IDENTIFY PHYSIOLOGICAL TRAITS TO INCREASE YIELD POTENTIAL THROUGH ENHANCED BIOMASS, SPIKE FERTILITY AND OPTIMIZED SOURCE-SINK BALANCE IN WHEAT (*Triticum aestivum* L.) GENOTYPES

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SEPTEMBER 2015

ABSTRACT

Increased demand for food, climate change and greater dependence on food imports in less developed countries represent a challenge to achieve global food security. Wheat (*Triticum aestivum L*.) is the most widely grown crop and an essential component in ensuring global food security. Therefore, breeders need to develop new cultivars with higher grain yield potential. One physiological avenue to raise yield potential is by improving the allocation of assimilate to the spike during stem elongation to enhance grains m^{-2} and harvest index (proportion of above-ground dry matter at harvest in grain; HI). Partitioning to spikes could be increased to enhance floret survival by reducing competition from alternative sinks, including roots, leaves, stems and infertile tillers (Foulkes et al., 2011). However, attention must also be paid to maintaining post-anthesis source (photosynthetic capacity) to ensure grain growth of new cultivars is not source-limited. Although yield grains in wheat have historically been associated with traits influencing the capacity of the grains to store assimilate (sink), rather than influencing potential assimilate production (source), there is some evidence that modern cultivars are moving closer towards source limitation of grain growth (Acreche and Slafer 2009). So it is important to quantify the source and sink balance in CIMMYT elite germplasm and its physiological determinants to guide further strategies for yield potential improvement.

The objectives of this study were to: i) Identify physiological traits determining enhanced above-ground biomass per unit area and radiation-use-efficiency (above-ground biomass per unit photosynthetically active radiation (PAR) interception; RUE_{PAR}), spike fertility and HI in modern high biomass CIMMYT spring wheat germplasm, ii) quantify post-anthesis source: sink balance according to responses to a degraining to source-sink manipulation treatment, and iii) identify marker-trait associations for grain weight response to degraining (as an indication of source-sink balance) and senescence-related traits.

In this study, a panel of twenty six elite CIMMYT spring wheat cultivars and a wheat association mapping (WAMI) panel of 294 genotypes comprising advanced lines and cultivars were evaluated in replicated field experiments under full irrigation in NW Mexico. The 26 cultivar panel was grown in in three seasons (2010-11, 2011-12 and 2012-13) and the WAMI panel in two seasons (2012-13 and 2013-14). In the 26 cultivar panel, growth analysis was carried at key development stages to assess above-ground dry matter (AGDM), DM partitioning and green canopy area. Assessment of fractional PAR interception by the canopy was carried out during the stem-elongation phase (GS31-GS65+7d) and RUE_{PAR} was calculated over this phase in 2011-12 and 2012-13. Flag-leaf stomatal conductance was measured at around anthesis and senescence parameters were assessed at the canopy level and at leaf level. In both the 26 cultivar panel and the WAMI panel experiments, a degraining treatment was carried out at anthesis (GS65) +7-14 days by removing half of the spikelets to assess grain weight responses compared to control spikes as an indicator of source-sink balance and leaf and canopy senescence parameters were quantified in the control treatment.

In the 26 cultivar panel, grain yield variation was associated more strongly with AGDM at harvest than HI; and biomass was positively associated with year of release. Radiation-use efficiency during stem elongation (GS61 – GS65+7d) showed genetic variation in one year out of two in which this trait was measured and a positive association with crop growth rate (AGDM g m^{-2} d^{-1}) and with flag-leaf nitrogen (N) content. Furthermore, both flag-leaf stomatal conductance at anthesis and RUE_{PAR} showed a positive association with grain yield among the 26 cultivars. There was genetic variation in each of spike partitioning index (proportion of AGDM as spike at GS65+7d) and fruiting efficiency (grain per g spike DM at GS61+7d) positively associated with grains m^{-2} amongst the 26 genotypes, and there was no trade-off between SPI and FE. Fruiting efficiency was associated with a greater proportion of lemma DM within the spike morphological components (glume, rachis, lemma, palea and

awn). Results showed there is scope to increase biomass through RUE_{PAR} and RUE_{PAR} could be enhanced by selecting for increased stomatal conductance and/or flag-leaf N content.

Grain weight responses to the degraining treatment in both the 26 cultivar panel and the WAMI panel showed that grain growth was either sink limited or co-limited by sink and source in the genotypes. In both panels, genotypes with higher grain yield showed higher grain weight responses to degraining (indicating a greater extent of source limitation); and greater grain weight responses were associated with faster senescence rate, supporting a co-limitation of source and sink in modern high yielding CIMMYT spring wheat cultivars. The genetic association mapping analysis identified a marker-trait association for grain weight response to degraining (an indicator of source-sink balance) on chromosome 7A in the WAMI panel.

Grain m^{-2} was not associated with grain yield indicating that grain weight at some extent is driving gains to grain yield due to its positive association with year of release. Grain yield positively correlated with response to degraining over years, grain weight response to degraining was negatively associated with Flag leaf senescence in the WAMI population. Genetic variation in radiation interception by the canopy during stem elongation from GS31 to GS65+7d positively associated with accumulated above-ground dry matter amongst the CIMCOG genotypes. There was genetic variation in radiation-use efficiency (in 2013) during stem elongation and RUE was positively related with above-ground biomass at anthesis amongst the CIMCOG genotypes.

Therefore, in order to increase grain yield is necessary to increase simultaneously source and sink traits.

DEDICATION

To my beloved wife Krista Lynae Trujillo

ACKNOWLEDGMENTS

This project and PhD studies were funded by Consejo Nacional de Ciencia y Tecnología (CONACYT), Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA), CIMMYT and The University of Nottingham.

I would like to express my sincere thanks and gratitude to my supervisors, Dr John Foulkes, Dr. Matthew Reynolds, for their support, and time investment thought the research study.

Many thanks to Dr. Candido Lopez Castañeda and Dr. Gemma Molero for their assistance in the field work. I want to thank Sivakumarfor the GWAS analysis on the WAMI panel.

I have to thank too many people that contribute with this achievement. Eugenio, Israel, Arturo, Fernando, Araceli, Mayra, Nayelli and Jose Maria for technical support with equipment at CIMMYT.

Many thanks to my colleagues and friends Alejandro Quintero, Francisco Chavez, Carolina Rivera, Viridiana Perez, and Oscar Gonzalez, my best wishes for all of them.

Thanks for family and friends from Reynosa Tamaulipas and Ciudad Obregon Sonora.

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LIST OF ABRREVIATIONS

Amax	Saturated Point
ANOVA	Analysis Of Variance
°C	Degrees Celcius
CENEB	Norman E. Borlaug Experimental Center
CGR	Crop Growth Rate
CIMCOG	CIMMYT Mexico Core Germplasm
CIMMYT	International Maize And Wheat Improvement Center
CO2	Carbon Dioxide
CTD	Canopy Temperature Depression
cv	Cultivar
Cv	Coefficient Of Variation
DF	Degrees of Freedom
DM	Dry Matter
DR	Double Ridge
DTA	Date of Anthesis
DW	Dry Weight
Em	Emergence
FE	Fruiting Efficiency (Grains g Spike DM^{-1})
FLS	Flag Leaf Senescence
FI	Fraction Of Radiation Intercepted
FW	Fresh Weight
GAI	Green Area Index
GS	Growth Stage
g_s	Stomatal Conductance
GWAS	Genome Wide Association
GWR	Grain weight Response to de-graining (%)
GWDEG	Grain Weight of De-graining treatment
GWCON	Grain Weight Control
HD	Heading
HI	Harvest Index
HTPPs	High-Throughput Phenotyping Technologies Or Platforms
k	Extinction Coefficient Of The Canopy
LAI	Leaf Area Index
LI	Radiation Interception
LSD	Least Significant Difference
LTM	Long-Term Means
NDVI	Normalized Differenced Vegetation Index
MAT	Marker Trait Association
nm	Nanometer
ns	Non-significant
m^{-2}	Square metre
N	Nitrogen

NW	Northwest
P	Probability
PAR	Photosynthetically Active Radiation
PCA	Principal Component Analysis
PGW	Potential Grain Weight
PM	Physiological Maturity
QTL	Quantitative Trait Loci
RAD	Incident Radiation (MJ m^{-2})
RCC	Reflectance Chlorophyll Content
RH	Relative Humidity
Rht	Semi-Dwarfing Genes
RI	Fraction Of That Radiation Intercepted
Rs	Solar Radiation
RuBP	Ribulose-1,5-Bisphosphate
RUE	Radiation-Use Efficiency (g MJ^{-1})
SED	Standar Error of the Difference of means
SLW	Specific Leaf Weight Nitrogen
SNPs	Single Nucleotide Polymorphisms
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
WAMI	Wheat Association Mapping Initiative
YoR	Year Of Release

CHAPTER 1 INTRODUCTION

1.1 Global wheat production and demand

Wheat (*Triticum* aestivum L.) is important for economic and nutritional reasons, since wheat provides 20% of the calories and 20% of protein intake for world's population (Reynolds et al., 2012; White 2004). Wheat is the most traded food product in the world (Dixon et al., 2009; Shiferaw et al., 2011). Globally, about 150 to 160 million tonnes of wheat are traded a year at a value of roughly £110 per ton (Tony Fischer 2014).

Globally, demand for wheat has been forecast to be 1,050 million tonnes by 2020 (Kronstad 1997). Although grain yield has been increasing in the last decades, there is a higher requirement to increase wheat yields per unit land area, since arable land area is restricted or less likely to increase (Figure 1.1;1.2).

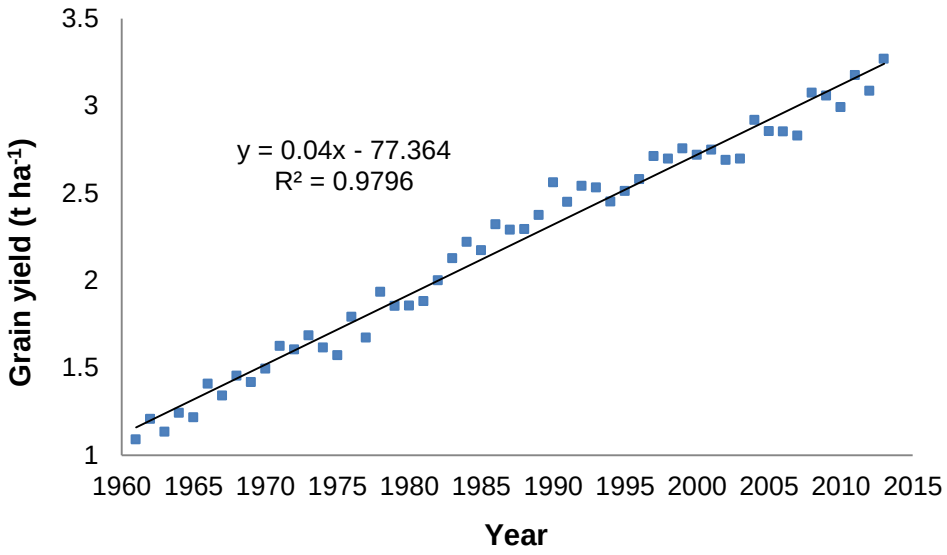


Figure 1.1. Trend in global grain yield over time (data from FAOSTAT database 2013)

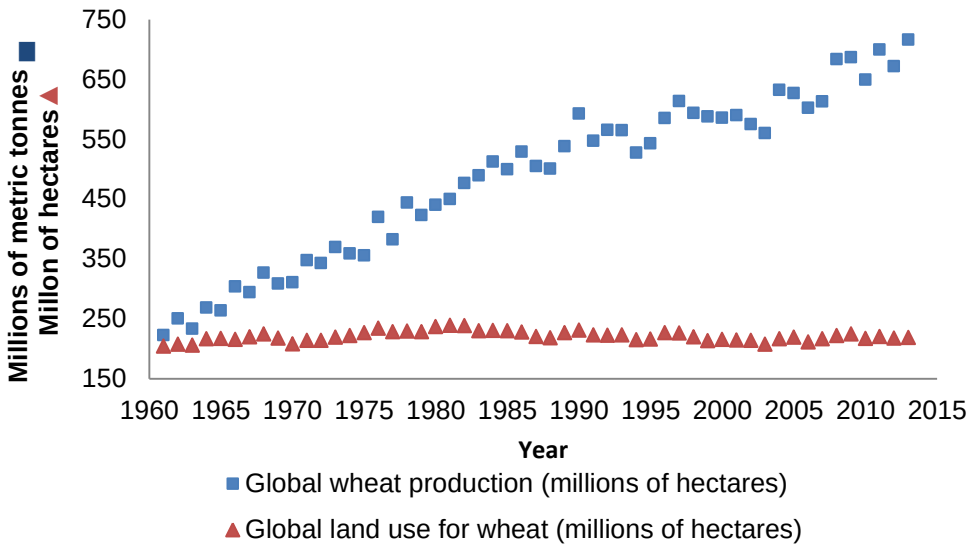


Figure 1.2. Global wheat production in terms of land used and tonnes of product *Source*: FAOSTAT database, 2013)

The current world population of 7.3 billion is expected to reach 8.5 billion by 2030, 9.7 billion in 2050 and 11.2 billion in 2100, (United Nations 2015). By 2050 the three most populous countries will be India, China and Nigeria.. Currently the growth rate of the population is 1.1% p.a. and for the less developed countries is 2.2% p.a. Therefore global food security is becoming more important for the future. Foley et al. (2011) reported that between 1965 and 1985 average crop yield increased by 47% worldwide, but between 1985 to 2005 average yields increased only by 20%. However, global agricultural area has actually decreased during the past decade and may continue to do so in the future (Cassman et al., 2003). Worldwide, cereal yields have risen nearly linearly since the advent of the Green Revolution in the 1960s and 1970s through improved cultivars and management techniques, and the use of chemical fertilizers, irrigation, and pesticides (Evans 1996). However, there are some indications that wheat yield gains have levelled off in France (Brisson et al., 2010) and Switzerland (Finger 2010), and it has been suggested that other nations also show signs of yield stagnation (Dixon et al., 2009; Hafner 2003) or very low gains compared to the past gains. Lin and Huybers (2012) tested for yield stagnation in 50 regions and found that yields in 27 regions

levelled off when performing the test at the 80% confidence level, including the Western US, the majority of Western Europe, India, Bangladesh, Romania, Colombia, Albania, Egypt, Hungary, Japan, Pakistan, South Korea and Zambia. However, at the international maize and wheat improvement center (CIMMYT) breeding programme, studies suggest that yield progress through breeding in spring wheat introduced from 1975 to 2010 has not declined, but the rate of yield progress is only 0.5% p.a in low productivity environments and 0.9% in high productivity environments (Lopes et al., 2012b).

Irrigated spring wheat of CIMMYT Mega Environment I (Irrigated high yield potential) occupies ~34 Mha in the developing world (especially in northern South Asia, southern China and Egypt) and is probably the most important wheat mega environment for world food security. The Yaqui Valley in the state of Sonora in north-west Mexico is representative of WME1 and has been the site of the wheat breeding operations of CIMMYT and its predecessor organisation for more than 50 years. In developed countries around 90% of the wheat area is rainfed, while in developing countries more than half of the wheat area is irrigated, especially in India and China (Dixon et al., 2009). In addition, the great majority of developing countries are wheat net importers, some very large ones, for instance Mexico, Saudi Arabia, the Republic of Korea, Egypt, Algeria and Taiwan Province of China (Alexandratos and Bruinsma 2012). Therefore, for global food security it is necessary to look for enhanced grain yield in potential conditions.

1.2 Impacts of breeding on global wheat production

Breeding success from the 1960s to 1990s in increasing yield potential has been largely due to introduction of the gibberellic acid-insensitivity (Rht alleles Rht-B1b and Rht-D1b on chromosome 4B and 4D, respectively) (Evans 1998; Gale andand Marshall 1973). These genes, reduced plant height, lodging, and also reduced competition from the

growing stem resulting in greater spike growth, more grains per unit area (Sayre et al., 1997; Slafer et al., 1994) and higher harvest index (HI) (Fischer andand Stockman 1986).

Austin (1980) modelled partitioning of biomass in wheat (grain DM/above ground DM) and predicted that there was scope to improve yields further by increasing HI. Austin predicted a theoretical maximum HI of 0.62, and, partly based on Austin's calculations, Foulkes et al. (2011) suggested a theoretical maximum of 0.64 for HI. For spring wheat, current values are typically around 0.50 (Foulkes et al., 2011). Therefore, it seems there is scope to increase HI. However, more recent investigations have demonstrated that above ground biomass has been increased through breeding since 1985 in the UK (Shearman et al., 2005) and in 1980 in China (Xiao et al. 2012) and similarly since 1980 in Mexico (Aisawi et al., 2015). However, gains in wheat yield have slowed down compared to the last decades of breeding as mentioned above. So, new germplasm or selection tools are necessary in order to breed new cultivars with higher grain yield. In this respect, CIMMYT in 2010 established a panel of genotypes called CIMCOG (CIMMYT Mexico Core Germplasm) for research purposes, in consultation with wheat breeders and physiologists (Reynolds et al., 2010). This germplasm includes advanced lines and cultivars representing the full range of genetic diversity available in CIMMYT modern elite backgrounds with high yield potential and high biomass.

1.3 The role of physiology in wheat breeding

Understanding the physiological processes determining yield is essential for breeders to design strategies for yield potential improvement. Studying a trait from the physiological level to the gene level may be a more effective way to improve such a yield-enhancing trait rather the opposite way (Fischer 2011a).

A new wheat ideotype must combine a number of physiological traits shown to be associated with higher grain yield potential. For example, traits such as grain size, high stomatal conductance, canopy cooling, lodging tolerance during grain filling, adaptation to canopy environment, and erect flag leaves, prioritising for wheat yield potential (Rajaram and van Ginkel 1996). It was recognise that in selecting for these traits, cultivar x environment interaction was one of the most difficult problems.

Two decades after, research on wheat physiology is undertaken usually with a stated view to impact, whether from improved crop agronomy or better cultivars. However, more knowledge is needed as showing the way forward (Fischer 2011a). Reynolds et al. (2012) suggested that there is potential to increase grain yield through the improvement of radiation-use efficiency (RUE) by 50% and suggested that the strategies include modifying specificity, catalytic rate and regulation of Rubisco, up-regulating Calvin cycle enzymes, introducing chloroplast CO_2 concentrating mechanisms, optimizing light and N distribution of canopies while minimizing photoinhibition, and increasing spike photosynthesis.

Gains in yield potential from plant breeding have plateaued in some countries and slowed in others. However, in the recent years, there has been increased interest in high throughput phenotyping platforms (HTPPs) to increase the efficiency of breeding programs. The most relevant traits for evaluation integrate in time and space at the canopy level are those relating to use radiation, water, nutrients etc. Different methodological approaches have been proposed to evaluate these traits in the field including remote sensing and near-infrared reflectance spectroscopy analysis (Araus and Cairns 2014). Overall, high-throughput phenotyping platforms (HTPPs) will be more common in the near future and are becoming more available already at low cost, and more reliable and accurate (Araus and Cairns 2014).

Examples of important physiological tools which could be used as selection criteria are canopy temperature depression (difference between ambient temperature and canopy temperature; CTD) using an infra-red thermometer indicative of photosynthetic capacity and spectral reflectance indices using spectral radiometers indicative of canopy size and biomass (Babar et al., 2006). Several spectral reflectance indices have been developed for estimating green part i.e. NDVI, R-NDVI, G-NDVI, SR, RARSa, NPQI, SIMI (Peñuelas et al., 1995).

Under favourable conditions in the last decades plant breeders have mainly increased grain yield (sink), indicating that older wheat cultivars were severely sink limited (Slafer and Savin 1994). But now maybe towards source limitation. Therefore, it is important to quantify before identify source and sink type traits for future yield gains.

1.4 The evolution of bread wheat

Bread wheat (*Triticum* aestivum L.) was one of the first domesticated food crops and for 8,000 years has been the basic staple food of the major civilizations of Europe, West Asia and North Africa (Curtis 2002). The process of continuous diversification was initiated by domestication of emmer wheat and driven by several events of natural hybridization and allopolyploid speciation that took place in agro-ecological environments (Matsuoka 2011) in the Levant region of the Near East (Lev-Yadun 2000; Matsuoka 2011). There are three types of wheat based on cytogenetics (i.e. chromosome number) namely: Einkorn group, diploid, 2n = 14, Emmer group, tetraploid, 4n = 28 and Vulgare group, hexaploid, 6n = 42 (Sears 1954) (Figure1.3)

Bread wheat is an allohexaploid (2n=6x=42) with a genome size of 16 billion base pairs of DNA organized into 21 pairs of chromosomes, seven pairs belonging to each of the genomes A, B and D (Sears 1954). Wheat possesses one of largest and most complex genomes, and because of its hexaploid nature and economic importance as a food source, bread wheat is the most cytogenetically studied of the crop species.

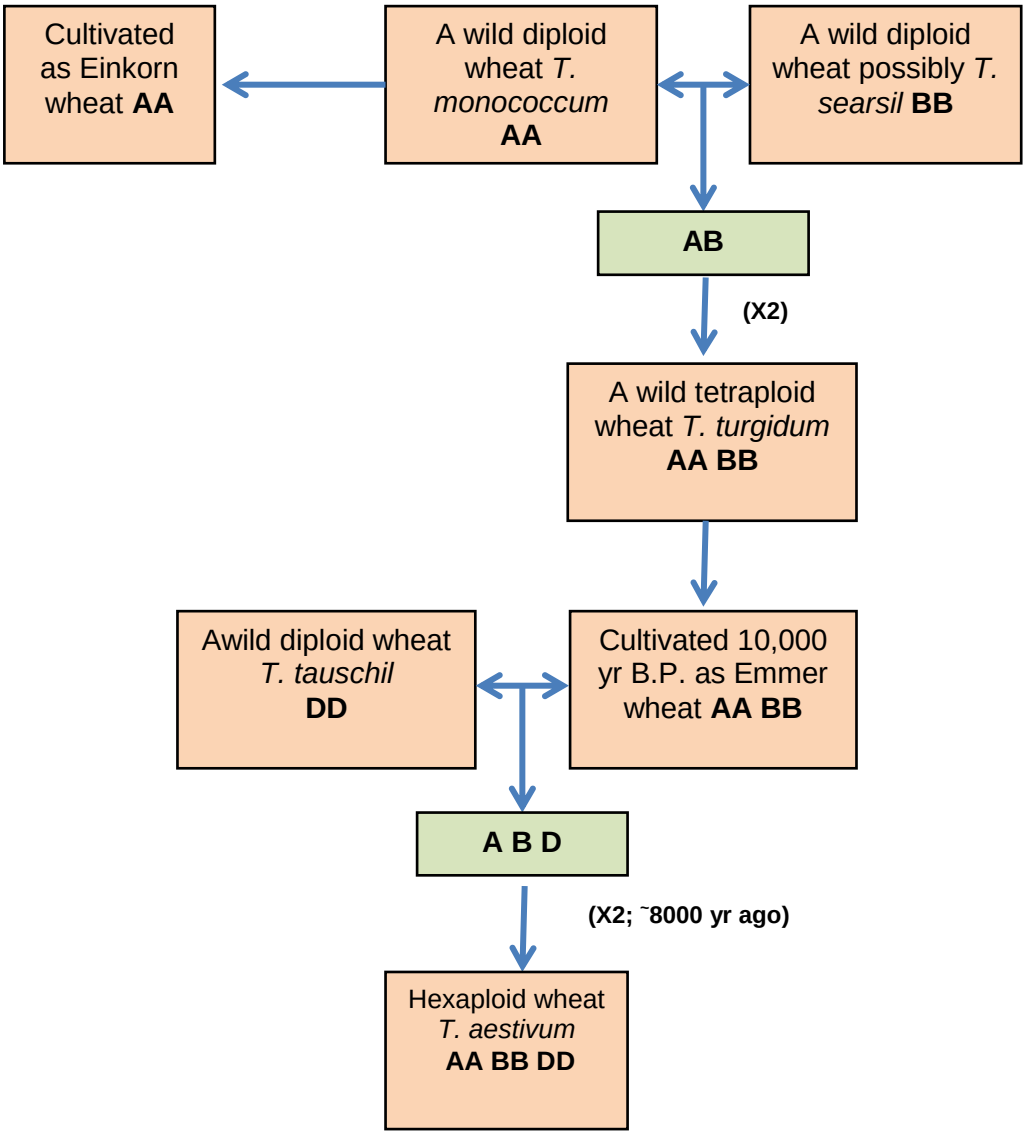


Figure 1.3. Hybridisations events involved in the evolution of bread Wheat, *Triticum aestivum*. The 'X2' refers to the doubling of the chromosome complement which gives rise in fertile hybrids. Source

(Adapted from

http://www.cerealsdb.uk.net/cerealgenomics/WheatBP/Documents/DOC_Evolution.php)

1.5 Objectives

The overall objectives of the present study in field experiments at CIMMYT Cd. Obregon, at CENEB (Centro Experimental Norman E Borlaug) is:

1. To identify physiological traits determining optimized DM partitioning for spike fertility, enhanced above-ground biomass per unit area, and enhanced HI in adapted CIMMYT wheat germplasm.

2. To quantify post-anthesis source: sink balance according to responses to degraining to source/sink manipulation treatment in adapted CIMMYT wheat germplasm and

3. To quantify genetic variation in radiation-use efficiency (RUE) and senescence-related traits and their physiological bases associations with source/sink balance in adapted CIMMYT wheat germplasm.

4. To Identify marker-trait associations for grain weight response (as an indication of source-sink balance) to degraining, and senescence-related traits

CHAPTER 2 LITERATURE REVIEW

2.1 The physiological basis of genetic progress in yield potential

Yield potential is defined as the yield of a cultivar when grown in an environment to which it is adapted, with nutrients and water non-limiting and with pests, diseases, weeds, lodging, and other stresses effectively controlled (Evans and Fischer 1999). This definition of yield potential builds upon the concept that there are yield potential genes and stress-resistance genes, and that a yield potential measurement attempts to quantify the effects of the former (Evans and Fisher 1999).The physiological components of yield potential are defined in equation 2.1:

Grain Yield = I x f x RUE X HI (Monteith and Moss 1977) **Equation 2.1**

Where I is incident radiation, f is fractional interception of radiation, RUE is radiation–use efficiency and HI is harvest index. Fractional interception is the proportion of incident solar radiation intercepted by the active photosynthetic surfaces of a crop. Radiation-use efficiency is the amount of above ground dry matter production per unit radiation intercepted or per unit radiation absorbed (if reflectance is subtracted from interception). Harvest index is the proportion of above-ground dry matter (AGDM) in grain at harvest.

During the green revolution of the 1960s and 1970s, yield potential was improved in wheat worldwide by increasing the HI (Reynolds et al., 2012; Slafer and Andrade 1993). Yield increases with the introduction of semi-dwarf cultivars were associated with reduction in stature, reduction in lodging, and also reduced competition between the growing stem and the spike resulting in greater spike growth, more grains per unit area, and higher HI (Fischer and Stockman 1986). Semi-dwarfing genes reduced lodging risk and favoured spike partitioning at anthesis and increased grains m^{-2} (Evans and Fisher 1999). In hexaploid wheat, semi-dwarfing has been achieved mainly through the introduction of the gibberellic acid-

insensitivity Rht alleles *Rht-B1b* and *Rht-D1b*, now found in the majority of varieties grown worldwide (Evans 1998; Gale and Marshall 1973). Different reduced height (*Rht*) genes and combinations gave optimal yields depending on the height of the background variety (Flintham et al., 1997). However, not all combinations of Rht genes produced high yields and not all varieties with high HI are high yielding. The photoperiod insensitivity genes (PpD-D1 and PpD-B1 formerly Ppd1, Ppd2) on chromosomes 2D and 2B were introgressed into spring wheats in the CIMMYT breeding program at the same time as the two homologous semi-dwarfing genes (*Rht-B1* or *Rht-D1* formerly Rht1, Rht2, respectively) on chromosome 4B and 4D, respectively were first utilized (Gale and Marchall 1973). Most current high-yielding CIMMYT lines have only one Ppd gene and either the *Rht-B1* or *Rht-D1* gene (Rajaram and van Ginkel 1996).

Table 2.1 Wheat rates of genetic gains in yield potential reported in sets of historic cultivars worldwide

Study	Country	Year of Release	Genetic gain (%)	No. Cultivars
Aisawi et al., (2015)	Mexico (CIMMYT)	1966-2009	0.4% p.a.	12
(Clarke et al., 2012)	UK + France	1953-2008		10
Lopes et al., 2012	Mexico (CIMMYT)	1997-2008	0.9% p.a.	26
(Brisson et al., 2010)	France	1980-2010		3 sets of references
Sener et al., 2009	Medi-terranean	1976-1999	0.5% pa.	16
(Shearman et al., 2005)	UK	1972-1995	1.2% p.a.	8
(Underdahl et al., 2008)	USA	1968-2006	1.3% p.a.	33

Table 2.1 shows values for the rate of genetic improvement in wheat yield from recent studies. Although in the last decades several investigations have reported gains in grain yield potential, others have reported that grain

yield has plateaued, for instance, in France (Brisson et al., 2010) and in the UK (Clarke et al., 2012). In France, a stagnation of bread wheat yield has been observed since the middle of the 1990s (Oury et al., 2012), in part attributed to effects of increasing temperature (increasing climate change).

Austin (1980) modelled partitioning of biomass in wheat and predicted there was scope to improve yields further by increasing HI. Austin predicted a theoretical maximum HI of 0.62, and, partly based on Austin's calculations, Foulkes et al. (2011) suggested a theoretical maximum of 0.64 for HI. Harvest index has already reached 0.61 for UK winter wheat (Foulkes et al., 2007); however, for spring wheat current values are typically around 0.50 (Foulkes et al., 2011). Shearman et al. (2005) in a set of 8 winter wheat cultivars in the UK released from 1972 to 1995 found that yield improvement was associated with above-ground biomass increase from about 1985. Similarly, Xiao et al. (2012), who reported gains in wheat grain yield in cultivars released from 1972 to 1995, found from about 1980 onward grain yield was associated with biomass. More recently Aisawi et al., (2015) found that yield potential progress was associated mainly with the increment of biomass in 12 semi-dwarf historic CIMMYT cultivars in NW Mexico released from 1966 to 2009. This evidence suggests that breeders have been selecting directly or indirectly for above-ground biomass which in turn has increased grain yield in recent decades. Parry et al. (2011) concluded that photosynthesis efficiency in the field in modern wheat cultivars is less than one third of the theoretical maximum.

2.2 Physiological determinants of yield potential

2.2.1 Physiological determinants of grains m^{-2}

Plant breeding in CIMMYT spring wheat on cultivars released from 1962 to 1988 increased grain number per unit area (Fischer et al., 1998) and recent investigations show that grain weight has been increased in a set of 12 CIMMYT semi-dwarf cultivars from 1966 to 2009 in irrigated, high-potential conditions in NW Mexico (Aisawi et al., 2015). Some studies have indicated a negative relationship between genetic progress in the grain weight and grains m^{-2} (Foulkes et al., 2010; Slafer et al., 1994). This relationship makes it difficult sometimes for breeders to increase one component independently of the other.

Grain sink size (capacity of the grains to accumulate available assimilates) rather than grain source size (capacity of plant to photosynthate assimilates) post-anthesis is reported to limit grain growth for the majority of wheat crops under favourable conditions (Borrás et al., 2004; Fischer 2008; Zhang et al., 2010). To date, the yield potential of wheat has been considered to be principally limited by the sink size (Fischer 2007; Reynolds et al., 2007; Shearman et al., 2005). In order to increase the grain sink size, the grain number per unit area and/or the potential grain weight must be increased. In addition to boosting grains m^{-2}, optimizing the balance between source and sink is therefore a critical factor in enhancing grain yield potential.

Many studies indicated that the proportion of above-ground dry matter partitioned to the spike pre-anthesis was positively correlated amongst genotypes with the grain number m^{-2}. For example, Slafer et al., (1990) reported genetic progress in grain yield in Argentina through HI was due to the improvement of pre-anthesis biomass partitioned to the spike. Fischer (2007) concluded that total assimilate accumulation in growing spikes, as reflected in spike weight per unit area at flowering, is a major part of the

determination of grain number m^{-2}. Maximizing growth during the rapid spike-growth phase from booting to anthesis is highly associated with genetic improvement in grains m^{-2} (Fischer 2011; Foulkes et al., 2011). Most studies report that modern wheat cultivars have a higher grain number per spike than their predecessors (Slafer and Andrade 1989). Evidence from historical sets of cultivars indicated that grains m^{-2} was the yield component that has mainly been modified by wheat breeding (Slafer et al., 1994). It has been suggested that extending the duration of the stem-elongation phase as a proportion of the thermal time to anthesis could bring about improvements in spike dry matter (DM) at anthesis and grains m^{-2} (Slafer et al*.,* 2001). Since pre-anthesis growth determines grain number m^{-2} (Slafer et al., 1994), increased pre-anthesis RUE is a mechanism that could increase grains m^{-2} combined with a better assimilate partitioning to the spike (Foulkes et al., 2011).

In addition to increase above-ground biomass in the pre-anthesis phase, grains m^{-2} may be increased by increasing spike partitioning index (spike DM / aboveground DM at anthesis; SPI) to boost spike DM at anthesis or by improving the fruiting efficiency (grains per gram of spike DM at anthesis; FE).

Grain number per unit area (GM2) can be analysed according to the following equation (Eqn. 2.2).

GM2 = RI x RUE x SPI x FE **Equation 2.2**

Where RI = amount of radiation interception by the crop (MJ m^{-2}) in the pre-anthesis period from emergence to anthesis, RUE = radiation-use efficiency (above-ground DM per unit radiation interception; g MJ^{-1}) in the pre-anthesis period, SPI = spike partitioning index (proportion of above-ground DM partitioned to spike at anthesis) and FE = fruiting efficiency (grains per g spike DM at anthesis).

Dry matter partitioning is the allocation of assimilate produced by photosynthesis of green tissues amongst plant components for growth and storage processes (Gardner et al., 1985). A wheat plant can be divided into the main plant organs: roots, leaf lamina, leaf sheath, true stem and ear. These organs have a competition between them, and alternative organs to the spike may compete to differing extents for assimilate during the stem-elongation phase. Increased partitioning to the spike could be increased by reducing competition for assimilate for spike growth from alternative sinks (roots, leaves, stems, structural and soluble carbohydrate) (Foulkes et al., 2011), especially during stem elongation when grain number is determined (Fischer 1985; Kirby 1988).

The fractional light interception is determined by the green area index and the light extinction coefficient (K); above-ground DM is the product of radiation interception and RUE (Shearman et al., 2005; Siddique et al., 1989). Spike partitioning index of wheat reported in the literature at anthesis ranges from 0.12-0.29 (Foulkes et al., 2011; Shearman et al., 2005; Siddique et al., 1989). Genetic ranges of DM partitioning at anthesis are reported for the leaf lamina from 0.19 to 0.31 and for the leaf sheath and stem from 0.31 - 0.63 (Gaju 2007; Shearman 2001).

The complementary trait to spike partitioning index influencing genetic variation in GM2 is the fruiting efficiency (grains per unit spike DM at anthesis; FE). Genetic variation in FE is reported in spring wheat in the range 35-87 grains g^{-1} (Abbate et al., 1998) and 78-196 grains g^{-1} in Argentina (González et al., 2011), 86-136 grains g^{-1} in Chile (Bustos et al., 2013), 162-201 grains g^{-1} in Australia (Dreccer et al., 2009) and 42-91 grains g^{-1} in Mexico (García et al., 2014). These studies were carried out in contrasting environments. E.g. in high radiation environment of Chile and UK.

Fruiting efficiency would be irrelevant as a strategy to increase grain yield if increases in FE are constitutively related to decreases in spike dry

weight at anthesis (Slafer et al., 2015). Some studies have reported negative relationships between these traits (Dreccer et al., 2009; Ferrante et al., 2012; Lazaro and Abbate 2012) while others found no significant associations (Bustos et al., 2013; García et al., 2014; González et al., 2011), supporting the idea of independence between these traits. Therefore, more knowledge is needed on the association of genetic variation in FE and spike dry weight at anthesis. However, strategies to increase SPI and FE should contribute to increasing grain number per m^2 and HI (grain DM /above-ground DM). Studies are now needed to identify traits to increase RUE and to identify the optimised plant DM partitioning at anthesis in order to increase spike fertility, HI and lodging resistance.

2.2.2 Potential grain weight

Grains fill with starch and protein during a relatively brief period and the seed matures. The grain is predominantly composed of starch deposited in the grain (endosperm cell number represents the potential of the grain), but filling includes a very important storage protein component plus the protein in the embryo. The grain components are shown in Figure 2.1

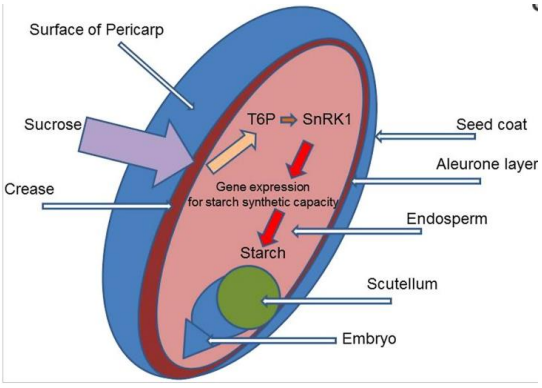


Figure 2.1 Diagram of a wheat grain identifying the main structural components from Lawlor and Paul,(2014)

Final grain weight in wheat is mainly dependent on the rate of accumulation of dry matter, which in turn is governed by the number of endosperm cells formed. Cell number in the endosperm seems to be

regulated by the supply of assimilates available to the grain during the first 14 days after anthesis (Brocklehurst 1977). Endosperm cell number represents the potential of the grain to accumulate starch (Brocklehurst 1977; Singh and Jenner 1982). The regulation of endosperm cell number by assimilate supply allows the source and sink to be maintained in a balance, with the potential grain weight geared to the likely subsequent assimilate supply (Singh and Jenner 1982). The period immediately before anthesis is important for the determination of potential grain weight as it is during this period that the structures of the ovary are formed, which are potentially important in determining grain development (Rijven and Banbury 1960). Calderini and Reynolds (2000) similarly found carpel weight at anthesis to be partially associated with the regulation of grain weight potential in synthetic hexaploid lines of wheat. Calderini et al., (2001) reported that temperature between booting and anthesis was closely related to final grain weight differences due to differences in carpel weight at anthesis. It has been demonstrated that high pre-anthesis temperatures can detrimentally affect the determination of carpel size (Calderini et al., 1999).

2.2.3 Crop development

Crop development is a continuous succession of changes, frequently divided into component phases (vegetative, reproductive and grain-filling phases). The most widely used scale for describing development of cereals is the scale designed by Zadoks et al. (1974). The different developmental stages in wheat are summarized in Figure 2.2.

2.2.3.1 Pre-emergence: After seed imbibition the shoot apex re-starts the initiation of leaf primordia. When soil moisture is not limiting germination, the rate of development depends only on temperature.

2.2.3.2 Post–emergence vegetative: When all remaining leaves on the main shoot are initiated the apex becomes reproductive and starts to

initiate spikelet primordia. During this phase leaves start to appear at a regular thermal interval (Phyllochron) and tillering begins: The appearance of the first tiller coincides with the appearance with the fourth leaf, and the subsequent primary tillers appear at regular intervals of one phyllochron (Masle 1985).

2.2.3.3 Early reproductive: When all the spikelets and many florets are initiated. Leaves continue to appear and in most cases tillering will reach its maximum rate. Depending on the length of the phase, and on agronomic conditions, the maximum number of tillers may be reached by the end of this phase. When the rate of development of this phase is accelerated by photoperiod and vernalization requirements, the shorter period results in less spikelets being initiated, (Slafer and Rawson 1994).

2.2.3.4 Late reproductive: When the number of fertile florets is determined simultaneously along with the active growth of stems and spikes. During this phase leaves continue to appear until the last initiated leaf appears (flag leaf). During this phase the demand for assimilate increases dramatically, with increases in the competition for resources among plant organs. Therefore, the period from terminal spikelet initiation to anthesis is considered to be critical in determining yield potential. Due to the competition, the resources are insufficient to maintain all the young tillers that have appeared and, some of these tillers will die. At the end of the late reproductive phase, when the terminal spikelet is initiated, the final number of spikelets per spike is determined and several floret primordia have been already initiated, particularly in the spikelets initiated in the middle third of the spike (González et al., 2003; Kirby 1988).

2.2.3.5 Post anthesis: this is the phase of grain filling, when the grains firstly develop their potential size at about 10-14 days after anthesis (Lawlor and Paul 2014) and then grow to reach their maximum dry weight by maturity. The initial stage of this period is characterized by the development of most endosperm cells, and the generation for grain

volume (the maximum grain water content is achieved). During the later stage, grains actively grow linearly with thermal time until the end of the phase. There is a steady reduction in grain water content from flowering to maturity and the water content at maturity is fairly constant across genotypes and conditions (Calderini et al., 2000; Schnyder and Baum 1992)

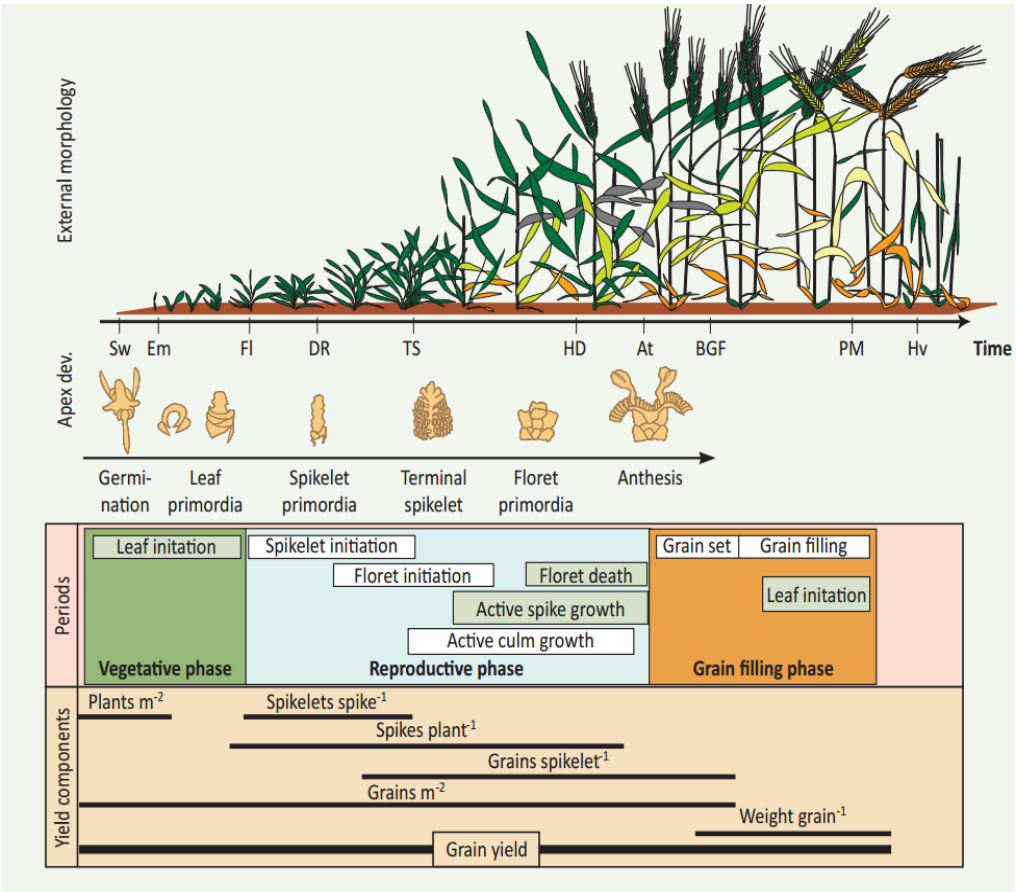


Figure 2.2 Schematic diagram of wheat growth and development adapted from (Slafer and Rawson 1996), showing the stages of sowing (Sw), emergence (Em), floral initiation (FI), first double ridge appearance (DR), terminal spikelet initiation (TS), heading (HD), anthesis (At), beginning of the grain-filling period (BGF), physiological maturity (PM), and harvest (Hv). Apex development is shown under each phenological stage. The periods of initiation or growth of specific organs and those of when different components of grain yield are determined are represented in the bottom boxes.

2.2.4 Tiller production and survival

Tillering is the emergence of side shoots in the leaf axils. It can continue until after the start of stem extension. Cereals are highly plastic in the number of tillers they produce. However, more than 50% of shoots produced by wheat can fail to produce grain-bearing spikes (Whaley et al., 2000). A wheat plant grows a number of tillers, depending on factors such as plant population, sowing date, mineral nutrition and the application of plant growth regulators. The first tiller emerges in the axil of the first leaf (or coleoptile) as the fourth leaf emerges. The next tiller develops in the second leaf junction one phyllochron (the time between emergence of two successive leaves, measured in thermal time) later, and so on. Secondary tillers develop in leaf axils of primary tillers (Huel and Hucl 1996).

The timing of tillering depends on sowing date, plant number established and temperature. Genotypes with large leaves, or that reach stem extension early, tend to have lower maximum shoot numbers. Tiller survival is usually more important than tiller production in determining final shoot number. Tiller survival varies significantly between varieties from under 40% to over 70% (Ellis et al., 2009).

In environments with adequate water supply there is conjecture as to whether non-surviving shoots are beneficial or detrimental to crop performance. However, Simons (1982) concluded that non-surviving shoots are likely to be detrimental for crop performance, even within environments with adequate water. Berry *et al.* (2003) found that none of the dry matter accumulated in non-surviving shoots is re-distributed to fertile shoots when they die. In addition, they showed that genetic differences in the amount of dry matter wasted ranged from 0.1 to 1.2 t ha^{-1} in four UK winter wheat cultivars and suggested that tiller economy would be a valuable trait for drought tolerance and that non-surviving shoots will reduce the yield potential in the majority of situations by competing for assimilate with the developing spikes in fertile shoots.

2.2.5 Stem water soluble carbohydrates

Water-soluble carbohydrates (WSC; composed mainly of fructans, sucrose, glucose, and fructose, with the main reserve as fructans at the late stages of WSC accumulation) deposited in wheat stems are important carbon sources for grain filling (Ruuska et al., 2006). Genetic variation in stem WSC amount in the range 220 – 300 g m^{-2} was observed by Foulkes et al. (2002) amongst six winter wheat cultivars grown in the UK. Genetic variation in the percentage of WSC in the stem at anthesis is reported from 5-50% in wheat (Ehdaie et al., 2006; Foulkes et al., 1998; Rebetzke et al., 2008; Reynolds et al., 2007a; Ruuska et al., 2006). The percentage contribution of stem water soluble carbohydrate (WSC) to the grain yield was at least 21% in spring wheat in Australia (Borrel et al., 1989) and 30-40% on winter wheat in the UK (Foulkes et al., 2002). Ehdaie et al. (2006) found a strong positive correlation between pre-anthesis stem WSC content and the utilized amount of WSC, indicating cultivars which accumulate more WSC pre-anthesis seem able to mobilize this storage as efficiently as cultivars with lower stem WSC accumulation, WSC is associated with grain yield usually under abiotic stress (Gallagher et al., 1976). However, the association of stem WSC and grain yield has also been reported in favourable conditions (Foulkes et al., 2007; Foulkes et al., 2002; Gebbing et al., 1999)).

2.3 Radiation interception and radiation-use efficiency (RUE)

2.3.1 Radiation interception

2.3.1.1 Canopy architecture

Photosynthetically active radiation (PAR) designates the spectral range of solar radiation from 400 to 700 nm that photosynthetic organisms are able to use in the process of photosynthesis. The ratio of PAR to total radiation in the direct solar beam is between 0.44 to 0.45 when the sun is more than 30^{o} above the horizon (Moon, 1940). However, the contribution of diffuse radiation, which is scattered by gas molecules in the atmosphere and which contains a much higher proportion of PAR to total radiation and the diffuse component is about 0.60. Combining the direct and diffuse components in appropriate proportions, the radio of PAR to total solar radiation in close to 0.5. Monteith (1972) suggests an average of 0.5 is probably appropriate in the tropics as well in the temperate latitudes.

The light extinction coefficient (K) is defined as:

$K = -\ln (I/I_O)/L$ (Monsi and Saeki 1953) **Equation 2.3**

Where Io is incident radiation, I is the amount of radiation transmitted below, L is the green area index, (ratio of crop green area to ground surface area).

The K_{PAR} is the rate at which PAR is absorbed down the canopy profile, in relation to green area index (eqn 2.3). Size and insertion angle of leaves influence K_{PAR}. In canopies with large portrait leaves K_{PAR} is higher and in canopies with narrow and erect leaves it is lower. Gallagher and Biscoe (1978) reported the conservative nature of radiation-use efficiency (RUE) in cereal crops. Crop growth is dependent on the ability of the canopy to intercept incoming radiation, which is a function of green area index and canopy architecture, and to convert it into new biomass (Gifford et al., 1984). The efficiency of crop production is defined in thermodynamic

terms as the ratio of energy output (carbohydrate) to energy input (solar radiation) (Monteith and Moss 1977).

Most incident radiation is already intercepted during the season in modern cultivars, but there may scope for increasing fractional interception at start and end of the season.

2.3.1.2 Senescence and stay green

All green t*i*ssues of a wheat plant are capable of photosynthesis, although the flag leaf is the most important photosynthetic apparatus during the post-anthesis period (Pourreza et al., 2009). During this period, light interception decreases with the onset of senescence. However, an increasing fractional absorption at the start of senescence would help to increase radiation use efficiency at educed plant densities (Whaley et al., 2000). The initiation and rate of senescence is dependent on a wide range of environmental factors and hormonal signaling effects (Nooden et al., 1997; Sharabi-Schwager et al., 2010), including nitrogen availability, water supply, and incidence of leaf diseases; however, nutrient deficiencies are not central senescence programme components (Nooden et al., 1997). Senescence is considered an internally programmed degeneration leading to death, but is nevertheless an important developmental process occurring in many tissues (Nooden et al., 1997). Senescent leaves become a source of nitrogen and minerals for developing grain. The process of senescence is triggered once the plant reaches a certain age, even when environmental conditions may be favourable for continued growth (Buchanan-Wollaston et al., 2003).

The rate of senescence is dependent mainly on accumulated temperature; higher temperatures increase the rate due to quicker re-mobilisation of nitrogen, which is related to the nitrogen nutrition status of the plant and source/sink relations (Crafts-Brandner et al., 1998; Masclaux et al., 2000; Ono et al., 1999). Slower senescence (stay green) has been associated

with less efficient post-anthesis remobilization of N from stems to grain (Foulkes et al., 2009). In addition, several investigations have concluded that the genetic control of N remobilization is linked to the regulation of leaf senescence (Masclaux et al., 2001; Uauy et al., 2006).

Senescence rate is linked to canopy N dynamics; either a plant has accumulated more nitrogen at anthesis that could delay senescence or the plant has a slower dynamic to remobilize N after anthesis during the grain filling stage (Gaju et al., 2011). In addition, in the latter study it was concluded that under low nitrogen supply the delayed senescence post-anthesis is an important trait to increase the grain yield in UK and French-winter wheat cultivars.

Five classes of delayed senescence or 'stay-green' have been described in the literature (Thomas 2000).

Type A: Late initiation of senescence, but a normal senescence rate.
Type B: Normal initiation of senescence, but a slower rate of senescence.
Type C: Lesion in chlorophyll degradation, leaving the rest of the senescence process unaffected. Example Mendel's locus in pea (*Pisum sativa*) (Armstead et al., 2007).
Type D: Rapid death (freeze, boil, dry) ensures maintenance of leaf colour in a dead leaf.
Type E: Enhanced greenness but unchanged initiation and rate of senescence. As a result the overall process of senescence will take longer to complete.
Types A, B, and possibly E are functionally stay-green: they maintain the photosynthetic capacity in their tissues.

There is evidence for genetic variation in stay-green in wheat and the stay-green phenotype has been shown to improve yields under abiotic stress (Harris *et al.* 2006; Lopes and Reynolds 2012). Christopher et al. (2008) found that the stay-green phenotype in spring wheat SeriM82 was

associated with extraction of deep soil water in Australia. Lopes and Reynolds (2012) reported under heat and heat combined with drought environments canopy temperature measured at the mid-grain filling stage and stay-green variables accounted for around 30% of yield variability in two panels/populations (291 advanced lines and recombinant inbred line population of 169 genotypes derived from a cross of Seri-Babax from CIMMYT).

Stay-green phenotypes and genetic variation in senescence have been reported in wheat under abiotic stress conditions (Blake et al., 2007; Bogard et al., 2011; Christopher et al., 2008; Gong et al., 2005; Lopes and *Reynolds* 2012; Luo et al., 2006; Naruoka et al., 2012). Genetic variation in the stay-green trait has been reported to increase wheat yields (Christopher et al., 2008; Gong et al., 2005; Lopes and Reynolds 2012; Luo et al., 2006), and there were positive correlations with nitrogen use efficiency (Gaju et al., 2011).

The specific mechanisms by which senescence is regulated are not fully understood, although there is evidence that it is driven by many specific genes which are either highly expressed or down regulated during the senescence process (Gepstein 2004). Degradation of Rubisco and chlorophyll loss, with simultaneous yellowing of leaves, slowly reduces photosynthetic capacity. Furthermore, radiation interception is reduced as the plant ages. Varieties with lower extinction coefficients tend to senesce more slowly as light penetrates further down the canopy. Genotypes with erect leaves are therefore more likely to have an advantage over prostrate ones in capturing more light and delaying senescence (Innes and Blackwell 1983; Thomas and Smart 1993).

Wheat above-ground crop components such as leaf lamina, leaf sheath and true stem contribute to produce source for the grains yield. These components accumulate nitrogen at earlier stages before anthesis to fill the grains during the filling grain stage. With a large physical size the true

stem has an important role in accumulation and storage of nitrogen reserves more than the ear (Pask et al., 2012b). High stem N reserves accumulated at anthesis may be associated with stay green effects.

Indices used for estimating greenness or canopy area

Genetic variation for stay-green traits can be selected either visually or using spectral reflectance techniques (Reynolds et al., 2005), and several spectral reflectance indices have been developed for estimating green leaf or canopy area, e.g. NDVI (Normalized Difference Vegetation Index), R-NDVI (Relative Normalized Difference Vegetation Index), G-NDVI (Green Normalized Difference Vegetation Index),, SR (Simple ratio), GI (Greenness), GLAI (Green leaf area index. RVI (Radio Vegetation), DVI (Difference Vegetation), PVI (Perpendicular vegetation index) etc.(Broge and Mortensen 2002; Penuelas et al., 1995; Wiegand et al., 1991). NDVI has been widely reported to be correlated with senescence traits in cereals.

NDVI = (R910-R680)/(R910+R680)

Where R910 is the reflectance at Near Infra-Red (910 nm) and R680 is the reflectance at red (680 nm).

2.3.1.3 High-throughput phenotyping

In the recent years, there has been increased interest in high throughput phenotyping canopy performance platforms (HTPPs). The most successful traits for evaluation integrate in time and space and across leaf layers canopy. Different methodological approaches have been proposed to evaluate these traits in the field (remote sensing and imaging, lab analysis of samples and near-infrared reflectance spectroscopy analysis) (Araus and Cairns 2014).

Recently, high throughput methods have been developed using NDVI measurements from devices such as the hand-held Greenseeker (NTech

Industries, Ukiah, CA, USA) (Kipp et al., 2014; Lopes and Reynolds 2012). The advantage of such methods is that they provide objective, integrated measurements of canopy greenness and only take a few seconds to measure a field plot. In addition they can provide measurements of hundreds of field plots per hour and thus can be used to screen large numbers of plots. As they are non-destructive and relatively rapid, repeated measurements can be made on the same plots over crop development. In particular, studies for senescence kinetics, visual assessments can be performed for greenness of the flag leaf (Gaju et al., 2014a) the flag level and peduncle (Joshi et al., 2007). More objective methods can be applied, with measurements of relative chlorophyll content of individual leaves using instruments such as the Minolta SPAD meter. High-throughput phenotyping platforms will be more common in the near future and are becoming more available already at low cost (Araus and Cairns 2014).

2.3.2 Radiation-use efficiency

2.3.2.1 Definition of Radiation-use Efficiency

Radiation-use efficiency (RUE) is defined as the ratio of above-ground dry matter that a crop accumulates per unit area to the radiation interception or absorption (Sinclair and Muchow 1999). Monteith derived the concept of RUE and demonstrated that that biomass production in cereal crops correlated more closely with light interception than with time.

$$\varepsilon_S \text{ (g MJ}^{-1}) = \Delta W / (\Delta t \times f \times I_0) \qquad \text{Equation 2.4}$$

Where ε is conversion efficiency, W is biomass, t is time, f is fractional interception and I0 is incident radiation. There are many methods for estimation of RUE. Calibrated sensors are required to measure daily incident radiation. Fractional interception can be measured continuously using tube solarimeters in uniform crop stands to avoid temporal errors

(Sinclair and Muchow 1999). Radiation-use efficiency can be calculated from difference in biomass between two consecutive harvests divided by the corresponding amount of radiation intercepted. However, a more appropriate measure is to fit a linear relationship between cumulative biomass accumulation and cumulative radiation interception, with RUE calculated as the slope of the linear relationship (Monteith 1977).

Estimates of RUE_{PAR} are approximately twice as large as those based on incident solar radiation. Calculation of RUE_{PAR} can also be adjusted for radiation interception where the reflected radiation is deducted. For closed canopies about 6% of the light incident at the canopy surface is reflected to the sky (Goudriaan and Van Laar, 2012). Calculation for RUE usually does not include root biomass. Sinclair and Muchow (1999) reported roots in cereals between 10-20 % of the total crop mass. A recent paper reported between 6 and 17% of total biomass in roots in wheat (Reynolds *et al.* 2009a). Variable losses of mass due to leaf senescence and even shoot death under some stress situations can lead to an underestimate of RUE (Sinclair and Muchow 1999). In wheat crops percentage light interception is generally very close to 100% when the canopy is closed, but losses occur before canopy closure and towards physiological maturity due to senescence as mentioned above (Reynolds 2000).

2.3.2.2 Genetic variation in RUE

Genetic variation in RUE has been found in bread wheat. For instance, Shearman et al. (2005) reported variation in a range of 2.33 to 2.64 g MJ^{-1} amongst eight bread wheat cultivars when RUE_{PAR} increased by 0.012 g MJ^{-1} yr^{-1} from 1972 to 1995 in winter wheat in UK. Yunusa et al. (1993) reported a range for RUE_{PAR} in the pre-anthesis period between 1.52-2.40 g MJ^{-1} for three spring cultivars released from 1986-1960 in Australia. Miralles and Slafer (1997) found a range for pre-anthesis RUE from 1.09 to 1.34 g MJ^{-1} for three near isogenic lines of spring wheat for Rht dwarfing alleles using solar radiation in Argentina. Calderini et al. (1997) reported a

range of 0.98 to 1.28 g MJ^{-1} for seven cultivars, released in Argentina from 1920 to 1990.Fischer and Edmeades (2010) estimated that RUE can be increased to 5.4 g MJ^{-1} in C3 crops by increasing the efficiency of photosynthesis. In addition, Reynolds et al. (2012) stated that there is potential to increase RUE by 50% and suggested that the strategies include modifying specificity, catalytic rate and regulation of Rubisco, up-regulating Calvin cycle enzymes, introducing chloroplast CO_2 concentrating mechanisms, optimizing light and N distribution of canopies while minimizing photoinhibition, and increasing spike photosynthesis. It is generally observed that post-anthesis RUE decreases after anthesis plus 10 days (Fischer 1993). Another route to increase post-anthesis RUE is by increasing the grain sink strength (Slafer et al., 1996). Reynolds et al. (2005) demonstrated that by increasing the sink strength at the booting stage RUE post-anthesis increased due to an upregulation effect.

2.3.2.3 Physiological determinants of genetic variation in RUE

2.3.2.3.1 Crop height and phenology

Optimal crop height for yield potential in wheat is in the range 80-100 cm (Foulkes *et al.* 2011). Crop height and phenology affect RUE (Miralles and Slafer 1997). In three spring wheats near-isogenic Rht lines in Argentina it was found that shorter plants had lower RUE pre-anthesis, but RUE post-anthesis declined post-anthesis more in taller plants than shorter plants (Miralles and Slafer 1997).

2.3.2.3.2 Canopy level traits

Leaf angle

Erect leaves use light more efficiently than prostrate leaves (Humphries and Long 1995), because with lower K_{PAR} light is able to penetrate deeper into a crop canopy, so photosynthesis of upper leaves is less light saturated. Therefore, the capacity of the leaf to use light is less likely to be

exceeded and photo protection is less likely to occur under saturation light intensity (Murchie and Niyogi 2011). More erect leaf canopy types are characteristic of many of CIMMYT's best yielding wheat lines (Araus et al., 1993; Fischer 1996). While different wheat cultivars absorbed similar amounts of solar radiation, RUE is higher before anthesis in cultivars with lower light extinction coefficients (Araus et al., 1993; Green 1989).

Leaf angle as a selected criterion was first highlighted by Donald (1968) and has been linked to progress in RUE in Australian wheat varieties (Siddique et al., 1989). Leaf angle influences photosynthesis in individual leaves, in canopies with higher values of GAI a more erect leaf intercepts light more efficiently, because light is spread less densely across the leaf surface, so that light saturation of photosynthesis is minimal (Murchie and Niyogi 2011). Long et al. (2006) suggested that photosynthetic efficiency can be increased by 45% by optimizing leaf angle. In the 1970's CIMMYT germplasm screening resulted in the introgression of an erect leaf gene from *T. aestivum* subsp. Sphaerococum (Reynolds 2000). Most modern cultivars however, already have erect leaves so there may limited scope for future improvements in RUE through modifying leaf angle.

Nitrogen distribution in leaf layers

Vertical gradients of leaf nitrogen (N) content are commonly observed in canopies (Shiraiwa and Sinclair 1993). In a vegetative wheat canopy, leaf N distribution closely followed the light gradient and thus maximized canopy photosynthesis (Dreccer 2000). Radiation-use efficiency can be improved if incoming radiation is shared amongst several leaf layers in the canopy resulting in less light saturation of upper leaves and higher photosynthetic conversion efficiency (Monteith 1965). In addition, reduced light saturation and higher RUE may be associated with smaller, thicker flag leaves (Shearman et al., 2005). Therefore, selection for a smaller flag leaf of greater leaf specific weight (dry matter per unit leaf area; SLW) could offer scope for increasing RUE pre- and post- anthesis (Foulkes et

al., 2007). Positive associations between N content per unit leaf area and A_{MAX} have been observed in 48 lines (wild and cultivated species of rice) in a controled environment study (Cook and Evans 1983).

Distributing N so that leaves receiving the greatest photon flux densities have the largest specific leaf N content (N per unit leaf area; SLN) is also predicted to increase canopy photosynthesis (Moreau et al., 2012). Moreau et al. (2012) concluded that the acclimation of the leaf N gradient to the light gradient is a whole-plant process that depends on canopy size. This study on 16 cultivars demonstrated that modelling leaf N distribution and canopy expansion based on the assumption that leaf N distribution parallels that of the light is inappropriate. A relationship accounting for vertical leaf N gradient with respect to vertical light gradient as a function of canopy size was proposed.

2.3.2.3.3 Leaf level traits

Chlorophyll concentration a/b ratio

Chlorophyll is a pigment molecule with a key role in photosynthesis and can be measured directly using spectrophotometry (Porra 2002) or approximated using a SPAD meter (Hoel 1998). Leaf chlorophyll content may be used as an indirect indicator of crop nitrogen status. Although, total chlorophyll concentration of leaves at different canopy levels is similar, there is usually variation through the vertical canopy profile in the radio of two types of chlorophyll; chlorophyll a and chlorophyll b (Reynolds 2000). Chlorophyll a is located in the photosynthetic reaction centre and chlorophyll b is confined to light harvesting complexes, serving the reaction centre; the two chlorophylls are similar in structure (Horton et al. 1996). However, they absorb light differently, chlorophyll a showed the maximum absorbance at 662 nm and chlorophyll b at 646 nm (Lichtenthaler and Wellburn 1983). Chlorophyll ratio has changed with

breeding in the last decades and typical a/b ratios are in the range 3:1 to 3:2 (S Rajaram and M van Ginkel 1996).

Quantum yield is the maximum possible carbon uptake per unit light intensity; therefore quantum yield limits photosynthesis at low light intensities. The chlorophyll a/b ratio can be a useful indicator of N partitioning within a leaf, because this ratio should be positively correlated with the ratio of PSII cores to light harvesting chlorophyll-protein complex (LHCII) (Terachimaand Hikosaka 1995). Higher ratio chlorophyll a/b increases the quantum yield in low light intensity. So modifying a/b ratio could increase RUE.

Flag leaf specific weight, specific nitrogen concentration and stomatal conductance

Fischer et al. (1998) reported that flag-leaf specific weight ranged from 45-54 g m^{-2} in CIMMYT in eight spring wheat cultivars released from 1962-1988. Foulkes et al. (2007) found that flag-leaf specific weight was associated with RUE in winter wheat in UK amongst eight UK wheat released from 1972 to 1995. However, Pask et al. (2012) found that in the UK winter wheat Istabraq, flag leaf specific nitrogen concentration at anthesis correlated linearly with pre-anthesis RUE only up to 2 g N m^{-2} above which there was no further increase in RUE.

Leaf photosynthesis rate

Fischer et al. (1998) found associations between flag-leaf light saturated photosynthesis rate (A_{max}) and stomatal conductance in a set of historic CIMMYT spring wheat cultivars released from 1962-1988. In addition, Sadras and Lawson (2011) reported an association between stomatal conductance (during stem extension) and RUE in a set of Australian spring wheat varieties. Increases in A_{max} in synthetic wheats were associated with reduced flag-leaf area, increased stomatal and mesophyll conductance and lower canopy temperature (del Blanco et al., 2000).

Metabolic level traits

Ribulose bisphosphate carboxylase oxygenase (Rubisco) is the key enzyme in photosynthesis. The concentration of Rubisco in leaves plays an important role in the determination of photosynthetic efficiency (Murchie et al., 2009). With the evidence that genetic yield progress is related with biomass production (Peng et al., 2000; Reynolds 2000; Shearman et al., 2005), improving leaf CO_2 assimilation rate through modifying Rubisco has been suggested as an avenue to yield progress. Rubisco catalyses the reaction that fixes CO_2 into a three carbon compound in C_3 photosynthesis. The amount and *in vivo* activity of Rubisco are considered as rate-limiting factors for carbon fixation (Parry, 2003). In order to tackle this limitation, increasing the Rubisco content in leaves is one simple possibility. This has to be with the optimal concentration of leaf N, which is determined partly by the leaf thickness limitation driven by inter-leaf shading (Murchie et al., 2009). In addition, a limit to the amount of protein that can be accumulated in the chloroplast, and the number of chloroplasts in the mesophyll cell may restrict Rubisco leaf concentration (Pyke and Leech 1987). An alternative for Rubisco amount improvement could be increase its specificity for CO_2 relative to O_2 by direct manipulation of the enzyme to reduce photorespiration (Parry 2003).

Approaches to increase RUE in wheat through modifying Rubisco properties will be by modifying catalytic rate and regulation of Rubisco, upregulating the Calvin cycle enzymes, introducing mechanisms for CO_2 concentrating in chloroplasts, and increasing the role of the spike photosynthesis (Reynolds et al., 2012).

2.3.3 Environmental determinants of RUE

Abiotic stresses are often interrelated, either individually or in combination; they cause morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity, and ultimately yield (Bita and Gerats 2013). The RUE can be affected by environmental

factors such as: temperature, atmospheric concentration of CO_2, light quantity, availability of nutrients, pest and diseases. In C3 plants, the optimum temperature range for photosynthesis is usually 20-35^{o}C (Sage and Kubien 2007). In wheat, there is evidence that N deficit affects leaf area index and consequently affects RUE (Sadras and Calderini 2015). Light quantity can also affect RUE; if the light has a high intensity proportionally less may be used by photosynthesis because of light saturation and RUE decreases. Incident radiation with a larger fraction of diffuse radiation is likely to have greater RUE than under direct radiation because the relative contribution of the shade leaves to biomass accumulation is increased as the fraction of diffuse radiation increased (Sinclair et al., 1992). Water stress also affects RUE, because dry conditions result in stomatal closure which reduces the rate of stomatal conductance (Foulkes et al., 2001; Tardieu and Simonneau 1998).

2.4 Source-sink balance

The photosynthetically active organs that are net exporters of photosynthate are classified as source. On the other hand, the photosynthetically inactive organs that are net importers of fixed carbon are classified as sink (Thomas 2000). For instance, active leaves (tissues) can be classified as source and inactive tissues can be classified as sink.

All production of biomass relies on the efficiency of light capture and of conversion of the intercepted light into biomass (Long et al., 2006). The actual contribution of carbon assimilation to grain yield depends on distribution of the photoassimilate to various organs and the balance between the source and sink (Cruz-Aguado et al., 2000). Sink strength may be defined as the competitive ability of an organ to import photoassimilates and is a product of sink size and sink activity (Gupta and Kaur 2000). Grain number positively influences post-anthesis RUE in wheat in relation to grain sink strength (Calderini et al., 1997; Miralles and Slafer 1997; Reynolds et al., 2005).

The final grain yield will be limited by either the supply of carbohydrate determined by the photosynthetic capacity (source) or the capacity of the grains to accumulate available carbohydrates (sink). Carbohydrates for grain filling are supplied concurrently from photosynthetic activities and from soluble carbohydrate reserves in the stem that are maximal about 10 days after anthesis (Zhang et al., 2010). It may be possible to increase simultaneously grain source and sink sizes through increased spike partitioning index (SPI) and/or pre-anthesis RUE, which would increase spike dry mass (g m^{-2}) and grain number per unit area, and hence enhance post-anthesis RUE through increased grain sink strength (Slafer and Andrade 1993).

2.4.1 Grain sink limitation

Dry matter partitioning is the end result of the flow of assimilates from source organs via a transport path to the sink organs (Marcelis 1996). Under optimal growing conditions and sufficient assimilate supply, grains can be fully developed. Potential grain sink size, however, may not be large enough to use all available assimilates efficiently, especially during early grain growth (Slafer et al., 1996). The extent of sink limitation can be tested by imposing source-sink manipulation treatments. For example, in a degraining (spikelet removal) treatment at anthesis + 10 days in potential conditions grain yield would be limited by the sink if grain weight did not increase significantly relative to the unmanipulated control. However, an increase in grain size relative to the control would indicate source limitation of grain growth.

The grain sink size depends on the number of grains per unit area (i.e. spikes per unit area and grains per spike) and the potential grain size. Bingham et al. (2007) estimated the sink size using Equation 2.4.

Equation 2.4

Sink size (gm^{-2}) = Grain $number^{-2}$ x PGW (Potential grain weight g)

It has been reported that grain growth of wheat crops is mainly sink limited under favourable conditions (Borras et al., 2004; Reynolds et al., 2005). In the last decades plant breeders have mainly increased grain yield (sink) without changing biomass (source), indicating that older wheat cultivars were severely sink limited (Slafer and Savin, 1994). However, future progress is likely to require improvement in assimilation capacity alongside continued improvement in grains m^{-2} and potential grain weight.

Source limitation during grain filling can result in shrivelled grains because of smaller endosperm cells containing less starch than corresponding cells in plump grains (Brocklehurst et al., 1978). The contribution of stem soluble carbohydrate reserves, which are maximal shortly after flowering, must also be considered when post-anthesis photosynthates do not meet the requirements of grain growth (Bonnett and Incoll 1993; Schnyder 1993). The grain source size is determined by the canopy architecture influencing the fractional radiation interception per unit of green area, canopy green area and senescence, RUE and the stem WSC accumulation and remobilisation efficiency. Under favourable conditions stay-green traits may not be expected to correlate with yield, as grain growth is reported to be sink limited, but this may be changing in most recent cultivars which may be co-limited by source and sink (Acreche and Slafer 2009; Ma et al., 1996).

Bingham et al 2007 estimated the grain source size using equation 2.5

Equation 2.5

Source size = Stem WSC at GS65 + 7 d (g m^{-2}) + (RUE (g MJ^{-1}) x RI (MJ m^{-2}).

Where Stem WSC = is the water-soluble stem carbohydrate reserves at anthesis +7 days, RUE = pre-anthesis RUE (GS31-GS65+7d), RI = Amount of radiation intercepted post-anthesis.

2.4.2 Evidence from source-sink manipulation experiments

Source-sink balance during the grain growth period is one of the key traits that needs to be better understood in modern wheat in order to identify combinations of traits to increase simultaneously grain sink and grain source size and ultimately enhance grain yield. Grain yield progress was highly associated with grain number per unit area in wheat during the Green Revolution (Brancourt-Hulmel et al., 2003; Canevara et al., 1994; Peltonen-Sainio et al., 2007; Sayre et al., 1997; Shearman et al., 2005) . Current evidence in the last decades suggests grain sink strength remains a critical yield-limiting factor (Borrás et al., 2004; Mirallesand Slafer 2007; Fischer 2009) and that improving the balance between source and sink is a promising approach for raising yield, biomass and post-anthesis radiation-use efficiency (Reynolds et al., 2001, 2005, 2012).

There is little information in the literature directly to test whether grain yields of wheat cultivars are source or sink limited. However, indirect evidence from the manipulation of source-sink ratios during grain filling and the analysis of changes in weight of grains from particular spikelet positions can be used as an indirect indicator of the extent of source or sink limitation (Slafer et al., 1996). Slafer and Savin (1994) found that altering the source-sink ratio in two wheat cultivars (Australian semi-dwarf and a Canadian standard-height cultivars) by removing all spikelets from one side of the spike 10 days after anthesis did not affect the grain weight at apical, central or basal positions, and grain yield was sink limited. Borrás et al. (2004) concluded in a review of 18 studies with different types of source-sink manipulation treatments that grain growth of wheat under favourable conditions was almost always sink limited, as the availability of the assimilates during the grain filling period was usually at a saturation level. On the other hand, Acreche and Slafer (2009) reported that in bread wheat genotypes released from 1940 to 2005 in Spain, when removing all the upper-half of the spike, grain weight was not changed in the old cultivars and increased in the newest cultivars. They concluded that

modern genotypes have a co-limitation of grain growth by source and sink rather than source limitation. Zhang et al. (2010) by imposing a degraining and shading treatment at anthesis found in wheat cultivars in SW Australia that grain growth of two from three spring wheat cultivars was sink limited. Ma et al., (1996) studying six red winter wheat varieties in the USA reported that in response to spikelet removal at anthesis two cultivars showed no increase in grain growth, but four cultivars showed increases in grain growth, indicating grain growth was co-limited by sink and source. In general, the above degraining studies tended to show that grain yield potential was limited under optimal conditions by the grain sink size rather than grain source size, and that there are some indications that more modern cultivars may be in closer source-sink balance than their predecessors.

A balance of large source size and large sink size would increase the grain yield significantly. Therefore, this balance can bring significantly gains in yield. The increment of sink size by the GM2 has been considered a key determinant of yield in wheat crops limited by sink (Fischer 1993; González et al., 2003; Savin and Slafer 1991; Sayre et al., 1997). However, Sinclair and Jamieson (2006) stated that grain number is only a consequence of grain yield rather as a determinant of grain yield, indicating that grain number and grain yield are controlled by the same factor rather grain number being the cause of determinant of yield, and concluded that carbon and nitrogen availability in the plant that set the grains are the crucial factors in determining both grain number and yield, and comparing to a brewery, it is not the number of bottles shipped from the brewery that is the determinant of productivity but the raw resources inputted to the brewing process. However, total dry weight at anthesis, as an index of resource accumulation, does not always correlate well with GM2, or yield and the mass of spikes at anthesis (SDW, g m^{-2}) is often a good predictor of GM2. Good evidence on the increase in the grain filling photosynthetic rate with increased sink strength, such as is likely to occur with increased GM2 with breeding progress (Fischer 2008). In addition,

studies with tall and semidwarf wheat cultivars in potential conditions, including near isogenic pairs, have shown that the shorter wheat cultivars, which have less biomass at anthesis but larger grain number values because of better partitioning to the spikes, gave clearly higher post-anthesis dry matter accumulation and grain yields (Fischer 1984; Siddique *et al.* 1989). Therefore, the sink and source need to be maintained in high balance for breeding progress.

2.5 Genetic marker systems in wheat breeding

In recent years, considerable emphasis has been placed on the development of molecular markers to be used for marker-assisted selection (Collard and Mackill 2008; Gupta et al., 1999). Molecular markers are a powerful tool for breeding. Due to their linkage to genes that are associated with traits in crops, they allow a reliable selection of breeding lines that is not concealed by environmental factors.

A genetic marker can be defined as an identifier of a particular aspect in the genome (a short DNA sequence). It describes a variation, which can be observed and may have arisen due to mutation or alteration at the genomic loci. The inheritance of a genetic marker can easily be tracked from generation to generation (Dreisigacker et al., 2012). DNA carries all genetic information needed for the organism to function; the entire string of nucleotides forms a double helix and is structured in chromosomes. Wheat has a total of 21 chromosomes and the genome contains approximately 16,000 mega base (Mb) pairs. In the DNA, there are non-coding sequences, including large amounts of repetitive sequences. Wheat contains 99% of repetitive regions; in addition, wheat is allohexaploid, which means that the wheat genome is derived from different species (diploid species A, B and D), as described in chapter 1.

In wheat breeding the most common markers used can be divided into two categories: 1) Markers that are based on a PCR (Polymerase chain reaction), for instance; microsatellites or Simple sequence repeats (SSRs),

Sequence-tagged site (STS) markers, Sequence characterized amplified regions (SCARs), Cleaved amplified polymorphic sequences (CAPS), Nuclear binding site (NBS) profiling, insertion based polymorphism (ISBP), and 2) markers for high-throughput platforms which are; Diversity array technology (Dart), Single nucleotide polymorphism (SNP), Golden Gate and infinitum SNP genotyping assays, and TaqMan and KASPar assays.

2.5.1 Mapping of genes

Genetic linkage maps area based upon the frequency of recombination in segregation populations and provide breeders with the position of genes on chromosomes and markers for tagging genes of desired traits.

2.5.2 Marker-assisted selection (MAS)

Many wheat breeding programs use the approach of marker-assisted selection (MAS). Once a marker is identified through quantitative trait loci (QTL) or association mapping analysis, its utility as an indirect selection tool must be validated in appropriate breeding populations.

Quantitative trait gene or locus (QTL) mapping has become a routine approach for genetic studies of complex traits in plants, QTL linkage mapping in plants it is based on bi-parental genetic populations, especially in practical breeding programs (Li et al., 2010). For example QTLs and marker development for complex traits have been detected for grain width on chromosome 2D for wheat cultivars in current elite soft winter germplasm in USA (Breseghello and Sorrells 2006). Snape et al., (2007) reported a QTL for yield on the short arm of chromosome 6A in winter wheat and a colocation in spring wheat has recently been reported (Lopes et al., 2015). Ashikari (2005) reported a QTL that increases grain number in rice, (Gn1a), which is a gene for cytokinin oxidase/dehydrogenase (OsCKX2).

The strategy of a Genome-wide association (GWA) is to genotype enough markers (discover a large number of genetic markers, tipically single nucleotide polymorphisms (SNPs), as a reference resource) across the genome so that functional alleles will likely be in linkage disequilibrium with at least one of the genotyped markers (Myles et al., 2009). The association mapping method requires collections of germplasm instead of bi-parental populations, to find genes underlying quantitative traits (Hästbacka et al., 1992; Yu et al., 2006). Identification of marker–trait associations is the first step towards marker-assisted selection in plant breeding (Wang et al., 2014). Association mapping or linkage disequilibrium mapping is an approach to dissect genetically complex traits based on the non-random association between alleles at a locus (or loci) and the phenotypic traits of interest across a diverse germplasm set (Weir 1996). Currently, AM has been used to identify the associations between molecular marker loci and complex traits of interest in wheat, such as grain size and milling quality (Breseghello and Sorrells 2006). Furthermore, AM has been successfully applied in on increasing number of wheat breeding programs (Gupta et al., 2005; Zhu et al., 2008). As a complementary approach to traditional bi-parental QTL mapping in wheat genetic studies, association mapping has three main advantages. Firstly, AM does not require the development of bi-parental progenies to find genes underlying quantitative traits (Hästbacka et al., 1992; Yu et al., 2006). Secondly, AM can have higher resolution power for mapping marker trait associations and greater capacity for detecting more alleles (Yu and Buckler 2006). Additionally, AM has a wide range of applications in plant breeding (Kruglyak 1999; Nordborg et al., 2002; Remington et al., 2001). And there has been increased interest in identifying novel marker–trait associations using AM in wheat (Gupta et al., 2005; Zhu et al., 2008).

2.6 Objectives and hypothesis

2.6.1 Objectives

The aims of the PhD study are:

1) To quantify the relationships between physiological traits, HI and grain yield at harvest amongst the 26 CIMCOG cultivars.
2) To identify interactions for grain yield and associated traits with the season amongst the CIMCOG genotypes.
3) To quantify rates of genetic gain in yield and associated traits with year of release of CIMCOG cultivars.
4) To quantify genetic variation in pre and post-anthesis RUE and senescence traits and associations with biomass and grain yield amongst ten CIMCOG genotypes -
5) To quantify the genetic variation in responses to a degraining source-sink treatment at GS65+7/10 days in the 26 CIMCOG genotypes. To determine in a subset of ten genotypes the responses of the basal, central and apical grains to degraining at GS65+7/10d and whether there are interactions with genotype.
6) To evaluate the genetic variation in senescence parameters in the 26 CIMCOG genotypes according to measurements of canopy NDVI, flag-leaf chlorophyll content (SPAD) and flag-leaf visual senescence score and correlations with grain growth responses to degraining.
7) To quantify the genetic variation in responses to a degraining source-sink treatment imposed at GS65+10/15 days on the 294 genotypes of the WAMI panel genotypes and to evaluate if the grain weight of modern high yielding WAMI spring wheat cultivars differs in the degree of sink limitation of grain growth.
8) To evaluate the genetic variation in senescence parameters according to measurements of NDVI, flag-leaf visual senescence score and quantify correlations with grain growth responses to degraining in a subset of genotypes of the WAMI panel.

9) To determine if the genetic variation in leaf and stem dry weight is related with the grain growth response to degraining at GS65+10/15d in the WAMI panel.
10) To identify marker-trait associations for grain weight response to degraining, senescence-related traits and leaf and stem weight responses to degraining (as an indication of stem WSC utilization) in the WAMI panel.

2.6.2 Hypothesis

- Genetic variation in grain yield is positively correlated with both HI and above-ground biomass; and there is no trade-off between these physiological yield components.
- Genetic variation in grain yield is positively correlated with both grains m^{-2} and grain weight; and there is no tradeoff between these numerical yield components.
- There are no effects of plant height and anthesis date on genetic variation in HI and grain yield in the CIMCOG panel.
- There is genetic variation in radiation interception by the canopy during stem elongation from GS31 to GS61+7d positively associated with above-ground dry matter (AGDM) accumulated during stem elongation and at anthesis amongst the CIMCOG genotypes.
- There is genetic variation in radiation-use efficiency during stem elongation and RUE is positively related with AGDM accumulated during stem elongation at anthesis amongst the CIMCOG genotypes.
- There is genetic variation in post-anthesis radiation-use efficiency positively related with grain yield amongst the CIMCOG genotypes.

- Genetic variation in each of spike partitioning index and fruiting efficiency is positively related with grains m^{-2} and HI amongst the 26 CIMCOG genotypes, and there is no trade-off between these two components.
- Post-anthesis source-sink balance differs amongst the 26 CIMMYT spring wheat cultivars in the CIMCOG panel.
- Grain growth of modern high-yielding cultivars in the CIMCOG panel is closer to source limitation than their predecessors.
- The grain weight response to degraining does not differ between spikelet positons in the spike (basal, central and apical) and there is no interaction with genotype
- Genetic variation in senescence durations and/or rates (NDVI, flag-leaf SPAD and flag-leaf visual score methodologies) are associated with grain yield potential and source/sink balance in the 26 CIMMYT spring wheat cultivars in the CIMCOG panel.
- Post-anthesis source-sink balance differs amongst the 294 WAMI genotypes.
- Genetic variation in senescence durations and/or rates (NDVI, and visual FLS assessment) is associated with grain yield and source/sink balance in the 294 WAMI spring wheat genotypes.
- Marker trait associations can be identified for grain weight response to degraining (as an indicator of source sink balance) and senescence related parameters in the WAMI panel.

CHAPTER 3 MATERIALS AND METHODS

This chapter describes the methodology used in the field experiments conducted in the study. Field experiments were carried out at the CIMMYT Norman E. Borlaug Experimental Station in Ciudad Obregon NW Mexico in the crop cycles of 2010-11, 2011-12, 2012-13 and 2013-14 (henceforth referred as 2011, 2012, 2013 and 2014, respectively). The experimental design and the crop and environmental measurements for the field experiments are described below.

3.1 Experiments on CIMMYT Core Germplasm (CIMCOG) panel

3.1.1 Site and experimental design

The field experiments were conducted in the 2011, 2012 and 2013 seasons at the Yaqui Valley, Ciudad Obregón, Norman E. Borlaug experimental station of the International Maize and Wheat Improvement Center (CIMMYT) in Sonora, north western Mexico (27°N, 110°W, 38 m above sea level) under fully irrigated conditions. 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 (Fischer et al*.,* 2014).

Twenty-six bread wheat cultivars were evaluated in a raised-beds system. A lattice design was used, with two replicates in 2011, and three replicates in 2012 and 2013; in each replicate 26 genotypes of CIMMYT spring bread wheat germplasm were randomized on plots. Plots were 5 x 1.6 m in 2011 and 1.6 x 8.5 m in 2012 and 2013. Plots were irrigated using a gravity-based system, each bed had two rows, and the rows were planted 24 cm apart and the width of a raised beds was 80 cm (Fischer et al., 2005; Figure 3.1).

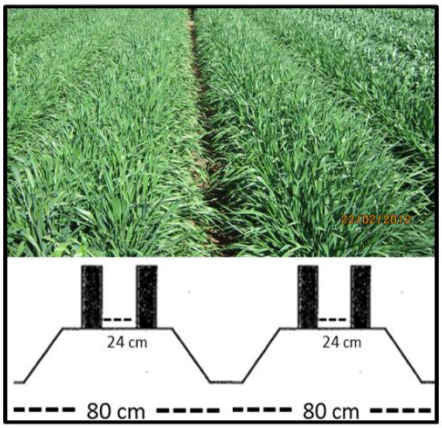


Figure 3.1 Transversal view of field plots under raised beds for experiments in NW Mexico. Picture from CIMCOG trials in 2012. Adapted from (Fischer et al., 2005)

Twenty six spring bread wheat genotypes from the CIMMYT Core Germplasm panel were used for the field experiments (Table 3.1). This germplasm (CIMCOG) includes advanced lines representing the full range of genetic diversity available in CIMMYT modern elite backgrounds with high yield potential and also several historical CIMMYT widely grown cultivars. The CIMCOG panel was established in 2010 in consultation with wheat breeders and physiologists (Reynolds et al., 2011). Within the CIMCOG panel of 26 genotypes, a subset of ten genotypes with similar date of anthesis was selected in the present study for a more detailed analysis of growth including pre anthesis radiation-use efficiency and senescence kinetics In addition, of spike structural partitioning (glumes, lemmas, paleas, and rachis) was carried out for this subset of cultivars.

Table 3.1. List of CIMMYT spring bread wheat genotypes evaluated in the three seasons (2011, 2012 and 2013).

ENTRY	Rht	Year of release	CROSS NAME
1	Rht1	2008	BABAX/LR42//BABAX/3/VORB
2 [s]	Rht1	1988	BACANORA T 88 (Hist 4)
3	Rht2	2008	BCN/RIALTO
4	Rht1	2009	BECARD/KACHU
5[s]	Rht1	2005	BRBT1*2/KIRITATI (HIST 14)
6	Rht1	2009	SAHUAL/4/CROC_1/AE.SQUARROSA(205)//
7	Rht1	2009	KAUZ/3/ATTILA/5/SAUAL SAHUAL/WHEAR//SAUAL
8	Rht2	2009	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/
10	Rht1	2009	NAC/6/RIALTO CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7
11	Rht1	2009	CROC_1/AE.SQUARROSA (205)//BORL95/3/
12	Rht1	2009	PRL/SARA//TSI/VEE#5/4/FRET2 (Hist 13) KBIRD//INQALAB 91*2/TUKURU
13	Rht1	2001	MILAN/KAUZ//PRINIA/3/BAV92
16	Rht2	1976	PAVON F 76
17 [s]	Rht1	2009	PBW343*2/KUKUNA*2//FRTL/PIFED
18	Rht1	2005	PFAU/SERI.1B//AMAD/3/WAXWING
19	Rht1	1982	SERI M 82 (Hist 3)
20 [s]	Rht1	1966	SIETE CERROS T66 (Hist 1)
21	Rht1	2008	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR
22	Rht1	2009	TACUPETO F2001/SAUAL/4/BABAX/LR42//BABAX*23/
23[s]	Rht2	2009	KURUKU TACUPETO F2001/BRAMBLING*2/KACHU
24 [s]	Rht1	2006	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1
25[s]	Rht1	2008	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU
26	Rht1	2009	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/
27 [s]	Rht1	2005	MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/ BECARD
28[s]	Rht1	2009	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/
30[s]	Rht1	2009	AE.SQUARROSA(213)//PGO/4/HUITES YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/

[s] these genotypes were selected for detailed analysis. Rht alleles was provided by Oscar Gonzalez from the John Innes Centre, UK.

3.1.2 Degraining treatment

A degraining treatment was imposed in 2011 at GS65+ 7 days in which 20 spikes per plot randomly selected were manipulated by removing the top half of the spike. In 2012 and 2013, a degraining treatment was imposed at GS65 + 10 days in which spikes were manipulated by removing half of the spike randomly selected (all spikelets down one side of the spike (Fig.3.2) in 20 spikes in 2012 and 12 in 2013 season. In each experiment a control treatment with 20/12 unmanipulated shoots was included. Shoots were selected at random and tagged at around anthesis.

a)



b)

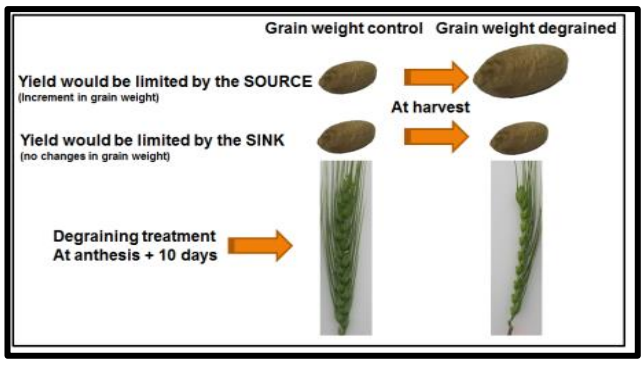


Figure 3.2 a) Control and degraining treatment at GS65+7 d in 2011 (lhs) and GS65+10 days (rhs) in 2012 and 2013 in the CIMCOG panel subset in 2013 b) Graphic definition of sink and source limitation

3.1.3 Plot management in field experiments

Experiments were sown on 7 December 2010, 8 December 2011 and 23 November of 2012. Seed rates of 10.1 g m^{-2}, 7.8 g m^{-2} and 10.8 g m^{-2} were sown, respectively. In order to maintain the crop free of stress, practices as fertilization, pest, and disease and weed control were implemented. Sesbania (*Sesbania punicea*) was sown in plots in the previous season.



Figure 3.3 CIMCOG Field experiments in raised beds at CENEB NW Mexico Post anthesis stage in 2012.

3.1.4 Irrigation

The irrigation was done using gravity based system where water was applied until soil was saturated. The crop was maintained free of any water stress from emergence to harvest. During the first crop cycle plots were irrigated after sowing (7 December 2010, 8 January, 9 February, 6 March and 8 April 2011). In 2012 the trial was irrigated seven times with the first irrigation after sowing (9 December 2011, 14 January, 20 January, 9 February, 2 March, 16 March, and 31 March 2012). In 2013, six irrigations were applied including the first irrigation after sowing (24 Nov 2012, 8 January, 1 February, 22 February, 15 March and 4 April of 2013).

3.1.5 Fertilization

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 the first irrigation.

3.1.6 Fungicide and pesticide and herbicide application

Application of chemicals was applied using a tractor or airplane in the three seasons. Herbicides, insecticides, and fungicides were applied in 2011, 2012 and 2013 as in Table 3.2

Table 3.2. Herbicides, insecticides and fungicides applied a) 2011, b) 2012 and c) 2013

a) 2011

Input	Date	Dosage
Herbicides	4 January 2011	Buctril (Bromoxinil)1300 ml ha-1) Estarane (Fluroxipir 20% p/v) 750 ml ha^{-1}
Insecticides	1 April 2011	Muralla Max (Imidacloprid and betacyflutrin) 300ml ha^{-1}
Fungicides	25 January 2011 21 February 2012 8 March 2011	Folicur (Tebuconazol) 500 ml ha^{-1}

b) 2012

Input	Date	Dosage
Herbicides	4 Jan 2012	Buctril (Bromoxinil)1000 ml ha^{-1} Estarane (Fluroxipir 20% p/v) 300 ml ha^{-1}
Insecticides	9 February 2012, 2 March 2012	Muralla Max (Imidacloprid and betacyflutrin) 300ml ha^{-1}
Fungicides	12 March 2012	Folicur (Tebuconazol) 500 ml ha^{-1}

c) 2013

Input	Date	Dosage
Herbicides	13 Dec 2012 20 Dec 2012	AXIAL 500 ml ha^{-1} and AGIDOR 1000 ml ha^{-1} Buctril (Bromoxinil)1300 ml ha^{-1}) Estarane (Fluroxipir 20% p/v) 750 ml ha^{-1}
Insecticides	22 Jan 2013	Muralla Max (Imidacloprid and betacyflutrin) 300ml ha^{-1}
Fungicides	25 January 2013 15 February 2013 8 March 2013	Folicur (Tebuconazol) 500 ml ha^{-1}

3.1.7 Crop measurements

3.1.7.1 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 stage. Final plant height and height to the spike collar were measured approximately in the second half of the grain filling in each plot in the experiments. Measurements were taken from the ground for 12 randomly selected shoots per plot.

3.1.7.2 Growth analysis of GS31, GS41 and GS65+7d

In experiments in 2012 and 2013, growth analysis samples were taken at anthesis +7 days (GS65+7d) for the 26 cultivars. Growth of the aboveground plant material was analysed in two adjacent 50 cm row lengths in each plot (0.4 m^2), situated at least 50 cm from the end of the plot to avoid border effects, at GS31, GS41 and GS65+7 days. Plants were cut at ground level in all cases and according to the calendar dates that they reached the specific stage (i.e., genotypes were sampled on different dates).

3.1.7.2a GS31 and GS41

Crop growth and DM partitioning analyses were carried out at GS31 and GS41 in 2012 and 2013 for the subset of ten cultivars. In each plot, the total sample was weighed for fresh weight and a 50% subsample (by fresh weight) 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). After recording total fresh weight, a random subsample of 100 fertile shoots was taken and weighed (spikes were counted) and then dried for 48 h at 70°C to determine moisture content. Moisture content was applied to the total sample fresh weight to determine above-ground DM in g m^{-2}. From the rest of the sample, 10 representative fertile shoots were taken to conduct DM partitioning analysis. At GS31, the plant material was 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 just 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. With the following exceptions growth analysis was carried out at GS31 and GS41.

3.1.7.2b GS65+7d

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. Infertile shoots were counted as those without an emerged spike. Plant components were separated as 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. Green leaf lamina area and true stem + sheath, and spike were measured using a leaf area meter (LI3050A/4, Li-Cor, Lincoln, NE, USA) and the dry weight was recorded for each plant component after drying for 48 h at 75^{o}C:

3.1.7.2c Stem water soluble carbohydrates

Water soluble carbohydrate (WSC) content of the true stem and leaf sheath was measured at GS65+7d and at harvest for the 10 cultivars. At GS65+7d a subsample 20 fertile shoots was analysised for WSC content. At harvest, 20 fertile shoots were sampled directly from the plot. Water soluble carbohydrate was examined in the true-stems and leaf-sheaths. After drying at 70^{o}C for 48 h, the true stem and leaf sheath was weighed, 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 (%). WSC amount was calculated as follows: fertile shoots m^{-2} x DM of true-stem and leaf sheath per fertile shoot (mg $shoot^{-1}$) x true-stem WSC concentration (%)/100). In the ten genotypes WSC was measured only in two replicates at Anthesis +7 days. Therefore, calculations for the source size averaged two repetitions to have three repetitions.

For the subset of ten cultivars, WSC content was measured in 2012 and 2013 in the true stem at physiological maturity in the degrained and control shoots following the same methodology for sampling and analysis as described above.

3.1.7.3 Radiation interception, radiation-use efficiency and extinction coefficient carried out on a subset of 10 cultivars

Fractional radiation interception was measured at GS31, GS41, GS65+7 days and at the middle of grain filling phase in each plot in each year using a ceptometer AccuPAR model LP-80 (Decagon Devices, Inc. 2365 NE Hopkins Ct. Pullman, WA 99163 – USA). Three transversal readings per plot were taken at different locations within the plot avoiding readings 50 cm near to the end of the plot. Photosynthetically active radiation (PAR) was measured above the canopy and at ground level below the canopy avoiding readings 50 cm near to the end of the plot. Readings of the reflected PAR were taken by inverting the ceptometer approximately 5 cm above the crop.

At GS65+7 days, In addition, PAR was measured in different layers within the canopy: below the spike, below the flag leaf (at ligule), below the second leaf (at ligule), below the third leaf at ligule. At all stages, readings of the reflected PAR were also taken by inverting the ceptometer approximately 5 cm above the crop. Post-anthesis readings were also taken below the green part of the canopy in addition at ground level. Measurements were made between the hours 11:00 and 14:00. Fractional interception (f) of photosynthetically active radiation (400-700 nm) (PAR) was calculated by using the equation:

f = 1- ((Transmitted PAR + Reflected PAR)/Incident PAR) **Equation 3.1**

Fractional PAR interception was interpolated between readings and above-ground dry matter (AGDM) from sequential samplings was then plotted against accumulated intercepted PAR calculated as the product of fractional interception and daily incident solar radiation. A linear regression of accumulated PAR interception on accumulated AGDM was fitted from GS31 to GS65+7d setting the intercept at zero with the slope of the line estimating RUE_{PAR} during stem-elongation for each plot (Sinclair and Muchow 1999). RUE_{PAR} was also calculated from the increments from

GS31 to GS41 and GS41 to GS65+7d in each plot as the ratio of AGDM increment to PAR interception over the respective periods. Post-anthesis RUE_{PAR} was calculated over the period from GS65+7 days to GS89 as the ratio of AGDM increment to PAR interception over the same period. Plot values for RUE_{PAR} were subjected to analysis of variance.

A modified version of Beer's law was used to determine, the extinction coefficient, k_{PAR} using Equation 3.2.

$$k_{PAR} = -\ln (I/I_0)/L \qquad \textbf{Equation 3.2}$$

Where I_0 is incident PAR and I is transmitted PAR below a GAI value of L (Monsi and Saeki 1953).

3.1.7.4 Senescence parameters

3.1.7.4a Flag-leaf SPAD senescence

A SPAD meter (SPAD-502, Minolta, Tokyo, Japan) was used in non-destructive measurements to assess the flag-leaf chlorophyll content. SPAD values were taken approximately every 7 days from flag-leaf emergence (GS39) to physiological maturity (1 reading per leaf) for three randomly selected flag leaves per plot (8 assessment dates in 2011, 12 in 2012 and 9 in 2013). SPAD values were linearly regressed against thermal time (base temp. 0°C) from anthesis (GSG5) to calculate the rate of senescence and the calculated SPAD value at PM (taken as an indicator of stay-green). The linear slope was originally negative, but was converted to a positive value (higher value = faster senescence).

3.1.7.4b NDVI senescence

The Normalized Difference Vegetative Index (NDVI) spectral reflectance index was measured in 2011, 2012 and 2013 as:

NDVI = ($R_{NIR} - R_{RED}$) /($R_{NIR}+R_{RED}$), with a range of -1 to 1 **Equation 3.3**

Where R_{NIR} is the reflectance at Near Infra-Red (910 nm) and R_{RED} is the reflectance at red (680 nm). NDVI is a ratio, so it is unit-less and it has a value ranging between -1 to 1.
This measurement provides an estimate of the canopy size based on the reflectance from the canopy surface in the red and near- infrared regions.

In this study, NDVI readings were taken throughout the crop cycle from onset of stem elongation to late grain filling. The NDVI was measured approximately every 7 d using a spectroradiometer GreenSeeker (N-Tech Industries). The sensor was held 50 cm above the canopy of each plot avoiding at least 50 cm at the end of the plot. NDVI values were regressed against thermal time (base temp. 0°C) from heading (GS59) to calculate the rate of senescence and the calculated NDVI value at PM taken as an indicator of stay-green. The linear slope was originally negative, but was converted to a positive value (higher value = faster senescence). The units for NDVI senescence rate are NDVI (unitless) per unit thermal time ($^{o}Cd^{-1}$).

3.1.7.4c Flag-leaf senescence visual score

Flag-leaf senescence was also scored visually approximately every three days by visual assessment. In 2012 and 2013, assessment on six tagged flag leaves per plot, after anthesis and until flag leaves had advanced to complete senescence. Visual measurements of flag leaf score (FLS) of senescence were taken using a diagnostic key (from 1 for flag-leaf 100% green) to 10 (fully senesced) (see appendix 1).

A logistic curve was fitted to the data for each plot with the thermal time (base temp $0^{\circ}C$) from anthesis (GS65).

Y= A + C/(1 + EXP(-B*(X - M))) **Equation 3.4**

Were X is the accumulated thermal time post-anthesis ($^{o}Cd^{-1}$), and Y= the value of the senescence score from 1-10 for the flag leaf using a visual score, A = Minimum asymtote, M = Inflection point, B = Hill's slope (faster rate) and C = Maximun asymptote.

The onset senescence was calculated using the Equation 3.3 substituting the value of y when the FLS visual score was 2 and The End senescence when the FLS visual score was 9. B is the flag leaf rate of senescence.

3.1.7.5 Nitrogen content

In 2013 flag leaves from 10 shoots at GS65+7d from the subset for 10 cultivars were submitted for analysis of nitrogen content using the Dumas method. This was determined using Technicon II (Technicon Autoanalyzer II, SEAL Analytical Limited) at CIMMYT lab in El Batan Texcoco. The total nitrogen content (% dm.) was calculated using the formula in Table 3.3. in section 3.1.7.7

3.1.7.6 Leaf stomatal conductance

Stomatal conductance was measured using a leaf porometer (Decagon Devices INC). Stomatal conductance was measured in the subset of 10 genotypes in 2013 at approximately GS31, GS41 and GS65 on the newest fully expanded leaf or the flag-leaf of fertile shoots. Measurements were taken on three randomly selected fertile shoots per plot at around 12.00 h on bright sunny days assessing three leaves (Three measurements per plot).

3.1.7.7 Crop growth and dry matter (DM) partitioning analysis at Harvest (GS91)

Grain yield was measured in each plot after physiological maturity was reached in each year, by machine-harvesting in the first two seasons and by hand harvest in 2013 with an average plot area of 6.2, 5.7,and 4.8 m^2,

respectively in 2011, 2012, 2013. The grain harvested was taken to the lab and weighed. A subsample was taken and weighed, dried at 70 ^{o}C for 24 hr and weighed again to calculate the moisture content. Grain yield was reported as 100% DM. Before combine harvest, a random sample of 100 fertile shoots was taken 1-2 days prior to harvest for all the plots, cutting each shoot at ground level (avoiding 50 cm at the plot ends). The plant material was dried, weighed and threshed and the grains weighed, so that HI could be estimated. From this sample, 200 grains were randomly counted and weighed. Using these data, and an estimate of individual grain weight, yield components, above-ground biomass and FE (based on anthesis spike DM and chaff) were calculated as shown in Table 3.3.

Table 3.3 Formulas for calculating grain yield, HI and yield components (Pask et al., 2012a). Grain yield in g m^{-2}, grain number m^{-2}, thousand grain weight (TGW, g), spike number per 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 70 ^{o}C for 48 h) in addition Leaf lamina Nitrogen Content.

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

3.1.7.8 Measurements on degrained and control shoots

The dry matter partitioning was carried out for the subset of ten genotypes in each year. The samples consisted of 20 randomly selected fertile shoots in 2913 and 12 in 2013 taken from the field 1-2 days prior to harvest. The same methodology for general DM partitioning analysis (spike, true-stem, leaf-sheath and leaf lamina DM determination) as at GS65+7d (section 3.1.7.2b) was followed. Analysis for WSC content was also carried out at harvest in the 12 fertile shoots for the true-stem. The methodology for analysis of WSC content is described in section 3.1.7.2c.

In addition from the 12 fertile shoot samples for the ten cultivars, three spikes were randomly selected from the field and dissected into: i) glumes, ii) lemmas, iii) paleas, iv) rachis, v) awns and vi) grains in each year. The different spike structural components and grains from the three spikes

were weighed after drying at 70 ^{o}C for 48 h. The number of grains for the three spikes was also counted. In addition, seven degrained and control spikes were separated in three spike sections (Basal, central and apical spikelets) threshed and weighed separately after drying at 70^{o}C for 48 h. The number of grains for each three sections s was also counted.

3.1.9 Meteorological data

Meteorological data were obtained from the closest meteorological station in the CENEB (Centro experimental Norman Borlaug) in Cd Obregon. Weather conditions (daily mean temperature, relative humidity, windspeed, rainfall and solar radiation) are presented for seasons 2011, 2012, 2013 and 2014 in addition a long term means of 23 years (see Chapter 4).

3.2 Experiments on Wheat Association Mapping Initiative (WAMI) panel

3.2.1 Plant material

The WAMI panel consists of 294 elite spring bread wheat advanced lines selected from the CIMMYT program based on 30 years of research data (Sivakumar et al., 2013). The genotypes were obtained from CIMMYT nurseries (26th, 27th and 28th ESWYT, Elite Spring Wheat Yield Trial, 1st to 16th SAWYT, Semiarid Wheat Yield Trial and from the 1st to 12th HTWYT, High Temperature Wheat Yield Trial) (Lopes et al., 2012a). The WAMI panel was assembled to detect genetic marker associations in spring wheat. (See Table 7.1 in chapter 7). The panel was selected for minimum variation in anthesis date and plant height to avoid confounding effects (Lopes et al., 2012).

3.2.2 Site and experimental design

Phenotyping was carried out at the Centro Experimental Norman E. Borlaug (CENEB) at Cd. Obregon, Sonora, Mexico (see above 3.1.1) from 2012-2013 and 2013-2014. A lattice design was used in both years with two replicates. Plot size in 2013 was 1.6 x 4 m and in 2014 was 0.8 x 0.25 m. In 2014 not for full yield and yield components measurements.

A degraining treatment was imposed to modify the grain sink size by manipulating the number of spikelets per spike at GS65+(10-15) days in which 20-10 spikes per plot were manipulated and 20-10 shoots per plot were labelled as control shoots, respectively, in 2013 (20 shoots) and 2014 (10 shoots). In the experiments, half of the spikelets (all spikelets down one side of the spike, terminal spikelet not removed and first fertile basal spikelet removed) at GS65+10 in 2013 and GS65+14 in 2014.

3.2.3 Plot management

Seed rate was 12.5 g m^{-2} in each year sown on 27 November 2012 and 18 December 2013, respectively. Fertilization was as described for the experiments in 2011-13 in 3.1.5 section. Fungicide and pesticide and herbicide application as described in Table 3.4 below. In 2013, five irrigations were applied with the first irrigation after sowing (24 Nov 2012, 8 January, 1 February, 22 February, and 15 March). In 2014 eight Irrigations were carried out with the first irrigation after sowing (19 and 30 December 2013, 13 January 2014, 8 and 28 February 2014, 14 and 18 March 2014.

Table 3.4. Herbicides, insecticides and fungicides applied in WAMI trials a) 2013 and b) 2014

a)

Input	Date	Dosage
Herbicides	13 Dec 2012 20 Dec 2012	AXIAL 500 ml ha^{-1} and AGIDOR 1000 ml ha^{-1} Buctril (Bromoxinil)1300 ml ha^{-1}) Estarane (Fluroxipir 20% p/v) 750 ml ha^{-1}
Insecticides	22 Jan 2013	Muralla Max (Imidacloprid and betacyflutrin) 300ml ha^{-1}
Fungicides	25 January 2013 15 February 2013 8 March 2013	Folicur (Tebuconazol) 500 ml ha^{-1}

b)

Input	Date	Dosage
Insecticides	6 February 2014 21 February 2014 28 February 2014	Allectus (imidacloprid bifenthrin)175 ml ha^{-1} Allectus (imidacloprid bifenthrin)175 ml ha^{-1} Lorsban 50EC (500ml ha^{-1})
Fungicides	19 February 2014	Folicur (Tebuconazol) 500 ml ha^{-1}

3.2.4 Crop measurements

3.2.4.1 Harvest growth analysis and degraining treatments

In 2013 grain yield in the unmanipulated area on the plot was analyzed according to the protocol describe in Section 3.1.7.7 and the area harvested was between 1.5 and 2.5 m^{-2}. At harvest for all lines in two replicates the control and degrained shoots were sampled by cutting at ground level and the spikes were threshed by hand in 2013 and using a thresher machine in 2014 (Wintersteiger A-1910 Ried/Austria). The grains were dried at 75 ºC for 48 hours and the weight recorded using a balance (Adventurer China with 0.001g of precision) and counted using a seed counter machine (Pfewffer, Contador).

3.2.5 Senescence measurements

In 2013 for all lines flag leaf visual score for senescence was measured in two replicates every 3-4 days using a diagnostic key (see appendix 1) from anthesis until complete flag-leaf senescence in the 294 lines in the control flag leaves. A logistic curve was fitted to estimate the senescence parameters as described above in 3.1.7.4c.

In 2013, reflectance chlorophyll content (RCC) was measured using the FieldScout CM 1000 Chlorophyll Meter (Spectrum Technologies Inc. Plainfield, IL), which measured ratios of reflected red and far-red light to calculate relative chlorophyll content. The measurements were carried out by placing the Field scout over the canopy at 20 cm height in each plot avoiding the borders. This equipment senses light at wavelengths of 700 nm and 840 nm to estimate the quantity of chlorophyll in leaves. The ambient and reflected light at each wavelength is measured. Chlorophyll a absorbs 700 nm light and, as a result, the reflection of that wavelength from the leaf is reduced compared to the reflected 840 nm light. Light having a wavelength of 840 nm is unaffected by leaf chlorophyll content and serves as an indication of how much light is reflected. A chlorophyll

index value (0 - 999) is calculated from the measured ambient and reflected light data (Bunderson *et al.* 2009). From heading to physiological maturity five readings were taken. The senescence rate and the canopy greenness value at physiological maturity were estimated in each plot by fitting RCC linearly against thermal time (base temp. 0^{o}Cd) post GS59 (value at PM taken as an indicator of stay-green).The linear slope was originally negative, but was transformed to a positive value (higher value = faster senescence).

3.2.6 Leaf and stem dry matter at harvest

In both years in the degraining and control treatment shoots (see section 3.1.7.2b), leaf + stem DM was measured in two replicates in a subset of 45 genotypes (see Table 7.1 in chapter 7). Leaf and stem DM was dried at 75°C for 48 h and recorded its weight. In 2013 leaf and stem DM in control and degraining treatment was assessed in all genotypes.

3.2.7. Association mapping

This study used an existing map for the WAMI panel (Sukumaran et al*.,* 2015). WAMI was genotyped at CIMMYT Illumina using the iSelect 90K SNP array. A consensus map of the 90K SNP array already exists with genetic positions of the markers and the sequence information of the SNPs and the analysis as described in Sukumaran et al. (2015).

Marker-trait association (MTAs) analysis was done with 9K SNP and 1992 DArT markers following the unified mixed model (Yu et al., 2006) method and comparisons was done in TASSEL (Bradbury et al., 2007) and R software (GAPIT package) for consistency of the MTAs (Lipka et al., 2012). The threshold for defining a marker to be significant was taken at 10^{-04}, considering the number of markers and the deviation of the observed F test statistics from the expected F test distribution (Sukumaran et al., 2012). In collaboration with CIMMYT GWAS analysis were carried at El Batan Texoco Mexico by Dr Sivakumar Sukuraman.

3.3 Statistical analysis

Statistical analysis was carried out in SAS 9.0 for analysis of variance for a lattice design in individual years and across the 2 years. Replicates were considered as random effects and genotypes and years as fixed effects in the ANOVA model.

The significance of the treatment effects was determined comparing the variation due to the main effects (cultivars) and the residual variation within the cultivars between replicates (blocks). A probability value of 0.05 or less was taken to be significant and consistent values between 0.05 and 0.10 could be considered as trends.

Significance of each source of variation was determined by application of the t test. Least significant difference (LSD) tests were performed to determine the significant differences between individual means at the 0.05 level of probability. Genstat 17th Edition (https://www.vsni.co.uk/webstore/software/genstat/) was used for correlation and regression analysis using the genotype means in individual experiments or the cross-year genotype means. R statistical software ver 3.0.1 was used for Principal Component Analysis amongst the traits.

Analysis for the interaction between the degraining treatment and the spikelet location (basal, central and apical), was carried out with a cross – year factorial ANOVA using Genstat 17th Edition.

CHAPTER 4 CHAPTER 4 GRAIN YIELD, YIELD COMPONENTS AND PHYSIOLOGICAL COMPONENTS AT HARVEST IN CIMCOG PANEL

4.1 Introduction

This chapter describes the results of the field experiments at harvest carried out in three seasons (2010/11, 2011/12 and 2012/13) at CIMMYT Norman E. Borlaug Experimental Center (CENEB), Ciudad Obregon, Mexico. The genetic variation of grain yield, yield components and physiological components is presented for 26 spring wheat genotypes from the CIMMYT Mexico Core Germplasm panel (CIMCOG) in individual years and across years. A subset of ten genotypes with similar date of anthesis was selected for more detailed analysis of dry matter partitioning including leaf sheath, leaf lamina, and true stem. In addition, an analysis of spike structural component DM (glumes, lemmas, paleas, and rachis) was carried out for this subset of cultivars.

Grain yield progress in studies on sets of historic cultivars was highly associated with grain number per unit area in wheat (Brancourt-Hulmel et al., 2003; Canevara et al., 1994; Peltonen-Sainio et al., 2007; Sayre et al., 1997; Serrago and Miralles 2014; Shearman et al., 2005).

Breeding progress in potential yield in wheat at CIMMYT started in the 1960s with an increase in HI and grain number m^{-2} (GN) contributed from the semi-dwarfing genes during the Green Revolution (Fischer and Stockman 1986). Although HI in spring wheat at ~0.45 has not reached the theoretical maximum of ca. 0.62 (Austin 1980), there is evidence that the latest progress yield potential of CIMMYT spring wheat in the Yaqui Valley is associated with greater above-ground dry matter (AGDM) and greater grain weight (GW) rather than gains in HI (Aisawi et al., 2015), in turn, associated with increases in height since the early 1990s amongst semi-dwarf cultivars. Indeed, Aisawi et al. (2015) reported that HI had decreased in recent CIMMYT spring wheat cultivars released since the early 1990's up to 2009. In this scenario, it seems that AGDM and GW

are important determinants of recent genetic gains in yield potential but it will be important to ensure there are not trade-offs between AGDM and HI. While boosting photosynthesis is expected to increase above-ground biomass, increases in yield will only follow if the extra biomass is invested in those structures that lead to maximization of grain number and weight, as opposed to 'non useful' biomass.

The aims of this chapter are:

- To quantify the relationships between physiological traits, HI and grain yield at harvest amongst the 26 CIMCOG cultivars.
- To identify optimum combinations of partitioning traits in order to maximize HI and grain yield potential.
- To identify any interactions for grain yield and associated traits with the season amongst the genotypes.
- To quantify rates of genetic gain in yield and associated traits with year of release

The hypotheses tested using the CIMCOG panel are:

- Genetic variation in grain yield is positively correlated with both HI and above-ground biomass; and there is no trade-off between these physiological yield components.
- Genetic variation in grain yield is positively correlated with both grains m^{-2} and grain weight; and there is no tradeoff between these numerical yield components.
- There are no effects of plant height and anthesis date on genetic variation in HI and grain yield in the CIMCOG panel.

The results presented in this chapter set the context for the detailed analyses in following chapters examining the physiological basis of the effects at harvest.

4.2 Materials and Methods

Twenty six spring bread wheat cultivars were evaluated under fully irrigated conditions in raised beds (2 beds per plot; two rows per bed).. A lattice design was used, with two replicates in 2011 and three replicates in 2012 and 2013. The 26 CIMMYT spring wheat genotypes were randomized on plots within replicates.

A subsample of 100 fertile shoots was taken at random from the harvested area. The fresh weight was recorded and the plant material was dried at 75°C for 48 h, weighed and data used for the calculation for number of spikes per square meter, individual grain weight, HI and above-ground dry matter.

Measurements were carried out at GS91 in all field experiments. Anthesis (GS65) date was recorded at the date when 50% of spikes have extruded 50% of their anthers. For a subset of 10 cultivars, dry matter partitioning analysis was done using 10 spike-bearing shoots at harvest tagged since anthesis. These shoots were cut at physiological maturity and dissected into leaf lamina, leaf sheath, true stem and spike. The spikes were dissected into the morphological components: awn, glume, lemma, palea, rachis and grain for each year. Each component was weighed after drying for 48 h at 75^{o}C.

Mean daily temperature, relative humidity, solar radiation and accumulated monthly rainfall were monitored in the three seasons.

Statistical analysis was carried out in SAS 9.0 (SAS Institute, Cary, NC) for analysis of variance for a lattice design in individual and across the 3 years. Genstat 17^{th} Edition was used for correlation and regression analysis using the genotype means in individual experiments or the cross-year genotype means. Multivariate analysis was carried out using biplot procedures in R ver 3.0.1

4.3 RESULTS

4.3.1. Growing conditions

The monthly means for the daily mean temperature, relative humidity, wind speed, rainfall, and solar radiation are presented in Table 4.1 for the three seasons and for the long-term means (LTM).

Table 4.1. Monthly and long-term means for mean daily temperature (T.) daily relative humidity (RH), monthly rainfall (mm), and daily solar radiation.

Month	T (^{o}C)	RH (%)	Rainfall (mm)	Solar Rad. (MJ m^{-2})
		2010-2011		
Dec	15.8	72.1	0.2	14.1
Jan	14.0	62.8	0.2	16.0
Feb	13.8	58.2	0	20.5
March	18.2	58.4	0	25.4
April	20.3	58.1	0	26.5
		2011-2012		
Dec	13.7	55.7	0.25	14.4
Jan	15.4	62.8	0	15.0
Feb	15.1	70.7	13.7	18.4
March	16.9	63.1	0	22.9
April	20.2	57.3	0.25	26.2
		2012-2013		
Dec	16.4	70.0	10.62	12.4
Jan	14.1	63.5	1.76	14.8
Feb	14.0	69.6	0.5	16.9
March	18.5	66.6	1.5	20.6
April	19.6	67.4	1.0	23.6
Long-terms means for the field site 1990-2013				
Dec	16.2	69.6	13.7	13.6
Jan	15.5	72.3	10.9	14.4
Feb	16.3	74.6	11.0	17.5
March	18.0	70.6	2.9	23.2
April	21.0	59.2	0.7	26.4

Radiation during grain filling in March and April was lower than the LTM in 2013 but similar to the LTM in 2012 and 2013. Rainfall from December to April 2010 was drier that LTM; and temperature during stem-elongation (January-February) in 2011 was colder than 2012 or 2013 and also than

the LTM. Temperature during the first half of grain filling in March was also lower than LTM in 2012.

4.3.2 Plant establishment

Plant establishment did not differ 2011 or 2012 amongst the 26 CIMMYT cultivars. In 2013, plant establishment ranged from 136 to 235 plants m^{-2}. Averaging over years, plant establishment differed amongst the 26 spring wheat cultivars from 147 to 221 plants m^{-2} ($P < 0.001$). There was a year effect ($P < 0.001$) but no year x cultivar interaction (Table 4.2).

Table 4.2 Plants m^{-2} for 26 CIMMYT spring wheat cultivars in 2011, 2012, 2013 and means.

	Plants m^{-2}			
Cultivar	2011	2012	2013	Mean
Babax/l	190.0	108.3	142.1	146.8
Bacanorat88	271.3	153.3	180.4	201.7
Bcn/rialto	255.0	165.0	235.4	218.5
Becard/k	175.0	139.2	164.2	159.4
Brbt1*2/k	188.8	134.2	144.2	155.7
Sahual/4	196.3	118.3	154.6	156.4
Sahual/w	216.3	128.3	135.8	160.1
Cmh79a.9	210.0	131.7	175.0	172.2
Cno79//p	196.3	130.8	155.0	160.7
Croc_1/	226.3	168.3	189.6	194.7
Kbird//i	240.0	154.2	170.4	188.2
Milan/k	222.5	154.2	170.4	182.4
Pavonf76	271.3	190.0	202.5	221.3
Pbw343*2	177.5	148.3	165.0	163.6
Pfau/seri	182.5	156.7	177.9	172.4
Serim82	241.3	146.7	167.5	185.1
Siete cerros	287.5	168.3	200.0	218.6
Sokoll//p	191.3	141.7	149.6	160.8
Tacupeto/s	180.0	149.2	142.9	157.4
Tacupeto/b	195.0	125.0	155.0	158.3
Tc870344/	190.0	135.8	155.0	160.3
Trap#1	212.5	136.7	161.3	170.1
Up2338*2	182.5	173.3	198.8	184.9
Becard	216.3	150.8	161.7	176.3
Wbll1*2/	185.0	126.7	140.4	150.7
Yav_3/sc	193.8	155.0	140.4	163.1
Mean	211.3	145.8	166.7	174.6
Genotype	ns	ns	***	***
LSD(0.05)	53.62	42.59	30.58	23.69
Year				***
LSD(0.05)				8.19
Year*Genotype				ns

Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability. Subset of 10 cultivars in bold letters.

4.3.3 Date of anthesis and plant height

The average emergence date for the 26 genotypes was 14 December 2010, 15 December 2011 and 1 December 2012. In 2011, the 26 genotypes differed in date of anthesis from 79 (Pfau/Seri) to 96 (Bcn/Rialto) days after emergence (DAE) ($P < 0.001$). The genotypes ranged from 81 (Pfa/Seri) to 94 (Bcn/Rialto) DAE in 2012 ($P < 0.001$) and from 73 (Pfa/Seri) to 100 (Bcn/Rialto) DAE in 2013 ($P < 0.001$). Generally, the same genotypes were earlier or later to anthesis in each year, although the year x genotype interaction was significant.

Plant height in 2011 differed amongst the 26 genotypes from 73.4 (Bcn/Rialto) to 124.3 (Tacupeto/s) cm ($P < 0.001$), in 2012 from 84.5 (Bcn/Rialto) to 121.4 (Tc870344/) cm ($P < 0.001$) and in 2013 from 83.8 (Bcn/Rialto) to 117.5 (Tc870344/) cm ($P < 0.001$). The year x genotype interaction was significant; the strongest interactions were observed between 2011 and 2012 in which Bcn/Rialto, Pfau/Seri, Croc_1/ae, Milan/k, Cno79//p and Tacupeto/b were only 10-11 cm shorter in 2011 and Serim82, Babax/l, Pbw343*2 and Sahual/4 were 1-2 cm shorter in 2011 respectively (Table 4.3).

Averaging over years anthesis date was not associated with grain yield, or with AGDM (GS91). Averaging across years, plant height was not correlated with grain yield or with AGDM but negatively associated with anthesis date ($P < 0.05$; Table 4.3).

Table 4.3 Anthesis date (GS65, days after emergence) and plant height at physiological maturity in 2011, 2012 and 2013 and correlations with grain yield, anthesis date and above ground biomass (GS91) for 26 CIMCOG spring wheat genotypes

Genotype	Anthesis date (DAE)				Plant height (cm)			
	2011	2012	2013	Mean	2011	2012	2013	Mean
Babax/l	90.5	87.3	86.0	87.9	117.8	118.6	116.8	117.7
Bacanorat8	89.5	86.7	86.0	87.4	90.8	94.9	93.0	92.9
Bcn/rialto	96.0	94.0	100.0	96.7	73.4	84.5	83.8	80.6
Becard/k	94.0	88.3	93.3	91.9	102.0	108.0	108.2	106.1
Brbt1*2/k	87.5	84.7	81.3	84.5	106.0	115.2	110.3	110.5
Sahual/4	93.5	91.3	93.7	92.8	110.1	115.3	110.3	111.9
Sahual/w	89.5	85.3	86.7	87.2	104.5	106.5	109.3	106.8
Cmh79a.9	95.0	93.3	96.0	94.8	97.6	106.8	101.7	102.0
Cno79//p	88.0	86.7	83.7	86.1	106.9	116.7	112.8	112.1
Croc_1/	86.0	86.0	80.0	84.0	102.4	112.7	106.3	107.1
Kbird//i	93.5	91.3	95.3	93.4	103.8	109.4	107.0	106.7
Milan/k	86.5	86.0	80.0	84.2	104.0	114.1	107.8	108.6
Pavonf76	90.5	86.7	86.7	87.9	103.8	106.3	107.3	105.8
Pbw343*2	87.5	83.7	84.7	85.3	105.5	106.8	108.3	106.9
Pfau/seri	79.0	81.0	73.0	77.7	99.8	111.2	105.5	105.5
Serim82	91.5	88.0	87.0	88.8	98.2	98.8	98.0	98.3
Siete cerros	87.0	85.3	79.7	84.0	99.7	108.8	103.0	103.8
Sokoll//p	89.0	86.3	86.3	87.2	112.3	114.7	104.8	110.6
Tacupeto/s	86.0	84.3	84.7	85.0	124.3	114.0	115.2	117.8
Tacupeto/b	91.0	89.0	87.3	89.1	100.8	107.3	107.2	105.1
Tc870344/	86.5	84.7	84.3	85.2	117.5	121.4	117.5	118.8
Trap#1	88.0	83.3	86.0	85.8	107.8	113.3	112.0	111.1
Up2338*2	91.5	88.0	91.0	90.2	104.5	109.9	105.8	106.8
Becard	88.0	82.3	83.7	84.7	103.5	108.3	109.0	106.9
Wbll1*2/	90.5	87.3	95.3	91.1	107.4	113.7	108.7	109.9
Yav_3/sc	88.5	86.7	90.3	88.5	109.8	113.4	110.5	111.2
Mean	89.4	86.8	87.0	87.7	104.4	109.6	106.9	107.0
Genotype	***	***	***	***	**	***	***	***
LSD(0.05)	2.897	1.620	2.125	1.169	10.825	4.906	3.878	2.959
Year				***				***
LSD(0.05)				0.404				1.024
Year*Genotype				***				***
r (with Yield)	-0.004	0.120	-0.013	0.053	0.114	-0.037	0.054	0.043
r (with anthesis date)					-0.35	-0.41*	-0.32	-0.40*
r (with AGDM)	0.25	0.11	0.15	0.21	0.13	0.16	0.30	0.21
r (with TGW)	-0.30	-0.43*	-0.26	-0.34	0.74***	0.78***	0.79***	0.81***
r (with HI)	-0.38	-0.22	0.08	-0.19	-0.001	-0.31	-0.31	-0.22

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability. Subset of 10 cultivars in bold letters.

4.3.4 Grain yield, above-ground DM and HI

Grain yield differed amongst genotypes in the range 4.48 (Pavonf76) to 7.20 t ha^{-1} (Tacupeto/b) in 2011 ($P < 0.01$), 5.94 [1] (Pavonf76) to 7.29 t ha^{-1} (Yav_3/sc) ($P < 0.001$) in 2012 and 5.91 (Pavonf76) to 7.56 t ha^{-1} (Kbird//i) in 2013 ($P < 0.001$).

Averaging years, grain yield ranged from 5.45 (Pavonf76) to 7.03 (Tacupeto/b) t ha^{-1} ($P < 0.001$). The year x genotype interaction was significant ($P < 0.05$), with the highest interaction between 2011 and 2013; the genotype difference between these two years ranged from 0.04 t ha^{-1} (Up2338*2/) to 1.43 t ha^{-1} (Pavonf76) (Fig 4.1; Table 4.4). Therefore, cultivars that caused G x year interaction were Pavonf76, Kbird//I, Trap#1/b and Bcn/Rialto. Nevertheless, genotypes generally ranked similarly in the three years and there were few cross-over interactions.

Above-ground biomass at physiological maturity (GS91) ranged amongst the 26 genotypes in 2011 from 1107 (Pavonf76) to 1560 (Tacupeto/b) g m^{-2} ($P < 0.05$), in 2012 from 1310 (Serim82) to 1623 (Croc_1/) g m^{-2} ($P < 0.001$) and in 2013 from 1257 (Serim82) to 1493 (Tacupeto/b) g m^{-2} ($P < 0.05$). The year x genotype interaction was not significant (Fig 4.2; Table 4.4).

Differences in HI were observed amongst genotypes in 2011 from 0.404 (Pavonf76) to 0.486 (Pfau/seri) ($P < 0.01$), in 2012 from 0.433 (Sokoll/) to 0.494 (Pfau/Seri) and in 2013 from 0.457 (Sokoll/) to 0.530 (Seri) ($P < 0.001$). The year x genotype interaction was not significant (Fig 4.3; Table 4.4).

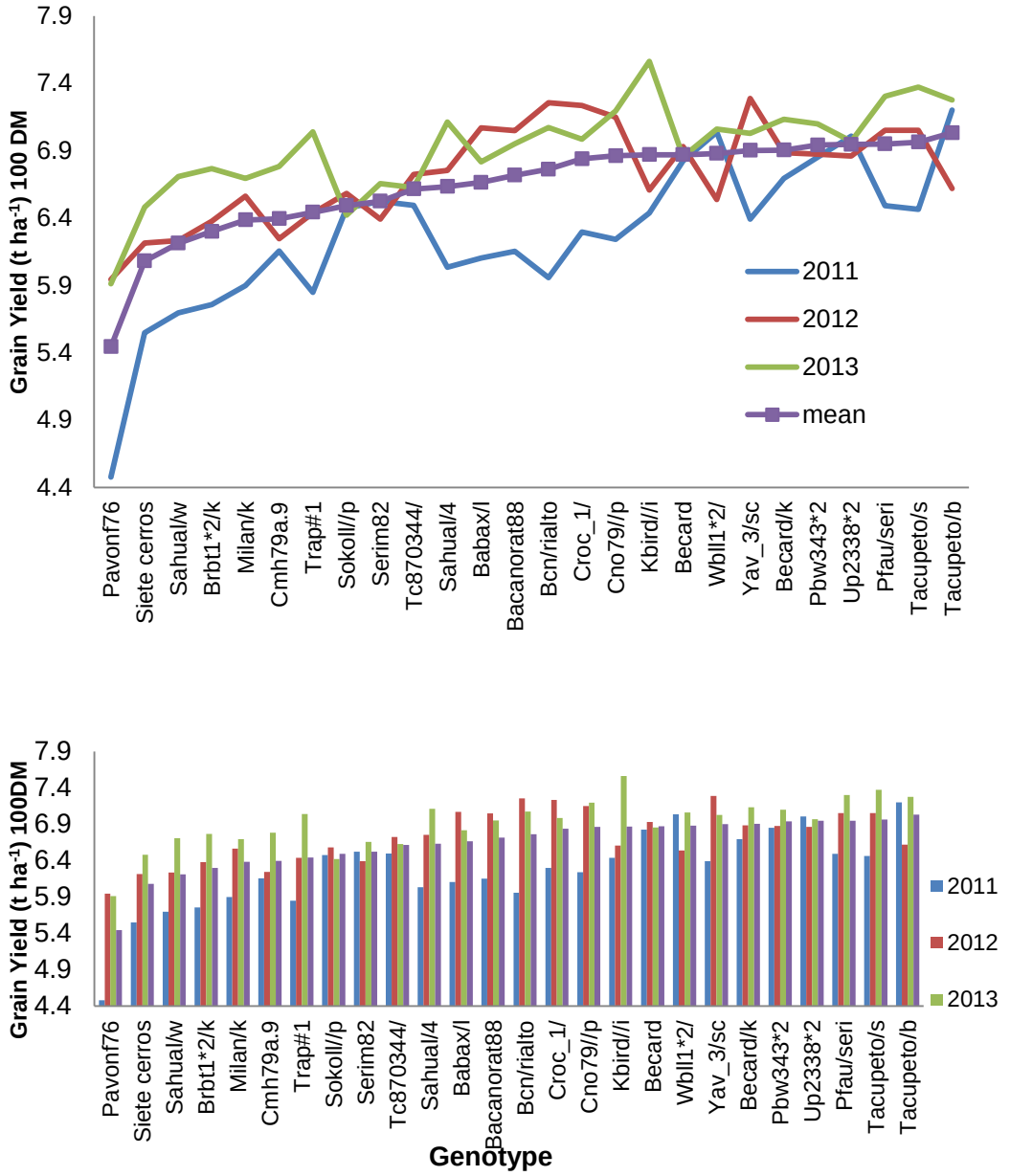


Figure 4.1. Grain yield for 26 CIMCOG spring wheat genotypes in 2011, 2012, 2013 and overall mean (LSD are in Table 4.3).

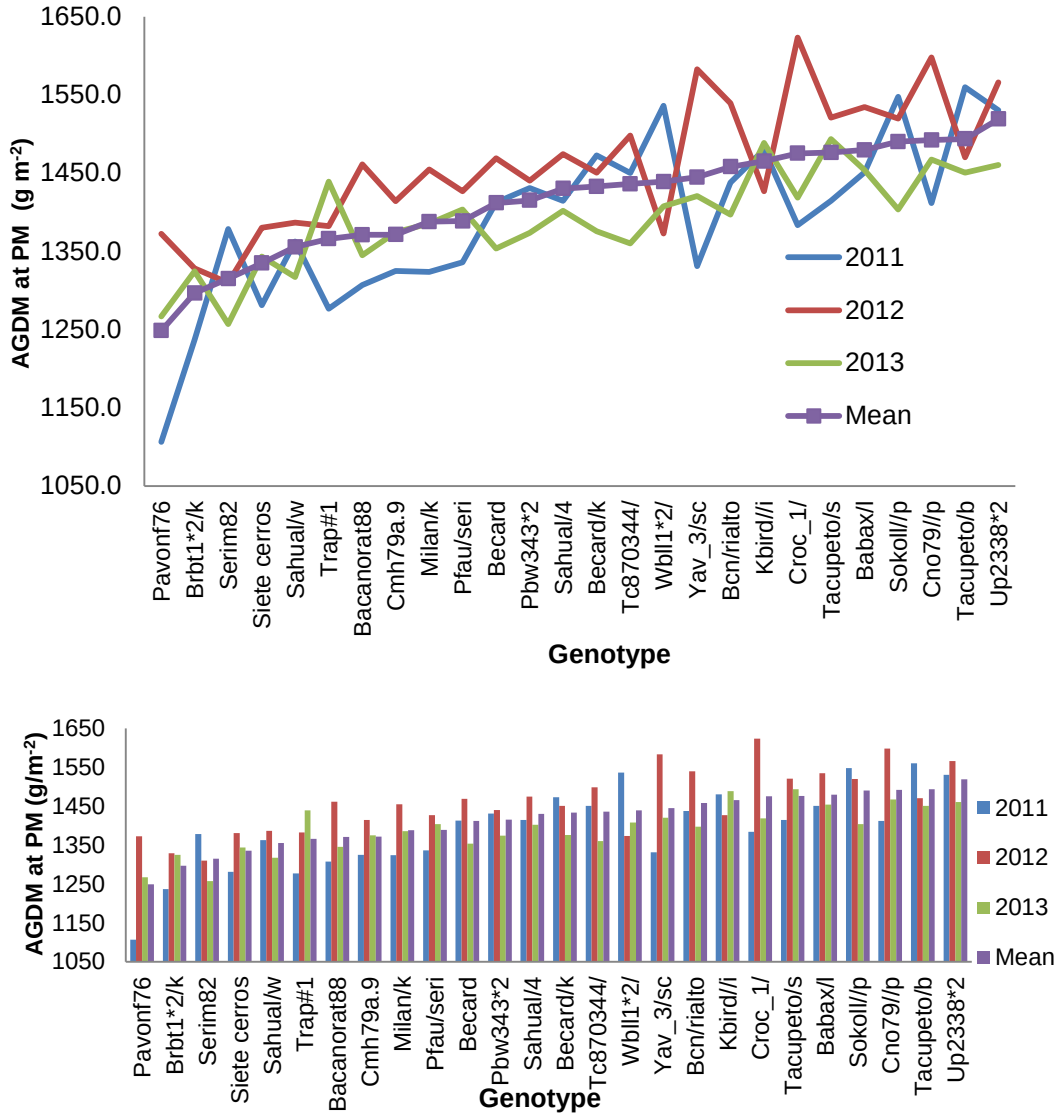


Figure 4.2. Above ground dry matter for 26 CIMCOG spring wheat genotypes in 2011, 2012, 2013 and overall mean (LSD are in Table 4.3)

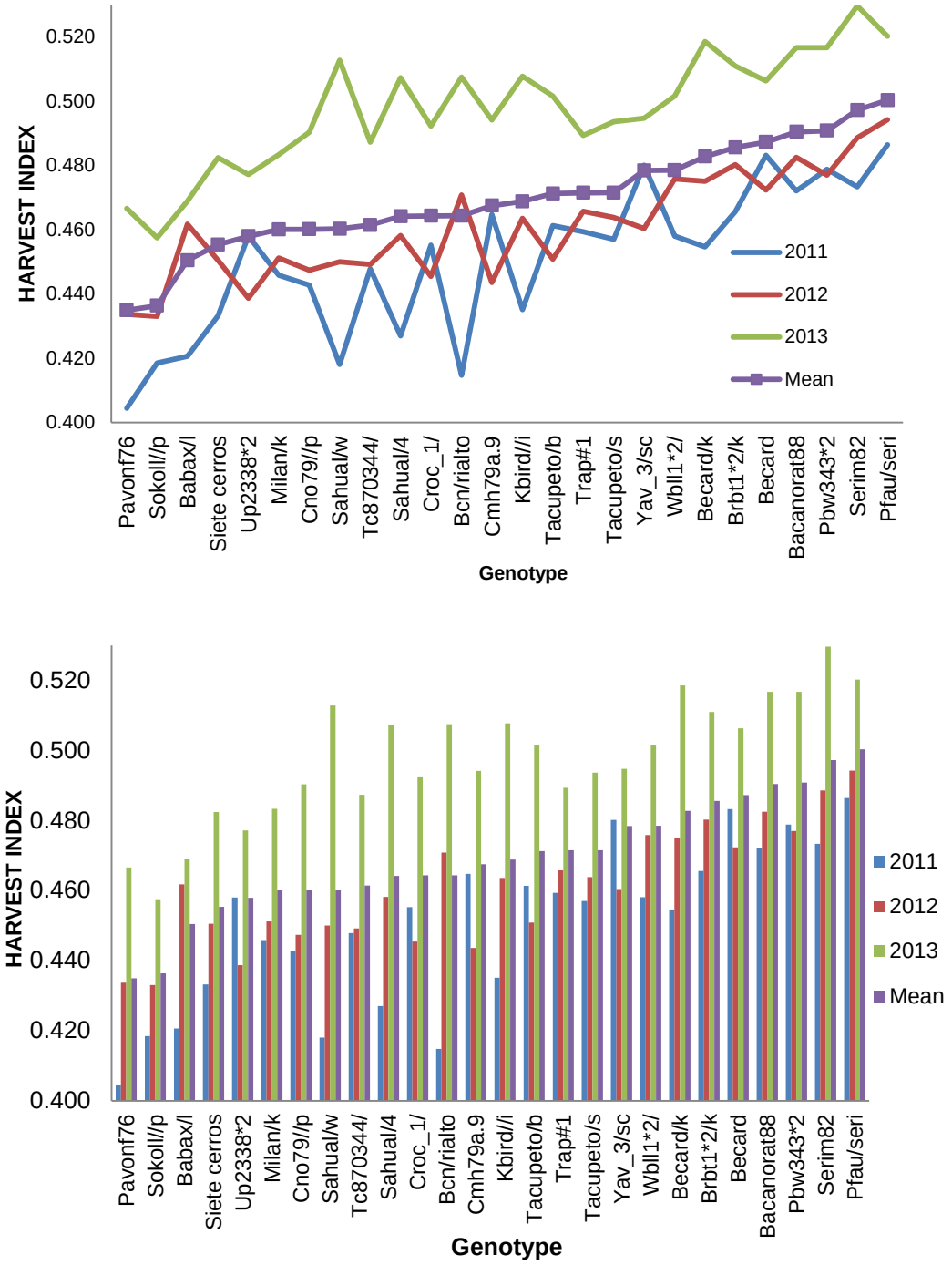


Figure 4.3. Harvest index for 26 CIMCOG spring wheat genotypes in 2011, 2012, 2013 and overall mean (LSD are in Table 4.3).

4.3.5 Grain weight, grain m^{-2} spikes m^{-2} and grains $spike^{-1}$

In 2011, grains m^{-2} differed amongst the 26 genotypes from 11,976 (Milan/k) to 21,387 (Bcn/Rialto), in 2012 from 13,324 (Tc870344/) to 23,071 (Bcn/rialto) and in 2013 from 12,494 (Tc870344/) to 23,761 (Bcn/Rialto) ($P < 0.001$). The year x genotype interaction was not statistically significant (Table 4.5).

In 2011, spikes m^{-2} differed amongst the genotypes from 244 (Cmh79a.9) to 380 (Kbird//i) ($P < 0.001$), and in 2012 and 2013 from 212 (Wbll1*2/) to 396 (Bcn/Rialto) and 239 (Cmh79a.9) to 407 (Bcn/rialto) ($P < 0.001$), respectively. The year x genotype interaction was not significant (Table 4.5).

Genotype differences were observed in grains $spike^{-1}$ in 2011 from 38.5 (Babax/l) to 66.7 (Cmh79a.9) ($P < 0.001$), in 2012 from 45.8 (Tc870344/) to 72.1 (Sahual/4) ($P < 0.001$) and in 2013 from 42.7 (Pavonf76) to 74.7 (Cmh79a.9) ($P < 0.001$). The year x genotype interaction was not significant (Table 4.5).

In 2011, grain weight differed amongst the genotypes in the range of 27.9 (Bcn/Rialto) to 50.5 (Babax/l) mg ($P < 0.001$), in 2012 from 31.4 (Bcn/Rialto) to 50.0 (Babax/l) mg ($P < 0.001$) and in 2013 from 29.8 (Babax/l) to 53.2 mg (Tc870344/) mg ($P < 0.001$). Averaging across years the range was 29.7- 50.6 mg, and there was no interaction for year x genotype (Table 4.5).

Table 4.4 Grain yield, above-ground dry matter (AGDM) at GS91 and Harvest Index in 2011, 20112 and 2013 for 26 CIMCOG spring wheat genotypes.

Cultivar	YIELD 100%DM (t ha^{-1})				AGDM GS91 (g m^{2})				HI			
	2011	2012	2013	Mean	2011	2012	2013	Mean	2011	2012	2013	Mean
Babax/l	6.10	7.07	6.82	6.66	1451	1534	1454	1480	0.421	0.462	0.469	0.450
Bacanorat88	6.15	7.05	6.95	6.72	1307	1461	1345	1371	0.472	0.483	0.517	0.490
Bcn/rialto	5.96	7.26	7.07	6.76	1438	1539	1397	1458	0.415	0.471	0.507	0.464
Becard/k	6.69	6.89	7.13	6.90	1473	1451	1376	1433	0.455	0.475	0.519	0.483
Brbt1*2/k	5.76	6.38	6.77	6.30	1237	1329	1325	1297	0.466	0.480	0.511	0.486
Sahual/4	6.03	6.75	7.11	6.63	1415	1474	1402	1430	0.427	0.458	0.507	0.464
Sahual/w	5.70	6.23	6.71	6.21	1363	1387	1317	1356	0.418	0.450	0.513	0.460
Cmh79a.9	6.16	6.25	6.78	6.39	1325	1414	1375	1371	0.465	0.444	0.494	0.467
Cno79//p	6.24	7.15	7.19	6.86	1412	1598	1467	1492	0.443	0.447	0.490	0.460
Croc_1/	6.30	7.24	6.98	6.84	1384	1623	1419	1475	0.455	0.445	0.492	0.464
Kbird//i	6.44	6.61	7.56	6.87	1480	1427	1489	1465	0.435	0.464	0.508	0.469
Milan/k	5.90	6.56	6.69	6.39	1324	1455	1386	1388	0.446	0.451	0.483	0.460
Pavonf76	4.48	5.94	5.91	5.45	1107	1373	1267	1249	0.404	0.434	0.467	0.435
Pbw343*2	6.85	6.87	7.10	6.94	1431	1440	1374	1415	0.479	0.477	0.517	0.491
Pfau/seri	6.49	7.05	7.30	6.95	1336	1427	1404	1389	0.486	0.494	0.520	0.500
Serim82	6.52	6.39	6.66	6.52	1379	1310	1257	1315	0.473	0.489	0.530	0.497
Siete cerros	5.55	6.22	6.48	6.08	1281	1380	1343	1335	0.433	0.451	0.482	0.455
Sokoll//p	6.47	6.58	6.42	6.49	1547	1520	1403	1490	0.418	0.433	0.457	0.436
Tacupeto/s	6.46	7.05	7.37	6.96	1415	1521	1493	1476	0.457	0.464	0.494	0.472
Tacupeto/b	7.20	6.62	7.28	7.03	1560	1470	1451	1494	0.461	0.451	0.502	0.471
Tc870344/	6.50	6.72	6.63	6.62	1450	1498	1360	1436	0.448	0.449	0.487	0.461
Trap#1	5.85	6.44	7.04	6.44	1277	1382	1439	1366	0.459	0.466	0.489	0.471
Up2338*2	7.01	6.86	6.97	6.95	1530	1566	1461	1519	0.458	0.439	0.477	0.458
Becard	6.82	6.93	6.86	6.87	1413	1469	1354	1412	0.483	0.472	0.506	0.487
Wbll1*2/	7.04	6.54	7.06	6.88	1536	1373	1408	1439	0.458	0.476	0.502	0.479
Yav_3/sc	6.39	7.29	7.03	6.90	1331	1583	1421	1445	0.480	0.460	0.495	0.478
CV	5.5	3.67	4.60	4.59	5.2	4	5.15	5.02	2.2	3.361	2.892	3.268
Genotype	**	***	***	***	*	***	*	***	**	***	***	***
LSD(0.05)	0.684	0.444	0.520	0.306	145.60	113.14	117.0	70.8	0.0115	0.0263	0.0235	0.0154
Year				***				***				***
LSD(0.05)				0.106				24.50				0.0053
Year*Genotype				*				ns				ns
r (with Yield)				-	0.73 ***	0.51 **	0.66 ***	0.78 ***	0.53 **	0.38 †	0.37 †	0.51 **
r (wth GW)	0.33 †	0.04	0.03	0.29	0.33 †	0.06	0.19	0.28	0.11	**-0.02**	**-0.19**	-0.02

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability Subset of 10 cultivars in bold letters.

Table 4.5 Grains m^{-2}, thousand grain weight (g), spikes m^{-2} and grains $spike^{-1}$ in 2011, 20112 and 20113 and cross-year mean for 26 CIMGOG spring wheat genotypes

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability Subset of 10 cultivars in bold letters.

Genotype	Grains m^{-2}				TGW g				Spikes m^{-2}				Grains per spike			
	2011	2012	2013	Mean	2011	2012	2013	Mean	2011	2012	2013	Mean	2011	2012	2013	Mean
Babax/l	12065	14132	13312	13170	50.5	50.0	51.3	50.6	314	273	302	296	38.5	51.9	44.1	44.8
Bacanorat88	17701	20275	18758	18911	34.8	35.4	37.1	35.7	379	384	339	368	46.7	52.7	55.5	51.6
Bcn/rialto	21387	23071	23761	22739	27.9	31.4	29.8	29.7	374	396	407	392	57.2	58.5	58.5	58.0
Becard/k	15345	17005	17231	16527	43.7	40.5	41.5	41.9	323	292	316	310	47.5	58.1	54.7	53.5
Brbt1*2/k	12853	14314	14994	14054	44.9	44.6	45.1	44.9	266	268	253	262	48.2	53.5	59.6	53.8
Sahual/4	12948	15832	15471	14750	46.6	42.7	46.0	45.1	265	221	263	250	48.8	72.1	58.8	59.9
Sahual/w	12832	14306	14995	14045	44.4	43.6	44.8	44.3	317	259	259	278	40.4	56.7	58.1	51.7
Cmh79a.9	16198	15828	17813	16613	38.1	39.7	38.2	38.6	244	233	239	239	66.7	69.4	74.7	70.3
Cno79//p	13032	15701	14729	14487	47.9	45.5	48.9	47.4	283	255	264	267	46.1	61.7	56.1	54.6
Croc_1/	15293	18714	16300	16769	41.2	38.6	42.9	40.9	358	362	340	354	42.7	51.7	48.6	47.7
Kbird//i	18561	18342	21214	19372	34.7	36.0	35.7	35.5	380	294	385	353	48.8	62.7	55.2	55.6
Milan/k	11976	14751	13907	13545	49.3	44.6	48.2	47.4	260	243	265	256	46.1	60.8	52.5	53.1
Pavonf76	13441	17054	15849	15448	33.3	35.0	37.3	35.2	345	348	354	349	39.0	49.1	45.0	44.4
Pbw343*2	17525	16349	17391	17088	39.2	42.2	41.0	40.8	370	349	306	342	47.4	46.9	57.1	50.4
Pfau/seri	16085	16855	16049	16330	40.4	41.9	45.5	42.6	318	313	312	314	50.5	54.2	51.5	52.1
Serim82	16895	16840	15919	16551	38.6	38.0	41.8	39.5	322	290	271	294	52.4	58.1	58.8	56.5
Siete cerros	15777	16778	17716	16757	35.2	37.5	36.7	36.4	345	277	297	307	45.7	61.8	60.1	55.9
Sokoll//p	14722	15263	14092	14692	44.0	43.1	45.6	44.2	336	318	304	320	43.8	48.0	46.3	46.0
Tacupeto/s	13824	16205	15598	15209	46.8	43.5	47.3	45.9	303	299	268	290	45.6	54.9	58.3	52.9
Tacupeto/b	15790	15404	16859	16018	45.6	43.0	44.1	44.3	308	250	292	283	51.2	61.8	57.7	56.9
Tc870344/	13255	13719	12494	13156	49.0	49.0	53.2	50.4	292	304	293	296	45.4	45.8	42.7	44.7
Trap#1	14201	14651	15910	14921	41.2	43.9	44.3	43.2	315	293	317	308	44.9	50.2	50.3	48.5
Up2338*2	17476	17007	17569	17351	40.1	40.3	39.7	40.0	358	332	335	342	48.9	51.3	52.5	50.9
Becard	15215	15467	14729	15137	44.8	44.8	46.6	45.4	351	321	306	326	43.4	48.5	48.6	46.8
Wbll1*2/	14483	13324	14265	14024	48.6	49.0	49.5	49.0	273	212	271	252	53.0	63.2	52.7	56.3
Yav_3/sc	14834	17588	15972	16132	43.1	41.4	44.1	42.9	300	285	281	289	49.5	62.5	57.2	56.4
Mean	15143	16337	16265	15915	42.1	41.7	43.3	42.4	319	295	301	305	47.6	56.4	54.4	52.8
Genotype	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
LSD(0.05)	1847.26	1840.47	1831.86	1057.7	1.96	3.42	3.6	2.1	26.65	41.08	36.55	21.1	4.9	9.10	8.03	4.76
Year				***				***				***				***
LSD(0.05)				365.9				0.7				7.3				1.6
Year*Genotype				ns				ns				*				ns

4.3.6 Relationships between grain yield and yield components, biomass and HI

There was a positive linear relationship between grain yield and AGDM amongst the 26 genotypes in 2011 (R^2 = 0.86, P < 0.01) and amongst the subset of 10 genotypes (R^2 = 0.68, P < 0.01) (Figure 4.4 (a)) and in 2012 (R^2 = 71, P < 0.01; R^2 = 0.62, P < 0.001; Figure 4.4 (b), respectively) and 2013 (R^2 = 52, P < 0.001;Figure 4.4 ; R^2 = 55, P < 0.05, respectively).

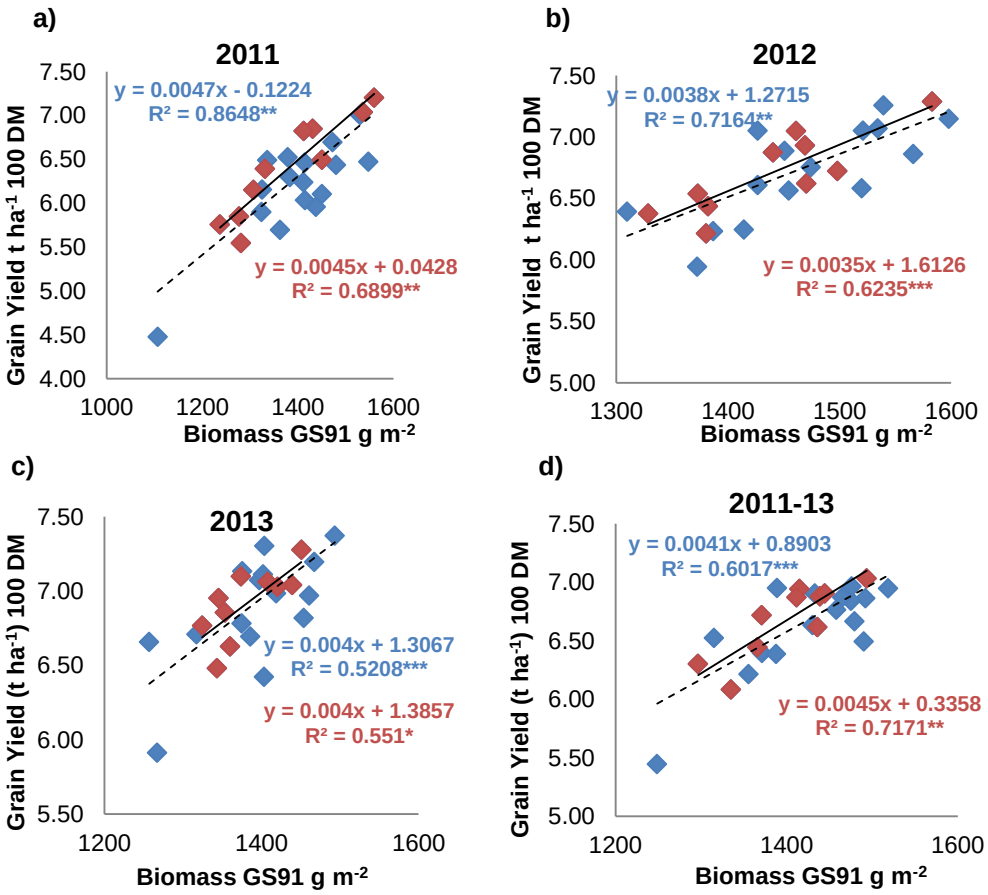


Figure 4.4. Relationship between grain yield (t ha^{-1}, 100% DM) and above-ground DM at physiological maturity in a) 2011, b) 2012, c) 2013, and d) cross-year mean for 26 CIMCOG cultivars. (Continuous line corresponds to 10 genotypes, broken line corresponds to 26 genotypes for regressions). Red symbols indicate subset for 10 cultivars.

Averaging over the three seasons, there was a positive linear association between grain yield and AGDM (R^2 = 0.60, P < 0.001) amongst the 26 genotypes and the subset of 10 genotypes (R^2 = 0.71, P < 0.01) (Figure 4.4 (d)).

Grain yield was positively associated with HI amongst the 26 genotypes in 2011 (R^2 = 0.33, P < 0.01; Figure 4.5 (a). However, there was no significant association in the subset of 10 genotypes in 2011 with a restricted range of anthesis date. Grain yield was not associated with HI amongst the 26 genotypes or amongst the 10 genotypes in 2012 (Figure 4.5 (b)). Grain yield in 2013 was positively associated with HI (R^2 = 24, P < 0.05) amongst the 26 genotypes, although there was no association amongst the subset of 10 genotypes (Figure 4.5 (c)). Averaging over the three seasons grain yield was nonlinearly associated with HI (R^2 = 0.38, quadratic, P < 0.01) amongst the 26 genotypes, although there was no association amongst the subset of 10 genotypes (Figure 4.7 (b).

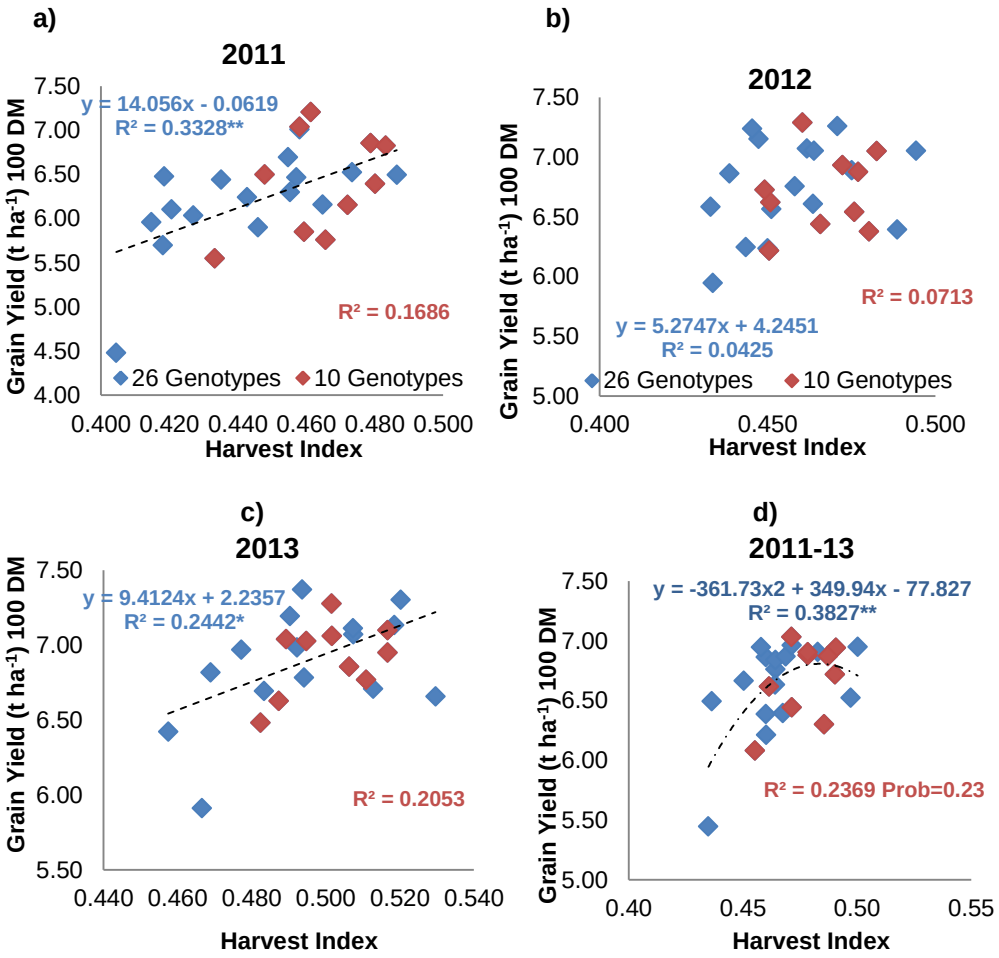


Figure 4.5 Relationship between grain yield and harvest index at maturity in a) 2011, b) 2012, c) 2013, and d) means for 26 CIMCOG cultivars (Continuous line corresponds to 10 genotypes, broken line corresponds to 26 genotypes for regressions). Red symbols indicate subset for 10 cultivars.

There was a trend for a positive linear relationship between grain yield and 1,000 grain weight (R^2 = 0.28, *P* = 0.09) amongst the 26 genotypes in 2011 but no association amongst the subset of ten genotypes (Figure 4.6 (a)). However, this trend has to be interpreted cautiously since it was only observed in one year.

Grain yield was not significantly associated with 1,000 grain weight amongst the 26 genotypes or the subset of 10 cultivars in 2012, 2013 or the means across years (Figure 4.6 (b, c d)).

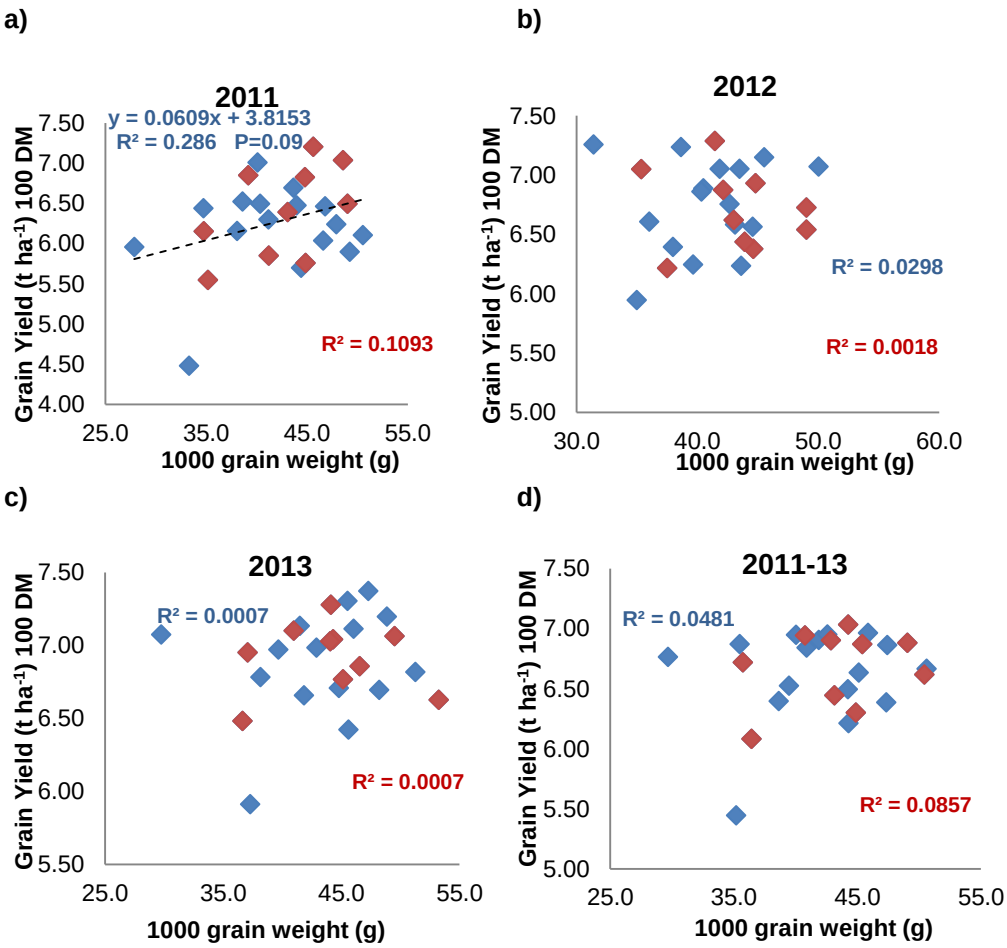


Figure 4.6 Relationship between grain yield and thousand grain weight, in a) 2011, b) 2012, c) 2013, and d) means for 26 CIMCOG cultivars. (Continuous line corresponds to 10 genotypes, broken line corresponds to 26 genotypes for regressions). Red symbols indicate subset for 10 cultivars.

In 2011, grain yield was not associated with grains m^{-2} amongst the 26 genotypes, or the subset of 10 genotypes (Figure 4.7 (a)). In 2012, grain yield was associated (R^2 = 0.29, P < 0.05) with grains m^{-2} amongst the 26 genotypes, but, no association was observed amongst the10 genotypes (Figure 4.7 (b)). Grain yield was not associated with grains m^{-2} amongst the 26 genotypes in 2013 (Figure 4.6 (d)). However, when omitting one cultivar (Pavonf76) in 2013 the regression was significant (R^2=0.16, P < 0.05) for 25 genotypes. Averaging over the three years, grain yield was not associated with grains m^{-2} amongst the 26 genotypes or amongst the subset of 10 genotypes (Figure 4.7 (d)).

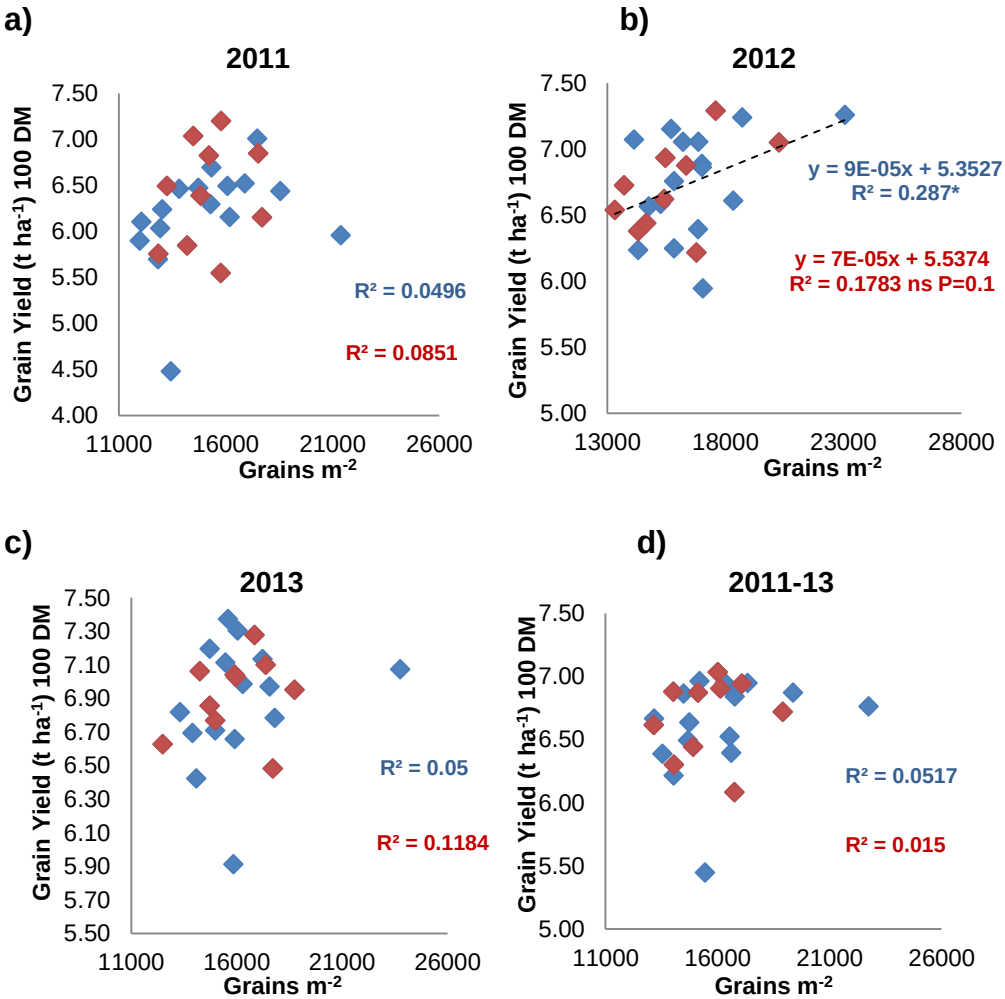
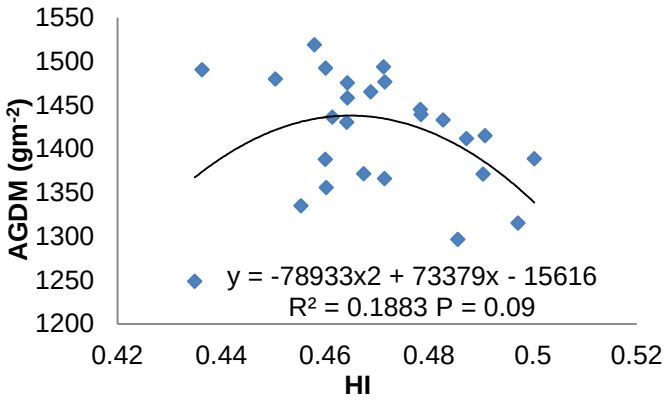


Figure 4.7 Relationship between grain yield and Grain m^{-2}, in a) 2011, b) 2012, c) 2013, and d) means for 26 CIMCOG cultivars. (Continuous line corresponds to 10 genotypes, broken line corresponds to 26 genotypes for regressions). Red symbols indicate subset for 10 cultivars.

4.3.7. Relationship between AGDM and HI and between grains m^{-2} and GW

Averaging over the three seasons, a nonlinear association was observed between AGDM and HI in the 26 CIMCOG genotypes (P = 0.09)and a trade-off was observed between grain weight and grains m^{-2} in the CIMCOG panel (Figure 4.8a). However, excluding Pavonf76, a trade-off (*P* < 0.05) was observed between AGDM and HI.

a)



b)

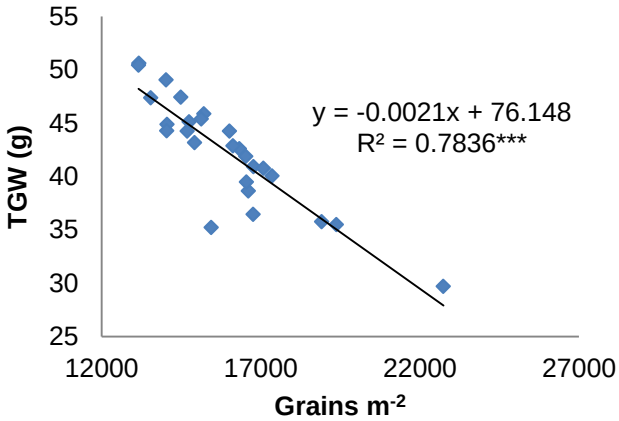


Figure 4.8 a) Relationship between AGDM at GS91 and HI. b) Relationship between 1000 grain TGW and grains m^{-2}. Values are means of 3 years for 26 CIMCOG cultivars.

4.3.8 Plant component DM partitioning at harvest in 2012 and 2013 for 10 genotype subset

In the subset of 10 genotypes, averaging over 2012 and 2013, grain yield differed amongst the genotypes from 6.35 (Siete cerros) to 7.16 (Yav_3/sc) t ha^{-1} (*P* < 0.01), AGDM from 1326.5 (Brbt1*2/k) to 1501.7

(Yav_3/sc) g m^{-2} (P < 0.01). The year x genotype interaction was not significant for grain yield but significant for AGDM.

Table 4.6 Grain yield (t ha^{-1}) Above ground dry matter (AGDM) and DM partitioning (Leaf lamina, Leaf sheath, True stem, Chaff, Grain DM). Values represent the means of the seasons, 2012 and 2013 for a subset of 10 genotypes.

Genotype	Grain Yield (t ha^{-1})	AGDM GS91 g m^{-2}	Leaf lamina DM g m^{-2}	Leaf Shelth DM g m^{-2}	True Stem DM g m^{-2}	Chaff DM g m^{-2}	Grain DM g m^{-2}
Bacanorat88	7.00	1403.2	136.7	123.1	264.2	178.1	700.0
Brbt1*2/k	6.57	1326.5	128.2	111.3	285.7	143.9	657.2
Pbw343*2	6.99	1407.1	139.9	124.6	292.8	150.8	698.6
Siete cerros	6.35	1362.0	126.4	111.3	299.9	189.0	634.8
Tacupeto/b	6.95	1460.5	147.1	130.7	278.2	208.9	694.9
Tc870344/	6.68	1429.1	139.4	126.8	328.5	165.2	667.5
Trap#1	6.74	1410.7	125.9	122.1	331.6	157.5	673.9
Becard	6.89	1411.4	128.7	115.0	312.9	164.2	689.3
Wbll1*2/	6.80	1390.4	138.7	127.2	291.9	153.0	680.0
Yav_3/sc	7.16	1501.7	151.5	129.4	321.3	182.3	715.8
Mean	6.81	1410.3	136.2	122.1	300.7	169.3	681.2
Max	7.16	1501.7	151.5	130.7	331.6	208.9	715.8
Min	6.35	1326.5	125.9	111.3	264.2	143.9	634.8
Cv	4.71	5.6	16.0	5.9	6.3	17.6	4.7
Genotype	**	**	ns	**	***	**	**
LSD (5%)	0.382	78.10	19.57	10.55	20.08	31.61	38.25
Year	*	**	**	ns	***	***	*
LSD (5%)	0.171	34.93	8.75	4.72	8.98	14.14	17.15
GenxYear	ns	*	*	ns	**	*	ns
Corr with Yield	-	0.74*	0.72*	0.69*	-0.11	0.15	-

† Significant at P = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

Averaging across 2012 and 2013, the true stem DM ranged amongst the 10 genotypes from 264.2 (Bacanorat88) to 331.6 (Trap#1) gm^{-2} (P < 0.001). Leaf lamina DM m^{-2} din not differ, whereas leaf sheath DM m^{-2} differed (P < 0.01) in the range 111.3 (Siente cerros) to 130.7 (Tacupeto/b). There were differences in chaff DM (P < 0.01) in the range 143.9 (Brbt1*2/k) to 208.9 (Tacupeto/b) amongst the 10 genotypes; the genotype x year interaction was significant for leaf lamina DM, true stem DM and chaff DM but not for leaf sheath DM (Table 4.6). Above-ground DM allocated to the plant components at physiological maturity in the subset ranged from 46.6 to 50% for grain yield, 10.7 to 13.9% for Chaff,

from 18.8 to 23.5% for true stem, from 8.1 to 9.2% for leaf sheath and from 8.9 to 10.1% for leaf lamina (Fig 4.9).

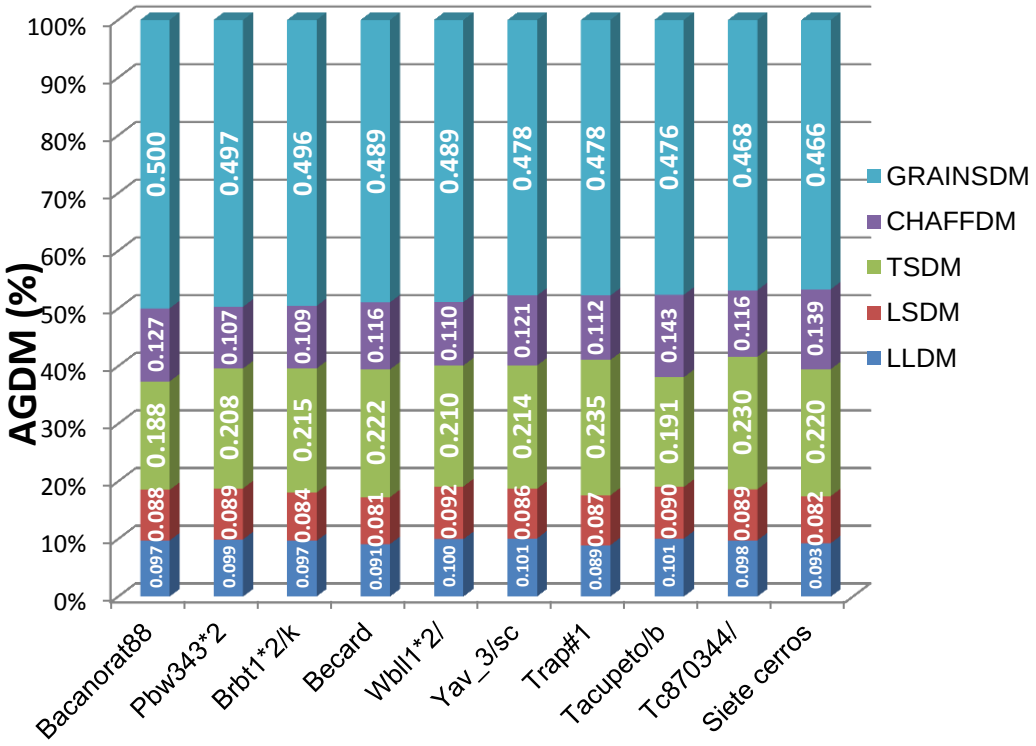


Figure 4.9 Percentage of above-ground DM allocated to the plant components (TS = true stem, LS = leaf sheath and LL= leaf lamina) at physiological maturity. Values represent the means of the seasons, 2012 and 2013 for a subset of 10 genotypes. (LSD $_{(0.05)}$ for Grain DM =18.053, Chaff DM = 16.814, TSDM = 10.567, LSDM = 4.069, LLDM = 12.248, AGDMGS91 = 44.101)

Averaging over 2012 and 2013, a trend for a linear and negative association was observed between true stem DM per unit area and HI (R^2 = 0.33, *P* = 0.06). Similarly, a trend for a negative association was observed between chaff DM per unit area and HI (R^2 = 25, *P* = 0.11) amongst the 10 genotypes (Figure 4.10).

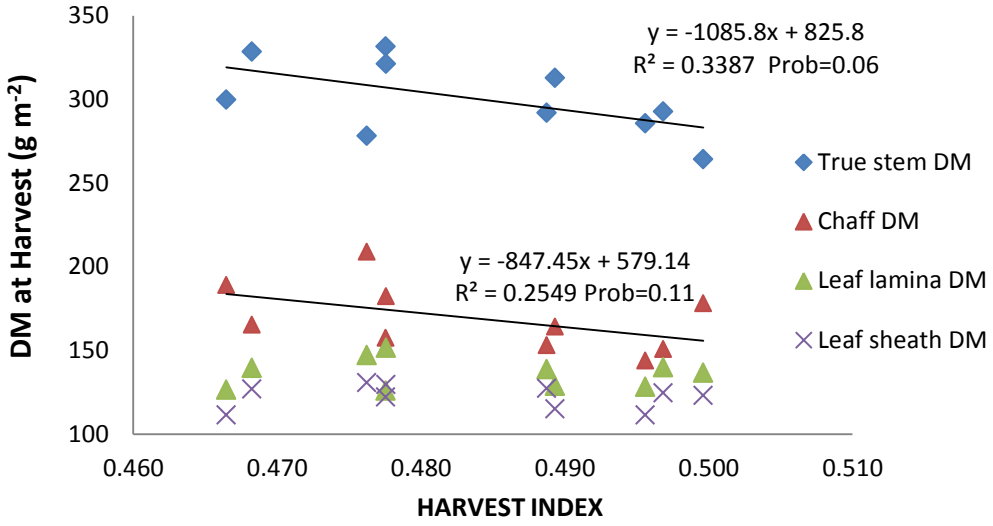
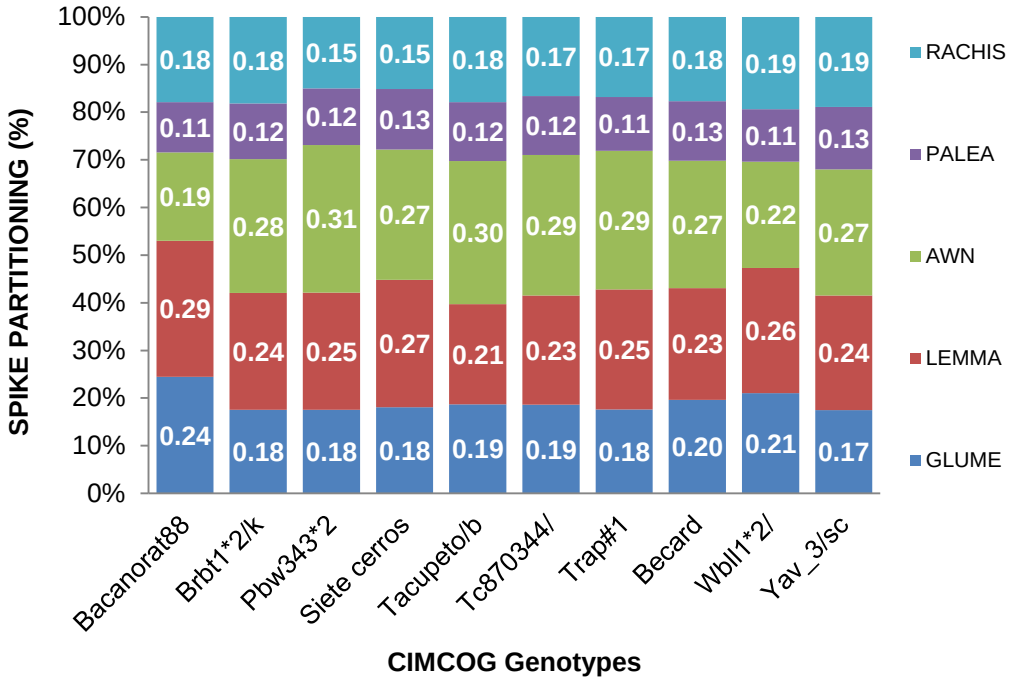


Figure 4.10 Relationship between harvest index and partitioning components (LLDM, TSDM, LSDM and Chaff DM)

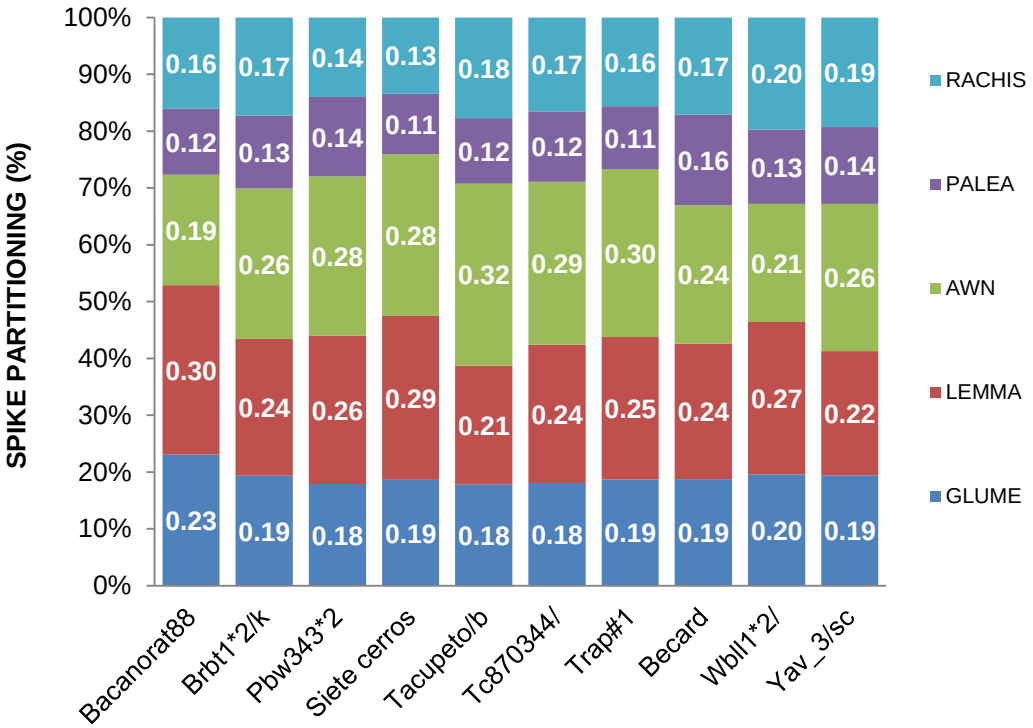
4.3.9 Spike morphological component DM partitioning for the subset of 10 genotypes

Genetic variation was observed in glume DM from 0.134 to 0.211 g spike^{-1}, in lemma DM spike^{-1} from 0.176 to 0.275, in awns DM spike^{-1} from 0.141 to 0.319, in rachis DM spike^{-1} from 0.111 to 0.202 and palea from 0.082 to 0.124 g spike^{-1} ($P < 0.01$) (Table 4.6). The genotype x year interaction was significant for glumes, rachis and grain DM spike^{-1}. However, the year x genotype interaction was not significant for lemmas, awns and paleas (Table 4.6). Averaging over two years, the percentage of spike DM allocated to the spike morphological components ranged from 14-20% for rachis, from 11-14% for paleas, from 19-31% for awns, from 21-29% for lemmas and from 18-24% for glumes (Figure 4.11c).

a)


SPIKE PARTITIONING (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.18 0.18 0.15 0.15 0.18 0.17 0.17 0.18 0.19 0.19
0.11 0.12 0.12 0.13 0.12 0.12 0.11 0.13 0.11 0.13
0.19 0.28 0.31 0.27 0.30 0.29 0.29 0.27 0.22 0.27
0.29 0.24 0.25 0.27 0.21 0.23 0.25 0.23 0.26 0.24
0.24 0.18 0.18 0.18 0.19 0.19 0.18 0.20 0.21 0.17
Bacanorat88
Brbt1*2/k
Pbw343*2
Siete cerros
Tacupeto/b
Tc870344/
Trap#1
Becard
Wbll1*2/
Yav_3/sc
CIMCOG Genotypes
RACHIS
PALEA
AWN
LEMMA
GLUME

b)


SPIKE PARTITIONING (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.16 0.17 0.14 0.13 0.18 0.17 0.16 0.17 0.20 0.19
0.12 0.13 0.14 0.11 0.12 0.12 0.11 0.16 0.13 0.14
0.19 0.26 0.28 0.28 0.32 0.29 0.30 0.24 0.21 0.26
0.30 0.24 0.26 0.29 0.21 0.24 0.25 0.24 0.27 0.22
0.23 0.19 0.18 0.19 0.18 0.18 0.19 0.19 0.20 0.19
Bacanorat88
Brbt1*2/k
Pbw343*2
Siete cerros
Tacupeto/b
Tc870344/
Trap#1
Becard
Wbll1*2/
Yav_3/sc
RACHIS
PALEA
AWN
LEMMA
GLUME

c)

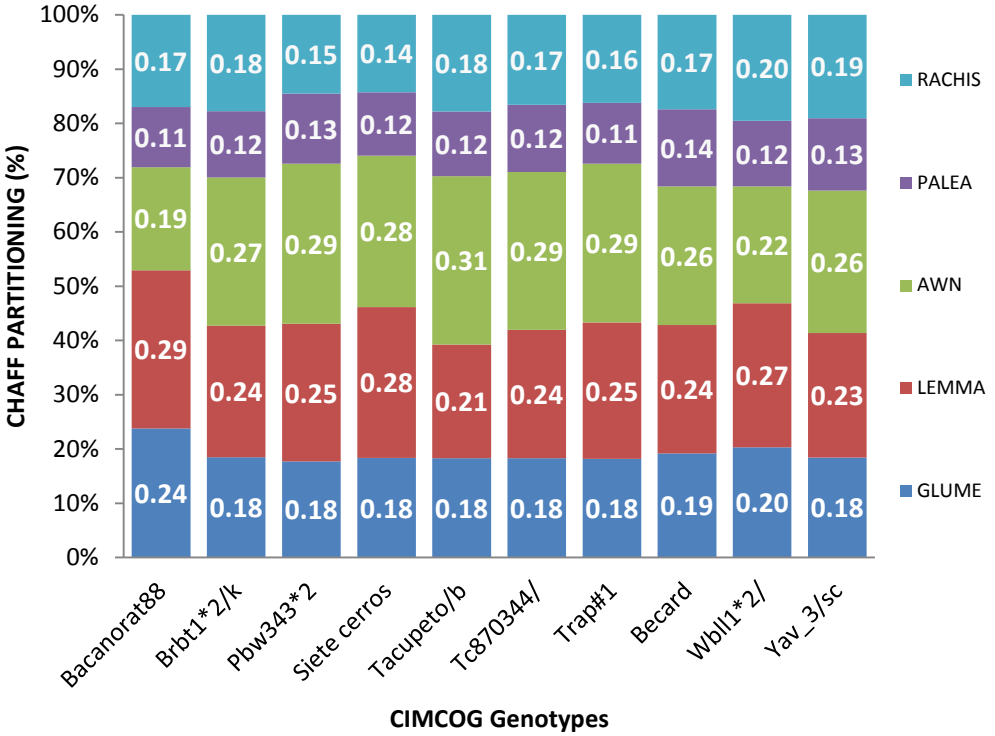


Figure 4.11 Percentage of spike DM allocated to the spike morphological components. Values represent the means of each season a) 2012 and b) 2013 and c) cross-year means for a subset of 10 genotypes. LSD (5%) (Glumes = 0.0174, Lemmas = 0.0375, Awns = 0.0390, Paleas = 0.0190, Rachis = 0.0165).

Table 4.7 Dry matter for glumes (g spike^{-1}), lemmas (g spike^{-1}), awns (g spike^{-1}), paleas, rachis, grain, and number of grains per spike. Values represent the means of the seasons, 2012 and 2013 for a subset of 10 genotypes.

Genotype	GLUME DM g spike^{-1}	LEMMAS DM g spike^{-1}	AWNS DM g spike^{-1}	PALEAS DM g spike^{-1}	RACHIS DM g spike^{-1}	GRAIN DM g spike^{-1}	Grains DM spike^{-1}
Bacanorat88	0.178	0.217	0.141	0.082	0.128	2.370	65.6
Brbt1*2/k	0.157	0.208	0.235	0.104	0.152	3.215	66.5
Pbw343*2	0.134	0.194	0.227	0.098	0.111	2.843	65.9
Siete cerros	0.167	0.254	0.254	0.108	0.131	2.840	74.7
Tacupeto/b	0.189	0.216	0.319	0.124	0.183	3.039	65.6
Tc870344/	0.154	0.196	0.247	0.104	0.139	2.998	56.9
Trap#1	0.174	0.242	0.282	0.108	0.156	3.095	66.9
Becard	0.143	0.176	0.191	0.104	0.130	2.817	59.8
Wbll1*2/	0.211	0.275	0.225	0.122	0.202	3.633	68.7
Yav_3/sc	0.162	0.207	0.234	0.118	0.169	3.161	69.0
Mean	0.167	0.219	0.235	0.107	0.150	3.001	66.0
Genotype	***	***	***	**	***	***	***
LSD(0.05)	0.017	0.038	0.039	0.019	0.017	0.345	6.85
Year	***	***	*	*	***	***	***
LSD(0.05)	0.026	0.029	0.044	0.020	0.009	0.242	5.96
Year*Genotype	***	ns	ns	ns	**	**	ns
LSD(0.05)	0.031	0.055	0.062	0.029	0.023	0.494	10.2

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

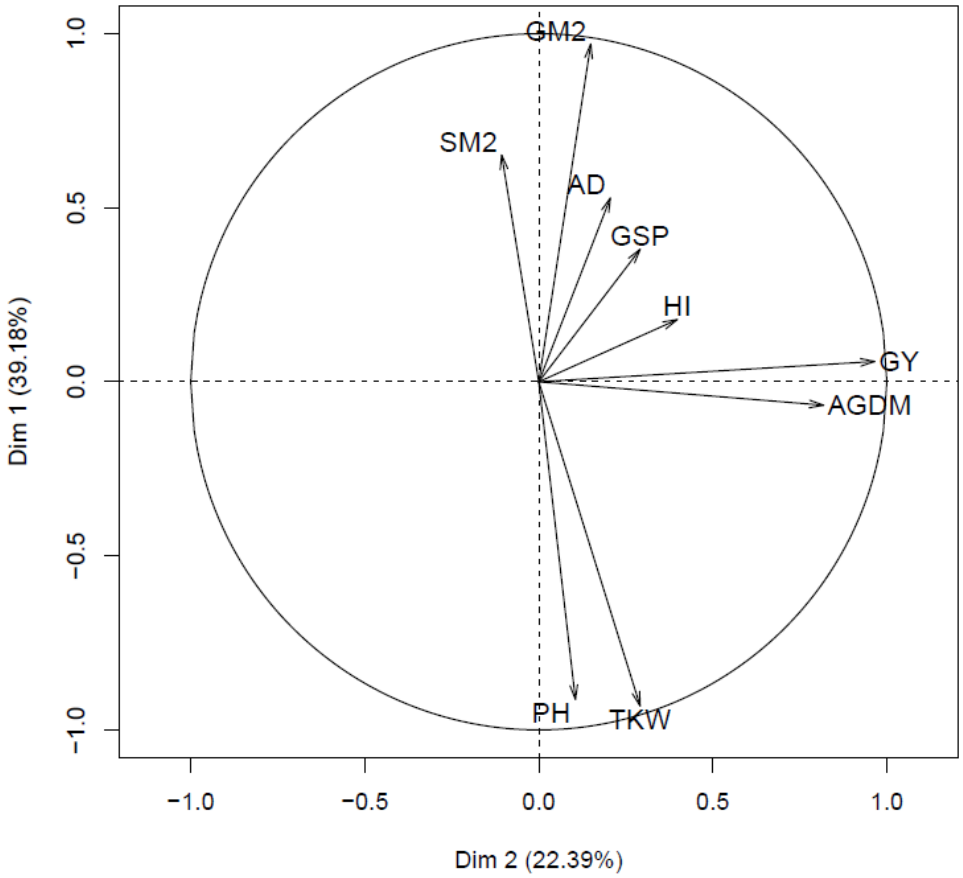
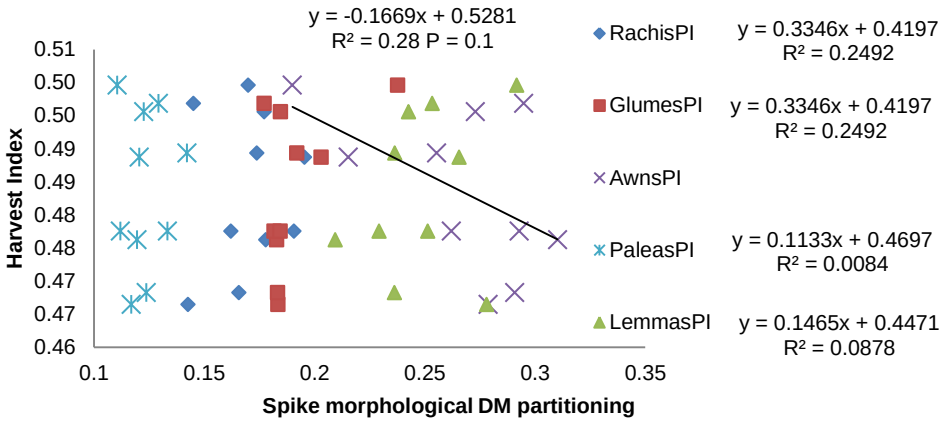


Figure 4.12 Biplot of GY (Grain yield), AGDM (Above ground dry matter at harvest), HI (Harvest index), TGW (Thousand grain weight), GPS (Grains per spike), SM2 (Spikes m^{-2}), AD (Anthesis date) and PH (Plant height) for 26 genotypes.

In the biplot analysis, the first two principal components explained 61.5% of the variation amongst the 26 genotypes. Over all, the biplot confirmed that grain yield is strongly associated with biomass at harvest. On the other side grain weight is positively associated with plant height and grain m^{-2} with spike per m^2 and anthesis date. Negative associations between plant height and SM2, and grain m^{-2} were observed.

Averaging over years, awns partitioning index (PI) showed a trend for a negative association amongst the 10 genotypes with HI (R^2 = 0.28, *P* = 0.1) and similarly with awns g m^{-2} vs HI (R^2 = 0.36, *P* = 0.06). Glume, lemma, palea and rachis PI did not show any association with HI amongst the 10 genotypes in two years.

a)



b)

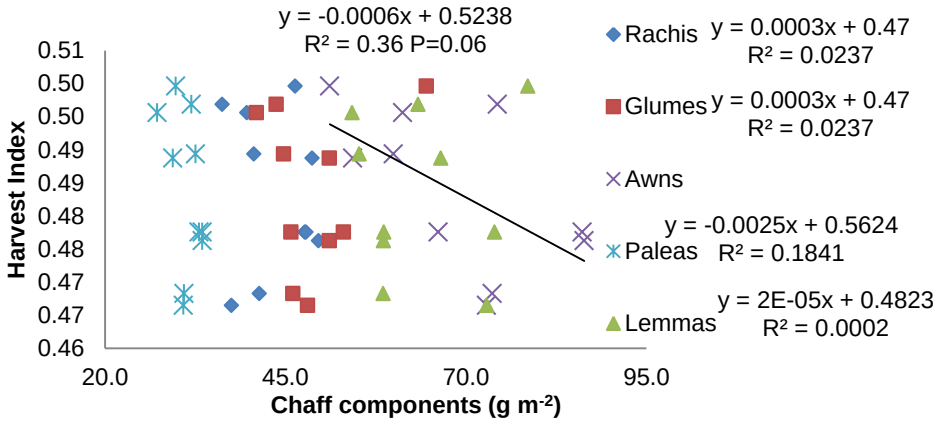


Figure 4.13 Association between Harvest index and Chaff components in a) spike morphological DM partitioning and b) chaff components (g m^{-2})

Averaging over years, awns partitioning index (PI) showed a trend for a negative association amongst the 10 genotypes with HI (R^2 = 0.28, *P* = 0.1) and similarly with awns g m^{-2} vs HI (R^2 = 0.36, *P* = 0.06). Glume,

lemma, palea and rachis PI did not show any association with HI amongst the 10 genotypes in two years.

Table 4.8 Relationships between year of release (YoR) realeased from 1966 to 2009 and Grain yield (YLD), Grain weight (GW), Harvest Index (HI), biomass at GS91 (BM), grains m^{-2}(GM2), Spike m^{-2} (SM2), grain $spike^{-1}$ (GSP), grain filling % (%GF), anthesis date (AD), plant height and year of release (YoR). Values represent the means of 2011, 2012 and 2013 for 26 genotypes.

Yield components	Year of release
YLD (tha^{-1})	0.64***
GW(g)	0.42*
HI	0.10
BM	0.67***
GM2	-0.11
SM2	-0.19
GSP	0.08
%GF	-0.01
AD (days)	0.19
Plant height (cm)	0.29

Grain yield was positively associated with year of release ($P < 0.001$) amongst the 26 genotypes. Grain weight was positively associated with year of release ($P < 0.05$) and biomass at harvest was positively associated with year of release ($P < 0.001$) amongst the 26 CIMCOG genotypes over years.

4.4 Discussion

4.4.1 Relationships between Genetic variation in grain yield, HI and above-ground biomass

Most yield potential progress in wheat has historically been associated with increased HI (Fischer and Stockman 1986). However, no systematic progress has been made since the early 1990s (Foulkes et al., 2010; Reynolds et al., 1999) from values around 50% for spring wheat (Reynolds et al., 1999; Sayre et al., 1997). Reports for HI for the best winter wheat in the UK reached around 0.53 for cultivars released in the mid 1990 to 1995 (Foulkes et al., 2002; Shearman et al., 2005). The present results showed HI from 0.43 to 0.50 confirming that there is considerable scope for improvement in HI in order to increase grain yield as the theoretical maximum is in the region of 0.64 (Foulkes et al., 2011).

In the present study the maximum value of HI achieved over the three years was 0.50 by Pfau/Seri yielding 6.94 t ha^{-1}. However, the highest yield was obtained by Tacupeto/b with 7.03 t ha^{-1} with a HI of 0.47. This was a consequence of a negative linear relationship between HI and AGDM amongst the highest yielding cultivars in the CIMCOG. In this respect, Tacupeto/b showed different distribution of the DM allocation compared to the rest of the subset cultivars analyzed, with the highest investment in stem DM (23%).

Some investigations of genetic grain progress under high-yielding conditions have shown no correlation between biomass and yield (Brancourt-Hulmel et al., 2003; Slafer et al., 1994). However, recent studies have demonstrated greater biomass at PM for modern cultivars in USA (Donmez et al., 2001), UK (Shearman et al., 2005) and Mexico (Aisawi et al., 2015). In present results above-ground dry matter showed a positive association with year of release in the 26 cultivars released from 1966 to 2009, indicating that modern varieties of CIMMYT spring wheat have increased their biomass. Bustos et al. (2013) studied a progeny of a

cross between two elite parents (Bacanora and Weebil) that contrasted in grain number and grain weight and found that grain yield was highly correlated with above-ground biomass and grain number, but no correlation was found with either harvest index or grain weight.

Overall genetic variation in grain yield showed stronger correlation with biomass than HI in the three years. Similar results have been found for spring wheat in Brazil with seven wheat cultivars released between 1940 and 1992 (Rodrigues et al., 2007). Similarly Aisawi et al. (2015) found that grain yield correlated more strongly for biomass than HI in 12 historic cultivars released from 1966 to 2009. The trade-off between HI and biomass amongst the cultivars indicates that breeders must identify traits associated with enhanced partitioning to 'useful' in contrast to 'non-useful' biomass. The enhancement of biomass as strategy to increase grain yield will require improvement in radiation-use efficiency (Parry and Hawkesford 2010; Reynolds et al., 2012), which must in part be related with increased leaf photosynthesis rate (see Chapter 5).

Results showed cultivars had increased AGDM positively through year of release, indicating that modern cultivars have more biomass, but the extra biomass produced has not been allocated efficiently to the grains. In order to reduce the trade-off between HI and AGDM at harvest, useful traits must be identified to increase HI. In the present results the cultivars with higher values of HI (12 cultivars with range 0.47-0.50) showed trends for positive associations with grains m^{-2} and negative association with plant height, and grain weight. In addition negative association with year of release indicated clearly that HI is decreasing in modern high biomass cultivars. Although the semi-dwarfs genes (RhtD1 or RhtB1) were presented in the CIMCOG cultivars, it seems that still there is still some potential for reductions of plant height, since cultivars are generally at the upper end of the optimum range of plant height from 70-100 c.m.

Optimal height has been considered between 70 to 90 cm (Richards 1992; Flintman et al., 1997 and Ortiz-Monasterio 2001). Present genotypes had either RhtB1 or RhtD1 dwarfing genes; the average height over the three years was 107 cm indicating that there is scope for reducing height.

Although a trend for a positive association between grain weight and a positive association between grain weight and plant height was observed, there may be potential to reduce height in CIMMYT spring wheat to increase HI whilst maintaining biomass. The challenge is to identify variation in canopy and leaf traits that can be utilised for maximize radiation-use efficiency. Higher biomass may be achieved by greater interception of radiation incident on the crop, and/or increased radiation-use efficiency; permitting higher sink demand and/or a higher partitioning of assimilate to yield (See chapter 5 results).

Austin (1980) predicted a theoretical maximum HI of 0.62 assuming a constant above ground DM and reducing the stem and leaf sheath by 50%. Austin reported 13% of AGDM allocate to chaff for his theoretical HI. The CIMCOG subset ranged from 11-14% for the chaff partitioning, this must be as an avenue to increase HI in high biomass cultivars held while adding more assimilate to the grains that will come with source traits that are discussed in the next chapter.

Analyses for a subset of 10 genotypes showed that HI was negatively associated with true stem at harvest and negatively associated with awns partitioning index. It is possible that the true stem relationship with HI comes from competition with the spike growth at pre-anthesis where the main competition amongst the plant components is the true stem.

In addition, the association between HI and chaff partitioning turns in the trade-off between the AwnsPI and HI (Figure 4.13) this indicates that the reduction of awns may be an avenue for increasing HI.

In addition the trend for a negative association observed between HI and awns PI indicated another potential component that need to be studied further in relation to improvement in FE. Relatively the awns were the heaviest component amongst the chaff (glume, lemma, palea and rachis). However, it has been reported that awns have a photosynthetic contribution to grain filling (Molero et al., 2013), so care must be taken in reducing partitioning to awns in some environments where awn photosynthesis may be important for post-anthesis assimilate supply, e.g. under terminal drought. In conclusion, the genetic variation in grain yield was positively correlated with both HI and above-ground biomass. However, and there was a trade-off between these physiological yield components. There were no effects of plant height and anthesis date on genetic variation in HI and grain yield in the CIMCOG panel.

4.4.2 Genetic variation between grain yield, grains m^{-2} and grain weight

In the present results, the nonlinear association between HI and grain yield amongst the 26 cultivars indicated that, for higher yielding cultivars the partitioning to the grains was not optimised. Turning to consider yield components, most studies worldwide showed that grain yield progress in wheat was mainly associated with grains m^{-2} (Abbate et al., 1998; Ortiz-Monasterio R. et al., 1997; Shearman et al., 2005; Waddington et al., 1986) rather than individual grain weight. However, in the present results grain yield in the three years was not positively associated overall with grains m^{-2} in the 26 cultivars; although there was a positive association amongst the 26 cultivars in 2012, and a significant association in 2013 for 25 CIMCOG cultivars. With regard to genetic variation amongst the 26 cultivars there was an association between grain weight and grain yield amongst the 26 cultivars in 2011, but not for 2012 and 2013. Also variation in GW was not associated with GY, so apparently both traits need to be considered equally in present plant breeding strategies. Thus, future yield gains in modern semi-dwarf wheat will require further gains in grain number per unit area (Foulkes et al., 2011) and/or potential grain weight.

This may be partly achieved by increased partitioning to spikes during stem elongation (Fischer 1985). The CIMCOG panel are elite cultivars selected for enhanced for yield and biomass and grain weight. Therefore, this may be one reason that in this panel yield is not associated with grains m^{-2}.

Recent studies show that grain weight has been increasing in CIMMYT for spring wheat (Aisawi et al., 2015; Lopes et al., 2012b) in part as a mechanism to increase biomass. In the present results grain weight, progress was 0.18 mg^{-1} $year^{-1}$ from 1976 to 2009. In general, these results indicate that grain weight was an important numerical yield component associated with genetic yield progress. Therefore, increasing potential grain weight is an important target for plant breeders to increase yield in these spring wheat cultivars. In conclusion, the hypothesis: 'Genetic variation in grain yield is positively correlated with both grains m^{-2} and grain weight; and there is no tradeoff between these numerical yield components' is rejected.

4.4.3. Trends with year of release

Over the 3 years, the subset of 10 cultivars showed a significant yield gain with year of release from 1966 to 2009. Potential grain yield increased by 2.9 g m^{-2} $year^{-1}$ or 0.45% $year^{-1}$. Similar values were reported by Aisawi et al. (2015) for potential yield when spring wheat varieties released between 1966 and 2009, giving an increment of 0.4% p.a. In addition, Fischer (2007) reported 0.41% increase p.a. for CIMMYT varieties released from 1966 to 2003. Fischer et al. (2014) reported 2.8 g m^{-2} $year^{-1}$ (a rate of 0.3 % p.a.) as estimate of the current potential yield progress at CIMMYT in the Yaqui valley for eight varieties released from 1979 to 2008 in bread wheat. However, higher rates were reported by Lopes et al. (2012) for yield potential progress for the best CIMMYT advanced bread wheat lines tested in the Yaqui Valley with varieties released from 1994 to 2008 giving a slope of 0.6% p.a of the estimated 2008. Over all, these results have

indicated that progress in potential yield has not plateaued in the breeding program in the Yaqui Valley in agreement with our results, but certainly it has been slower in recent years compared with rates greater that 1% p.a. a few decades after the green revolution (Fischer and Edmeades 2010). Therefore, the actual rates of progress in potential yield are not enough for the demands that has been estimated of 1.1% p.a. to ensure global food security in 2050 (Fischer et al., 2014).

The trade-off between grains m^{-2} and GW has been considered as a limitation of source, and for future progress in grain yield it will be important to minimize effects of increasing grains m^{-2} on potential grain weight. The potential grain weight will be quantified in chapter 7. In addition, the trade-off between FE and PGW will be examined in chapter 5 and 6.

CHAPTER 5 PHYSIOLOGICAL TRAITS ASSOCIATED WITH GRAIN YIELD, ABOVE-GROUND DM AND YIELD COMPONENTS IN CIMCOG PANEL

5.1 Introduction

This chapter describes results for physiological traits measured during the season with a focus on radiation interception and radiation-use efficiency and associations with grain yield and above-ground biomass in the field experiments in two seasons (2011-12 and 2012-13) for the 26 spring wheat genotypes in the CIMCOG panel.

In order to increase the grain sink size, the grain number per unit of area and/or the potential grain size must be increased. Grain number per unit area (GM2) can be analysed according to the following equation (Eqn. 1).

$$GM2 = RI \times RUE \times SPI \times FE \qquad \textbf{Equation 5.1}$$

Where RI = radiation interception by the crop (MJ m^{-2}) in the pre-anthesis period from emergence to anthesis, RUE = radiation-use efficiency (above-ground DM per unit radiation interception; g MJ^{-1}) in the pre-anthesis period, SPI = spike partitioning index (proportion of above-ground DM partitioned to spike at anthesis) and FE = fruiting efficiency (grains per g spike DM at anthesis).

There is evidence for a strong association amongst genotypes between spike dry weight per unit area (SDW) and GM2 at anthesis in wheat (Fischer, 2011). However, the other component determining genetic variation in GM2 is the fruiting efficiency.

In this chapter, for the 26 CIMCOG genotypes results on above-ground DM and spike dry matter partitioning at anthesis + 7 days as well as the fruiting efficiency will be presented as determinants of grains m^{-2} and grain yield. For a subset of 10 cultivars, selected as far as possible to express the full range of grains m^{-2} with a restricted range of anthesis date,

detailed growth analysis measurements at GS31 and GS41 were carried out. These measurements included green area index, above-ground DM, fractional photosynthetically active radiation interception and RUE calculated for relevant phases during the stem elongation phase. The relationship between genetic variation in pre-anthesis RUE and biomass is analysed.

In addition, for the subset of ten cultivars the tiller production and survival is assessed, as determinants of genetic variation in spikes m^{-2} and SDW. Flag-leaf stomatal conductance, canopy NDVI at key growth stages, and flag-leaf senescence are also described as determinants of grain source size. In addition, results for spike partitioning at harvest (awns, rachis, glumes paleas, and lemmas) are presented and discussed as determinants of FE.

The hypotheses tested in this chapter are:

- There is genetic variation in radiation interception by the canopy during stem elongation from GS31 to GS61+7d positively associated with above-ground dry matter (AGDM) accumulated during stem elongation and at anthesis amongst the CIMCOG genotypes.
- There is genetic variation in radiation-use efficiency during stem elongation and RUE is positively related with AGDM accumulated during stem elongation at anthesis amongst the CIMCOG genotypes.
- There is genetic variation in post-anthesis radiation-use efficiency positively related with grain yield amongst the CIMCOG genotypes.
- Genetic variation in each of spike partitioning index and fruiting efficiency is positively related with grains m^{-2} and HI amongst the 26 CIMCOG genotypes, and there is no trade-off between these two components.

5.2 Materials and Methods

5.2.1 Site and location

The field experiments were conducted in the 2011-12 and 2012-13 seasons at the CIMMYT Norman E. Borlaug Experimental Station in the Yaqui Valley, Ciudad Obregón northwest Mexico. Twenty-six bread wheat cultivars were evaluated in a raised beds planting system (see Chapter 3.1). A lattice design was used with two replicates in 2011, and three replicates in 2012 and 2013. In each replicate 26 genotypes of the CIMCOG panel were randomized on plots. Plots were fully irrigated and diseases and pests were controlled, (see Chapter 3.1. for full details of fungicide, pesticide and fertilizer inputs).

5.2.2 Crop measurements

Measurements of above-ground DM and DM partitioning at anthesis were carried out for the 26 CIMGOG genotypes in each season as described in Chapter 3. For the subset of 10 genotypes, above-ground DM and fractional photosynthetically active radiation (PAR) interception was measured at GS31, GS41, GS61+7 d and near to physiological maturity in 2012 and 2013. Photosynthetically active radiation was assumed to be 50% solar radiation (Monteith 1972). Radiation-use efficiency (RUE_{PAR}) for each plot was calculated as the linear relationship between above-ground dry matter and PAR interception over the relevant phase between development stages (see chapter 3 for full details of the methodology used). Plot values of RUE were subjected to ANOVA. At harvest, for the subset cultivars three spikes per plot were randomly sampled and their chaff dry matter components were analysed (glumes, lemmas paleas, rachis and awns).

5.3 Results

5.3.1 Development stages

5.3.1.1 Date of anthesis (GS65), physiological maturity (GS91) and duration between GS65-GS91 for 26 genotypes

The 26 genotypes reached anthesis from 81(Pfau/Seri) to 94 (Bcn/Rialto) days after emergence (DAE) in 2012 ($P < 0.001$), and from 73 (Pfau/Seri) to 100 (Bcn/Rialto) DAE in 2013 ($P < 0.001$). Averaging over years, the 26 genotypes differed in anthesis date ($P < 0.001$) from 77 (Pfau/Seri) to 97 (Bcn/Rialto) DAE. No year effect was observed, but there was a year x genotype interaction ($P < 0.001$) (Table 5.1).

Amongst the 26 genotypes physiological maturity was reached from 124 (Trap#1) to 136 (Cmh79a.9) DAE in 2012 and from 123 (Pfau/Seri) to 138 ($P < 0.001$) DAE (Bcn/Rialto) in 2013. Averaging over years, the 26 genotypes differed in PM date ($P < 0.001$) from 124 (Pfau/Seri) to 136 (Bcn/Rialto) DAE. No year effect was observed, but the genotype x year interaction was significant ($P < 0.001$) (Table 5.1).

The duration from anthesis to PM ranged from 38.7 (Bcn/Rialto) to 46.7 (Milan/k) DAE ($P < 0.001$) in 2012 and from 37.7 (Bcn/Rialto) to 49.7 (Pfau/Seri) DAE ($P < 0.001$) in 2013. Averaging over years, the 26 genotypes differed in GS65-GS91 duration ($P < 0.001$) from 38.5 (Bcn/Rialto) to 48.0 (Milan/k) days. No year effect was observed, but the year x genotype interaction was significant ($P < 0.01$) (Table 5.1).

5.3.1.2. Date of GS31, GS41 and GS65 for subset of 10 genotypes

The subset of 10 genotypes reached GS31 from 43-49 DAE in 2012 ($P < 0.001$). In 2013, GS31 ranged from 44 to 50 DAE ($P < 0.001$). Averaging over years, genotypes showed a range of 5.6 days from 20 (43.5 DAE) to 26 (49.3 DAE) January ($P < 0.001$). At GS41, the genotypes ranged from 66 to 71 DAE in 2012 ($P < 0.001$), from 59 - 72 DAE in 2013 ($P < 0.001$) and averaging over years from 63 - 72 DAE (9 to 18 February, respectively) ($P < 0.001$).

Anthesis date (AD; GS65) ranged amongst the genotypes from 82 to 89 DAE in 2012, from 80 to 95 DAE in 2013 ($P < 0.001$); averaging over years AD ranged from 28 February (82.5 DAE) to the 8 March (91.3 DAE) ($P < 0.001$).The duration of the stem-elongation phase from GS31 to GS65 ranged from 36.3 to 46.0 days in 2012 from 35.7 to 45.7 days ($P < 0.001$) in 2012 and from 36.3 to 44.7 days averaging over years ($P < 0.001$) (Table 5.1).

Table 5.1 Date of anthesis (GS65) and physiological maturity (GS89) (days after emergence, DAE) for 26 cultivars in 2012, 2013 and mean for 2012-3.

	ANTHESIS DAE			MATURITY DAE			GS65-GS91 DURATION		
Cultivar	2012	2013	2012-13	2012	2013	2012-13	2012	2013	2012-13
Babax/l	87.3	86.0	86.7	131	128	130	44.0	41.7	42.8
Bacanorat88	86.7	86.0	86.3	125	126	126	38.7	40.0	39.3
Bcn/rialto	94.0	100.0	97.0	133	138	136	39.3	37.7	38.5
Becard/k	88.3	93.3	90.8	131	135	133	42.7	41.7	42.2
Brbt1*2/k	84.7	81.3	83.0	128	125	127	43.3	44.0	43.7
Sahual/4	91.3	93.7	92.5	134	135	134	42.7	41.0	41.8
Sahual/w	85.3	86.7	86.0	126	129	128	40.7	42.3	41.5
Cmh79a.9	93.3	96.0	94.7	136	134	135	42.3	38.0	40.2
Cno79//p	86.7	83.7	85.2	133	129	131	46.0	45.7	45.8
Croc_1/	86.0	80.0	83.0	132	128	130	45.7	48.0	46.8
Kbird//i	91.3	95.3	93.3	134	136	135	43.0	40.7	41.8
Milan/k	86.0	80.0	83.0	133	129	131	46.7	49.3	48.0
Pavonf76	86.7	86.7	86.7	131	126	129	44.7	39.7	42.2
Pbw343*2	83.7	84.7	84.2	127	128	128	43.0	43.7	43.3
Pfau/seri	81.0	73.0	77.0	125	123	124	44.3	49.7	47.0
Serim82	88.0	87.0	87.5	129	130	129	40.7	42.7	41.7
7cerros	85.3	79.7	82.5	128	123	125	42.3	43.3	42.8
Sokoll//p	86.3	86.3	86.3	127	128	128	41.0	41.7	41.3
Tacupeto/s	84.3	84.7	84.5	128	129	128	43.3	44.0	43.7
Tacupeto/b	89.0	87.3	88.2	131	130	131	42.3	43.0	42.7
Tc870344/	84.7	84.3	84.5	128	126	127	43.0	42.0	42.5
Trap#1	83.3	86.0	84.7	124	126	125	41.0	40.0	40.5
Up2338*	88.0	91.0	89.5	131	130	131	43.0	39.0	41.0
Becard	82.3	83.7	83.0	124	127	125	41.3	43.0	42.2
Wbll1*2/	87.3	95.3	91.3	132	134	133	45.0	38.7	41.8
Yav_3/sco	86.7	90.3	88.5	132	130	131	45.0	39.7	42.3
Means	86.8	87.0	86.9	130	129	130	42.9	42.3	42.6
Max	94.0	100	97.0	136	138	136	46.7	49.7	48.0
Min	81.0	73.0	77.0	124	123	124	38.7	37.7	38.5
CV	1.03	1.46	1.28	1.92	0.90	1.48	5.75	4.29	5.00
Cult	***	***	***	***	***	***	*	***	***
LSD(0.05)	1.620	2.125	1.284	4.463	1.984	2.212	4.153	2.964	2.453
Year			ns			ns			ns
LSD(0.05)			0.356			0.6136			0.6802
Year*Cult			***			***			**

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability. The subset of 10 genotypes are in bod font.

Table 5.2 Dates of GS31, GS41 and GS65 (days after emergence, DAE) for 10 cultivars in 2012, 2013 and mean for 2012-3.

DAE	GS31			Date	GS41			Date	GS65			Date	Days GS31-GS65		
Cultivars	2012	2013	Mean	Mean	2012	2013	Mean	Mean	2012	2013	Mean	Mean	2012	2013	Mean
Bacanorat88	47.0	47.3	47.2	24-Jan	70.3	67.3	68.8	15-Feb	86.7	86.0	86.3	04-Mar	39.7	38.7	39.2
Brbt1*2/k	46.0	44.0	45.0	22-Jan	67.0	62.0	64.5	10-Feb	84.7	81.3	83.0	28-Feb	38.7	37.3	38.0
Pbw343*2	47.0	45.3	46.2	23-Jan	66.3	66.3	66.3	13-Feb	83.7	84.7	84.2	02-Mar	36.7	39.3	38.0
7cerros	43.0	44.0	43.5	20-Jan	67.0	58.7	62.8	09-Feb	85.3	79.7	82.5	28-Feb	42.3	35.7	39.0
Tacupeto/b	43.0	44.0	43.5	20-Jan	69.7	66.7	68.2	15-Feb	89	87.3	88.2	05-Mar	46.0	43.3	44.7
Tc870344/	47.0	44.0	45.5	22-Jan	66.3	67.0	66.7	13-Feb	84.7	84.3	84.5	02-Mar	37.7	40.3	39.0
Trap#1	47.0	48.3	47.7	24-Jan	68.3	69.3	68.8	15-Feb	83.3	86.0	84.7	02-Mar	36.3	37.7	37.0
Becard	45.3	48.0	46.7	23-Jan	68.3	66.3	67.3	14-Feb	82.3	83.7	83.0	28-Feb	37.0	35.7	36.3
Wbll1*2/	49.0	49.7	49.3	26-Jan	71.0	72.0	71.5	18-Feb	87.3	95.3	91.3	08-Mar	38.3	45.7	42.0
Yav_3/sco	46.0	48.0	47.0	24-Jan	71.0	70.7	70.8	17-Feb	86.7	90.3	88.5	06-Mar	40.7	42.3	41.5
Mean	46.0	46.3	46.2	23-Jan	68.5	66.6	67.6	14-Feb	85.4	85.9	85.6	03-Mar	39.3	39.6	39.5
P-value(Cult)	***	***	***		***	***	***		***	***	***		***	***	***
SED(18[a], 36[b])	0.298	1.21	0.625		0.72	0.81	0.542		0.96	0.92	0.665		1.005	1.855	1.055
Year			ns				**				ns				ns
SED(18[a], 36[b])			0.229				0.34				0.243				0.313
Year*Cult.			***				***				***				***
SED(18[a], 36[b])			0.869				0.803				0.925				1.449

Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

[a]=Degrees of freedom for individual years, [b]= Degrees of freedom for cross-year mean.

5.3.2 Fertile shoots per m^2 and green area index

For the 26 genotypes at GS65+7d, fertile shoots m^{-2} differed in 2012 from 269 (Wbll1*2/) to 437 (Bacanorat88) ($P < 0.001$) and in 2013 from 317 (Cno79//p) to 508 (Pavonf76) ($P < 0.001$; Table 5.3). Averaging over years, fertile shoots m^{-2} ranged from 299 (Cmh79a.9) to 453 (Pavonf76) ($P < 0.001$). The year effect ($P < 0.001$) and the year x genotype interaction ($P < 0.001$) were significant. Overall fertile shoots m^{-2} was 10.2% higher in 2013 than 2012 (Table 5.4)

For the 10 genotypes, fertile shoots m^{-2} at GS31 ranged from 496 (Brbt1*2/k) to 743 (Bacanorat88) ($P < 0.05$) in 2012. However, no differences were observed in 2013. Averaging over years, genotypes ranged from 519 (Brbt1*2/k) to 736 (Bacanorat88) shoots m^{-2} ($P < 0.05$). No effect of year or of year x genotype was observed (Table 5.3).

No differences in fertile shoots m^{-2} at GS41 amongst the 10 genotypes were observed in 2012. However, in 2013 fertile shoots m^{-2} ranged from 392 (Brbt1*2/k) to 652 (Wbll1) ($P < 0.05$). Averaging over years, genotypes did not differ amongst the 10 genotypes. No effect of year was observed; but there was an effect of year x genotype ($P < 0.05$) (Table 5.3). Averaging over years, the range in fertile shoots m^{-2} at GS65+7d for the subset of 10 genotypes was 306 (Wbll1*2/) to 422 (Bacanorat88). Tiller loss averaged across years from GS31 to GS41 was 10.2% and from GS41 to GS65+7d 32.2%.

There was a trend for differences in green area index at GS65+7d amongst the 26 genotypes in 2012 (5.91 – 9.50; $P = 0.06$). Differences were observed in 2013 from 4.36 (Brbt1*2/k) to 7.81 (Tacupeto/b) ($P < 0.05$; Table 5.4). Averaging over years, however, the 26 genotypes did not differ in GAI at GS65 +7d ($P = 0.18$). There was a year effect ($P < 0.001$), but the year x genotype interaction was not significant (Table 5.3; Figure 5.1).

The subset of 10 genotypes at GS31 differed in GAI in the range 2.98 (Tacupeto/b) to 5.36 (Tc87034) in 2012 ($P < 0.05$). However, in 2013 no significant differences were observed. Averaging over years, GAI at GS31 differed from 4.86 (Tacupeto/b) to 7.37 (Yav_3/sco/) ($P < 0.05$). The year effect was significant ($P < 0.001$) but the year x genotype interaction was not significant (Table 5.3).

At GS41 the 10 genotypes did not differ in GAI in 2012. However, in 2013 GAI ranged from 4.08 to 9.65 ($P < 0.01$). Averaging over years, the 10 genotypes ranged from 5.41 (Brbt1) to 9.19 (Yav_3/sco/) ($P < 0.01$). There was an effect of year ($P < 0.05$), but no year x genotype interaction At GS65+7d averaging over years, GAI ranged from 5.42 – 8.02 amongst the 10 genotypes (Table 5.3).

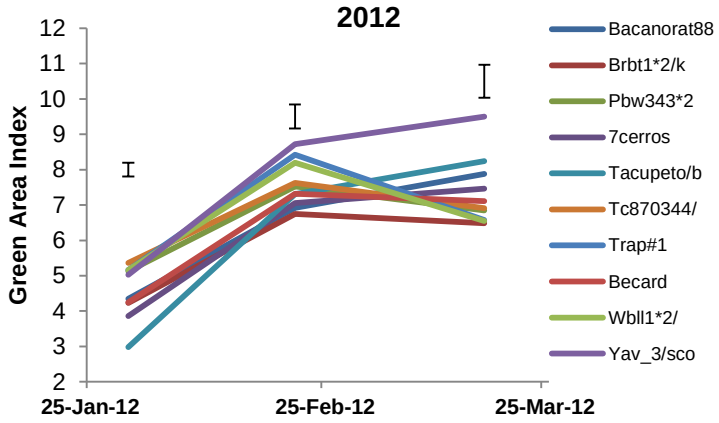
Table 5.3 Green area index (GAI) and fertile shoots m^{-2} at GS31, GS41 and GS65+7 days for 10 cultivars in 2012, 2013 and means of the two seasons.

	Green Area Index									Fertile shoots per m^2								
	GS31			GS41			GS65+7d			GS31			GS41			GS65+7d		
Cultivar	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
Bacanorat	4.35	6.26	5.30	6.92	6.23	6.57	7.87	5.49	6.68	743	729	736	652	635	644	437	408	422
Brbt1*2/k	4.23	6.88	5.56	6.74	4.08	5.41	6.48	4.36	5.42	496	542	519	540	392	466	352	320	336
Pbw343*2	5.13	7.75	6.44	7.52	8.69	8.11	6.85	5.71	6.28	616	650	633	562	455	509	413	407	410
7cerros	3.86	7.70	5.78	7.06	6.27	6.66	7.46	4.80	6.13	598	576	587	559	480	520	383	392	388
Tacupeto/	2.98	6.73	4.86	7.31	7.27	7.29	8.24	7.81	8.02	497	600	548	469	542	506	300	353	326
Tc870344	5.36	7.29	6.33	7.63	8.34	7.98	6.91	5.36	6.13	577	554	566	540	488	514	337	368	352
Trap#1	5.16	7.66	6.41	8.42	7.46	7.94	6.58	5.62	6.10	723	622	673	642	510	576	380	455	417
Becard	4.25	7.10	5.67	7.32	6.76	7.04	7.11	5.74	6.42	623	636	629	644	615	630	385	413	399
Wbll1*2/	5.17	8.28	6.72	8.20	7.27	7.73	6.54	6.28	6.41	563	649	606	509	652	581	269	344	306
Yav_3/sc	5.02	9.71	7.37	8.72	9.65	9.19	9.50	5.46	7.48	532	684	608	511	560	535	354	347	350
Mean	4.55	7.54	6.04	7.58	7.20	7.39	7.35	5.66	6.51	597	624	610	563	533	548	361	381	371
Max	5.36	9.71	7.37	8.72	9.65	9.19	9.50	7.81	8.02	743	729	736	652	652	644	437	455	422
Min	2.98	6.26	4.86	6.74	4.08	5.41	6.48	4.36	5.42	496	542	519	469	392	466	269	320	306
P-value (Cult)	*	ns	ns	ns	**	**	ns	ns	ns	*	ns	*	ns	*	ns	*	*	ns
SED (18^a, 35^b)	0.385	1.259	0.658	0.679	0.736	0.501	0.928	0.677	0.569	42.7	44.0	30.7	56.1	46.3	36.5	20.6	21.7	15.0
Year			***			*			**			ns			ns			ns
SED (18^a, 35^b)			0.43			0.278			0.389			23.29			9.140			8.170
Year*Cult			ns			ns			ns			ns			*			ns
SED (18^a, 35^b)			0.98			0.727			0.857			47.27			49.8			43.05

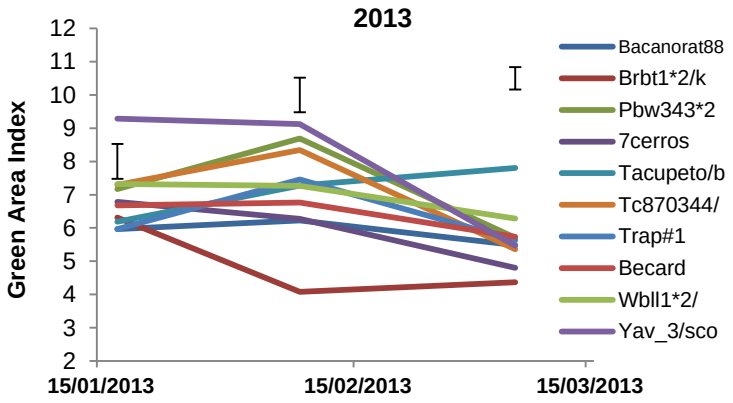
† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

Note a=Degrees of freedom for individual years, b= Degrees freedom for across years.

a)



b)



c)

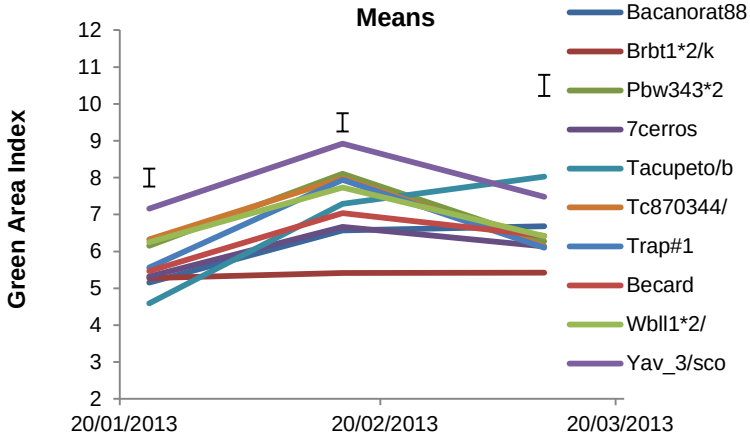
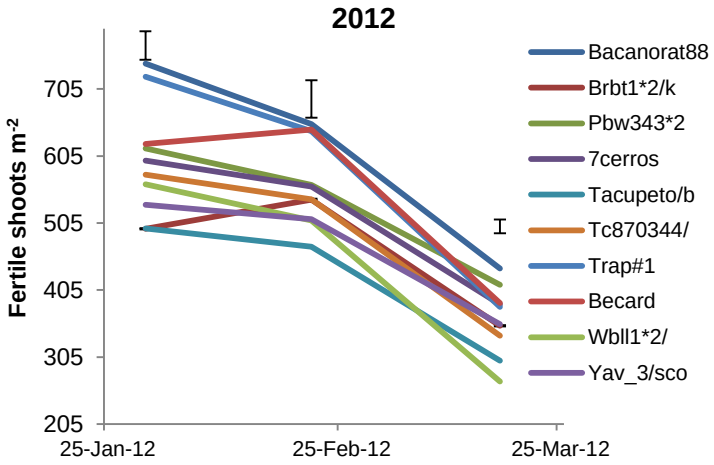
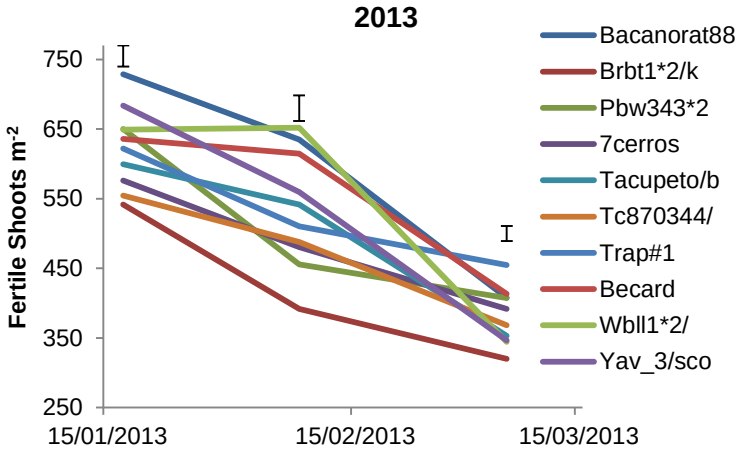


Figure 5.1 Green area index at GS31, GS41, GS65+7 days in a) 2012, b) 2013 and c) Mean of 2012-3 for ten genotypes. Error bars = SED genotype (df = 8)

a)



b)



c)

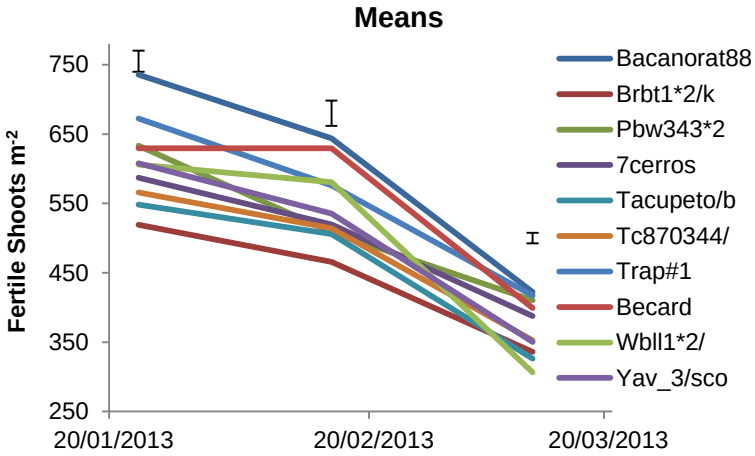


Figure 5.2 Shoots m^{-2} at GS31, GS41, GS65+7 days in a) 2012, b) 2013 and c) Mean of 2012-3 for ten genotypes. Error bars = SED genotype (df = 8)

Table 5.4 Green area index (GAI) and fertile shoots m^{-2} at GS65+7 days for 26 cultivars in 2012, 2013 and mean 2012-3.

	Green Area Index			Fertile shoots m^{-2}		
Cultivar	2012	2013	Mean	2012	2013	Mean
Babax/l	8.63	6.09	7.36	350	397	374
Bacanorat88	7.87	5.49	6.68	437	408	422
Bcn/rialto	9.50	6.75	8.12	387	505	446
Becard/k	8.83	6.43	7.63	358	376	367
Brbt1*2/k	6.48	4.36	5.42	352	320	336
Sahual/4	7.95	6.17	7.06	302	326	314
Sahual/w	6.59	6.09	6.34	284	345	314
Cmh79a.9	6.78	6.28	6.53	281	317	299
Cno79//p	7.38	5.74	6.56	298	337	318
Croc_1/	7.60	4.64	6.12	379	406	392
Kbird//i	9.50	5.68	7.59	379	469	424
Milan/k	7.98	5.00	6.49	296	335	316
Pavonf76	5.91	6.39	6.15	398	508	453
Pbw343*2	6.85	5.71	6.28	413	407	410
Pfau/seri	8.09	5.54	6.81	408	374	395
Serim82	8.04	5.92	6.98	334	358	346
7cerros	7.46	4.80	6.13	383	392	388
Sokoll//p	8.34	5.33	6.84	370	377	373
Tacupeto/s	7.16	5.46	6.31	332	381	357
Tacupeto/b	8.24	7.81	7.98	300	353	332
Tc870344/	6.91	5.36	6.13	337	368	352
Trap#1	6.58	5.62	6.10	380	455	417
Up2338*	9.49	5.55	7.52	351	439	395
Becard	7.11	5.74	6.42	385	413	399
Wbll1*2/	6.54	6.28	6.39	269	344	314
Yav_3/sco	9.50	5.46	7.48	354	347	350
Mean	7.74	5.76	6.75	351	387	369
Cult	ns P=0.1	*	ns	***	***	***
LSD(0.05)	2.366	1.411	1.46	60.4	49.6	38.9
Year			***			***
LSD(0.05)			0.41			10.78
Year*Cult			ns			**

† Significant at $P = 0.10$; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

5.3.3 Above-ground dry matter and dry matter partitioning

Above-ground dry matter at GS65+7d did not differ amongst the 26 genotypes in 2012. However, in 2013 AGDM ranged from 879 (Pfau/Seri) to 1582 (Bcn/Rialto) g m^{-2} ($P < 0.001$). Averaging over seasons, the 26 genotypes differed in AGDM at GS65+7d from 926 (Pfau/Seri) to 1388 (Bcn/Rialto) g m^{-2} ($P < 0.05$). A year effect was not observed for AGDM at

GS85+7d. However, the year x genotype interaction was significant (P < 0.05) (Table 5.5).

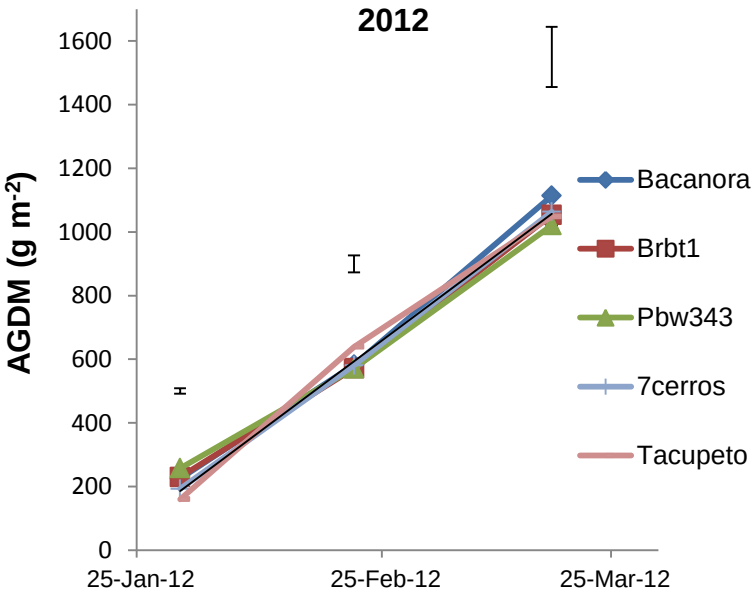
Spike DM per unit area did not differ amongst genotypes in 2012. However, in 2013 spike DM m^{-2} ranged from 218 (Milan/k) – 370 (Bcn/Rialto) gm^{-2} (P < 0.01). Averaging over years, spike DM m^{-2} ranged from 221 (Sahual/w) – 365 (Bcn/Rialto) 365 gm^{-2} (P < 0.05), an effect of the year was observed (P < 0.05) but no year x genotype interaction.

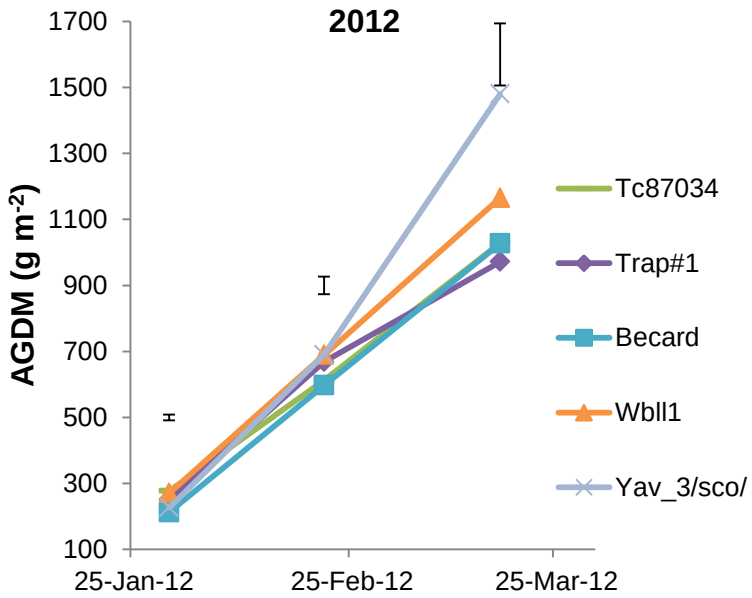
In 2012, the subset of 10 genotypes differed in AGDM at GS31 in the range 160 (Tacupeto/b) to 276 (Tc87034) g m^{-2} (P < 0.001). In 2013, the genotypes did not differ in AGDM at GS31. Averaging over seasons, the genotypes ranged from 181 (Tacupeto/b) to 261 (Wbll1) g m^{-2} (P < 0.001). No year effect was observed. However, the genotype x year interaction was significant (P < 0.05).

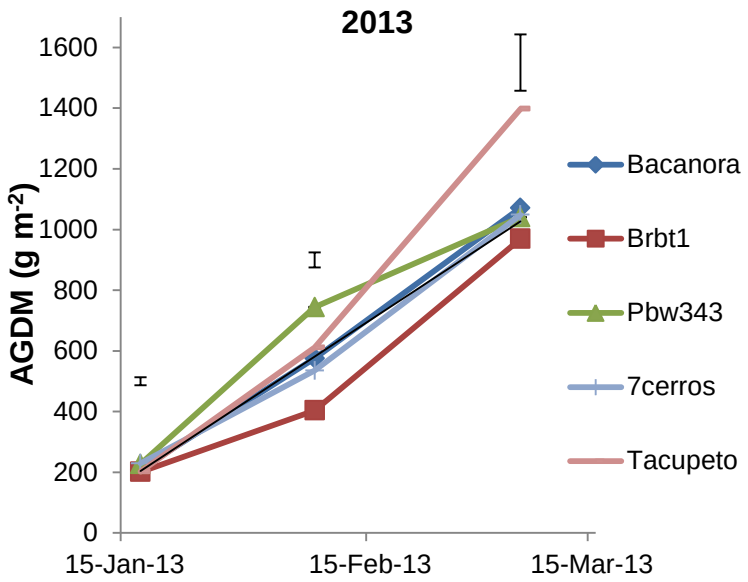
At GS41 AGDM did not differ amongst the 10 genotypes in 2012, but in 2013 ranged from 404 (Brbt1) to 765 (Yav_3/sco/) g m^{-2} (P < 0.01). Averaging over years, AGDM differed amongst genotypes at GS41 from 487.7 (Brbt1) to 727.5 (Yav_3/sco/) g m^{-2} (P < 0.01). No year effect was observed but there was a year x genotype interaction (P < 0.01) (Figure 5.2).

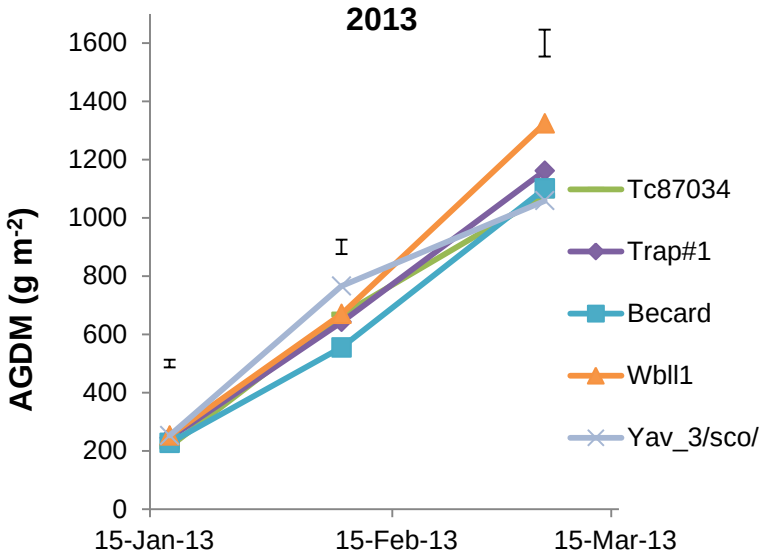
At GS65 +7d averaging over years for the 10 cultivars, AGDM at GS61+7d ranged from (Brbt1*2/k)1012 – (Yav_3/sc) 1269 (P < 0.05). Averaging over years, spike DM at GS65+7d ranged from 238.5 (Brbt1*2/k) to 342.0 (Yav_3/sc) g m^{-2} (P < 0.05) (Table 5.5).

a)


2012
AGDM (g m-2)
1600
1400
1200
1000
800
600
400
200
0
25-Jan-12
25-Feb-12
25-Mar-12
Bacanora
Brbt1
Pbw343
7cerros
Tacupeto


2012
AGDM (g m-2)
1700
1500
1300
1100
900
700
500
300
100
25-Jan-12
25-Feb-12
25-Mar-12
Tc87034
Trap#1
Becard
Wbll1
Yav_3/sco/


2013
AGDM (g m-2)
1600
1400
1200
1000
800
600
400
200
0
15-Jan-13
15-Feb-13
15-Mar-13
Bacanora
Brbt1
Pbw343
7cerros
Tacupeto


2013
AGDM (g m-2)
1600
1400
1200
1000
800
600
400
200
0
15-Jan-13
15-Feb-13
15-Mar-13
Tc87034
Trap#1
Becard
Wbll1
Yav_3/sco/

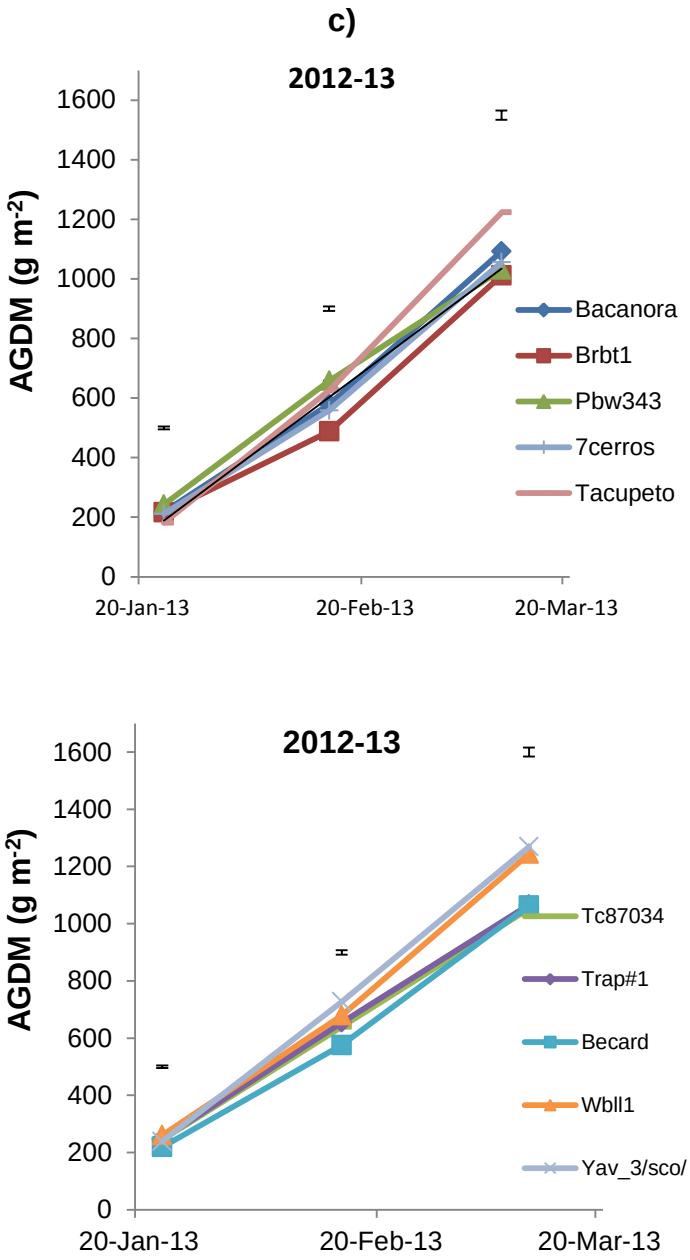


Figure 5.3 Above ground dry matter at GS31, GS41, GS65+7 days in a) 2012, b) 2013 and c) mean 2012-3 for ten genotypes. Error bars = SED genotype (df =8)

Table 5.5 Above-ground dry matter and spike DM per m^2 at GS65+7d for 26 cultivars) for 2012, 2013 and mean 2012-13

	2012		2013		2012-2013	
Cultivar	AGDM	Spike	AGDM	Spike	AGDM	Spike
Babax/l	1188	311	1107	258	1147	285
Bacanorat88	1114	338	1071	291	1092	314
Bcn/rialto	1195	360	1582	370	1388	365
Becard/k	1073	326	1243	305	1158	316
Brbt1*2/k	1054	244	970	233	1012	238
Sahual/4	1124	334	1285	314	1204	324
Sahual/w	918	187	1160	255	1039	221
Cmh79a.9	1137	381	1256	325	1197	353
Cno79//p	1138	317	1120	264	1129	290
Croc_1/	1123	322	966	222	1045	272
Kbird//i	1118	326	1162	277	1140	302
Milan/k	1130	244	902	218	1016	231
Pavonf76	1019	293	1068	268	1044	280
Pbw343*2	1021	301	1040	275	1031	288
Pfau/seri	973	312	879	244	926	278
Serim82	1011	214	1070	293	1040	254
Siete cerros	1063	283	1048	277	1056	280
Sokoll//p	1124	331	923	251	1024	291
Tacupeto/s	1025	277	1165	316	1095	297
Tacupeto/b	1049	238	1397	319	1223	278
Tc870344/	1029	283	1077	260	1053	271
Trap#1	972	215	1161	304	1066	259
Up2338*	1195	351	1135	263	1165	307
Becard	1027	229	1100	264	1063	246
Wbll1*2/	1165	313	1324	303	1244	308
Yav_3/sc	1480	410	1058	274	1269	342
Mean	1095	298	1126	279	1110	288
Prob.-	ns	ns	***	**	*	*
LSD(0.05)	282.5	94.1	199.6	61.6	183.4	52.5
Prob.					ns	*
LSD (0.05)					50.9	14.5
Prob Yr*Cult.					*	ns
LSD (0.05) Yr*Cult.					242.6	119.04

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

5.3.4 PAR interception and radiation-use efficiency pre-anthesis

5.3.4.1 PAR interception

Photosynthetically active radiation intercepted from GS31-GS41 differed amongst the 10 genotypes in the range 129.7 (Pbw343) to 190.8 (Tacupeto/b) MJ m^{-2} in 2012 (P = 0.001) but in 2013 did not differ significantly amongst the genotypes. Averaging across seasons, the genotypes ranged from 115.5 (Pbw343) to 167.9 (Yav_3/sco/) MJ m^{-2} (P < 0.001); the year x genotype interaction was not significant, although there was an effect for the year (P < 0.001). In 2012 from GS41 to GS65+7d PAR interception ranged amongst genotypes from 195.1 (Wbll1) to 238.6 (Tacupeto/b) MJ m^{-2} (P < 0.01) and in 2013 from 136.2 (Siete cerros) to 217.7 (Wbll1) MJ m^{-2} (P < 0.05). Averaging across years, differences amongst the 10 genotypes were not significant (P = 0.14). The effect of year (P < 0.001) and year x genotype (P < 0.01) were significant. Radiation intercepted from GS31 to GS65+7d in 2012 differed amongst the genotypes from 340.8 (Pbw343) to 429.4 (Tacupeto/b) MJ m^{-2} (P < 0.001) and in 2013 from 250.7 (Brbt1) to 347.4 (Wbll1) MJ m^{-2} (P < 0.001). Averaging over seasons, the genotypes ranged from 298.0 (Pbw343) to 369.6 (Yav_3/sco) MJ m^{-2} (P < 0.001). The year effect and the year x genotype interaction were significant (P < 0.001) (Table 5.6). Overall genotypes intercepted more radiation in the 2012 than in 2013, similarly GAI was larger in 2012 than in 2013. However, Wbll and Trap#1 responded similarly in both years but Siete cerros performed much better in 2012 intercepting radiation from GS31-GS65+7d.

No differences were observed in the PAR extinction coefficient at GS65+7d amongst the 10 genotypes in 2012 and in 2013 or averaging over years. This could be because of an overestimation GAI due to selecting a subsample of relatively larger shoots from the green area assessment form the total plant sample. The year effect and the genotypes x year interaction for K_{PAR} were also not statistically significant (Table 5.7).

Table 5.6 Photosynthetically active radiation (PAR) intercepted by the canopy from GS31-GS4, from GS41-GS65+7days and from GS31-GS65+7 days in 2012, 2013 and mean 2012-3 for 10 CIMCOG genotypes.

Cultivar	PAR MJ m^{-2} intercepted by the canopy								
	GS31 to GS41			GS41 to GS65+7d			GS31 to GS65+7d		
	2012	2013	2012-3	2012	2013	2012-3	2012	2013	2012-3
Bacanora	165.5	104.3	134.9	211	159.9	185.4	376.6	264.2	320.4
Brbt1	149.1	90.4	119.8	217.6	160.2	188.9	366.7	250.7	308.7
Pbw343	129.7	101.3	115.5	211.1	154	182.6	340.8	255.3	298
7cerros	173.1	114.8	143.9	236.8	136.2	186.5	409.9	251	330.4
Tacupeto	190.8	116.4	153.6	238.6	184.8	211.7	429.4	301.2	365.3
Tc87034	135	119.3	127.2	232.6	147.5	190.1	367.6	266.9	317.2
Trap#1	153.3	124.5	138.9	209	170	189.5	362.3	294.6	328.4
Becard	160.9	95.7	128.3	203.1	157.4	180.2	364	253.1	308.5
Wbll1	184.2	129.7	157	195.1	217.7	206.4	379.3	347.4	363.3
Yav_3/sco/	189.9	145.9	167.9	221.1	182.2	201.6	411	328.1	369.6
Mean	163.2	114.2	138.7	217.6	167	192.3	380.7	281.2	331
Prob Cult	***	ns	***	**	*	P=0.14	***	***	***
SED (df = 18)	9.83	19.40	10.87	10.46	21.08	11.77	16.12	18.8	12.38
Prob, Year			***			***			***
SED(df =18)			6.13			5.51			7.1
Prob. Year*Cult			ns			**			***
SED(18[a], 35[b])			15.83			16.72			18.07

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

Note [a]=Degrees of freedom for individual years, [b]= Degrees freedom for across years.

Table 5.7 PAR extinction coefficient (K_{PAR}) at GS65+7 days in 2012, 2013 and mean 2012-3 for 10 CIMCOG genotypes.

Cultivar	2012	2013	2012-3
Bacanorat88	0.271	0.305	0.288
Brbt1*2/k	0.303	0.387	0.345
Pbw343*2	0.228	0.320	0.274
Siete cerros	0.291	0.371	0.331
Tacupeto/b	0.294	0.347	0.321
Tc870344/	0.336	0.448	0.392
Trap#1	0.353	0.428	0.390
Becard	0.348	0.361	0.354
Wbll1*2/	0.313	0.338	0.325
Yav_3/sco	0.324	0.399	0.361
Mean	0.306	0.370	0.338
Max	0.353	0.448	0.392
Min	0.228	0.305	0.274
Genotype	ns	ns	ns
SED(18^a, 35^b)	0.0675	0.0553	0.0434
Year			ns
SED(18^a, 35^b)			0.0256
Year*Genotype			ns
SED(18^a, 35^b)			0.0636

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

Note ^{a}=Degrees of freedom for individual years, ^{b}= Degrees freedom for across years.

5.3.4.2 Radiation-use efficiency

Radiation-use efficiency was calculated for three phases during the pre-anthesis period (GS31-GS41, GS41-GS65+7d and GS31-GS65+7d) for the 10 genotypes. From GS31 to GS41 in 2012, no significant differences were found amongst the genotypes in RUE_{PAR}, but in 2013 genotypes ranged from 2.24 (Brbt1) to 5.27 g MJ^{-1} (Pbw343) (*P* = 0.053). Averaging across years, the genotypes differed in RUE_{PAR} from 2.27 (Siete cerros) to 3.84 (Pbw343) g MJ^{-1} (*P* = 0.053). The year effect (*P* < 0.01) and the year x genotype interaction were significant (*P* < 0.05) (Table 5.6). Radiation-use efficiency from GS41 to GS65+7d did not differ amongst the

genotypes in 2012, however, in 2013 ranged from 1.61 (Yav_3/sco) to 4.25 (Tacupeto/b) ($P < 0.05$) and averingin over years ranged from 1.91 (Yav_3/sco)to 2.98 (Tacupeto/b) ($P < 0.05$)). There was a year effect ($P < 0.001$) and the year x genotype interaction ($P < 0.01$) was significant.

During the stem-elongation period from GS31 to GS65+7d in 2012, RUE_{PAR} did not differ amongst the genotypes, but in 2013 cultivars ranged from 2.63 (Yav_3/sco/) to 3.94 g MJ^{-1} (Tacupeto/b) ($P = 0.06$). Averaging over years, the differences between genotypes in RUE ranged from 2.41 (Yav_3/sco) to 3.04 (Tacupeto/b) ($P = 0.07$); the year effect was significant ($P < 0.01$), but the year x genotype interaction was not significant (Table 5.8).

Table 5.8 Pre-anthesis radiation-use efficiency (RUE_{PAR}) from GS31 -GS41, GS41-GS65+7 days and GS31- GS65+7days for 10 genotypes in 2012 and 2013 and for the mean across seasons, and Pearson's correlation coefficiency amongst genotypes with AGDM and spike DM per m^2 at GS65+7d and AGDM accumulated from GS31 to GS65+7 days.

	PAR radiation-use efficiency (g MJ^{-1})								
Cultivar	GS 31-41			GS41-65+7d			GS31-65+7d		
	2012	2013	Means	2012	2013	Means	2012	2013	Means
Bacanorat88	2.46	3.68	3.07	2.29	3.10	2.70	2.33	3.33	2.83
Brbt1*2/k	2.30	2.24	2.27	2.22	3.55	2.88	2.26	2.97	2.62
Pbw343*2	2.41	5.27	3.84	2.14	1.91	2.03	2.27	3.44	2.85
7cerros	2.41	3.13	2.77	2.04	2.97	3.42	2.14	3.10	2.62
Tacupeto/b	2.51	3.51	3.01	1.71	4.25	2.98	2.14	3.94	3.04
Tc870344/	1.92	3.79	2.86	1.79	2.76	2.27	1.89	3.32	2.60
Trap#1	2.73	3.19	2.96	1.46	3.07	2.27	2.11	3.13	2.62
Becard/k	2.40	3.46	2.93	2.12	3.46	2.79	2.26	3.45	2.86
Wbll1*2/	2.27	3.24	2.76	2.43	3.04	2.74	2.34	3.15	2.74
Yav_3/sco	2.44	3.55	3.00	2.22	1.61	1.92	2.19	2.63	2.41
Mean	2.38	3.51	2.95	2.04	3.15	2.66	2.19	3.25	2.71
P-value(Cult)	ns	†	†	ns	*	*	ns	†	† P=0.07
SED(18[a], 36[b])	0.3115	0.686	0.3769	0.356	0.600	0.349	0.4215	0.3182	0.189
Year			**			**			**
SED(18[a], 36[b])			0.1934			0.199			0.168
Year*Cult			*			**			ns
SED(18[a], 36[b])			0.5414			0.508			0.305
Correl SDW GS65+7d	0.29	-0.15	0.42	0.48	0.54	0.56†	0.49	0.48	0.56†
Correl AGDM GS65+7d	0.21	-0.01	-0.04	0.78*	0.48	0.39	0.49	0.55†	0.40
Correl. AGDM 31-65+7d	0.05	-0.03	-0.08	0.59	0.57	0.55	0.48	0.64*	0.53

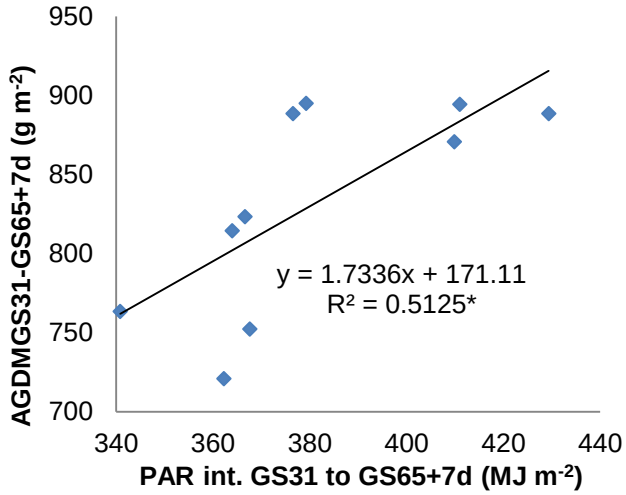
† Significant at $P = 0.10$; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

[a]=Degrees of freedom for individual years, [b]= Degrees of freedom for across-year mean.

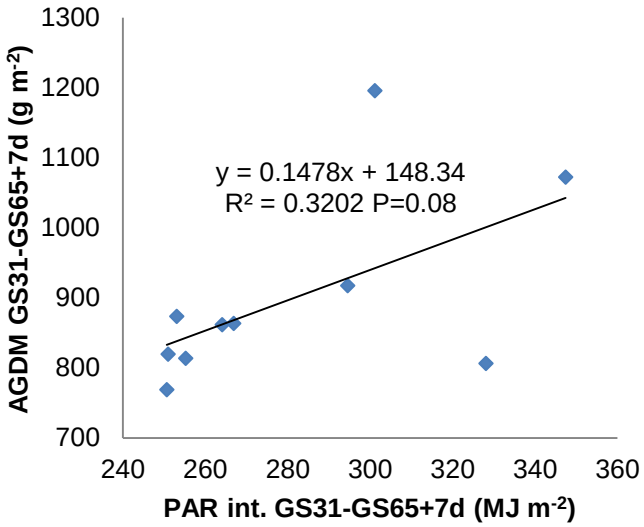
5.3.5 Relationships between above-ground dry matter, radiation interception and RUE.

Accumulated AGDM from GS31 to GS65+7d showed a positive association amongst the 10 genotypes with accumulated PAR intercepted over the same phase (Fig. 5.4) in 2012 (R^2 = 0.37, *P* = 0.05), 2013 (R^2 = 0.32, *P* = 0.08) and for the means of 2012 and 2013 (R^2 = 0.56, *P* < 0.05).

a)



b)



c)

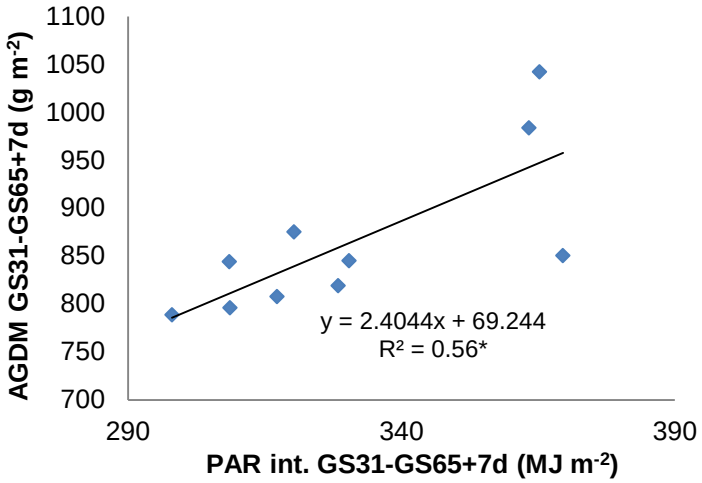
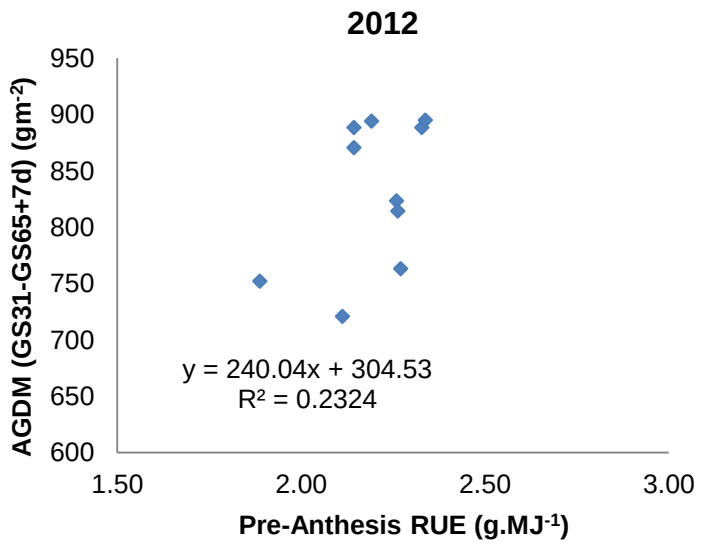


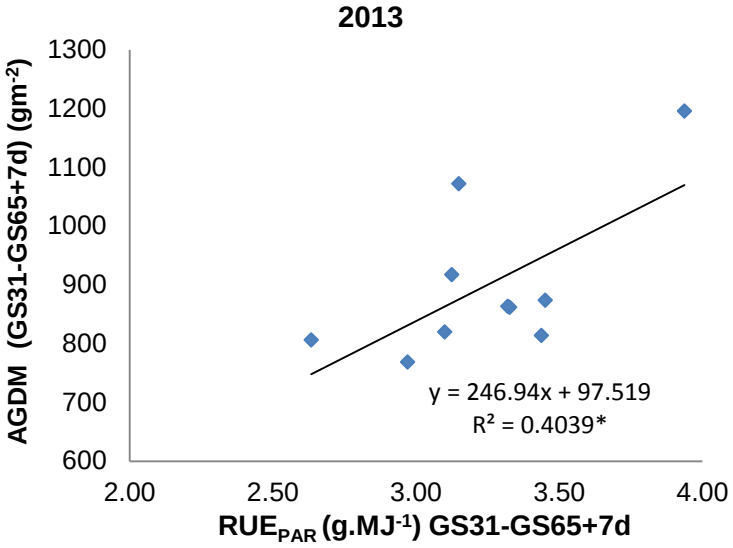
Figure 5.4 Linear regression of above-ground dry matter (AGDM) accumulated GS31-GS65+7d (gm^{-2}) on photosynthetically active radiation intercepted (PAR int.) GS31-GS65+7d (MJ m^{-2}) in a) 2012, b) 2013 and c) mean 2012-2013.

Accumulated AGDM from GS31 to a GS65+7d did not show an association with RUE_{PAR} from GS31 to GS65+7d amongst genotypes in 2012, but in2013 a linear and positive association was observed (R^2 = 0.40, P < 0.01) and for the mean across years (R^2 = 0.31, P = 0.09) (Figure 5.5). Overall the PAR interception (R^2 = 0.56; Figure 5.4) explained relatively more of the genetic variation in accumulated AGDM during stem elongation than RUE_{PAR} (R^2 = 0.31; Figure 5.5).

a)



b)



c)

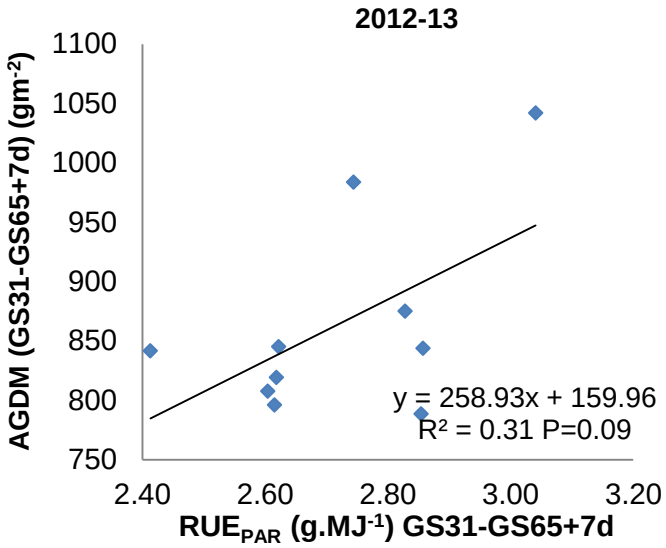


Figure 5.5 Linear regression of the above-ground dry matter accumulated from GS31-GS65+7 days (gm^{-2}) on PAR radiation-use efficiency (RUE_{PAR})from GS31 to GS65+7 days ($g\ MJ^{-1}$) for a) 2012, b) 2013 and c) Averaged over years for ten genotypes.

Averaging across the two years, RUE from GS31 to GS65+7d did not show an association with grain yield amongst the subset of ten genotypes. Similarly, RUE from GS31 to GS65+7d did not show an association with AGDM at harvest (R^2 = 0.25, *P* = 0.13) (Fig. 5.6). However, avoinding Yav_3/sco/ that has the higher amount of DM at

harvest; the association was significant for RUE_{PAR} vs grain yield and for RUE_{PAR} vs Biomass at PM.

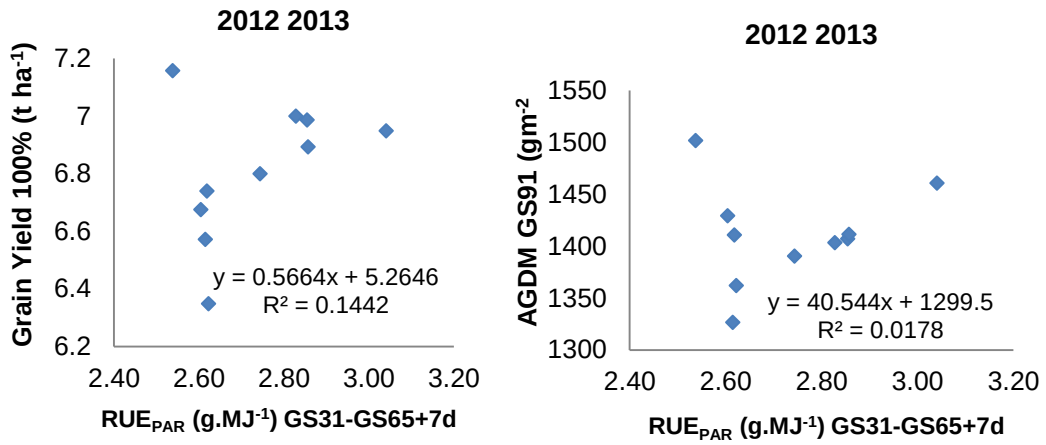


Figure 5.6 Linear regression of radiation-use efficiency from GS31 to GS65+7 days versus a) grain Yield (100% DM b) above ground dry matter at harvest for ten genotypes. Values are means of 2012 and 2013.

5.3.6 Crop growth rate

In 2012, for the subset of 10 genotypes, crop growth rate during GS31-GS41 (18.9 g m^{-2} d^{-1}) was overall lower than during GS41-GS65+7d (25.3 g m^{-2} d^{-1}) (P = 0.26;P = 0.32) respectively. In 2012 CGR from GS31-GS41 did not differ amongst the genotypes, but in 2013 CGR ranged from 11.3 (Brbt1) to 25.0 (Pbw343) g m^{-2} d^{-1} (P < 0.01). Averaging over seasons, genotypes differed from 14.2 (Brbt1) to 21.4 (Yav_3/sco/) g m^{-2} d^{-1}(P < 0.01). No year effect was observed. However, the year x genotype interaction was significant (P < 0.01).

Crop growth rate from GS41-GS65+7d did not differ amongst the 10 genotypes in 2012, but in 2013 ranged from 15.0 (Yav_3/sco/) to 37.7 (Tacupeto/b) g m^{-2} d^{-1} (P < 0.01). Averaging over seasons, a trend was observed amongst the genotypes (P = 0.07) with a range from 20.4 (Pbw343*2) to 29.8 (Becard/k) g m^{-2} d^{-1}. The year effect was not significant, but there was a year x genotype interaction (P < 0.01).

Crop growth rate from GS31 to GS65+7d did not differ amongst genotypes in 2012, but in 2013 ranged from 19.0 (Yav_3/sco/) to 27.6 (Tacupeto/b) g m^{-2} d^{-1} (*P* = 0.06). Averaging over years, however, no significant differences were observed amongst the genotypes. The year effect and the year x genotype effects were not significant (Table 5.8). Crop growth rate from GS31-41 was associated amongst genotypes with RUE_{PAR} (GS31-41) in 2013 (*P* < 0.001) and for the 2012-3 mean (*P* < 0.05) (Figure 5.6b). In addition, averaging over years CGR was positively associated at GS41-65+7d and from GS31-GS65+7d with RUE_{PAR} from GS41-65+7d and from GS31-GS65+7d respectively (Table 5.8)

Table 5.7 Crop Growth rate for GS31-41, GS41-65+7d and GS31 - GS65+7days for ten CIMCOG genotypes in 2012, 2013 and mean 2012-3.

Cultivar	CGR 31-41 g m^{-2} d^{-1}			CGR41-65 g m^{-2} d^{-1}			CGR31-65 g m^{-2} d^{-1}		
	2012	2013	Means	2012	2013	Means	2012	2013	Means
Bacanorat88	18.7	18.2	18.5	26.8	26.3	26.5	22.4	22.3	22.4
Brbt1*2/k	17.1	11.3	14.2	25.9	29.3	27.6	21.3	20.6	21.0
Pbw343*2	17.0	25.0	21.0	24.6	16.2	20.4	20.8	20.6	20.7
7cerros	17.4	21.0	19.2	24.2	24.5	24.4	20.6	23.0	21.8
Tacupeto/b	19.1	18.0	18.6	19.7	37.7	28.7	19.4	27.6	23.5
Tc870344/	18.9	19.7	19.3	21.0	23.5	22.3	20.0	21.4	20.7
Trap#1	21.6	18.9	20.3	17.9	31.3	24.6	19.9	24.4	22.1
Becard/k	17.7	17.9	17.8	28.1	31.5	29.8	22.0	24.4	23.2
Wbll1*2/	19.6	18.7	19.2	28.0	28.2	28.1	23.3	23.7	23.5
Yav_3/sco	20.1	22.7	21.4	27.3	15.0	21.2	22.1	19.0	20.6
Mean	18.7	19.2	18.9	24.2	26.3	25.3	21.2	22.7	21.9
Cult	ns	**	**	ns	**	P=0.07	ns	P=0.06	ns
SED(18[a], 36[b])	1.767	2.367	1.477	4.54	5.10	3.412	2.30	2.296	1.627
Year			ns			ns			ns
SED(18[a], 36[b])			0.646			1.531			0.797
Year*Cult			**			**			ns
SED(18[a], 36[b])			2.084			4.836			2.32
Correl.									
RUE_{PAR} 31-41	0.35	0.81**	0.71*	-0.20	-0.51	-0.51	-0.11	-0.15	-0.13
RUE_{PAR} 41-65	-0.44	-0.72*	-0.57†	0.94***	0.96***	0.67*	0.88**	0.79*	0.53
RUE_{PAR} 31-65	-0.24	-0.01	-0.07	0.69*	0.57†	0.49	0.72*	0.74*	0.63*

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability. Note a=Degrees of freedom for individual years, b= Degrees of freedom for across years

5.3.7 Flag-leaf stomatal conductance

Flag-leaf stomatal conductance was measured in 2013 in the 10 genotypes. At GS31 conductance ranged from 300.1 (Yav_3/sco/) to 449.8 (Brbt1) mmol m^{-2} s^{-1} (P = 0.08), at GS41 from 512.9 (Siete cerros) to 687.6 mmol m^{-2} s^{-1} (Tacupeto/b) (P < 0.001) and at GS65 from 368.9 (Siete cerros) to 665.1 (Wbll1) mmol m^{-2} s^{-1} (P < 0.001). Stomatal conductance was overall slightly higher at initiation of booting than at anthesis. There was a positive linear association between each of stomatal conductance at GS41 and GS65 and grain yield among the 10 genotypes (P < 0.05) (Table 5.9). In addition, there was a positive association between stomatal conductance at GS41 and GS65 and AGDM at GS65+7d (P < 0.05) (Table 5.9).

Table 5.8 Flag-leaf stomatal conductance (mmol m^{-2} s^{-1}) at around GS31 (29 January), GS41 (27 February) and GS65 (11 March); and flag-leaf specific dry weight (FLSW), flag-leaf N content (FN) and flag-leaf specific leaf N content (FLSN) at GS65+7d for 10 CIMCOG genotypes in 2013, and Pearson's correlation coefficient amongst genotypes with RUE_{PAR}.

	Stomatal cond. (mmol m^{-2} s^{-1})			FLSW	FN	FLSN
Genotype	**GS31**	**GS41**	**GS65**	**g m^{-2}**	**(mg N flag $leaf^{-1}$)**	**g m^{-2}**
Bacanorat88	370	536	523	52.4	5.49	2.87
Brbt1*2/k	450	616	547	74.5	10.68	2.93
Pbw343*2	371	633	579	65.0	7.10	3.58
7cerros	346	513	369	73.1	7.82	3.44
Tacupeto/b	362	688	661	82.3	10.36	3.59
Tc870344/	363	567	572	78.7	9.50	3.38
Trap#1	363	590	542	81.4	7.83	2.91
Becard/k	384	622	593	70.4	8.49	3.18
Wbll1*2/	352	623	665	79.8	10.80	3.11
Yav_3/sco	300	578	545	77.1	9.54	3.54
Mean	366	597	559	73.5	8.76	3.25
Max	450	688	665	82.3	10.80	3.59
Min	300	513	369	52.4	5.49	2.87
P-value(Cult)	P=0.08	***	***	*	***	*
SED(18)	44.49	18.42	75.5	7.38	0.666	0.226
Corr RUE_{31-41}	-0.35	0.13	0.17	-0.36	-0.47	0.53
Corr $RUE_{41-65+7d}$	0.31	-0.06	-0.30	0.14	0.10	-0.12
Corr $RUE_{31-65+7d}$	0.21	0.54 †	0.43	-0.05	-0.08	0.22
Corr CGR_{31-41}	-0.76**	-0.16	-0.13	-0.11	-0.40	0.65*
Corr $CGR_{41-65+7d}$	0.43	0.42	0.33	0.30	0.27	-0.37
Corr $CGR_{31-65+7d}$	0.05	0.44	0.33	0.30	0.12	-0.03
Corr Gs41	0.25	1	0.83***	0.39	0.53 †	0.24

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability.

At GS65+7d flag-leaf specific dry weight ranged from 52.4 (Bacanorat88) to 82.3 (Tacupeto/b) g m^{-2} (*P* < 0.05) amongst the 10 genotypes and N per flag-leaf from 5.49 (Bacanorat88) to 10.8 mg (Wbll1*2/) (*P* < 0.001). Flag-leaf specific N content ranged from 2.87 (Bacanorat88) to 3.59 g N m^{-2} (Tacupeto/b) (*P* < 0.05). There was a positive trend (*P* = 0.10) for an association between pre-anthesis RUE_{PAR} (GS31-GS65+7d) and stomatal conductance at GS41 and a positive association between CGR (GS31-GS41) and FLSN amongst the 10 genotypes (Fig 5.7).

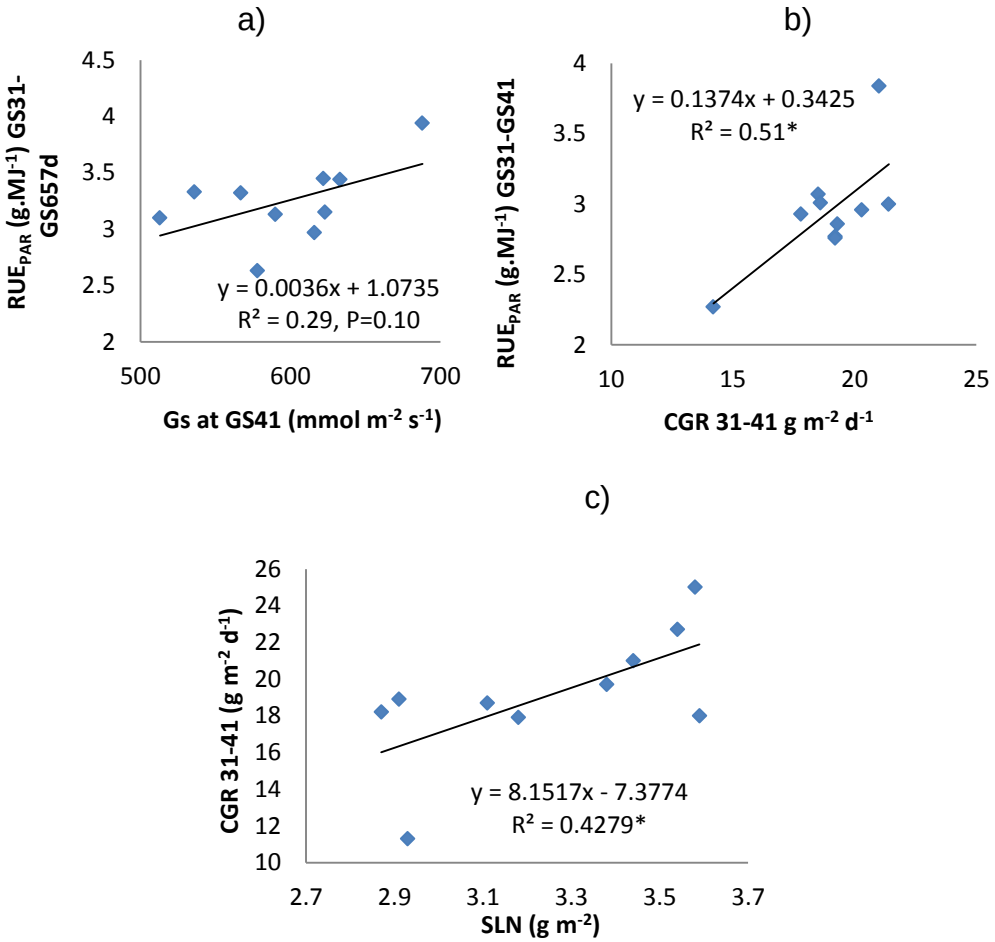


Figure 5.7 Relationship between a) RUE_{PAR} (GS31-GS65+7d) and stomatal conductance, b) RUE_{PAR} (GS31-GS41) and crop growth rate (CGR) (GS31-41) values are mean of two years and c) CGR (GS31-GS41) and flag-leaf specific N (FSLN) (g N cm^{-2} of flag leaf green area) in 10 genotypes in 2013.

5.3.8 Spike partitioning index and fruiting efficiency

Spike partitioning index ranged from 0.220 (Sahual/w) to 0.276 (Pavonf76) in 2012 ($P < 0.001$), from 0.199 (Babax/l) to 0.272 (Pfau/Seri) in 2013 ($P < 0.001$) and averaging over years from 0.212 (Sahual/w) to 0.268 (Pfau/Seri) ($P < 0.001$). There was a year effect ($P < 0.001$), but the year x genotype interaction was not significant (Table 5.10). Fruiting efficiency ranged amongst the 26 genotypes from 62.2 (Cmh79a.9) to 109.9 (Bcn/Rialto) grains g^{-1} DM in 2012 ($P < 0.01$), from 70.8 (Sokoll//p) to 136.3 (Bcn/Rialto) grains g^{-1} in 2012 ($P < 0.001$) and averaging over years from 67.9 (Sokoll//p) to 123.1 grains g^{-1} DM (Bcn/Rialto) ($P < 0.001$). There was an effect of year ($P < 0.001$), but the year x genotype interaction was not significant (Table 5.11).

A positive linear relationship was observed between SPI and grains m^{-2} ($R^2 = 0.59$, $P < 0.01$) in the ten genotypes, and a weak positive nonlinear (quadratic) regression amongst the 26 genotypes ($R^2 = 0.148$, $P = 0.15$) over years (Fig 5.8 c). However, in 2013 a significant nonlinear association was observed between GM2 a SPI ($R^2 = 0.27$, $P < 0.05$). For 25 genotypes (omitting Bcn/Rialto) there was a positive linear relationship between SPI and grains m^{-2} averaging over years ($R^2 = 0.24$, $P < 0.001$) and also a positive non-linear regression ($R^2 = 0.28$, $P = 0.053$) for 25 cultivars.

Fruiting efficiency showed a strong linear association with grains m^{-2} amongst the 26 genotypes in 2012 ($R^2 = 0.80$, $P < 0.001$), in 2013 ($R^2 = 0.77$, $P < 0.001$) and averaging over years ($R^2 = 0.82$, $P < 0.001$; Fig 5.7d).

Table 5.9 Spike partitioning index and fruiting efficiency (grains g^{-1} DM) for 26 CIMCOG genotypes in 2012 and 2013 and mean 2012-3.

	Spike partitioning Index			Fruiting efficiency grains g^{-1}		
Genotype	**2012**	**2013**	**2012-3**	**2012**	**2013**	**2012-3**
Babax/l	0.232	0.199	0.215	66.7	80.5	73.6
Bacanorat88	0.271	0.238	0.255	86.2	100.5	93.3
Bcn/rialto	0.233	0.237	0.235	109.9	136.3	123.1
Becard/k	0.246	0.237	0.242	78.6	103.2	90.9
Brbt1*2/k	0.239	0.232	0.236	72.9	93.6	83.2
Sahual/4	0.243	0.233	0.238	73.3	83.7	78.5
Sahual/w	0.220	0.204	0.212	70.2	84.3	77.2
Cmh79a.9	0.260	0.263	0.261	62.2	85.4	73.8
Cno79//p	0.235	0.218	0.227	70.4	84.4	77.4
Croc 1/	0.229	0.222	0.225	86.5	96.6	91.5
Kbird//i	0.238	0.238	0.238	91.7	115.0	103.4
Milan/k	0.241	0.217	0.229	69.1	76.6	72.9
Pavonf76	0.276	0.244	0.260	79.9	94.9	87.4
Pbw343*2	0.273	0.220	0.246	72.5	103.5	88.0
Pfau/seri	0.264	0.272	0.268	77.2	89.3	83.3
Serim82	0.273	0.238	0.255	79.1	101.6	90.3
7cerros	0.273	0.247	0.260	78.1	91.8	85.0
Sokoll//p	0.224	0.212	0.218	64.9	70.8	67.9
Tacupeto/s	0.240	0.211	0.225	72.2	86.4	79.3
Tacupeto/b	0.261	0.234	0.248	64.9	82.1	73.5
Tc870344/	0.232	0.211	0.222	64.7	76.5	70.6
Trap#1	0.239	0.227	0.233	73.7	88.8	81.3
Milan/k	0.229	0.213	0.221	72.9	96.8	84.8
Becard	0.259	0.222	0.240	71.6	88.9	80.3
Wbll1*2/	0.246	0.237	0.241	67.8	85.3	76.5
Yav 3/sc	0.249	0.223	0.236	77.5	93.5	85.5
Mean	0.247	0.229	0.238	75.2	91.9	83.6
Cult Prob.	***	***	***	***	***	***
LSD(0.05)	0.0252	0.0195	0.0174	10.17	9.66	7.15
Year Prob.			***			***
LSD(0.05)			0.005			1.981
Year*Cult Prob.			ns			ns
LSD(0.05)			0.0233			9.860
Corr with SPI	1	1	1	0.04	0.31	0.23

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

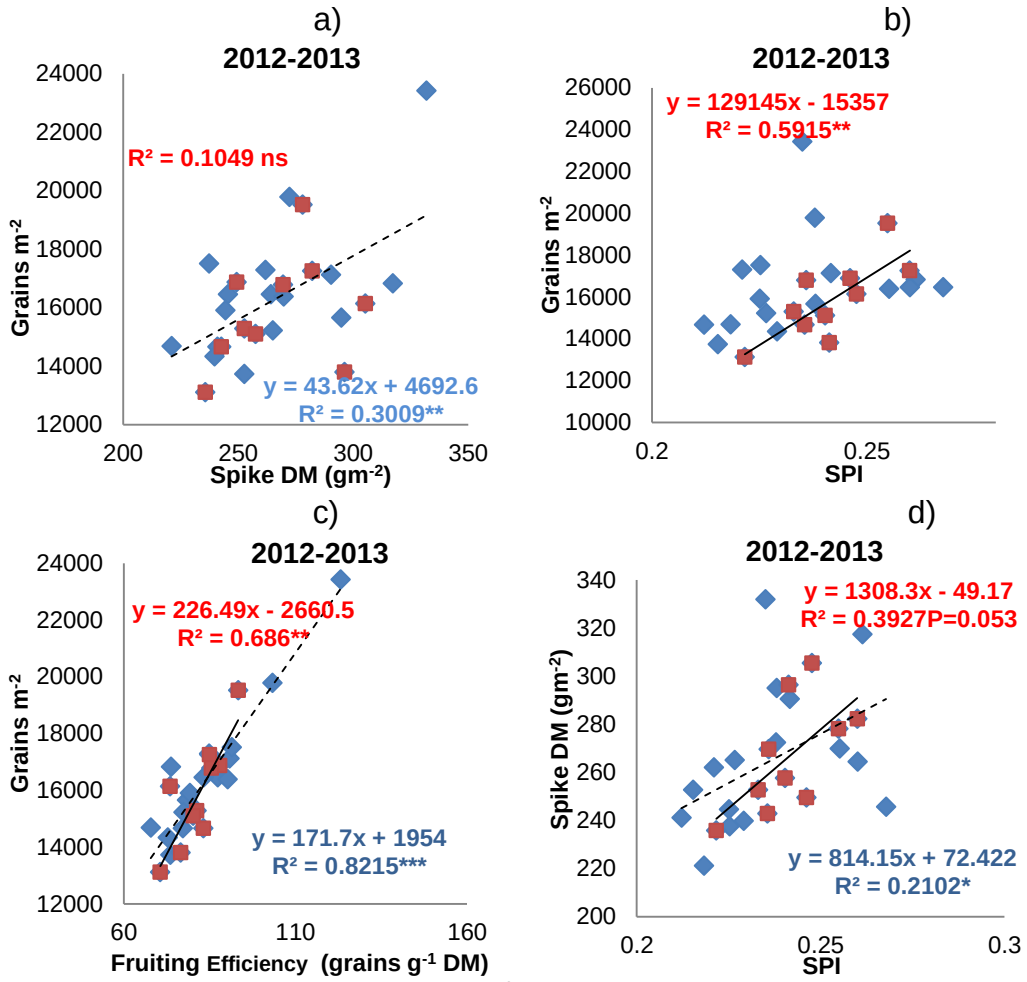
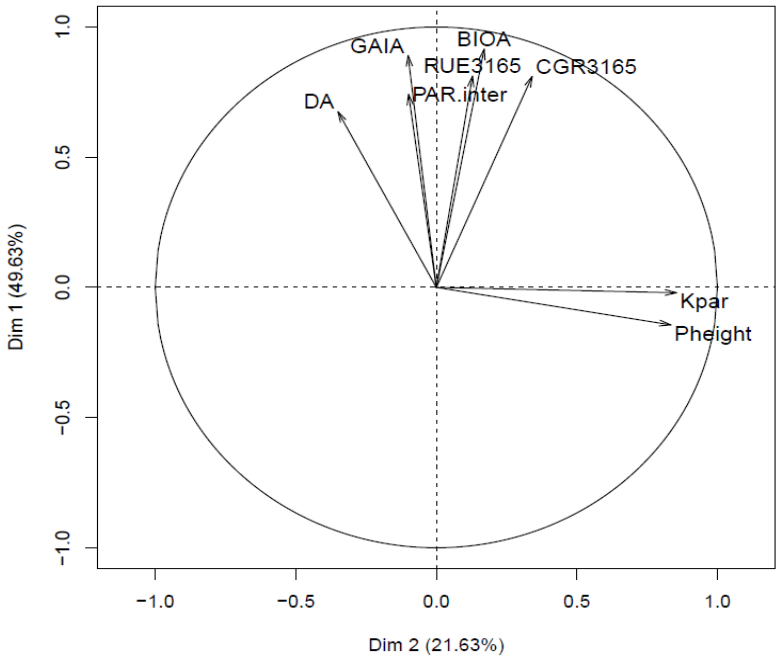


Figure 5.8 Linear regression of grains m^{-2} on a) spike DM (g m^{-2}) b) spike partitioning Index at GS65+7d (SPI), c) fruiting efficiency (grains g^{-1} DM) and d) Spike DM (g m^{-2}) on spike partitioning index (SPI) at GS65+7d for 26 (diamonds and squares) and ten (squares) genotypes. Values are means of 2011-2 and 2012-3. (Continuous line corresponds to 10 genotypes, broken line corresponds to 26 genotypes for regressions). Red symbols indicate subset for 10 cultivars.

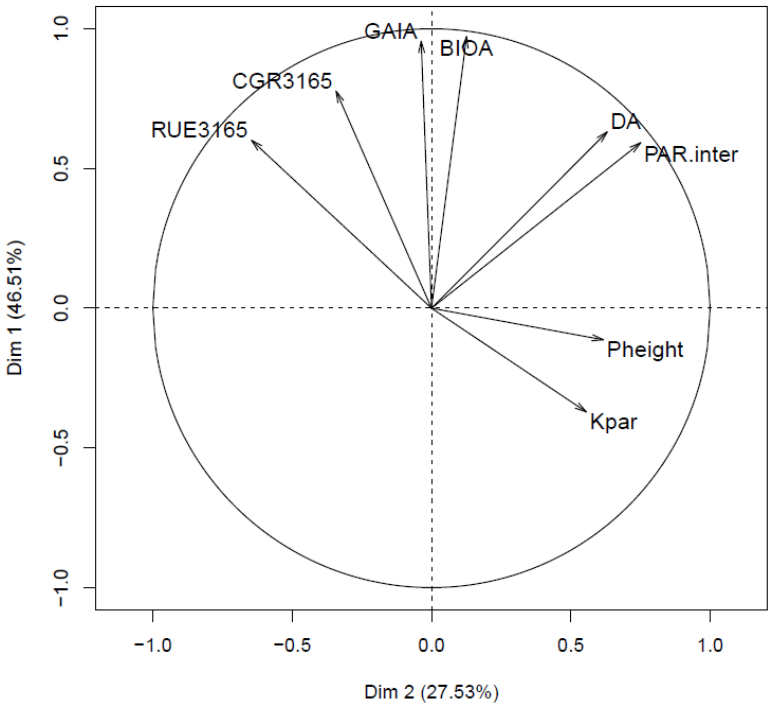
The biplot analysis in 2012 for the 10 genotypes confirmed that AGDM at GS65 +7d was positively associated with CGR (GS31-GS65+7d) and RUE_{PAR}; the two principal components explained 71.3% of the variation amongst traits. Similarly AGDM at anthesis +7d in 2013 was also positively associated with CGR (GS31-65+7d). The two principal components explained 74.0% of the trait variation.

Averaged across the two years, the PCA confirmed the positive association of biomass at anthesis with crop growth rate and date of anthesis; the two principal components explained 80.98% of the trait variation (Fig. 5.8 c). Kpar was associated (R^2 = 0.57, P < 0.05) with plant height, but neither of these traits were associated with AGDM at GS65+7d.

a)



b)



c)

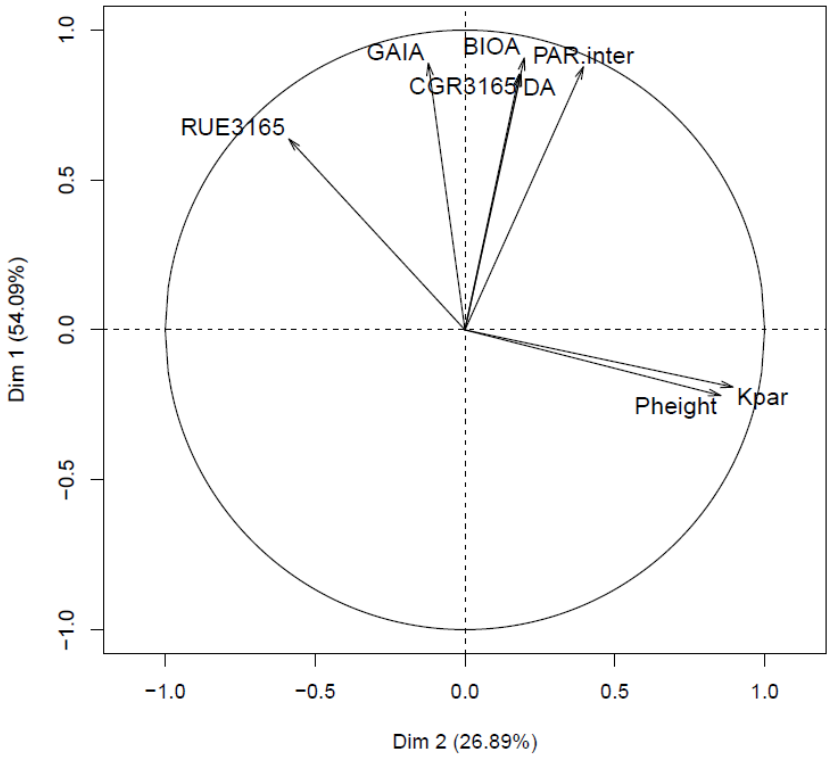


Figure 5.9 Principal component analysis for pre-anthesis traits in NW Mexico for a) 2012, b) 2013 and c) means of two years for 10 CIMCOG genotypes. Variables are: DA = Date of anthesis (DA), Above-ground DM per m^2 at GS65+7d (BIOA), Plant height (Pheight), Green are index at GS65 +7d (GAIA), Crop growth rate from GS31 to GS65+7d (CGR3165), radiation-use efficiency from GS31 to GS65+7d (RUE3165), extinction coefficient (Kpar) and radiation interception (PAR Inter).

5.3.9 Association of spike structural components with FE

Averaging over years, lemma PI showed a trend for positive association with fruiting efficiency (R^2 = 0.39, *P* = 0.053), whereas glumes, palea and rachis PI showed no association with FE amongst the ten genotypes. An increased lemma DM partitioning could be indicative of a high sugar concentration in the lemma, in turn, associated with high floret fertility due to the lemma's proximity to the floret.

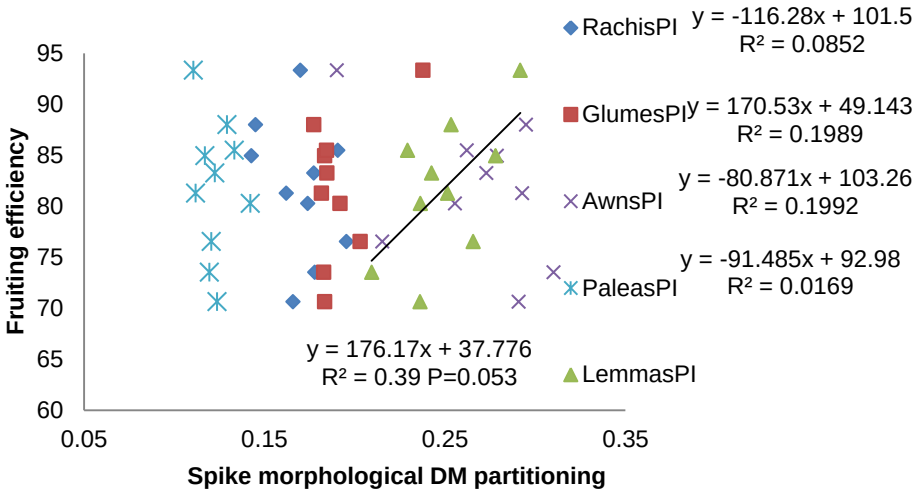


Figure 5.10 Association between spike structural components and Fruiting efficiency (FE)

5.3.10 Relationship between changes in traits and year of release

Year of release showed a weak positive trend with plant height (r= 0.50, *P* = 0.13) and a negative association with SPI (R^2 = 0.48, *P* < 0.05), and a positive association with stomatal conductance at GS41 and GS65 amongst the subset of 10 CIMCOG genotypes (Table 5.11).

Table 5.10 Physiological traits and year if release for above ground dry matter at GS65+7d (AGDM65+7d), grains m-2 (GM2), Pre-anthesis radiation-use efficiency from GS31-GS65+7d (RUE pre), crop growth rate from (CGR), days from GS31-GS65+7d, Spike partitioning Index (SPI), Fruiting efficiency (FE), Green area index (GAI), extinction coefficient (Kpar) Plat height, stomatal conductance at GS65 (Gs GS65) and stomatal conductance at GS41 (GsGS41) for 10 genotypes. Based on means across 2012 and 2013, except for stomatal conductance (Gs, measured only in 2013)

Traits	Correlation with YoR	Linear slope yr^{-1}	Prob. Linear reg.
AGDM 65+7d gm^{-2}	0.19	1.03 ± SE 1.87	0.597
GM2	-0.51	-68.8 ± SE 40.6	0.129
RUE pre g MJI^{-1}	0.28	0.002 ± SE 0.00350	0.45
CCG g m^{-2} d^{-1}	0.18	0.02± SE 0.0348	0.599
Days 31-65+7d days	0.15	0.03 ± SE 0.0640	0.675
SPI	-0.69*	-0.0005 ± SE	0.026
FE grains g^{-1}	-0.41	-0.20 ± SE 0.158	0.244
GAI	0.20	0.01 ± SE 0.0184	0.576
Kpar	0.20	0.0006 ± SE	0.549
Plant height cm	0.50	0.23 ± SE 0.141	0.138
Gs GS65 mmol m^{-2} s^{-1}	0.85**	5.05 ± SE 0.856	0.002
GsGS41 mmol m^{-2} s^{-1}	0.75*	2.73 ± SE 1.09	0.013

5.4 Discussion

5.4.1. Genetic variation in anthesis biomass in CIMCOG panel RUE

An increased biomass can result from increased radiation interception, or increased RUE_{PAR} associated with changes in canopy architecture, photosynthetic metabolism, or more optimal source:sink balance (Monteith, 1977; Reynolds et al., 2001, 2005). Genetic progress in biomass has been observed and modern varieties at CIMMYT (Aisawi et al., 2015).

In the present study genetic variation in AGDM at GS65+7d of 926.1 to 1388.1 g m^{-2} in the 26 CIMCOG genotypes was greater than that reported by Reynolds et al. (2007) for 86 CIMMYT spring wheat genotypes from 704 to 818 g m^{-2}. However, a similar range was reported by Aisawi et al., (2015) from 980 to1287 g m^{-2} at anthesis + 7d in 12 historic CIMMYT spring wheat cultivars. In the present study AGDM at anthesis +7d was positively associated with anthesis date and plant height. However, future increases in biomass in CIMMYT spring wheats must likely be obtained without increases in plant height. Future gains in yield potential must also largely be achieved by developing cultivars with greater biomass whilst maintaining or ideally increasing HI (Slafer et al., 1999). However, HI has decreased in CIMCOG cultivars with higher biomass in this study as discussed in chapter 4. Therefore, it is necessary to identify traits that will increase biomass production and also improve HI in high biomass cultivars in order enhance grain yield.

5.4.2 Traits to increase biomass

Fractional radiation interception is a function of the green area index and light extinction coefficient (Gifford et al., 1984). In the present study, genetic variation was observed in radiation intercepted from GS31-

GS65+7d and this was explained mainly by GAI at anthesis +7d (R^2 = 0.51) amongst the 10 genotypes rather that Kpar (R^2 = 0.01).

Fischer (1984) reported that CGR genetic variation in wheat is mainly explained by the PAR absorbed by the canopy. In the present results, the CGR from GS31-GS65+7d was highly associated with the amount of PAR intercepted by the canopy over the same period explaining 65% of the genetic variation. However, CGR from GS31-41 was also related to RUE_{PAR} over this period explaining 50.9% of the biomass variation.

Genetic variation in RUE_{PAR} was observed in 2013 but not in 2012. Averaging over years for RUE_{PAR} from GS31 – GS61+7d the maximum value of 3.04 g MJ^{-1} in the present study was slightly higher than the maximum value of 2.76 g MJ^{-1} from GS41-GS61 reported by Gaju et al., (2009) in larger phenotype CIMMYT spring wheats in NW Mexico or 2.64 g MJ^{-1} that (Shearman et al., 2005) reported from GS31-GS61 in a set of eight UK winter wheats released from 1972-1995.

In both years, genetic variation in RUE_{PAR} was significantly associated with biomass growth during the stem-elongation phase. This suggests increasing RUE_{PAR} is an important breeding target that can be applied to increase SDW at anthesis. In these results, RUE_{PAR} explained 30% of the genetic variation of spike dry weight per unit area. In addition, RUE_{PAR} explained 23% of the genetic variation in biomass at harvest and 48% of variation for grain yield amongst the subset of 10 genotypes. Higher RUE_{PAR} could drive increases in grain yield either through increasing crop growth rate and biomass in the pre-anthesis period or through associations with increased RUE_{PAR} and grain growth post anthesis. Shearman et al. (2005) found that genetic gains in grain yield were based on improved pre-anthesis RUE and in grain number per unit area. In the present results, grain number per m^2, however, was not associated with RUE_{PAR} during stem elongation. It is also possible that pre-anthesis RUE was associated with final grain yield in the present study through

increasing soluble stem carbohydrate storage, or through correlations with post-anthesis RUE.

The association between leaf stomatal conductance and pre-anthesis RUE in the present study indicated RUE_{PAR} could be associated with higher leaf photosynthesis rate amongst the cultivars, because stomatal conductance is indicative of rate of CO_2 diffusion into leaf for photosynthesis (Rebetzke et al., 2000). This trait was associated with genetic variation in grain yield in wheat in an investigation reported by Condon et al. (2008) who measured leaf porosity in five experimental breeding populations under irrigated conditions in NW Mexico and found 50% of the grain yield variability was explained by Gs before anthesis. In the present results, Gs at around anthesis explained 52% of the grain yield variation amongst 10 cultivars. In irrigated environments, high Gs indicates high CO2 conductance rates and thus the increased potential for biomass accumulation. In addition, higher stomatal conductance may be indicative of a higher grain sink strength demand for photo-assimilates. Present results are in agreement with previous investigations where positive relationships were observed between Gs pre-anthesis and grain yield under irrigated environments for CIMMYT spring wheat (Fischer et al., 1998; Reynolds et al., 1994).

In the present results, the maximum value of pre-anthesis RUE was only 56.29% of the theoretical maximum of 5.4 g MJ^{-1} proposed by Fischer and Edmeades (2010).

Theoretically RUE_{PAR} can be increased by 50% in wheat for instance, by optimizing light and N distribution of canopies while minimizing photoinhibition, and increasing spike photosynthesis. (Reynolds et al., 2012; Zhu et al., 2010). In the present results the PCA showed that biomass at GS65+7d was associated with CGR and CGR was associated with flag-leaf SLN. So a greater concentration of N or Rubisco per cm^2 leaf may favour enhanced RUE or biomass; In addition, stomatal conductance

at GS41 showed a positive trend with flag leaf nitrogen content (mg flag leaf^{-1}) amongst the 10 genotypes. This is in agreement with Reynolds et al. (2012). Therefore FSLN, could be potential selection trait grain yield amongst CIMMYT spring wheat cultivars to reduce the light saturation of photosynthesis in upper leaves of canopy and in RUE.

Another strategy to increase photosynthetic capacity is through delayed onset or a slower rate of post-anthesis senescence to increase grain yield (Bogard et al., 2011). Post anthesis senescence and prospects for applying high-throughput phenotyping for senescence traits will be discussed in the chapter 6 and 7.

5.4.3. Physiological basis of grains m^{-2} variation in CIMCOG

Grain number per m^2 can be analysed as the product of: radiation interception, RUE_{PAR}, SPI and FE (see Equation 5.1). In relation to these physiological components, genetic variation in grains m^{-2} in the 10 CIMCOG genotypes was explained in the following order: fruiting efficiency (R^2 = 0.68), spike partitioning index (R^2 = 0.59), radiation-use efficiency (R^2 = 0.13) and radiation interception (R^2 = 0.001); and for 26 genotypes, grains m^{-2} was again a more strongly associated with fruiting efficiency (R^2 = 0.82) than spike partitioning index (R^2 = 0.10). However, applying a multiple regression analysis, the best model explaining grains m^{-2} was obtained with three variables (FE, RUE and SPI) which explained 79% of the variation in GM2 amongst the subset of 10 genotypes.

Similar to present results, Acreche et al. (2008) found that grains m^{-2} was more associated with the spike dry weight at anthesis than with FE amongst nine cultivars in Spain. Gaju et al., (2014) also found that SPI explained a higher proportion of variation in grain m^{-2} in 57 DH lines derived from a cross between a UK winter wheat Rialto and CIMMYT spring wheat LSP2 with in NW Mexico. An increased fruiting efficiency may be associated with an improved partitioning within spike

morphological components (rachis, glumes, awns) in modern cultivars (Slafer and Andrade 1993) or a lower threshold for assimilate requirement per grain (Foulkes et al., 2011).

Fruiting efficiency varies largely and whenever different cultivars are compared a wide range of variation can be found (Slafer et al., 2015). The genetic range obtained in present results 68-123 grains g^{-1} is comparable with that reported by Acreche et al. (2008) of 45-153 grains g^{-1} under high yielding conditions in Spain for 8 genotypes released from 1940-1998 and that reported by Shearman et al. (2005) of 84-128 grains g^{-1} for 8 UK cultivars released from 1972 to 1995 (Slafer et al., 2015). In the present results, grains m^{-2} was associated with spike dry weight per unit area wich in turn was associated with SPI amongst the 26 genotypes. Acreche et al.*(* 2008) showed grains per m^2 was associated with spike growth before anthesis, and Shearman et al (2008) also reported a trend between grain number per m^2 and spike dry weight.

The non linear association between SPI and grain m^{-2} indicates that greater SDW was not being efficiently converted into additional grain sites, and improvement in FE is required to exploit fully high SDW cultivars.

Genetic variation in grains m^{-2} in the CIMCOG lines was more strongly associated with fruiting efficiency than with SPI or even with spike dry weight (g m^{-2}) in the 26 genotypes. Although spike dry weight at flowering has been reported as the main physiological component for grains m^{-2} (Abbate et al., 1997; Demotes-Mainard et al., 1999; Fischer 2011; González et al., 2003; Slafer and Andrade 1993), fruiting efficiency is amongst the traits which could be exploited to achieve future gains in wheat (Slafer et al., 2015). Present results indicated FE may be an important physiological determinant related to grains per unit area for modern CIMMYT spring wheat. These results agree with a recent investigation of García et al. (2014) who found in an elite double-haploid population for spring wheat a stronger correlation between FE and grains

m^{-2} than between SPI. It is encouraging that no trade-off between SPI and FE was observed in this study, and present results support the idea that FE is partially independent of SPI (Bustos et al., 2013; García et al., 2014; González et al., 2011). Therefore, more knowledge is needed on traits underlying FE and spike dry weight and their genetic control for application in breeding.

In two years, analysis of spike morphological component DM partitioning showed a trend for a positive association between lemma PI and FE, explaining 39% of variation of FE. The positive association between lemma PI and FE may be related to larger lemmas being linked with increased assimilate supply to florets within the spikelets, as florets are subtended by the lemmas within the spikelet.

Overall the conclusions are that:
There is genetic variation in radiation interception by the canopy during stem elongation from GS31 to GS65+7d positively associated with accumulated above-ground dry matter amongst the CIMCOG genotypes.

There was genetic variation in radiation-use efficiency (in 2013) during stem elongation and RUE was positively related with above-ground biomass at anthesis amongst the CIMCOG genotypes. Therefore, there is some scope to increase biomass through RUE and this could be enhanced by selecting for increased stomatal conductance and/or flag-leaf specific N content.

There is genetic variation in each of spike partitioning index and fruiting efficiency positively related with grains m^{-2} and HI amongst the 26 CIMCOG genotypes, and there is no trade-off between these two components. Improving FE could be achieved by selecting for lemma PI with the spike morphological components.

CHAPTER 6 CHAPTER 6 CIMGOG PANEL FIELD EXPERIMENTS: RESPONSES TO SOURCE-SINK MANIPULATION TREATMENT

6.1 Introduction

This chapter describes the results of the source-sink manipulation degraining treatment carried out in three seasons (2010-11, 2011-12 and 2012-13) at CIMMYT Norman E. Borlaug experimental center, Ciudad Obregon, Mexico. The results are presented in individual years and across years for the 26 spring bread wheat CIMCOG cultivars. A subset of 10 cultivars with a restricted range of anthesis date was studied in relation to spikelet position of grain responses ; and using pre-anthesis RUE and other physiological traits the model described by Bingham et al. (2007) was applied to estimate source-sink balance. In this model, the grain sink size was calculated as:

Individual grain weight in degrained treatment x grains m^{-2} **Equation 6.1**

Where degrained individual grain weight was taken as an estimate of potential grain weight.

Grain source size was calculated as:

Stem WSC at GS65 + 7 days (g m^{-2}) + (RUE (g MJ^{-1}) x RI (MJ m^{-2}).

Equation 6.2

Where, Stem WSC = water soluble carbohydrate of stems and leaf sheaths at GS65 +7d (g m^{-2}); RUE_{PAR} = PAR radiation-use efficiency (above-ground DM per unit radiation interception) pre-anthesis, GS31-GS65+7d (g MJ^{-1}) and RI = the amount of radiation intercepted (photosynthetically active radiation, 400-700 nm) post-anthesis (GS65-GS89) by the canopy (MJ m^{-2}).

Source-sink balance during the grain growth period and its determinants are key traits that must be better understood in modern spring wheat in order to identify combinations of traits to increase simultaneously grain

sink and grain source size and enhance grain yield. Grain yield progress was highly associated with grain number per unit area in wheat during the Green Revolution (Brancourt-Hulmel et al., 2003; Canevara et al., 1994; Peltonen-Sainio et al., 2007; Sayre et al., 1997; Shearman et al., 2005) . Slafer and Savin (1994) found that altering the source-sink ratio in two wheat cultivars (Australian semi-dwarf and a Canadian standard-height cultivar) by removing all spikelets from one side of the ear 10 days after anthesis did not affect the grain weight at apical, central or basal positions, and grain yield in the two cultivars was either sink limited or co-limited by source and sink. Borrás et al. (2004) concluded in a review of 18 studies with different types of source-sink manipulation treatments that grain growth of wheat under favourable conditions was almost always sink limited, as the availability of the assimilates during the grain filling period was usually at a saturation level. On the other hand, Acreche and Slafer (2009) reported that in bread wheat genotypes released from 1940 to 2005 in Spain, when removing all the upper-half of the spike, grain weight was not changed in the old cultivars and increased in the newest cultivars. They concluded that modern genotypes have a co-limitation of grain growth by source and sink rather than sink limitation. Zhang et al. (2010) in a degraining and a shading treatment imposed at anthesis found in wheat cultivars in SW Australia that grain growth of two from three spring wheat was sink limited. Ma et al. (1996) studying six red winter wheat varieties reported that in response to spikelet removal at around anthesis two cultivars showed no increase in grain growth, but four cultivars showed increases in grain growth, indicating grain growth was co-limited by source and sink. In general, degraining source-sink manipulation studies tend to show that grain yield potential is limited under optimal conditions by the grain sink size rather than grain source size (Slafer and Savin 1994), but that there are some indications that more modern cultivars may be in closer source/sink balance than their predecessors.

In this study, the source-sink balance was manipulated by removal of the spikelets at GS65+7/10 days. Grain growth responses to degraining were

quantified to estimate whether the limitation to grain growth is due to grain storage capacity (grain sink size) or the photosynthetic capacity of the crop to supply assimilate to grains (grain source size).

The aims of this chapter are:

- To quantify the genetic variation in responses to a degraining source-sink treatment at GS65+7/10 days in the 26 CIMCOG genotypes. To determine in a subset of ten genotypes the responses of the grains in the basal, central and apical spikelets to degraining at GS65+7/10d and whether there are interactions with genotype.
- To evaluate the genetic variation in senescence parameters in the 26 CIMCOG genotypes according to measurements of canopy NDVI, flag-leaf chlorophyll content (SPAD) and flag-leaf visual senescence score in the unmanipulated crop and correlations with grain growth responses to degraining.

The specific hypotheses tested in this chapter were:

- Post-anthesis source-sink balance differs amongst the 26 CIMMYT spring wheat cultivars in the CIMCOG panel.
- Grain growth of modern high-yielding cultivars in the CIMCOG panel is closer to source limitation than their predecessors.
- The grain weight response to degraining does not differ between spikelet positons in the spike (basal, central and apical) and there is no interaction with genotype.
- Genetic variation in senescence durations and/or rates (NDVI, flag-leaf SPAD and flag-leaf visual score methodologies) are associated with grain yield potential and source/sink balance in the 26 CIMMYT spring wheat cultivars in the CIMCOG panel.

The physiological analysis of degraining responses and associated traits in the CIMCOG panel is presented in this chapter. In the next chapter the

genetic analysis of degraining responses and associated senescence traits will be examined in a Wheat Association Mapping Initiative (WAMI) panel.

6.2 Materials and Methods

6.2.1 Experimental site treatment

The same experiments as described in Chapter 4 and 5 were used. The treatment was carried out within 1.5 m length of the plot. A degraining treatment was imposed to modify the grain sink size by manipulating the number of spikelets per spike at GS65+7 days in 2011 and at GS65+10 days in 2012 and 2013. In 2011, the top half of the spike was removed at GS65+7d. In 2012, half of the spikelets (all spikelets down one side of the spike, terminal spikelet not removed and first fertile basal spikelet removed) were removed at GS65+10d in 20 randomly selected shoots; and in 2013 the same treatment was applied as in 2012 in 12 randomly selected shoots at GS65+10d.

6.2.2 Crop measurements

The degraining treatment was applied to the 26 genotypes in three years. In each year, for all 26 genotypes, at harvest grain weight per shoot, grains per spike and individual grain weight were determined in the degrained and control shoots. The spikelets were hand threshed and grains were dried at 70^{o}C for 48 h and the dry weight recorded. For a subset of 10 genotypes, in each treatment, seven of the sampled spikes were separated into three spike sections (proportionally according to the number of spikelets) basal, central and apical spikelets. These three spike sections were threshed separately and grains were dried at 70^{o}C for 48 h, counted and the dry weight recorded.

At physiological maturity DM partitioning of leaf lamina, leaf sheath and true stem was assessed in the degrained and control shoots. In addition water soluble carbohydrates were measured in the true-stem in 2012 (by NIRS) and in 2013 (by the anthrone method (Yemm and Willis 1954).
In the sections of the plots not allocated for source-sink manipulation treatments, measurements of flag-leaf and canopy senescence were carried out.

Flag-leaf visual senescence parameters: For the subset of 10 genotypes in 2012 and 2013, measurement of flag-leaf senescence parameters was carried out. Flag-leaf senescence was scored approximately every three days by visual assessment on 10 flag leaves per plot on tagged shoots, after anthesis until leaves were fully senesced. Visual measurements of flag-leaf score (FLS) of senescence were taken along the plot using a diagnostic key (see Chapter 3.1.7.4 Material and Methods).. A logistic curve was fitted with the thermal time (base temp 0°C) from anthesis (GS65).

$$Y= A + C/(1 + EXP(-B*(X - M))) \quad \textbf{Equation 6.3}$$

Where Y= the value of the senescence score from 1-10 for the flag leaf and X is the amount of ^{o}Cd. Using Eqn 6.3 the onset senescence was calculated by substituting the value of y when the FLS visual score was 2 and for the end of senescence when the FLS visual score was 9. The rate of senescence was taken as the parameter B (Hills'slope) of the logistic curve.

Flag-leaf SPAD senescence parameters: Flag-leaf chlorophyll content (SPAD-502, Minolta, Tokyo, Japan) was used as a non-destructive measurement to assess the leaf lamina senescence.

NDVI senescence parameters: In each year, NDVI readings were taken from spike emergence (GS59) to near PM using a (Greenseeker N-Tech

Industries, USA). (See Chapter 3 for more details). Senescence rate was calculated as the slope of the linear regression with the thermal time from spike emergence.

Table 6.1 List of the 26 CIMCOG panel with Identifiers used in Figs 6.1-6.10.The 26 genotypes as identified by number are indicated in the figures in this chapter. (Cultivars in bold are the subset of 10 cultivars)

Cultivar	Short name	GID	Cross Name
1	Babax/l	5999777	BABAX/LR42//BABAX/3/VORB
2	**Bacanorat88**	16122	BACANORA T 88
3	Bcn/rialto	6056245	BCN/RIALTO
4	Becard/k	6174886	BECARD/KACHU
5	**Brbt1*2/k**	5397958	BRBT1*2/KIRITATI
6	Sahual/4	6176523	SAUAL/4/CROC_1/AE.SQUARROSA(205)//KAUZ/3/ATTILA/5/SAUAL
7	Sahual/w	6178783	SAUAL/WHEAR//SAUAL
8	Cmh79a.9	6171893	CMH79A.955/5/AGA/4/4*PI/CHR//2*SN64/3/INIA66/6/NAC/7/RIALTO
10	Cno79//p	6178401	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7
11	Croc_1/	5343246	CROC_1/AE.SQUARROSA(205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2
12	Kbird//i	6177599	KINGBIRD#1//INQALAB91*2/TUKURU
13	Milan/k	4556647	MILAN/KAUZ//PRINIA/3/BAV92
16	Pavonf76	2465	PAVON F 76
17	**Pbw343*2**	6179222	PBW343*2/KUKUNA*2//FRTL/PIFED
18	Pfau/seri	5390612	PFAU/SERI.1B//AMAD/3/WAXWING
19	Serim82	3895	SERI M 82
20	**Siete cerros**	775	SIETE CERROS T66
21	Sokoll//p	6000921	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR
22	Tacupeto/s	6179128	TACUPETOF2001/SAUAL/4/BABAX/LR42//BABAX*2/3/KURUKU
23	**Tacupeto/b**	6175024	TACUPETOF2001/BRAMBLING*2//KACHU
24	**Tc870344/**	5423688	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1
25	**Trap#1**	5995410	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1
26	Up2338*2	6176178	UP2338*2/3/SNI/TRAP#1//KAUZ*3/TRAP/4/MILAN/KAUZ//CHIL/CHUM18/5/UP2338*2/3/SNI/
27	**Becard**	5325839	BECARD
28	**Wbll1*2/**	6176346	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES
30	**Yav_3/sco**	6175172	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA(498)/5/LINE1073/6/KAUZ*3/4/CAR//KAL/

6.3 Results

6.3.1 Grain growth responses to degraining for 26 genotypes

In 2011, there was an effect of the degraining treatment ($P < 0.001$) and an interaction between degraining treatment and genotype ($P < 0.05$; Fig. 6.1). Grain weight responses to degraining ranged from 9.2 (Babax/l) to 43.6 (Bcn/Rialto) % ($P < 0.05$). Grain weight in the control differed amongst the 26 genotypes from 27.8 (Bcn/Rialto) to 51.9 (Babax/l) mg ($P < 0.001$). Similarly, the potential grain weight (as indicated by the grain weight in the degrained spikes) ranged from 39.9 (Bcn/Rialto) to 59.3 (Tc870344/) mg ($P < 0.001$). A linear and positive association was found between the grain weight in the degrained treatment and the control ($R^2 = 0.77$, $P < 0.001$) (Figure 6.1).

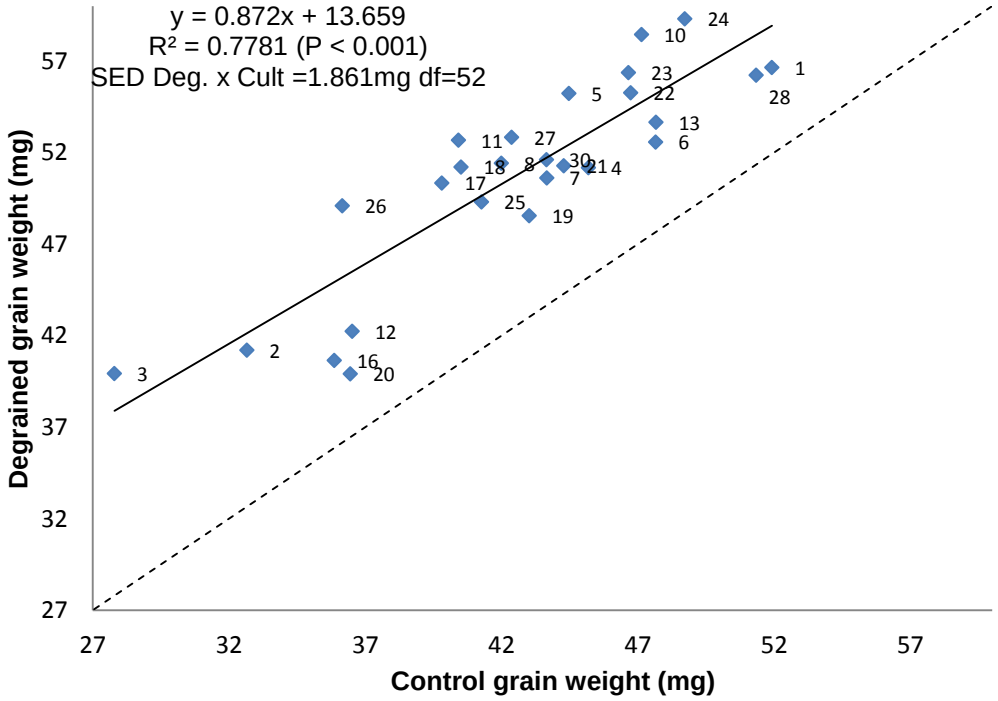


Figure 6.1 Individual grain weight in control shoots versus degrained shoots for 26 CIMCOG genotypes in 2011. Dashed line represents the 1:1 ratio.

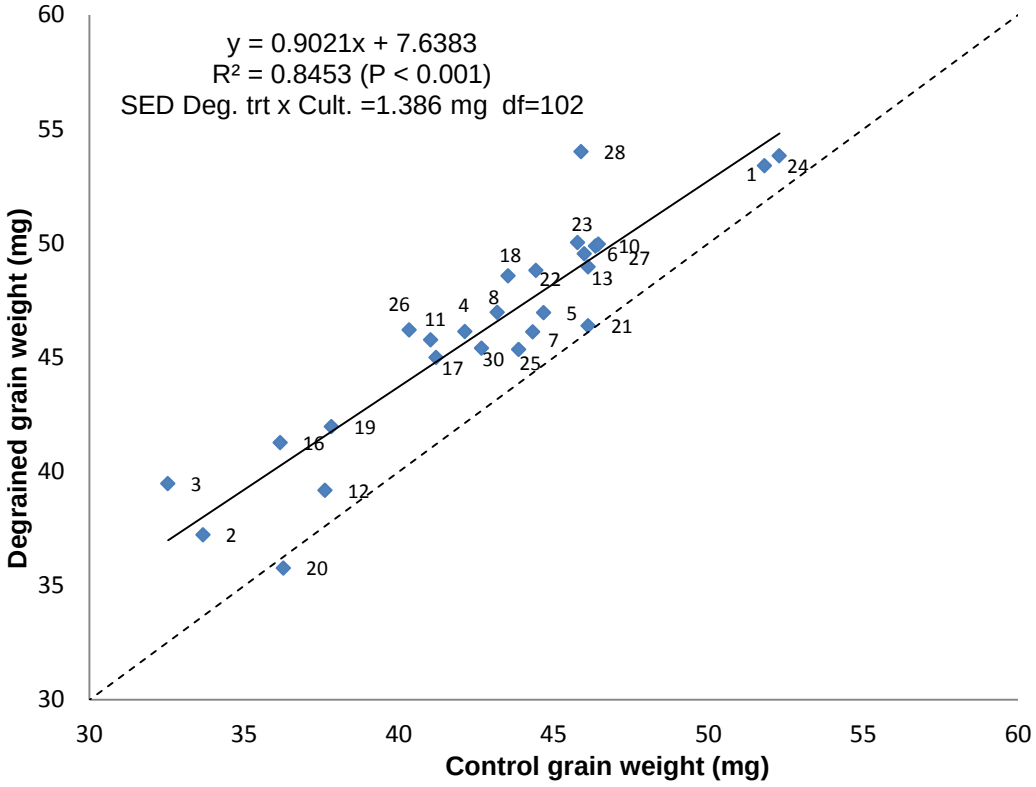


Figure 6.2 Individual grain weight in control shoots versus degrained shoots for 26 CIMCOG genotypes in 2012. Dashed line represents the 1:1 ratio.

In 2012, there was an effect of the degraining treatment ($P < 0.001$) on grain weight and a degraining treatment x genotype interaction ($P < 0.01$). Grain growth response to degraining ranged from -0.5 (Siete cerros) to 21.4 (Bcn/Rialto) % ($P < 0.05$). Grain weight in the control differed amongst the 26 genotypes from 32.5 (Bcn/Rialto) to 52.3 (Babax/l) mg and in the degraining treatment from 35.8 (Siete cerros) to 54.0 (Wbll1*2/) mg ($P < 0.001$; Fig. 6.2). A positive linear association was found between the grain weight in the degraining treatment and the control ($R^2 = 0.84$, $P < 0.001$) (Fig. 6.2).

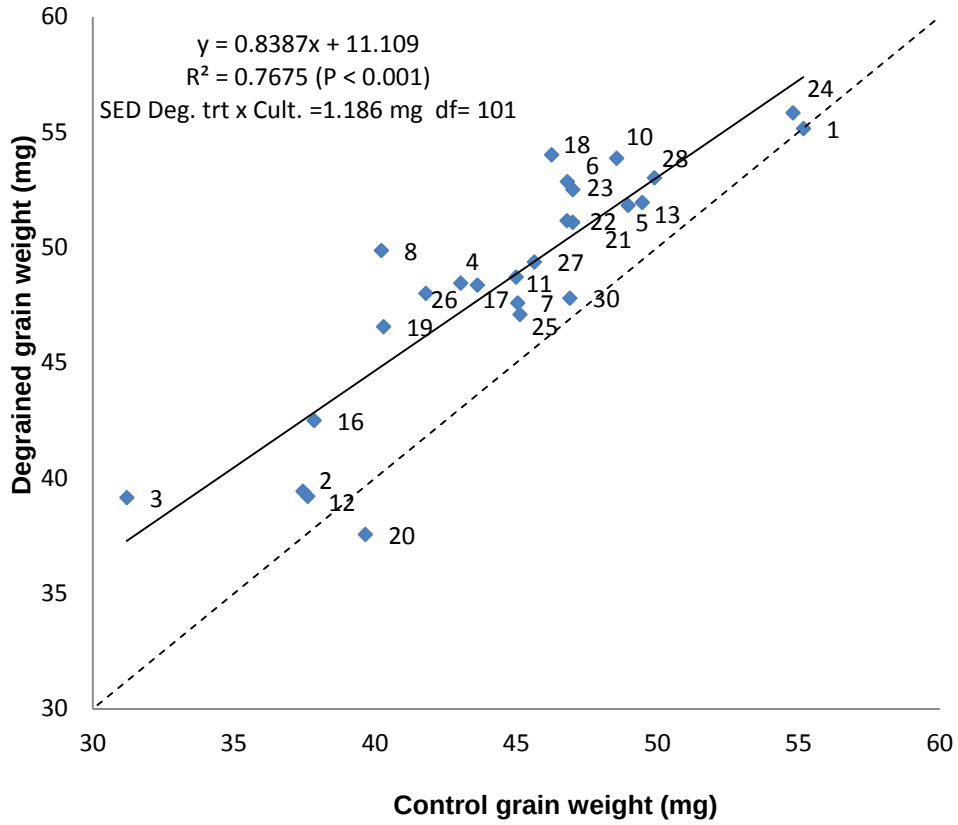


Figure 6.3 Individual grain weight in control shoots versus degrained shoots for 26 CIMCOG genotypes in 2013. Dashed line represents the 1:1 ratio.

In 2013, there was an effect of the degraining treatment on grain weight ($P < 0.001$) and a degraining treatment x genotype interaction ($P < 0.001$; Fig. 6.3). Grain weight to response to degraining ranged from -5.3 (Siete cerros) to 25.4 (Bcn/Rialto) % ($P < 0.001$). Grain weight in control shoots differed amongst the 26 genotypes in the range 31.2 (Bcn/Rialto) to 55.2 (Babax/l) mg ($P < 0.001$) and in the degrained shoots from 37.6 (Siete cerros) to 55.8 (Tc870344/) mg ($P < 0.001$). A positive linear association was found amongst the genotypes between the degraining treatment and the control ($R^2 = 0.76$, $P < 0.001$) (Fig. 6.3).

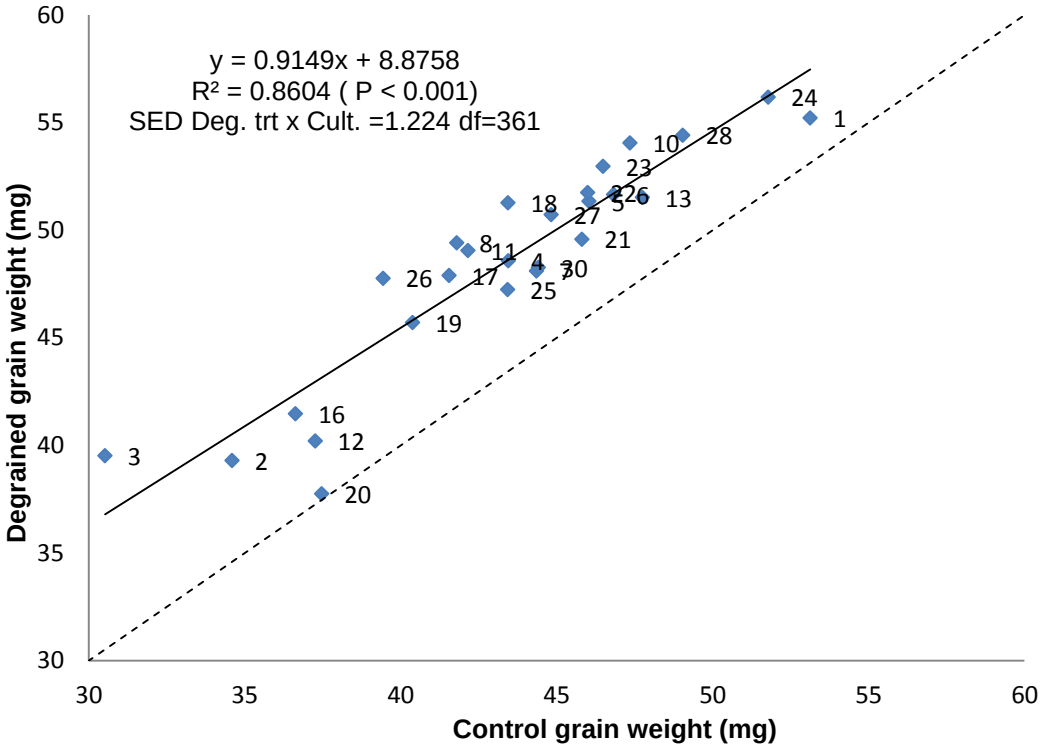


Figure 6.4 Individual grain weight in control shoots versus degrained shoots for 26 genotypes. Values represent means across 2011, 2012 and 2013. Dashed line represents the 1:1 ratio.

Averaging over the three years, there was an effect of the degraining treatment ($P < 0.001$) and the degraining treatment x genotype interaction ($P < 0.001$). Averaging over years and 26 cultivars, final grain weight increased in response to degraining by 5.19 mg (12.6%) ($P < 0.001$). Grain weight response to degraining ranged from 1.0 (Siete cerros) to 30.1% (Bcn/Rialto) compared to the control ($P < 0.001$). The grain weight increase ranged from 0.28 to 8.99 mg ($P < 0.001$). Grain weight in the control treatment differed amongst the 26 genotypes in the range 31.2 (Bcn/Rialto) to 55.2 (Babax/l) mg ($P < 0.001$), the grain weight in the degraining treatment ranged from 37.6 (Siete cerros) to 55.8 (Tc870344/) mg ($P < 0.001$) Figure 6.4). A linear positive association was found amongst cultivars between the grain weight in the degraining treatment and the control ($R^2 = 0.86$, $P < 0.001$) (Fig. 6.4). Averaging across the

three years, higher yielding cultivars also had larger responses to degraining; and the grain weight response to degraining was positively linearly associated amongst the 26 cultivars with grain yield (R^2 = 0.167, *P* < 0.05; Fig. 6.5).

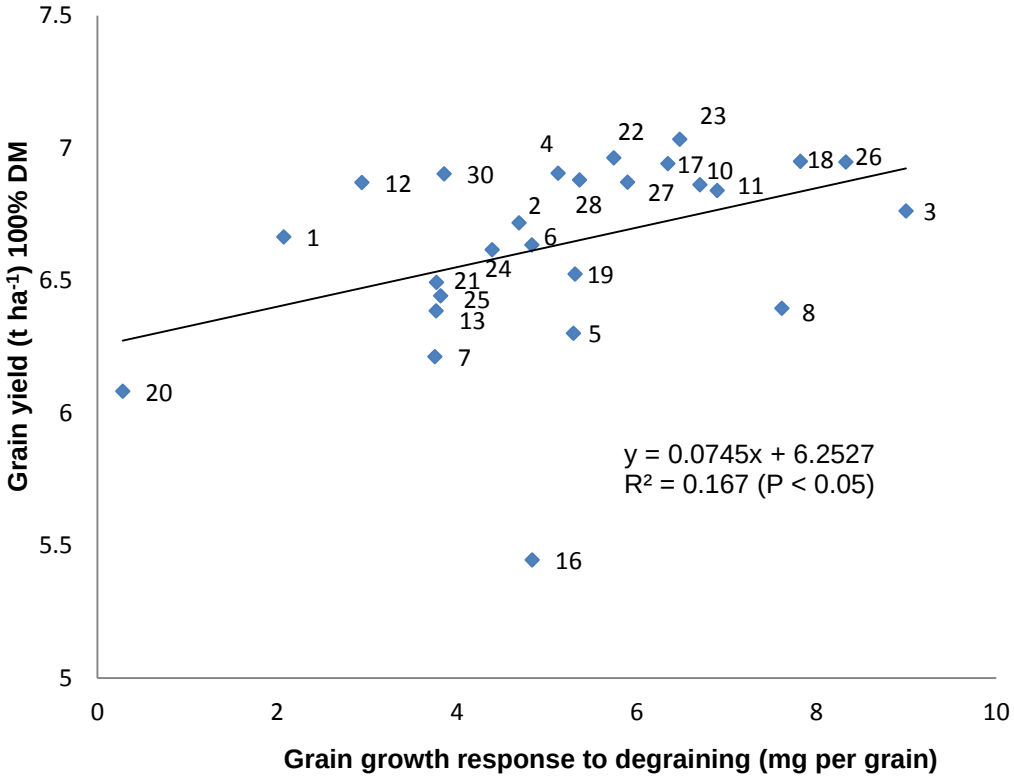
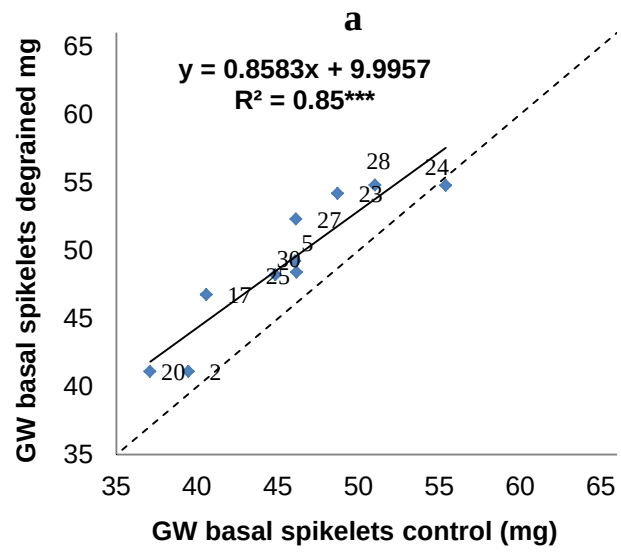
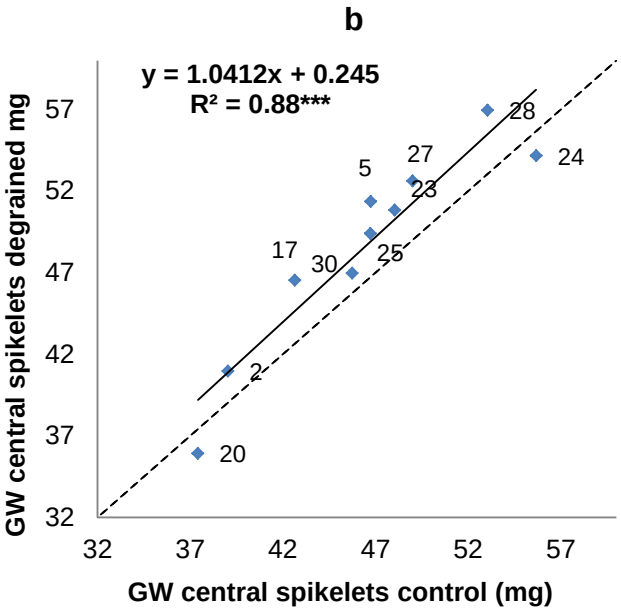


Figure 6.5 Linear relationship between grain weight response to degraining treatment (mg) and grain yield (100% DM) for 26 genotypes. Values represent means across 2011, 2012 and 2013.

6.3.2 Grain growth in basal, central and apical spikelets for 10 genotypes

Grain growth responses in basal, central and apical spikelets were assessed in 2012 and 2013 in 10 genotypes (Fig. 6.6).





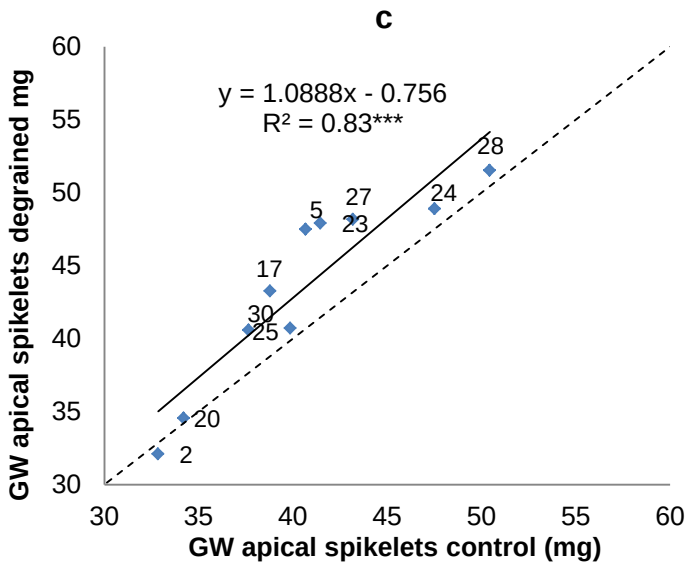


Figure 6.6 Relationship of grain weight (GW) in control treatment versus degraining treatment for (a) basal spikelets, (b) central spikelets (c) apical spikelets for 10 genotypes in 2012. Dashed line represents the 1:1 ratio.

In 2012, an effect of the degraining treatment on grain weight was observed in basal, central and apical ($P < 0.001$) spikelets, but there was no interaction for degraining treatment x genotype for basal or central spikelets. However, a trend was observed for an interaction between degraining treatment and genotype for apical spikelets ($P = 0.13$) in 2012.

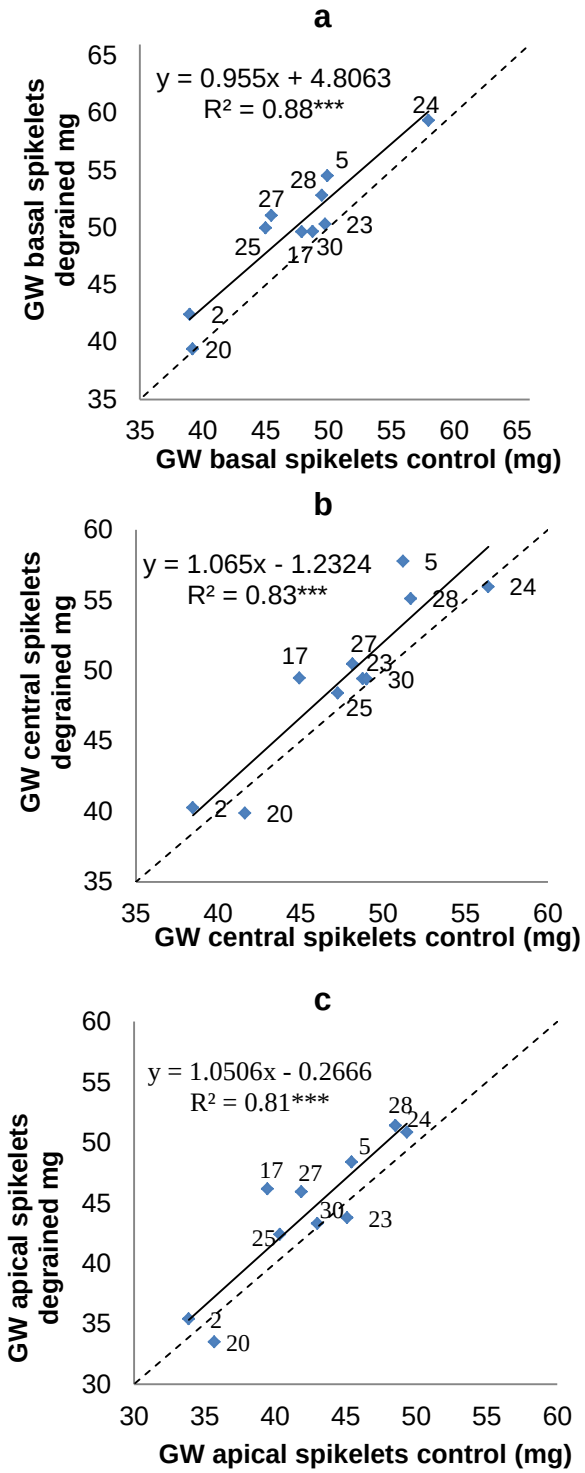


Figure 6.7 Linear relationship between grain weight (GW 100% DM) in the control treatment and degraining treatment for (a) basal spikelets, (b) central spikelets (c) apical spikelets for 10 genotypes in 2013. Dashed line represents the 1:1 ratio.

In 2013, an effect of the degraining treatment was observed for basal spikelets ($P < 0.001$), central spikelets ($P < 0.05$) and apical spikelets ($P < 0.01$). No degraining treatment x genotype interaction was observed for

grain weight in basal, or central spikelets, but a trend for an interaction was observed in apical spikelets (*P* = 0.08) in 2013.

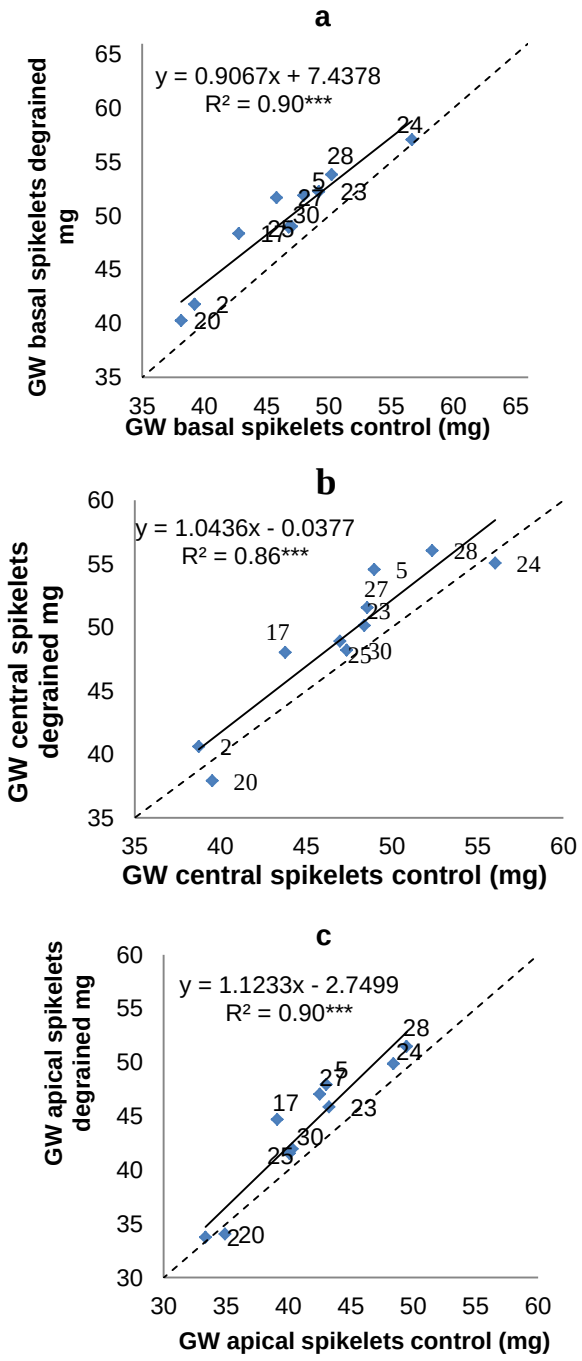


Figure 6.8 Relationship of grain weight (GW, 100% DM) in control treatment versus degraining treatment for (a) basal spikelets, (b) central spikelets (c) apical spikelets for 10 genotypes. Values represent means of 2012 and 2013. Dashed line represents the 1:1 ratio.

6.3.2.1 Degraining treatment x spikelet position interaction

The grain weight in the spikelet positions differed in 2012 ($P < 0.001$). The difference between basal and apical spikelet was 5.24 mg and between central and apical spikelets was 5.32 mg. There was an interaction between spikelet position and cultivar ($P < 0.01$). There was no interaction between degraining treatment and spikelet position (P=0.2), or cultivar x degraining treatment x spikelet position amongst the 10 genotypes.

The grain weight of the spikelet positions amongst the cultivars differed in 2013 ($P < 0.001$). There was an interaction between spikelet position and cultivar ($P < 0.001$). However no interaction was observed for degraining treatment x cultivar (P=0.14) or cultivar x degraining treatment x spikelet position amongst the 10 genotypes.

Averaging over years, there was an overall effect of spikelet position ($P < 0.001$), with basal and central spikelets 13% and 12% heavier that apical spikelets. The cultivar x spikelet position interaction was significant ($P < 0.001$) and the degraining treatment x spikelet position was significant; the increase in grain weight compared to control treatment with degraining was 6.4%, 4.2% and 5.3% in basal, central and apical spikelets, respectively, ($P < 0.05$). However, the degraining treatment x cultivar x spikelet position interaction was not significant.

6.3.3 Leaf lamina, leaf sheath and true stem DM responses to degraining for 10 genotypes

In 2012, a positive effect of the degraining treatment was observed on leaf lamina (+10.6%; $P < 0.01$), leaf sheath (+10.7%; $P < 0.001$) and true stem DM shoot^{-1} (+8.87%; $P < 0.001$), but there were no degraining treatment x genotype interactions (Figure 6.10). The increase in leaf lamina DM shoot^{-1} with degraining ranged from 0.4% (Bacanora88) to 34.3% (Brbt1*2/k), in leaf sheath from 4.7% (Tc870344/) to 22.6% (Siete cerros) and in true stem DM shoot^{-1} from 3.14% (Becard) to 13.2% (Yav_3/sco).

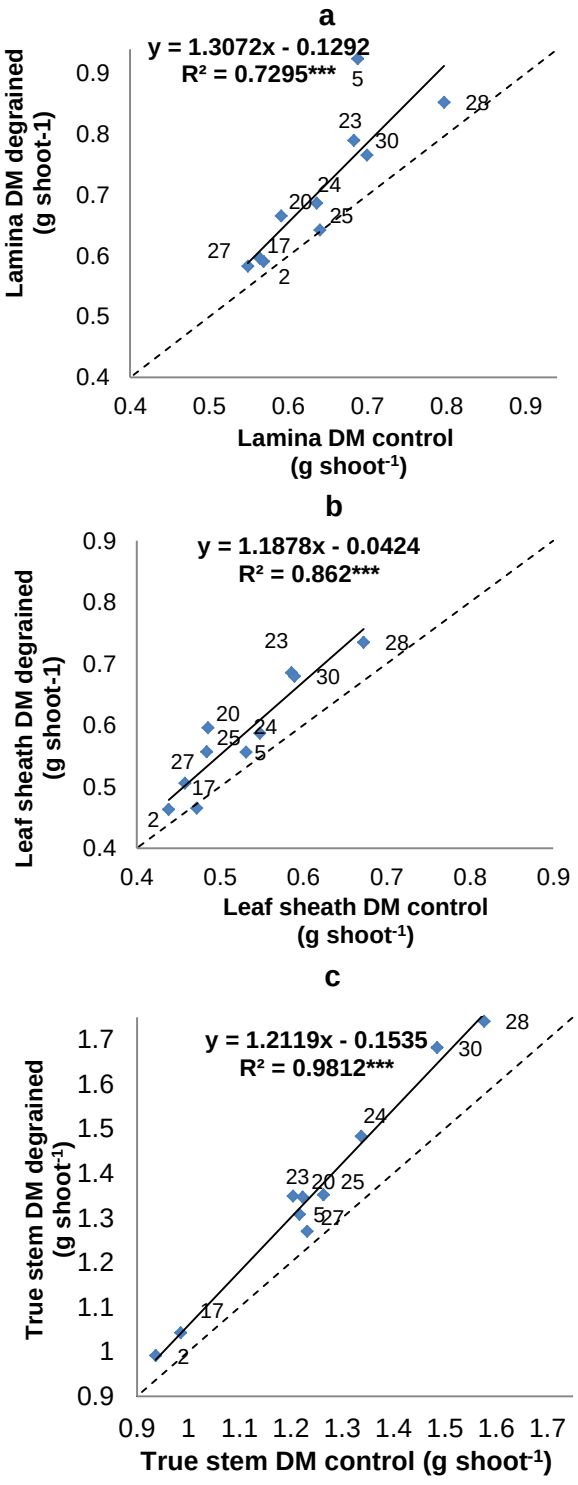


Figure 6.9 Relationship for (a) leaf lamina dry weight, (b) leaf sheath dry weight and (c) true stem dry weight per shoot in degrained versus control shoots for 10 genotypes in 2012. Dashed line represents the 1:1 ratio.

In 2013, a positive effect of the degraining treatment on the lamina (+7.98%; $P < 0.05$), leaf sheath (+9.03%; $P < 0.001$) and true-stem (+10.9%; $P < 0.001$; Fig. 6.10) DM shoot^{-1} was observed. There was no interaction between degraining treatment and genotype for leaf lamina and leaf sheath, but a significant interaction for the true stem DM shoot^{-1} ($P < 0.001$). For the true stem, the response to degraining ranged amongst genotypes from 0.6% (Becard) to 29.5% (Tacupetof2).

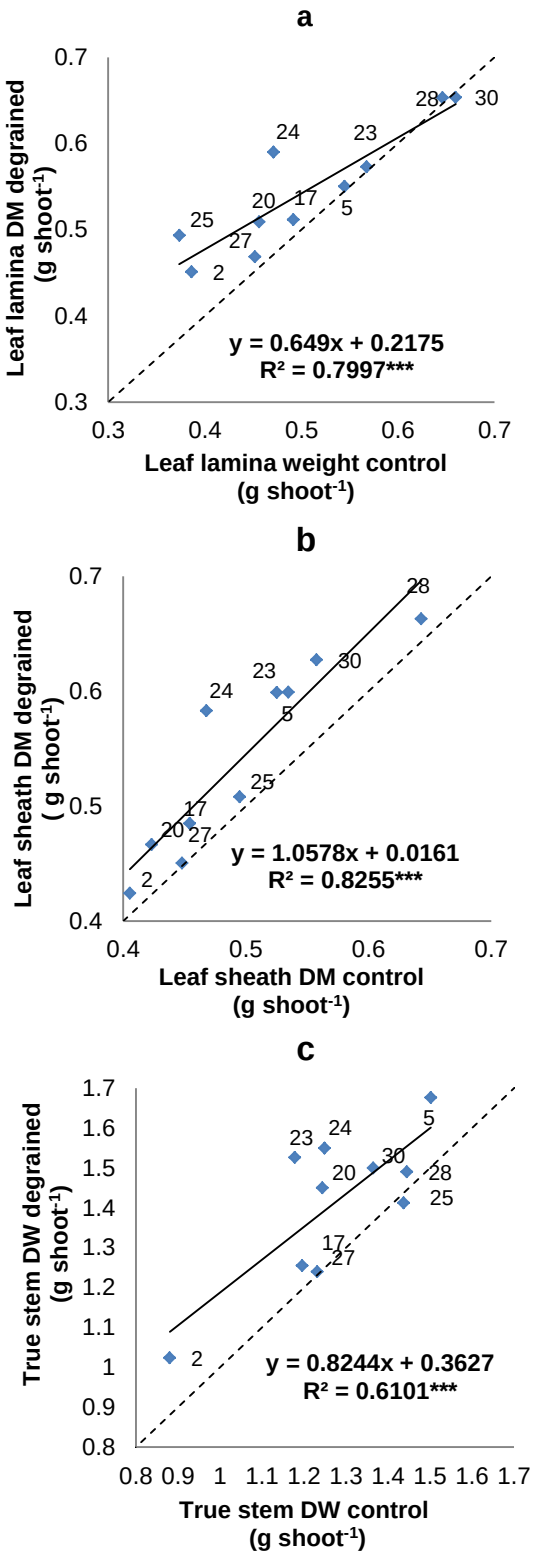


Figure 6.10 Relationship for (a) leaf lamina dry matter, (b) leaf sheath DM and (c) true-stem DM per shoot in degrained versus control shoots for 10 genotypes in 2013. Dashed line represents the 1:1 ratio.

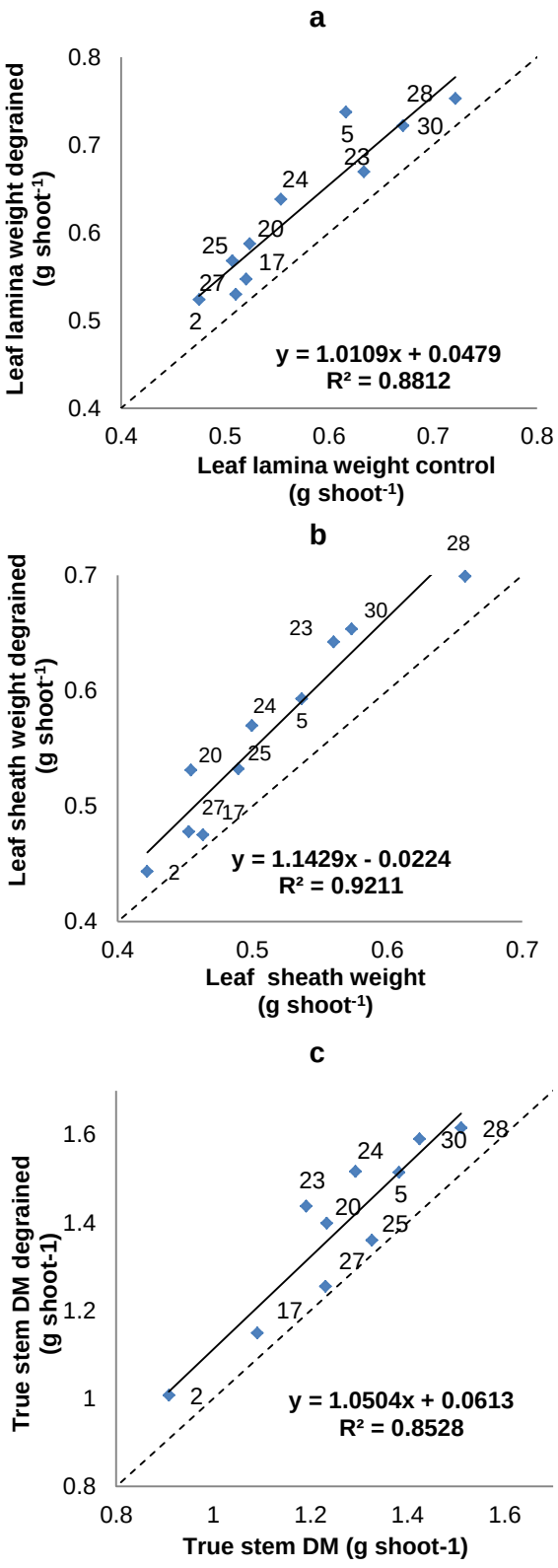


Figure 6.11 Relationship for (a) leaf lamina dry matter (DM), (b) leaf sheath DM and (c) true-stem DM per shoot in degrained versus control shoots for 10 genotypes. Values represent the means of 2012 and 2013. Dashed line represents the 1:1 ratio.

6.3.4 Stem WSC responses to degraining for 10 cultivars

In 2012, there was a positive effect of the degraining treatment (+83.3%; *P* < 0.01) on true-stem WSC (g shoot^{-1}) at PM. The responses of genotype to degraining ranged from 29.1% (Tc870344/) to 205% (Yav_3/sco) (*P* < 0.01). In 2013 there was a strong trend for an increase in stem WSC (+125%; *P* = 0.06). However, the cultivar x treatment interaction was not significant. Averaging over years the effect of the degraining treatment on stem WSC was significant (*P* = < 0.001), but the genotype x treatment interaction was not significant (Table 6.2).

Table 6.2 Water soluble carbohydrates in stem and leaf sheath at physiological maturity in the control and in degrained shoots in 2012, 2013 and mean of two years for 10 CIMCOG genotypes. (g WSC shoot^{-1})

	Stem Water soluble carbohydrate (g WSC shoot^{-1})					
	2012		2013		Means	
Cultivar	Control	Treatment	Control	Treatment	Control	Treatment
Bacanorat88	0.044	0.069	0.003	0.044	0.023	0.059
Brbt1*2/k	0.048	0.101	0.005	0.109	0.027	0.104
Pbw343*2	0.048	0.074	0.004	0.049	0.026	0.064
7cerros	0.081	0.142	0.010	0.099	0.045	0.125
Tacupeto/b	0.060	0.122	0.009	0.071	0.034	0.101
Tc870344/	0.111	0.144	0.011	0.115	0.061	0.132
Trap#1	0.051	0.136	0.005	0.046	0.028	0.100
Becard/k	0.055	0.076	0.003	0.009	0.029	0.049
Wbll1*2/	0.071	0.129	0.016	0.123	0.043	0.127
Yav_3/sco	0.042	0.127	0.014	0.112	0.028	0.121
Means	0.061	0.112	0.008	0.078	0.034	0.098
P-value(ENT)		**		**		***
SED(df=18[a], 48[b])		0.01		0.02316		0.01
Year						***
SED						0.01
Year*Gen						ns
SED						0.0184
Cult x Trat		**		ns		ns
SED		0.01184		0.03329		0.0184
Trat		*		P=0.06		***
SED		0.00631		0.01195		0.00582

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level. [a]=Degrees of freedom for individual years, [b]= Degrees of freedom for cross-year mean.

6.3.5 Senescence parameters

Senescence parameters were measured in the unmanipulated plot areas. NDVI senescence rate in 2012 ranged from 0.00037 to 0.00056 $^{o}Cd^{-1}$ ($P < 0.001$) and from 0.00072 to 0.00084 $^{o}Cd^{-1}$ ($P < 0.001$) in 2013. A year effect was observed ($P < 0.001$) and the year x genotype interaction was significant ($P < 0.001$).

SPAD senesce rate in 2012 ranged from 0.025 to 0.049 $^{o}Cd^{-1}$ ($P < 0.05$) and from 0.00014 to 0.02813 $^{o}Cd^{-1}$ ($P < 0.001$) in 2013. No year effect was observed and the year x genotype interaction was not significant. Flag-leaf senescence rate from the visual senescence scores in 2012 ranged amongst genotypes from 0.008 to 0.015 $^{o}Cd^{-1}$ ($P < 0.05$) and there were no differences in 2013. Averaging over years, visual score senescence rate ranged from 0.009 to 0.014 $^{o}Cd^{-1}$ ($P < 0.05$); no year effect was observed and the year x genotype interaction was significant ($P < 0.05$) (Table 6.3).

In 2012 start of senescence ranged from 319.3 (Brbt1*2/k) to 600.4 (Wbll1*2/) ^{o}Cd ($P < 0.001$) and in 2013 from 344.5 (Brbt1*2/k) to 504.6 (Siete cerros) ^{o}Cd ($P < 0.05$). End of senescence ranged from 747 (Bacanorat88) to 879.3 (Tc870344/) ^{o}Cd and from 750.7 (Bacanorat88) to 915 (Yav_3/sco) ^{o}Cd in 2012 and 2013, respectively ($P < 0.05$). Averaging over years, start of senescence ranged from 325.6 (Brbt1*2/k) to 525.6 (Wbll1*2/) ^{o}Cd ($P < 0.001$). An effect of year was observed ($P < 0.05$) and an interaction between year x cultivar ($P < 0.001$). Similarly, averaging over years, end of senescence ranged from 748.8 (Bacanorat88) to 891.5 (Tc870344/) ^{o}Cd in the two years. No effect of year or year x cultivar was observed (Table 6.4).

Table 6.3 Rate of senescence in unmanipulated crop estimated from NDVI from spike emergence (GS59) to physiological (PM) ($^{o}Cd^{-1}$), from flag-leaf SPAD from GS65 to PM (SPAD units $^{o}Cd^{-1}$) and from flag-leaf senescence visual score (FLS) from anthesis (GS65) to PM for 10 wheat genotypes. Values represent means across 2012 and 2013.

	NDVI Sen Rate $^{o}Cd^{-1}$			SPAD Sen Rate $^{o}Cd^{-1}$			FLsen Rate $^{o}Cd^{-1}$		
Genotype	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
Bacanorat88	0.00052	0.00084	0.00068	0.04423	0.01852	0.03137	0.013	0.015	0.014
Brbt1*2/k	0.00055	0.00073	0.00064	0.04905	0.02575	0.03740	0.008	0.012	0.010
Pbw343*2	0.00056	0.00075	0.00066	0.04055	0.01444	0.02749	0.011	0.012	0.012
7cerros	0.00045	0.00076	0.00061	0.03942	0.02345	0.03144	0.011	0.015	0.013
Tacupeto/b	0.00037	0.00076	0.00057	0.03669	0.01264	0.02466	0.009	0.010	0.009
Tc870344/	0.00052	0.00077	0.00064	0.04096	0.02813	0.03455	0.010	0.011	0.010
Trap#1	0.00054	0.00077	0.00065	0.04235	0.02506	0.03370	0.015	0.012	0.014
Becard/k	0.00055	0.00073	0.00064	0.04719	0.02737	0.03728	0.013	0.010	0.011
Wbll1*2/	0.00043	0.00072	0.00058	0.02577	0.00014	0.01296	0.014	0.008	0.011
Yav_3/sco	0.00055	0.00078	0.00066	0.03229	0.01081	0.02155	0.010	0.009	0.010
Means	0.00050	0.00076	0.00063	0.03985	0.01863	0.02924	0.011	0.011	0.011
P-value(ENT)	***	***	***	*	***	***	*	ns	*
SED (18^{a}, 36^{b})	0.0000295	0.0000186	0.0000174	0.0055500	0.0049900	0.0037340	0.0017	0.0025	0.0015
Year			***			***			ns
SED			0.0000071			0.002131			0.00084
Year*Gen			***			ns			*
SED			0.00002443			0.005444			0.00219

† Significant at $P = 0.10$; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level. ^{a}=Degrees of freedom for individual years, ^{b}= Degrees of freedom for cross-year mean.

Table 6.4 Duration of flag leaf senescence: Start of senescence (^{o}Cd post GS65) and End of senescence (^{o}Cd) for 10 genotypes in two years.

Cultivars	2012	2013	2012	2013	Mean	Mean
	Start of Sen ^{o}Cd		End of senescence ^{o}Cd		Start of Sen ^{o}Cd	End of Sen ^{o}Cd
Bacanorat88	411.4	368.3	747.0	750.7	400.6	748.8
Brbt1*2/k	319.3	344.5	785.1	863.9	325.6	824.5
Pbw343*2	484.6	365.0	845.3	865.7	454.7	855.5
Siete cerros	466.0	504.6	795.2	836.9	485.3	816.1
Tacupeto/b	417.5	458.7	812.1	859.9	438.1	836.0
Tc870344/	509.8	385.3	879.3	903.6	460.0	891.5
Trap#1	503.3	453.1	781.2	831.9	478.2	806.6
Becard/k	507.7	464.3	837.2	872.9	486.0	855.0
Wbll1*2/	600.4	450.3	860.2	865.2	525.3	862.7
Yav_3/sco	496.0	472.4	833.8	915.0	484.2	874.4
Means	471.6	426.7	817.6	856.6	453.8	837.1
Max	600.4	504.6	879.3	915.0	525.3	891.5
Min	319.3	344.5	747.0	750.7	325.6	748.8
P-value(ENT)	***	*	*	*	***	***
SED	27.49	42.34	34.39	35.78	23.97	24.81
Year					*	ns
SED (df)					16.01(4)	33.18(4)
Year*Gen					***	ns
SED (df)					31.24 (29)	47(36)

6.3.6 Correlation between grain yield, degraining responses and source-type traits

Averaging across years, a trend was observed for a positive association amongst genotypes between grain response to degraining and SPAD value at physiological maturity in the 26 genotypes (R^2 = 0.36, *P* = 0.08). Higher grain growth response to degraining was also associated with greater flag-leaf chlorophyll content amongst the 10 CIMCOG genotypes (Table 6.5).

Averaging over years, grain weight response to degraining showed a positive association with grain yield (R^2 = 0.37, *P* = 0.06). Similarly, a trend for a positive association was observed with anthesis date (R^2 = 0.36, *P* = 0.06). Grain weight response to degraining was also positively associated with pre-anthesis RUE_{PAR} (R^2 = 0.44, *P* < 0.05). Visual flag leaf

senescence rate showed a trend for a negative association with TGW. A trend for a positive association was observed between SPAD at PM and GW responses to degraining amongst the ten genotypes (Table 6.5).

Table 6.5 Correlation coefficients between physiological traits (Grain weight (GW) for degraining treatment, GW control, Grain yield (GY), Grains m-2 (GM2), anthesis date GS65 (AD), plant height (cm), Post anthesis radiation intercepted (MJ m-2), Pre-anthesis RUEPAR, Senescence traits (SPAD rate, NDVI rate, Visual FLS rate, start of senescence, end of senescence, NDVI and SPAD calculated at physiological maturity) versus Grain weight responses to degraining (GW Resp D), grain yield (GY), Anthesis date (AD), above ground dry matter (AGDM) at GS91 (gm-2) and grain weight (TGW)) for 10 wheat genotypes. Values represent means across 2012 and 2013.

Traits	GW Resp D A+10 (mg)	GW Resp D A+10 (%)	GY	AD	AGDM91	TGW
GW degraining A+10	0.57 p=0.08	0.49	0.17	0.34	0.22	0.94***
GW Control (mg)	0.27	0.18	-0.03	0.17	0.19	0.97***
GY (t/ha)	0.57 p=0.08	0.61 p=0.06	-	0.52	0.74*	-0.03
GM2	-0.18	-0.09	0.31	-0.06	0.11	-0.95***
AD	0.58 P=0.07	0.60 p=0.06	0.52	-	0.49	0.25
Plant height	-0.08	-0.18	-0.24	-0.02	0.10	0.83**
Post GS65 ad. Int.	-0.37	-0.42	0.04	0.10	0.47	0.34
Pre-Anthesis RUE	0.66*	0.67*	0.70*	0.39	0.51	-0.19
SPAD sen. rate	-0.48	-0.50	0.12	-0.90***	-0.39	-0.13
NDVI sen, rate	-0.32	-0.27	0.30	-0.39	0.08	-0.29
Visual FLS sen, rate	-0.38	-0.32	-0.31	-0.36	-0.44	-0.566†
Start FLS senescence	0.03	0.01	0.12	0.37	0.42	0.22
End FLS Senescence	0.16	0.08	0.09	0.17	0.37	0.75*
NDVI at PM	-0.44	-0.45	-0.44	-0.18	0.01	-0.41
SPAD at PM	0.59p=†	0.57 †	0.12	0.732*	0.08	0.26

† Significant at *P* = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level

6.3.7 Source sink balance model

The model components for grain sink size were grains m^{-2} and PGW (grain weight in degraining treatment). Grains m^{-2} ranged from 13,107 to 19,517 and PGW ranged from 36.7 to 54.g mg (P < 0.001) amongst genotypes. Grain source size did not differ amongst the cultivars (Table 6.6).

Table 6.6 Grains m^{-2} (GM2), potential grain weight (PGW), grain sink size, stem water soluble carbohydrate (WSC) at GS65+7 days, PAR radiation-use efficiency pre-anthesis from GS31- GS71+7d (RUE Pre A), PAR interception post-anthesis (GS64) (PAR int. Post A), grain source size and source-sink balance for 10 wheat genotypes. Values represent means across 2012 and 2013. See equation 6.1 and 6.2 in section 6.1.

Genotype	GM2	PGW (mg)	Sink Size (gm^{-2})	WSC A+7 (gm^{-2})	RUE Pre A (gMJ^{-1})	PAR inter Post A	Source Size (gm^{-2})	S-Sink Balance (gm^{-2})
Bacanorat88	19517	38.3	747.5	85.2	2.83	216.9	688	59.8
Brbt1*2/k	14654	49.4	723.9	77.0	2.62	234.2	683	40.6
Pbw343*2	16870	46.7	789.1	74.9	2.85	219.6	695	93.8
7cerros	17247	36.7	632.4	93.5	2.62	236.1	706	-73.7
Tacupeto/b	16132	51.3	827.5	86.5	3.04	229.6	776	51.3
Tc870344/	13107	54.6	715.5	73.8	2.60	241.4	687	28.3
Trap#1	15281	46.2	706.7	80.9	2.62	242.2	706	0.49
Becard/k	15098	49.7	749.8	60.1	2.86	239.5	743	7.27
Wbll1*2/	13795	53.5	737.9	76.4	2.74	232.8	702	36.0
Yav_3/sco	16780	46.6	780.7	83.4	2.41	262.0	734	47.2
Mean	15848	47.3	741.1	79.2	2.72	235.4	712	29.1
Max	19517	54.6	827.5	93.5	3.04	262.0	776	93.8
Min	13107	36.7	632.4	60.1	2.41	216.9	683	-73.7
P-value(ENT)	***	***	**	**	0.07	ns	ns	ns
SED(36)	835.70	0.767	39.320	7.150	0.189	16.98	64.70	68.00
Year	ns	*	ns	**	**	*	*	*
SED(4)	429.50	0.482	24.91	2.45	0.168	10.03	33.80	29.50
Year*Gen	ns	*	ns	***	ns	ns	*	*
SED(39.66)	1200.7	1.136	58.34	9.90	0.304	24.90	93.1	95.8
Corr with GY	0.31	0.17	0.85***	-0.21	0.22	0.04	0.39	0.75*
Corr with YoR	-0.51	0.82**	0.70*	-0.57	0.10	0.20	0.22	0.69*

† Significant at P = 0.10; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level. [a]=Degrees of freedom for individual years, [b]= Degrees of freedom for cross-year mean.

There was a positive association between percentage grain weight response (%, mg) to degraining at GS65+10 days and the source-sink balance according to the model (Fig 6.12).

a) b)

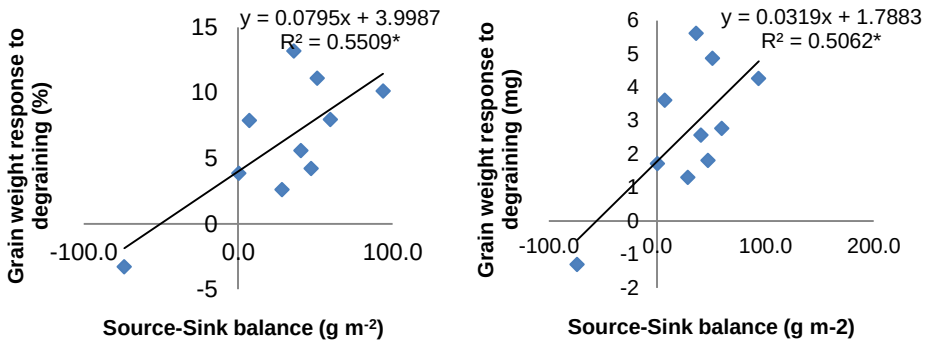


Figure 6.12 a,b) Relationship between grain weight response to degraining (% and mg) and Source sink balance (g m^{-2}) estimated according to source-sink balance model (Bingham et al., 2007). Values represent the means of 2012 and 2013.

6.3.8 Grain yield versus source-sink balance estimated according to the model

A positive and linear association was observed between source-sink balance and grain yield amongst the 10 genotypes (R^2=0.56, P < 0.05). Indicating that genotypes with higher yields were less sink limited (Figure 13).

a)

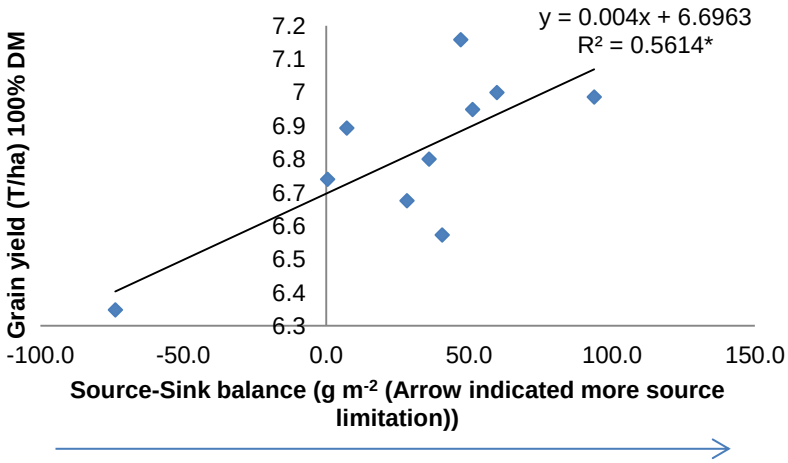


Figure 6.13 Relationship between grain yield (t ha^{-1} 100% DM) and source-sink balance (g m^{-2}) estimated according to source-sink balance model (Bingham et al., 2007) for 10 genotypes. Values represent means of 2012 and 2013.

6.3.9 Changes in degraining responses and source and sink type traits with year of release

Grain weight was linearly positively associated with YoR (r = 0.68 *P* < 0.05) with a gain of 0.246 mg year^{-1}. Potential grain weight showed a gain of 0.346 mg year $^{-1}$ (r = 0.81, *P* < 0.01). Grain response to degraining increased with year of release by 0.221% year^{-1}. NDVI at physiological maturity estimated (from the linear regression with thermal time post GS59) showed a negative association with YoR. Therefore, NDVI at PM it is indicative of stay-green, and if stay green is decreasing this may be associated with source limitation in modern cultivars.

Table 6.7 Source sink type traits versus year of release (YoR) for 10 wheat genotypes. Values represent means across 2012 and 2013.

Traits	Correlation with YoR	Linear slope yr^{-1}	Prob. Linear reg.
GY (t ha^{-1} 100% DM)	0.574	0.010	0.083
GW mg	0.684*	0.246	0.029
PGW mg	0.818**	0.346	0.004
Post-anthesis RUE_{PAR} g MJ^{-1}	0.294	0.003	0.41
SPAD Sen rate $^{o}Cd^{-1}$	-0.231	-0.002	0.52
NDVI Sen rate $^{o}Cd^{-1}$	0.002	04.56E-09	0.996
Visual FLS Sen rate $^{o}Cd^{-1}$	-0.602†	-7.05E-09	0.066
GW resp to deg (%)	0.646*	0.22180	0.044
GW resp to deg (mg)	0.692*	0.099	0.027
SPAD at PM	0.190	0.02994	0.599
NDVI at PM	-0.638*	-0.00143	0.047
Start of FLS sen ^{o}Cd [a]	0.014	5.81E-02	0.968
End of FLS sen ^{o}Cd [b]	0.508	1.48	0.134

[a]= When flag leaf senescence score was 2 [b]= when flag leaf senescence score was 9

6.4 Discussion

6.4.1. Evidence for changes in source-sink balance with plant breeding

The balance of the source and sink size in the post-anthesis period in a crop is an important factor influencing the yield potential of wheat (Reynolds et al., 2005). Grain growth responses to degraining (where assimilate supply per grain is effectively increased by 100%) of ca. 0–10% are indicative of sink limitation of grain growth, whereas those of ca.10–20% are indicative of co-limitation of grain growth by sink during the earlier phase of grain filling and source during the latter phase of grain filling (Acreche and Slafer 2009). In this study, the 26 genotypes showed positive grain weight responses to degraining ranging from 1.0 – 30.2% indicating a level of either sink limitation of grain growth (< 10%) or co-limitation by source or sink (> 10%). The results for the subset of ten genotypes again showed positive responses up to 13.17%. A similar degraining treatment was carried out by Aisawi et al. (2015) at anthesis + 14d on 12 CIMMYT spring wheat genotypes released from 1966 to 2009 in NW Mexico where the increase in grain growth response to degraining ranged from 0.5 to 13.2%. Gaju et al., (2014) also reported grain weight responses to degraining in a degraining treatment at anthesis +14d with increases from 10.6 to 14.7% comparing DH lines derived from a cross between UK winter wheat Rialto and a CIMMYT large-spike phenotype advanced line LSP2. So there is evidence that current CIMMYT spring wheats may have some co-limitation of yield by source and sink. Nevertheless, Aisawi et al. (2015) found no change in degraining responses with year of release for 12 CIMMYT spring wheat genotypes.

The present results showed that grain weight response to degraining was positively associated with grain yield in the control crop amongst the 26 genotypes. So breeders may now need to pay more attention to increasing grain source size alongside sink size. For example, in order to increase

source and sink simultaneously, breeders could aim to increase pre-anthesis RUE hence grain number m^{-2} and post-anthesis source in relation to remobilized stem WSC simultaneously. Another avenue to increase post-anthesis source is the stay green trait; this trait could be enhanced by maximizing the canopy N accumulation at anthesis and/or slowing post-anthesis N remobilization during grain filling (Derkx et al., 2012; Gaju et al., 2014b).

The negative association between NDVI calculated at physiological maturity and year of release indicated that modern cultivars have less green canopy area duration post-anthesis. This is in agreement with the positive relationship between grain weight response to degraining and year of release indicating the extent of source limitation increased in modern cultivars. Therefore, one of the potential reasons for a lack of increase of grain weight to its potential capacity is due to post-anthesis competition for assimilates (Flitham et al., 1997) and the increased sink size in modern cultivars. However, in the present study other senescence parameters such as flag-leaf VS rate of senescence and flag-leaf SPAD rate of senescence were not associated with year of release. SPAD senescence rate actually showed a weak non-significant negative association with response to degraining but caution is needed to interpret this trend as there may be autocorrelation with anthesis date. In chapter 7 relationships between senescence parameters and source-sink balance will be discussed further in a wider association panel.

There was a positive association between plant height and biomass in the present study. Future increases in plant height, however, may be limited because of lodging risk (Berry et al., 2007). Therefore, it will be necessary to identify strategies for increasing grain source and biomass while maintaining plant height for future gains in yield potential in (Foulkes et al., 2010; Reynolds et al., 2012).

Grain weight and potential grain weight (as indicated by the final grain weight in the degrained treatment) increased with year of release by 0.246 mg year^{-1} and 0.346 mg year^{-1}, respectively, based in the subset of 10 genotypes (with release dates from 1966-2009). Furthermore, a trend for a positive association was found between the grain weight degraining response and the potential grain weight amongst the 26 CIMCOG cultivars. This suggests that breeders have been increasing grain sink size in recent years manly by increasing the potential grain size. Similar findings for recent CIMMYT spring wheat releases were reported by Lopes et al. (2012) and Aisawi et al. (2015). Future strategies for gains in PGW may include modification of structural properties of the ovary and pericarp cell walls before anthesis (Foulkes et al., 2010).

6.4.2. Effect of spikelet position

Slafer and Savin (1994) found that altering the source-sink ratio by removing all spikelets from one side of the spike 10 days after anthesis did not affect the grain weight at apical, central or basal spikelet positions, and grain yield in the studied cultivars was either sink or co-limited by source and sink. In the present results, the spikelet positions responded slightly differently to the degraining treatment, higher grain weight responses were observed in basal and apical than in central spikelets. These results suggest that to some extent the basal and apical spikelets are less sink limited than central spikelets. Therefore, increases in PGW may be relatively more beneficial in the central rather than the distal spikelets in future breeding strategies to increase grain sink size.

6.4.3. Source-sink balance at whole crop scale

Calculations of source-sink balance per unit area following the model of Bingham et al. (2007) generally indicated a close balance between the grain sink size and the source size in the 10 genotypes. The results showed a positive association between source-sink balance and grain yield indicating that the extent of sink limitation was reduced (i.e. source

limitation increased) in the higher yielding CIMCOG cultivars. This was in agreement with the positive association between grain yield and grain growth response to degraining amongst the 26 CIMCOG genotypes indicating co-limitation by source and sink in the higher yielding cultivars. In the 10 genotypes, there was also a positive association between grain weight response to degraining (%) and year of release. The increment in PGW with YoR was 0.34 mg year^{-1} from 1966 to 2009 suggesting that increases in PGW have contributed to the increases in extent of source limitation in the CIMMYT breeding for spring wheat program in recent years. It appears that PGW is the main trait responsible for the increase in the extent of source limitation in modern CIMMYT cultivars according to the present results.

More generally the association between grain weight response to degraining and the source-sink balance estimated from the model must be interpreted cautiously since the final grain weight in the degraining treatment was used in the model to estimate potential grain weight, so these two estimates for source-sink balance are not completely independent.

6.4.4. Relationships between source type traits and source-sink balance

Lopes et al. (2012) reported a positive association between NDVI at PM and grain yield under heat and heat combined with drought environments in NW Mexico in two populations, and NDVI senescence rate was negatively associated with grain yield in one population. The present results generally showed senescence rate parameters were not closely associated amongst cultivars with grain yield in potential conditions. Similarly, Lopes et al. (2012) did not find associations between stay-green parameters and grain yield under full irrigation conditions. The increase in the extent of sink limitation in higher yielding cultivars according to the present results may however, to some extent be explained by the negative association between stay-green as indicated by the NDVI at PM and year

of release. In this scenario, as the grain sink size has increased amongst the CIMCOG genotypes, the source size in terms of post-anthesis radiation interception may have decreased causing the grain growth of the higher yielding cultivars to be co-limited by source and sink.

Overall these results suggested that in the highest yielding genotypes in the CIMCOG the source still needs to be enhanced in order to exploit fully the sink capacity. Increasing pre-anthesis RUE and stem WSC may offer promise in this respect (Fischer and Edmeades 2010). Stem WSC at anthesis was generally low compared with values reported in the literature. Therefore, there may be scope to increase this component of source in future years in the CIMMYT spring wheat breeding program. Stem WSC is a trait reported to have high heritability in wheat (Ruuska et al., 2006).

Present results showed NDVI at PM with year of release is decreasing. So breeders may need to pay more attention to maintaining green canopy area in the latter stages of grain filling. Traits that may favour stay green could be deeper roots. Lopes and Reynolds (2010) found that relatively deeper distribution of roots was associated with delay of senescence in CIMMYT synthetic wheat derivatives compared to check parental bread wheats. It has been reported that reduced efficiency of leaf lamina N remobilisation is associated with greater flag leaf senescence duration under high N in bread wheat cultivars grown in UK and France (Gaju et al., 2014a). Higher post-anthesis N uptake and greater root density at depth could also be used as a potential traits to enhance stay-green and the grain source size for grain filling (Foulkes et al., 2009).

The present results apparently showed flag-leaf SPAD at PM and flag-leaf SPAD senescence rate) had unexpected trends for associations with grain growth response to degraining such that prolonged green area persistence of canopy or flag leaf was associated with greater response to grain growth (increased source limitation). These trends, however, may be an autocorrelation between traits that are each related with anthesis date

as mentioned above; thus, grain yield was positively associated with anthesis date and AD was negatively associated with SPAD senescence rate and positively associated with SPAD at physiological maturity.

6.4.5 Conclusions

With regard to the first hypothesis it is confirmed that post-anthesis source-sink balance differs amongst the 26 CIMMYT spring wheat cultivars in the CIMCOG Panel.

Grain weight response to de-graining was greater in higher yielding cultivars in the CIMCOG panel indicating grain yield progress has resulted in cultivars closer to source limitation than their predecessors. Furthermore, the genetic variation in source sink balance amongst the 26 CIMMYT spring wheat cultivars appeared to be explained by a combination of increase in potential grain weight and a decrease in flag-leaf green area duration with plant breeding in the CIMMYT spring wheat program.

CHAPTER 7 WAMI PANEL FIELD EXPERIMENT POST-ANTHESIS RESULTS

7.1 Introduction

This chapter describes the results of the source-sink manipulation degraining treatment carried out on the Wheat Association Mapping Initiative (WAMI) panel in 2012-3 and 2013-4 at the CIMMYT Norman E. Borlaug experimental field station, Ciudad Obregon, Mexico. The results are presented for individual years and across years. In the present study in 2013 senescence kinetics was measured in the 294 genotypes using the flag-leaf visual score and canopy RCC methodologies (see section 3.2.5). Responses of grain weight to a degraining treatment and associated senescence traits were examined in the WAMI panel.

In the experiments, the source-sink balance was manipulated by removal of spikelets (all spikelets down one side of the spike) at GS65+10/15 days. Grain weight responses to degraining can indicate whether the limitation to grain growth is due to grain storage capacity (grain sink size) or the photosynthetic capacity of the crop to supply assimilate to grains (grain source size), as discussed in Chapter 6. In addition, indirect measurement of stem WSC utilization was assessed in degrained and control shoots in WAMI lines by measuring the leaf and stem dry weight at harvest.

The aims of this chapter are:

- To quantify the genetic variation in responses to a degraining source-sink treatment imposed at GS65+10/15 days on the 294 WAMI panel genotypes and to evaluate if the grain weight of modern high yielding WAMI spring wheat genotypes differs in the degree of sink limitation of grain growth.
- To evaluate the genetic variation in senescence parameters according to measurements of NDVI, flag-leaf visual senescence score and quantify correlations with grain growth responses to degraining in genotypes of the WAMI panel.

- To determine if the genetic variation in in response for leaf and stem dry weight to degraining at harvest is related with the grain growth response to degraining at GS65+10/15d in the WAMI panel.
- To Identify marker-trait associations for grain weight response to degraining, senescence-related traits and leaf and stem weight responses to degraining (as an indication of stem WSC utilization)

The specific hypotheses tested were:

- Post-anthesis source-sink balance differs amongst the 294 WAMI genotypes.
- Genetic variation in senescence durations and/or rates (NDVI, and visual FLS assessment) is associated with grain yield and source/sink balance in the 294 WAMI spring wheat genotypes.
- Marker trait associations can be identified for grain weight response to degraining (as an indicator of source sink balance) and senescence related parameters in the WAMI panel.

7.2 Materials and Methods

7.2.1 Experimental treatments

A degraining treatment was imposed to modify the grain sink size by manipulating the number of spikelets per spike at GS65+10 days in 2013 and at GS65+15 days in 2014. In each year, half of the spikelets (all spikelets down one side of the spike, terminal spikelet not removed and first fertile basal spikelet removed) were removed in 20 randomly selected shoots in 2013 and 10 randomly selected shoots in 2014. In control shoots, spikes were tagged at GS65+10/15 d and harvested for comparison.

294 WAMI spring wheat cultivars and advanced lines were used in this experiment (Table 7.1).

7.2.2 Crop measurements

7.2.2.1. Grain dry matter and partitioning and yield components

In each year, for all 294 genotypes at harvest the degraining and control shoots were cutting at ground level. In each plot the spikelets of degrained and control shoots were threshed by hand in 2013 and by using a thresher machine in 2014 (Wintersteiger A-1910 Ried/Austria). Grains were dried at 70 ^{o}C for 48 h and the dry weight recorded. A grain counter (Pfewffer, Contador) was used to count the number of grains in the degrained and control spikes in each year.

At harvest, stem and leaf (leaf lamina, leaf sheath and true stem) weight (20 stems per plot in 2013 for 294 genotypes and 10 stems per plot for a subset of 45 genotypes in 2014) was recorded after drying at 70 ^{o}C for 48 h. In 2013 in the sections of the plots not allocated for source-sink manipulation treatments, measurements of flag-leaf and canopy senescence were carried out.

7.2.2.2 Measurements in unmanipulated crop area in 2013

In 2013 in the unmanipulated plot area designated for combining, a subsample of 100 fertile shoots (those with a spike) were taken at random and cut at ground level, 1-2 days prior to harvest. The fresh weight was recorded and the plant material was dried at 75°C for 48 h and weighed. A sample of 200 grains was counted and weighed after drying for 48 h at 75^{o}C. These data were used for the calculation of grain yield and yield components (See section 3.5.4.1).

7.2.2.3 Senescence traits

7.2.2.3.1 Flag leaf score (Senescence)

In 2013 for all lines flag leaf visual score for senescence was measured in two replicates every 3-4 days using a diagnostic key (see appendix 1) from anthesis until complete flag-leaf senescence in the 294 genotypes in the control flag leaves. A logistic curve was fitted to estimate the senescence parameters as described in M&M (section 3.1.7.4c.). In addition NDVI was assed five times from heading to phisiological maturity as described in section 3.1.4.7b.

7.2.2.3.2 Reflectance chlorophyll content

In 2013, reflectance chlorophyll content was measured using the FieldScout CM 1000 Chlorophyll Meter (Spectrum Technologies Inc. Plainfield, IL) which measured ratios of reflected red and far-red light to calculate relative chlorophyll content as described in Chapter 3 (section 3.5.4.2). The measurements were carried out by placing the Field scout over the canopy at 20 cm height in each plot avoiding the borders. The senescence rate and the canopy greenness value at physiological maturity were estimated in each plot by fitting RCC linearly against thermal time (base temp. 0^{o}Cd) post GS59 (value at PM taken as an indicator of stay-green). The linear slope was originally negative, but was transformed to a positive value (higher value = faster senescence).

7.2.3 Genetic analysis

This study used an existing map for the WAMI panel (Sukumaran et al., 2015). WAMI was genotyped at CIMMYT Illumina using the iSelect 90K SNP array. A consensus map of the 90K SNP array already exists with genetic positions of the markers and the sequence information of the SNPs and the analysis as described in Sukumaran et al. (2015).

Marker-trait associations (MTAs) analysis was done with 90K SNP and 1992 DArT markers following the unified mixed model (Yu et al., 2006) method and comparisons were done in TASSEL (Bradbury et al., 2007) and R software (GAPIT package) for consistency of the MTAs (Lipka et al., 2012). The threshold for defining a marker to be significant was taken at 10^{-04}, considering the number of markers and the deviation of the observed F test statistics from the expected F test distribution (Sukumaran et al., 2012).

Table 7.1 List of the 294 genotypes in the WAMI panel with Identifiers

Cultivar	Genotype Name (WAMI population)	Subset 45 genotypes for leaf and stem DM assessment in 2014
1	COOK/VEE//DOVE/SERI/3/BJY/COC	
2	JUP/ZP//COC/3/PVN/4/GEN	
3	PFAU/VEE#5	
4	SERI M 82	•
5	VORONA/GEN	
6	KAUZ/GEN	
7	KEA/TOW//LIRA	
8	LIRA/URES//MILO 9G19-2-26	
9	MYNA/VUL//PRL	
10	JUPARE C 2001	
11	TIA.3	
12	CHOIX M 95	•
13	KAUZ*2/FN//KAUZ	
14	KAUZ*2/MNV//KAUZ	
15	PAT10/ALD//PAT72300/3/PVN/4/URES/5/PFAU	
16	PRINIA	•
17	CAR422/ANA//URES	

18	CIANO T 79	•
19	HD2206/HORK//BUC/BUL	
20	PAT10/ALD//PAT72300/3/PVN/4/BOW	
21	VORONA/CNO79	
22	AZ//KAL/BB/3/PGO	
23	BACANORA T 88	
24	FCT/3/GOV/AZ//MUS/4/DOVE/BUC	
25	INIFAP M 97	
26	KAUZ*2//DOVE/BUC/3/KAUZ	
27	KAUZ*2/TRAP//KAUZ	
28	LIRA/BUC	
29	PARA2//JUP/BJY/3/VEE/JUN/4/2*KAUZ	•
30	RHEA	
31	TOBARITO M 97	•
32	TRAP#1/BOW	
33	TURACO/CHIL	
34	KAUZ*3//TC*6/RL5406(RL6043)	•
35	KAUZ*2//TC*6/RL6081/3/KAUZ	
36	KAUZ*2/YACO//KAUZ	
37	PRL/VEE#6	
38	UP 2338	
39	ATTILA//ALTAR 84/AOS/3/ATTILA	
40	FANG60/7C	
41	HP 1761	
42	HYBRID DELHI 2172	
43	KAUZ//ALTAR 84/AOS	•
44	MNCH/3*BCN	
45	RAYON F 89	
46	SERI/7C	
47	SERI/NKT//2*KAUZ	
48	STAR//KAUZ/STAR	•
49	TILHI	•
50	JUPARE C 2001	•
51	URES/RAYON	
52	COMARA/TEG//WEAVER/3/LAJ3302	•
53	PICUS/4/CS(5A)/5RL-1//BUC/BJY/3/ALD/PVN/5/LAJ3302	
54	TIA.2/KAUZ	
55	HP 1731	
56	IAS62/ALDAN//2*SKAUZ	
57	KAUZ/RAYON	
58	KEA/TAN/4/TSH/3/KAL/BB//TQFN/5/PAVON/6/SW89.3064	
59	PASTOR/2*SITTA	
60	BHRIKUTI	
61	BL 1724	
62	BOW/PRL*3/6/WRM/4/FN/3*TH//K58/2*N/3/AUS-6869/5/PELOTAS-ARTHUR/7/HE1/3*CNO79//2*SERI	
63	BUC/PRL//WEAVER	
64	CHUM18/5*BCN	
65	LAJ3302/2*MO88	

66	MILAN/3/JUP/BJY//URES	
67	NL 750	
68	PUNJAB 96	
69	RABE/2*MO88	
70	SW89-5124*2/FASAN	
71	TIA.4/WL6572//RL6043/3*GEN/3/LUAN	
72	CAZO/KAUZ//KAUZ	
73	CHIR1//SHA5/WEAVER	
74	HUITES/4/CS/TH.SC//3*PVN/3/MIRLO/BUC	
75	KAUZ//BOW/NKT	
76	KAUZ/WEAVER	
77	MINO	
78	OASIS/SKAUZ//4*BCN	
79	SHA3/SERI//SHA4/LIRA/3/CHIR1/4/SHA7//PRL/VEE#6/3/FASAN	
80	SW89.5181/KAUZ	
81	W462//VEE/KOEL/3/PEG//MRL/BUC	•
82	GUAM92/KAUZ	
83	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/2*KAUZ	
84	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/KAUZ	
85	CMH84.3379/CMH78.578//MILAN	
86	HUAYTU CIAT	
87	OTUS	
88	TARACHI F 2000	
89	SITE/MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC	•
90	SKAUZ*2/FCT	
91	SURUTU-CIAT	
92	TAURUM	
93	INQALAB 91	
94	SKAUZ*2/FCT	
95	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	
96	KETUPA*2/PASTOR	
97	WEAVER/3/SAPI/TEAL//HUI/4/CROC_1/AE.SQUARROSA (213)//PGO/5/SKAUZ*2/SRMA	
98	KAUZ*2/TRAP//KAUZ/3/PASTOR/4/SKAUZ*2/SRMA	
99	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	
100	JUPARE C 2001	
101	ATTILA/3*BCN//BAV92/3/TILHI	
102	CROC_1/AE.SQUARROSA (205)//BORL95/3/PASTOR	•
103	CHEN/AE.SQ//WEAVER/3/SSERI1	
104	BAV92/3/OASIS/SKAUZ//4*BCN/4/PASTOR	
105	CROC_1/AE.SQUARROSA (205)//KAUZ/3/2*KAUZ*2/YACO//KAUZ	
106	WBLL1*2/KUKUNA	
107	ROELFS F2007	
108	HD2687	
109	PBW450	
110	MILAN/S87230//BAV92	
111	ATTILA/3*BCN*2//BAV92	
112	TOBA97/PASTOR	
113	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	•

114	ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1	
115	TUKURU//BAV92/RAYON	
116	SUNSU/CHIBIA	
117	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ	•
118	WBLL1*2/VIVITSI	
119	F6.74/BUN//SIS/3/YR/PAM	
120	PASTOR	
121	GALVEZ S 87	
122	PAVON	
123	VEE#8/5/VEE/4/KLTO//S12/J9281.67/3/MO/JUP	
124	IRENA	•
125	BB//TOB/CNO67/3/HUAC/4/TI-R/3/BB/PL//SX	
126	CULIACAN T 89	
127	KITE/PGO	
128	PSN/BOW//SERI	
129	GIM/LIRA	
130	URES/JUN//KAUZ	
131	PROINTA FEDERAL	
132	HI.1077	
133	SITTA	
134	URES//BUC/FLK/3/KAUZ	
135	ARIVECHI M 92	
136	TIA.1	
137	RL6043/4*NAC	
138	F60314.76/MRL//CNO79	•
139	ATTILA	
140	PFAU/VEE#9	•
141	BAU/OPATA	•
142	PRINIA	•
143	GRANERO INTA	
144	HUITES F 95	
145	KEA/BUC//FCT	
146	KITE/GLEN	
147	CHIL/BUC	
148	FILIN	
149	PRL/SARA//TSI/VEE#5	
150	JUPARE C 2001	
151	PJN/BOW//OPATA	•
152	VEE/PJN//TUI	
153	PARA2//JUP/BJY/3/VEERY#5.4/JUN/4/TUI	
154	VEE/PJN//2*TUI	
155	URES/BBL//KAUZ/3/KAUZ	
156	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA	•
157	DUCULA//HUI/TUB/3/CAZO	
158	SHUHA	
159	PROINTA GRANAR	
160	TZPP/SERI//BUC	
161	PIFED/DERN	

162	HIDHAB	•
163	W3918A/JUP	
164	JUN/GEN	
165	K134(60)/VEE//BOW/PVN	
166	ESDA/4/BD120/3/GTA/MXP//RUFF/FGO	•
167	VEE#8//JUP/BJY/3/F3.71/TRM/4/BCN/5/KAUZ	
168	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER	
169	HXL-F86/2*BAU	
170	VI/PIFED//VEE#8	
171	GOV/AZ//MUS/3/SARA	•
172	MON/IMU//ALD/PVN	
173	TUI*2/MILAN	
174	KAUZ*2/BOW//KAUZ	•
175	SIMORGH	
176	CAR853/COC//VEE/3/BOW/4/TUI/5/TUI	
177	NANJING 8646/KAUZ//BCN	
178	TUI/3/TMP64/TWN//SDY/4/RAYON	•
179	VEE#5/SARA//DUCULA	
180	CLC89//ESDA/KAUZ/3/BJY/COC//PRL/BOW	
181	NS-732/HER//KAUZ	•
182	3VASKAR/G303.1M.1.3.2.2.2//KAUZ/3/SKAUZ/4/KAUZ	•
183	TODY/3/JUP/BJY//SARA/4/TRAP#1/BOW/5/NL456/VEE#5	
184	CROC_1/AE.SQUARROSA (205)//JUP/BJY/3/SKAUZ/4/KAUZ	
185	PASTOR/3/VEE#5//DOVE/BUC	•
186	VEE#5//PF70354/MUS/3/PIFED/4/OR791432/VEE#3.2	
187	KAUZ/5/PAT10/ALD//PAT72300/3/PVN/4/BOW	
188	MRL/BUC//LIRA/5/BB//TOB/CNO67/3/HUAC/4/TI-R/3/BB/PL//SX	
189	SAAR	
190	MNCH/3*BCN	
191	JUP/BJY//URES/3/HD2206/HORK//BUC/BUL	
192	SITE/PIOS	
193	PASTOR//SITE/MO/3/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN	•
194	FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER	
195	CROC_1/AE.SQUARROSA (205)//KAUZ/3/ENEIDA	
196	F60314.76/MRL//CNO79/3/CHIL/PRL	
197	PASTOR/BAV92	
198	BARBET1	
199	MILVUS2	•
200	JUPARE C 2001	
201	WEEBILL1	
202	KAMBARA1	
203	BABAX.1B.1B*3/PRL	
204	PEWIT1	
205	FRET2	•
206	WEEBILL4	
207	ATTILA*2/9/KT/BAGE//FN/U/3/BZA/4/TRM/5/ALDAN/6/SERI/7/VEE#10/8/OPATA	
208	ATTILA*2/PBW65	
209	BABAX/KS93U76//BABAX	

210	SUJATA/SERI	
211	PASTOR/3/MUNIA//CHEN/ALTAR 84/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA	
212	URES/PRL//BAV92	
213	VOROBEY	
214	SOROCA	
215	FILIN/2*PASTOR	
216	URES/JUN//KAUZ/3/BAV92	
217	NAI60/HN7//BUC/3/PSN/BOW//TUI	
218	ATTILA*2/4/CAR//KAL/BB/3/NAC	
219	KAMBARA2	
220	PRL/2*PASTOR	•
221	PBW65/2*PASTOR	
222	ATTILA*2/PASTOR	
223	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	
224	PASTOR//HXL7573/2*BAU	
225	SOKOLL	
226	CROC_1/AE.SQUARROSA (213)//PGO/3/BAV92	
227	MILAN/KAUZ//PRINIA/3/BAV92	
228	ALTAR 84/AE.SQUARROSA (221)//PASTOR/3/PASTOR	
229	MILAN/KAUZ//PASTOR	
230	FLORKWA-1/DHARWAR DRY	
231	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PASTOR	•
232	VEBOW/IRENA	
233	PASTOR/DHARWAR DRY	
234	BJY/COC//PRL/BOW/3/FRTL	
235	PASTOR//HXL7573/2*BAU	
236	SRMA/TUI//PASTOR	
237	SKAUZ/PASTOR/3/CROC_1/AE.SQUARROSA (224)//OPATA	
238	CNO79//PF70354/MUS/3/PASTOR/4/BAV92	
239	MILAN/KAUZ/3/URES/JUN//KAUZ/4/CROC_1/AE.SQUARROSA (224)//OPATA	
240	KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA	
241	BOW//BUC/BUL/3/KAUZ/4/BAV92/5/MILAN/KAUZ	
242	PASTOR//MILAN/KAUZ/3/VEE/PJN//2*TUI	
243	BJY/COC//PRL/BOW/3/MILAN/KAUZ/4/BAV92	
244	KAUZ/BAV92/3/BJY/COC//PRL/BOW	•
245	FRAME/BUCHIN	•
246	TEMPORALERA M 87*2/KONK	
247	FRAME*2/3/URES/JUN//KAUZ	
248	CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR/4/PASTOR*2/OPATA	
249	RL6043/4*NAC//2*PASTOR	
250	JUPARE C 2001	
251	CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR/4/JARU	
252	ALTAR 84/AE.SQ//2*OPATA/3/PIFED	
253	KRICHAUFF/2*PASTOR	
254	KABY//2*ALUBUC/BAYA	
255	CNO79//PF70354/MUS/3/PASTOR/4/CROC_1/AE.SQUARROSA (224)//OPATA	•
256	BUC/MN72253//PASTOR/3/BAV92	
257	SCA/AE.SQUARROSA (409)//PASTOR/3/PASTOR	

258	CROC_1/AE.SQUARROSA (224)//OPATA/3/B1Y/COC//PRL/BOW/4/B1Y/COC//PRL/BOW	
259	CHEN/AE.SQ//2*OPATA/3/BAV92/4/JARU	
260	TIE CHUAN 1*2/3/HE1/3*CNO79//2*SERI	
261	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA/3/ATTILA	•
262	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI	
263	PASTOR//TRAP#1/BOW/3/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN	
264	PRL/2*PASTOR	
265	ND643/2*WAXWING	
266	ND643//2*PRL/2*PASTOR	
267	KIRITATI//2*PRL/2*PASTOR	
268	KIRITATI//2*ATTILA*2/PASTOR	
269	SAAR/WBLL1	
270	CHONTE	
271	CHEWINK	
272	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1	
273	WHEAR/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/C80.1/3*BATAVIA//2*WBLL1	
274	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1	
275	WHEAR/JARU/3/C80.1/3*BATAVIA//2*WBLL1	
276	WHEAR/TUKURU/3/C80.1/3*BATAVIA//2*WBLL1	
277	WHEAR/KURUKU/3/C80.1/3*BATAVIA//2*WBLL1	
278	WHEAR//2*PRL/2*PASTOR	
279	WHEAR//2*PRL/2*PASTOR	•
280	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/MEAVER/5/2*KAUZ/6/PRL/2*PASTOR	
281	PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI	
282	PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI	
283	PFAU/MILAN/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR	
284	PRL/SARA//TSI/VEE#5/3/TILHI/4/ATTILA/2*PASTOR	
285	ELVIRA/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/VEE/PJN//KAUZ/3/PASTOR	
286	HEILO//MILAN/MUNIA	•
287	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	
288	QUAIU #1	
289	PAURAQUE	
290	FRET2*2/BRAMBLING	
291	BECARD	•
292	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	
293	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	
294	BETTY/3/CHEN/AE.SQ//2*OPATA	•

7.3 Results

7.3.1 Genetic variation in grain weight and physiological traits in WAMI panel

In 2013, grain yield in the unmanipulated crop ranged amongst WAMI genotypes from 4.49 to 9.16 t ha^{-1} ($P < 0.05$). Grain weight in the control treatment ranged from 36.0 to 58.9 mg ($P < 0.001$). Grain weight in the degraining treatment ranged from 45.1 to 58.8 ($P < 0.001$). Leaf and stem DM in degrained shoots ranged from 1.18 to 2.90 g ($P < 0.001$) and from 1.05 to 2.66 g in control shoots. Date of anthesis ranged from 76 to 96 days in 2013 (DAE) in the control treatment.

In 2013 in the unmanipulated crop, post-heading RCC senescence rate ranged amongst WAMI genotypes from 0.42 to 1.38 ($P < 0.001$), RCC at PM ranged from -13.5 to 241.1 ($P < 0.001$), rate of RCC senescence ranged from 0.004 to 0.0031 $^{o}Cd^{-1}$ ($P < 0.001$). In the control shoots, based on flag-leaf visual score measurements, the start of flag-leaf senescence ranged from -27.9 to 585.6 ^{o}Cd ($P < 0.001$) and the end of flag-leaf senescence from 627.9 to 955.1 ^{o}Cd ($P < 0.001$) amongst the 294 WAMI genotypes (Table 7.1).

In 2014 grain yield per unit area was not measured since the treatment was imposed in small plots consisting of two spike rows. Potential grain weight (grain weight in degraining treatment) ranged from 34.05 to 59.37 mg ($P < 0.05$). A trend was observed for grain weight in the control treatment to range from 30.6 to 56.7 mg ($P = 0.08$) in 2014. Leaf and Stem DM was assessed only for a subset of 45 genotypes chosen for differences in stay green and response to degraining. Leaf and stem DM in degrained shoots ranged from 2.45 to 4.88 g ($P < 0.05$), and in control shoots from 2.41 to 3.94 g ($P < 0.01$). Anthesis date in the control treatment did not differ significantly amongst the 294 WAMI genotypes.

Table 7.2 Grain yield (GY 100% DM), grain weight response to degraining (GWR), grain weight degraining treatment (GWDEG), grain weight control (GWCON), Leaf + stem DM per shoot in degraining treatment (L+SDMSHDEG), Leaf and stem dry matter per shoot in control (L+SDMSHCON), date of anthesis (days after emergence, DAE), Post heading RCC senescence rate (PHEADRCCI), RCC calculated at physiological maturity (RCCPM), Flag leaf score senescence) rate (Sen Rate), start of senescence 2 (SENonset) and end of senescence (SENend) for 294 WAMI cultivars for a) 2013, b) 2014 and c) mean.

a) 2013

294 Cult.	GY t ha^{-1}	GW DEG mg	GW CON mg	L+SMSH DEG g $shoot^{-1}$	L+SDMSH CON g $shoot^{-1}$	Anthesis date
MEAN	6.79	45.16	42.69	2.04	1.87	86.71
MAX	9.16	58.86	55.43	2.90	2.66	96.00
MIN	4.49	35.96	32.65	1.18	1.05	76.00
P VALUE	*	***	***	***	***	***
SED (DF)	1.0563 (293)	1.7704 (178)	1.8467 (186)	0.17334 (198)	0.15339(199)	1.6538 (291)

294 Cult.	PHEAD RCC $^{o}Cd^{-1}$	RCC PM	(Sen rate) $^{o}Cd^{-1}$	SEN onset ^{o}Cd	SEN end ^{o}Cd
MEAN	0.929	159.0	0.011	258.5	809.3
MAX	1.376	241.1	0.031	585.6	955.1
MIN	0.416	-13.5	0.004	-27.9	627.9
P VALUE	***	***	***	***	***
SED (DF)	0.15281 (293)	35.02 (293)	0.005345 (218)	61.79 (170)	42.74 (218)

b) 2014

294 Cult	GW DEG mg	GW CON mg	AGDMSH DEG g shoot^{-1}	AGDMSH CON g shoot^{-1}	Anthesis date
AVERGE	43.11	41.02	3.14	2.94	68.88
MAX	59.37	56.77	4.88	3.94	74.00
MIN	34.05	30.60	2.45	2.41	54.00
Pvalue	*	P=0.08	*	**	ns p=0.5
SED (DF)	5.033 (177)	4.943 (196)	0.4159 (40)	0.3224 (40)	2.675 (293)

c) Mean of 2013 and 2014

	GW DEG Mg	GW CON mg	L+SDMSH DEG g shoot^{-1}	L+SDMSH CON g shoot^{-1}	Anthesis date
Mean	44.17	41.85	2.13	1.95	77.77
Max	58.97	55.88	3.89	3.30	83.25
Min	36.92	33.57	1.18	1.05	72.00
Pvalue	***	***	***	***	***
SED(DF)	2.664 (355)	2.664 (382)	0.159 (238)	0.129 (236)	1.5735 (584)
Year	*	ns	***	*	**
SED	0.45	0.43	0.1027	0.1017	1.06
Env*Cult	***	ns	*	*	***
SED	3.79	3.79	0.2472	0.2094	2.46

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

Averaging over years, potential grain weight (degrained treatment) ranged from 36.9 to 58.8 mg (P > 0.001). A year effect (P > 0.05) and an interaction for year x genotype (P > 0.001) were observed. Grain weight in the control raged from 33.6 to 55.9 mg (P > 0.001). No year effect or year x genotype interaction was observed. Leaf and stem DM for the degrained shoot treatment ranged from 1.18 to 3.89 g (P > 0.001). Leaf and stem DM for the control shoots treatment ranged from 2.41 to 3.94 g (P > 0.001). A year and year x cultivar interaction was observed (P > 0.05). Finally, averaging over years, date of anthesis ranged from 72.0 to 83.3 DAE (P > 0.001). A year effect was observed (P > 0.01) and there was an interaction between year x cultivar (P > 0.001) amongst the WAMI genotypes.

7.3.2 Grain weight responses to degraining for 294 genotypes

In 2013 an effect of the degraining treatment was observed ($P < 0.001$; Fig.7.1). However, no interaction was observed for cultivar x degraining treatment amongst the WAMI genotypes. In 2014, an effect was observed for the degraining treatment ($P < 0.001$). However, no effect of the cultivar x degraining treatment interaction was observed amongst the WAMI genotypes. Averaging over years, an effect of the degraining treatment and year was observed ($P < 0.001$). However no interaction was observed for cultivar x degraining treatment neither for year x cultivar x degraining treatment interaction.

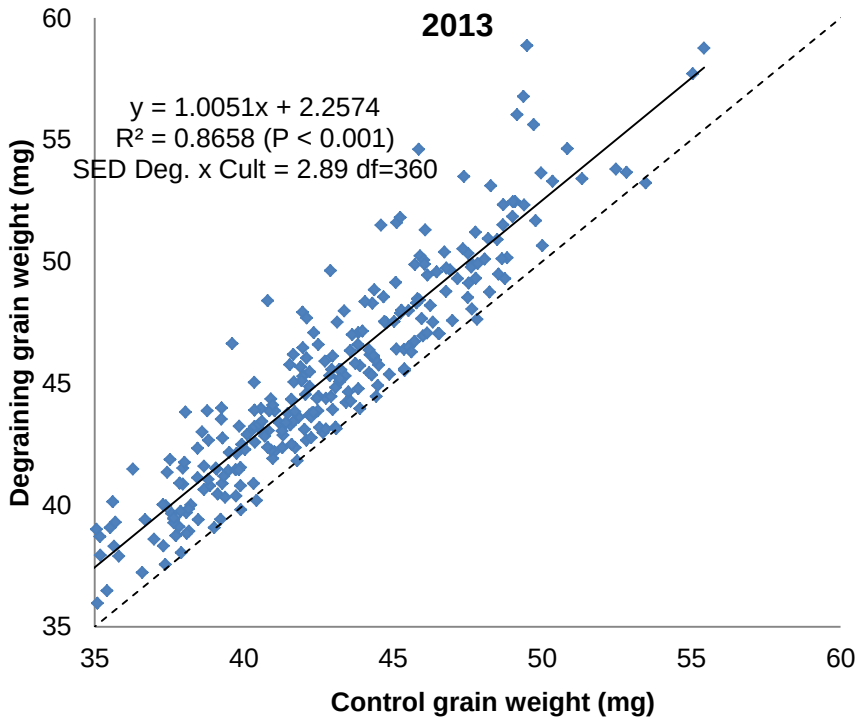


Figure 7.1 Individual grain weight in control shoots versus degrained shoots for 294 WAMI genotypes in 2013. Dashed line represents the 1:1 ratio.

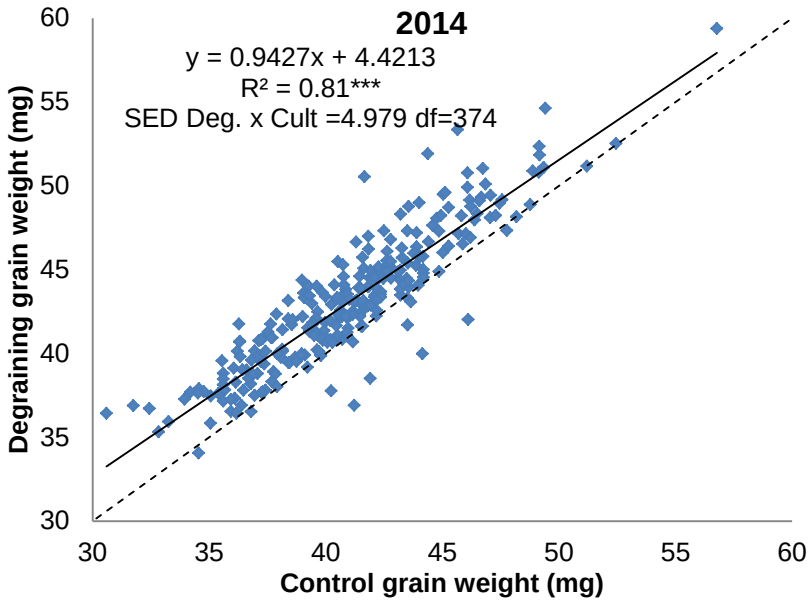


Figure 7.2 Individual grain weight in control shoots versus degrained shoots for 294 WAMI genotypes in 2014. Dashed line represents the 1:1 ratio.

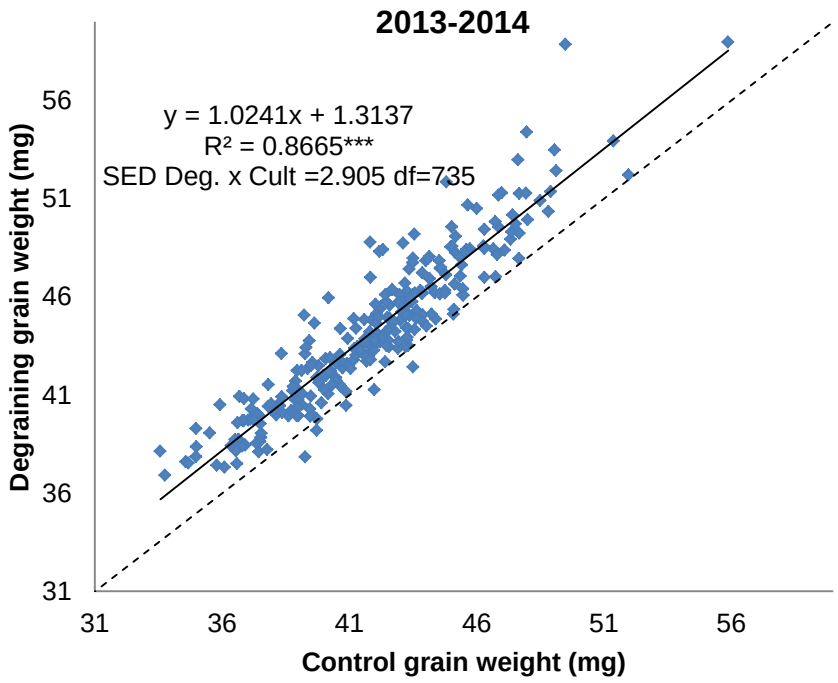


Figure 7.3 Individual grain weight in control shoots versus degrained shoots for 294 WAMI genotypes. Values are mean of 2013 and 2014 season. Dashed line represents the 1:1 ratio.

7.3.3 Leaf and stem dry weight responses to degraining for 45 genotypes

An effect of the degraining treatment on leaf and stem dry weight per shoot was observed in 2013 with an increase of 9.2% in the degraining treatment ($P < 0.001$; Fig. 7.4). However no cultivar x degraining treatment interaction was found. Similarly, an effect of the degraining treatment on the shoot weight was observed in 2014 with an increase of 4.05% in the degraining treatment ($P < 0.001$) but no cultivar x degraining treatment interaction amongst the 45 cultivars (Subset in 2014) (Figure 7.5).

Averaging over years for the 45 cultivars, an effect of the degraining treatment on shoot weight was observed with an increase of 5.47% in the degraining treatment ($P < 0.001$). However, no cultivar x degraining treatment on shoot weight was observed in 2014 (Fig 7.6).

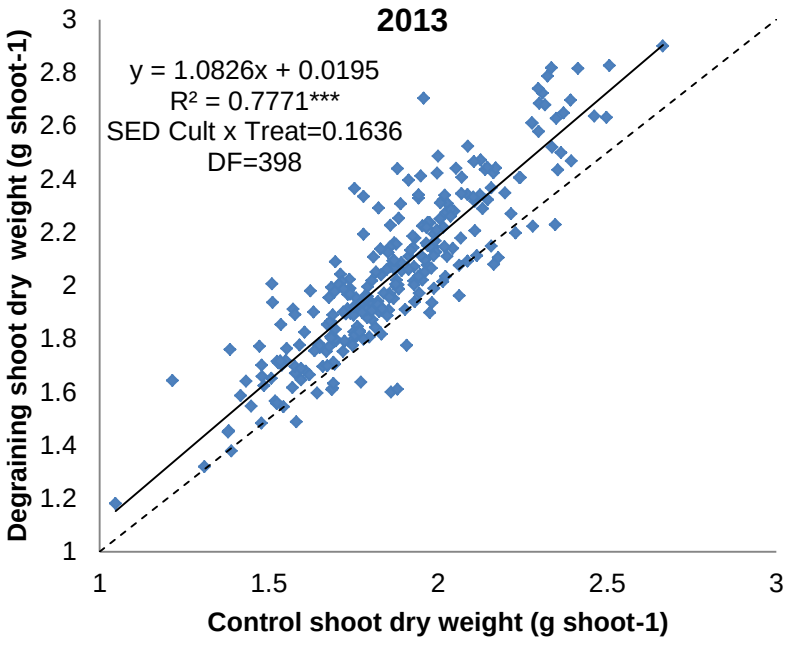


Figure 7.4 Leaf and stem dry weight per shoot in control versus degrained shoots for 294 genotypes in 2013. Dashed line represents the 1:1 ratio.

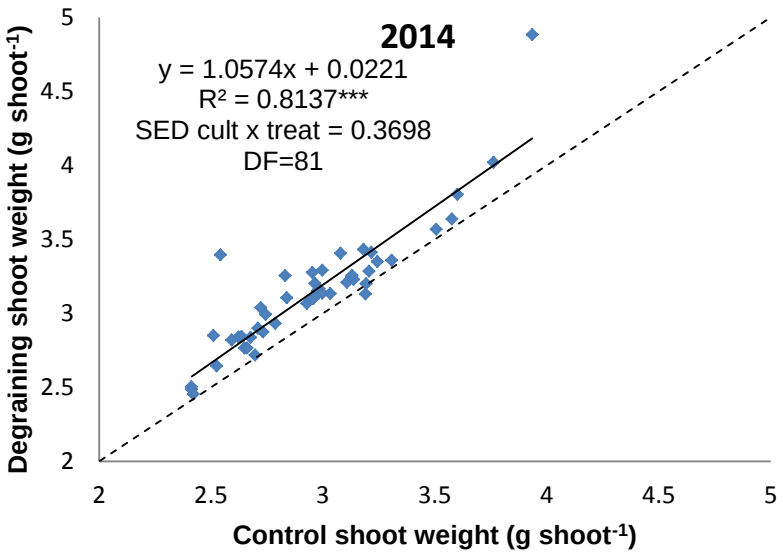


Figure 7.5 Leaf and stem DM per shoot in control versus degrained shoots for 45 genotypes in 2014. Dashed line represents the 1:1 ratio.

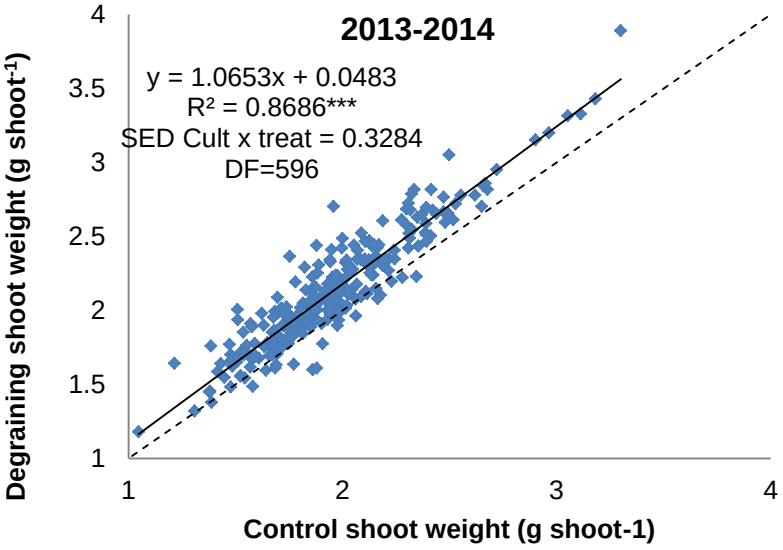


Figure 7.6 Leaf and stem dry weight per shoot in control versus degrained shoots for 45 genotypes. Values are mean of 2013 and 2014 season. Dashed line represents the 1:1 ratio.

7.3.4 Trait correlations for 294 and 47 genotypes in 2013

7.3.4.1 294 genotypes

Grain yield in the unmanipulated crop showed a trend amongst genotypes for a positive association with grain weight in the control treatment (P=0.053). Grain weight response to degraining showed a positive association with flag leaf senescence rate ($P < 0.001$). Grain weight in the control and in the degraining treatment each showed a positive association with flag-leaf senescence rate *($P < 0.01$;$P < 0.001$)* respectively amongst the 294 WAMI genotypes (Table 7.3 a). A positive association was observed between leaf and stem DM and the end of flag-leaf senescence ($P < 0.01$).

7.3.4.2 47 genotypes (with responses of 10-20% indicating co-limitation

Forty seven genotypes were selected for further correlation analysis as those with grain weight responses between 10-20%, indicating co-limitation of grain growth by source and sink. For these 47 genotypes, grain yield was positively associated with flag-leaf SENonset ($P < 0.01$). Grain weight response to degraining showed a positive association with the rate of senescence ($P < 0.001$) with higher grain weight responses associated with faster senescence. In addition grain weight response showed a positive association with SENonset ($P < 0.05$) amongst the 47 genotypes)

Potential grain weight showed a negative association with DTA ($P < 0.05$), and a positive association with senescence rate ($P < 0.001$) and with SENend ($P < 0.05$). Grain weight in the control was negatively associated with DTA ($P < 0.05$), and positively associated with senescence rate ($P < 0.001$) and SENend ($P < 0.05$). Leaf and stem DM of degrained shoots showed a positive association with flag-leaf end of senescence ($P < 0.05$). Anthesis date showed a negative association with flag-leaf start of

senescence ($P < 0.05$) and a negative association with flag-leaf end of senescence ($P < 0.001$) (Table 7.3b). A positive association was observed between leaf and stem DM and the end of flag-leaf senescence ($P < 0.05$).

Table 7.3 Matrix of trait correlations in 2013 for grain yield (GY), grain weight response to degraining (GWR (%)), grain weight degraining treatment (GWDEG), grain weight control (GWCON), Leaf and stem DM in degraining shoots (L+SSHDEG), Leaf and stem DM in control shoots (L+SDMSHCON), Date of anthesis days after emergence (DTA), Post heading RCC senescence rate (PHEADRCC), RCC at physiological maturity (RCCPM), flag leaf score senescence rate, (FLS Sen rate), start of senescence (Sen onset) and end of senescence)(Sen end) for **a**) 294 genotypes and **b**) 47 genotypes with responses to degraining ≥10%.

a)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
GY (1)	1	-0.09	0.09	0.12 P=0.053	0.09	0.07	0.05	0.04	-0.09	-0.01	0.05	0.10
GWR (2)		1	0.22 ***	-0.14 *	-0.19 **	-0.17 **	0.02	-0.03	-0.14 *	0.21 ***	-0.02	-0.09
GWDEG (3)			1	0.93 ***	0.44 ***	0.47 ***	-0.18 **	-0.01	-0.03	0.22 ***	0.19 **	0.22 ***
GWCON (4)				1	0.52 ***	0.54 ***	-0.19 ***	-0.01	0.02	0.15 *	0.20 **	0.27 ***
L+SDMSHDEG (5)					1	0.88 ***	0.00	0.00	-0.06	0.04	0.17 **	0.18 **
L+SDMSHCON (6)						1	0.01	0.01	-0.06	0.11	0.21 ***	0.12
DTA (7)							1	0.49 ***	-0.36 ***	-0.01	-0.21 ***	-0.60 ***
PHEADRCC (8)								1	-0.14	0.18 **	0.12	-0.31 ***
RCCPM (9)									1	0.04	-0.05	0.04
FLS Sen rate (10)										1	0.69 ***	-0.43 ***
Sen onset (11)											1	-0.08
Sen end (12)												1

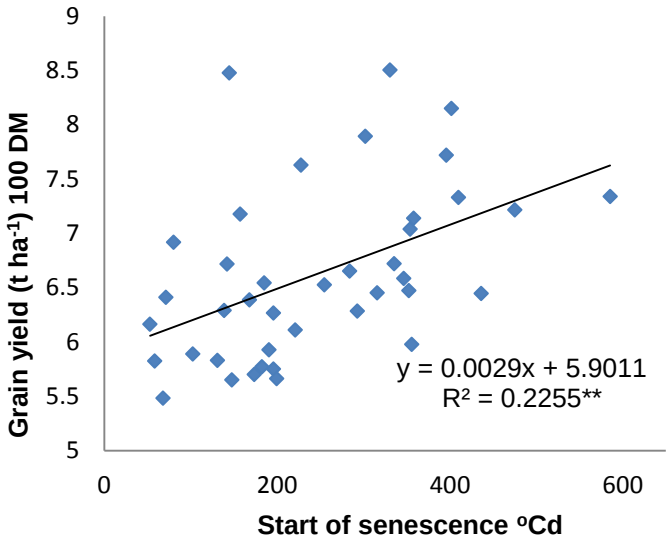
*Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

b)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
GY (1)	1	0.08	0.15	0.11	0.04	0.06	-0.19	0.13	-0.13	0.11	0.47**	0.24
GWR (2)		1	0.44**	0.25	0.12	0.17	-0.08	-0.06	-0.02	0.55***	0.38*	-0.02
GWDEG (3)			1	0.96***	0.45**	0.60***	-0.33*	0.01	0.06	0.53***	0.25	0.33*
GWCON (4)				1	0.49***	0.63***	-0.33*	-0.01	0.04	0.44**	0.17	0.36*
AGDMSHDEG (5)					1	0.90***	-0.12	0.11	-0.41**	0.07	0.18	0.34*
AGDMSHCON (6)						1	-0.18	0.13	-0.26	0.26	0.18	0.26
DTA(7)							1	0.44**	-0.49***	-0.20	-0.40*	-0.71***
PHEADRCC (8)								1	-0.41**	0.13	0.14	-0.30*
RCCPM (9)									1	0.20	-0.18	0.07
FLS Sen rate (10)										1	0.60***	-0.23
FLS STARTsen (11)											1	0.27
FLS ENDsen (12)												1

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

a)



b)

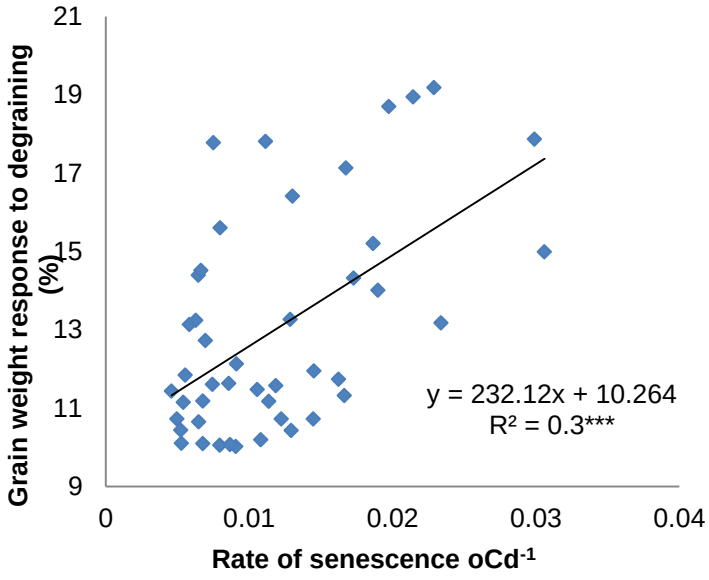


Figure 7.7 Relationship between a) grain yield and start of senescence in control shoots and b) Grain weight response to degraining (%), and flag leaf rate of senescence ($^{o}Cd^{-1}$) for 47 cultivars with higher response to degraining treatment in 2013.

7.3.5 Trait correlations for 294 genotypes in 2014

In 2014, grain weight response to degraining was positively associated with grain weight in the control (P < 0.001). Grain weight in degraining

treatment was positively associated with grain weight in control treatment ($P < 0.001$) and negatively associated with anthesis date ($P < 0.05$) amongst the 294 genotypes.

Table 7.4 Matrix of trait correlations in 2014 for grain weight response to degraining (GWR (%)), grain weight degraining treatment (GWDEG), grain weight control (GWCON), and Date of anthesis days after emergence (DTA), for 294 genotypes.

	1	2	3	4
GWR (1)	1	0.04	-0.31***	0.10
GWDEG(2)		1	0.90	-0.09
GWCON (3)			1	-0.14*
DTA (4)				1

- *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

7.3.6 Trait correlations for 294 genotypes (mean across 2013 and 2014)

Grain response to degraining was positively associated with potential grain weight ($P < 0.001$), negatively associated with grain weight control ($P < 0.05$), and negative associated with grain yield ($P < 0.05$). Potential grain weight was positively associated with control grain weight ($P < 0.001$) and negatively associated with date of anthesis ($P < 0.05$). Grain weight in the control was negatively associated with date of anthesis ($P < 0.05$) amongst the WAMI cultivars in two years for 294 genotypes.

Table 7.5 Matrix of correlations (means for 2013 and 2014) for grain weight response to degraining (GWR), Potential grain weight (GWDEG), Grain weight control (GWCON), and date of anthesis days after emergence (DTA). Values represent means for 2013 and 2014 in the WAMI population.

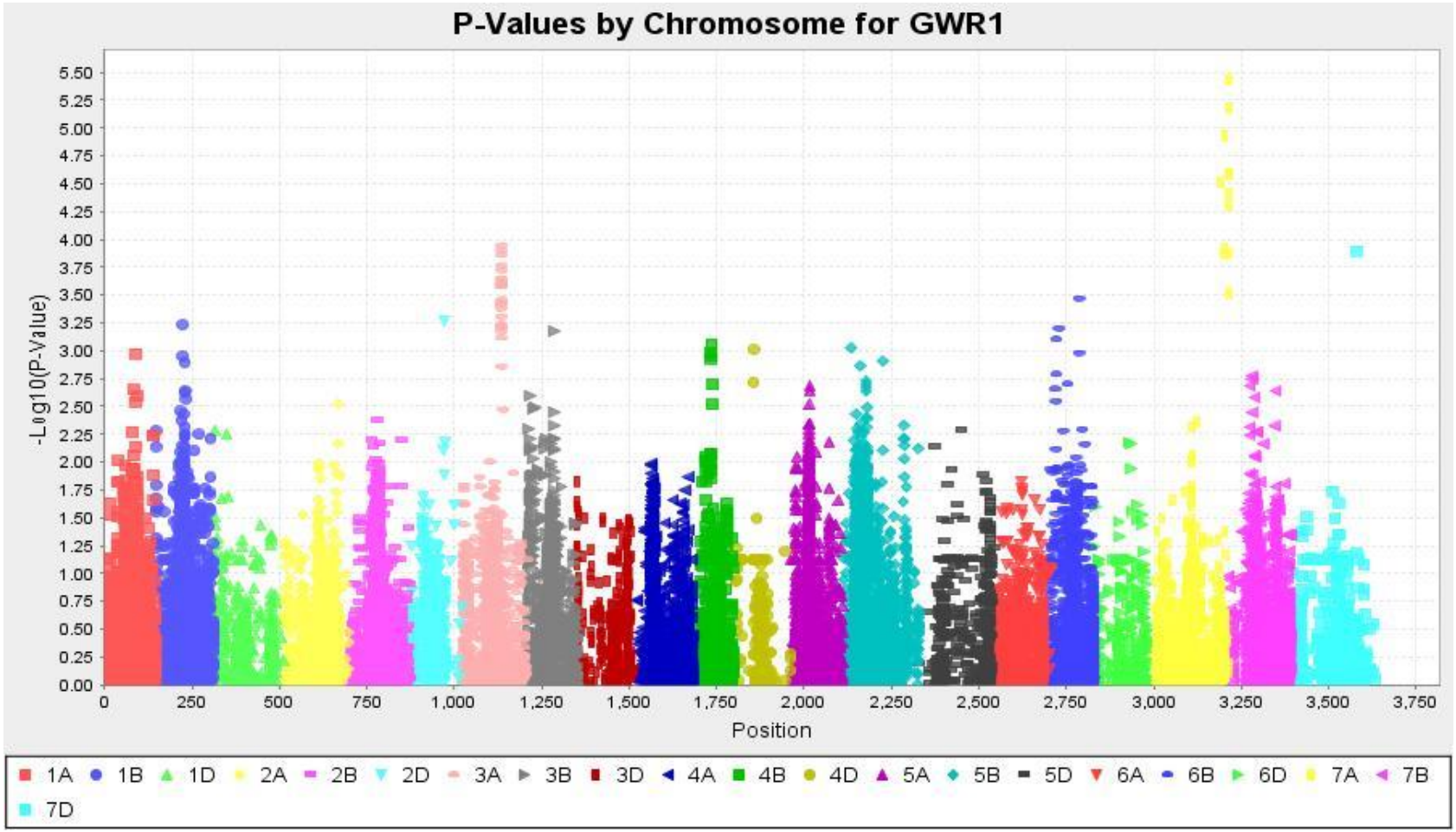
	(1)	(2)	(3)	(4)
GWR (1)	1	0.20***	-0.14*	0.02
GWDEG(2)		1	0.93***	-0.16**
GWCON (3)			1	-0.17**
DTA (4)				1

- *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability

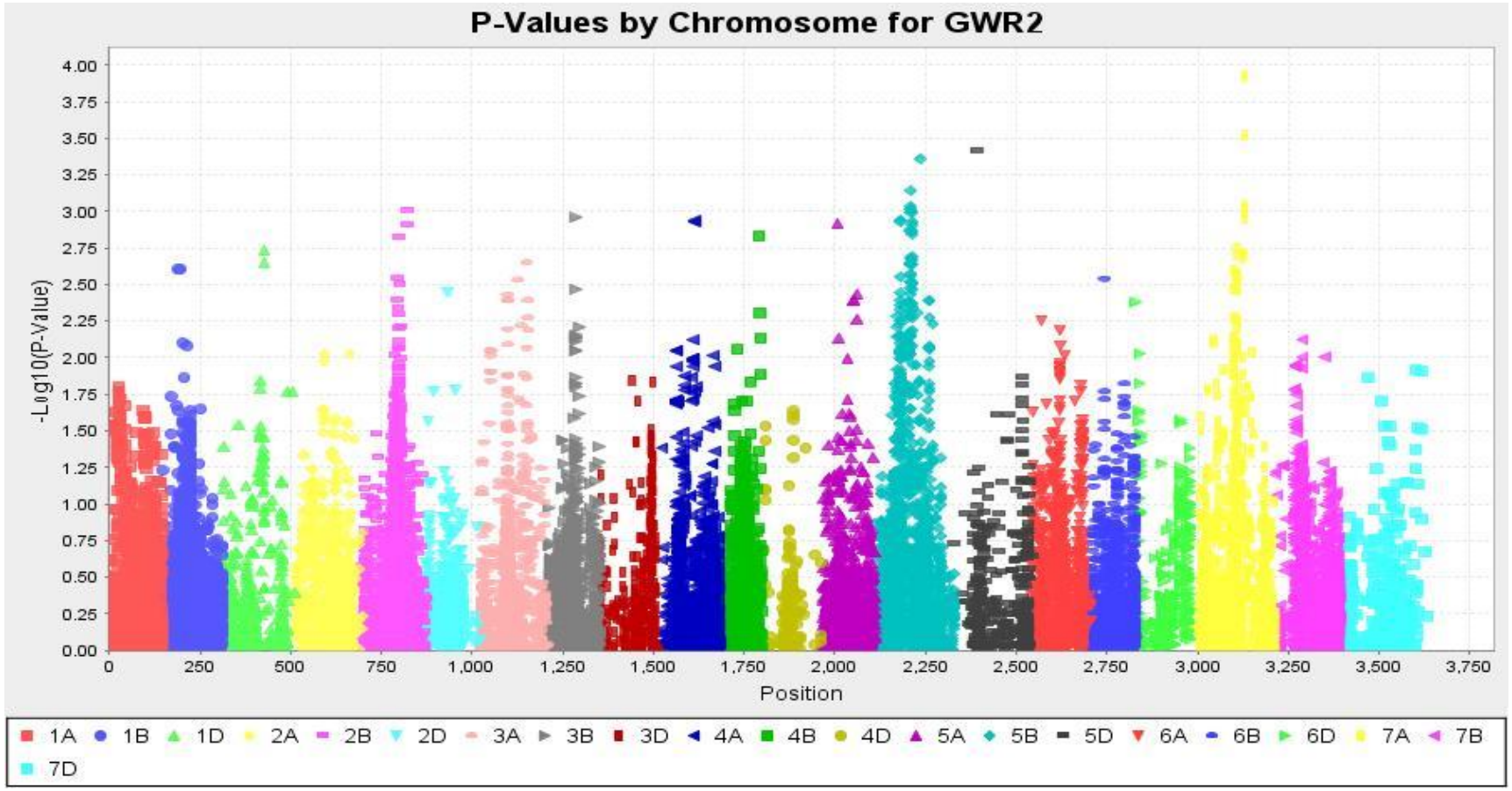
7.3.7 Genetic analysis for 2013 experiment

Genome wide association (GWAS) study analysis identified a marker for grain weight response to degraining treatment on chromosome 7A at the probability level of 10^{-04} (Figure 7.8abc). A marker was detected for leaf and stem DM response to degraining on chromosome 5A at the probability of 10^{-03} (Figure 7.9). Analysis of GWAS was not carried out for 2014.

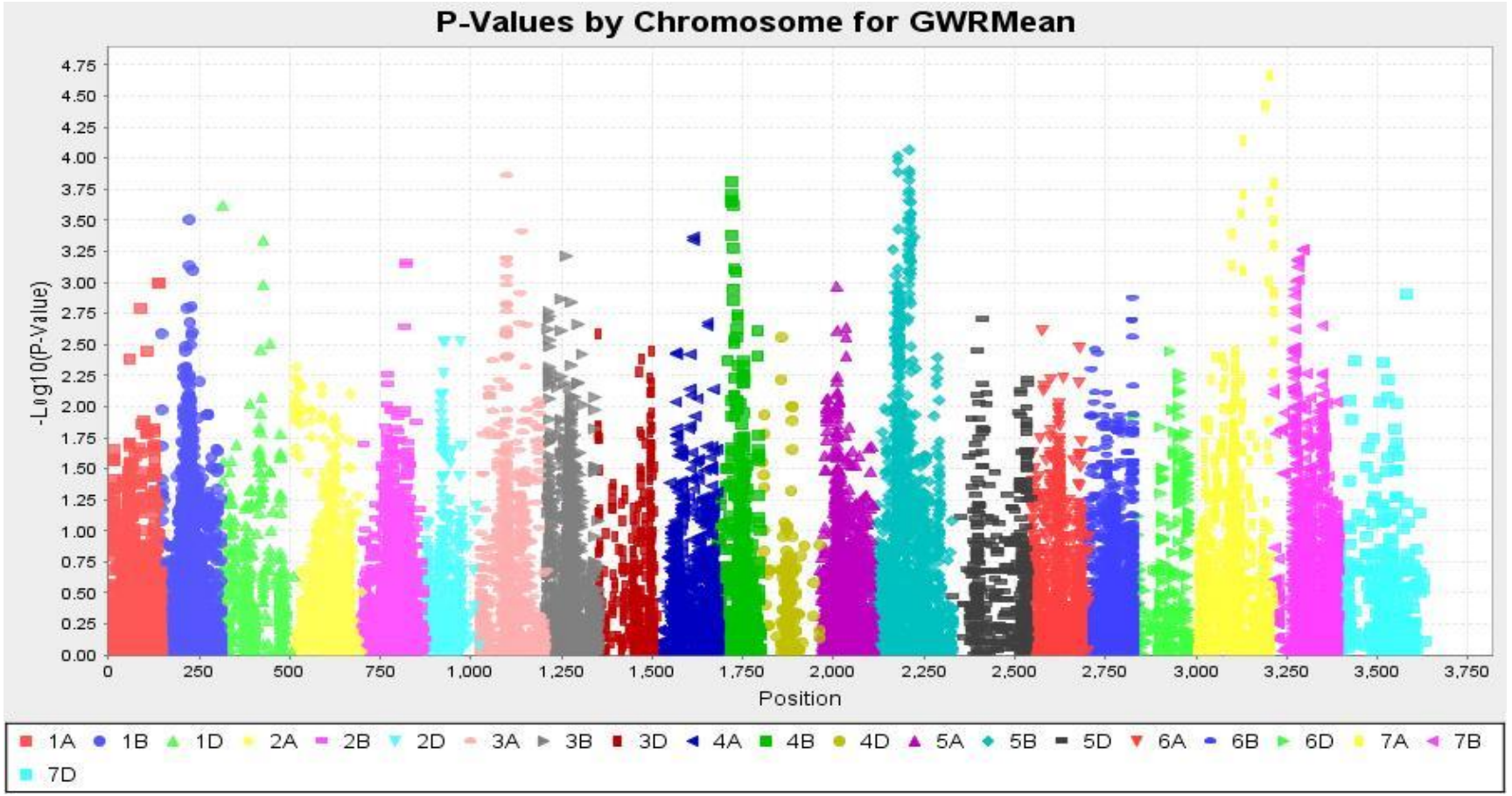
a)


P-Values by Chromosome for GWR1
-Log10(P-Value)
5.50
5.25
5.00
4.75
4.50
4.25
4.00
3.75
3.50
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1,000
1,250
1,500
1,750
2,000
2,250
2,500
2,750
3,000
3,250
3,500
3,750
Position
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D

b)


P-Values by Chromosome for GWR2
-Log10(P-Value)
4.00
3.75
3.50
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1,000
1,250
1,500
1,750
2,000
2,250
2,500
2,750
3,000
3,250
3,500
3,750
Position
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D

c)


P-Values by Chromosome for GWRMean
-Log10(P-Value)
4.75
4.50
4.25
4.00
3.75
3.50
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1,000
1,250
1,500
1,750
2,000
2,250
2,500
2,750
3,000
3,250
3,500
3,750
Position
1A
1B
1D
2A
2B
2D
3A
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7A
7B
7D

d)

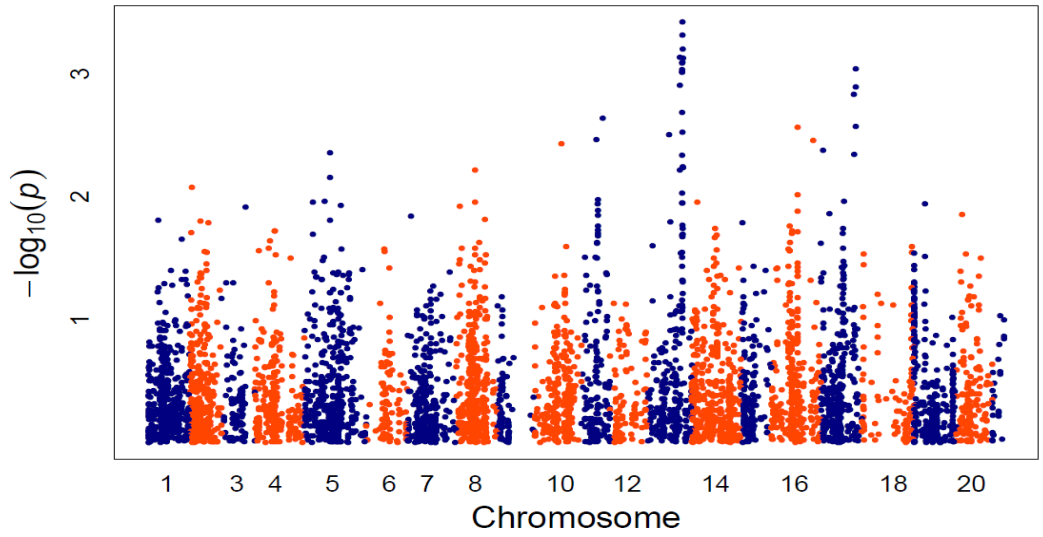


Figure 7.8 GWAS marker trait associations using 90K SNP and 1992 DArT markers based on least quare means of 2013, 2014 and means data for a) grain weight response to degrainin 2013 and b) grain weight response to degrainin 2013 c) grain weight response to degrainin over years and d) Leaf and stem DM response to degrainin 2013.

7.4 Discussion

The balance of the source and sink size in the post-anthesis period in a crop is a very important factor influencing the yield potential of wheat (Reynolds et al., 2005). Grain growth responses to degraining (where assimilate supply per grain is effectively increased by 100%) of ca. 0–10% are indicative of sink limitation of grain growth, whereas those of ca.10–20% are indicative of co-limitation of grain growth by sink during the earlier phase of grain filling and source during the latter phase of grain filling (Acreche and Slafer 2009). In this study, averaging over year the 294 genotypes showed positive grain weight responses to degraining ranging from 0.08 – 18.95% indicating a level of either sink limitation of grain growth (< 10%) or co-limitation by source or sink (> 10%). However, grain weight responses were not significantly different according to the cultivar x degraining interaction in any year.

Sink limitation or co-limitation by the sink and source has been reported in potential conditions for wheat (Borrás et al., 2004). In present results the positive association between senescence rate and response to de-graining amongst 294 cultivars and more strongly amongst 47 genotypes in the WAMI panel with ≥10% of response to degraining has indicated that the sink capacity for grain weight is not fully satisfied during the grain filling phase in at least some of the WAMI panel. Therefore, the source size needs to be enhanced to supply assimilates in order to exploit fully the potential sink size. Yield progress will depend crucially on enhancing the synergy between increases in photosynthetic capacity and grain sink capacity (Foulkes et al., 2011).

Positive association between GWR (%) and senescence rate indicates that faster senescence rate is contributing to increasing the extent of source limitation in modern CIMMYT spring wheat genotypes. One of the avenues to increase the source would be with the functional stay green associated with extended canopy duration. This trait has been suggested

as an important component of yield potential gains in wheat under high-input conditions (Shearman et al., 2005; Reddy et al., 2007). In addition, the positive association may be moving towards co-limitation of grain growth by source and sink. This is in agreement with CIMCOG cultivars discussed in chapter 6 where the ranges of response to degraining ranged from 1.0 – 30.2% similar to the responses obtained in the WAMI panel. Therefore, at present results demonstrated that modern higher yielding cultivars are becoming more source limited to some extent probably in the last third of grain filling period when the canopy senescence was observed to occur at a faster rate. Aisawi et al. (2015) reported a non-linear change in senescence duration with YoR, with senescence duration increasing from approximately 1980, indicating that delayed senescence in modern cultivars may be related with higher yielding

On the other hand, Reynolds et al. (2005) demonstrated that increasing the grain sink strength of field-grown wheat, by artificially boosting grain number per spike through opening rows pre-anthesis can stimulate flag-leaf photosynthesis during grain-filling. Nevertheless, it appears that current photosynthetic capacity during the grain filling period is not enough to match the sink strength according the potential grain weight in some of genotypes of the WAMI population.

Although the range for anthesis date was restricted in the WAMI panel, a negative association between grain weight on the control treatment and anthesis date was observed. It is possible that cultivars that reached flowering earlier had a longer grain filling duration due to cooler temperatures during grain filling.

The association between grain weight and leaf and stem DW was higher in the control than the degraining treatment, indicating that stem WSC contributed more assimilate to grain filling in the control in 2013. In addition, grain weight response to degraining was positively correlated with potential grain size and with senescence rate, indicating that

reduction in extent of sink limitation is associated with increases in potential grain weight and increases in senescence rate, in agreement with findings in the CIMCOG cultivars. In this respect, several investigations have concluded that the genetic control of N remobilization is linked to the regulation of leaf senescence (Masclaux et al., 2001; Uauy et al., 2006). The stem has an important role in accumulation storage of nitrogen reserves more than the ear, leaf lamina, or leaf sheath (Pask *et al.* 2012b). The reduction of the efficiency of nitrogen from canopy to the grains could be useful for grain filling post-anthesis.

GWAS

Genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping are the two main approaches used to detect complex traits (Risch et al., 1996). At CIMMYT, detection of QTLs was improved in mapping populations where flowering time and height was controlled (Pinto et al., 2010; Reynolds et al., 2009b). The WAMI panel has wide differences in morphological characters and agronomic traits that makes a good panel to test marker traits associations (Edae et al., 2014). The present study has identified a marker for grain weight response to degraining on chromosome 7A and a marker for leaf and stem DM response to degraining on chromosome 5A.

According to the literature, no QTLs or marker-trait associations (MTA) have been previously reported for grain weight response to degraining (indicative of source-sink balance). However, QTLs for complex traits have been reported for grain yield in spring and winter wheat by Snape et al. (2007) and Lopes et al. (2015), respectively, on the short arm of chromosome 6A. Su et al. (2006) reported a QTL for biomass increment post-anthesis on chromosome 7A in wheat. On the other hand, a major MTA has recently been reported in CIMMYT spring wheat for TGW and grain number on chromosome 5A (Sukumaran et al., 2015). Therefore QTL and MTA are important for developing markers for marker-assisted

selection for complex traits related to yield potential in the CIMMYT wheat breeding program and worldwide wheat breeding programs. Although only two markers were detected in the present results in the WAMI panel, these MTAs, if validated in future site-seasons, will be useful for developing markers for marker-assisted selection for optimised source-sink balance and enhanced biomass and grain yield in the CIMMYT breeding program.

CHAPTER 8 GENERAL DISCUSSION

This chapter discusses the main findings of this study. Source-sink balance results from experiments in 4 years in high elite CIMMYT germplasm are discussed in relation to evidence of limitations for grain yield potential. Physiological traits related to biomass production, spike partitioning index, and fruiting efficiency are discussed and an optimum plant ideotype with desirable traits that breeders should aim for to raise biomass and HI simultaneously is proposed.

8.1 Introduction

During the season, limitations due to the sink or source can vary at different phases of the lifecycle. Understanding source or sink limitations on crop yield is critical for the rational design of wheat breeding strategies (Gambín and Borrás 2007). In the present study, source-sink balance was quantified in a degraining treatment in 26 CIMCOG cultivars in order to test if grain growth of the latest CIMMYT bread wheat germplasm is sink or source limited or co-limited by both source and sink.

8.2 Is grain yield of CIMCOG wheat cultivars sink, source or co-limited?

The balance of the source and sink size in the post-anthesis period in a crop is a very important factor influencing the yield potential of wheat (Reynolds et al., 2005). Under favourable conditions in the last decades wheat breeders have mainly increased grain yield (sink), indicating that older wheat cultivars were severely sink limited (Slafer and Savin, 1994). Now recent studies have reported gains in grain sink size via potential grain weight amongst CIMMYT cultivars (Aisawi et al., 2015). Therefore, grain yield is possibly moving towards source limitation. In this study, the 26 CIMCOG genotypes showed positive grain weight responses to degraining treatment ranging from 1.0 – 30.2% indicating a level of either sink limitation of grain growth (< 10%) or co-limitation by source or sink (>

10%). The present results showed that grain weight response to degraining was positively associated with grain yield in the control crop amongst the 26 genotypes over three years. This evidence suggests that breeders have been increasing grain sink size in recent years mainly by increasing the potential grain size. Similar findings for recent CIMMYT spring wheat releases were reported by Lopes et al. (2012) and Aisawi et al. (2015). In addition, calculations of source-sink balance per unit area following the model of Bingham et al. (2007) generally indicated a close balance between the grain sink size and the source size in the a subset of 10 genotypes in agreement with the degraining response results.

The present results showed a trend for a positive association between source-sink balance and grain yield indicating that the extent of sink limitation was reduced (i.e. source limitation increased) in the higher yielding CIMCOG cultivars. Furthermore, the negative association between NDVI calculated at physiological maturity and year of release in the CIMCOG genotypes indicated that modern cultivars may have less green canopy area duration post-anthesis their predecessors. This was supported in a separate degraining treatment in a wider association panel (WAMI), where genotypes with higher grain weight response to degraining were associated with higher senescence rate.

In summary the evidence in the present study suggested that in the highest yielding genotypes in the CIMCOG panel there is a co-limitation of grain growth by the source and sink. Therefore, there may be scope to increase grain yield by increasing simultaneously source and sink traits in the CIMMYT spring wheat breeding program in future years. A plant ideotype to increase source and sink traits simultaneously hence raise HI in high biomass spring wheat cultivars is now discussed.

8.3 Plant ideotype to optimize source and sink in CIMMYT spring bread wheat

A new wheat ideotype must combine a number of physiological traits shown to be associated with higher grain yield potential. For example, traits such as grain size, high stomatal conductance, canopy cooling, lodging tolerance during grain filling, adaptation to canopy environment, and erect flag leaves have been prioritised for wheat yield potential (Rajaram and van Ginkel 1996). Reynolds et al. (2012) suggested that there is potential to increase grain yield through the improvement of radiation-use efficiency by 50% and suggested that the strategies include modifying specificity, catalytic rate and regulation of Rubisco, up-regulating Calvin cycle enzymes, introducing chloroplast CO_2 concentrating mechanisms, optimizing light and N distribution of canopies while minimizing photoinhibition, and increasing spike photosynthesis.

According to present results, an ideotype spring wheat plant for yield potential (i.e. combining higher biomass and higher HI) should include increased pre-anthesis RUE_{PAR}, through higher flag-leaf stomatal conductance as well flag-leaf stay green properties, according to increased nitrogen content in the flag leaf. The latter traits may contribute to an increased crop growth rate from GS31-65. In addition, grain sink size should be increased through increased potential grain weight particularly in the central spikelets, in which grains appear to be most sink limited according to the responses to the degraining treatment in the present study. Greater gain weight increase with degraining indicates more source limitation of grain growth. Grain growth response to degraining for the central spikelet was less compared to the other spikelet positions. So these spikelets had the least source limitation, i.e. were the most sink limited. In addition, spike partitioning index could be increased as well as the fruiting efficiency through increased lemma partitioning within the spike morphological components. These target traits will now be discussed in further detail below.

8.4 Radiation interception and RUE

Future gains in yield potential must largely be achieved by developing cultivars with greater biomass whilst ideally increasing HI towards the theoretical maxim of ca, 0.62. In order to produce more biomass, photosynthesis capacity needs to be enhanced (Sage and Sage 2009). In the present studies, above-ground dry matter at PM showed a positive association with year of release in the 26 cultivars released from 1966 to 2009, in agreement with Aisawi et al. (2015), Bustos et al. (2013) and Rodrigues et al. (2007).

An increased biomass can result from increased radiation interception and/or increased RUE_{PAR} associated with changes in canopy architecture and photosynthetic metabolism (Monteith, 1977; Reynolds et al., 2001, 2005). In the present study, genetic variation was observed in radiation intercepted from GS31-GS65+7d and this was explained mainly by GAI at anthesis +7d (R^2 = 0.51).

The enhancement of biomass as a strategy to increase grain yield will require improvement in radiation-use efficiency (Parry and Hawkesford 2010; Reynolds et al., 2012), which must in part be related with increased leaf photosynthesis rate. Crop growth rate from GS31-41 was related to RUE_{PAR} over this period explaining 50.9% of the biomass variation amongst the subset of 10 CIMCOG genotypes.

The association between leaf stomatal conductance and pre-anthesis RUE in the present study indicated that RUE_{PAR} could be associated with higher leaf photosynthesis rate amongst the cultivars. Present results are in agreement with previous investigations where positive relationships were observed between pre-anthesis flag-leaf stomatal conductance and grain yield under irrigated environments for wheat (Fischer et al., 1998; Reynolds et al., 1994).

Pre-anthesis flag-leaf stomatal conductance was also associated with genetic variation in grain yield in wheat, in an investigation reported by Condon et al. (2008) who measured leaf porosity in five breeding populations under irrigated conditions in NW Mexico and found 50% of the grain yield variability was explained by stomatal conductance. In the present results, flag-leaf stomatal conductance at around anthesis explained 52% of the grain yield variation amongst the subset of 10 cultivars. In irrigated environments, high stomatal conductance indicates high CO_2 conductance rates and thus increased potential for enhanced leaf photosynthesis rate and biomass accumulation. In addition, higher stomatal conductance may be indicative of a higher grain sink strength demand for photo-assimilates.

Crop growth rate was associated with flag-leaf specific N content amongst the CIMCOG genotypes so a greater concentration of N or Rubisco per cm^2 leaf may be a strategy to favour enhanced RUE in an optimized plant ideotype; In addition, stomatal conductance at GS41 showed a positive trend with flag-leaf nitrogen content (mg N flag $leaf^{-1}$) amongst the 10 genotypes; this is in agreement with Reynolds et al. (2012). Therefore FLSN could be a potential selection trait to enhance biomass and grain yield amongst CIMMYT spring wheat cultivars with increased FSLN presumably reducing the light saturation of photosynthesis in the flag leaf. Furthermore, genetic variation in radiation-use efficiency in 2013 during stem elongation was identified amongst the 10 CIMCOG genotypes and RUE was positively related with above-ground biomass at anthesis.

A positive association between percentage grain weight response to degraining (%) in the CIMCOG and senescence rate suggested that faster senescence rate may possibly be contributing to an increase in extent of source limitation in modern CIMMYT spring wheat genotypes at the end of grain filling. In addition, the association between senescence rate and response to de-graining amongst 294 WAMI genotypes and more strongly

amongst 47 genotypes in the WAMI panel with ≥10% of grain weight response to degraining indicated that there is scope to reduce the extent of source limitation in CIMMYT spring wheat through selecting for stay-green traits. It appears that enhancement of source-type traits is needed in order to match the increases in potential grain weight in modern CIMMYT genotypes and one of the avenues to increase the source is the functional 'stay green' associated with extended green canopy area duration. The 'stay-green' trait has been suggested as an important component of yield potential gains in wheat under high-input conditions (Reddy 2007; Shearman et al., 2005) and reported in heat and drought (Lopes and Reynolds 2012). In the present study according to 'stay-green' as estimated by the NDVI calculated at PM there was a negative association with year of release, indicating that modern varieties may be less efficient at capturing radiation post anthesis than their predecessors.

In practice, lower rates of senescence would increase grain yield but reduce grain N concentration, because the slower senescence may be associated with reduced nitrogen remobilization to the grain, so the stay-green trait needs to be crossbreed carefully in relation to the end-use market of the wheat cultivar, e.g. this trait may be less applicable in bread-making cultivars than in feed wheat varieties.

Stay-green genotypes with optimized canopy N dynamics have been reported in maize (Echarte et al., 2008) and wheat (Gaju et al., 2011). A stay green phenotype was reported in wheat associated with the rye chromosome arm 1RS that improved grain yield of cultivars developed in China combining a functional stay green phenotype with increased grain yield and biomass (Chen et al., 2010). Therefore, it seems it may be necessary to target functional stay green in future CIMMYT breeding in order to match the potential grain capacity in the CIMCOG panel for prolonged photosynthetic capacity (Foulkes et al., 2011). Moreover, grain weight responses to degraining were positively correlated with potential

grain size in the present study, indicating that reduction in extent of sink limitation (increased source limitation) is also associated with increases in potential grain weight in the CIMCOG cultivars.

8.5 Fruiting efficiency and Spike Partitioning Index

In the present study, grains m^{-2} was more strongly associated with fruiting efficiency than spike partitioning index. The nonlinear association between SPI and grain m^{-2} indicates that cultivars with the highest spike DM at anthesis were not efficiently converting the extra spike DM into additional grain sites, and improvement in FE in these cultivars may help to exploit fully high spike DM cultivars. Encouragingly, the present results did not show a trade-off between SPI and FE. Therefore, present findings support the idea that FE is partially independent of SPI (Bustos et al., 2013; García et al., 2014; González et al., 2011). More knowledge is needed on traits underlying FE and their genetic control for application in breeding. The positive association between lemma PI and FE in the present study may be related to larger lemmas being associated with increased assimilate supply to florets within the spikelets, as florets are subtended by the lemmas within the spikelet. It can be speculated that larger lemma fraction within the spike morphological components could favour floret survival through enhanced lemma photosynthesis increasing assimilate supply within the spike to developing florets which are initiated in the axils of lemmas. In future, breeders may need to focus on simultaneously enhancing FE and PGW. There have been some reports of a negative trade-off between these two traits (Gaju et al., 2011). Encouragingly, in the present results there appeared to be no negative association between PGW and FE indicating that ideally an increment of grains m^{-2} through FE whilst maintaining or ideally increasing the potential size seems feasible in modern CIMMYT spring wheat germplasm.

The average plant height of the CIMCOG genotypes was 107 cm indicating that further increases in biomass may have to be without

increment in plant height as further increases may be associated with increased lodging risk and reductions in SPI associated with increased partitioning to the stem during the critical window for floret survival from onset of booting to anthesis.

8.6 Development of molecular markers for source-sink balance and grain yield

The present study identified a molecular marker for grain weight response to degraining on Chromosome 7A and a marker for leaf and stem DM response to degraining on chromosome 5A. QTLs for complex traits in wheat have been previously reported, e.g. for grain yield in spring and winter wheat by Snape et al. (2007) and Lopes et al. (2015), respectively, on the short arm of chromosome 6A. Su et al. (2006) reported a QTL for biomass increment post-anthesis in chromosome 7A, in addition grain number has been reported for Quarrie et al. (2006). The WAMI population had wide differences in morphological and agronomic characters that makes a good population to test QTL (Edae et al., 2014). The present results, show that there is scope to identify molecular markers related to source and sink balance in wheat. This information will be useful for future breeding for wheat.

8.7 Effects of G x E

The Cultivar x Environment interaction is recognised as one of the most difficult problems when breeders are selecting for grain yield. At CIMMYT the approach of multi-environments has been practised. Traits were identified in this study in fully irrigated high potential conditions relating to CIMMYT Mega Environment I (Irrigated high yield potential) that occupies ~34 Mha in the developing world (Fischer et al., 2014). Irrigation was applied in the present study using the raised bed planting system which has some advantages to farmers as an agronomic practice to save water usage. However, this system may favour tall genotypes compared to flat

beds plant system under which most irrigated wheat worldwide is grown, since tall, high tillering plant capture light better in the raised bed systems in the gaps between beds. Therefore, the present target traits should be tested across a range of environments and plant systems including rain fed systems to test their general applicability in wheat yield potential breeding programs. For example no trade-off was identified between SPI and FE in the present experiments. However, other environments where cultivars are the lower end of the optimum range of plant height of 70-100 cm rather the upper end of the range as was the case in the present study, the SPI may be generally increased (in shorter crops) and larger spikes may be associated higher chaff costs per grain. Also stem WSC reserves may be relatively more important in other environments than in irrigated crops NW, e.g. in rainfed crops in NW Europe.

8.8 Conclusions

Genetic variation in grain yield was positively correlated with HI but more strongly with above-ground biomass amongst the CIMCOG cultivars. A trade off was observed between biomass and HI in the CIMCOG cultivars
The production of biomass was related with the genetic variation in RUE_{PAR} during stem elongation from GS31 to GS61+7d in one season out of two. Genetic variation in RUE_{PAR} was associated with flag leaf traits including flag-leaf stomatal conductance and flag-leaf specific N content.

Genetic variation in grains m^{-2} was more strongly correlated with FE than SPI amongst the CIMGOG genotypes. There was no trade-off between these two physiological components of grains m^{-2}. Genetic variation in FE was associated with spike morphological components including the lemma PI. In addition the awn PI was associated amongst the CIMCOG genotypes with HI

There was genetic variation in grain weight response to degraining amongst CIMCOG cultivars and grain growth of modern high-yielding cultivars were closer to source limitation than their predecessors. The

grain weight response to degraining of the CIMCOG genotypes was apparently associated with faster senescence, and faster rate senescence could be associated with a later onset of senescence.

A novel marker-trait association was detected for the complex trait of source sink balance on chromosome 7A.

8.9 Future work

Further work must address the use of present findings as follows:

1. Physiologists and plant breeders should collaborate to cross parental lines with contrasting high expression of source type and sink type traits identified in the present study for selection in trait-based breeding in a range of environments and planting systems. This work is required to validate the traits in terms of their practical application in plant breeding in a range of spring wheat environments.

2. The biomass and RUE_{PAR} traits identified should be further quantified and validated in wider range of genetic backgrounds with contrasting canopy architecture and plant height. Also the contribution of genetic variation in spike photosynthesis to RUE_{PAR} and associations with SPI and spike DM per unit area and spike photosynthesis should be quantified.

3. Further work should use crop simulation modelling for joint optimization of traits in new ideotypes to predict outcomes under different environmental conditions and/or in a wider range of genotypes.

4. Further work is required to understand the physiological basis of the genetic variation in the stay-green traits identified in the present study, including analysis of N accumulation at anthesis in stems and leaves and post-anthesis remobilization to the grain. The target stay-green traits and their determinants should be further phenotyped in the WAMI panel to identify marker-trait associations and co-locations with marker-trait associations for source-sink balance as targets for development of markers for marker-assisted selection.

5. High-throughput phenotyping systems should be further developed to facilitate genetic analysis for the complex traits of biomass and RUE_{PAR}. High-throughput phenotyping related to biomass may relate multispectral and hyperspectral reflectance imaging including the use of canopy sensors mounted on aerial platforms. Canopy temperature using infrared thermometry on aerial platforms should also be developed for high throughput phenotyping of photosynthetic capacity indicative of RUE_{PAR}.

6. Further genetic analysis of the WAMI panel for the key target traits (including stay-green traits and their determinants) should be carried out. Once marker-trait associations are validated across multiple site-seasons, the development of backcross lines for target genetic loci and fine mapping of locations on the chromosomes may be implemented. These markers can then be developed as targets for marker-assisted selection for optimized source-sink balance and enhanced biomass and grain yield, and candidate genes established.

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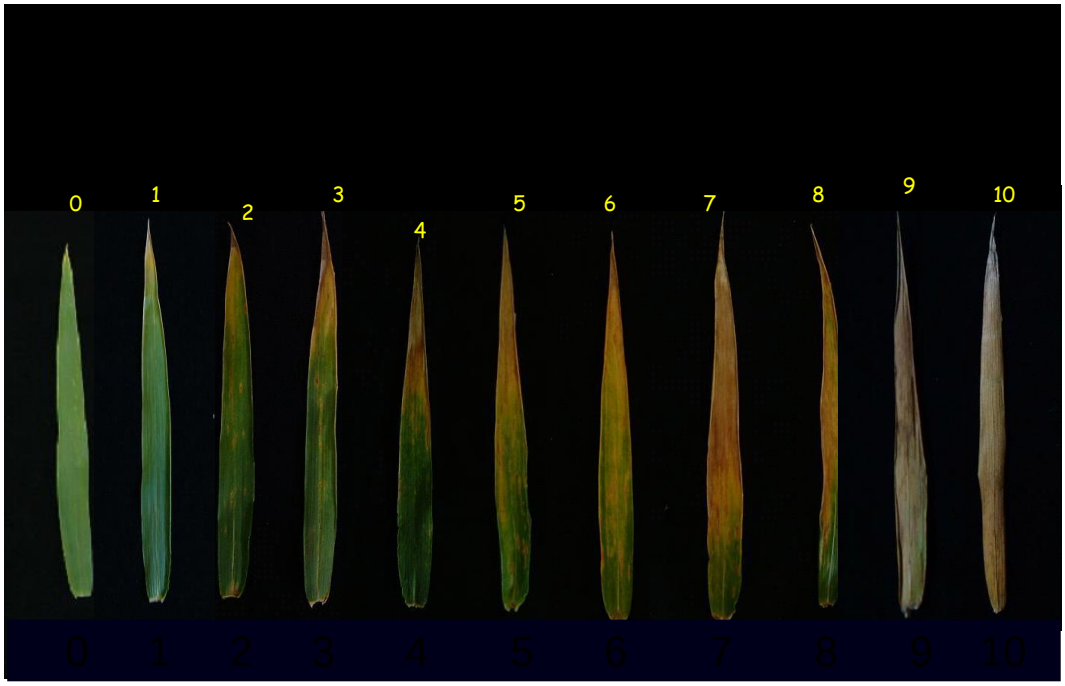
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APENDIX 1



Leaf senescence score key

APENDIX 2

Analysis of variance to test the interaction between the degraining treatment and spikelet location.

Variate: allspike

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
repyear stratum						
Year_1	1		191.342	191.342	16.83	0.015
Residual	4		45.469	11.367	0.59	
repyear.PLOT_1 stratum						
Cult_1	9		9187.301	1020.811	52.99	<.001
Year_1.Cult_1	9		368.459	40.940	2.13	0.053
Residual	36		693.545	19.265	1.50	
repyear.PLOT_1.batch stratum						
DegrainingTrat_1	1		509.499	509.499	39.62	<.001
Year_1.DegrainingTrat_1	1		4.881	4.881	0.38	0.542
Cult_1.Deg Trat_1	9		173.050	19.228	1.50	0.193
Year_1.Cult_1.DegrainingTrat_1	9		146.454	16.273	1.27	0.294
Residual	31	(9)	398.626	12.859	5.03	
repyear.PLOT_1.batch.*Units* stratum						
location	2		2296.677	1148.338	448.92	<.001
Year_1.location	2		0.466	0.233	0.09	0.913
Cult_1.location	18		253.616	14.090	5.51	<.001
Degraining Trat_1.Spikelet location	2		15.940	7.970	3.12	0.048
Year_1.Cult_1.location	18		67.453	3.747	1.46	0.112
Year_1.Deg Trat_1.Spikelet location	2		1.020	0.510	0.20	0.819
Cult_1.Deg Trat_1. Spikelet location	18		48.682	2.705	1.06	0.402
Year_1.Cult_1.DegTrat_1.Spik loc	18		46.284	2.571	1.01	0.458
Residual	136	(24)	347.886	2.558		
Total	326	(33)	13102.721			