023	**-0.371***	0.114	**0.337***	0.146	0.175	0.111	**0.729*****	**0.316**†	**0.395***	**0.724*****	-	**0.664*****	**0.573*****	**0.747*****	**-0.381***
MS fertile spikelets ear^{-1}	**-0.334***	**-0.526*****	-0.244	0.215	**-0.285**†	-0.155	**0.427****	**0.501****	**0.701*****	**0.337***	**0.403***	**0.607*****	-	-0.231	**0.658*****	-0.184
MS GN spikelet^{-1}	0.214	-0.169	**0.277**†	**0.317**†	**0.335***	**0.293**†	-0.105	**0.623*****	0.015	**0.271**†	**0.667*****	**0.893*****	0.188	-	0.250	**-0.309**†
MS RL	0.155	**-0.301**†	0.001	-0.023	**0.465****	**0.598*****	0.165	**0.510****	0.220	**0.554*****	**0.603*****	**0.501****	**0.437****	**0.372***	-	-0.056
Ears m^{-2} COUNT	**0.321**†	**0.378***	**0.394***	-0.110	0.253	0.091	-0.005	-0.111	-0.121	-0.198	-0.164	-0.136	**-0.282**†	-0.020	-0.131	-

† *P≤0.10*, * *P≤0.05*, ** *P≤0.01*, *** *P≤0.001*

Table A3-1 Table for yield and yield components in spring wheat lines in 2013.

No.	Line no.	BC_3S_5 Genotype (WMS136)	YIELD (t ha^{-1} 100%DM)	Grains m^{-2}	AGDM (t ha^{-1} 100%DM)	Ears m^{-2}	AGDM $plant^{-1}$	HI	TGW (g)	GN ear^{-1}	Plant height (PH)	Anthesis date (AD)
1	Ashby	Ash	9.13	26353.85	16.81	654.94	9.76	0.54	34.84	40.85	73.39	24/06/13
2	5	Ash/L14	8.95	21310.96	14.86	348.89	13.80	0.61	42.09	62.07	75.22	23/06/13
3	19	Ash/L14	6.76	16983.39	12.22	434.79	10.06	0.55	39.74	39.06	69.47	25/06/13
4	20	Ash/L14	7.30	19891.11	13.46	515.75	6.54	0.54	37.12	38.31	68.70	25/06/13
5	21	Ash/L14	9.48	28991.78	19.21	590.70	12.27	0.49	32.92	50.72	76.65	25/06/13
6	25	Ash/L14	8.16	22042.26	13.49	374.17	13.76	0.60	37.09	58.20	69.57	24/06/13
7	33	L14	1.34	4097.62	2.31	68.29	5.07	0.58	32.61	60.00	55.30	24/06/13
8	36	L14	3.74	8575.48	6.22	110.24	12.85	0.61	42.99	78.43	69.23	24/06/13

Table A3-1 (2) Table for yield and yield components in spring wheat lines in 2013.

No.	Line no.	BC_3S_5 Genotype (WMS136)	Fertile shoots $plant^{-1}$	Infertile shoots $plant^{-1}$	GDW (g) MS^{-1}	GDW (g) FS^{-1}	GDW (g) $plant^{-1}$	GN MS^{-1}	GN FS^{-1}	GN $plant^{-1}$	AGDM (g) MS^{-1}	AGDM (g) FS^{-1}
1	Ashby	Ash	3.65	0.15	1.90	1.28	5.31	52.60	37.26	151.10	3.40	2.40
2	5	Ash/L14	3.25	0.00	2.80	2.46	8.24	66.00	58.89	196.50	4.44	4.17
3	19	Ash/L14	3.58	0.00	2.13	1.33	5.57	50.58	34.61	139.96	3.68	2.47
4	20	Ash/L14	2.50	0.00	1.77	1.18	3.52	51.38	30.25	94.13	3.19	2.22
5	21	Ash/L14	3.43	0.00	2.10	1.52	6.31	60.15	45.73	180.40	3.99	3.10
6	25	Ash/L14	4.00	0.00	2.33	2.12	8.20	67.50	55.25	221.00	3.43	3.73
7	33	L14	1.00	0.00	1.86	2.14	2.93	45.50	89.00	90.00	2.78	4.59
8	36	L14	2.25	0.00	3.83	1.55	7.69	86.25	35.05	173.88	6.35	2.60

Table A3-1 (3) Table for yield and yield components in spring wheat lines in 2013.

No.	Line no.	BC_3S_5 Genotype (WMS136)	(MS) spikelets ear^{-1}	(plant) spikelets ear^{-1}	(MS) fertile spikelets ear^{-1}	(plant) fertile spikelets ear^{-1}	(MS) GN spikelets ear^{-1}	(plant) GN spikelets ear^{-1}	(MS) RL (cm)	(plant) RL (cm)
1	Ashby	Ash	19.65	18.75	17.65	16.20	2.97	2.46	8.28	7.85
2	5	Ash/L14	19.50	20.00	18.75	19.50	3.52	3.24	8.63	8.75
3	19	Ash/L14	19.58	18.30	17.50	15.30	2.89	2.46	8.25	7.51
4	20	Ash/L14	19.38	17.75	17.00	14.00	3.02	2.53	7.71	6.87
5	21	Ash/L14	19.70	19.40	17.98	17.20	3.34	2.87	8.98	8.82
6	25	Ash/L14	21.00	19.50	20.00	18.00	3.38	3.14	9.60	8.72
7	33	L14	20.00	21.00	20.00	21.00	2.28	2.95	10.80	10.75
8	36	L14	20.75	20.40	20.75	20.40	4.15	3.82	10.41	10.18

Table A3-2 Table for yield and yield components in spring wheat lines in 2014.

No.	Line no.	BC_3S_6 Genotype (WMS136)	YIELD (t ha^{-1} 100%DM)	Grains m^{-2}	AGDM (t ha^{-1} 100%DM)	Ears m^{-2}	AGDM $plant^{-1}$	HI	TGW (g)	GN ear^{-1}	Plant height (PH)	Anthesis date (AD)
1	Ashby	Ash	9.03	22959.63	16.12	510.07	16.39	0.56	39.33	44.97	71.82	11/06/14
2	5	L14	2.54	5182.98	4.56	65.79	18.92	0.55	44.94	80.30	61.98	11/06/14
3	19	Ash/L14	4.72	10815.67	8.45	180.09	26.19	0.56	44.39	64.27	64.93	11/06/14
4	20	Ash/L14	5.59	12782.51	10.14	226.50	13.86	0.55	43.75	56.31	63.34	11/06/14
5	21	Ash/L14	3.66	7383.56	6.16	123.75	11.94	0.59	49.51	59.67	62.18	12/06/14
6	25	L14	1.83	3599.00	3.68	56.74	9.08	0.50	50.72	63.43	56.04	11/06/14
7	33	L14	3.50	7704.36	5.99	101.42	21.27	0.59	45.86	76.91	65.53	11/06/14
8	36	L14	0.97	1961.93	1.66	19.56	15.72	0.58	48.88	102.63	65.18	11/06/14

Table A3-2 (2) Table for yield and yield components in spring wheat lines in 2014.

No.	Line no.	BC_3S_6 Genotype (WMS136)	Fertile shoots $plant^{-1}$	Infertile shoots $plant^{-1}$	GDW (g) MS^{-1}	GDW (g) FS^{-1}	GDW (g) $plant^{-1}$	GN MS^{-1}	GN FS^{-1}	GN $plant^{-1}$	AGDM (g) MS^{-1}	AGDM (g) FS^{-1}
1	Ashby	Ash	5.10	0.10	2.35	1.63	9.17	51.30	43.43	232.35	3.67	3.03
2	5	L14	2.40	0.38	4.86	2.76	10.48	96.63	68.46	242.88	7.67	5.86
3	19	Ash/L14	4.00	0.00	3.89	2.63	14.77	78.43	60.69	328.37	6.68	4.73
4	20	Ash/L14	3.10	0.00	3.13	2.02	7.71	65.70	50.79	176.10	5.56	3.71
5	21	Ash/L14	4.50	0.60	4.06	2.16	7.09	74.20	49.29	143.20	6.57	3.84
6	25	L14	3.30	0.00	4.08	1.07	4.50	77.60	28.00	88.80	7.12	4.90
7	33	L14	3.40	0.25	4.81	3.02	12.56	92.50	70.38	271.13	8.97	4.71
8	36	L14	1.80	0.00	5.80	4.08	9.05	110.70	93.57	185.40	9.13	8.22

Table A3-2 (3) Table for yield and yield components in spring wheat lines in 2014.

No.	Line no.	BC_3S_6 Genotype (WMS136)	(MS) spikelets ear^{-1}	(plant) spikelets ear^{-1}	(MS) fertile spikelets ear^{-1}	(plant) fertile spikelets ear^{-1}	(MS) GN spikelets ear^{-1}	(plant) GN spikelets ear^{-1}	(MS) RL (cm)	(plant) RL (cm)
1	1	Ash	21.15	22.05	19.15	20.20	2.68	2.25	7.97	8.22
2	5	L14	23.50	22.37	23.50	22.03	4.13	3.55	10.99	10.80
3	19	Ash/L14	23.75	22.84	23.33	22.40	3.36	2.86	10.22	9.71
4	20	Ash/L14	21.60	22.59	19.98	22.60	3.29	2.63	8.43	8.30
5	21	Ash/L14	22.00	20.38	21.80	19.88	3.40	2.89	9.92	9.45
6	25	L14	24.40	25.00	24.40	24.00	3.18	2.61	11.88	12.34
7	33	L14	24.13	22.70	24.13	22.70	3.84	3.32	10.64	9.98
8	36	L14	25.20	24.10	25.20	24.10	4.39	4.14	12.44	12.26

Table A3-3 Table for yield and yield components in winter wheat lines in 2013.

No.	Line no.	BC_3S_5 Genotype (WMC428)	YIELD (t ha^{-1} 100%DM)	Grains m^{-2}	AGDM (t ha^{-1} 100%DM)	Ears m^{-2}	AGDM $plant^{-1}$	HI	TGW (g)	GN ear^{-1}	Plant height (PH)	Anthesis date (AD)
1	Humber	Hum	11.50	36290.30	21.43	782.82	11.51	0.54	31.68	46.59	66.10	27/06/13
2	67	Hum/L14	10.78	31422.33	25.11	859.05	13.23	0.44	34.30	36.52	70.32	26/06/13
3	68	Hum/L14	10.94	30738.22	19.77	664.49	13.84	0.55	35.56	46.90	71.78	26/06/13
4	72	L14	10.33	29468.89	18.55	610.94	12.28	0.56	35.18	48.41	72.17	27/06/13
5	73	L14	11.12	31989.91	20.95	718.43	13.41	0.53	34.80	44.60	71.78	26/06/13
6	74	L14	8.77	24480.83	15.53	525.01	10.26	0.57	35.98	46.38	72.05	26/06/13
7	75	L14	9.84	26898.53	17.61	517.78	19.73	0.56	36.61	51.93	70.42	26/06/13
8	76	L14	11.12	30094.79	20.12	625.65	14.41	0.55	36.96	48.71	73.42	25/06/13
9	77	L14	11.96	31867.47	21.32	683.59	11.35	0.56	37.84	46.49	73.67	25/06/13
10	78	L14	11.08	33419.33	23.90	742.32	15.91	0.49	33.33	47.35	68.18	25/06/13
11	79	L14	8.43	24945.12	15.44	540.51	10.84	0.55	33.88	47.00	74.32	26/06/13
12	80	L14	10.05	29694.50	18.46	708.44	10.99	0.55	33.82	41.88	67.95	25/06/13
13	81	Hum/L14	11.99	38068.32	22.94	813.74	11.48	0.52	31.44	46.91	70.48	25/06/13
14	82	L14	11.41	31965.74	19.79	618.63	12.17	0.58	35.72	51.68	72.33	24/06/13
15	150	L14	10.83	33482.01	20.09	736.10	12.72	0.54	32.34	46.01	69.70	27/06/13
16	151	L14	12.72	38384.77	23.69	809.11	13.18	0.54	33.16	47.44	73.70	27/06/13
17	152	Hum/L14	11.57	37978.66	21.74	847.57	10.77	0.53	30.47	44.90	67.85	27/06/13
18	167	L14	12.23	35045.14	21.92	750.06	13.42	0.56	35.04	46.73	71.60	27/06/13
19	170	L14	11.43	31503.25	21.70	662.07	14.36	0.53	36.21	47.59	76.78	29/06/13
20	171	L14	12.39	34968.81	23.67	796.13	11.18	0.52	35.44	44.71	76.67	29/06/13
21	172	L14	10.72	31470.92	21.04	658.45	16.74	0.51	33.90	48.96	79.80	29/06/13
22	173	L14	12.24	33757.29	23.60	675.61	16.10	0.52	36.21	50.49	82.38	29/06/13
23	174	L14	12.86	34259.41	24.37	670.48	13.80	0.53	37.55	51.11	81.73	29/06/13
24	175	L14	11.77	33368.33	22.15	684.27	9.82	0.53	35.30	50.27	79.35	29/06/13
25	176	L14	11.53	30959.06	21.71	627.76	14.22	0.53	37.15	50.05	86.92	29/06/13
26	177	L14	14.07	41630.34	27.28	790.07	14.50	0.52	33.79	52.73	79.40	29/06/13
27	178	Hum/L14	13.08	34404.53	24.29	695.07	12.44	0.54	38.27	49.62	74.58	28/06/13
28	179	L14	11.93	32015.48	22.16	685.07	10.51	0.54	37.28	47.26	77.75	29/06/13
29	180	Hum/L14	11.32	32721.34	21.77	705.27	11.51	0.52	34.62	47.57	74.20	28/06/13
30	181	Hum/L14	12.30	34938.35	22.66	708.14	14.74	0.54	36.17	48.67	76.55	28/06/13
31	184	Hum/L14	11.60	31430.35	21.56	664.09	12.98	0.54	37.02	47.86	74.05	27/06/13
32	185	Hum/L14	12.58	32360.64	21.72	690.81	12.25	0.58	38.97	46.80	72.53	27/06/13
33	191	Hum/L14	11.80	31756.09	21.47	643.12	13.73	0.55	37.18	49.40	75.10	29/06/13
34	194	Hum/L14	13.13	34367.87	25.00	889.88	10.48	0.53	38.61	38.53	78.67	29/06/13
35	195	Hum/L14	12.76	32215.10	23.05	734.00	11.68	0.55	39.78	43.94	75.93	29/06/13
36	196	Hum/L14	13.76	34261.92	25.42	767.60	16.22	0.54	40.34	44.58	75.22	29/06/13
37	223	Hum/L14	10.22	32853.64	18.77	656.85	12.56	0.55	31.23	50.05	68.18	27/06/13
38	229	L14	11.82	36568.46	21.75	753.81	13.30	0.54	32.53	48.33	69.75	28/06/13

Table A3-3 (2) Table for yield and yield components in winter wheat lines in 2013.

No.	Line no.	BC_3S_5 Genotype (WMC428)	Fertile shoots plant^{-1}	Infertile shoots plant^{-1}	GDW (g) MS^{-1}	GDW (g) FS^{-1}	GDW (g) plant^{-1}	GN MS^{-1}	GN FS^{-1}	GN plant^{-1}	AGDM (g) MS^{-1}	AGDM (g) FS^{-1}
1	Humber	Hum	4.15	0.00	1.62	1.44	6.17	50.15	45.73	195.25	2.95	2.70
2	67	Hum/L14	4.60	0.00	1.05	1.29	5.75	31.70	37.36	167.60	3.56	2.70
3	68	Hum/L14	4.50	0.30	1.73	1.66	7.71	48.70	46.35	213.90	3.10	3.00
4	72	L14	4.10	0.30	1.98	1.61	6.87	56.10	46.15	193.30	3.46	2.92
5	73	L14	4.60	0.20	1.46	1.59	7.12	43.70	45.09	204.80	2.77	2.99
6	74	L14	3.80	0.00	1.67	1.67	5.83	47.70	45.82	161.60	2.84	2.98
7	75	L14	5.80	0.40	1.83	1.92	11.02	49.40	52.44	301.40	3.21	3.44
8	76	L14	4.30	0.20	1.98	1.76	7.99	53.00	47.64	214.30	3.42	3.23
9	77	L14	3.60	0.00	1.79	1.73	6.37	46.30	46.28	166.40	3.14	3.11
10	78	L14	4.90	0.00	1.67	1.57	7.75	50.70	46.51	231.20	3.84	3.10
11	79	L14	3.70	0.00	1.74	1.55	5.94	50.40	45.76	174.40	3.05	2.87
12	80	L14	4.20	0.10	1.61	1.42	5.97	46.60	42.36	176.30	2.87	2.63
13	81	Hum/L14	4.10	0.00	1.69	1.39	5.99	50.00	45.84	193.70	3.07	2.73
14	82	L14	3.80	0.00	1.84	1.85	7.01	50.60	52.06	196.70	3.05	3.25
15	150	L14	4.50	0.00	1.64	1.43	6.85	49.00	44.83	212.40	2.85	2.71
16	151	L14	4.50	0.00	1.47	1.60	7.07	45.20	48.08	213.50	2.63	3.02
17	152	Hum/L14	4.20	0.00	1.57	1.31	5.73	51.00	43.04	188.20	2.81	2.50
18	167	L14	4.60	0.10	1.86	1.58	7.47	58.90	42.56	214.90	3.18	2.86
19	170	L14	4.40	0.10	1.79	1.70	7.58	50.10	46.85	209.40	3.23	3.27
20	171	L14	3.70	0.00	1.51	1.61	5.88	42.20	45.63	166.00	2.74	3.11
21	172	L14	4.80	0.20	1.97	1.61	8.66	56.60	46.44	245.70	3.72	3.17
22	173	L14	4.60	0.10	2.03	1.77	8.35	55.20	49.10	230.40	3.71	3.47
23	174	L14	3.80	0.10	1.95	1.91	7.29	52.30	50.68	194.20	3.63	3.63
24	175	L14	3.00	0.00	1.82	1.75	5.24	53.10	48.54	148.40	3.31	3.32
25	176	L14	4.10	0.00	2.04	1.80	7.56	50.90	49.55	203.20	3.57	3.45
26	177	L14	4.20	0.10	1.83	1.76	7.47	56.10	51.53	221.00	3.49	3.44
27	178	Hum/L14	3.40	0.10	1.98	1.87	6.68	51.80	48.22	170.00	3.63	3.49
28	179	L14	3.20	0.20	1.99	1.97	5.67	53.70	52.71	153.40	3.45	3.83
29	180	Hum/L14	3.60	0.00	1.54	1.92	6.05	43.60	55.69	172.70	2.88	3.73
30	181	Hum/L14	4.70	0.20	1.96	1.68	8.03	55.00	46.81	221.10	3.53	3.11
31	184	Hum/L14	4.00	0.30	1.89	1.86	7.04	54.70	48.86	187.90	3.36	3.51
32	185	Hum/L14	3.90	0.10	2.00	1.82	7.11	46.00	48.79	182.60	2.98	3.31
33	191	Hum/L14	4.10	0.20	1.98	1.79	7.54	51.10	48.84	202.60	3.50	3.29
34	194	Hum/L14	3.70	0.10	1.58	1.51	5.52	42.20	38.86	142.30	2.95	2.89
35	195	Hum/L14	3.60	0.00	1.69	1.77	6.47	40.70	45.18	162.60	3.00	3.21
36	196	Hum/L14	4.90	0.10	2.00	1.74	8.79	51.00	42.92	218.20	3.56	3.25
37	223	Hum/L14	4.40	0.00	1.59	1.55	6.83	57.00	48.07	220.00	2.85	2.86
38	229	L14	4.60	0.00	1.66	1.55	7.23	51.00	47.74	221.20	2.95	2.88

Table A3-3 (3) Table for yield and yield components in winter wheat lines in 2013.

No.	Line no.	BC_3S_5 Genotype (WMC428)	(MS) spikelets ear^{-1}	(plant) spikelets ear^{-1}	(MS) fertile spikelets ear^{-1}	(plant) fertile spikelets ear^{-1}	(MS) GN spikelets ear^{-1}	(plant) GN spikelets ear^{-1}	(MS) RL (cm)	(plant) RL (cm)
1	Humber	Hum	18.70	19.50	16.40	17.95	3.05	2.65	7.64	7.88
2	67	Hum/L14	19.10	20.30	16.10	17.70	1.94	2.11	7.56	8.02
3	68	Hum/L14	19.30	20.10	16.70	18.30	2.91	2.60	8.08	8.25
4	72	L14	20.90	21.80	19.10	20.20	2.94	2.45	8.83	9.02
5	73	L14	19.70	21.60	18.00	20.30	2.44	2.25	8.27	8.86
6	74	L14	19.60	21.30	17.10	19.30	2.79	2.48	8.03	8.42
7	75	L14	20.90	22.30	18.20	20.90	2.72	2.54	8.90	9.52
8	76	L14	20.80	21.80	18.30	19.40	2.88	2.54	8.23	8.49
9	77	L14	19.50	20.60	16.50	18.80	2.80	2.56	8.06	8.44
10	78	L14	19.90	21.10	17.00	19.40	2.98	2.49	7.70	8.11
11	79	L14	20.10	21.20	17.40	18.80	2.89	2.55	7.98	8.25
12	80	L14	18.90	19.80	16.60	17.80	2.80	2.40	7.19	7.46
13	81	Hum/L14	18.90	20.20	16.70	18.10	3.00	2.64	7.78	8.01
14	82	L14	19.40	20.80	17.10	19.10	2.96	2.78	7.83	8.21
15	150	L14	18.90	20.20	16.80	18.80	2.91	2.50	7.74	8.23
16	151	L14	19.20	20.60	16.00	19.30	2.82	2.56	7.96	8.51
17	152	Hum/L14	18.70	20.00	16.10	18.30	3.18	2.53	8.04	8.28
18	167	L14	20.20	21.40	17.90	19.80	3.32	2.42	8.16	8.50
19	170	L14	19.40	20.90	16.30	19.20	3.07	2.57	8.35	9.18
20	171	L14	18.70	20.10	14.90	17.80	2.82	2.62	7.64	8.43
21	172	L14	20.00	20.70	17.20	18.80	3.28	2.64	9.65	9.92
22	173	L14	19.90	21.00	17.60	19.30	3.13	2.67	9.03	9.50
23	174	L14	19.30	20.30	16.80	18.50	3.11	2.83	8.95	9.40
24	175	L14	19.00	19.70	16.60	17.50	3.18	2.91	8.34	8.71
25	176	L14	19.70	20.50	16.50	18.70	3.08	2.75	8.92	9.63
26	177	L14	19.30	20.20	17.50	18.60	3.20	2.87	8.68	9.13
27	178	Hum/L14	19.10	20.00	16.80	17.80	3.08	2.83	8.43	8.77
28	179	L14	18.90	20.00	16.60	18.00	3.24	2.69	9.04	9.34
29	180	Hum/L14	19.35	20.30	16.58	18.80	2.63	2.61	8.63	9.16
30	181	Hum/L14	19.70	19.80	17.40	17.70	3.15	2.75	8.57	8.79
31	184	Hum/L14	19.20	20.20	16.70	18.70	3.29	2.63	7.75	8.30
32	185	Hum/L14	19.40	20.30	16.20	19.10	2.83	2.55	7.98	8.40
33	191	Hum/L14	19.40	20.60	17.50	19.40	2.92	2.61	8.10	8.43
34	194	Hum/L14	18.60	19.10	15.20	17.40	2.78	2.29	7.95	8.27
35	195	Hum/L14	17.90	19.30	14.90	16.90	2.73	2.69	7.40	7.81
36	196	Hum/L14	19.60	19.80	17.20	17.60	2.96	2.54	8.94	9.01
37	223	Hum/L14	19.50	20.60	17.30	19.50	3.29	2.65	7.84	8.21
38	229	L14	19.60	20.90	17.40	19.50	2.93	2.54	8.11	8.46

Table A3-4 Table for yield and yield components in winter wheat lines in 2014.

No.	Line no.	BC_3S_6 Genotype (WMC428)	YIELD (t ha^{-1} 100%DM)	Grains m^{-2}	AGDM (t ha^{-1} 100%DM)	Ears m^{-2}	AGDM $plant^{-1}$	HI	TGW (g)	GN ear^{-1}	Plant height (PH)	Anthesis date (AD)
1	Humber	Hum	9.06	23853.73	16.09	478.67	12.59	0.56	38.34	50.00	62.69	11/06/14
2	67	Hum/L14	10.17	23318.37	17.15	410.64	18.42	0.59	43.59	56.79	68.35	11/06/14
3	68	Hum/L14	10.26	23173.35	17.77	463.08	18.06	0.58	44.38	50.16	69.10	11/06/14
4	72	L14	9.64	23155.76	17.44	434.00	15.87	0.55	41.47	55.64	69.15	11/06/14
5	73	L14	9.66	23213.71	16.94	411.90	18.50	0.57	41.63	56.40	68.30	11/06/14
6	74	L14	8.08	20119.08	14.20	378.96	16.52	0.57	40.22	53.02	65.05	11/06/14
7	75	L14	8.95	21653.47	15.31	353.45	19.62	0.58	41.15	61.38	69.00	11/06/14
8	76	L14	9.81	23133.47	16.85	375.02	19.32	0.58	42.36	61.61	73.60	11/06/14
9	77	L14	10.03	25166.82	17.64	438.56	18.90	0.57	39.86	57.34	67.95	11/06/14
10	78	L14	9.12	23482.67	16.20	435.00	15.64	0.56	38.84	54.00	67.75	11/06/14
11	79	L14	10.35	24296.93	17.94	423.31	21.63	0.58	42.62	57.32	70.55	11/06/14
12	80	L14	9.04	23089.34	16.66	436.12	15.17	0.54	39.16	53.63	66.20	11/06/14
13	81	Hum/L14	9.87	23487.15	17.58	473.42	13.48	0.56	42.30	49.28	66.95	11/06/14
14	82	L14	9.19	22153.24	16.08	377.25	20.75	0.57	41.43	58.92	69.65	11/06/14
15	150	L14	9.78	24514.58	17.08	441.31	17.02	0.57	39.94	55.56	64.00	11/06/14
16	151	L14	9.42	24358.77	16.61	404.44	19.30	0.57	38.83	60.09	65.25	11/06/14
17	152	Hum/L14	9.34	24616.79	16.58	479.58	15.20	0.57	38.31	51.00	62.20	11/06/14
18	167	L14	11.17	27950.36	19.35	501.09	18.88	0.58	39.92	55.76	63.40	13/06/14
19	170	L14	10.76	29081.33	19.97	496.98	16.84	0.54	36.99	58.62	71.45	14/06/14
20	171	L14	10.50	28129.63	19.27	493.62	19.20	0.54	37.32	56.99	71.80	14/06/14
21	172	L14	9.93	25273.84	18.12	404.57	17.80	0.55	39.40	62.33	76.85	13/06/14
22	173	L14	11.51	29275.13	21.12	476.46	20.71	0.54	39.36	61.43	74.15	14/06/14
23	174	L14	12.27	28663.16	22.23	506.03	17.93	0.55	42.75	56.61	72.95	14/06/14
24	175	L14	11.68	27744.42	20.87	518.90	15.59	0.56	42.13	53.69	74.08	14/06/14
25	176	L14	10.15	25122.52	17.98	410.77	16.91	0.56	40.42	61.41	75.00	14/06/14
26	177	L14	11.02	28074.28	20.35	519.65	16.96	0.54	39.24	54.24	73.77	14/06/14
27	178	Hum/L14	10.29	26815.71	19.12	500.29	16.45	0.54	38.52	53.70	73.40	14/06/14
28	179	L14	7.69	19863.18	14.17	302.03	24.31	0.54	38.93	65.50	76.30	13/06/14
29	180	Hum/L14	9.83	24004.93	17.36	443.95	16.99	0.57	40.96	55.45	70.93	14/06/14
30	181	Hum/L14	11.14	29568.59	20.35	494.95	19.35	0.55	37.85	59.84	68.42	13/06/14
31	184	Hum/L14	10.07	25371.00	18.10	433.10	20.49	0.56	40.12	58.44	66.95	12/06/14
32	185	Hum/L14	10.50	24176.57	17.97	433.92	19.11	0.58	43.19	55.56	66.70	12/06/14
33	191	Hum/L14	9.27	24506.74	17.04	479.66	12.64	0.54	37.71	51.11	67.90	14/06/14
34	194	Hum/L14	8.95	21147.69	15.86	410.23	18.15	0.56	41.98	51.95	71.45	14/06/14
35	195	Hum/L14	10.74	24046.16	18.70	465.80	16.06	0.57	44.66	51.62	72.70	14/06/14
36	196	Hum/L14	9.30	22878.55	16.68	415.50	20.86	0.56	40.48	55.42	70.03	14/06/14
37	223	Hum/L14	9.83	25624.00	17.19	444.16	17.72	0.57	38.38	58.17	62.50	11/06/14
38	229	L14	9.48	24643.31	16.78	447.33	17.40	0.57	38.37	56.46	65.35	12/06/14

Table A3-4 (2) Table for yield and yield components in winter wheat lines in 2014.

No.	Line no.	BC_3S_6 Genotype (WMC428)	Fertile shoots plant^{-1}	Infertile shoots plant^{-1}	GDW (g) MS^{-1}	GDW (g) FS^{-1}	GDW (g) plant^{-1}	GN MS^{-1}	GN FS^{-1}	GN plant^{-1}	AGDM (g) MS^{-1}	AGDM (g) FS^{-1}
1	Humber	Hum	3.70	0.15	2.64	1.65	7.13	62.05	45.50	185.05	4.02	2.99
2	67	Hum/L14	4.40	0.20	3.36	2.20	10.91	71.00	52.32	249.60	4.97	3.76
3	68	Hum/L14	4.70	0.00	3.04	2.00	10.43	64.70	46.22	236.10	4.60	3.50
4	72	L14	3.90	0.20	3.21	1.97	8.77	71.70	50.04	211.30	5.00	3.66
5	73	L14	4.50	0.40	3.25	2.09	10.56	72.10	51.89	253.60	4.92	3.72
6	74	L14	4.40	0.00	2.99	1.88	9.38	69.00	48.32	233.30	4.54	3.36
7	75	L14	4.60	0.20	3.36	2.32	11.43	76.20	57.30	278.10	5.01	4.00
8	76	L14	4.30	0.10	3.53	2.34	11.25	80.20	56.00	265.20	5.40	4.02
9	77	L14	4.70	0.00	3.23	2.03	10.75	74.60	52.65	269.70	4.94	3.62
10	78	L14	4.20	0.10	3.02	1.80	8.80	70.90	48.70	226.60	4.65	3.26
11	79	L14	5.10	0.00	3.29	2.24	12.46	73.00	53.49	292.40	5.06	3.91
12	80	L14	4.20	0.00	2.77	1.88	8.22	68.50	48.74	210.30	4.44	3.51
13	81	Hum/L14	3.60	0.10	2.63	1.86	7.58	60.20	44.88	180.70	4.09	3.36
14	82	L14	4.90	0.30	3.32	2.21	11.88	74.30	54.78	285.50	5.03	3.93
15	150	L14	4.40	0.20	2.98	1.99	9.77	69.90	51.32	244.50	4.63	3.49
16	151	L14	4.70	0.10	3.19	2.09	10.95	76.40	55.54	283.00	4.91	3.71
17	152	Hum/L14	4.40	0.00	2.74	1.72	8.58	65.10	46.81	225.20	4.16	3.10
18	167	L14	4.80	0.30	2.98	2.03	10.89	70.60	51.94	273.10	4.46	3.57
19	170	L14	4.20	0.10	2.92	1.94	9.09	68.70	55.64	244.90	4.64	3.68
20	171	L14	4.90	0.10	2.83	1.95	10.46	69.20	53.92	279.70	4.53	3.61
21	172	L14	4.00	0.00	3.31	2.15	9.82	75.90	57.55	248.10	5.27	3.99
22	173	L14	4.70	0.00	2.95	2.26	11.27	68.80	59.06	290.00	4.65	4.21
23	174	L14	4.10	0.10	3.05	2.22	9.88	65.10	53.91	231.50	4.90	4.05
24	175	L14	3.90	0.20	2.87	2.06	8.72	63.00	50.84	206.50	4.45	3.75
25	176	L14	3.80	0.00	3.44	2.13	9.52	78.10	55.35	235.30	5.23	3.89
26	177	L14	4.30	0.10	3.00	1.86	9.19	68.60	49.85	234.20	4.78	3.50
27	178	Hum/L14	4.30	0.00	2.92	1.81	8.86	69.20	48.91	230.60	4.60	3.45
28	179	L14	5.10	0.00	3.26	2.38	13.27	75.40	63.18	339.50	5.12	4.43
29	180	Hum/L14	4.20	0.10	3.13	2.02	9.70	68.60	51.32	234.60	4.71	3.60
30	181	Hum/L14	4.70	0.20	3.07	2.03	10.59	74.30	55.89	283.60	4.87	3.74
31	184	Hum/L14	4.90	0.00	3.15	2.13	11.42	73.30	54.62	286.80	4.89	3.85
32	185	Hum/L14	4.70	0.10	3.09	2.21	11.14	66.60	52.38	259.20	4.66	3.83
33	191	Hum/L14	3.60	0.20	2.80	1.60	6.87	63.30	46.39	183.10	4.33	3.08
34	194	Hum/L14	4.70	0.00	2.99	1.95	10.23	64.90	48.41	243.50	4.57	3.52
35	195	Hum/L14	4.00	0.00	3.09	2.04	9.22	63.30	47.56	206.40	4.69	3.62
36	196	Hum/L14	5.30	0.20	3.09	2.04	11.65	68.30	52.37	288.70	4.72	3.72
37	223	Hum/L14	4.50	0.00	2.96	2.03	10.17	72.30	53.94	263.20	4.48	3.58
38	229	L14	4.30	0.30	3.15	1.86	9.87	70.40	52.20	258.60	4.78	3.37

Table A3-4 (3) Table for yield and yield components in winter wheat lines in 2014.

No.	Line no.	BC_3S_6 Genotype (WMC428)	(MS) spikelets ear^{-1}	(plant) spikelets ear^{-1}	(MS) fertile spikelets ear^{-1}	(plant) fertile spikelets ear^{-1}	(MS) GN spikelets ear^{-1}	(plant) GN spikelets ear^{-1}	(MS) RL (cm)	(plant) RL (cm)
1	Humber	Hum	21.85	21.30	20.70	19.80	3.00	2.49	9.12	8.86
2	67	Hum/L14	23.20	22.80	22.00	20.90	3.23	2.68	10.26	9.81
3	68	Hum/L14	22.20	22.00	20.90	20.30	3.10	2.46	9.60	9.38
4	72	L14	24.00	23.70	23.20	22.30	3.09	2.46	10.35	9.93
5	73	L14	24.00	23.30	23.50	22.30	3.07	2.50	10.43	9.93
6	74	L14	23.80	23.40	23.30	22.80	2.96	2.31	10.11	9.98
7	75	L14	24.60	24.00	23.80	22.70	3.20	2.67	10.99	10.62
8	76	L14	24.00	23.40	23.80	22.50	3.37	2.70	10.86	10.39
9	77	L14	24.20	23.70	23.60	22.80	3.16	2.50	11.01	10.64
10	78	L14	22.90	22.50	22.10	21.60	3.21	2.49	9.50	9.35
11	79	L14	24.30	23.70	23.40	22.50	3.12	2.53	10.51	10.17
12	80	L14	22.20	22.00	21.80	20.80	3.14	2.54	9.62	9.22
13	81	Hum/L14	22.80	22.20	21.20	20.30	2.83	2.38	9.12	8.86
14	82	L14	23.50	22.90	22.80	21.80	3.26	2.67	10.28	9.89
15	150	L14	23.00	21.60	22.10	19.60	3.16	2.75	10.04	9.32
16	151	L14	23.20	22.50	23.00	21.70	3.32	2.73	10.56	10.11
17	152	Hum/L14	22.70	21.70	21.80	19.80	2.98	2.52	9.90	9.24
18	167	L14	24.30	24.20	23.30	23.10	3.03	2.41	10.38	10.38
19	170	L14	23.00	22.20	21.60	20.80	3.18	2.79	10.37	9.87
20	171	L14	22.40	22.30	21.00	21.60	3.29	2.66	9.73	9.76
21	172	L14	22.30	21.80	21.40	20.80	3.55	2.97	10.72	10.58
22	173	L14	21.80	23.00	20.00	21.60	3.44	2.88	9.82	10.32
23	174	L14	22.20	22.40	20.60	20.50	3.16	2.76	10.54	10.36
24	175	L14	22.00	21.60	19.60	19.40	3.21	2.77	9.34	9.32
25	176	L14	22.10	21.90	21.00	20.70	3.71	2.95	10.28	10.17
26	177	L14	22.00	22.80	20.30	20.80	3.38	2.62	9.72	9.61
27	178	Hum/L14	22.50	22.10	21.20	20.50	3.26	2.60	9.58	9.43
28	179	L14	22.60	23.30	21.90	22.50	3.44	2.93	11.44	11.43
29	180	Hum/L14	22.70	22.10	21.70	20.90	3.16	2.61	10.49	10.13
30	181	Hum/L14	22.40	22.00	21.80	20.70	3.40	2.85	10.33	10.04
31	184	Hum/L14	23.10	22.50	22.50	21.50	3.26	2.69	10.26	9.96
32	185	Hum/L14	21.80	21.50	21.00	20.90	3.17	2.65	9.47	9.40
33	191	Hum/L14	21.30	21.30	20.00	19.80	3.17	2.57	8.54	8.10
34	194	Hum/L14	21.60	20.30	20.80	19.10	3.12	2.67	10.05	9.59
35	195	Hum/L14	21.00	20.50	19.50	19.00	3.25	2.70	8.75	8.72
36	196	Hum/L14	21.30	21.40	20.60	20.10	3.31	2.75	9.71	9.52
37	223	Hum/L14	22.90	22.40	22.40	21.50	3.23	2.68	9.68	9.37
38	229	L14	22.60	22.40	21.90	20.90	3.21	2.66	9.70	9.38