IDENTIFYING PHYSIOLOGICAL TRAITS TO OPTIMIZE ASSIMILATE PARTITIONING AND SPIKE FERTILITY FOR YIELD POTENTIAL IN WHEAT (*Triticum aestivum* L.) GENOTYPES

BY

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DEDICATION

To Alma and Manuela

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ABSTRACT

Although wheat demand is expected to increase along with a projected growing population, the global rate of wheat yield increase appears to be declining. Genetic progress in yield potential in the past has been achieved mainly through increases in grains m^{-2} and harvest index with no major changes in above-ground biomass; however, HI has not increased since about 1990 indicating that further increases in yield potential will rely on increases in biomass while optimizing grain DM partitioning. A panel of 26 high biomass CIMMYT elite spring wheat cultivars (CIMMYT Mexico Core Germplasm; CIMCOG) was evaluated for grain yield, above-ground dry-matter (AGDM), DM partitioning and spike fertility in three field experiments (2011, 2012 and 2013) in NW Mexico. An additional field experiment was carried out in 2014 on a subset of four cultivars to examine source-sink related traits by imposing source manipulation treatments (leaf-lamina removal and leaf-sheath shading) on the crop. The main objective was to identify the physiological determinants of grains m^{-2} and HI in relation to DM partitioning among organs and to prioritise traits for application in breeding to increase HI in high biomass genotypes.

Genetic variation in grain yield among genotypes was explained mainly by differences in AGDM. Fruiting efficiency (grains per unit spike DM at anthesis) was affected by high rachis specific weight and appeared to be important in grains m^{-2} determination. Trends for positive associations were observed between HI and spike partitioning index (Spike DM / AGDM - at anthesis; SPI). Stem DM proportion (mainly structural DM in internodes 2 and 3) was negatively associated with SPI; peduncle length and peduncle DM as a proportion of the stem DM on the other hand were more strongly associated to spike DM per unit area. In 2014, reductions in final grain weight in response to source reduction treatments were relatively low compared to the estimated reductions in light interception. No up-regulation of photosynthesis rate was observed by the flag-leaf or the spike in response to source reductions, suggesting that grain growth was overall sink limited but close to a co-limitation by source and sink. These results indicated scope for designing a plant ideotype to raise HI in high biomass spring wheat cultivars with reductions in stem structural DM (in upper internodes) without major effects on water soluble carbohydrate accumulation (in lower internodes). Finally, results from the source-sink manipulation treatments indicated limited scope for reductions in leaf-sheath and lamina DM partitioning during stem elongation to favour spike growth.

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LIST OF ABBREVIATIONS

AGDM	Above-ground dry matter
A_{max}	Saturated point
ANOVA	Analysis of variance
At	Anthesis
BGF	Beginning of the grain filling
CENEB	Norman E. Borlaug Experimental Center (Spanish Acronym)
CIMMYT	International Maize and Wheat Improvement Center (Spanish Acronym)
CGR	Crop growth rate (g m^{-2} d^{-1})
CIMCOG	Mexico Core Germplasm
CO_2	Carbon dioxide
CTD	Canopy temperature depression
cv	Cultivar
Cv	Coefficient of variation
DM	Dry matter
DR	Double ridge
Ds	Days from emergence to anthesis
DW	Dry weight
Em	Emergence
FE	Fruiting efficiency (grains at harvest per g spike DM at anthesis)
FI	Fraction of radiation intercepted
F_m	Maximal chlorophyll fluorescence

F_v	Variable chlorophyll fluorescence
FW	Fresh weight
GS	Growth stage
HD	Heading
HI	Harvest index
HTPPs	High-throughput phenotyping technologies or platforms
k	Extinction coefficient of the canopy
LI	Radiation interception
NDMI	Normalized difference moisture index
NDVI	Normalized differenced vegetation index
ns	Non-significant
NW	Northwest
P	Probability
PCA	Principal component analysis
PAR	Photosynthetically active radiation
PM	Physiological maturity
PPFD	Photosynthetic photon flux density (Mmol m^{-2} s^{-1})
PSII	Efficiency of photosystem II
PTQ	Photo-thermal quotient
QTL	Quantitative trait loci
RAD	Incident radiation (MJ m^{-2})
Rht	Semi-dwarfing genes
RI	Fraction of total radiation intercepted
Rs	Solar radiation
RuBP	Ribulose-1,5-bisphosphate
RUE	Radiation use efficiency (g m^{-2} MJ^{-1})

SPI	Spike partitioning index
SS	Subsample
Sw	Stages of sowing
T	Temperature
t ha^{-1}	Ton per hectare
TGW	Thousand grain weight
TS	Terminal spikelet
UN	United nations
WSC	Water soluble carbohydrates
WSC_A	Water soluble carbohydrates amount (g m^{-2}).
WSC_C	Water soluble carbohydrates concentration (% DM)
YoR	Year of release
$\Delta_{13}C$	Carbon isotope ($_{13}C$) discrimination

CHAPTER 1.

INTRODUCTION

1.1 Wheat demand globally

The United Nations (UN) Population Division (2004) forecast that the global human population will reach 9.1 billion by 2050. This represents an increase of 20.1% from the 7 billion reached in 2014 according to the Department of Economics and Social Affairs of the UN, mostly occurring in developing countries (UN Population Division, 2009), where income growth will also increase per-capita demand (Fischer et al., 2014). Globally, wheat is the most widely grown crop with 220 million hectares worldwide with approximately half of this grown in developing countries (Lantican, 2003), where wheat provides 19% of calorie and 20% of protein intake (Braun et al., 2010).

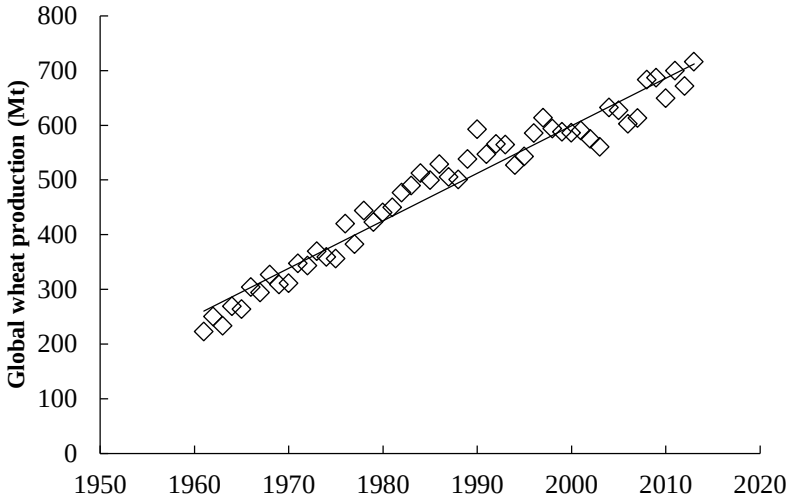


Figure 1.1 Linear regression of global wheat production (million tonnes) on year from 1961 to 2013 ($y = 8.6901x - 16781$, $R^2 = 0.95$).

Global wheat production has increased linearly in the past four decades (Figure 1.1), related to increases in harvested area until *ca.* 1980 (Figure 1.2 b) but mainly associated with linear increases in grain yield per unit area over the last four decades (1961 – 2013, Figure 1.2 a) (FAOSTAT, 2015). However, although

wheat yield has increased linearly over the past 40 years, the global rate of wheat yield increase appears to be declining in the last 20 years and has reached 1.0% per annum in 2010 while global crop area is likely to increase by 10% from 2010 to 2050 (Fischer et al., 2014) but just in specific areas (Rejesus et al., 1999). These facts coupled with the estimated negative projections of climate change impacts on crop productivity for most developing countries (Reynolds and Ortiz, 2010) indicate the urgent need for further increases in wheat yield through genetic improvement.

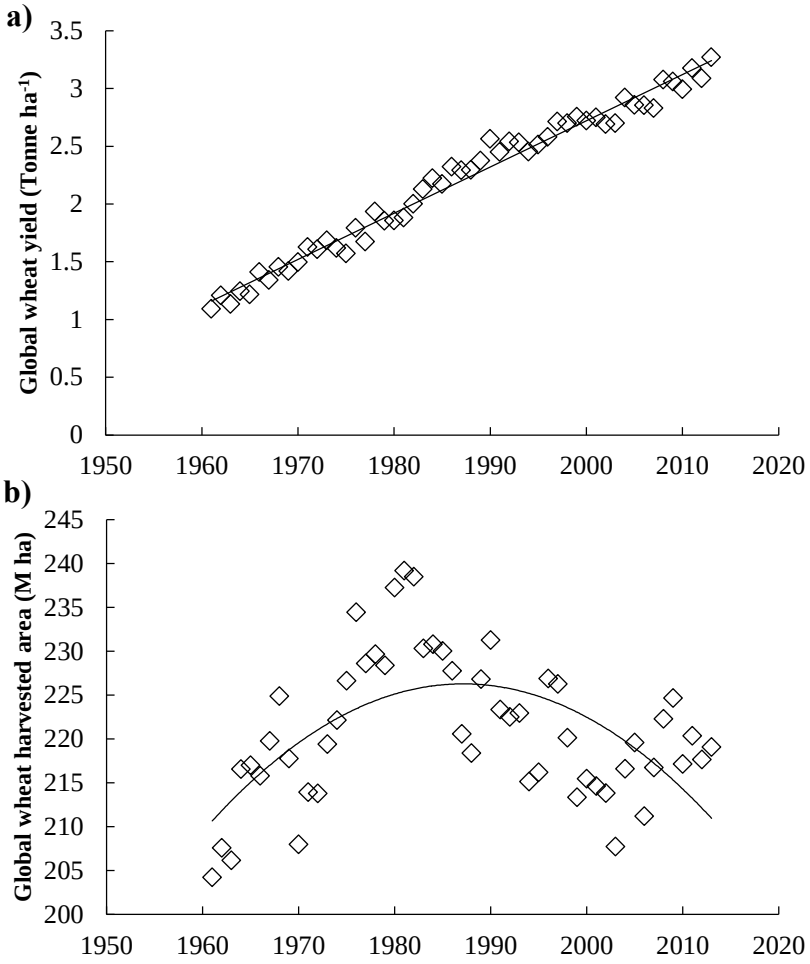


Figure 1.2 Regressions of a) Global wheat yield (ton ha^{-1}) and b) Global wheat harvested area (million hectares) on year from 1961 to 2013 ($y = -0.04x - 77.364$, $R^2 = 0.98$ and $y = -0.0229x2 + 90.992x - 90180$, $R^2 = 0.35$, respectively).

1.2 **Contribution of breeding to wheat production**

Bread wheat (*Triticum aestivum* L.) accounts for 90% of the global wheat production in more than 70 countries (Dixon et al., 2009). The birth place of cultivated wheat is considered to be the Fertile Crescent about 8,000 to 10,000 years ago (Gill and Friebe, 2002) and since then it has been the basic staple food of the major civilizations of Europe, West Asia and North Africa (Curtis, 2002). Three different diploid donor species were involved in the evolution of the common hexaploid wheat (*T. aestivum* L.) (Zohary et al., 1969). The first evolutionary event leading to hexaploid wheat was the hybridization of *T. Urartu* (genome A) and a wild grass (*Aegilops speltoides*) providing the B genome, becoming a fertile tetraploid wheat (AABB) known as emmer wheat or *T. turgidum* (Luo et al., 2007) occurring ~12,000 years ago (Chantret, 2005). The second evolutionary event occurred when emmer wheat crossed with *Ae. tauschii*, a wild grass carrying the D genome occurring ~ 10,000 years ago (Feuillet et al., 2008). This cross will normally yield sterile hybrids but a doubling of the chromosomes in the progeny resulted in fertile hexaploid species (AABBDD), organized into 21 pairs of chromosomes, seven pairs belonging to each of the genomes (Sears, 1954) (Figure 1.3).

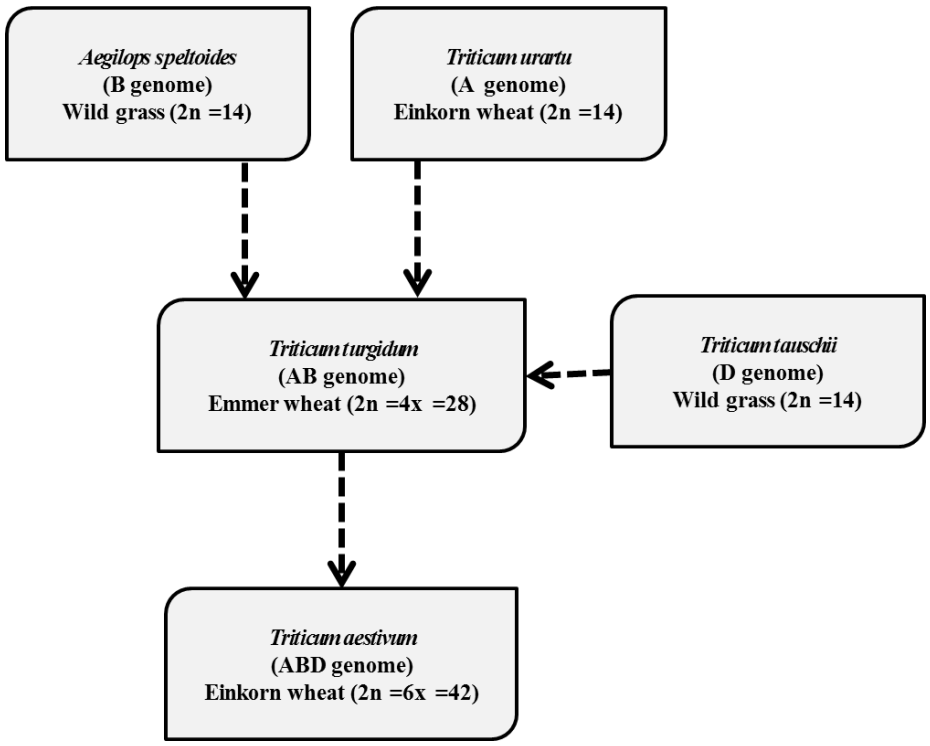


Figure 1.3 Phylogeny of hexaploid wheat. Adapted from Feuillet et al. (2008).

The '*Green Revolution*' is known as a widespread adoption of technology developed jointly with genetically improved varieties of higher yielding crops in the late 1960s; before this gains in global wheat production per unit area were slow and based mostly on area expansion (Rejesus et al., 1999). Modern varieties were developed by the International Maize and Wheat Improvement Center (CIMMYT) in Mexico based on selecting genetic traits for increased yield stability and wide-scale adaptability (Khush, 2001). Genetic improvements in yield were achieved mainly by the introduction of semi-dwarfing genes (*Rht-B1b* and *Rht-D1b*, to reflect their chromosomal location on chromosomes 4B and 4D, formerly *Rht1* and *Rht2*) into cereal crops by reducing lodging and increasing the proportion of assimilate partitioned to the grain as a result of reductions in stature. These genes were introduced in American high-yielding varieties through crosses with a Japanese semi-dwarf wheat variety, producing Norin-10 which was later used in spring wheat by CIMMYT, producing high-yielding lines that could grow in tropical and sub-tropical climates (Hedden, 2003). The basis of reductions in height was the gibberellin (GA)-insensitive character of Norin-10 (Gale and Youssefian, 1985).

The crop area occupied by modern wheat high yielding varieties increased rapidly from 12 million ha in 1970 to 50 million ha in 1990 (Reynolds and Borlaug, 2006), almost doubling cereal production in developing countries between 1961 to 1985 (Conway, 1997). Roughly 45% of the area designated to wheat in developing countries is under irrigation conditions (excluding China), and accounts for more than 55% of the total wheat production (Reynolds et al., 1999). These increases in wheat productivity are associated with genetic improvements in yield potential, adaptation to abiotic stress and disease resistance and better agronomic practices (Reynolds et al., 2009). However, the global population grew at a rate of approximately 1.5% from 1993 to 2000, whereas the growth rate of wheat production increased by 0.9% from 1985 to 1995 (Dixon et al., 2009). Furthermore, gains in genetic wheat yield in irrigated areas have increased at a rate of approximately 1% per year since the mid-1960s with similar results in other areas, indicating that wheat demand is growing faster than gains in genetic yield potential (Reynolds et al., 1999). Global grain demand is expected to double

by 2050, also related to increases in per capita income and from dietary shifts towards a higher proportion of meat (much of it grain-fed) (Tilman et al., 2002).

The wheat cultivars developed by CIMMYT in the high radiation irrigated environment of Northwest Mexico (Yaqui Valley) represent more than 40% of the wheat production in developing countries (Bell et al., 1995) and they are widely distributed to those countries (Fischer et al., 1998).

Yield potential is the yield of a cultivar grown in environments to which it is adapted and nutrients, water, lodging and other stressed are controlled (Evans and Fischer, 1999) and is a major component of yield progress, since it is well expressed in a wide range of environments (Fischer, 2007). Recent evidence showed that although increases in yield potential of CIMMYT spring wheat cultivars have not ceased, they had slowed down to 0.3% per annum in the Yaqui Valley (Fischer et al., 2014). Most of the studies investigating yield progress in CIMMYT spring wheat cultivars have reported linear genetic gains in yield potential (Waddington et al., 1986; Sayre et al., 1997) with cultivars released between 1950 and 1988 averaging genetic yield gains at *ca.* 1% per annum. More recently and using a wider time span, Aisawi et al. (2015) reported gains in CIMMYT spring wheat semi-dwarf cultivars released from 1966 to 2009 of around 0.59% per annum. Similar trends have been observed in other countries, such as the UK (Shearman et al., 2005; Clarke et al., 2012), France (Brancourt-Hulmel et al., 2003), China (Xiao et al., 2012), and lower but significant genetic gains in wheat yield potential were reported in South Australia (Sadras and Lawson, 2011). On the other hand, other studies have shown that genetic gains in wheat yield have been approaching a ceiling (Acreche et al., 2008; Graybosch and Peterson, 2010; Matus et al., 2012; Beche et al., 2014) in Spain, the North plains of North America, Chile and Brazil, respectively. The physiological basis of genetic gains in yield potential has been mostly associated with increases in harvest index (grain DM/above-ground DM) and grains m^{-2}, explained in some cases by greater fruiting efficiency (grains per g of spike DM at anthesis) (Abbate et al., 1998; Acreche et al., 2008). In contrast, some studies have reported that more recent yield gains have been associated with greater above-ground dry matter (Shearman et al., 2005; Aisawi et al., 2015), photosynthetic-related traits

(Fischer et al., 1998), greater stem WSC (Foulkes et al., 2002; Shearman et al., 2005) and increased grain weight (Lopes et al., 2012b; Aisawi et al., 2015).

1.3 **Physiological tools that improve wheat breeding**

Crop breeding is usually based on attributes of economic interest such as yield (Jackson et al., 1996). Most of the yield progress through breeding during the last century was achieved by selecting for yield *per se* empirically (Loss and Siddique, 1994) but yield gains appear to be slowing down in the last decades (Fischer, 2007). Furthermore, as yield possesses relatively low heritability due to high genotype x environment interactions (Slafer et al., 2005), exacerbated by unpredictable weather conditions due to climate change (Richards et al., 2002), the use of physiological selection criteria plays an important role by making empirical selection more efficient and defining useful traits and enabling the identification of the most suitable parental germplasm (Richards, 1996). On the other hand, trends for potential and actual yields tend to be parallel (Slafer et al., 2005; Fischer et al., 2014), yield potential remains a main component of yield progress (Fischer, 2007). Potential approaches in the search for traits for application as selection criteria in breeding could be based on identifying physiological processes determining productivity, divided into three major processes: i) interception of incident solar radiation by the canopy, ii) conversion of intercepted radiation into chemical energy and iii) optimized partitioning of assimilate among plant organs (Hay and Walker, 1989; Araus et al., 2001).

A potential bottle-neck in the application of physiology in wheat breeding is the availability of field-based high-throughput phenotyping technologies or platforms (HTPPs) (Andrade-Sanchez et al., 2014; Araus and Cairns, 2014). Examples of the most recent tools used for physiological breeding are determination of carbon isotope ($_{13}$C) discrimination (Δ_{13}C), stomatal conductance, canopy temperature depression (CTD) by infrared thermometry and normalized differenced vegetation index (NDVI) by spectroradiometry (Reynolds et al., 2001b). Spectral reflectance indices such as NDVI provide information on the physiological status of a crop, including estimation of green area index, pigments and water and nutrient status (Reynolds et al. 2001). For instance, Li et al. (2011) reported significant relationships between NDVI with HI and radiation use-efficiency. Lobos et al.

(2014) reported significant correlations between spectral reflectance indices (SRI) (e.g. Normalized Difference Moisture Index; NDMI) and grain yield and $\Delta_{13}C$, particularly during grain filling. In C_3 cereals such as wheat, a greater ^{13}C discrimination is positively associated with intracellular CO_2 levels (Farquhar et al., 1982) and negatively related to water-use efficiency (WUE) or transpiration efficiency (Ehdaie et al., 1991; Condon et al., 1993); therefore, cultivars with higher WUE will discriminate less against ^{13}C and accumulate this heavy isotope in their tissues (Araus et al., 2001). Canopy temperature depression is an integrative technique that has been shown to correlate with stomatal conductance, WUE, leaf area index and grain yield (Fischer et al., 1998); under water stress conditions it can indicate better water uptake (deeper rooting), and under potential conditions it provides information on photosynthetic capacity and sink strength (Reynolds et al., 1994). For instance, Lopes and Reynolds (2012) showed that canopy temperature during grain-filling and stay-green (prolonged onset of senescence, calculated with NDVI) accounted for 30% of yield variability under heat and heat combined with drought environments in populations of advanced and recombinant inbreed spring wheat lines.

Overall, future benefits of the application of physiological tools in breeding programs could be achieved through: i) focussing on specific objectives on appropriate range of germplasm, ii) use of large populations to enable extrapolation of techniques, iii) identification of traits to be used as direct selection criteria in addition to traits used already in breeding programs, iv) use of traits in introgression programs, v) conducting experimentation or selection trials in representative environments and vi) developing high-throughput and easy to use tools (Reynolds et al. 2001), such as aerial spectroradiometry platforms (Araus and Cairns, 2014).

1.4 Enhanced pre-anthesis dry-matter partitioning to raise yield potential in wheat

As introduced above, it is generally agreed that increases in wheat yield were achieved due to increased partitioning of assimilate to grains as an immediate result of to the introgression of *Rht* genes (Gale and Youssefian, 1985) and also as a result of continuous selection for yield in the post-anthesis green revolution period

(Reynolds et al., 2005). Moreover, several studies have shown that increases in grains m^{-2} and harvest index were related to greater spike DM at anthesis (Brooking and Kirby 1981; Fischer and Stockman 1986; Miralles and Slafer 1995) and a higher above-ground DM proportion partitioned to the spike at anthesis (spike partitioning index; SPI) (Slafer et al., 1990). Other studies in contrast have shown that increases in grains m^{-2} were explained by increased fruiting efficiency (grains g^{-1} spike DM at anthesis) (Abbate et al., 1998; Acreche et al., 2008) or by a combination of greater FE and spike growth during stem elongation to anthesis (González et al., 2011). The basis of the positive association between grains m^{-2} and SPI and spike DM at anthesis has been linked to competition for assimilate between the growing spike and stem during the stem elongation period, *i.e.* higher SPI represents less competition for the spike and a greater amount of assimilate for floret survival and grain set (Fischer and Stockman 1980; Siddique et al. 1989).

Although the highest HI value published for spring wheat is approximately 0.50 (Foulkes et al., 2011), its theoretical maximum has been estimated at 0.62 (Austin, 1980). Increases in HI through further reductions in plant height seem unlikely, since evidence indicates that optimum plant heights of *ca.* 70-90 cm have been already achieved in most wheat breeding programmes worldwide (Miralles and Slafer, 1995a; Reynolds et al., 2005). Moreover, as i) recent research indicates positive associations between genetic gains in wheat yields with biomass and ii) there is evidence of some extent of source-limitation during grain filling in modern wheat cultivars (Acreche and Slafer 2009) ; approaches to increase yield potential will therefore require increases in biomass while ensuring extra assimilates are partitioned optimally among organs to benefit grain growth (Foulkes et al., 2011).

In this respect, assimilate partitioning to the spike could be increased by reduced competition from alternative sinks (Foulkes et al., 2011) and particularly during stem elongation when grain number is being determined (Fischer 1985). However, reductions in leaf lamina may have negative effects on photosynthetic capacity affecting biomass production (Reynolds et al., 2009; Foulkes et al., 2011) and only a moderate increase may be achieved in spike assimilate partitioning through improved economy of tillering in spring wheat; as the proportion of DM in non-spike bearing shoots at anthesis appears to be relatively low in modern elite wheat cultivars (Reynolds et al., 2009). Thus, potential avenues for increasing spike

partitioning seem to rely on reducing partitioning to the structural stem DM (Foulkes et al., 2015), since it has been reported that stem water soluble carbohydrates (WSC) do not compete strongly with the spike for assimilate during the pre-anthesis period (Beed et al., 2007) and furthermore other studies have reported a positive association between floret survival and stem WSC, linked to greater spike DM and spike WSC content at anthesis (Dreccer et al. 2014). However, potential trade-offs between reduction of structural stem DM and lodging resistance must be taken into account (Berry et al., 2007; Foulkes et al., 2011). Moreover, potential increases in sink strength (grain number per unit area and/or potential grain size) will need to be matched by increases in source, i.e. enough photosynthetic capacity during grain filling to fill extra grains, to avoid possible source limitation. Within this context, a dynamic optimization of DM partitioning into reproductive and structural organs at anthesis and optimized source-sink balance are required for greater yield expression under potential conditions.

CHAPTER 2.

LITERATURE REVIEW

2.1 Genetic gains in wheat yield potential

Yield potential has been defined as "the yield of a cultivar grown in environments to which it is adapted, when nutrients and water are non-limiting, and when pests, diseases, weeds, lodging and other stresses are effectively controlled" (Evans and Fischer, 1999). As it has been shown that yield potential is well expressed in a wide range of environments (Reynolds et al., 2009), it remains a major component of yield progress (Fischer, 2007). Genetic gains in yield potential have been studied widely in CIMMYT and worldwide for spring wheat. In Northwest (NW) México, the work by Waddington et al. (1986) showed that the grain yield potential of cultivars released from 1950 to 1982 rose by 59 kg ha^{-1} yr^{-1} or about 1.1% yr^{-1}. In a similar study in NW Mexico, yield potential progress measured in eight semi-dwarf cultivars released between 1962 and 1988 increased by 67 kg ha^{-1} y^{-1} (0.88% yr^{-1}, r =0.99, P <0.001), indicating that linear progress in yield continued at least until the late 1980's within semi-dwarf germplasm (Sayre et al., 1997). More recently, yield potential progress in CIMMYT bread wheat varieties released in CIMMYT from 1996 to 2005 showed an increase of 0.51% yr^{-1} (r =0.78, P <0.01) with year of release under raised beds and 0.20% yr^{-1} (r =0.53, ns with year) under flat planting system with full irrigation (Fischer, 2007). Finally, the latest study on yield potential progress under potential conditions found a genetic gain of 0.59% per year in CIMMYT spring semi-dwarf wheat cultivars released from 1966 to 2009 in NW Mexico (Aisawi et al., 2015).

Genetic gains in yield in CIMMYT's spring bread wheat have been also quantified in different growing conditions in advanced lines. Examples include investigations by Sharma et al. (2012) showing a mean annual gain of 27.8 kg ha^{-1} (0.65%) over a period of 15 years (1995 – 2009) across 919 environments in 69 countries and Lopes et al. (2012) who found a linear increase in genetic yield progress of 0.7% yr^{-1} in 26 spring wheat lines released by CIMMYT (1977 to

2008), across different sites with a range of yield potential in Mexico, Asia and Africa. Manès et al. (2012) also reported grain yield increases of 1% yr^{-1} (31 kg ha^{-1} yr^{-1}) over a 17 year period in low and high yielding CIMMYT semi-arid wheat trials around the world. These studies exhibit continuing genetic yield gains in CIMMYT spring bread wheat.

Similar trends for genetic gains in yield potential have been shown in the rest of the world. Yields in the UK for winter wheat have increased progressively from 1972 to 2005 at a rate of 100 kg ha^{-1} yr^{-1} (Shearman et al., 2005) and 67 kg ha^{-1} yr^{-1} for bread making varieties released from 1953 to 2008 (Clarke et al., 2012). In France, wheat yields under favourable conditions increased at a rate of 63 kg ha^{-1} yr^{-1} in 14 winter wheat cultivars released from 1946 to 1992 (Brancourt-Hulmel et al., 2003). In China, genetic gain in grain yield for 13 cultivars and two advanced lines released from 1969 to 2006 was 62 kg ha^{-1} yr^{-1} (Xiao et al., 2012). A study examining 13 South Australian wheat varieties released from 1958 to 2007 showed linear increases with year of release at a rate of 3.4 kg ha^{-1} yr^{-1} (Sadras and Lawson, 2011). Although most of the above studies showed a linear progress in wheat yield, there are some studies in which genetic yield gains seem to show a plateau (Acreche et al., 2008; Graybosch and Peterson, 2010; Matus et al., 2012; Beche et al., 2014). For instance, Acreche et al. (2008) reported genetic gains in wheat yields only from 1940 to 1970 with no further increases thereafter under Mediterranean conditions in Spain, using seven representative bread wheat cultivars and two advanced breeding lines.

2.2 **Physiological basis of genetic gains in wheat yield**

Most of the studies investigating the physiological traits associated with yield potential progress in spring and winter wheat suggest that yield potential gains have been achieved through increases in HI and grains per unit land area (grains m^{-2}) (Waddington et al., 1986b; Austin et al., 1989; Slafer et al., 1990; Sayre et al., 1997; Fischer et al., 1998; Shearman et al., 2005). For six spring bread semi-dwarf cultivars under potential conditions in NW Mexico, Sayre et al. (1997) reported that yield potential progress was associated with greater grains m^{-2} and HI with no increases in above-ground dry matter (AGDM) or grain weight. In France, Brancourt-Hulmel et al. (2003) showed that grains m^{-2} increased from

1946 to 1992 with no changes in grain weight in 14 winter bread wheat cultivars. Clarke et al. (2012) recently reported that genetic gains in winter bread wheat released from 1953 to 2008 in the UK were associated with non-linear increases in HI without changes in grains m^{-2}; they showed a linear increase in grains per spike with no change in grain weight. Genetic yield gains through increases in grains m^{-2} have been associated with a greater fruiting efficiency ; for instance, Abbate et al. (1998) showed that increases in grains m^{-2} were achieved through greater FE, rather than increased partitioning to the spikes in Argentinian spring wheat cultivars. Similarly under Mediterranean conditions increases in grains m^{-2} were associated with greater grains per spike, spike DM at anthesis and FE in seven representative bread wheat cultivars released in Spain from 1940 to 1998 (Acreche et al., 2008).

Furthermore, photosynthetic-related traits were associated with yield progress in eight representative spring wheat cultivars released from 1962 to 1988 in NW Mexico, such as higher flag-leaf stomatal conductance, pre-anthesis radiation-use efficiency (RUE), maximum light-saturated flag-leaf photosynthetic rate (A_{max}) and canopy temperature depression (CTD) (Fischer et al., 1998). Similarly in the UK, Shearman et al. (2005) showed significant genetic changes with time and relations with gains in grain yield for pre-anthesis radiation use efficiency (RUE) and stem water soluble carbohydrate (WSC) and a linear increase in leaf specific weight with year of release. Genetic gains in grain yield were overall associated with increases in HI in the early phase of a 23-year period (1972 – 1995) and AGDM was linearly associated with yield gains from *ca.* 1983 (Shearman et al., 2005). They concluded that an increased pre-anthesis growth rate had resulted in more grains m^{-2} and a larger source for grain filling through increased stem WSC. Similarly, a recent study with historic CIMMYT spring wheat semi-dwarf cultivars found yield progress from 1966 to 2009 to be associated with increases in overall AGDM and grain weight and not grains m^{-2} (Aisawi et al., 2015). Lopes et al. (2012) also found linear grain yield progress in Mexico to be associated with greater grain weight, cooler canopies during grain filling and increased stay-green.

The physiological basis of increases in yield through the introgression of semi-dwarfing genes (*Rht*) is based on increased HI (Waddington et al., 1986) and a

proportionately smaller decrease in biomass; greater grains m^2 results from high spikelet fertility and more grains per spike (Flintham et al., 1997). However, not all combinations of *Rht* genes produce high grain yield and not all cultivars with high HI are high yielding, indicating that other factors are needed to achieve more yield (Rajaram and van Ginkel, 1996). Notwithstanding a hypothetical limit of HI of 0.62 in wheat, genetic HI progress over time indicates no systematic improvement since the early 1990s, when values were approximately 0.50 for spring wheat (Sayre et al., 1997; Reynolds et al., 1999) and 0.55 for winter wheat (Shearman et al. 2005). However, there might be scope for further increases in HI towards the hypothetical limit through improved understanding of the physiological basis of limits to partitioning assimilate to grain in high biomass cultivars (Foulkes et al., 2011).

2.3 General models for yield potential determination

One of the first studies mentioning a general model for yield determination was mentioned by Bingham (1969), describing yield as a product of the number of grains and the weight of those grains. Moreover, it was considered that grains m^{-2} is a product of following numerical subcomponents (Slafer et al, 1996) (Figure 2.1). Nonetheless, the approach of analysing grain yield in terms of grains m^{-2} and other numerical components has been recognized as not being completely effective for identifying the physiological basis of increases in yield potential (Slafer et al., 1996) because:

i) It is difficult to isolate the effect of a given yield subcomponent on grains m^{-2} due to compensation effects (Acevedo et al., 2002).

ii) Trade-offs between grains m^{-2} and average grain weight (Foulkes et al., 2009) although some exceptions have been reported in the literature (Gaju et al., 2009; Bustos et al., 2013).

iii) The magnitude of the parameters of these relationships (intercept and slope) is variable (Slafer et al., 1996).

iv) The numerical components of yield often exhibit a high GxE interaction.

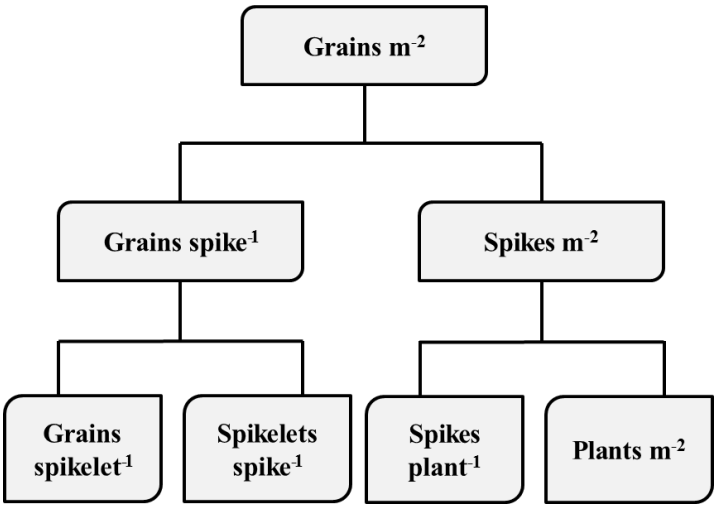


Figure 2.1 Diagram of the main numerical components of grains m^{-2}. Adapted from Slafer et al. (1996).

Therefore, models explaining yield from a physiological perspective (e.g. resource capture and use efficiency and plant development) may be more effective in identifying the critical traits which can enhance yield potential.

Within this context, models turned to explain yield potential generally as the transformation of solar energy into harvestable plant parts, covering three main processes: first, interception of solar radiation by the canopy, conversion of that light into chemical energy and partitioning dry matter between harvested parts and the rest of the plant at any given period of time (Araus, 1996).

Firstly and in terms of general crop dry matter production, Gallagher and Biscoe (1978) identified (assuming this is proportional to radiation absorption) that this is a product of high radiation absorbed by the crop (light interception) and the growth efficiency, considering photosynthesis and respiration.

One of the first models involving concepts such as harvest index within yield potential determination models was Hay and Walker (1989). More recently, Reynolds et al. (2005) developed a more integrative model for the physiological explanation of yield potential, as described in equation 2.1. They stated that further genetic progress in yield potential will depend on increases in AGDM, focusing on constraints to RUE and the physiological determinants of grains $^{-2}$ alongside continued increases in HI.

$$YP = LI\ x\ RUE\ x\ HI \qquad [2.1]$$

Where, LI is radiation interception, RUE is radiation-use efficiency (above-ground DM per unit interception) and HI is harvest index or the partitioning of biomass to grain yield.

2.4 Physiological determinants of yield potential

2.4.1 Crop development

In order to facilitate the understanding of the physiological determination of yield potential it is important to distinguish between crop development and crop growth, as they are driven by different environmental factors thus they can begin and progress at different rates; the first is mainly determined by temperature and day length whilst crop growth responds to the availability of resources (Weightman et al., 1997).

2.4.1.1 Developmental stages

The differentiation of the respective organs is what defines the various stages of wheat development and subsequently the phasing over which yield components are determined (Acevedo et al., 2002). Briefly, the following stages are usually distinguished: germination, leaf emergence, tillering, floral initiation or double ridge, terminal spikelet, first node or beginning of stem elongation, booting, spike emergence or heading, anthesis and maturity and the duration in time of each one depends on genotype, temperature, day-length and sowing date (Acevedo et al., 2002). These phases were earlier described by Kirby and Appleyard (1984), as well as Waddington et al. (1986) among others. For practical purposes, the most widely used scale to measure wheat developmental stages is the one proposed by Zadoks et al. (1974). This scale determines ten main developmental stages as growth stage (GS) 0 (germination), GS10 (Seedling growth), GS20 (Tillering), GS30 (Stem elongation), GS40 (Booting), GS50 (Inflorescence emergence or heading), GS60 (Anthesis or flowering), GS70 (Grain milk development), GS80 (Grain dough development) and GS90 (Ripening or physiological maturity).

During seed germination (GS0), the coleoptile grows after the seminal roots (GS10) protecting the emergence of the first leaf (GS11) (Acevedo et al., 2002). The first tiller to emerge is the one formed between the axil of the coleoptile and the first true leaf (GS21) (Kirby, 2002). Floral initiation occurs when the first

spikelet is initiated on the apex and the double ridge stage signals when about half of the spikelets have been initiated. The terminal spikelet phase coincides with the cessation of tiller production (GS30); then along with leaf emergence the onset of stem elongation introduces a new sink for assimilate (GS31) (Weightman et al., 1997). Rahman and Wilson (1977) showed that an increase in spikelet number is related with longer durations of the vegetative and spikelet phases and longer apices at floral initiation. Slafer and Rawson (1994) summarised some of the relationships between morphological changes and yield component determination with the different developmental stages (Figure 2.2).

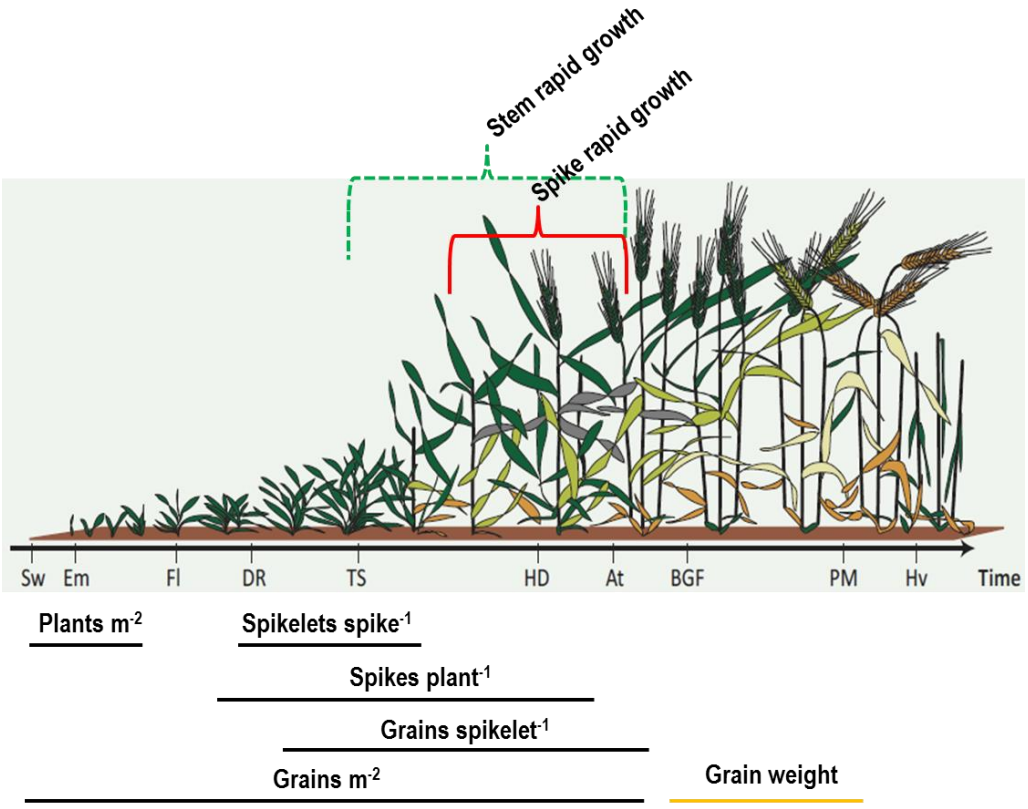


Figure 2.2 Diagram of wheat development and relationships between morphological changes and yield components showing stages of sowing (Sw), emergence (Em), floret initiation (FI), double ridge (DR), terminal spikelet (TS), heading (HD), anthesis (At), beginning of the grain filling (BGF), physiological maturity (PM) and harvest (HV) through time. Adapted from Slafer and Rawson (1994) and Reynolds et al. (2001).

2.4.1.2 Crop development and determination of grain number

As shown in Figure 2.2, some stages are more important in determining particular numerical components of grains m^{-2}. The period between terminal spikelet and

anthesis is of paramount importance in determining grains m^{-2} and yield potential (Slafer and Rawson, 1994) as confirmed experimentally by several studies (Fischer, 1985; Fischer and Stockman, 1986; Kirby, 1988; Siddique et al., 1989; Slafer et al., 1990). For instance, Slafer and Andrade (1993) showed that the maximum number of fertile florets was related to spike DM at heading rather than at anthesis, suggesting that the period of floret primordia death is crucial for determining the final number of fertile florets at anthesis, as differences between cultivars in the fate of florets were related to spike growth during a short period (approximately 25 days) before anthesis. This in turn has been investigated by comparing tall and semi-dwarf cultivars, showing that increases in grain number in semi-dwarf cultivars are associated with reduced competition between growing stems and the spike during the pre-anthesis phase (Fischer and Stockman, 1986; Siddique et al., 1989). In a recent analysis of a wide range of data sets on cultivars and near-isogenic lines (winter, spring, tall and semi-dwarf), Gonzalez et al. (2011) reported that the onset of floret death was related with the beginning of rapid spike growth and that rate of floret death (the main determinant of floret survival) showed a negative relationship with spike DM at anthesis (i.e. less spike DM induced greater floret mortality). Furthermore, a greater sugar concentration in the spike has been linked with greater floret fertility (Ghiglione et al., 2008; Dreccer et al., 2014b).

Fischer (1985) stated that the number of grains m^{-2} was also influenced by solar radiation (Rs) and temperature (T) under potential conditions in the 30 days preceding anthesis, summarised in the photo-thermal quotient (PTQ; MJ m^{-2} °C; equation 2.2):

$$PTQ = \frac{Rs}{T - 4.5} \qquad [2.2]$$

Where 4.5 is the base temperature for wheat growth and the numerator represents the positive association of R_S with crop growth rate during that period, while the denominator represents the negative effects of high temperature that shorten the duration of the spike growth period (Acevedo et al., 2002). The base temperature usually used in wheat when considering development stages around leaf emergence is 0 °C (Ritchie and NeSmith 1991; Lázaro and Abbate, 2011); however, the values for base temperature increase with the successive

developmental stages (Fischer 1985). In the case of the equation to calculate the PTQ in the 30 days preceding anthesis (eq. 2.2), the base temperature is 4.5 °C.

Bindraban et al. (1998) stated that PTQ takes into consideration the environmental conditions (radiation and temperature) while neglecting crop condition and found no association between PTQ from initiation of booting and the end of anthesis (GS40 to GS70) and grains m^{-2}. Other studies in wheat have shown significant associations between PTQ and grains m^{-2} (Savin and Slafer, 1991; Magrin et al., 1993; Ortiz-Monasterio et al., 1994; Abbate et al., 1995). The latter is in agreement with Fischer and Stockman (1980), who showed associations between pre-anthesis shading (10 – 13 days before anthesis) in spring wheat and reductions in grain number per spike through effects on grains per spikelet; this was related with reductions in spike DM at anthesis and reductions in the number of competent florets (florets with full anther development) per unit of spike DM. Furthermore, this study showed that shading before or after this period reduced grain number less, reducing spike DM but not competent florets per unit of spike DM and concluded that the most sensitive period to solar radiation was 20 days before anthesis when spike and stem were growing at their fastest rates.

In summary, the period after terminal spikelet to anthesis is when the whole process of floret initiation and death takes place, followed by ovule fertilization or grain set, resulting in final grains m^{-2} at one week after anthesis (Bindraban et al., 1998). Furthermore, the final stage of this period is when rapid spike and stem growth takes place; early booting (GS40) to final anthesis (GS70) as described by Bindraban et al. (1998); or the last 20 or 30 days before anthesis, starting when the penultimate leaf has emerged and ending soon after 50% of the spikes have started to flower, as described by Fischer (2011). This period is concurrent with floret mortality partly due to competition for assimilates between these organs (Fischer and Stockman, 1986; Kirby, 1988).

Tillering is another process influencing grains m^{-2} as it determines maximum shoots m^{-2}; tiller survival then determines final spikes m^{-2}. Tillering starts when the first tiller bud is mature to grow. When growth resources become limiting to maintain all tillers, some die in the opposite order of their appearance. The process of tiller mortality stabilises immediately before anthesis, when the number of spikes is defined (Slafer et al., 2009). Hucl and Baker (1989) reported that

tillers 1 to 3 did not differ from the main stem in culm length, spikelets per spike or harvest index, but produced fewer and lighter grains. Sharma (1995) in a study evaluating a set of 20 spring wheat cultivars under Nepal rain-fed conditions showed that cultivars differed in maximum number of tiller produced, number of reproductive tillers and tiller mortality. Furthermore, he reported that non-reproductive tillers contributed negatively to grain yield. Berry et al. (2003) showed significant cultivar differences in the percentage of shoots which died (32 to 63%) in winter wheat in the UK and concluded that non-surviving shoots will reduce yield potential in the majority of situations by competing for assimilate with the developing spike but the negative effects are most likely to occur under water limited conditions. Furthermore, reduced tillering in cereals has been proposed to increase assimilate supply to fertile tillers (Duggan et al., 2005). The tiller inhibition gene (*tin*) was shown to inhibit tillering in wheat plants, leading to larger spikes and thicker stems and leaves, known as 'Gigas' characteristics (Richards, 1988). Yield advantages of wheat lines containing the *tin* genes have been reported under terminal drought conditions, where grain protein content was not affected and in some cases favoured (Duggan et al., 2005). Moreover, Gaju et al. (2009) suggested that CIMMYT spring wheat large-spike phenotype cultivars (LSP) could offer positive departures from negative associations between fruiting efficiency and grain weight, as shown by increases in spikelets per spike and grains per spike along with increases in average grain weight of up to 20%, compared to one check cultivar (Bacanora).

2.4.2 Crop growth

2.4.2.1 Radiation capture and use-efficiency

The general ratio of photosynthetically active radiation (PAR) to total solar radiation, averages 50% from tropics to temperate latitudes (Monteith, 1965). In modern wheat varieties, light interception under favourable conditions from canopy closure until the onset of senescence is close to 100%, therefore light interception can only be increased by improving the rate of canopy closure or prolonging green canopy area in the final stages of grain filling (stay-green) (Reynolds et al., 2005). In this respect, achieving good canopy closure rates depends partly on specific leaf area and embryo size (López-Castañeda et al.,

1996; Richards, 2000). Leaf-area development is also responsive to nitrogen and so is RUE and crop growth rate (CGR; above-ground DM g m^{-2} d^{-1}) (Acevedo et al., 2002). Leaf senescence is a critical process for the fitness of plants and is regarded as an evolutionary acquired genetic process (Nam, 1997). Prolonged stay-green refers to slower senescence rates and there is evidence for genetic variation in these traits (Lopes and Reynolds, 2012). The onset of senescence has been negatively associated with the efficiency of above-ground N remobilization to the grain at anthesis (Gaju et al., 2011) and associated with decreases in photosynthetic rates and start or mobilization of stem reserves (Araus and Tapia, 1987). Another factor influencing light capture and radiation-use efficiency is the light extinction coefficient (K_{PAR}), which is mostly influenced by leaf angle and overall canopy architecture (Foulkes et al., 2009). Optimum values for wheat modern cultivars are related with semi erect to erect leaf angles that help reduce light saturation in the upper canopy hence boosting RUE (Reynolds et al., 2005).

Crop growth also depends on radiation-use efficiency (RUE), defined as the ratio of above-ground biomass to the radiation interception or absorption by the crop (Sinclair and Muchow, 1999). Genetic gains in wheat yields have been previously associated with increases in pre-anthesis RUE in the UK in winter wheat cultivars released from 1972 to 1995 (Shearman et al., 2005). Other studies have suggested that RUE has not been significantly improved, showing no associations between yield progress and overall above-ground dry-matter (Slafer et al., 1994; Calderini et al., 1995, 1999b). As for many other complex traits, RUE depends on environmental and physiological factors such as temperature, linked with optimum temperatures for photosynthesis (20-35 ºC) in C3 plants (Sage and Kubien, 2007). Moreover, Miralles and Slafer (1997) in a study comparing near-isogenic wheat lines differing in *Rht* alleles composition (dwarf, semi-dwarf and standard height) showed that light extinction coefficient and leaf thickness were higher in dwarf lines but this was related to a better capture of incoming radiation, the dwarf lines showing the lowest value of RUE in the pre-anthesis phase and the highest in the post-anthesis period.

2.4.2.2 Photosynthesis

The rate of photosynthesis increases as light (photon flux density) increases, reaching a saturated point (A_{max}). The slope of the initial linear portion of this curve is the maximum quantum yield (efficiency). The efficiency of photosystem II (PSII) can be estimated by the ratio of variable chlorophyll fluorescence to maximal fluorescence (F_v/F_m) (Figure 2.3 Light response curve. From Murchie and Niyogi (2011).

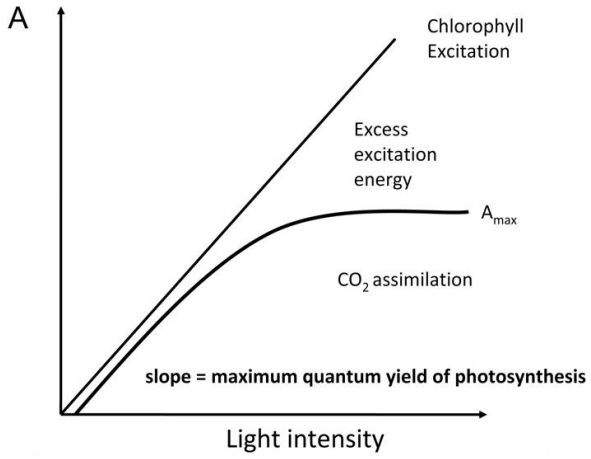


Figure 2.3 Light response curve. From Murchie and Niyogi (2011).

The primary determinant of crop biomass is the cumulative rate of photosynthesis over the growing season, which depends on:

i) The ability of the canopy to intercept light.
ii) The duration of light capture.
iii) The photosynthetic capacity and efficiency (Parry et al., 2011).

Regarding the latter, most studies conducted with the aim of relating improved yields and crop photosynthesis have been based on measuring light-saturated net carbon exchange rate or A_{max} of individual leaves (Reynolds, 2000). For instance, Fischer et al. (1998) reported that genetic progress in yield potential in semi-dwarf spring wheat cultivars released in NW Mexico from 1962 to 1988 was associated to greater flag-leaf stomatal conductance and A_{max}, as indirect evidence of increased CGR during stem elongation. On the other hand, nitrogen economy and photosynthesis are linked since Rubisco accounts for a large fraction of the N found in the leaves and other photosynthetic active tissues (Horton, 2000). Once canopy architecture and light interception have been optimized total

photosynthesis can be improved by increasing the photosynthetic capacity per unit leaf area (Parry et al., 2011). Rubisco (ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase) enables net carbon fixation through the carboxylation of RuBP (Parry et al., 2013) and, at a cellular level, it might be possible to modify genetically Rubisco CO_2 specificity thus decreasing photorespiration and raising net photosynthetic rate at the leaf level (Reynolds et al., 2005) as there is evidence for genetic variation in the catalytic properties of rubisco for different species (Parry et al., 2013).

In wheat and other C_3 cereals, grain filling is sustained by current photosynthesis of the upper parts of the plant, including the spike (Maydup et al., 2012), particularly in dry areas where source limitation can occur (Tambussi et al., 2007). The contribution of spike photosynthesis to grain filling has been estimated in wheat with ranges of 20 - 40%, depending on cultivar and growing conditions (Araus et al., 1993; Tambussi et al., 2007; Maydup et al., 2010), with significant genotype effects (Abbad et al., 2004). Moreover, Maydup et al. (2012) reported positive associations between spike contribution to grain filling (%) with year of release in 10 spring wheat bread cultivars released between 1920 and 2008. The spike contribution to grain filling has also been associated with its capacity to intercept a great proportion of light (25 to 40%) in a dense wheat canopy, depending on spike architecture (Reynolds et al., 2012). Another important characteristic of the spike during grain filling is the partial use of the CO_2 respired by the grains, increasing transpiration efficiency compared with leaves, as the CO_2 fixed in the spikes represents relatively less water losses (Evans and Rawson, 1970; Araus et al., 1993). Comparisons between spike and flag-leaf photosynthesis contribution to grain growth, yield and/or biomass have been reported in wheat (Rawson and Evans, 1971; Abbad et al., 2004; Sanchez-Bragado et al., 2014). For instance, Abbad et al. (2004) reported that whole-spike photosynthesis (organ) at anthesis was better correlated with grain yield and biomass under water-stressed conditions but also correlated in the absence of stress in six durum wheat genotypes studied in South Morocco, representing a wide range of variation in the shape and size of spikes and flag-leaf blades. While spike photosynthesis and its contribution to grain yield have been reported, the leaf-sheath photosynthesis and its contribution to grain growth remains unclear.

However, the role of leaf-sheath storage and subsequent remobilization of assimilates to the developing grains seems to be more important than its photosynthetic function after the onset of senescence occurs (Araus and Tapia, 1987). Evans and Rawson (1970) reported that at 12 to 15 days after anthesis, net photosynthesis in stem plus leaf-sheath was comparable to that observed by the flag-leaf blade, 41.7 and 49.7mg CO_2 day^{-1}, respectively.

2.5 Grain number model

2.5.1 Description of grain number model and its basis

Above-ground dry matter accumulation at anthesis (as an indicator of resource accumulation) does not usually explain variation in grains m^{-2} or grain yield, even under well irrigated conditions (Slafer et al., 1990; Gomez-Macpherson and Richards, 1995; Fischer, 2008). On the other hand, grains m^{-2} is more responsive to variation in radiation, temperature and the duration of the rapid spike growth period before anthesis, rather than anthesis date or the AGDM accumulated at this point (Fischer, 2008). Therefore, Fischer, (1983) proposed a more integrative model for grains m^{-2} determination (equation 2.3).

$$\textbf{Grains m}^{\textbf{-2}} = \text{Spike DM at anthesis (g m}^{-2}\text{) x FE} \qquad [2.3]$$

where, FE is fruiting efficiency (number of grains per g of spike DM at anthesis).

This model is supported by the general agreement that genetic variation grains m^{-2} is closely associated with greater spike DM accumulation (g m^{-2}) at anthesis (Fischer, 1983, 2007; Miralles and Slafer, 2007; Reynolds et al., 2009). Fischer (2011) further described the equation for the determination of spike DM at anthesis (Equation 2.4).

$$\textbf{Spike DM at anthesis (g m}^{\textbf{-2}}\textbf{)} = \text{Ds x CGR x SPI} \qquad [2.4]$$

where, Ds is the period from emergence to anthesis; CGR is the crop growth rate (g m^{-2} d^{-1}) in the same period and SPI is the spike DM as a proportion of AGDM at anthesis (Spike partitioning index).

This model is still one of the most widely accepted for grains m^{-2} determination. Fischer (2008) stated that this model is useful if the components are relatively independent and can be measured and understood. Therefore, quantifying the

available variation in the traits associated with grains m^{-2} and the degree of independence between them may be useful to further improve wheat yield potential (González et al., 2011).

In field experiments, several studies have shown that genetic variation in grains m^{-2} is closely related to spike DM at anthesis (g m^{-2}), related to competition for assimilate between growing spikes and stems (i.e. to floret survival) (Fischer, 1985; Fischer and Stockman, 1986; Slafer et al., 1990; Miralles and Slafer, 2007; Savin and Slafer, 1991). For example, Slafer et al. (1990) showed that both spike DM per unit area and SPI at anthesis were closely associated with each of grains m^{-2} and HI, rather than overall biomass production at that stage in six Argentinian bread wheat cultivars released from 1912 to 1980. Similarly, Bindraban et al. (1998) reported that AGDM at anthesis varied across different sowing dates and cultivars, but differences in grains m^{-2} were explained by spike DM per unit area at a week before anthesis and not by FE. Moreover, Fischer (1983; 2008) stated that the strong association between grains m^{-2} and spike DM per unit area was supported by associations between competent floret number, grain number and spike mass across different spike sizes (many or fewer grains) within a plot. With respect to the effect of Nitrogen (N) on grains m^{-2}, Fischer (2011) stated that under potential conditions (where yield is relatively unresponsive to N) there is no evidence to support the notion that N acquisition ability by crops is more important to yield determination than that of assimilate from photosynthesis driving GN, as variation in FE is not associated with spike N concentration. For instance, close associations between grains m^{-2} and spike DM per unit area at anthesis have also been observed in durum wheat, with no evidence for a direct involvement of N in grain number determination, beyond a general effect of N availability on spike DM per unit area at anthesis (Ferrante et al., 2012).

Along with the rate of spike growth, the duration of the stem elongation period as a proportion of the thermal time to anthesis is a key determinant of spike DM per unit area at anthesis (González et al., 2011). Several studies have previously found associations between grains m^{-2} and the duration of the stem-elongation period (Miralles et al., 2000; González et al., 2003, 2005, 2011; Serrago et al., 2008) and genetic variation for the duration of this period (related to photoperiod/vernalisation sensitivity) has also been reported, even among

cultivars showing the same anthesis date (Whitechurch et al., 2007; González et al., 2011). Slafer et al. (1996) suggested that further increases in spike DM per unit area at anthesis could be attained by lengthening the duration of this phase with the reduction of the previous phases and without major changes in anthesis date (as optimal anthesis date is usually already achieved depending on the region), resulting in increased fertile florets at anthesis and therefore grains m^{-2} (Fischer, 1985). As the duration of the stem-elongation period is sensitive to photoperiod (even more than the vegetative phase in some very photoperiod sensitive cultivars) (Slafer and Rawson, 1994; Slafer et al., 2001), one way to alter the duration of this phase is through changes in photoperiod environment (González et al., 2011). González et al. (2003) showed that the stem-elongation phase increased its duration with induced shorter photoperiods, resulting in more fertile florets at anthesis hence grains m^{-2} in three bread wheat cultivars, differing in height and spike to stem DM partitioning during the stem elongation phase. Serrago et al. (2008) reported that the number of fertile florets was reduced by extended photoperiod under optimum sowing dates; however, this was reversed by reducing sink size at booting (spike de-graining); they concluded the negative effects of extended photoperiod were also mediated by changes in assimilate supply (source-sink balance) to the developing florets. Similarly, González et al. (2005) showed that when the stem elongation was lengthened, spike DM at anthesis increased resulting in more fertile florets and that this was mediated by assimilate supply to the growing spike when comparing negative effects of shading and extended photoperiod on number of fertile florets.

The duration of the stem-elongation period and the crop growth rate (CGR; g m^{-2} d-1) capture the important environmental determinants of grains m^{-2} (Fischer, 2011). Wheat is a C3 crop, and Gardner et al. (1985) considered a typical CGR value for C3 plants to be 20 g m^{-2} d^{-1}; nevertheless, the theoretical maximum CGR for wheat is 71 g m^{-2} d^{-1} (Loomis and Williams, 1963). Most studies to date found no genetic variation in wheat for CGR during stem elongation (Sayre et al., 1997; Abbate et al., 1998; Lázaro and Abbate, 2011) with values between 10.8 to 29 g m^{-2} d^{-1}. Similarly, Slafer et al. (1990) showed no genetic gains with breeding in the efficiency of conversion of pre-anthesis intercepted radiation to dry-matter in six Argentinian bread-wheat cultivars.

2.5.2 Spike partitioning index and fruiting efficiency

The proportion of assimilate partitioning to the spike is not constant but it reaches a plateau around 10 days before anthesis. Moreover, it does not appear to be very influenced by radiation but it is more dependent on the cultivar, and is greater in shorter cultivars (Fischer, 1983) (see section 2.6). Abbate et al., 1997 reported that SPI was negatively related to the crop growth rate (CGR) and that the main effect of radiation on spike DM per unit area at anthesis was related to crop and spike growth rate, rather than SPI, using two Mexican and one Argentinian spring bread wheat cultivars. In contrast Slafer et al. (1990) showed that greater spike DM per unit area was explained by genetic variation in SPI, rather than overall biomass production at anthesis. Gaju et al. (2009) reported that a larger SPI at anthesis (0.27) in a large-spike-phenotype spring wheat line (longer rachis and more spikelets per spike), compared to other genotypes (0.22 – 0.23) resulted in greater spike DM per unit area; however, this resulted in fewer grains m^{-2}, since the fruiting efficiency was lower. Similarly, in a study with six representative Argentinian semi-dwarf spring wheat cultivars, Abbate et al. (1998) showed that spike DM per unit area at anthesis was closely related to the SPI; however, the cultivars with greatest Spike DM at anthesis did not produce the highest grains m^{-2}. Genetic variation in grains m^{-2} was instead explained by increased FE.

Genetic variation in FE has been widely reported in wheat and furthermore its importance in determining grains m^{-2} has been highlighted in recent investigations (Abbate et al., 1998; Gaju et al., 2009; Dreccer et al., 2009; González et al., 2011; Lázaro and Abbate, 2011; Gonzalez et al., 2011; Bustos et al., 2013; García et al., 2014; Aisawi et al., 2015). In contrast, Ferrante et al. (2012) showed that cultivar differences in grains m^{-2} were related to either spike DM per unit area at anthesis or fruiting efficiency; and Shearman et al. (2005) found cultivar differences for FE but no association with grains m^{-2} in modern winter wheat cultivars in the UK.

Some authors have hypothesised that the physiological limiting factors for increases in FE (*i.e.* low FE) could be related to a greater allocation of spike DM to non-productive sinks (glumes, awns and rachis) and/or to a lesser ability of developing flowers to benefit from available assimilates (e.g. Abbate et al., 1998). Gaju et al. (2009) suggested that an increase in rachis length per spikelet favoured

spikelet photosynthesis, and minimized an apparent trade-off between FE and potential grain weight. A greater WSC content in the growing spike has been associated with greater floret survival and presumably greater FE (Fischer and Stockman, 1980; Ghiglione et al., 2008). Moreover, Dreccer et al. (2014b) reported high stem WSC lines showed more grains per spike associated with more florets per spike, in turn, associated with a positive relationship between stem WSC and glucose amount in spikes.

With respect of the usefulness of FE to achieve gains in yield potential, Slafer et al. (2015) suggested that the most important potential trade-offs are with spike DM per unit area at anthesis and grain weight. From direct experimental evidence, some studies have reported negative associations between SPI and the FE (Dreccer et al., 2009; González et al., 2011; Lázaro and Abbate, 2011; Ferrante et al., 2012) while others showed a lack of association between these two determinants of grains m^{-2} (Abbate et al., 1998; González et al., 2011; Bustos et al., 2013; García et al., 2014). For instance, Abbate et al. (1998) reported differences in FE among cultivars with similar values of spike DM per unit area at anthesis in Argentinian spring wheat cultivars.

Fruiting efficiency is usually calculated with the spike DM per unit area at anthesis but can also be expressed as grains per g of chaff DM (structural parts of the spike) at harvest. When calculating FE with spike DM at anthesis it is important to consider the presence of any initial grain growth, and if present grains need to be removed to estimate properly spike DM (Fischer, 2011). Although in this respect, using chaff DM at harvest seems a simpler method to estimate FE, its calculation also requires particular attention. Fischer and Stockman (1980) previously reported that greater chaff DM at harvest than spike DM at anthesis, later estimated to be 20 to 50% more (Fischer, 2011), probably as a consequence of environmental factors or sampling methods. Furthermore, in a population of doubled-haploid lines García et al. (2014) reported that using chaff DM underestimated FE, showing a larger underestimation with greater values of FE. However, Abbate et al. (1997) reported that although chaff DM was higher, significant correlations were found between chaff DM at harvest and spike DM at the end of the spike growth period (r =0.84). Similarly, Abbate et al. (2013) found significant associations between genetic variation in FE based on spike DM at the

beginning of grain filling and chaff DM at maturity, even though chaff DM was greater than Spike DM at anthesis.

Regarding FE as a selection criteria in breeding programs, García et al. (2014) reported positive transgressive segregation (i.e. doubled-haploid lines that exceed phenotypic values of the parents) for all the grains m^{-2} model determinants; and moreover showed that FE was explaining most of the differences in grains m^{-2} among the highest yielding doubled-haploid lines. Martino et al. (2015) also found relatively high heritability and positive transgressive segregation for FE, despite decreases in spike DM per unit area, suggesting that early selection for high FE is possible. In this respect, both Abbate et al. (2013) and Mirabella et al. (2015) reported low genotype x environment interaction for FE, suggesting that this trait is mostly genetically controlled.

2.6 **Dry matter partitioning**

Dry matter partitioning is the end result of co-ordinated processes acting on dry matter (i.e. distribution of DM between organs of a plant) (Marcelis, 1996). Furthermore, it is more likely that a series of events rather than single limiting events control partitioning (Marcelis, 1996), as regulation that may occur in one region of the plant is not isolated from other parts of the plant (Wardlaw, 1990).

It is well known that reductions in plant height through the introgression of the *Rht-B1b (Rht1)* and *Rht-D1b* (*Rht2*) alleles in wheat increased grain yield through increased lodging resistance allowing higher N fertilizer application (Gale and Youssefian, 1985) and increasing DM partitioning to the developing spike (Brooking and Kirby, 1981). The *Rht* semi-dwarfing alleles caused a reduced response to the gibberellin class growth plant hormones, that participate in developmental processes determining cell wall elasticity during stem elongation (Hooley, 1994; Hedden, 2003). Hoogendoorn et al. (1990) showed that the semi-dwarfing genes *Rht1* and *Rht2* reduced flag-leaf and peduncle length by 15 %, while *Rht3* caused a reduction of 50%, mainly through reductions in cell length, related to decreases in osmotic potential of the cell solutes. They concluded that effects on cell length rather than number could explain why under favourable conditions, these genes have been found to reduce plant height with only slight reductions in biomass with the effect on biomass proportionally less than the

increases in HI. For instance, leaves (and especially younger leaves) in semi-dwarf cultivars may be reduced in size, but compensate with increased photosynthetic rates, so that overall biomass is typically only slightly reduced compared to tall (*rht*) lines (Flintham et al., 1997). Thus, breeders have increased grain yields with small decreases in biomass at anthesis (Slafer and Andrade, 1993) linked to proportionally larger improvements pre-anthesis DM partitioning to the spikes and increases in HI (Slafer et al., 1990).

The physiological basis of a more efficient DM partitioning among plant organs, as an effect of the introduction of *Rht* alleles in wheat, has been related to relatively greater allocation of DM to the developing spike than to the developing stem at the time of maximum spike growth (shortly before anthesis); resulting in greater spike DM, more fertile florets and grains per spikelet and higher HI and yield; as shown in winter wheat (Brooking and Kirby, 1981) and in near-isogenic spring wheat lines (Borrell et al., 1991). Miralles et al. (1998) showed that increased number of fertile florets in semi-dwarf compared to tall isogenic wheat lines was due to increased floret survival in relatively distal primordia. On the other hand, Youssefian et al. (1992) found that differences in spike to stem DM ratio among near-isogenic spring and winter wheat short and tall lines were apparent even before terminal spikelet formation. Similarly, Fischer and Stockman (1986) and Brooking and Kirby (1981) observed that competition between stem and spike could be also due to small differences in apex size which may have existed from the early stages of floral differentiation. Thus, since stem and spike growth overlaps during the stem-elongation phase especially during rapid spike growth shortly before anthesis (Brooking and Kirby, 1981), true-stem growth must be associated with final DM allocation to the spike at anthesis differently among true-stem internodes according to the extent of overlap.

2.6.1 True-stem development

Stem elongation is an acropetal process (Bonnett and Incoll, 1992) that starts prior to terminal spikelet formation in the apex, and continues until after anthesis with a constant interval in thermal time between the start of elongation of successive internodes and faster elongation rates (internode length per unit thermal time) for the more distal internodes in wheat stems (Youssefian et al., 1992a). Bonnett and

Incoll (1992) reported that the extention of each internode in winter barley was initiated as the internode below it was about to enter its period of maximum growth rate. Moreover, Borrell et al. (1989) reported that maximum length as well as rate of extension of stem internodes decreased progressively down the stem; whereas internode specific weight (mg cm^{-1}) was reported to be progressively greater down the stem (Borrell et al., 1993; Ehdaie et al., 2006a). Maximum growth rates of stem during stem extension have been reported to vary from 11 to 17 days before anthesis to around anthesis (Borrell et al., 1989, 1991; Youssefian et al., 1992a), when both peduncle and penultimate internode were rapidly extending (Youssefian et al., 1992a); however, maximum stem length is usually reached after anthesis (Borrell et al., 1989; Youssefian et al., 1992a; Bonnett and Incoll, 1992; Ehdaie et al., 2006a), with stem extension after anthesis attributed solely to peduncle extension. Borrell et al. (1989) reported that the period between the attainment of maximum length and maximum mass was minimal; for the peduncle but was 38 days for internodes 6 to 8 (top-down) in the semi-dwarf spring wheat cultivar Condor.

Genetic variation in partitioning of stem length into peduncle, penultimate and lower internodes, as well as in the timing for the peduncle to reach its maximum length, has been reported among spring bread and durum wheat cultivars (Ehdaie et al., 2006a). In this respect, Youssefian et al. (1992) showed that *Rht* did not affect the number of elongated internodes in near-isogenic lines, demonstrating that in all genotypes only the six uppermost internodes elongated. However, they found that *Rht* genes reduced lengths of all elongated internodes, although the greatest absolute effects were on the upper internodes with constant percentages of reduction in length for all internode positions, so that relative contribution of each internode to total stem length was unaffected by *Rht* alleles. They also concluded that semi-dwarfing genes did not affect the timing of stem developmental events but reduced the rate of stem elongation and vegetative dry-matter accumulation, resulting in reduced competition by stem for assimilate, leading to a greater proportion of dry-matter partitioned to spikes.

2.6.2 Genetic variation in DM partitioning among plant organs

For a given genotype under controlled conditions, spike DM partitioning at anthesis tends to remain quite stable across crops with different above-ground biomass, as caused by early photoperiod effects on spikelet number (Fischer, 2007, 2011). Genetic variation in spike DM partitioning index (spike DM as a proportion of AGDM; SI) at anthesis has been reported to range from 0.12 to 0.21 (Shearman et al., 2005) and from 0.15 to 0.20 (Pask, 2009) in winter wheat in the UK. In spring wheat, SPI has been shown to range more widely from 0.16 to 0.29 in Mexico (Reynolds et al., 2001a; Gaju, 2007; Aisawi et al., 2015) and Australia (Siddique et al., 1989). In Argentinian spring wheat, Abbate et al. (1998) reported values of mean SPI during the rapid spike growth period to range from 0.28 to 0.35 and of SPI at anthesis to range from 0.19 to 0.21. Comparing older (taller) with newer (shorter) cultivars, Fischer (1983) showed ranges for SPI from 0.15 to 0.24, respectively, for old and modern Mexican spring wheat cultivars. Finally, Slafer et al. (1990) reported SPI values for spring wheat ranging from 0.11 to 0.19 for the oldest tall and the newest semi-dwarf cultivars.

Values for DM partitioning to other organs, such as lamina, true-stem and leaf-sheath have been less frequently reported in the literature. Aisawi et al. (2015) reported ranges for true-stem plus leaf-sheath DM partitioning seven days after anthesis of 0.52 to 0.57 within a set of 12 historic CIMMYT spring wheat cultivars; and an increase from 0.53 to 0.56 over a 43-year period (1966 – 2009), with increases mainly after ca. 1980. The same study showed also that SPI increased from 1966 up to *ca*. 1980 and then decreased to 2009, with overall a small decrease from 0.25 to 0.23 over the 43-year period. Leaf lamina PI was shown to range amongst genotypes from 0.19 to 0.21 in winter wheat in the UK (Shearman, 2001) and from 0.25 to 0.31 in spring wheat in Mexico (Gaju, 2007). Linear increases in leaf lamina specific dry weight (leaf lamina dry weight per unit leaf lamina area) have been observed with breeding in the UK (Shearman et al., 2005). Aisawi et al. (2015) found no association between lamina PI at anthesis plus seven days and the year of release in CIMMYT historic spring wheat cultivars from 1966 to 2009.

2.6.3 Stem water soluble carbohydrates (WSC)

The stem DM is divided between structural (mainly cellulose and lignin in the stem walls) (Wang et al., 2012) and soluble components (glucose, fructose and sucrose but mainly fructans) in grasses (Blum, 1998; Slewinski, 2012) that in wheat are produced and stored in parenchyma cells encircling the vascular bundles of stem internodes (Dreccer et al., 2014a). Stem storage is commonly analysed as total non-structural carbohydrates (TNC) or water-soluble carbohydrates (WSC) (Blum, 1998). Water soluble carbohydrates start accumulating in the pre-anthesis period up to 20 days before anthesis (Gebbing and Schnyder, 1999). Moreover, WSC contributing to grain growth can originate either from current assimilation (*i.e.* direct translocation to the grain) or from temporary storage pools that serve to accommodate photosynthate during periods of high production, for subsequent relocation to grain when the rate of photosynthetic production is less than demand to the grains (Borrell et al., 1989; Schnyder, 1993). This carbohydrate allocation (how, much, when and where) is mainly driven by source-sink dynamics within the plant (Slewinski, 2012). Significant genetic variation in stem WSC accumulation has been found among wheat cultivars (Ruuska et al., 2006; Saint Pierre et al., 2010). Ruuska et al. (2006) examined stem WSC concentration (%) at anthesis in a diverse set of wheat genotypes across multiple environments, in Australia reporting high but repeatable environmental differences in WSC % and showing genetic variation was mainly related to variation in the fructans component with little variation in sucrose and hexose content. Associations between genetic gains in yield potential and changes in stem WSC have been reported in the literature (Shearman et al., 2005; Foulkes et al., 2007; Xiao et al., 2012), and also associated with increases in pre-anthesis RUE (Shearman et al., 2005).

The extent of contribution of stem WSC reserves to grain yield depends on two factors, the ability to store assimilate and the efficiency of assimilate translocation to the grain (Ehdaie et al., 2006b). Several studies have investigated the contribution of stem reserves to grain yield, typically being lower under favourable conditions (10 to 20%) (Bidinger et al., 1977; Schnyder, 1993; Gebbing et al., 1999; Foulkes et al., 2002; Shearman et al., 2005) than under water limited or heat stressed conditions (van Herwaarden et al., 1998; Rebetzke et al.,

2008; Ehdaie et al., 2008), where assimilate previously stored in the stems can represent as high as 50% of the total grain weight. In the latter scenario, pre-anthesis reserves are particularly important in buffering grain yield against unfavourable conditions for photosynthesis during the grain filling period (Gebbing et al., 1999). Interestingly, Dreccer et al. (2009) reported that high WSC lines had similar or greater yield than low WSC lines (a set of recombinant inbreed lines contrasting for stem WSC %); as a consequence of lower grains m^{-2}, linked to lower spike density, greater grain weight and stems with less structural biomass. In addition to non-structural carbohydrates (or WSC), proteins remobilized from leaves and glumes can also contribute pre-anthesis C to grain filling (Simpson et al., 1983; Gebbing and Schnyder, 1999). Palta et al. (1994) showed that total contribution of pre-anthesis C and N to grain was 64 and 81%, respectively, and also reported that although post-anthesis C assimilation was reduced (57%) by water deficit, remobilization of pre-anthesis stored C was increased by 36%. The amount of WSC accumulated in the stems can be estimated by monitoring post-anthesis changes in stem dry-matter (DM) (Cruz-Aguado et al., 2000; Ehdaie et al., 2006a) or by directly measuring stem WSC % (Blum et al., 1994; Ehdaie et al., 2006b, 2008). Under field conditions, several studies have reported strong correlations between stem WSC % and stem DM (Ehdaie et al., 2008; Xue et al., 2009). Direct WSC % determination is usually done by destructive methods, sampling, drying, milling and then using the spectra-photometrical anthrore method (Yemm and Willis, 1954) or by near infrared reflectance spectroscopy (NIRS) calibrated with the anthrone method (Wang et al., 2011). The latter method allows more rapid and high-throughput estimations and selections of WSC content than the anthrone method. The consistency of rankings is generally lower for WSC amounts (g m^{-2}) than for WSC %, as calculations of WSC per unit area include variation in biomass (Dreccer et al., 2014a). Moreover, it is important to consider that WSC % is negatively correlated (genetically and environmentally) with N % in the stem tissues at anthesis (Ruuska et al., 2006; McIntyre et al., 2013), and also trade-offs between N-uptake and WSC accumulation have been reported (van Herwaarden et al., 1998).

In a very extensive study, Rebetzke et al. (2008) identified 7 to 16 QTLs for stem WSC % and reported high transgressive segregation for WSC % and wide ranges among the progeny contributing to moderate to high narrow sense heritability within environments (lower for WSC amounts); concluding that small effects of many WSC QTLs may limit the use of markers in marked-assisted selection in breeding programs for stem WSCs. As phenotypic selection for WSC % is presently costly and its complex genetic control requires further investigation targeting many alleles of small individual effects (McIntyre et al., 2013), another option for non-destructively monitoring WSC in the field is using canopy reflectance in the 350-2500 nm range with a hyperspectral radiometer as has been proposed recently by Dreccer et al. (2014a).

2.6.3.1 WSC and true-stem morphological properties

Developmentally, potential stem WSC accumulation and subsequent mobilization in wheat depends on stem length and stem specific weight (*i.e.* linear density, stem DM per unit length) (Ehdaie et al., 2006b). Blum et al. (1994) stated that storage capacity increases with longer stems and greater stem linear density. The last two internodes below the spike (peduncle and penultimate internode) have been shown to store the greatest amount of WSC (Wardlaw and Willenbrink, 1994; Cruz-Aguado et al., 2000; Gebbing, 2003; Dreccer et al., 2014a). Moreover, differences in WSC accumulation differ between the exposed and leaf-sheath enclosed parts of the peduncle, being greater in the enclosed part (Wardlaw and Willenbrink, 1994; Gebbing, 2003) and accounting for 88% of the fructans in the total peduncle at the time of maximum fructan content (Gebbing, 2003). Stem WSC storage in wheat has been shown to increase later in the enclosed part of the peduncle than in the penultimate internode, but the subsequent loss occurred at the same time (Wardlaw and Willenbrink, 1994), suggesting that some sort of signal for mobilization of stem reserves had a simultaneous effect upon all of the internodes. In this respect, evidence suggests that while WSC accumulate first in the lower internodes during pre-anthesis and continue to accumulate in these internodes as they fully extend (Bonnett and Incoll, 1992), true-stem upper internodes tend to accumulate WSC more rapidly once the demands for structural stem and spike growth are fulfilled (Bonnett and Incoll, 1992; Gebbing, 2003).

As a consequence of a 21% reduction in stem length, semi-dwarfing genes have been found to reduce stem WSC storage by 39% (Borrell et al., 1993) and although the latter study did not show differences in the amount of absolute stored stem reserves (defined as the sum of the decreases in mass in each internode between time of maximum accumulation and maturity), the efficiency of use stem reserves as a proportion of deposition was greater in semi-dwarf cultivars compared to tall cultivars. In contrast, Rawson and Evans (1971) reported no differences between tall and short Mexican spring wheat cultivars in the capacity of stem reserves for grain filling under limited photosynthesis conditions. Furthermore, the latter study reported that stem reserve mobilization increased particularly from the lower internodes, when post-anthesis photosynthesis was reduced artificially.

Genetic variation in true-stem internode morphological attributes, related with WSC accumulation capacity, has been previously reported in wheat (Borrell et al., 1993; Cruz-Aguado et al., 2000; Ehdaie et al., 2006a; Saint Pierre et al., 2010), as well as high heritability values for stem morphological attributes, such as stem length and internode 2 length and thickness under water deficit and irrigated environments (Saint Pierre et al., 2010). Furthermore, genotypic differences in mean and maximum stem weight were not related to anthesis date in bread spring wheat cultivars, indicating that stem weight can be changed in wheat breeding without significantly affecting the phenological periods (Ehdaie et al., 2006a).

2.7 **Potential grain size**

Most studies analysing the effects of plant breeding on yield potential have concluded that increases in grains m^{-2} have been more important than changes in grain weight, the latter appearing to be a more stable character (Slafer et al., 1994). Furthermore, there is evidence for a trade-off between grains m^{-2} and grain weight (Slafer and Andrade, 1989, 1993; Miralles and Slafer, 1995a), suggesting that further increases in grains m^{-2} can be partially offset by reductions in average grain weight (Miralles and Slafer, 2007; Bustos et al., 2013). However, as evidence suggests that grain weight under favourable conditions in wheat is most commonly sink limited (Borrás et al., 2004), competition among grains for assimilate may not necessarily be the cause of the trade-off between grain number

and weight (Miralles and Slafer, 2007). Acreche and Slafer (2006) reported that although average grain weight was reduced by increases in grains m^{-2}, this negative association was less significant for grains in different positions within the spikelet; concluding that the trade-off between these two yield components is mainly due to increased proportional contribution of distal grains with low potential weight, reducing average grain weight. Therefore, it is important to determine possible causes limiting grain weight potential in order to increase potential grain weight in all grain positions within the spikelet and/or in all spikelet positions within the spike, to break the negative linkage between grains number per unit area and grain weight yield.

In this respect, it has been reported that grains in more distal positions from the rachis have less access to macronutrient and micronutrient concentrations (Calderini and Ortiz-Monasterio, 2003), therefore grains in basal positions within the spike and within the spikelets tend to have greater grain weight potential (Calderini and Reynolds, 2000; Hasan et al., 2011). Reductions in grain weight potential by introgression in *Rht* alleles have been shown to be related with grain volume at the beginning of linear grain growth, associated with reductions in total cell number in the pericarp (Miralles et al., 1998a). Moreover, potential grain weight in wheat is largely determined during the 14 days after anthesis, as endosperm cell division ends within this period (Briarty et al., 1979) and coincides with the rapid net water uptake (Schnyder and Baum, 1992). The total number of cells in the endosperm at the end of the cellularization phase is closerly associated with final grain weight, this presumably being the main factor controlling the rate of starch accumulation during the linear grain-filling phase (Foulkes et al., 2011).

Developmentally, final grain weight in wheat is traditionally considered as the product of the duration of linear growth and the rate of this growth during grain filling (Fischer, 2011), where temperature is identified as the main environmental factor affecting the physiological determinants of final grain weight (Sofield et al., 1977), mainly related with reductions in the grain filling duration (Slafer and Miralles, 1992). However, Calderini et al. (2001) showed that the best physiological determinant of final grain weight post-anthesis was the rate of grain filling during the linear growth phase, rather than the duration of the grain filling

period. Evidence suggests that pre-anthesis conditions are also involved in the determination of grain weight potential (Wardlaw, 1994; Calderini et al., 1999a, 2001; Calderini and Reynolds, 2000). For instance, Calderini et al. (1999) reported that high temperatures between heading and anthesis (coinciding with the period of rapid growth of the carpel) affected final grain weight, having a different effect depending on the grain position within the spikelet. Similarly, Hasan et al. (2011) reported that carpel weight and its dimensions (volume and length), as well as water content at anthesis, were highly related to final grain weight, irrespective of the grain position. Therefore, potential grain weight is partly determined around anthesis by ovary growth of fertile florets occurring during pre-anthesis and endosperm cell number per grain during post-anthesis (Fischer, 2011; González et al., 2014). Moreover, associations between final grain size and the volume of floret cavity may suggest that early grain development could be influenced by glumes physical restrictions (Millet, 1986; Foulkes et al., 2011). Gaju et al. (2009) suggested that altered glume characteristics in large-spike phenotype spring wheat cultivars may have facilitated greater potential grain weight through alleviation of physical restrictions.

Some studies have suggested that the trade-off between grains m^{-2} and average grain weight could be related to trade-offs between potential grain weight and the fruiting efficiency (FE) (Fischer and HilleRisLambers, 1978; Fischer, 2007; Gaju et al., 2009; Ferrante et al., 2012, 2015), resulting in low yield advantages from increases in grains m^{-2} due to decreased FE (Slafer et al., 2015). However, as the trade-off between grains m^{-2} and average grain weight has been related with increases in more distal grains with lower grain weight potential rather than to an increased competition for resources (Acreche and Slafer, 2006) and grain weight is apparently not source limited (Borrás et al., 2004), Slafer et al. (2015) hypothesized that one cause of the potential trade-off between average grain weight and FE might be reduced supply of assimilate per floret (*i.e.* according to minimum floret survival assimilate requirements), causing reductions in the size of the fertile florets (carpels). However, González et al. (2014) reported no associations between FE and potential grain weight in wheat cultivars with great genetic variation in FE, concluding that improvements in FE do not represent a trade-off if with potential grain weight.

2.8 **Source-sink relationships**

A source can be defined as an organ that is a net exporter of carbon assimilate (e.g. photosynthetically active tissues), while source strength refers to the rate at which carbon assimilates are produced (Marcelis, 1996). However, although the main sink organ during grain filling is the grain, the source-sink status of other structures can vary during crop development, such as the peduncle (which still extends after anthesis) that can represent a minor permanent sink during the first part of grain filling (Fischer, 1983), but after that it contributes to post-anthesis photosynthesis and soluble DM remobilisation to the grain (Gebbing, 2003), representing a source within the plant. The capacity of the source to fill the potential sink is an essential component of yield potential and it is generally accepted that the actual rates of carbon partitioned to the yield sink (e.g. spike) at any given time also depends on competition for assimilate by other sinks (Blum, 1996). In this respect, yield during grain filling can be limited either by source (source-limitation) or sink strength (not enough grains or low grain weight potential). Another possibility is that grain growth is co-limited during grain filling, i.e. limited to a certain extent by both, the source capacity and the sink strength (Acreche and Slafer, 2009).

While sink limitation relies more on the physiological determinant of grains m^{-2} (pre-anthesis) and potential grain weight (pre and post-anthesis) (see sections 2.5 and 2.7, respectively), post-anthesis source can be responsive to increases in sink strength (grains m^{-2}) determined during pre-anthesis, as shown by Reynolds et al. (2005) with spring wheat lines containing the 7DL.7Ag translocation where post-anthesis RUE was increased indirectly by increasing sink strength (grains m^{-2}) due to greater light interception at booting stage. Another example of greater sink strength influencing post-anthesis RUE was shown by Calderini et al. (1997) who reported that after anthesis older cultivars accumulated less biomass and their RUE and CGR were smaller than those of modern cultivars, the latter maintaining almost the same levels of post-anthesis RUE reached during pre-anthesis. In contrast, some studies have suggested that increases in sink size promoted a greater post-anthesis source-limitation in modern bread wheat cultivars (Fischer and HilleRisLambers, 1978; Kruk et al., 1997). Other ways to reduce risk of source limitation during grain filling are related to mantaining or increasing RUE,

by optimized leaf architecture, prolonged stay-green, enhanced spike photosynthesis or genetic modifications of rubisco CO_2 specificity (see section 2.4.2). Moreover, stem WSC accumulation and remobilization (from pre to post-anthesis) play an important role in grain weight determination, especially during unfavourable conditions as described in 2.6.3. Manipulations in assimilate availability (source-sink treatments) can provide indirect evidence on whether yield may be either limited by its source strength, the sink capacity or co-limited by both (Borrás et al., 2004). Moreover, the degree of source-limitation will be sensitive to interplant competition and therefore source limitations to grain growth must be investigated under typical field conditions (Fischer and HilleRisLambers, 1978).

2.8.1 Source-sink manipulation treatments

In general, most studies performing source-sink manipulation treatments (shading, defoliation and de-graining) suggest that grain growth of wheat is mostly sink limited during grain filling under favourable conditions (Borrás et al., 2004). For instance, Slafer and Savin (1994) in a study altering the source-sink relationship by removing half of the spikelets reported that average grain weight was insensitive to increases in the availability of assimilates for both a semi-dwarf and a standard-height cultivar, implying that assimilate availability was sufficient to satisfy fully grain growth. Similarly, Ahmadi et al. (2009) showed that post-anthesis defoliation did not affect grain yield or individual grain weight in one high yielding winter bread wheat cultivar. Other studies have shown evidence for co-limitation of grain growth by source and sink during grain filling in modern wheat varieties (Shearman et al., 2005; Álvaro et al., 2008; Acreche and Slafer, 2009; Aisawi et al., 2015), which is defined as grain growth limited by both the source capacity and sink capacity (*i.e.* grain growth limited by sink size during early and mid-grain filling and by source size during late grain filling). Similarly, Cruz-Aguado et al. (1999) showed that, in spring wheat semi-dwarf cultivars under tropical conditions at different sowing dates, grain growth appeared to experience source limitation during early grain filling. Furthermore, Acreche and Slafer (2009) showed that grain growth during grain filling was significantly increased in trimmed spikes in modern lines but differently across different spikelet positions and also reported reductions in post-anthesis RUE. They

concluded that the magnitude of the responses reflected a co-limitation of grain growth by source and sink rather than source limitation in most modern cultivars, as a consequence of reduced strength of sink-limitation during post-anthesis by breeding.

2.8.2 Pre-anthesis stem assimilate and post-anthesis photosynthesis up-regulation

The interpretation of grain growth responses to source reduction treatments (e.g. defoliation) should take into account potential compensations by other photosynthetic active organs (e.g. spike) (Maydup et al., 2012) or by increased pre-anthesis assimilate contribution to grain growth during grain filling (Savin and Slafer, 1991; Cruz-Aguado et al., 1999). In this respect, although the flag-leaf lamina is the principal source of contribution of photo-assimilate imported by grains during grain filling, the removal of this organ can lead to increased photosynthetic activity of other green parts avoiding negative effects of limitations in source (Cruz-Aguado et al., 1999). However, the activity of other photosynthetic tissues, such as the leaf-sheath (Araus and Tapia, 1987) and the degree of source-limitation has been suggested to be more evident under warmer environments due to earlier senescence (Fischer, 1983). For instance, Cruz-aguado et al. (1999) reported that defoliation performed under tropical conditions in three spring wheat cultivars showed evidence of grain growth source-limitation during late grain filling.

Previous studies have reported increases in contribution of pre-anthesis assimilates to grain yield when photosynthetic capacity during grain filling is reduced artificially reduced (Rawson and Evans, 1971; Savin and Slafer, 1991; Cruz-Aguado et al., 1999), while others have reported no changes in stem assimilate remobilization (Álvaro et al., 2008; Ahmadi et al., 2009). For instance, Savin and Slafer (1991) showed that contribution of pre-anthesis assimilates to grain yield increased when by shading in one semi-dwarf Argentinian spring wheat cultivar. In contrast, Álvaro et al. (2008) showed that post-anthesis defoliation in 24 durum-wheat cultivars released in different periods did not affect dry-matter translocation (estimated as a difference between anthesis and maturity stem weight; DMT) and reported that reductions in translocation of leaf lamina

was compensated by translocation increases from the chaff. They concluded that DMT was rather controlled by the sink demand. While Maydup et al. (2012) reported that pre-anthesis DMT as a response to post-anthesis defoliation decreased with year of release in ten bread wheat cultivars released from 1920 to 2008, Rawson and Evans (1971) previously showed that tall cultivars were not more dependent that short ones on stem reserves and no more able to draw on reserves during grain filling in conditions limiting photosynthesis, in a study evaluating grain weight responses by artificial reductions in spike photosynthesis in six spring wheat cultivars varying in their number of dwarf genes. However, they also estimated loses in stem weight due to respiration at about one third, attributing the rest to remobilization and furthermore observed cultivar differences in this proportion of post-anthesis stem weight loss due to respiration. Interestingly, Bonnett and Incoll (1993a) showed that de-eared winter barley plants retained 43 to 55% of the WSC stored in their penultimate internode and did not lose any stored WSC from their fourth internode, suggesting that mobilization stem stored WSC is highly dependent on the sink demand.

Furthermore, interpretation of grain growth responses to source-sink manipulation treatments which reduce source size (particularly defoliation) must take into account potential up-regulation (i.e. compensation) of remaining photosynthetic tissues (Maydup et al., 2012), such as the spike or the leaf-sheath. Up-regulation of leaf or spike photosynthesis to artificial reductions in source has been infrequently reported. The contribution of spike photosynthesis (SP) in wheat ranges from 10 to almost 80% of the assimilates deposited in grains, depending on cultivar, growing conditions, and even on the methodology used to derive the estimates (Kriedemann, 1966; Evans and Rawson, 1970; Araus et al., 1993; Maydup et al., 2010; Sanchez-Bragado et al., 2014), observing an increase of spike contribution to grain yield with year of release (Maydup et al., 2012). For instance, Rawson and Evans (1971) reported that although spike photosynthesis contribution to grain filling during grain growth was estimated to vary from 16.3 to 24.9% in Mexican spring wheat cultivars, spike photosynthesis inhibition using 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea (DCMU) reduced grain yield only by 3.3%; implying low yield reductions occurred due to increases in flag-leaf net photosynthesis (up-regulation). In the case of leaf-sheath photosynthesis, its

contribution to grain filling remains unclear. For instance, Araus and Tapia (1987) reported that patterns shown by the rate of net CO_2 assimilation were basically similar in flag-leaf blades and leaf-sheath irrespective of differences in their absolute values (flag-leaf blade net CO_2 uptake was four times greater than in the flag-leaf sheath at 2 days after anthesis). Furthermore, they concluded that the role of leaf-sheath storing and later transporting assimilates to the developing grains seems to be more important than its photosynthetic function after the onset of senescence occurs.

2.9 **Objectives and hypotheses**

The main gaps identified in the literature review are related to i) the physiological basis of limitations in raising HI in modern spring wheat cultivars (especially in those showing high biomass or exposing relationships between increases in biomass with year of release moreover related with greater yields); ii) genetic variation and heritability of physiological determinants of spike fertility (e.g. spike partitioning index and fruiting efficiency) in modern spring wheat cultivars; iii) genetic variation and optimal DM partitioning among organs at anthesis in terms of spike growth, WSC accumulation and structural support and to iv) the extent of source limitation in modern spring wheat cultivars, as well as avenues to overcome it such as non-exploited leaf sheath and spike photosynthetic capacity and/or WSC remobilization to grain growth. Particular information not reported in the literature relates to i) genetic variation in true-stem and leaf sheath DM partitioning and morphology at the phytomer level (peduncle (internode1), internode 2, internode 3 etc.), as well as interactions with spike growth, plant height and phenology and to ii) leaf sheath WSC accumulation at anthesis and photosynthesis contribution during grain filling.

The overall aim of this thesis was to examine novel genetic variation and physiological determinants of grain yield and HI and identify candidate traits for increasing HI in a set of 26 CIMMYT elite high biomass spring bread wheat cultivars (CIMCOG panel) under high radiation conditions in NW Mexico.

2.9.1 Objectives

1. To quantify genetic variation and physiological determinants of grain yield and HI in a set of 26 CIMMYT elite high biomass spring bread wheat cultivars (CIMCOG panel) under high radiation conditions in NW Mexico.
2. To investigate associations between above-ground dry-matter (AGDM) and HI and physiological limits for further increases in HI in high biomass spring bread wheat cultivars.
3. To investigate genotypic variation in grains m^{-2} based on its physiological determinants, with a particular focus on spike DM as a proportion of AGDM (SPI) at anthesis +7 days (GS65+7d) and fruiting efficiency (grains per g of spike DM at anthesis; FE).
4. To identify novel genetic variation in DM partitioning among plant organs at GS65+7d and to investigate the physiological basis of potential competition for assimilate among spike and other sink organs (*e.g.* true-stem).
5. To quantify genetic variation in stem phytomer morphological attributes at GS65+7d in order to identify promising traits associated with trade-offs between spike and stem growth in the pre-anthesis phase, leading to increases in SPI, HI and grain yield to be used in breeding programs.
6. To investigate extent of source limitation of grain growth during the post-anthesis period (grain filling) in a subset of four spring wheat cultivars and to estimate leaf-sheath and spike photosynthesis contribution to grain growth by measuring grain growth and leaf-lamina and spike photosynthesis responses to source-sink manipulation treatments.

2.9.2 Hypotheses

1. Overall, variation in grain yield in the CIMCOG panel is explained by differences in AGDM at harvest and HI and there is a non-linear negative association between AGDM and HI.
2. Differences in grains m^{-2} found among CIMMYT elite spring wheat cultivars are related to greater SPI spike DM at GS65+7d and fruiting efficiency (FE).

3. There is high genetic variation in DM partitioning among plant organs at GS65+7d, with greater competition for assimilates with the spike by the true-stem than by the leaf lamina or leaf sheath.
4. There is high genetic variation in soluble DM accumulation and partitioning among plant organs at GS65+7d related to spike growth and HI within the CIMCOG sub-set of nine cultivars.
5. There is genetic variation in stem soluble DM remobilization (from GS41 to maturity) within the CIMCOG sub-set of nine cultivars.
6. There is wide genetic variation and high heritability in true-stem and leaf-sheath morphological characteristics (*i.e.* length, DM and specific weight (SW)) in different phytomers (*i.e.* peduncle, internode 2, 3 and remaining) at GS65+7d among the 26 CIMCOG cultivars.
7. Competition for assimilate between the spike and the true-stem during stem elongation is better explained by DM partitioning to true-stem upper internodes rather than to true-stem lower internodes.
8. Genetic variation in true-stem DM morphological traits (such as DM and SW) at GS65+7d is at least partly independent of plant height and anthesis date.
9. The extent of source limitation of grain growth differs among modern CIMMYT spring wheat cultivars, as indicated by grain weight responses to artificial reductions in photosynthetic capacity during grain filling.
10. Reductions in leaf-sheath photosynthesis (through leaf-sheath shading) reduce general post-anthesis photosynthetic capacity, resulting in source limitation of grain growth; as indicated by reductions in final grain weight compared to non-manipulated shoots and there is variation in responses among four CIMCOG cultivars.
11. There is an increase in spike photosynthesis and stem pre-anthesis DM translocation as responses (*i.e.* up-regulation) to lamina-lamina defoliation and/or leaf-sheath shading treatments (*i.e.* source reduction) during grain filling and these responses differ among four CIMCOG cultivars.

CHAPTER 3.

MATERIAL AND METHODS

This chapter describes the methodology used in four field experiments carried out in NW Mexico. Four field experiments were conducted in NW Mexico in 2010/11, 2011/12, 2012/13 and 2013/14 (henceforth referred as 2011, 2012, 2013 and 2014) under high potential conditions. Firstly in 2011, a set of 60 elite spring wheat cultivars (58 *T. aestivum* L. and 2 *T. turgidum* var. *durum*) was selected from different CIMMYT breeding programs on the basis of (i) representing historical genetic gains, (ii) cultivars derived from the most recent CIMMYT selection since 1999 including some, (iii) synthetic derived wheat material and (iv) two durum wheats as checks, showing high yields and/or high adaptation (Appendix 1). The panel was named 'CIMCOG' after CIMMYT Mexico Core Germplasm panel. These cultivars were characterized according to dry matter (DM) growth and partitioning at harvest and phenology. From the 60 cultivars grown in 2011, 26 were selected to be grown in the following two seasons (2012 and 2013). The basis for the selection of the 26 cultivars was: contrasting DM partitioning indices (e.g. DM partitioned to the spike and/or true-stem), high yield and biomass, narrower range of anthesis date, similar plant height, high spike photosynthesis, lodging resistance related traits and contrasting potential grain weight (See section 3.1). From the 26 cultivars, a subset of nine cultivars was selected for more detailed DM partitioning analyses during the crop growth cycle (the basis for this selection is described in section 3.5). Finally, in 2014, grain growth and photosynthesis DM responses to source-sink manipulation treatments were examined in a field experiment for four of the 26 cultivars. These four cultivars were selected from within the subset of nine to show contrasting true-stem and leaf-sheath DM partitioning indices. The experiments examined elite CIMMYT spring wheat material from the 'Mexico Core Germplasm' (CIMCOG) panel.

3.1 **Experimental field site**

The field experiments were carried out at the International Maize and Wheat Improvement Center (CIMMYT) experimental station within the Norman E. Borlaug Experimental Center (CENEB) in the Yaqui Valley near Ciudad Obregon (27°N, 110°W and 38 m above the sea level) in the state of Sonora, NW Mexico (Figure 3.1). The Yaqui Valley comprises about 255,000 ha of arable land grown under fully irrigated conditions, 60% of which is used to grow irrigated wheat, most commonly under the raised-beds system, each winter-spring season (Limon-Ortega et al., 2002). The Yaqui Valley is representative of CIMMYT world mega-environment one (WME1, irrigated temperate spring wheat), with a low average total rainfall of 60 mm, areas proximal to the coast providing a cooler grain filling and a high solar radiation environment (Fischer et al., 2014). The soil type in the research station has been classified as coarse sandy clay, mixed montmorillonitic typic calciorthid, low in organic matter (<1%) and slightly alkaline (pH 7.7) (Limon-Ortega et al., 2002). More recently, Verhulst et al (2009) classified the soil in the experimental station according to the World Reference Base (IUSS Working Group WRB, 2007), as a Hyposodic Vertisol (Calcaric, Chromic).



Figure 3.1 Location of the Yaqui Valley irrigation area and CENEB (Norman E. Borlaug Experimental Center) research station in the state of Sonora, NW Mexico.

3.2 **Experimental design and treatments**

3.2.1 Field experiment 2011

In 2010-11, 26 spring bread wheat cultivars were grown under full irrigation and high radiation conditions (Table 3.1). The cultivars were chosen from several CIMMYT breeding programs based on one or more of the following criteria: widely grown, wide adaptation, high grain number per m^2, high biomass, high light interception and radiation-use efficiency (RUE), large seed, large spike and high yield. Cultivar nine (Cirno C2008) was omitted from the analyses as it is a durum wheat, showing a very high grains m^{-2} and very low individual grain weight with relatively higher yield. A complete α-lattice design was implemented

with two replications (the experimental plan can be found in Appendix 2, highlighting the 26 cultivars analysed and presented in this thesis).

Table 3.1 List of CIMMYT elite bread spring wheat cultivars sown in the field experiments in 2012 and 2013 (selection from the 60 cultivars sown in 2011). Shaded rows represent the sub-set of nine cultivars selected for detailed DM partitioning analyses and source-sink treatments carried out in 2012 and 2013. This subset of cultivars examined for detailed source-sink manipulation responses in 2014 are marked with an asterisk.

Entry	**Name**	**YoR**	***†Rht***	**Traits considered**
1	BABAX/LR42//BABAX/3/VORB	2008	*Rht1*	Lodging related traits
2^{H}	BACANORA T 88	1988	*Rht1*	High grains m^{-2}
3	BCN/RIALTO	2008	*Rht1*	High spike photosynthesis and grains m^{-2}
4^{H}*	BECARD/KACHU	2009	*Rht1*	Lodging related traits
5	BRBT1*2/KIRITATI	2005	*Rht1*	Source limited
6	SAUAL/4/CROC_1/AE.SQUARROSA (205)	2009	*Rht1*	Sink limited
7	SAUAL/WHEAR//SAUAL	2009	*Rht1*	
8*	CMH79A.955/4/AGA/3/4*SN64/CNO67	2009	*Rht2*	Low SPI and high spike photosynthesis
10	CNO79//PF70354/MUS/3/PASTOR/4	2009	*Rht1*	Low HI
11^{H}	CROC_1/AE.SQUARROSA(205)//BORL95	2005	*Rht1*	High floret number
12	KINGBIRD #1//INQALAB 91*2/TUKURU	2009	*Rht1*	Sink limited
13	MILAN/KAUZ//PRINIA/3/BAV92	2001	*Rht1*	Closed and closed canopy
14^{H}	PAVON F 76	1976	*Rht2*	Source limited
15	PBW343*2/KUKUNA*2//FRTL/PIFED	2009	*Rht1*	High SPI
16	PFAU/SERI.1B//AMAD/3/WAXWING	2005	*Rht1*	
17*^{H}	SERI M 82	1982	*Rht1*	Open and erect canopy
18^{H}	SIETE CERROS T66	1966	*Rht1*	Early development
19	SOKOLL//PBW343*2/KUKUNA/3/ATTILA	2008	*Rht1*	Source limited
20^{H}	TACUPETO F2001/SAUAL/4/BABAX/LR42	2009	*Rht1*	Benchmark
21^{H}	TACUPETO F2001/BRAMBLING*2//KACHU	2009	*Rht2*	
22	TC870344/GUI//TEMPORALERA M 87/AGR	2006	*Rht1*	Closed and erect canopy
23	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92	2008	*Rht1*	Low HI, high yield
24*	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP	2009	*Rht1*	Source limited
25	BECARD	2005	*Rht1*	Widely adapted
26	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN	2009	*Rht1*	High biomass
27	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE	2009	*Rht1*	High HI

The abbreviations in this table can be found in the list of abbreviations in page II in this thesis. *†Information on the presence of Rht alleles was provided by Oscar Gonzalez from the John Innes Centre. The letter H stands for historic CIMMYT cultivars.*

The 26 CIMCOG spring wheat cultivars were genotyped for the presence of single nucleotide polymorphism in *Rht1* (RhtB1) and *Rht2* (RhtD1) using perfect markers available for these genes (Ellis et al., 2002) at John Innes Centre (data provided by Simon Griffiths, pers comm).

3.2.2 Field experiment 2012 and 2013

The 26 cultivars characterized in 2010-11 (Table 3.1) were sown again in the field experiments in 2011-2 and 2012-3 (Figure 3.2 and Figure 3.3 for 2012 and 2013, respectively), using a complete α-lattice design with three replications under raised beds (These experimental plans can be found in Appendices 3 and 4, respectively). Each plot consisted of two raised beds, each of which was 8.5 m long and 0.80 m wide with two rows (24 cm gap between rows), and with a 56 cm gap between beds.



Figure 3.2 The experiment site at CENEB in 2012 during the vegetative stage.



Figure 3.3 The experiment site at CENEB in 2013 with full canopy closure.

3.2.3 Field experiment 2014

From the 26 cultivars, four cultivars were selected for source-sink manipulation treatments and spike/flag-leaf photosynthesis measurements in 2014 (marked with an asterisk in Table 3.1). The experiment was arranged in a complete randomized block design with three replications (The field plan can be found in Appendix 5). The plots consisted of two raised beds each 5 m long and 0.8 m wide with two rows per bed (24 cm gap between rows) and with a 56 cm gap between beds (Figure 3.4). Details on the application of source-sink manipulation treatments are given in 3.5.5.1.



Figure 3.4 The experiment site at CENEB in 2014 during grain filling.

3.3 **Plot management**

The field experiments were sown on 7 December 2010, 8 December 2011, 23 November 2012 and 23 November 2013. Seed rates were 10.1 kg ha^{-1} in 2011, 10.8 kg ha^{-1} in 2012, 11.0 kg ha^{-1} in 2013 and 10.2 kg ha^{-1} in 2014. The cropping system used was raised-beds. Each plot consisted of two raised beds, each of which was 5 m long and 0.80 m wide with two rows (24 cm gap between rows); and a 56 cm gap between beds (Figure 3.5). The summer cropping used in every season was *sesbania sesban*, grown from late June to late August with two purposes; the first is related to the low organic matter content in the Yaqui Valley soils (<1%), promoting the incorporation of organic matter and Nitrogen into the soil. The second reason is to allow all the seeds from the previous experiment to grow. All the cultivation practices followed a prophylactic crop management with full tillage (mouldboard ploughing), appropriate fertilization, weed disease and pest control to avoid yield limitations. Application of fertilizers was the same in each experiment. The first application of nitrogen (N) (50 kg N ha^{-1}) was applied as urea during land preparation, followed by 40 kg ha^{-1} of phosphorous (P) as triple super phosphate at sowing. The second application of N (150 kg N ha^{-1}) as urea was applied at the time of first irrigation. In each experiment, herbicides for broad and narrow leaf weeds, fungicide and insecticide were applied as indicated in Appendix 4.

In each year, irrigation was applied five to seven times during the crop cycle at 3 to 4 weeks intervals using a gravity-based system (**2011:** 7 Dec, 8 Jan, 9 Feb, 6 Mar and 8 Apr; **2012:** 9 Dec, 14 Jan, 20 Jan, 9 Feb, 2 Mar, 16 Mar and 31 Mar; **2013:** 24 Nov, 8 Jan, 1 Feb, 22 Feb, 15 Mar and 4 Apr and **2014:** 23 Nov, 9 Jan, 31 Jan, 21 Feb, 14 Mar and 4 Apr).

The average dates of emergence (50% of the plot emerged) in the experiments were 15 December 2010, 17 December 2011, 2 December 2012 and 1 December 2013.

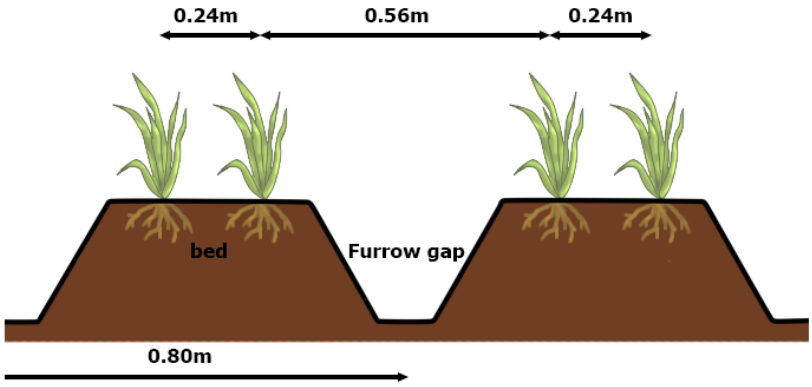


Figure 3.5 Transversal view of the field plots under raised beds. Adapted from (Fischer et al., 2005).

3.4 Meteorological measurements

Mean daily temperature (ºC), relative humidity (%), solar radiation (MJ m^{-2}) and accumulated monthly rainfall (mm) were obtained from the meteorological station 'CENEB CIMMYT ISONORAC3' (N 27° 22' 17", W 109° 55' 40" and 118 m above the sea level), approximately 1 km away from the Norman E. Borlaug Experimental Center. The hardware used was Davis Vantage Pro2Plus and data were processed with the software Wunderground v.1.15. A summary of the weather conditions prevailing in the four seasons can be found in Chapter 4 in Figure 4.1.

3.5 Crop measurements

A subset of nine cultivars was selected for detailed DM partitioning analyses and analysis in 2012 and 2013 (shaded cultivars in Table 3.1). These cultivars were selected to represent a full range of harvest index (HI) with a restricted range of anthesis date and a relatively narrower range of plant height, based on values observed in 2011 in the 26 cultivars. Unless otherwise stated, measurements were carried out in all 26 cultivars.

3.5.1 Plant establishment, crop development and plant height

In each experiment, plants were counted in each plot after emergence in a 0.5 x 0.8 m area. Dates of onset of stem elongation (GS31), initiation of booting (GS41), booting (GS45), anthesis (GS65) (Zadoks et al., 1974) and physiological

maturity (GS89) (50% of the peduncle, top-down, is completely dry and with a yellow colouration) were recorded for each plot, when 50% of the plot was at the corresponding growth stage. Final plant height and height to the spike collar were measured approximately in the second half of the grain filling period in each plot in the four experiments. Measurements were taken from the ground for 12 shoots per plot, randomly selected.

3.5.2 Crop growth and dry matter (DM) partitioning analysis at GS31, GS41 and GS65+7 days

In the experiments in 2012 and 2013, growth analysis samples were taken at anthesis +7 days (GS65+7) for the 26 cultivars, and for the four cultivars grown in 2014. Additionally, growth analysis was carried out at onset of stem extension (GS31) and onset of booting (GS41) in 2012 and 2013 for the subset of nine cultivars. Each genotype was sampled on the actual date of reaching the stage. Plant material was sampled in an area of 0.4 m^2 (four 50-cm length rows, 0.8 m total width) by cutting at ground level, at least 50 cm from the end of the plot to avoid edge effects (Figure 3.6). At GS65+7d, infertile shoots were counted as those without an emerged spike; the remaining shoots were classified as fertile. For DM partitioning analysis 10 fertile shoots were randomly selected from the growth analysis samples; the specific methodology utilised in each stage is outlined below.



Figure 3.6 Sampling for in-season biomass (Pask et al., 2012).

3.5.2.1 GS31 and GS41

Crop growth and DM partitioning analyses were carried out at GS31 and GS41 in 2012 and 2013 for the subset of nine cultivars. In each plot, the total sample was weighed for fresh weight and a 50% subsample (by fresh weight; SS 1) was used to determine infertile and fertile shoot number m^{-2}. Infertile shoots were classified as those that either had no green area or those for which the newest fully expanded leaf was completely senesced and counted; the remaining shoots were classified as fertile (Gaju et al., 2009). Above-ground DM was determined by drying SS1 for 48 h at 70°C. From the 50% remainder (SS 2), ten fertile shoots were separated into: i) leaf lamina and ii) true stem plus attached leaf sheath and at GS41 into: i) leaf lamina, ii) true stem and iii) leaf sheath (in 2013 only true-stem+leaf-sheath DM was determined). The green area was measured for each component using a leaf area meter (LI3050A/4; LICOR, Lincoln, NE). Dry matter of each plant component was obtained by weighing components after drying for 48 h at 70°C. Additionally at GS41, a subsample of 20 fertile shoots was taken from the whole sample for analysis of water soluble carbohydrate (WSC) content in the true stem. The methodology for analysis of WSC content determination is described in section (3.5.4).

3.5.2.2 GS65 + 7 days

Crop growth and DM partitioning analyses were carried out also at GS65+7d in 2012 and 2013 for the 26 cultivars. At GS65+7d, the total sample from each plot was weighed for fresh weight and a subsample of 100 randomly selected shoots was taken (SS 1). Above-ground DM was determined by drying SS 1 for 48 h at 70°C. Dry-matter infertile and fertile shoot number m^{-2} was determined by counting spike bearing shoots in SS1 before drying. From the 50% remainder (SS 2), ten fertile shoots were separated and DM partitioning analysis was carried out as described for GS31 and GS41, with the following extra measurements:

i) Plant components were separated into: i) green leaf lamina, ii) dead leaf lamina, iii) true stem, iv) leaf sheath and v) spike for the 26 cultivars from 10 spike-bearing shoots.

ii) WSC was analysed in the whole true stems at GS65+7d for the 26 cultivars.

iii) WSC was analysed in: i) spikes and ii) leaf sheaths at GS65+7d for the sub-set of nine cultivars.

iv) The 10 fertile shoots were further separated into true-stem and leaf-sheath phytomers in the 26 cultivars. Lengths were recorded for the following true-stem internodes and leaf-sheath sections: i) peduncle (t-s ped; l-sh ped), ii) internode 2 (t-s 2; l-sh 2), iii) internode 3 (t-s 3; l-sh 3) and iv) remaining internodes (true-stem internode 4 and leaf-sheath section 4 downwards; t-s rem; l-sh rem). The true-stem internodes were separated at the middle of each node (Figure 3.7 a) and the leaf-sheath sections were considered to start at the node and to end at the ligule (Figure 3.7 b) for the true-stem and the leaf-sheath, respectively). Dry weights for each component were obtained after drying at 70^{o}C for 48 h. Specific weight or linear density for each true-stem internode and leaf sheath section was also calculated as mg per cm. The stem phytomer traits measured at GS65+7d will be abbreviated hereafter as indicated below (*e.g.* t-s ped length; t-s ped WSC_C). Shaded rows indicate measurements of WSC in true-stem internodes.

True-stem:

t-s ped	True-stem peduncle: Dry matter (DM; mg shoot^{-1} and g m^{-2}), lengths and specific weight (SW).
t-s 2	True-stem internode 2 DM, lengths and SW.
t-s 3	True-stem internode 3 DM, lengths and SW.
t-s rem	True-stem internodes remaining DM, lengths and SW.
t-s ped WSC	True-stem peduncle WSC_C (concentration; %) and WSC_A (amount; mg shoot^{-1} and g m^{-2}).
t-s 2 WSC	True-stem internode 2 WSC_C and WSC_A
t-s 3+r WSC	True-stem internode 3 plus true-stem remaining internode WSC_C and WSC_A

Leaf-sheath:

l-sh ped	Leaf-sheath in the peduncle section DM (mg shoot^{-1} and g m^{-2}), lengths and SW.
l-sh 2	Leaf-sheath in the internode 2 section DM, lengths and SW.
l-sh 3	Leaf-sheath in the internode 3 section DM, lengths and SW.
l-sh rem	Leaf-sheath in the internodes remaining section DM, lengths and SW.

v) In addition for the nine cultivars, WSC concentration (WSC_C; %) and amount (WSC_A; g m^{-2}) were assessed in the following true-stem internodes: i) peduncle (t-s ped WSC), ii) internode 2 (t-s 2 WSC) and iii) internode 3 plus remaining internodes (t-s 3+r WSC). At GS65+7d, estimations of WSC amounts per unit area (WSC_A; g m^{-2}) were calculated as follows: above-ground fertile-shoot DM (g m^{-2}) x plant component DM partitioning x plant component WSC_C (%/100). The methodology for analysis of WSC content is described in section 3.5.4.

vi) True-stem internode DM partitioning indices were calculated as true-stem internode DM as a proportion of the above-ground shoot DM (*e.g.* peduncle true-stem DM partitioning index = t-s ped DM in mg $shoot^{-1}$/spike bearing shoot AGDM in mg).

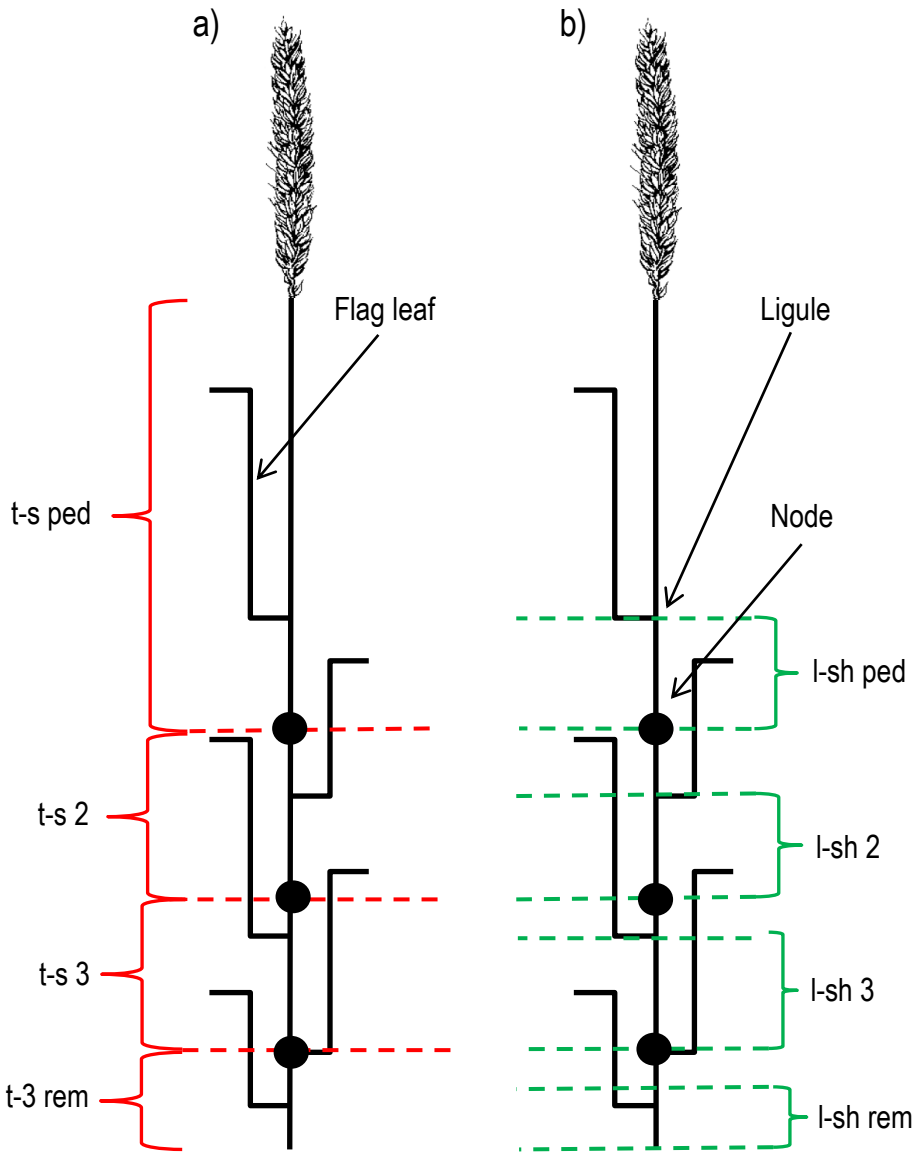


Figure 3.7 Diagram of a) true-stem and b) leaf-sheath sectioning at GS65+7 days for phytomer morphological analysis in the 26 spring wheat cultivars.

3.5.3 Crop growth and dry matter (DM) partitioning analysis at harvest

After physiological maturity was reached in each experiment, grain yield was measured in each plot by machine-harvesting an average plot area of 6.2, 5.7, 4.8 and 3.5 m^2, respectively for 2011, 2012, 2013 and 2014. Prior to that, a random sample of 100 fertile shoots was taken 1-2 days prior to harvest for all the cultivars, cutting each shoot at ground level randomly throughout the plot (avoiding 50 cm of edge at the plot ends). The plant material was dried, weighed and threshed, so that HI could be estimated. From this lot, 200 grains were randomly counted and weighed. Using these data, and an estimate of individual

grain weight, yield components, above-ground biomass and FE (ant and chaff) were calculated as shown in Table 3.2.

Table 3.2 Formulas for calculating grain yield, HI and yield components (Pask et al., 2012). Grain yield in g m^{-2}, grains m^{-2}, thousand grain weight (TGW, g), spike number m^{-2} and above-ground dry matter (AGDM at harvest (GS95), g m^{-2}). GW: grain weight, GM (%): grain moisture, SS: grain sub-sample (ca. 30 g) from the area harvested, FW: fresh weight and DW: dry weight (after drying at 70oC for 48 h).

Trait	**Formula**
Grain yield g m^{-2}	[(Plot GW x GM) + (100 culms DW x HI / Harvested area)]
Grains m^{-2}	Yield (g m^{-2}) / (TGW g / 1000)
TGW (g)	200 grains DW (g) x 5
Spike number m^{-2}	AGDM (g m^{-2}) / Single culm DW (g)
HI	Grain yield (g m^{-2}) / AGDM (g m^{-2})
GPS	Grains m^{-2} / Spikes m^{-2}
AGDM g m^{-2}	Grain yield (g m^{-2}) / HI
GM	(SS FW – SS DW) / (SS FW) x 100
FE ant	Grains m^{-2} / Spike DM at GS65+7d (g m^{-2})
FE chaff	Grains m^{-2} / Chaff DM at harvest (g m^{-2})

In the experiment in 2012 and 2013, additional DM partitioning analysis was done for the subset of nine cultivars, as described below.

The samples consisted of 20 randomly selected fertile shoots taken from the field 1-2 days prior to harvest (on the same day for all plots). The same methodology for general DM partitioning analysis (spike, true-stem, leaf-sheath and leaf lamina DM determination) as at GS65+7d (section 3.5.2.2) was followed. Analysis for WSC content was also carried out at harvest in the 20 fertile shoots, separately for the true-stem and leaf-sheath. The methodology for analysis of WSC content is described later in section 3.5.4. In addition from the 20 fertile shoot samples for the nine cultivars, three spikes were randomly selected and dissected into: i) glumes, ii) lemmas, iii) paleas, iv) rachis, v) awns and vi) grains (Figure 3.8). The different spike structural components and grains were bulked for the three spikes and weighed after drying at 70^{o}C for 48 h. The number of grains for the three spikes was also counted.

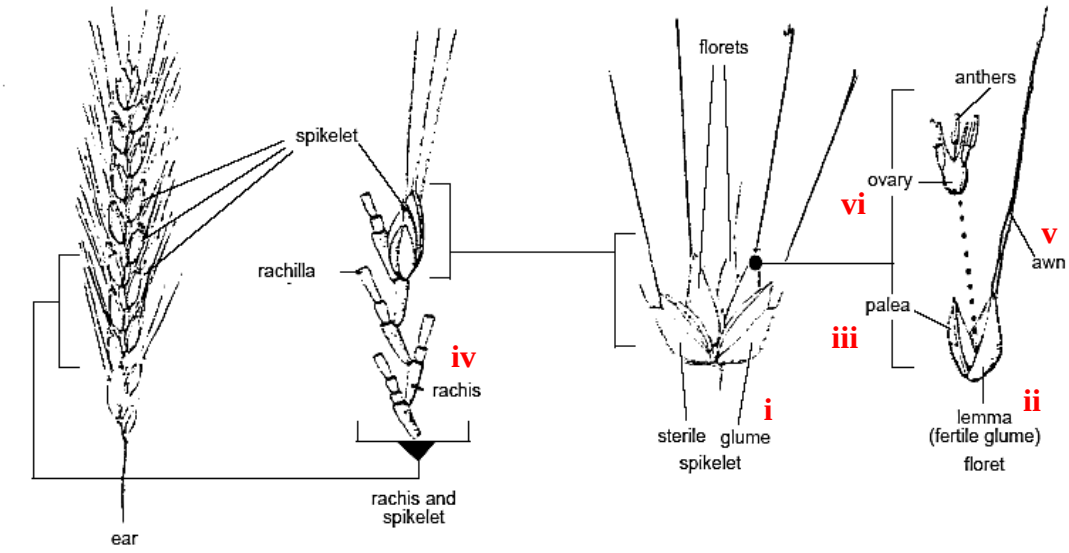


Figure 3.8 Wheat spike and structure of the spikelets and florets (Setter and Carlton, 2000) (Spike structural units separated and weighed at harvest are indicated in red.

3.5.4 Water soluble carbohydrates (WSC)

As described above for relevant cultivars and plant components at GS61+7d and harvest, water soluble carbohydrate (WSC) content was examined in the true-stem, leaf-sheath and spike. Total shoot fresh weight was firstly determined. After drying at 70^{o}C for 48 h, the relevant plant component(s) was weighed again, milled (IKA-WERKE mill, model IKA MF 10, Staufen, Germany) and passed through a 0.5 mm screen. The method used for WSC determination was the 'anthrone' method, as described by Yemm and Willis (1954) and following specific procedures described in Galicia et al (2009). Concisely, the anthrone method is based on the reaction of anthrone (9,10-dihydro- 9-oxoantraceno) with the furfural conformation of carbohydrates (treatment of carbohydrate in strong sulphuric acid) to give a coloured hemi-acetal, which is determined spectroscopically at 630 nm. The results were expressed as total WSC concentration on a DM basis (%).

For the nine cultivars, WSC content was additionally examined in the true-stem at spike emergence (GS55) and during grain filling (GS65+14d, GS65+21d, GS65+28d) following the same methodology for sampling and analyses as described above. WSC_A was calculated as: fertile shoots m^{-2} x DM of true-stem per fertile shoot (mg $shoot^{-1}$) x true-stem WSC_C (%)/100).

3.5.5 Source-sink manipulation treatments in 2014

3.5.5.1 Application of source-sink manipulation treatments

Source-sink manipulation treatments were applied at GS65+10 days in 2014 in four selected cultivars within the subset of nine cultivars (marked with an asterisk in Table 3.1). The selection was based on cultivars contrasting for values of DM partitioning among plant organs at GS65+7d. All the treatments were applied in 12 randomly selected spike-bearing shoots per plot across 1.5 m plot length (Figure 3.9 a), six shoots in the two outer rows and six in the two inner rows. Fertile shoots were tagged at GS65, taking into account different phenological strata within the plot (i.e. primary shoots were selected, *i.e.* those with spikes in the top 10 cm of the canopy).

The following source-sink treatments were applied to the tagged shoots:

1. ***Defoliation treatment***
2. ***Leaf-sheath covering treatment***
3. ***Leaf-sheath covering + defoliation***
4. ***Control***

The ***defoliation treatment*** (**DEF**) consisted of removing the lamina of the top three leaves (flag leaf, leaf 2 and leaf 3) at the ligule using a pair of scissors (Figure 3.10 a).

For the ***leaf-sheath covering treatment*** (**LSCOV**), pieces of light fabric were attached to cover the leaf-sheaths using a stapler from the spike collar downwards and leaving all the leaf lamina uncovered in a normal position. The fabric used was two-sided and was attached with the black side inside and white side on the outside to avoid overheating (Figure 3.9 and Figure 3.10 b). The extent of reduction in solar radiation by the fabric was roughly assessed by measuring the light under the fabric with a ceptometer (*ca*. 10%), resulting in a reduction of approximately 90%.

The treatment ***leaf-sheath covering + defoliation*** **(LSCOV+DEF)** consisted of a combination of defoliation (top three leaves) and leaf sheath covering also at GS65+10 days.

Twelve ***control*** (**CTRL**) shoots per plot were also tagged at GS65 to be sampled at maturity with the manipulated shoots.

In addition, a ***defoliation treatment was imposed on an area basis*** (**DEFarea**) consisting of removing the top three leaves at GS65 +10 days with scissors in an area of 1.2 x 0.8 m^2 (2 beds =0.96 m^2: 0.4 m wide and 1.2 m long each bed) (Figure 3.9 c).

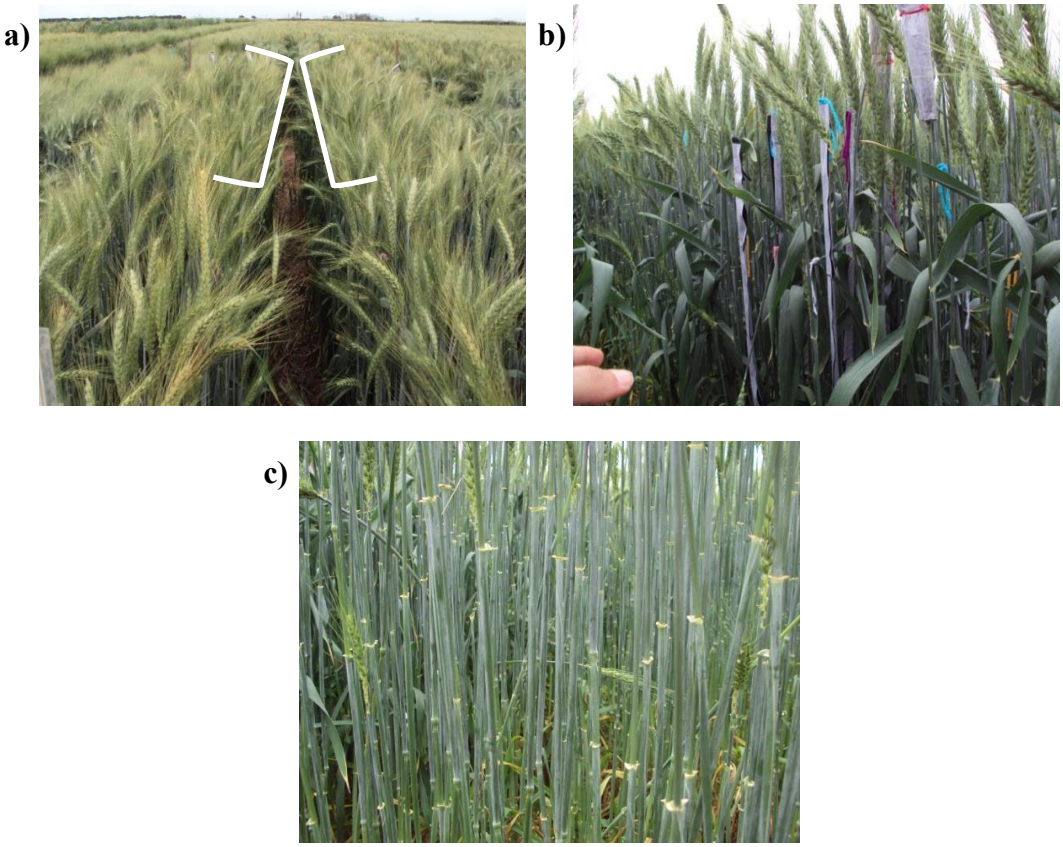


Figure 3.9 Field experiment pictures (2014): a) plot indicating the area designated for the manipulation treatments application (single shoot basis); b) LSCOV and LSCOV+DEF treatments and c) DEFarea treatment.

Moreover, approximately a 2 m plot-length was designated for a general un-manipulated area in each plot, in order to record crop development, AGDM at anthesis, grain yield, final plant height, HI and yield components following the methodology explained for previous seasons.

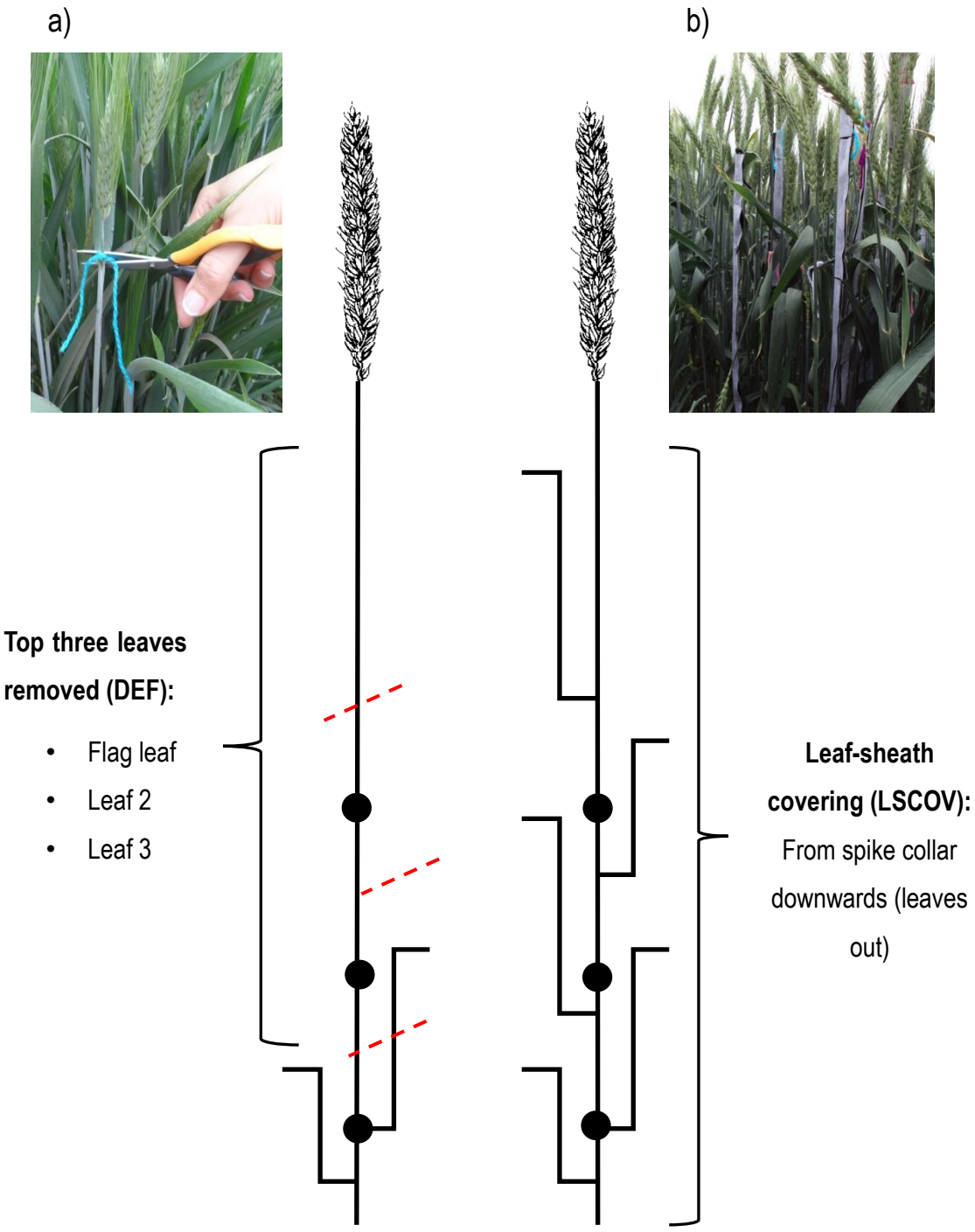


Figure 3.10 Diagram for a) defoliation (DEF) and b) leaf-sheath covering (LSCOV) applied to individual shoots (12 shoots per plot).

3.5.5.2 Dry-matter responses at physiological maturity

At physiological maturity (GS89), the 12 tagged shoots for source-sink manipulation treatments (including control shoots) were sampled from plots by cutting at ground level. The shoots were separated into the following components: i) spikes, ii) true-stem DM and iii) leaf-sheath. The spikes were threshed and the

grains per spike were counted. The plant components (bulked for the 12 shoots) were dried for 48 h at 80°C in order to determine their DM. Grain, true-stem and leaf-sheath DM responses to the manipulation treatments were calculated as DM losses or gains in relation to the control.

For the defoliation imposed on an area basis (DEFarea), 12 shoots were also sampled from the field to conduct analysis on DM responses and after that an area of 1 m^{-2} (avoiding an edge of 20 cm within the un-manipulated part of the plot) was harvested by hand in order to determine grain yield per unit area. Responses (% reductions) in grain yield for the defoliated area were calculated as the difference in grain yield compared to the general non-manipulated area.

As an estimate of C remobilization to the grains, stem DM translocation (DMT) was calculated as the difference between true-stem plus leaf-sheath DM at seven days after anthesis (GS65+7d) and physiological maturity (GS89) (Maydup et al., 2012). Dry-matter translocation efficiency (DMT_e, %) and the contribution of pre-anthesis stem plus leaf sheath reserves to grain yield (CPA) were calculated as in Álvaro et al. (2008):

$$DMT_e = \frac{DMT}{DM_{GS65+7}} \times 100 \quad [3.1]$$

$$CPA = \frac{DMT}{GW_{spike}} \times 100 \quad [3.2]$$

where DM_{GS65+7} is the DM per shoot at seven days after anthesis and GW_{spike} is the total grain weigh per spike at harvest.

3.5.5.3 Spike and flag-leaf photosynthesis responses during grain filling

Direct spike and flag-leaf photosynthesis rate measurements were taken during grain filling in different stages, treatments and cultivars in 2014 (as indicated in Table 3.3). Photosynthetic and respiration rates were measured as net CO_2 uptake using a LI-6400XT portable gas-exchange photosynthesis system (LICOR, Lincoln, NE, USA; Figure 3.11). To ensure steady-state conditions inside the chamber, the system was left to stabilize for 2-5 minutes. The flag-leaf photosynthesis rate was measured at a saturating photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1} and 30 °C.

Spike photosynthetic assimilation rate was estimated using a hand-made chamber connected to the LI-6400XT. The spikes were placed inside the chamber passing air through the chamber (Figure 3.12) at a rate of 1 L min^{-1} and measuring the molar fractions of CO_2 assimilated by the spike and humidity with the infra-red gas analyser (IRGA) of the LI-6400XT (Aranjuelo et al., 2009; Sanchez-Bragado et al., 2014). The CO_2 partial pressure was maintained constant with the infrared gas analyser-controlled CO_2 injection system. To ensure steady-state conditions inside the chamber, the system was left to stabilize for 2-5 minutes. An external light source composed of LED lights was placed around the chamber during the measurement achieving a saturating PPFD of approximately 1200 μmol m^{-2} s^{-1} measured inside the chamber (Figure 3.13).

Dark respiration of flag-leaves and spikes was measured immediately after the photosynthetic measurements at a temperature of 30 °C and maintaining the PPFP inside the chamber at 0 μmol m^{-2} s^{-1} by covering it with a two-sided piece of fabric (black side on inside and white side on the outside to avoid overheating). In order to calculate flag-leaf and spike photosynthetic assimilation rates per organ, the area of leaves and spikes was recorded using a leaf area meter (LI3050A/4; LICOR, Lincoln, NE, USA) after the field photosynthesis measurements.

Table 3.3 Summary of photosynthesis (Ps) measurements carried out for each cultivar in the flag-leaf and spike at 15 and 25 days after anthesis (GS65+15d and +25d). No photosynthesis measurements were conducted in cultivar CMH79A and in treatment DEFarea (shaded)

Cultivar	**Treatment**	**Flag-leaf Ps**		**Spike Ps**	
		GS65+15d	GS65+25d	GS65+15d	GS65+25d
BECARD/K	CTRL	√	√	√	√
	DEF	-	-	√	√
	LSCOV	√	√	√	√
	LSCOV+DEF	-	-	√	√
	DEFarea	*No*	*No*	*No*	*No*
CMH79A	CTRL				
	DEF				
	LSCOV			*No*	
	LSCOV+DEF				
	DEFarea				
SERI M82	CTRL	√	√	√	√
	DEF	-	-	√	√
	LSCOV	√	√	√	√
	LSCOV+DEF	-	-	√	√
	DEFarea	*No*	*No*	*No*	*No*
UP2338	CTRL	-	√	-	√
	DEF	-	-	-	√
	LSCOV	-	√	-	√
	LSCOV+DEF	-	-	-	√
	DEFarea	*No*	*No*	*No*	*No*



Figure 3.11 LI-6400XT portable photosynthesis system used to measure flag-leaf and spike photosynthesis in the field in 2014.

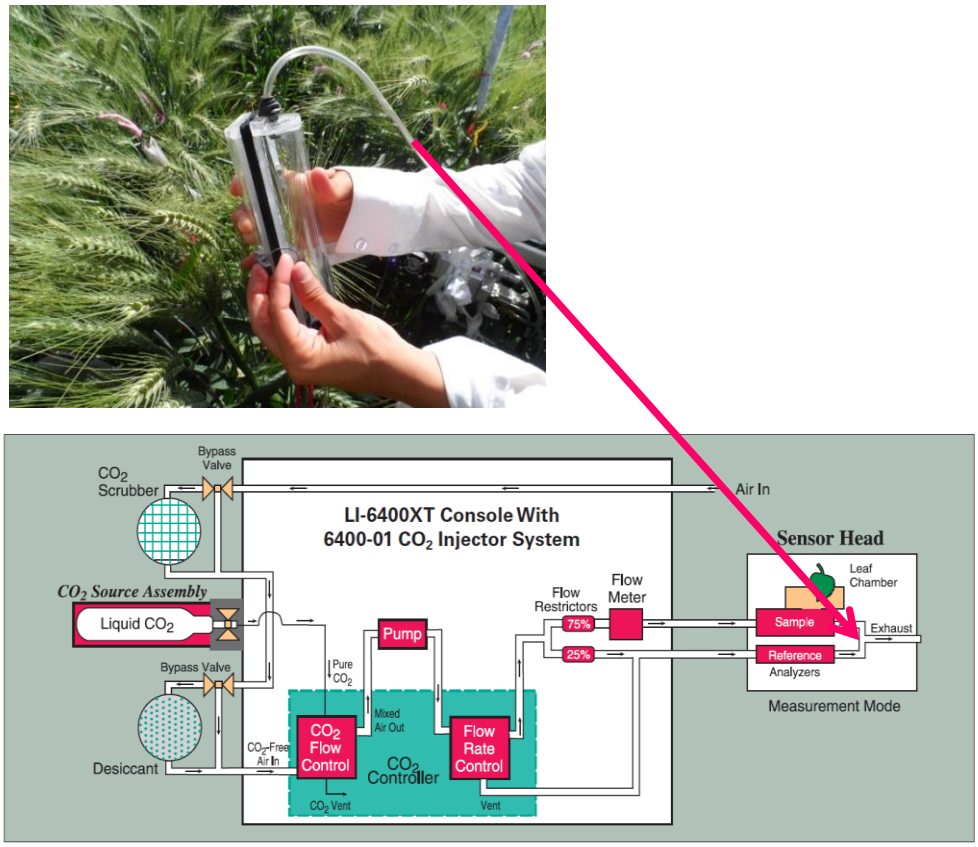


Figure 3.12 Spike chamber attached to the LI-6400XT used to calculate spike photosynthesis in the field in 2014. Diagram of the LI-6400XT system taken from the product brochure (http://www.licor.com/env/pdf/photosynthesis/LI6400XTBrochure.pdf).



Figure 3.13 Measurements of spike photosynthesis in the field using spike chamber and external light source with 10% Blue Light and a maximum PPFD of 1200 µmol m^{-2} s^{-1}.

3.6 **Statistical analysis**

Data collected in field experiments were subjected to analysis of variance (ANOVA) using a general linear model (GLM). For individual analysis in each experiment replications were regarded as random effects and cultivars as fixed effect. For Chapters 4 – 6, ANOVAs were carried out in 26 cultivars and additionally for Chapters 5 and 6 in a sub-set of nine cultivars. Finally for Chapter 7 (2014), ANOVAs were performed in a sub-set of four cultivars to investigate effects of source-sink manipulation treatments (defoliation and shading) on DM and photosynthesis responses. To investigate the effects of manipulation treatments and cultivar x treatment interactions (see Chapter 7), manipulation treatments were regarded as random effects.

For cross-year ANOVAs, years were regarded as random effects, combining three seasons (2011, 2012 and 2013) in Chapter 4 and two seasons (2012 and 2013) in Chapters 4 to 6. Additionally, cross-year ANOVAs were carried out in the sub-set of nine cultivars using data from 2012 and 2013 in Chapters 5 and 6. Standard error of differences between means (SED) and significant differences were summarised for seasons and cultivars in all cases. Additionally, in Chapter 7 cultivar means, source-sink manipulation treatment means and interaction means were compared using the least significant difference of the means (LSD) of Fisher. Linear and non-linear regression analyses and Pearson's correlation coefficient were calculated between cultivar means in each season (Chapters 4 – 6) and in addition for mean data across three (2011, 2012 and 2013; Chapter 4) and two seasons (2012 and 2013; Chapters 5 to 6). All the statistical procedures mentioned before were carried out using GenStat version 15.1 (VSN International, Hemel Hempstead UK).

Procedures to estimate broad-sense heritability of 26 cultivars across 2011-12 and 2012-13 for Chapter 6 and principal component analysis for the cross-year mean of 26 cultivars were also implemented. Broad-sense heritability (H^2) of a given trait within years was calculated as described in Equation 3.1 (Cooper and DeLacy, 1994) using META-R, an R-program for multi-environment analysis (Lopez-Cruz et al, 2015 in press):

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{re}} \quad [3.3]$$

where σ_g^2 and σ_e^2 are the genotypic and error variance, σ_{ge}^2 is the genotype x environment interaction. The number of environments and number of replicates are represented by *e* and *r*, respectively.

Principal component analysis (PCA) was used to examine associations between the cross-year means of true-stem internode morphological traits and main agronomical or physiological traits of 26 cultivars across seasons 2012 and 2013 (Chapter 6). PCA uses a multi-dimensional dataset and reduces the dimensions into the smallest number of components that account for the most variation among the variables (Jolliffe, 2002). The PCAs were done in R (https://www.r-project.org/) using the package FactoMineR (Lê et al., 2008) (http://factominer.free.fr/).

CHAPTER 4.

HARVEST TRAITS IN CIMCOG PANEL

4.1 Introduction

Understanding physiological processes under potential conditions is crucial to attain higher and more stable yields through breeding (Fischer 2007). Gains in yield potential (YP) are well documented for CIMMYT cultivars grown in NW Mexico; and as continued genetic progress in yield potential has been found for modern cultivars, it is important to understand the physiological basis of improved yields, grain number per unit area and HI. Although most of the studies associate gains in YP with increased grains m^{-2} and HI, some studies have reported associations between genetic gains in YP and AGDM in Mexico and the UK (Shearman et al., 2005; Aisawi et al., 2015), related to a combination of improved growth rate in the pre-anthesis period (increasing grains m^{-2}) and a larger source for grain filling and no apparent source limitation in CIMMYT spring wheat. Within this context, the present chapter attempts to describe novel genetic variation found for grain yield, AGDM, HI and yield components among the 26 CIMCOG cultivars in the three seasons, as well as to investigate the physiological basis of grain yield. In addition to the 3-year averages for 2011, 2012 and 2013, results averaged across 2012 and 2013 are also shown in this chapter to allow comparison of results for growth and DM partitioning carried out in 2012 and 2013 (Chapters 5 and 6).

Specific hypotheses tested in this chapter:

1. There is genetic variation in grain yield, yield components, HI and AGDM at harvest amongst a set of 26 CIMMYT spring bread wheat cultivars (CIMCOG) under high radiation conditions in NW Mexico.
2. There have been genetic linear gains in grain yield, HI, AGDM, grains m^{-2} and grain weight within eight historic cultivars selected from the set of 26 cultivars.

3. Variation in grain yield in the CIMCOG panel is explained by differences in grains m^{-2} and HI rather than AGDM at harvest and final grain weight.
4. There is a non-linear negative association between AGDM at harvest and HI amongst the 26 CIMCOG cultivars.
5. Grain number m^{-2} is positively associated with both number spikes m^{-2} and grains $spike^{-1}$ among the 26 CIMCOG cultivars
6. There is a positive association between grain yield and plant height among the 26 CIMCOG cultivars.

4.2 **Results**

The present Chapter presents results for harvest traits in 2011, 2012 and 2013. Harvest data in 2014 is presented in Chapter 7. Results for the sub-set of nine cultivars (see Chapter 3, section 3.5) are also presented to allow comparisons between results of harvest traits and detailed DM partitioning analyses carried out only for this subset of cultivars presented in the following chapters. In the present chapter, the sub-set of nine cultivars is represented by shaded rows in results tables.

4.2.1 Growing conditions in the experimental site

The prevailing weather conditions in the field experiments (mean daily temperature, relative humidity, solar radiation and accumulated monthly rainfall) are presented in Figure 4.1 for the three seasons, together with the long term-means (LTM; 1990 - 2013). Weather data are also presented for 2014, in which a source-sink manipulation treatment experiment was carried out (Chapter 7).

The mean air temperature from sowing (December) to harvest (April) was similar in 2011 (16.4 ºC), 2012 (16.3 ºC), 2013 (16.5 ºC), but warmer in 2014 (18.2 ºC). In general, just 2014 was considerably warmer that the LTM; and apart from 2011, March was warmer during the experimental seasons that the LTM. April was cooler than the LTM than during 2011, 2012 and 2013 (Figure 4.1 a).

Mean daily relative humidity (RH) was higher during the entire crop cycle in 2011 and 2012 than in 2013 and 2014 (*ca.* +6%), but no constant pattern was found for the relative humidity to differ among months, although it was lower in

April for all the seasons. The LTM was considerably higher than the RH observed in 2011 and 2012 (+7%) but similar to the average RH recorded in 2012 and 2013 (+1%) (Figure 4.1 b).

Overall, rainfall was negligible during the four seasons, with significant rainfall occurring just in December 2013 and 2014 (10.6 and 23.8 mm, respectively), February 2012 (13.7 mm) and March 2014 (17.7 mm). In general, apart from specific events in 2014, the monthly accumulated rainfall during the experimental seasons was generally lower than the LTM (Figure 4.1 c).Total incident solar radiation (SR) from sowing to harvest was slightly higher in 2011 (2708 MJ m^{-2}) and 2012 (2699 MJ m^{-2}) compared to 2013 (2367 MJ m^{-2}) and 2014 (2308 MJ m^{-2}). Mean daily solar radiation during post-anthesis (March and April) was lower than the LTM in 2013 and 2014 (Figure 4.1 d).

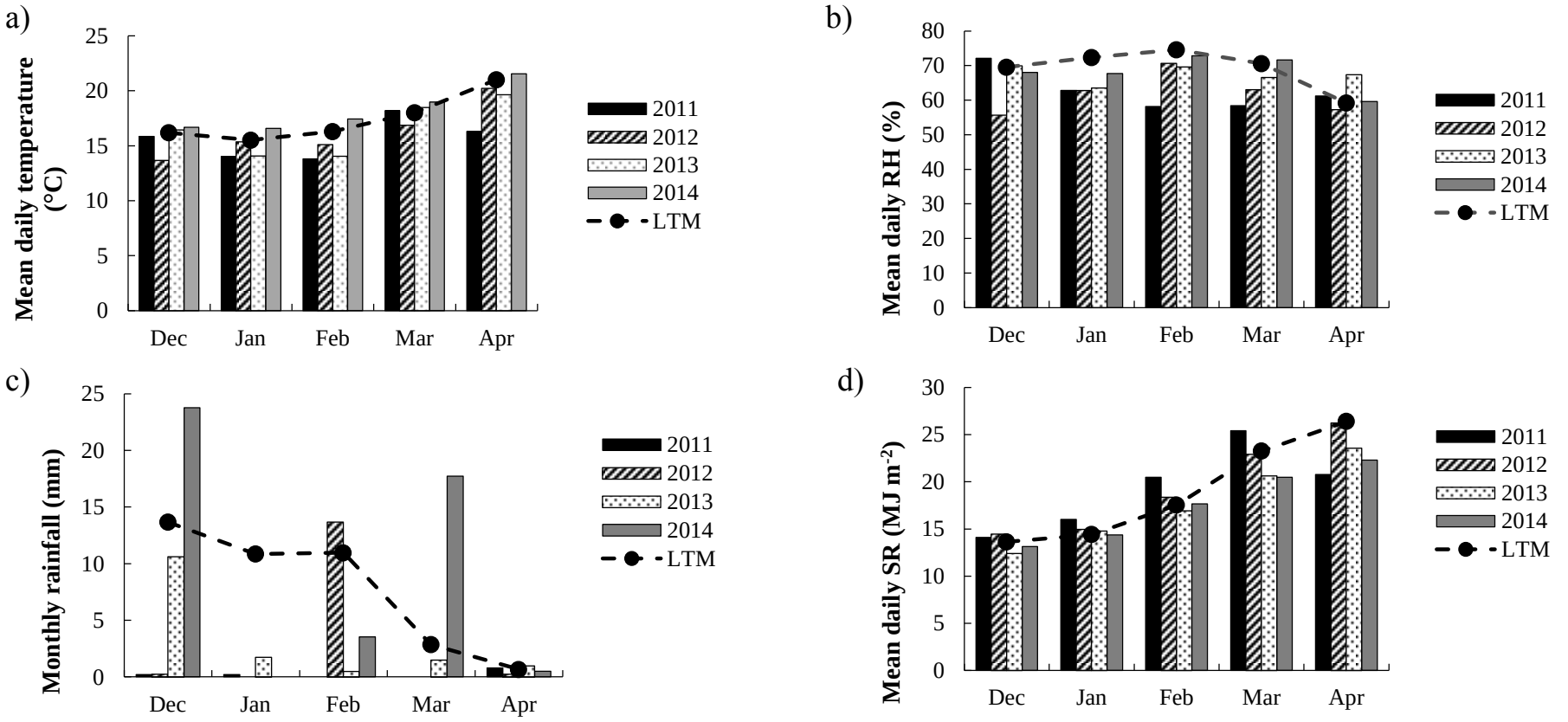


Figure 4.1Environmental conditions in the experimental site for a) mean daily temperature (°C), b) relative humidity (RH, %), c) rainfall accumulated monthly (mm) and d) mean daily solar radiation (MJ m^{-2}) for each field season and 20-year long-term mean (LTM, 19 Environmental conditions in the experimental site for a) mean daily temperature (°C), b) relative humidity (RH, %), c) rainfall accumulated monthly (mm) and d) mean daily solar radiation (MJ m^{-2}) for each field season and 20-year long-term mean (LTM, 1990 - 2010) at the Norman E. Borlaug Experimental Centre (CENEB) in NW Mexico.

4.2.2 Plant establishment

Plant establishment in 2011 ranged among the 26 cultivars from 175 – 288 plants m^{-2} (P <0.001; mean 211 plants m^{-2}, Table 4.1). In 2012, mean plant establishment was 149 plants m^{-2} and there were no significant differences among the 26 CIMMYT cultivars (P =0.117). In 2013, plant establishment ranged among cultivars (P <0.001) from 136 to 235 plants m^{-2}. Averaging over years plant establishment differed amongst the 26 spring wheat cultivars from 147 to 221 (P <0.001), as well between years (P <0.001). No year x cultivar interaction was found for plant establishment.

Table 4.1 Plants m^{-2} for 26 CIMMYT spring wheat cultivars in 2011, 2012, 2013 and overall mean.

Cultivar	**Plants m^{-2}**			
	2011	**2012**	**2013**	**Mean**
BABAX/LR42	190.0	108.3	142.1	146.8
BACANORA T88	271.3	153.3	180.4	201.7
BCN/RIALTO	255.0	165.0	235.4	218.5
BECARD/KACHU	175.0	139.2	164.2	159.4
BRBT1*2/KIRITATI	188.8	134.2	144.2	155.7
SAUAL/4/CROC_1	196.3	118.3	154.6	156.4
SAUAL/WHEAR	216.3	128.3	135.8	160.1
CMH79A.955/4/AGA/3	210.0	131.7	175.0	172.2
CNO79//PF70354	196.3	130.8	155.0	160.7
CROC_1/AE.SQUAR	226.3	168.3	189.6	194.7
KINGBIRD #1//INQA	240.0	154.2	170.4	188.2
MILAN/KAUZ//PRINIA	222.5	154.2	170.4	182.4
PAVON F 76	271.3	190.0	202.5	221.3
PBW343*2/KUKUNA*2	177.5	148.3	165.0	163.6
PFAU/SERI.1B/	182.5	156.7	177.9	172.4
SERI M 82	241.3	146.7	167.5	185.1
SIETE CERROS	287.5	168.3	200.0	218.6
SOKOLL//PBW343	191.3	141.7	149.6	160.8
TACUPETO F2001/S	180.0	149.2	142.9	157.4
TACUPETO F2001/B	195.0	125.0	155.0	158.3
TC870344/GUI//TEMP	190.0	135.8	155.0	160.3
TRAP#1/BOW/3/VEE	212.5	136.7	161.3	170.1
UP2338*2/4/SNI	182.5	173.3	198.8	184.9
BECARD	216.3	150.8	161.7	176.3
WBLL1*2/KURUKU	185.0	126.7	140.4	150.7
YAV_3/SCO//JO69	193.8	155.0	140.4	163.1
Mean	**211.3**	**145.8**	**166.7**	**174.6**
SED Cultivar (df)	26.92 (25)	21.55 (52)	15.49 (52)	11.91 (128)
***SED* Year (df)**				**7.22 (2)**
***SED* Interaction (df)**				**24.45 (128)**
Prob. Cultivar	0.006	0.117	<0.001	<0.001
***Prob*. Year**				**<0.001**
***Prob*. Interaction**				**0.246**
CV (%)	12.7	18.1	11.4	14.0

4.2.3 Crop phenology

Emergence date was 15 December in 2010 (50% of the plot emerged), 17 December in 2011 and 2 December in 2013, reflecting slight differences in sowing dates (Table 4.2). Anthesis date was measured in days after emergence (DAE); calendar dates are presented in appendices 6 to 8, respectively for the three seasons. There were differences amongst genotypes for anthesis date (GS65) in the range 79 – 96 in 2011, 81 – 94 in 2012 and 73 – 100 DAE in 2013 (P <0.001), with Pfau/seri/1 as the earliest cultivar to reach anthesis and Bcn/rialto the latest in the three seasons among the 26 cultivars. Days to anthesis ranged from 78 to 97 DAE overall (P <0.01) for the 26 spring wheat cultivars, and there was a year x cultivar interaction (P <0.001). Days to maturity differed among the 26 cultivars in the range 121 (Siete cerros) – 131 (Bcn/rialto) DAE in 2011, 124 (Trap#1/bow/3/vee) – 136 (Cmh79A.955) DAE in 2012 and 123 (Pfau/seri/1) – 138 (Bcn/rialto) DAE in 2013 (P <0.001). Averaging across years, days to maturity ranged from 124 (Pfau/seri/1) to 134 (Bcn/rialto) DAE (P <0.001).

For the sub-set of nine cultivars, anthesis date range was reduced overall from 78 – 97 to 84 – 95 DAE, but still differed among cultivars (P <0.001). However, the range for days to maturity was similar for both the set of 26 cultivars and the sub-set of nine cultivars (124 – 134 and 125 – 134 days, respectively).

Table 4.2 Days after emergence (DAE) for anthesis (GS65) and physiological maturity (GS89) for 26 CIMMYT spring wheat cultivars in 2011, 2012 and 2013 and the overall mean. See appendices 4 to 6 for the equivalent calendar dates.

Cultivar	**2010-11**		**2011-12**		**2012-13**		**Overall mean**	
	Ant	**Mat**	**Ant**	**Mat**	**Ant**	**Mat**	**Ant**	**Mat**
BABAX/LR42	91	126	87	131	86	128	88	129
BACANORA T88	90	123	87	125	86	126	87	125
BCN/RIALTO	96	131	94	133	100	138	97	134
BECARD/KACHU	94	129	88	131	93	135	92	132
BRBT1*2/KIRITATI	88	123	85	128	81	125	84	126
SAUAL/4/CROC_1	94	129	91	134	94	135	93	133
SAUAL/WHEAR	90	123	85	126	87	129	87	126
CMH79A.955	95	130	93	136	96	134	95	134
CNO79//PF70354	88	124	87	133	84	129	86	129
CROC_1/AE.SQUAR	86	125	86	132	80	128	84	129
KINGBIRD #1//INQA	94	130	91	134	95	136	93	134
MILAN/KAUZ//PRINIA	87	126	86	133	80	129	84	130
PAVON F 76	91	125	87	131	87	126	88	128
PBW343*2/KUKUNA*2	88	125	84	127	85	128	85	127
PFAU/SERI.1B	79	122	81	125	73	123	78	124
SERI M 82	92	127	88	129	87	130	89	129
SIETE CERROS	87	121	85	128	80	123	84	124
SOKOLL//PBW343	89	125	86	127	86	128	87	127
TACUPETO F2001/S	86	124	84	128	85	129	85	127
TACUPETO F2001/B	91	127	89	131	87	130	89	130
TC870344/GUI//TEMP	87	124	85	128	84	126	85	126
TRAP#1/BOW/3/VEE	88	123	83	124	86	126	86	125
UP2338*2/4/SNI	92	128	88	131	91	130	90	130
BECARD	88	123	82	124	84	127	84	125
WBLL1*2/KURUKU	91	130	87	132	95	134	91	132
YAV_3/SCO//JO69	89	123	87	132	90	130	89	129
SED Cultivar (df)	1.45 (25)	0.963 (52)	0.942 (52)	2.50 (52)	1.07 (52)	1.02 (52)	0.640 (128)	1.05 (128)
***SED* Year (df)**							**0.371 (2)**	**0.78 (2)**
***SED* Interaction (df)**							**1.31 (128)**	**2.21 (128)**
Prob. Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
***Prob*. Year**							**0.001**	**0.004**
***Prob*. Interaction**							**<0.001**	**0.016**
CV (%)	1.6	0.8	1.3	2.3	1.5	1.0	1.5	1.6

4.2.4 Plant height

The 26 cultivars differed in plant height in each season, as well as when averaging across the seasons (*P* <0.001; Table 4.3). Overall, plant height ranged from 80 (Bcn/rialto) to 116 cm (Babax/LR42). A significant effect of season on plant height was found (*P* <0.001) and there was a year x cultivar interaction (*P* <0.001).

Table 4.3 Plant height (cm) for the 26 CIMMYT elite spring wheat cultivars in 2011, 2012, 2013 and the overall mean.

Cultivar	2011	2012	2013	Mean
BABAX/LR42	117.8	112.4	116.8	115.7
BACANORA T88	90.8	90.2	93.0	91.3
BCN/RIALTO	73.4	83.3	83.8	80.2
BECARD/KACHU	102.0	102.5	108.2	104.2
BRBT1*2/KIRITATI	106.0	109.4	110.3	108.6
SAUAL/4/CROC_1	110.1	110.8	110.3	110.4
SAUAL/WHEAR	104.5	102.4	109.3	105.4
CMH79A.955/4/AGA/3	97.6	101.0	101.7	100.1
CNO79//PF70354	106.9	110.8	112.8	110.2
CROC_1/AE.SQUAR	102.4	107.3	106.3	105.4
KINGBIRD #1//INQA	103.8	104.6	107.0	105.1
MILAN/KAUZ//PRINIA	104.0	108.0	107.8	106.6
PAVON F 76	103.8	100.1	107.3	103.7
PBW343*2/KUKUNA*2	105.5	99.8	108.3	104.6
PFAU/SERI.1B/	99.8	105.4	105.5	103.6
SERI M 82	98.2	93.8	98.0	96.7
SIETE CERROS	99.7	102.4	103.0	101.7
SOKOLL//PBW343	112.3	107.4	104.8	108.2
TACUPETO F2001/S	124.3	108.5	115.2	116.0
TACUPETO F2001/B	100.8	101.3	107.2	103.1
TC870344/GUI//TEMP	117.5	114.9	117.5	116.6
TRAP#1/BOW/3/VEE	107.8	107.6	112.0	109.1
UP2338*2/4/SNI	104.5	105.1	105.8	105.1
BECARD	103.5	102.3	109.0	104.9
WBLL1*2/KURUKU	107.4	108.1	108.7	108.0
YAV_3/SCO//JO69	109.8	107.8	110.5	109.4
SED Cultivar (df)	5.75 (52)	2.45 (52)	1.97 (25)	1.77 (128)
***SED* Year (df)**				**0.554 (2)**
***SED* Interaction (df)**				**3.52 (128)**
Prob. Cultivar	<0.001	<0.001	<0.001	<0.001
***Prob.* Year**				0.001
***Prob.* Interaction**				<0.001
CV (%)	5.5	2.9	2.3	3.4

4.2.5 Grain yield, harvest index and above-ground biomass

Grain yield ranged from 447.9 (Pavon F76) to 720.9 (Tacupeto F2001/B) g m^{-2} amongst the 26 cultivars in 2011 (P <0.001; Table 4.4), from 594.4 (Pavon F76) to 742 (Tacupeto F2001/S) g m^{-2} (P <0.001) in 2012 and from 591.2 (Pavon F76) to 756.3 (Kingbird#1//inqa) g m^{-2} (P <0.001) in 2013. Averaging across cultivars, grain yield was slightly lower in 2011 (672.2 g m^{-2}) and 2012 (674.3 g m^{-2}) than 2013 (684.2 g m^{-2}) (P =0.01) and ranged from 556.6 (Pavon F76) to 716.3 (Tacupeto F2001/S) g m^{-2} (P <0.001) with a year x cultivar interaction (P =0.001). The relative grain yield of the cultivars differed between 2011 and 2012 and between 2011 and 2013 but not between 2012 and 2013. Genotypes BCN/Rialto and Pavon F76 showed the largest interaction between 2011 and 2012 and also between 2011 and 2013. When comparing just the last two seasons (2012 and 2013), results showed no significant year x cultivar interaction.

Averaging across cultivars, HI was greater in 2013 (0.50) than in 2011 (0.45) and 2012 (0.46) (P <0.001, Table 4.4). HI in 2011 ranged amongst cultivars from 0.41 (BCN/Rialto and Pavon F76) to 0.49 (Pfau/seri.1b) (P <0.001) in 2012 from 0.43 (Pavon F76) to 0.49 (Pfau/seri.1b and Seri M82) (P <0.001) and in 2013 from 0.46 (Sokoll//Pbw343) to 0.53 (Seri M82) (P <0.001). Averaging over years, HI ranged from 0.44 (Sokoll//Pbw343) to 0.50 (Pfau/seri.1b and Seri M82) (P <0.001), showing a year x cultivar interaction (P <0.05).

In 2011, AGDM at harvest ranged from 1106.6 (Pavon F76) to 1559.9 (Tacupeto F2001/B) g m^{-2} (P <0.001; Table 4.4), in 2012 from 1309.7 (Seri M82) to 1623.3 (Croc_1/ae.squar) g m^{-2} (P <0.001) and in 2013 from 1241.2 (Saual/whear) to 1493.4 (Tacupeto F2001/S) g m^{-2} (P <0.001). Averaging over the seasons, cultivars differed in the range 1266.5 (Pavon F76) to 1517.5 (UP2338*2/4SNI) g m^{-2} (P <0.001); there was an effect of season (P <0.05) and a year x cultivar interaction (P <0.01).

Table 4.4 Grain yield (g m^{-2}), harvest index (HI) and above-ground dry matter (AGDM, g m^{-2}) at harvest for the 26 CIMMYT spring wheat cultivars in 2011, 2012 and 2013 and overall means. Degrees of freedom for individual years are 25 for 2011 and 52 for 2012 and 2013.

Cultivars	2011			2012			2013			Overall mean			2012-13 mean		
	Grain yield g m^{-2}	HI	AGDM g m^{-2}	Grain yield g m^{-2}	HI	AGDM g m^{-2}	Grain yield g m^{-2}	HI	AGDM g m^{-2}	Grain yield g m^{-2}	HI	AGDM g m^{-2}	Grain yield g m^{-2}	HI	AGDM g m^{-2}
BABAX/LR42	610.3	0.42	1451	707.0	0.46	1534	681.8	0.47	1454	673.4	0.455	1483	694.4	0.47	1494
BACANORA T88	615.4	0.47	1307	704.9	0.48	1461	656.8	0.52	1271	664.5	0.493	1351	680.8	0.50	1366
BCN/RIALTO	595.9	0.41	1438	725.7	0.47	1539	707.2	0.51	1397	686.3	0.469	1461	716.4	0.49	1468
BECARD/KACHU	669.4	0.45	1473	688.6	0.48	1451	713.3	0.52	1376	693.1	0.486	1428	701.0	0.50	1413
BRBT1*2/KIRITATI	575.8	0.47	1237	637.7	0.48	1329	676.7	0.51	1325	636.9	0.489	1304	657.2	0.50	1327
SAUAL/4/CROC_1	603.5	0.43	1415	675.4	0.46	1474	711.2	0.51	1402	670.8	0.470	1432	693.3	0.48	1438
SAUAL/WHEAR	569.6	0.42	1363	623.4	0.45	1387	634.3	0.51	1241	614.1	0.468	1326	628.9	0.48	1314
CMH79A.955/4/AGA/3	615.5	0.46	1325	624.6	0.44	1414	678.3	0.49	1375	642.4	0.469	1377	651.4	0.47	1394
CNO79//PF70354	624.1	0.44	1412	714.9	0.45	1598	719.5	0.49	1467	693.9	0.461	1502	717.2	0.47	1532
CROC_1/AE.SQUAR	629.7	0.46	1384	723.6	0.45	1623	698.4	0.49	1419	690.7	0.465	1487	711.0	0.47	1521
KINGBIRD #1//INQA	643.9	0.44	1480	660.7	0.46	1427	756.3	0.51	1489	692.3	0.473	1463	708.5	0.49	1458
MILAN/KAUZ//PRINIA	589.9	0.45	1324	656.3	0.45	1455	669.4	0.48	1386	644.6	0.461	1396	662.8	0.47	1420
PAVON F 76	447.9	0.41	1107	594.4	0.43	1373	591.2	0.47	1267	556.6	0.438	1266	592.8	0.45	1320
PBW343*2/KUKUNA*2	685.1	0.48	1431	687.4	0.48	1440	709.9	0.52	1374	695.2	0.491	1413	698.6	0.50	1407
PFAU/SERI.1B/	649.3	0.49	1336	705.2	0.49	1427	647.7	0.52	1245	669.6	0.503	1336	676.4	0.51	1336
SERI M 82	652.3	0.47	1379	639.2	0.49	1310	665.7	0.53	1257	652.4	0.503	1307	652.4	0.51	1283
SIETE CERROS	554.8	0.43	1281	621.6	0.45	1380	648.1	0.48	1343	614.8	0.458	1342	634.8	0.47	1362
SOKOLL//PBW343	647.4	0.42	1547	658.3	0.43	1520	642.2	0.46	1403	649.5	0.440	1483	650.2	0.45	1462
TACUPETO F2001/S	646.4	0.46	1415	742.0	0.46	1602	737.1	0.49	1493	716.3	0.474	1515	739.6	0.48	1548
TACUPETO F2001/B	720.2	0.46	1560	662.0	0.45	1470	686.3	0.50	1368	685.7	0.471	1454	674.2	0.48	1419
TC870344/GUI//TEMP	649.5	0.45	1450	672.4	0.45	1498	662.6	0.49	1360	663.0	0.464	1434	667.5	0.47	1429
TRAP#1/BOW/3/VEE	584.9	0.46	1277	643.8	0.47	1382	704.0	0.49	1439	651.6	0.473	1377	673.9	0.48	1411
UP2338*2/4/SNI	700.8	0.46	1530	686.1	0.44	1566	697.0	0.48	1461	693.9	0.458	1518	691.6	0.46	1513
BECARD	682.4	0.48	1413	693.1	0.47	1469	685.5	0.51	1354	687.6	0.488	1412	689.3	0.49	1411
WBLL1*2/KURUKU	703.8	0.46	1536	653.8	0.48	1373	706.1	0.50	1408	685.9	0.481	1427	680.0	0.49	1390
YAV_3/SCO//JO69	639.1	0.48	1331	728.8	0.46	1583	702.8	0.49	1421	696.6	0.479	1459	715.8	0.48	1502
SED Cv	34.79	0.012	72.8	25.24	0.013	61.2	33.03	0.0126	67.8	17.9 (128)	0.008 (128)	38.9 (128)	20.8 (102)	0.009 (102)	45.7 (102)
***SED* Year (df)**										**12.6 (2)**	**0.004 (2)**	**30.2 (2)**	**11.4 (1)**	**0.001 (1)**	**23.0 (1)**
***SED* Interaction (df)**										**37.3 (128)**	**0.015 (128)**	**82.1 (128)**	**31.0 (102)**	**0.013 (102)**	**67.4 (102)**
Prob. Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
***Prob*. Year**										**0.01**	**<0.001**	**0.035**	**0.434**	**<0.001**	**0.018**
***Prob*. Interaction**										**0.001**	**0.034**	**0.008**	**0.118**	**0.821**	**0.261**
CV (%)	5.5	2.7	5.2	4.6	3.5	5.1	5.9	3.1	6.0	5.4	3.2	5.5	5.3	3.3	5.6

4.2.6 Yield components

In 2011, grains m^{-2} ranged from 11,976 (Milan/kauz/prinia) to 21,387 (Bcn/rialto) (P <0.001; Table 4.5). In general, the majority of cultivars produced more grains m^{-2} in 2012 than 2011. In 2012, the 26 cultivars ranged from 13,324 (Wbill1*2/kuruku) to 23,071 (Bcn/rialto) grains m^{-2}. Grains m^{-2} in 2013 was overall slightly lower (16,078 grains m^{-2}; P <0.001) than in 2012 and ranged from 12,495 (TC870344/gui//temp) to 23,761 (Bcn/rialto) (P <0.001). Overall grains m^{-2} ranged from 13,144 (TC870344/gui//temp) to 22,909 (Bcn/rialto). There was an effect of season (P <0.05), as well as a year x cultivar interaction (P <0.001).

Spikes m^{-2} (SN) ranged amongst the 26 cultivars from 244 (Cmh79A.955) to 380 (Kingbird#1//inqa) m^{-2} in 2011 (P <0.001), from 212 (Wbill1*2/kuruku) to 396 (Bcn/rialto) in 2012 (P <0.001) and from 239 (Cmh79A.955) to 407 (Bcn/rialto) in 2013 (P <0.001). Averaging across seasons, SN ranged from 238 (Cmh79A.955) to 394 (Bcn/rialto) (P <0.001). Although SN did not differ between years, a significant year x cultivar interaction was found (P < 0.01; Table 4.5).

More grains per spike (GPS) were produced in 2012 (56.4) and 2013 (54.4) than in 2011 (47.6) (P <0.001; Table 4.5). The genetic range in GPS was 38.5 (Babax/LR42) to 66.7 (Cmh79A.955) in 2011, 45.8 (TC870344/gui//temp) to 72.1 (Saual/4/croc_1) in 2012 and 42.7 (TC870344/gui//temp) to 74.7 (Cmh79A.955) in 2013 (P <0.001). Overall, cultivar differences were found for GPS (P <0.001) in the range 44.6 (TC870344/gui//temp) to 70.7 (Cmh79A.955). GPS differed between seasons (P <0.01), and there was a year x cultivar interaction (P <0.05).

Although thousand grain weight (TGW) was similar across seasons for the 26 spring wheat cultivars, differences among cultivars were found in each season (P <0.001), as well as averaging across the years (P <0.001; Table 4.5). Thousand grain weight among the 26 cultivars ranged from 27.9 (Bcn/rialto) to 50.5 g (Babax/LR42) in 2011, from 31.4 (Bcn/rialto) to 50.0 g (Babax/LR42) (P <0.001) in 2012 and from 29.8 (Bcn/rialto) to 53.2 g (TC870344/gui//temp) in 2013. Overall, TGW ranged from 29.9 (Bcn/rialto) to 50.6 (Babax/LR42) g. There was an effect of the season (P <0.05) for TGW, but no year x cultivar interaction (P =0.193).

Table 4.5 Grains m^{-2} (GN), thousand grain weight (TGW; g), spikes m^{-2} (SN) and grains per spike (GPS) for the 26 CIMMYT spring wheat cultivars in 2011, 2012 and 2013 and overall means. Degrees of freedom for individual years are 25 for 2011 and 52 for 2012 and 2013.

Cultivars	2011				2012				2013				Overall mean			
	GN	**TGW**	**SN**	**GPS**	**GN**	**TGW**	**SN**	**GPS**	**GN**	**TGW**	**SN**	**GPS**	**GN**	**TGW**	**SN**	**GPS**
BABAX/LR42	12065	50.5	314	38.5	14132	50.0	273	51.9	13312	51.3	302	44.1	13308	50.6	294	45.6
BACANORA T88	17701	34.8	379	46.7	20275	35.4	384	52.7	17717	37.1	322	55.5	18672	35.9	360	52.3
BCN/RIALTO	21387	27.9	374	57.2	23071	31.4	396	58.5	23761	29.8	407	58.5	22909	29.9	394	58.2
BECARD/KACHU	15345	43.7	323	47.5	17005	40.5	292	58.1	17231	41.5	316	54.7	16675	41.7	309	54.2
BRBT1*2/KIRITATI	12853	44.9	266	48.3	14314	44.6	268	53.6	14994	45.1	253	59.6	14204	44.9	262	54.5
SAUAL/4/CROC_1	12948	46.6	265	48.8	15832	42.7	221	72.1	15471	46.0	263	58.8	14975	44.9	248	61.3
SAUAL/WHEAR	12832	44.4	317	40.4	14306	43.6	259	56.5	14130	44.8	244	58.1	13872	44.3	268	53.1
CMH79A.955	16198	38.1	244	66.7	15828	39.7	233	69.4	17813	38.2	239	74.7	16665	38.7	238	70.7
CNO79//PF70354	13032	47.9	283	46.1	15701	45.5	255	61.7	14729	48.9	264	56.1	14669	47.4	265	55.7
CROC_1/AE.SQUAR	15293	41.2	358	42.7	18714	38.7	362	51.7	16300	42.9	340	48.6	16954	40.9	353	48.3
KINGBIRD #1//INQA	18561	34.7	380	48.8	18342	36.0	294	62.7	21214	35.7	385	55.2	19474	35.6	349	56.4
MILAN/KAUZ//PRINIA	11976	49.3	260	46.1	14751	44.6	243	60.8	13907	48.2	265	52.5	13741	47.1	255	54.0
PAVON F 76	13441	33.3	345	39.0	17054	35.0	348	49.1	15849	37.3	354	45.0	15699	35.4	349	45.0
PBW343*2/KUKUNA*2	17525	39.2	370	47.4	16349	42.2	349	46.9	17391	41.0	306	57.1	17034	41.0	338	50.8
PFAU/SERI.1B	16085	40.4	318	50.5	16855	41.9	313	54.2	14229	45.5	277	51.5	15678	42.9	301	52.3
SERI M 82	16895	38.6	322	52.4	16840	38.0	290	58.1	15919	41.8	271	58.8	16508	39.6	291	57.0
SIETE CERROS	15777	35.2	345	45.7	16778	37.5	277	61.8	17716	36.7	297	60.1	16879	36.6	302	57.1
SOKOLL//PBW343	14722	44.0	336	43.8	15263	43.1	318	48.0	14092	45.6	304	46.3	14689	44.3	317	46.3
TACUPETO F2001/S	13824	46.8	303	45.6	17052	43.5	316	54.9	15598	47.3	268	58.3	15700	45.7	295	53.9
TACUPETO F2001/B	15790	45.6	308	51.2	15404	43.0	250	61.8	15718	44.1	275	57.7	15619	44.1	274	57.6
TC870344/GUI//TEMP	13255	49.0	292	45.4	13719	49.0	304	45.8	12494	53.2	293	42.7	13144	50.6	297	44.6
TRAP#1/BOW/3/VEE	14201	41.2	315	44.9	14651	43.9	292	50.4	15910	44.3	317	50.3	15011	43.4	307	49.0
UP2338*2/4/SNI	17476	40.1	358	48.9	17007	40.3	333	51.2	17569	39.7	335	52.5	17335	40.0	340	51.1
BECARD	15215	44.8	351	43.4	15467	44.8	321	48.5	14729	46.6	306	48.6	15127	45.5	323	47.3
WBLL1*2/KURUKU	14483	48.6	273	53.0	13324	49.0	212	63.2	14265	49.5	271	52.7	13967	49.1	250	56.7
YAV_3/SCO//JO69	14834	43.1	300	49.5	17588	41.4	285	62.5	15972	44.1	281	57.2	16294	42.8	287	57.3
SED Cv	941.3	1.27	13.32	2.45	960.2	1.71	23.55	4.65	870.2	1.83	22.09	4.12	544.2 (128)	1.01 (128)	12.85 (128)	2.47 (128)
***SED* Year (df =2)**													**288.7**	**0.46**	**9.41**	**1.59**
***SED* Interaction (df =128)**													**1105.7**	**2.04**	**26.91**	**5.10**
Prob. Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
***Prob*. Year**													**0.014**	**0.021**	**0.083**	**0.005**
***Prob*. Interaction**													**<0.001**	**0.193**	**0.006**	**0.039**
CV (%)	6.2	3.0	4.2	5.1	7.2	5.0	9.8	10.1	6.6	5.2	9.1	9.3	6.8	4.8	8.5	9.2

4.2.7 General correlations between harvest traits

Overall for the three seasons, ranges for key harvest traits were similar among the 26 and the nine cultivars, with the exception of TGW and grains m^{-2} (Table 4.6). Minimum TGW was greater among the nine cultivars (35.4 g) compared to the 26 cultivars (29.9 g). Grains m^{-2} ranges differed just for the maximum values between the 26 (22,909) and the nine cultivars (17,335). Grain yield, HI and AGDM presented very similar ranges among the 26 and the nine cultivars.

Phenotypic correlations among harvest traits averaged across the three seasons are shown for the 26 and the nine cultivars in Table 4.7 a and b, respectively. Differences in the extent of correlation among key harvest traits between the two sets of cultivars were observed for spikes m^{-2} and anthesis date. For instance, spikes m^{-2} among the 26 cultivars was strongly and positively associated with grains m^{-2} (r =0.73, P <0.001) but negatively related to TGW (-0.69, P <0.001), whereas for the nine cultivars these relationships were not significant. Moreover, a significant association between grain yield and HI was observed for the 26 cultivars (r =0.42, P <0.05) but this was not significant among the nine cultivars.

No associations were found between plants m^{-2} and either HI or AGDM at harvest for either the 26 or the nine cultivars. Although there were trends for negative associations between plants m^{-2} and grain yield, linear regressions showed no significant association between these traits. Regressions between yield, yield components and harvest traits are shown in the following sections. Although some of the traits are not independent (*e.g.* AGDM and HI) they are shown as these key associations are relevant to the hypotheses presented at the end of the literature review.

Table 4.6 Ranges shown for grain yield (100% DM), above-ground dry matter (AGDM) at harvest, grains m^{-2} and thousand grain weight (TGW) among the 26 CIMMYT spring wheat cultivars and for the subset of 9 cultivars. Values represent mean of the three seasons.

Trait	**Grain yield**	**TGW**	**HI**	**AGDM**	**Grains**
Units	g m^{-2}	g		g m^{-2}	m^{-2}
26 cultivars					
Max	716.3	50.6	0.50	1518	22909
Min	556.6	29.9	0.44	1266	13144
Nine cultivars					
Max	693.9	50.6	0.50	1518	17335
Min	556.6	35.4	0.44	1267	13308

Table 4.7 Phenotypic correlations among key harvest traits and yield components for the 3 season means of a) 26 and b) 9 CIMMYT spring wheat cultivars.

Trait	1.Grain yield	2.TGW	3.HI	4.AGDM	5.Grains	6.Spikes	7.GPS	8.Anthesis	9.Maturity	10.Plant height	11.Plants
Units	g m^{-2}	g		g m^{-2}	m^{-2}	m^{-2}		DAE	DAE	cm	m^{-2}
a) 26 cultivars											
1. GY	1.00										
2. TGW	0.26	1.00									
3. HI	0.42*	-0.02	1.00								
4. AGDM	0.76***	0.32	-0.26	1.00							
5. GN	0.13	-0.90***	0.15	0.00	1.00						
6. Spikes	0.00	-0.69***	-0.08	0.03	0.73***	1.00					
7. GPS	0.11	-0.27	0.26	-0.07	0.32	-0.41*	1.00				
8. Ant	0.12	-0.38	-0.21	0.29	0.50**	0.04	0.62***	1.00			
9. Mat	0.27	-0.21	-0.20	0.43*	0.37	-0.09	0.61***	0.89***	1.00		
10. PH	0.11	0.85***	-0.26	0.32	-0.86***	-0.55*	-0.38	-0.41*	-0.26	1.00	
11. Plants	-0.44*	-0.91***	-0.18	-0.37	0.77***	0.75***	0.02	0.09	-0.01	-0.76***	1.00
b) nine cultivars											
1. GY	-										
2. TGW	0.53	-									
3. HI	0.58	0.13	-								
4. AGDM	0.84**	0.58	0.08	-							
5. GN	0.15	-0.75*	0.29	-0.02	-						
6. Spikes	-0.13	-0.23	-0.26	-0.01	0.16	-					
7. GPS	0.14	-0.31	0.32	-0.04	0.48	-0.80**	-				
8. Ant	0.11	-0.41	0.01	0.15	0.63	-0.50	0.86*	-			
9. Mat	0.21	-0.34	-0.02	0.27	0.63	-0.45	0.78*	1.00	-		
10. PH	0.18	0.77*	-0.56	0.57	-0.73*	0.18	-0.61	-0.33	-0.29	-	
11. Plants	-0.67*	-0.91***	-0.28	-0.62	0.53	0.68*	-0.20	-0.12	-0.14	-0.51	-

4.2.8 Regressions between grain yield, AGDM and harvest index

In 2011, grain yield was non-linearly (quadratic) and positively associated with HI, the association being stronger among the nine cultivars (R^2 = 0.83, P <0.01) than for the 26 cultivars (R^2 =0.42, P <0.01; Figure 4.2 a). No significant relationships were observed between grain yield and HI in 2012 for either the 26 cultivars or the subset of nine cultivars (Figure 4.2 b). In 2013; grain yield and HI were related positively and non-linearly (quadratic) amongst the 26 cultivars, (R^2 =0.37, P <0.01; Figure 4.2 c), but not amongst the subset of nine cultivars. Overall, there was a positive and non-linear (quadratic) association between grain yield and HI amongst the 26 spring wheat cultivars (R^2 =0.36, P <0.01) and a trend for the same relationship among the nine cultivars (R^2 =0.54, P =0.095) (Figure 4.2 d).

In general, grain yield was more strongly associated with AGDM than with HI, showing strong, linear and positive associations in all individual seasons among the 26 cultivars (Figure 4.3 a, b and c respectively for 2011, 2012 and 2013). There were similar associations (linear and positive) among the nine cultivars (Figure 4.3 closed symbols). Averaging across years, grain yield was strongly and linearly related with greater AGDM at harvest among both the 26 cultivars (R^2 =0.61, P <0.001) and the sub-set of nine cultivars (R^2 =0.70, P <0.01) (Figure 4.3 d). However, HI and AGDM were generally weakly associated. In 2012, there was a trend for a negative linear association among the 26 cultivars between these two traits (R^2 =0.16, P <0.05; Figure 4.4 b), as well as for a non-linear relationship for the overall means (R^2 =0.22, P =0.056) (Figure 4.4 d). In the non-linear association it was observed that HI started declining for those cultivars with the highest values of AGDM (1400 – 1600 g m^{-2}).

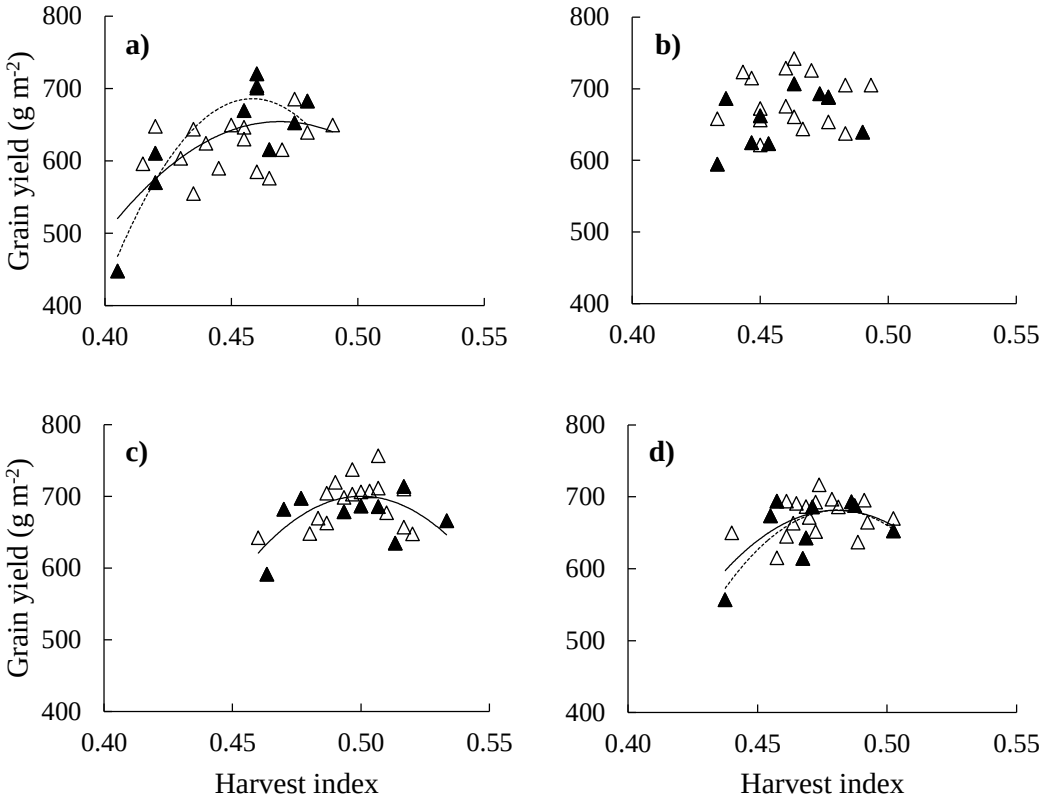


Figure 4.2 Regression of grain yield (100% DM) on harvest index for the subset of 26 cultivars (Δ, —) and nine cultivars (▲, ----) in a) 2011: (Δ) y =-32453x^2+30456x-6491 (R^2 =0.42, *P* <0.01), (▲) y =-76696x^2+70299x-15423 (R^2 =0.83, *P* <0.01); b) 2012: (○) R^2 =0.10, *P* =0.288, (▲) R^2 =0.3152, *P* =0.321; c) 2013: (Δ) y = -48986x^2+49005x-11556 (R^2 =0.37, *P* <0.01), (▲) R^2 =0.31, *P* =0.335 and d) overall mean: (Δ) y = -45237x^2+43475x-9764 (R^2 =0.36, *P* <0.01), (▲) R^2 =0.54, *P* =0.095.

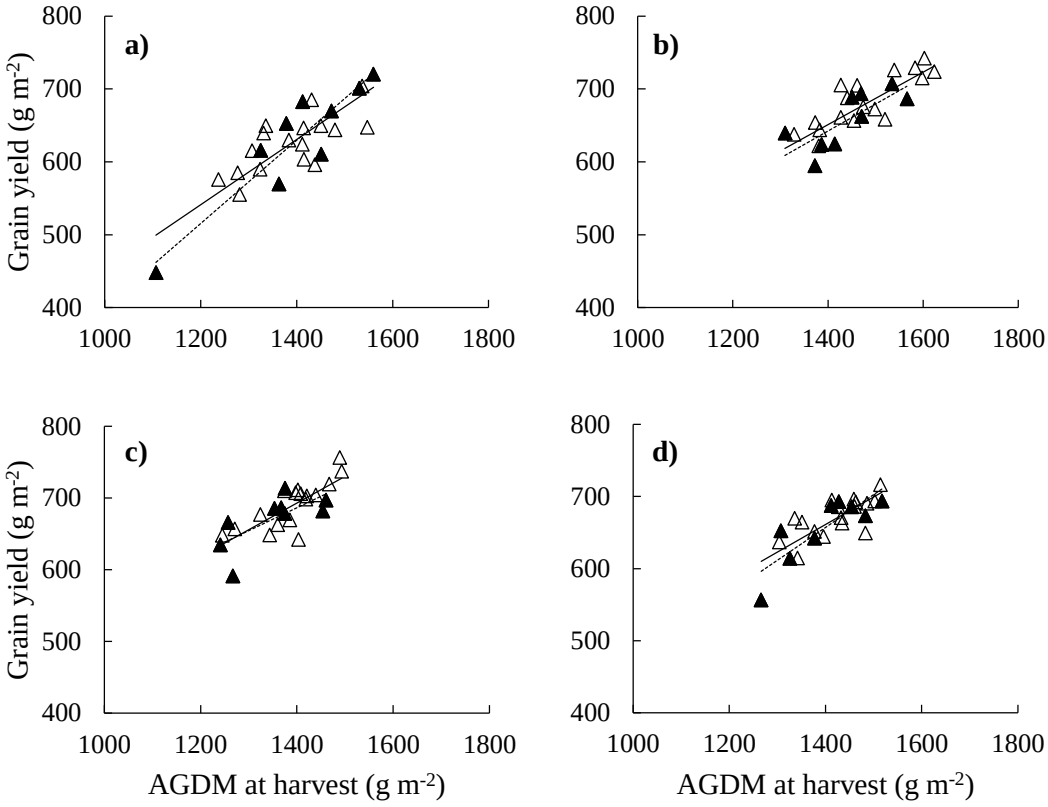


Figure 4.3 Regression of grain yield (100% DM) on above-ground dry matter at harvest for the subset of 26 cultivars (Δ, —) and nine cultivars (▲, ----) in a) 2011: (Δ) y =0.4474x+4.3 (R^2 =0.69, *P* <0.001), (▲) y =0.5721x-171.09 (R^2 = 0.85, *P* <0.001); b) 2012: (Δ) y = 0.3612x+145.1 (R^2 = 0.65, *P* <0.001), (▲) y =0.3697x+124.63 (R^2 = 0.58, *P* <0.05); c) 2013: (Δ) y =0.3705x+174.1 (R^2 = 0.60, *P* <0.001), (▲) y =0.3214x+236.48 (R^2 = 0.50, *P* =0.034) and d) overall mean: (Δ) y =0.3807x+128.1 (R^2 = 0.61, *P* <0.001), (▲) y =0.4553x+19.494 (R^2 = 0.70, *P* <0.01).

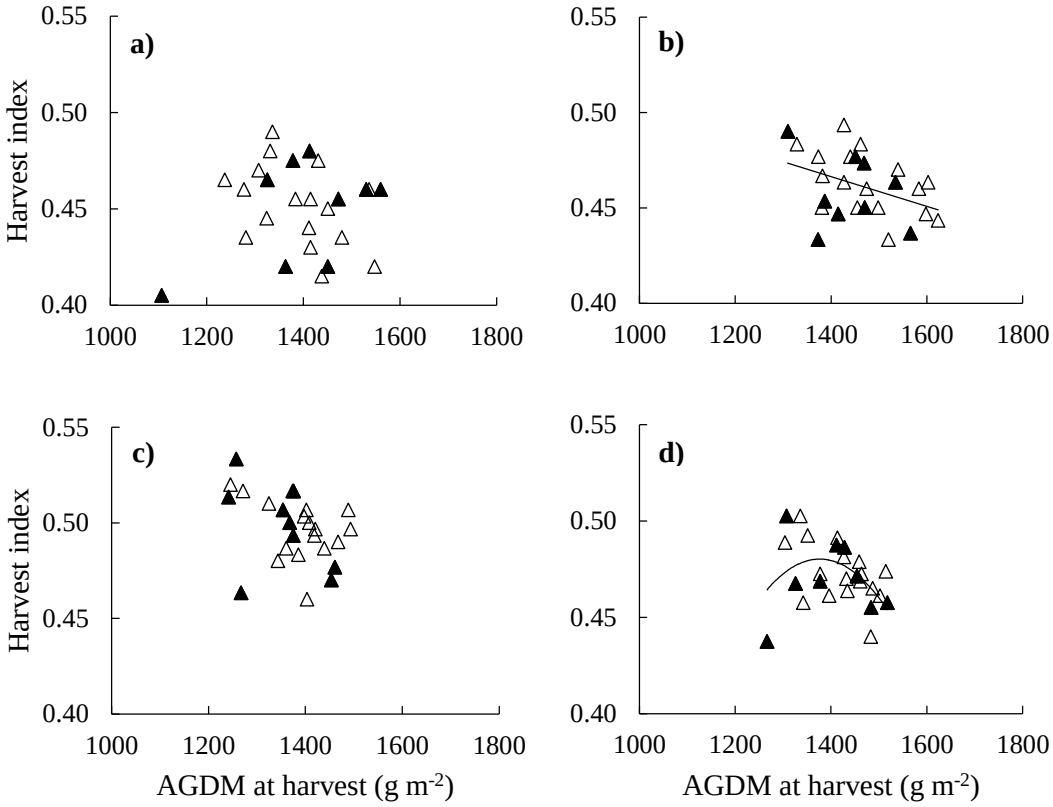


Figure 4.4 Regression of harvest index on above-ground dry matter at harvest for the subset of 26 cultivars (Δ, —) and nine cultivars (▲, ----) in a) 2011: (Δ) R^2 =0.1401, *P* =0.176, (▲) R^2 =0.26, *P* =0.159; b) 2012: (Δ) y = -0.0000782x+0.05758 (R^2 =0.16, *P* <0.05), (▲) R^2 =0.09, *P* =0.424; c) 2013: (Δ) R^2 =0.17, *P* =0.116, (▲) R^2 =0.2102, *P* =0.215 and d) overall mean: (Δ) y = -0.000001318x^2+0.00363x-2.02 (R^2 =0.22, *P* =0.056), (▲) R^2 =0.41, *P* =0.206.

4.2.9 Regressions between grain yield and its components

Grain yield was positively associated with TGW only among the nine cultivars, showing a non-linear relationship in 2011 (R^2 = 0.68, *P* <0.05; Figure 4.5 a) and linear strong relationship in 2012 (R^2 =0.68, *P* <0.05; Figure 4.5 b). No associations were observed for these traits in 2013. Averaging across the seasons, there was a trend for a positive non-linear association between grain yield and TGW among the nine cultivars (R^2 =0.54, *P* =0.1; Figure 4.5 d).

In 2011, there were trends for positive associations between grain yield and grains m^{-2}, the association being non-linear (quadratic) among the 26 cultivars (R^2 =0.22, *P* =0.056) and linear among the nine cultivars (R^2 =0.39, *P* =0.074) (Figure 4.6 a). In 2012, there was a trend for a lineal positive association between these traits amongst the 26 cultivars (R^2 =0.18, *P* <0.05), but no association was found for the nine cultivars (Figure 4.6 b). These traits were not related in 2013 (Figure 4.6 c) or when averaging across the three seasons (Figure 4.6 d) for either the set of 26 cultivars or the sub-set of nine cultivars.

Overall and in individual years, there was a strong trade-off between greater TGW and grains m^{-2} among the 26 and the nine cultivars (Figure 4.7). All the associations among the 26 cultivars were linear; stronger in 2012 (R^2 =0.75, *P* <0.001; Figure 4.7 a) and 2013 (R^2 =0.82, *P* <0.001; Figure 4.7 b) than in 2011 (0.62, *P* <0.001; Figure 4.7 c). Generally, cultivars from the sub-set also exhibited strong linear negative associations between grains m^{-2} and TGW; with the exception of 2011 where a better fit was obtained using a quadratic equation (R^2 =0.62, *P* =0.057; Figure 4.7 a). Averaging across the three seasons, the negative linear association between grains m^{-2} and grain weight was stronger in the set of 26 cultivars (R^2 =0.79, *P* <0.001) than among the nine cultivars (R^2 =0.54, *P* <0.05) (Figure 4.7 d).

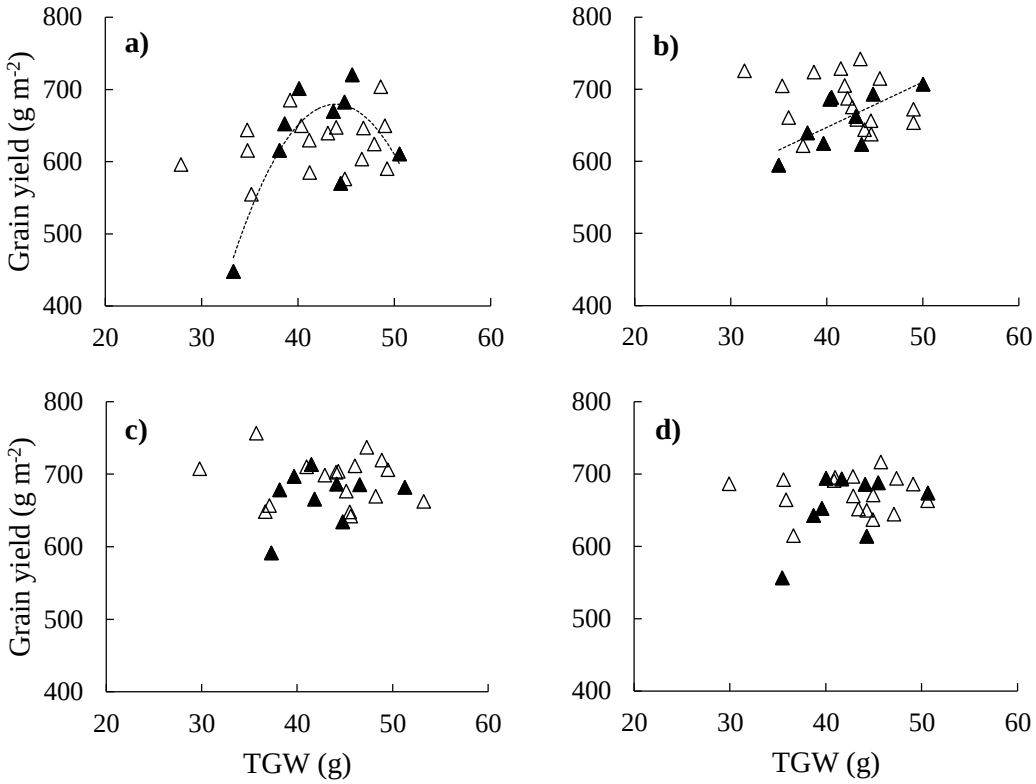


Figure 4.5 Regression of grain yield (100% DM) on thousand grain weight (TGW) for the subset of 26 cultivars (Δ, —) and nine cultivars (▲, ----) in a) 2011: (Δ) R^2 =0.16, *P* =0.142, (▲) y =-1.888x^2+165.8x-2961.5 (R^2 =0.68, *P* <0.05); b) 2012: (Δ) R^2 =0, (▲) y =6.2712x+396.34 (R^2 =0.49, *P* <0.05); c) 2013: (Δ) R^2 =0, (▲) R^2 =0.18, *P* =0.556 and d) overall mean: (Δ) R^2 =0.04, *P* =0.315, (▲)R^2 =0.54, *P* =0.099.

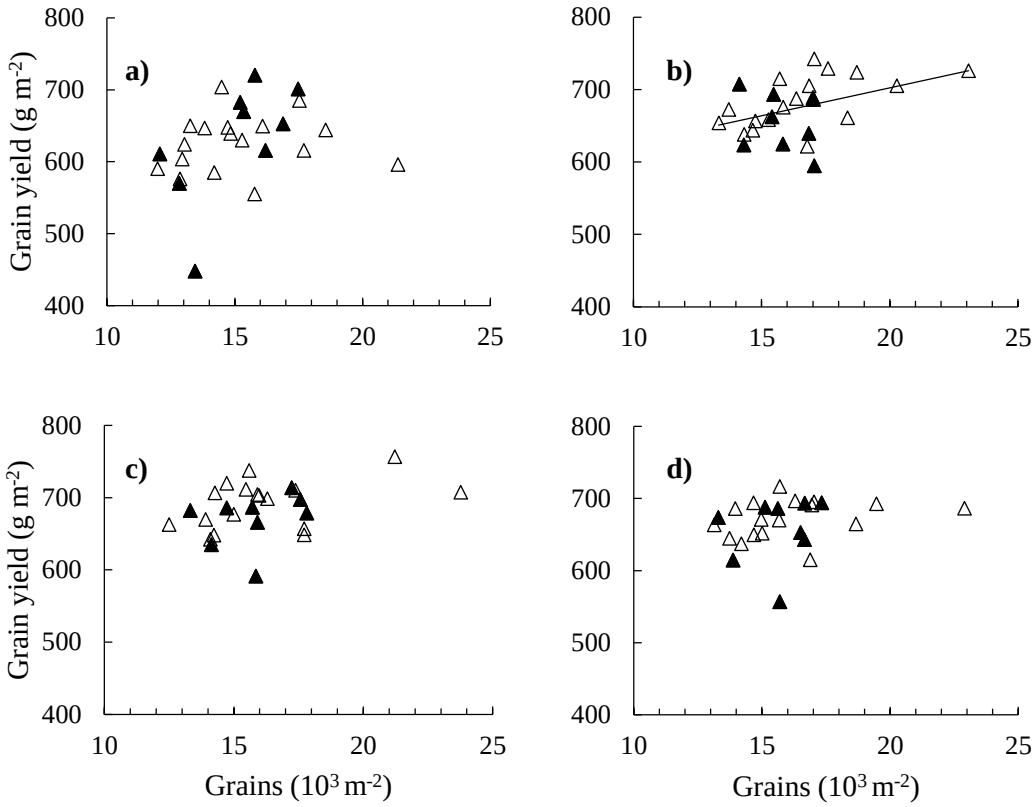


Figure 4.6 Regression of grain yield (100% DM) on grains m^{-2} for the subset of 26 cultivars (Δ, —) and nine cultivars (▲, ----) in a) 2011: (Δ) R^2 =0.22, *P* =0.056, (▲)R^2 =0.39, *P* =0.074; b) 2012: (Δ) y =0.00773x+547.8 (R^2 = 0.18, *P* <0.05), (▲) R^2 =0.04, *P* =0.605; c) 2013: (Δ) R^2 =0.14, *P* = 0.063, (▲) R^2 =0.22, *P* =0.477 and d) overall mean: (Δ) R^2 =0.05, *P* =0.253, (▲) R^2 =0.14, *P* =0.633.

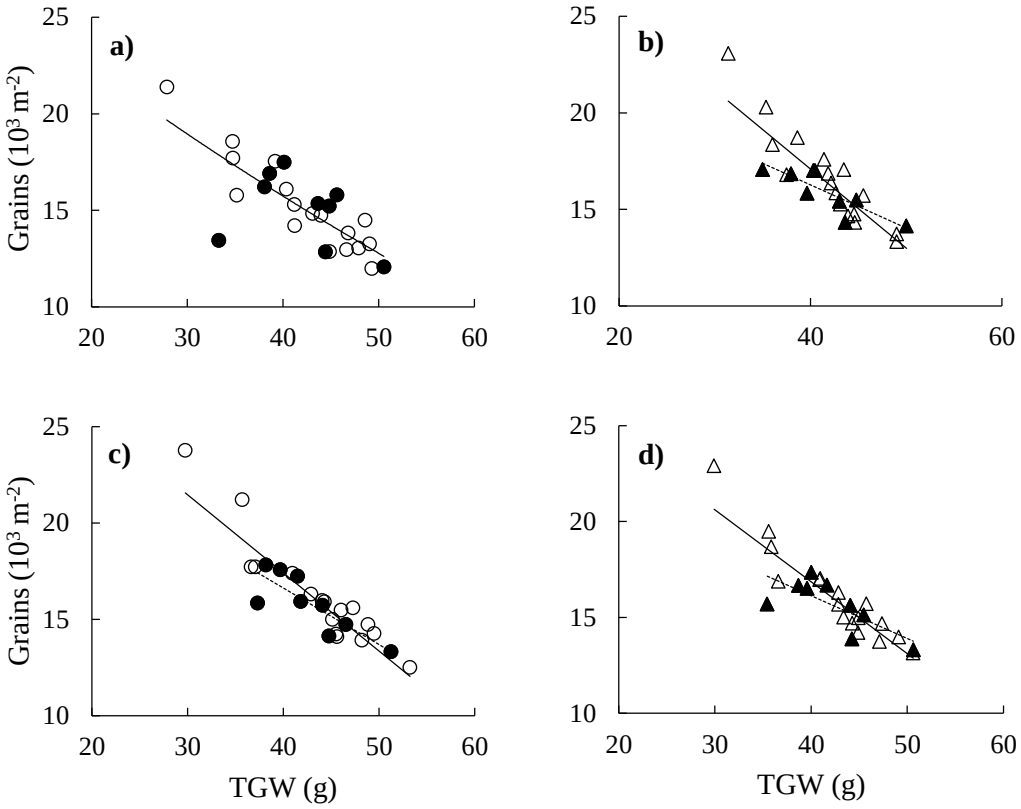


Figure 4.7 Regression of grains m^{-2} on thousand grain weight (TGW) for the subset of 26 cultivars (Δ, —) and nine cultivars (▲, ----) in a) 2011: (Δ) y =-308.1x+28103 (R^2 =0.62, *P* <0.001), (▲) R^2 =0.62, *P* =0.057; b) 2012: (Δ) y =-409.4x+33463 (R2 =0.75, *P* <0.001), (▲) y = -223.64x+25211 (R^2 =0.70, *P* <0.01); c) 2013: (Δ) y =-405.6x+33642 (R^2 = 0.82, *P* = <0.001), (▲) y = -293.29x+28360 (R^2 = 0.69, *P* <0.01) and d) overall mean: (Δ) y =-374.6x+31842 (R^2 =0.79, *P* <0.001), (▲) y = -222.9+25053 (R^2 =0.54, *P* <0.05)

4.2.10 Effect of plant height and anthesis date on key harvest traits

Plant height was not significantly related to grain yield or HI in either the set of 26 cultivars or the sub-set of nine cultivars when averaging across the seasons (Figure 4.8 a and e, respectively). There was a non-linear (quadratic) association between plant height and AGDM among the 26 cultivars (R^2 =0.23, P <0.05; Figure 4.8 b). Biomass at harvest initially decreased with increased plant height up to *ca.* 90 – 95 cm, but then increased with plant height above 95 cm.

Plant height was linearly negatively associated with grains m^{-2} (Figure 4.8 c), more strongly among the 26 cultivars (R^2 =0.65, P <0.001) than the sub-set of nine cultivars (R^2 =0.47, P <0.05). On the other hand, taller cultivars had heavier grains, as shown by a strong positive linear association between TGW and plant height among the 26 (R^2 =0.68, P <0.001) and the nine cultivars (R^2 =0.54, P<0.05) (Figure 4.8 d).

Regression analysis showed anthesis date was not associated with either grain yield, AGDM or HI among the 26 CIMMYT elite cultivars (Figure 4.9 a, b and e, respectively). Grains m^{-2} decreased initially with later anthesis date (*ca.* 80 – 90 DAS) but then increased up to *ca.* 95 – 97 DAE amongst the 26 cultivars (R^2 = 0.41, P <0.01; Figure 4.9 c). In contrast, TGW increased with anthesis date up to *ca.* 80 – 85 DAE but then decreased above *ca.* 87 DAE (non-linearly) amongst the 26 cultivars (R^2 =0.26, P <0.05; Figure 4.9 d). A similar non-linear association was also found between anthesis date and plant height among the 26 cultivars (R^2 =0.31, P <0.05; Figure 4.9 f), with plant height decreasing with later anthesis date in the range *ca.* 85 – 95 DAE. Regressions of anthesis date on key harvest traits in the sub-set of nine cultivars were not significant for averaged data across the three seasons.

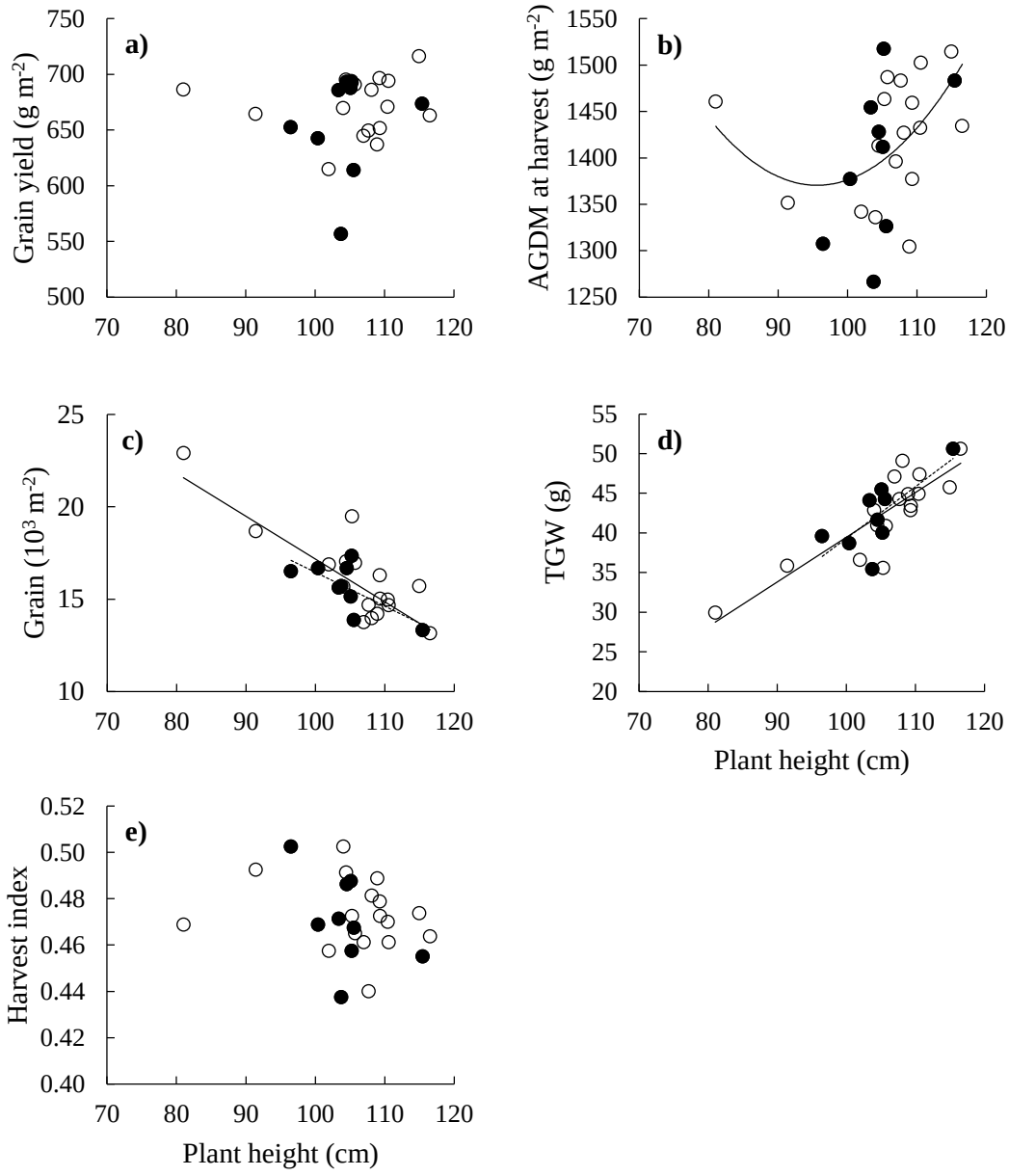


Figure 4.8 Regression of plant height on key harvest traits (values represent means of 2011, 2012 and 2013) among 26 cultivars (○, —) and nine cultivars (●, ----). a) Grain yield; b) Above-ground dry matter (AGDM) at harvest: (○) y =0.298x^2-56.9x+4093 (R^2 =0.23, *P* =0.048), (●) R^2 =0.28, *P* =0.144; c) Grain number m^{-2}: (○) y =-232.2x+40398 (R^2 =0.65, *P* <0.001), (●) y =184.04x+34863 (R^2 =0.47, *P* <0.05); d) Thousand grain weight: (○) y =0.5639x-16.94 (R^2 =0.68, *P* <0.001), (●) y =0.6502x-25.695 (R^2 =0.54, *P* <0.05) and e) Harvest index: (●) R^2 =0.25, *P* =0.170).

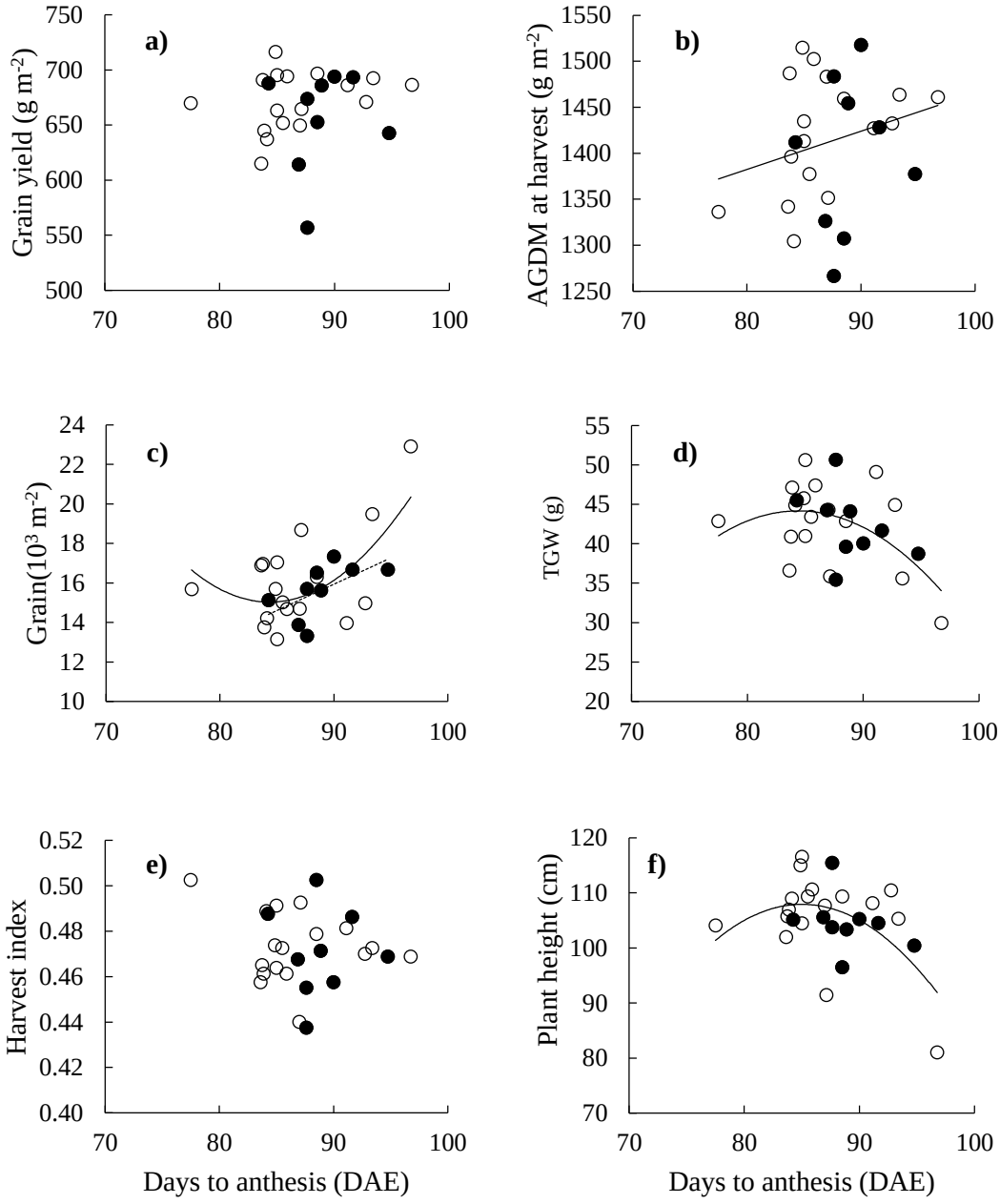


Figure 4.9 Regression of Days to anthesis (DAE = days after emergence) on key harvest traits (overall means of 2011, 2012 and 2013) among 26 cultivars (○, —) and nine cultivars (●, ----). a) Grain yield; b) Above-ground dry matter (AGDM) at harvest; c) Grain number m^{-2}: (○) $y = 34.7x^2 - 5853x + 261890$ ($R^2 = 0.41$, $P = 0.002$), (●) $y = 266.65x - 8061.1$ ($R^2 = 0.35$, $P = 0.093$); d) Thousand kernel weight: (○) $y = -0.0663x^2 + 11.2x - 428$ ($R^2 = 0.26$, $P = 0.031$), (●) $R^2 = 0.18$, $P = 0.263$; e) Harvest index and f) Plant height: (○) $y = -0.1164x^2 + 19.78x - 733$ ($R^2 = 0.31$, $P = 0.015$).

4.2.11 Year of release and key harvest traits in historic cultivars

The key harvest traits were regressed against year of release (YoR) for eight historic CIMMYT spring wheat releases (marked with H in Table 3.1 in Chapter 3, section 3.2.1) in order to quantify rates of genetic gain.

Results showed that from 1966 to 2009 there was a linear rate of genetic gain in grain yield of 25.3 kg ha^{-1} yr^{-1} (R^2 =0.72, *P* <0.01; Figure 4.10 a). There was a trend for grain yield progress to be associated with greater HI in the period from 1966 to 1990 increasing from 0.43 to 0.50, but decreasing with YoR thereafter (Figure 4.10 b). A linear genetic gain in AGDM of 44.8 kg ha^{-1} yr^{-1} was observed, from *ca.* 1300 g m^{-2} to *ca.* 1466 over the 43-year period (R^2 =0.74, *P* <0.01; Figure 4.10 c).

There was also a weak trend for a non-linear association between plant height and the YoR over the 43 years (R^2 =0.56, *P* =0.127; Figure 4.10 d), decreasing from 1966 (102 cm) to 1988 (91.4 cm), but then increasing rapidly to 2009 (104.5 cm).

Linear genetic gains in TGW were evident among the eight historic cultivars, with values of 36.6 for 1966 and *ca.* 47.6 for 2009 (R^2 =0.71, *P* <0.01; Figure 4.10 e). On the other hand, there was no association between grains m^2 and YoR in the historic cultivars (R^2 =0.23, *P* =0.523; Figure 4.10 f). No associations were found between YoR with either grains per spike (GPS) or spikes m^{-2} at harvest.

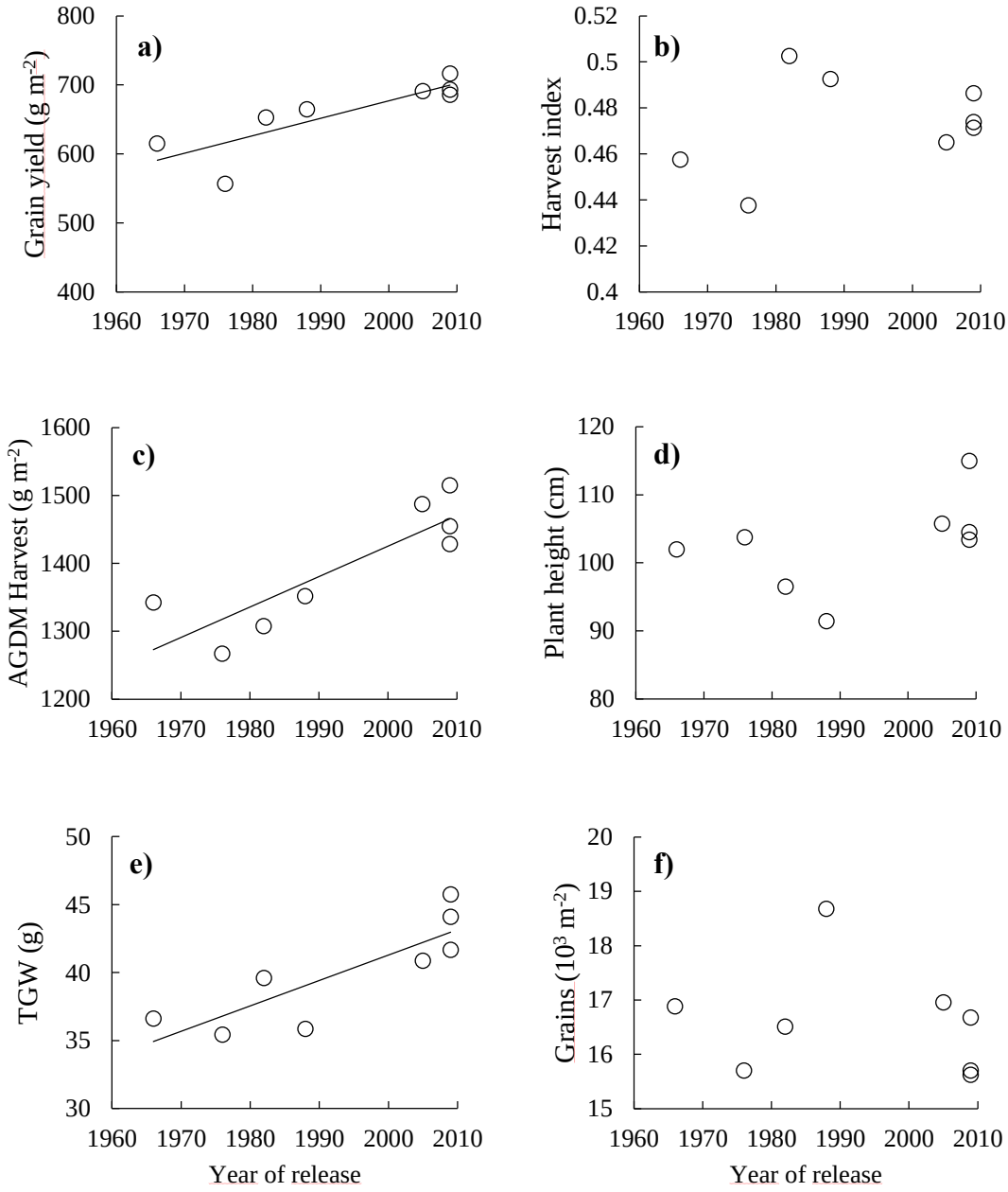


Figure 4.10 Linear regressions of Year of release on a) Grain yield: y =2.528x-4379 (R^2 =0.71, *P* <0.01); b) Harvest index: R^2 = 0.28, *P* =0.438; c) AGDM at harvest: y =4.4775x-7529.8 (R^2 =0.74, *P* <0.01); d) Plant height: R^2 =0.56, *P* =0.127; e) Thousand grain weight (TGW): y =0.1873x-333.27 (R^2 =0.71, *P* <0.01) and on f) Grains m^{-2}: R^2 =0.23, *P* =0.523 among 8 CIMCOG historic cultivars across the three seasons.

4.3 **Discussion**

No systematic progress in HI has been shown in recent decades (Reynolds et al., 2009) and recent studies have suggested that increases in grain yield have been driven mostly by AGDM in modern wheat cultivars (Shearman et al., 2005; Aisawi et al., 2015). However, in order to translate improvements in biomass production into gains in yield potential, HI must be maintained or ideally increased in high biomass cultivars (Reynolds et al., 2012). Within this context, limitations to HI in modern high yielding cultivars will need to be understood from a physiological perspective (Fischer, 2011). This discussion aims to consider variation in grain yield among the 26 CIMMYT spring wheat cultivars in terms of AGDM and HI and their potential limitations, while taking into account effects of days to anthesis and plant height. Moreover, implications for wheat breeding in high biomass cultivars will be discussed.

4.3.1 Physiological basis of grain yield: Above-ground DM and harvest index

Yield potential can be physiologically explained as the product of AGDM and HI (Reynolds et al., 2005). In the present study, significant variation in HI and AGDM was found among the 26 spring wheat cultivars. Grain yield ranged from 5.6 to 7.2 t ha^{-1}, as well as wide ranges were found for HI (0.44 – 0.50) and AGDM (1266 – 1518 g m^{-2}) amongst the 26 cultivars (P <0.001). However, present HI values, and those found elsewhere for winter (*ca.* 0.50) and spring wheat (*ca.* 0.45) (Fischer, 2007, 2011), are much less than the theoretical limit for HI of *ca.* 0.62 (Austin, 1980), indicating scope to raise HI in current CIMMYT wheat cultivars (Foulkes et al., 2011).

Overall in the present study, grain yield was explained mainly by greater AGDM amongst the 26 cultivars (R^2 =0.61, P <0.001), as well as in the subset of nine cultivars (R^2 =0.70, P <0.01). This is consistent with recent studies showing positive associations between grain yield progress and biomass production as well as with and RUE related traits (Waddington et al., 1986; Donmez et al., 2001; Shearman et al., 2005; Xiao et al., 2012; Beche et al., 2014; Aisawi et al., 2015). However, there was a lack of association between grain yield and AGDM among cultivars showing the highest values of AGDM (association not shown). The latter

effect could be possibly related to a non-linear association between grain yield and HI observed among the 26 cultivars, showing increases in grain yield (~7 ton ha^{-1}) with HI up to *ca.* 0.48 and then declining at HI > 0.48 (R^2 = 0.36, P <0.01). This suggests that differences in grain yield among CIMCOG cultivars were only partly explained by differences in HI. Similarly, Aisawi et al. (2015) found an association between grain yield and HI in historic CIMMYT spring wheat cultivars, where yield increased only up to HI values of *ca.* 0.45; and genetic gains in grain yield were mostly explained by increases in AGDM over a 43-year period. The fact that increases in grain yield are not maintained when high HI values are reached could be linked to limitations in photosynthetic capacity (AGDM), as the crop may allocate less biomass for photosynthetic structures to support assimilation for reproductive structures (Slafer et al., 1999). Furthermore, there was a trend for a trade-off between AGDM and HI (R^2 =0.22, P =0.056), it is possible that some cultivars had inefficient investment of extra biomass in spike growth resulting in a lack of demand by the sink (not enough grain number set). Alternatively, the extra biomass could be associated with additional partitioning to the chaff within the spike, reducing fruiting efficiency, as hypothesised previously by Abbate et al. (1998) who suggested that fruiting efficiency can be affected by greater spike DM partitioning to the rachis.

Overall, no associations between anthesis date and harvest traits (grain yield, AGDM or HI) were found within the CIMCOG panel; nevertheless, there was a positive correlation between the total duration of the crop cycle to physiological maturity and AGDM at harvest among the 26 cultivars (r =0.43, P <0.05). Previously, genetic progress in harvest biomass has been related to increases in post-anthesis AGDM accumulated from anthesis to harvest, rather than AGDM accumulated from sowing to anthesis (*e.g.* Aisawi et al. 2015). However, Calderini et al. (1997) reported that modern Argentinian wheat cultivars exhibited almost the same levels of crop growth rates during post-anthesis than in the pre-anthesis period, contrary to their older counterparts. Other studies have reported positive associations between the duration of pre-anthesis stages (especially the rapid spike growth phase) and grain yield, explained in turn by greater tillering and more DM partitioned to spike growth during the pre-anthesis phase resulting in more grains m^{-2} (Slafer et al., 1999). In summary, the lack of association of the

duration of the pre-anthesis phase (*i.e.* sowing to anthesis) and/or the post-anthesis phase (anthesis to physiological maturity) with grain yield and AGDM suggest that other physiological processes related to crop growth rate in key specific phases and/or DM partitioning could be explaining greater yields.

Plant height differed amongst the 26 CIMCOG cultivars, ranging from 0.8 to 1.16 m. These values were generally within the range of plant height proposed to optimize yield (0.7 to 1.0 m; (Miralles and Slafer, 1995a), although the upper end of the range among CIMCOG cultivars was slightly higher than the proposed optimum maximum value. In regard of the non-expected negative correlation between plant height and plant density (plants m^{-2}), there is no apparent physiological explanation reported in the literature for this association under potential conditions (non-nutrient/water limited). It is suggested in the present work that this association was a product of an autocorrelation related to a strongly negative association between TGW and plants m^{-2} and a positive relationship between TGW and plant height (as discussed below in 4.3.2). The negative association between plants m^{-2} and TGW might have been further influenced by differences in seed rate used in sowing; i.e. as different seed rates were used for each variety (seed rate determined by equal weight for all plots); cultivars with lighter seeds had higher seed rates therefore high plant densities, affecting TGW (positively related to plant height) by final differences in shoot number m^{-2}.

On the other hand, some studies have reported positive associations between plant height and stem biomass in modern cultivars (Slafer and Andrade, 1989; Calderini et al., 1995; Aisawi et al., 2015).The fact that plant height was not related to grain yield or AGDM in the present study suggests that plant heights among CIMCOG cultivars are within the optimum range for the environment of NW Mexico. Regarding association between plant height and biomass, Miralles and Slafer (1997) examined effects of different plant height in radiation interception and radiation use efficiency reporting that RUE during pre-anthesis was lower in short lines but from anthesis to maturity RUE was improved in semi-dwarf lines, associated with thicker leaves and increased attenuation coefficients. Plant height was not related to HI in the present study and although reductions in plant height within CIMMYT semi-dwarf cultivars introduced during 1960s improved grains m^{-2} and HI further major reductions in plant height to favour spike growth may

not be feasible due to trade-offs with biomass production (Foulkes et al., 2011) and pre-anthesis RUE (poor canopy architecture during this phase) (Miralles and Slafer, 1997). Thus, decreases in stem DM to favour spike growth may rely on changes in stem morphology while maintaining overall plant height.

4.3.2 Grain yield agronomical basis: yield numerical components

It has been recognised that historically genetic gains in grain yield have been achieved mostly through grains m^{-2} (Calderini et al. 1999) by increased floret survival and grains per spike (Slafer and Andrade, 1993). Overall in the present study, grain yield was not consistently related to either grains m^{-2}, GPS or TWG amongst the 26 cultivars; however, trends for positive associations between grain yield and grains m^{-2} were observed in individual years for the 26 cultivars as well as trends for positive associations between grain yield and TGW in the sub-set of nine cultivars. The lack of consistent association between grain yield and its numerical components could imply that both were equally important in the determination of grain yield among CIMCOG cultivars and that future yield gains will depend on simultaneous improvement of grain number and grain weight. However, there was a strong trade-off between grain number m^{-2} and TGW among the 26 (R^2 =0.79, P <001) and nine cultivars (R^2 =0.54, P <0.05). Many studies have shown a negative association between genetic variation in grains m^{-2} and the final grain weight (Miralles and Slafer 1995; Slafer et al. 1994; Acreche and Slafer 2009). Although this trade-off could be associated with increased competition for assimilate between growing grains as grains m^{-2} suggesting source limitation, the physiological mechanisms driving this negative association have been shown to be much more complex and related to the determination of potential grain weight, fruiting efficiency, increased proportion of grains in distal positions, within the spike, *Rht* alleles effects, among others. For instance, Acreche and Slafer (2009) showed that average grain weight was reduced when grain number was increased due to genetic and/or environmental factors; however, grain weight in specific positions within the spike was not significantly related to the grain number, concluding that negative associations between grains m^{-2} and TGW was due to contribution of lighter grains in more distal grains within the spikelet and/or more distal spikelets, reducing average grain weight potential independently of competitive relationships between growing grains.

Moreover, positive departures to break this negative association have been shown (Gaju et al., 2009; Bustos et al., 2013) and will be discussed further in the general discussions.

Regarding the numerical determination of grains m^{-2}, significant cultivar differences were found for grain number m^{-2} and grains $spike^{-1}$ (GPS) in the present study (P <0.001). Spikes m^{-2} explained most of the seasonal variation in grains m^{-2} (r =0.73, P <0.001), rather than GPS (r =0.32, ns) amongst the 26 cultivars. Moreover, more days to anthesis were positively correlated with more grains m^{-2} (r =0.50, P <0.01) within the 26 CIMCOG cultivars, but this association was not observed for the nine cultivars, as the rationale for the selection of this subset included reduced range of anthesis date. This association could be related the positive relationship often reported between the duration of stem elongation and spike growth (Gonzalez et al., 2011), as moreover for high latitude spring wheats this period is inevitably short (Fischer, 2011). Further discussion on physiological basis of grains m^{-2} determination will be presented in Chapter 5.

Significant genetic variation was also found for TGW amongst the 26 CIMCOG cultivars (P <0.001). As the CIMCOG panel was chosen from a wide genetic background, it is not surprising to find highly significant genetic variation for TGW (29.9 to 50.6 g). As it has been shown that in modern spring wheat cultivars final grain weight is unlikely to be source limited (Slafer and Savin 1994), differences found in TGW among CIMCOG cultivars could be presumably related to grain weight potential. Similarly, Aisawi et al. (2015) reported that differences in TGW were mostly associated with differences in potential grain weight rather than by source limitation, as shown by the lack of grain weight response to de-graining treatments in most modern historic CIMMYT cultivars.

The duration of the grain filling period among CIMCOG cultivars was not associated with the final grain weight achieved at harvest. In this respect, the rate and duration of the grain filling period has not been consistently related to grain weight in previous studies (e.g. Calderini and Reynolds 2000; Simmons et al. 1982; Miralles and Slafer 1995), but it is the temperature during grain filling that captures the major environmental factor affecting final grain weight (Sofield et al., 1977). Moreover, there was a trend for a non-linear negative association

between anthesis date and TGW (R^2 =0.26, P <0.05) amongst the 26 cultivars. This could be related to higher temperatures during pre-anthesis due to prolonged time for flowering, as temperature between booting and anthesis is related to grain weight differences due to detrimental effects of high pre-anthesis temperatures on carpel weight at anthesis (Calderini et al., 1999a, 2001), as the weight of carpels at anthesis has been reported to condition potential grain weight and grain growth after anthesis (Calderini and Reynolds 2000).

Although there were no significant effects of plant height on grain yield, AGDM or HI, yield components showed different associations with this trait. Final plant height was strongly and positively associated with TGW amongst the 26 (R^2 =0.68, P <0.001) and the nine cultivars (R^2 =0.54, P <0.05). On the other hand, grain number m^{-2} was linearly and negatively related to plant height amongst the 26 (R^2 =0.65, P <0.001) and the nine cultivars (R^2 =0.47, P <0.05). The reason for opposite effects of plant height on TGW and grains m^{-2} could be related to the strong trade-off observed among these traits, possibly linked to a greater proportion of lighter distal grains in shorter cultivars. This was suggested by Miralles and Slafer (1995b) who concluded that reduction in average grain weight produced by *Rht* alleles was due to a combination of effects on potential size of each grain and on contribution of grains from distal positions within the spike with lower potential grain weight. In this respect, reductions in grain weight potential by introgression in *Rht* alleles have been related with reductions in total cell number in the pericarp (Miralles et al. 1998). However, most studies investigating effects of *Rht* genes on yield components are performed in isogenic lines where effects can be entirely attributed to plant height. Although all CIMCOG cultivars possess alleles for *Rht* genes, they are also high yielding cultivars selected for a wide range of traits and it only can be suggested that within semi-dwarf adapted spring wheat cultivars, TGW appeared to be favoured in taller plants. On the other hand, a negative association between plant height and grains m^{-2} can be taken as a starting point to investigate the associations between a greater competition for assimilate between growing stems and spikes shortly before anthesis and floret survival, hence grain set (Brooking and Kirby, 1981; Fischer and Stockman, 1986).

4.3.3 Year of release and key harvest traits in a sub-set of eight historic cultivars

In order to investigate genetic gains in yield potential with time and the associated physiological basis of these gains within the CIMCOG panel, a sub-set of eight representative historic cultivars was selected for regression analysis with year of release (YoR). Overall for the three seasons, there was a significant linear genetic grain yield of 25.3 kg ha^{-1} yr^{-1} or 0.38 % yr^{-1} among eight CIMMYT historic spring wheat cultivars released from 1966 to 2009. Genetic gains in yield potential have been reported widely in CIMMYT cultivars (Aisawi et al. 2015; Sayre, Rajaram, and Fischer 1997; Waddington et al. 1986). Rate of yield progress observed in the present study was lower than previously reported for CIMMYT spring wheat cultivars. For instance, Waddington et al. (1986) showed that the grain yield potential of cultivars released from 1950 to 1982 in NW Mexico rose at an estimated average of 59 kg ha^{-1} yr^{-1} or about 1.1% yr^{-1}. Similarly, a study in the same area measured yield potential progress in eight short cultivars released 1962 and 1988 and reported an average rate of increase of 67 kg ha^{-1} y^{-1} or 0.88% yr^{-1}, indicating that linear progress in yield continued at least until the late 1980's within short germplasm (Sayre et al., 1997). However, the range of YoR for the cultivars considered in the present study is wider, including recent CIMMYT releases up to 2009. In this respect, the latest study in yield potential progress in CIMMYT spring wheat cultivars (same time span than for historic CIMCOG cultivars) reported slightly lower linear grain yield increases of 30 kg ha^{-1} yr^{-1} or 0.59% yr^{-1} (Aisawi et al., 2015), compared to studies with older cultivars. Moreover, other studies evaluating genetic yield gains in CIMMYT spring wheat cultivars grown under different environments have been also reported continuous genetic yield gains in CIMMYT modern cultivars (Lopes et al., 2012a; Manès et al., 2012; Sharma et al., 2012).

Present results also showed that genetic gains in yield potential were mostly explained by linear gains in AGDM at harvest (4.5 g m^{-2} or 0.32% yr^{-1}) and TGW (0.19 g or 0.47% yr^{-1}) with time. On the other hand and although not significant, there was a trend for a non-linear relationship between HI and YoR, increasing to values up to *ca.* 0.50 in 1988 and decreasing thereafter and no significant associations were found between grains m^{-2} and YoR in the present study.

Shearman et al. (2005) found that a quadratic function fitted to the data showed that progress in HI was most apparent during an early phase of the 23-year period, whereas biomass contributed most since about 1983 for eight winter wheat varieties introduced from 1972 to 1995 in the UK. Similar to our results in the recent study of CIMMYT historic including 6 of the 8 cultivars in the present analysis at the same site, Aisawi et al. (2015) showed that genetic gains in grain yield were associated to linear increases in AGDM at harvest (6.67 g m^{-2} yr^{-1}) and TGW rather than HI or grain number m^{-2}. Moreover, the latter study reported increases in plant height with YoR and trends for decreases in HI. Although the YoR of the historic CIMCOG cultivars was not significantly related with plant height amongst the eight historic CIMCOG cultivars, there was a trend for an increased plant height in most modern cultivars (2009). Overall, these findings suggest evidence for decreases in HI with YoR in modern CIMMYT spring wheat cultivars.

4.3.4 Conclusions

The answers to the hypotheses presented at the beginning of this chapter are outlined below:

1. Wide genetic variation in grain yield, yield components, HI and AGDM at harvest was found among the 26 CIMCOG cultivars under high radiation conditions in NW Mexico.
2. Grain yield, AGDM and TGW appeared to increase linearly with year of release but this was not the case for HI, that showed a tendency for decreases in the last 20 years within eight historic cultivars selected from the set of 26 cultivars.
3. Variation in grain yield was not explained by differences in grains m^{-2} nor HI and was rather linearly associated with AGDM and TGW among the 26 CIMCOG cultivars. However, when just considering the cultivars showing the highest AGDM production, there was no association between grain yield and AGDM, suggesting physiological restrictions for further increases in AGDM to be translated into grain. Grain yield was not consistently explained by its numerical components (grain number and average weight), implying that physiological processes determining these

components are both important in grain yield determination. Moreover, strong trade-offs were observed between these components, possibly determined by trade-offs between intrinsic physiological processes determining grains m^{-2} and potential grain weight.

4. There was a non-linear association between AGDM at harvest and HI amongst the 26 CIMCOG cultivars indicating assimilate partitioning was not optimised in high biomass cultivars.
5. Genetic variation in grain number m^{-2} was mainly determined by greater spikes m^{-2} rather than by grains per spike.
6. Grain yield was not associated with plant height among the 26 CIMCOG cultivars, implying that the CIMCOG cultivars were within the optimal range of heights in elite CIMMYT spring wheat material.

CHAPTER 5.

OPTIMIZED DM PARTITIONING AND ASSOCIATIONS WITH SPIKE GROWTH, GRAIN YIELD AND YIELD COMPONENTS IN CIMCOG PANEL

5.1 Introduction

Recent studies indicate that genetic gains in grain yield since about 1990 have been associated mainly with above-ground biomass in spring (Aisawi et al., 2015) and winter wheat (Shearman et al., 2005), and also show a trend for decreases in HI in the latest CIMMYT spring wheat releases (Aisawi et al., 2015). Moreover, recent studies have reported some extent of co-limitation of grain growth during grain filling in modern wheat varieties (Slafer and Savin, 1994; Shearman et al., 2005; Acreche and Slafer, 2009; Aisawi et al., 2015), emphasising the need for simultaneous improvements in post-anthesis source and sink strength in modern wheat cultivars.

In this respect, boosting spike fertility and an optimal assimilate partitioning among organs are two promising avenues to overcome the physiological limits in biomass conversion into grain yield. The main idea behind this approach is to decrease assimilate partitioning to alternative sinks, such as structural DM and/or infertile tillers whilst taking into consideration potential trade-offs with lodging resistance, WSC accumulation and/or photosynthetic capacity. Within this context, the main objective of the present chapter is to identify key pre-anthesis traits associated with grains m^{-2}, HI and grain yield among the 26 CIMCOG cultivars, particularly those traits directly related to SPI at anthesis and fruiting efficiency (FE).

Specific hypotheses tested in this chapter:

1. Differences in grains m^{-2} found among CIMMYT elite spring wheat cultivars studied in this thesis (CIMCOG panel) are related to greater FE, SPI and spike DM at GS65+7d.
2. The FE is partially independent to changes in SPI and DM at GS65+7d within the CIMCOG panel.
3. Genetic variation for FE among CIMCOG cultivars is associated with variation in DM partitioned to the different spike structural components (*i.e.* rachis, glumes, lemmas, paleas and awns) and/or to spike WSC concentration at GS65+7d.
4. Competition for assimilates between the spike and the true-stem at GS65+7d is stronger than between the spike and the leaf lamina or leaf sheath among CIMMYT elite spring wheat cultivars.
5. The late stem elongation phase or rapid spike growth phase (GS41 and GS65+7d) is crucial in determining the competition between true-stem and spike assimilate partitioning at GS65+7d.
6. Genetic variation in true-stem soluble DM or WSC at GS65+7d is determined by WSC concentration rather than true-stem DM amount (g m^{-2}).
7. A negative association between SPI and true-stem partitioning index (T-stem PI) at GS65+7d is stronger when considering just structural true-stem DM among the nine cultivars, mainly associated with the structural DM increment from GS41 to GS65+7d.
8. Genetic variation in HI and SPI at GS65+7d are positively related to true-stem soluble DM (WSC) concentration at GS65+7d among the 26 CIMCOG cultivars.

5.2 **Results**

Firstly, this results section describes the genetic variation and associations found for the physiological determinants of grains m^{-2} among the 26 CIMCOG cultivars. Results for the DM partitioning analysis are presented for the 26 cultivars at

GS65+7d, and additionally for the nine cultivars at GS31, GS41 and harvest. Finally, a detailed analysis of soluble DM partitioning is presented for the nine cultivars, including associations with yield and yield components and true-stem WSC accumulation dynamics.

5.2.1 Physiological determinants of grains m^{-2}

Genetic variation in grains m^{-2} for the 26 cultivars is presently analysed in relation to the following determinants: (i) the duration in days of the rapid spike growth phase from GS41 to GS65+7d, (ii) the crop growth rate (CGR, g m^{-2} d^{-1}) during the rapid spike growth phase, (ii) the spike partitioning index (SPI) and (iv) the fruiting efficiency (grains at harvest per g spike DM at anthesis; FE).

Cultivar values for grains m^{-2} and its physiological components are shown in Table 5.1 for 2012, 2013 and averaging across the seasons. Grains m^{-2} differed amongst the 26 cultivars overall in the range 13,107 (TC870344) – 23,416 (BCN/Rialto) (P <0.001) and there was a year x cultivar interaction (P <0.01). The genotypes differed in SPI in both years (P <0.001), ranging from 0.220 (Saual/whear) to 0.276 (Pavon F76) in 2012 and 0.199 (Babax/LR42) to 0.272 (Pfau/Seri.1b) in 2013. Averaging across years, cultivars ranged from 0.212 (Saual/whear) to 0.266 (Pfau/Seri.1b). Differences for the subset of nine cultivars were not observed for CGR from GS41-GS65+7d in either 2012, 2013 or averaging across the seasons. Overall, CGR ranged from 17.9 (Cmh79a.955) to 21.7 (Tacupeto F2001/B) g m^{-2} d^{-1}. Overall, the fruiting efficiency (FE; calculated with the spike DM per unit area at GS65+7d) differed amongst cultivars (P <0.001) and years (P <0.01) but showed no significant year x cultivar interaction (P =0.251). The FE was slightly greater in 2013 (64.4 grains g^{-1} DM) than 2012 (60.1 grains g^{-1} DM). In 2012 cultivars differed in the range 79.8 (Bcn/rialto) to 48.8 (Wbill1*2) grains g^{-1} DM and in 2013 from 46.3 (Wbill1*2) to 77.0 (Pbw343*2) grains g^{-1} DM in 2013. Overall, days from GS41 to GS65+7 ranged from 22.3 (Becard) to 27.8 (Tacupeto F2001/B) (P <0.001) among the 26 CIMCOG cultivars.

In 2012, grains m^{-2} was not associated with SPI (Figure 5.1 a) among the 26 cultivars or with CGR among the nine cultivars, but was linearly and positively associated with FE (R^2 =0.71, P <0.001; Figure 5.1 b). In 2013 on the other hand,

grains m^{-2} was non-linearly associated with SPI (R^2 =0.27, P <0.05; Figure 5.2 a) and with spike DM per unit area at GS65+7d (R^2 =0.37, P <0.001; Figure 5.2 d). No relationship was found between grains m^{-2} and FE in 2013 (Figure 5.2 b). Averaging across seasons and the 26 cultivars, regressions showed that differences in grains m^{-2} were more strongly related to FE (R^2 =0.40, P <0.001; Figure 5.3 b) than SPI (R^2 =0.15, P =0.126; Figure 5.3 a). No association was found between FE and SPI in either year or averaging over years.

Table 5.1. Grain number model components: grains m^{-2}, spike partitioning index at GS65 +7 days (SPI), crop growth rate (between GS41 and GS65 +7 days; CGR; g m^{-2} d^{-1}) and fruiting efficiency (FE; grains g^{-1} spike DM) for the 26 CIMMYT spring wheat genotypes in 2012, 2013 and 2012-2013 means. Shaded rows represent the sub-set of nine cultivars. Degrees of freedom for individual years were 52, except for CGR (16).

	2012					2013					2012-13 mean				
Entry	**Grains** m^{-2}	**SPI**	**CGR** GS41-65+7d g m^{-2} d^{-1}	**Days** GS41-65+7d	**FE** grains g^{-1} spike DM	**Grains** m^{-2}	**SPI**	**CGR** GS41-65+7d g m^{-2} d^{-1}	**Days** GS41-65+7d	**FE** grains g^{-1} spike DM	**Grains** m^{-2}	**SPI**	**CGR** GS41-65+7d g m^{-2} d^{-1}	**Days** GS41-65+7d	**FE** grains g^{-1} spike DM
BABAX/LR42	14132	0.232	23.5	27	49.8	13312	0.199	19.1	25	61.6	13722	0.215	21.3	26	55.7
BACANORA T88	20275	0.271	-	25	67.2	17717	0.238	-	26	73.8	18996	0.255	-	25	70.5
BCN/RIALTO	23071	0.233	-	20	79.8	23761	0.237	-	27	63.5	23416	0.235	-	24	71.6
BECARD/KACHU	17005	0.246	18.3	24	60.0	17231	0.237	19.8	26	59.8	17118	0.242	19.0	25	59.9
BRBT1*2/KIRITATI	14314	0.239	-	26	55.0	14994	0.232	-	26	66.8	14654	0.236	-	26	60.9
SAUAL/4/CROC_1	15832	0.243	-	24	55.4	15471	0.233	-	31	52.4	15651	0.238	-	28	53.9
SAUAL/WHEAR	14306	0.220	20.2	26	58.9	14130	0.204	22.2	26	64.1	14218	0.212	21.2	26	61.5
CMH79A.955	15828	0.260	17.6	21	52.4	17813	0.263	18.3	31	54.2	16821	0.261	17.9	26	53.3
CNO79//PF70354	15701	0.235	-	28	55.0	14729	0.218	-	26	60.4	15215	0.227	-	27	57.7
CROC_1/AE.SQUA	18714	0.229	-	26	71.9	16300	0.222	-	24	76.2	17507	0.225	-	25	74.1
KINGBIRD #1	18342	0.238	-	22	68.2	21214	0.238	-	23	77.0	19778	0.238	-	22	72.6
MILAN/KAUZ	14751	0.241	-	25	55.1	13907	0.217	-	26	71.4	14329	0.229	-	26	63.2
PAVON F76	17054	0.276	13.8	25	64.1	15849	0.244	17.0	27	61.1	16451	0.260	15.4	26	62.6
PBW343*2/KUK	16349	0.273	-	25	60.7	17391	0.220	-	25	77.0	16870	0.246	-	25	68.9
PFAU/SERI.1B	16855	0.264	-	25	64.0	14229	0.272	-	24	67.9	15542	0.266	-	24	64.9
SERI M 82	16840	0.273	17.1	24	59.1	15919	0.238	21.9	24	63.8	16380	0.255	19.5	24	61.5
SIETE CERROS T66	16778	0.273	-	27	55.0	17716	0.247	-	28	68.4	17247	0.260	-	28	61.7
SOKOLL//PBW343*2	15263	0.224	-	26	64.1	14092	0.212	-	26	74.0	14678	0.218	-	26	69.1
TACUPETO F2001/SAUAL	17052	0.240	-	26	67.6	15598	0.211	-	26	64.4	16325	0.225	-	26	66.0
TACUPETO F2001/BRAM	15404	0.261	15.3	28	55.2	15718	0.234	28.2	28	51.5	15561	0.248	21.7	28	53.3
TC870344/GUI//TEMPOR	13719	0.232	-	27	56.3	12494	0.211	-	24	55.4	13107	0.222	-	26	55.9
TRAP#1/BOW/3/VEE	14651	0.239	-	24	60.6	15910	0.227	-	24	60.6	15281	0.233	-	24	60.6
UP2338*2/4/SNI/TRAP#1	17007	0.229	20.7	24	61.1	17569	0.213	16.0	28	73.2	17288	0.221	18.3	26	67.2
BECARD	15467	0.259	19.6	22	57.2	14729	0.222	22.4	24	60.7	15098	0.240	21.0	23	58.9
WBLL1*2/KURUKU*2	13324	0.246	-	24	48.8	14265	0.237	-	30	46.3	13795	0.241	-	27	47.6
YAV_3/SCO//JO69	17588	0.249	-	25	59.9	15972	0.230	-	27	67.8	16780	0.240	-	26	63.8
Mean	**16370**	**0.247**	**18.4**	**25**	**60.1**	**16078**	**0.229**	**20.5**	**26**	**64.4**	**16224**	**0.238**	**19.5**	**26**	**62.2**
SED Cultivar (df)	960	0.013	4.82	1.1	13.1	870	0.01	5.40	1.3	6.5	655 (102)	0.01 (102)	3.57 (34)	0.9 (102)	4.8 (102)
SED Year (df)											**182 (1)**	**0.002 (1)**	**1.68 (1)**	**0.24 (1)**	**1.3 (1)**
SED Interaction (df)											**927 (102)**	**0.01 (102)**	**5.05 (34)**	**1.2 (102)**	**6.8 (102)**
CV (%)	7.2	6.2	32.0	5.4	13.5	6.6	5.3	32.2	6.0	4.2	7.0	6.2	31.7	5.8	13.4

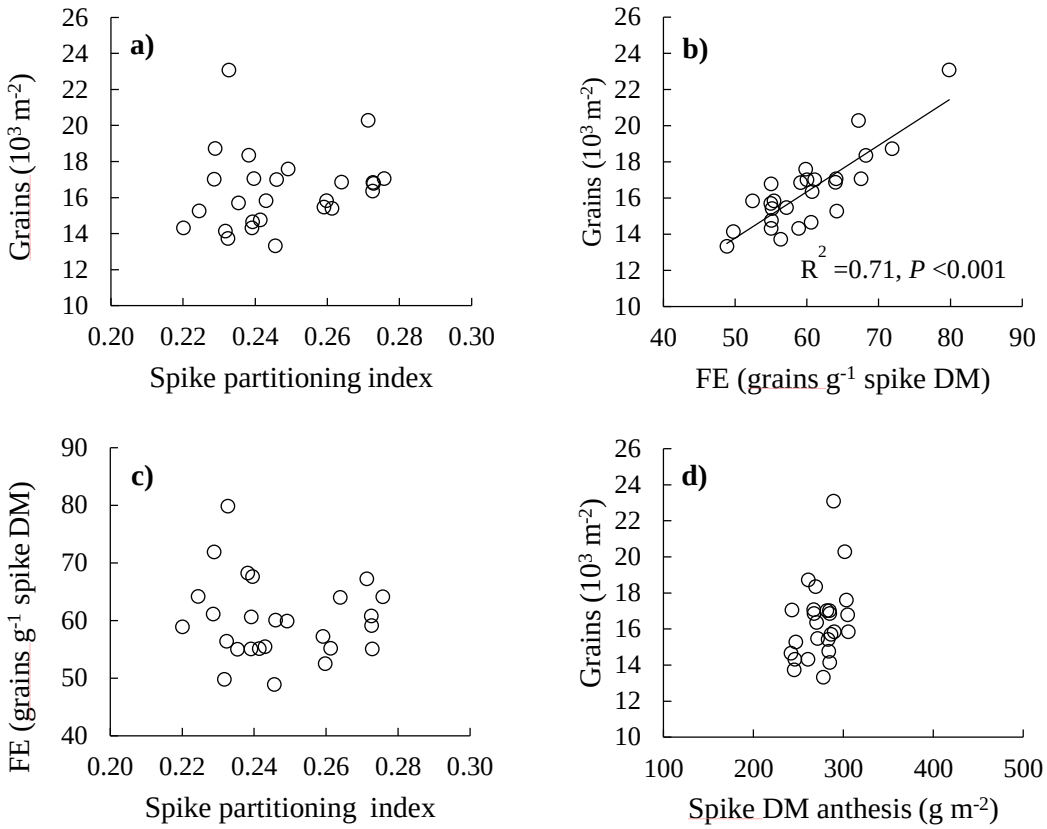


Figure 5.1 Regressions of a) grains m^{-2} on spike partitioning index; b) grains m^{-2} on fruiting efficiency (grains g^{-1}spike DM at GS65+7d; FE) (y =256.4x+964.6); c) FE on SPI and e) grains m^{-2} on spike DM (g m^{-2}) at GS65+7d among the 26 spring wheat CIMMYT cultivars in 2012.

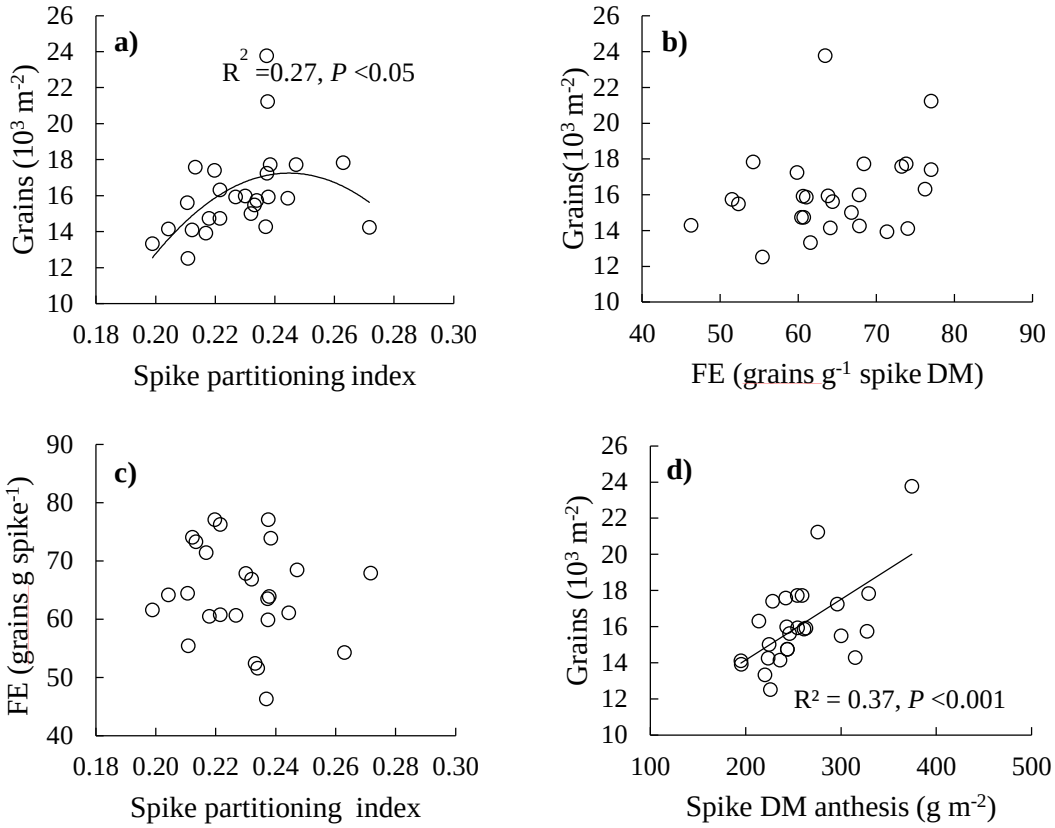


Figure 5.2 Regressions of a) grains m^{-2} on spike partitioning index (y =2E+06x2+1E+06x-116863); b) grains m^{-2} on fruiting efficiency (grains g^{-1}spike DM at GS65+7d; FE); c) FE on SPI and d) grains m^{-2} on spike DM (g m^{-2}) at GS65+7d (y =33.64x+7413.8) among the 26 spring wheat CIMMYT cultivars in 2013.

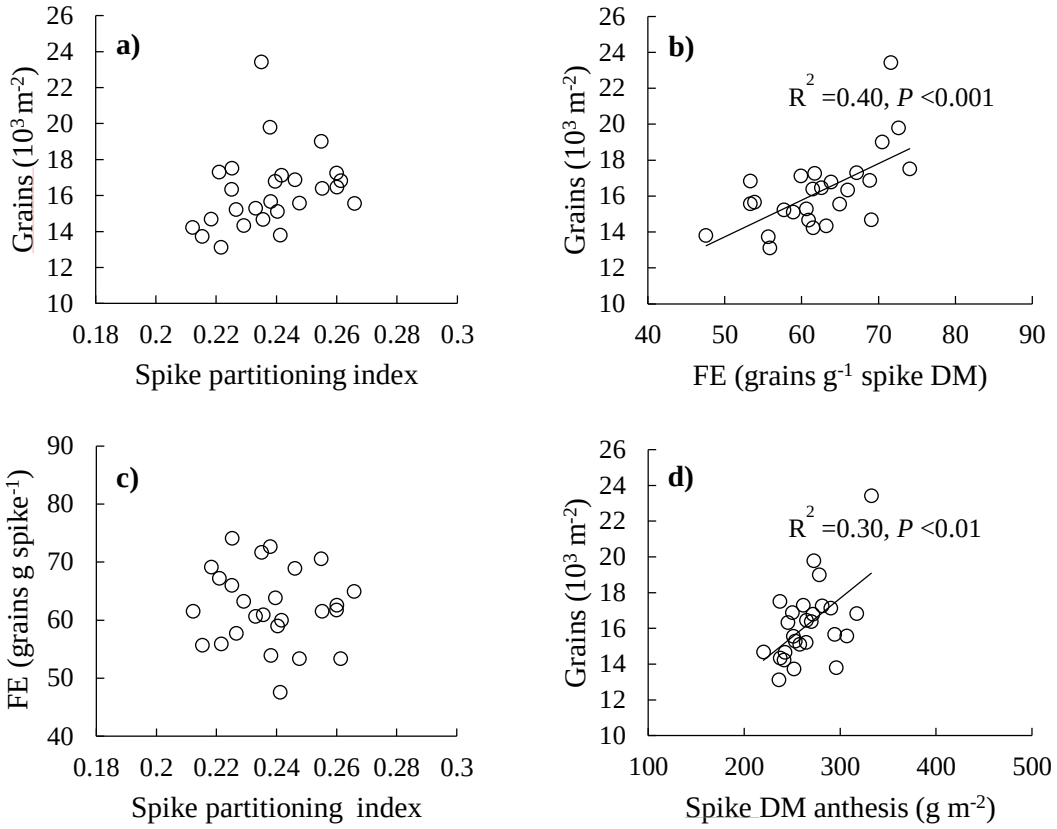


Figure 5.3 Regressions of **a)** grains m^{-2} on spike partitioning index; **b)** grains m^{-2} on fruiting efficiency (grains g^{-1}spike DM at GS65+7d; FE) (y =203.85x+3546.5); **c)** FE on SPI and **d)** grains m^{-2} on spike DM (g m^{-2}) at GS65+7d (y =43.4x+4658.5) among the 26 spring wheat CIMMYT cultivars in the two years (mean of 2012 and 2013).

The spike DM at harvest was dissected into its morphological components in 2012 and 2013 in seven of the nine subset cultivars (see section 5.3.3.2, Table 5.5). Moreover rachis length was measured in 2012 and rachis specific weight (rachis DM per cm rachis length; RSW) was calculated. Also in 2012, the DM allocated to the components (as a proportion of the spike DM at harvest, 100% without grains) was calculated. There were differences in RSW in the range 11.0 to 15.1 mg cm^{-1} among the seven cultivars (P <0.01, 2012). The FE (calculated based on the chaff DM at harvest) was linearly and negatively associated with RSW (R^2 =0.66, P <0.05;

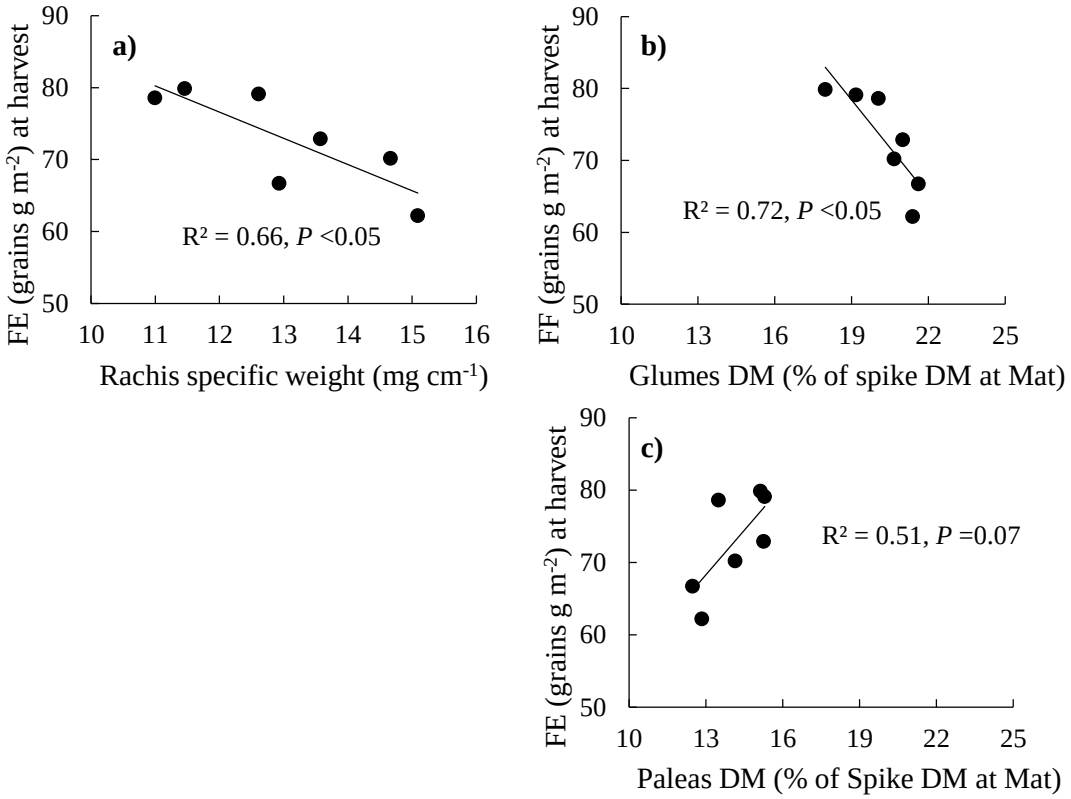


Figure 5.4. Linear regressions of fruiting efficiency (FE, grains per g of chaff at harvest) on a) rachis specific weight (mg cm^{-1}) (y =-3.64x+120.3), b) glumes % of DM (y =-4.42x+162.4) and c) Paleas % of DM (y =4.108x+14.88) (as a proportion of spike DM at harvest, 100% without grains) among seven cultivars (within the subset of nine cultivars) in 2012.

5.2.2 Crop growth at GS65+7 days and for developmental stages

5.2.2.1 GS65+7d days (26 cultivars)

Across the 26 cultivars, AGDM at GS65+7d ranged from 970 (Pavon F76) - 1249 (Bcn/rialto) g m^{-2} in 2012 and from 879 (Pfau/Seri) - 1582 (Bcn/Rialto) g m^{-2} in

2013 (P <0.001; Table 5.2). Averaging across seasons, AGDM at GS65+7d ranged from 945 (Pfau/Seri.1b) - 1415 (Bcn/rialto) g m^{-2}. There was a significant year x cultivar interaction (P <0.01), mainly due to the cultivars Bcn/rialto and Milan/Kauz showing relatively different performances across years.

In 2012, fertile shoots m^{-2} ranged from 333 (Cmh79a.955) to 579 (Bcn/rialto) (P <0.001), in 2013 from 362 (Brbt1*2/Kiritati) to 568 (Bcn/rialto) among the 26 cultivars (P <0.001) and averaging across seasons from 348 (Cmh97a.955) to 574 (Bcn/rialto) (P <0.001) (Table 5.2). Fertile shoots m^{-2} were slightly higher in 2012 than in 2013 (P <0.01), although no year x cultivar interaction was present. In 2012, spikes m^{-2} ranged from 275 (Tacupeto F2001) to 446 (Bacanora T88) (P <0.001) and in 2013 from 362 (Cmh79a.955) to 508 (Pavon F76) in 2013 (P <0.001). There was a cultivar x year interaction (P <0.001). Infertile shoot DM at GS65+7d (g m^{-2}) as a proportion of aerial biomass ranged widely in 2012 from 7.3 (75.2 g m^{-2}) to 31% (381.8 g m^{-2}) (P <0.001 and <0.01, respectively). In 2013, infertile shoot DM ranged from 4.7 (49.3 g m^{-2}) to 22.0% (234.9 g m^{-2}) (P <0.05 and P =0.065, respectively) (Table 5.2).

Cross-year means for the AGDM as infertile shots are presented in Figure 5.5 (P <0.01), where percentages of AGDM as infertile shoots are shown for each cultivar.

Table 5.2. Above-ground dry-matter (AGDM), shoots and spikes number m^{-2} at anthesis +7 days (GS65+7d) for the 26 CIMMYT spring wheat cultivars in 2012, 2013 and the overall means. Shaded rows represent the sub-set of nine cultivars. Degrees of freedom for individual years are 52.

Cultivar	2012			2013			2012-13 mean		
	AGDM ant g m^{-2}	**Shoot No.** m^{-2}	**Spike No.** m^{-2}	**AGDM ant** g m^{-2}	**Shoot No.** m^{-2}	**Spike No.** m^{-2}	**AGDM ant** g m^{-2}	**Shoot No.** m^{-2}	**Spike No.** m^{-2}
BABAX/LR42//BABAX/3/VORB	1233	415	364	1107	418	397	1170	416	380
BACANORA T 88	1112	540	445	1071	429	408	1091	485	426
BCN/RIALTO	1249	579	402	1582	568	505	1415	574	453
BECARD/KACHU	1158	496	377	1243	405	376	1200	451	377
BRBT1*2/KIRITATI	1090	443	370	970	362	320	1030	403	345
SAUAL/4/CROC_1	1187	408	314	1285	380	326	1236	394	320
SAUAL/WHEAR//SAUAL	1117	367	313	1160	399	345	1138	383	329
CMH79A.955/4/AGA/3	1173	333	289	1256	363	317	1215	348	303
CNO79//PF70354/MUS/3	1216	388	312	1120	367	337	1168	377	324
CROC_1/AE.SQUARROSA	1140	520	423	966	467	406	1053	493	414
KINGBIRD #1//INQALAB 91*2	1129	560	389	1162	523	469	1146	541	429
MILAN/KAUZ//PRINIA/3/BAV92	1172	388	306	902	379	335	1037	383	321
PAVON F 76	970	579	411	1068	534	508	1019	556	459
PBW343*2/KUKUNA*2	994	477	423	1040	432	407	1017	454	415
PFAU/SERI.1B//AMAD/3/	1011	457	424	879	410	374	945	433	404
SERI M 82	1046	451	348	1070	458	358	1058	455	353
SIETE CERROS T66	1116	503	400	1048	447	392	1082	475	396
SOKOLL//PBW343*2/KUKUNA	1093	481	372	923	416	377	1008	449	374
TACUPETO F2001/SAUAL/4/	1018	416	346	1165	427	381	1092	421	364
TACUPETO F2001/BRAMBLING*2	1083	338	275	1397	429	353	1240	384	314
TC870344/GUI//TEMPORALERA	1055	406	345	1077	412	368	1066	409	357
TRAP#1/BOW/3/VEE/PJN	1013	513	396	1161	511	455	1087	512	425
UP2338*2/4/SNI/TRAP#1/3	1234	484	363	1135	506	439	1184	495	401
BECARD	1047	468	393	1100	449	413	1073	459	403
WBLL1*2/KURUKU*2/5/REH	1131	372	275	1324	377	344	1227	374	310
YAV_3/SCO//JO69/CRA/3	1209	434	352	1058	388	347	1134	411	349
Mean	**1115**	**455**	**363**	**1126**	**433**	**387**	**1120**	**444**	**375**
SED Cultivar (df)	103.3	28.1	22.9	99.9	32.8	24.82	71.4 (102)	26.4 (102)	19.07 (101)
SED Year (df)							**19.8 (1)**	**7.3 (1)**	**5.29 (1)**
***SED* Interaction (df)**							**101.03 (102)**	**37.4 (102)**	**26.96 (102)**
CV (%)	12.2	11.0	2.0	10.9	9.3	7.9	11.0	10.3	8.8

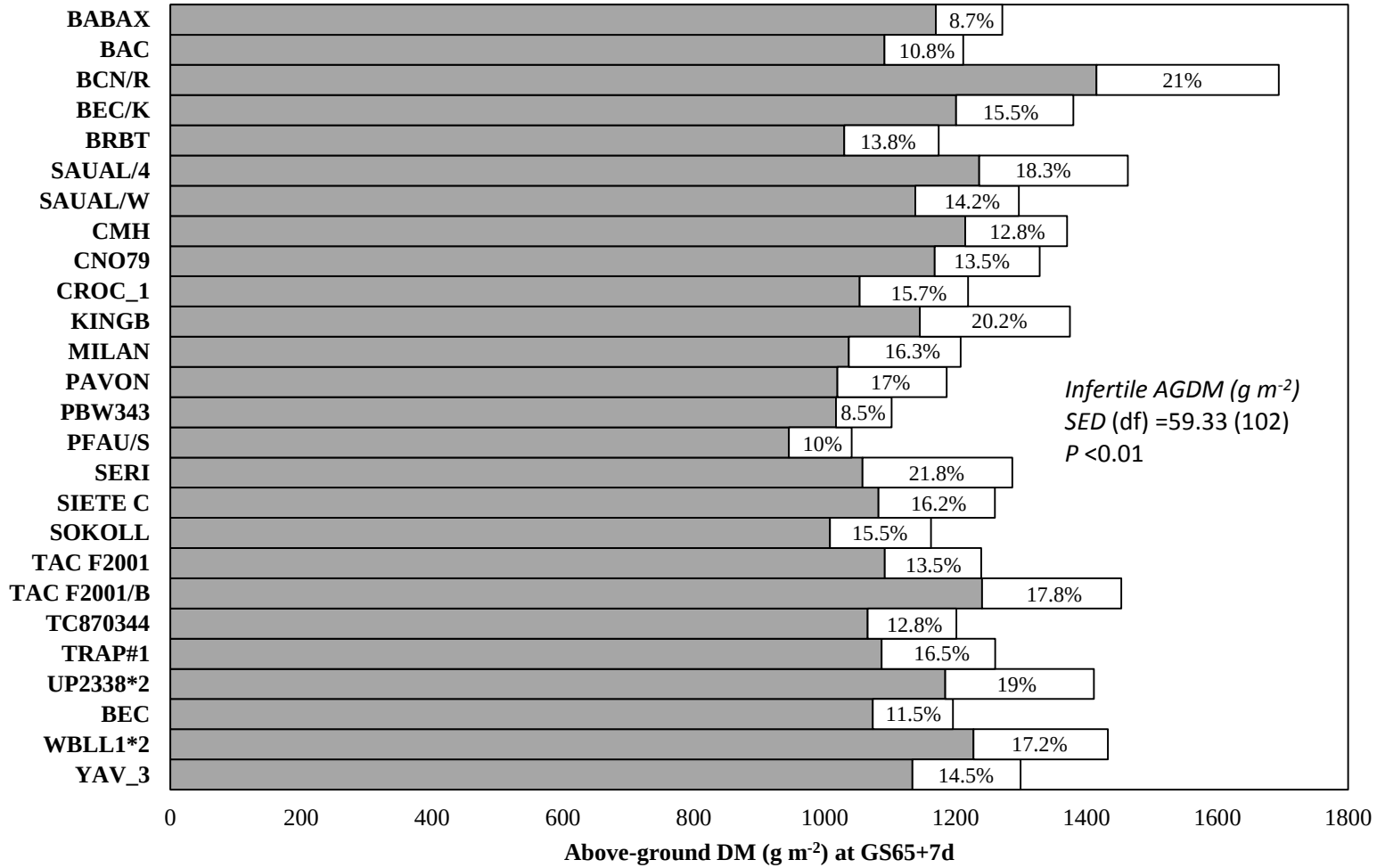


Figure 5.5. Above-ground and infertile shoot DM (g m^{-2}) at anthesis + 7 days (GS65+7d) among the 26 cultivars across the two seasons (2012 and 2013). Percentages indicate the proportion of AGDM in infertile shoots in each cultivar.

5.2.2.2 Crop growth in developmental stages (nine cultivars)

The time course for AGDM accumulation from GS31 to harvest among the nine cultivars is shown in Figure 5.6. The AGDM at GS31 differed between the cultivars in 2012 from 155.1 (Tacupeto F2001/B) to 266.2 g m^{-2} (Pavon F76) (P <0.01) and in 2013 from 169.9 (Seri M82) to 276.5 g m^{-2} (UP2338*2) (P <0.001). Averaging over years, the above-ground DM ranged from 171.1 (Seri M82) to 261.7 (UP2338*2) g m^{-2} amongst the nine cultivars (P <0.001). The AGDM did not differ between years (P =0.056) and the year x cultivar interaction was not significant.

In 2012 AGDM at GS41 ranged from 598.5 (Saual/W) to 788.0 (Cmh79a.955); however, cultivars were not statistically different for AGDM at GS31 (P =0.102). In 2013, AGDM ranged from 541.9 (Seri M82) to 726.4 (Becard/K) (P <0.01). Overall, the AGDM at GS41 ranged from 581.6 (Becard/K) to 737.9 (Cmh79a.955) g m^{-2}. AGDM at GS41 did not differ significantly between years and there was no year x cultivar interaction.

Compared to the 26 cultivars, the AGDM at GS65+7d showed also a narrower range (1019.2, Pavon F76 – 1240.3, Tacupeto F2001/B g m^{-2}; P =0.067), *cf*. 26 cultivars (945.0 – 1415.4 g m^{-2}; P <0.001). The effect of the year was not significant and there was no significant year x cultivar interaction. Overall, the crop at GS61+7d accumulated 929.5 g m^{-2} from GS31 and 498.9 g m^{-2} from GS41 among the nine cultivars.

Overall, AGDM at harvest differed among the nine cultivars (P <0.001) from 1283.4 (Seri M82) to 1513.2 (UP2338*2) g m^{-2}. The crop gained 251.6 g m^{-2} between GS65+7d and (harvest). Years differed for AGDM at harvest (P <0.001), with 91.3 g m^{-2} more biomass accumulated in 2012 than 2013; however there was no significant year x cultivar interaction. In 2012, AGDM showed a strong trend to differ between the nine cultivars (P =0.056) from 1309.7 (Seri M82) to 1565.9 g m^{-2} (UP2338*2), and in 2013 cultivars differed (P <0.05) from 1241.2 (Saual/W) to 1460.6 g m^{-2} (UP2338*2).

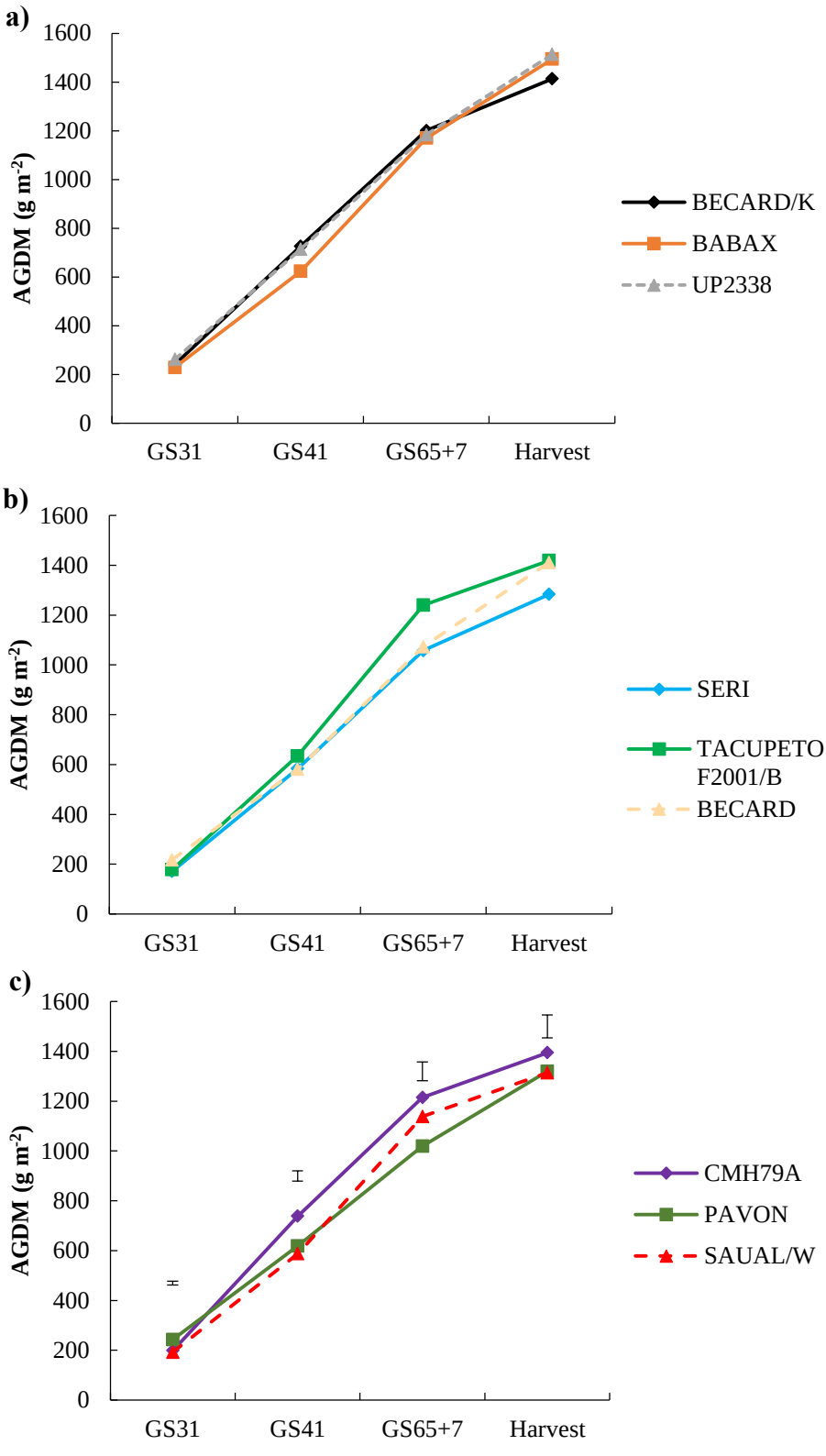


Figure 5.6 AGDM (g m^{-2}) through time (GS31, GS41, GS65+7d and harvest for the nine cultivars): a) 3 highest yielding cultivars (691 - 701 g m^{-2}), b) 3 mid-yielding cultivars (652 - 689 g m^{-2}) and c) lowest yielding cultivars (592 - 651 g m^{-2}). Error bars in c) represent SED for each stage (df = 34).

5.2.3 DM partitioning among plant organs

5.2.3.1 DM partitioning among plant organs at GS65+7d (26 cultivars)

In 2012, plant component DM at GS65+7d (Table 5.3) differed for the leaf lamina (P <0.05) and leaf-sheath (P <0.01) among the 26 cultivars; but not for spike and true-stem. However, cultivars in 2013 differed for all the plant components DM amounts (P <0.001). The genetic range for spike DM in 2012 was 242 (Sokoll//pbw343*2) to 305 (Bcn/rialto) g m^{-2} and from 195 (Sokoll//pbw343*2) to 374 (Bcn/rialto) in 2013. True-stem DM ranged from 306 (Pavon F76) to 460 (Babax/LR42) g m^{-2} in 2012 and from 300 (Pfau/Seri.1b) - 567 (Bcn/rialto) g m^{-2} in 2013. Values for lamina DM ranged from 189 (Pfau/Seri) - 316 (Bcn/rialto) g m^{-2} (Bcn/rialto) in 2012 and 157 (Brbt1*2/Kiritati) – 336 g m^{-2} (Bcn/rialto) in 2013 and leaf-sheath DM from 148 (Pavon F76) to 246 g m^{-2} (Bcn/rialto) in 2012 and from 141 (Pfau/Seri.1b) to 304 g m^{-2} (Bcn/rialto) in 2013. Year x cultivar interactions were found for all plant components (P <0.05). For reference, results for plant components DM in mg $shoot^{-1}$ are presented in Table 5.4.

Table 5.3. Plant component dry matter (g m^{-2}) at anthesis +7 days (GS65+7d) for the 26 CIMMYT spring wheat cultivars in 2012, 2013 and the overall means. Shaded rows represent the sub-set of nine cultivars. Degrees of freedom for individual years are 52.

Cultivar	2012				2013				2012-13 mean			
	Spike DM g m^{-2}	**True-stem** DM g m^{-2}	**Lamina** DM g m^{-2}	**Leaf-sheath** DM g m^{-2}	**Spike** DM g m^{-2}	**True-stem** DM g m^{-2}	**Lamina** DM g m^{-2}	**Leaf-sheath** DM g m^{-2}	**Spike** DM g m^{-2}	**True-stem** DM g m^{-2}	**Lamina** DM g m^{-2}	**Leaf-sheath** DM g m^{-2}
BABAX/LR42//BABAX/3/VORB	284.9	459.9	271.7	216.1	220.5	480.8	208.7	197.1	252.7	470.3	240.2	206.6
BACANORA T 88	301.6	376.7	242.1	191.1	254.4	431.0	196.1	189.7	278.0	403.9	219.1	190.4
BCN/RIALTO	289.2	397.4	316.2	246.4	374.4	567.4	336.1	303.6	331.8	482.4	326.1	275.0
BECARD/KACHU	284.5	393.0	257.9	222.5	296.3	494.3	241.3	211.1	290.4	443.7	249.6	216.8
BRBT1*2/KIRITATI	260.7	414.5	227.4	187.1	224.7	391.6	157.0	196.2	242.7	403.1	192.2	191.6
SAUAL/4/CROC_1	289.6	421.4	266.5	209.9	300.3	480.2	262.4	242.1	294.9	450.8	264.4	226.0
SAUAL/WHEAR//SAUAL	245.9	407.0	258.2	205.9	236.3	492.6	219.2	211.7	241.1	449.8	238.7	208.8
CMH79A.955/4/AGA/3	305.3	368.2	276.7	222.8	329.3	410.3	267.0	249.7	317.3	389.2	271.8	236.2
CNO79//PF70354/MUS/3	286.2	445.3	265.4	218.9	243.9	473.3	199.9	202.6	265.1	459.3	232.7	210.8
CROC_1/AE.SQUARROSA	260.9	426.1	245.7	207.6	213.8	412.6	168.4	171.5	237.4	419.3	207.1	189.6
KINGBIRD #1//INQALAB 91*2	269.2	406.6	269.8	183.4	275.6	445.9	253.1	187.5	272.4	426.3	261.4	185.4
MILAN/KAUZ//PRINIA/3/BAV92	283.8	417.2	281.8	188.8	195.6	377.7	179.6	149.0	239.7	397.4	230.7	168.9
PAVON F 76	267.1	305.7	249.4	147.8	261.5	405.4	222.1	179.5	264.3	355.6	235.7	163.7
PBW343*2/KUKUNA*2	270.2	337.4	222.7	163.7	228.5	443.2	192.9	175.7	249.4	390.3	207.8	169.7
PFAU/SERI.1B//AMAD/3/	267.4	384.1	189.0	170.5	223.7	300.4	158.5	140.6	256.5	363.2	181.4	163.1
SERI M 82	284.9	326.9	234.5	199.6	254.8	410.8	209.8	194.4	269.8	368.8	222.1	197.0
SIETE CERROS T66	304.9	399.9	225.4	186.1	259.5	442.7	168.0	178.2	282.2	421.3	196.7	182.1
SOKOLL//PBW343*2/KUKUNA	247.2	391.3	252.3	202.1	195.0	378.6	183.5	166.1	221.1	385.0	217.9	184.1
TACUPETO F2001/SAUAL/4/	242.9	352.5	236.7	185.6	246.3	518.1	206.4	194.6	244.6	435.3	221.5	190.1
TACUPETO F2001/BRAMBLING*2	283.1	377.7	231.9	190.4	327.6	525.9	276.0	268.0	305.3	451.8	254.0	229.2
TC870344/GUI//TEMPORALERA	245.2	396.7	226.5	186.4	226.2	468.9	188.9	192.6	235.7	432.8	207.7	189.5
TRAP#1/BOW/3/VEE/PJN	241.8	381.8	207.7	181.3	263.5	504.3	196.4	196.9	252.7	443.0	202.1	189.1
UP2338*2/4/SNI/TRAP#1/3	281.5	439.5	281.9	230.6	242.3	482.8	208.4	201.1	261.9	461.2	245.1	215.8
BECARD	271.2	386.1	214.8	174.5	243.9	490.0	195.2	170.9	257.6	438.0	205.0	172.7
WBLL1*2/KURUKU*2/5/REH	277.7	401.9	262.8	188.4	314.9	543.2	243.3	222.2	296.3	472.6	253.1	205.3
YAV_3/SCO//JO69/CRA/3	303.4	440.3	246.9	218.7	243.3	462.2	170.5	182.1	273.4	451.3	208.7	200.4
Mean	**275.0**	**394.4**	**248.5**	**197.2**	**257.5**	**455.2**	**211.9**	**199.0**	**266.7**	**425.6**	**230.5**	**198.4**
SED Cultivar (df)	30.14	42.67	26.68	19.17	25.17	46.67	23.8	23.22	19.9 (102)	31.8 (102)	17.8 (102)	14.96 (102)
SED Year (df)									**5.5 (1)**	**8.8 (1)**	**4.9 (1)**	**4.2 (1)**
SED Interaction (df)									**28.1 (102)**	**44.95 (102)**	**25.2 (102)**	**21.2 (102)**
CV (%)	13.4	13.3	13.1	1.6	12.0	12.8	13.8	1.8	12.9	13.0	13.4	13.1

Table 5.4. Plant components dry matter per shoot (mg) at anthesis +7 days (GS65+7d) for the 26 CIMMYT spring wheat cultivars in 2012, 2013 and the overall means. Shaded rows represent the sub-set of nine cultivars. Degrees of freedom for individual years are 52.

Cultivars	2012				2013				2012-13 mean			
	Spike	**True-stem**	**Lamina**	**Leaf-sheath**	**Spike**	**True-stem**	**Lamina**	**Leaf-sheath**	**Spike**	**True-stem**	**Lamina**	**Leaf-sheath**
	mg	Mg	Mg	Mg	Mg	mg	mg	mg	mg	Mg	mg	mg
BABAX/LR42//BABAX/3/VORB	829	1337	789	625	659	1433	621	588	744	1385	705	606
BACANORA T 88	815	1018	654	517	663	1115	503	491	739	1066	579	504
BCN/RIALTO	679	928	734	574	669	1015	599	542	674	971	667	558
BECARD/KACHU	851	1172	771	672	881	1480	725	638	866	1326	748	655
BRBT1*2/KIRITATI	878	1398	768	631	914	1582	639	806	896	1490	703	718
SAUAL/4/CROC_1	1037	1507	952	751	907	1453	796	736	972	1480	874	744
SAUAL/WHEAR//SAUAL	762	1268	802	642	717	1489	666	639	740	1379	734	641
CMH79A.955/4/AGA/3	1142	1388	1008	832	952	1185	753	694	1047	1286	881	763
CNO79//PF70354/MUS/3	953	1482	884	730	883	1716	720	733	918	1599	802	732
CROC_1/AE.SQUARROSA	771	1259	725	613	673	1298	530	539	722	1278	627	576
KINGBIRD #1//INQALAB 91*2	771	1172	770	525	692	1121	636	471	731	1146	703	498
MILAN/KAUZ//PRINIA/3/BAV92	903	1321	904	607	790	1528	727	602	846	1425	816	604
PAVON F 76	719	814	658	402	621	966	528	426	670	890	593	414
PBW343*2/KUKUNA*2	826	1025	677	499	670	1300	565	515	748	1163	621	507
PFAU/SERI.1B//AMAD/3/	896	1285	632	571	564	757	407	354	813	1153	542	517
SERI M 82	916	1053	743	636	721	1162	596	549	819	1107	670	592
SIETE CERROS T66	934	1222	689	570	844	1446	544	580	889	1334	617	575
SOKOLL//PBW343*2/KUKUNA	704	1117	715	574	692	1335	649	590	698	1226	682	582
TACUPETO F2001/SAUAL/4/	737	1057	706	558	778	1650	650	619	757	1353	678	588
TACUPETO F2001/BRAMBLING*2	1004	1333	814	670	770	1253	646	629	887	1293	730	649
TC870344/GUI//TEMPORALERA	779	1261	720	593	767	1580	634	649	773	1420	677	621
TRAP#1/BOW/3/VEE/PJN	820	1290	699	612	820	1577	609	616	820	1434	654	614
UP2338*2/4/SNI/TRAP#1/3	718	1121	713	584	734	1467	632	609	726	1294	673	596
BECARD	825	1174	654	531	698	1406	559	488	762	1290	606	509
WBLL1*2/KURUKU*2/5/REH	1088	1576	1027	742	943	1625	724	664	1016	1600	875	703
YAV_3/SCO//JO69/CRA/3	1029	1497	832	743	842	1605	601	631	936	1551	717	687
Mean	**861**	**1234**	**771**	**615**	**764**	**1367**	**625**	**592**	**816**	**1305**	**699**	**606**
SED Cultivar (df)	113.3	146.3	52.13	72.6	74.2	143.7	52.0	61.81	68.12 (102)	102.7 (102)	37.5 (102)	48.07 (102)
SED Year (df)									**18.9 (1)**	**28.5 (1)**	**10.4 (1)**	**13.33 (1)**
SED Interaction (df)									**96.34 (102)**	**145.2 (102)**	**53.04 (102)**	**67.99 (102)**
CV (%)	16.1	14.5	8.3	1.3	11.9	12.9	10.2	4.0	14.5	13.7	9.3	13.8

Dry-matter partitioning at GS65+7d differed amongst the 26 cultivars in the two years for all plant components (Figure 5.7). The SPI averaged over years ranged from 0.199 (Babax/LR42) - 0.272 (Pfau/Seri.1b) (P <0.001). Moreover, the true-stem PI ranged from 0.328 (Cmh79a.955) - 0.446 (Tacupeto F2001) (P <0.001) and there was a year x cultivar interaction (P <0.001). Cultivars differed in the lamina PI from 0.160 (Siete cerros) - 0.217 (Kingbird#1) (P <0.001) and in leaf-sheath PI from 0.155 (Becard) - 0.204 (Brbt1*2/kiritati) (P <0.01). The year x cultivar interaction was not significant for either lamina (P =0.536) or leaf-sheath PI (P =0.827). In 2012, SPI ranged from 0.212 (Sokoll//pbw343*2) - 0.266 (Croc_1/ae.squarrosa) and in 2013 from 0.220 (Saual/whear) - 0.276 (Pavon F76). The true-stem PI ranged from 0.321 (Babax/LR42) - 0.407 (Yav_3/sco) in 2012 and from 0.313 (Seri M82) - 0.380 (Brbt1*2/kiritati) in 2013. The lamina PI ranged from 0.181 (Pfau/seri.1b) - 0.232 (Bacanora T88) in 2012; and from 0.187 (Pfau/seri.1b) - 0.256 (Pavon F76) in 2013. Finally, leaf-sheath PI ranged from 0.161 (Yav_sco) - 0.194 (Babax/LR42) in 2012 and from 0.153 (Pavon F76) - 0.197 (Bcn/rialto) in 2013.

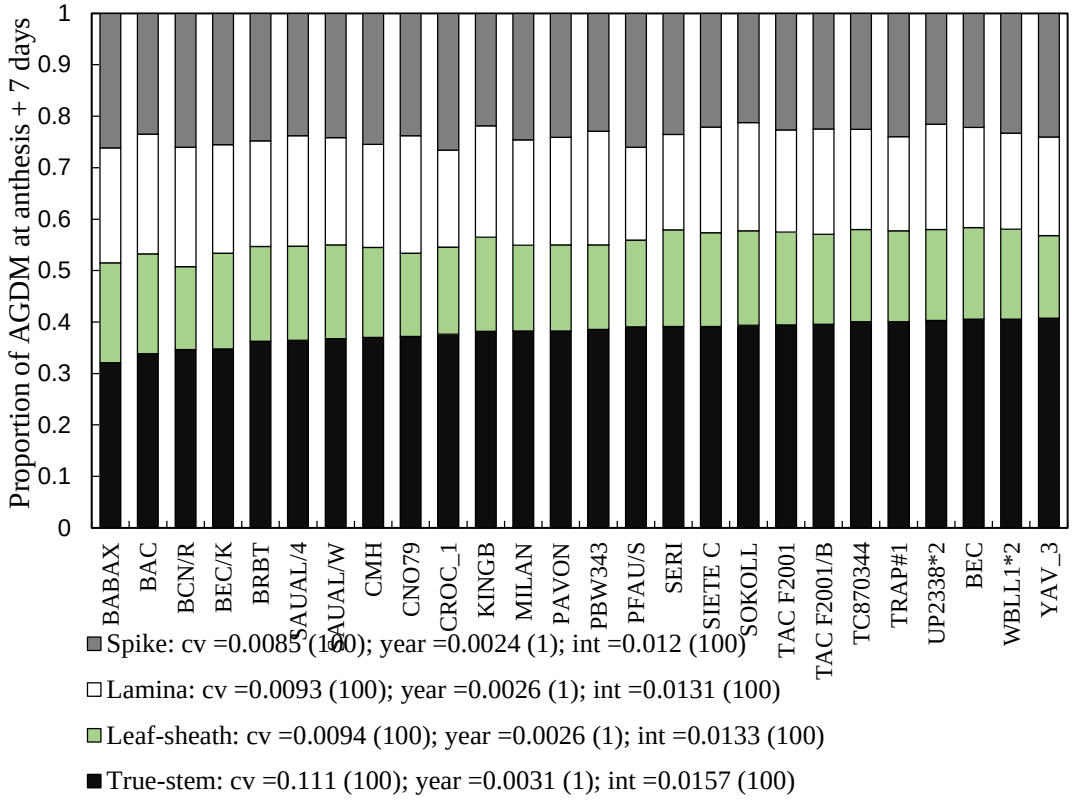


Figure 5.7. Plant components DM partitioning (proportion of shoot AGDM in fertile shoots) at anthesis +7 days (GS65+7d) for the 26 CIMMYT spring wheat cultivars (overall means from 2012 and 2013). SED (df) for cultivars, year and year x cultivar interaction.

5.2.3.2 DM partitioning among plant organs through time in the subset of nine cultivars

Overall, lamina PI and leaf-sheath PI at GS31 ranged from 0.676 (Saual/Whear) to 0.722 (Becard/Kachu) and 0.278 (Becard/Kachu) to 0.324 (Saual/Whear), respectively among the nine cultivars (P <0.05). Leaf-sheath PI at GS31 showed differences between seasons (P <0.001) but no significant year x cultivars interactions. Averaging across seasons at GS41, the nine cultivars differed in lamina PI in the range 0.441 (Seri M82) – 0.516 (Pavon F76) (P <0.001). The true-stem PI and leaf-sheath PI at GS41 were only measured separately in 2012, ranging from 0.166 (Pavon F76) – 0.235 (Saual/Whear) for the true-stem PI and from 0.280 (Saual/Whear) – 0.344 (Seri M82) for the leaf-sheath PI but not differing between cultivars (Figure 5.8 a). There was a year effect for lamina PI at GS31 and at GS41 (P <0.001), but no significant year x cultivar interactions were observed.

Overall, DM partitioning at GS65+7d among the nine cultivars showed similar ranges to those observed for the 26 cultivars (Figure 5.8 a), achieving a wide range of DM partitioning values within a narrow range of anthesis date and relatively narrow range of plant height. There were no significant year x cultivar interactions for the different DM partitioning indices at GS65+7d.

Harvest DM partitioning for the nine cultivars was measured only in 2013 and differed between cultivars for all the plant components. The lamina PI ranged from 0.091 (Becard) to 0.116 (Saual/Whear) and leaf-sheath PI from 0.09 (Becard) to 0.104 (Cmh79a.955) (P <0.05). The true-stem PI ranged from 0.196 (Cmh79a.955) to 0.268 (Babax) (P <0.001) (Figure 5.8a). The spike DM at harvest was dissected into its morphological components in 2012 and 2013 in seven of the nine cultivars, differing for each spike structural DM partitioning (i.e. awns, lemmas, paleas, rachis and glumes; Table 5.5). The awns accounted for most of the DM allocated to the spike morphological components at harvest (223 mg spike^{-1}, 26%; P <0.001). The lemmas accounted for 201 mg spike^{-1} (23.7%) P <0.01) followed by the glumes (174 mg spike^{-1}, 20.4%; P <0.001) and the rachis (147 mg spike^{-1}, 17.1%; P <0.001). Finally, the paleas had the least spike DM at harvest (110 mg spike^{-1}; P <0.01) and accounted for 12.8% of the spike DM (P <0.05). There was an effect of year for the spike component DM amounts (P

<0.01); however, only the awns, lemmas and paleas DM proportions of spike differed between years. No cultivar x year interaction was shown for the spike components DM or partitioning.

Overall for the two seasons of GS31, DM in g m^{-2} differed for lamina and leaf-sheath, averaging 149 and 65.7 g m^{-2} (P <0.001), respectively. At GS41, the nine cultivars also differed in lamina DM (302.3g m^{-2}, P <0.001) across the two seasons. In 2012, the true-stem and leaf-sheath DM averaged 132.6 (P <0.05) and 196.4 (P <0.001) g m^{-2}, respectively. Averaging across seasons at GS65+7d, DM differed for all the plant components among the nine cultivars (P <0.01) except for the lamina (P =0.085), showing mean values for the spike, lamina, true-stem and leaf-sheath of 273.4, 240.3, 425.4 and 205.2 g m^{-2}, respectively (Figure 5.8 b). Years differed for lamina and leaf-sheath DM at GS31 (P <0.001). No significant year x cultivars interactions were observed for the plant components DM in any stage.

At harvest in 2013, the true-stem showed the greatest DM amount after the spike, averaging 326.1 g m^{-2} among the nine cultivars (P <0.01). Lamina DM averaged 143.7 g m^{-2} (P <0.001) and the leaf-sheath 136.0 g m^{-2} (P <0.01) (Figure 5.8 b). At harvest, chaff DM (spike structural components) ranged from 227 to 148 g m^{-2} among the nine cultivars (P <0.05) (data not shown).

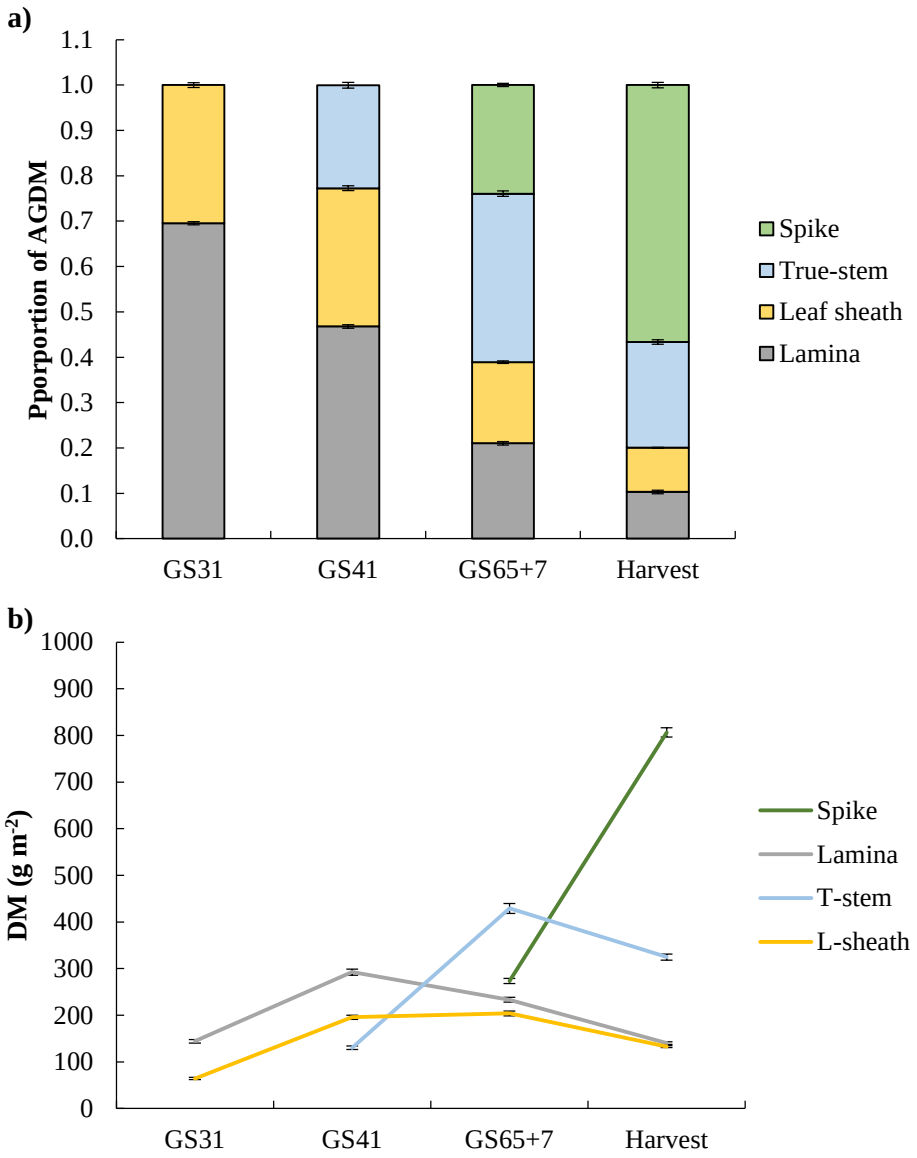


Figure 5.8. a) Percentage of AGDM in fertile shoots (%) and b) AGDM in fertile shoots (g m^{-2}) through time (GS31 (df =34), GS41 (df =16), GS65+7d (df =34) and harvest (df =16)) for spike, lamina, true-stem and leaf-sheath. Mean of 2012 and 2013 for the nine cultivars. Error bars represent the standard error of the mean.

Table 5.5. Spike morphological units at harvest (DM per spike and DM partitioning within the spike, as 100% of DM without grains). Values in each year for each cultivar for rachis (Rac), glumes (Glum), Lemmas (Lem), Paleas (Pal) and Awns (Aw).

Cultivar	Rac	Glum	Lem	Pal	Aw	Rac	Glum	Lem	Pal	Aw
	DM (mg spike^{-1})					(% of DM spike without grains)				
2012										
BABAX/LR42	171	210	200	121	269	17.7	21.6	20.5	12.5	27.3
BECARD/K	133	170	189	116	236	15.7	20.0	22.7	13.5	27.7
SAUAL/W	183	204	223	139	238	18.5	20.7	22.5	14.1	23.8
CMH79A.955	202	247	251	148	311	17.5	21.4	21.6	12.8	26.4
PAVON F76	148	169	217	142	265	15.7	18.0	23.1	15.1	27.8
SERI M82	147	177	191	142	268	15.9	19.2	20.7	15.3	28.6
UP2338*24	162	200	239	146	206	17.0	21.0	25.1	15.3	21.3
Mean	**164**	**197**	**216**	**136**	**256**	**16.9**	**20.3**	**22.3**	**14.1**	**26.5**
Max	**202**	**247**	**251**	**148**	**311**	**18.5**	**21.6**	**25.1**	**15.3**	**28.6**
Min	**147**	**170**	**189**	**116**	**206**	**15.7**	**18.0**	**20.5**	**12.5**	**21.3**
2013										
BABAX/LR42	120	138	157	58	175	18.5	21.3	24.2	9.1	26.9
BECARD/K	113	145	169	77	186	17.3	21.9	23.5	10.9	26.5
SAUAL/W	159	193	225	105	192	18.2	22.1	25.7	11.8	22.1
CMH79A.955	176	198	224	110	271	18.0	20.3	22.9	11.3	27.5
PAVON F76	97	107	154	73	167	16.1	18.1	25.8	12.1	27.9
SERI M82	121	144	189	92	189	16.6	19.6	25.6	12.5	25.8
UP2338*24	113	140	186	77	142	17.3	21.3	28.2	11.5	21.7
Mean	**129**	**152**	**186**	**85**	**189**	**17.4**	**20.6**	**25.2**	**11.3**	**25.4**
Max	**176**	**198**	**225**	**110**	**271**	**18.5**	**22.1**	**28.2**	**12.5**	**27.9**
Min	**97**	**107**	**154**	**58**	**142**	**16.1**	**18.1**	**22.9**	**9.1**	**21.7**
Combined ANOVA										
Overall mean	147	174	201	110	223	17.1	20.4	23.7	12.8	26.0
SED cv (df =26)	9.5	10.1	15.7	10.2	18.1	0.549	0.682	0.813	0.830	1.07
SED year (df =1)	**5.1**	**5.4**	**8.4**	**5.5**	**9.7**	**0.293**	**0.364**	**0.435**	**0.444**	**0.570**
SED interaction (df =26)	**13.5**	**14.3**	**22.1**	**11.4**	**25.7**	**0.776**	**0.964**	**1.15**	**1.17**	**1.51**
CV (%)	11.3	10.0	13.5	16.0	14.1	5.6	5.8	5.9	11.3	7.1

5.2.3.3 Associations between plant component DM partitioning indices at GS65+7d

Relationships between the spike and the other plant organs at GS65+7d on a per unit area basis and proportional basis are shown in Figure 5.9 (a-c for g m^{-2} and d-f for DM partitioning indices) for the 26 cultivars. Averaging across seasons, there was no significant association between spike and true-stem DM but positive linear associations with lamina DM (R^2 =0.51, P <0.001) and leaf-sheath DM (R^2 =0.51, P <0.001) amongst the 26 cultivars (Figure 5.9 c).

There was a negative linear association between SPI and the true-stem PI in 2012 (R^2 =0.22, P <0.05; Figure 5.9 d) and 2013 (R^2 = 0.56, P <0.001; Figure 5.9 e) and for the overall mean (R^2 =0.35, P <0.01; Figure 5.9 c). In 2012, there was a negative linear association between SPI and the lamina PI (R^2 =0.17; P <0.05)

amongst the 26 cultivars, but this association was not found in 2013 or for the cross-year mean. No relationships were found between the SPI and the leaf-sheath PI in either year or averaging over years.

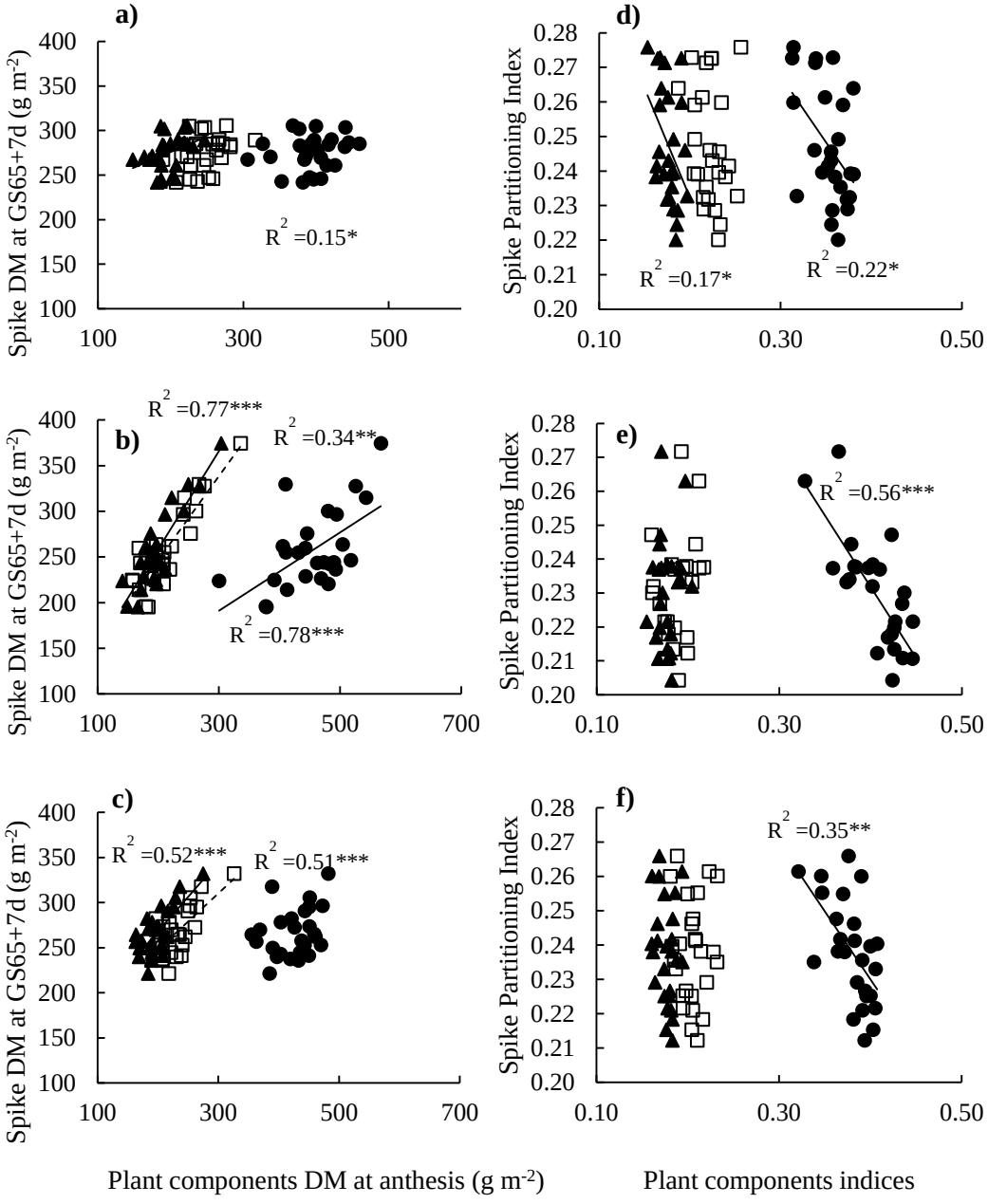


Figure 5.9. Linear regression of spike DM (g m^{-2}) on plant components DM (g m^{-2}) at anthesis +7 days (GS65+7d) (left hand side in **a)** 2012 (leaf-sheath (▲): y =0.345x+207, R^2 =0.15, *P* <0.05), **b)** 2013 (true-stem (●): y =0.429x+62.27; lamina (□): y =0.918x+62.96; leaf-sheath (▲): y =1.072x+44.27) and **c)** overall mean (lamina (□): y =0.624x+122.9; leaf-sheath (▲): y =0.77x+133.76) **and** of SPI on plant component PI (right hand side) in **d)** 2012 (true-stem (●): y =-0.383x+0.383 and leaf-sheath (▲): y =-0.635x+0.359), **e)** 2013 (true-stem (●): y =-0.421x+0.4) and **f)** overall mean (true-stem (●): y =-0.395x+0.388); among the 26 cultivars.

5.2.4 Soluble and structural DM partitioning among plant organs at GS65+7d

5.2.4.1 True-stem WSC in the 26 cultivars at GS65+7d

True-stem water soluble carbohydrate concentration (WSC_C) (%; Figure 5.10 a) was measured at GS65+7d for the 26 cultivars and the WSC amount (WSC_A) (g m^{-2}; Figure 5.10 b) was calculated. In 2012, true-stem WSC_C ranged from 5.4 to 24.0 % amongst the 26 cultivars (P <0.001) and WSC_A from 20.1 to 90.3 g m^{-2} (P <0.05). In 2013, true-stem WSC_C and WSC_A were higher, ranging from 12.6 to 29.2 % and from 31 to 172 g m^{-2} (P <0.001), respectively. Overall, true-stem WSC concentrations and amounts averaged 15.1% (10.5 – 26.6%) and 63.7 g m^{-2} (39.7 – 131.1 g m^{-2}) (P <0.001); differing between years (P <0.001) and showing a year x cultivar interaction (P <0.05).

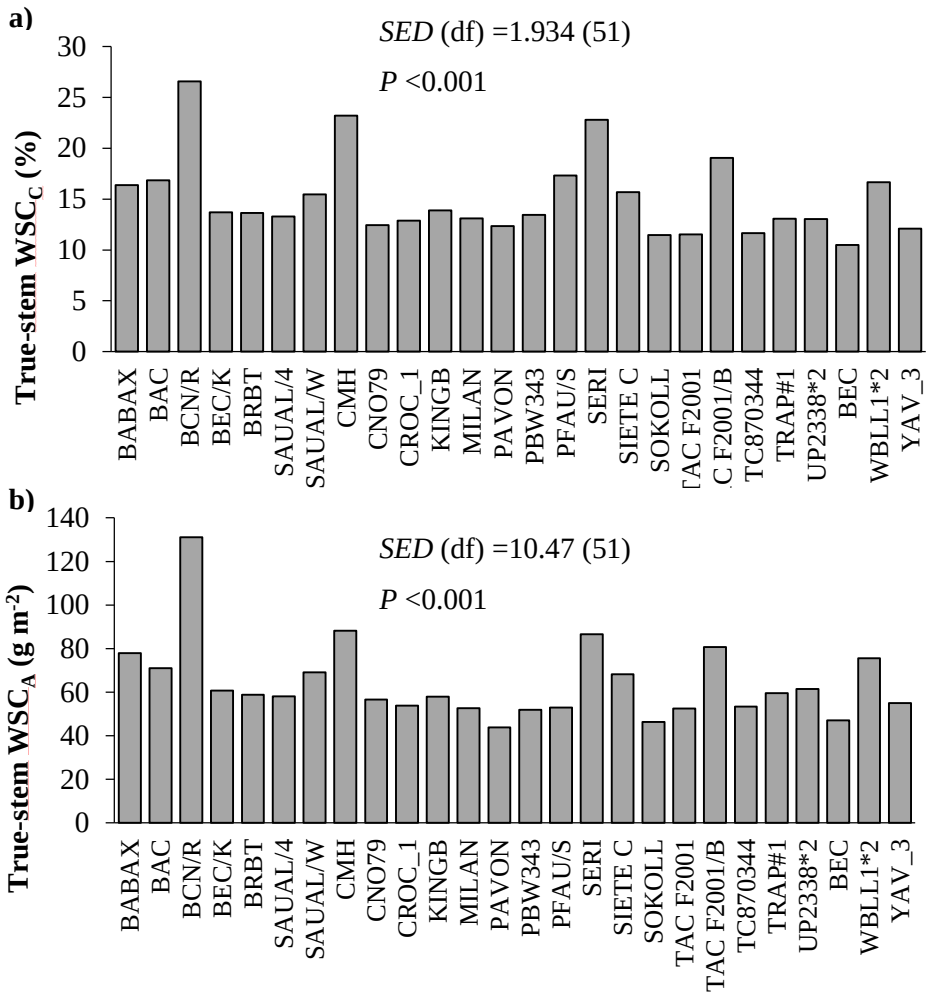


Figure 5.10. True-stem WSC a) concentrations (WSC_C, %) and b) amounts (WSC_A, g m^{-2}) at anthesis + 7 days (GS65+7d) for the 26 CIMMYT cultivars, means of 2012 and 2013.

5.2.4.2 Associations between true-stem WSC_C and WSC_A and t-stem DM

In 2012, differences amongst the 26 cultivars in t-stem WSCA at GS65+7d were mostly explained by true-stem WSCC (R2 =0.89, P <0.001; Figure 5.11 d) rather than t-stem DM (g m-2) (Figure 5.11 a). However, in 2013, t-stem WSCA (g m-2) was explained by both true-stem DM (R2 = 0.30, P <0.01; Figure 5.11 b) and true- Averaging across seasons, true-stem WSCA amounts were positively and linearly associated with true-stem WSCC (R2 =0.77, P <0.001) and a weaker positive relationship was observed between true-stem WSCA and true-stem DM (R2 =0.15, P <0.05). stem WSCC (R2 =0.59, P <0.001; Figure 5.11 e), by positive linear associations.

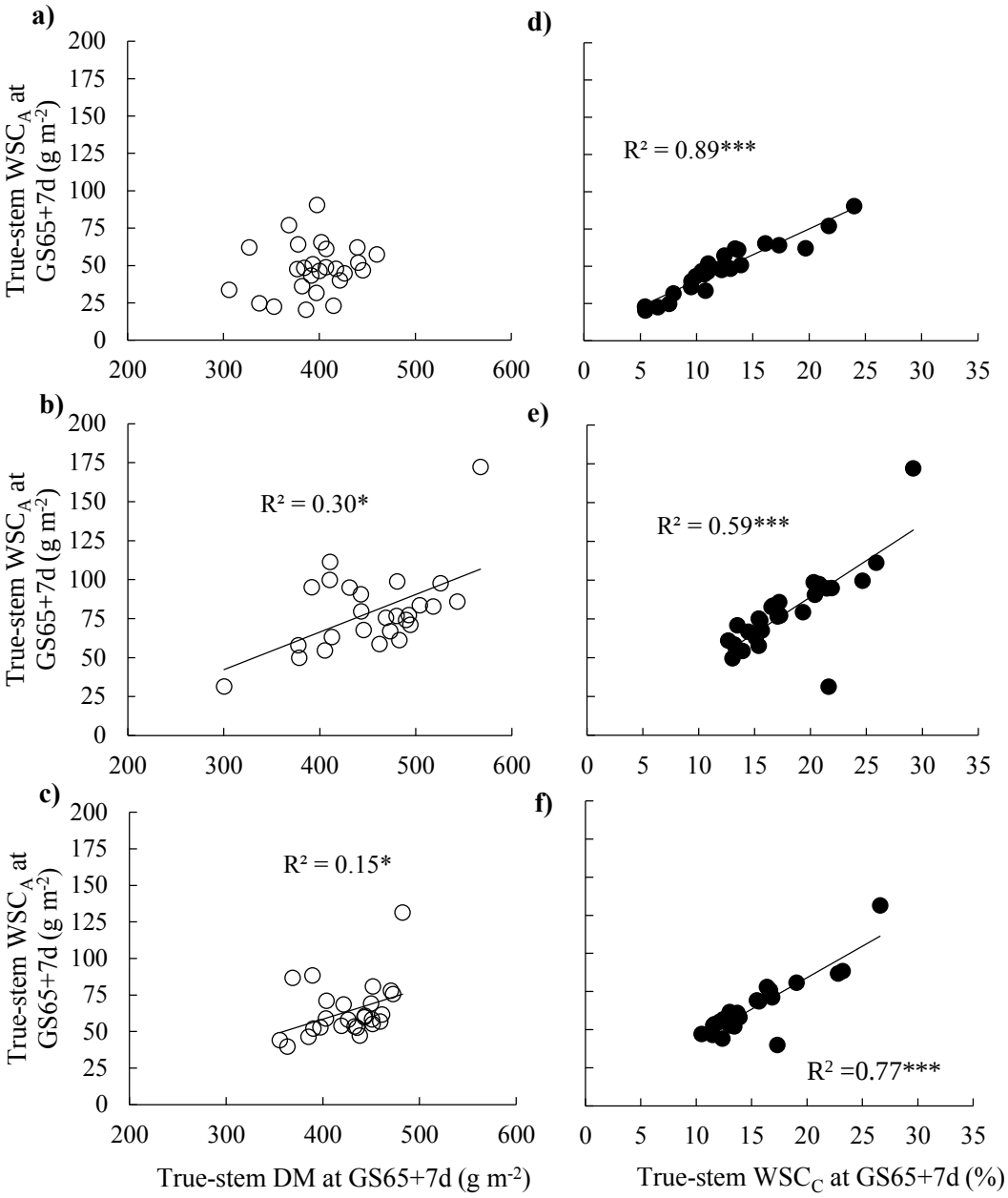


Figure 5.11. Regressions of true-stem WSC amounts (WSC_A, g m^{-2}) on true-stem total DM at GS65+7d: **a)** 2012; **b)** 2013 (y =0.242x-30.27) and **c)** overall mean (y =0.208x-24.56) **and** on true-stem WSC concentrations (WSC_C, %) at GS65+7d: **d)** 2012 (y =3.482x+5.396); **e)** 2013 (y =4.703x-4.9) and **f)** overall mean (y =4.127x+1.49) at anthesis + 7 days (GS65+7d) amongst the 26 cultivars.

5.2.4.3 WSC_C and WSC_A in the different plant organs at GS65+7d (nine cultivars)

WSC_C and WSC_A in the true-stem, spike and leaf sheaths (Table 5.6) differed amongst the nine cultivars in individual years and averaged across seasons; the highest accumulation overall was in the true-stem (16.3%, P <0.001 and 68.4 g m^{-}

^{2}, P <0.05; respectively). WSC_C for plant components ranged among the nine cultivars from 10.5 (Becard) to 23.2% (Cmh79a.955) in the true-stem, from 10.7 (Pavon F76) to 18.9% (Babax/LR42) in the spike (P <0.05) and from 7.8 (Becard) to 14.5% (Seri M82) in the leaf-sheath (P <0.01). Year x cultivar interactions were not observed for WSC_C amongst the nine cultivars.

Cultivar differences were also shown for WSC_A for all the plant components; ranging from 43.9 (Pavon F76) to 88.2 (Cmh79a.955) g m^{-2} for the true-stem (P <0.05); from 28.3 (Pavon F76) to 53.9 (Babax/LR42) g m^{-2} for the spike (P <0.05) and from 12.9 (Pavon F76) to 28.4 g (Seri M82) m^{-2} for the leaf-sheath (P <0.001). There were no significant year x cultivar interactions for WSC_A (cultivar rankings were similar within seasons).

Table 5.6. Water soluble carbohydrates (WSC) concentration (WSC_C; %) and amount (WSC_A; g m^{-2}) in the spike, true-stem and leaf-sheath at anthesis + 7 days (GS65+7d). Overall means in 2012 and 2013 for the nine spring wheat CIMMYT cultivars.

Cultivar	Shoot DM	WSC_C (%)			AGDM	WSC_A (g m^{-2})		
	mg $shoot^{-1}$	Spike	T. Stem	L. Sheath	g m^{-2}	Spike	T. Stem	L. Sheath
BABAX/LR42	3440	18.9	16.4	9.9	1169.8	53.9	77.9	20.9
BECARD/KACHU	3595	14.4	13.7	8.4	1200.5	40.4	60.7	18.6
SAUAL/WHEAR	3493	13.7	15.5	9.3	1138.4	35.4	69.0	19.4
CMH79A.955	3977	11.7	23.2	11.8	1214.6	33.1	88.2	27.2
PAVON F 76	2566	10.7	12.4	8.0	1019.2	28.3	43.9	12.9
SERI M 82	3188	18.3	22.8	14.5	1057.8	50.4	86.5	28.4
TAC F2001/B	3559	12.6	19.0	11.5	1240.3	35.2	80.6	25.1
UP2338*2	3289	11.5	13.0	8.5	1184.1	34.1	61.4	18.3
BECARD	3167	11.5	10.5	7.8	1073.3	29.9	47.0	13.4
Mean	3364	13.7	16.3	9.96	1144.2	37.9	68.4	20.5
SED Cultivar (df)	241.2(34)	2.213(8)	2.031(17)	0.965(34)	75.9(34)	8.07(8)	13.14(17)	3.271(34)
SED Year (df)	**113.7(1)**	**-**	**0.957(1)**	**0.455(1)**	**35.8(1)**	**-**	**6.19(1)**	**1.542(1)**
SED Interaction (df)	**341.1(34)**	**-**	**2.872(17)**	**1.365(34)**	**107.3(34)**	**-**	**18.58(17)**	**4.626(34)**
CV (%)	12.4	16.1	17.6	16.8	11.5	21.3	0.9	2.0

5.2.4.4 Soluble and structural (non-soluble) DM partitioning among plant organs (true-stem, sheaths and spike)

The soluble DM (WSC) partitioned to plant components as a proportion of AGDM differed between the nine cultivars for most components, with the exception of the spike at GS65+7d (

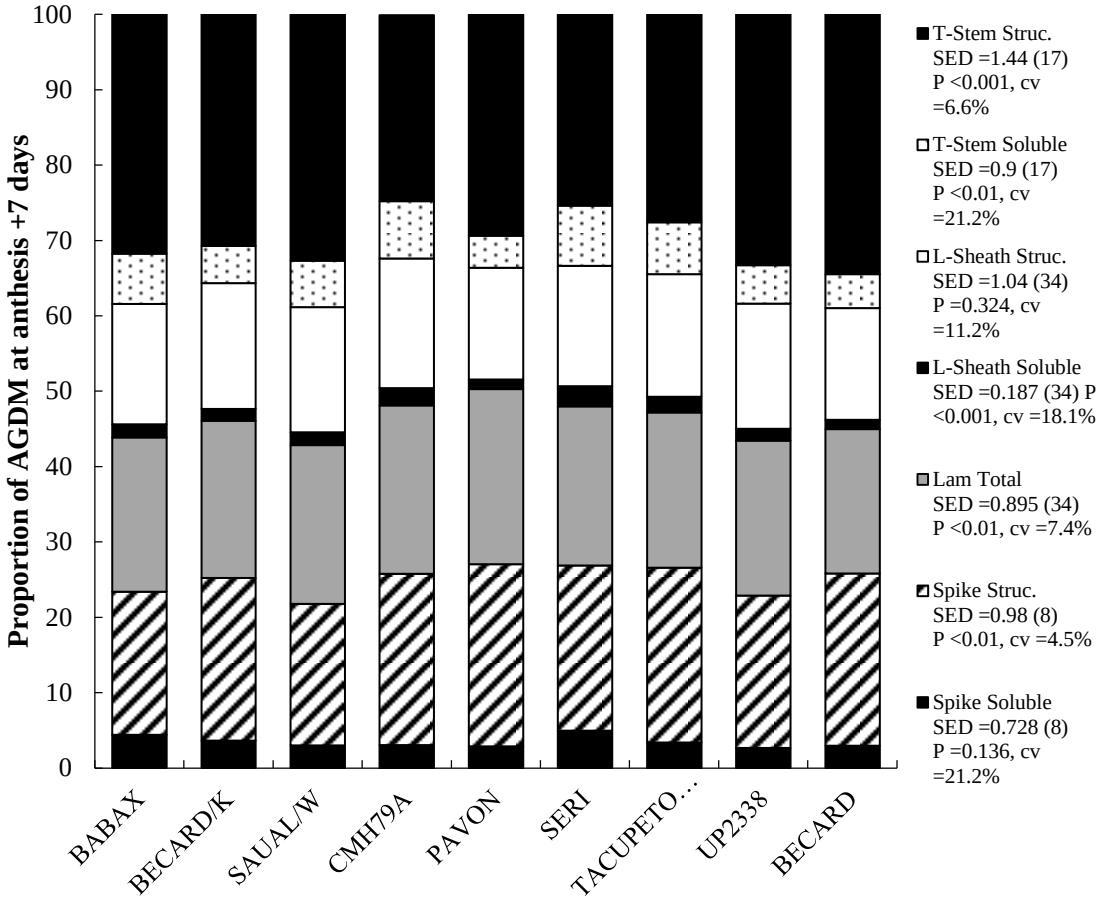


Figure 5.12). On the other hand, the structural DM partitioned to the components showed no differences between cultivars except for the leaf-sheath. The amount of soluble spike DM did not differ between the nine cultivars in 2012 (P =0.136). The amount of soluble and structural true-stem DM was as expected much higher than in the other plant components (P <0.01 and P <0.001, respectively). The amount of leaf-sheath soluble DM showed significant differences between the nine cultivars (P <0.001), but cultivars did not differ in the amount of structural DM in the leaf sheath (P =0.324). Analysis for WSC was not carried out for the leaf lamina in any season; for that reason, for the lamina, the total DM (soluble and structural) is shown in Figure 5.13.

Associations between the SPI and the structural partitioning indices at GS65+7d amongst the nine cultivars showed a negative linear relationship with the true-stem structural PI (R^2 =0.50; P <0.05). No association was found between the SPI and the leaf-sheath structural PI (Figure 5.13).

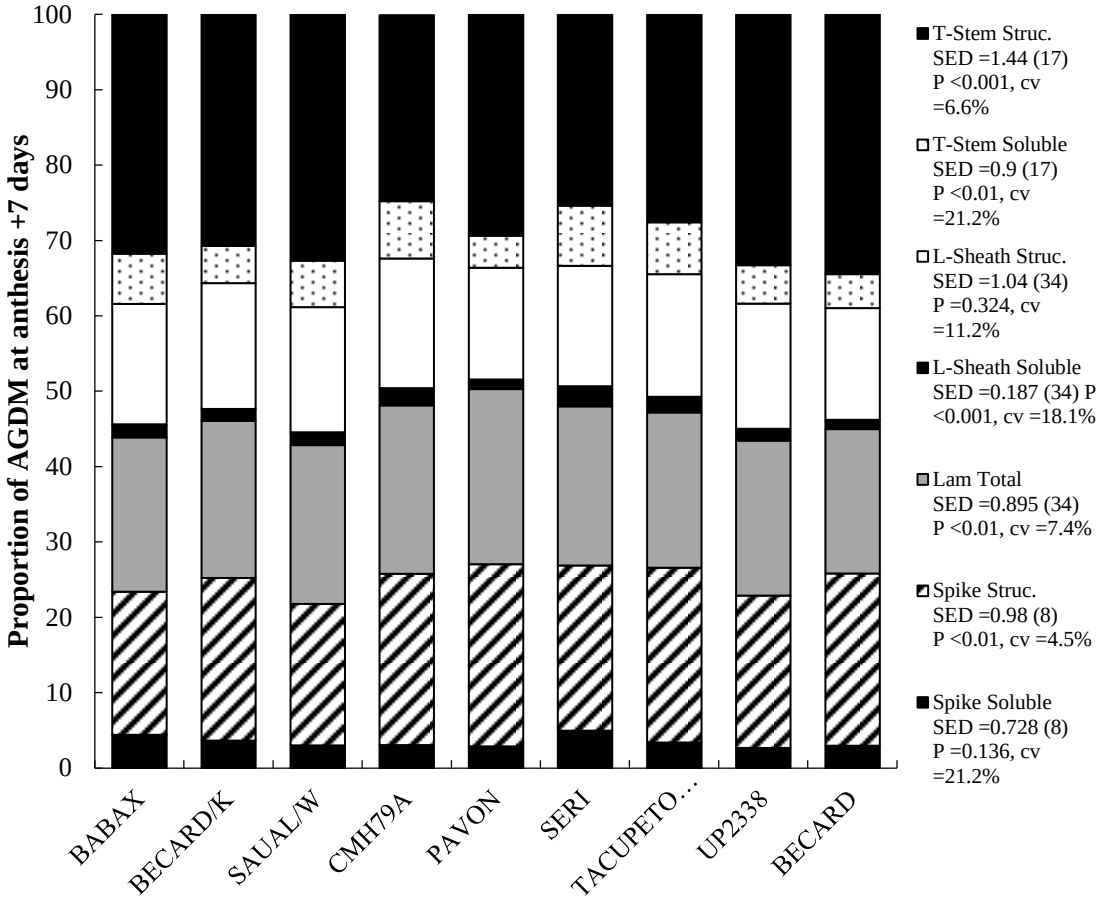


Figure 5.12. Soluble and structural DM as a proportion of total AGDM for each plant component and cultivar at anthesis + 7 days (GS65+7d), means of 2012 and 2013.

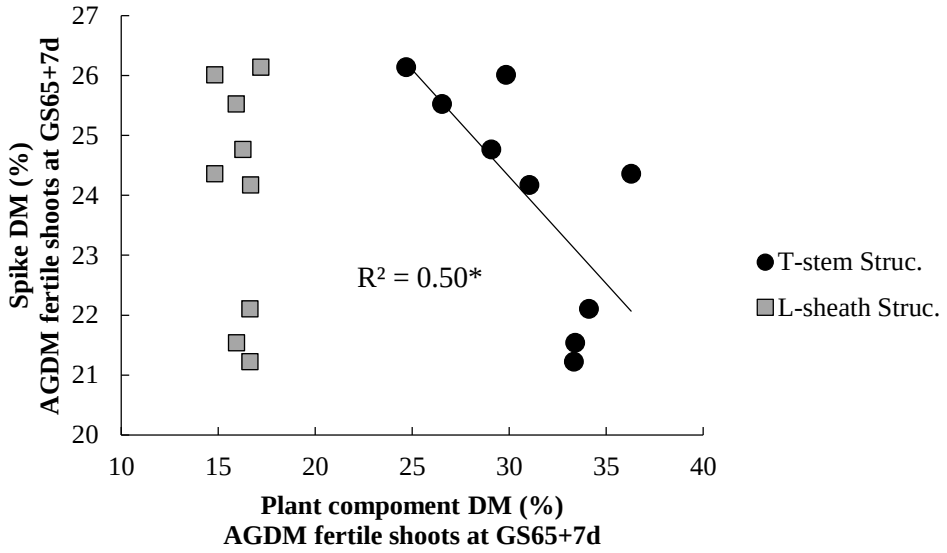


Figure 5.13. Regression of spike partitioning index on true-stem (y =-0.355x+0.35) and leaf-sheath structural DM as a proportion of total AGDM at anthesis + 7 days (GS65+7d) among the nine cultivars (values represent means of 2012 and 2013).

5.2.5 Associations between soluble and structural DM at anthesis and pre-anthesis traits

5.2.5.1 Correlations between soluble and structural DM at anthesis and key pre-anthesis traits

Water soluble carbohydrate concentrations and amounts in the true-stem and leaf-sheath were strongly and positively associated with days from GS31 (start of stem elongation) to GS65 (anthesis) (Table 5.7). True-stem PI was negatively associated with true-stem WSC_C (r = -0.64, P <0.05) but not with leaf-sheath WSC_C. Leaf-sheath PI was strongly and positively associated with true-stem and leaf-sheath WSC_C and WSC_A. The shoot number m^{-2} at anthesis was negatively associated with true-stem WSC_A (r = -0.64, P <0.05) and WSC_C (r = -0.77, P <0.05) and with leaf-sheath WSC_A (r = -0.69, P <0.05). No significant association was found between plant component soluble DM amounts or concentrations and SPI, plant height, anthesis date or crop growth rate (CGR; GS31-65+7d; g m^{-2} d^{-1}).

Table 5.7. Phenotypic correlations between WSC concentrations (WSC_C, %) and amounts (WSC_A, g m^{-2}) at anthesis +7 days (GS65+7d) in the plant organs and pre-anthesis traits, shoot number and plant height for the subset of nine cultivars, based on mean values in 2012 and 2013.

	Spike	**True-stem**	**Leaf-sheath**	**Spike**	**True-stem**	**Leaf-sheath**
	WSC concentration (WSCc)			WSC amounts (WSC_a)		
Anthesis date	-0.10	0.56	0.33	0.01	0.51	0.55
CGR (GS31-65+7)	0.23	-0.11	-0.12	0.28	0.22	0.12
Days (GS31-65)	-0.08	0.84**	0.65*	-0.03	0.74*	0.78*
SPI	-0.29	0.36	0.35	-0.30	0.04	0.21
T. Stem PI	0.19	-0.64*	-0.53	0.17	-0.36	-0.49
Lamina PI	-0.27	0.26	0.10	-0.27	0.00	0.05
L. Sheath PI	0.29	0.78*	0.66*	0.35	0.86**	0.86**
Plant height (cm)	0.17	-0.47	-0.57	0.20	-0.21	-0.41
Shoot No. /m^2	-0.22	-0.64*	-0.48	-0.22	-0.77*	-0.69*

* Significant at 0.05 probability level

** Significant at 0.01 probability level

*** Significant at 0.001 probability level

5.2.5.2 Relationships between true-stem structural DM increment from GS41 to GS65+7d and spike growth and fertility

Relationships between true-stem structural DM increment from GS41 to GS65+7d and spike growth and fertility are shown for the nine cultivars averaging across the two seasons. The true-stem structural DM increment (g m^{-2}; from GS41 to GS65+7d) was linearly and negatively associated with days from GS31 to GS65+7d (stem elongation period) (R^2 =0.71, *P* <0.001; Figure 5.14 a) and grains per spike at harvest (GPS) (R^2 =0.53, *P* <0.05; Figure 5.14 b).

Moreover, in agreement with the negative association found between the SPI and the true-stem PI at GS65+7d, the structural DM increment per shoot (GS41 to GS65+7d) was strongly negatively associated to the SPI at GS65+7d (R^2 =0.68, *P* <0.01; Figure 5.15 a). No significant associations were found between the SPI and the true-stem structural DM increment in g m^{-2} (Figure 5.15 b).

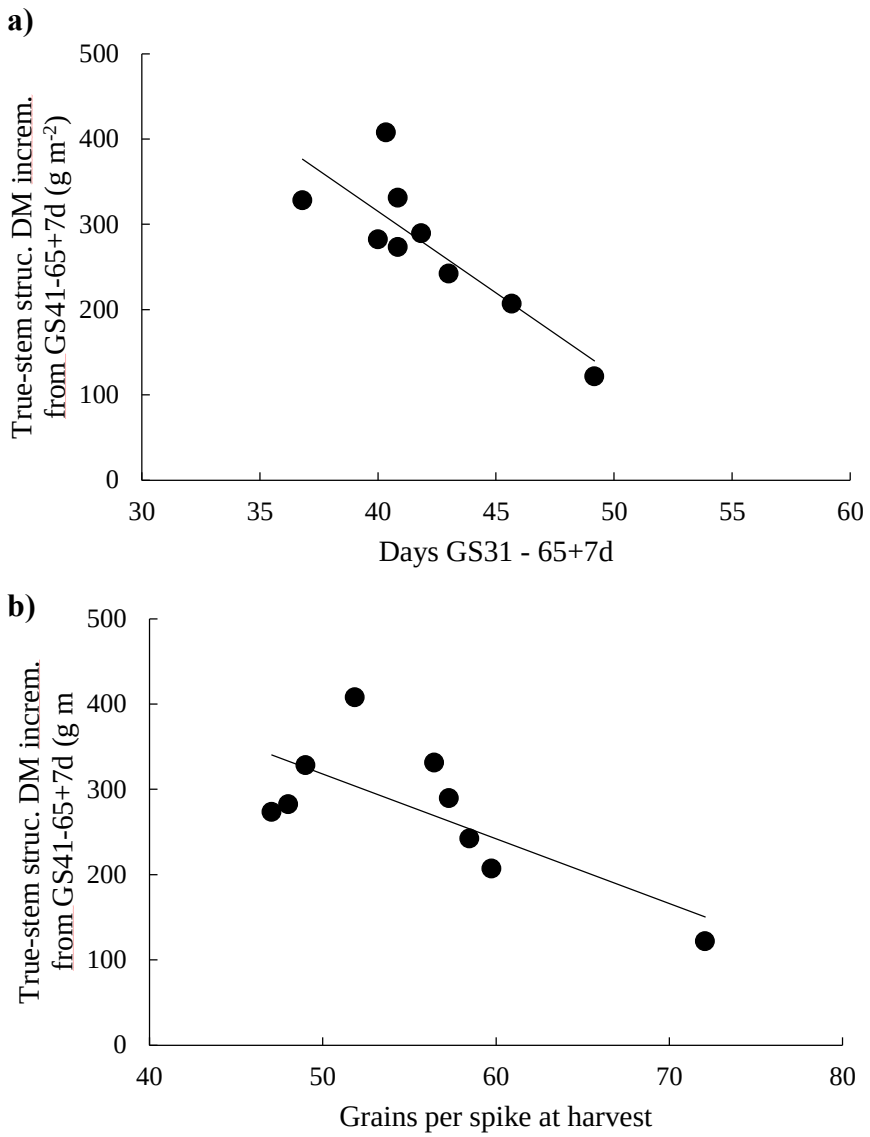


Figure 5.14. Regressions of true-stem structural DM increment from GS41 to GS65+7d in g m^{-2} on a) days from GS31 to GS65+7d (R^2 =0.71, *P* <0.001; y =-19.134x+1080.5) and on b) grains per spike at harvest (GPS) (R^2 =0.53, *P* <0.05; y =-0.0701x+74.873). Values represent means of 2012 and 2013 among the nine cultivars.

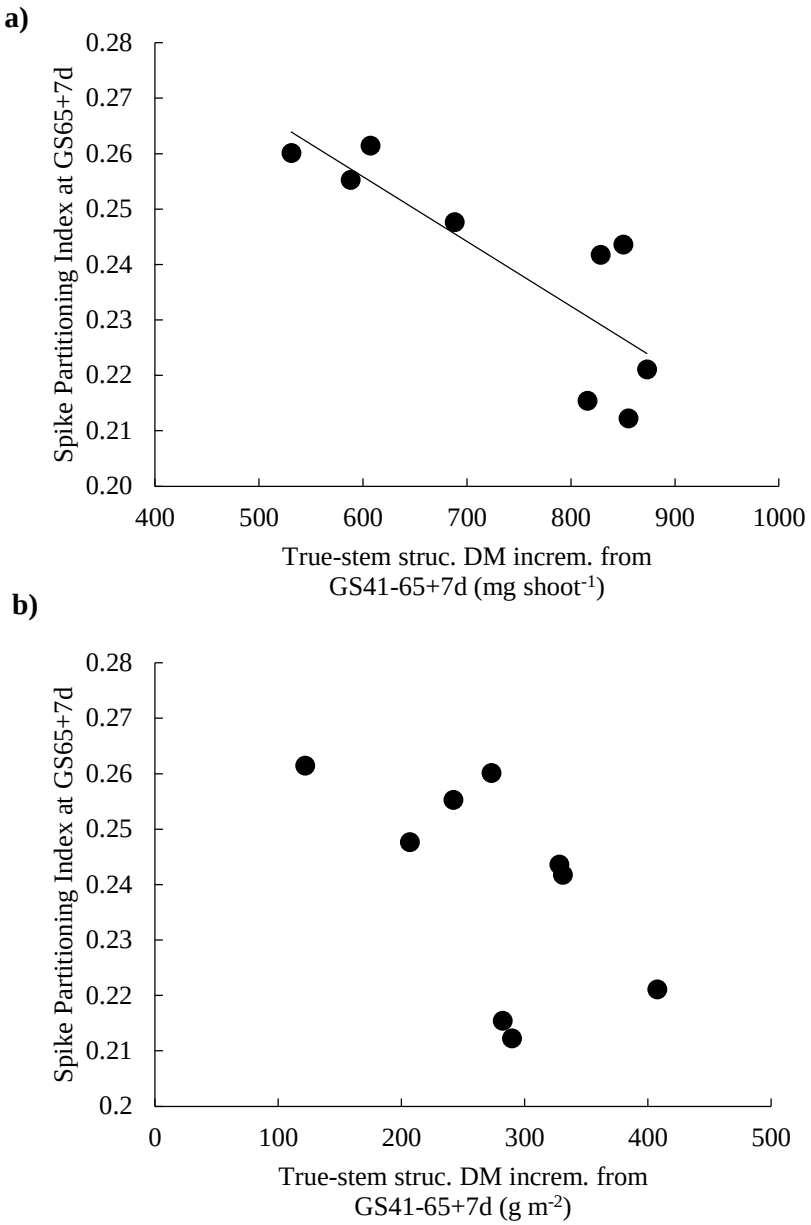


Figure 5.15. Relationships between spike partitioning index (SPI; spike DM as a proportion of shoot AGDM) on a) true-stem structural DM increment from GS41 to GS65+7d (mg shoot^{-1}) (R^2 =0.68, P <0.01; y =-0.0001x+0.326) and on b) true-stem structural DM increment from GS41 to GS65+7d in g m^{-2} (ns). Values represent means of 2012 and 2013 among the nine cultivars.

5.2.6 Associations between soluble and structural DM at anthesis and harvest traits and contribution to grain yield

5.2.6.1 Correlations between soluble and structural DM at anthesis and key harvest traits

Phenotypic correlations between harvest traits and WSC_A or WSC_A at GS65+7d in the spike, true-stem and leaf-sheath are presented in Table 5.8. No significant association was found between plant organ WSC_A or WSC_C and grain yield, TGW, AGDM or grains m^{-2}. Harvest index, however, was positively correlated with the true-stem WSC_A (r = 0.35, P <0.05). There were also no significant associations between WSC_C or WSC_A and grain yield or yield components among the 26 cultivars.

Table 5.8. Phenotypic correlations between WSC concentration (WSC_C, %) and amount (WSC_A) at GS65+7d in the plant organs and key harvest for the subset of nine cultivars, based on mean values in 2012 and 2013.

	Spike	**True-stem**	**Leaf-sheath**	**Spike**	**True-stem**	**Leaf-sheath**
	WSC concentration (WSC_C)			WSC amounts (WSC_A)		
Grain yield (t ha^{-1})	-0.20	-0.15	-0.32	-0.17	0.07	-0.21
TGW (g)	-0.02	0.06	-0.03	-0.02	0.31	0.02
HI	-0.29	0.27	-0.07	-0.29	0.35*	-0.09
AGDM harvest	0.11	-0.40	-0.20	0.13	-0.29	-0.09
Grain number m^{-2}	-0.12	-0.16	-0.18	-0.10	-0.30	-0.18
Spike number m^{-2}	-0.24	-0.49**	-0.51**	-0.27	-0.42*	-0.52**
Grains per spike	0.19	0.47*	0.47*	0.23	0.29	0.47*

* Significant at 0.05 probability level
** Significant at 0.01 probability level
*** Significant at 0.001 probability level

5.2.6.2 True-stem WSC dynamics

The temporal pattern of true-stem WSC amount from GS41 to harvest is shown in Figure 5.16 for each of the nine cultivars. The true-stem WSC_A increased from 84.7 g m^{-2} at GS65+7d to 147.6 g m^{-2} at GS61+21d. For eight of the cultivars, the maximal true-stem WSC_A occurred at GS61+21d (127.9-164.5 g m^{-2}) and for one cultivar (Sahual/W) at GS65+28d (193 g m^{-2}). Loss of true-stem WSC_A occurred only slowly between GS65+21d (147.6 g $m^{-2)}$ and GS65+28d (127.4 g m^{-2}), with rapid utilization between GS61+28d and harvest (7.3 g m^{-2}).

At GS41 (8 Feb) WSC_A accumulated in the true-stem was small (22.4 g m^{-2}, P <0.001). At GS65+7d (6 March), true-stem WSC_A had increased to 62.3 g m^{-2} (range 38.7 – 91.4 g m^{-2}, P <0.05).

At harvest, WSC_A in true-stem was small for all cultivars in the range 3.0 – 22 g m^{-2}. Overall of the 148 g m^{-2} soluble DM accumulated in the true-stem at GS65+21d (time of maximum WSC accumulation) only 7.3 g m^{-2} (5 %) remained at harvest. Cultivars differed in utilization of true-stem WSC in the range 84.2 – 97.7 % (P < 0.01) and of leaf-sheath WSC in the range 39.7 – 77.6 % (P < 0.001; data not shown). The true-stem WSC utilization (difference between amount of true-stem WSC at GS65+7d and harvest) was strongly positively linearly related with the amount of true-stem WSC at GS65+7d (R^2 = 0.94, P < 0.001).

The true-stem structural DM at GS65+7d (6 March) was 441.7 g m^{-2} and remained relatively unchanged at harvest at 530.4 g m^{-2} (30 April) (Figure 5.17).

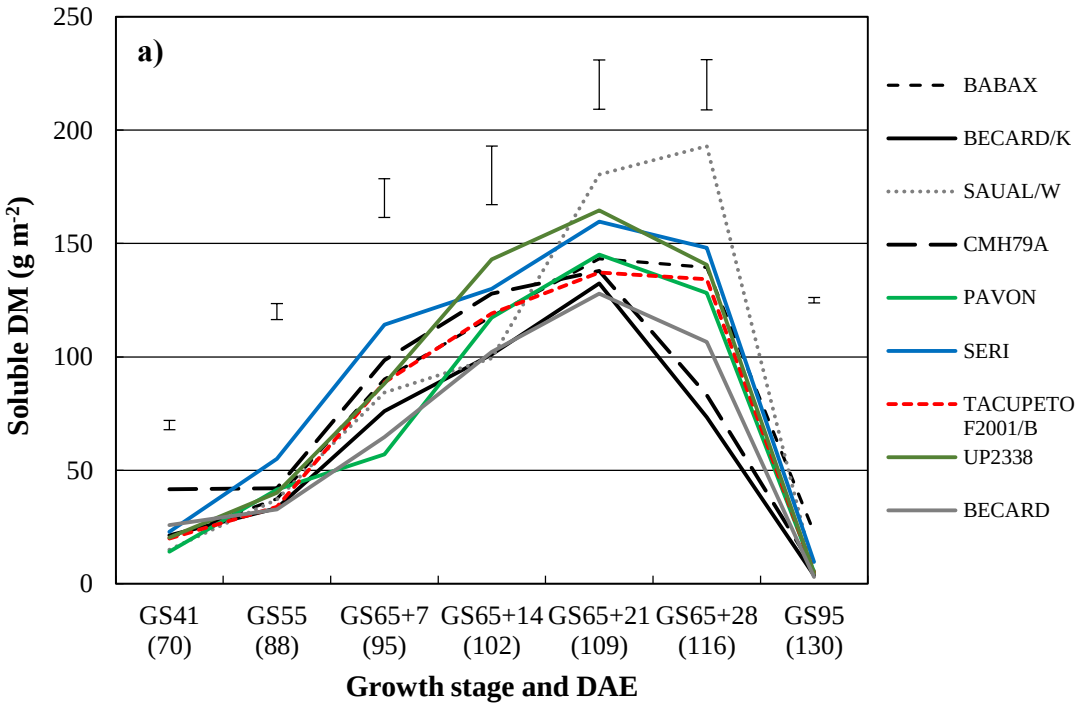


Figure 5.16. True-stem soluble DM (g m^{-2}) from initiation of booting (GS41) to maturity for the nine CIMCOG cultivars. Values represent means (2012 and 2013). Error bars represent the SED (df =16) for each sampling date.

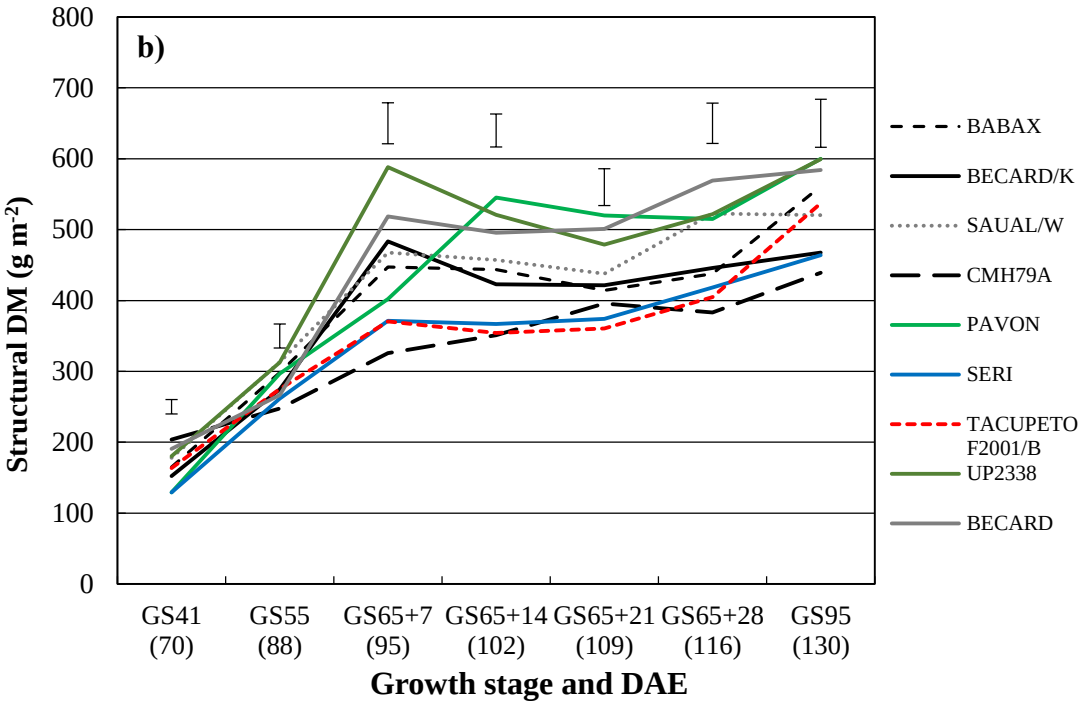


Figure 5.17. True-stem structural DM (g m^{-2}) from initiation of booting (GS41) to maturity for the nine CIMCOG cultivars. Values represent means (2012 and 2013). Error bars represent the SED (df =16) for each sampling date.

5.3 **Discussion**

Although differences in grain yield were explained mostly by the harvest AGDM among CIMCOG cultivars, a non-linear association between grain yield and HI indicated assimilates were partitioned less efficiently in high biomass cultivars; thus, physiological limitations to DM partitioned to harvestable grain need to be investigated. In this respect, this section will discuss the physiological basis of grains m^{-2} among CIMCOG cultivars (according to the grain number model (equation 3.1) focusing on spike DM partitioning at anthesis and spike fertility or fruiting efficiency. Moreover, the hypothesis that a more optimized pre-anthesis DM partitioning among organs could benefit spike growth (translated into more grains m^{-2}) will be discussed in terms of associations between DM partitioning among organs at seven days after anthesis (GS65+7d). For this, genetic variation in soluble DM (WSC) partitioning in the plant among the sub-set of nine cultivars will be discussed, focusing on true-stem WSC accumulation and associations between structural DM and spike partitioning at GS65+7d.

5.3.1 Physiological basis of variation in grains m^{-2}

Overall, cultivar variation in grains m^{-2} was better explained by the fruiting efficiency (FE) than SPI. Nevertheless, grains m^{-2} was positively related to the Spike DM (g m^{-2}) at GS65+7d among CIMCOG cultivars. Genetic variation in grains m^{-2} has been related to spike DM at anthesis (g m^{-2}) in wheat (Fischer, 1985, 2007; Fischer and Stockman, 1986; Slafer et al., 1990; Miralles and Slafer, 2007; Reynolds et al., 2009). The present positive non-linear (quadratic) association between SPI and the grains m^{-2} related to a positive effect of SPI on Spike DM. A pair of cultivars with the highest expression of grains m^{-2} and average SPI affected associations between these traits, as when removing them from the analysis there was a significant linear association between these traits (R^2 =0.24, P <0.05). Higher values of SPI at anthesis have been related to a greater spike DM at anthesis (g m^{-2}) in previous studies (Abbate et al., 1998; Gaju et al., 2009), indicating SPI to be a relevant trait to consider for improvements in spike fertility.

Trade-offs between the FE and spike DM or SPI at GS65+7d were not observed among CIMCOG cultivars, indicating on extent of independence between these traits. As the spike DM at anthesis is one of the two numerical components of the FE, it is logical to think that high FE requires low spike DM at anthesis. However Slafer et al. (2015) reviewed studies showing associations between grains m^{-2} and spike DM at anthesis when the variation was imposed by genetic factor, different management or artificial manipulations. Some previous studies indicate evidence for trade-offs between spike DM at anthesis and FE in bread wheat cultivars and/or advanced lines. On the other hand, (González et al., 2011) found that grains m^{-2} was highly associated with both FE and spike DM and Abbate et al. (1998) reported differences in FE for comparable spike DM values. The physiological basis of the cultivar differences found for SPI will be discussed further in section 5.4.2.

In the present study, values of FE calculated with spike DM at GS65+7d (genetic range 47.6 to 74.1 grains g^{-1}) were lower than those for FE calculated with chaff DM at harvest (genetic range 67.9 to 123.8 grains g^{-1}; data not shown). Different studies have found genetic variation for FE and associations with grains m^{-2} across a wide range of environments (Abbate et al., 1998; Gaju et al., 2009, 2014;

González et al., 2011; Lázaro and Abbate, 2011; Bustos et al., 2013; Aisawi et al., 2015). Moreover, Abbate et al. (2013) and Mirabella et al. (2015) suggested that this trait is mostly genetically controlled with low genotype x environment interaction. Differences in reported genetic ranges depend partially on the method of calculation. Fruiting efficiency in spring wheat calculated with spike DM one week after anthesis (without removing grains) ranged from 42 to 91 grains g^{-1} (Gaju et al., 2014; García et al., 2014) and from 35 to 137 grains g^{-1} when FE was calculated with the chaff DM at harvest (González et al., 2011; Abbate et al., 2013). The maximum value for FE (chaff DM at harvest) found in the present study was similar to these previous studies; however, the maximum FE (spike DM at GS65+7d) was *ca.* 25 grains g^{-1} lower than found in the literature.

With regard to the physiological basis of cultivar differences in FE, Abbate et al. (1998) found differences in the proportion of rachis in spike DM in six varieties of semi-dwarf awned spring wheat cultivars but no association with FE. They hypothesized that higher FE could be due to allocating a lower proportion of spike DM to non-productive sinks (glumes, awns and rachis) or to an enhanced ability of developing flowers to benefit from available assimilates. Present results showed a negative association between the FE and rachis specific weight and glume proportion of spike DM in 2012. Moreover, there was a trend for greater palea proportion of spike DM to favour higher FE. Gaju et al. (2009) similarly hypothesized that an increase in rachis length per spikelet favoured spikelet photosynthesis, increasing assimilate supply to the florets. It has been reported that floret survival is a positively associated with the sugar content of the spike (Ghiglione et al., 2008; Dreccer et al., 2014b), suggesting that high WSC content of spikes might be associated with increased FE. However, no association was found between WSC% of the spike and the fruiting efficiency or grains per spike in the present study.

Overall, the only component of the grain number model that did not differ significantly among the cultivars was the crop growth rate (CGR). Generally this trait has been shown to be constant among wheat cultivars in previous studies. For instance, no genetic variation in CGR was reported among Argentinian spring wheat cultivars (Abbate et al., 1998; Lázaro and Abbate, 2011) with values between 10.8 to 29 g m^{-2} d^{-1}. Similarly, Slafer et al. (1990) showed no genetic

gains with breeding in the efficiency of conversion of pre-anthesis intercepted radiation to dry-matter in six Argentinian bread-wheat cultivars.

In the present study, there was a trend for a negative linear association between days from GS41 to GS65+7d and grains m^{-2} and similarly a significant negative linear relationship between this period and the FE among the 26 cultivars. Although the duration of the period was not significantly related to spike DM (g m^{-2}); there was positive linear association between the period from GS31 – GS65+7d and spike DM at GS65+7d (R^2 = 0.61, P <0.05) and grains per spike (R^2 =0.80, P <0.001) among the nine cultivars. González et al. (2011) showed no associations between thermal time from GS31 to GS65 and the FE; however, thermal time from GS31 to GS65 was the main determinant of the spike DM at anthesis. This will be further discussed in section 5.4.3 in terms of true-stem soluble and structural DM accumulation between GS41 and GS65+7d.

In summary, trends for positive associations between SPI at GS65+7d and grains m^{-2} and strong linear relationships between grains m^{-2} and the FE indicate that determinations of grains m^{-2} strongly relies on greater spike DM during pre-anthesis; therefore avenues for enhanced DM partitioning to the spikes at anthesis were examined and are discussed below.

5.3.2 DM partitioning among plant organs and relationships with spike growth, HI and grain yield.

Overall among the 26 CIMCOG cultivars, genetic variation in spike DM (g m^{-2}) at GS65+7d was associated with both anthesis AGDM (R^2 =0.62, P <0.001) and the SPI (R^2 =0.36, P <0.01). However, Spike DM at anthesis has been generally more associated with SPI, than AGDM at anthesis (Slafer et al., 1990). SPI is reported to range from 12 to 21% for winter wheat cultivars in the UK (Shearman et al., 2005) and more widely from 16 to 29% for spring wheat genotypes in Australia and Mexico (Siddique et al., 1989; Reynolds et al., 2001a; Gaju, 2007). In the present study, SPI ranged from 21 to 27% among the 26 CIMCOG cultivars, showing values just slightly lower than the maximum found previously for spring wheat (Siddique et al., 1989). Slafer et al. (1990) found a significant positive trend between SPI and the year of release in six Argentinian bread-wheat cultivars released between 1912 and 1980. However, a recent study on CIMMYT

spring wheat cultivars reported decreases in SPI over a 43-year period from 0.25 to 0.23, increasing from 1966 up to *ca.* 1980, then decreasing to 2009 (Aisawi et al., 2015). Similar ranges for SPI were found in the present study to the latter study (using eight cultivars found in the CIMCOG panel) (0.21 to 0.28). Decreases in SPI with breeding could be associated with increases in plant height within semi-dwarf cultivars after the green revolution (Aisawi et al., 2015). Overall, the stem partitioning index (true stem and leaf sheath) was greater among the 26 CIMCOG cultivars (genetic range 0.48 to 0.65) than reported previously for CIMMYT cultivars. Aisawi et al. (2015) recently reported values of stem PI to range from 0.52 to 0.57, furthermore showing overall an increase from 0.53 to 0.56 over the 43-year period and being positively and a positive association with plant height. Finally, present results for genetic variation in lamina PI (0.16 to 0.22) were overall similar to values reported in the UK for winter wheat (0.19 – 0.21) (Shearman, 2001), and lower than spring wheat in Mexico (0.25 – 0.31) (Gaju, 2007). In order to understand the physiological basis of the competition for assimilates around anthesis between the spike and true-stem, discussions based on pre-anthesis spike and true-stem development are outlined below.

Regressions between SPI and competitive sinks at GS65+7d indicated that genetic variation in SPI was mostly explained by a negative association with true-stem PI among the 26 CIMCOG cultivars and SPI was not associated with either lamina or leaf-sheath partitioning index. The true-stem PI at maturity was significantly and negatively (linearly) associated with the SPI at GS65+7d, implying that structural true-stem DM (as almost all the WSC was remobilised or lost at maturity in CIMCOG cultivars) is the main driver of the negative associations observed at anthesis (see further discussion below in section 5.4.3). Borrell et al. (1991) in a study examining the influence of *Rht* alleles on the partitioning of DM between the spike and stem showed that greater allocation of DM to the developing spike than to the developing stem in shorter cultivars was evident at the time of maximum stem growth (*ca.* 17 b before anthesis), associated with greater spike DM (g m^{-2}) and that this stage coincides with the start of floret death, the rapid-spike-growth period and the rapid extension of the peduncle.

Moreover, in the present study the SPI was negatively related with true-stem DM increment from GS41 to GS65+7d among the nine cultivars. In an extensive study

examining the effects of dwarfing genes on the growth and DM partitioning in wheat Youssefian et al. (1992, a and b) reported that differences in the ratio of spike to stem DM between *Rht* and tall genotypes were evident even before terminal spikelet formation up to ear emergence. Nonetheless, previous studies concluded that although greater spike DM partitioning in semi-dwarf cultivars was apparent soon after terminal spikelet (possibly due to differences in apex size), overall differences were more related to the period of rapid spike growth (10 – 14 days before anthesis) (Brooking and Kirby, 1981; Siddique et al., 1989), in agreement with Fischer and Stockman (1986) who suggested that advantages of dwarf cultivars in spike DM partitioning could be fully exploited by reducing stem growth in the last 20 days before anthesis. Based on this evidence, it is likely that negative associations between SPI and true-stem PI observed in the present study are linked to greater partitioning to upper internodes since they are extended shortly after and before anthesis. In Chapter 6, results of DM partitioning within the stem (true-stem internodes and leaf-sheath phytomers) will be presented within the context of optimizing true-stem competition during stem elongation.

5.3.3 Optimizing soluble and structural DM partitioning among plant organs and relationship with spike growth, HI and yield

Overall in the present study, there was wide genetic variation in true-stem and leaf-sheath soluble DM accumulated at GS65+7d; however the values observed were relatively small (15.1% of total true-stem DM, range 10.5 – 26.6%, and 10% of total leaf-sheath DM, range 7.8 - 14.5%) compared to values previously reported in the literature. Cultivars differences were also observed for true-stem WSC amounts per unit area, ranging from 39.7 to 131.1 g m^{-2} among the 26 CIMCOG cultivars and from 13.4 to 28.4 g m^{-2} in the leaf-sheaths among the nine cultivars. These amounts are low compared to ranges in the literature for favourable conditions. For example, in the UK, Shearman et al. (2005) reported values of 244 to 391 g m^{-2} in the true-stem plus leaf-sheaths at GS61 for winter wheat cultivars in the UK.

Contribution of stem reserves to grain yield varies greatly with environmental and growing conditions and cultivars; ranging from 10 to 20% in favourable conditions (Bidinger et al., 1977; Schnyder, 1993; Gebbing et al., 1999; Shearman

et al., 2005). Moreover, WSC reserves can represent as high as 50% of the total grain weight under stress conditions (van Herwaarden et al., 1998; Rebetzke et al., 2008; Ehdaie et al., 2008). Previous work has also reported a positive association between stem soluble DM and HI in wheat (Foulkes et al., 2002). Present results showed a trend for a positive linear association between true-stem soluble DM partitioning at anthesis and HI ($R^2 = 0.13$, $P = 0.07$).

The genetic variation in true-stem and leaf-sheath WSC amount (g m^{-2}) was not significantly correlated with grain yield in the present study. In contrast, genetic variation in stem WSC amount has been shown to be positively correlated with grain yield in rain-fed conditions in the UK (Shearman et al., 2005), in irrigated conditions in NW Mexico (Saint Pierre et al., 2010) and under drought conditions in NW Mexico (Lopes and Reynolds, 2010; Saint Pierre et al., 2010) and Australia (van Herwaarden et al., 1998). It is possible that grain growth of the CIMCOG cultivars in the irrigated, high radiation conditions in the experiment was less source-limited than in these investigations. Grain growth of wheat crops may be more source-limited under lower radiation conditions during grain filling in UK and with accelerated senescence under drought than in the present study; the genetic variation in stem WSC was also less in the present study than in these investigations. Present results demonstrated that true-stem WSC continued to accumulate throughout the first 21 days of grain filling, suggesting that grain growth was not restricted by source supply during this phase of grain filling. The cultivars generally showed similar patterns of accumulation and loss of WSC with respect to time. Nevertheless, amounts of true-stem WSC remaining at harvest were small in the range 0.5 - 3.8% of total true-stem DM. It is uncertain how much of the loss in stem WSC from GS65+21 days to harvest related to grain DM growth and how much was potentially lost by respiration. In a field study in the UK with six winter wheat genotypes, Austin et al. (1977) estimated losses of dry matter from the stems between 10 days after anthesis to maturity averaged 172 g m^{-2} (range 82-236), and that respiration from the stems during the same period amounted to 106 g m^{-2} (range 104-225). It is possible that losses of WSC to respiration were relatively large given that the onset of the utilization of WSC in the true stem was about 21 days after anthesis.

In the present study the SPI was not associated with true-stem WSC % at GS65+7d among the nine cultivars. The study of Beed et al. (2007) of winter wheat in the UK demonstrated shading from flag-leaf emergence to heading reduced non-structural (water soluble carbohydrate, WSC) stem DM proportionally more than the structural stem DM, implying that stem WSC do not compete strongly with the spike for assimilate. This evidence indicates that stem WSC may have a lower intrinsic sink strength during stem elongation than the general spike growth. This apparent lack of competition between stem WSC and SPI at GS65+7d suggests that WSC tends to accumulate mainly once the sink demand of the spike and structural stem has been satisfied, so selecting for large stem WSC may not be detrimental to spike partitioning and yield potential. It is possible that stem WSC may be more important in the future if CIMMYT releases start to become co-limited by source and sink.

5.3.4 Breeding for high stem WSC concentration

Breeding for high WSC% depends on the heritability as well as available genetic diversity for this trait (Ruuska et al., 2006). Ruuska et al. (2006) and Saint Pierre et al. (2010) reported high heritability values (> 0.75) for stem WSC%. Saint Pierre et al. (2010) observed high heritability for stem morphological traits such as pith volume of the upper stem internode which were associated with stem WSC% indicating their potential use in breeding for high WSC. Although genetic variation in anthesis date was not correlated with WSC%, WSC per area or WSC per shoot in the present study, true-stem WSC% showed a positive association between days from GS31 to GS65+7d (stem elongation phase). However, Ehdaie et al. (2006) reported a lack of association between WSC-related traits and phenology and concluded selections for stem and WSC traits could be conducted without significantly affecting maturity classes in wheat breeding programs. In an analysis of the key carbohydrate metabolic enzymes relevant to WSC metabolism, the genetic variation in stem WSC was shown to be associated with enhanced fructan deposition, reduced sucrose hydrolysis, and reduced carbon partitioning into cell wall polysaccharides (Xue et al., 2008). Present results showed that there was a strong positive association between the WSC% in the true-stem and the leaf-sheath at GS65+7d amongst the nine genotypes (R^2 = 0.88, P< 0.01). and further work may test whether the fructan synthetic and sucrose hydrolysis

enzymes influencing WSC% in these two plant components are regulated by the same genes.

Genetic variation in WSC% and WSC per area has also been shown to be associated with tillering, with reduced tillering genotypes accumulating 20% more WSC in Australia (Van Herwaarden and Richards 2002). In the present study fertile shoot number at GS65+7d had a negative effect on true-stem WSC% but not on the leaf-sheath WSC%; however, greater shoot number reduced both true-stem and leaf-sheath WSC amounts per unit area. Plant height was negatively associated with true-stem soluble DM partitioning. Genetic variation and heritability for stem morphological traits and WSC% in different internodes and leaf sheaths within CIMCOG cultivars and associations with SPI and spike DM per unit area, grains m^{-2} and HI will be examined further in Chapter 6.

5.3.5 Conclusions

The answers to the hypotheses presented at the beginning of this chapter are outlined below:

1. Differences in grains m^{-2} were found among the 26 CIMCOG cultivars studied in this thesis, mostly associated with greater FE, but also with SPI and spike DM at anthesis + 7 days.
2. There was no trade-off between FE and SPI at anthesis + 7 days among the 26 CIMCOG cultivars.
3. Genetic variation in FE among eight CIMCOG cultivars was related to rachis specific weight and to DM partitioning to spike structural components (glumes and paleas) but not to spike WSC concentration.
4. The DM partitioned to the true-stem showed the greatest competition for assimilate with the DM partitioned to the spike at anthesis + 7 days while there was no apparent competition between the spike and the lamina or leaf-sheath DM partitioning among the 26 CIMCOG cultivars.
5. The rapid spike growth phase (booting to anthesis) was the most important phase in determining competition between the true-stem and spike at anthesis + 7 days among the sub-set of nine CIMCOG cultivars.

6. True-stem soluble DM or WSC amount (g m^{-2}) at anthesis + 7 days was determined by WSC concentration rather than total true-stem DM (g m^{-2}) among the sub-set of nine CIMCOG cultivars.
7. Negative associations between SPI and true-stem partitioning index (T-stem PI) at anthesis + 7 days among the nine CIMCOG cultivars were not stronger when just considering structural true-stem DM but SPI was mainly associated to the structural DM increment from GS41 to GS65+7d.
8. There were trends for positive associations between SPI at anthesis + 7 days and true-stem soluble (WSC) concentration at the same stage among the 26 CIMCOG cultivars, possibly explaining part of the genetic variation in grains m^{-2} and HI.

CHAPTER 6.

GENETIC VARIATION IN STEM MORPHOLOGICAL TRAITS AND ASSOCIATIONS WITH GRAIN SINK STRENGTH IN CIMCOG PANEL

6.1 Introduction

Results presented in Chapter 5 suggested that decreasing assimilate partitioning to the true-stem is a promising avenue to increase spike growth. Thus, it is crucial to identify promising stem morphological traits (such as internode length, DM and specific weight) determining competition for assimilates between growing stems and spikes, while taking into account potential trade-offs. The physiological basis of the competition between stem and spike growth is related to the latter coinciding with stem extension and dry-matter (DM) accumulation (Brooking and Kirby, 1981). Moreover, although studies suggest that stem WSC accumulation does not compete directly with the spike for assimilate and that it is actually beneficial for floret survival (Beed et al., 2007; Dreccer et al., 2009), it is important to consider that breeding for high WSC can involve greater investment in true-stem DM. Decreases in the stem structural DM on the other hand could benefit spike growth to a greater extent as they appear to a have less negative effects on the overall plant integrity than those of stem WSC, except for potential trade-offs with the minimum DM requirements for stem lower internodes in relation to wall thickness and material strength to assure lodging resistance (Berry et al. 2007; Foulkes et al. 2011).

Therefore, in order to investigate strategies for decreasing structural stem DM to benefit SPI and HI it is crucial to explore genetic variation among cultivars for stem phytomer morphological properties (true-stem and leaf-sheath), and associations with soluble DM accumulation and spike growth, which is the main

objective of this chapter. Furthermore, as plant height and phenology are commonly optimised in most regions, their intrinsic effects on stem morphological attributes should be taken into account for the identification of useful traits to be used in breeding programs.

Specific hypotheses tested in this chapter:

1. There is wide genetic variation and high heritability in stem phytomer morphological characteristics at GS65+7d (length, dry-matter (DM) and specific weight (SW) in the peduncle, internode 2, 3 and remaining) for the true-stem and the leaf-sheath among 26 high yielding CIMMYT spring wheat cultivars (CIMCOG panel) under irrigated high radiation conditions in NW Mexico.
2. Individual true-stem internode DM is determined by their lengths rather than SW among the 26 CIMCOG cultivars.
3. Competition for assimilate between the spike and the true-stem at GS65+7d is better explained by assimilate partitioning to true-stem upper internodes rather than to true-stem lower internodes.
4. Greater WSC concentration (%) in lower internodes is positively related to spike DM and SPI at GS65+7d and grains per spike at harvest among the sub-set of nine CIMCOG cultivars.
5. There is scope in breeding for true-stem morphological traits (such as DM and SW) at GS65+7d to enhance spike DM growth with minimum effects on plant height, anthesis date and the duration of the stem elongation period.

6.2 **Results**

Initially, this results section describes the genetic variation for stem-phytomer morphological attributes at GS65+7d among the 26 spring wheat cultivars. Then, associations between true-stem internodes morphological traits and spike-related traits at GS65+7d are described. Additionally, genetic variability for true-stem internodes WSC concentrations and amounts were examined in the nine cultivars, as well as the associations with their morphological attributes. Finally, the effects of plant height and phenology on the stem morphology at GS65+7d are described.

6.2.1 Genetic variation in stem-phytomer traits at GS65+7d

6.2.1.1 True-stem

Averaging over 2012 and 2013, the t-s ped DM ranged from 236 (BCN/Rialto) to 538 (Milan/Kauz) mg shoot^{-1}; internode that represented most of the true-stem DM (mean of 405 mg shoot^{-1} (31 %), P <0.001). The average true-stem DM content was similar for t-s 2 and t-s rem, 318 and 333 mg shoot^{-1}, respectively (P <0.001); however, the t-s rem DM showed a wider range from 132 (Pavon F76) to 474 (Cmh79a.955) mg shoot^{-1} compared to the t-s 2, from 224 (BCN/Rialto) to 394 (Wbll1*2) mg shoot^{-1}. The t-s 3 was the lightest phytomer, ranging from 165 (Pavon F76) to 299 (Wbll1*2) mg shoot^{-1} (P< 0.001) amongst the 26 cultivars. There was a year x cultivar interaction for the t-s 2, t-s 3 and t-s rem DM shoot^{-1} (P < 0.01), but not for the t-s ped DM (Table 6.1 a).

The true-stem internode lengths decreased linearly with lower internode positions. The t-s ped was the longest internode with an average length of 36.7 cm for the 26 cultivars (P <0.001), ranging widely from 25.7 (BCN/Rialto) to 43.3 (TC870344) cm. The t-s 2 length showed a narrower range from 15.8 (BCN/Rialto) to 23.3 (Trap#1) cm (P <0.001). The t-s 3 and t-s rem were similar in length, 14.1 and 14.8 cm, respectively (P <0.001), with a narrower range for the t-s 3 from 11.1 (BCN/Rialto) to 16.1 cm (Tacupeto F2001), compared to the t-s rem from 8.1 (Pavon F76) to 19.5 (Saual/4) cm. There was a year x cultivar interaction for all the internode true-stem lengths (P <0.01 for the peduncle and P <0.001 for the rest) (Table 6.1 a).

The true-stem specific weight (SW) increased linearly with lower internode position, from 11.0 mg cm^{-1} for the t-s ped to 15.4 mg cm^{-1} for the t-s 2 to 17.6 mg cm^{-1} for the t-s 3 and to 22.3 mg cm^{-1} (P <0.001) for the t-s rem. Overall, the SW for the t-s ped ranged from 9.2 (Pbw343*2 and Pfau/Seri.1b) to 13.2 mg cm^{-1} (CNO79) (P< 0.001) and for the t-s 2 from 12.3 (Pavon F76) to 18.0 mg cm^{-1} (Wbll1*2) (P <0.001). Ranges for true-stem SW were wider for lower internodes, from 13.3 (Pavon F76) to 21.0 mg cm^{-1} (Cmh79a.955) for the t-s 3 and from 16.7 (Pavon F76) to 27.6 mg cm^{-1} (Wbll1*2) for the t-s rem. Years differed in true-stem SW for all the internodes (P <0.001), but year x cultivar interactions were observed only for t-s 2 and t-s 3 (P <0.05) (Table 6.1 a).

No consistent pattern among true-stem internode position was observed regarding the broad sense heritability among the 26 cultivars. The true-stem DM per shoot had higher heritability for the t-s ped (0.85) than for the t-s 3 (0.77), followed by the t-s 2 (0.65) and the t-s rem (0.58). The heritability for the true-stem internode length was highest for t-s 2 (0.93), followed by the t-s ped (0.88), t-s 3 (0.70) and t-s rem (0.68). Finally, for true-stem SW the lowest heritability was observed for t-s 2 (0.24), whereas the heritability was considerably higher for the t-s ped (0.83) and similar between the t-s 3 (0.63) and the t-s rem (0.67) (Table 6.1 a).

6.2.1.2 Leaf-sheath

Overall, the l-sh ped section constituted most of the leaf sheath DM per shoot (261 mg shoot^{-1}), decreasing considerably for lower sections; 154, 99 and 94 mg shoot^{-1}, respectively, for the l-sh 2, l-sh3 and l-sh rem among the 26 cultivars (P <0.001). The genetic range was considerably wider for the l-sh rem (40 mg shoot^{-1}, Pavon F76 – 171 mg shoot^{-1}, Cmh79a.955) than for the l-sh 3 section (69 mg shoot^{-1}, Pavon F76 – 124 mg shoot^{-1}, Wbll1*2). Years differed just for the l-sh ped (P <0.01) and l-sh 3 DM (P <0.001). There were year x cultivar interactions for the l-sh 3 and l-sh rem sections DM (P <0.05 and <0.001, respectively) (Table 6.1 b).

Averaging across seasons, leaf-sheath length decreased linearly for lower sections, and differed among the 26 cultivars (P <0.001). The length of the l-sh ped section ranged widely from 17.5 (BCN/Rialto) to 22.7 cm (Sokoll). The lengths of the rest of the leaf-sheath sections showed narrower ranges than the peduncle; l-sh 2 from 15.1 (BCN/Rialto, Kingbird #1 and Becard) to 18.0 cm (Sokoll), l-sh 3 from 13.5 (Tacupeto F2001) to 16.0 cm (Wbll1*2) and l-sh rem from 11.1 (Pavon F76) to 16.3 cm (Wbll1*2). There was a significant effect of year for all the leaf-sheath section lengths (P <0.001), as well as year x cultivar interactions (P <0.001 for the l-sh ped and l-sh 3, P <0.05 for the l-sh 2 and P <0.01 for the l-sh rem) (Table 6.1 b).

The leaf-sheath SW differed for all the sections between the 26 cultivars (P <0.001). However, in contrast to the true-stem SW, leaf-sheath SW decreased linearly down the shoot (*i.e.* leaf-sheath in lower internodes was less dense than in the upper internodes). The leaf-sheath SW in the peduncle (l-sh ped SW) ranged

from 9.6 (Pavon F76) to 14.9 mg cm^{-1} (Yav_3), from 6.2 (Pavon F76) to 12.6 mg cm^{-1} (Becard/Kachu) for l-sh 2, from 4.9 (Pavon F76) to 8.3 mg cm^{-1} (CNO79) for l-sh 3 and from 4.2 (Siete cerros) to 9.0 mg cm^{-1} (Cmh79a.955) for l-sh rem. Years did not differ significantly in SW for any of the leaf-sheath sections, but a year x cultivar interaction was observed for the lower two leaf-sheath sections of the shoot (P <0.05 and <0.01, respectively, for the l-sh 3 and l-sh rem) (Table 6.1 b).

Heritability for the leaf-sheath DM per shoot was higher for the l-sh ped (0.87) than for l-sh 2 (0.62), l-sh 3 (0.59) or l-sh rem (0.65). Among leaf-sheath sections lengths, there was very high heritability for l-sh ped and l-sh 2 (0.92 and 0.90, respectively) and lower heritability for l-sh 3 and l-sh rem (0.72 and 0.70, respectively). Broad sense heritability for the leaf-sheath SW was lower for l-sh 2 (0.62) and l-sh 3 (0.52), and higher for l-sh ped (0.71) and l-sh rem (0.70) (Table 6.1 b).

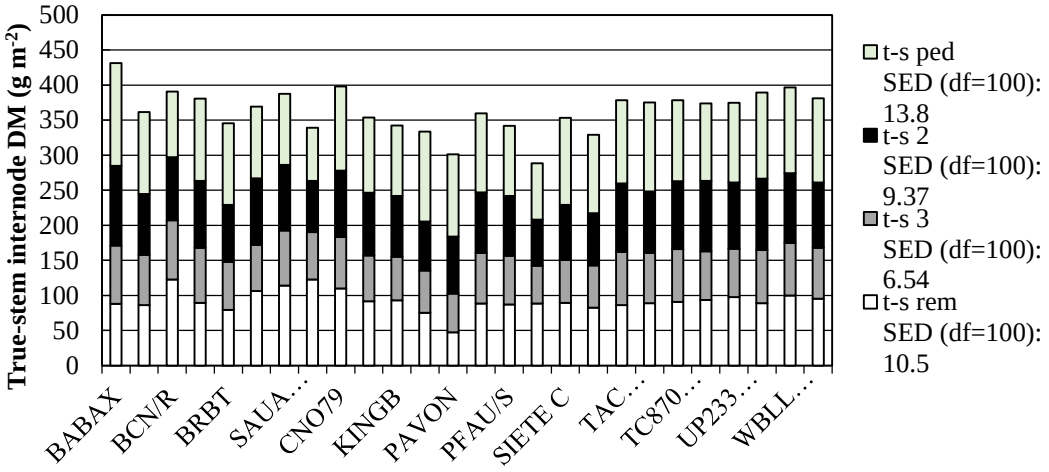
Although t-s ped accounted for most of the true-stem DM $shoot^{-1}$ (31%), it showed the weakest linear association with total true-stem DM $shoot^{-1}$ amongst the 26 genotypes (R^2 =0.59, P <0.001). Thus t-s ped DM had more genetic variation independent of the total true-stem DM $shoot^{-1}$ than other internodes (Figure 6.2 a). The total leaf-sheath DM $shoot^{-1}$ was more strongly associated with l-sh 2 DM $shoot^{-1}$ (R^2 = 0.83, P <0.001) than with l-sh ped (R^2 = 0.78, P <0.001), l-sh 3 (R^2 =0.75, P <0.001) or l-sh rem DM $shoot^{-1}$ (R^2 =0.56, P <0.001) (Figure 6.2 b).

The genetic variation in true-stem DM per unit area was best explained by l-sh 2 (R^2 =0.73, P <0.001) and l-sh 3 (R^2 =0.74, P <0.001) DM (g m^{-2}), rather than by l-sh ped or l-sh rem DM (g m^{-2}) (R^2 =0.25, P <0.01 and R^2 =0.22, P <0.05, respectively; Figure 6.3 a). Total leaf-sheath DM (g m^{-2}) was linearly associated with each leaf-sheath section DM (g m^{-2}) with similar R^2 values from 0.56 to 0.77 (P <0.001; Figure 6.3 b).

Table 6.1. Genetic variation and broad-sense heritability for above-ground dry matter per shoot, lengths and specific weights in a) true-stem internodes and b) leaf-sheath sections among the 26 cultivars. Values represent the mean for 2012 and 2013.

	Dry matter (mg shoot^{-1})				**Length (cm)**				**Specific weight (mg cm^{-1})**			
a) True-stem	**t-s ped**	**t-s 2**	**t-s 3**	**t-s rem**	**t-s ped**	**t-s 2**	**t-s 3**	**t-s rem**	**t-s ped**	**t-s 2**	**t-s 3**	**t-s rem**
Mean	405	318	249	333	36.7	20.5	14.1	14.8	11.0	15.4	17.6	22.3
Max	538	394	299	474	43.3	23.3	16.1	19.5	13.2	18.0	21.0	27.6
Min	236	224	165	132	25.7	15.8	11.1	8.1	9.2	12.3	13.3	16.7
SED cv (df=100)	**39.69**	**33.40**	**18.62**	**39.49**	**1.609**	**0.433**	**0.381**	**1.142**	**0.78**	**1.50**	**1.29**	**1.75**
SED year (df =1)	**11.01**	**9.26**	**5.16**	**10.95**	**0.446**	**0.120**	**0.106**	**0.317**	**0.22**	**0.42**	**0.36**	**0.49**
SED cxy (df =100)	**56.13**	**47.24**	**26.33**	**55.85**	**2.275**	**0.613**	**0.539**	**1.615**	**1.10**	**2.12**	**1.83**	**2.47**
CV (%)	**17.1**	**18.2**	**13.0**	**20.6**	**7.6**	**3.7**	**4.7**	**13.4**	**12.4**	**16.9**	**12.7**	**13.6**
Heritability	**0.85**	**0.65**	**0.77**	**0.58**	**0.88**	**0.93**	**0.70**	**0.68**	**0.83**	**0.24**	**0.63**	**0.67**
b) Leaf-sheath	**l-sh ped**	**l-sh 2**	**l-sh 3**	**l-sh rem**	**l-sh ped**	**l-sh 2**	**l-sh 3**	**l-sh rem**	**l-sh ped**	**l-sh 2**	**l-sh 3**	**l-sh rem**
Mean	261	154	99	94	20.2	16.2	14.4	13.7	12.8	9.5	6.9	6.3
Max	321	217	124	171	22.7	18.0	16.0	16.3	14.9	12.6	8.3	9.0
Min	200	103	69	40	17.5	15.1	13.5	11.1	9.6	6.2	4.9	4.2
SED cv (df =99)	**19.53**	**23.27**	**8.78**	**12.34**	**0.348**	**0.297**	**0.280**	**0.522**	**1.12**	**1.33**	**0.60**	**0.56**
SED year (df =1)	**5.42**	**6.45**	**2.43**	**3.42**	**0.096**	**0.082**	**0.078**	**0.145**	**0.31**	**0.37**	**0.17**	**0.16**
SED year x cv (df =99)	**27.62**	**32.91**	**12.41**	**17.45**	**0.492**	**0.420**	**0.396**	**0.738**	**1.59**	**1.88**	**0.85**	**0.79**
***CV* (%)**	**13.0**	**26.2**	**15.4**	**22.9**	**3.0**	**3.2**	**3.4**	**6.6**	**15.3**	**24.2**	**15.0**	**15.5**
Heritability	**0.87**	**0.62**	**0.59**	**0.65**	**0.92**	**0.90**	**0.72**	**0.70**	**0.71**	**0.62**	**0.52**	**0.70**

a)



b)

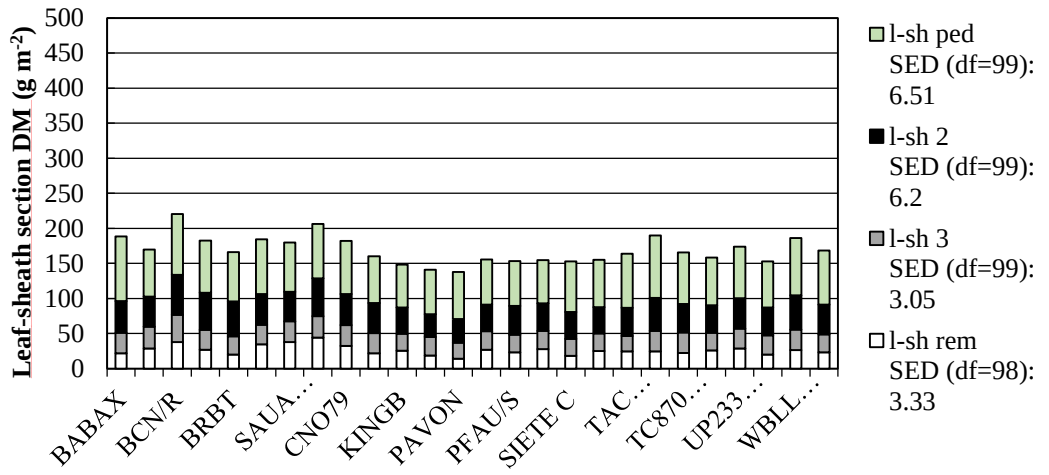


Figure 6.1. a) True-stem internodes and b) Leaf-sheath sections DM per unit area (g m^{-2}) from fertile AGDM at GS65+7d. Values represent the mean for 2012 and 2013.

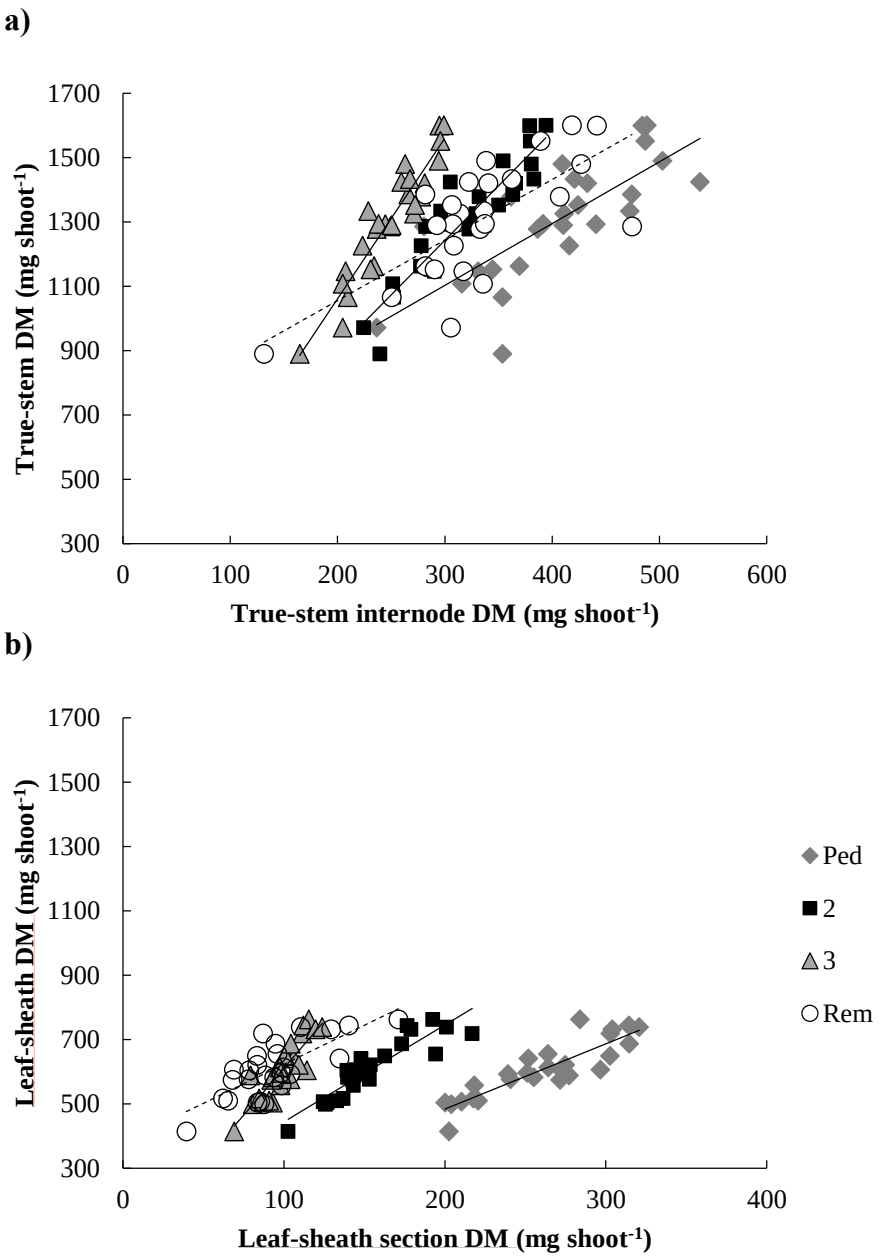


Figure 6.2. Linear regressions of a) total true-stem DM shoot^{-1} on each t true-stem internode DM shoot^{-1} (t-s ped: y =1.9x+526, R^2 =0.59, *P* <0.001; t-s 2: y =3.39x+227, R^2 =0.84, *P* <0.001; t-s 3: y =5.0x+61, R^2 =0.88, *P* <0.001 and t-s rem: y =1.88x+680, R^2 =0.51, *P* <0.001) and b) total leaf-sheath DM shoot^{-1}on each leaf-sheath section DM shoot^{-1} (l-sh ped: y =2.03x+78. R^2 =0.78, *P* <0.001; l-sh 2: y =3.0x+143, R^2 =0.83, *P* <0.001; l-sh 3: y =5.8x+28, R^2 =0.75, *P* <0.001 and l-sh rem: y =2.4x+381, R^2 =0.56, *P* <0.001) among the 26 cultivars. Values represent the mean for 2012 and 2013.

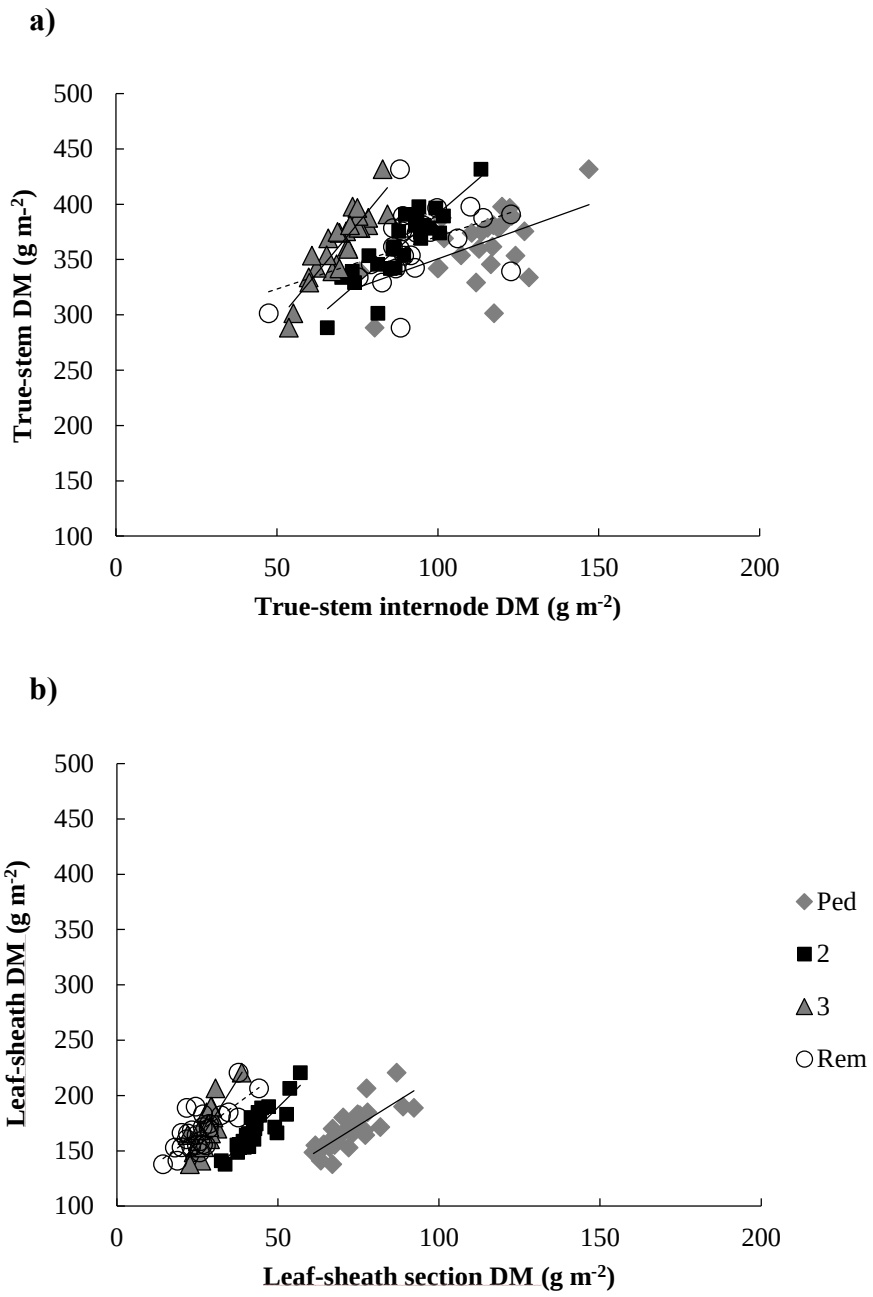


Figure 6.3. Linear regressions of a) total true-stem DM per unit area (g m^{-2}) on each true-stem internode DM per unit area (g m^{-2}) from fertile AGDM at GS65+7d (t-s ped: y =1.04x+246, R^2 =0.25, *P* <0.01; t-s 2: 2.5x+141, R^2 =0.77, *P* <0.001; t-s 3: y =3.5x+118, R^2 =0.80, *P* <0.001 and t-s rem: y =0.95x+276, R^2 =0.21, *P* <0.05) and b) total leaf-sheath DM per unit area (g m^{-2}) on each leaf-sheath DM per unit area (g m^{-2}) from fertile AGDM at GS65+7d (l-sh ped: y =1.82x+36.9, R^2 =0.61, *P* <0.001; l-sh 2: y =2.87x+45.7, R^2 =0.77, *P* <0.001; l-sh 3: y =4.68x+39.4, R^2 =0.66, *P* <0.001 and l-sh rem: y =2.15x+112, R^2 =0.56, *P* <0.001) among the 26 cultivars. Values represent the mean for 2012 and 2013.

6.2.2 Relationship between phytomers DM, specific weights (SW) and lengths

Overall, genetic variation in t-s ped DM shoot^{-1} was less dependent on length (R^2 =0.58, *P* <001; Figure 6.4 a) than t-s 2 DM shoot^{-1} (R^2 =0.76, *P* <0.001). The regression between the t-s 3 DM shoot^{-1} and its length showed the weakest linear association amongst internodes (R^2 = 0.48, *P* <0.001). The second highest linear relationship was between t-s rem DM shoot^{-1} and its length (R^2 =0.71, *P* <0.001). The true-stem SW for each internode was not explained by their respective lengths, with just a trend observed for t-s 2 SW and length (Figure 6.4 b). As a summary, phenotypic correlations between true-stem internodes DM, lengths and SW are presented in Table 6.2, showing positive and significant associations between true-stem internode DM and SW for all the internodes and no significant relationships between SW and lengths (except for t-s 2).

Among the 26 cultivars, leaf-sheath DM across the different sections was slightly related to their respective lengths, stronger for the l-sh ped (Figure 6.5 a). On the other hand, leaf-sheath SW across the different section was not explained by their respective lengths (Figure 6.5 b). However, leaf-sheath SW was linearly and positively associated with leaf-sheath DM across all the sections, stronger for l-sh 2 and l-sh 3 (data not shown).

Table 6.2. Summary of phenotypic correlations among true-stem internode dry-matter, lengths and specific weight (SW) among the 26 CIMCOG cultivars. Values represent mean of 2012 and 2013.

	a) Dry-matter (mg; DM)				b) Length (cm)			
	t-s ped	**t-s 2**	**t-s 3**	**t-s rem**	**t-s ped**	**t-s 2**	**t-s 3**	**t-s rem**
T-s ped DM	-							
T-s 2 DM	0.69	-						
T-s 3 DM	0.66	0.89	-					
T-s rem DM	0.15	0.55	0.66	-				
T-s ped length	0.76***	0.54	0.41	-0.22	-			
T-s 2 length	0.57	0.87***	0.67	0.24	0.69	-		
T-s 3 length	0.56	0.81	0.69***	0.21	0.62	0.88		
T-s rem length	-0.11	0.38	0.36	0.85***	-0.32	0.24	0.23	-
T-s ped SW	0.83***	0.59	0.63	0.44	0.28	0.30	0.34	0.13
T-s 2 SW	0.62	0.87***	0.87	0.74	0.24	0.52**	0.52	0.45
T-s 3 SW	0.40	0.47	0.74***	0.73	0.01	0.12	0.04	0.31
T-s rem SW	0.50	0.48	0.73	0.64***	0.09	0.09	0.08	0.15

* Significant at 0.05 probability level
** Significant at 0.01 probability level
*** Significant at 0.001 probability level

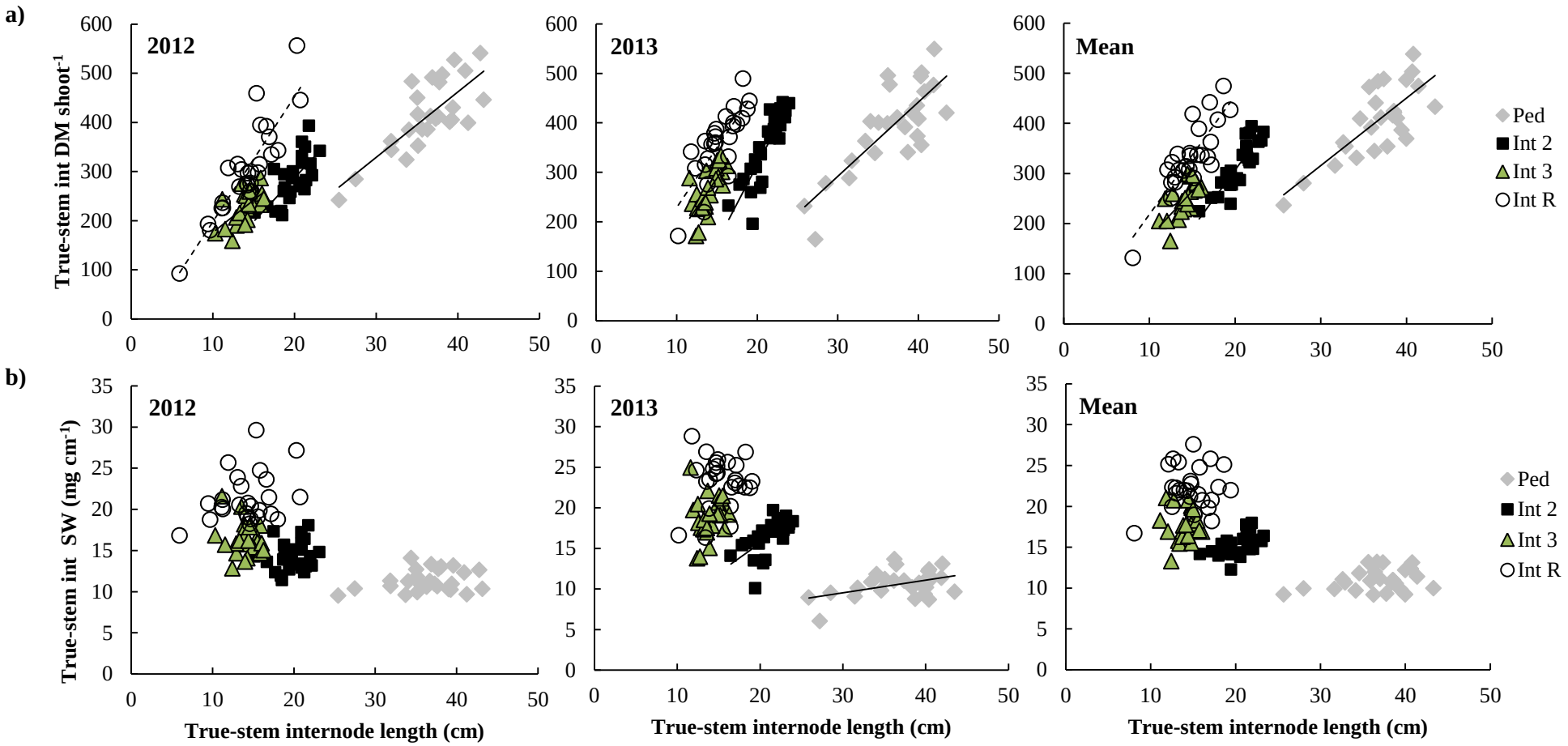


Figure 6.4. Linear regressions of true-stem internode length (cm) on **a)** true-stem internode DM shoot^{-1} in 2012 (t-s ped: y =13.3x-70.6, R^2 =0.59, *P* <0.001; t-s 2: y =17.7x-67.4, R^2 =0.49, *P* <0.001; t-s 3: y =13.2x+44.5, R^2 =0.40, *P* <0.001 and t-s rem: y =25.5x-57.4, R^2 =0.59, *P* <0.001), 2013 (t-s ped: y =15x-157.6, R^2 =0.59, *P* <0.001; t-s 2: y =31.3x-310.3, R^2 =0.76, *P* <0.001; t-s 3: y =24x-71.6, R^2 =0.50, *P* <0.001 and t-s rem: y =24.2x-13.6, R^2 =0.62, *P* <0.001) and for the overall mean (t-s ped: y =13.5x-88.6, R^2 =0.58, *P* <0.001; t-s 2: y =23.2x-157.9, R^2 =0.76, *P* <0.001; t-s 3: y =18.6x-13.6, R^2 =0.48, *P* <0.001 and t-s rem: y =23.7x-17.9, R^2 =0.71, *P* <0.001) and on **b)** true-stem internode SW (mg cm^{-1}) in 2012, 2013 (t-s ped: y =0.15x+4.9, R^2 =0.19, *P* <0.05 and t-s 2: y =0.73x+1.09, R^2 =0.43, *P* <0.001) and for the overall mean (t-s 2: y =0.39x+7.46, R^2 =0.27, *P* <0.01) among the 26 cultivars.

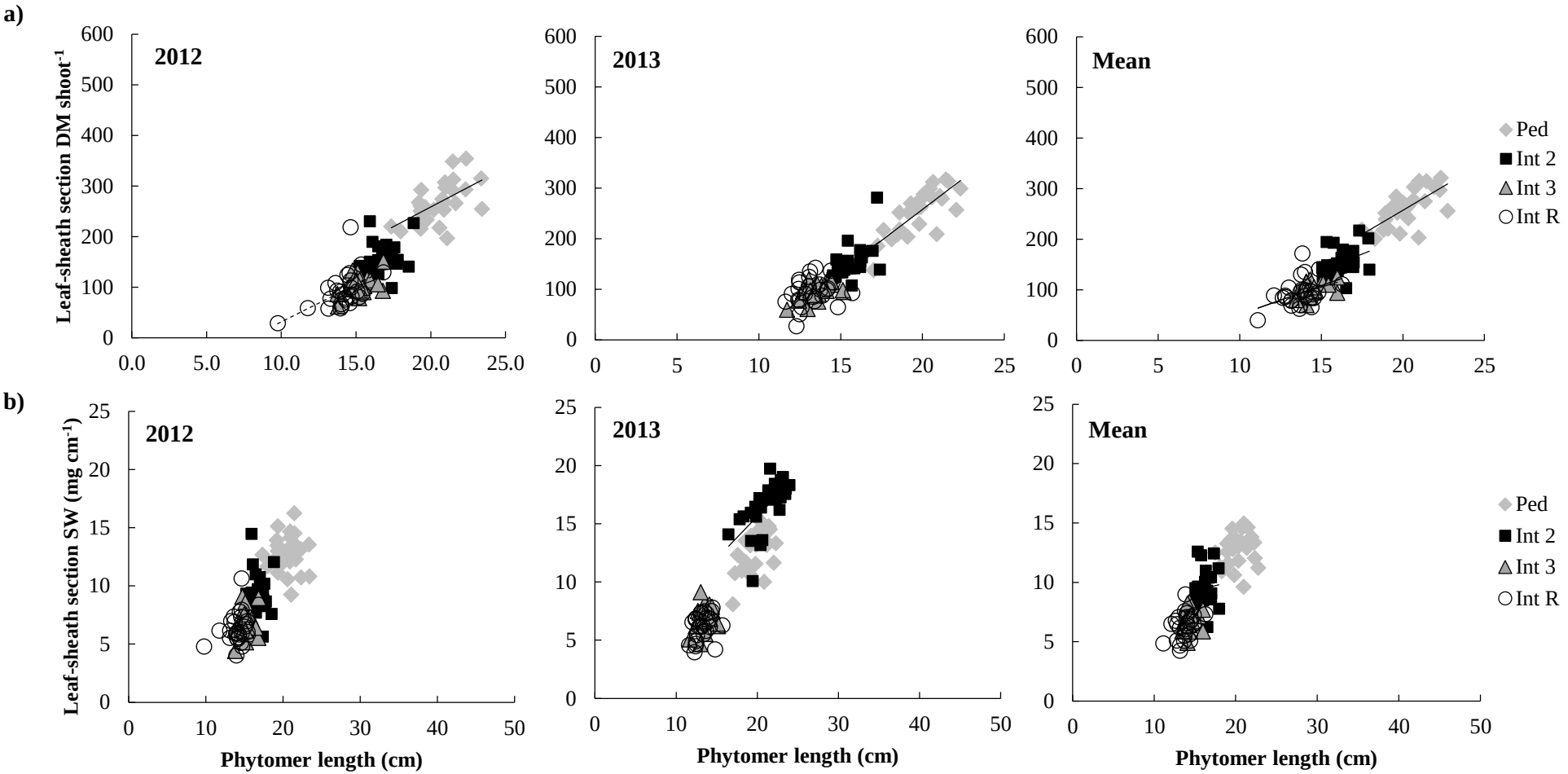


Figure 6.5. Linear regressions of leaf-sheath section lengths (cm) on **a)** leaf-sheath section DM shoot^{-1} in 2012 (l-sh ped: y =15.5x-51.9, R^2 =0.32, *P* <0.01 and l-sh rem: y =14.8x-116.1, R^2 =0.27, *P* <0.01), 2013 (l-sh ped: y =24.6x-234.3, R^2 =0.62, *P* <0.001 and l-sh 2: y =23.6x-216, R^2 =0.37, *P* <0.001) and for the overall mean (l-sh ped: y =19.1x-124, R^2 =0.47, *P* <0.001; l-sh 2: y =12.5x-49.1, R^2 =0.15, *P* =0.05; l-sh 3: y =9.8x-41.8, R^2 =0.23, *P* <0.05 and l-sh rem: y =11.4x-63.2, R^2 =0.18, *P* <0.05) and on **b)** leaf-sheath section SW (mg cm^{-1}) in 2012 and 2013 (l-sh 2: y =0.73x+1.09, R^2 =0.43, *P* <0.05) among the 26 cultivars.

6.2.3 Associations between true-stem internode morphology and spike growth at GS65+7d in subset of nine cultivars

Results from Chapter 5 suggested that among plant organs the true-stem is competing the most for assimilates with the spike at anthesis. Although the association between spike and true-stem DM partitioning at GS65+7d was not stronger when just considering the t-stem structural DM among the nine cultivars, the true-stem structural DM increment from GS41 to GS65+7d was negatively associated with grains per spike (GPS) and spike DM among the nine cultivars (Figure 5.4 in Chapter 5). It has been hypothesized that structural DM accumulation in the upper internodes could be more strongly negatively associated with the spike DM accumulation in the late rapid spike growth phase, as its timing overlaps more with rapid spike growth.

The spike and true-stem DM per shoot at GS65+7d were positively and linearly associated among the 26 cultivars (R^2 =0.51, P <0.001, data not shown); however, this relationship was not significant between the spike and true-stem DM on an area basis (g m^{-2}). Furthermore, the present results showed that the strength of the association between spike and true-stem DM per shoot at GS61+7d differed among true-stem internodes for the 26 cultivars (Figure 6.6 a). The t-s ped DM $shoot^{-1}$ was not significantly related to the spike DM $shoot^{-1}$. The t-s 2 and t-s 3 DM $shoot^{-1}$ were weakly but positively and linearly associated with the spike DM $shoot^{-1}$ (R^2 =0.22, P <0.05; R^2 =0.33, P <0.01, respectively). Moreover, at GS65+7d the cultivars with heavier spikes per shoot seemed to have greater t-s rem DM $shoot^{-1}$ (R^2 =0.57, P <0.001), with a non-linear relationship showing spike DM started increasing more rapidly at above values of t-s rem DM above ca. 350 mg $shoot^{-1}$. Similarly, there was a linear positive association between spike DM (g m^{-2}) and t-s rem DM (g m^{-2}) at GS65+7d (R^2 =0.24, P <0.05; data not shown).

The spike DM $shoot^{-1}$ was not related to any internode true-stem length at GS65+7d (Figure 6.6 b), but was positively associated with internode true-stem SW (Figure 6.6 c). Greater internode true-stem linear densities in the t-s 2, t- 3 and t-s rem seemed to be equally positively related with spike DM $shoot^{-1}$ (R^2 =0.48, 0.49, and 0.48, P <0.001, respectively).

On the other hand, spike DM (g m^{-2}) was strongly negatively related to t-s ped length (R^2 =0.50, *P* <0.001) and slightly negatively associated to t-s 2 and 3 lengths (R^2 =0.22 and 0.24 respectively, *P* <0.05; data not shown) but not significantly related to t-s rem length. Spike DM (g m^{-2}) was not associated with true-stem SW for any internode (data not shown).

The genetic variation in spike DM $shoot^{-1}$ was not associated with the length of any leaf-sheath section, but it was strongly positively and linearly related to the l-sh DM $shoot^{-1}$ in all sections, most strongly for the t-s 2 DM $shoot^{-1}$ (data not shown). The spike DM per unit area (g m^{-2}) was also positively and linearly associated to leaf-sheath DM (g m^{-2}) and lengths, similarly for each section (data not shown). Furthermore, leaf-sheath SW across the different sections was strongly positively related to the spike DM $shoot^{-1}$ but not to Spike DM per unit area (g m^{-2}) (data not shown).

The spike DM partitioning (SPI) was not explained by either the true-stem internode lengths, DM or SW among the 26 cultivars. However, the SPI was linearly negatively associated to the t-s 2 and t-s 3 DM partitioning (as a proportion of AGDM at GS65+7d) among the 26 (Figure 6.7 a) and the nine cultivars (Figure 6.7 b), this association was strongest for the t-s 3 in the subset of nine cultivars (R^2 =0.66, *P* <0.01).

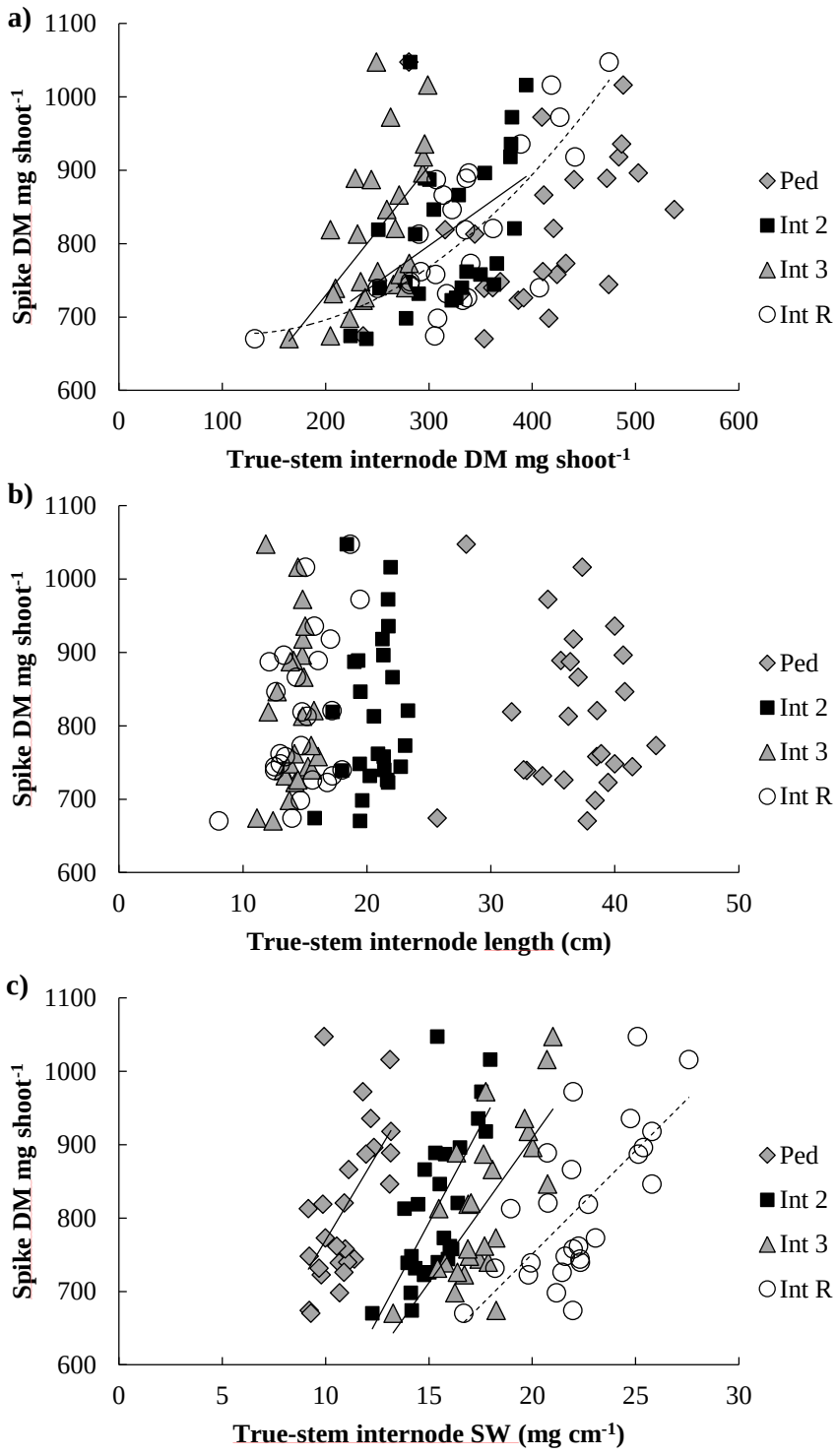


Figure 6.6. Regressions of spike DM (mg shoot^{-1}) at GS65+7d on **a)** true-stem internode DM shoot^{-1} (t-s 2: y =1.003x+496, R^2 =0.22, *P* <0.05; t-s 3: y =1.763x+377, R^2 =0.33, *P* <0.01 and t-s rem: y =0.003x^2-0.62x+713, R^2 =0.57, *P* <0.001), **b)** true-stem internode length (cm) and **c)** true-stem internode specific weight (SW; mg cm^{-1}) (t-s ped: y =47.3x+297, R^2 =0.35, *P* =0.001; t-s 2: y =52.8x+1.4, R^2 =0.48, *P* <0.001; t-s 3: y =39.4x+120, R^2 =0.49, *P* <0.001 and t-s rem: y =28.1x+189, R^2 =0.48, *P* <0.001) at GS65+7d. Values represent mean for 2012 and 2013.

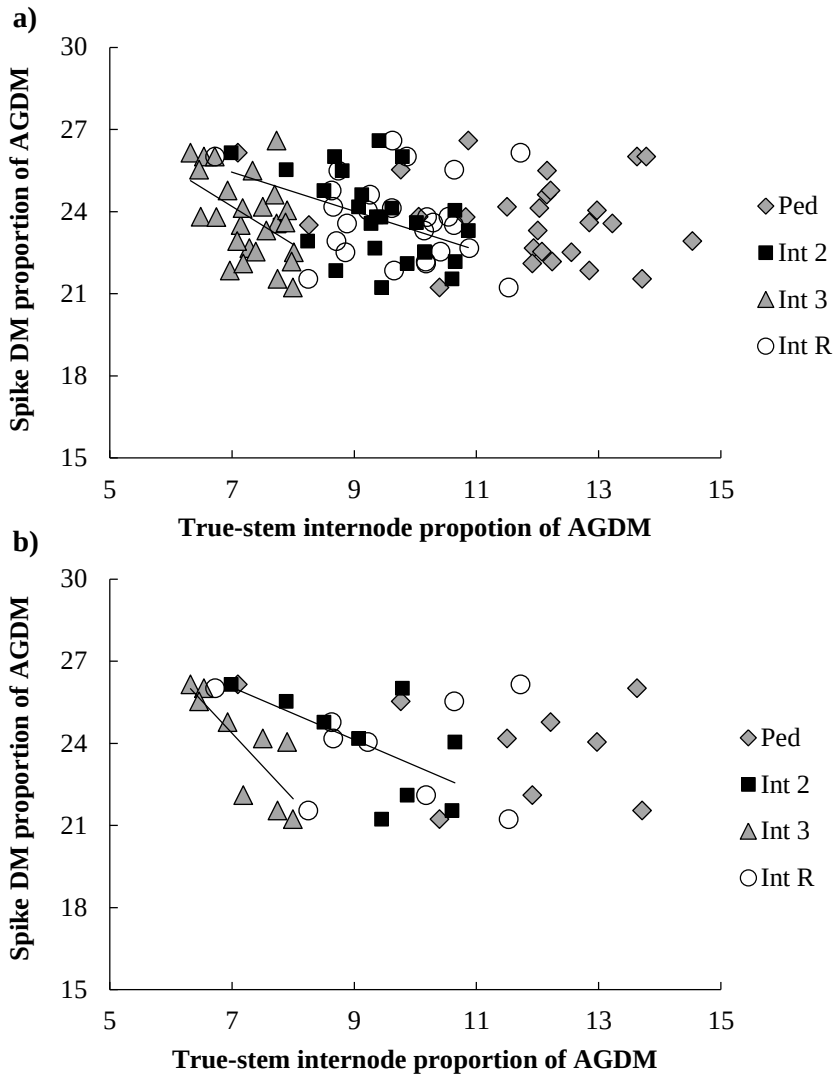


Figure 6.7. Regressions of Spike Partitioning Index (SPI) at GS65+7d (spike DM per shoot as a proportion of shoot AGDM) on true-stem internode PI (true-stem internode DM per shoot as a proportion of shoot AGDM) among **a)** 26 cultivars (t-s 2: -0.71x+30.4, R^2 =0.20, P <0.05 and t-s 3: -1.38x+33.9, R^2 =0.23, P <0.05) and **b)** nine cultivars (t-s 2: -0.95x+32.7, R^2 =0.38, P =0.078 and t-s 3: -2.39x+41.1, R^2 =0.66, P <0.01) at GS65+7d. Values represent mean for 2012 and 2013.

6.2.4 Genetic variation in true-stem internodes WSC and associations with spike growth in subset of nine cultivars

Means across the seasons for all the WSC analyses performed in the shoot at GS65+7d are shown in Figure 6.8 (averages for the nine cultivars).

Overall, results for the distribution of WSC reserves in the pre-anthesis phase showed that spike WSC concentration (WSC_C) differed in the range 7.6 – 12.3% among the nine cultivars (P <0.001), averaging 9.3% with differences between the

two seasons (P <0.001). In 2012, WSC analysis of spikes was carried out in samples containing small amounts of grain (WSC_C =18.9%, considering analysis was at seven days after anthesis) but in 2012 the grains were removed before milling the spikes (WSC_C =5.4%). Furthermore, there was a year x cultivar interaction for the spike WSC_C (P <0.05) (Table 6.3 a).

As expected, the true-stem WSC was more concentrated in the lower internodes at GS65+7d. The t-s ped WSC_C differed among the nine cultivars (P <0.05), averaging 10.9% and ranging from 8.3 – 13.5%. The t-s 2 WSC_C was greater among the nine cultivars (14.8%, P <0.001), but showed a considerably wider range from 11.0 – 21.4%. The t-s 3 and t-s rem internode were combined for the WSC analysis (t-s 3+r) and had the highest WSC_C (18.4%, P <0.001), also ranging widely amongst cultivars from 13.4 – 27.9%. Seasons differed for all the true-stem internodes WSC_C at GS65+7d (P <0.001) but no significant year x cultivar interactions were found. As described in Chapter 5, the nine cultivars differed in their leaf-sheath WSC_C at GS65+7d (P <0.001); however, WSC_C was not analysed separately for the different leaf-sheath sections (Table 6.3 a).

Spike WSC amount (WSC_A) per unit area at GS65+7d ranged from 15.3 to 28.6 g m^{-2} amongst the nine cultivars and the two seasons (P <0.01, Table 6.3 b); however, values were considerably higher in 2012 (20 – 46.5 g m^{-2}) compared to 2013 (7.6 – 18.1 g m^{-2}), presumably for the reasons mentioned above when describing spike WSC_C at GS65+7d (related to the methodology).

Overall, the t-s ped WSC_A differed significantly among the nine cultivars from 9.5 to 18.0 g m^{-2} (P <0.01). On the contrary, cultivar differences were not found for t-s 2 WSC_A per unit area. WSC_A per unit area in the lower true-stem internode (t-s 3+r) ranged from 13.9 to 53.3 g m^{-2} among the nine cultivars (P <0.001). Although WSC % was not examined in the leaf lamina, total leaf lamina DM amount at GS65+7d (per shoot and per unit area) is shown for the nine cultivars in the two seasons to allow comparisons with the true-stem DM and leaf-sheath DM (Table 6.3 b).

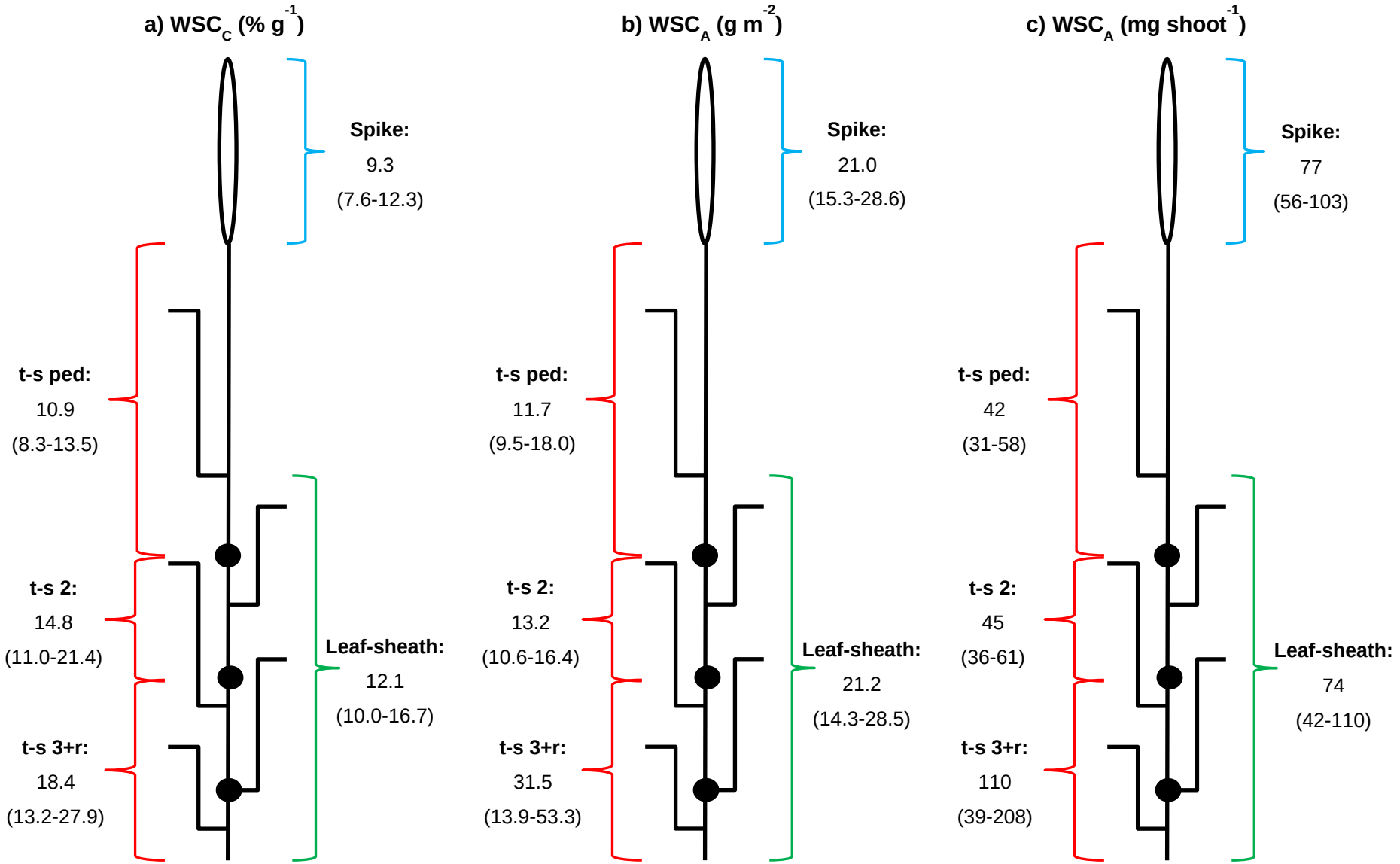


Figure 6.8. Results for water soluble carbohydrates (WSC) as: **a)** concentration (WSC$_C$: %); **b)** amounts per unit area (WSC$_A$ area: g m^{-2}) and c) amounts per shoot (WSC$_A$ shoot: mg shoot^{-1}) among plant organs and true-stem internode. Values represent the mean and ranges of 2012 and 2013 and the nine cultivars.

Table 6.3. Water soluble carbohydrates (WSC) as concentration (WSC_C: %), amounts per unit area (WSC_A area: g m^{-2}) and amounts per shoot (WSC_A shoot: mg $shoot^{-1}$) for spike, true-stem peduncle (t-s ped), true-stem internode 2 (t-s 2), true-stem internode 3 + remaining (t-s 3+r), lamina and leaf-sheath for the nine cultivars. Values represent the mean of 2012 and 2013.

	Spike		t-s ped		t-s 2		t-s 3+r		Lamina	Leaf-sheath	
a)WSC_C	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Total**	**Sol.**
BABAX	-	12.1	-	12.1	-	13.6		16.9	-	-	12.0
BECARD/K	-	9.4	-	9.9	-	11.0		14.7	-	-	10.6
SAUAL/W	-	9.0	-	9.5	-	13.8		19.9	-	-	11.5
CMH79A	-	9.2	-	13.5	-	21.4		27.9	-	-	14.0
PAVON	-	7.6	-	8.8	-	13.9		13.2	-	-	10.1
SERI	-	12.3	-	12.8	-	20.3		25.0	-	-	16.7
TAC/F2001/B	-	8.5	-	11.8	-	14.1		17.6	-	-	13.7
UP2338	-	7.7	-	11.2	-	13.3		16.6	-	-	10.6
BECARD	-	8.1	-	8.3	-	11.2		13.4	-	-	10.0
Mean	-	9.3**	-	10.9*	-	14.8***		18.4***	-	-	12.1***
SED df =34	-	1.025	-	1.62	-	2.073		2.284	-	-	0.976
b)WSC_A area	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Total**	**Sol.**
BABAX	230.2	28.6	146.9	18.0	113.4	16.4	171.2	30.3	218.2	188.5	22.9
BECARD/K	246.7	21.3	117.3	11.3	95.6	10.6	167.9	26.5	210.9	182.7	19.3
SAUAL/W	207.5	19.3	101.3	9.5	93.6	13.4	192.6	40.0	205.3	179.7	20.8
CMH79A	276.9	23.6	75.7	9.7	73.4	15.5	190.1	53.3	236.8	206.1	28.5
PAVON	219.2	15.3	117.5	9.6	81.4	12.1	102.6	13.9	194.0	137.6	14.3
SERI	211.1	26.7	80.4	10.3	65.7	13.4	142.3	36.7	174.7	154.8	25.6
TAC/F2001/B	252.8	19.6	127.0	13.8	87.8	12.6	160.5	28.5	209.5	189.7	25.5
UP2338	211.4	16.7	113.7	12.6	94.6	13.0	166.6	29.0	196.5	174.0	18.2
BECARD	227.4	18.0	123.0	10.1	101.8	11.8	164.6	24.9	181.0	153.0	15.3
Mean	231.5*	21.0**	114.4***	11.7**	89.7**	13.2ns	162***	31.5***	203.0ns	174.0*	21.2***
SED df =34	21.85	3.048	13.44	2.048	10.06	2.296	7.35	5.71	19.26	18.41	2.484
c)WSC_A shoot	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Sol.**	**Total**	**Total**	**Sol.**
BABAX	744	92	474	58	363	52	548	96	705	606	73
BECARD/K	866	80	411	41	329	36	585	89	748	655	70
SAUAL/W	740	72	362	34	332	47	685	140	734	641	74
CMH79A	1047	103	281	37	282	61	724	208	881	763	110
PAVON	670	47	354	31	240	34	296	39	593	414	42
SERI	819	100	316	42	251	51	540	137	670	592	99
TAC/F2001/B	887	81	441	54	301	43	552	98	730	649	89
UP2338	726	56	392	44	327	45	576	101	673	596	64
BECARD	762	63	410	35	337	39	543	79	606	509	50
Mean	807***	77**	382***	42*	307**	45ns	561***	110***	699***	603***	74***
SED df =34	75.0	13.83	38.21	8.53	32.79	9.22	59.2	24.82	39.15	48.8	10.53

* Significant at 0.05 probability level
** Significant at 0.01 probability level
*** Significant at 0.001 probability level

Overall, the t-s ped WSC_C showed a strong positive correlation with t-s 2 WSC_C (r =0.77, P <0.05) and with the t-s 3+r WSC_C (r =0.80, P <0.01), but was the strongest correlation between WSC_C in t-s 2 and t-s 3+r (r =0.93, P <0.001) (Table 6.4).

The t-s ped WSC_C was not significantly correlated with t-s ped DM shoot^{-1} or SW among the nine cultivars, and just a trend for a negative correlation with t-s ped length (r =-0.50, P =0.173) was observed (Table 6.4). A negative correlation was found between t-s 2 WSC_C and its length (r =-0.78, P <0.05) and a trend for a positive correlation with its DM shoot^{-1} (r =0.59, P =0.09). The t-s 3+r WSC_C appeared to be positively correlated with DM shoot^{-1} (r =0.61, P =0.08) and SW (r =0.67, P <0.05) (Table 6.4).

Table 6.4. Phenotypic correlations between t-stem phytomer WSC % and DM, SW and lengths among the nine cultivars and the two seasons.

		1	2	3	4	**5**	6	7	8	**9**	10	11	12
1	**t-s ped WSC_C**	-											
2	t-s ped DM	-0.27	-										
3	t-s ped SW	0.07	0.76	-									
4	t-s ped length	-0.50	0.88	0.37	-								
5	**t-s 2 WSC_C**	**0.77***	**-0.77***	-0.48	**-0.80****	-							
6	t-s 2 DM	-0.16	0.70	0.74	0.47	**-0.59**	-						
7	t-s 2 SW	0.27	0.38	0.71	0.02	-0.08	0.75	-					
8	t-s 2 length	-0.43	0.69	0.52	0.65	**-0.78***	0.85	0.30	-				
9	**t-s 3+r WSC_C**	**0.80****	**-0.70***	-0.24	**-0.87****	**0.93*****	-0.33	0.19	**-0.62**	-			
10	t-s 3+r DM	0.44	-0.20	0.38	-0.56	0.30	0.44	0.74	0.08	**0.61**	-		
11	t-s 3+r SW	0.60	-0.17	0.37	-0.55	0.43	0.30	0.78	-0.17	**0.67***	0.91	-	
12	t-s 3+r length	0.22	-0.10	0.41	-0.42	0.08	0.57	0.65	0.33	0.41	0.92	0.69	-

* Significant at 0.05 probability level
** Significant at 0.01 probability level
*** Significant at 0.001 probability level

True-stem internode WSC_C was not related to SPI GS65+7d (Figure 6.9 a). However, positive and linear associations were observed between grains per spike and true-stem internode WSC_C, stronger for t-s 3+r WSC_C (R^2 =0.70, P <0.001) than for t-s 2 (R^2 =0.51, P <0.05) and t-s ped (R^2 =0.42, P =0.059) (Figure 6.9 b). Associations between true-stem internode WSC_C and key anthesis and harvest traits are described later in this chapter.

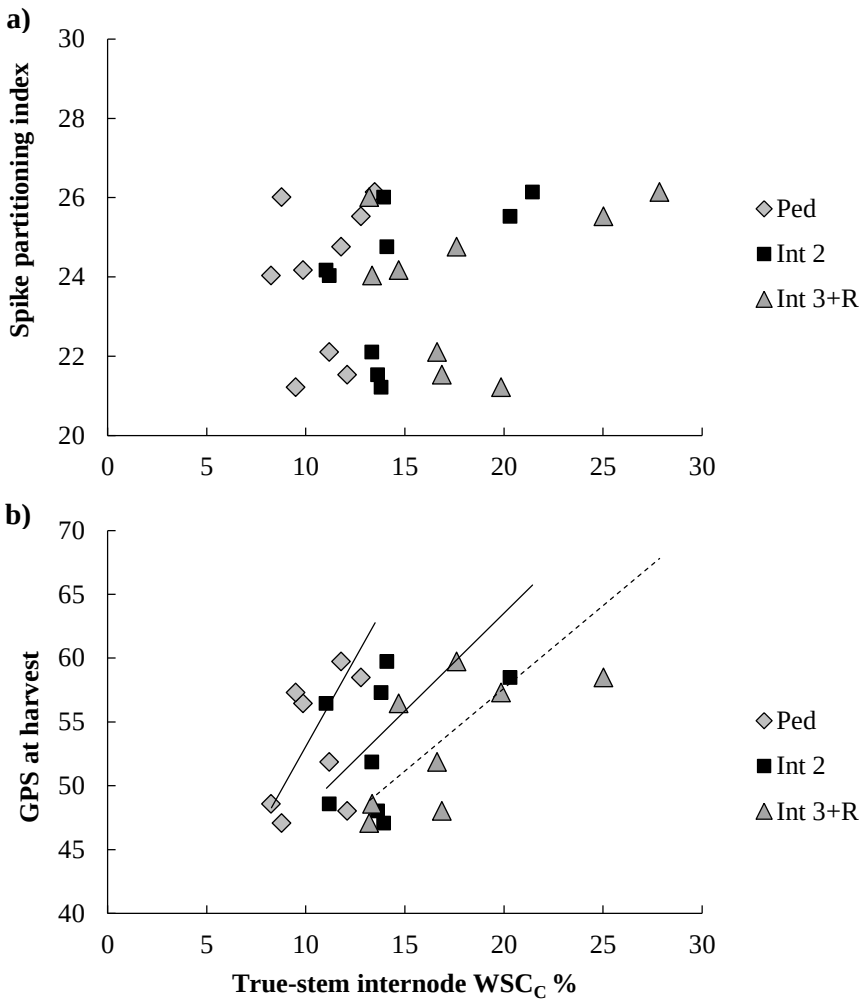


Figure 6.9. Regression of true-stem internode WSC_C (%) on a) Spike PI and on b) grains per spike at harvest (GPS; t-s ped: y =2.77x+25.39, R^2 =0.42, *P* =0.059; t-s 2: y =1.53x+32.9, R^2 =0.51, *P* <0.05 and t-s 3+r: y =1.29x+31.74, R^2 =0.70, *P* <0.01) at GS65+7d among the nine cultivars. Values represent the mean for 2012 and 2013.

6.2.5 Relationships between stem-phytomer traits and stem height and phenology

Associations between stem-phytomer length and the final plant height and stem height indicated the extent of genetic variation in phytomer length that is independent of plant height at GS65+7d (*i.e.* the stronger the association the less is the proportion of the genetic variation in the internode length independent of plant height).

Strong correlations were found between true-stem internode lengths and either plant height (*i.e.* considering the spike) or height to spike collar (*i.e.* stem height) (Table 6.5 a), slightly stronger for t-s 2 than for other internodes. Associations between true-stem DM shoot^{-1} and plant height were also positive and similar for all the internodes. On the contrary no significant correlations were observed between plant or stem height and true-stem SW for any internode.

The effect of plant height on the leaf-sheath morphology at GS65+7d (Table 6.5 b) was only apparent for the upper internodes. Final plant height was positively correlated with l-sh ped and l-sh 2 lengths (r =0.69, P <0.001 and r =0.53, P <0.01, respectively) and with the l-sh ped DM shoot^{-1} (r =0.56, P <0.001). No significant correlations were observed between plant or stem height and leaf-sheath SW for any section.

Phenotypic correlations between stem-phytomer morphological attributes and key phenological stages showed that true-stem internode SW was not affected by days to GS41 (days from emergence to initiation of booting) (Table 6.5 a). In contrast, t-s ped and t-s 3 length were negatively correlated with days to GS41 (r =-0.62, P <0.001; r =-0.46, P <0.05; respectively); but t-s 3 and t-s rem lengths were not affected significantly by duration of this period. Days to GS41 appeared to be negatively correlated with true-stem internode DM shoot^{-1} just for the peduncle (r =-0.54, P <0.05). Days to GS65 (days from emergence to anthesis) had a greater effect on the peduncle, as inferred from a negative correlation between anthesis date and t-s ped DM shoot^{-1} (r =-0.42, P <0.05) and length (r =-0.61, P <0.001). Positive correlation coefficients were observed between the period of rapid spike growth (from GS41 to GS65) and most of the true-stem internodes SW (r =0.62, P <0.001; r =0.40, P <0.05

and r =0.48, P <0.05, respectively, for the t-s ped, t-s 2 and t-s rem), except for the t-s 3 (r =0.30, ns).

With respect to leaf sheath morphology, dates of GS41 and GS65 were not correlated with any leaf-sheath section length and correlated positively with the l-sh rem DM shoot^{-1} and SW (Table 6.5 b). The period of rapid spike growth (GS41 to GS65) was positively correlated with the l-sh ped morphology (or flag-leaf sheath), DM shoot^{-1}, length and SW. Results also showed positive correlations between the duration of this period and l-sh 2 DM shoot^{-1} and length and with l-sh 3 length.

Table 6.5. Phenotypic correlations between plant height, height to spike collar, date of GS41 (initiation of booting), anthesis date (GS65) and period of rapid spike growth (days from GS41 to GS65+7d) and a) true-stem internodes and b) leaf-sheath sections morphological traits among the 26 spring wheat cultivars using means of 2012 and 2013.

	Plant height	**Height to spike collar**	**Days to GS41**	**Days to GS65**	**Days GS41 – GS65**
a) True-stem					
t-s ped DM shoot^{-1}	**0.70*** **	**0.68*** **	**-0.54***	**-0.42***	**0.48***
t-s ped SW (mg cm^{-1})	0.39	0.33	-0.26	-0.08	**0.62*** **
t-s ped length (cm)	**0.78*** **	**0.83*** **	**-0.62*** **	**-0.61*** **	0.13
t-s 2 DM shoot^{-1}	**0.80*** **	**0.77*** **	-0.20	-0.12	0.28
t-s 2 SW (mg cm^{-1})	**0.53****	**0.46***	-0.04	0.08	**0.40***
t-s 2 length (cm)	**0.87*** **	**0.88*** **	-0.30	-0.29	0.09
t-s 3 DM shoot^{-1}	**0.65*** **	**0.59****	-0.20	-0.11	0.33
t-s 3 SW (mg cm^{-1})	0.19	0.10	0.12	0.22	0.30
t-s 3 length (cm)	**0.77*** **	**0.80*** **	**-0.46***	**-0.43***	0.17
t-s rem DM shoot^{-1}	0.24	0.16	0.18	0.29	0.32
t-s rem SW (mg cm^{-1})	0.22	0.13	0.01	0.15	**0.48***
t-s rem length (cm)	0.15	0.12	0.22	0.25	0.05
b) Leaf-sheath					
l-sh ped DM shoot^{-1}	**0.56****	**0.47***	-0.08	0.12	**0.67*** **
l-sh ped SW (mg cm^{-1})	0.30	0.20	0.06	0.21	**0.45***
l-sh ped length (cm)	**0.69*** **	**0.66*** **	-0.34	-0.18	**0.61*** **
l-sh 2 DM shoot^{-1}	0.26	0.18	0.13	0.26	0.38
l-sh 2 SW (mg cm^{-1})	0.11	0.03	0.25	0.34	0.24
l-sh 2 length (cm)	**0.53****	**0.51****	-0.29	-0.14	**0.57****
l-sh 3 DM shoot^{-1}	0.21	0.13	0.07	0.21	**0.45***
l-sh 3 SW (mg cm^{-1})	0.11	0.03	0.07	0.19	0.35
l-sh 3 length (cm)	0.32	0.32	-0.02	0.10	**0.41***
l-sh rem DM shoot^{-1}	-0.02	-0.11	**0.43***	**0.54****	0.27
l-sh rem SW (mg cm^{-1})	-0.20	-0.29	**0.42***	**0.54****	0.32
l-sh rem length (cm)	0.19	0.16	0.15	0.25	0.28

* Significant at 0.05 probability level
** Significant at 0.01 probability level
*** Significant at 0.001 probability level

6.2.6 Associations between true-stem internode morphological characteristics with key pre-anthesis and harvest traits (principal component analysis).

6.2.6.1 Associations between SPI and spike DM (g m^{-2}) and true-stem internode morphological traits

Firstly, with the aim of investigating associations between true-stem internode morphological traits and key pre-anthesis and harvest traits at the crop level (*i.e.* per unit area), principal component analysis (PCA) was conducted for the 26 cultivars and the two seasons, including true-stem morphological traits at GS65+7d, true-stem internode PI, spike DM (g m^{-2}), SPI, grains m^{-2}, HI and shoots m^{-2} at GS65+7d (Figure 6.10). Shoots m^{-2} was negatively associated with true-stem internode SW, less strongly for the peduncle. Spike DM (g m^{-2}) was negatively associated with t-s ped DM (g m^{-2}) and t-s ped PI (t-s ped DM as a proportion of total AGDM) and t-s 2 PI (t-s 2 DM as a proportion of total AGDM). However, SPI was not associated with t-s ped DM or PI but was rather negatively associated to t-s 2 DM (g m^{-2}) and t-s 3 PI (t-s 3 DM as a proportion of total AGDM). The t-s ped SW appeared to affect negatively the SPI at GS65+7d. There were positive associations between t-s rem DM (g m^{-2}) and t-s rem PI and spike DM (g m^{-2}) among the 26 cultivars. Grains m^{-2} (GN) appeared to be negatively related to t-s 3 PI and t-s 2 DM (g m^{-2}).

6.2.6.2 Associations between true-stem internode WSC per m^{-2} and true-stem internode morphological traits

The same analysis was performed for true-stem internode WSC amounts (WSC_A; g m^{-2}), true-stem internode morphological traits, shoots m^{-2}, spike DM (g m^{-2}), SPI and grains m^{-2} among the nine cultivars and the two seasons (Figure 6.11). Greater shoots m^{-2} appeared to reduce true-stem internode WSC_A in t-s 2 and 3+r, but it was not related to the t-s ped WSC_A. Interestingly, the t-s ped WSC_A and t-s 3 PI at GS65+7d seemed to have a negative effect on grains m^{-2} and on SPI at GS65+7d. On the other hand, a greater t-s 3+r PI (t-s 3 DM as a proportion of total AGDM) appeared to promote higher WSC_A for the same true-stem internode segment.

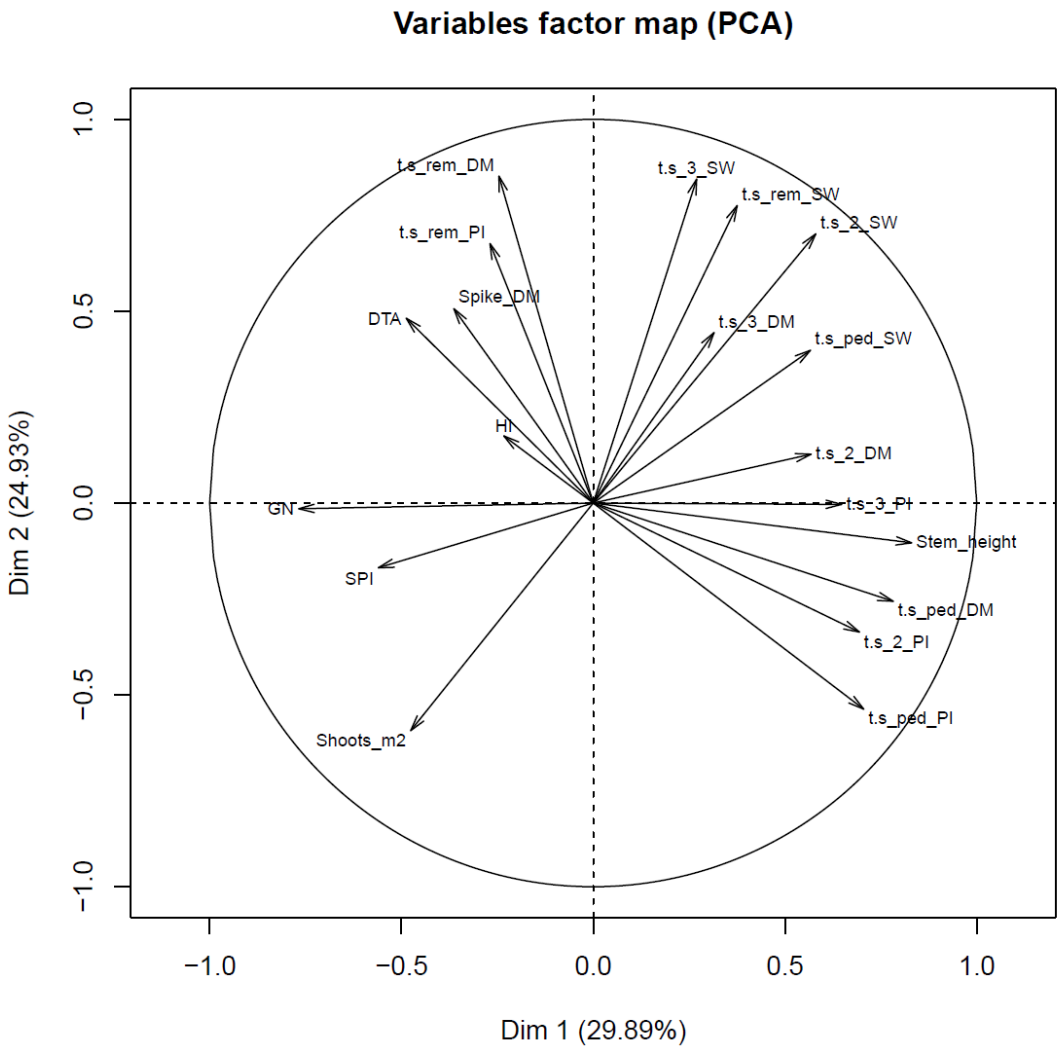


Figure 6.10Principal component analyses (PCA) for true-stem internodes DM per unit area, specific weight (SW) and DM as a proportion of total AGDM (partitioning index; PI), spike DM per unit area at GS65+7d, SPI, days to anthesis (DTA), fertile shoot number per unit area at GS65+7d, stem height at physiological maturity and grain number per unit area among the 26 CIMMYT spring wheat cultivars. Based on means for 2012 and 2013.

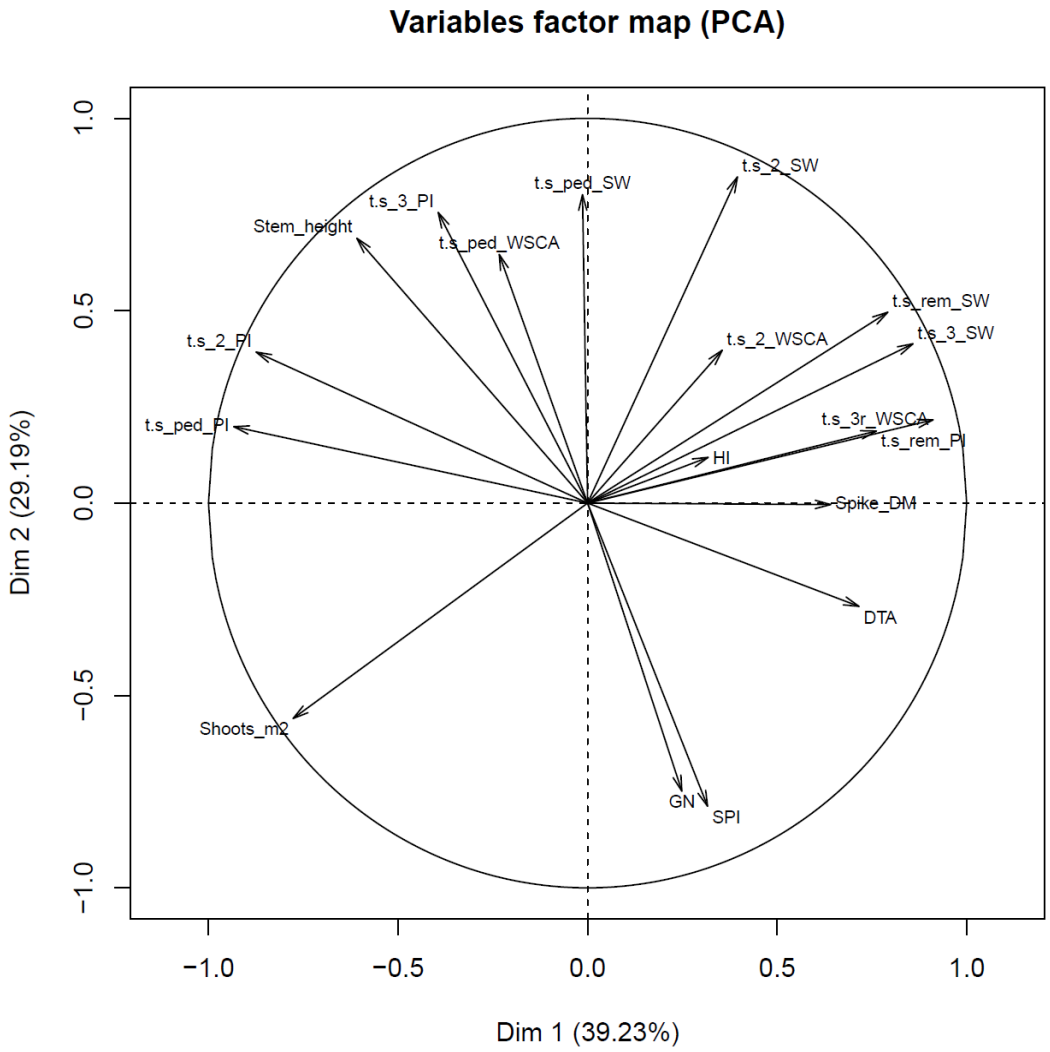


Figure 6.11 Principal component analyses (PCA) for true-stem internodes WSC amounts (WSC_A; g m^{-2}), specific weight (SW) and DM as a proportion of total AGDM (partitioning index; PI), spike DM per unit area at GS65+7d, SPI, days to anthesis (DTA), shoot number per unit area at GS65+7d, stem height at physiological maturity and grain number per unit area among the 26 CIMMYT spring wheat cultivars. Based on means for 2012 and 2013.

6.3 **Discussion**

The discussion will focus initially on the genetic variation found for true-stem and leaf-sheath morphological characteristics in relation to competition for assimilates between the true-stem and spike and determination of true-stem WSC accumulation. Then, the scope to identify stem-phytomer morphological attributes that are at least partly independent of stem height will be discussed.

6.4.1 Genetic variation in true-stem and leaf-sheath morphological attributes

Significant genetic variation was found in all stem and leaf sheath morphological attributes among the 26 CIMCOG cultivars. Previous studies have reported genetic variation among true-stem internode morphological attributes and accumulation and mobilization of non-structural DM in wheat (Borrell et al., 1993; Cruz-Aguado et al., 2000; Ehdaie et al., 2006a; Saint Pierre et al., 2010) and barley (Bonnett and Incoll, 1992). The stem length at GS65+7d gradually decreased from the peduncle to the lower internodes. The peduncle was the longest true-stem internode, presumably reaching its maximum length around GS65+7d, accounting for 42.6% of the total stem length. Whether or not the peduncle was fully extended by seven days after anthesis could partly explain the wide genetic range observed in its length in the CIMCOG genotypes. The time for the peduncle to reach its maximum length differs widely among wheat cultivars, occurring over several days after anthesis (Borrell et al., 1989; Cruz-Aguado et al., 2000; Gebbing, 2003; Ehdaie et al., 2006a). The peduncle was also the heaviest true-stem internode at GS65+7d and its maximum DM accumulation was likely to coincide with the time of maximum stem DM accumulation at around GS65+21 days in the CIMCOG panel.

The t-s 2 was the longest internode after the t-s ped. As the lower stem internodes are fully extended before anthesis (Ehdaie et al., 2006a), it is likely that maximum mass was attained earlier in lower internodes than in the penultimate internode among CIMCOG cultivars. Previous studies in wheat reported that t-s 2 showed the greatest DM at the time of maximum stem DM accumulation (24 – 27 d after anthesis) (Borrell et al., 1989; Borrell et al., 1993). Maximum true-stem DM was attained 21-28 days after anthesis for the subset of nine cultivars and full stem height was

attained around 7-10 days after anthesis; indicating that the period between full stem height and maximum stem weight was *ca.* 10 days for the whole stem but likely to fluctuate among true-stem internodes. Borrell et al. (1989) reported that the period between the attainment of maximum length and maximum mass was minimal for the peduncle and was 38 days for internodes 6 to 8 (top-down) in the semi-dwarf spring wheat cultivar Condor. True-stem internode capacity to accumulate DM can be considered as a function of its length and specific weight. At GS65+7d the true-stem SW increased gradually from the peduncle to lower internodes. Borrell et al. (1993) in a study comparing accumulation and use of stem reserves in three *Rht* near-isogenic wheat lines also found maximum SW (24 – 27 d after anthesis) was greater down the stem.

Leaf-sheath DM at GS65+7d comprised mostly the flag-leaf sheath (*i.e.* peduncle leaf-sheath) (42.9%). Lengths of the leaf-sheaths decreased linearly down the shoot, ranging amongst cultivars more widely for lower leaf-sheaths than for flag-leaf sheath. In contrast to the true-stem SW, the leaf-sheath SW decreased linearly down the shoot. There are no previous reports on leaf-sheath DM partitioning at the phytomer level in wheat, but lower leaf-sheaths (particularly l-s rem) are likely to be senesced or close to senescence at GS65+7d. Therefore, upper leaf-sheaths are more relevant in terms of photosynthetic capacity or assimilate remobilization during grain filling. In this respect, significant genetic variation was found for flag-leaf sheath length among the 26 cultivars, presumably causing differences in the proportion of the true-stem internode which is either enclosed by the leaf sheath or exposed; the peduncle (leaf-sheath and exposed true-stem) contributes significantly to photosynthesis during grain filling (Evans and Rawson, 1970) and soluble DM accumulation differs considerably between the leaf-sheath enclosed and true-stem exposed parts (88% of fructans found in the enclosed part) (Gebbing, 2003). Furthermore, low values for SW in lower leaf-sheaths could be related to earlier onset of senescence, earlier accumulation and loss of WSC compared to upper sections and shading by the upper canopy.

In general, good broad sense heritability was shown for all true-stem internodes and morphological characteristics among the 26 CIMCOG cultivars. The peduncle

generally showed the greatest broad sense heritability values for t-s internode traits. High broad sense heritability values were also shown for leaf-sheath morphological attributes across all the leaf sheaths. Previous studies have also shown high heritability values for stem morphological attributes, such as stem length and internode 2 length and thickness under water deficit and irrigated environments (Saint Pierre et al., 2010).

6.3.1 Associations between true-stem morphological attributes and spike growth

It was hypothesized that the competition for assimilates between growing stems and spikes differs among true-stem internodes, related to the synchrony of upper internodes extension and the rapid spike growth phase. A negative association was found between the DM partitioned to the true-stem and the spike at GS65+7d among the 26 cultivars presumably related to structural DM in the true-stem from GS41 to GS65+7d competing with assimilate supply to the spike. Assimilates partitioned to the spike determine the proportion of floret primordia as competent florets at anthesis (Fischer, 1985) since timing for rapid growth of stem (stem elongation) and spike coincide (Kirby, 1988). Moreover, true-stem internodes elongate sequentially up the stem in wheat (Borrell et al., 1993), so competition with spike growth differs according to upper and lower stem internodes. For instance, Borrell et al. (1991) concluded that a greater allocation of assimilate to the developing spike than to the stem in near-isogenic wheat lines containing the *Rht* gene was particularly greater at the time of maximum stem growth (17 days before anthesis), suggesting that a negative association between SPI and true-stem PI is more likely to occur during upper internodes DM accumulation. Present results for the 26 cultivars showed cultivars partitioning less DM to the t-s 2 and t-3 tended to have greater SPI at GS65+7d. This suggested that reduced partitioning to the t-s ped and t-s rem may be less important targets for wheat breeding to enhance SPI. Although it has been suggested that reductions in peduncle length may favour spike partitioning (as this organ extends most rapidly before anthesis when the grain number is determined) (Richards, 1996), the present results may reflect that the peduncle was still elongating

and accumulating structural DM after anthesis, probably coinciding less with floret mortality shortly before anthesis than t-s 2 and t-s 3. However, any attempt to reduce height of specific stem internodes to enhance SPI must likely be done without affecting overall plant height greatly, as the optimum values have been reached for modern cultivars and are estimated to range from ca. 70 – 100 cm (Richards, 1992; Miralles and Slafer, 1995a). Moreover, since t-s 2 and t-s 3 PIs are difficult to measure it is important to identify other stem-phytomer morphological attributes indicative of DM in these internodes that breeders could select for to reduce partitioning to these internodes. In this respect, present results showed that reduced internode length and reduced SW each contributed to reduced internode DM shoot^{-1}, and in addition there were no associations between SW and length, So selection for these two traits (reduced internode length and SW) may have additive effects allowing targeted changes in true-stem DM to increase SPI.

6.3.2 Interrelationships between stem-phytomer morphological attributes and effects of plant height and phenology

Results shown in the previous chapter indicate that decreases in true-stem DM are a key target to attain greater DM proportion to the spike during pre-anthesis. Moreover, as it may not be not feasible to achieve this by further major decreases in plant height, it is important to determine key stem morphological traits determining true-stem internode DM variation (*e.g.* length or SW). The fact that total stem length was not strongly related to stem length among the 26 CIMCOG cultivars suggested that stem morphology varies among its different phytomers partly independent of plant height. The t-s ped DM shoot^{-1} showed the greatest genetic variation independent of the total true-stem DM shoot^{-1} (per shoot and per unit area basis) Morphological attributes explaining true-stem internode DM among the 26 cultivars are discussed below.

True-stem internode DM was similarly explained by greater length and SW, with the exception of t-s DM rem which was more strongly associated to its length (r =0.85, P <0.001) than to its SW (r =0.64, P <0.001). True-stem SW decreased progressively down the stem and its genetic variation among internodes was not generally explained by internode length. Ehdaie et al. (2006) in a study evaluating genotypic

variation for internode morphological characteristics in bread and durum wheat, reported that true-stem internode DM showed a greater effect on the determination of SW than stem length, suggesting that selection for stem weight is more efficient to increase stem SW than selection for shorter stems.

In summary, given true-stem internode DM is highly related with both respective SW and length and genetic variation in SW appeared to be independent of length, reductions in true-stem DM without affecting stem length could be achieved by changes in true-stem internode SW. Reductions in length of t-s 2 and t-s 3 could also contribute to reduction in true-stem DM if genetic variation in these internode lengths can be identified independent of overall plant height. In contrast, leaf-sheath DM was almost entirely explained by their respective SW and only the l-s ped DM showed associations with length, indicating that overall changes in leaf-sheath DM could only be achieved by changes in leaf-sheath SW. Further work will require the investigation of the associations between simultaneous changes in true-stem internode DM and leaf-sheath sections DM to examine the extent of independence between the morphological attributes of these organs.

Most of the studies examining stem morphology are aimed to achieve greater stem WSC% and mobilization for grain growth (post-anthesis). However, as shown in chapter 5, true-stem soluble DM accumulation appeared not to be competing sink with the spike at GS65+7d, thus to increase SPI it is necessary to consider mostly true-stem structural DM accumulation across its different internodes. Furthermore, there is need to investigate if a reduced capacity to store structural DM will diminish the capacity to store soluble DM among the internodes (*i.e.* is capacity to store soluble DM independent of maximum structural DM accumulation?).

Considering true-stem DM reductions in upper internodes (and reductions in SW) as key traits to be used in breeding programs, it is crucial to examine overall effects of plant height and phenology on true-stem internode morphological attributes. The rationale is that major changes in plant height and/or phenology are unlikely since most growing wheat regions have optimized these traits. Final plant height is the result of the true-stem internodes extension following a logistic pattern of growth

(acropetally up the stem) (Bonnett and Incoll, 1992) and true-stem internode length appears to be greater gradually for upper internodes. Overall among the CIMCOG cultivars in the present study, final plant height appeared to have a greater effect in the determination of DM in upper internodes (t-s ped, t-s2 and t-s 3) but was not related to any morphological trait in t-s rem. Encouragingly, plant height was not related to t-s ped SW and t-s 3 SW, although it had a positive effect on t-s 2 SW. On the other hand, plant height was positively associated with leaf-sheath DM and length just for the peduncle but not with any leaf-sheath section SW, including the peduncle. These results suggest potential decreases in SW among true-stem internodes without any major effects on final plant height are possible.

Regarding effects of key phenological traits on stem-phytomer morphology, date of initiation of booting (GS41) or anthesis (GS65) did not affect SW for any internode. However, a longer period of stem elongation (days GS41 to GS65) seemed to benefit greater SW for all the internodes, except for t-s 3.

6.3.3 True-stem internode WSC accumulation at GS65+7d and relations with internode characteristics

Upper internodes have been highlighted as an important source of assimilates accumulated after anthesis and lower internodes as the main potential contributors of pre-anthesis reserves (Bonnett and Incoll, 1993). In order to examine potential trade-offs between reductions in true-stem DM to particular internodes and true-stem WSC accumulation at GS65+7d, it is important to determine genetic variation in WSC accumulation among the different true-stem internodes,. Previous studies have shown that true-stem WSC accumulation differs among true-stem internodes (Wardlaw and Willenbrink, 1994; Cruz-Aguado et al., 2000; Ehdaie et al., 2006a) in wheat (Rawson and Evans, 1971). In the present study, there were significant differences in WSC concentration among true-stem internodes, averaging 10.3% for the peduncle, 14.8% for the internode 2 and 18.4% for the remaining internodes among the nine cultivars. In a study evaluating the effect of internode characteristics on WSC related traits, Ehdaie et al. (2006b) reported WSC % similar to present results, with WSC % considerably greater for the lower internodes (10.3, 14.2 and 27.9%, respectively for

the peduncle, internode 3 and the remaining section). Ehdaie et al. (2006a) also showed that differences in WSC accumulation among internodes were related to internode morphological characteristics. As mentioned in 6.4.1, this study demonstrated substantial genetic variation for true-stem morphological characteristics (DM, length and SW) among the different internodes, possibly having an effect on internode capacity to accumulate WSC among the 26 CIMCOG cultivars.

Developmentally, potential stem reserve accumulation and subsequent mobilization in wheat depends on stem length and stem specific weight (Blum, 1998; Ehdaie et al., 2006b). Concerning associations between stem WSC accumulation and morphological attributes, patterns for the t-s 2 and t-s 3+r were opposite. Genetic variation in t-s 2 WSC_C at GS65+7d was significantly and negatively related to its length and a trend for negative association with its DM was apparent but not with its SW. This was similar to what observed for the peduncle. On the other hand, t-s 3+r WSCc was positively associated with its SW (r =0.67, P <0.05) and trends were observed for a positive association with its DM and length. Overall, genetic variation in WSC_C at GS65+7d was more dependent on SW for lower internodes and more dependent on internode length (negative correlation) for internode 2 and the peduncle. However, as maximum accumulation point for stem WSC occurred at *ca.* GS65+21 days, it is also possible that SW attained in upper internodes could be an important morphological trait to maximize WSC accumulation during grain filling.

The weak association between grains per spike and the peduncle WSC_C (just a trend for a positive linear relationship) compared to lower internodes WSC_C (significant positive linear relationships) could imply that remobilized WSC reserves accumulated in the peduncle could be more important during grain filling and potentially remobilized reserves in the lower t-stem internodes (accumulated in the pre-anthesis phase) are related with floret survival. It has been observed that fructans are accumulating while the internodes (mainly upper internodes) are still extending (Gebbing, 2003), turning upper internodes into an important source of post-anthesis reserves and lower internodes as the main potential contributors of pre-anthesis reserves (Bonnett and Incoll, 1993).

6.3.4 Conclusions

The answers to the hypotheses presented at the beginning of this chapter are outlined below:

1. Wide genetic variation and high heritability was found for true-stem and leaf sheath phytomer morphological characteristics among the 26 CIMCOG cultivars under irrigated high radiation conditions in NW Mexico.
2. Individual true-stem internode DM was highly correlated with and determined by internode SW rather than lengths among the 26 CIMCOG cultivars.
3. The negative relationship between the SPI and the true-stem DM partitioning at anthesis + 7 days was explained by DM partitioning to internode 2 and 3 rather than to the lower internodes among the 26 cultivars. However, spike DM amount at anthesis + 7 was related with the peduncle DM partitioning rather than other internodes among the 26 CIMCOG cultivars. In fact, the analysis showed that lower t-s ped PI and reduced peduncle length were more strongly associated with increased spike DM per unit area. The reasons for this require further investigation, in part they reflect that overall there was a relatively weak correlation between SPI and Spike DM per unit area amongst the 26 cultivars. However, if t-s ped PI is affecting spike DM per unit area, it would be expected that this would be reflected in greater SPI which was not the case in the present study. There may also be confounding effects of shoot density on internode SW.
4. Greater WSC concentration (%) in lower internodes at anthesis + 7 days was positively related to GSP at harvest but not to SPI at GS65+7d among the nine CIMCOG cultivars.
5. Genetic variation in true-stem DM morphological traits (such as DM and SW) at GS65+7d was partly independent of total stem length and of the onset of key phenological stages, but this varied among the internodes. Therefore, there is scope for breeding for true-stem internode morphological traits with minimal effects on plant heigt, anthesis date and duration of stem-elongation period among elite CIMMYT spring wheat cultivars.

6. Further work in more precise genetic material (mapping populations with a more uniform genetic background and/or isogenic lines) are needed to investigate further how the stem morphological traits scale up from effects at the single shoot scale to the crop scale. Overall, it is concluded that there is scope to breed for stem morphological attributes to enhance grain yield potential without affecting plant height.

CHAPTER 7.

SOURCE-SINK MANIPULATION TREATMENTS IN THE CIMCOG PANEL

7.1 Introduction

Historically, increases in yield potential have been mainly related to increases in grains m^{-2} rather than to greater individual grain weight, implying that grain yield in wheat is sink-limited during grain filling (Slafer and Savin, 1994). This source-sink balance status can be investigated through source-sink manipulation treatments. Although most of these studies have indicated grain yield of wheat crops is sink limited, some modern wheat cultivars have shown evidence of source limitation or co-limitation. These findings therefore point out the importance of achieving simultaneous improvements in source size during grain filling and sink strength during pre-anthesis, a crucial task in breeding for higher yield potential. However, further increases in grain sink strength must be accompanied with enough post-anthesis source capacity to fill extra grains. Avenues for this could be enhanced contribution of stem pre-anthesis assimilates to grain yield and photosynthesis up-regulation (*i.e.* response to reduced photosynthetic capacity relative to grain sink strength. Within this context, the main objective of the present chapter is to quantify variation in grain growth and photosynthesis responses to imposed reductions in post-anthesis photosynthetic capacity in four CIMCOG cultivars (indicating the extent of source limitation). More specific objectives involve investigating leaf-lamina spike photosynthesis up-regulation and leaf-sheath photosynthesis contribution to grain growth.

Specific hypotheses tested in this chapter:

1. The extent of source limitation differs between CIMCOG genotypes as indicated by grain weight responses to lamina defoliation and leaf-sheath shading treatments at GS65+10d.
2. Reductions in leaf-sheath photosynthesis (leaf-sheath shading) during grain filling result in source limitation of grain growth; as indicated by reductions in final grain weight compared to non-manipulated shoots.
3. There is an increase in pre-anthesis dry-matter translocation (DMT, mg), efficiency (DMTe, %) and contribution of pre-anthesis assimilates to final grain weight (CPA, %), as a response to lamina defoliation and/or leaf-sheath shading treatments.
4. There is an increase in spike photosynthesis rate as a response (*i.e*. up-regulation) to leaf-lamina defoliation and/or leaf-sheath shading treatments (*i.e.* source reduction) during grain filling, significantly differing among the four CIMCOG cultivars.

7.2 **Results**

Firstly, the present results section describes general crop growth and development at seven days after anthesis (GS65+7d) and key harvest traits, such as grain yield, yield components, harvest index (HI), above-ground dry-matter (AGDM) and plant height in the unmanipulated plot area for the four CIMMYT CIMCOG spring wheat cultivars. Then, grain yield responses to the defoliation treatment applied per unit area are described. Finally, dry-matter responses to treatments (defoliation and/or leaf-sheath covering) at physiological maturity, as well as flag-leaf and spike photosynthesis responses, are shown for treatments applied on a single shoot basis.

7.2.1 Crop development, growth and yield in unmanipulated plot area

Emergence date for all plots was 1 December 2013. Anthesis date differed between the four cultivars and was on average 85 days after emergence (P <0.001; Table 7.1). Above-ground dry-matter (AGDM) at seven days after anthesis (GS65+7d) was not significantly different among the four cultivars (P =0.055) while differences where observed for AGDM measured at harvest (Table 7.1). Spikes m^{-2} at GS65+7d ranged

from 322 (CMH79A) to 421 at GS65+7d (P <0.05). From the harvest growth analysis, trends for cultivars differences were observed for grain yield (P =0.061) and harvest index (HI) (P =0.096) but not for grains per spike (GPS) (P =0.109) and grains m^{-2} (P =0.187). There were cultivar differences for thousand grain weight (TGW) with a range of 7.3 (CMH79A) – 42.6 g (BECARD/K) (P <0.05). There were no trends for a negative relationship between grains m^{-2} and TGW, as the higher grains m^{-2} values did not resulted in lower TGW in the four selected cultivars. Spikes m^{-2} at harvest averaged slightly lower (345, P <0.01) than at GS65+7d (378). Finally, significant differences among the four cultivars were found for final plant height from 85 to 90 cm (P <0.01) (Table 7.1).

7.2.2 Responses to defoliation applied per unit area

Grain yield and spikes m^{-2} at harvest were also measured in the defoliated area (DEFarea) and results are shown in Table 7.2. There were statistically significant cultivar differences in spikes m^{-2} at harvest in the unmanipulated area (P <0.01) and in DEFarea (P <0.05). However as expected, spikes m^{-2} did not differ between unmanipulated and defoliated areas (P =0.913). This finding allowed a reliable comparison between DM responses in the control (CTRL) and DEF area measured on a single shoot basis samplings (Table 7.3).

In contrast with grain yield in the unmanipulated area, grain yield in the defoliated area differed between the four cultivars (P <0.01), ranging from 463 (SERI M82) to 556 g m^{-2} (BECARD/K) (100% DM) (Table 7.2). Analysis of variance for grain yield in both areas showed an effect of the treatment (P <0.001) but no cultivar x treatment interaction (P =0.467). The greatest reduction in grain yield in DEFarea was shown by the cultivar UP2338*2 (-25.4%) while the rest of cultivar showed similar reductions (-20.3 to -20.6%).

Table 7.1. Crop development, growth and yield in unmanipulated plot area for the subset of four cultivars in the field experiment in 2014. Grain yield (GY), yield components, harvest index (HI), plant height (PH), above-ground DM (AGDM) and spikes m^{-2} at harvest and at seven days after anthesis (GS65+7), days to anthesis (DTA in days after emergence).

Cultivars	**GY**	**TGW**	**HI**	**Grains**	**Spikes**	**GPS**	**PH**	**AGDM Harvest**	**AGDM GS65+7**	**Spikes GS65+7**	**DTA**
	g m^{-2}	**g**		**m^{-2}**	**m^{-2}**	**#**	**cm**	**g m^{-2}**	**g m^{-2}**	**m^{-2}**	**Days**
BECARD/K	699	42.6	0.50	16417	339	48.6	90.1	1385.5	1276.9	410.5	86.3
CMH79A.95	583	37.3	0.47	15630	282	55.9	84.8	1230.6	1324.8	322	89.7
SERI M82	661	41.9	0.56	15812	337	46.9	84.7	1179.5	1107.4	358	79.7
UP2338*2	717	40.1	0.49	17895	421	42.5	89.8	1469.2	1125.3	420.8	82.3
Mean	**665**	**40.5**	**0.51**	**16439**	**345**	**48.5**	**87.3**	**1316.2**	**1208.6**	**377.8**	**84.5**
P-value	**0.061**	**0.014**	**0.096**	**0.187**	**0.002**	**0.109**	**0.008**	**0.026**	**0.055**	**0.023**	**<0.001**
***SED* (df =6)**	**40.5**	**1.1**	**0.03**	**976.9**	**19.3**	**4.45**	**1.3**	**74.5**	**72.1**	**24.95**	**0.36**
***CV* (%)**	**7.5**	**3.4**	**7.2**	**7.3**	**6.9**	**11.3**	**1.9**	**6.9**	**7.3**	**8.1**	**0.5**

Table 7.2. Grain yield (GY 100% DM) and spikes m^{-2} at harvest in defoliated area (DEFarea) and GY responses (reductions, %) compared to unmanipulated area for four cultivars in the field experiment in 2014.

Cultivars	**GY DEFarea**	**GY reduction**	**Spikes DEFarea**
	g m^{-2}	**%**	**m^{-2}**
BECARD/K	556	20.4	354
CMH79A.95	463	20.3	298
SERI M82	523	20.6	320
UP2338*2	534	25.4	413
Mean	**524**	**21.8**	**350**
P-value	**0.004**	**0.603**	**0.018**
SED (df =5)	**11.39**	**5.12**	**22.91**
CV (%)	**2.7**	**29.5**	**8.1**
Unmanipulated and DEFarea			
Overall mean	**593**	**-**	**346**
***P-value* Cultivar**	**<0.001**	**-**	**<0.001**
***P-value* Treat.**	**<0.001**	**-**	**0.913**
***P-value* CxT**	**0.467**	**-**	**0.627**
***SED* (df =13) Cv**	**21.06**	**-**	**15.38**
***SED* (df =1) Treat.**	**14.89**	**-**	**10.88**
***CV* (%)**	**6.2**	**-**	**7.7**

7.2.3 Dry-matter responses to defoliation and leaf-sheath covering on a single shoot basis

Grains per spike (GPS) and individual grain weight (GW) at harvest, stem dry-matter translocation (DMT, g) from seven days after anthesis (GS65+7d) to physiological maturity (GS89), stem DM translocation efficiency (DMTe, %) and the contribution of stem pre-anthesis assimilate to grain (CPA, %) are shown in Table 7.3 for the control and the manipulation treatments on a single shoot basis. There were highly significant differences among cultivars and treatments for all these traits (P <0.001). Results for GPS in descending order averaged 69 for CTRL, 65 for LSCOV+DEF, 64 for LSCOV and DEF and 58 for DEFarea. The number of GPS in the CTRL (62 to 73

GPS) for the single shoot basis manipulations was higher than for the unmanipulated area (43 to 56 GPS), presumably due to a different methodology for its calculation; *i.e.* for GPS in the unmanipulated area, all the shoots were considered and the GPS is representative of the whole plot (yield components analysis, see section 3.5.3) while the GPS in the CTRL for the single shoot basis manipulation was done just using 12 main shoots. Although treatments differed for GPS (P <0.01), there was no significant cultivar x treatment interaction (P =0.601). Individual GW showed effects of treatments (P <0.001), as well as cultivar x treatment interaction (P <0.05). Results for individual GW, averaged as follows among the four cultivars: CTRL (40.8 mg), LSCOV (36.1 mg), DEF (33.5 mg), DEFarea (33.1 mg) and LSCOV+DEF (26.2 mg).

Weak trends for treatment differences were observed for stem DMT (g) and DMTe (%) (P =0.126 and P= 0.082, respectively) with no significant cultivar x treatment interaction. Treatments differed significantly for the percentage of stem contribution of pre-anthesis assimilate (CPA) (P <0.01) among the four cultivars; but no significant cultivar x treatment interaction was observed (P =0.659). The highest values of DMT (g), DMTe (%) and CPA (%) were shown by the DEFarea treatment and the lowest by the CTRL.

A significant effect of treatment was found for GPS (P <0.01). Individual GW differed significantly among treatments (P <0.001). Responses (reduction in manipulated compared to CTRL, %) in final individual GW differed among the four cultivars (P <0.01) but no particular pattern for more responsive cultivars was observed; *i.e.* some cultivars were more responsive in one treatment than other. For instance, CMH79A.955 showed the greatest reductions in GW within defoliation treatments (DEF and DEFarea) but not within LSCOV or LSCOV+DEF treatments. Overall among the four cultivars the greatest reductions in individual GW were observed for LSCOV+DEF (-36.2%), followed by DEFarea (-18.1%), DEF (-18.7%) and LSCOV (-10.3%). Reductions in individual GW were not statistically different between DEF (single shoot basis) and DEFarea. As a summary of individual GW responses, averaged individual GW reductions (%) for each treatment can be found in Figure 7.1 a. Similarly, CPA (%) averaged for each treatment is presented in Figure 7.1 b. No differences of CPA were found between CTRL, LSCOV and LSCOV+DEF

while an increase in pre-anthesis assimilates contribution was observed for DEF and DEF area.

Table 7.3. Grains per spike (GPS) and grain weight (GW) at harvest, dry-matter translocation from pre-anthesis reserves (DMT), dry mater translocation efficiency (DMTe) and contribution of pre-anthesis reserves (CPA) for the control and treatments, and their respective responses to the treatments (DEF, LSCOV, LSCOV+DEF and DEFarea) for the subset of four cultivars in the field experiment in 2014.

Dry matter	**Treatment**	**GPS**	**GW**	**DMT**	**DMTe**	**CPA**
Cultivars	*Units*	*#*	*mg*	*g*	*%*	*%*
BECARD/K	CTRL	64.5	42	0.275	15.5	10.1
CMH79A.955	CTRL	73.1	41.4	0.304	14.6	10.7
SERI M82	CTRL	67.6	41.2	0.101	6.2	3.7
UP2338*2	CTRL	61.8	38.5	0.145	8.4	6.0
Mean	**CTRL**	**66.8**	**40.8**	**0.206**	**11.2**	**7.6**
BECARD/K	DEF	-	34.6	0.340	19.2	17.4
CMH79A.955	DEF	71.8	32.5	0.381	18.3	23.7
SERI M82	DEF	60.3	36.3	0.324	19.8	11.9
UP2338*2	DEF	59.4	30.7	0.082	4.8	4.4
Mean	**DEF**	**63.8**	**33.5**	**0.348**	**15.5**	**14.4**
BECARD/K	LSCOV	63	37.5	0.299	16.9	12.7
CMH79A.955	LSCOV	71.2	36.1	0.454	21.7	17.7
SERI M82	LSCOV	62.6	36.1	0.279	17.1	12.4
UP2338*2	LSCOV	59.5	34.6	0.203	11.9	9.7
Mean	**LSCOV**	**64.1**	**36.1**	**0.309**	**16.9**	**13.1**
BECARD/K	LSCOV+DEF	60.6	24.7	0.282	15.9	19.2
CMH79A.955	LSCOV+DEF	76.1	27.5	0.352	16.8	17.5
SERI M82	LSCOV+DEF	63.2	27.7	0.181	11.0	10.5
UP2338*2	LSCOV+DEF	59.2	24.8	0.125	7.3	8.8
Mean	**LSCOV+DEF**	**64.8**	**26.2**	**0.234**	**12.8**	**13.6**
BECARD/K	DEFarea	56.6	34.6	0.276	15.5	17.5
CMH79A.955	DEFarea	-	30.4	0.319	15.2	22.8
SERI M82	DEFarea	60.7	35.8	0.278	17.0	13.0
UP2338*2	DEFarea	55.9	31.7	0.233	14.1	14.1
Mean	**DEFarea**	**57.7**	**33.1**	**0.291**	**15.5**	**16.8**
Overall mean		**63.4**	**33.9**	**0.263**	**14.4**	**12.7**
***P-value* Cv**		**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**
***P-value* Treat.**		**0.002**	**<0.001**	**0.126**	**0.082**	**0.004**
***P-value* CxT**		**0.601**	**0.014**	**0.451**	**0.271**	**0.659**
***SED* (df) Cv**		**1.25 (30)**	**0.544 (31)**	**0.0353 (32)**	**1.877 (32)**	**1.968 (25)**
***SED* (df) Treat.**		**1.398 (4)**	**0.608 (4)**	**0.0395 (4)**	**2.099 (4)**	**2.2 (4)**

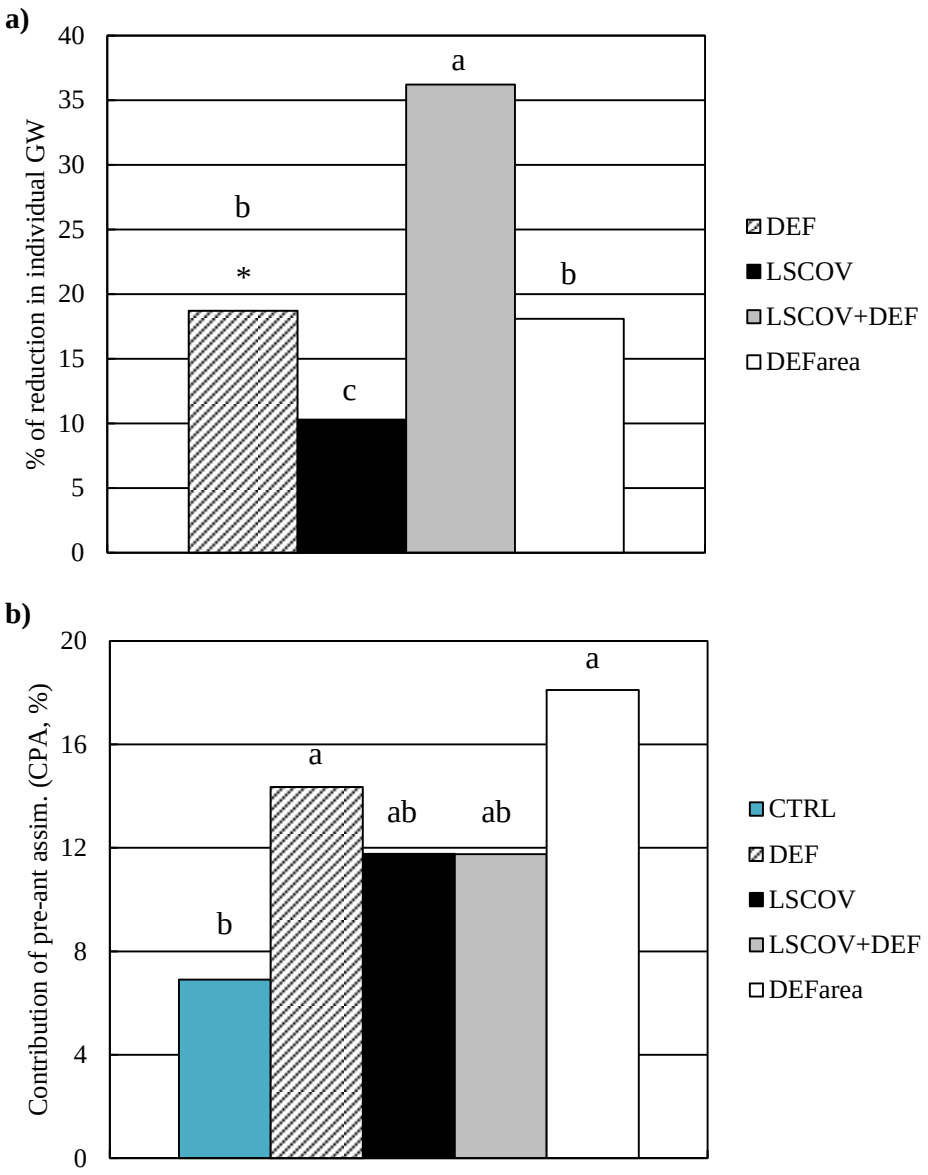


Figure 7.1. Summary of a) grain weight (GW) responses (% of reduction) in DEF (SED =2.033; df =4), LSCOV (SED =3.37; df =4), LSCOV+DEF (SED =4.12; df =5) and DEFarea (SED =2.95; df =4) compared to CTRL and b) contribution of true-stem plus leaf-sheath pre-anthesis assimilate (CPA; %) to the grain in CTRL (SED =4.13; df =6), DEF (SED =6.02; df =1), LSCOV (SED =2.438; df =5), LSCOV+DEF (SED =2.777; df =4) and DEFarea (SED =7.59; df =3). Values represent means for the four CIMCOG cultivars. Bars represent SED for each treatment. * = significant differences among the four cultivars at P <0.05. Different letters indicate differences among treatments.

7.2.4 Flag-leaf and spike photosynthesis responses

Two different readings of flag-leaf and spike photosynthesis were performed at 15 and 25 days after anthesis and are presented in Table 7.4 for each cultivar and treatment measured. Photosynthesis readings were significantly higher at GS65+15d than at GS65+25d (analysis not shown). It was not physically possible to measure the two stages evenly for all cultivars, treatments and organs, so stages analysed are presented separately. No photosynthesis measurements were conducted in cultivar CMH79A and in treatment DEFarea.

While spike photosynthesis per unit area (as net CO_2 uptake, μmol CO_2 m^{-2} s^{-1}) at GS65+15d differed between cultivars BECARD/K and SERI M82 (P <0.01), cultivar differences were not observed at GS65+25d (P =0.76). On the other hand, spike photosynthesis per organ (as net CO_2 uptake, μmol CO_2 $Organ^{-1}$ s^{-1}) showed differences among cultivars at both stages, at GS65+15d (P <0.001) and at GS65+25d (P <0.05), between BECARD/K and SERI M82 at GS65+15d and between BECARD/K, SERI M82 and UP2338*2 at GS65+25d. A summary of results for spike photosynthesis per organ at GS65+25d averaged for each treatment is shown in Figure 7.2 a.

Flag-leaf photosynthesis per unit area (as net CO_2 uptake, μmol CO_2 m^{-2} s^{-1}) did not differ between cultivars at either GS65+15d (P =0.56) or GS65+25d (P =0.289). When expressing flag-leaf photosynthesis per organ (as net CO_2 uptake, μmol CO_2 $Organ^{-1}$ s^{-1}), marginal differences were observed between cultivars (BECARD/K, SERI M82 and UP2338*2) just at GS65+25d (P =0.062), but not in the earlier stage (GS65+15d, P =0.138) among CTRL and LSCOV. A summary of flag-leaf photosynthesis per organ at GS65+25d averaged for each treatment is shown in Figure 7.2 b.

Although no significant effects of treatment or cultivar x treatment interactions were observed for photosynthesis in either combination of organ, stage or unit basis (area or organ), there was a tendency for spike photosynthesis to be upregulated in LSCOV compared to the CTRL for the cultivar SERI M82.

Table 7.4. Flag-leaf and spike photosynthesis (A_{sat}) per area (Gross; µmol CO_2 m^{-2} s^{-1}) and per organ (µmol CO_2 $organ^{-1}$ s^{-1}) at 15 (GS65+15) and 25 days after anthesis (GS65+25) in CTRL, DEF10, LSCOV and LSCOV+DEF for four CIMCOG cultivars in the field experiment in 2014.

A_{sat}	**Gross Photos**				**Organ Photos**			
Units	(µmol CO_2 m^{-2} s^{-1})				(µmol CO_2 $Organ^{-1}$ s^{-1})			
	GS65+15		**GS65+25**		**GS65+15**		**GS65+25**	
	Flag-leaf	Spike	Flag-leaf	Spike	Flag-leaf	Spike	Flag-leaf	Spike
BECARD/K								
CTRL	27.3	15.7	20.7	10.2	0.097	0.049	0.073	0.029
DEF	-	16.1	-	12.2	-	0.050	-	0.034
LSCOV	29.1	15.5	24.4	12.2	0.104	0.048	0.090	0.039
LSCOV+DEF	-	12.6	-	10.5	-	0.050	-	0.033
Mean	**28.2**	**15.0**	**22.5**	**11.3**	**0.101**	**0.049**	**0.081**	**0.034**
SERI M82								
CTRL	28.2	9.6	28.1	11.0	0.117	0.031	0.112	0.039
DEF	-	9.1	-	11.4	-	0.029	-	0.042
LSCOV	26.4	14.6	23.8	9.1	0.110	0.048	0.095	0.033
LSCOV+DEF	-	10.3	-	11.2	-	0.033	-	0.040
Mean	**27.3**	**10.9**	**26.0**	**10.7**	**0.1137**	**0.035**	**0.104**	**0.039**
UP2338*2								
CTRL	-	-	22.4	11.5	-	-	0.07	0.034
DEF	-	-	-	9.6	-	-	-	0.027
LSCOV	-	-	21.7	10.8	-	-	0.074	0.031
LSCOV+DEF	-	-	-	12.4	-	-	-	0.032
Mean	**-**		**22.1**	**11.1**			**0.072**	**0.031**
Overall mean	**27.74**	**12.94**	**23.5**	**11.01**	**0.107**	**0.042**	**0.086**	**0.035**
***P-value* Cv (Df)**	**0.56 (6)**	**0.002 (13)**	**0.289 (10)**	**0.76 (22)**	**0.138 (6)**	**<0.001 (14)**	**0.062 (10)**	**0.023 (22)**
***P-value* Treat. (Df)**	**0.969 (1)**	**0.134 (3)**	**0.836 (1)**	**0.901 (3)**	**1 (1)**	**0.263 (3)**	**0.912 (1)**	**0.983 (3)**
***P-value* CxT (Df)**	**0.268 (6)**	**0.159 (13)**	**0.330 (10)**	**0.287 (22)**	**0.371 (6)**	**0.121 (14)**	**0.404 (10)**	**0.194 (22)**

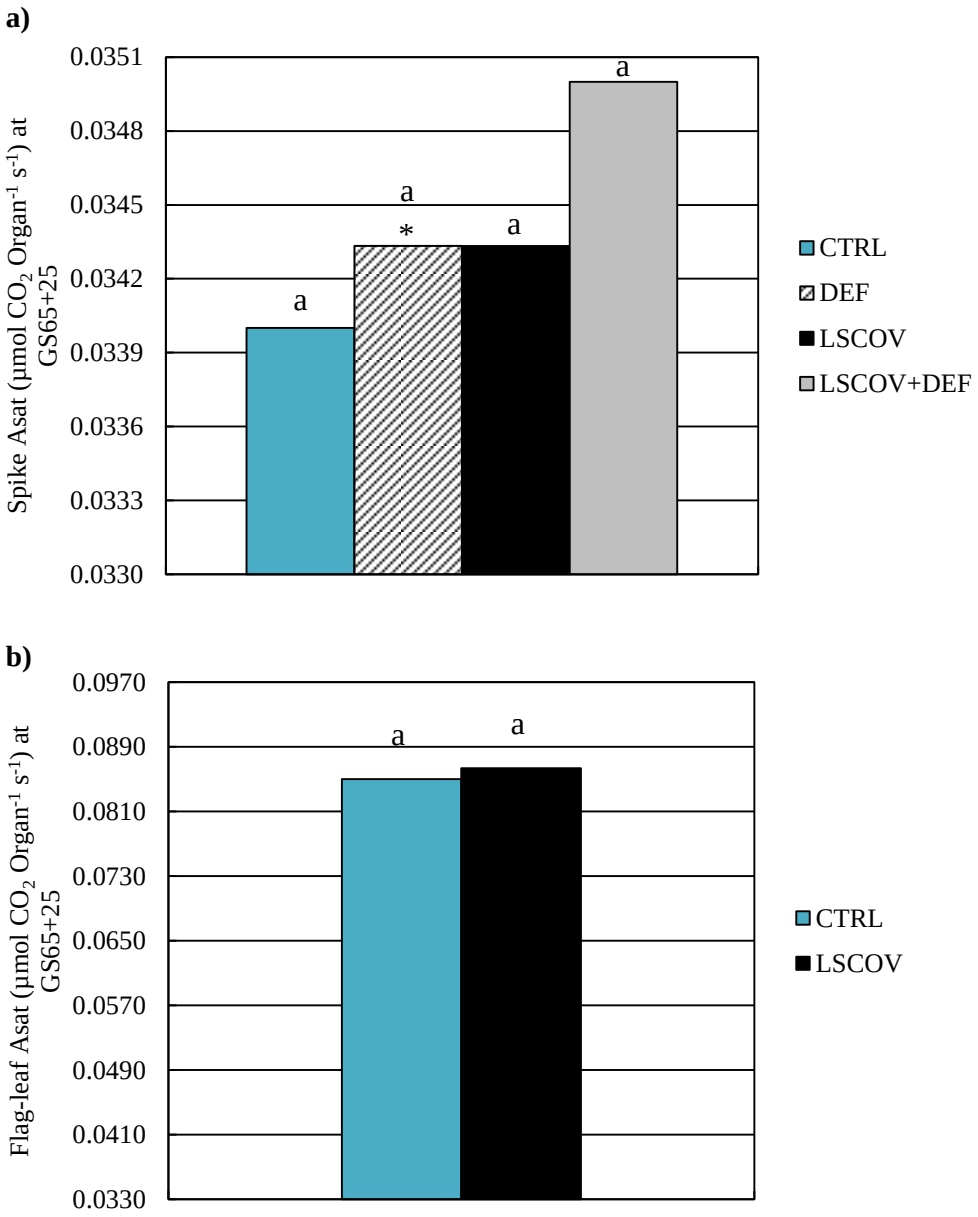


Figure 7.2. Summary of a) spike photosynthesis per organ in CTRL (SED =0.0077), DEF (0.003), LSCOV (SED =0.00413) and LSCOV+DEF (SED =0.0048) (df =4) and b) Flag-leaf photosynthesis per organ in CTRL (SED =0.0132) and LSCOV (SED =0.0205) (df =4). Values represent means for the cultivars measured for each treatment (see table 7.5). Different letters indicate differences among treatments * = significant differences among the four cultivars at P <0.05.

7.3 **Discussion**

A trade-off between the grain number per unit land area and average grain weight is commonly reported (Slafer et al., 1994, 2014; Miralles and Slafer, 1995b; Sadras and Lawson, 2011) and the most common interpretation is increased competition for assimilate during grain filling as grains m^{-2} increases (Miralles and Slafer, 2007; Acreche and Slafer, 2009). In this respect, artificial manipulations in assimilate availability (source-sink treatments: defoliation and leaf-sheath shading) can provide indirect evidence on whether yield may be either limited by its source strength, the sink capacity or co-limited by both (Borrás et al., 2004). A large number of studies analysing source and sink balance during grain filling in wheat agreed that increases in assimilate availability by de-graining the spike did not result in considerably greater grain weight (Miralles and Slafer 1995, Borrás et al., 2004; Calderini et al., 2006), indicating that source strength is not the major factor limiting grain growth under favourable conditions. However, some reports disagree with this conclusion, and examples in the literature with grain weight responding to source–sink manipulations during grain filling can be also found in wheat (Bremner and Rawson, 1978; Fischer and HilleRisLambers, 1978; Bindraban, 1996; Sandaña et al., 2009). As explained in section 3.5.5 in Chapter 3, the source was reduced a few days after anthesis in four CIMCOG cultivars. This was done through the application of different shading and excision treatments, in order to quantify genetic variation in final grain weight responses and to determine source and/or sink limitations to grain growth during grain filling under yield potential conditions.

7.3.1 Grain weight responses to source-sink manipulation treatments

In the present study, grain yield was considerably reduced in the defoliation per unit area treatment (20% average) (due to the reduction in grain weight) as a response to reductions in light interception, therefore in photosynthesis. However, even with the removal of all photosynthetically active leaves (flag leaf, 2 and 3) at GS65+10d, the extent of source-limitation (as indicated by reductions in grain weight) was moderate. Previous studies have reported estimates of flag-leaf photosynthesis contribution to be an average of 5 to 10% to final grain yield under irrigated conditions (Carr and

Wardlaw, 1965; Evans and Rawson, 1970; Evans et al., 1975; Aggarwal et al., 1990; Sanchez-Bragado et al., 2014). On the other hand, contribution of assimilates partitioned to the grains by the top three leaves have been estimated at ca. 20% (Thorne, 1982; Kruk et al., 1997). As expected, reductions in grains m^{-2} and GPS in defoliated (area and individual basis) and shaded plants were not significant, since the treatments were applied at GS65+10d when grain number is already set (Foulkes et al., 2009). Reductions in average grain weight by defoliation on a single shoot basis differed significantly among the four cultivars, ranging from 13% to 24% and were probably affected by differences in potential grain weight (as suggested by cultivar differences in TGW in the unmanipulated area). Although differences in contribution of pre-anthesis assimilate and photosynthesis compensation of non-affected organs were not clearly observed, it is possible that these organs might have contributed to lessen the effect of the artificial source reduction. In this respect, Fischer and HilleRisLambers (1978) stated that potential grain weight is mostly independent of factors affecting post-anthesis assimilate supply but it may be affected directly by the cultivar and other environmental factors.

It was expected that reductions in grain weight in defoliated shoots on a single shoot basis were going to be greater than those defoliated on a per unit basis, as the latter could presumably have higher amounts of light intercepted by the canopy (stems) as shading from neighbour plants was reduced. However, grain weight reductions for these two treatments were very similar (-18.7% single shoot basis and -18.1% area basis), suggesting there was no compensation by an increased photosynthesis of the leaf sheath and peduncle induced by extra incident radiation. In this sense, the photosynthetic contribution of the top three leaves to final grain yield was consistently close to 20%, with significant differences among the four genotypes. Leaf-sheath shading (included exposed peduncle true-stem) did significantly reduce grain weight compared to control shoots; estimating that this organ contributed an average of 10.3% to final grain weight with no differences between cultivars. As previously mentioned in the literature review, leaf-sheath photosynthesis is more relevant during grain filling, especially during the second half of grain filling when temperatures are higher and leaf-blades have started to senesce. The highest levels of

flag-leaf lamina and sheath photosynthesis during the crop cycle coincide with maximum accumulation of water soluble carbohydrates in both organs (Araus and Tapia, 1987). On the other hand, the upper part of the peduncle has a more autotropic carbohydrate metabolism as it is more exposed to irradiance and possesses a different light environment (Gebbing, 2003). Thus, it is possible that grain weight reductions (in shaded stems) were partly related to decreases in photosynthesis in the exposed part of the peduncle, as this internode has been shown to contribute to overall photosynthesis during grain filling (Kong et al., 2010). For instance, Wang et al. (2001) suggested that photosynthesis in the exposed peduncle and flag leaf sheath contribute about 9-12% of grain dry mass with cultivar differences in 26 winter wheat genotypes contrasting in spike morphology but similar in anthesis date.

On the other hand, more drastic reductions in post-anthesis assimilate availability among the four CIMCOG cultivars were shown by combining leaf-lamina defoliation and leaf-sheath covering treatments. In this treatment, final grain weight was reduced by 36.2%. However, when leaf sheath covering and defoliation covering were applied separately, the grain weight was reduced in total almost by 30% (-10% and -19%, respectively). The small difference between the two (*ca.* 8%) could be related to a lack of overall stem assimilate accumulation and translocation to the grain in the more drastic treatment (leaf-sheath covering + defoliation) compared to the summed effect of both treatments applied separately; implying that a reduction in leaf photosynthesis in source demand did only affect assimilate translocation from the stems to the grains without having any effect on current photosynthesis (Álvaro et al., 2008) as suggested by the results.

In summary, these results highlight the importance of the source strength during late grain filling as indicated by reductions in final grain weight when shading the leaf-sheaths. Although this could suggest a possible source or co-limitation during grain filling, the extent of reductions in final grain weight was relatively low compared to the reduction in light interception by the treatments (*ca.* 40% in the defoliation treatment; comm. Pers. Sylvester-Bradley, 2013). However, potential grain growth co-limitation by source and sink during grain filling could be offset through up-regulation of photosynthesis, as suggested by Reynolds et al. (2005) who showed that

increased sink demand (by increases in radiation interception during booting stage through opening rows) caused higher post-anthesis crop growth and radiation use efficiency (RUE).

7.3.2 Stem dry-matter responses to source-sink manipulation treatments

During the grain filling period, assimilate supply can be provided by three different sources: (i) remobilization of stem reserves (Schnyder, 1993), (ii) current post-anthesis lamina and stem photosynthesis (Parry et al., 2011) and (iii) spike photosynthesis (Maydup et al., 2010). Previous studies have reported that carbohydrates stored in the stem prior to anthesis may contribute between 5 to 10% to final grain weight (Wardlaw & Porter 1967; Serrago et al, 2013). Similarly in the present study, reductions in final grain weight ranged from X (LScov) to X% (DEF+LScov). However, studies showing reductions in final grain weight due to leaf sheath photosynthesis inhibition are scarce. Moreover, genetic variation in the extent of response (final grain weight reductions) to source reduction treatments was clearly shown among the four cultivars studied in 2014. Regarding the stem DM responses to the treatments, contribution of pre-anthesis assimilate (CPA) was 7 to 10% higher in the DEF treatment compared to the control. However, this was not enough to compensate for full grain weight in the treatments, suggesting moderate source limitation. Previous studies imposing source-sink manipulation treatments have also reported increases in remobilization of stem pre-anthesis reserves (Bonnett and Incoll, 1993; Beed et al., 2007; Merah and Monneveux, 2015); in contrast no effects were observed in other defoliation studies (Álvaro et al., 2008). Nonetheless, different results in the contribution of pre-anthesis reserves among the studies could be attributable to environmental differences (Gebbing and Schnyder, 1999; Cruz-Aguado et al., 2000; Álvaro et al., 2008; Ahmadi et al., 2009; Sanchez-Bragado et al., 2014). Overall, a higher stem assimilate remobilization in defoliated shoots indicated potential for improvement in re-translocation efficiency through breeding and in the source-sink balance. This will become a crucial aspect to investigate in the case that modern cultivars may become more source limited. For instance, Álvaro et al. (2008) showed that the efficiency of translocation (DMTe, *i.e.* the ratio between translocated

stem dry mass to stem dry mass at anthesis) has increased in recent cultivars of durum wheat, presumably due to increases in grain sink strength and demand. However, among the four CIMCOG cultivars analysed in 2014, neither DMT nor DMT*e* were statistically higher in the manipulation treatments compared to the control.

7.3.3 Flag-leaf and spike photosynthesis responses to source-sink manipulation treatments

The respective contribution of photosynthesis of a given organ (spike or flag leaf), as well as the stem assimilate remobilization to grain yield, showed considerable genetic variation among CIMCOG cultivars (as indicated by the final grain weight response). The removal of leaves has been reported to enhance the photosynthetic capacity of other green parts and these mechanisms can avoid the restriction of grain filling in such a manner that often no source limitation occurs (Cruz-Aguado et al., 1999). However, in the present results high reductions in light interception, therefore in photosynthesis did not result in significant photosynthesis compensations from unaffected photosynthetic organs (flag leaf and/or spike), in contrast with other studies (Aggarwal et al. 1990; Zhu et al. 2004; Cruz-Aguado et al. 1999). For example, in an experiment involving a drastic photosynthesis inhibition (whole canopy below the spike shaded) and stem +leaf sheaths shading performed in one wheat cultivar and one barley cultivar, Serrago et al. (2013) showed that spike photosynthesis in shaded shoots was consistently higher than in unshaded shoots in both species. Results from the present study on the other hand, suggest that although some extent of source or co-limitation was indicated by reductions in final grain weight, there was no apparent up-regulation of gas exchange in manipulated shoots, contrary to the responses observed in pre-anthesis assimilate translocation. Nonetheless, there was a trend for flag-leaf photosynthesis in the leaf sheath covering treatment in the cultivar SERI M82 at GS65+25 days.

7.4 Conclusions

The answers to the hypotheses presented at the beginning of this chapter are outlined below:

1. Different extent of source limitation was shown among the four CIMCOG cultivars as indicated by grain weight responses to lamina defoliation and leaf sheath shading treatments at GS65+10d.
2. Leaf sheath shading reduced general post-anthesis photosynthetic capacity among the four cultivars, limiting normal grain growth (10%); indicating that leaf sheath photosynthesis contributes effectively to grain filling, presumably most actively during its late stages.
3. Pre-anthesis dry-matter translocation (DMT, mg) and translocation efficiency (DMTe, %) were not significantly greater in the treatments compared to the manipulated shoots, but there was a significant increase in contribution of pre-anthesis assimilates to final grain weight (CPA, %) in the defoliation (DEF and DEFarea) treatments compared to control shoots.
4. Spike photosynthesis differed significantly among the four cultivars but did not show significant increases in manipulated shoots (response or up-regulation) compared to control shoots.

Overall, results from Chapter 7 highlight the importance of maintaining source strength during grain filling, even considering that sink strength is the main limitation to yield in modern cultivars. In addition, contributions of leaf lamina, leaf sheath (+true-stem) and pre-anthesis reserves were estimated for different cultivars showing differences among genotypes. Moreover, the contribution of leaf-sheath photosynthesis was important for grain growth during grain filling among elite CIMCOG spring wheat cultivars, contrary to the often assumed idea that this organ does not actively contribute post-anthesis photosynthesis.

CHAPTER 8.

GENERAL DISCUSSION

8.1 Introduction

Wheat grain yield is generally reported to be sink limited during grain filling under favourable conditions (Borrás et al., 2004), and genetic progress in yield potential in the past has been achieved mainly through increases in sink strength (grains m^{-2} and HI) with no major changes in biomass (Siddique et al., 1989; Slafer et al., 1990; Sayre et al., 1997). However, HI has not increased in the recent decades (Reynolds et al., 1999) and the most recent evidence indicates increases in biomass accompanied with decreases in HI associated with yield grains in the modern CIMMYT spring wheat cultivars (Aisawi et al., 2015). Moreover, recent studies have shown grain yield in modern wheat cultivars to be closer to source-limitation or co-limitation by source and sink during grain filling (Cruz-Aguado et al., 1999; Acreche and Slafer, 2009; Aisawi et al., 2015). Therefore, further increases in yield potential will require simultaneous increases in biomass while optimizing DM partitioning into the reproductive organs.

Within this context, the present chapter focuses firstly on discussing a wheat cultivar ideotype for optimized DM partitioning and increased HI, including yield components, optimized DM partitioning among plant organs for better expression of grains m^{-2} and potential trade-offs with changes in DM partitioning through key growth periods. Then, this chapter highlights the practical applications of partitioning-related traits in breeding programs in NW Mexico and other representative wheat-growing environments.

8.2 High biomass cultivars and implications for wheat breeding

Although most of the studies on genetic progress in grain yield in spring wheat reported they are a consequence of increases in grains m^{-2} , there is recent evidence

for increases in AGDM, grain weight along with decreases in HI (Aisawi et al., 2015). A selected sub-set of eight representative historic cultivars in the present study showed linear increases in grain yield (25.3 kg ha^{-1} yr^{-1} or 0.38 % yr^{-1}), mainly related to increases in AGDM with year of release. The positive associations between grain yield and AGDM and trade-offs between AGDM and HI observed among CIMCOG cultivars suggested grain yield was mainly associated with AGDM. However, the association between grain yield and AGDM among the highest AGDM cultivars was not significant in the CIMCOG genotypes), probably associated with limitations to DM partitioning to grains or HI. Assuming constant biomass, yield and a reduction in competitive sinks (stems and sheaths), Austin (1980) calculated a hypothetical limit to HI of 0.62 with an increase in yield of 26%. Moreover, Foulkes et al. (2011) suggested that decreasing the chaff to grain ratio could further increase HI to ca. 0.64; nevertheless, HI values for spring wheat are currently around 0.45 for spring wheat and 0.50 for winter wheat (Fischer, 2007).

No systematic genetic progress in HI has been achieved over time in spring wheats since the 1990s (Reynolds et al., 2005), and further increases in HI (towards 0.60) will require altered partitioning to reproductive organs, *e.g.* reduced structural stem DM partitioning to benefit the spike but taking into account implications for trade-offs with lodging resistance, photosynthetic capacity and stem water soluble carbohydrate (WSC) accumulation

8.3 Ideotype with optimized DM partitioning for increased HI

Increases in harvest index in high biomass cultivars must take into consideration the main processes determining DM partitioning among plant organs during the crop cycle and having influence on the main yield components; as well as the source-sink balance relationships determining the sink strength and source during grain filling.

8.3.1 Yield components and positive departures for their optimization

Yield potential is the product of grains m^{-2} and final average grain weight. In the present study, there were no consistent associations between grain yield and its numerical components among the 26 cultivars, implying that both were equally

important in the determination of grain yield. Moreover, a strong trade-off between these yield components was observed in each season, indicating that further increases in grains m^{-2} might be partially offset by reductions in grain weight (Miralles and Slafer, 2007). Acreche and Slafer (2006) reported that trade-off was associated with increased proportion of distal grains with lower grain weight potential. Therefore, assuming there is no source limitation during grain filling, it seems that potential avenues to break the trade-off between the numerical components of grain yield will rely on increases in the intrinsic determinants of grain weight potential. On the other hand, a trade-off between the FE and potential grain weight has been reported (Gaju et al., 2009; Ferrante et al., 2012). Although this could be associated with the same physiological basis as the one suggested for grains m^{-2} and grain weight (greater FE resulting in more grains with less potential grain weigh), another hypothesis was proposed by Slafer et al. (2015). They hypothesized that this trade-off could be related to higher FE through lower minimum requirements for floret development and survival which, in turn, has a negative effect on carpel size before anthesis. Trade-offs between spike DM per unit area at GS65+7d or SPI and fruiting efficiency were not observed in the present study, indicating scope for simultaneously selecting for higher FE and Spike DM at anthesis.

Grains m^{-2} is also a key target for further improvements in yield potential and its physiological determination involves events mostly occurring during pre-anthesis. In the present study, genetic variation in grains m^{-2} was mostly associated with increases in fruiting efficiency, although a trend for non-linear association with SPI was apparent. Moreover, increases in spike DM were associated with greater SPI. Several studies have related genetic variation in spike DM per unit with grains m^{-2} (Fischer, 1985; Fischer and Stockman, 1986; Slafer et al., 1990; Miralles and Slafer, 2007) or with SPI at anthesis (Slafer et al., 1990). This association has its physiological basis in effects on floret survival and grain set. Several previous studies have reported strong associations between the fruiting efficiency and grains m^{-2} (Abbate et al., 1997; Fischer, 2011; Lázaro and Abbate, 2011), as well as a negative association between FE and DM partitioning to the spike at anthesis (Gaju et al., 2009; Lázaro and Abbate, 2011). However, there is no clear evidence for a constitutive trade-off

between these traits (Slafer et al., 2015), as other studies indicate evidence for independence between these traits (González et al., 2011; Ferrante et al., 2012; Bustos et al., 2013; García et al., 2014) and the cause of trade-offs is uncertain (Foulkes et al., 2015). The physiological basis of this trade-off could be related to the DM partitioning within the spike; *i.e.* non-optimized DM partitioning to the structural components of the spike (glumes, lemmas, paleas, rachis etc.). It has been hypothesized that reductions in DM partitioning within the spike to structural organs could benefit FE (Abbate et al., 1998). In the present study, FE was negatively associated with rachis specific weight and glumes DM partitioning among seven cultivars in 2012. On the other hand, some studies have reported greater spike WSC content at anthesis to be associated with improved floret survival and increased FE (Ghiglione et al., 2008; Dreccer et al., 2014b). However, in the present study there was no association between spike WSC % at GS65+7d and FE. Also in the present study genetic variation in crop growth rate during the stem-elongation period was not associated with more grains m^{-2}. The wide genetic variation found for FE indicates there is scope for selection for this trait within a wide range of other optimized physiological traits in high biomass CIMMYT spring wheat germplasm.

8.3.2 Potential avenues to increase spike DM partitioning

Dry-matter partitioning could be decreased at expense of alternative sinks in order to favour spike growth during stem elongation and spike DM partitioning at anthesis (Foulkes et al., 2011). Other competing sinks include roots, leaf laminae and sheaths, stems and infertile tillers (Bingham, 1969; Reynolds et al., 2009; Foulkes et al., 2011). However, it is important to consider potential trade-offs, for instance, reductions in leaf-laminae and sheaths may have negative effects on photosynthetic capacity-related traits. Wide genetic variation was found for SPI at GS65+7d among CIMCOG cultivars, showing values just slightly lower than previously reported for spring wheat. Dry-matter partitioning into the lamina and leaf-sheath (separately) showed no association with SPI among the 26 CIMCOG cultivars. Consistent with the present results, changes in DM partitioning to the lamina with plant breeding have been less important for increasing grains m^{-2} than those in the spike partitioning index

(Fischer et al., 1998). However, as further increases in AGDM are expected to meet requirements of increases in sink strength in modern spring wheat cultivars, decreases in leaf-lamina are not suggested in the present study. Spike partitioning index, however, showed strong negative associations with the true-stem DM, consistently among the 26 CIMCOG cultivars, the sub-set of nine cultivars with restricted anthesis date in all the seasons. True-stem soluble DM partitioning index was not associated with the SPI at GS65+7d, indicating that true-stem soluble DM accumulation was not competing with spike growth at anthesis; hence structural true-stem DM was mainly explaining the competition with spike growth and association with SPI. This was confirmed by a negative association between SPI and true-stem DM partitioning at harvest. A study- on UK winter wheat, shading field plots from leaf-emergence to heading, showed stem WSC was reduced proportionally more than the structural stem DM also concluded that stem WSC does not compete strongly with the spike for assimilate during stem elongation (Beed et al., 2007). Therefore, reductions in stem DM to favour spike growth should be directed towards structural DM. Recent evidence suggests small decreases in SPI with time in CIMMYT spring wheat cultivars since *ca.* 1980 to 2009, (Aisawi et al., 2015), so there is an urgent requirement for selection traits to increased SPI. Genetic variation in SPI was positively associated with HI in the present study.

The period shortly before anthesis (*ca.* 17 days before anthesis) coincides with floret death apparently associated with greater competition between growing stems and spikes (Fischer and Stockman, 1986; Borrell et al., 1991; Gonzalez et al., 2011). In this respect, it was hypothesised in the present study that the competition observed between SPI and true-stem DM partitioning was related to the structural DM accumulation in the upper internodes. Upper internodes extend shortly before and after anthesis coinciding with the rapid stem elongation phase. In summary, associations between SPI and HI and between spike DM at GS65+7d and grains m^{-2} among CIMCOG cultivars suggested that increased DM partitioned to the spike may favour overall expression of grain yield potential. Furthermore it is suggested that decreases in true-stem structural DM, particularly in upper internodes might be a more targeted avenue to benefit spike growth, rather than overall true-stem DM

decreases. Reduction in DM partitioning to the leaf-sheath might also be feasible as this was not related with SPI; however, potential trade-offs with photosynthetic capacity during grain filling need to be considered.

Wide genetic variation was found for true-stem and leaf-sheath morphological attributes among the different phytomers (internodes or sections). Moreover, all stem morphology related traits exhibited high heritability values. Associations between true-stem internode DM partitioning and SPI were explained by true-stem DM partitioning to t-s 2 and t-s 3. In addition, SPI was negatively associated with true-stem length for internode 2 and 3 and specific weight for each internode. Nevertheless, spike DM per unit area was negatively related to ped true-stem partitioning index and length rather than attributes of true-stem internode 2 and 3. Further work is required to confirm the mechanism associated with the association found between peduncle length and SPI and spike DM per unit area, since it would be anticipated to conclude that these traits would affect SPI, as this was not observed in the present study.

Encouragingly genetic variation for SW was independent of internode length, so reductions in true-stem DM without affecting stem length seem feasible by modifying true-stem internode SW. Genetic variation in leaf-sheath DM was almost entirely explained by leaf SW. Plant height did not affect leaf-sheath SW for any section. Present results suggest potential to decrease SW among true-stem internode and leaf-sheaths without any major effects on final plant height and/or changes in anthesis date. Potential trade-offs between reductions in structural stem DM and lodging resistance, however, may need to be addressed, considering the minimum stem DM requirements for plant support (Foulkes et al., 2011). However, as internode failure is more prone to occur in lower internodes (Berry et al., 2007), reductions in upper true-stem internode DM may not affect lodging resistance significantly. Changes in true-stem morphological attributes could also affect WSC accumulation, as potential stem reserve accumulation and subsequent mobilization in wheat depends on stem length and stem specific weight (Blum, 1998; Ehdaie et al., 2006a). Overall, genetic variation in WSC % at GS65+7d was more dependent on SW for lower internodes (positively) and negatively associated with length for upper internode (t-s 2 and t-s

ped) in the CIMCOG genotypes. However, as maximum accumulation point for stem WSC occurred at *ca.* GS65+21 days, it is possible that SW in upper internodes could be an important morphological trait to maximize WSC accumulation during grain filling.

Overall among CIMCOG cultivars, ranges for true-stem and leaf-sheath WSC at GS65+7d were small in the range 10.5 – 26.6% and 7.8 - 14.5%, respectively. These amounts are low compared to ranges in the literature for favourable conditions. For example, in the UK, Shearman et al. (2005) reported values of 244 to 391 g m^{-2} in the true-stem plus leaf-sheaths at GS61 for winter wheat cultivars in the UK. The genetic variation in true-stem and leaf-sheath WSC amount (g m^{-2}) was not significantly correlated with grain yield in the present study. In contrast, genetic variation in stem WSC amount has been shown to be positively correlated with grain yield in rain-fed conditions in the UK (Shearman et al., 2005), in irrigated conditions in NW Mexico (Saint Pierre et al., 2010) and under drought conditions in NW Mexico (Lopes and Reynolds, 2010; Saint Pierre et al., 2010) and Australia (van Herwaarden et al., 1998). It is possible that grain growth of the CIMCOG cultivars in the irrigated, high radiation conditions in the experiment was less source-limited than in these investigations. Moreover, stem WSC accumulated during grain filling up to 21 to 28 days after anthesis; suggesting that grain growth was not restricted by source supply during this phase of grain filling. In summary, although stem WSC was not associated to grain yield, it is possible that stem WSC may be more important in the future if CIMMYT releases start to become co-limited by source and sink.

8.3.3 Contribution of leaf-sheath photosynthesis during grain filling

Increases in sink strength (grain number) will need to be matched by increases in source, *i.e.* enough photosynthetic capacity during grain filling to fill extra grains, to avoid possible source limitation (Reynolds et al., 2012). Most of the studies analysing source and sink balance during grain filling in wheat have reported that increases in assimilate availability did not result in considerably greater grain weight (Borrás et al., 2004), indicating that source strength is not the factor limiting grain growth. However, some others have suggested that grain yield during grain filling in most

recent releases is getting closer to a source or co-limitation (Cruz-Aguado et al., 1999; Acreche and Slafer, 2009), especially during late grain filling. This could be a consequence of constant selection for greater sink strength (grains m^{-2} and average grain weight). Avenues to prevent source-limitation during filling under potential conditions are mainly related to assuring that photosynthetic capacity is sufficient to achieve full expression of grain weight potential during grain filling. Examples include prolonged stay-green (delayed onset of senescence), post-anthesis stem WSC accumulation and remobilization, increased post-anthesis RUE and flag-leaf/leaf-sheath/spike post-anthesis photosynthesis up-regulation. Stem pre-anthesis dry-matter translocation to grain growth can also be important, especially under unfavourable conditions during grain filling.

Source-sink manipulation treatments carried out in 2014 in four representative CIMCOG cultivars showed that grain yield reductions in defoliation on a per unit area basis were considerable (-20.3 to -25.4%), but the extent of source-limitation of grain growth appeared overall not to be high considering the removal of all photosynthetically active lamina at GS65+10d. Based on the reduction in green leaf area per shoot light interception was reduced by *ca.* 40% compared to the control; however, reductions in grain yield by defoliation during post-anthesis were not considerably less (21.8%). Leaf-sheath shading did reduced GW compared to control shoots by 10.3% similarly for the four cultivars. Moreover, there were trends for greater pre-anthesis stem pre-anthesis dry-matter translocation as a consequence of reductions in post-anthesis source. There was a weak trend for spike photosynthesis up-regulation as a consequence of leaf-sheath shading for one cultivar. Overall from the results on source-sink manipulation treatments, it is suggested that leaf sheath appears to make an important contribution to photosynthesis for grain growth, so there may be limited scope to reduce DM partitioning to leaf sheath pre-anthesis to increase SPI. In summary, present results may indicate that cultivars are still mainly sink limited, but modern high yielding CIMCOG cultivars may be closer to source limitation than their predecessors since there appeared to be relatively little excess current photosynthetic capacity (*i.e.* ability to up-regulate lamina and spike photosynthesis and pre-anthesis DM translocation) when relative grain sink strength

increases. Therefore, breeders may need to pay more attention to source-type traits in future in high biomass cultivars alongside increases in grain sink strength.

8.4 Practical application of optimized DM partitioning-related traits in breeding programs

According to the results from the present study and existing work, the present section describes the main potential applications of optimized DM partitioning-related traits use in breeding programs.

1. Grains m^{-2} was significantly associated with greater FE. Genetic variation in FE independent of spike DM per unit area among CIMCOG cultivars indicated that there is scope to select for FE without trade-offs with spike DM per unit area. Cultivar ranking was similar determining FE using spike DM per unit area at GS65+7d or with chaff DM at harvest. As determination of chaff DM per unit area is relatively simpler than spike DM per unit area at anthesis, these results suggested that estimation of FE with chaff DM could help trait-based breeding for FE allowing relatively high-throughput determination of this trait. According to the present results, scope for increases in FE might be related to the selection for lower rachis specific weight and spike DM proportion to the palea.
2. Greater DM partitioning to the spike could be attained by selecting for lower true-stem structural DM, considering minimum requirements for plant support to avoid stem lodging. There is scope to breed for stem morphological attributes to increase SPI without affecting plant height by reductions in stem specific weight (true-stem and/or leaf-sheath). Additionally, an extended duration of the rapid-spike growth phase (days GS41 to GS65) seemed to benefit SW. However, further work is required to refine true-stem internode targets to reduced true-stem structural DM in breeding programs (see section 8.7).
3. Genetic variation was found for AGDM at GS65+7d and it was associated with greater spike DM per unit area at the same stage, thus, to a certain extent

and depending on the environment, direct selection for AGDM at GS65+7d could be implemented as an avenue for increases in spike DM per unit area at anthesis. Indirect selection methods for anthesis biomass could include spectral reflectance and canopy temperature.

4. The present results indicated that higher WSC% could be selected for in lower internodes by selecting for high stem SW and increased in upper internodes by selecting for increased internode length.

5. The plant height in the CIMCOG lines was towards the upper end of the optimum plant height range, so there may be some scope to reduce height slightly to increase SPU, whilst maintaining radiation-use efficiency. However, this may depend on the plant system with taller plant apparently favouring high biomass in raised beds planting systems (Aisawi et al., 2015)

The above traits could be implemented when designing parental crosses in plant breeding programs aimed at raising spike fertility, HI and yield in plant breeding programs and/or when screening progeny in spike-rows or small plots in the later stages of the breeding program.

8.5 Use of the plant ideotype traits in other environments

The optimized-DM partitioning related traits investigated in the present study gave improved performance in the fully irrigated, high potential conditions of the study in NW Mexico. In other environments the optimization of the partitioning related traits could be different, depending on different duration of season and/or sowing date, planting system, extent of source-limitation during grain filling among other factors. For instance, cultivars sown under the raised beds planting system in NW Mexico are usually taller than cultivars sown under flat planting systems (*e.g.* 10 – 20 cm more than reported for modern wheat cultivars in many environments worldwide on flat planting systems) (Aisawi et al., 2015). Increased plant height allows better radiation interception in the gap between beds during vegetative growth before canopy closure and also during the critical period determining grain number from booting to anthesis (Fischer et al., 2005), possibly favouring grain number set as the pre-anthesis phase is

inevitably short in spring wheat under high radiation conditions. Therefore, wheat grown under raised beds may offer more possibilities for decreases in plant height as mechanism to reduce structural true-stem DM whilst maintaining biomass. In addition, grain growth in more source-limited environments during grain filling might rely more on pre-anthesis stem reserves that at the present site at Cd Obregon in NW Mexico. Therefore, there may be reduced scope for changes in stem morphology to achieve reductions in stem DM in more source-limited environments.

8.6 **Overall conclusions**

Present results showed that genetic progress in a sub-set of eight historic CIMMYT cultivars was associated with linear increases in AGDM and TGW, possibly related to increases in photosynthetic efficiency related traits and direct selection for heavier grains. Grain yield was explained mainly by differences in AGDM at harvest among the 26 CIMMYT spring wheat cultivars, but this was not the case for cultivars with the highest values of AGDM. Moreover, there were trade-offs between AGDM and HI. On the other hand, it seemed that genetic variation in grain yield was not explained by either variation grains m^{-2} and TGW, implying that physiological process determining these traits are equally important for yield potential expression. Genetic variation in grains m^{-2} was explained by greater FE and spike DM per unit area at anthesis and trends for positive associations were observed between HI and spike partitioning index at anthesis (SPI). Encouragingly, there was a lack of a trade-off between spike DM per unit area and SPI. Traits associated with FE were rachis specific weight and palea DM partitioning within the spike (negatively and positively, respectively). Associations between SPI and proportion of DM partitioned into alternative sinks at anthesis showed that the true-stem DM was competing most strongly for assimilates with the spike. However, although true-stem DM partitioned to internode 2 and 3 appeared to be competing most strongly with the spike at GS65+7d and true-stem internode 2 and 3 PI were well correlated with spike partitioning index, these traits were not well correlated with spike DM per unit area. Reductions in length and DM partitioning to the peduncle on the other hand were more strongly associated with increased spike DM per unit area. Further work is

required to confirm the mechanisms underlying these associations. Moreover, the present results showed scope for designing a plant ideotype with reductions in true-stem DM without major effects on WSC accumulation in lower internodes, which is important if grain yield is source limited at later stages during grain filling during unfavourable grain filling seasons. Reductions in final grain weight as a response of source-sink manipulation treatments (defoliation and leaf-sheath shading) were low compared to the estimated reduction in light interception by the treatments indicated that grain growth the CIMCOG lines were overall likely sink limited. However, the lack of up-regulation in leaf lamina and spike photosynthesis and pre-anthesis DM translocation to grain growth indicated limited scope for reductions in leaf-sheath and lamina DM partitioning to favour spike growth. Finally, although stem WSC was not associated to grain yield in the present study, it is possible that stem WSC may be more important in the future if grain yield of CIMMYT spring wheat releases start to become co-limited by source and sink.

8.7 **Future work**

Further work must address the use of present findings in the joint optimization of traits in new ideotypes, mainly with the use of crop simulation modelling methods to predict outcomes under different environmental conditions or in a wider range of cultivars.

Further work in more precise genetic material (mapping populations with a more uniform genetic background and/or isogenic lines) is needed to validate traits and investigate further how the stem morphological traits scale up from effects at the single shoot scale to the crop scale. Suitable bi-parental doubled-haploid populations for future QTL analysis segregating for partitioning traits are Kauz/Weebil and Seri Babax). Furthermore, the practical application of optimized DM related traits needs to be actually tested in trait-based breeding in a range of environments, particularly in genetic backgrounds differing in plant height or canopy architecture (and grown under different planting systems than the present raised beds plant system). With regard to association mapping analysis (e.g. Genome Wide Association Studies (GWAS)), one suggested panel is the High Biomass Association Panel ‘HiBAP’

comprising 150 high biomass lines, assembled from CIMMYT spring wheat germplasm (Wheat yield consortium yield trial (WYCYT), CIMCOG and the Wheat Association Mapping Initiative (WAMI) panel. The material includes agronomically acceptable spring wheat CIMMYT backgrounds lines with high expression of spike and stem DM partitioning related traits.

Further work investigating optimized DM partitioning to increase spike growth in high biomass cultivars must also quantify genetic variation in root or infertile tiller DM partitioning, as well as trade-offs between true-stem DM reductions and lodging type traits. In addition, changes in stem length partitioning must investigate effects on light distribution into the canopy and potential effects on radiation-use efficiency and biomass.

Reduced DM partitioning to t-s 2 and t-s 3 appeared to favour higher SPI. However, these traits were not well correlated with spike DM per unit area. In fact, the analysis showed that lower t-s ped PI and reduced peduncle length were more strongly associated with increased spike DM per unit area. The effects may in part reflect confounding effects of shoot density and their physiological basis requires further investigation using precise genetic stocks such as DH populations segregating for true-stem internode partitioning traits and SPI appropriate near-isogenic lines. In the present study, FE was negatively associated with rachis specific weight and glumes DM partitioning among seven cultivars in 2012. So further work to examine associations between FE and morphological components of the spike and any related effects on spike photosynthesis seems justified.

Further work is required to quantify leaf-sheath photosynthesis during grain filling and contribution to grain growth in cultivars differing in source-sink balance, measuring a wider range of cultivars, also considering that leaf-sheaths are important for N storage and N remobilization to lamina to maintain lamina green area post-anthesis.

Present results showed that there was a strong positive association between the WSC% in the true-stem and the leaf-sheath at GS65+7d amongst the nine genotypes ($R^2 = 0.88$, $P < 0.01$). Further work may test whether the fructan synthetic and sucrose

hydrolysis enzymes influencing WSC% in these two plant components are regulated by the same genes. High-throughput phenotyping systems should be further developed to facilitate genetic analysis for the complex traits of biomass, spike partitioning and FE. For example, high-throughput phenotyping related to biomass may related to canopy photosynthesis and its determinants, *e.g.* hyperspectral reflectance imaging including the use of canopy sensors mounted on aerial platforms.

Further genetic analysis of mapping populations segregating for the key target traits should be carried out with the development of genetic marker maps for these populations. This will allow identification of novel QTL together with the development of backcross lines for target QTLs and fine mapping of QTLs to precise locations on the chromosomes. These QTLs can then be developed as targets for marker-assisted selection for spike fertility, HI and grain yield, and candidate genes established.

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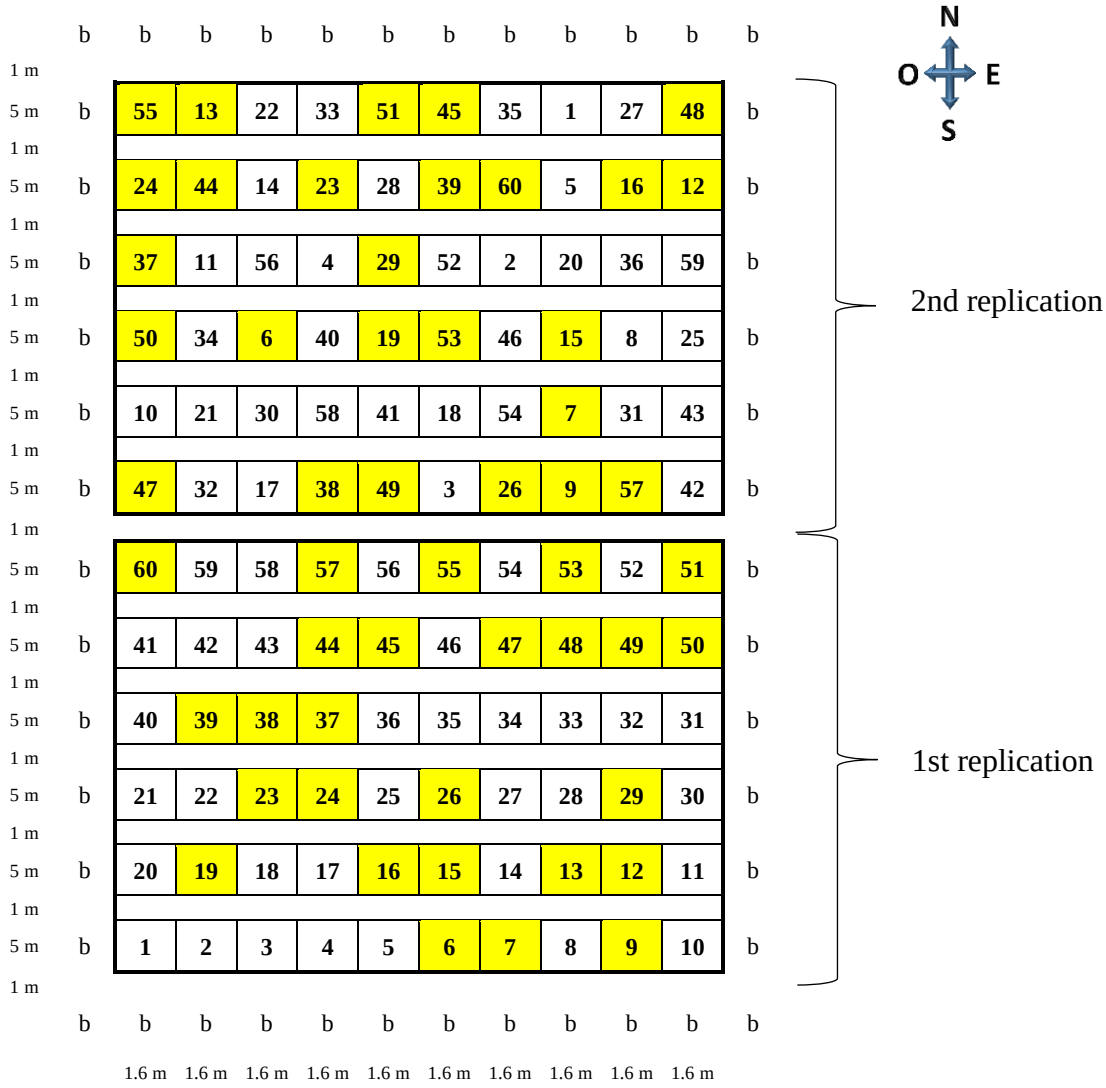
APPENDICES

1. Cultivars

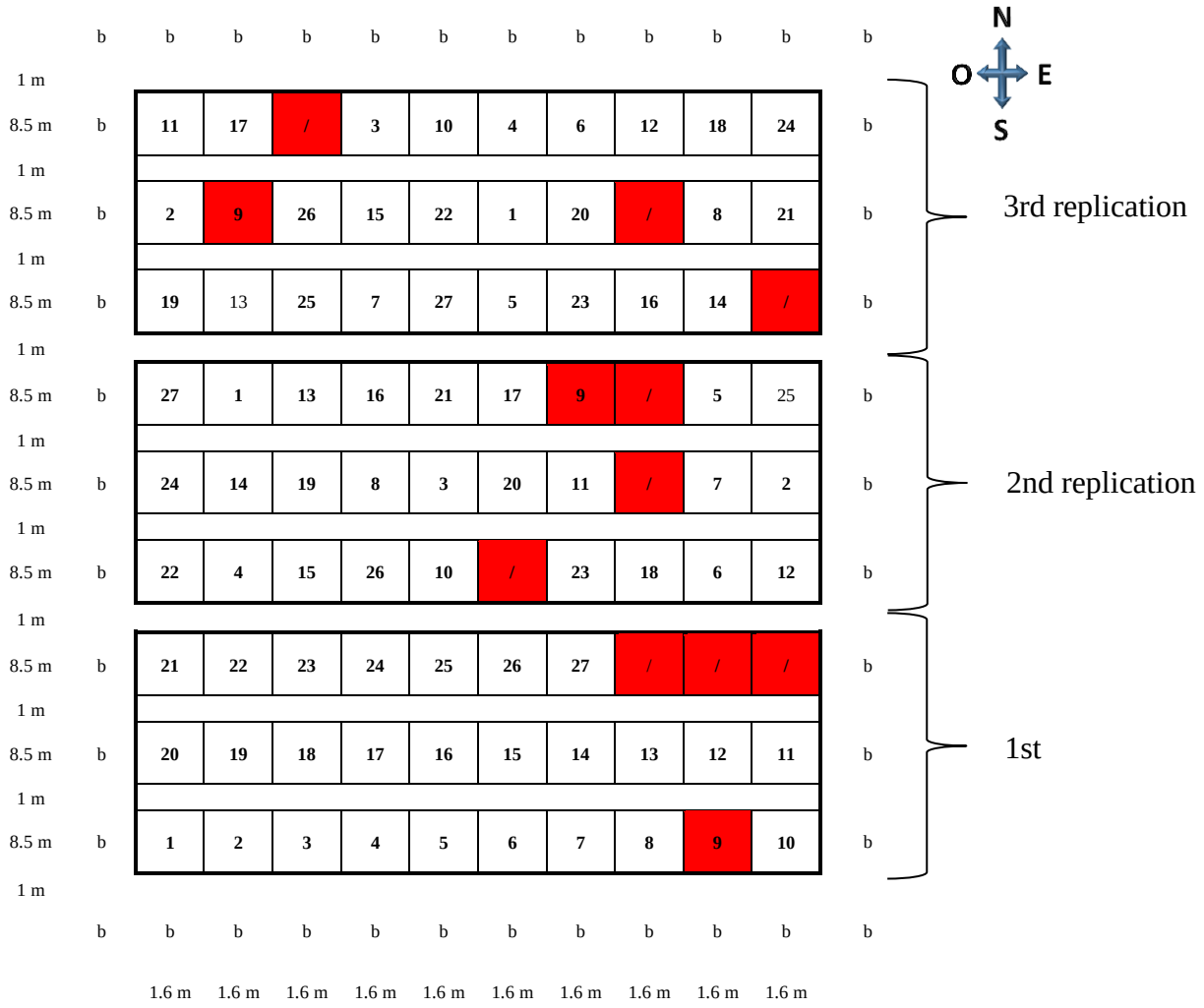
Entry 2011	Entry 2012 &2013	Year	Name	Traits considered for selection
1		1966	ATTILA (PBW 343)	Widely grown
2		1976	ATTILA*2/PBW65	
3		1982	ATTILA*2/PBW65*2/5/REH/HARE	
4		1988	ATTILA//PGO/SERI/3/PASTOR	
5		1990	BABAX/LR42//BABAX/3/ER2000	
6	1	1992	BABAX/LR42//BABAX/3/VORB	
7	2	1999	BACANORA T 88	High grains m^{-2}
8		2000	BAVIACORA M 92	High biomass
9	3	2001	BCN/RIALTO	High RUE
10		2002	BCN/WBLL1	High LI and RUE
11		2003	BECARD (Wbll 1*2/Kiritati)	Widely adapted
12	4	2003	BECARD/5/KAUZ//ALTAR 84	
13	5	2003	BRBT1*2/KIRITATI	Large seed
14		2004	C80.1/3*BATAVIA//2*WBLL1	Widely adapted
15	6	2005	CAL/NH//H567.71/3/SERI/4_1	
16	7	2005	CAL/NH//H567.71/3/SERI/4_2	
17		2005	CHIR3/4/SIREN//ALTAR 84	
18		2005	CHWL86/6/FILIN/IRENA	Long coleoptile/presence of Rht1
19	8	2005	CMH79A.955/4/AGA	Large spike and high biomass
20	**9**	**2005**	**CIRNO (durum wheat)**	
21		2005	CNDO/R143//ENTE/MEXI_2/3_1	Long coleoptile non-Rht1
22		2005	CNDO/R143//ENTE/MEXI_2/3_2	High yield and heat adaptation
23	10	2006	CNO79//PF70354/MUS/3	
24	11	2006	CROC_1/AE.SQUARROSA	High biomass
25		2006	GK ARON/AG SECO 7846	
26	12	2006	KBIRD//INQALAB 91*2	
27		2006	KFA/3/PFAU/WEAVER/	
28		2006	MEX94.27.1.20/3/SOKOLL	High yield and heat adaptation
29	13	2007	MILAN/KAUZ//PRINIA	High yield and heat adaptation
30		2007	MUNAL #1	Widely adapted
31		2008	Navojoa M2007 (=ATTILA/PASTOR)	
32		2008	OASIS/5*BORL95/5/CNDO	High yield and heat adaptation
33		2008	OASIS/SKAUZ//4*BCN/3/2*PASTOR	Widely adapted
34		2008	OASIS/SKAUZ//4*BCN/3/2*PASTOR/5/FRET2	
35		2008	PANDORA//WBLL1*2	
36		2008	PASTOR/3/URES/JUN//KAUZ	
37	14	2008	PAVON F 76	
38	15	2008	PBW343*2/KUKUNA*2	
39	16	2008	PFAU/SERI.1B//AMAD/3	Widely adapted
40		2008	ARMENT//2*SOOTY_9/RASCON	
41		2009	QUAIU #3//MILAN/AMSEL	
42		2009	RL6043/4*NAC//2*PASTOR	High yield and heat adaptation
43		2009	ROLF07*2/5/REH/HARE/	
44	17	2009	SERI M 82	Widely adapted
45	18	2009	SIETE CERROS T66	Benchmark
46		2009	SOKOLL*2/3/BABAX/	
47	19	2009	SOKOLL//PBW343*2	
48	20	2009	TACUPETO F2001/7/CAL/NH	
49	21	2009	TACUPETO F2001/BRAMBLING	

50	22	2009	TC870344/GUI	
51	23	2009	TRAP#1/BOW/3/VEE/PJN	Widely adapted
52		2009	TRCH/SRTU/5/KAUZ	Widely adapted
53	24	2009	UP2338*2/4/SNI/TRAP#1/3	
54		2009	W15.92/4/PASTOR//	
55	25	2009	WBLL1*2/ KIRITATI	Widely adapted
56		2009	WBLL1*2/4/BABAX/LR42	
57	26	2009	WBLL1*2/KURUKU*2/5/REH	
58		2009	WBLL1*2/TUKURU*2/4	
59		2009	WHEAR/SOKOLL	Widely adapted
60	27	2009	YAV_3/SCO//JO69/CRA/3	

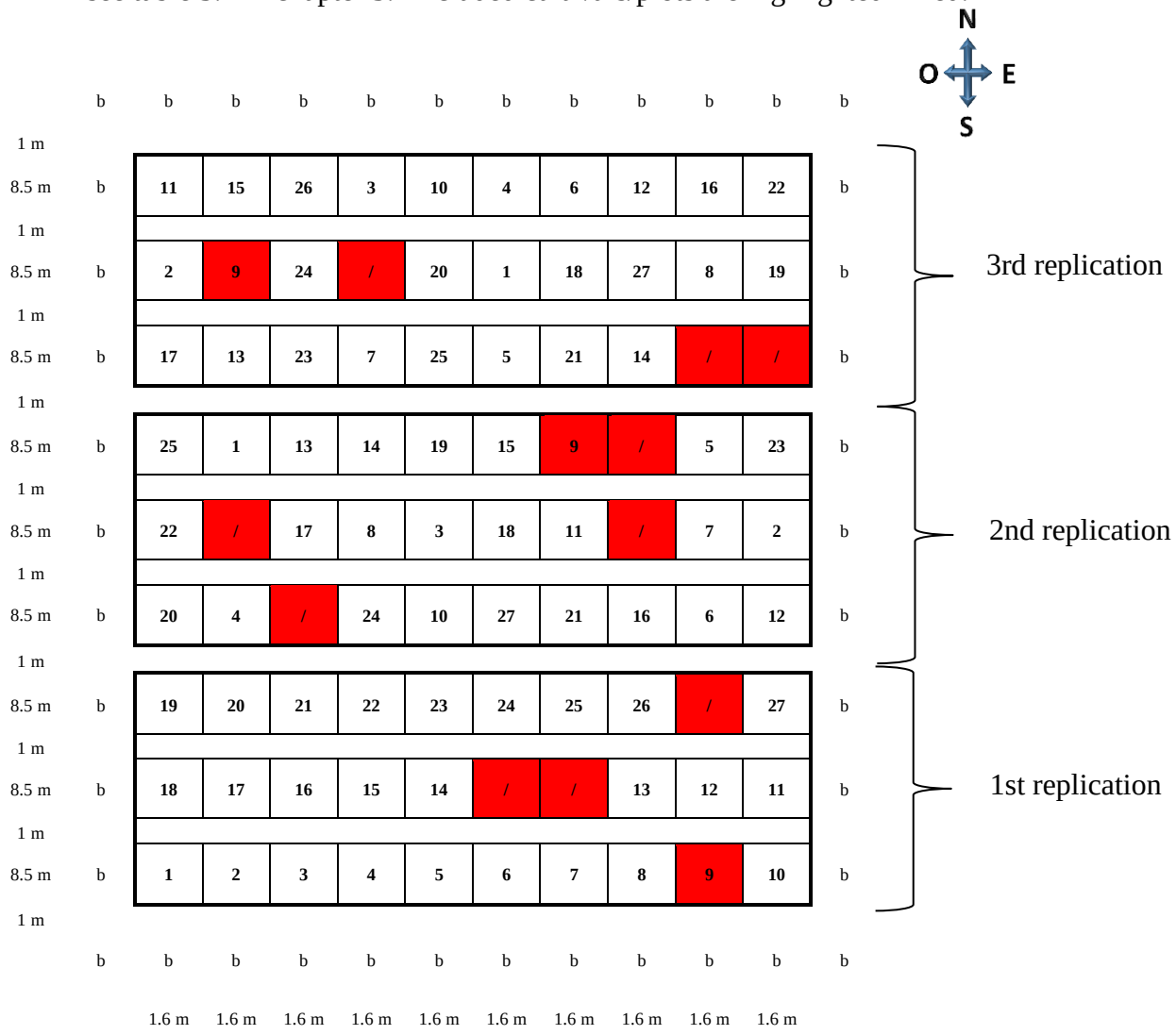
2. Field arrangement in the field in 2010-11 (2011). The 26 cultivars (selected from 2011 and sown in 2012 and 2013 are highlighted in yellow).



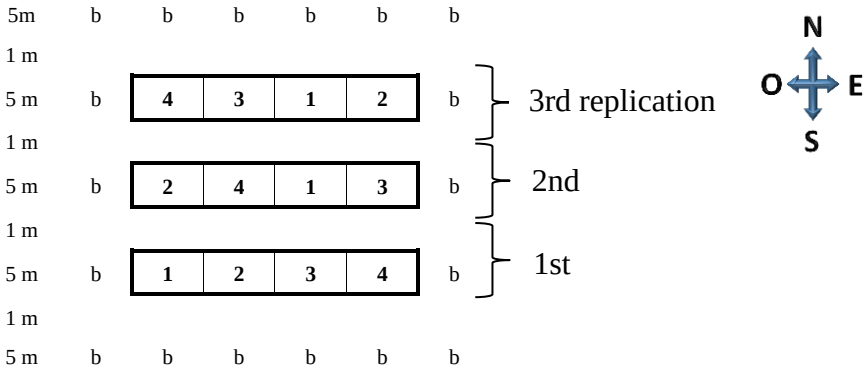
3. Field arrangement in the field in 2011-12 (2012). For the name of the cultivars see table 3.1 in chapter 3. Excluded cultivars/plots are highlighted in red.



4. Field arrangement in the field in 2012-13 (2013). For the name of the cultivars see table 3.1 in chapter 3. Excluded cultivars/plots are highlighted in red.



5. Field arrangement in the field in 2013-14 (2014). For the name of the cultivars see table 3.1 in chapter 3.



6. Herbicides, fungicide and insecticide applied during field seasons 2011, 2012, 2013 and 2014.

Season	Broad leave weeds herbicide	Narrow leave weeds herbicide			Fungicide	Insecticide
2010-11	**Buctril (1300 ml ha^{-1})**	**Strane (750 ml ha^{-1})**			**Folicur (500 ml ha^{-1})**	**Muraya (300 ml ha^{-1})**
	04 January 2011				25 January 2011	01-Jan-11
					21 February 2011	
					18 March 2011	
2011-12	**Buctril (1000 ml ha^{-1})**	**Strane (300 ml ha^{-1})**			**Folicur (500 ml ha^{-1})**	**Muraya (175 ml ha^{-1})**
	04 January 2012				12 March 2012	09 February 2012
						02 March 2012
2012-13	**Buctril (1000 ml ha^{-1})**	**Strane (300 ml ha^{-1})**	**Axial (500 ml ha^{-1})**	**Adigor (1000 ml ha^{-1})**	**Folicur (500 ml ha^{-1})**	**Muraya (250 ml ha^{-1})**
	20 December 2012		13 December 2012		25 January 2013	22 January 2013
					15 February 2013	
					08 March 2013	
2013-14	**Buctril (1000 ml ha^{-1})**	**Strane (350 ml ha^{-1})**			**Folicur (500 ml ha^{-1})**	**Muraya (250 ml ha^{-1})**
	16 December 2013				27 January 2014	24 January 2014
					19 February 2014	
					06 March 2014	

7. Calendar dates and days after emergence (DDE) and in 2010-11 (2011) (Emergence date: 15 December 2010).

Calendar date	DDE	Calendar date	DDE	Calendar date	DDE
15/12/10	1	**03/02/11**	51	**25/03/11**	101
16/12/10	2	**04/02/11**	52	**26/03/11**	102
17/12/10	3	**05/02/11**	53	**27/03/11**	103
18/12/10	4	**06/02/11**	54	**28/03/11**	104
19/12/10	5	**07/02/11**	55	**29/03/11**	105
20/12/10	6	**08/02/11**	56	**30/03/11**	106
21/12/10	7	**09/02/11**	57	**31/03/11**	107
22/12/10	8	**10/02/11**	58	**01/04/11**	108
23/12/10	9	**11/02/11**	59	**02/04/11**	109
24/12/10	10	**12/02/11**	60	**03/04/11**	110
25/12/10	11	**13/02/11**	61	**04/04/11**	111
26/12/10	12	**14/02/11**	62	**05/04/11**	112
27/12/10	13	**15/02/11**	63	**06/04/11**	113
28/12/10	14	**16/02/11**	64	**07/04/11**	114
29/12/10	15	**17/02/11**	65	**08/04/11**	115
30/12/10	16	**18/02/11**	66	**09/04/11**	116
31/12/10	17	**19/02/11**	67	**10/04/11**	117
01/01/11	18	**20/02/11**	68	**11/04/11**	118
02/01/11	19	**21/02/11**	69	**12/04/11**	119
03/01/11	20	**22/02/11**	70	**13/04/11**	120
04/01/11	21	**23/02/11**	71	**14/04/11**	121
05/01/11	22	**24/02/11**	72	**15/04/11**	122
06/01/11	23	**25/02/11**	73	**16/04/11**	123
07/01/11	24	**26/02/11**	74	**17/04/11**	124
08/01/11	25	**27/02/11**	75	**18/04/11**	125
09/01/11	26	**28/02/11**	76	**19/04/11**	126
10/01/11	27	**01/03/11**	77	**20/04/11**	127
11/01/11	28	**02/03/11**	78	**21/04/11**	128
12/01/11	29	**03/03/11**	79	**22/04/11**	129
13/01/11	30	**04/03/11**	80	**23/04/11**	130
14/01/11	31	**05/03/11**	81	**24/04/11**	131
15/01/11	32	**06/03/11**	82	**25/04/11**	132
16/01/11	33	**07/03/11**	83	**26/04/11**	133
17/01/11	34	**08/03/11**	84	**27/04/11**	134
18/01/11	35	**09/03/11**	85	**28/04/11**	135
19/01/11	36	**10/03/11**	86	**29/04/11**	136
20/01/11	37	**11/03/11**	87	**30/04/11**	137
21/01/11	38	**12/03/11**	88	**01/05/11**	138
22/01/11	39	**13/03/11**	89	**02/05/11**	139
23/01/11	40	**14/03/11**	90	**03/05/11**	140

24/01/11	41	**15/03/11**	91	**04/05/11**	141
25/01/11	42	**16/03/11**	92	**05/05/11**	142
26/01/11	43	**17/03/11**	93	**06/05/11**	143
27/01/11	44	**18/03/11**	94	**07/05/11**	144
28/01/11	45	**19/03/11**	95	**08/05/11**	145
29/01/11	46	**20/03/11**	96	**09/05/11**	146
30/01/11	47	**21/03/11**	97	**10/05/11**	147
31/01/11	48	**22/03/11**	98	**11/05/11**	148
01/02/11	49	**23/03/11**	99	**12/05/11**	149
02/02/11	50	**24/03/11**	100	**13/05/11**	150

8. Calendar dates and days after emergence (DDE) and in 2011-12 (2012) (Emergence date: 17 December 2011).

Calendar date	DDE	Calendar date	DDE	Calendar date	DDE
17/12/11	1	**05/02/12**	51	**26/03/12**	101
18/12/11	2	**06/02/12**	52	**27/03/12**	102
19/12/11	3	**07/02/12**	53	**28/03/12**	103
20/12/11	4	**08/02/12**	54	**29/03/12**	104
21/12/11	5	**09/02/12**	55	**30/03/12**	105
22/12/11	6	**10/02/12**	56	**31/03/12**	106
23/12/11	7	**11/02/12**	57	**01/04/12**	107
24/12/11	8	**12/02/12**	58	**02/04/12**	108
25/12/11	9	**13/02/12**	59	**03/04/12**	109
26/12/11	10	**14/02/12**	60	**04/04/12**	110
27/12/11	11	**15/02/12**	61	**05/04/12**	111
28/12/11	12	**16/02/12**	62	**06/04/12**	112
29/12/11	13	**17/02/12**	63	**07/04/12**	113
30/12/11	14	**18/02/12**	64	**08/04/12**	114
31/12/11	15	**19/02/12**	65	**09/04/12**	115
01/01/12	16	**20/02/12**	66	**10/04/12**	116
02/01/12	17	**21/02/12**	67	**11/04/12**	117
03/01/12	18	**22/02/12**	68	**12/04/12**	118
04/01/12	19	**23/02/12**	69	**13/04/12**	119
05/01/12	20	**24/02/12**	70	**14/04/12**	120
06/01/12	21	**25/02/12**	71	**15/04/12**	121
07/01/12	22	**26/02/12**	72	**16/04/12**	122
08/01/12	23	**27/02/12**	73	**17/04/12**	123
09/01/12	24	**28/02/12**	74	**18/04/12**	124
10/01/12	25	**29/02/12**	75	**19/04/12**	125
11/01/12	26	**01/03/12**	76	**20/04/12**	126
12/01/12	27	**02/03/12**	77	**21/04/12**	127
13/01/12	28	**03/03/12**	78	**22/04/12**	128

14/01/12	29	**04/03/12**	79	**23/04/12**	129
15/01/12	30	**05/03/12**	80	**24/04/12**	130
16/01/12	31	**06/03/12**	81	**25/04/12**	131
17/01/12	32	**07/03/12**	82	**26/04/12**	132
18/01/12	33	**08/03/12**	83	**27/04/12**	133
19/01/12	34	**09/03/12**	84	**28/04/12**	134
20/01/12	35	**10/03/12**	85	**29/04/12**	135
21/01/12	36	**11/03/12**	86	**30/04/12**	136
22/01/12	37	**12/03/12**	87	**01/05/12**	137
23/01/12	38	**13/03/12**	88	**02/05/12**	138
24/01/12	39	**14/03/12**	89	**03/05/12**	139
25/01/12	40	**15/03/12**	90	**04/05/12**	140
26/01/12	41	**16/03/12**	91	**05/05/12**	141
27/01/12	42	**17/03/12**	92	**06/05/12**	142
28/01/12	43	**18/03/12**	93	**07/05/12**	143
29/01/12	44	**19/03/12**	94	**08/05/12**	144
30/01/12	45	**20/03/12**	95	**09/05/12**	145
31/01/12	46	**21/03/12**	96	**10/05/12**	146
01/02/12	47	**22/03/12**	97	**11/05/12**	147
02/02/12	48	**23/03/12**	98	**12/05/12**	148
03/02/12	49	**24/03/12**	99	**13/05/12**	149
04/02/12	50	**25/03/12**	100	**14/05/12**	150

9. Calendar dates and days after emergence (DDE) and in 2012-13 (2013) (Emergence date: 2 December 2012).

Calendar date	DDE	Calendar date	DDE	Calendar date	DDE
02/12/12	1	**21/01/13**	51	**12/03/13**	101
03/12/12	2	**22/01/13**	52	**13/03/13**	102
04/12/12	3	**23/01/13**	53	**14/03/13**	103
05/12/12	4	**24/01/13**	54	**15/03/13**	104
06/12/12	5	**25/01/13**	55	**16/03/13**	105
07/12/12	6	**26/01/13**	56	**17/03/13**	106
08/12/12	7	**27/01/13**	57	**18/03/13**	107
09/12/12	8	**28/01/13**	58	**19/03/13**	108
10/12/12	9	**29/01/13**	59	**20/03/13**	109
11/12/12	10	**30/01/13**	60	**21/03/13**	110
12/12/12	11	**31/01/13**	61	**22/03/13**	111
13/12/12	12	**01/02/13**	62	**23/03/13**	112
14/12/12	13	**02/02/13**	63	**24/03/13**	113
15/12/12	14	**03/02/13**	64	**25/03/13**	114
16/12/12	15	**04/02/13**	65	**26/03/13**	115
17/12/12	16	**05/02/13**	66	**27/03/13**	116
18/12/12	17	**06/02/13**	67	**28/03/13**	117

19/12/12	18	**07/02/13**	68	**29/03/13**	118
20/12/12	19	**08/02/13**	69	**30/03/13**	119
21/12/12	20	**09/02/13**	70	**31/03/13**	120
22/12/12	21	**10/02/13**	71	**01/04/13**	121
23/12/12	22	**11/02/13**	72	**02/04/13**	122
24/12/12	23	**12/02/13**	73	**03/04/13**	123
25/12/12	24	**13/02/13**	74	**04/04/13**	124
26/12/12	25	**14/02/13**	75	**05/04/13**	125
27/12/12	26	**15/02/13**	76	**06/04/13**	126
28/12/12	27	**16/02/13**	77	**07/04/13**	127
29/12/12	28	**17/02/13**	78	**08/04/13**	128
30/12/12	29	**18/02/13**	79	**09/04/13**	129
31/12/12	30	**19/02/13**	80	**10/04/13**	130
01/01/13	31	**20/02/13**	81	**11/04/13**	131
02/01/13	32	**21/02/13**	82	**12/04/13**	132
03/01/13	33	**22/02/13**	83	**13/04/13**	133
04/01/13	34	**23/02/13**	84	**14/04/13**	134
05/01/13	35	**24/02/13**	85	**15/04/13**	135
06/01/13	36	**25/02/13**	86	**16/04/13**	136
07/01/13	37	**26/02/13**	87	**17/04/13**	137
08/01/13	38	**27/02/13**	88	**18/04/13**	138
09/01/13	39	**28/02/13**	89	**19/04/13**	139
10/01/13	40	**01/03/13**	90	**20/04/13**	140
11/01/13	41	**02/03/13**	91	**21/04/13**	141
12/01/13	42	**03/03/13**	92	**22/04/13**	142
13/01/13	43	**04/03/13**	93	**23/04/13**	143
14/01/13	44	**05/03/13**	94	**24/04/13**	144
15/01/13	45	**06/03/13**	95	**25/04/13**	145
16/01/13	46	**07/03/13**	96	**26/04/13**	146
17/01/13	47	**08/03/13**	97	**27/04/13**	147
18/01/13	48	**09/03/13**	98	**28/04/13**	148
19/01/13	49	**10/03/13**	99	**29/04/13**	149
20/01/13	50	**11/03/13**	100	**30/04/13**	150