Physiological and genetic analyses for the determination of grain number in wheat

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Abstract

Grain number is a key target of wheat (*Triticum aestivum* L.) breeding for increasing grain yield. To study the determination of grain number in wheat, four crucial questions arose during the time of my PhD period. To answer these yield-related questions, a series of experiments were carried out using various approaches, growth conditions and germplasm with a clear emphasis on a better elucidation of the pre-anthesis spike development of wheat plants.

Question 1: what are the connections between anther size, ovary size and spike dry weight at anthesis (SDW_a)? Importance of question 1: SDW_a and the development of anther and ovary are critical factors for the determination of grain number in wheat. We selected 30 central European winter wheat genotypes based on reduced height (*Rht*) and photoperiod (*Ppd*) genes with variable genetic backgrounds. We found that correlations between SWD_a and anther and ovary size in both greenhouse and field were strong, suggesting that anther and ovary size were good predictors of each other, as well as SWD_a in both conditions. Relationships between SWD_a and anther and ovary size at F3/4 (i.e. the third and fourth florets from the base of a spikelet) positions were stronger than at F1, suggesting that F3/4 anther and ovary size were better predictors of SWD_a. Generally, ovary size showed a closer relationship with SWD_a than anther size, suggesting that ovary size was a more reliable predictor of SWD_a.

Question 2: what is the time window of floral degeneration/demise? Importance of question 2: grain number is the outcome of floral development and degeneration; also, the time window of floral degeneration is critical to determine the maximum number of floret primordia per spikelet (MFS, grain yield potential). Moreover, after floral degeneration of apical floret primrodia within individual spikelets, anther and ovary size of living florets (basal positions) increases rapidly due to increased assimilate supply. We selected 12 spring wheat accessions to conduct a detillering experiment under both field and greenhouse conditions. We consistently observed the MFS at green anther stage (GA), while visible floral degeneration occurred at different developmental stages (e.g. from GA to yellow anther stage (YA), from YA to tipping stage (TP) in controlled and detillered plants under both field and greenhouse conditions. Detillering can delay floral demise which may be attributed to increased allocation of assimilates to the spike. Meanwhile, we found great plasticity of spike fertility-related traits induced by detillering in wheat (e.g. increased infertile spikelets and grain number per spikelet in the center of spike).

Question 3: what are the associations between ovary size, MFS, number of fertile florets per spikelet (FFS, at anthesis) and number of grains per spikelet (GS)? Importance of question 3: the difference between MFS (yield potential) and GS is the gap between ambition and realization, while FFS represents a central hub between both. Ovary size is a promising tool to narrow the gap between the “ambition” and “reality”. Using the 30 genotypes mentioned in experiment one, we detected that

FFS was closely associated with floret survival and only weakly related to MFS. We also found that the post-anthesis process of grain set or abortion was important in determining genotypic variation in GS; an increase in GS was mainly associated with improved grain set survival (post-anthesis). Ovary size at anthesis was associated with both floret survival (pre-anthesis) and grain set survival (post-anthesis), and was thus believed to 'connect' the two traits. In this work, proximal florets (i.e. the first three florets from the base of a spikelet: F1, F2, and F3) produced fertile florets and set grains in most cases. The ovary size of more distal florets (F4 and beyond) seemed to act as a decisive factor for grain setting and effectively reflected pre-anthesis floret development. In both growth conditions, GS positively correlated with ovary size of florets in the distal position (F4), suggesting that assimilates allocated to distal florets may play a critical role in regulating grain set.

Question 4: what are the genetic determinants of grain number (floret fertility), assimilate partitioning and spike morphology? Importance of question 4: as proved by our own work (mentioned above) and previous other studies assimilate distribution is important in determining grain number (floret fertility) and changed assimilate partitioning is always accompanied with modification of spike morphology. By quantifying 54 traits associated with floret fertility, assimilate distribution (above-ground parts) and spike morphology, we conducted a genome-wide association study (GWAS) using 210 winter wheat accessions. The GWAS results revealed potential Quantitative Trait Loci (QTLs) and candidate genes for floret fertility. The shared QTLs suggested a pivotal role of assimilate partitioning and spike morphology in the determination of floret fertility.

Summarily, through my PhD work, we clarified the floral development and demise process in wheat, displayed the relationships between ovary size, FFS, grain number per spikelet/spike and grain survival/abortion, but also, for the first time, discovered the underlying genetic factors of floret fertility, and their relations with assimilate partitioning and spike morphology in wheat.

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Abbreviations

AFLP	amplified fragment length polymorphism
AN	anthesis
BBCH	**B**iologische **B**undesanstalt, Bundessortenamt and **Ch**emical industry
BRI1	*BRASSINOSTEROID INSENSITIVE1*
D3	*DWARF 3*
CEN	*CENTRORADIALS*
CO	*CONSTANS*
DW	dry-weight
EMMA	Efficient Mixed Model Association
eps	earliness *per se*
F1	floret 1, the first floret from the base of spikelet
F3	floret 3, the third floret from the base of spikelet
F4	floret 4, the forth floret from the base of spikelet
FAO	Food and Agriculture Organization of the United Nations
FDR	false discovery rate
FE	fruiting efficiency
FFS	the number of fertile florets per spikelet
FT2	*FT-like protein gene 2*
GA	green anther
G × E	genotype × environment interaction
GLM	general linear model
GS	number of grains per spikelet
GWAS	genome-wide association study
HD	heading
HXK9	*HEXOKINASE9*
HI	harvest index
IDRC	International Development Research Centre Act
LD	linkage disequilibrium
MAF	minor allele frequency

MFS	maximum number of floret primordia per spikelet
MLM	mixed linear model
MYA	million years ago
PCA	principle component analysis
PCR	polymerase chain reaction
PhyC	*Phytochrome C*
PM	physiological maturity
Ppd	photoperiod
QTL	quantitative traits locus
RAPD	random amplified polymorphism
REML	restricted maximum likelihood
RFLP	restriction fragment length polymorphism
Rht	reduced height
SDW_a	spike dry weight at anthesis
SNP	single-nucleotide polymorphism
SS1	*SUCROSE SYNTHASE1*
SSR	simple sequence repeat
SUSIBA2	*SUGAR SIGNALLING IN BARLEY 2*
TASSEL	**T**rait **A**nalysis by a**SS**ociaiton **E**volution and **L**inkage
TIN	tillering inhibition
TKW	thousand kernel weight
TP	tipping
TS	terminal spikelet
USDA	United States Department of Agriculture
Vrn	*vernalization*
Vrs1	*SIX-ROWED SPIKE 1*
W	waddington
WA	white anther
YA	yellow anther
Z	Zadoks scale

1. Introduction

1.1. Worldwide wheat production and consumption

Wheat is the most widely grown cereal grain, occupying 17% of the total cultivated land in the world (IDRC, 2016). Wheat is the staple food for 35% of the world's population, and provides more than 20% of the total food calories and protein in human nutrition (IDRC, 2016). By 2050 the world's population will reach 9.6 billion, nearly 40% higher than today (FAO, 2016). Demand for wheat is predicted to increase in the future as the global population increases and developing countries adapt more meat-intensive diets. Wheat production will play an important role in food security and global economy in the coming decades. Wheat breeders around the world are in a serious situation and have to achieve improvement of wheat yield using constrained resources (e.g. land, water, nutrients) and other factors (e.g. climate change and rising energy costs). An important example is the sharp increase of international market prices for wheat in 2007/08, which hit a record due to the drought struck (http://iwyp.org/the-global-challenge/). The high wheat price caused political and economical instability and social unrest in both poor and developed nations. The food risk is likely to reoccur if wheat production continues to fall short of demand.

The introduction of dwarfing genes, known as reduced height (*Rht*) genes, has greatly increased grain yields in the Green Revolution after the 1960s (Peng et al., 1999; Hedden, 2003; Saville et al., 2012). However, worldwide, especially in developed countries, after 1990 the growth rate of wheat yield has slowed down (FAO, 2016). It was estimated that global wheat production will have to rise by 60% until 2050 to meet rising population (http://iwyp.org/the-global-challenge/). According to the report of the United States Department of Agriculture (USDA), global wheat supplies in 2015/16 are reduced by 3.3 million tons primarily due to decreased production. Of all the reduction, the largest

production change is a 2.4-million-ton decrease for India. In addition, due to the sharp reduction in harvested area Australian production declined by 1.5 million tons. An increase of 0.5 million ton was observed in European Union production. Global wheat production in 2015/16 remains a record. World wheat consumption in 2015/16, however, decreases to 709.4 million tons, by 2.0 million tons (http://www.usda.gov/oce/commodity/wasde/). FAO (2016) puts its first forecast for world cereal production in 2016 at around 2521 million tons, which represent the third highest performance on record. Global wheat utilization in 2016/17 is projected to remain nearly unchanged, at around 723 million tons, as a slight increase in total food consumption is expected to offset a decline in feeding and other uses of wheat. Wheat inventories could decline to nearly 194 million tons, by around 11 million tons, and most of the decrease is from the Russian Federation and Ukraine. Global wheat trade in 2016/17 is predicted to 154 million tons, an increase of 0.7 percent (1 million tons) compared with 2015/16 (http://www.fao.org/worldfoodsituation/csdb/en/).

Nowadays, wheat species are widely planted across the temperate, Mediterranean, and subtropical parts of both hemispheres of the world, from 67 °N in Norway, Finland, and Russia, to 45 °S in Argentina (Nevo et al., 2002). The harvested area of wheat worldwide in 2015/16 is estimated to be 224,278,000 hectares. This compares to an average world harvested wheat acreage estimate of 238,911,000 hectares since the 1981/82 marketing year, with a decline of 14,633,000 hectares over the most recent 34 marketing years (USDA, 2016). Major wheat exporters worldwide are Argentina, Australia, Canada, the EU, Kazakhstan, Russian Federation, Ukraine and the United States (FAO, 2016). World wheat exports in the 2015/16 marketing year are estimated to be 162,734,000 metric tons. This compares to average world wheat exports of 100,376,000 metric tons since the 1981/82

marketing year, with an average increase of 62,358,000 metric tons over the 34 years period (http://www.fas.usda.gov/psdonline/psdQuery.aspx).

Due to the serious situation of food shortages and the important role of wheat in the food supply worldwide mentioned above, increasing grain yield in wheat is still the main goal for wheat breeding. Grain number is the most decisive factor for the determination of grain yield in wheat, as at least 50% of grain yiel potential has been lost during floral development process (Reynolds et al., 2009; Fischer, 2011; Pedro et al., 2012; Guo and Schnurbusch, 2015). Therefore, it is necessary to clarify the floral development process, find the key traits and developmental stages for the improvement of grain yield in order to find new breeding strategies to increase the survival rate of grain yield potential.

Germany is the European Union's biggest member state, in terms of population and the size of its economy. Germany's climate is moderate and quite suitable for winter wheat cultivation. During summer months, the average temperature across the country is 17°C and it is 1°C during winter period. Gernerally, most regions in Germany receive between 700 and 800 mm rainfall per year, precipitation in the Eastern part is significantly lower with only 400-600 mm (http://www.agribenchmark.org/cash-crop/sector-country-farm-information/country-profiles/germany.html). Germany is the second-ranked wheat grower in the European Union. With a 53% proportion of the total area under grain cultivation, winter wheat tops the list of grain cultivated in 2015. Based on a first estimate, the Federal Statistical Office reports that, winter wheat is cultivated for harvest on 3.25 million hectares in Germany in 2015. This is an increase of 3% compared with a year earlier (Germany's statistics office, 2016). Total wheat production in Germany are 25,019,100 metric tons in 2013, 27,784,700 metric tons in 2014, 26,549,500 metric tons in 2015 (Germany's statistics office, 2016). Meanwhile, yield per hectare of wheat are 8.00 metric tons (2013), 8.63 metric tons (2014) and 8.09 metric tons

(2015), which are rather good scores worldwide (Germany's statistics office, 2016). However, according to our work (Guo and Schnurbusch, 2015; Guo et al., 2016) more than 60% of grain yield potential has been lost in both modern and old wheat genotypes released in Germany. Therefore, grain number is an important target for wheat breeding and there is a great potential for the improvement of grain yield in Germany.

1.2. Wheat evolution and domestication

1.2.1. Distribution of wheat genomes

Studies of wheat evolution have attracted great attention over the past 100 years. Wheat species can be devided into three groups based upon their ploidy level according to cytogenetic work: (1) diploid 2n = 2x =14 =einkorn wheat; (2) tetraploid 2n = 4x =28 =emmer wheats; (3) hexaploid 2n = 6x =42 =bread wheats (Sax and Sax, 1924).

Modern wheat cultivars belong primarily to two polyploid species: hexaploid bread wheat and tetraploid wheat. The cultivated diploid species *T. monococ-cum* L. einkorn wheat is a relic and is only found in some mountainous Mediterranean regions (Killian et al., 2010). The genus *Triticum* consists of six species: *T. monococcum* L. (AA genome); *T. urartu* (AA genome); *T. turgidum* L. (AABB genome); *T. timopheevii* (AAGG genome); *T. aestivum* L. (AABBDD genome); and *T. zhukovskyi* (AAAAGG genome). Of these species, *T. urartu* exists only in its wild form, whereas *T. aestivum* and *T. zhukovskyi* exist only as cultivated forms; the other species, *T. monococcum*, *T. turgidum* and *T. timopheevii*, have both a wild and a domesticated form (Matsuoka, 2011). All *Triticum* species are native to the "Fertile Crescent" of the Near East (Matsuoka, 2011). The Fertile Crescent is the region in the Middle East, which curves like a quarter-moon shape (Fig. 1.1). This region is generally considered as the birthplace of agriculture, the high variety in topography of this region gave rise to many

species of edible plants for early experiments in cultivation. In current usage, all definitions of the Fertile Crescent include Mesopotamia, the land in and around the Tigris and Euphrates rivers; and the Levant, the eastern coast of the Mediterranean Sea. Nowadays, the countries with significant territory within the Fertile Crescent are Iraq, Syria, Lebanon, Jordan, Israel and Palestine, beside the southeastern fringe of Turkey and the western fringes of Iran (https://en.wikipedia.org/wiki/Fertile_Crescent)

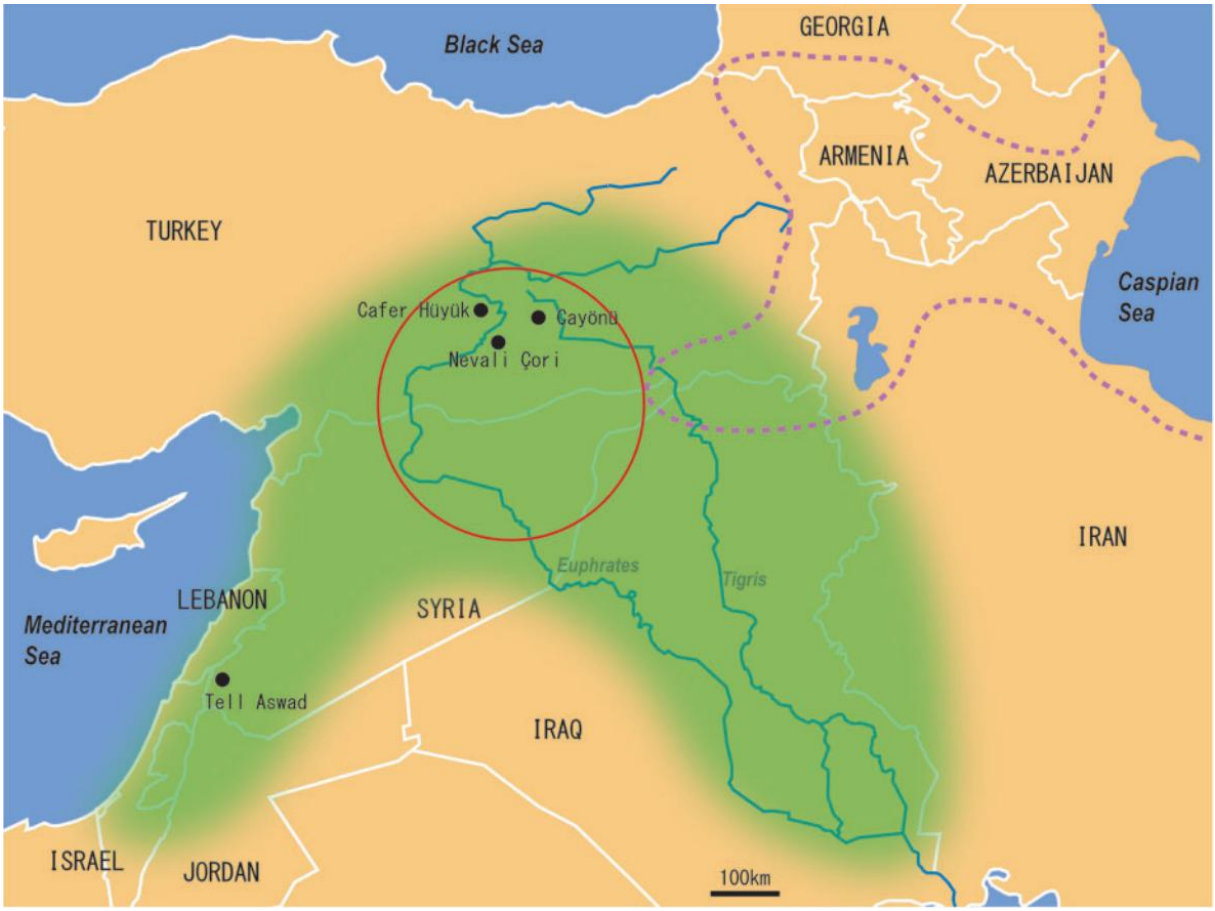

Fig.1.1. Map of the Near East that includes the Fertile Crescent (shaded in green). The red circle and dashed purple line respectively denote the 'core area', the birthplace of agriculture (Lev-Yadun et al., 2000)(source: Matsuoka, 2011).

1.2.2. Domestication and evolution of wheat genomes

Previous studies led to the genome distinctions A, B, D, G, S, and so forth, that are still used today in wheat research. The classic wheat evolutionary history is one of adaptive radiation of the diploid *Triticum*/*Aegilops* species (A, S, D), genome convergence and divergence of the tetraploid (*T. turgidum* AABB, and *T. timopheevii* AAGG) and hexaploid (*T. aestivum*, AABBDD) species. Domestication and evolution of wheat genomes have been investigated using different approaches in previous work.

Huang et al. (2002) used *Acc-1* (plastid acetyl-CoA carboxylase) and *Pgk-1* (plastid 3-phosphoglycerate kinase) genes to establish the timeline of wheat evolution. It was concluded that the A genome of polyploid wheat diverged from *T. urartu* less than half a million years ago (MYA) and the diploid *Triticum* and *Aegilops* progenitors of the A, B, D, G, and S genomes all radiated 2.5-4.5 MYA. Devos et al. (2005) used the *Phytochrome C* (*PhyC*) gene involved in photomorphogenesis for phylogenetic analyses. According to the level of sequence divergence between the three wheat *PhyC* homoeologs, they concluded that the divergence of the diploid wheat ancestors occurred some 6.9 MYA, which is considerably earlier than the previously estimated 2.5-4.5 MYA by Huang et al. (2002). Gornicki et al. (2014) traced the evolutionary history of the wheat species through relationships between chloroplast genomes. They sequenced 25 chloroplast genomes, and genotyped 1127 plant accessions, representing 13 *Triticum* and *Aegilops* species. The results suggested that the *Ae. speltoides* (SS genome) diverged before the divergence of *T. urartu* (AA), *Ae. tauschii* (DD) and the *Aegilops* species of the Sitopsis section. *Ae. speltoides* forms a monophyletic clade with the polyploid Emmer and Timopheevi wheats, which originated within the last 0.7 and 0.4 MYA, respectively.

The origin of the B and G genomes remains controversial. Much evidence suggests that an ancestor *Ae. speltoides* species (S genome) was the donor of what became the B genome of the bread and durum wheats. Blake et al. (1999) suggested that these Sitopsis species are diverged forms of the ancestral B genome donor. It was also concluded that *Ae. speltoides* (S genome) is the B and G genome donor of all polyploid wheats (Wang et al., 1997; Maestra and Naranjo, 1998) and the B genome in *T. turgidum* and the G genome in *T. timopheevii* were proposed to be closely related to each other (Dvorak and Appels, 1982), but also it was suggested that the G genome of *T. timopheevii* is more closely related to the S genome of *Ae. speltoides* than to the B genome of *T. turgidum* (Dvorak et al., 1989).

1.3. Inflorescence development in wheat

The difference between wheat yield potential (MFS, maximum number of floret primordia per spikelet) at green anther stage (GA) and grain number per spikelet (GS) at physiological maturity (PM) display that more than half of the yield potential has been lost, suggesting a great potential of grain yield for improvement (Fig. 1.2). To increase grain number per spike/spikelet, it is critical to investigate the mechanism of loss of yield potential (i.e. floral degeneration), and the clarification of floral degeneration process will be helpful to investigate floral abortion. Therefore, one of the purposes of my PhD study is to clarify the floral development and degeneration process.

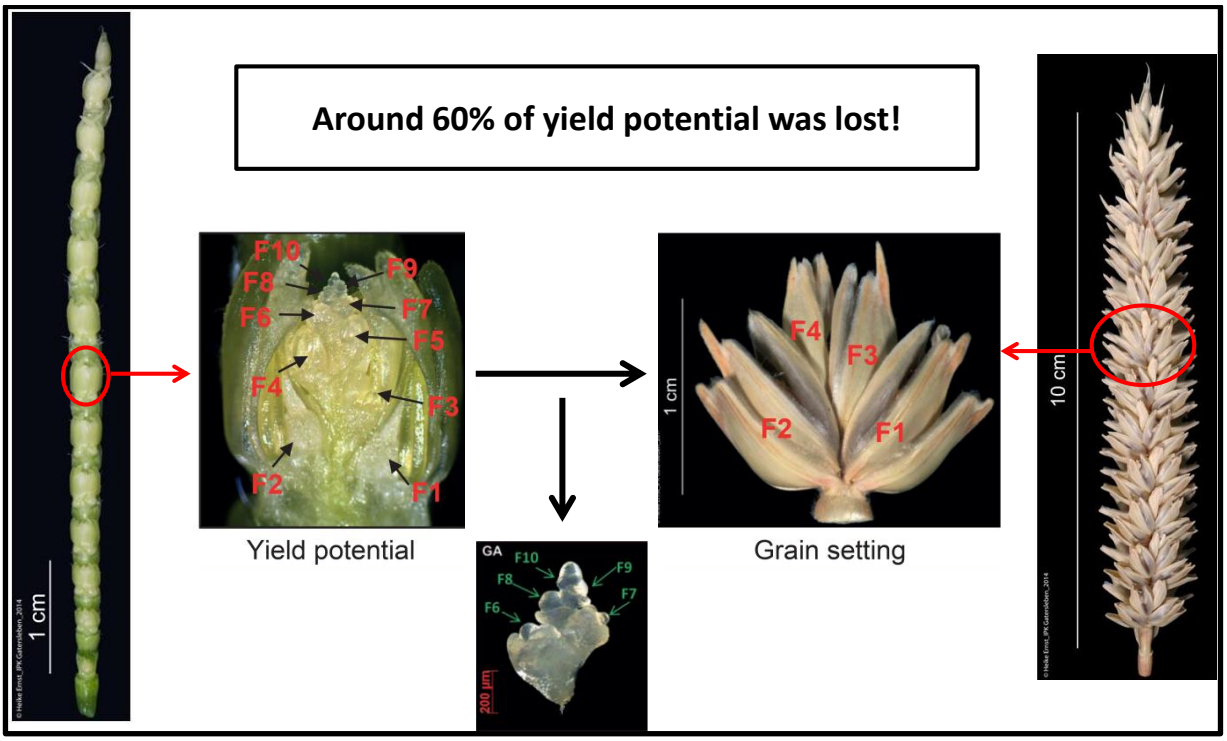

Fig.1.2. Yield potential (MFS), grain setting (GS) and loss of grain yield potential within individual spikelet of wheat.

As spikelet structure is critical for the study of floral development and abortion process, it is mandatory to learn spikelet structure of wheat. In the developing plants, there are three anthers and one ovary within one floret. Each spikelet consists of up to 10 floret primordia and more than half of the floret primrodia (apical part within the spikelet) will abort (Fig. 1.2, 1.3). In mature plants of wheat, one spikelet contains several grains, which are enclosed by two large and strong glumes.

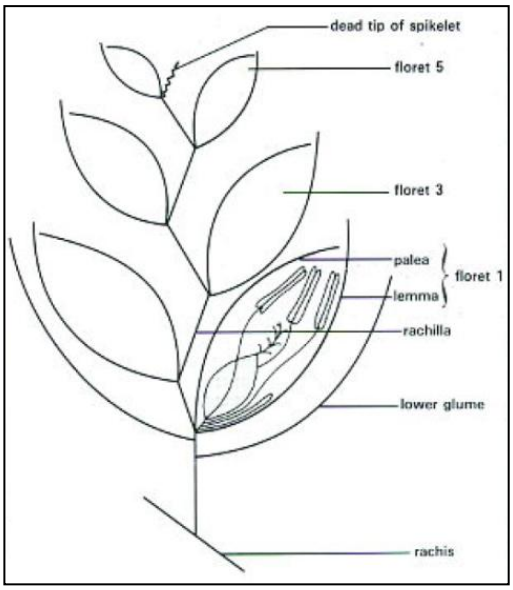

Fig. 1.3. Schematic spikelet structure in developing plants of wheat (Source: Kirby and Appleyard, 1987).

Kirby and Zadoks scales have been verified to be effective tools to monitor the spike development in wheat. Using the Kirby scale, there is a long period between yellow anther (YA) stage and anthesis (AN), so we introduce the tipping (TP) and heading (HD) stages from Zadoks scale to devide the long period. Here we describe the stages in wheat following Kirby and Appleyard (1987), and Zadoks et al. (1974).

Vegetative stage in wheat (after Kirby and Appleyard, 1987). The wheat plant at the vegetative stage is a seedling; this vegetative stage starts from germination until four to eight leaves (depending on sowing datas and varieties) emerge on the main shoot.

Double ridge stage in wheat (after Kirby and Appleyard, 1987). At double ridge stage, the spikelet and leaf primordia are visible. Generally, below each spikelet primordium ridge, there is one leaf primordium ridge which is less prominent. Almost one half of the spikelet primordia have initiated.

Glume primordia stage in wheat (after Kirby and Appleyard, 1987). The glume primordia can be found under spikelet primorida. The glume primordia in wheat at this stage are larger and more easily seen than in barley.

Lemma primordia stage in wheat (after Kirby and Appleyard, 1987). At lemma primordia stage, the lemma primordia of floret 1 and 2 at the bottom of one spikelet can be found between glume primordia and floret meristem. The lemma primordia initiate firstly, and then the axillary meristems differentiate to form the other floral structures. Meanwhile, the development of the spikelets proceeds the meristematic dome of the shoot apex and continues to initiate more spikelets primordia.

Floret primordium stage in wheat (after Kirby and Appleyard, 1987). At this stage the multi-floret nature of the spikelet is clearly visible. A floret meristem grows in the axil of each lemma. The spikelet meristem initiates more florets. The region in the axil of the lemma has grown to form a smoothly rounded meristematic dome. Within each spikelet, the alternate arrangement of the florets can be seen and the smoothly rounded meristematic dome of the floret is prominent.

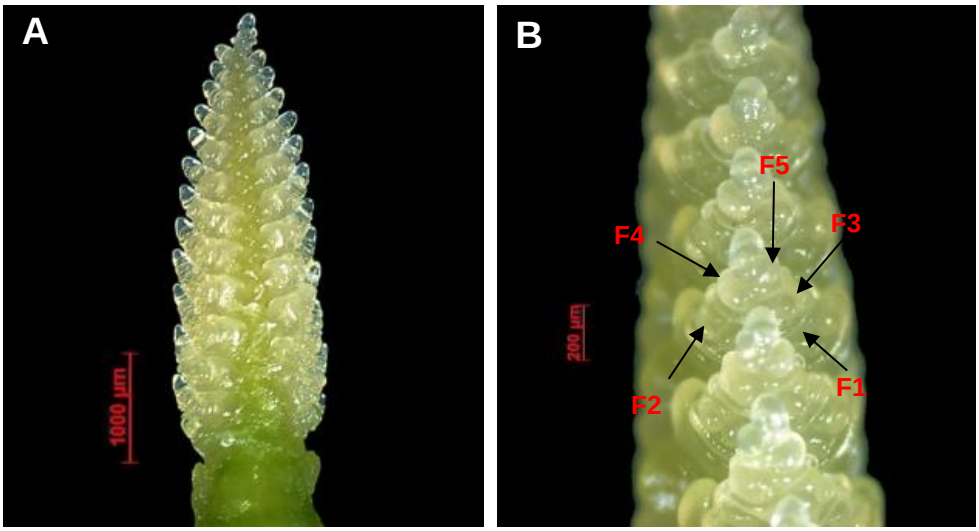

Fig. 1.4. Spikelet and floret primordia at the terminal spikelet (TS) stage. Fig. 1.4 A is a spike at TS stage, and Fig. 1.4B is the floret primordia within one spikelet.

Terminal spikelet (TS) stage in wheat (Fig. 1.4, after Kirby and Appleyard, 1987). At this stage, the embryo spike almost completely forms; no more spikelets will initiate. At this stage, the last few primordia initiated by the dome of the shoot apex do not develop into spikelets but become the glumes and floret primordia of a terminal spikelet.

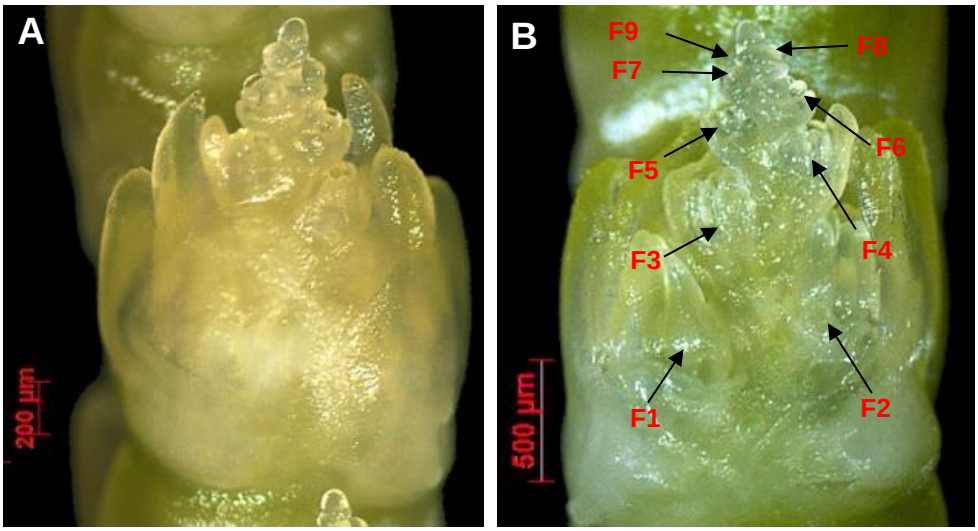

Fig. 1.5. Floret primordia within individual spikelet at the white anther (WA) stage. Fig. 1.5A is a spikelet at WA stage, and Fig. 1.5B is the longitudinal section of a corresponding spikelet in Fig. 1.5A.

White anther (WA) stage in wheat (Fig. 1.5, after Kirby and Appleyard, 1987). The meristematic dome of each spikelet has initiated more than eight floret primordia. The glumes partially enclose the florets, the stamens and other structures cannot be seen as floret 1 and 2 at the bottom of spikelet are completely enclosed by their lemmas. Small awns are visible on lemmas of floret 2 and 3 at the bottom of spikelet. The anthers can be checked by removing glumes and the lemma of floret 1.

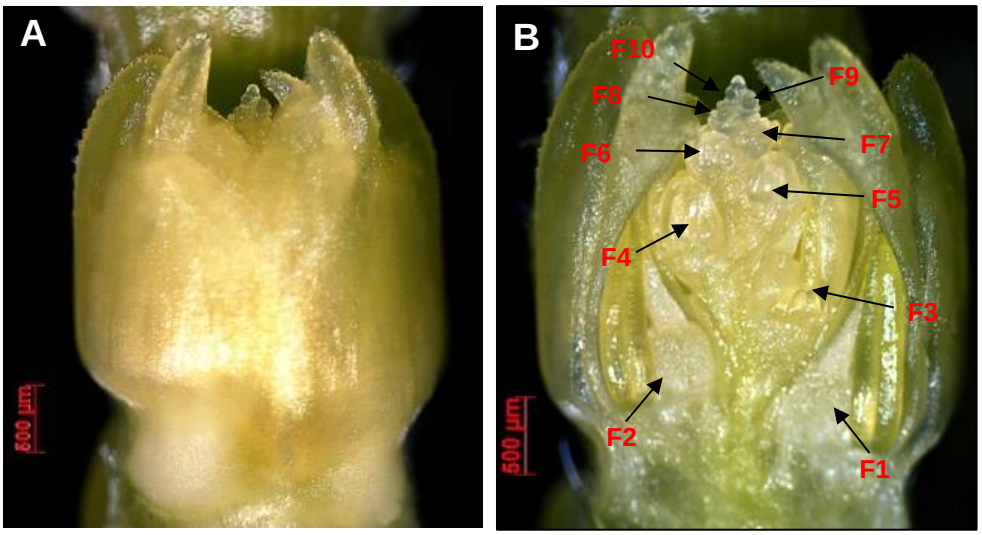

Fig. 1.6. Floret primordia within individual spikelet at the green anther (GA) stage. Fig. 1.6A is a spikelet at GA stage, and Fig. 1.6B is the longitudinal section of a corresponding spikelet in Fig. 1.6A.

Green anther (GA) stage in wheat (Fig. 1.6, after Kirby and Appleyard, 1987). The dome probably would not produce any more primordia according to our work (Guo and Schnurbusch, 2015). The most obvious evident trait at this stage is that: the glumes cover the entire spikelet except the tips of the florets. At the base of the floret, two fleshy lodicules are prominent. The wings of the palea fold over to partly enclose the three stamens.

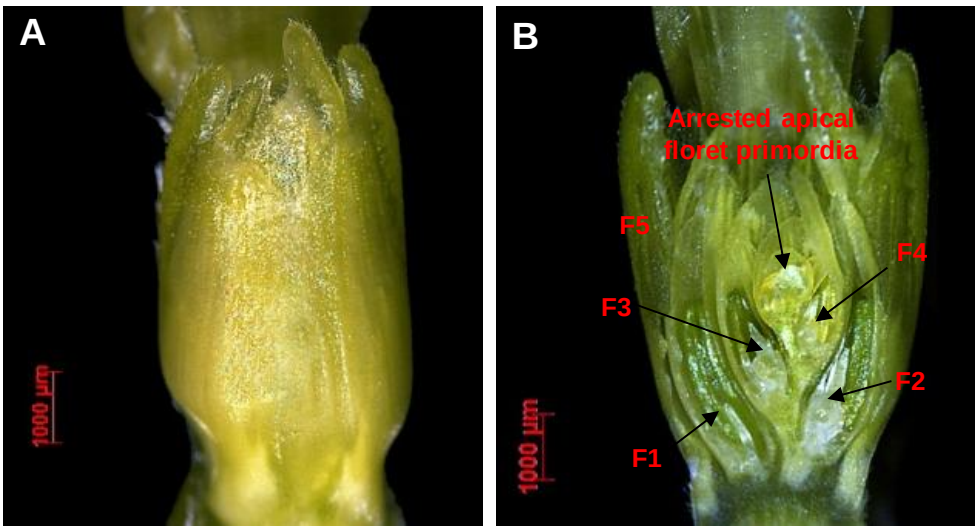

Fig. 1.7. Floret primordia within individual spikelet at the yellow anther (YA) stage. Fig. 1.7A is a spikelet at YA stage, and Fig. 1.7B is the longitudinal section of a corresponding spikelet in Fig. 1.7A.

Yellow anther (YA) stage in wheat (Fig. 1.7, after Kirby and Appleyard, 1987). The glumes are fully formed and the lemmas of the first three florets are visible. The lemma of floret 1 has a short awn point; the awns of floret 2 and 3 at the base of spikelet are longer. Floret 1, 2 and 3 at the base of spikelet have well developed. The anthers are bright greenish yellow. Floret 4 at the base of spikelet has large anthers and a well developed carpel but is less mature than the lower florets.

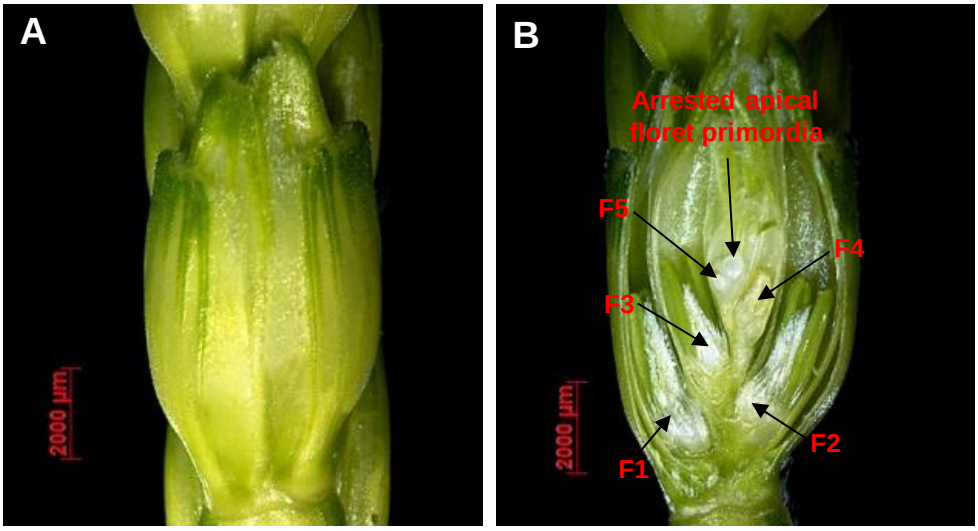

Fig. 1.8. Floret primordia within individual spikelet at the tipping (TP) stage. Fig. 1.8A is a spikelet at TP stage, and Fig. 1.8B is the longitudinal section of a corresponding spikelet in Fig. 1.8A.

Tipping (TP) stage in wheat (Fig. 1.8, Zadoks scale, Z49). At TP stage, first awns are visible. At the TP stage florets with visible anthers and ovaries developed further; whereas apical floret primordia remain arrested. Anther colour is greenish yellow, and the colour of glumes shifted from light green to yellow at TP.

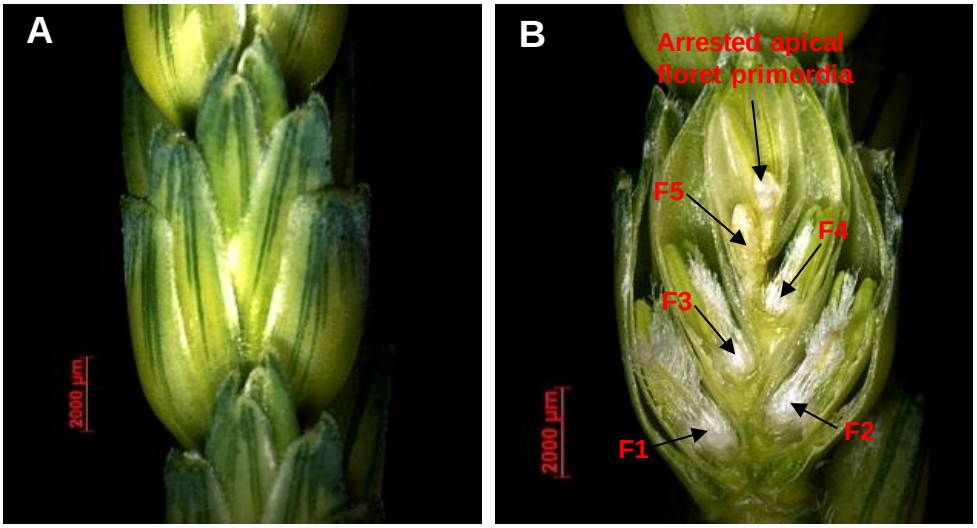

Fig. 1.9. Floret primordia within individual spikelet at the heading (HD) stage. Fig. 1.9A is a spikelet at HD stage, and Fig. 1.9B is the longitudinal section of a corresponding spikelet in Fig. 1.9A.

Heading (HD) stage in wheat (Fig. 1.9, Zadoks scale, Z55). At HD stage, half of individual spike is visible. F1 anthers reach maximum size; glumes become stiffer.

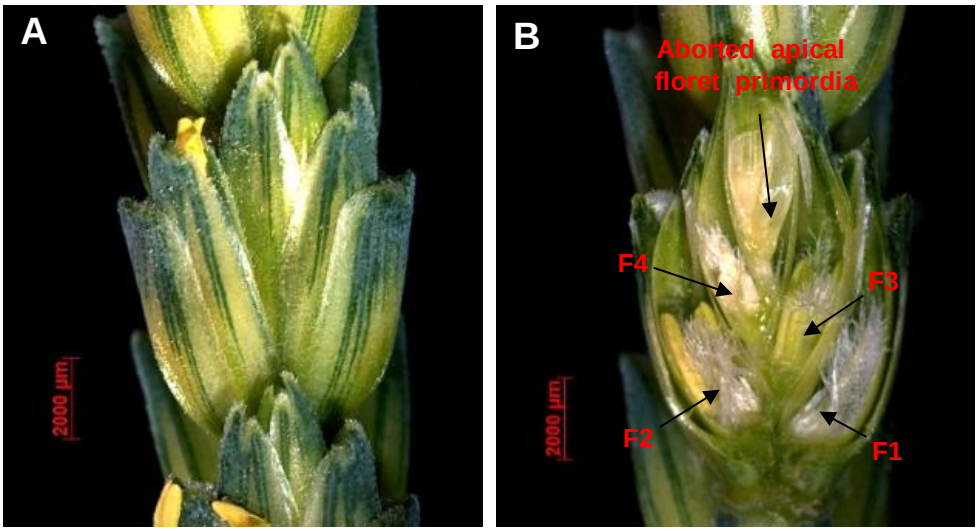

Fig. 1.10. Floret primordia within individual spikelet at anthesis (AN). Fig. 1.10A is a spikelet at AN stage, and Fig. 1.10B is the longitudinal section of a corresponding spikelet in Fig. 1.10A.

Anthesis (AN) in wheat (Fig. 1.10, after Kirby and Appleyard, 1987). The yellow anthers extrude from floret 1, 2 at the base of the spikelet. At anthesis, those florets, which are still alive, become fertile florets. F1 ovaries develop rapidly from HD to AN. The aborted apical

floret parts disappear completely. Anther colour turn completely yellow, stigmatic hair is well developed.

Grain development immediately after fertilization is also an important phase where grain number is set and potential seed size is determined. Grain development can be devided into three stages: milk development, dough development and ripening (Zadoks et al., 1974). In the milk developmental stage, a marked increase in solids of liquid endosperm can be observed when the grains are crushed between fingers; the chlorophyll of the inflorescence is lost during the dough development; the grains is becoming harder when they are ripening, meanwhile, the straw is dead and collapsing (Zadoks et al., 1974).

In this thesis, we will predominantly focus on the floral development during the pre-anthesis phase, since most of the grain yield potential (MFS) will be lost during this phase. Here, we plan to investigate the process of floral abortion using variable approaches based on previous work which provides some hints (Calderini et al., 1999; Castillo et al., 2015; Hanif and Lager, 1972; Whingwiri et al., 1981).

Of all the previous studies, the two dealing with the vascular system winthin spikelet and spike seem very valuable for the explanation of floral demise. Hanif and Langer (1972) observed that the lower three florets in a spikelet of wheat were supplied by the principal vascular bundles of the rachilla, while the system of more distal florets consisted of subvascular elements derived from the vascular cylinder formed at the disc of insertion of these florets. This pattern appeared to be consistent accross all spikelets irrespective of position, apart from the terminal one. The vascular system is contrasted with grain number per spikelet and it suggested that if a floret is connected directly with the main vascular system, and assimilates are not limiting, then competition for assimilates does not appear to be the main limitation of grain setting. In another study, Whingwiri et al. (1981) examined

the vascular system within the rachis of wheat spikes. The number of both central and peripheral bundles declined acropetally (i.e. towards the tip) along the length of the rachis. The decline of peripheral bundles was observed mainly between internodes 1 and 6, numbered from the base. Three central bundles consistently reached the terminal spikelet. The decline in total vascular size was determined by three factors: (i) some bundles branched and to reducted in size, (ii) the diversion or dropping of bundles into spikelets, or (iii) a combination of (i) and (ii).

Beside vascular connectivity, the anther and carple development is also quite important for floral development. Carple size is associated with grain weight (Calderini et al., 1999; Xie et al., 2015) and number (Guo et al., 2016). Boron deficiency displays great effects on anther development and floret fertility in Wheat (Huang et al., 2000). It was also reported that several important genes are involved in the regulation of anther development, which suggests the influence of phytohormone and other factors on anther development (Song et al., 2012; Wang et al., 2012; Castillo et al., 2015).

Although lots of excellent work associated with the determination of grain number has been conducted, it appears important to develop a hypothesis which displays the connections of previous studies and will similarly include comprehensive information about the determinants of grain yield in wheat, e.g. assimilate partitioning, vascular system, phytohormone, ovary size.

1.4. Models for wheat development.

Both variation and consistency of development exsit in different grasses, many scientists have tried to summarize these variations and consistences by developing different growth models. All the models for growth of crops demonstrate the advantages and limitations in

terms of different investigation purposes. Here, five most widely used models (Zadoks scale, BBCH scale, Haun Scale, Kirby Scale, and Waddington Scale) are introduced to describe the wheat development on different levels.

1.4.1. Zadoks scale

The Zadoks scale is used to describe growth stages of cereals (Zadoks et al., 1974). Developmental stages are described with an adapted Zadoks codes listed in the Table 1.1. The Zadoks scale devide the plant life cycle into ten principal phases: Emergence (0-10), seedling growth (11-19), tillering (20-29), stem elongation (30-36), flag leaf to booting (37-49), heading (51-60), flowering (anthesis) (61-69), kernel and milk development (70-79), dough development (81-89), and finally ripening (91-94).

Table 1.1. Adapted Zadoks decimal codes for cereals.

0 Emergence	52 20% of spikes visible
00 Dry seed sown	53 30% of spikes visible
01 Seed absorbs water	54 40% of spikes visible
03 Germination, seed swollen	55 50% of spikes visible
05 Radicle emerged from seed	56 60% of spikes visible
07 Coleoptile emerged from seed	57 70% of spikes visible
09 Leaf at coleoptile tip	58 80% of spikes visible
10 First leaf through coleoptile and tip visible	59 90% of spikes visible
1 Seedling growth	60 Whole spike visible, no yellow anthers
11 1st leaf more then half visible	**6 Flowering (anthesis)**
12 2nd leaf more then half visible	61 Early– 10% of spikes with yellowanthers
13 3rd leaf more then half visible	62 20% of spikes with yellowanthers
14 4th leaf more then half visible	63 30% of spikes with yellow anthers
19 6th leaf more then half visible	64 40% of spikes with yellowanthers
17 7th leaf more then half visible	65 Mid– half of spikes with anthers
18 8th leaf more then half visible	66 60% of spikes with yellowanthers
19 9 or more leaves visible and stem not elongating.	67 70% of spikes with anthers
	68 80% of spikes with yellowanthers
2 Tillering	69 Late– 90% of spikes with anthers
20 Main shoot only	**7 Kernel and Milk development**
21 Main shoot and 1 tiller	70.2 Kernels middle spike extended 20%
22 Main shoot and 2 tillers	70.5 Kernels middle spike half formed
23 Main shoot and 3 tillers	70.8 Kernels middle spike extended 80%
24 Main shoot and 4 tillers	71 Watery ripe, clear liquid
25 Main shoot and 5 tillers	73 Early milk, liquid off-white

26 Main shoot and 6 tillers
27 Main shoot and 7 tillers
28 Main shoot and 8 tillers
29 Main shoot and 9 or more tillers

3 Stem elongation

30 stem starts to elongate, 'spike at 1cm'
31 swelling 1st node detectable
32 swelling 2nd node detectable
33 swelling 3rd node detectable
34 swelling 4th node detectable
35 swelling 5th node detectable
36 swelling 6th node detectable

4 Flag leaf to Booting

37 Flag leaf tip visible
38 Flag leaf half visible
39 Flag leaf ligule just visible
41 Early boot, flag sheath extending
43 Mid-boot, boot opposite ligule of 2nd last leaf
45 Full-boot, boot above ligule of 2nd last leaf
47 Flag leaf sheath opening
49 First awns visible

5 Heading

51 10% of spikes visible (ear peep)
75 Medium milk, contents milky liquid
77 Late milk, more solids in milk
79 Very-late milk, half solids in milk

8 Dough development

81-85 spikes turn colour from light-green to yellow-green to yellow
81 Very early dough, more solids and slides when crushed
83 Early dough, soft, elastic and almost dry, shiny
85 Soft dough, firm, crumbles but fingernail impression not held
87 Hard dough, fingernail impression held, spike yellow-brown
89 Late hard-dough, difficult to dent

9 Ripening

91 Kernels hard, difficult to divide by thumb-nail
92 Harvest ripe, kernels can no longer be divided by
93.ernels loosening in daytime
94.ver-ripe, straw brittle

(Source: Dr Maarten Stapper, CSIRO Plant Industry. http://www.biologicagfood.com.au/wp-content/uploads/STAPPER-Crop-Monitoring-v21.pdf)

1.4.2. BBCH scale

The abbreviation BBCH derives from **B**iologische **B**undesanstalt, Bundessortenamt and **Ch**emical industry (http://www.jki.bund.de/fileadmin/dam_uploads/_veroeff/bbch/BBCH-Skala_englisch.pdf). The decimal code is divided into principal and secondary growth stages; the structure of the individual scales was in general based on the cereal code developed by Zadoks et al. (1974). The entire life cycle of the plants is divided into ten developmental phases in BBCH scales. These principal growth stages are described using numbers from 0 to 9. Within each principal growth stage, the greater codes suggest the plants at later growth stages. An advantage of BBCH coding system compared with the Zadoks coding system is

that it includes monocotyledons and dicotyledons. The general scale for all the crops are shown in Table 1.2.

Table 1.2. Principal growth stages in BBCH scale.

Stage	Description
0	Germination / sprouting / bud development
1	Leaf development (main shoot)
2	Formation of side shoots / tillering
3	Stem elongation or rosette growth / shoot development (main shoot)
4	Development of harvestable vegetative plant parts or vegetatively propagated organs / booting
5	Inflorescence emergence (main shoot) / heading
6	Flowering (main shoot)
7	Development of fruit
8	Ripening or maturity of fruit and seed
9	Senescence, beginning of dormancy

(Source: Dr Uwe Meier, Federal Biological Research Centre for Agriculture and Forestry, http://www.jki.bund.de/fileadmin/dam_uploads/_veroeff/bbch/BBCH-Skala_englisch.pdf)

1.4.3. Haun scale

Haun scale makes use of the regular appearance of leaves at the growing point (Haun, 1973). Each new leaf displays a unit of development. After the emergence of flag leaf, the remaining visible morphological development is developed into four additional units: extension of flag leaf, enlargement of boot, emergence of spike, elongation of culm. Each growth unit beginns with the appearance of the next leaf. The development of the first leaf of wheat is defined using 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 units from emergence of the leaf from the coleoptile (0.0) to appearance of the second leaf (1.0). The extension of the flag leaf is defined using x.2, x.4, x.6, x.8, and 1+x.0 units. This phase is completed with the first signs of swelling of the boot. The enlargement of the boot is determined by 1+x.2, 1+x.4, 1+x.6, 1+x.8, and 2+x.0 units. This phase is completed when the spike begins to emerge. The emergance of the spike is discribed by 2+x.2, 2+x.4, 2+x.6, 2+x.8, and 3+x.0 units. X refers to the leaf number of the flag leaf.

1.4.4. Kirby scale

As spike and floral development in Kirby scale has already been mentioned above, we only describe tiller development and stem elongation for the Kirby scale (Kirby and Appleyard, 1987). Tiller development of wheat and barley in Kirby scale is described using four stages: tiller bud initiation, prophyll initiation, tiller bud formation, tiller leaf emergence. The first visible stage in the formation of a tiller is the growth of the ridge of meristematic tissue in the axil of a basal leaf (Tiller bud initiation). As the ridge of meristem grows, it becomes dome-shaped. This ridge grows to form prophyll which is a sheathing structure, similar to the coleoptile of the main shoot (Prophyll initiation). The prophyll grows to enclose the dome and leaf primordia and a bud is formed. Dissection of the tiller bud at this stage will reveal a shoot apex with leaf primordia and meristematic dome. The dome initiates leaves and then spikelets in the same way as the main shoot (Tiller bud formation). When the tiller has grown up within the sheath of the leaf and its tip protrudes above the level of the ligule, the prophyll ceases growth and the first true leaf emerges (Tiller leaf emergence). Stem elongation starts when spike is around 1 cm. Stem elongation is closely associated with the stage in barley or floret stage in wheat. Fast stem elongation occurs at maximum number of primordia stage at TS stage. Stem growth is the result of elongation of the internodes (the regions between the nodes). An internode may or may not elongate depending on its position on the shoot. The wheat stem is formed by the elongation, in sequence, of the last 5 or 6 internodes.

1.4.5. Waddington scale

Waddington scale is another critical tool to discribe spike development in wheat (Waddington et al., 1983). Quantitative scales of development in wheat and barley from

seedling emergence (0) to pollination (10) were proposed, based on the morphogenesis of the spike initial, then the floret and finally the pistil. These scales can be used to quantify developmental progress (Table 1.3).

Table 1.3. Spike development in wheat (and barley).

Waddington scales	Description
1.5	Transition apex
2	Early double ridge stage
2.5	Double ridge stage
3	Glume primordium present
3.25	Lemma primordium present
3.5	Floret primordium present
4	Stamen primordium present
4.25	Pistil pnmordium present
4.5	Carpel primordium present
5	Carpel extending round three sides of ovule
5.5	Stylar canal closing; ovarian cavity enclosed on all sides but still open
6	Stylar canal remaining as a narrow opening; two short round style
6.5	Styles begin elongating
7	Stigmatic branches just differentiating as swollen cells on styles
7.5	Unicellular hairs just differentiating on ovary wall; stigmatic branches
8	Stigmatic branches and hairs on ovary wall elongating
8.5	Stigmatic branches and hairs on ovary wall continue to elongate; stigmatic
9	Styles and stigmatic branches erect;stigmatic hairs differentiating
9.5	Styles and stigmatic branches spreading outwards. Stigmatic hairs well
10	Styles curved outwards and stigmatic branches spread wide; pollen grains

(Source: Waddington et al, 1983)

1.5. Determinants of grain number in wheat

Grain number per spike is determined by the number of fertile spikelets (spikelet fertility) and grain number per spikelet (floret fertility). Assimilate distribution and the utilization efficiency of assimilates are critical for the determination of spikelet and floret fertility. Spike fertility index, harvest index (HI) and fruiting efficiency are three crucial indicators for displaying the assimilate distribution and the utilization efficiency of assimilates. Here, the important roles of the five indicators would be introduced and strategies are shown how we can use them to increase grain number per spike.

1.5.1. Spike fertility index

The grain number per spike between cultivars was shown to be highly associated with SDW_a and spike fertility index (i.e. grain number per gram of non-grain or chaff spike at anthesis or harvest) (Gonzalez et al., 2011). Using different strategies, it has been proven that SDW_a is positively associated with FFS and GS. Slafer et al. (1990) used six Argentinian bread-wheat cultivars released between 1912 and 1980 and found SDW_a is closely associated with both grains number per m^2 and harvest index. Fischer and Stockman (1980) observed that shading reduced SDW_a which further decreased grain number per spike. Stockman et al. (1983) detected that shading decreased floret number and grain yield by reducing assimilate partitioning to the spike. Gonzalez et al. (2003) lengthened the duration of stem elongation by exposuring wheat plants to less inductive photoperiod; and then a higher number of fertile florets at anthesis was achieved leading to an increased grain number and higher yield. As SWD_a is critical for the determination of grain number, increase of SDW_a is crucial for the improvement of grain number. Lots of previous work has suggested variable strategies for the increase of SDW_a. For example, SDW_a could be achieved by lengthening duration of the stem elongation phase (which encloses spike growth period) without changing anthesis date (which is close to optimum in modern wheat cultivars) (Slafer, 1996; Slafer et al., 2001). SDW_a could also be altered by shading the canopy during stem elongation (Stockman et al., 1983; Gonzalez et al., 2005), or by altering the dwarfing genes (Miralles et al., 1998). Gonzalez et al. (2011) reported that the spike fertility index and duration of stem elongation phase were not related; therefore, it seemed more promising to increase grain number in a cultivar with high spike fertility index, meanwhile, lengthened duration of the stem elongation phase which may result in higher SWD_a.

1.5.2. Spikelet fertility

Pinthus and Millet (1978) investigated the interactions among spikelet number, grains number and weight by removing spikelets in wheat. Removal of spikelets resulted, in most cases, in a small increase in grain number and in a considerable increase in the individual grain weight of the remaining spikelets. Removal of the upper florets in each spikelet resulted in a certain increase in the weight of the two basal grains. It was concluded that an increase in spikelet number per spike may reduce grain weight but will still contribute to yield. The number of grains per spikelet was cultivar dependent but not causally associated with grain weight. It appears that spikelet fertility is associated with grain size and assimilate supply to individual spikelets.

Although little work about spikelet fertility (i.e. spikelet survival, ratio between fertile spikelet and total spikelet number) in wheat was reported. Lots of work has been done to investigate the factors that effect initiated spikelet number and develop strategies for the increase of initiated spikelet number. An increase in spikelet number per ear is usually associated with longer growth duration and slower rate of spikelet initiation. Vernalization has been verified to be an important approach for the manipulation of the duration (Rawson, 1970; Rahman and Wilson, 1977). It was reported that the number of spikelets on the differentiating inflorescence and the spike at anthesis was highest at high light intensities and at low temperatures; the length of the developing inflorescence and the spike, the height of the main stem, and the total plant dry weight at the time of anthesis were also greatest under these conditions.These results are related to differential effects of temperature and light intensity on the rates and duration of apical elongation, morphological development of the spike, and spikelet formation in wheat (Friend, 1965). Wheat spike development is also sensitive to day length; a large number of spikelets can be

achieved by long duration and high rates of production, which are in excess of those for wheat under corresponding photoperiods (Rawson, 1971). Toyota et al. (2001) observed that nitrogen supply at the double ridge stage could affect neither the rate nor duration of spikelet initiation, the number of spikelets remaining unchanged. These results suggested that under field conditions, nitrogen supply at the double ridge stage is too late to have a significant effect on spikelet number. They also found shading could decrease the rate of spikelet initiation, resulting in a significant decrease in spikelet number. Lower, non-structural carbohydrate content at TS stage suggested that the limited assimilate supply by shading was likely responsible for the decreases in the initiation of spikelets.

1.5.3. Floret fertility

Wheat spikelets produce reproductive structures called florets; the determination of floret number per spikelet is a primary factor of spikelet architecture in the grass family. The indeterminate nature of the wheat spikelets may enable more than eight florets to be formed within one spikelet. Floret fertility (i.e. GS, grain setting) at PM is one of the most decisive factors for final grain yield (Fischer, 2011; Pedro et al., 2012). However, before the final grain number is set at PM, floral structures undergo a sophisticated development and abortion process. After reaching the MFS, representing wheat's yield potential, a floral degeneration process is initiated determining the fertile florets at anthesis. Following this pre-anthesis floral degradation, another one to three florets are usually lost during post-anthesis grain setting events until final grain number is reached at physiological maturity. MFS, FFS and GS are three crucial points during this floral developmental process. Previous studies monitored the floral degradation process according to the Waddington scale (Waddington *et al.*, 1983). For example, Craufurd and Cartwright (1989) reported that floret

death began when the floret 1 (F1, the first floret from the spikelet base) of central spikelets reached a stage of Waddington score 8 (W8). Bancal (2009) observed the onset of floret abortion during a period ranging between W7 (styles elongating) to W8. González et al. (2011) reported the initiation of floret death, when F1 ranged from W8 to W9. As far as it could be ascertained, the only work to determine the onset of floral abortion following the Kirby scale was conducted by Kirby and Appleyard (1987). They showed that floret primordia reach a maximum at the white anther stage (Kirby and Appleyard, 1987). Although previous studies have shown that exogenous and endogenous factors can affect final grain yield in wheat (McGrath *et al.*, 1997; Richards *et al.*, 2002; Sadras and Denison, 2009; Richards *et al.*, 2010; Potgieter *et al.*, 2013; Sadras and Richards, 2014), more work is needed to enrich our knowledge of how these factors influence floral development and abortion processes, especially the initiation of floral degradation, which will determine final grain number.

1.5.4. Harvest index

Donald (1962) first defined HI in wheat and the utility of the HI concept is further clarified to be associated with agronomic improvement in crop yield by Donald and Hamblin (1976). Nowadays, it has been widely accepted that HI (i.e. the ratio of grain weight to total aboveground sun-dried weight *100) has been one of the principal factors contributing to genetic yield improvements in wheat cultivars. Although high HI exhibits high-yielding ability when cultivars are compared, it also suggests challenges to improvement of yield under different growth conditions (Peltonen-Sainio et al., 2008). The values for modern varieties of most intensively-cultivated grain crops fall within the range 0.4 to 0.6. The improvement in HI is a consequence of increased grain population density coupled with stable individual grain weight (Hay, 1995). González et al. (2011) observed trait relations between HI, spike

fertility index, SDW_a and duration of stem elongation phase and suggested strategies for the improvement of grain yield based on the relations: the high yield potential was associated with increased HI and grain number, which was determined by spike fertility index and SDW_a. They also observed that duration of the stem elongation phase showed stable variation between years for two pairs of cultivars, supporting the idea that increased SDW_a could be achieved by lengthening duration of the stem elongation phase. As spike fertility index and duration of the stem elongation phase were not related, it seems promising to increase grain number and yield potential using the two traits respectively.

In the wheat breeding history, most success was achieved based on the increase of HI, without altering the biomass produced at harvest (Austin et al., 1989; Siddique et al., 1989; Slafer and Andrade, 1991). Wheeler et al. (1996) displayed the variations of response of HI to temperature and CO_2. Zhang et al. (2012) observed that HI was positively related to water soluble carbohydrates at anthesis and the proportion of spike dry weight to above-ground dry matter, but negatively related to the proportion of stem dry weight to above-ground dry matter. Aranjuelo et al. (2013) highlighted that elevated CO_2 only increased plant growth in the genotype with the largest HI.

1.5.5. Fruiting efficiency

In the past decades, higher grain number was achieved through increased assimilates partitioning to spikes before anthesis, due to the reduced competition for assimilates from stem. The introduction of dwarfing genes, known as reduced height (*Rht*) genes during Green Revolution after the 1960s, has been the most important success (Peng et al., 1999; Hedden, 2003; Saville et al., 2012). In this process, plant type was modified to optimize its height and current high-yield cultivars possess a height with the optimum range to maximize

yield (Richards, 1992; Flintham et al., 1997; Miralles and Slafer, 1997). This indicates taller plants would have poorer partitioning towards to the spike and a higher possibility of lodging; shorter plants would increase assimilate distribution to the spike, and reduce the risk of lodging (Miralles and Slafer, 1995).

As modern wheat cultivars have reached an optimum height, alternatives must be identified for further increasing grain number (Slafer et al., 2015). One option is increasing utilization efficiency of assimilates as the low possibility to increase assimilates allocation to spike by decreasing stem length. Fruiting efficiency (i.e. grains set per unit of SWD_a) is an important indicator for the utilization efficiency of assimilates. Fruiting efficiency is the final outcome of floral development and degeneration. Variation of fruiting efficiency within modern cultivars would explain the variable survival of floret primordia. Slafer et al. (2015) suggested two alternative physiological pathways to improve fruiting efficiency by allowing a normal development of most vulnerable floret primordia: (1) an increased allocation of assimilates for the developing florets before anthesis, and (2) reduced demand of the florets for maintaining their normal development. When considering this trait in breeding process, we must be aware of potential trade-offs and therefore it must be avoided that increases in fruiting efficiency is not compensated by decreases in SWD_a or grain weight.

During my PhD study, genome-wide association study (GWAS) was used to investigate the genetic regulation of floret fertility and floral abortion in wheat.

1.6. Introduction of genome-wide association study

1.6.1. Principles of GWAS

Genome-wide association study (GWAS) is an examination of association between genetic variants in different individuals and a trait that help scientists to identify genes involved in it. The first successful GWAS was published by Klein et al. (2005). It investigated patients

with age-related macular degeneration by genotyping 116,204 human single nucleotide polymorphisms (SNPs) in 96 cases and 50 controls and detected an intronic and common variant in the complement factor H gene which is strongly associated with age-related macular degeneration. GWAS searches the genome for small variations (e.g. SNPs) that occur more frequently in one population with a particular trait than in other populations without the trait. Each study can look at hundreds, thousands or millions of SNPs at the same time. Researchers use data from this type of study to identify genes that may contribute to the target trait. Because GWAS examine SNPs across the genome, they represent a promising way to study complex traits. This approach has already identified SNPs related to several complex traits in plants and animals.

1.6.2. Advantages and limitations of GWAS

Both linkage analysis and association studies rely on co-inheritance of functional polymorphisms and neighboring DNA variants. However, in linkage analysis, there are only a few opportunities for recombination to occur within families and pedigree with known ancestry, resulting in relatively low mapping resultion; in association mapping, high resolution mapping can be achieved by exploiting historical recombination and natural genetic diversity (Fig. 1.11). Lingkage disequilibrium between a functional locus (yellow diamond for mutated allele) and molecular markers is low except for those within very short distance.

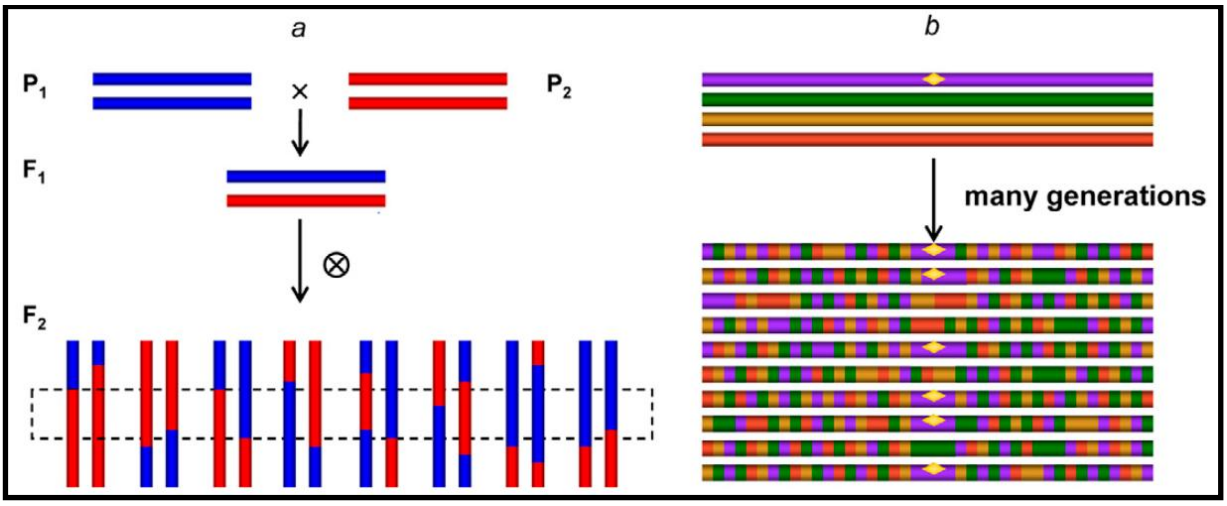

Fig. 1.11. Schematic comparison of linkage analysis with designed mapping populations and association mapping with diverse collections (Source: Zhu et al., 2008).

Due to the difference of the principle between linkage and GWAS, there are some clear anvantages and limitations for the two mapping approaches, for example, linkage analysis needs the experimental crossing population which takes some time, while GWAS works with existing germplasm which will save time; linkage analysis display high detection power, and spurious association may occur in GWAS due to the population structure; in linkage analysis, there are only two alleles which are essentially tested, whereas more than two alleles are tested in GWAS. The summary of the advantages and limitations is shown in Table 1.4.

Table 1.4. The differences between linkage analysis and GWAS.

Linkage analysis	GWAS
Experimental cross required	No cross required, works with existing germplasm
Phenotypes to be collected	Phenotypic data stored in databases can be used
Limited mapping resolution	High resolution
Essentially 2 alleles are tested	More than 2 alleles are tested
Constraints to segregating loci between parental lines.	Many loci for a single trait can be analyzed simultaneously.
High detection power	Population structures may result in spurious association
Relatively small sample sizes	Large sample sizes required

A major limitation of GWAS is that a large number of false positives may occur when GWAS are conducted in some complex traits with population structure (Devlin and Roeder, 1999;

Zablocki et al., 2014). GWAS has largely failed to identify substantial portions of the genetic basis of complex traits (Manolio et al., 2009; Collins, 2010).

Bonferroni correction and false discovery rate (FDR) correction have been validated rather effective for the control of false positives (Benjamini and Hochberg, 1995). Bonferroni correction is a stringent method, which adjusts p-values by multiplying each p-value with the total number of tests; FDR corrections is less stringent and tolerates more false positives (Qu et al., 2010). The FDR method first ranks all p-values from the smallest to the largest, and then adjusts each p-value accordingly, i.e. a FDR=0.05 allows 5% of reported positives are false positives, while the Bonferroni correction α=0.05 requires the whole family of positives to be true positives with the certainty of 95% (Qu et al., 2010). However, for the smallest p-value in one study, the Bonferroni corrected p-value is equal to the FDR corrected p-value (Qu et al., 2010).

Population stratification, which can occur in ethnically mixed populations can lead to significant associations being detected at loci that have nothing to do with target traits — spurious associations in GWAS studies (Curtis, 1996; Reich and Goldstein, 2001; Lohmueller et al., 2003; Freedman et al., 2004; Price et al., 2006). Stratification is an important problem in GWAS, which analyze thousands of samples to detect common genetic variants of weak effect.

Principal components analysis (PCA) is widely used to detect population structure (Patterson et al., 2006). Structured association uses a program such as STRUCTURE to assign the samples to discrete subpopulation clusters based on PCA (Pritchard et al., 2000). The inferred principal components capturing the genetic ancestry of each individual are often included as fixed effects in a regression-based test of association in order to account for population structure (Price et al., 2006; Price et al., 2010). More recently, kinship (a linear

mixed model) was proposed to explain population structure by considering the genome-wide similarity between all pairs of individuals (Kang et al., 2008; Kang et al., 2010; Hoffman, 2013), and recent work has proven that this linear mixed model is quite efficienct (Segura et al., 2012; Svishcheva et al., 2012; Zhou and Stephens, 2012; Pirinen et al., 2013).

1.6.3. Tools for GWAS

Due to the wide use of GWAS for complex traits, some tools have been developed in the past ten years. The GWAS tools have displayed variable advantages and limitations for different target traits in the different populations. Of all the tools, TASSEL, PLINK, GenABEL, GWASTools and EMMA are specific and widely used for GWAS, while Genstat and ASReml are statistical tools which not only provide a vast range of statistical techniques, but also quite useful for the GWAS. All the seven GWAS tools mentioned above are introduced.

TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) implements general linear model and mixed linear model approaches for controlling population and family structure (Bradbury et al., 2007). For the interpretation of the results, the program allows graphical visualization for calculation of linkage disequilibrium statistics. In TASSEL, the Q method, a general linear model (GLM) for structured association analysis was implemented with the same function of using a Q-matrix of population membership estimates, these false associations approach can be partially corrected (Bradbury et al., 2007). The Q + K method, a mixed linear model (MLM) function was also implemented in TASSEL. Since kinship (K) estimates can display the average relationship between individuals or lines. A composite approach, Q + K, has been shown to be superior (Yu et al., 2006) to these former methods.

PLINK is a tool for genome-wide association and population-based linkage analyses (Purcell et al., 2007). Using PLINK, we can rapidly manipulate and analyze a large amount of markers

genotyped for thousands of individuals. PLINK not only provides tools to carry out the basic analysis efficiently, but also supports some novel approaches for the analysis of genome-wide data. The five main domains of function in PLINK are data management, summary statistics, population stratification, association analysis, and identity-by-descent estimation. With PLINK, we can also detect and correct for population stratification and to identify extended chromosomal segments that are shared identical by descent between very distantly related individuals (Purcell et al., 2007).

GenABEL is a R package for GWAS between quantitative or binary traits and SNPs (Aulchenko et al., 2007). It implements effective storage and handling of GWA data, fast procedures for genetic data quality control, testing of association of SNPs with binary or quantitative traits, visualization of results and also provides easy interfaces to standard statistical and graphical procedures implemented in base R and special R libraries for genetic analysis (Aulchenko et al., 2007). We can evaluate GenABEL using one simulated and two real data sets. We can conclude that GenABEL enables the analysis of GWAS data on desktop computers.

GWASTools is an R/Bioconductor package for quality control and analysis of GWAS (Gogarten et al., 2012). GWASTools brings the interactive capability and extensive statistical libraries of R to GWAS. Data are stored in NetCDF format to accommodate extremely large datasets that cannot fit within R's memory limits. GWASTools provides a convenient interface for linking genotypes and intensity data with sample and single nucleotide polymorphism annotation (Gogarten et al., 2012).

Efficient Mixed Model Association (EMMA) was developed by Kang et al. (2008). It is a statistical test for model organisms association mapping correcting for the confounding from population structure and genetic relatedness. EMMA takes advantage of the specific nature of the optimization problem in applying mixed models for association mapping, which allows

us to sustantially increase the computational speed and the reliability of the results (Kang et al., 2008). The current implementation of EMMA is available in an R package.

Genstat is quite useful for GWAS, although it is not specific for GWAS (http://www.scc.ms.unimelb.edu.au/genstat.html). It is a comprehensive statistics system offering ease-of-use for the beginning user, or power and flexibility for the more experienced user. The vast range of statistical techniques in GenStat have been tested in previous work by practicing statisticians, through many applications and disciplines. Genstat was originally conceived and developed at Rothamsted, an experimental station in the UK, GenStat has been going strong in the past 30 years.

ASReml has been verified a poweful tool for big data analysis, and is also quite effective for the GWAS (https://www.vsni.co.uk/downloads/asreml/release3/UserGuide.pdf). According to ASReml user guide: ASReml provides a stable platform for delivering well established procedures while also delivering current research in the application of linear mixed models. The strength of ASReml fits the linear mixed model by using the Average Information algorithm and sparse matrix methods. ASReml is used to fit linear mixed models to quite large data sets with complex variance models. It extends the range of variance models available for the analysis of experimental data.

1.6.4. Current progress of GWAS

Molecular markers are the basis of the GWAS. To learn the progress of GWAS, it is necessary to know the progress of molecular markers. The development of molecular markers is quite valuable for the detection and exploitation of DNA polymorphisms. The detection and analysis of genetic variation is helpful to understand the molecular basis of various biological phenomena (Agarwal et al., 2008; Song et al., 2015). Assigning molecular markers to linkage

groups and constructing genetic maps is an important step for the genome analysis in different species (Song et al., 2015). The first report about molecular marker technique in the detection of DNA sequence polymorphism is the publication about genetic mapping for temperature-sensitive mutations of adenoviruses using restriction fragment length polymorphism (RFLP) (Grodzicker et al., 1974). In RFLP (non-PCR-based techniques), DNA polymorphism is detected by hybridizing a chemically labelled DNA probe to a Southern blot of DNA digested by restriction endonucleases, resulting in differential DNA fragment profile (Semagn et al., 2006). After the invention of polymerase chain reaction (PCR) technology (Mullis and Faloona, 1987), a range of PCR-based markers, such as random amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP), Single nucleotide polymorphism (SNP), and simple sequence repeat (SSR), have been introduced during the 20th century (Marczewski, 1995; Kumar et al., 2009). RAPD and AFLP are arbitrarily primed PCR-based markers; while SNP and SSR are sequence specific PCR based markers. Of all the types of markers, SNPs are most widely distributed throughout the genome and constitute the most abundant molecular markers in the genome (Agarwal et al., 2008). It is suitable for the fine mapping of genes and association studies, which aim at identifying alleles potentially affecting important agronomic traits (Clark, 2010; Huang et al., 2015; Crowell et al., 2016). Therefore, the SNP marker gradually became preferred markers for many applications in genetics and genomics of different crops (Zhao et al., 2011; Riedelsheimer et al., 2012; Morris et al., 2013; Zanke et al., 2014a). Due to the relatively small genome sizes of rice (389 Mb), barley (5.1 Gb) and maize (2.3 Gb) compared with haxploid wheat (17 Gb) (Matsumoto et al., 2005; Schnable et al., 2009; Brenchley et al., 2012; Mayer et al., 2012), more SNP markers are avaible and more advanced progress of SNP marker development has been achieved in rice, barley and maize than in wheat, resulting in

great progress for GWAS analyses in the past ten years in crops (Tian et al., 2011; Pasam et al., 2012; Chen et al., 2014). On the other hand, it suggests the great potential of GWAS in wheat. Recently, a genotyping array including about 90000 gene-associated SNPs was developed and can be used to characterize genetic variation in wheat populations (Wang et al., 2014). In GWAS analysis during my PhD study, all 210 varieties were genotyped based on a novel 90k Infinium chip (90k iSELECT) for SNP-analysis (Zanke et al., 2014a,b; Wang et al., 2014). This resulted in a total of 21742 scorable and polymorphic markers on our association panel by considering all polymorphic markers with a minor allele frequency (MAF) >0.03 (Zanke et al., 2014a,b). Of these markers, only 7934 mapped markers were included in the association analysis, while the unmapped markers were not used for GWAS analysis (Zanke et al., 2014a,b).

2. Association between ovary size, anther size and SDW_a

Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat (Journal of Experimental Botany 66:3099-3111)

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2.1. Abstract

Anther and ovary development play an important role in grain setting, a crucial factor determining wheat (*Triticum aestivum* L.) yield. One aim of this study was to determine the heritability of anther and ovary size at different positions within a spikelet at seven floral developmental stages and conduct a variance components analysis. Relationships between anther and ovary size and other traits were also assessed. The thirty central European winter wheat genotypes used in this study were based on reduced height (*Rht*) and photoperiod sensitivity (*Ppd*) genes with variable genetic backgrounds. Identical experimental designs were conducted in a greenhouse and field simultaneously. Heritability of anther and ovary size indicated strong genetic control. Variance components analysis revealed that anther and ovary sizes of floret 3 (i.e. F3, the third floret from the spikelet base) and floret 4 (F4) were more sensitive to the environment compared with those in floret 1 (F1). Good correlations were found between spike dry-weight and anther and ovary size in both greenhouse and field, suggesting that anther and ovary size are good predictors of each other, as well as spike dry-weight in both conditions. Relationships between spike dry-weight and anther and ovary size at F3/4 positions were stronger than at F1, suggesting that F3/4 anther and ovary size are better predictors of spike dry-weight. Generally, ovary size showed a closer

relationship with spike dry-weight than anther size, suggesting that ovary size is a more reliable predictor of spike dry-weight.

Key words: wheat, anther size, ovary size, spike dry-weight, heritability, sensitivity.

2.2. Introduction

Bread wheat (*Triticum aestivum L.)* is one of the most widely planted crops worldwide. Increasing wheat yield remains one of the main goals of wheat breeding efforts, and wheat yield is a particularly complex trait. Grain number per spike plays an important role in wheat yield improvement and is closely related to floret survival (Sreenivasulu and Schnurbusch, 2012). Because the rate of floral survival in most wheat varieties is low (Langer and Hanif, 1973; Gonzalez et al., 2003, 2005; Ferrante et al., 2010; Gonzalez et al., 2011; Ferrante et al., 2013; Dreccer et al., 2014), there is great potential for improvement. Vegetative, reproductive, and grain filling phases are three main phases of the wheat life cycle (Fig. 2.1); floral development and differentiation is an important part of the pre-anthesis stage (Fig. 2.1). Anther and ovary growth is a vital factor determining grain number per spike and grain size, and further affects wheat grain yield (Kherde et al., 1967; Khan et al., 1973; Dorion et al., 1996; Blum, 1998; Koonjul et al., 2005; Ji et al., 2010). Despite this, studying anther and ovary growth and development in wheat over time has been widely neglected possibly, simply because of the challenging and demanding labour associated with measuring these organs *in planta*. More recently, however, new efforts in developing hybrid-wheats have been flamed up again (Longin et al., 2013; Whitford et al., 2013; Longin and Reif, 2014), and with this field of research a more detailed understanding of how anthers and ovaries develop is of fundamental interest.

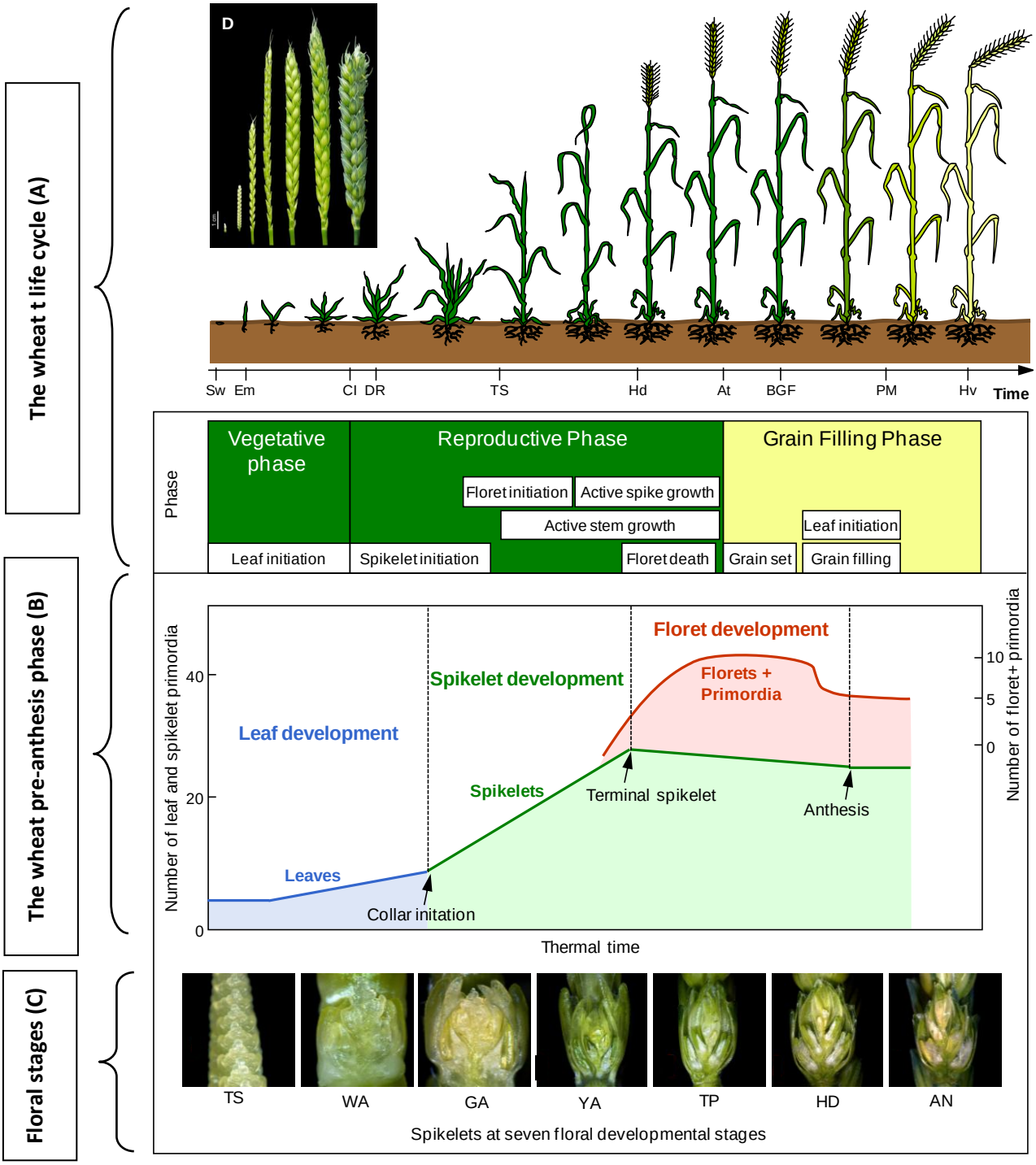


Fig. 2.1. Different developmental stages in the wheat life cycle. **A**, The wheat life cycle. **B**, The wheat pre-anthesis phase, including leaf, spikelet, and floret initiation. **C**, The spikelets at the seven floral developmental stages (after Kirby and Appleyard 1987): TS (terminal spikelet stage), WA (white anther stage), GA (green anther stage), YA (yellow anther stage), TP (tipping stage), HD (heading stage), and AN (anthesis) (from left to right). **D**, The spikes at the seven floral developmental stages: TS, WA, GA, YA, TP, HD, and AN (from left to right) during floral development (floret initiation).

Many studies have been previously conducted on anthers and ovaries in different species. One study reported a significant positive correlation between kernel dry-weight at maturity and ovary volume at anthesis in sorghum (Yang et al., 2009). They concluded that pre-anthesis ovary growth determines genotypic diversity in sorghum kernel weight. Another study showed that ovary swelling in bell pepper flowers benefited from low nighttime temperatures or a high source–sink ratio. Influence of low nighttime temperatures and a high source–sink ratio on ovary swelling were additive. (Darnell et al., 2012). In another experiment, all sweet pepper genotypes measured exhibited increased ovary size at 12 ^{o}C compared with 20 ^{o}C nighttime temperatures, indicating that low nighttime temperatures can increase ovary size in sweet peppers (Cruz-Huerta et al., 2011). Measurement of anther length in field-grown wild barley (*Hordeurn vulgare* ssp. *spontaneum*) showed strong positive correlations between anther length, head length, and grain weight, while a negative correlation was found between anther length and spike number per plant (Giles and Bengtsson, 1988). Inheritance of anther length and width in a cross of two oat (*Avena sativa* L.) genotypes exhibited dihybrid ratios with incomplete dominance (Kim et al., 1968). Moreover, olive (*Olea europaea* L.) fruit weight has been related to ovary weight (Rosati et al., 2009); tissue size, and cell number in the olive ovary was shown to determine tissue growth and partitioning in the fruit (Rosati et al., 2012).

In maize (*Zea mays* L.), drought stress was found to cause considerable delay in female plant organ development, while the male inflorescence was less influenced (Barnabas et al., 2008). Ovaries in maize generally grow rapidly and contain much glucose and starch, with a glucose gradient favoring glucose movement into the developing ovary. Shade can block photosynthesis and decrease ovary size and weight, as well as glucose and starch content. However, sucrose fed to the maize plant stem was shown to reverse these losses, and

kernels were as large as the controls (Hiyane et al., 2010). The differences in sucrose and starch responses under water-limited conditions suggests alteration of ovarian carbohydrate metabolism in maize (Zinselmeier et al., 1995). In wheat, it was documented that pre-anthesis stem dry-mass accumulation influences floral development and grain filling under stressed conditions (Bidinger et al., 1977; Kiniry, 1993; Blum et al., 1994; Blum, 1998). The ability to control and maintain sink strength and carbohydrate supply to anthers may be the key to maintaining pollen fertility and grain number per spike in wheat (Ji et al., 2010). When one Australian wheat variety was treated with high temperature (30 °C) during the meiosis phase, a third of the ovaries were found to exhibit abnormal development (Saini et al., 1983). Unfortunately, there are only a limited number of previous studies related to heritability. One report estimated the heritability of anther length to be above 0.65 from wheat anthers sampled from the first or second florets in the central position of a spike just before flowering (Komaki and Tsunewaki, 1981).

In wheat, spike dry weight at anthesis (sdw_a), including dry weight of ovary and anther, is crucial for grain yield determination; sdw_a has been confirmed to have strong correlations with fertile floret number and final grain number in wheat (Fischer and Stockman, 1980; Fischer, 1985; Thorne and Wood, 1987; Savin and Slafer, 1991; Fischer, 1993; Abbate et al., 1995; Abbate et al., 1997; Demotes-Mainard and Jeuffroy, 2001, 2004; Fischer, 2007; Serrago et al., 2008; Gonzalez et al., 2011). Thus, it is necessary to assess the relationships between spike dry weight and anther/ovary size.

Although a number of studies on wheat have been reported, only a few related to anther size can be found, most being measured at limited floret positions and stages (Kherde et al., 1967; Khan et al., 1973; Komaki and Tsunewaki, 1981; Trione and Stockwell, 1989). Furthermore, wheat ovary size is also not well documented. Therefore, one aim of this study

is to determine the heritability of anther and ovary size at different spikelet and floret positions as well as developmental stages to further understand floral development and grain setting in wheat. To this end, variance component analysis was conducted to assess genetic and environmental influence on anther and ovary growth. Moreover, relationships between spike dry weight and ovary and anther size were assessed in both field and greenhouse. Finally, it was found that (i) anther and ovary size at different positions and developmental stages are under strong genetic control; (ii) anther and ovary size are good predictors for spike dry weight as well as to each other; and (iii) anther and ovary size at F1 (i.e. Floret 1 from the spikelet base) are more stable to the environment compared with florets at F3/4 positions.

2.3. Materials and methods

Plant material and growth conditions

Experiments were conducted at the Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany; 51° 49’ 23” N, 11° 17’ 13” E, altitude 112 m) during the 2012-2013 growing season under greenhouse and field conditions. Thirty European hexaploid winter wheat cultivars were grown, including 23 photoperiod-sensitive and seven photoperiod-insensitive cultivars. These cultivars can also be classified into 24 semi-dwarfed and six tall cultivars. Marker information for all 30 cultivars is found in Table S2.1.

For both greenhouse and field experiments, seeds were sown in 96-well trays on the same day (January 1, 2013) and germinated under greenhouse conditions (16/8 h day/night; ~20/~16 ^{o}C) for 14 d. Seedlings at the two- to three-leaf stage were transferred to 4 ^{o}C to vernalize for 63 d. Vernalized seedlings were transferred to a hardening stage (12/12 h day/night, and ~15 ^{o}C) for 7 d to gradually acclimatize. Finally, half of the plants were

transplanted (April 15, 2013) into 0.5 L pots (one plant per pot; 9 cm length x 9 cm width x 9 cm height) under greenhouse conditions (16/8 h day/night; ~20/~16 ^{o}C). Supplemental light (~250 mE $m^{-2}s^{-1}$ PAR (photosynthetically active radiation) was supplied with low intensity incandescent light, and plants were irrigated when required. Another half of the plants were directly transplanted into the field (April 15, 2013) into silty loam soil (10 plants per row; 50 cm long with 20 cm between rows). All plants were manually irrigated.

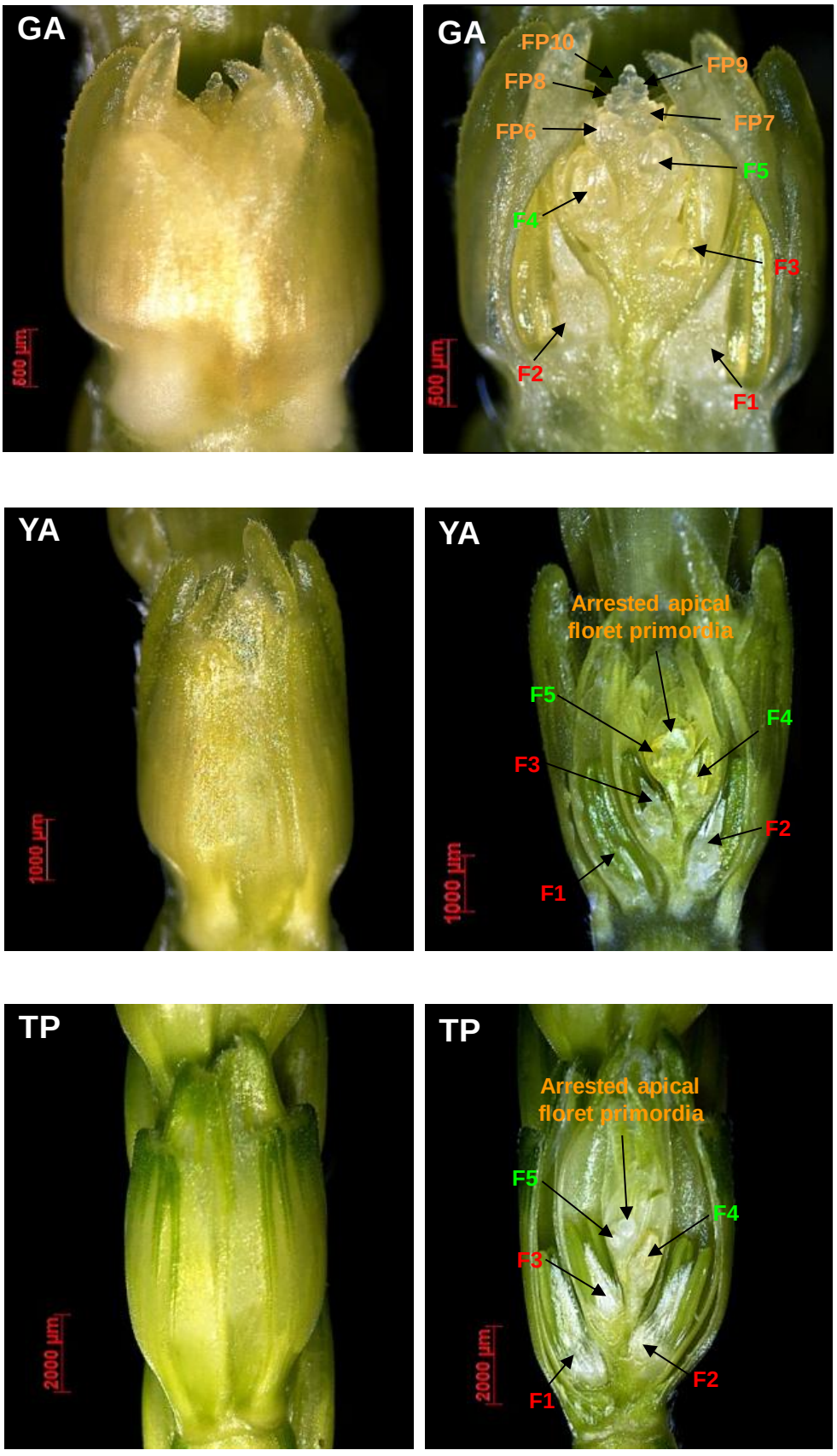
Phenotypic staging and measurements

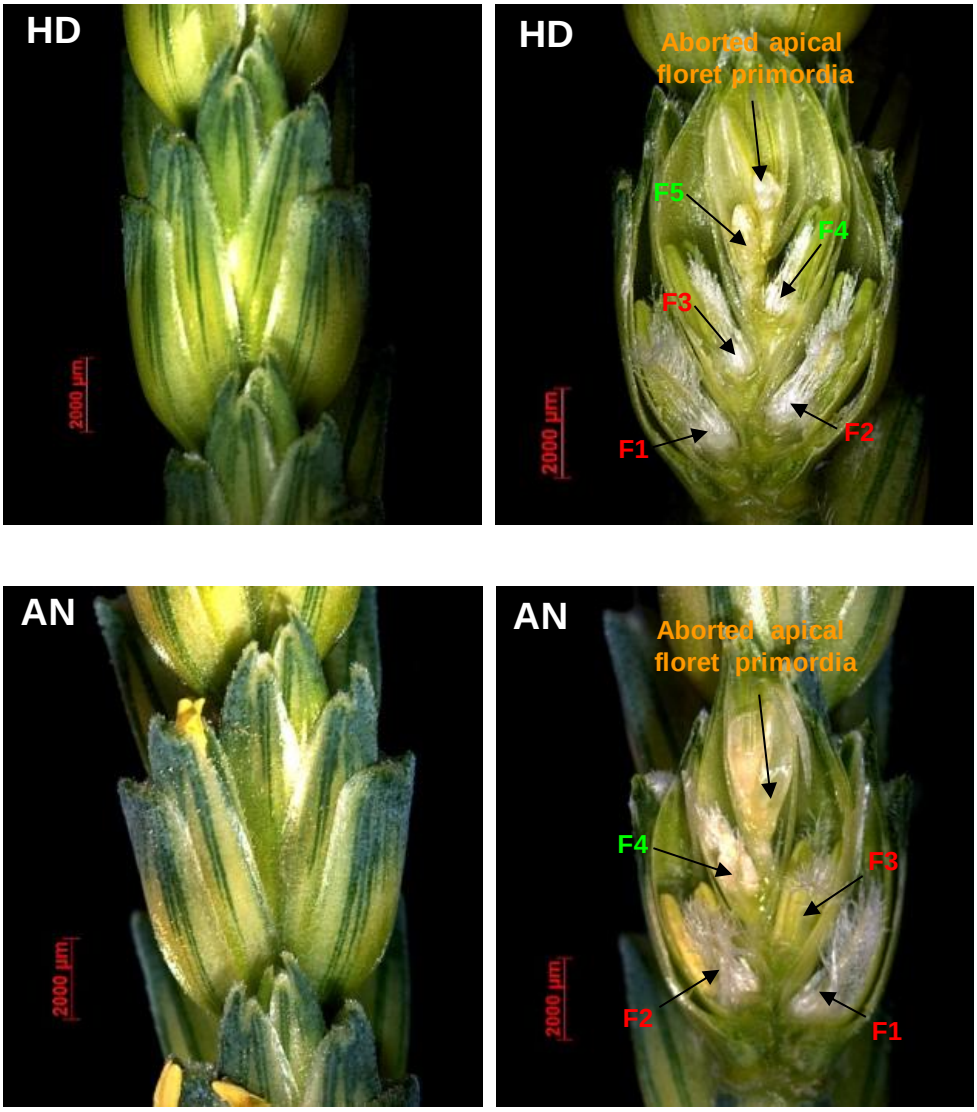
To study floral development in detail, seven stages were selected: the terminal spikelet (TS) stage, completion of spikelet initiation (Kirby and Appleyard, 1987); white anther (WA) stage, lemmas of florets 1 (F1) and 2 (F2) completely enclosing stamens and other structures (Kirby and Appleyard, 1987); green anther (GA) stage, glumes cover all but the floret tips (Kirby and Appleyard, 1987); yellow anther (YA) stage, glumes are fully formed and the lemmas of first three florets are visible (Kirby and Appleyard, 1987); tipping (TP) stage (Z49), first awns visible (Zadoks, 1974); heading time (HD) stage (Z55), 50% of spikes visible (Zadoks, 1974); and anthesis (AN) stage (Z65), 50% of spikes with anthers (Zadoks, 1974). The spikes and spikelets at the seven floral developmental stages can be found in Fig. 2.1.

To detect the TS, WA, GA, and YA stages, cultivars were examined every 2 d under a stereomicroscope (Stemi 2000-c, Carl Zeiss Micro Imaging GmbH, Gottingen, Germany). For TP, HD, and AN stages, the day of onset was recorded as the point at which 50% of plants reached the respective stage. Thermal time was used to identify the duration of each stage and was calculated as the sum of the daily average temperature [$(T_{max}+T_{min})/2$; base temperature assumed as 0 ^{o}C].

During each stage, three plants for each cultivar were randomly selected for all phenotypic measurements. Leaf area was measured immediately after dissection of fresh main culm leaf material using an area meter (LI-3100, LI-COR Ltd, Lincoln, Nebraska, USA). Main culm shoots and spikes were dried separately in two cellophane bags at 60 ^{o}C for 3-5 d for dry-weight measurement. Stem dry-weight refers to the dry-weight of one main culm shoot, including leaves without spikes. Anther and ovary size measurements were only conducted on the main culm spikes, and they were not measured on spikes of tillers. From GA to AN stages (Fig. 2.2A), spikelets from the center of the main culm spike were dissected to obtain digital images of anthers and ovaries for three plants in each stage (Fig. 2.2B). When the anther or ovary were aborted, the size was not measured. Anther length (anther size) and ovary width (ovary size) were also measured using the stereomicroscope and the Carl Zeiss Imaging System AxioVision Rel. 4.8.2. Anthers and ovaries below 0.2 mm in size were difficult to measure. For most plants, the visible structure of the anther and ovary can only be found at the first four florets from the base (F1-F4). According to our previous experiments, anther and ovary size at F1 and F2 positions are very similar; therefore, F2 anther and ovary size were not measured. The ovary and anther positions at each developmental stage measured in the experiment are shown in Table 2.1.

A


GA
GA
FP10
FP9
FP8
FP7
FP6
F5
F4
F3
F2
F1
600 µm
500 µm
YA
YA
Arrested apical floret primordia
F5
F4
F3
F2
F1
1000 µm
1000 µm
TP
TP
Arrested apical floret primordia
F5
F4
F3
F2
F1
2000 µm
2000 µm


HD
2000 µm
HD
Aborted apical floret primordia
F5
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B

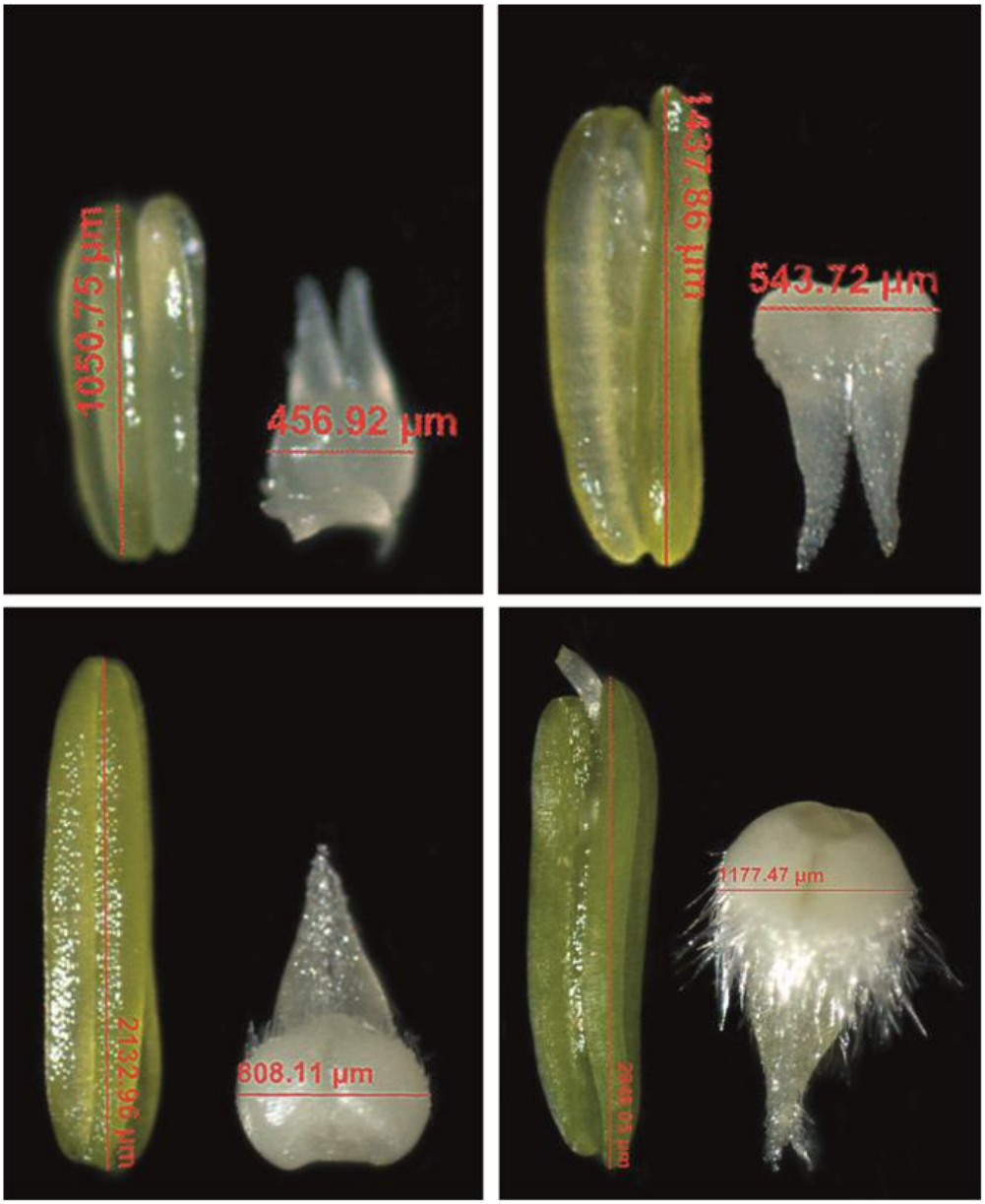


Fig. 2.2. A, Details of spikelets structure at GA,YA, TP, HD and AN developmental stages in wheat according to Kirby and Appleyard (1987); B, the four examples display the measurements of ovary and anther size in this experiment, the top two images show the anther and ovary at F1 positions at GA stage, the bottom two images show the anther and ovary at F1 positions at YA stage.

Table 2.1. Anther and ovary positions at each developmental stage measured in the experiment.

	GA	YA	TP	HD	AN
Anther size	F1	F1,F3,F4	F1,F3,F4	F1,F3,F4	n.a.
Ovary size	F1	F1	F1,F3	F1,F3,F4	F1,F3,F4

Statistical analysis

We performed an additive main-effects and multiplicative interaction (Matsumoto et al.) model analysis to determine differences among genotype (G), environment (E), and the interaction between GxE for each measured trait. The AMMI analysis combines the analysis of variance (Kostkanova et al.) and singular value decomposition (SVD). The AMMI mode partitions the overall variation into G main effects, E main effects, and G×E interactions, defined as:

$$y_{ij} = \mu + g_i + e_j + \sum_{k=0}^{n} \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij}$$

where y_{ij} denotes the yield for genotype i in environment j ; μ is the grand mean; g_i is the mean for i - μ; e_j is the mean in environment j - μ; λ_k is the singular value for principal component (PC) k ; α_{ik} is the eigenvector (or PC score) for i and k ; γ_{jk} is the eigenvector for j and k ; and ε_{ij} is the residual for i and j . As the singular value is the square root of the eigenvalue, the above model can be written as:

$$y_{ij} - g_i - e_j + \mu = \sum_{k=0}^{n} \left(\sqrt{\lambda_k}\alpha_{ik}\right) \cdot \left(\sqrt{\lambda_k}\gamma_{jk}\right) + \varepsilon_{ij} = \sum_{k=0}^{n} G_{ik} \cdot E_{jk} + \varepsilon_{ij}$$

In this way, the additive interaction (the data minus the i and j means; the left side of the formula) in the ANOVA model is obtained by multiplication of genotype PC scores (G_{ik}) by environmental PC scores (E_{jk}). Hence, the AMMI model applies SVD to the interaction from the additive model. The AMMI model is therefore called doubly-centered PCA.

With the ANOVA model, the total variation of each trait was partitioned into four parts: G effects, E effects, G×E interactions, and residual; results were visualized in pie plots. To graphically show clear insight into the G×E interaction effect and the which-won-where patterns of the data, a triplot via the PC scores for G and E was done for some important traits. In the triplot, a reduced number of PCs is used ($n = 3$) and dimensionality reduction

was achieved with just a small loss in the descriptive ability of the model. The first three PCs (PC1-PC3) represent the three components (x-, y-, and z-axes) in the triangular plot, respectively. All statistical analyses in this study were performed using the R statistical package (http://www.r-project.org/; release 2.14.1).

To quantify the contributions of direct and indirect genetic effects to trait variation, phenotypic data were analyzed using the following full linear mixed model:

$$y = 1\mu + X_r r + Z_g g + Z_l l + Z_{gl} gl + e$$

where y denotes a vector of individual plant observations of a given trait; X and Z are incidence matrices associating phenotypic observations with fixed and random factors, respectively; effects of fixed factors, which include μ (overall trait means) and r (replicate effects within the E), were assessed with approximate F-tests; and random factors (followed by their phenotypic variance components) include g [G effect following $g \sim (0, \mathbf{I}\sigma_G^2)$, where $\mathbf{I}$ is the identity matrix], l [E effect, $l \sim (0, \mathbf{I}\sigma_E^2)$], gl (G×E effect, $gl \sim (0, \mathbf{I}\sigma_{GE}^2)$], and e [residuals, $e \sim (0, \mathrm{I}\sigma_e^2)$].

The model was fitted with ASReml-R software using restricted maximum likelihood to estimate variance parameters and their standard errors. Likelihood-ratio tests (LRT) were used to assess the significance of variance parameter estimation. The test statistic for the LRT (denoted by D) is twice the difference in the log-likelihoods of two models:

$$D = 2(\log(likelihood_{alt}) - \log(likelihood_{null}))$$

where $\log(likelihood_{alt})$ is the log-likelihood of the alternative model (with more parameters) and $\log(likelihood_{null})$ is the log-likelihood of the null model, and both log-likelihoods can be calculated from the ASReml mixed model. Under the null hypothesis of zero correlation, the test statistic was assumed to be χ^2-distributed with degrees of freedom equal to the

difference in number of covariance parameters estimated in the alternative versus null models.

Variance components estimated in the models defined above were used to calculate broad-sense heritability (H^2) as follows:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2/2 + \sigma_e^2/2r}$$

where r is the average number of replications.

2.4. Results

Sensitivity and stability of ovary size at different stages under greenhouse and field conditions

Ovary size at the F1 position between greenhouse and field conditions was not significantly different at GA, YA, TP, and HD stages, while F1 ovary size was substantially greater in the greenhouse at the AN stage ($p<0.01$, Fig 2.3A). F3 ovary size was significantly higher in the field at TP ($p<0.001$) and HD ($p<0.001$) stages, but identical in both environments at the AN stage (Fig 2.3A). F4 ovary size was substantially higher in the field at both HD ($p<0.001$) and AN ($p<0.01$) stages. Interestingly, F1 ovary size at AN showed the opposite trend, with significantly higher ($p<0.01$) ovary sizes under greenhouse conditions. Broad-sense heritability of ovary size at all positions was above 0.70, and F4 ovary size at the HD stage had the highest heritability (0.89; Fig 2.3B). As it could be assumed if the heritability was high, the increased anther/ovary size would be reflected across all florets positions within individual genotypes. We selected one genotype 'Tukan' to calculate the correlations between anther/ovary size at different floret positions across different floral developmental stages under field and greenhouse conditions (Fig. S2.1, S2.2). Clearly, anther/ovary at

different positions display close relationships in most of the cases, but there are not strong correlations in some case due to the variation within genotypes. Environment (i.e. σ^2_E green bar; Fig 2.3B) had a large impact on F3 (19.1%) and F4 (35.6%) ovary size at the HD stage, indicating that ovary size at positions F3 and F4 is sensitive to the environment. However, the relatively small residual proportion suggests low within-genotype variation for F3 (24.0%) and F4 (20.2%) ovary size at HD (i.e. σ^2_e gray bar; Fig 2.3B). On the contrary, ovary size at position F1 was generally more stable than F3 and F4 at all examined stages (i.e. σ^2_E green bars, GA, 1.8%; YA, 2.3%; TP, 2.2%; HD, 0.0%; Fig 3B); however, the large residual proportion (i.e. σ^2_E gray bars, GA, 46.7%; YA, 30.4%; TP, 36.5%; HD, 27.8%; Fig. 2.3B) indicated that within-genotype variation was rather high.

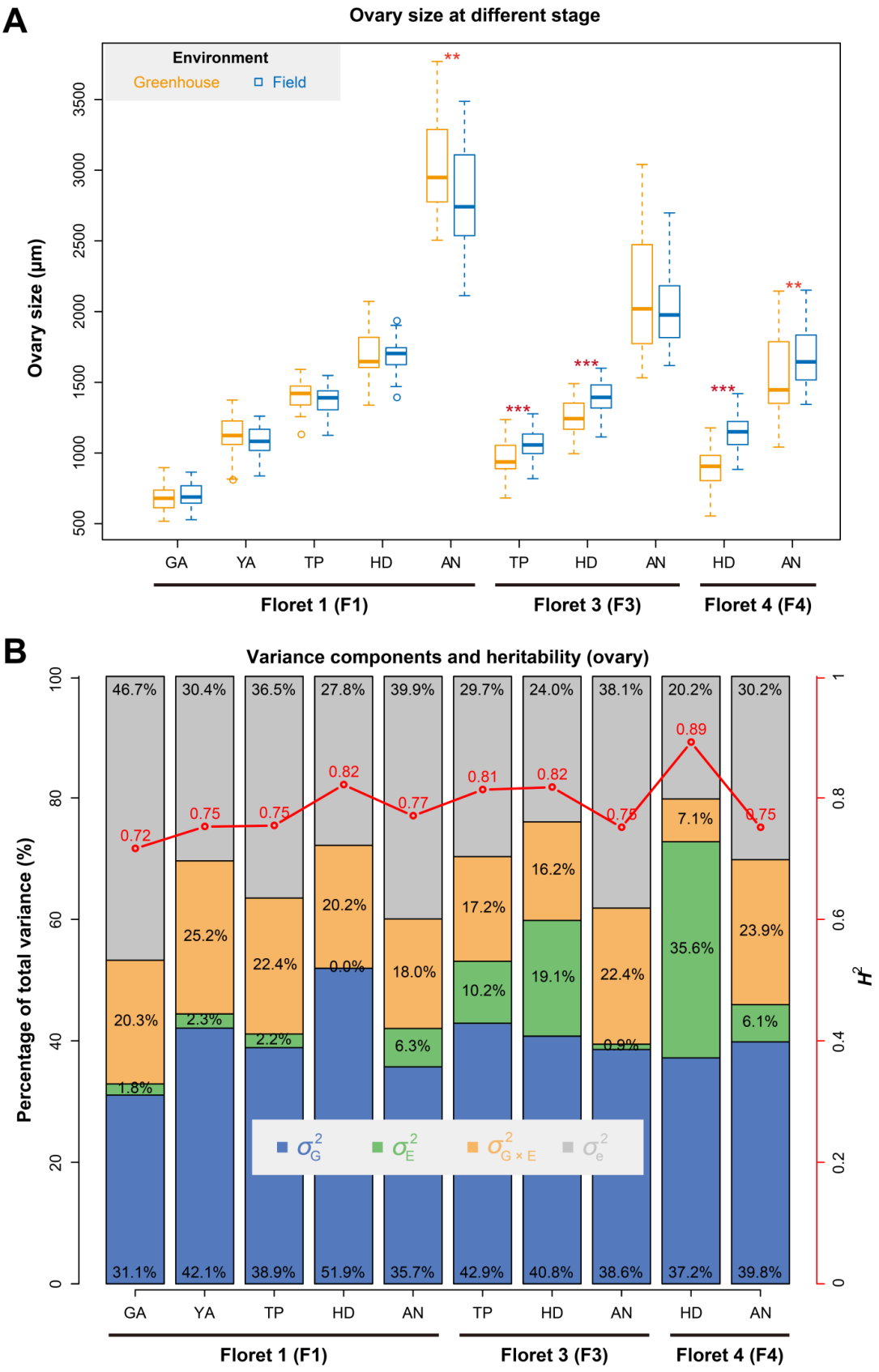


Fig. 2.3. A, Ovary size (μm) at different positions at floral developmental stages under greenhouse and field conditions; **B**, variance components and heritability (red point) of ovary size. **p<0.01; ***p<0.001.

Sensitivity and stability of anther size at different stages under greenhouse and field conditions

F1 anther size in the field was significantly higher than in the greenhouse at GA, TP, and HD stages at 0.05, 0.05, and 0.001 levels, respectively (Fig 2.4A). Similarly, F3 and F4 anther size in the field was substantially greater than in the greenhouse at YA (F3, $p<0.01$; F4, $p<0.001$), TP (F3, $p<0.001$; F4, $p<0.001$), and HD (F3, $p<0.001$; F4, $p<0.001$) stages (Fig 2.4A). Broad-sense heritability of anther size at all positions was above 0.68, and F1 anther size at the HD stage had the highest heritability (0.92; Fig 2.4A). Environment contributed largely to F1 anther size at the HD stage (24.3%) and F4 anther size at YA (18.5%), TP (29.0%), and HD (37.4%) stages, with the greatest contribution to F3 anther size at TP (21.3%) and HD (23.8%) stages (σ^2_E green bars; Fig 2.4B). Generally, the residual proportions for anther size (i.e. σ^2_e gray bars; Fig 4B) ranging from 14.7% to 40.5% were not as big as ovary size ranging from 20.2% to 46.7% (Fig 2.3B), which indicates that within-genotype variation of anther size is more stable compared with ovary size. The proportion of genotypic influence on anther ranging from 30.0% to 50.4% and ovary ranging from 31.1% to 51.9% size was relatively large at most stages.

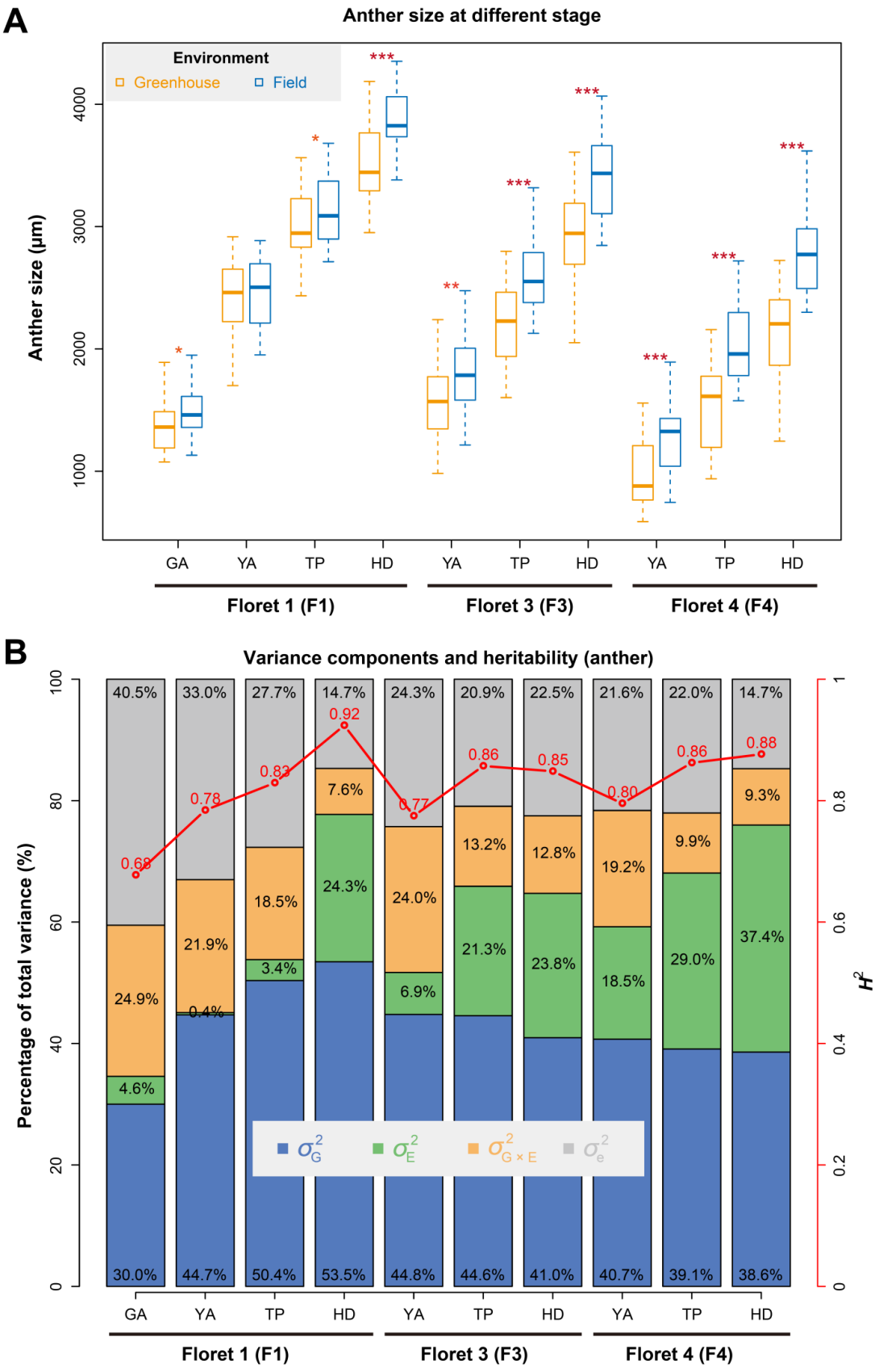


Fig. 2.4. A, Anther size (μm) at different positions at floral stages under greenhouse and field conditions; **B**, variance components and heritability of anther size.*p<0.05; **p<0.01; ***p<0.001.

Relationships between spike dry weight, anther size, ovary size and thermal time of floral developmental stages.

As expected, the thermal time for all seven floral developmental stages in the greenhouse was substantially higher than in the field ($p<0.001$, Fig. 2.5). However, absolute growing time (i.e. in days) for all seven floral developmental stages in the field was significantly higher than in the greenhouse ($p<0.001$, Fig. 2.5). Large temperature fluctuations were observed in the field, whereas greenhouse temperatures were more stable for plant growth (Fig. S2.3). Clearly, there was a significant difference in temperature amplitude between the greenhouse and field, indicating different environmental influences on ovary and anther growth during floral development. Fig. S2.4 and S2.5 show that most of the correlations between thermal time and ovary and anther size were strong under both greenhouse and field conditions, although thermal time weakly correlated with F3 anther size, F4 anther size, and F4 ovary size under greenhouse conditions. The relationship (value of R^2) between thermal time and ovary/ anther size in the field were markedly higher than in the greenhouse.

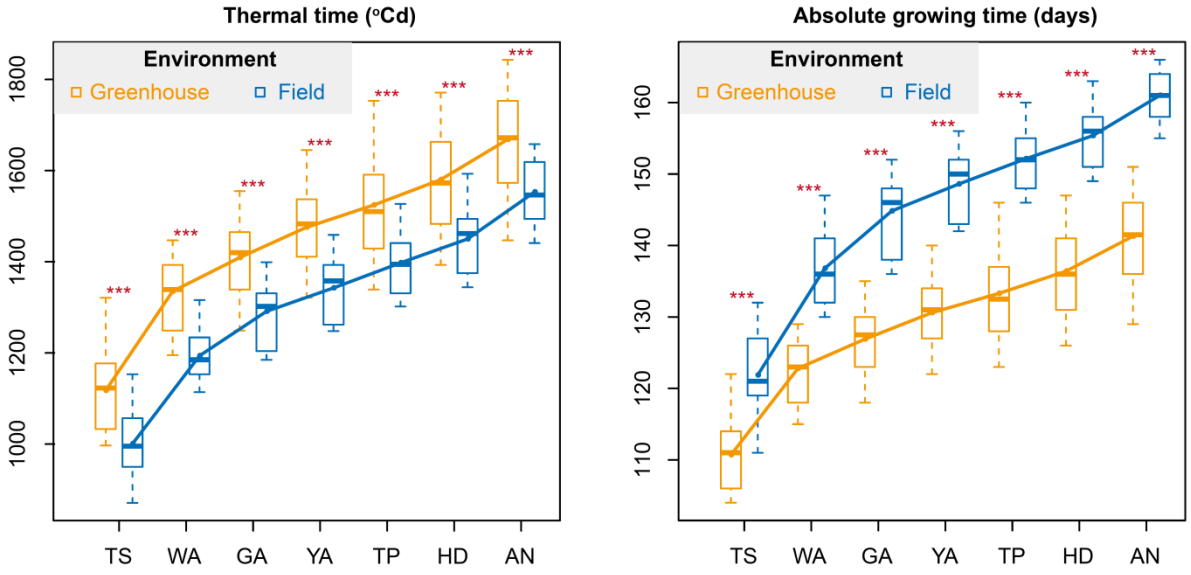


Fig. 2.5. The range and difference of thermal time (°Cd) and absolute growing time (days) between greenhouse and field conditions at seven floral developmental stages. ***p<0.001.

As shown in Fig. 2.6 and 2.7, F1 anther and ovary size was closely related to spike dry-weight at the YA stage, but their correlation at GA, TP, and HD stages were rather weak under greenhouse conditions. However, F1 anther and ovary size strongly correlated with spike dry-weight at all developmental stages in the field, except for F1 anther size at the HD stage. Clearly, the relationship between F1 anther size and spike dry-weight in the field was not as strong as that between F1 ovary size and spike dry-weight. At most stages, F3 and F4 anther and ovary size were closely related to spike dry-weight under both greenhouse and field conditions, although greenhouse correlations were not as strong as those in the field. Hence, we conclude that anther and ovary size are good predictors of spike dry-weight in response to G and E variables, respectively. On the other hand, correlations between anther/ovary size at F3 and F4 positions were stronger than at F1, suggesting that F3/F4 anther and ovary sizes are better predictors of spike dry-weight than F1 anther or ovary size. In fact, F3/F4 ovary sizes were more closely related with spike dry-weight than F3/F4 anther sizes, implying that ovary size is a more reliable predictor of spike dry-weight during pre-anthesis floral development.

Overall, we found strong relationships between anther/ovary size at different positions, correlations at the YA stage were strongest, and that correlations between F3 and F4 were better than those between F1-F4 and F1-F3 (Fig. S2.6, S2.7). Moreover, anther and ovary size at the same position also strongly correlated (Fig. S2.8), suggesting that anther/ovary grow consistently at different positions, as well as in the same position. Similarly, greenhouse correlations were not as strong as those in the field.

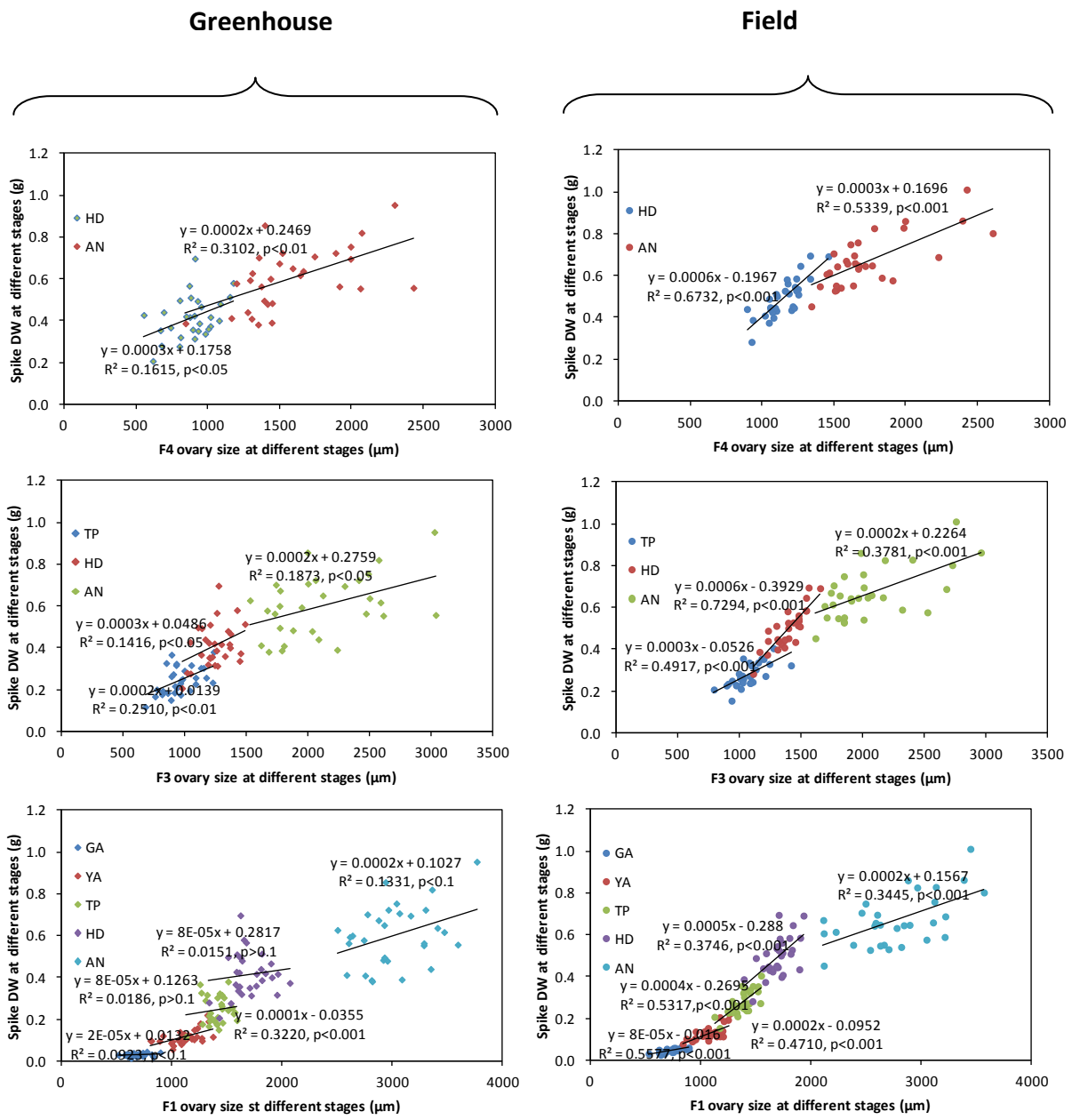


Fig. 2.6. Relationship between ovary size (µm) and spike dry-weight (g) at different floral developmental stages under greenhouse and field conditions. Solid lines were fitted by linear regression.

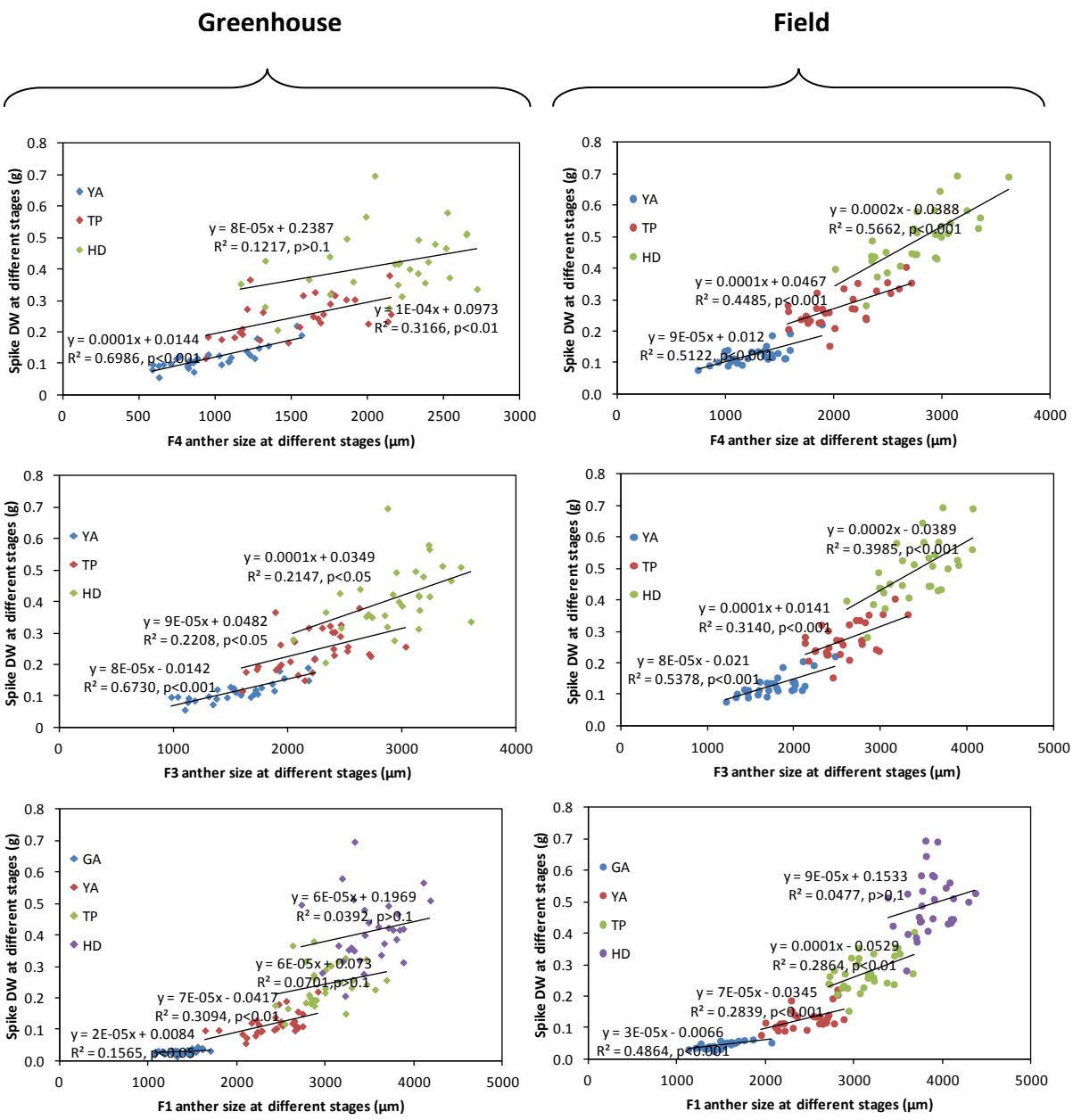


Fig. 2.7. Relationship between anther size (µm) and spike dry-weight (g) at different floral developmental stages under greenhouse and field conditions.

2.5. Discussion

Anther and ovary size under strong genetic control

Previously, much work related to anthers and ovaries in different species has been conducted (Barnabas et al., 2008; Rosati et al., 2009; Engelke et al., 2010; De Storme and

Geelen, 2014), whereas work focusing on anther and ovary size, especially in wheat, is limited. Genotypes used in this study were based on *Rht* and *Ppd* genes which showed variable genetic backgrounds (Table S2.1). Broad-sense heritability of anther and ovary size were consistently high (range, 0.7-0.9; average, 0.8) at different positions and stages. In previous studies, anthers were sampled from the first or second florets in the central position of a spike, just before flowering, and heritability of anther length was estimated above 0.65 (Komaki and Tsunewaki, 1981). This is consistent with our results, which suggest anther and ovary growth is stable. Conversely, according to variance component analysis, environment had a greater influence on F3 and F4 anther and ovary size than those at F1, indicating that F3 and F4 anther and ovary growth is more sensitive. Genotype, on the other hand, had the smallest influence on F1 anther and ovary size at the GA stage, while the residual proportion was largest, though not significant, which suggests a relatively large variation in F1 anther and ovary size in the GA stage. Furthermore, the residual proportion was higher for ovaries, suggesting larger variation in ovary size. Overall, although heritability, sensitivity, and variation were variable across floret positions and developmental stages, we can infer that wheat anther and ovary size is under strong genetic control based on the high heritability (Fig. 2.3B, 2.4B) and close relationships between anther/ovary at different floret positions within genotypes (Fig. S2.1, S2.2).

We also found there are strong relationships between spike dry weight and anther and ovary size, especially, anther and ovary at F3 and F4 positions. In previous studies, it was reported that wheat carpel size and weight was closely related to grain weight (Calderini et al., 1999; Hasan et al., 2011). According to our experience, the florets with large anther and ovary have more opportunities to set seeds. The anther and ovary size at F3 and F4 positions is possibly associated with grain number per spike which further determines the grain weight

per spike/spike dry weight. Hence, F3/4 anther and ovary size can be used as an indicator to breed and select cultivars with high grain number per spike, grain weight per spike and spike dry weight.

Effect of temperature and dry-mass accumulation on anther and ovary growth

Fluctuating temperatures during the day/night cycle in the field revealed a strong temperature oscillation while greenhouse temperatures were comparably stable (Fig. S2.3). The stable greenhouse temperatures resulted in a substantially increased thermal time for each floral developmental stage in the greenhouse versus the field, although growing time in days was significantly longer in the field. As expected, low night-time temperatures, strong temperature fluctuation, and longer growing time (days) lead to more spike dry-mass accumulation at different stages of the field-grown plants. Fig. 2.8 reveals that spike dry-weight in the field was significantly higher than in the greenhouse at different floral developmental stages. Previously it was reported that ovary swelling in bell pepper flowers is favored by low night-time temperatures (Darnell et al., 2012) and can increase ovary size in sweet peppers (Cruz-Huerta et al., 2011). Hence, we concluded that F3 and F4 ovary size, which was higher in the field than in the greenhouse, could be attributed to low night-time temperatures and higher spike dry-mass accumulation. Similarly, F1, F3, and F4 anther size, which was also higher in the field, might have been positively affected by low night-time temperatures and higher spike dry-mass accumulation.

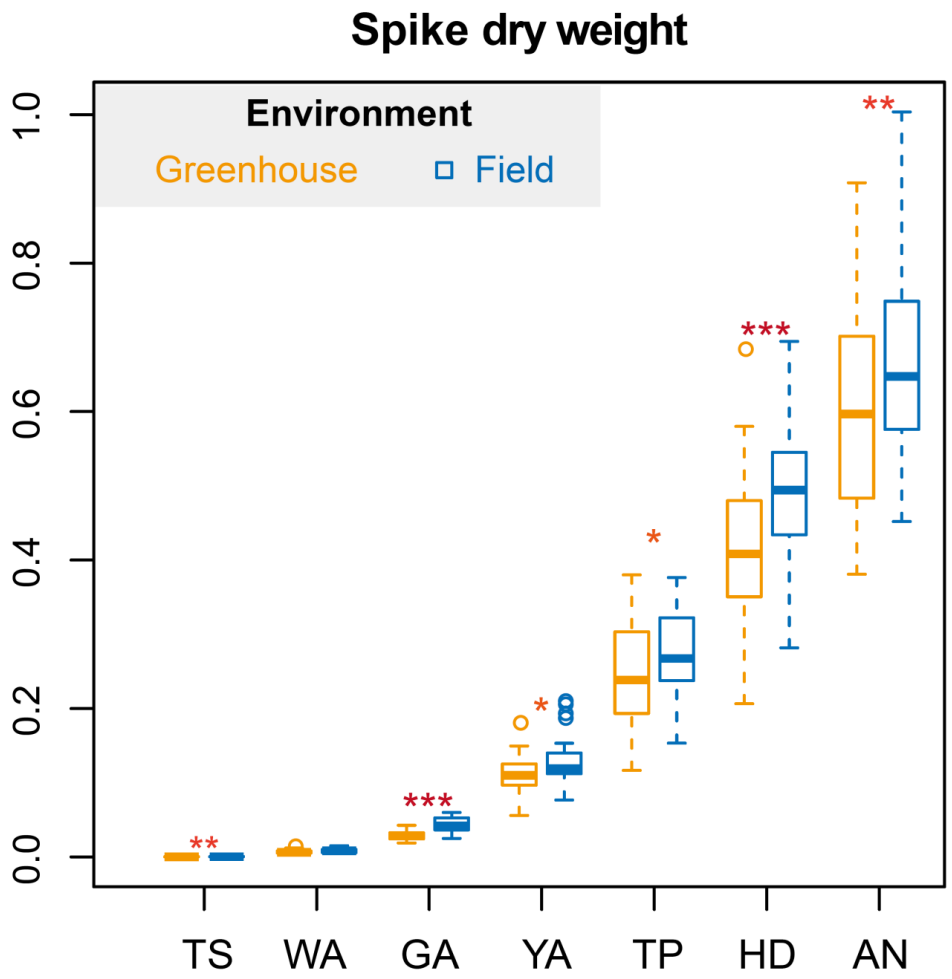


Fig. 2.8. Spike dry-weight (g) at the seven floral developmental stages under greenhouse and field conditions. *p<0.05. **p<0.01. ***p<0.001.

Stem dry weight is also considered a crucial factor affecting anther and ovary growth. Fig. 2.9 reveals that main-stem dry-weight in the field was significantly higher than in the greenhouse, suggesting that low night-time temperatures, large temperature fluctuations, and longer growing time (days) are probably responsible for higher dry-mass accumulation within the main-stem. In previous studies, it was documented that pre-anthesis stem dry-mass accumulation influenced floral development and grain filling under stressed conditions (Bidinger et al., 1977; Kiniry, 1993; Blum et al., 1994; Blum, 1998). In the present study, F3 and F4 were found to vary in size, and F1, F3, and F4 anther size in the field were higher than in the greenhouse, indicating that more dry-matter accumulation within the main-stem in

the field may also contribute to anther and ovary size differences between field and greenhouse conditions.

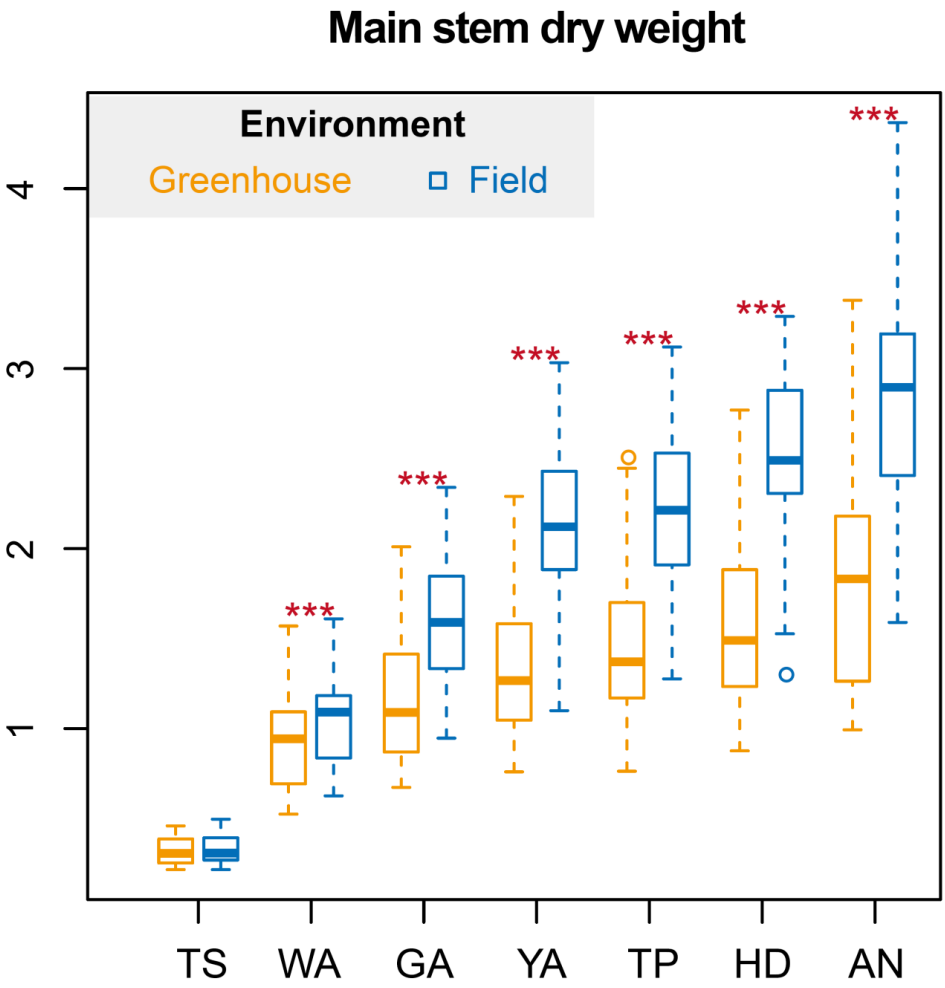
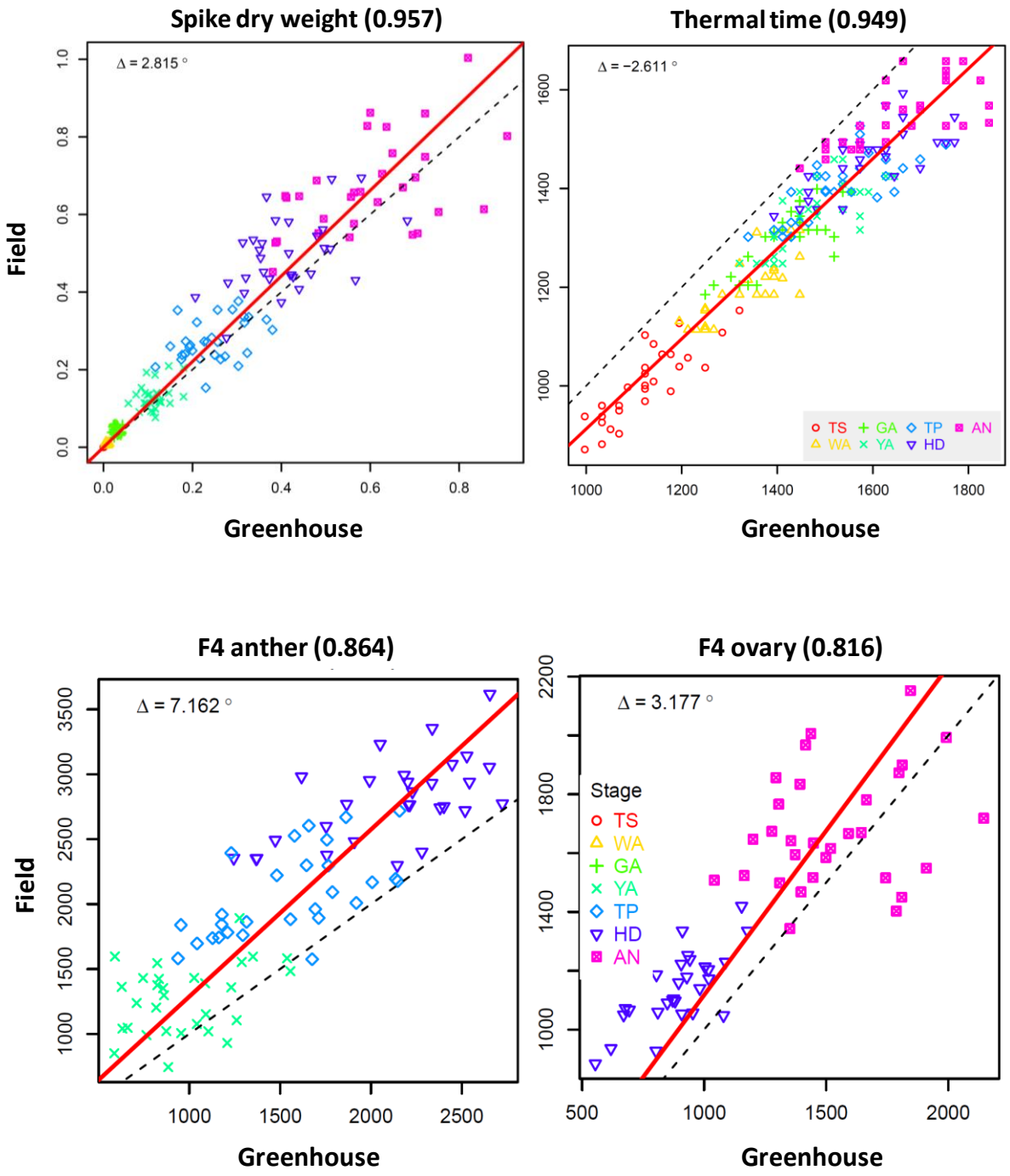


Fig. 2.9. Main-stem dry-weight (g) at the seven floral developmental stages under greenhouse and field conditions. *p<0.05; **p<0.01; ***p<0.001.

Contribution from sensitivity of different traits to correlation differences between greenhouse and field environments

Correlation analyses in this study (Fig. 2.6, 2.7, S2.8) revealed that (1) anther and ovary size are closely associated with spike dry-weight, indicating that anther and ovary size are good predictors of spike dry-weight; (2) there are strong relations between anther/ovary size at different positions, as well as at the same position, allowing us to conclude that anther and ovary size are good predictors of each other within the germplasm studied. However,

correlations were substantially different within the two environments (Fig. 2.2, S2.1-S2.4). As shown in Fig. 2.10, the correlations above each graph suggest the close relationship between field and greenhouse for these traits, meanwhile, spike dry-weight, thermal time, and anther/ovary size at F1, F3, and F4 positions exhibited different sensitivity levels, with F4 anther size expectedly exhibiting the greatest sensitivity, which was 2-4 times higher than that of the other traits (i.e., greater angle between the red and dashed lines Fig. 2.10). It is evident that spike dry-weight, F1, F3, and F4 anther size, and F4 ovary size benefited from field conditions (i.e., red line above dashed line and closer to field; Fig. 2.10), whereas F1 and F3 ovary size benefited from greenhouse conditions (i.e., red line below dashed line and closer to greenhouse; Fig. 2.10). In addition, thermal time to all seven floral developmental stages under greenhouse conditions was substantially higher than in the field, while absolute growing time (days) to reach all seven floral developmental stages in the field was significantly higher than in the greenhouse (Fig. 2.5). Hence, the difference in sensitivity not only concerns value (angles), but also direction (values of angles are positive or negative; the traits benefit from greenhouse or field). The variable sensitivity levels of these traits may explain the correlation differences between greenhouse and field conditions.


Spike dry weight (0.957)
Δ = 2.815 °
Field
Greenhouse
Thermal time (0.949)
Δ = −2.611 °
TS GA TP AN
WA YA HD
Greenhouse
F4 anther (0.864)
Δ = 7.162 °
Field
Greenhouse
F4 ovary (0.816)
Δ = 3.177 °
Stage
TS
WA
GA
YA
TP
HD
AN
Greenhouse

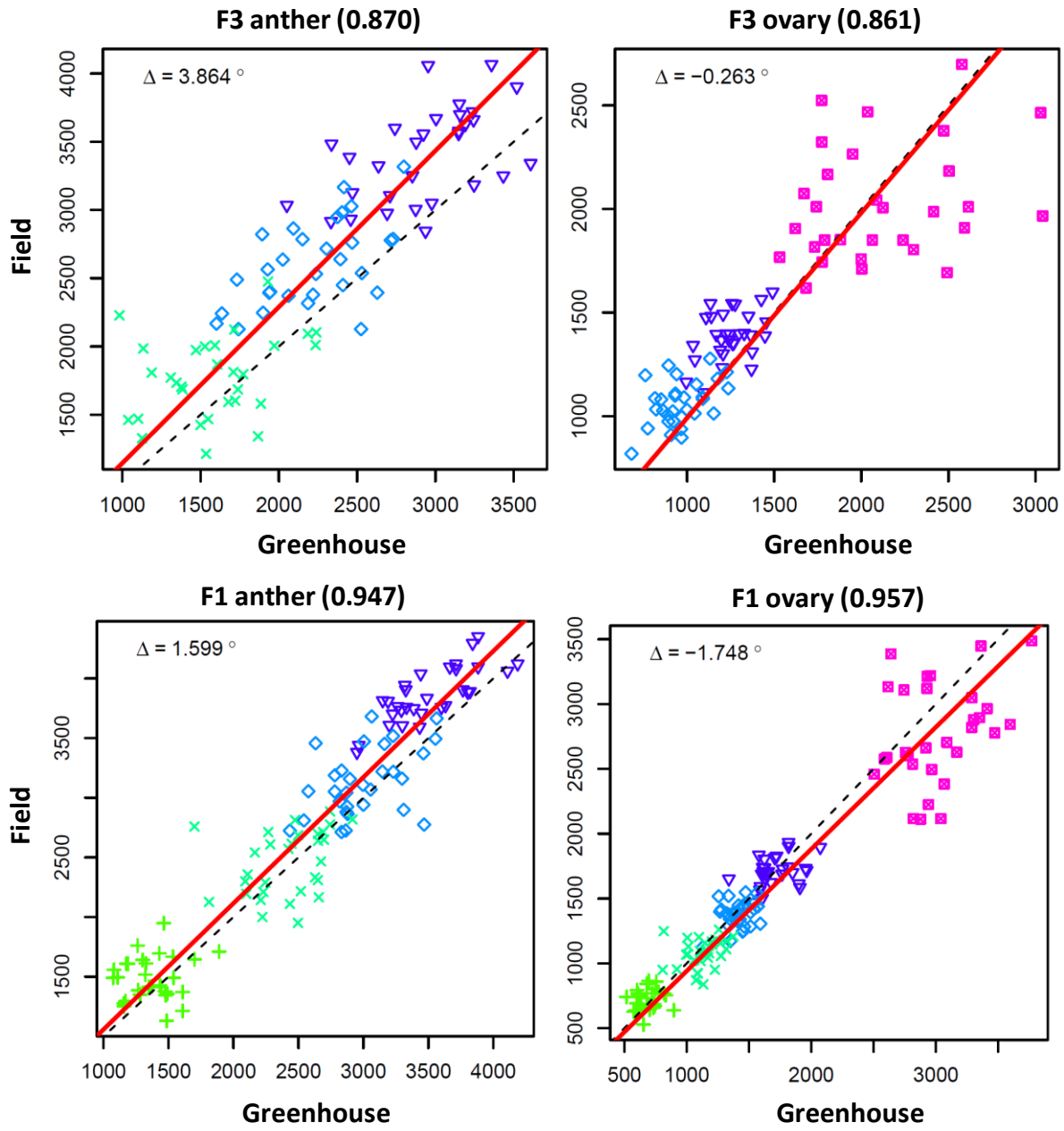


Fig. 2.10. Sensitivity analysis of the correlation between spike dry-weight (g), thermal time (^{o}Cd), and anther and ovary size (µm) at the seven floral developmental stages between greenhouse and field conditions.Red line, trend line between the greenhouse and field; the traits are equivalent between the greenhouse and field on the dashed line (1:1). The angles between red and dashed lines are used to show the sensitivity levels. If the red line is above the dashed line, the angles are positive and suggests these traits are higher (favoured) in the field, whereas a negative angle indicates these traits are higher (favoured) in the greenhouse.

Conclusions

This study showed that broad-sense heritability of anther and ovary size at different positions and stages, as well as all traits examined, showed a high level of genetic control. Meanwhile, variance component analysis of anther and ovary size exhibited a residual proportion, G, E, and GxE effects, suggesting that F3 and F4 anthers and ovaries are more sensitive than those at F1. Relationships between spike dry-weight and anther and ovary size were established, showing a close relationship of spike dry-weight-anther size, spike dry-weight-ovary size, and anther-ovary size. This implies anther and ovary size are good predictors of each other and spike dry-weight. However, the correlations under greenhouse conditions are not as strong as those in the field. Correlations at F3 and F4 positions were stronger than at F1, suggesting F3 and F4 anther and ovary size are better predictors of spike dry-weight. In fact, ovary size had a closer relationship with spike dry-weight than anther size, which indicates that ovary size is a more reliable predictor of spike dry-weight. The residual proportions for anther size were not as big as ovary size, indicating that within-genotype variation of anther size is more stable compared with ovary size. The different sensitivities of these traits are related to the correlation differences. Higher F1, F3, and F4 anther and F3 and F4 ovary size, low nighttime temperature, large temperature fluctuations, more spike dry-mass and stem reserve accumulation in the field are thought to be responsible for the detected difference of anther and ovary size.

3. Time window of floral degeneration/demise

Variation of floret and spikelet fertility in wheat revealed by tiller removal (Journal of Experimental Botany 66: 5945-5958)

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3.1. Abstract

Grain number per spike, which is greatly influenced by floret fertility, is an important trait of wheat (*Triticum aestivum* L.) yield. Maximum floret primordia, fertile floret and final grain number per spikelet are three crucial factors of floret fertility. Floral degradation plays a critical role in determining these three floret fertility-related traits. Twelve hexaploid spring wheat genotypes were selected to investigate the influence of detillering on floral degradation and floret fertility-related traits in the field and greenhouse. Notably, the green anther stage was found to consistently have the maximum floret primordia number. Visible floral degradation, however, was observed to occur at several floral developmental stages, specifically from green anther stage to anthesis. Detillering, on the other hand, was able to delay floral degradation in most cases and was evidently highly associated with increased maximum floret primordia, fertile floret and final grain number per spikelet, with only a few exceptions. Thermal time required for each floral developmental stage was overall not influenced by detillering. Our data hereby reveals a predominant spikelet fertility pattern along the spike in which the number of fertile florets per spikelet at anthesis becomes developmentally confined.

Key words: floral degradation, maximum floret primordia, fertile floret, final grain.

3.2. Introduction

Grass spikelets produce reproductive structures called florets; the determination of floret number per spikelet is a primary factor of spikelet architecture in the grass family. The indeterminate nature of wheat spikelets may enable more than eight florets to be formed within one spikelet and is in contrast with other cereals that have a fixed number (rice and corn) (Skibbe *et al*., 2008; Yoshida and Nagato, 2011) or effectively are fixed at one floret (Mayer et al.) (Bonnett, 1967). Final grain number per spikelet (grain setting) at physiological maturity is one of the most decisive factors for final grain yield in wheat (Reynolds et al., 2009; Fischer, 2011; Pedro et al., 2012). However, before the final grain number is set at physiological maturity, floral structures undergo a sophisticated development and abortion process. After reaching the maximum number of floret primordia, representing wheat's yield potential, a floral degradation process is initiated determining the fertile florets at anthesis. Following this pre-anthesis floral degradation, another one to three florets are usually lost during post-anthesis events until final grain number is reached at physiological maturity. Maximum floret primordia number, fertile floret number and final grain number per spikelet are three crucial points during this floral developmental process. Previous studies monitored the floral degradation process according to the Waddington scale (Waddington et al., 1983). For example, Craufurd and Cartwright (1989) reported that floret death began when the floret 1 (F1, the first floret from the spikelet base) in the central spikelet reached a stage of Waddington score of 8 (W8). Bancal (2009) observed the onset of floret abortion during a period ranging between W7 (styles elongating) to W8. González *et al*. (2011) reported the initiation of floret death when F1 ranged from W8 to W9. To the best of our knowledge, the only work to determine the onset of floral degradation following the Kirby scale was conducted by Kirby and Appleyard. They showed that floret primordia reach a maximum at

the white anther stage (Kirby and Appleyard, 1987). Although previous studies have shown that exogenous and endogenous factors can affect final grain yield in wheat (McGrath et al., 1997; Richards et al., 2002; Sadras and Denison, 2009; Richards et al., 2010; Potgieter et al., 2013; Sadras and Richards, 2014), more work is needed to enrich our knowledge of how these factors influence floral development and abortion processes, especially the initiation of floral degradation, which will determine final grain number.

Tillering is a critical factor for wheat yield, which is regulated genotypically, but is also affected by the environment (Pinthus and Meiri, 1979; Spielmeyer and Richards, 2004; Duggan et al., 2005; Kuraparthy et al., 2007; Fujita et al., 2010; Mitchell et al., 2012; Dreccer et al., 2013). Tillering is closely associated with wheat yield because of its involvement in grain number and grain weight determination (Mohamed and Marshall, 1979; Kemp and Whingwiri, 1980; Borras-Gelonch et al., 2012). An excessive tiller number in wheat can result in yield reductions because some tillers compete for assimilates with the main shoot but abort before reaching physiological maturity and thus do not contribute to the final grain yield (Ishag and Taha, 1974; Thorne and Wood, 1987; Davidson and Chevalier, 1990). Low tiller numbers can also lead to yield loss because of reduced spike and grain number. Although grain weight can increase in this case, it is not enough to compensate for the loss caused by tiller reduction.

Previously, a wheat mutant with strongly reduced tillering was identified. This so called *tin* (*tiller inhibition*) mutant had thicker stems, a higher kernel number per unit stem weight, a lower leaf to stem weight ratio at maturity, and larger spikes with more and larger grains (Atsmon and Jacobs, 1977; Richards, 1988). Research was conducted to determine the physiological characteristics of *tin* mutants across environments and treatments. The growth of *tin* was found to be stunted after long photoperiods and at low temperatures (Atsmon et

al., 1986; Duggan et al., 2002). Their internodes are solid rather than hollow, suggesting that tiller bud growth is arrested due to sucrose transfer from the bud to support internode elongation (Kebrom et al., 2012; Kebrom and Richards, 2013; Kebrom et al., 2013; Kebrom and Mullet, 2014). Interestingly, the effects of *tin* on grain yield were found to vary with environment and genetic background. In some *tin* lines, grain yield was unchanged, but reduced in others (Mitchell et al., 2012). Although nitrogen increased spike numbers in *tin* lines, these numbers were still around 20% less than those of free-tillering cultivars (Duggan et al., 2005). The higher grain weight of *tin* mutant lines under stress conditions was related to more biomass accumulation at anthesis and increased water-soluble carbohydrates in the stem which can ensures more assimilate for later diversion to grain filling (Mitchell et al., 2012). In addition to studies on reduced-tillering (*tin*) wheat lines, a number of work have been conducted to investigate tiller removal. Results of detillering experiments in wheat and barley have shown considerable increase in grain yield (including grain number and weight), main stem dry weight and total biomass. This overall response after tiller removal indicates that tillers compete with the main shoot for resources which significantly restricts improvements in the potential biomass and yield (Mohamed and Marshall, 1979; Kemp and Whingwiri, 1980; Elalaoui et al., 1988; Gu and Marshall, 1988; Kirby et al., 1994).

Although the influence of tiller removal on grain yield-related traits has been shown, there is a paucity of information regarding its effects on the maximum number of floret primordia and fertile floret number in wheat. Furthermore, the work related to the effects of tiller removal on floral degradation is not well documented.

Field growing conditions usually induce a complex environmental response in plants due to steadily fluctuating growing conditions; whereas the greenhouse environment is comparably stable. Thus, it is necessary to determine the effects of detillering on the maximum number

of floret primordia, fertile floret number, final grain number and floral degradation under field and greenhouse growth conditions. To this end, floret primordia number was measured at seven floral developmental stages to determine the timing of maximum floret primordia number. Comparisons between control and tiller removal experiments are conducted to show the effects of detillering on the timing of floral degradation, maximum floret primordia number, fertile floret and final grain number.

3.3. Materials and methods

Plant materials and growth conditions

Experiments were carried out at the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (51° 49′ 23″ N, 11° 17′ 13″ E, altitude 112 m) during the 2014 growing season under greenhouse and field conditions. Twelve German hexaploid spring wheat cultivars were selected according to their years of release (Table 3.1). Control and tiller removal experiments were conducted in the field and greenhouse simultaneously. Tillers were removed two to three times per week. Eighty plants per cultivar (forty plants for control and the other forty plants for tiller removal) were planted under field and greenhouse conditions.

Table 3.1. German wheat cultivars studied and their years of release to the market.

Running number	Cultivar Name	Year of release
1	Adlung's Alemannen	1931
2	NOS Nordgau	1933
3	Peragis Garant	1946
4	Heine's Peko	1947
5	Hohenheimer Franken II	1951

6	Probat	1953
7	Breustedt's Lera	1959
8	Arin	1962
9	Kolibri	1966
10	Ralle	1980
11	Nandu	1988
12	Fasan	1997

For both greenhouse and field experiments, seeds were sown in 96-well trays on the same date (February 11, 2014) and germinated under greenhouse conditions (photoperiod, 16 h :8 h, light: dark; temperature, 20 ^{o}C : 16 ^{o}C, light : dark) for 14 d. Seedlings at the two to three leaf stage were transferred to 4 ^{o}C to vernalize for 63 d. Vernalized seedlings were transferred to a hardening stage (photoperiod, 12 h:12 h, light: dark; temperature, 15^{o}C) for 7d to gradually acclimatize. Finally, half of the plants were transplanted into 0.5 liter pots (one plant per pot; 9 x 9 x 9 cm) under greenhouse conditions (photoperiod, 16 h :8 h, light: dark; temperature, 20 ^{o}C : 16 ^{o}C, light : dark) (Fig. S3.1a). Supplemental light (~250 mE $m^{-2}s^{-1}$ PAR, Photosynthetically Active Radiation) was supplied with low intensity incandescent light and plants were irrigated when required. The other half of the plants were directly planted into a field with silty loam soil (20 plants per 2m long row with 20 cm between rows) (Fig. S3.1b). All plants were manually irrigated on requirement. The temperature and global solar radiation in the 2014 field growing season are presented in Table S3.1.

Phenotypic staging and measurements

To study detillering on floral degradation, maximum number of floret primordia, fertile floret number and final grain number, seven floral developmental stages were selected: terminal

spikelet, TS stage (completion of spikelet initiation; Kirby and Appleyard, 1987); white anther (WA) stage (lemmas of florets 1 and 2 completely enclose stamens and other structures; Kirby and Appleyard, 1987); green anther (GA) stage (glumes cover all but the tips of florets; Kirby and Appleyard, 1987); yellow anther (YA) stage (glumes are fully formed and the lemmas of first three florets are visible; Kirby and Appleyard, 1987); tipping (TP) stage (Z49, first awns visible; Zadoks, 1974); heading time (HD) stage (Z55, 50% of spikes visible; Zadoks, 1974); anthesis (AN) stage (Z65, 50% of spikes with anthers; Zadoks, 1974). The corresponding Waddington stages of floret1 (i.e. F1) at the seven floral developmental stages are shown in Table S2 to be better able to compare between this study and others.

In order to detect the TS, WA, GA and YA stages, every cultivar was examined every two days under a stereomicroscope (Stemi 2000-c, Carl Zeiss Micro Imaging GmbH, Gottingen, Germany). For the TP, HD and AN stages, the day of onset was recorded as the point at which 50% of plants reached the particular stage. Thermal time was used to identify the duration of each stage and was calculated as the sum of the daily average temperature [(Tmax+Tmin)/2] (a base temperature was assumed as 0 ^{o}C).

During each stage, main shoots of three plants for each cultivar were randomly selected to measure floret primordia and fertile floret number per spikelet; while main shoots of six plants were used for determining final grain number per spikelet. Floret primordia, fertile floret and final grain number per spikelet were measured in spikelets at three positions: apical (the third spikelet from the top of spike), central (the spikelet in center of the spike) and basal positions (the third spikelet from the bottom of the spike) (Fig. 3.1) at different floral developmental stages (Fig. 3.2). Here, it should be noted that apical and basal spikelets of the spike in this experiment are high-middle and low-middle positions of the spike and do not include the extreme apical and basal spikelets at the top and bottom of the spike. Floret

primordia number per spikelet at apical parts of spikelets is not visible in Fig. 3.2 from YA to HD stage compared with basal parts of spikelets, so we prepared the apical part out to display apical floret primordia number (Fig. 3.3). Due to genotypic variation in floral development, visible floral degradation (as seen in Fig. 3 from TP to HD) can also occur at other stages between GA to AN. To determine the fertile floret number per spikelet at anthesis, it is necessary to distinguish between living and aborted florets. The living and aborted florets at the same developmental stages were shown in Fig. 4, the anthers in the aborted florets are always small and dry, the stigmatic hairs are not well-developed. Leaf area of the main stem at GA and AN stage was measured immediately after dissection of fresh leaf material using an area meter (LI-3100, LI-COR Ltd, Nebraska, USA). Main stem, tillers and spike on the main shoot at GA and AN stage were dried in two cellophane bags at 60 ^{o}C for 3-5 d for dry weight measurement and they were measured directly after harvesting for PM stage. Stem dry weight refers to the dry weight of shoots without spikes. In this experiment, floret and grain number, leaf area, spike dry weight were only measured on the main shoot.

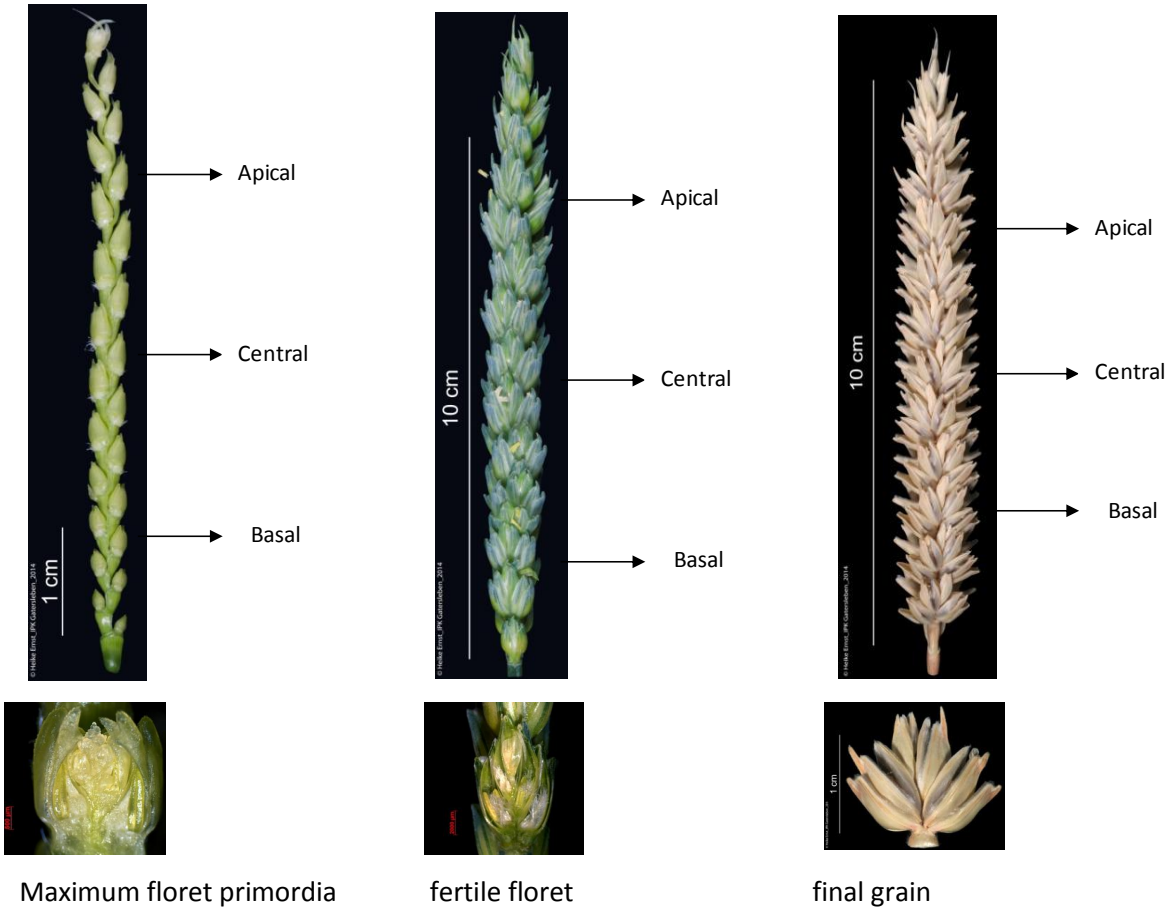
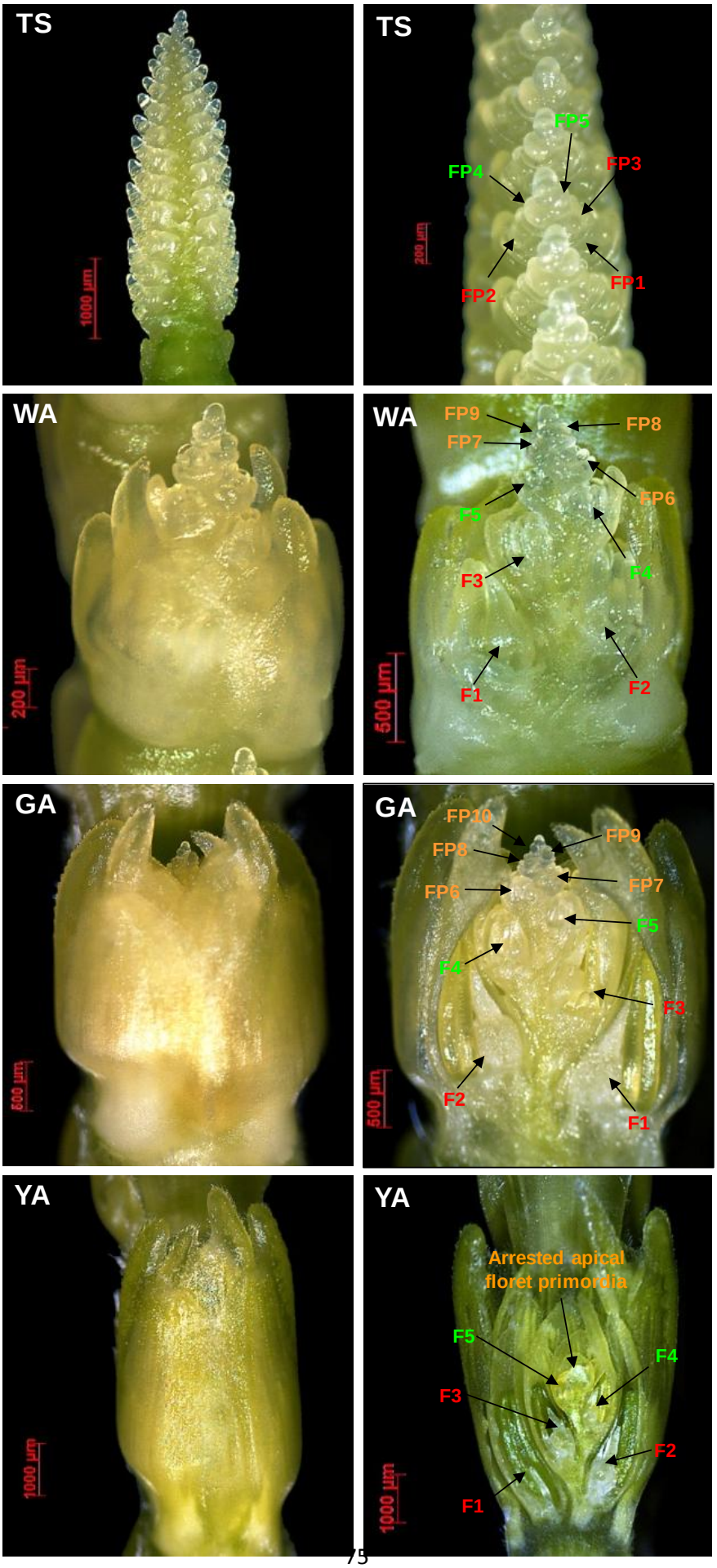


Fig. 3.1. Apical, central and basal spikelets for measurement of maximum number of floret primordia (GA), fertile floret number (AN) and final grain number (PM) per spikelet.


TS
1000 µm
TS
FP5
FP4
FP3
200 µm
FP2
FP1
WA
200 µm
WA
FP9
FP8
FP7
FP6
F5
F4
F3
500 µm
F1
F2
GA
500 µm
GA
FP10
FP9
FP8
FP7
FP6
F5
F4
F3
500 µm
F2
F1
YA
1000 µm
YA
Arrested apical floret primordia
F5
F4
F3
F2
1000 µm
F1

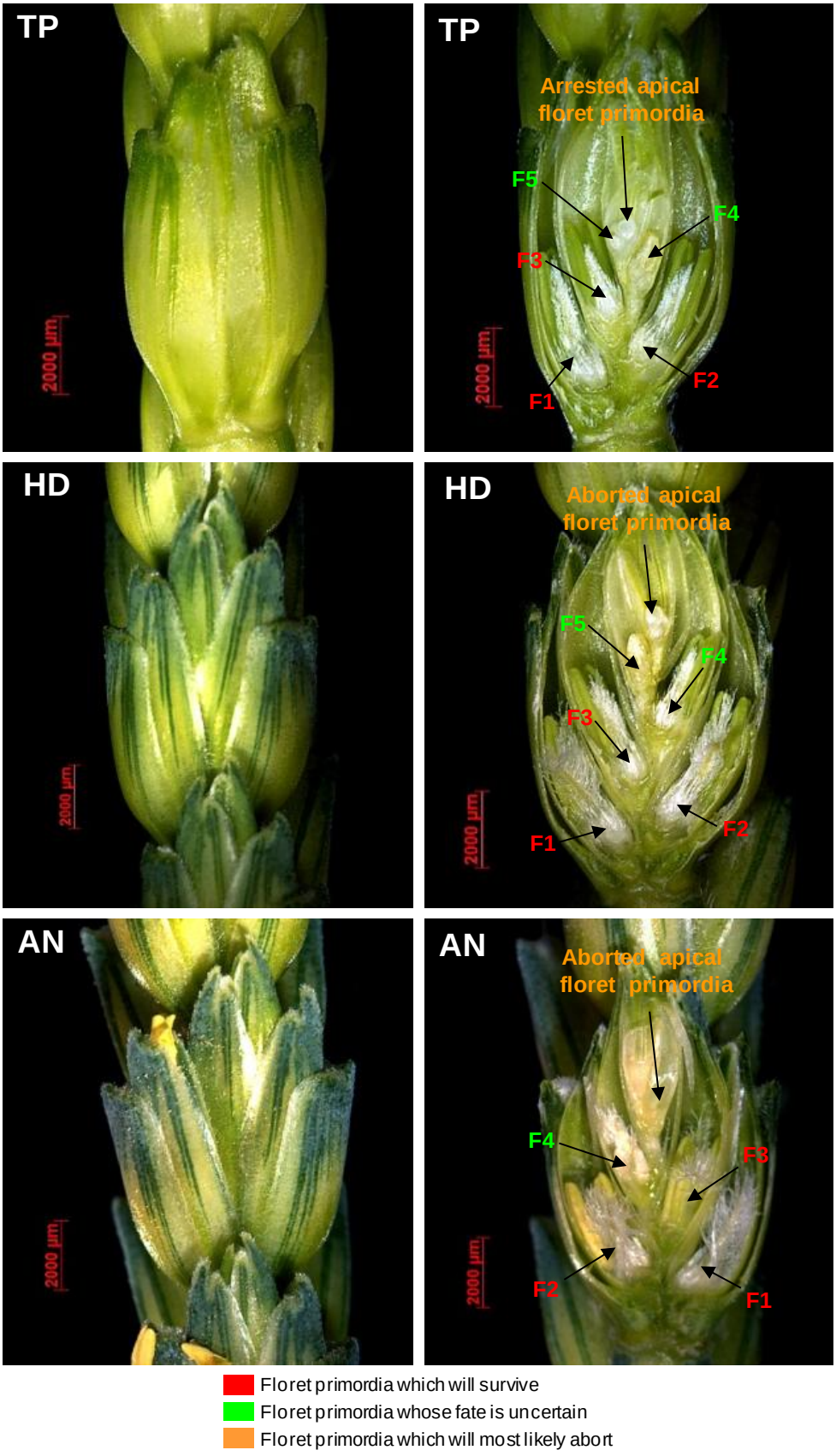


Fig. 3.2. Details of floral development and abortion processes at seven developmental stages in wheat following the staging of Kirby and Appleyard (1987). The left panels are spikelets at the seven

floral developmental stages, and the right panels are the longitudinal section of corresponding spikelets.

3.4. Results

Influence of detillering on visible floral degradation

The timing of visible floral primordia degradations are shown in gray frames in Table 3.2, 3.3, 3.4, and 3.5 and are indicated from TP to HD stage in Fig. 3.3. As shown in Table 3.2, visible floral degradation in the four exemplified genotypes, NOS Nordgau, Adlung's Alemannen, Peragis Garant and Nandu, occured from GA-YA, YA-TP, TP-HD and HD-AN stages, respectively; indicating that visible floral degradation can occur at all floral developmental stages between YA to AN. Table 3.3 and 3.5 show that visible floral degradation in three genotypes (NOS Nordgau, Adlung's Alemannen, Peragis Garant) grown in field and greenhouse was delayed by tiller removal relative to their respective environmental controls (Table 3.2 and 3.4). Additionally, Table 3.4 (gray frames) shows that visible floral degradation without tiller removal was delayed by greenhouse growth conditions compared with control plants in the field (Table 3.2). A delay in floral degradation was not found in Nandu plants because it occurred from HD to AN, which is the last stage for fertile florets. Thus, floral degradation can be delayed by both tiller removal and greenhouse growth conditions (Table 3.2, 3.3, S3.3 and S3.4). It should also be noted that GA stage contained the maximum floret primordia number (bold numbers) across the two environments (field and greenhouse), the two treatments (control and tiller removal) and all 12 tested genotypes (Table S3.5, S3.6, S3.7, and S3.8). Generally, the difference between floret primordia and fertile floret number in spikelet positions of the same developmental stage is below two, in most cases, it is one or zero (the standard deviation is below two). Moreover, visible floral degradation in apical, central and basal spikelets occurred at the same stage within a genotype but in a few cases

the standard deviations of floret primordia number became very large, indicating large differences between plants within a genotype (Table S3.5, S3.6, S3.7, and S3.8). The explanation is that the floral degradation already occurred in one selected plant suggesting almost half of floret primordia lost; but not in the other two selected plants. In extreme cases, the three selected positions (apical, central, and basal) within one spike may also display different degradation situation: degradation may occur in apical and middle spikelets, but not in basal spikelets (Ralle, HD stage, Table S3.6).

Table 3.2. Living floret primordia number at apical, central and basal spikelet positions at seven floral developmental stages in four selected, free-tillering genotypes (control) grown in the field.

Cultivars	Position	TS	WA	GA	YA	TP	HD	AN	PM
NOS Nordgau	Apical	2.00±0.00	8.67±0.58	**10.00±0.00**	4.00±0.00	4.00±0.00	4.00±0.00	3.00±0.00	2.83±0.75
	Central	3.67±0.58	9.00±0.00	**10.33±0.58**	5.67±0.58	5.00±0.00	5.00±0.00	4.33±0.58	3.67±0.82
	Basal	3.00±0.00	9.67±0.58	**10.33±0.58**	5.67±0.58	5.00±0.00	5.00±0.00	4.33±0.58	3.67±0.52
Adlung's Alemannen	Apical	2.33±0.58	9.00±1.00	**9.67±0.58**	9.67±0.15	4.33±0.58	4.33±0.58	3.00±0.00	2.50±0.55
	Central	3.33±0.58	10.00±0.58	**10.67±0.58**	10.00±0.00	5.33±0.58	5.33±0.58	4.33±0.58	3.67±0.82
	Basal	3.33±0.58	9.33±0.58	**10.33±0.58**	11.00±0.00	5.33±0.58	5.00±1.00	4.33±0.58	3.50±0.55
Peragis Garant	Apical	4.00±0.00	8.67±1.15	**10.00±0.00**	9.33±1.15	9.67±1.15	4.00±0.00	3.67±0.58	2.67±0.52
	Central	5.33±0.58	10.33±0.58	**11.00±1.00**	11.00±1.00	10.67±1.15	5.67±0.58	5.00±1.00	3.50±0.84
	Basal	5.33±0.58	10.33±0.58	**11.33±0.58**	10.67±0.58	11.67±0.58	6.00±0.00	5.00±1.00	3.00±1.26
Nandu	Apical	3.33±0.58	9.00±0.00	**10.67±0.58**	10.33±0.58	10.00±0.00	10.00±0.00	3.33±0.58	2.50±0.55
	Central	5.33±0.58	10.00±0.00	**11.67±0.58**	11.33±0.58	11.00±0.00	11.00±0.00	4.33±0.58	3.67±0.52
	Basal	5.00±1.00	10.00±0.00	**11.67±0.58**	12.00±0.00	11.67±0.58	12.00±1.00	4.33±0.58	3.33±1.03

Data are presented as the mean ± SD, n=6 for PM stage, and n=3 for the remaining stages. The bold text suggests maximum floret primordia number stage is GA stage, and the grey boxes indicate the time windows of floral degradation occrued.

Table 3.3. Living floret primordia number at apical, central and basal spikelets at seven floral developmental stages in the four selected, detillered genotypes (tiller removal) grown in the field.

Cultivars	Position	TS	WA	GA	YA	TP	HD	AN	PM
NOS Nordgau	Apical	2.00±0.00	8.33±0.58	**10.67±0.58**	10.33±0.58	9.67±0.58	5.67±0.58	3.67±0.58	3.00±1.55
	Central	3.33±0.58	9.00±1.00	**10.67±0.58**	11.00±0.00	11.00±1.00	6.33±0.58	6.00±0.00	4.50±0.84
	Basal	3.33±0.58	9.67±0.58	**10.67±0.58**	10.67±0.58	11.00±0.00	7.33±2.31	6.00±0.00	4.50±0.84
Adlung's Alemannen	Apical	3.50±0.71	8.67±0.58	**10.67±0.58**	10.67±0.58	10.33±0.58	5.67±0.58	5.00±1.00	2.33±1.75
	Central	4.50±0.71	9.67±0.58	**10.67±0.58**	11.33±0.58	11.67±0.58	6.00±1.00	6.00±0.00	4.67±0.52
	Basal	4.00±0.00	9.67±0.58	**11.00±0.00**	11.33±0.58	12.00±0.00	5.67±0.58	6.00±0.00	4.33±1.21
Peragis Garant	Apical	4.00±1.73	9.67±0.58	**10.67±0.58**	10.67±0.58	10.67±0.58	9.00±3.46	3.33±0.58	2.50±1.05

	Central	5.33±0.58	10.33±0.58	**11.67±0.58**	12.00±0.00	11.33±0.58	10.00±3.46	5.00±0.00	3.33±1.03
	Basal	6.67±0.58	9.67±0.58	**11.67±0.58**	12.00±0.00	12.33±0.58	10.00±3.46	5.00±1.00	3.33±1.03
	Apical	3.67±1.15	8.00±0.00	**10.67±0.58**	11.00±0.00	11.00±0.00	11.00±1.00	4.67±0.58	3.29±0.76
Nandu	Central	5.67±0.58	9.00±0.00	**11.00±0.00**	12.33±0.58	12.00±0.00	12.33±0.58	6.67±0.58	4.00±0.82
	Basal	6.00±1.00	10.00±0.00	**11.33±0.58**	11.67±0.58	12.33±0.58	13.00±0.00	6.67±0.58	3.43±1.13

Data are presented as the mean ±SD, n=6 for PM stage, and n=3 for the remaining stages. The bold text suggests that maximum floret primordia number stage occurred during GA stage, while grey boxes indicate the time windows when visible floral degradation occurred.

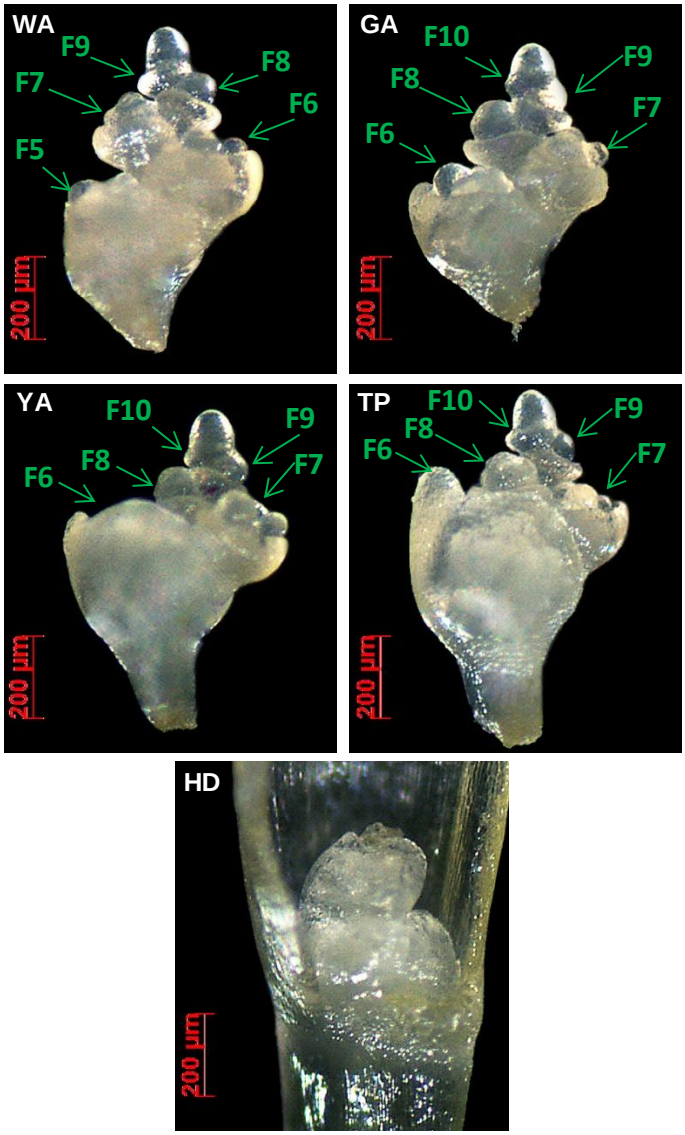


Fig. 3.3. Floret primordia growth, arrest and visible degradation at WA, GA, YA, TP and HD stages. At WA stage, the first four florets at the bottom were removed; at GA, YA, TP and HD stages, the first five florets at the bottom were removed. Exemplified is the process of floret primordia arrest (GA to YA) and visible degradation (TP to HD); due to genotypic variation, the visual degradation can vary from GA, YA or HD.

Generally, visible floral degradation in apical, central or basal spikelets occurs simultaneously between the same two stages of the same genotypes. In the field, six free-tillering (i.e. control) genotypes underwent visible floral degradation between GA-YA, four between YA-TP, one between TP-HD, and one between HD-AN (Table 3.4); for detillered plants, one genotype underwent floral degradation in GA-YA, three in YA-TP, five in TP-HD, and three in HD-AN (Table 3.4). In the greenhouse, no control plant genotype underwent floral degradation in GA-YA, while seven showed degradation during YA-TP, four during TP-HD, and one during HD-AN (Table 3.5); for detillered plants, zero genotypes underwent floral degradation from GA to YA, one from YA to TP, seven from TP to HD, and four from HD to AN (Table 5).

After tiller removal, 11 genotypes grown in the field and 10 grown in the greenhouse showed delayed floral degradation (Table 3.4 and 3.5). The cultivar 'Nandu' did not show delayed floral degradation after tiller removal in both greenhouse and field. In the greenhouse, besides Nandu, there was only one genotype (Arin), which did not display delayed floral degradation after tiller removal (Table 3.5). Nandu plants did not show delayed floral degradation after tiller removal in both field and greenhouse conditions because it occurred in HD-AN, which is the last possible stage for producing fertile florets (Table 3.4 and 3.5).

Table 3.4. Stages of visible floral degradation in twelve spring wheat cultivars grown in the field (control and tiller removal).

Cultivars	Field Control (Free-tillering)				Field Detillering			
	GA-YA	YA-TP	TP-HD	HD-AN	GA-YA	YA-TP	TP-HD	HD-AN
1- Adlung's Alemannen		†					†	
2- NOS Nordgau	†						†	
3- Peragis Garant			†					†
4- Heine's Peko		†					†	

5- Hohenheimer Franken II	†					†		
6- Probat		†						†
7- Breustedt's Lera		†					†	
8- Arin	†						†	
9- Kolibri	†				†			
10- Ralle	†					†		
11- Nandu				†				†
12- Fasan	†					†		
Number of genotypes per stages	6	4	1	1	1	3	5	3

† indicates the stages of visible floral degradation.

Table 3.5. Stages of visible floral degradation in twelve spring wheat cultivars grown in the greenhouse (control and tiller removal).

Cultivars	Control (Free-tillering)				Detillering			
	GH				GH			
	GA-YA	YA-TP	TP-HD	HD-AN	GA-YA	YA-TP	TP-HD	HD-AN
1- Adlung's Alemannen		†					†	
2- NOS Nordgau		†					†	
3- Peragis Garant			†					†
4- Heine's Peko		†					†	
5- Hohenheimer Franken II		†					†	
6- Probat			†					†
7- Breustedt's Lera		†					†	
8- Arin			†				†	
9- Kolibri		†				†		
10- Ralle		†					†	
11- Nandu				†				†
12- Fasan			†					†
Number of genotypes per stages	0	7	4	1	0	1	7	4

† indicates the stages of visible floral degradation.

Under greenhouse growth conditions, eight control genotypes demonstrated delayed floral degradation compared with field growth conditions. For the detillered treatment, on the other hand, only three genotypes (Peragis Garant, Probat, and Nandu) exhibited delayed floral degradation because they were already in the last stage before fertile florets (Table 3.4).

Influence of detillering on thermal time required for seven floral developmental stages

Although floral degradation was delayed by tiller removal and greenhouse condition, tiller removal did not significantly affect the thermal time required for each floral developmental

stage relative to controls (Table S3.9 and S3.10), with four exceptions. Exceptions included average thermal time required for GA (1069), YA (1121), and AN (1316) stages, which were significantly increased by tiller removal in the field (GA, 1104, $p<0.001$; YA, 1149, $p<0.05$; AN, 1349, $p<0.01$) (Table S3.9). Moreover, the average thermal time required for AN was significantly higher with tiller removal in the greenhouse (1285, control; 1337, detillering; $p<0.05$) (Table S3.10). When we consider specific genotypes, there are seven, six and five cultivars at GA, YA and AN stages with significantly higher thermal time requirements after detillering in the field (Table S3.9); eight culitvars at AN stage in the greenhouse (Table S3.10).

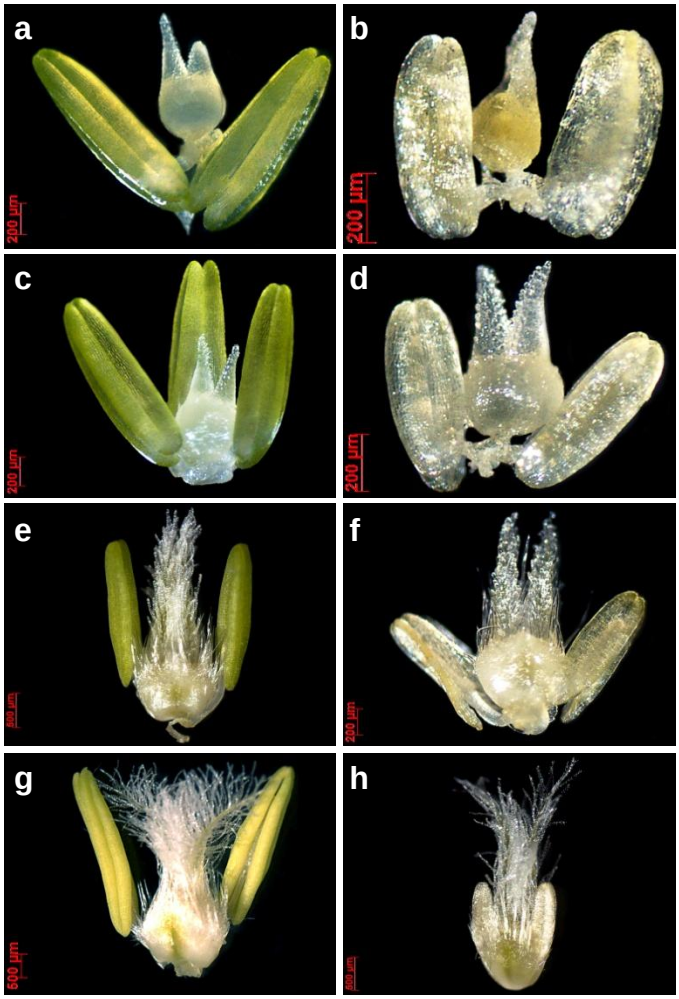


Fig. 3.4. Fertile (left, a, c, e, g) and aborted florets (right, b, d, f, h) at the same floral developmental stages.Please note that these fertile and aborted florets can be found at different floret positions

within the same spikelets or at different floral developmental stages at the same floret positions in different spikelets.

Influence of detillering on maximum floret primordia number, fertile floret number and final grain number per spikelet

Under greenhouse conditions, average maximum floret primordia number per spikelet for all twelve genotypes in control plants at apical (9.23), central (10.20) and basal (10.09) spikelet positions is significantly lower than in detillered plants (apical, 9.60, $p<0.05$; central, 10.60, $p<0.05$; basal, 10.77, $p<0.001$) (Fig. 3.5). Under field conditions, average maximum floret primordia number per spikelet for all twelve genotypes in control plants at apical (9.72), central (10.64) and basal (10.67) spikelet positions is significantly lower than in detillered plants (apical, 10.17, $p<0.01$; central, 10.94, $p<0.05$; basal, 10.94, $p<0.1$) (Fig. S3.2). Generally, tiller removal increases the maximum floret primordia number at apical, central, and basal spikelets under both field and greenhouse conditions (Fig. 3.5 and S3.2). The most obvious increase related to detillering, however, occurred at central spikelet in the greenhouse (Fig. 3.5). Although maximum floret primordia number is increased in most genotypes, it is relatively stable across positions, genotypes, treatments and environments, with around 10 floret primordia, ranging from nine to 12 compared with fertile floret number and final grain number. Central and basal spikelet positions generally have higher number of floret primordia (Fig. 3.5).

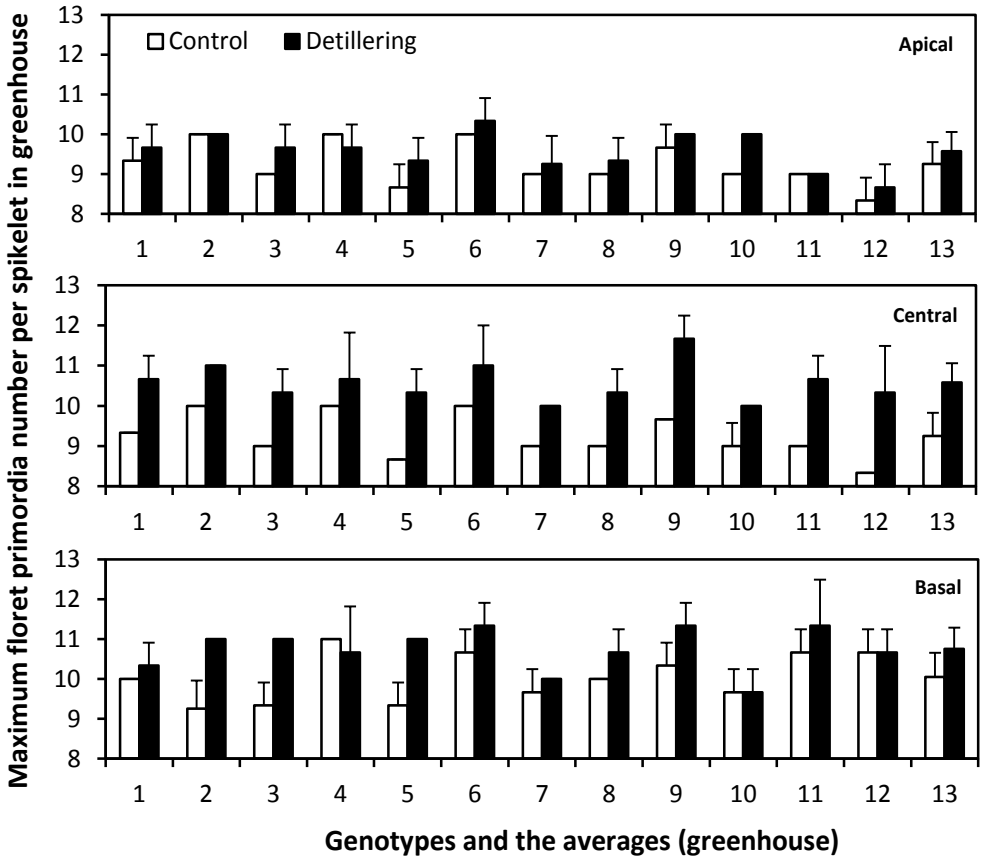


Fig. 3.5. Maximum number of floret primordia per spikelet at apical, central and basal spikelets in twelve genotypes (1-12) and averages of genotypes (13) under control and detillering treatments in the greenhouse (mean ± SD, n=3).

Under greenhouse conditions, average fertile floret number per spikelet for all twelve genotypes in control plants at apical (3.11), central (3.94) and basal (3.78) spikelet positions is significantly lower than in detillering plants (apical, 3.51, $p<0.001$; central, 4.80, $p<0.001$; basal, 4.80, $p<0.001$) (Fig. 3.6). Under field conditions, average fertile floret number per spikelet for all twelve genotypes in control plants at apical (3.56), central (4.69) and basal (4.64) spikelets is significantly lower than in detillering plants (apical, 4.00, $p<0.05$; central, 5.59, $p<0.001$; basal, 5.56, $p<0.001$) (Fig. S3.3). Tiller removal significantly increased fertile floret numbers at apical, basal and central positions in all twelve genotypes under both field and greenhouse conditions (Fig. 3.6, S3.3). The increase of fertile floret number is much more significant and consistent than for the maximum floret primordia number. However,

there is strong fluctuation of fertile floret number across spikelet positions, genotypes, treatments and environments (Fig. 3.6, S3.3), ranging from three to six. This indicates that fertile floret number per spikelet underlies positional effects within the spike. The significant increase and positional effects suggest large opportunities for improving fertile floret number; in particular for apical spikelets because central and basal spikelets generally have higher fertile floret number per spikelet (Fig. 3.6, S3.3).

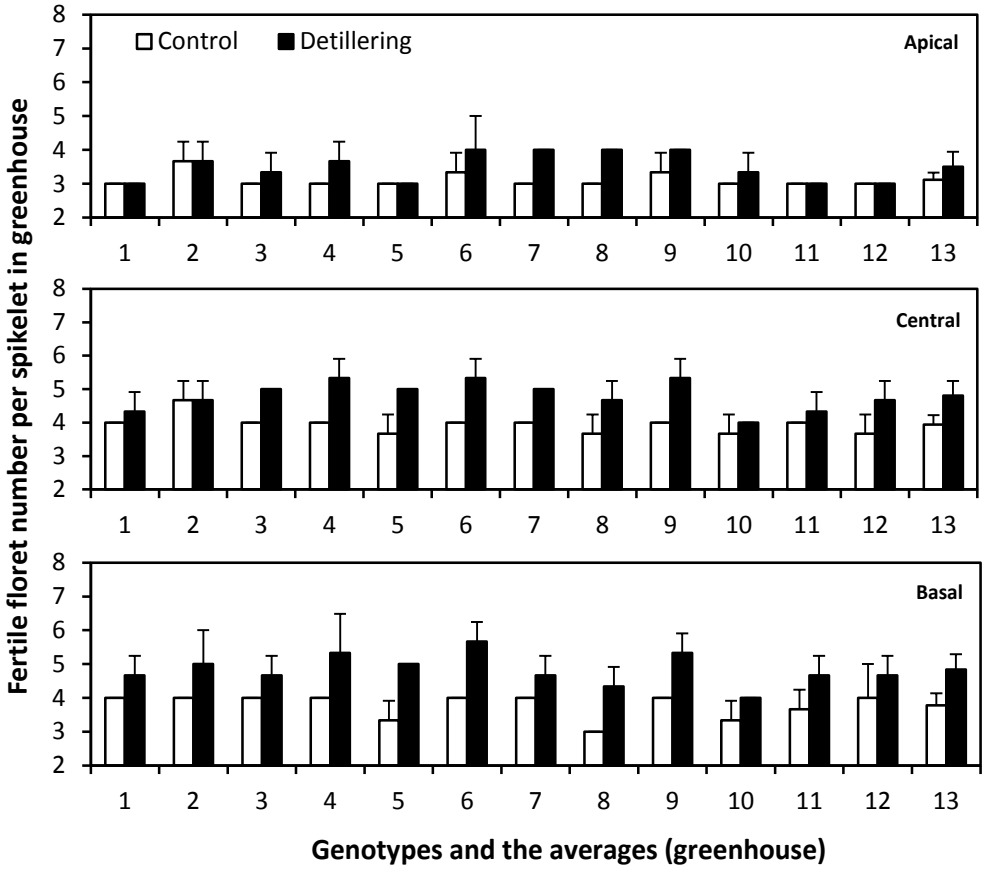


Fig. 3.6. Fertile floret number per spikelet at apical, central and basal spikelets in twelve genotypes (1-12) and averages of genotypes (13) under control and detillering treatments in greenhouse (mean ± SD, n=3).

For the average final grain number per spikelet for all twelve genotypes, the significant difference only occurred at basal spikelets in the greenhouse (2.92 control, 3.78 detillering, $p<0.001$) and at central spikelet positions in the field (3.47 control, 3.82 detillering, $p<0.05$)

(Fig. 3.7, S3.4). In the greenhouse, apical (1.34) and central (2.87) spikelets, have no significant difference with detillered plants (apical, 1.04, $p>0.1$; central, 3.24, $p>0.1$); in the field, apical (2.53) and basal (3.49) spikelets in control plants have no significant difference with detillered plants (apical, 2.25, $p>0.1$; basal, 3.63, $p>0.1$) (Fig. 3.7, S3.4). Generally, in most plants, the final grain number per spikelet was also increased by detillering under both greenhouse and field conditions (Fig. 3.7, S3.4). The most significant and consistent increase occurred at basal spikelets in the greenhouse where the final grain number per spikelet is markedly increased in all twelve genotypes (Fig. 3.7). The increase in final grain number per spikelet is not seen at apical spikelets in some genotypes of detillered plants. The lower apical spikelet fertility becomes more evident under the detillering treatment in the greenhouse (Fig. 3.7). The most important difference of final grain number per spikelet compared with maximum floret primordia and fertile floret number is that apical spikelets show a large variation because, in some cultivars, apical spikelets did not set grain at all, which means that the final grain number per spikelet was zero. It was concluded that the increase of final grain number per spikelet in basal and central spike positions caused that more apical spikelets failed to set seeds because of the competition between spikelets at different positions. Therefore, the large variation of final grain number and reduced spikelet fertility at apical spikelets is due to preferential resource allocation to the mid-bottom part of the spike.

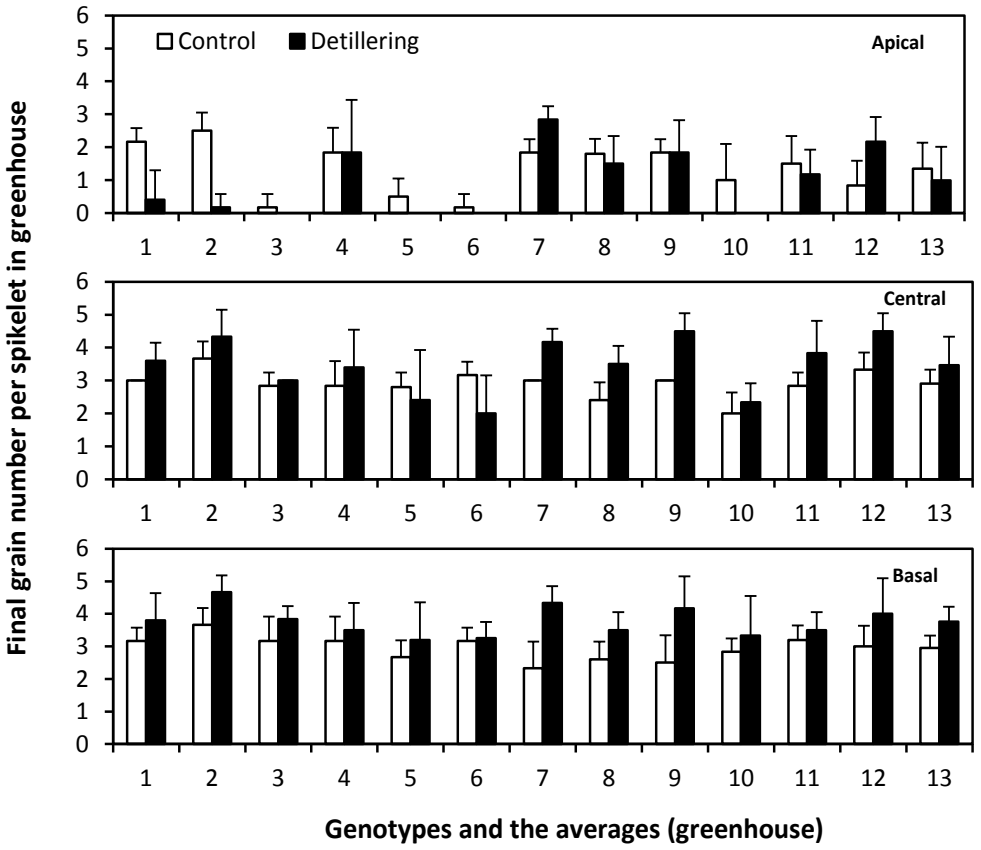


Fig. 3.7. Final grain number per spikelet at apical, central and basal spikelets in twelve genotypes (1-12) and averages of genotypes (13) under control and detillering treatments in greenhouse (mean ± SD, n=6).

Influence of detillering on spikelet number, grain number per spike, spikelet fertility

As shown in Table 3.6, detillering has no significant effects on total spikelet number per spike, but markedly decreased fertile spikelet number. This caused the considerable decrease of spikelet fertility (the ratio of fertile and total spikelet number). Under greenhouse conditions, the average spikelet fertility rate for all the twelve genotypes in control plants (78.50%) was markedly higher than in detillered (70.81%) plants (p<0.01). Similarly, under field conditions it is significantly higher in control plants (94.96%) than detillered (88.19%) plants (p<0.001). Grain number per spike was considerably increased by detillering in greenhouse (detillering 53.34, control 45.33, p<0.05), but not significantly influenced in field. We generally did not find an association between spikelet fertility and

year of release based on the twelve genotypes in this study (Table S3.11). All these results suggest that the overall impact of detillering is complicated, since detillering can increase grain number per spikelet, but decreased the fertile spikelet number and spikelet fertility. In order to better understand the influence on grain number per spike and yield, the relationship between different traits should be studied.

Table 3.6. Total spikelet number, fertile spikelet number, spikelet fertility (%), grain number per spike and grain number per spikelet (apical, central, basal positions) in twelve genotypes under control and tiller removal treatments under the greenhouse and field conditions at harvest.

traits	greenhouse		field	
	control	detillering	control	detillering
Total spikelet number/spike	23.48±2.79a	23.29±2.69a	21.86±2.28a	21.53±2.25a
Fertile spikelet number/spike	18.24±3.02a	16.54±4.57b	20.77±2.63a	19.10±3.33b
Spikelet fertility (%)	78.50±15.16a	70.81±18.52b	94.96±6.05a	88.19±11.85b
Grain number /spike	45.33±9.16b	53.34±15.84a	59.18±17.21a	57.35±15.84a

Data are presented as the mean±SD, n=12; different letters per trait indicate significant differences between control and treated plants (p<0.05).

3.5. Discussion

Many previous studies have confirmed that tillers compete with the main shoot for resources (Mohamed and Marshall, 1979; Elalaoui et al., 1988; Gu and Marshall, 1988). When tillers are removed, more assimilates become available which will further enhance the growth potential of the main shoot. We measured leaf area (main shoot), spike dry weight (main shoot) and main stem dry weight at GA, AN and physiological maturity to determine the direct and indirect effects of tiller removal on the maximum number of floret primordia, fertile floret number and final grain number (grain setting).

Effects of tiller removal on the maximum floret primordia number per spikelet through growth of leaves, spikes and stems on the main shoot

The maximum floret primordia number per spikelet stage was consistently found at the GA stage (Table S3.5, S3.6, S3.7, and S3.8). Hence, leaf area, spike dry weight and stem dry weight of the main shoot were measured at this stage. As is shown in Fig. S3.5, main stem dry weight was significantly increased by tiller removal in both field and greenhouse. Leaf area was also considerably increased by tiller removal under greenhouse conditions, but there was no considerable difference between control and tiller removal plants in the field. In addition, there was a marked increase in spike dry weight after tiller removal in field-grown plants. Pre-anthesis stem dry mass accumulation has been documented to influence floral development and grain filling under stressed conditions (Bidinger et al., 1977; Kiniry, 1993; Blum et al., 1994; Blum, 1998). Thus, floret primordia may also benefit from an increase in main stem dry weight. Hence, these increases in the different structural parts of the main shoot can lead to increases in the maximum floret primordia number.

Effects of tiller removal on fertile floret number per spikelet through growth of leaves, spikes and stems on the main shoot

Because the fertile floret number per spikelet was determined at AN, leaf area, spike dry weight and stem dry weight of main shoot were also measured for this stage. Spike dry weight and stem dry weight of the main shoot were both markedly increased by tiller removal at AN in both field and greenhouse. Leaf area was also considerably increased by tiller removal in greenhouse, but the increase is not very significant in field growth conditions (Fig. 3.8). Here, the situation for the number of fertile florets is similar to that of maximum floret primordia number at GA stage. After tiller removal, more resources may

result in increased number of fertile florets per spikelet. This is consistent with the work by (Dreccer et al., 2014).

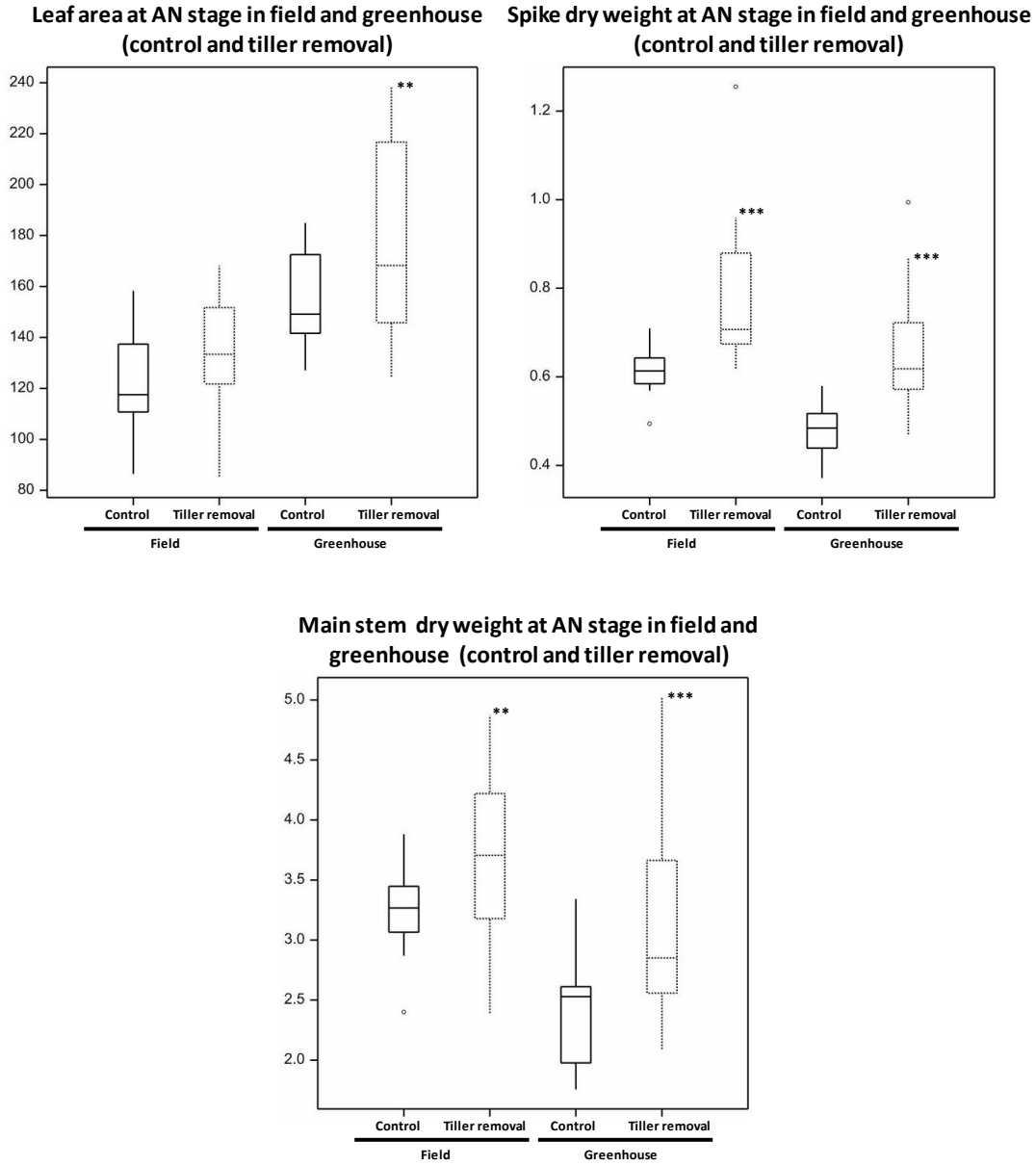


Fig. 3.8. Boxplots of leaf area (cm^2) (main shoot), spike dry weight (g) (main shoot), main stem dry weight (g) at AN stage and significant levels of difference between control and tiller removal. **p< 0.01; ***p< 0.001. Each boxplot displays the data of the twelve genotypes for corresponding traits at AN stage, the significances suggest comprehensive influence of detillering on different traits of the twelve genotypes.

Effects of tiller removal on final grain number per spikelet through growth of spikes and stems on the main shoot

Unlike for maximum floret primordia number and fertile floret number, some work has been done to understand the effects of detillering on final grain number per spike (grain setting). It was previously reported that detillering in wheat plants leads to more grains per spikelet. The increase in grain yield per spike was due to an increase in grain number per spikelet particularly in the mid and lower spikelets of a spike (Mohamed and Marshall, 1979; Kemp and Whingwiri, 1980). Here, we found a substantial increase in main stem dry weight and spike chaff after tiller removal under both field and greenhouse conditions (Fig. S3.8), suggesting that the significant increase in spike chaff and main stem dry weight after detillering was responsible for the improvement of final grain number in the same environment.

Effects of tiller removal on visible floral degradation by alleviating competition between tillers and the main shoot, spike and stem

Siddique et al. (1989) found an increase in grain number could be attributed to less competition from stem development. It was also reported that floret abortion occurs when the spike grows at its maximum rate (Fischer and Stockman, 1980; Kirby, 1988; Gonzalez et al., 2011). Because tillers compete with the main shoot for resources, and competition between the stem and spike is critical for determining grain number, detillering makes more resources available to spike and stem growth on the main shoot which in turn can alleviate competition, thereby diverting more resources toward floral development (Fig. 3.9). Furthermore, allocation of more resources toward floral development can delay visible floral degradation resulting in one or two more floret primordia or final grains, respectively.

Regrettably, more available resources cannot retain all developed floret primordia, and more than half of them still abort. Nevertheless, better understanding the underlying developmental and resource-allocation dependent limiting factors may shed more light on how to increase wheat's yield potential in the future.

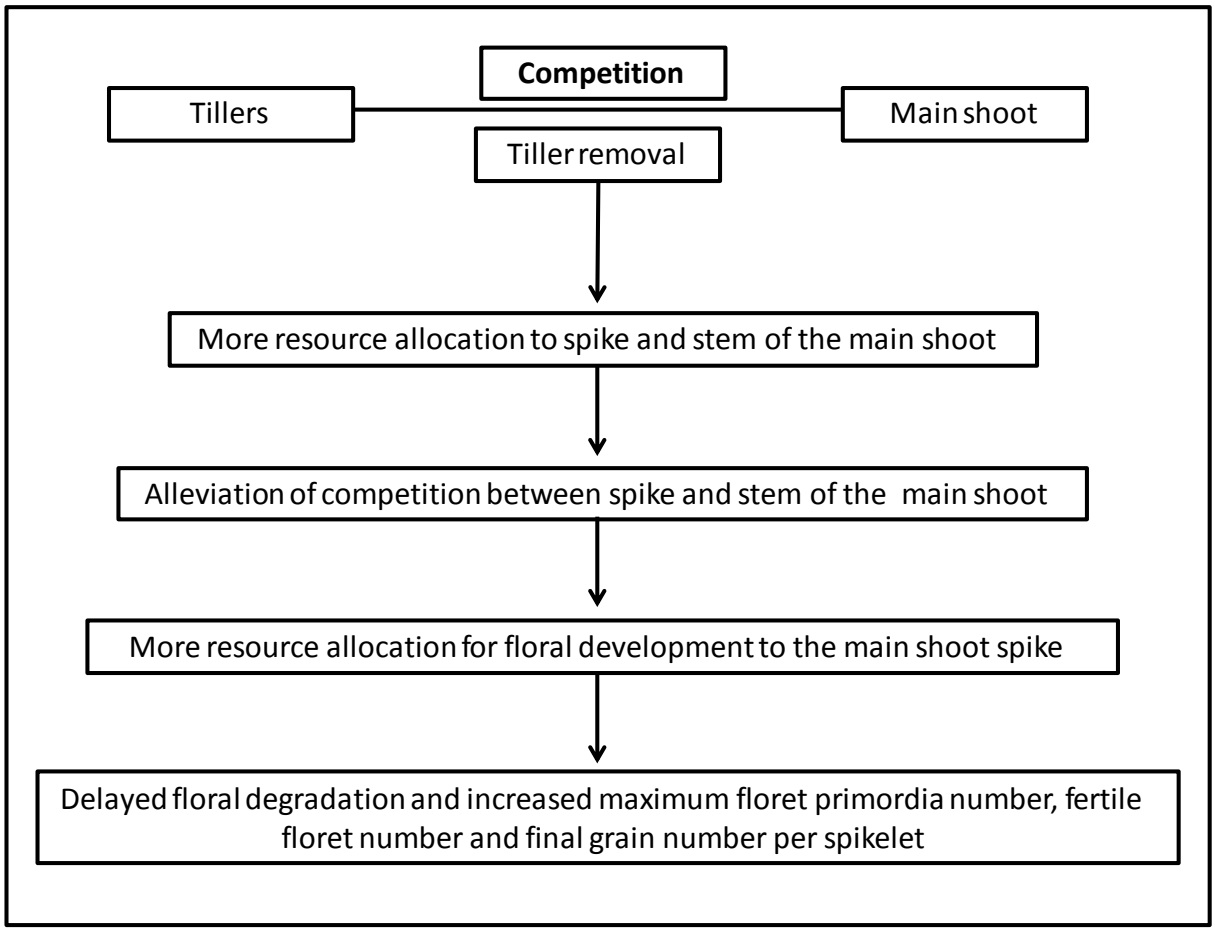


Fig. 3.9. Effects of tiller removal on floral degradation, the maximum floret primordia number, fertile floret number and final grain number by modifying competition between tillers and the main shoot, spike, and stem on main shoot.

The Green Revolution refers to the vast increases in grain yields after the 1960s, resulting from the introduction of dwarfing genes, known as *Reduced height* (*Rht*) genes (Peng et al., 1999; Hedden, 2003; Saville et al., 2012). Dwarfing genes markedly enhanced the partitioning of dry matter to spikes' growth in wheat (Abbate et al., 1998; Fischer, 2007;

Foulkes et al., 2011). The thought is that shorter stems compete less for limited assimilate with spikes (Fischer and Stockman, 1986; Bancal, 2008).

Breeding strategies have been discussed and a lot of factors which may influence wheat yield have been considered (Reynolds et al., 2009; Fischer, 2011; Foulkes et al., 2011). The general idea is to improve assimilate partitioning to growing spikes. Because grain yield is more dependent upon grain number than on grain weight, increasing available assimilates to spike growth is an important target for wheat breeding (Reynolds et al., 2009). Due to the wheat breeding efforts over the past decades, the spike: stem ratio has been greatly increased (Siddique et al., 1989; Slafer and Andrade, 1993) (Siddique and Whan, 1994). Hence, the floret and spikelet fertility related traits have undergone a great improvement, and significantly contributed to wheat's yield increase simply because more assimilates have been allocated to spikes (Fischer, 2011; Foulkes et al., 2011). To lengthen stem elongation has been widely hypothesized to be an alternative approach to increase the number of fertile florets. Previous studies showed that when the duration of stem elongation increased, under for example short photoperiod, the number of fertile florets was also increased due to the increased partitioning to the spike at anthesis (Slafer, 1996; Miralles et al., 2000) (Alqudah and Schnurbusch 2014). Our data suggests that delaying visible floral degradation may equally contribute to higher fertile floret numbers at anthesis. To which extent floral degradation is genetically or environmentally induced and independent to pre-anthesis phase durations remains to be shown.

Generally, around 6 to 12 floret primordia per spikelet are initiated across the different spikelet positions within the spike; however, not more than usually five to six fertile florets can be found at anthesis (Langer and Hanif, 1973; Kirby, 1974, 1988; Ferrante et al., 2010, 2013). This is consistent with our findings (Table S3.5, S3.6, S3.7, and S3.8). The number of

fertile florets at anthesis possibly depends upon two floral factors shaping the fate of apical floret primordia and more basal florets within a spikelet. One could be autophagy, regulated by day length or sugar supply, that might be involved in the abortion of apical floret primordia (Ghiglione et al., 2008). Consistently, Dreccer et al. (2014) found that wheat lines with high spike biomass and sugar content at booting can achieve more fertile florets. Whether autophagic processes are cause or consequence of apical floral abortion is not known. Development-dependent asynchronic floral progression between more basal florets (i.e. earliest florets) and younger apical primordia may lead to less time for further differentiation processes, such as vascularization, in apical primordia. Thus, we hypothesize that the vascular system within spikelets may limit the fertile floret number to maximally six. Consistent with our observation, Hanif and Langer (1972) also found that only the first six florets (F1-F6) at the bottom of a spikelet were sufficiently connected by the vascular system; there was no vascular connectivity after F6. They show that florets F1-F3 at the basal positions are supplied via the main vascular system from the rachilla; while the other three florets (F4-F6) share the branched-off veins from the main vasculature (Hanif and Langer, 1972). It seems that the final grain number per spikelet is predominantly associated with the connectivity of the main vascular system (F1-F3), while the fertile floret number is dependent upon the entirely developed vascular system (F4-F6). This observation should be further confirmed by studying the vascular system within spikelets.

4. Association between ovary size, MFS, FFS and GS

Genotypic variation in spike fertility traits and ovary size as determinants of floret and grain survival rate in wheat (Journal of Experimental Botany 67: 4221-4230)

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4.1. Abstract

Spike fertility traits are critical attributes for grain yield in wheat (*Triticum aestivum* L.). Here, we examine the genotypic variation in three important traits: maximum number of floret primordia, number of fertile florets, and number of grains. We determine their relationship in determining spike fertility in 30 genotypes grown under two contrasting conditions: field and greenhouse. The maximum number of floret primordia per spikelet (MFS), fertile florets per spikelet (FFS), and number of grains per spikelet (GS) not only exhibited large genotypic variation in both growth conditions and across all spikelet positions studied, but also displayed moderate levels of heritability. FFS was closely associated with floret survival and only weakly related to MFS. We also found that the post-anthesis process of grain set/abortion was important in determining genotypic variation in GS; an increase in GS was mainly associated with improved grain survival. Ovary size at anthesis was associated with both floret survival (pre-anthesis) and grain survival (post-anthesis), and was thus believed to 'connect' the two traits. In this work, proximal florets (i.e. the first three florets from the

base of a spikelet: F1, F2, and F3) produced fertile florets and set grains in most cases. The ovary size of more distal florets (F4 and beyond) seemed to act as a decisive factor for grain setting and effectively reflected pre-anthesis floret development. In both growth conditions, GS positively correlated with ovary size of florets in the distal position (F4), suggesting that assimilates allocated to distal florets may play a critical role in regulating grain set.

Key words: Fertile florets, floret abortion, fruiting efficiency, grain number.

4.2. Introduction

Raising wheat (*Triticum aestivum* L.) yield remains one of the main objectives of wheat breeding efforts (Dencic et al., 2000; Kirigwi et al., 2007; Edgerton, 2009; Shewry, 2009; Ainsworth and Ort, 2010). Because yield is a complex, multifactorial trait (Reynolds et al., 2009; Foulkes et al., 2011; Parry et al., 2011), continually achieving this aim has been become increasingly difficult (Reynolds et al., 2012). The better we understand the genetic factors determining yield the more likely are to effect we may be able to recover relatively large rates of genetic gains in yield (Slafer, 2003). Grain yield in wheat is commonly reported to be associated with grain number (Fischer, 2008; Lizana and Calderini, 2013), and seems impossible to achieve large gains in yield without increasing grain number is not further increased (Slafer et al., 2014). In this context, improving spike fertility seems critical (Foulkes et al., 2011; Reynolds et al., 2012). Most of what we know of spike fertility comes from studies analysing genetic and environmental factors that influence spike dry weight at anthesis (AN) and fruiting efficiency (FE) (Slafer et al., 2015). Although this approach has been extremely useful (Fischer, 2011), it considers only the final number of grains set, focusing on the final output of the reproductive biology process determining spike fertility.

An alternative/complementary approach to understand the grain number and spike fertility is to consider the complete process of reproductive biology that culminates in grain number (Fig. 4.1). First, a large number of floret primordia are initiated in each of the spikelets of the spike from approximately the terminal spikelet stage (TS) to around the stage of green anthers (GA) when the maximum number of floret primordia per spikelet (MFS) and per spike is frequently reached (Guo and Schnurbusch, 2015). Many (usually most) of the primordia do not continue to develop normally (that is, they die) and only a relatively small fraction of the initiated floret primordia survive to produce a number of fertile florets per spikelet (FFS) and per spike at AN. The ovaries of these florets are then fertilized and, during the lag-phase occurring immediately after AN, a proportion (variable, though normally small) of these fertilized ovaries abort and the rest set grains, thereby determining yield at maturity. Grain number is therefore the outcome of floret primordia initiation and survival during the stem elongation phase, which produces fertile florets at AN and grain setting immediately after AN (Slafer et al., 2015). The rate of floret survival is generally low (Miralles et al., 1998; Miralles et al., 1998; Ghiglione et al., 2008; Gonzalez et al., 2011; Ferrante et al., 2013); therefore, there might be great potential for improving grain yield in wheat by increasing the rate of floret survival (Sreenivasulu and Schnurbusch, 2012).

A widely accepted hypothesis supported by previous studies is that the variation in grain number is associated with changes in the availability of assimilates during the period of stem elongation when floret survival takes place (Siddique et al., 1989; Slafer and Andrade, 1993). Thus, wheat spikes over produce energetically inexpensive floret primordia and, when floret development requires increasing amounts of resources, the number of primordia that become fertile florets is adjusted to the actual assimilate availability (Sadras and Slafer, 2012). This seems in line with recent evidences that the loss of floret primordia (determining

the rate of floret survival) is resource-driven (Gonzalez et al., 2011; Ferrante et al., 2013). For instance, dwarfing genes have been found to increase spike fertility, due to reduced stem growth allowing for more assimilate translocation to the spike (Miralles et al., 1998). Similarly, a longer duration of stem elongation allowed greater assimilate translocation to spike growth increasing the FFS at AN in wheat (Gonzalez et al., 2003) and fertile spikelet in barley (Alqudah and Schnurbusch, 2014); likewise detillering plants allowing more resources to become available for the main shoot spike also increases FFS (Ferrante et al., 2013; Guo and Schnurbusch, 2015). Increased plant density reduces both the number of floret primordia initiated and floret survival, resulting in the typical agronomic reaction of reduced individual spike fertility in response to increased density. , A study analysing this issue in more detail found that a low red/far-red ratio (simulating dense stands) can reduce spike fertility due to a delayed spike growth and results in fewer floret primordia initiated and fewer fertile florets (Ugarte et al., 2010). High nitrogen levels seem to accelerate developmental rates of floret primordia by increasing spike growth (though not consistently altering phasic development; (Hall et al., 2014), allowing an increase in the number of fertile florets and in grain setting in durum wheat (Ferrante et al., 2010, 2013) and barley (Arisnabarreta and Miralles, 2010). Complementarily, shading treatments immediately before AN (during the period of floret mortality) significantly reduced spike fertility (Fischer and Stockman, 1980; Slafer and Savin, 1991); irrespective of the yield potential of the genotype (Slafer et al., 1994). High temperatures (up to 30 ^{o}C) during the pre-AN phase, especially from booting to AN, can result in a considerable reduction in the number of fertile florets at AN, possibly affecting sensitive stages of pollen meiosis (Saini et al., 1983, 1984; Dawson and Wardlaw, 1989), but also due to a reduction in assimilate availability for floret survival. The latter is evidenced by the fact that moderately high temperatures (not

damaging pollen viability) also reduce spike fertility by shortening the duration of stem elongation and consequently reducing assimilate availability per unit of developmental time (Fischer, 1985; Ugarte et al., 2007). High temperatures may also increase grain abortion (Prasad and Djanaguiraman, 2014).

Most of the few studies analysing the dynamics of floret development as determinant of grain number have focused on environmental effects. Very few have included genotypic variation, and when that variation was considered, the number of genotypes analysed was extremely low. Assuming a parallelism with the relationships uncovered with environmental effects (analogy that may not be strictly correct; (Slafer et al., 2014), it might be hypothesized that genetic differences in spike fertility are based on differences in floret survival (represented by the red dotted curves in the models on the right of Fig. 4.1). To the best of our knowledge, only Gonzalez-Navarro et al. (2015) analysed the dynamics of floret development with a reasonable number of genotypes and their conclusions provided preliminary support to the first part of this hypothesis (i.e. genotypic variation in fertile florets was more related to variation in floret survival than in maximum number of floret primordia; (Gonzalez-Navarro et al., 2015). Differences between genotypes must be uncovered and quantified for the selection of prospective parents in crosses aimed to further improve yield potential. Genotypes that can be readily used in breeding programmes aiming to increase yield potential (for which breeders pyramided genes during many generations) include virtually only elite material, like commercial cultivars. In this study we aimed to analyse genotypic variation in grain number by analysing not only the maximum number of floret primordia and floret survival to produce fertile florets but also grain abortion (i.e. failure of fertile florets to set grains) under two contrasting environmental conditions. We expanded considerably the genotypic variation explored so far by trialing 30

cultivars that showed large genotypic variation for thermal time (^{o}Cd), absolute growing time (days) and floral organ development (Guo et al., 2015).

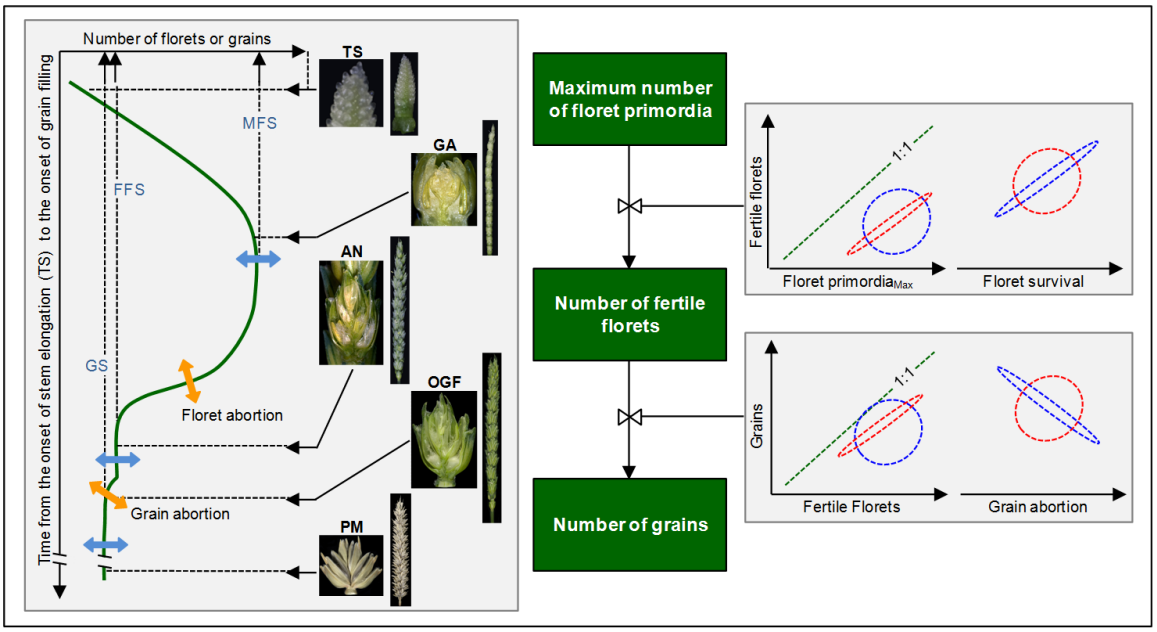


Fig. 4.1. Schematic diagram describing the determination of spike fertility in wheat. On the left part of the scheme the dynamics of floret generation/degeneration and grain set through different stages of development (TS: terminal spikelet; GA: green anther; AN: anthesis; OGF: onset of grain filling; PM: physiological maturity) is illustrated, with blue arrows indicating possible genotypic variation in the state variables and orange arrows standing for possible genotypic variation in the processes of floret survival and grain abortion (pictures displaying individual floret/grain and whole spike morphology at each stage are not in scale). On the right part a simple flow diagram in which the three critical state variables in the generation/degeneration of organs resulting in spike fertility (maximum number of floret primordia, number of fertile florets and number of grains) are linked with keys determined by the rates of processes of floret survival (top right) and grain abortion (bottom right): in the first key the differences in number of fertile florets may be the consequence of those in the maximum number of floret primordia (and the hypothetical relationships are illustrated in red) or in the level of floret survival (hypothetical relationships in blue); whilst in the second key the differences in number of grains may be the consequence of those in number of fertile florets (and the hypothetical relationships are illustrated in red) or in the level of grain abortion (hypothetical relationships in blue). Here, we used examples of two different regression trends between survived number (fertile florets and grains), survival (floret and grain survival) and abortion (floret and grain abortion).

4.3. Materials and methods

Plant material and growth conditions

Experiments were carried out at the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (51° 49′ 23″ N, 11° 17′ 13″ E, altitude 112 m) during the 2012/13 growing season in two contrasting environmental conditions: greenhouse and field. Thirty European hexaploid winter wheat cultivars were grown, including 23 photoperiod-sensitive and 7 photoperiod-insensitive cultivars. These genotypes can also be classified into 24 semi-dwarf and 6 tall cultivars. Marker information for all 30 cultivars is presented in Table S1. Forty plants per cultivar were planted in each of the growth conditions (a total of 2400 plants). Growth conditions for both experiments have previously been described (Guo et al. (2015); they matched in terms of temperature, day length, planting date and density.

Phenotypic staging and measurements

To determine the maximum number of floret primordia, plants around the GA stage (glumes cover all but the tips of florets; Kirby and Appleyard, 1987) were phenotyped (Guo and Schnurbusch, 2015). Every cultivar was examined every 2-3 days under a stereomicroscope (Stemi 2000-c, Carl Zeiss Micro Imaging GmbH, Göttingen, Germany). Samples were taken at AN, when yellow anthers were extruded and became visible in F1 and F2 florets. After the dissection of the floral developmental process, we confirmed the previous finding that the maximum number of floret primordia per spikelet (MFS) consistently occurred at around GA stage (Guo et al, 2015). The FFS and GS were obtained at AN and PM (physological maturity), respectively. Compared with fertile florets, aborted florets are dry and transparent. the clear distinction between fertile and aborted florets can be seen In Figure 4 of the previous work

(Guo and Schnurbusch, 2015). We defined fertile florets as those that reached the stage W10 of the Waddington scale as in a previous study (Ferrante et al., 2010).

Ideally, one should follow the stages of development of the selected 'fertile' florets until a few days after the most proximal florets are in W10, to make sure all the florets are fertile. Owing to the high number of genotypes and treatments in our study, this was not possible. As a compromise, we determined the number of fertile florets at AN (i.e. anthers extruded at F1 and F2), and then considered F3 and F4 to be 'fertile florets' if they were at that time in stages later than W8, as once florets get to stage W8 they can be considered irreversibly committed to producing a fertile floret (accepting that this is stage W10, a scale focused on carpel development), although it is possible for a fertile floret to abort after AN due to its size and/or its delayed condition. (In papers reporting the dynamics of floret development it is possible to see cases in which primordia may stop developing [and die] between W8 and W10, but it is only because the reported cases are averages of several florets measured for each particular treatment. Individually seen, it is virtually universal that florets developing beyond W8 would reach W10, even if few days later than AN of F1 and F2.) Even if a floret does not have viable pollen (male sterility), it can still be considered fertile if it reaches W10 because if it were fertilized with pollen from other florets it would produce a grain (and cross-pollination may be more likely with pollen from other florets).

Three plants from each cultivar were randomly selected for phenotypic measurements at GA and AN, and six plants at PM. The MFS, FFS, and GS were measured at three positions on the spikes from the main culms: apical (the third spikelet from the top), central (the spikelet in the centre of the spike), and basal positions (the third spikelet from the bottom) (Fig. 2). At AN, spikelets from the centre of the main culm spike were dissected to obtain digital images of ovaries of the first (F1), third (F3), and fourth (F4) florets from the base of the spikelet;

when the ovary was degenerated, the size was not measured. Ovary size was recorded as ovary width as indicated in Fig. 3. Ovary width was measured using a stereomicroscope and Carl Zeiss Imaging System version AxioVision Rel. 4.8.2. There are several reasons why we chose to examine florets in positions F1, F3, and F4. First, the ovary in the F1 position develops early and is stable; second, ovary size at F2 is similar to F1, so we expected similar results; finally, the F3 and F4 positions are important because these are most frequently the vulnerable floret primordia determining the final number of fertile florets. The spike and main culm were separately dried in two cellophane bags at 60°C for 3–5 days for dry weight measurement. Stem dry weight refers to the dry weight of one main shoot culm, including leaves but without spikes.

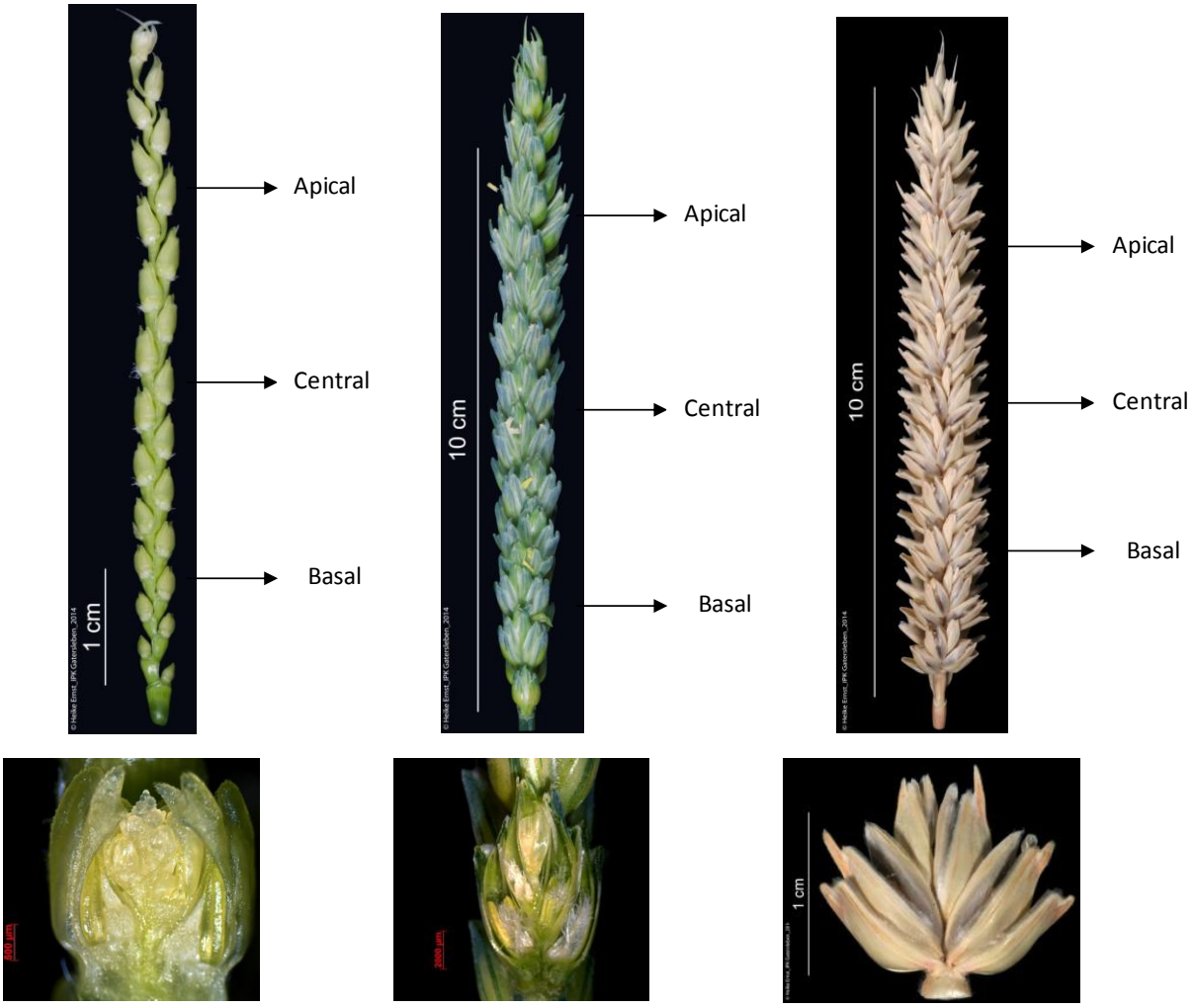


Fig. 4.2. The apical, central, and basal spikelets for the measurements of the maximum number of floret primordia per spikelet at GA stage, number of fertile florets per spikelet at AN, and number of final grains per spikelet at PM (Guo and Schnurbusch, 2015).

Floret survival was calculated as the proportion of the maximum number of floret primordia that reached the stage of fertile florets (FFS MFS^{-1}), and grain survival was determined as the proportion of the fertile floret number that set a normal grain (GS FFS^{-1}). Floret abortion was determined as the number of floret primordia that did not reach the stage of fertile florets, and grain abortion was calculated as the number of fertile florets that did not produce a normal grain. 'Normal grains' are defined as seeds that are completely developed, are not shrivelled, and have a size that is not particularly reduced.

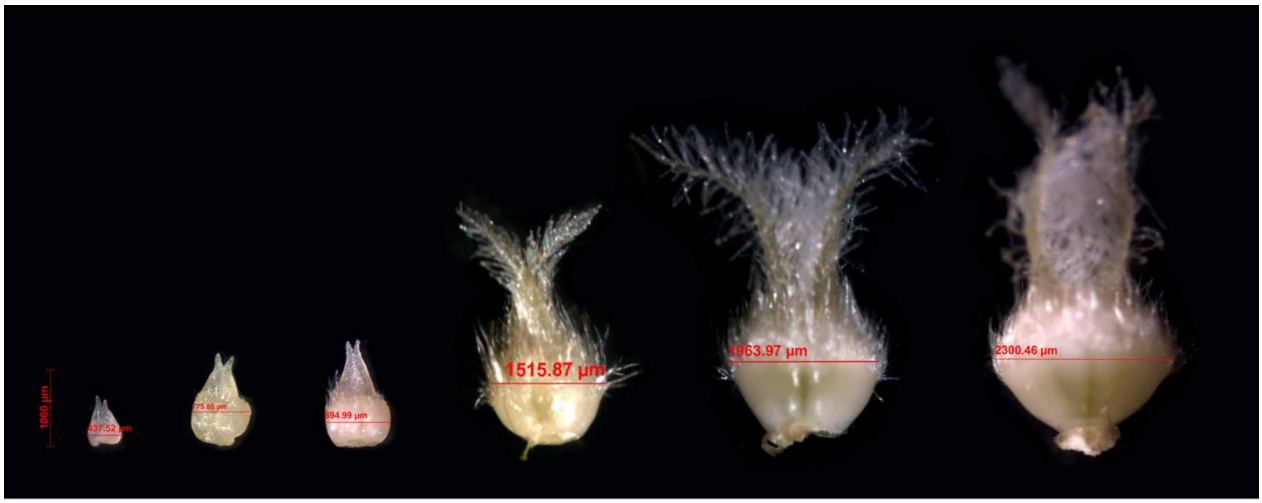


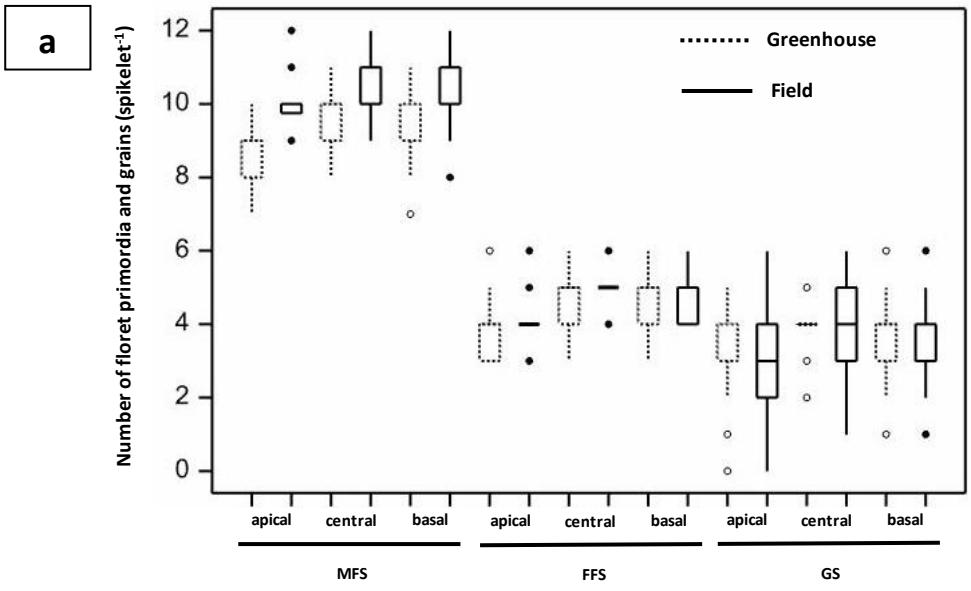
Fig. 4.3. Wheat ovary growth and development over time. This picture shows the development of ovary from early stages to anthesis and how ovary size was determined in this study. The first two ovaries are from F4 and F1 at green anther (GA) stage, the third one is from F3 at yellow anther (YA) stage (the stage was defined in (Guo and Schnurbusch, 2015), the fourth and fifth are from F3 and F1 at HD stage (the stage was defined in (Guo and Schnurbusch, 2015), the last one is from F1 at AN.

4.4. Results

Genotypic variation in, and stability of, floret fertility-related traits

MFS, FFS, and GS varied widely in both growth conditions and across all spikelet positions studied (Fig. 4.4a). Despite these strong variations, the ranges of the three traits at the apical, central, and basal spikelets of the spike were identical between all the genotypes and showed a reasonable degree of consistency between the two different growth conditions, as well as relatively high heritability (Fig. 4.4b). This indicates a common genetic base for the improvement of floret fertility and wheat breeding based on these traits. In addition, MFS displayed a high environmental sensitivity while GS showed large variation within individual genotypes (Fig. 4.4b). FE (here defined as grains set per unit chaff weight, i.e. the non-grain spike dry weight at physiological maturity) also displayed strong genotypic variation across the 30 genotypes, but there was no significant difference between greenhouse and field

conditions, indicating that FE is consistent across variable conditions (Fig. S4.1). The broad genotypic variation (Fig. S4.1) and relatively high heritability (Fig. 4.4b) indicate the large potential and a genetic basis for the increase in FE. Because the environmental effects (σ^2_E) and influence of the interaction between environment and genotype ($\sigma^2_{G:E}$) were large for most traits according to an ANOVA analysis (Fig. 4.4b), the regression values for most traits between greenhouse and field conditions were low (Fig. S4.2). In summary, there was a large degree of variation and also moderate to high heritability among the 30 cultivars analysed for all the traits determined; detailed data for individual genotypes are shown in supplementary Table S4.2, 4.3, 4.4, 4.5.



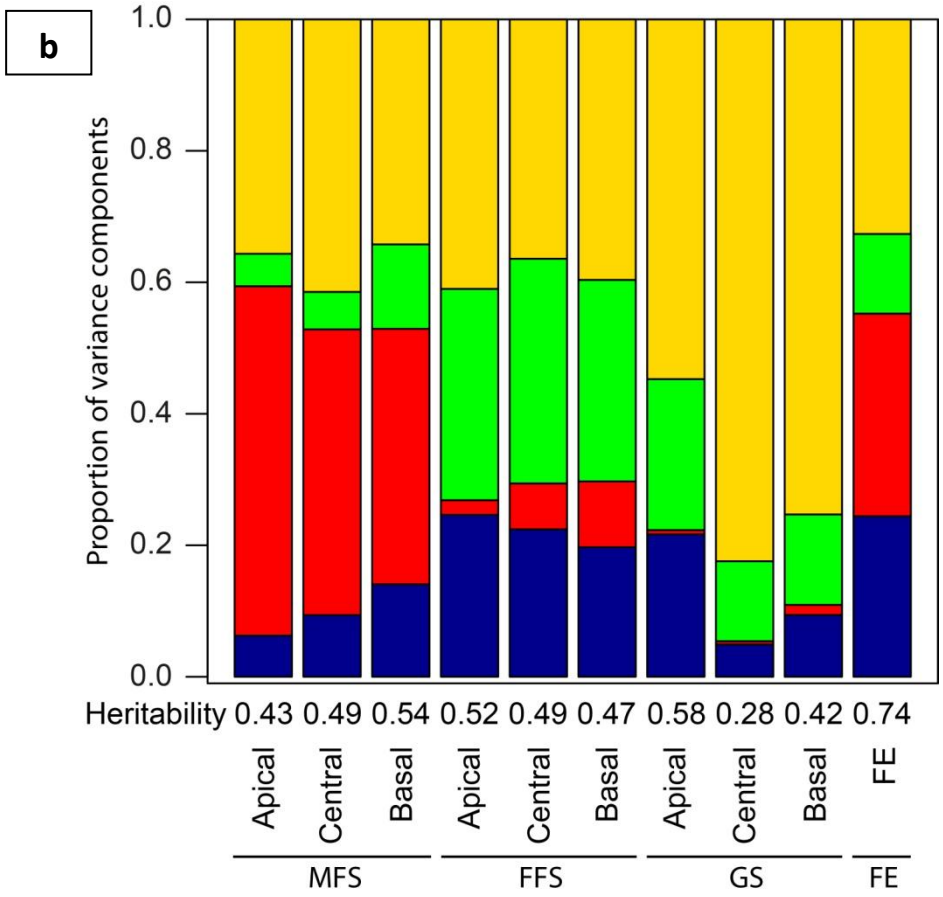


Fig. 4.4. Genotypic variation of the spike fertility traits. (a), range of maximum number of floret primordia (MFS), number of fertile florets (FFS), number of grains (GS) per spikelet in the apical, central and basal spikelets of the spikes (see Fig. 4.2) in two growth conditions. For a few positions within FFS and GS, the values are the same or identical, therefore they are displayed as a line (not a box). (b), proportions of variance components of MFS, FFS, GS and fruiting efficiency (FE). The numbers below Fig. 4.4b represent the broad sense heritability of the corresponding traits.

Importance of differences in MFS, floret abortion and floret survival in determining genotypic differences in FFS

Averaging across spikelets, it is quite clear that even though there was substantial phenotypic variation in MFS (average of the three spikelets), the genotypic differences in FFS (average of the three spikelets) were almost exclusively due to genotypic differences in the survival of floret primordia (average of the three spikelets) (Fig. 4.5a, b). This overwhelming importance of floret primordia survival compared with MFS as the main determinant of

genotypic differences in FFS was strongly consistent across the two contrasting environments (Fig. 4.5b). The fact that FFS was virtually unrelated to MFS was at least partly due to the fact that the larger the number of floret primordia initiated, the higher the tendency for primordia to be aborted (Fig. 4.5c). Part of the irrelevance of the differences in MFS between the 30 cultivars analysed in determining genotypic differences in FFS was due to the fact that there was no relationship between these two determinants (MFS and floret survival) of FFS (Fig. 4.5d), and that the relative variation in floret survival (~50% difference from <0.4 to ~0.55–0.6) was much higher than that in MFS (~20%, from 8–10 primordia in greenhouse or from 9–11 in the field). Analysing the results at the individual spikelet position level, the relationships between MFS and floret survival were weak in most cases (Fig. S4.3a, b, c), although there were a number of exceptions (although in these exceptions the relationships were significant, the values of R^2 were small).

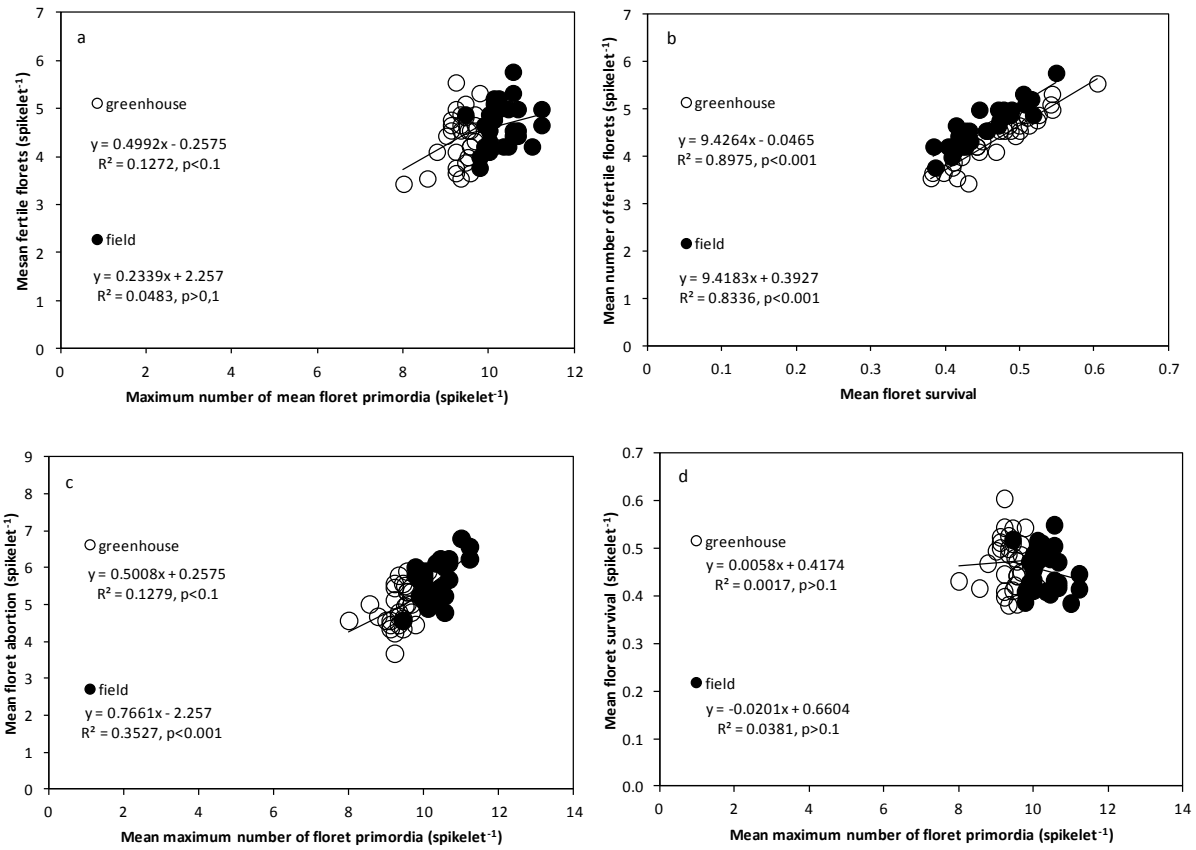


Fig. 4.5. Relationship between mean maximum number of floret primordia, mean number of fertile florets, mean floret survival and mean number of floret abortion within spikelet under field and greenhouse conditions, all the traits are displayed by averages of the apical, central and basal spikelets.

We found compelling evidence for the relative importance of floret survival compared with MFS in determining genotypic differences in FFS among modern cultivars after analysing the relationships for each spikelet position independently. At each particular spikelet position, and in both growth conditions, FFS was strongly significantly related to the proportion of primordia surviving (Fig. S4.4a, b, c) and unrelated to MFS (Fig. S4.4d, e, f). Again, at each spikelet position, it was evident that differences in MFS significantly induced parallel differences in floret abortion (Fig. S4.4g, h, i). While the relationship between FFS and floret survival was highly significant for all three spikelet positions in both field and greenhouse conditions (R^2 ranging between 0.76 and 0.89; $P < 0.001$ in all six cases), the relationship

between FFS and MFS was only significant in three of the six cases analysed. Even in those in which it was significant, R^2 was very small (MFS never explained more than 18% of the variation in FFS).

Importance of differences in FFS and grain abortion in determining genotypic differences in GS

Consistent across both environments, genotypic differences in GS were positively related to those in FFS (Fig. 4.6a). However, even though R^2 was statistically significant, the proportion of the variation explained was moderate. This was because GS was also related to the likelihood of a fertile floret to set a grain (Fig. 4.6b). In general, genotypes producing more FFS presented higher grain abortion (Fig. 4.6c) and lower grain survival (Fig. 4.6d), though the absolute differences in FFS overrode those in grain abortion.

Analysing the results at the individual spikelet position level showed that the relationships between GS and grain abortion were maintained (Fig. S4.5a, b, c), while those between GS and FFS were maintained in the greenhouse experiment but disappeared in apical and central spikelets (and were maintained only in basal spikelets) in the field experiment (Fig. S4.5e, f, g). The positive relationship (with slopes consistently smaller than 1) was also maintained between grain abortion and FFS at the spikelet level position (Fig. S4.5h, i, j).

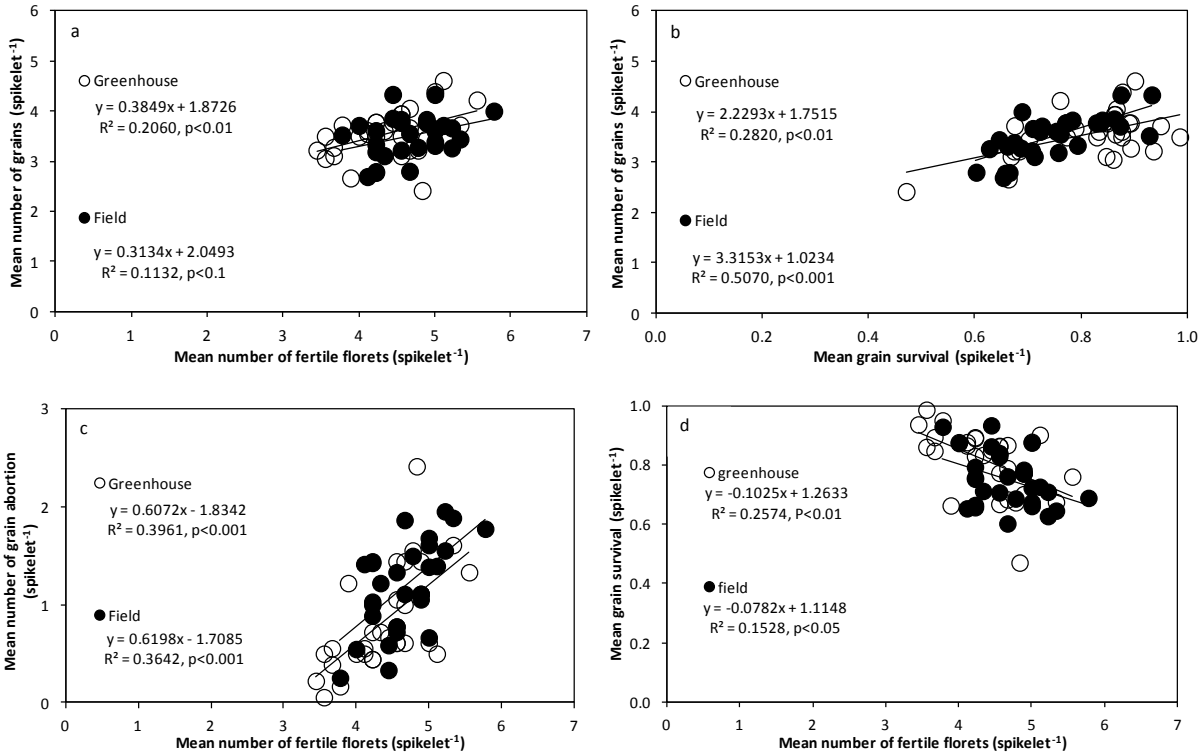


Fig. 4.6. Relationship between mean number of fertile florets, mean number of grains and grain abortion per spikelet under field and greenhouse conditions, all the traits are displayed by averages of the apical, central and basal spikelets.

Genotypic variation in spike fertility and size of the ovaries

Proximal florets became fertile in almost all spikelets and therefore differences in spike fertility depend on the likelihood of distal florets surviving to produce a fertile floret at, and set a grain immediately after, AN. It therefore seems relevant to determine the dependence/independence of phenotypic differences in spike fertility and the size of ovaries in distal florets. In this study, we measured ovary sizes of proximal florets and distal floret F4. In the greenhouse experiment, ovary size of F4 was related to FFS (Fig. 4.7a, open symbols), implying that in this condition cultivars with more resource allocation for growth to distal florets presented higher levels of spike fertility. However, in the field experiments (in which the size of ovaries was larger than in the greenhouse), there was only a weak relationship (Fig. 4.7a, closed symbols). In both field and greenhouse conditions, the number

of grains per spike was related to ovary sizes of distal florets at AN (Fig. 4.7b), suggesting that floret fertility was improved through the generation of more fertile distal florets. Distal florets with smaller ovaries were usually more prone to abortion, which in turn may have resulted in fewer grains being produced; this trend occurred in both environments (Fig. 4.7c, d) although it was statistically significant only in the greenhouse experiment (Fig. 4.7c).

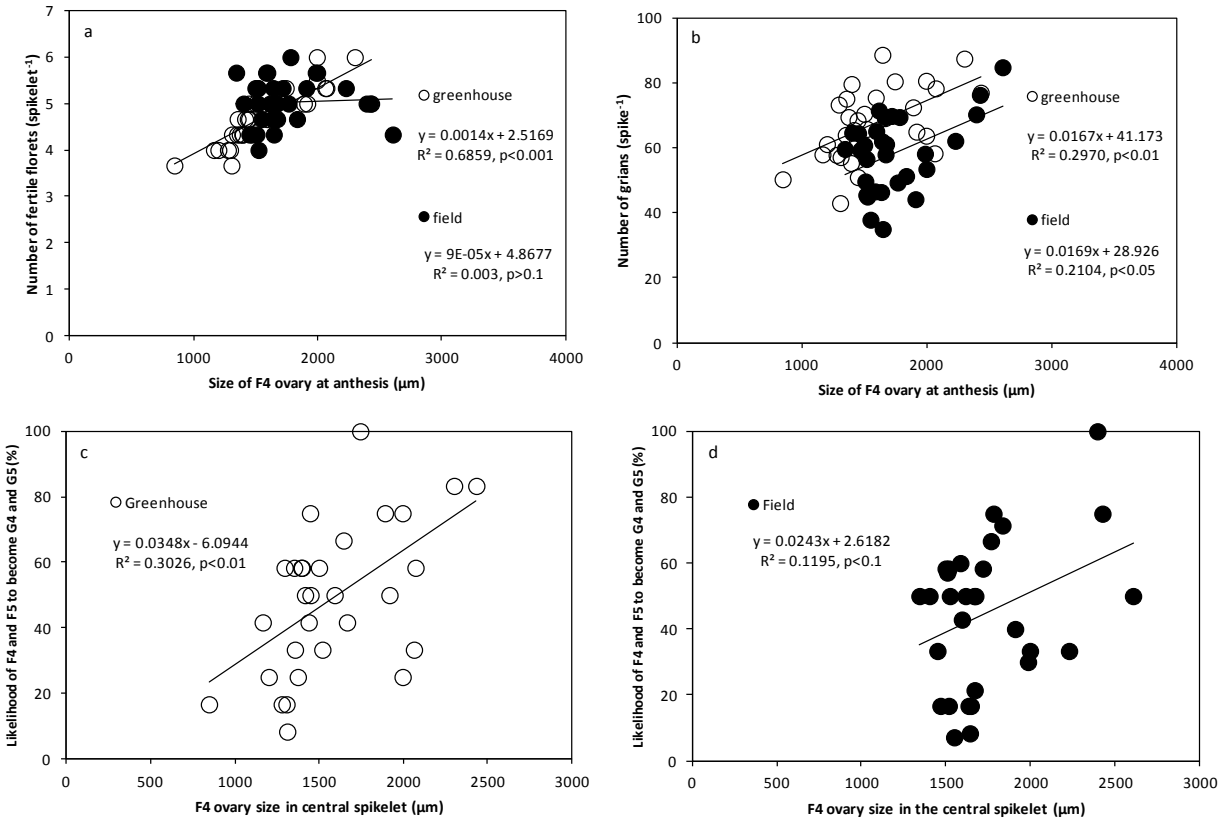


Fig. 4.7. Relationship between F4 (floret 4, the forth floret from the base of spikelet) ovary size in central spikelet and under field and greenhouse conditions. In Fig. 4.7a, b, we displayed the association between ovary size and number of fertile florets and grains, which can connect the pre-anthesis process (fertile florets) and post-anthesis (grains). In Fig. 4.7c, d, likelihood of F4 and F5 to become G4 (Grain 4, the forth grain from the base of spikelet) and G5 is considered as totally 100% (F4-G4, 0-50%; F5-G5, 51-100%). In our previous publication, we found that there are close relationships between ovary size at different positions (Guo et al., 2015). Hence, even though we did not measure ovary size at F5 position (generally it is too small to be measured), F4 ovary size also includes the likelihood of grain setting at F5 position. In Fig.4.7, if the likelihood is above 50%, it means that the F5 is also likely to set grain.

4.5. Discussion

In this study, we illustrated genotypic variation in floret development traits determining spike fertility, and presented relationships between them, in two contrasting environments (field and greenhouse). The analytical framework included three parameters in the dynamics of generation/degeneration of organs determining spike fertility: MFS, FFS, and GS. The MFS is the consequence of floret primordia initiation starting around TS stage and finishing approximately at GA; the FFS is the outcome of the floret primordia mortality/survival process occurring broadly from GA to AN; and GS is the result of grain set/abortion taking place in the 'lag phase' of roughly 7–10 days after AN to the onset of grain growth. Here, we not only quantified the outcomes of floret initiation, floret mortality/survival, and grain set/abortion in 30 modern cultivars (this analysis comprising for the first time such a large set of genotypes), but also tried, for the first time, to connect parts of these processes by describing the association between ovary size of distal florets with the number of fertile florets and grains. Interestingly, MFS, FFS, and GS exhibited not only large variation in both growth conditions and across all spikelet positions studied, but also displayed moderate levels of heritability. It has previously been reported that ovary size has high heritability (Komaki and Tsunewaki, 1981; Guo et al., 2015). Understanding that these spike fertility traits have moderate heritabilities will be of use in further exploring their genetic basis in experimental populations.

In agreement with most agronomic literature, we found that floret initiation was far less relevant than the subsequent process of primordia degeneration to determine the number of fertile florets: FFS was closely associated with floret survival, but had a weak relationship with MFS. This association is consistent with previous work in wheat (Miralles et al., 1998; Gonzalez et al., 2011; Ferrante et al., 2013). Gonzalez et al. (2005) reported that a period of

extended floret development, due to exposure to a short photoperiod during the stem elongation period, can increase the number of fertile florets at AN by improved floret survival while not influencing the maximum number of floret primordia initiated.

Furthermore, we also found that the post-AN process of grain set/abortion is also important for determining genotypic variation in grain number, although it was previously concluded that the number of grains per spike is mainly a consequence of floret initiation and degradation (fertile florets) (Kirby, 1988). Here we showed that the increase in GS was closely associated with improved grain survival. If spikelet positions along the spike are considered, it becomes clear that the central part of the spike dominates to produce fertile florets and to set grains (Rawson and Evans, 1970; Evans et al., 1972; Pinthus and Millet, 1978; Millet, 1986). In our work, the relationship between GS and grain abortion after AN was maintained, whereas associations between GS and FFS were maintained in the greenhouse experiment but disappeared in apical and central spikelets (and were maintained only in basal spikelets, 'basal' here being the low central spikelets of the spike, not the extreme bottom spikelets) in the field experiment. These variable correlations indicate that spikelets at different positions of the spike possess variable sensitivities to growth conditions. Hence, the effects of spikelet positions along spikes should be considered in further studies of spike fertility.

Ovary size at AN is the result of floret initiation and degradation, and represents a possible predictor for grain setting (Guo et al., 2015), so we consider it as the 'connector' between floret survival (pre-AN) and grain survival (post-AN). In this work, proximal florets (i.e. the first three florets from the base of a spikelet, F1, F2, and F3) did produce fertile florets and set grains in most cases. Ovary sizes of more distal florets (F4 and beyond) appeared to be a critical factor for grain setting and also effectively reflected the pre-AN floret development.

In both environments, GS displayed positive correlations with ovary sizes of distal florets (≥F4), suggesting a critical role of photoassimilates partitioned to distal florets in regulating grain set (although environmental factors that are involved in anther and ovary abortion should not be neglected). In other words, improving floret fertility at AN may be irrelevant when more resources are not allocated to the growth of these florets, because the small florets, albeit fertile, would become abortive even if after pollination (post-AN grain abortion). This is in agreement with previous findings where access to assimilates plays a crucial role for spike fertility (Gonzalez et al., 2011; Guo and Schnurbusch, 2015). Interestingly, we only found a positive association between ovary size of F4 and FFS in the greenhouse condition. The fact that the development of distal florets is relatively sensitive to environments (Guo et al., 2015) could be helpful in explaining the weak correlations found in the field. Besides the close correlation between ovary size and grain setting, one reason for highlighting the importance of ovary size is that the effects of some other factors such as assimilate allocation and anther size are reflected by ovary size. For example, anther size is closely associated with ovary size (Guo et al., 2015). In addition, increasing FE (i.e. grain set per unit of spike dry weight at AN) is another way to improve grain setting. Slafer et al. (2015) suggested two pathways to improve FE: (1) by increasing assimilate partitioning to floret primordia during pre-AN development, or (2) by reducing assimilate demand for floret development. The first option may lead to an increase in fertile florets without penalizing ovary size at AN (or to an increase in ovary size without penalizing the number of fertile florets); in option 2, the increase in number of fertile florets associated with higher FE would be achieved at the expense of reducing ovary size (because the amount of assimilates being consumed per developing primordium is decreased). Our data support option 1 because in the field we found an increase in spike dry weight that was associated with bigger ovary size

(Guo et al., 2015), which most likely was due to a longer duration (longer growth time, by days) of pre-AN phases. In a concurrent study, Elia et al (2016) also found that differences in FE between modern cultivars did not reflect penalties in ovary size when the number of fertile florets was increased. It was also detected that the maximum number of floret primordia under field conditions was generally increased compared with greenhouse conditions, further suggesting that a longer duration from floret primordia initiation to GA stage increases floret primordia in the field (Guo et al., 2015).

The observed non-grain setting even in F1 and F2 under 'ideal conditions' suggests other limiting factors for grain setting. For example, some stresses around meiosis (e.g. drought, B deficiency) can reduce grain set in these favoured positions, giving obvious spike 'sterility' despite no obvious growth limitations (Huang et al., 2000; Ji et al., 2010). Rawson and Evans (1970) found that F1 and F2 can inhibit grain formation in F3 and F4 even though the latter is perfectly competent to form grains. Clearly, spike fertility is a complex trait and displays strong genotypic variation; however, this study found ovary size to be a potentially promising factor in determining overall spike fertility.

5. Genetic relationships between floret fertility, assimilate partitioning and spike morphology

Genome-wide association studies of 54 traits identified multiple loci for the determination of spike fertility in wheat (New Phytologist 214: 257-270)

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5.1. Abstract

Increasing grain yield is still the main target of wheat breeding; yet today's wheat plants utilize less than half of their potential yield. Due to the difficulty of determining grain yield potential in a large population, no genetic factors regulating floret fertility (i.e. the difference between grain yield potential and grain number) have been reported so far. Here, we conducted a genome-wide association study (GWAS) by quantifying 54 traits (16 floret fertility traits and 38 traits for assimilate partitioning and spike morphology) in 210 European winter wheat accessions and detected shared Quantitative Trait Loci (QTLs) suggesting potential associations between floret fertility, assimilate partitioning and spike morphology. Several candidate genes involved in carbohydrate metabolism, phytohormones or floral development co-localized with such QTLs, thereby providing targets for selection. Based on our GWAS results we finally propose a genetic network underlying floret fertility and related traits, nominating determinants for improved yield performance.

Keywords: assimilates distribution, grain number, GWAS, QTL, spike fertility

5.2. Introduction

Wheat (*Triticum aestivum* L.*)* is one of the most widely planted crops worldwide (FAO, 2016). Increasing the grain yield remains the main goal of wheat breeding. Grain number is one crucial trait for determining the grain yield, and floret fertility plays an important role in the determination of grain number. Floret fertility is governed by the allocation of assimilates to spikes and the distribution of assimilates within spikes. The introduction of dwarfing genes, known as *Reduced height* (*Rht*) genes, has been the most successful example in the past decades. *Rht* genes improve grain yields by increasing the allocation of assimilates to the spike, which further modifies intra-spike partitioning (Peng et al., 1999; Khush, 2001; Hedden, 2003). It is thus a prerequisite to learn more about assimilate supply of grains; furthermore, it is also necessary to learn about the critical traits and genes regulating assimilate distribution. Here, we aimed to better understand assimilate partitioning by assessing five patterns of dry weight distribution (only considering above-ground parts). (1) Tiller-to-main shoot; the relationship between tillers and the main shoot has been studied historically. Tillering is a determinant of grain yield in many crops because tiller number is key to regulate competition for assimilates between tillers and main shoot. Many studies showing the control of tillering in different species have been performed (Li et al., 2003; Aguilar-Martinez et al., 2007; Crawford et al., 2010; Kebrom et al., 2012; Tavakol et al., 2015). (2) Spike-to-stem; the introduction of *Rht* genes has greatly alleviated competition for assimilates between spikes and stems (Austin et al., 1980; Sreenivasulu and Schnurbusch, 2012; Chandler and Harding, 2013). (3) Spikelet-to-spikelet within a spike; an unbalanced distribution of grains per spikelet along the spike (top, center, and bottom of a spike) has been widely reported (Ferrante et al., 2013; Guo and Schnurbusch, 2015). Spikelet fertility (i.e. ratio between fertile spikelet number and total spikelet number) can be used to explore

the competition for assimilates between spikelets in a spike; however, few genetic studies have been carried out to better understand and alleviate this competition. (4) Floret-to-floret within individual spikelets; it was hypothesized that competition for assimilates between florets possibly causes a large proportion of the loss in grain number (floral degeneration) (Guo and Schnurbusch, 2015). Until now, however, there is only little evidence for suggesting a mechanism of how floral degeneration occurs; nevertheless previous studies suggested possible avenues (Wang et al., 2001; Ghiglione et al., 2008). Finally, (**5**) grain-to-spike chaff; the spike fertility index (i.e. the ratio between grain number per spike and weight of spike chaff) is a critical indicator of spike dry weight distribution between grains and spike chaff, and many studies have been carried out on this factor (Foulkes et al., 2011; Gonzalez et al., 2011; Isidro et al., 2011; Reynolds et al., 2012). However, more genetic studies are still required to disclose the relationship between grains and spike chaff.

In this study, we systematically phenotyped 54 agronomic and fertility-related traits exhibiting assimilate distribution, and displayed their phenotypic and genotypic associations among them. By performing a GWAS on all 54 traits, we identified novel QTLs that are involved in assimilate partitioning, floret fertility, spike morphology and ultimately in the determination of grain number.

5.3. Materials and methods

Plant materials and growth conditions

Experiments were carried out at the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (51° 49′ 23″ N, 11° 17′ 13″ E, altitude 112 m) in 2014 and 2015 under greenhouse conditions. A total of 210 German hexaploid winter wheat cultivars

were selected based on *Rht* and photoperiod (*Ppd)* genes (Data S5.1). Forty plants were planted for each cultivar. The seeds were sown in 96-well trays on the same date and germinated under greenhouse conditions (photoperiod, 16 h/8 h, light/dark; temperature, 20°C/16°C, light/dark) for 14 days. Seedlings at the two- to three-leaf stage were transferred to 4°C to vernalize for 63 days. The vernalized seedlings were transferred to a hardening stage (photoperiod, 12 h/12 h, light/dark; temperature, 15°C) for 7 days to gradually acclimatize. Finally, all the plants were transplanted into 0.5 L pots (one plant per pot; 9×9×9 cm) under greenhouse conditions (photoperiod, 16 h/8 h, light/dark; temperature, 20°C/6°C, light/dark). Supplemental light (approximately 250 μmol m^{-2} s^{-1} photosynthetically active radiation) was supplied with low-intensity incandescent light and plants were irrigated when required.

Twelve German hexaploid spring wheat cultivars were selected according to their years of release (Table S5.1) for detillering experiment. Control and tiller removal experiments were conducted in the field and greenhouse simultaneously. Tillers were removed continuously every two to three days from the bottom of the plants, when tillers started to appear. Eighty plants per cultivar (forty plants for control and the other forty plants for tiller removal) were planted under field and greenhouse conditions.

Phenotyping details

The thermal time for anthesis was calculated as the sum of the daily average temperature [(Tmax + Tmin)/2] (the base temperature was assumed to be 0°C) from potting in the greenhouse. The maximum number of floret primordia was determined at the green anther stage according to dissection of the plants at seven floral developmental stages in previous work (Guo and Schnurbusch, 2015). The main shoots of three plants for each cultivar were

randomly selected to measure the maximum number of floret primordia per spikelet, while the main shoots of six plants were used for determining the grain number per spikelet at the following three positions: A (the third spikelet from the top of spike), C (the spikelet in the center of the spike), and B (the third spikelet from the bottom of the spike) (Fig. 5.2). It should be noted that A and B spikelets of the spike in this experiment were high-middle and low-middle positions of the spike and did not include the extreme A and B spikelets at the top and bottom of the spike. The leaf area of the main stem at the GA and AN stages was measured immediately after dissection of fresh leaf material using an area meter (LI-3100, LI-COR Ltd., Nebraska, USA).

The spike length (cm), spike chaff per spike (g), spike dry weight (g), main stem dry weight (g), grain weight per spike (g), grain number per spike, and total and fertile spikelet numbers were measured at physiological maturity. Six plants for each cultivar were randomly selected for trait measurements. The spike length was measured without the awn. The main stem was measured based on the weight of the main shoot without spikes but including leaves. The spike chaff refers to the rachis with empty spikelets after removal of grains. In addition, final grain number per spikelet was measured in spikelets at three positions of the spike: at the A (the third spikelet from the top of the spike), C (the spikelet in center of the spike), and B (the third spikelet from the bottom of the spike) positions. The spike fertility index, spikelet density, and fertility were determined based on spikelet number per centimeter of spike length, grain number (per spike) per gram of spike chaff (per spike) at harvest (Gonzalez et al., 2011), and the ratio of the fertile and total spikelet numbers. It should be noted that the basal spikelets of the spikes examined in this experiment were at a low-middle position on the spike and did not include the extreme basal spikelets at the bottom of the spike. Infertile spikelets were defined as spikelets that did not set any grain (i.e., were

completely empty), whereas fertile spikelets produced at least one grain. The grain width, length, area, and TKW were determined with a digital seed analyzer/counter MARVIN (GTA Sensorik GmbH, Neubrandenburg, Germany).

Population structure analyses

Principal component analysis plots were used to infer the structure of the wheat population. The hierarchical population structure was estimated with the ADMIXTURE program (Alexander et al., 2009), a model-based estimation of ancestry in unrelated individuals using the maximum-likelihood method. A recently adapted and widely used approach to assess the genetic differences/similarities between samples in large multilocus genotyping panels is to use structure-like analysis (Biswas et al., 2009). This increasingly diverse set of Bayesian (Patterson et al., 2006; Tishkoff et al., 2009) or maximum likelihood (ML) methods (Alexander et al., 2009; Weiss and Long, 2009) share a common principle in which population structure is inferred as differential membership of individuals in a specified number (K) of hypothetical ancestral populations (genetic clusters) characterized by ML estimates for allele frequencies at each loci. The term 'ancestry', as inferred from structure-like analyses used in the current study, refers to genetic relatedness. 'Ancestry' should be considered in terms of genetic 'similarity' and 'dissimilarity' irrespective of its genesis and does not reflect phylogenetic history, although it mirrors it. Of the possible ML approaches, we chose the algorithm that was assembled in the program Admixture (Alexander et al., 2009). This algorithm is fast and accurate due to a stringent convergence criterion.

Genome-wide association analyses

Only SNPs with minor allele frequencies ≥0.05 were used to carry out the GWAS. We conducted GWA mapping using four models based on 8,000 SNPs for the wheat population. First, the naive model used in this study referred to linear regression for continuous quantitative traits. Then, we used different approaches to detect and correct the population structure. Although it has been argued that a random effect in a linear mixed model (LMM) using the kinship (K) matrix would be sufficient for correction of cryptic relatedness and control of spurious associations, we also observed lower genomic inflation factors when we corrected additionally for the main directions of population structure by regressing on the several top principal components of the SNP information (e.g., Q5 and Q10 means Q matrix based on five and ten principal components). We performed the GWAS using linear regression, the K model, K+Q5 model, and K+Q10 model. Bonferroni's correction has been validated and is rather effective for the control of false positives (Benjamini and Hochberg, 1995). In this study, the genome-wide significance thresholds of the GWAS were determined using a Bonferroni's correction, and the calculated genome-wide significance threshold was 3.89 ($P = 1.30 \times 10^{-4}$) for the whole population.

Phenotype statistical analyses

The repeatabilities (W^2) were calculated as $W^2=\sigma^2_g/(\sigma^2_g+\sigma^2_e/r)$, where r is the number of field replications. Genotypic variance σ^2_g was estimated by restricted maximum likelihood (REML), assuming that $g_i=N(0, \sigma^2_g)$. REML-based additive estimates of heritabilities (H^2) were calculated using the function polygenic_hglm of GenABEL , assuming random genotype effects with a kinship matrix of proportion of shared SNP alleles as a variance-covariance matrix. *P*-values for Pearson's correlation coefficients were calculated with a two-sided *t*-test

using the cor.test() function in R. The best linear unbiased predictors (BLUPs) for genetic values were calculated using the mixed.solve() function in the R package. *P*-values for genetic correlation coefficients between BLUPs were calculated with a two-sided *t*-test using the cor.test() function in R.

We assessed the overall consistency for the phenotypic and genotypic correlations between different traits with a Mantel test. The Mantel test is a statistical test of the correlation between two matrices. The matrices must be of the same rank; in most applications, they are matrices of interrelations between the same vectors of objects. The test was first published by Nathan Mantel, a biostatistician at the National Institutes of Health, in 1967 (Mantel, 1967), and has been widely used in population genetics (Sokal, 1979; Smouse et al., 1986; Perez et al., 2010; Diniz et al., 2013).

5.4. Results

Association of floret fertility, assimilate partitioning and spike morphology traits

We measured 54 traits (above-ground part of plants, Table 5.1) according to the assimilate distribution (Fig. 5.1) and grouped these traits into seven clusters. The assimilate distribution between the tiller and main shoot, stem and spike, spikelet-spikelet, floret-floret, and spike chaff and grain yield formed five of the clusters. In addition, there were two other groups, i.e., harvest index-related and grain size-related traits (Table 5.1). For example, the tiller number, tiller dry weight (DW), main shoot DW and the ratio of tiller DW/main shoot DW were considered to describe the assimilate distribution between the tiller and main shoot. The harvest index is determined by the ratio between the grain number per plant and plant biomass, so the grain number per plant, plant biomass and the ratio of grain number per plant/plant biomass were clustered into group 6 (harvest index related traits), and grain size

is described by the seed length, width, area and thousand kernel weight (TKW). The number of traits per group was eight (group 1), four (group 2), five (group 3), 16 (group 4), five (group 5), four (group 6) and 12 (group 7; Table 1). We assessed the assimilate distribution between different parts of the wheat plant (above-ground only) and further developed a strategy for increasing the grain yield by regulating assimilate partitioning to floret/grain development. Some of the 54 traits also affect spike morphology (e.g. spike length, spikelet density) and floret fertility (e.g. the traits in the group C4).

Table 5.1. Traits for the different groups based on the assimilate distribution.

Tiller-main shoot (C1)	Stem-spike (C2)	Spikelet-spikelet (C3)	Floret-floret (C4)	Spike chaff-grain yield (C5)	Harvest index (C6)	Grain size (C7)
tiller number, DW	spike DW	fertile spikelet number	max floret A,C,B,average	grain number/spike (main shoot)	grain number/plant	TKW (tiller, main shoot, entire plant)
main DW	main stem DW	total spikelet number	floret primordia loss A, C, B, average	spike chaff	grain weight/plant	seed length (tiller, main shoot, entire plant)
grain number/spike (tiller)	spike DW/main stem DW	spikelet fertility	grain A,C,B,average survival A, C,B,	spike fertility index grain weight/spike	plant DW	seed width (tiller, main shoot, entire plant) seed area (tiller, main
grain weight/spike (tiller)	stem length	spike length	average	(main shoot)	harvest index	shoot, entire plant)
leaf number, DW		spikelet density		grain weight/spike chaff		
tiller DW/main DW						

Note: DW means dry weight; A and B indicate the third spikelet from the top (Apical) and bottom (Base) of the spike while C (Central) indicates spikelets from the middle of the spike; TKW is thousand kernel weight. All data are from the main shoot, except data which have been named by tiller. The traits in C4 group mean the numbers within one spikelet, e.g. grain A means "grain number per spikelet in the apical position of the spike".

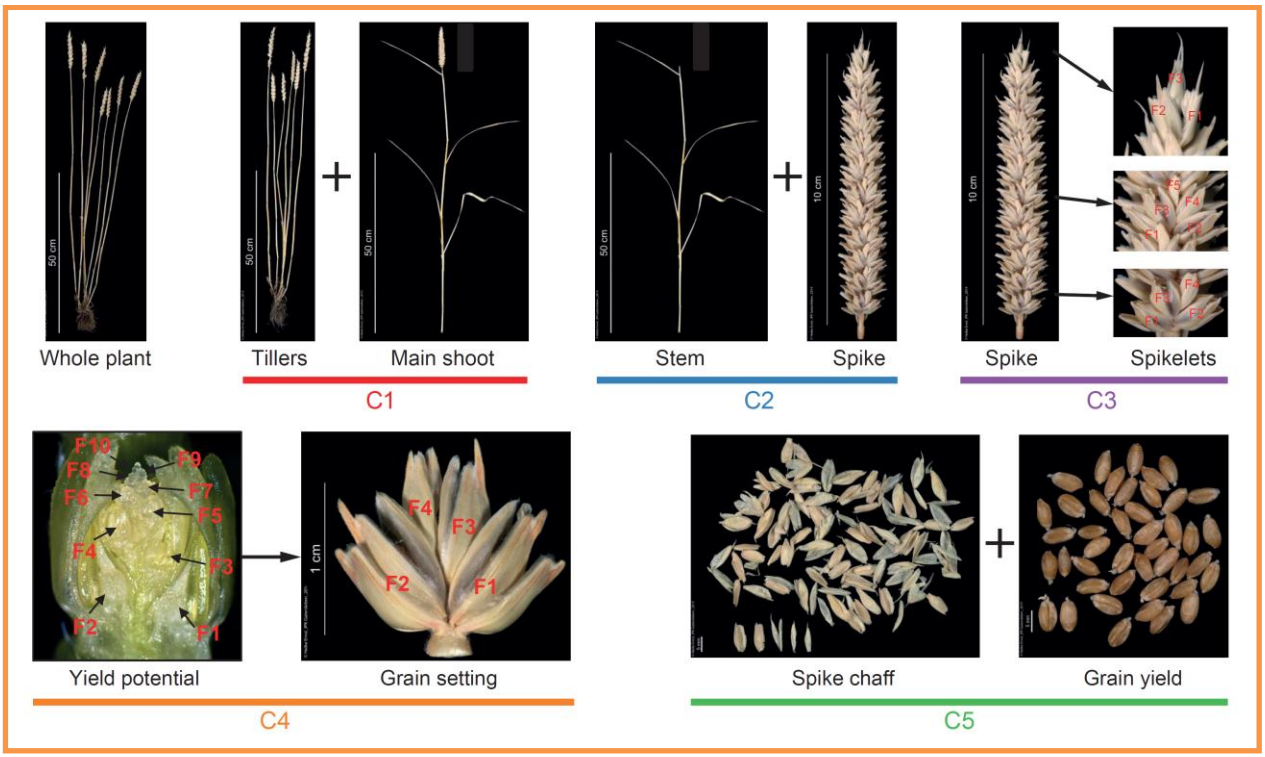


Fig. 5.1. The five clusters of assimilate partitioning across the entire plant (above-ground parts) in wheat. This figure displays assimilate distribution between tiller and main shoot (C1), stem and spike (C 2), spikelet-spikelet (C 3), floret-floret (C 4), spike chaff and grain yield (C 5). Group 1 and 2 determine the allocation of assimilates to the spike, while groups 3, 4, 5 exhibit the distribution of assimilates within spike. Harvest index and grain size related traits were considered as C 6 and C 7.

The "core group" of all these seven groups was "floret-floret (C4)" (Fig. 5.2). The traits in this group (floret fertility) exhibit yield potential, loss of yield potential and the assimilate partitioning between florets. Because no genetic studies have been carried out on these traits (C4) in wheat, GWAS analyses primarily focused on traits in this group. Here, we define the apical (A), central (C) and basal (B) spikelets of a spike (Fig. 5.2): the apical spikelet is the third one located at the top of a spike, the central spikelet is the one in the middle of a spike, and the basal spikelet is the one located at the bottom of a spike. The maximum number of floret primordia per spikelet at the three positions (max floret A, C, and B) was recorded at the green anther stage (Guo and Schnurbusch, 2015), and the difference between maximum number of floret primordia and grain number per spikelet was defined as floret primordia loss (floret primordia loss A, C, and B). From our analyses it becomes evident that these winter wheat accessions lose more than 50% of their grain yield potential (maximum number of floret primordia)(Fig. 5.2). The grain number per spikelet at physiological maturity (grain A, C, and B) and grain survival (survival A, C, and B) were also determined in spikelet positions A, C and B of the spikes. Grain survival (survival A, C, and B) was determined by the ratio between the grain number per spikelet and the maximum number of floret primordia per spikelet. The grain survival within individual spikelets was less than 50% (Fig. 5.2).

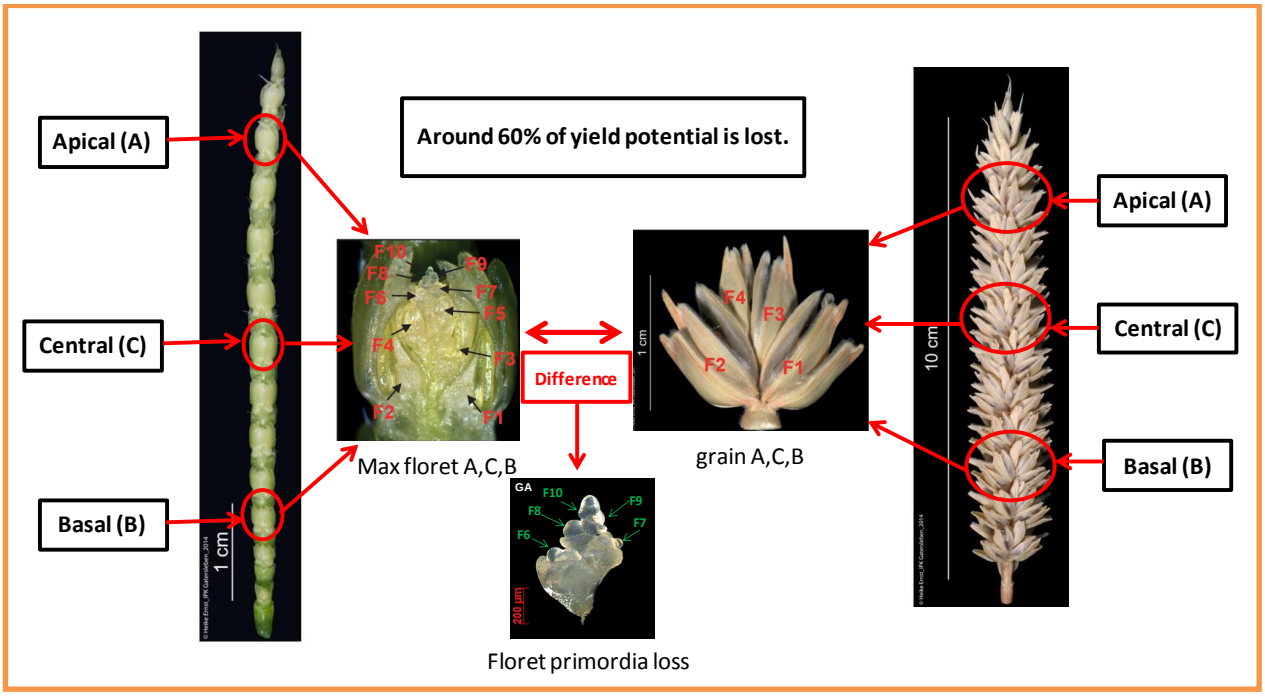


Fig. 5.2. The explanation of the traits in the "core group". The apical (A) and basal (B) spikelets are the three one from the top and bottom of spike, respectively. The central (C) spikelet is the one in the center of the spike.

Repeatabilities showed a broad range from 0.50–0.90. The additive estimates of heritabilities, ranging from 0.20–0.90, correlated strongly with the repeatabilities. Of all 54 traits, 81.48% (44 traits) displayed broad-sense heritability (H^2) above 0.4, and 31 traits showed broad-sense heritability (H^2) over 0.7 (Fig. 5.3A), providing the genetic basis for the genome-wide association study (GWAS). The 12 grain size-related traits in cluster 7 displayed the highest broad-sense heritability (>0.7), while 16 traits involved in assimilate supply between florets in cluster 4 showed relatively low broad-sense heritabilities compared with other groups.

We assessed the phenotypic and genetic relationships between all 54 traits through cluster and correlation analyses (Fig. 5.3). Generally, traits for the same group clustered closely and demonstrated a strong correlation among each other (Fig. 5.3B). As expected, four grain size related traits (i.e., grain width, length, area and TKW in the main shoot, tillers and entire

plant) showed high phenotype similarity (close to each other by cluster analysis and a strong correlation among themselves). In addition, the five traits suggested the direction of assimilate partitioning for spikes and grains: the ratio of grain weight/spike chaff, the ratio of spike DW/main stem DW, harvest index, spike fertility index, and spikelet fertility were clustered together and demonstrated a close relationship. Furthermore, the grain number per spike and grain weight per spike in both the tillers and main shoot displayed a close connection. Unexpectedly, the maximum number of floret primordia per spikelet (Guo and Schnurbusch, 2015) (grain yield potential) at A (max floret A, the third spikelet from the top of the spike), C (max floret C, the spikelet in the center of the spike) and B (max floret B, the third spikelet from the bottom of the spike) parts of the spike exhibited close positive associations with floret primordia loss at the three positions (floret primordia loss A, C, and B), suggesting that the increase in the grain yield potential may also result in greater loss of the yield potential. However, the negative correlation between loss of the grain yield potential (floret primordia loss A, C, and B) and grain survival A, C, and B and the positive correlation between the grain yield potential (max floret A, C, and B) and grain survival A, C, and B indicated that spikelets can maintain a higher grain survival despite the increased grain yield potential causing greater loss. The far cluster distance between grain number per spikelet (grain A, C, B, average) and the maximum number of floret primordia (max floret A, C, and B average, floret primordia loss A, C, and B average) indicated that the grain number per spikelet was relatively independent from the maximum number of floret primordia. All these phenotypic relationships should be verified by genetic studies before applying these results in wheat breeding and research.

We therefore assessed genetic associations to verify these phenotypic correlations. We observed consistent genetic relations with phenotypic associations. For example, four grain

size related traits; that is, grain width, length, area and TKW in the main shoot, tillers and entire plant showed high genetic similarity based on SNP markers (close to each other by cluster analysis and showed a strong genetic correlation between each other based on SNP markers). However, we also found some inconsistencies between the genetic and phenotypic correlations. For example, the genetic connections between max floret A and C and floret primordia loss A and C were weak and negative, while the phenotypic associations were strong and positive. The distance between the genetic cluster of grain number and survival per spikelet at A (grain A, survival A), C (grain C, survival C) and B (grain B, survival B) spikelets across the spike were far away from each other, while they were clustered together in the phenotypic cluster analysis.

We also determined the overall consistencies for the phenotypic and genotypic correlations between different traits with a Mantel test. As expected, the phenotypic and genotypic correlation matrices according to the Mantel test showed a close correlation (r=0.635, p<0.0001), suggesting moderate consistency between the phenotypic and genotypic correlations.

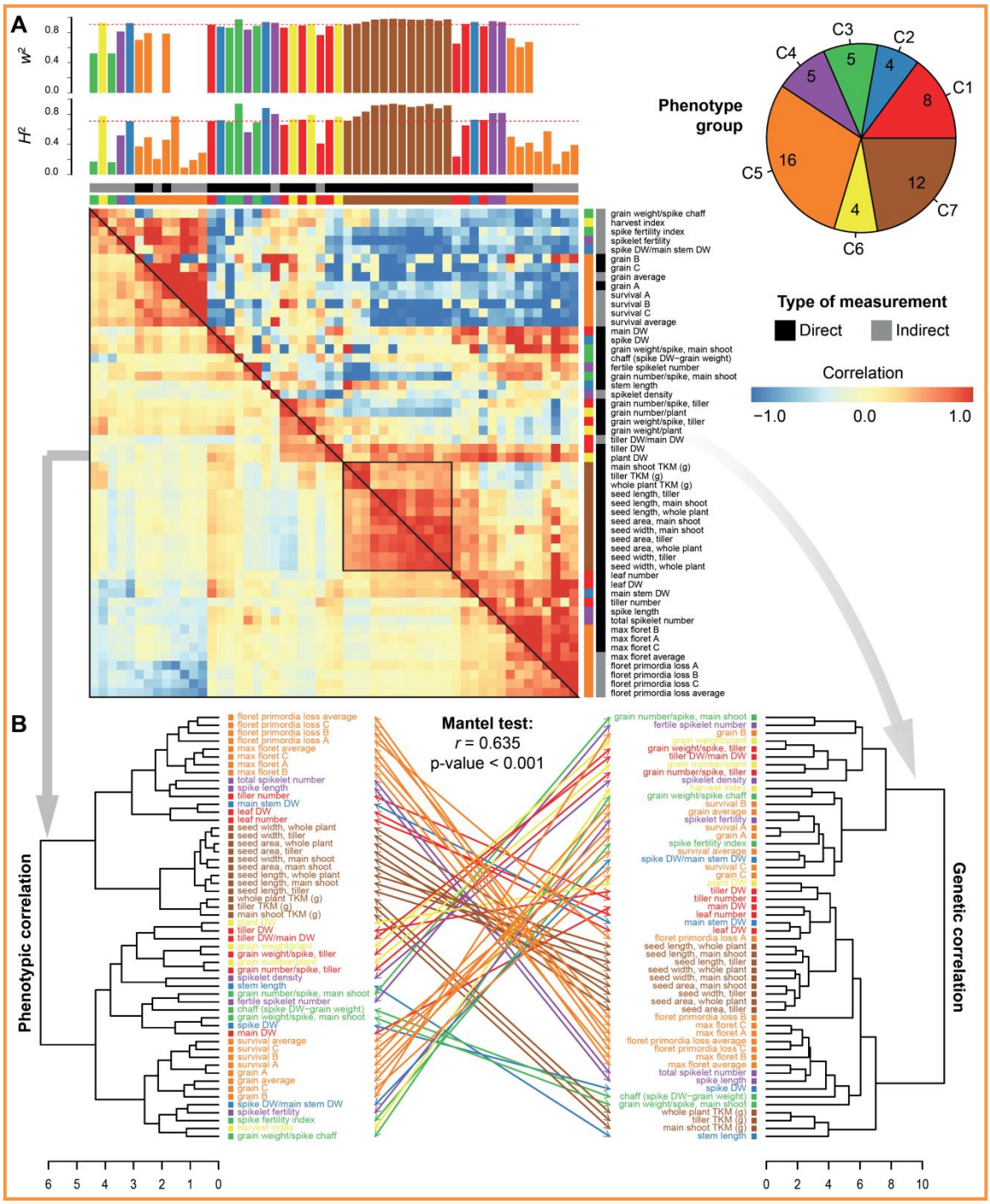


Fig. 5.3. Phenotypic and genotypic relationship between all the 54 traits across the population. (A) broad-sense heritability and phenotypic and genotypic correlations for all the 54 traits; A,C,B indicate apical (the third spikelet from the top of the spike), central (the spikelet in the center of the spike) and basal (the third spikelet from the bottom of the spike) spikelet positions were phenotyped (Guo and Schnurbusch, 2015); (B) phenotypic and genotypic cluster analysis to show the distance among the traits, also display the consistence and difference between phenotypic and genotypic cluster.

Population structure

To further explore the population structure, we performed principal component analyses (PCA) to capture the genetic variation of the entire population of 210 individuals (Fig. 5.4A). The eigenvalues for nine of the top 10 principal components (PCs) were above four. Notably, the eigenvalues for PC1 (more than 14) were approximately four times bigger than PC10 (less than 4). After PC10, there was a sharp decrease in the eigenvalues (Fig. 5.4A). The top 10 PCs explained approximately 30% of the total phenotypic variation (Fig. 5.4B). Obviously, PC1 distinguished one group (yellow points) from the rest, whereas PC2-9 could not distinguish different clusters, suggesting there was no significant population structure.

We performed the estimation of cross-validation error for Bayesian hierarchical clustering of wheat accessions based on 8,000 SNPs. The lowest cross-validation error was observed at K = 10 according to Fig. 5.4C, suggesting clustering the whole population into 10 groups was reliable for analyzing the subpopulation structure. After elucidation of these groupings by PCA, we turned to structure-like analysis with the algorithm ADMIXTURE to assign individuals proportionally to hypothetical ancestral populations. Variable ancestry proportions suggested the variations of morphological distribution and geographic regions. Furthermore, the identical ancestry proportions implied similarity of some groups (Fig. 5.4D). Finally, we inferred the population structure for the entire population in this study. The wheat panel was divided into ten subgroups, and the numbers of wheat samples accessed in each group were distributed in a wide range, from 12 to 30 (Fig. 5.4E).

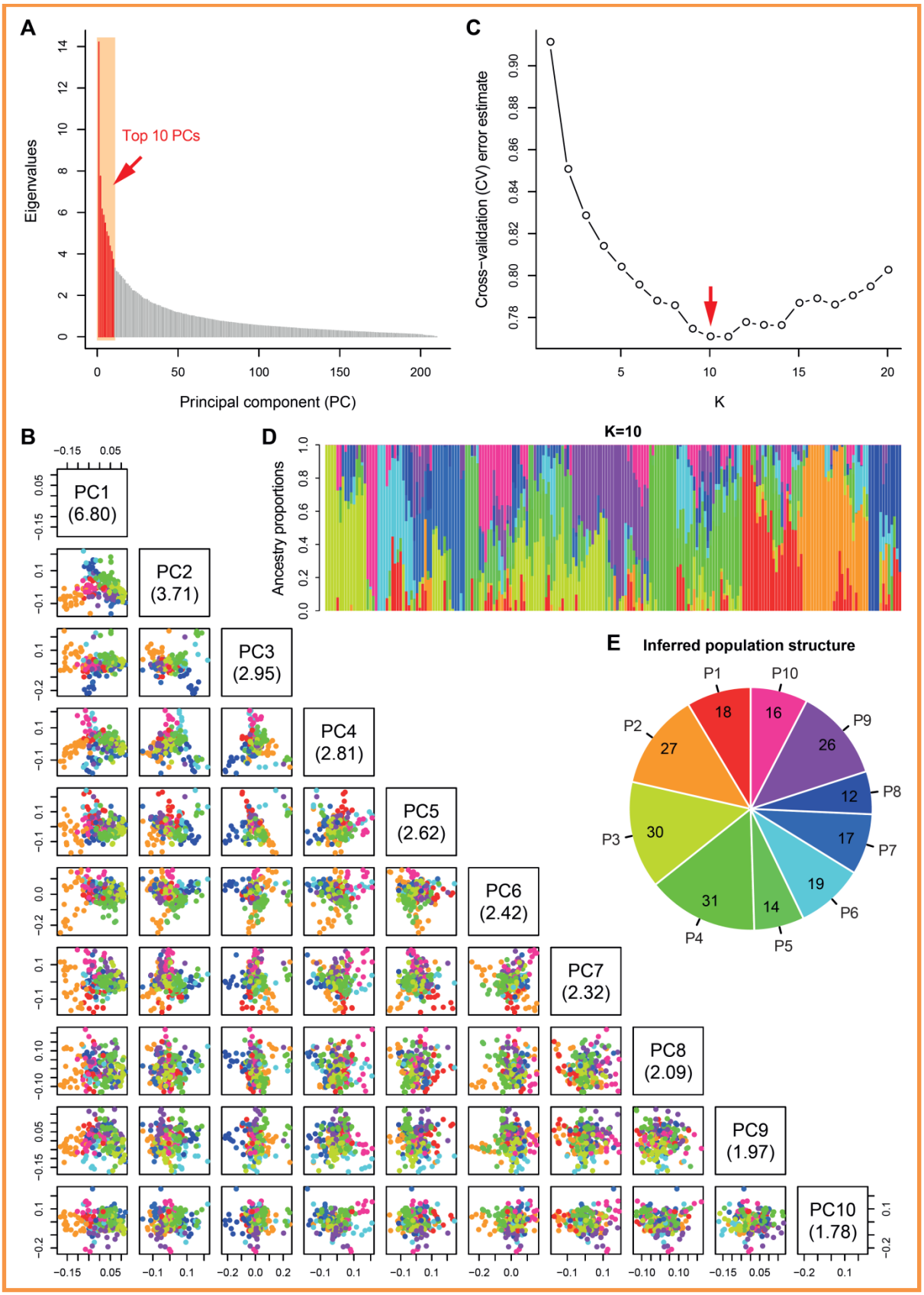


Fig. 5.4. The genetic population structure was determined by principle component analysis (PCA) on SNP markers. (A) The eigenvalues for eigenvectors in the PCA; the red part exhibits the first 10 PCs

with the highest eigenvalues. (B) The proportion of genetic variance (%) explained by the first 10 PCs. (C) The estimation of cross-validation error for Bayesian hierarchical clustering of wheat accessions based on 8,000 SNPs, indicating the lowest cross-validation error was observed at K = 10. (D) Ancestry proportions of the studied 210 individuals revealed by the ADMIXTURE program (Alexander et al., 2009) with K=10. Each individual is represented by a vertical (100%) stacked column of the genetic components proportion shown in color for K=10, with fractions indicated on the y-axis. The analysis assumed no grouping information. (E) Population structure inferred by ADMIXTURE analysis. Each individual is represented by a vertical (100%) stacked column of genetic component proportions shown in color for K=10, as the lowest cross-validation error was observed at K = 10 (Fig. 5.4C).

GWA mapping of assimilate partitioning traits

We carried out a GWAS on all 54 traits using different models (with and without population structure correction). First, a naive marker-trait association model without any correction of the population structure was carried out to search for associations between trait phenotypes and marker genotypes. The naive model used in this study referred to linear regression for continuous quantitative traits. Then, we used different approaches to detect and correct the population structure. Perhaps the most commonly accepted statistical method to detect population structure is a model-based cluster approach, which uses multi-locus genotype data to infer the subpopulation number K and creates a subpopulation membership matrix Q to represent the samples (Wang et al., 2012). The K+Q model was first developed and used by Yu et al. (2006), and then Cockram et al. (2010) used a K+Q model in a barley population and found it was quite effective.

Fig. 5.5 shows the QTLs based on the GWAS results for the grain number per plant. The distribution of the phenotypic values is critical in a GWAS, so we first created the frequency distribution of grain number per plant (Fig. 5.5A). As quantile-quantile plots between observed- and expected-$\log_{10}$ (p) can be used to show the effects of different models, we then created quantile-quantile plots for different GWAS models: K, K+Q [5], K+Q [10], and a

linear regression model (Fig. 5.5B). It was clear that the linear mixed models were more effective for the detection of significantly associated SNP markers due to the regression test between expected and observed $-\log_{10}(p)$ (Fig. 5.5B). Fig. 5.5C suggests distinguished and shared associated SNP markers between the three linear mixed models: K, K+Q [5], and K+Q [10]. It is clear there were more QTLs and candidate genes found in the model K+Q [10] than in K and K+Q [5]. We therefore used the results from the model K+Q [10], which was the most efficient for a GWAS analysis related to grain number per plant. Fig. 5.5D shows the most significant effect of the SNP marker kukri_rep_c106786_230 (N=C:125|T:65; P<2.65x10-2), which was most closely associated with grain number per plant on chromosome 2D. A *P* value $<2.65\text{x}10^{-2}$ suggested that this SNP marker had a significant influence on the grain number per plant. Finally, we assessed the grain number per plant detected by a linear mixed model (K+Q [10]) (Fig. 5.5E) and a linear regression model (Fig. 5.5F). However, different traits in this study showed variable subpopulation structures that fit different GWAS models to detect and correct the structures. For example, for the 12 grain size related traits, which included seed area, length, width and TKW in the tiller, main shoot and entire plant, we detected more QTLs and candidate genes when we used the K model than when other models were used. Based on the GWAS results of all 54 traits, we detected more associated SNP markers using the K model (127 SNPs) than using other models (63, K+Q [5]; 77, K+Q [10]), indicating that the K model is more efficient than the other models across the overall results for all traits (Fig. S5.1, S5.2).

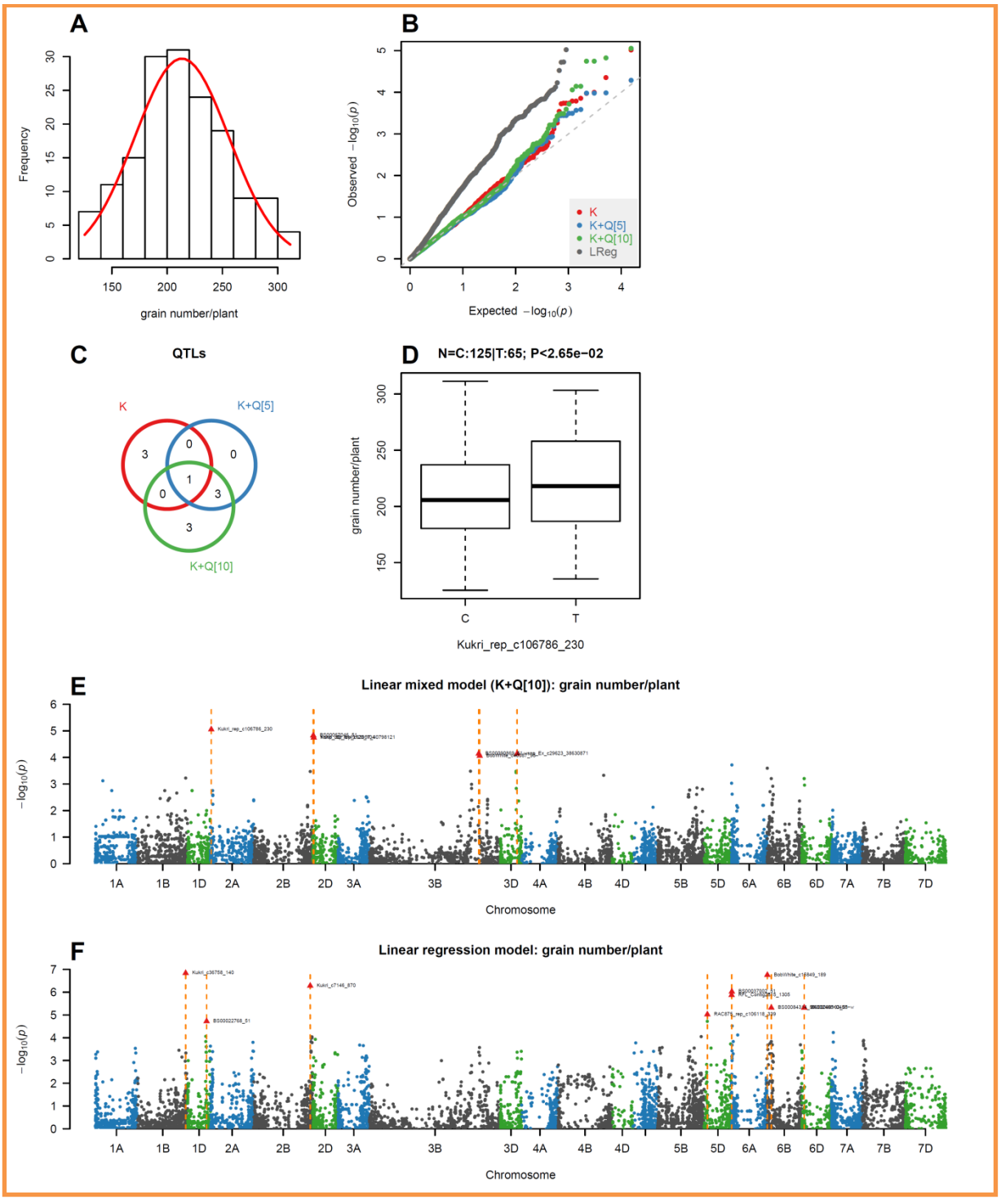


Fig. 5.5. Associated SNPs of the grain number per plant was facilitated by GWAS results based on different models. (A) The frequency distribution of grain number per plant. (B) Quantile-quantile plots for different GWAS models, before correcting for population structure. It was critical to detect and infer the hidden structure in a sample. We tried the relative kinship (K) in a GWAS and also fitted both population structure (Q) and K together using a linear mixed model. The Q [5] and Q [10] indicated the first 5 and ten components were used to correct the population structure, respectively.

Furthermore, the GWAS results with the linear regression model are shown for comparison purposes. (C) The distinguished and shared QTLs between the three linear mixed models. (D) The distinguished and shared associated genes between the three linear mixed models. (E) The most significantly associated SNP marker kukri_rep_c106786_230 (N=C:125|T:65) showed a significant effect on the grain number per plant; P<2.65x10-2 suggested the SNP marker had significant influence on the grain number per plant. (F) The significantly associated markers for grain number per plant detected by the linear mixed model (K+Q [10]), which showed more QTLs and significantly associated markers than the other two models. (G) Markers for the grain number per plant detected by the linear regression model.

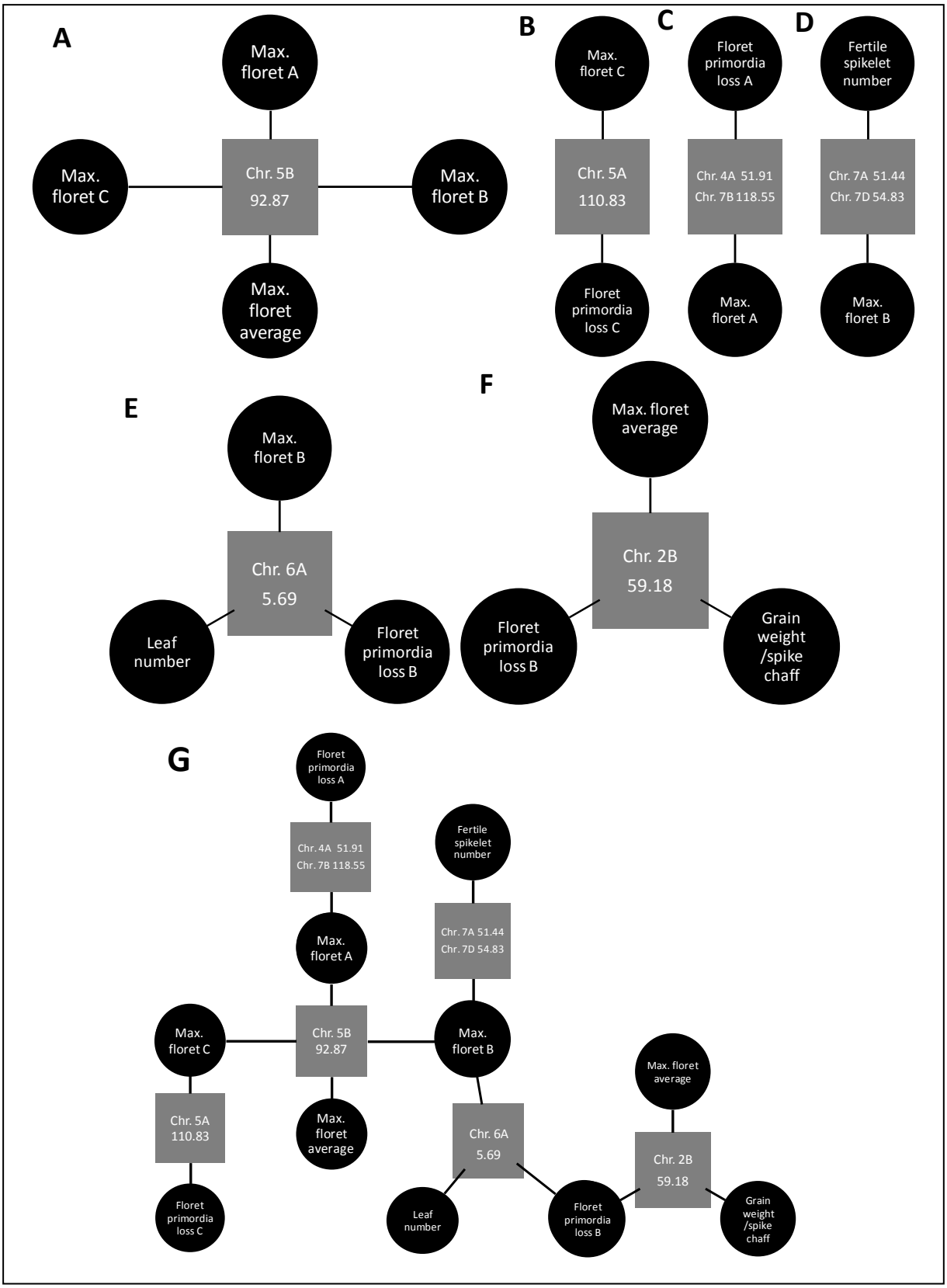


Fig. 5.6. Shared QTLs between different traits. (A) A QTL on chromosome 5B (92.57 cM) is shared by maximum number of floret primordia per spikelet (potential of grain number) at apical, central and basal spikelet, and the average of the three positions; (B) A QTL on 110.83 cM of chromosome 5A is

shared by maximum number of floret primordia per spikelet at central spikelet and floret primordia loss (difference between maximum number of floret primordia and grain number per spikelet) at central spikelet; (C) Two QTLs on chromosome 4A (51.91 cM) and 7B (118.55 cM) are shared by maximum number of floret primordia per spikelet at apical spikelet and floret primordia loss at apical spikelet; (D) Two QTLs on chromosome 7A (51.44 cM) and 7D (54.83 cM) are shared by maximum number of floret primordia per spikelet at basal spikelet and fertile spikelet number; (E) A QTL on chromosome 6A (5.69 cM) is shared by three traits: leaf number on the main shoot, maximum number of floret primordia at basal spikelet, floret primordia loss at basal spikelet; (F) A QTL on chromosome 2B (59.18 cM) is shared by three traits: average of maximum number of floret primordia at apical, central and basal spikelet, floret primordia loss at basal spikelet, ratio between grain weight and spike chaff within one spike; (G) the network based on the shared QTLs mentioned in figure 6A, B, C, D, E, F.

Assessing pleiotropy between floret fertility traits

To determine whether the relationships among floret fertility traits were the result of linkage or pleiotropism, we constructed association networks based on the shared SNP markers (QTLs) among all the 16 floret fertility traits (Fig. 5.6, S5.3, S5.4). Considering the genome-wide significance thresholds of the GWAS using a Bonferroni's correction ($P = 1.30 \times 10^{-4}$, threshold=3.890), we observed there are few overlaps between QTLs for different traits and realized that it might be too stringent for detecting the overlaps of QTLs (Data S5.2). Therefore, we decreased the threshold to 2.70 ($P = 2.0\times 10^{-3}$) since we observed that some associated markers can be regularly detected between $P = 1.30 \times 10^{-4}$ and $P = 2.0\times 10^{-3}$ for most traits. We observed 172 significant SNPs (52 QTLs) for more than one trait; 27 significant SNPs (15 QTLs) had associations for five or more traits (Data S5.3). In most cases, QTLs associated with more than one trait were identified for the 16 floret fertility traits (C4, table 5.1, Data S5.3). As expected, maximum number of floret primordia at apical, central and basal spikelets (max. floret primordia A, C, B) and the average at the three positions (max. floret primordia average) shared one QTL on chromosome 5B (92.87 cM) (Fig. 5.6A),

suggesting a consistent effect of this locus on the development of floret primordia in these three positions within one spike. Similarly, we also observed that one QTL (chr. 6A, 95.98-99.39 cM) is associated with grain number per spikelet (grain A, C, B, average) (Fig. S5.3A, Data S5.3), and two QTLs (chr. 5B, 80.92 cM; chr. 5D, 95.64 cM) are associated with grain survival in central and basal spikelets and the averages of the three postions (grain survival C,B, average), floret primordia loss in central and basal spikelets and the averages of the three postions (floret primordia loss C, B, average) (Fig. S5.4A, Data S5.2). This suggested a relative consistent effect of this QTL on flroet fertility at apical, central and basal positions. However, some QTLs are specific for only one position, e.g. one QTL on 3B (52.35 cM) is only associated with maximum number of floret primordia in apical spikelet (max. floret primordia A); one QTL on 7A (63.95 cM) is specific for grain number per spikelet in basal spikelet (grain B); one QTL on 5A (50.26 cM) is only associated with grain number per spikelet in central spikelet (grain C) (Data S5.3). The associations between floret fertility traits (C4) detected in Fig. 5.3 were validated by the shared QTLs. For example, four QTLs (on 5B, 80.92 cM; 5D, 95.64 cM; 6A, 99.39 cM; 7A, 79.86-84.41 cM) are associated with grain A, C, B, grain survival A, C, B and floret primordia loss A, C, B (Fig. S5.3D, S5.3H, S5.4A), suggesting that the maximum number of floret primordia per spikelet will increase the floret primordia loss per spikelet, without penalizingfinal grain number per spikelet. Very similar interactions between these traits had already been shown in Fig. 5.3, confirming previous investigations (Guo et al., 2016). However, we also noted that there was no overlap between QTLs associated with grain A, C, B, average and max. floret A, C, B, average; this fits the conclusion of the correlation and cluster analysis (Fig. 5.3): grain number per spikelet is relatively independent to the maximum number of floret primordia per spikelet.

Relationships between floret fertility and assimilate partitioning traits

Beside the relationships among the 16 floret fertility traits, we also assessed the associations between floret fertility and assimilate partitioning, since one of the goals of this study was to investigate how the assimilate flow regulates floret/grain development, grain yield potential, and grain setting. QTLs on 2B (59.18 cM) and 6A (5.69 cM) implied the involvement of leaf number in determining maximum number of floret primordia per spikelet and floret primordia loss per spikelet which further affect the ratio between grain weight and spike chaff (Fig. 5.6E, F). It was concluded that increased leaf number will improve the amount of assimilates which will further increase the maximum number of floret primordia per spikelet; this appears consistent with previous work (Guo et al., 2015). Finally the increased maximum number and floret primordia loss might increase the ratio between grain weight and spike chaff, suggesting the increased efficiency of spike chaff. The association of spike length on the main shoot with grain A, average, survival A, C, average, floret primordia loss C, average, harvest index and grain weight per spike (tiller) was revealed by the QTL on 1A (3.42 cM) (Fig. S5.3B). We detected three QTLs (2A, 66.14 cM; 3A, 58.21 cM; 3D, 55.95 cM) which implied the interaction among grain A, survival A, spikelet fertility, harvest index, grain weigh per spike (tiller), spike fertility index and the ratio between spike DW and main stem DW (Fig. S5.3C, E). As spikelet fertility, harvest index, spike fertility index and ratio between spike DW and main stem DW are indicators of the utilization efficiency of assimilates. Therefore, it highlighted the critical roles of grain A and survival A in increasing utilization efficiency of assimilates. The shared QTLs on 6A (95.41 cM), 6D (113.12 cM) and 7D (4.55 cM) (Fig. S5.3F, G) indicated important roles of grain C and survival C in the improvement of grain number per spike on the main shoot and decrease of the ratio between tiller DW and main DW. Not only grain A and survival A on the main shoot, but also grain average, survival average, floret

primordia loss average, survival B, floret and primordia loss B on the main shoot , shared QTLs with grain weight per spike on tillers (Fig. S5.3K, 4C). It suggests that increased floret fertility on the main shoot may also improve grain weight per spike on tillers. Interestingly, we detected one QTL on 7B (140.40 cM) being associated with survival B, floret primordia loss B and seven grain size traits. This implied that grain survival and floret primordia loss may also be involved in the determination of grain size. Finally, a network was constructed to show the relationships between different traits (Fig. 5.6G).

We also carried out GWAS analyses on plant height and flowering time (i.e. time to heading; Z55) to better determine a possible role on floret fertility traits (Data S5.3). We detected that one QTL on 7B (109.45 cM) associated with plant height was shared by three floret fertility traits: grain average, survival B and floret primordia loss B. No QTL overlapped between flowering time and any floret fertility trait, indicating that flowering time has negligible effect on floret fertility in this population.

Important novel candidate genes related to floret fertility in wheat

We used the NCBI and URGI Wheat Blast Server databases to identify wheat contigs of putative candidate genes (annotated in wheat, barley and other grass species) and localized their genetic positions utilizing population sequencing (POPSEQ) contig information in wheat (Mayer et al., 2014) (Table 5.2). One QTL (associated with floret primordia loss B and grain A) close to the barley ortholog of the wheat *Six-rowed spike 1* (*TaVrs 1*) gene mapped on 2A (59.23 cM). Previously, it was reported that barley *Vrs1* conferred the two-rowed spike phenotype. Here, expression of *Vrs1* was strictly localized to the lateral spikelet/floret primordia of immature spikes, suggesting that the VRS1 protein suppresses development of lateral florets (Komatsuda et al., 2007). Based on *Vrs1*'s function in barley, we hypothesized

that the wheat VRS1 protein may similarly suppress floret primordia development within wheat spikelets, which in turn may further influence the floret primordia loss and grain number. *SUGAR SIGNALLING IN BARLEY 2* (*SUSIBA2*) is a transcriptional activator in plant sugar signaling (Sun et al., 2003; Sun et al., 2005), expression of *SUSIBA2* in rice increased biomass and starch content in the seeds and stems, and suppressed methanogenesis (Su et al., 2015). The wheat (*TaSUSIBA2*) gene mapped closely to the QTLs (associated with grainA, average, survival A, grain weight/spike chaff, max. floret primordia, floret primordia loss B) on 2A (59.23 cM) and 2B (59.18 cM). *SUCROSE SYNTHASE 1* (*SS1*) plays an important role in sucrose synthase and is also possibly involved in the cellulose and starch biosynthesis (Zheng et al., 2011; Baroja-Fernandez et al., 2012). The wheat (*TaSS1*) gene was mapped closely to the QTLs on 7A (51.44 cM, associated with fertile spikelet number and max. floret B). The two candidate genes-*TaSUSIBA2 and TaSS1* suggested the metabolism of sugars in the determination of floret fertility . As expected, phytohormones may also be in involved in floret/grain development. Mutations in *BRASSINOSTEROID INSENSITIVE 1* (*BRI1*) severely affect plant growth and development (Yamamuro et al., 2000; Li et al., 2001). *BRI1* appears as a candidate for the QTL on 3A (58.21 cM; associated with grain A, survival A, spikelet fertility), implying that phytohormones play a role in the regulation of grain number and survival. Although flowering time has negligible effect on floret fertility in this population, we still detected some candidate genes related to flowering time, e.g. *CEN*, *CO4*, *CO6*, *FT2*. It was concluded that the influence of these flowering time genes may attribute to their effects on regulation of floral development, instead of the effects on flowering time.

Table 5.2. Candidate genes identified according to QTLs for different traits

Candidate gene	Accesion number	Associated traits	Best SNP marker	Co-locating wheat genes
CONSTANS 4 (CO4)	AF490475	grain A, spike DW/main stem DW, spike fertility index	RFL_Contig3780_644, 2A, 66.144 cM	*TaCO4,* ta_contig_2AL_6395731, 2A, 58.09 cM
SIX-ROWED SPIKE 1 (Vrs1)	AB259782	Same as *CO4*	RFL_Contig3780_644, 2A, 66.144 cM	*TaVrs1,* ta_contig_2AL_3630815, 2A, 74.887 cM
CENTRORADIALS (CEN)	JX844798	floret primordia loss A, B, average, grain A, avearge, survival A, grain weight/spike chaff, max floret C, B, average	wsnp_Ex_c15325_23565794, 2B, 55.773 cM	*TaCEN,* ta_contig_2BL_8091663, 2B, 59.184 cM
SUGAR SIGNALLING IN	KR935231	Same as *CEN*	wsnp_Ex_c15325_23565794, 2B,	*TaSUSIBA2,*

BARLEY 2 (SUSIBA2)			55.773 cM	ta_contig_2DS_5348573, 2D, 63.43 cM
HEXOKINASE9 (HXK9)	DQ116391	grain A, grain weight/spike (tiller), harvest index, spikelet fertility, survival A	RAC875_c8010_155, 3A, 58.217 cM	*TaHXK9,* ta_contig_3AL_3632952, 3A, 57.08 cM
BRASSINOSTEROID INSENSITIVE 1 (BRI1)	AB109215	Same as *SUSIBA2*	RAC875_c8010_155, 3A, 58.217 cM	*TaBRI1,* ta_contig_3AL_4448665, NA, NA
FT-like protein gene 2 (FT2)	DQ297407	Same as *SUSIBA2*	RAC875_c8010_155, 3A, 58.217 cM	*TaFT2,* ta_contig_3AS_3335977 , NA, NA
SUCROSE SYNTHASE 1 (SS1)	FN400939	fertile spikelet number, max floret B	Excalibur_rep_c101407_222, 7A, 51.442 cM	*TaSS1,* ta_contig_7AS_4255196, 7A, 61.673 cM
CONSTANS 6 (CO6)	AY082962	floret primordia loss A, max floret A	RAC875_c5744_115, 7B, 118.551 cM	*TaCO6,* ta_contig_7DL_3331672 , 7D , 119.745 cM
DWARF 3 (D3)	AK069429	grain C, grain average, tiller DW/main DW	RAC875_c11969_384, 7D, 4.551 cM	*TaD3,* ta_contig_7DS_3927231, 7D, 4.551 cM

Note: genetic positions (cM) of co-locating wheat genes are from wheat POPSEQ and corresponding contig number, NA means the information is not known.

Effects of detillering on floret fertility and assimilate partitioning

GWAS as well as correlation and cluster analysis have suggested that the assimilate flow to floret/grain development is critical for the determination of floret fertility. To further validate the effects of assimilates partitioning; we measured some floret fertility, assimilate partitioning and spike morphology traits in a detillering study. As shown in Table 5.3, detillering significantly decreased plant height in both the field and greenhouse growth conditions. The leaf blades are thicker after detillering in both the field and greenhouse conditions because leaf number was not significantly influenced in the greenhouse and was decreased in the field by detillering and leaf DW was increased in both the field and greenhouse. The marked decrease in the ratio of spike DW to main stem DW reveals after detillering was observed. The decreased spike fertility index in detillered plants in both the greenhouse and field indicates that detillering decreases the strength of competition for assimilates from the spike chaff. Unexpectedly, detillering decreased spikelet fertility, although more assimilate partitioning into spikes after detillering weakened the competition for assimilates between the spikelets. A possible explanation for this is that the central and basal spikelets have an advantage over the apical spikelets in terms of competition for assimilates (Table 5.3, grain A, C, B). However, the increase in assimilate partitioning into spikes of detillered plants (Table 5.2, spike DW) appeared to strengthen the advantage of the central and basal spikelets and did not help to increase spikelet fertility in the apical

region of the spikes in most cases. The decreased spikelet density of detillered plants was mainly caused by increased spike length (i.e., rachis internode length) as a part of the spike chaff. Detillering only increased the likelihood to set grains at the G3 and G4 positions in the greenhouse, not in the field. Similarly, detillering also only increased the four grain-size related traits (seed area, width, length and TKW) in the greenhouse, and an increase in these traits was not observed in the field. A possible explanation for the negligible effects of detillering under field conditions is that the sensitivity of the plants to the conditions of the field environment may offset the influence of detillering. It seems that the increase in assimilate partitioning into the spike of detillered plants (greater spike DW) helped to increase grain number, but the likelihood of grain setting was relatively more sensitive to the field environment, suggesting the potential of plasticity. Detillering markedly increased max. floret primordia A, C, B, but the effects on survival A, C, B and floret primordia loss A, C, B are not as significant as max. floret primordia A, C, B.

Table 5.3. Differences in the 34 examined traits between control and detillered plants in both field and greenhouse conditions.

Phenotype	**Greenhouse**		**Field**	
	control	detillering	control	detillering
1-plant height (cm)	111.00+14.53a	100.65+7.91b	112.32+9.82a	99.87+12.10b
2-leaf number	3.45+0.26a	3.41+0.32a	3.10+0.31a	2.70+0.58b
3-leaf DW (g)	0.42+0.07b	0.76+0.12a	0.42+0.05b	0.61+0.20a
4-spike DW (g)	2.52+0.38b	3.45+0.66a	3.26+0.38a	3.38+0.56a
5-main stem DW (g)	2.42+0.51b	5.14+1.53a	3.15+0.45b	3.79+0.75a
6-spike DW/main stem DW	1.08+0.15a	0.74+0.25b	1.06+0.16a	0.93+0.21b
7-grain weight/spike (g)	1.82+0.26b	2.42+0.57a	2.57+0.32a	2.51+0.45a
8-grain number/spike	45.33+6.91b	53.34+12.84a	59.18+14.60a	57.35+11.22a
9-spike chaff (g)	0.70+0.13b	1.07+0.15a	0.72+0.09b	0.94+0.12a
10-spike fertility index	66.45+11.45a	51.26+15.46b	84.25+19.03a	60.48+19.74b
11-grain weight/spike chaff	2.68+0.26a	2.36+0.54b	3.64+0.38a	2.68+0.41b
12-total spikelet number/spike	23.48+2.52a	23.29+2.33a	21.86+1.89a	21.53+1.87a
13-fertile spikelet number/spike	18.24+2.02a	16.54+3.79b	20.77+2.09a	19.10+2.70b
14-aborted spikelet number/spike	5.29+3.34b	6.86+4.06a	1.08+0.55b	2.47+1.70a
15-spike length (cm)	11.11+0.59b	12.68+0.51a	11.53+0.58b	13.07+0.67a
16-spikelet density	2.13+0.25a	1.86+0.20b	1.91+0.13a	1.67+0.17b
17-spikelet fertility	0.78+0.12a	0.71+0.16b	0.95+0.03a	0.88+0.09b
18-grain A	1.34+0.79a	0.99+1.02a	2.53+0.41a	2.27+0.80a
19-grain C	2.91+0.42b	3.46+0.87a	3.47+0.5a	3.84+0.54a

20-grain B	2.96+0.38b	3.76+0.46a	3.49+0.62a	3.62+0.44a
21-max. floret A	9.25+0.55b	9.58+0.48a	9.72+0.49b	10.17+0.48a
22-max. floret C	10.19+0.58b	10.58+0.47a	10.64+0.48b	10.94+0.45a
23-max. floret B	10.05+0.61b	10.75+0.53a	10.67+0.55a	10.94+0.58a
24- survival A	0.14+0.08a	0.11+0.11a	0.26+0.04a	0.22+0.08a
25-survival C	0.29+0.05a	0.33+0.08a	0.33+0.04a	0.35+0.05a
26-survival B	0.30+0.04b	0.35+0.05a	0.33+0.06a	0.33+0.05a
27-floret primordia loss A	7.91+0.79b	8.58+1.34a	7.19+0.58b	7.90+0.86a
28-floret primordia loss C	7.29+0.77a	7.12+0.87a	7.17+0.48a	7.10+0.77a
29-floret primordia loss B	7.09+0.70a	6.99+0.71a	7.18+0.73a	7.33+0.84a
30-likelihood (G3, G4)	0.23+0.17b	0.44+0.27a	0.56+0.21a	0.54+0.21a
31-TKW (g)	40.77+6.10b	47.94+6.41a	44.85+7.52a	43.39+7.15a
32-seed area (mm^2)	16.44+1.40b	18.73+1.77a	18.63+1.98a	19.04+2.04a
33-seed width (mm)	3.32+0.13b	3.54+0.17a	3.50+0.18a	3.54+0.21a
34-seed length (mm)	5.98+0.34b	6.34+0.34a	6.37+0.42a	6.44+0.39a

Note: DW suggests dry weight; G3, G4 indicates the third and forth grains from the bottom within spikelet; TKW implies thousand kernel weight. Data are presented as the mean ± stand deviation, n=6; different letters per trait indicate significant differences between control and treated plants (p<0.05).

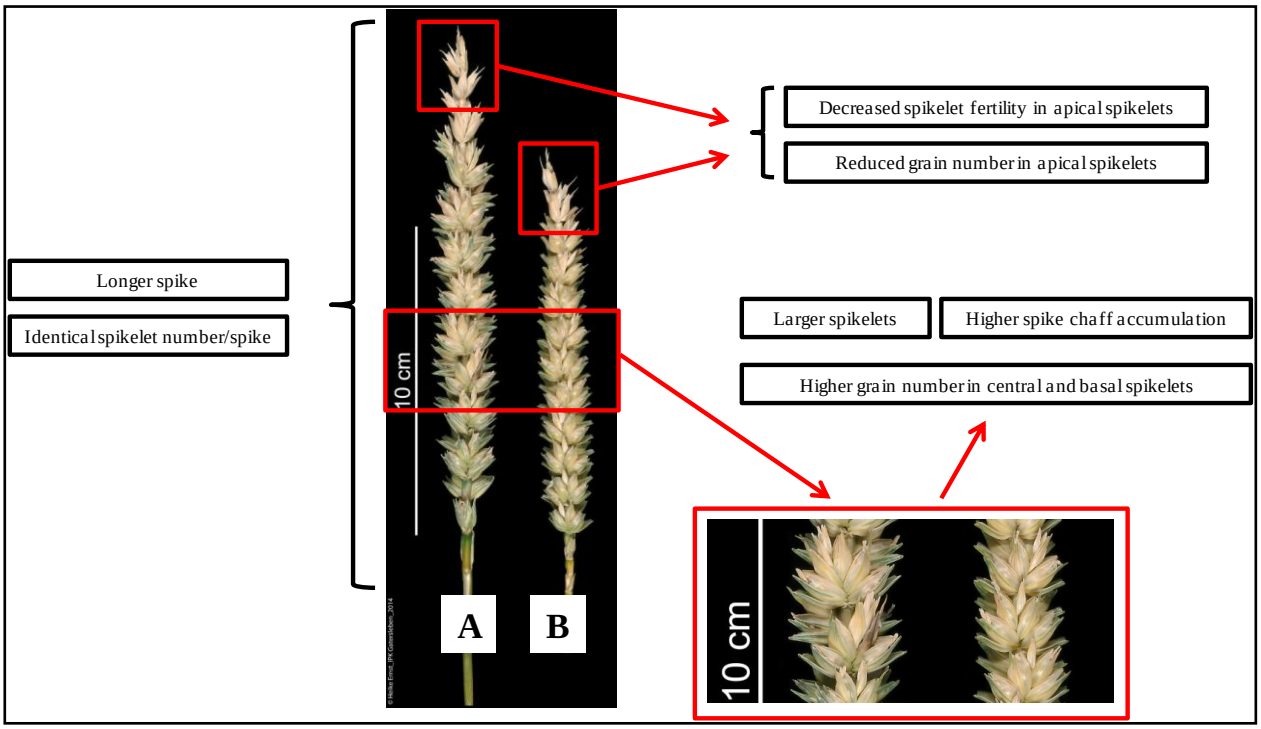


Fig. 5.7. Summary of the wheat spike's response to detillering. The spikes (three weeks after anthesis) in detillering (A) and control (B) treatments in the genotype 'Breustedt's Lera'. The traits under detillering treatment are described compared to control.

Fig. 5.7 shows the overall differences between the control and detillering treatments. The length of the spike was increased by detillering, but the total spikelet number per spike was unchanged. Therefore, spikelet density was decreased. This is consistent with previous work

in the wheat-*tin* (*tiller inhibition*) mutant (Kebrom et al., 2012; Kebrom and Richards, 2013). The decreased spikelet density provides more space (predominantly rachis internode length) for spikelet development, resulting in larger spikelets, higher spike chaff accumulation and a higher number of grains in the central and basal spikelets. Interestingly, the grain number per spikelet in the apical regions of the spikes was decreased after detillering, which may be attributable to strong competition for assimilates from the central and basal spikelets.

Summarily, we found the most obvious effects of detillering were the increase of floret fertility and the modification of spike morphology, suggesting a clear interaction of floret fertility and spike morphology. Combined with our GWAS results we concluded that shared QTLs between spike morphology related traits (e.g. spike length, harvest index, spike fertility index, grain width, length, area) and floret fertility traits are indicative of changed assimilate supply. However, the caused modification of spike morphology (e.g. more spacing) may also have influenced floret fertility.

5.5. Discussion

In this report, we present the GWA mapping of assimilate distributions within an entire plant (aboveground parts) and propose critical determinants of floret fertility in wheat. We determined QTLs and associated markers that are involved in the regulation of assimilate partitioning and floret fertility. Our results revealed the association between floret fertility and assimilate allocations in wheat.

As floret fertility traits (“core group” C4), especially the yield potential-maximum number of floret primordia, are difficult to determine in a large population, our work highlighted the experimental design strategies and challenges involved in detecting QTLs and potential genes associated with floret fertility determined by the assimilate distribution and grain set.

At the start of the experiment, we first used two types of assimilate partitioning, i.e., main shoot-tiller and spike-stem, which determined the allocation of assimilates to the spike. Considerable work has been carried out to study these two types of assimilate partitioning; the two important examples are the study of the *tiller inhibition* (*tin*) mutant (Kuraparthy et al., 2007; Kebrom et al., 2012; Kebrom and Richards, 2013) and introduction of dwarfing genes (Peng et al., 1999; Hedden, 2003) in wheat. Then, we aimed to study the assimilate distribution within a spike, that is, in the spikelets at the A, C and B parts of a spike, the florets within a spikelet, and the grain-spike chaff. No genetic studies related to assimilate distributions within a spike have been reported possibly mainly due to the large workload required to make the measurements (which also limits the population size in this study) and the sensitivity to environments. The GWAS of floret fertility related traits in relation to assimilate distribution within a spike is reported here for the first time. Taken together, these two steps showed the genetic determinants of floret fertility according to the five patterns of assimilate distributions for floret/grain development.

The environmental effects on spikes are very likely to be compensated by the modification of other parts within the same plant. The influence on spike components are possibly compensated by the responses of other components within the same spike (Dreccer et al., 2009; Gaju et al., 2009; Gonzalez et al., 2011; Lazaro and Abbate, 2012; Reynolds et al., 2012; Nagel et al., 2013; Martino et al., 2015). Therefore, grain yield-related traits do not always comprise the overall information concerning environmental effects because the environmental influence on the grain weight per spike is often partly offset by the "compensations." However, the modification of spike morphology can reveal overall "compensational effects." Consequently, we used the relationship between spike

morphology and floret fertility to display the potential “compensational effects” that could be ignored when focusing on the grain yield.

Our results demonstrated a close relationship between phenotypic and genotypic correlation matrices for all 54 traits in this study. This relationship revealed the moderate control of these correlations (r=0.635, p<0.0001). It was concluded that the moderate control may attribute to influence of wheat breeding work on SNP markers. Humans have selected for breeding-related traits in certain directions to adapt stressed growth conditions and increase the grain yield. Where breeding-related traits are involved (e.g., grain survival A and spikelet fertility), more associated SNPs with large effects were observed. Moreover, the SNPs with large effects are often shared across different breeding-related traits and are difficult to detect in cultivated wheat because they are nearly fixed in cultivated material; Other SNPs that are not shared and are only specific to some breeding-related traits, even those with only small effects, are important for the modification of specific traits.

According to the GWAS results, we found 141 significant associated SNP markers (49 QTLs) according to Bonferroni correction and we determined the associated genes by including the genes in the contig, which included the associated SNP markers. In this study, we observed a considerable number of SNP markers (QTLs) related to grain survival at the apical spikelet of a spike (grain survival A). This result was consistent with previous reports that grain survival A is vulnerable and many factors can be involved in the regulation of grain survival A (Guo and Schnurbusch, 2015). In addition, we detected that some associated genes contribute to both grain survival A and spikelet fertility, and this was supported by the fact that grain survival A is a crucial determinant of spikelet fertility (Guo and Schnurbusch, 2015). Notably, QTLs for grain yield potential (max floret A, C, and B and their average) were not associated with grain number per spikelet (grain A, C, B, and their average), suggesting that the grain

yield potential is relatively independent to the final grain number. However, the QTLs associated with floret primordia loss, grain number and grain survival within the spikelets were detected. The analysis of candidate genes suggested a critical role of carbohydrate metabolism and phytohormones in regulating the abortion of floret primordia, grain number and survival.

Previous work suggested that sugar may influence floral transition by activating or inhibiting genes that control floral transition, but the effects are variable according to the concentration of sugars, the genetic background of the plants, and when the sugar is introduced (Ohto et al., 2001; Rolland et al., 2002). In this study, the candidate genes-*SUSIBA2 and SS1* also indicate the involvement of sucrose in the regulation of floral development and degeneration. Hormones have been validated to be important determinants of floral development in Arabidopsis. For example, under short days, gibberellins activate transcription of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* and *LEAFY* at the shoot meristem, two genes encoding transcription factors involved in flowering (Porri et al., 2012); cytokinin promotes flowering of Arabidopsis via transcriptional activation of the *FT* paralogue *TWIN SISTER OF FT* (D'Aloia et al., 2011); crucial roles of brassinosteroid signals in the floral-induction network has been verified in Arabidopsis (Li et al., 2010). The candidate gene-*BRI 1* detected in this study is one potential candidate for the involvement of brassinosteroids in the floral development and demise in wheat.

The role of plant height has been highlighted by the introduction of dwarfing genes, known as *Rht* genes in the Green Revolution (Rebetzke and Richards, 2000; Borojevic and Borojevic, 2005; Pearce et al., 2011). As expected, plant height was involved in this improvement of grain yield due to the introduction of dwarfing genes during the wheat breeding process in the past decades. The most important contribution of the introduction of reduced height

(*Rht*) genes is to increase the allocation of assimilates to a spike, and the improved assimilates allowed more surviving spikelets at the apical part of a spike and increased the surviving distal florets. This role of plant height was confirmed by the shared QTLs with grain average, survival B (two), floret primordia loss B (Data S5.3). Flowering time has been considered historically as a critical factor for determining grain yield, as it is an important trait for the adaptation of bread wheat (*Triticum aestivum* L.) to diverse climatic environments. The adaptability of wheat to a wide range of environments has been favored by allelic diversity in genes regulating growth habits and photoperiod responses (Snape et al., 2001; Herndl et al., 2008; Greenup et al., 2009; Semenov et al., 2014). Unexpectedly, we observed that there is no overlap between flowering time and floret fertility QTL (Data S5.3). These results suggested that floret fertility was partially associated with plant height but independent to flowering time in this study.

The change of assimilate partitioning is almost always companied with the modification of spike morphology, the association of assimilate partitioning with floret fertility suggested by the shared QTLs and correlation analysis seems also clear. These findings raise important questions: How is the connection between spike morphology and floret fertility? How shall we use the connection if it is validated? The connection is of great interest for wheat breeding, since breeders can improve floret fertility/grain yield by selecting spike morphology. The near isogenic lines of plant height, spike length and *tin* gene might be helpful to answer these two questions.

Overall, through phenotypic and genotypic correlation analysis, as well as by considering the detected QTLs and associated genes in this study, we aimed to deepen the understanding of assimilate partitioning and to open the door to more efficient utilization of assimilate partitioning to modify floret fertility and further influence grain yield. Moreover, the

potential association between spike morphology and floret fertility is valuable for wheat breeding.

6. Summary and outlook

6.1. Summary

Floret size and number are two critical traits for monitoring the floral development and degeneration in wheat, so we measured floret size (anther and ovary size was used to display the floret size) and number according to the seven stages. SDW_a is considered as an important factor for the determination of grain number in wheat. Here we found connections between anther and ovary size and SDW_a. In addition, we also determined the influence of detillering on the floral abortion process and tried to explain the influence by increased assimilate partitioning to the spike. Moreover, we found an association between ovary size and grain setting, suggesting an important role of ovary size in the determination of grain number per spike/spikelet. After the physiological analysis of floret size and number, we conducted the GWA mapping of traits associated with floret fertility, assimilate partitioning and spike morphology to assess genetic factors for the regulation of floret fertility and its genetic associations between assimilate distribution and spike morphology.

In experiment one (association between ovary size, anther size and SWD_a), floret size was investigated using anther and ovary size from GA to AN. Broad-sense heritabilities of anther and ovary size were consistently high across positions and stages. The environment had a greater influence on F3 and F4 anther and ovary size than in F1, indicating that F3 and F4 anther and ovary growth is more sensitive to growth conditions. Genotype, on the other hand, had the smallest influence on F1 anther and ovary size at the GA stage, while the residual proportion was largest, which suggests a relatively large variation in F1 anther and ovary size at the GA stage. We also found strong relationships between SWD_a, anther and ovary size, especially, anther and ovary at F3 and F4 positions. The anther and ovary size at F3 and F4 positions is most likely associated with grain number per spike, which further

determines the grain weight per spike/spike dry weight at PM. Hence, F3/4 anther and ovary size can be used as an indicator to breed and select cultivars with high grain number per spike, grain weight per spike and spike dry weight at PM.

In experiment two (time window of floral degeneration/demise), MFS was consistently found at the GA stage; and not at WA stage as previously described (Kirby and Appleyard, 1987). After tiller removal, main stem DW was significantly increased, a marked increase was observed in SWD_a and leaf area at AN. These increases in the different structural parts of main shoot indicate that more available resources may result in an increase of MFS, FFS and GS. Because tillers compete with the main shoot for resources, and competition between the stem and spike is critical for determining grain number, detillering makes more resources available to spike and stem growth on the main shoot, which in turn can alleviate competition and thereby diverts more resources towards floral development. Furthermore, allocation of more resources toward floral development can delay visible floral demise, resulting in one or two more floret primordia or final grains, respectively. Regrettably, more available resources cannot retain all developed floret primordia, and more than half of them still abort. Nevertheless, a better understanding of the underlying developmental and resource-allocation–dependent limiting factors may shed more light on how to increase wheat's yield potential in the future.

In experiment three (association between ovary size, MFS, FFS and GS), MFS, FFS and GS exhibited large genotypic variation and moderate levels of heritability. FFS was closely associated with floret survival and only weakly related to MFS. Post-anthesis process of grain set/abortion was important for the determination of genetic variation in GS; the increase in GS was mainly associated with improved grain survival. Ovary size at anthesis was considered as an important link between floret survival (pre-anthesis) and grain survival

(post-anthesis). In this work, proximal florets (i.e. the first three florets from the base of a spikelet: F1, F2, F3) produced fertile florets and set grains in most cases. The ovary size of more distal florets (F4 and beyond) seemed to have acted as a decisive factor for grain setting and effectively reflected pre-anthesis floret development. GS displayed positive correlations with ovary size of florets in distal position (F4), suggesting that assimilates allocated to more distal florets may play a critical role in regulating grain set.

In experiment four (genetic relationships between floret fertility, assimilate partitioning and spike morphology), we conducted a GWAS by quantifying 54 traits (16 traits for floret fertility and 38 traits for assimilates partitioning and spike morphology) in 210 wheat accessions. We detected QTLs shared by different traits, it suggested the potential associations between floret fertility, assimilates partitioning and spike morphology. These associations were further observed and validated in a detillering experiment. Several candidate genes involved in the metabolism of carbohydrates, phytohormones and floral development were confirmed by the shared QTLs. Finally, we proposed a network underlying floret fertility related traits based on the GWAS results and discovered interesting, novel determinants of yield performance and potential target traits for wheat breeding.

6.2. Zusammenfassung

Blütchengröße und Anzahl sind zwei kritische Kenngrößen zur Verfolgung der Blütchenentwicklung und Degeneration in Weizen. Im Rahmen dieser Arbeit wurden diese beiden Merkmale in den sieben Entwicklungsstadien vermessen, wobei Antheren- und Ovargröße als Maß für die Blütchenfertilität verwendet wurde. Das Trockengewicht der Ähre zum Zeitpunkt der Blüte/Anthese ("Spike dry weight at anthesis", SDWa) gilt als wichtiger Faktor für die Bestimmung der Kornzahl im Weizen. In unseren Untersuchunfgen fanden wir

Verbindungen zwischen der Antheren- und Ovargröße und SDWa. Zudem haben wir auch den Einfluss der Entfernung von Seitentrieben auf den Prozess des Blütchen-Aborts bestimmt und versucht den Einfluss auf die Ähre durch eine erhöhte Assimilat-Verteilung zu erklären. Darüber hinaus fanden wir einen Zusammenhang zwischen Ovargröße und Kornansatz, was auf eine wichtige Rolle der Ovargröße bei der Bestimmung der Kornzahl pro Ähre/Ährchen hindeutet. Nach der physiologischen Analyse von Blütchengröße und -anzahl, führten wir eine genomweite Assoziationskartierung (GWAS) von Merkmalen durch, welche mit der Blütchenfruchtbarkeit, der Assimilat-Verteilung und der Ähren-Morphologie assoziiert sind. Hierdurch sollten genetische Faktoren für die Regulation der Blütchenfruchtbarkeit bestimmt und die genetischen Assoziationen zwischen Assimilat-Verteilung und Ähren-Morphologie evaluiert werden.

In Experiment 1 (Assoziation zwischen Antheren- und Ovargröße und SDWa) wurde die Blütchengröße unter Verwendung von Antheren- und Ovargröße in den Entwicklungsstadien von der Grünen Anthere („green anther“, GA) bis zur Anthese („anthesis“, AN) untersucht. Die "broad sense" Heritabilitäten von Antheren- und Ovargröße waren sowohl über Positionen, als auch über Entwicklungsstadien durchweg hoch. Die Umwelt hatte einen größeren Einfluss auf die Blütchen in Position 3 und 4 (F3 und F4), als auf die F1 Antheren- und Ovargröße, was darauf hindeutet, dass das Wachstum von F3 und F4 Antheren und Ovarien empfindlicher auf geänderte Wachstumsbedingungen reagiert. Der Genotyp hatte hingegen den geringsten Einfluss auf die F1 Antheren- und Ovargröße im GA-Stadium, während der Anteil der Restvarianz am höchsten war, was auf eine relativ große Variation der F1 Antheren- und Ovargröße im GA-Stadium hindeutet. Zudem fanden wir starke Wechselbeziehungen zwischen SWDa, Antheren- und Ovargröße, vor allem zwischen Antheren und Ovarien in F3 und F4 Position. Die Antheren- und Ovargröße der F3 und F4

Positionen ist höchstwahrscheinlich mit der Kornzahl pro Ähre assoziiert, die ferner das Korngewicht pro Ähre bzw. Ähren-Trockengewicht zum Zeitpunkt der physiologischen Reife („physiological maturity“, PM) bestimmt. Daher kann die F3/F4 Antheren- und Ovargrösse als Indikator für die Züchtung und die Auswahl von Sorten mit hoher Kornzahl pro Ähre, hohem Korngewicht pro Ähre und hohem Ähren-Trockengewicht zum Zeitpunkt der physiologischen Reife verwendet werden.

In Experiment zwei (Zeitfenster der Blütchendegeneration/Absterben) wurde die maximale Anzahl der Blütchenprimordien pro Ährchen („maximum number of floret primordia per spikelet“, MFS) durchgehend im GA-Stadium gefunden; und nicht im WA-Stadium, wie zuvor beschrieben (Kirby und Appleyard, 1987). Nach der Entfernung der Seitentriebe war das Trockengewicht des Hautptriebs signifikant erhöht. Zudem wurde ein signifikanter Anstieg von SWDa und der Blattfläche im AN-Stadium beobachtet. Dieser Zuwachs in den verschiedenen strukturellen Teilen des Haupttriebs deuten darauf hin, dass mehr verfügbare Ressourcen zu einer Zunahme von MFS, der Anzahl der fertilen Blütchen pro Ährchen („fertile florets per spikelet“, FFS) und Kornzahl pro Ähre („grain number per spike“, GS) führen können. Da Seitentriebe mit dem Haupttrieb um Ressourcen konkurrieren und Konkurrenz zwischen dem Halm und der Ähre entscheidend für die spätere Kornzahl ist, werden durch die Entfernung der Seitentriebe mehr Ressourcen für das Wachstum von Haupttrieb und Ähre verfügbar. Dies minderte den Wettbewerb und es wurden mehr Ressourcen in die Blütchenentwicklung umgeleitet. Darüber hinaus kann die Allokation von mehr Ressourcen in die Blütenentwicklung ein sichtbares Blütenabsterben verzögern, was zu einem oder zwei weiteren Blütchen-Primordien bzw. Körnern resultieren kann. Bedauerlicherweise sind die zusätzlich verfügbaren Ressourcen nicht ausreichend um alle sich entwickelten Blütchen-Primordien zu erhalten, sodass mehr als die Hälfte von diesen

dennoch absterben. Nichtsdestotrotz kann ein besseres Verständnis der zugrunde liegenden Limitierungsfaktoren von Entwicklung und Ressourcenzuteilung bei der Klärung der Frage helfen, wie das Ertragspotenzial von Weizen künftig gesteigert werden kann.

In Experiment 3 (Assoziation zwischen Ovargöße, MFS, FFS und GS) zeigten MFS, FFS und GS eine große genotypische Variabilität und moderate Heritabilitäten. FFS war eng mit dem Überleben von Blütchen, jedoch nur schwach mit MFS assoziiert. Der Prozess des Kornansatzes/Aborts, der nach der Anthrese erfolgt, war wichtig für die Bestimmung der genetischen Variation von GS; so war die Zunahme von GS hauptsächlich mit einem verbesserten Überleben des Korns verbunden. Die Ovargröße bei der Anthese wurde als ein wichtiges Bindeglied zwischen dem Überleben der Blütchen (Vorblüte/Pre-Anthese) und dem Überleben des Korns (Post-Anthese) betrachtet. In dieser Arbeit bildeten die proximalen Blütchen (d. H. die ersten drei Blütchen von der Basis eines Ährchens: F1, F2, F3) fruchtbare Blütchen, die in den meisten Fällen Körner ansetzten. Die Ovargröße von eher distal gelegenen Blütchen (F4 und darüber hinaus) schien als ein maßgebender Faktor für den Kornansatz zu agieren und reflektierte effektiv die Blütchenentwicklung vor der Anthese. GS wies eine positive Korrelationen mit der Ovargröße von Blütchen in der distalen Position (F4) auf, was darauf hindeutet, dass die Assimilatallokation, zu eher distalen Blütchen, eine kritische Rolle bei der Regulierung des Kornsatzes spielen können.

In Experiment 4 (genetische Zusammenhänge zwischen Blütchenfruchtbarkeit, Assimilat-Partitionierung und Ähren-Morphologie) wurde eine genomweite Assoziationsstudie (GWAS) durchgeführt, wobei 54 Merkmalen (16 Merkmale für Blütchenfruchtbarkeit und 38 Merkmale für Assimilat-Partitionierung und Ähren-Morphologie) in 210 Weizen-Akzessionen quantifiziert wurden. Wir detektieren QTLs, die bei verschiedenen Merkmalen detektiert wurden, was potentielle Assoziationen zwischen Blütchenfruchtbarkeit, Assimilat-

Partitionierung und Ähren-Morphologie nahelegt. Diese Assoziationen wurden in einem weiteren Versuch beobachtet und validiert, in welchem die Seitentriebe der Pflanzen entfernt wurden. Mehrere Kandidatengene, die am Stoffwechsel von Kohlenhydraten, Phytohormonen und der Blütchenentwicklung beteiligt sind, wurden durch die gemeinsamen QTLs bestätigt. Abschließend schlagen wir ein Netzwerk für blütchenfruchtbarkeits-bezogene Merkmale vor, welches auf den GWAS-Ergebnissen beruht und beschreiben interessante, neuartige Determinanten für die Ertragsleistung und potentielle Zielmerkmale für die Weizenzüchtung.

6.3. Outlook

6.3.1. Relationships between floret fertility, assimilate partitioning and spike morphology

It was concluded that the improvement of floret fertility (MFS, FFS and GS) and the delay of floral abortion increased assimilate partitioning to the spike based on our physiological work in the first three experiments. Meanwhile, spike morphology was also modified by detillering, e.g. increased spike length and spikelet size (Fig. 5.7). Moreover, the shared QTLs from our GWAS analyses of chapter four also suggest potential genetic connections between floret fertility, assimilate distribution and spike morphology. Furthermore, previous studies also indicated that the modification of spike morphology (mainly spike length) is one outcome of increased assimilate distribution (Miralles and Slafer, 1995; Duggan et al., 2005; Kebrom et al., 2012) which may further affect ovay size. Therefore, it is interesting to separately validate effects of assimilate partitioning and spike morphology on floret fertility, using existing germplasm resources. For example, near isogenic lines (NILs) for spike length can be used to determine the effects of spike morphology (mainly increased space for floret

development) on the floret development and abortion process as well as ovary size. NILs for awn development are useful to assess the effects of assimilate supply on the floret development and degeneration process and ovary size, since awn setting may compete for assimilates during the initiation and growth of floret primrodia. Plant height has been the most successful example of altering the assimilate allocation to the spike during wheat breeding process in the past decades. NILs for plant height (*Rht* gene) are good resources to determine the influence of assimilate partitioning on spike morphology and floret fertility. Tiller removal is an artificial approach for increasing assimilate distribution to the spike; notably, additional damage can be caused by using this approach. To avoid such damage, we plan to use NILs for tillering inhibition (*tin*) gene to further test the assimilate allocation on floral demise and ovary size. Therefore, we plan to use the NILs for spike length, awn development, *tin* gene and plant height (see introduction of NILs below) to further validate the associations between floret fertility, assimilate allocation and spike morphology.

NILs for spike length. Through the recurrent backcrossing method in the genetic background of common wheat (*Triticum aestivum*) cv. Chinese Spring, one NIL carrying a marker gene was reported by Tsujimoto (2001). It was observed that *C* (compact spike) gene shortened the spike and decreased the total spikelet number. However, it increased the number of grains per spike because the number of grains per spikelet increased from 2.6 to 3.7. This gene did not affect plant height, indicating that the rachis and culm length are controlled by independent genetic determinants. The modification of spike length and spikelet density without changing plant height in this NIL would be helpful to determine the spike morphology on floral development and demise.

NILs for awn. Awns are threadlike extensions of the lemma in most grasses. The seeds of many wild grass species have large and barbed awns, which can fend off seed-eating animals,

assist in seed dispersal, and help plants to keep the seeds (Elbaum et al., 2007). Awns may significantly increase grain yield due to greater water-use efficiency, high temperature-tolerance, and higher photosynthetic caused by awns (Evans et al., 1972; Blum, 1985; Weyhrich et al., 1995; Motzo and Giunta, 2002; Guo and Schnurbusch, 2016; Rebetzke et al., 2016). The positive influence of awns on grain yield in wheat can be offsetted by the costs of awn setting. As it is hypothesized by Rebetzke et al. (2016), allocation of assimilates to large and rapidly developing awns decreases fertile spikelet number and floret fertility and reduces grain number particularly in more distal florets. It appears that awn setting can decrease assimilate partitioning towards the wheat spike which is associated with a decreased number of fertile florets. Therefore, it is also interesting to investigate the effects of awn setting on the floral development and abortion.

NILs for *tin* gene. A wheat mutant with reduced tillering, thicker stems, darker leaves, and larger spikes with more and larger grains was identified and characterized (Fig. 6.1) (Atsmon and Jacobs, 1977; Richards, 1988). The reduced tiller number of *tin* mutant is probably due to early cessation of tillering (Duggan et al., 2002). Usin the NILs for *tin* gene, it was observed that the higher kernel weight of the *tin* lines under stress conditions was associated with greater anthesis biomass and increased stem water-soluble carbohydrates, ensuring more assimilate for later translocation to filling grain (Mitchell et al., 2012; Mitchell et al., 2013). It was also found that tiller inhibition in *tin* mutants is associated with the precocious elongation of basal internodes, which are solid rather than hollow, and arrest of bud growth is due to diversion of sucrose away from the axillary bud to support internode elongation (Kebrom et al., 2012). More recently, it was detected that *tin* gene can influence root–shoot carbon partitioning and pattern of water use to improve wheat productivity in rainfed environments (Hendriks et al., 2016) and canopy architectural and physiological

characterization of NILs for *tin* gene was also assessed (Moeller et al., 2014). Based on the excellecnt work in previous study, there is a good opportunity to further determine the effects of *tin* gene on the floral demise process and association among floret fertility assimilate distribution and spike morphology.

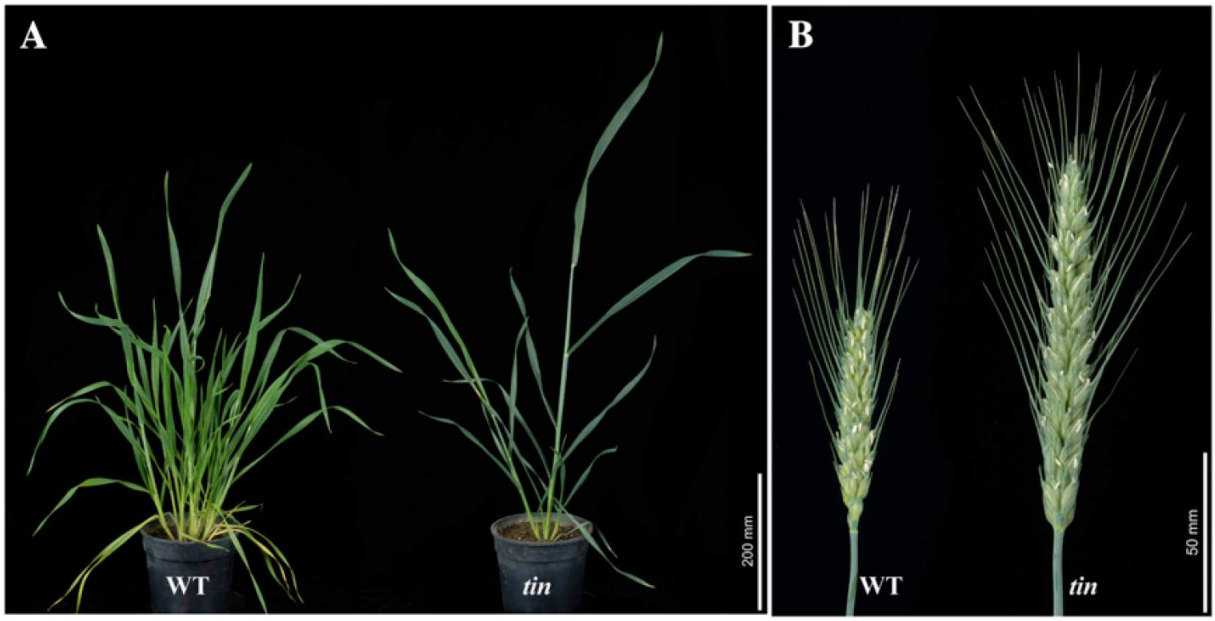


Fig. 6.1. A, Wild-type (WT; left) and *tin* mutant (right) wheat plants. **B**, The spike of the main stem of WT (left) and tin mutant (right) (source: Kebrom et al. 2012).

NILs for plant height. Plant height reduced (*Rht*) genes have greatly reduced the plant height and increased assimilates partitioning to spike (Fig. 6.2). The main achievement derived from the introduction of the *Rht-B1* dwarfing gene was an increase in the number of grains per spikelet, but it did not have any effect on the number of spikelets on the main spike (Alvaro et al., 2008). Miralles et al. (1998) concluded that *Rht*1 genes reduced the final sizes of vegetative organs (such as internodes and leaves) and of tissues (pericarp) associated with reproductive structures (grains). Therefore the NILs for *Rht* genes provide an opportunity to study the links among floret fertility, assimilate distribution and spike morphology.

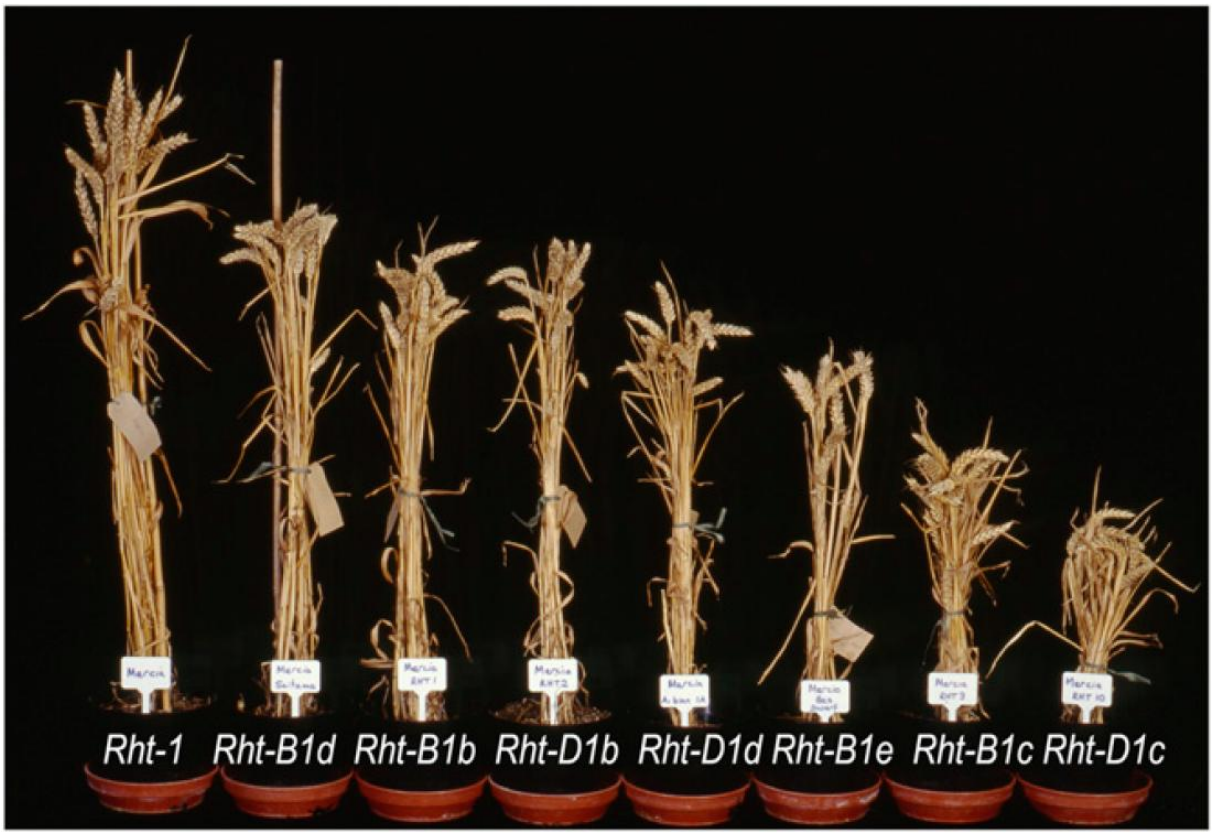


Fig. 6.2. Phenotypes of *Rht-B1* and *Rht-D1* dwarfing alleles in NILs (source:Pearce et al., 2011).

Summarily, these NILs provide us the opportunities to study the roles of assimilates and spike morphology on the floral development and abortion.

6.3.2. Flowering time genes

The control of flowering is critical to reproductive development, and has a major impact on grain yield in wheat (Bogard et al., 2011; Muhleisen et al., 2014). Flowering time is a crucical trait for the adaptation of wheat to diverse environments, variation in flowering time is crucial for the successful expansion of wheat during the domestication process (Snape et al., 2001; Kamran et al., 2014). The adaptability of wheat to a wide range of environments has been favored by allelic diversity in genes related to flowering time. The functions of several important flowering time genes have been validated. *Vernalization* (*Vrn*) genes determine

spring and winter wheat habits; *photoperiod* (*Ppd*) genes are critical for determining the sensitivity to photoperiod; earliness *per se* (*Eps)* genes are those that regulate flowering time independently of the effects derived from photoperiod and vernalization (Lewis et al., 2008). *FLOWERING LOCUS T* (*FT*) genes are crucial for the accelerated flowering in response to long days (Turck et al., 2008). *CONSTANS* (*CO*) genes may promote flowering and mediate interaction between the circadian clock and the control of flowering, also regulate the activities of *FT* genes (Putterill et al., 1995; Kardailsky et al., 1999; Suarez-Lopez et al., 2001). The QTLs detected in the GWAS analysis revealed that the flowering genes--*CO4*, *CO6*, *eps2*, *FT2,* are very likely involved in the regulation of floral degeneration and floret fertility. This is consistent with previous studies that the *CO* and *FT* genes are not only involved in the regulation of flowering time, but may play important roles in floral development/fertility which is also critical for grain yield improvement (Kardailsky et al., 1999; Samach et al., 2000). Although some genes about plant growth and flowering time were not detected in this study , they may also play an important role in floret fertility in wheat. Phytochromes are encoded by three genes (*phyA*, *phyB*, and *phyC*) in most monocot species, phytochrome genes play a major role in optimizing growth and development under different light environments (Sharrock, 2008; Mathews, 2010). The effect of the *EPS-A1* gene on flowering time is highly-dependent on temperature conditions and *EPS-A1* gene can increase spikelet number per spike by delaying the transition from vegetative to reproductive growth (an extension between the double ridge state and the terminal spikelet stage) in diploid wheat (Lewis et al., 2008; Gawronski et al., 2014). As spikelet number per spike can be increased by *EPS-A1* gene (Lewis et al., 2008), combining with the results in my PhD study: the potential interaction between spike morphology and floral development and abortion, it is concluded *EPS-A1* gene may influence the floret fertility and floral demise.

Although no phytochrome genes were detected as candidate genes for floret fertility, they can still be potential determinants, because it was reported that wheat PHYC forms active signaling homodimers that translocate into the nucleus in red light to mediate photomorphogenic responses (Chen et al., 2014). Ugarte et al. (2010) found that low red light/far-red light ratios decreased grain yield per plant, delayed spike growth and development, reduced the expression of spike marker genes, accelerated the development of florets already initiated, and reduced the number of fertile florets at anthesis in wheat. Taken together, it is concluded that the phytochrome genes may play important roles during floral development and demise in wheat which should be further validated.

6.3.3. Mechanisms of floral degeneration

The work on the floral development and abortion process in wheat is helpful to identify the key stages for the improvement of grain yield. But the mechanism of floral development and degeneration is still not clearly understood. Map-based cloning of genes is an effectively and widely used approach to genetically investigate phenotypes. According to our experience, however, the observed sensitivity of floral degeneration and its lack of genotypic variation in the used winter and spring wheat panels (i.e. apical floret primordia within a spikelet have always been aborted in all the genotypes) fairly limits this approach. To continue the work for detecting genetic factors involved in floral degeneration, we should consider other options. For example, the high-throughput microscopy to single cell resolution can be used to monitor cell morphology and activities accross the floral development and demise process in wheat. Meanwhile, RNAseq and omics technologies can also be applied during this process. Combined with mathematical modeling, we hopefully can detect some key genes / proteins and hypothesize about their regulation related to floral degeneration. In addition, it

is also interesting to assess roles of phytohormones by determining the dynamic change of phytohormones during this process. Even more, since we detected one candidate gene related to floret fertiltiy--*BRI1* which is a phytohormone associated gene and severely affect plant growth and development (Yamamuro et al., 2000; Li et al., 2001). Moreover, lots of studies in different species (e.g. Arabidopsis, rice, maize) have proved that epigenetic (e.g. DNA methylation, histone modification, microRNA) regulation is involved in cell differentiation and apoptosis (Jovanovic and Hengartner, 2006; Sun et al., 2014; Huo et al., 2015; Jeon et al., 2015; Wang et al., 2015), it is also necessary to assess the involvement of epigenetics during floral degeneration.

6.3.4. Potential SNP markers for improvement of floret fertility

The introduction of *Rht* has markedly increased assimilates to spikes' growth in wheat which further greatly increased grain yields (Abbate et al., 1998; Fischer, 2007; Foulkes et al., 2011), As current cultivars have already reached an optimum height to maximize grain yield (Richards, 1992; Miralles and Slafer, 1995; Flintham et al., 1997; Slafer et al., 2015), alternatives must be identified for further increasing grain yield. Improvement of floret fertility is an important option to increase grain yield for wheat breeding. According to the physiological and genetic work, increasing utilization efficiency of assimilates and developing spike morphology suitable for floret survival are two alternatives. The SNPs associated with floret fertility, utilization efficiency of assimilate (e.g. spike fertility index, harvest index) and spike morphology can be used in the wheat breeding. For example, the 15 SNPs on Chr. 3A (57.08-58.21 cM) associated with grain number and grain survival within individual spikelets (grain A, survival A) are valuable to increase grain number through surviving more florets to grains in apical spikelets (Data S5.3), which usually contribute most of the loss of grain yield

potential. In addition, 10 SNPs on Chr. 2B (55.77-58.14 cM) are linked to floret primordia loss B and the ratio between grain weight and spike chaff (Data S5.3), it suggests these SNPs can help to increase the ustilization of spike chaff (assimilates) by manipulating the floret primordia loss at basal spikelets. All in all, the SNPs associated with floret fertility detected in the GWAS are promising to be used in wheat breeding after further validation.

Summarily, NILs can be used to further varify the influence of assimilate distribution and spike morphology during floral development and degeneration, the importance of flowering time genes for the regulation of floral abortion can also be validated based on previous work. Moreover, by using latest omics technologies, the influence of hormones, epigenetic regulation and other factors in floral development and degeneration process will be assessed. The SNPs shared by floret fertility, assimilate partitioning and spike morphology may be used for the improvement of grain yield in wheat breeding.

7. Bibliography

Abbate PE, Andrade FH, Culot JP (1995) The Effects of Radiation and Nitrogen on Number of Grains in Wheat. Journal of Agricultural Science **124:** 351-360

Abbate PE, Andrade FH, Culot JP, Bindraban PS (1997) Grain yield in wheat: Effects of radiation during spike growth period. Field Crops Research **54:** 245-257

Abbate PE, Andrade FH, Lazaro L, Bariffi JH, Berardocco HG, Inza VH, Marturano F (1998) Grain yield increase in recent argentine wheat cultivars. Crop Science **38:** 1203-1209

Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Reports **27:** 617-631

Aguilar-Martinez JA, Poza-Carrion C, Cubas P (2007) Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. Plant Cell **19:** 458-472

Ainsworth EA, Ort DR (2010) How Do We Improve Crop Production in a Warming World? Plant Physiology **154:** 526-530

Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. Genome Research **19:** 1655-1664

Alqudah AM, Schnurbusch T (2014) Awn primordium to tipping is the most decisive developmental phase for spikelet survival in barley. Functional Plant Biology **41:** 424-436

Alvaro F, Isidro J, Villegas D, del Moral LFG, Royo C (2008) Old and modern durum wheat varieties from Italy and Spain differ in main spike components. Field Crops Research **106:** 86-93

Aranjuelo I, Sanz-Saez A, Jauregui I, Irigoyen JJ, Araus JL, Sanchez-Diaz M, Erice G (2013) Harvest index, a parameter conditioning responsiveness of wheat plants to elevated CO2. Journal of Experimental Botany **64:** 1879-1892

Arisnabarreta S, Miralles DJ (2010) Nitrogen and radiation effects during the active spike-growth phase on floret development and biomass partitioning in 2-and 6-rowed barley isolines. Crop & Pasture Science **61:** 578-587

Atsmon D, Bush MG, Evans LT (1986) Stunting in Gigas Wheat as Influenced by Temperature and Daylength. Australian Journal of Plant Physiology **13:** 381-389

Atsmon D, Jacobs E (1977) Newly Bred Gigas Form of Bread Wheat (Triticum-Aestivum L) - Morphological Features and Thermo-Photoperiodic Responses. Crop Science **17:** 31-35

Aulchenko YS, Ripke S, Isaacs A, Van Duijn CM (2007) GenABEL: an R library for genome-wide association analysis. Bioinformatics **23:** 1294-1296

Austin RB, Ford MA, Morgan CL (1989) Genetic-Improvement in the Yield of Winter-Wheat - a Further Evaluation. Journal of Agricultural Science **112:** 295-301

Austin RB, Morgan CL, Ford MA, Blackwell RD (1980) Contributions to Grain-Yield from Pre-Anthesis Assimilation in Tall and Dwarf Barley Phenotypes in 2 Contrasting Seasons. Annals of Botany **45:** 309-319

Bancal P (2008) Positive contribution of stem growth to grain number per spike in wheat. Field Crops Research **105:** 27-39

Bancal P (2009) Early development and enlargement of wheat floret primordia suggest a role of partitioning within spike to grain set. Field Crops Research **110:** 44-53

Barnabas B, Jager K, Feher A (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant Cell and Environment **31:** 11-38

Baroja-Fernandez E, Munoz FJ, Li J, Bahaji A, Almagro G, Montero M, Etxeberria E, Hidalgo M, Sesma MT, Pozueta-Romero J (2012) Sucrose synthase activity in the sus1/sus2/sus3/sus4 Arabidopsis mutant is sufficient to support normal cellulose and starch production. Proceedings of the National Academy of Sciences of the United States of America **109:** 321-326

Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B-Methodological **57:** 289-300

Bidinger F, Musgrave RB, Fischer RA (1977) Contribution of Stored Pre-Anthesis Assimilate to Grain-Yield in Wheat and Barley. Nature **270:** 431-433

Biswas S, Scheinfeldt LB, Akey JM (2009) Genome-wide Insights into the Patterns and Determinants of Fine-Scale Population Structure in Humans. American Journal of Human Genetics **84:** 641-650

Blake NK, Lehfeldt BR, Lavin M, Talbert LE (1999) Phylogenetic reconstruction based on low copy DNA sequence data in an allopolyploid: The B genome of wheat. Genome **42:** 351-360

Blum A (1985) Photosynthesis and Transpiration in Leaves and Ears of Wheat and Barley Varieties. Journal of Experimental Botany **36:** 432-440

Blum A (1998) Improving wheat grain filling under stress by stem reserve mobilisation (Reprinted from Wheat: Prospects for global improvement, 1998). Euphytica **100:** 77-83

Blum A, Sinmena B, Mayer J, Golan G, Shpiler L (1994) Stem Reserve Mobilization Supports Wheat-Grain Filling under Heat-Stress. Australian Journal of Plant Physiology **21:** 771-781

Bogard M, Jourdan M, Allard V, Martre P, Perretant MR, Ravel C, Heumez E, Orford S, Snape J, Griffiths S, Gaju O, Foulkes J, Le Gouis J (2011) Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. Journal of Experimental Botany **62:** 3621-3636

Borojevic K, Borojevic K (2005) The transfer and history of "reduced height genes" (Rht) in wheat from Japan to Europe. Journal of Heredity **96:** 455-459

Borras-Gelonch G, Rebetzke GJ, Richards RA, Romagosa I (2012) Genetic control of duration of pre-anthesis phases in wheat (Triticum aestivum L.) and relationships to leaf appearance, tillering, and dry matter accumulation. Journal of Experimental Botany **63:** 69-89

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics **23:** 2633-2635

Brenchley R, Spannagl M, Pfeifer M, Barker GLA, D/'Amore R, Allen AM, McKenzie N, Kramer M, Kerhornou A, Bolser D, Kay S, Waite D, Trick M, Bancroft I, Gu Y, Huo N, Luo M-C, Sehgal S, Gill B, Kianian S, Anderson O, Kersey P, Dvorak J, McCombie WR, Hall A, Mayer KFX, Edwards KJ, Bevan MW, Hall N (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature **491:** 705-710

Calderini DF, Abeledo LG, Savin R, Slafer GA (1999) Effect of temperature and carpel size during pre-anthesis on potential grain weight in wheat. Journal of Agricultural Science **132:** 453-459

Castillo AM, Sanchez-Diaz RA, Valles MP (2015) Effect of ovary induction on bread wheat anther culture: ovary genotype and developmental stage, and candidate gene association. Frontiers in Plant Science **6:** fpls.2015.00402

Chandler PM, Harding CA (2013) 'Overgrowth' mutants in barley and wheat: new alleles and phenotypes of the 'Green Revolution' Della gene. Journal of Experimental Botany **64:** 1603-1613

Chen A, Li CX, Hu W, Lau MY, Lin HQ, Rockwell NC, Martin SS, Jernstedt JA, Lagarias JC, Dubcovsky J (2014) PHYTOCHROME C plays a major role in the acceleration of wheat flowering under long-day photoperiod. Proceedings of the National Academy of Sciences of the United States of America **111:** 10037-10044

Chen W, Gao YQ, Xie WB, Gong L, Lu K, Wang WS, Li Y, Liu XQ, Zhang HY, Dong HX, Zhang W, Zhang LJ, Yu SB, Wang GW, Lian XM, Luo J (2014) Genome-wide association analyses provide genetic and biochemical insights into natural variation in rice metabolism. Nature Genetics **46:** 714-721

Clark RM (2010) Genome-wide association studies coming of age in rice. Nature Genetics **42:** 926-927

Cockram J, White J, Zuluaga DL, Smith D, Comadran J, Macaulay M, Luo ZW, Kearsey MJ, Werner P, Harrap D, Tapsell C, Liu H, Hedley PE, Stein N, Schulte D, Steuernagel B, Marshall DF, Thomas WTB, Ramsay L, Mackay I, Balding DJ, Waugh R, O'Sullivan DM, Consortium A (2010) Genome-wide association mapping to candidate polymorphism resolution in the

unsequenced barley genome. Proceedings of the National Academy of Sciences of the United States of America **107:** 21611-21616

Collins F (2010) Has the revolution arrived? Nature **464:** 674-675

Craufurd PQ, Cartwright PM (1989) Effect of Photoperiod and Chlormequat on Apical Development and Growth in a Spring Wheat (Triticum-Aestivum) Cultivar. Annals of Botany **63:** 515-525

Crawford S, Shinohara N, Sieberer T, Williamson L, George G, Hepworth J, Muller D, Domagalska MA, Leyser O (2010) Strigolactones enhance competition between shoot branches by dampening auxin transport. Development **137:** 2905-2913

Crowell S, Korniliev P, Falcao A, Ismail A, Gregorio G, Mezey J, McCouch S (2016) Genome-wide association and high-resolution phenotyping link Oryza sativa panicle traits to numerous trait-specific QTL clusters. Nature Communications **7:** 10527

Cruz-Huerta N, Williamson JG, Darnell RL (2011) Low Night Temperature Increases Ovary Size in Sweet Pepper Cultivars. Hortscience **46:** 396-401

Curtis D (1996) Genetic dissection of complex traits. Nature Genetics **12:** 356-357

D'Aloia M, Bonhomme D, Bouche F, Tamseddak K, Ormenese S, Torti S, Coupland G, Perilleux C (2011) Cytokinin promotes flowering of Arabidopsis via transcriptional activation of the FT paralogue TSF. Plant Journal **65:** 972-979

Darnell RL, Cruz-Huerta N, Williamson JG (2012) Night temperature and sourcesink effects on overall growth, cell number and cell size in bell pepper ovaries. Annals of Botany **110:** 987-994

Davidson DJ, Chevalier PM (1990) Preanthesis Tiller Mortality in Spring Wheat. Crop Science **30:** 832-836

Dawson IA, Wardlaw IF (1989) The Tolerance of Wheat to High-Temperatures during Reproductive Growth .3. Booting to Anthesis. Australian Journal of Agricultural Research **40:** 965-980

De Storme N, Geelen D (2014) The impact of environmental stress on male reproductive development in plants: biological processes and molecular mechanisms. Plant Cell and Environment **37:** 1-18

Demotes-Mainard S, Jeuffroy MH (2001) Incorporating radiation and nitrogen nutrition into a model of kernel number in wheat. Crop Science **41:** 415-423

Demotes-Mainard S, Jeuffroy MH (2004) Effects of nitrogen and radiation on dry matter and nitrogen accumulation in the spike of winter wheat. Field Crops Research **87:** 221-233

Dencic S, Kastori R, Kobiljski B, Duggan B (2000) Evaluation of grain yield and its components in wheat cultivars and landraces under near optimal and drought conditions. Euphytica **113:** 43-52

Devlin B, Roeder K (1999) Genomic control for association studies. American Journal of Human Genetics **65:** A83-A83

Devos KM, Beales J, Ogihara Y, Doust AN (2005) Comparative sequence analysis of the Phytochrome C gene and its upstream region in allohexaploid wheat reveals new data on the evolution of its three constituent genomes. Plant Molecular Biology **58:** 625-641

Diniz JAF, Soares TN, Lima JS, Dobrovolski R, Landeiro VL, Telles MPD, Rangel TF, Bini LM (2013) Mantel test in population genetics. Genetics and Molecular Biology **36:** 475-485

Donald CM (1962) In search of yield. J. Aust. Inst. Agric. Sci. **28:**171-178

Donald CM, Hamblin J (1976) **T**he biological yield and harvest index of cereals as agronomic and plant breeding criteria. Advances in Agronomy **28:** 361-405

Dorion S, Lalonde S, Saini HS (1996) Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. Plant Physiology **111:** 137-145

Dreccer MF, Chapman SC, Rattey AR, Neal J, Song YH, Christopher JT, Reynolds M (2013) Developmental and growth controls of tillering and water-soluble carbohydrate accumulation in contrasting wheat (Triticum aestivum L.) genotypes: can we dissect them? Journal of Experimental Botany **64:** 143-160

Dreccer MF, van Herwaarden AF, Chapman SC (2009) Grain number and grain weight in wheat lines contrasting for stem water soluble carbohydrate concentration. Field Crops Research **112:** 43-54

Dreccer MF, Wockner KB, Palta JA, McIntyre CL, Borgognone MG, Bourgault M, Reynolds M, Miralles DJ (2014) More fertile florets and grains per spike can be achieved at higher temperature in wheat lines with high spike biomass and sugar content at booting. Functional Plant Biology **41:** 482-495

Duggan BL, Richards RA, Tsuyuzaki H (2002) Environmental effects on stunting and the expression of a tiller inhibition (tin) gene in wheat. Functional Plant Biology **29:** 45-53

Duggan BL, Richards RA, van Herwaarden AF, Fettell NA (2005) Agronomic evaluation of a tiller inhibition gene (tin) in wheat. I. Effect on yield, yield components, and grain protein. Australian Journal of Agricultural Research **56:** 169-178

Dvorak J, Appels R (1982) Chromosome and Nucleotide-Sequence Differentiation in Genomes of Polyploid Triticum Species. Theoretical and Applied Genetics **63:** 349-360

Dvorak J, Zhang HB, Kota RS, Lassner M (1989) Organization and Evolution of the 5s-Ribosomal Rna Gene Family in Wheat and Related Species. Genome **32:** 1003-1016

Edgerton MD (2009) Increasing Crop Productivity to Meet Global Needs for Feed, Food, and Fuel. Plant Physiology **149:** 7-13

Elalaoui AC, Simmons SR, Crookston RK (1988) Effects of Tiller Removal on Spring Barley. Crop Science **28:** 305-307

Elbaum R, Zaltzman L, Burgert I, Fratzl P (2007) The role of wheat awns in the seed dispersal unit. Science **316:** 884-886

Engelke T, Hirsche J, Roitsch T (2010) Anther-specific carbohydrate supply and restoration of metabolically engineered male sterility. Journal of Experimental Botany **61:** 2693-2706

Evans LT, Bingham J, Roskams MA (1972) Pattern of Grain Set within Ears of Wheat. Australian Journal of Biological Sciences **25:** 1-8

Evans LT, Sutherla.J, Jackson P, Bingham J (1972) Effect of Awns and Drought on Supply of Photosynthate and Its Distribution within Wheat Ears. Annals of Applied Biology **70:** 67-&

Ferrante A, Savin R, Slafer GA (2010) Floret development of durum wheat in response to nitrogen availability. Journal of Experimental Botany **61:** 4351-4359

Ferrante A, Savin R, Slafer GA (2013) Floret development and grain setting differences between modern durum wheats under contrasting nitrogen availability. Journal of Experimental Botany **64:** 169-184

Ferrante A, Savin R, Slafer GA (2013) Is floret primordia death triggered by floret development in durum wheat? Journal of Experimental Botany **64:** 2859-2869

Fischer RA (1985) Number of Kernels in Wheat Crops and the Influence of Solar-Radiation and Temperature. Journal of Agricultural Science **105:** 447-461

Fischer RA (1993) Irrigated Spring Wheat and Timing and Amount of Nitrogen-Fertilizer .2. Physiology of Grain-Yield Response. Field Crops Research **33:** 57-80

Fischer RA (2007) Understanding the physiological basis of yield potential in wheat. Journal of Agricultural Science **145:** 99-113

Fischer RA (2008) The importance of grain or kernel number in wheat: A reply to Sinclair and Jamieson. Field Crops Research **105:** 15-21

Fischer RA (2011) Wheat physiology: a review of recent developments. Crop & Pasture Science **62:** 95-114

Fischer RA, Stockman YM (1980) Kernel Number Per Spike in Wheat (Triticum-Aestivum L) - Responses to Preanthesis Shading. Australian Journal of Plant Physiology **7:** 169-180

Fischer RA, Stockman YM (1986) Increased Kernel Number in Norin 10-Derived Dwarf Wheat - Evaluation of the Cause. Australian Journal of Plant Physiology **13:** 767-784

Flintham JE, Borner A, Worland AJ, Gale MD (1997) Optimizing wheat grain yield: Effects of Rht (gibberellin-insensitive) dwarfing genes. Journal of Agricultural Science **128:** 11-25

Foulkes MJ, Slafer GA, Davies WJ, Berry PM, Sylvester-Bradley R, Martre P, Calderini DF, Griffiths S, Reynolds MP (2011) Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. Journal of Experimental Botany **62:** 469-486

Freedman ML, Reich D, Penney KL, McDonald GJ, Mignault AA, Patterson N, Gabriel SB, Topol EJ, Smoller JW, Pato CN, Pato MT, Petryshen TYL, Kolonel LN, Lander ES, Sklar P, Henderson B, Hirschhorn JN, Altshuler D (2004) Assessing the impact of population stratification on genetic association studies. Nature Genetics **36:** 388-393

Friend DJC (1965) Ear Length and Spikelet Number of Wheat Grown at Different Temperatures and Light Intensities. Canadian Journal of Botany **43:** 345-353

Fujita D, Ebron LA, Araki E, Kato H, Khush GS, Sheehy JE, Lafarge T, Fukuta Y, Kobayashi N (2010) Fine mapping of a gene for low-tiller number, Ltn, in japonica rice (Oryza sativa L.) variety Aikawa 1. Theoretical and Applied Genetics **120:** 1233-1240

Gaju O, Reynolds MP, Sparkes DL, Foulkes MJ (2009) Relationships between Large-Spike Phenotype, Grain Number, and Yield Potential in Spring Wheat. Crop Science **49:** 961-973

Gawronski P, Ariyadasa R, Himmelbach A, Poursarebani N, Kilian B, Stein N, Steuernagel B, Hensel G, Kumlehn J, Sehgal SK, Gill BS, Gould P, Hall A, Schnurbusch T (2014) A Distorted Circadian Clock Causes Early Flowering and Temperature-Dependent Variation in Spike Development in the Eps-3A(m) Mutant of Einkorn Wheat. Genetics **196:** 1253-1261

Ghiglione HO, Gonzalez FG, Serrago R, Maldonado SB, Chilcott C, Cura JA, Miralles DJ, Zhu T, Casal JJ (2008) Autophagy regulated by day length determines the number of fertile florets in wheat. Plant Journal **55:** 1010-1024

Giles BE, Bengtsson BO (1988) Variation in Anther Size in Wild Barley (Hordeum-Vulgare Ssp Spontaneum). Hereditas **108:** 199-205

Gogarten SM, Bhangale T, Conomos MP, Laurie CA, McHugh CP, Painter I, Zheng XW, Crosslin DR, Levine D, Lumley T, Nelson SC, Rice K, Shen J, Swarnkar R, Weir BS, Laurie CC (2012) GWASTools: an R/Bioconductor package for quality control and analysis of genome-wide association studies. Bioinformatics **28:** 3329-3331

Gonzalez-Navarro OE, Griffiths S, Molero G, Reynolds MP, Slafer GA (2015) Dynamics of floret development determining differences in spike fertility in an elite population of wheat. Field Crops Research **172:** 21-31

Gonzalez FG, Miralles DJ, Slafer GA (2011) Wheat floret survival as related to pre-anthesis spike growth. Journal of Experimental Botany **62:** 4889-4901

Gonzalez FG, Slafer GA, Miralles DJ (2003) Floret development and spike growth as affected by photoperiod during stem elongation in wheat. Field Crops Research **81:** 29-38

Gonzalez FG, Slafer GA, Miralles DJ (2003) Grain and floret number in response to photoperiod during stem elongation in fully and slightly vernalized wheats. Field Crops Research **81:** 17-27

Gonzalez FG, Slafer GA, Miralles DJ (2005) Floret development and survival in wheat plants exposed to contrasting photoperiod and radiation environments during stem elongation. Functional Plant Biology **32:** 189-197

Gonzalez FG, Slafer GA, Miralles DJ (2005) Photoperiod during stem elongation in wheat: is its impact on fertile floret and grain number determination similar to that of radiation? Functional Plant Biology **32:** 181-188

Gonzalez FG, Terrile II, Falcon MO (2011) Spike Fertility and Duration of Stem Elongation as Promising Traits to Improve Potential Grain Number (and Yield): Variation in Modern Argentinean Wheats. Crop Science **51:** 1693-1702

Gornicki P, Zhu HL, Wang JW, Challa GS, Zhang ZZ, Gill BS, Li WL (2014) The chloroplast view of the evolution of polyploid wheat. New Phytologist **204:** 704-714

Greenup A, Peacock WJ, Dennis ES, Trevaskis B (2009) The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals. Annals of Botany **103:** 1165-1172

Grodzicker T, Williams J, Sharp P, Sambrook J (1974) Physical Mapping of Temperature-Sensitive Mutations of Adenoviruses. Cold Spring Harbor Symposia on Quantitative Biology **39:** 439-446

Gu J, Marshall C (1988) The Effect of Tiller Removal and Tiller Defoliation on Competition between the Main Shoot and Tillers of Spring Barley. Annals of Applied Biology **112:** 597-608

Guo Z, Chen D, Schnurbusch T (2015) Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat. Journal of Experimental Botany **66**:3099-3111.

Guo Z, Schnurbusch T (2015) Variation of floret fertility in hexaploid wheat revealed by tiller removal. Journal of Experimental Botany **66**: 5945-5958.

Guo Z, Schnurbusch T (2016) Costs and benefits of awns. Journal of Experimental Botany **67:** 2533-2535

Guo ZF, Slafer GA, Schnurbusch T (2016) Genotypic variation in spike fertility traits and ovary size as determinants of floret and grain survival rate in wheat. Journal of Experimental Botany: doi: 10.1093/jxb/erw200

Hall AJ, Savin R, Slafer GA (2014) Is time to flowering in wheat and barley influenced by nitrogen?: A critical appraisal of recent published reports. European Journal of Agronomy **54:** 40-46

Hanif M, Langer RHM (1972) Vascular System of Spikelet in Wheat (Triticum-Aestivum). Annals of Botany **36:** 721-&

Hasan AK, Herrera J, Lizana C, Calderini DF (2011) Carpel weight, grain length and stabilized grain water content are physiological drivers of grain weight determination of wheat. Field Crops Research **123:** 241-247

Haun JR (1973) Visual Quantification of Wheat Development. Agronomy Journal **65:** 116-119

Hay RKM (1995) Harvest Index - a Review of Its Use in Plant-Breeding and Crop Physiology. Annals of Applied Biology **126:** 197-216

Hedden P (2003) The genes of the Green Revolution. Trends in Genetics **19:** 5-9

Hendriks W, Kirkegaard JA, Lilley JM, Gregory PJ, Rebetzke GJ (2016) A tillering inhibition gene influences root-shoot carbon partitioning and pattern of water use to improve wheat productivity in rainfed environments. Journal of Experimental Botany **67:** 327-340

Herndl M, White JW, Hunt LA, Graeff S, Claupein W (2008) Field-based evaluation of vernalization requirement, photoperiod response and earliness per se in bread wheat (Triticum aestivum L.) (vol 105, pg 193, 2008). Field Crops Research **107:** 87-87

Hiyane R, Hiyane S, Tang AC, Boyer JS (2010) Sucrose feeding reverses shade-induced kernel losses in maize. Annals of Botany **106:** 395-403

Hoffman GE (2013) Correcting for Population Structure and Kinship Using the Linear Mixed Model: Theory and Extensions. Plos One **8:** e75707

Huang LB, Pant J, Dell B, Bell RW (2000) Effects of boron deficiency on anther development and floret fertility in wheat (Triticum aestivum L-'Wilgoyne'). Annals of Botany **85:** 493-500

Huang S, Sirikhachornkit A, Su XJ, Faris J, Gill B, Haselkorn R, Gornicki P (2002) Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the Triticum/Aegilops complex and the evolutionary history of polyploid wheat. Proceedings of the National Academy of Sciences of the United States of America **99:** 8133-8138

Huang XH, Yang SH, Gong JY, Zhao Y, Feng Q, Gong H, Li WJ, Zhan QL, Cheng BY, Xia JH, Chen N, Hao ZN, Liu KY, Zhu CR, Huang T, Zhao Q, Zhang L, Fan DL, Zhou CC, Lu YQ, Weng QJ, Wang ZX, Li JY, Han B (2015) Genomic analysis of hybrid rice varieties reveals numerous superior alleles that contribute to heterosis. Nature Communications **6**:6258

Huo XY, Wang C, Teng YB, Liu XY (2015) Identification of miRNAs associated with dark-induced senescence in Arabidopsis. Bmc Plant Biology **15:**266

Ishag HM, Taha MB (1974) Production and Survival of Tillers of Wheat and Their Contribution to Yield. Journal of Agricultural Science **83:** 117-124

Isidro J, Alvaro F, Royo C, Villegas D, Miralles DJ, del Moral LFG (2011) Changes in duration of developmental phases of durum wheat caused by breeding in Spain and Italy during the 20th century and its impact on yield. Annals of Botany **107:** 1355-1366

Jeon J, Choi J, Lee GW, Park SY, Huh A, Dean RA, Lee YH (2015) Genome-wide profiling of DNA methylation provides insights into epigenetic regulation of fungal development in a plant pathogenic fungus, Magnaporthe oryzae. Scientific Reports **5**

Ji XM, Shiran B, Wan JL, Lewis DC, Jenkins CLD, Condon AG, Richards RA, Dolferus R (2010) Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant Cell and Environment **33:** 926-942

Jovanovic M, Hengartner MO (2006) miRNAs and apoptosis: RNAs to die for. Oncogene **25:** 6176-6187

Kamran A, Iqbal M, Spaner D (2014) Flowering time in wheat (Triticum aestivum L.): a key factor for global adaptability. Euphytica **197:** 1-26

Kang HM, Sul JH, Service SK, Zaitlen NA, Kong SY, Freimer NB, Sabatti C, Eskin E (2010) Variance component model to account for sample structure in genome-wide association studies. Nature Genetics **42:** 348-U110

Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D, Daly MJ, Eskin E (2008) Efficient control of population structure in model organism association mapping. Genetics **178:** 1709-1723

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer FT. Science **286:** 1962-1965

Kebrom TH, Chandler PM, Swain SM, King RW, Richards RA, Spielmeyer W (2012) Inhibition of Tiller Bud Outgrowth in the tin Mutant of Wheat Is Associated with Precocious Internode Development. Plant Physiology **160:** 308-318

Kebrom TH, Mullet JE (2014) Photosynthetic Leaf Area Modulates Tiller Bud Outgrowth In Sorghum. Plant Cell Environ **38**: 1471-1478

Kebrom TH, Richards RA (2013) Physiological perspectives of reduced tillering and stunting in the tiller inhibition (tin) mutant of wheat. Functional Plant Biology **40:** 977-985

Kebrom TH, Spielmeyer W, Finnegan EJ (2013) Grasses provide new insights into regulation of shoot branching. Trends in Plant Science **18:** 41-48

Kemp DR, Whingwiri EE (1980) Effect of Tiller Removal and Shading on Spikelet Development and Yield Components of the Main Shoot of Wheat and on the Sugar Concentration of the Ear and Flag Leaf. Australian Journal of Plant Physiology **7:** 501-510

Khan MN, Heyne EG, Arp AL (1973) Pollen Distribution and Seedset on Triticum-Aestivum L. Crop Science **13:** 223-226

Kherde MK, Atkins IM, Merkle OG, Porter KB (1967) Cross Pollination Studies with Male Sterile Wheats of 3 Cytoplasms Seed Size on F1 Plants and Seed and Anther Size of 45 Pollinators. Crop Science **7:** 389-394

Khush GS (2001) Green revolution: the way forward. Nature Reviews Genetics **2:** 815-822

Kim HB, Atkins IM, Pawlisch PE (1968) Inheritance of Anther Size and Spikelet Characteristics - Their Interrelationship in Crossing Cultivars Bronco Avena Sativa L and a Sterilis L Ssp Macrocarpa (Moench) Briq. Crop Science **8:** 101-104

Kiniry JR (1993) Nonstructural Carbohydrate Utilization by Wheat Shaded during Grain-Growth. Agronomy Journal **85:** 844-849

Kirby EJM (1974) Ear Development in Spring Wheat. Journal of Agricultural Science **82:** 437-447

Kirby EJM (1988) Analysis of Leaf, Stem and Ear Growth in Wheat from Terminal Spikelet Stage to Anthesis. Field Crops Research **18:** 127-140

Kirby EJM, Appleyard M (1987) Cereal Development Guide. 2nd ed. Stoneleigh, UK: NAC Cereal Unit. 85pp

Kirby EJM, Appleyard M, Simpson NA (1994) Coordination of Stem Elongation and Zadoks Growth-Stages with Leaf Emergence in Wheat and Barley. Journal of Agricultural Science **122:** 21-29

Kirigwi FM, Van Ginkel M, Brown-Guedira G, Gill BS, Paulsen GM, Fritz AK (2007) Markers associated with a QTL for grain yield in wheat under drought. Molecular Breeding **20:** 401-413

Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J (2005) Complement factor H polymorphism in age-related macular degeneration. Science **308:** 385-389

Komaki MK, Tsunewaki K (1981) Genetic-Studies on the Difference of Anther Length among Common Wheat Cultivars. Euphytica **30:** 45-53

Komatsuda T, Pourkheirandish M, He CF, Azhaguvel P, Kanamori H, Perovic D, Stein N, Graner A, Wicker T, Tagiri A, Lundqvist U, Fujimura T, Matsuoka M, Matsumoto T, Yano M (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proceedings of the National Academy of Sciences of the United States of America **104:** 1424-1429

Koonjul PK, Minhas JS, Nunes C, Sheoran IS, Saini HS (2005) Selective transcriptional down-regulation of anther invertases precedes the failure of pollen development in water-stressed wheat. Journal of Experimental Botany **56:** 179-190

Kostkanova E, Stehno Z, Manev M (1992) Technological Grain Quality of Set of Spring Wheat Genetic-Resources. Rostlinna Vyroba **38:** 793-806

Kumar P, Gupta VK, Misra AK, Modi DR, Pandey BK (2009) Potential of Molecular Markers in Plant Biotechnology. Plant Omics **2:** 141-162

Kuraparthy V, Sood S, Dhaliwal HS, Chhuneja P, Gill BS (2007) Identification and mapping of a tiller inhibition gene (tin3) in wheat. Theoretical and Applied Genetics **114:** 285-294

Langer RHM, Hanif M (1973) Study of Floret Development in Wheat (Triticum-Aestivum L). Annals of Botany **37:** 743-751

Lazaro L, Abbate PE (2012) Cultivar effects on relationship between grain number and photothermal quotient or spike dry weight in wheat. Journal of Agricultural Science **150:** 442-459

Lewis S, Faricelli ME, Appendino ML, Valarik M, Dubcovsky J (2008) The chromosome region including the earliness per se locus Eps-A(m)1 affects the duration of early developmental phases and spikelet number in diploid wheat. Journal of Experimental Botany **59:** 3595-3607

Li J, Lease KA, Tax FE, Walker JC (2001) BRS1, a serine carboxypeptidase, regulates BRI1 signaling in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America **98:** 5916-5921

Li JH, Li YH, Chen SY, An LZ (2010) Involvement of brassinosteroid signals in the floral-induction network of Arabidopsis. Journal of Experimental Botany **61:** 4221-4230

Li XY, Qian Q, Fu ZM, Wang YH, Xiong GS, Zeng DL, Wang XQ, Liu XF, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li JY (2003) Control of tillering in rice. Nature **422:** 618-621

Lizana XC, Calderini DF (2013) Yield and grain quality of wheat in response to increased temperatures at key periods for grain number and grain weight determination: considerations for the climatic change scenarios of Chile. Journal of Agricultural Science **151:** 209-221

Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN (2003) Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nature Genetics **33:** 177-182

Longin CFH, Gowda M, Muhleisen J, Ebmeyer E, Kazman E, Schachschneider R, Schacht J, Kirchhoff M, Zhao YS, Reif JC (2013) Hybrid wheat: quantitative genetic parameters and consequences for the design of breeding programs. Theoretical and Applied Genetics **126:** 2791-2801

Longin CFH, Reif JC (2014) Redesigning the exploitation of wheat genetic resources. Trends in Plant Science **19:** 631-636

Maestra B, Naranjo T (1998) Homoeologous relationships of Aegilops speltoides chromosomes to bread wheat. Theoretical and Applied Genetics **97:** 181-186

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TFC, McCarroll SA, Visscher PM (2009) Finding the missing heritability of complex diseases. Nature **461:** 747-753

Mantel N (1967) Detection of Disease Clustering and a Generalized Regression Approach. Cancer Research **27:** 209-220

Marczewski W (1995) [Molecular markers in plant genetics and plant breeding]. Postepy Biochem **41:** 237-243

Martino DL, Abbate PE, Cendoya MG, Gutheim F, Mirabella NE, Pontaroli AC (2015) Wheat spike fertility: inheritance and relationship with spike yield components in early generations. Plant Breeding **134:** 264-270

Mathews S (2010) Evolutionary Studies Illuminate the Structural-Functional Model of Plant Phytochromes. Plant Cell **22:** 4-16

Matsumoto T, Wu JZ, Kanamori H, Katayose Y, Fujisawa M, Namiki N, Mizuno H, Yamamoto K, Antonio BA, Baba T, Sakata K, Nagamura Y, Aoki H, Arikawa K, Arita K, Bito T, Chiden Y, Fujitsuka N, Fukunaka R, Hamada M, Harada C, Hayashi A, Hijishita S, Honda M, Hosokawa S, Ichikawa Y, Idonuma A, Iijima M, Ikeda M, Ikeno M, Ito K, Ito S, Ito T, Ito Y, Ito Y, Iwabuchi A, Kamiya K, Karasawa W, Kurita K, Katagiri S, Kikuta A, Kobayashi H, Kobayashi N, Machita K, Maehara T, Masukawa M, Mizubayashi T, Mukai Y, Nagasaki H, Nagata Y, Naito S, Nakashima M, Nakama Y, Nakamichi Y, Nakamura M, Meguro A, Negishi M, Ohta I, Ohta T, Okamoto M, Ono N, Saji S, Sakaguchi M, Sakai K, Shibata M, Shimokawa T, Song JY, Takazaki Y, Terasawa K, Tsugane M, Tsuji K, Ueda S, Waki K, Yamagata H, Yamamoto M, Yamamoto S, Yamane H, Yoshiki S, Yoshihara R, Yukawa K, Zhong HS, Yano M, Sasaki T, Yuan QP, Shu OT, Liu J, Jones KM, Gansberger K, Moffat K, Hill J, Bera J, Fadrosh D, Jin SH, Johri S, Kim M, Overton L, Reardon M, Tsitrin T, Vuong H, Weaver B, Ciecko A, Tallon L, Jackson J, Pai G, Van Aken S, Utterback T, Reidmuller S, Feldblyum T, Hsiao J, Zismann V, Iobst S, de Vazeille AR, Buell CR, Ying K, Li Y, Lu TT, Huang YC, Zhao Q, Feng Q, Zhang L, Zhu JJ, Weng QJ, Mu J, Lu YQ, Fan DL, Liu YL, Guan JP, Zhang YJ, Yu SL, Liu XH, Zhang Y, Hong GF, Han B, Choisne N, Demange N, Orjeda G, Samain S, Cattolico L, Pelletier E, Couloux A, Segurens B, Wincker P, D'Hont A, Scarpelli C, Weissenbach J, Salanoubat M, Quetier F, Yu Y, Kim HR, Rambo T, Currie J, Collura K, Luo MZ, Yang TJ, Ammiraju JSS, Engler F, Soderlund C, Wing RA, Palmer LE, de la Bastide M, Spiegel L, Nascimento L, Zutavern T, O'Shaughnessy A, Dike S, Dedhia N, Preston R, Balija V, McCombie WR, Chow TY, Chen HH, Chung MC, Chen CS, Shaw JF, Wu HP, Hsiao KJ, Chao YT, Chu MK, Cheng CH, Hour AL, Lee PF, Lin SJ, Lin YC, Liou JY, Liu SM, Hsing YI, Raghuvanshi S, Mohanty A, Bharti AK, Gaur A, Gupta V, Kumar D, Ravi V, Vij S, Kapur A, Khurana P, Khurana P, Khurana JP, Tyagi AK, Gaikwad K, Singh A, Dalal V, Srivastava S, Dixit A, Pal AK, Ghazi IA, Yadav M, Pandit A, Bhargava A, Sureshbabu K, Batra K, Sharma TR, Mohapatra T, Singh NK, Messing J, Nelson AB, Fuks G, Kavchok S, Keizer G, Llaca ELV, Song RT, Tanyolac B, Young S, Il KH, Hahn JH, Sangsakoo G, Vanavichit A, de Mattos LAT, Zimmer PD, Malone G, Dellagostin O, de Oliveira AC, Bevan M, Bancroft I, Minx P, Cordum H, Wilson R, Cheng ZK, Jin WW, Jiang JM, Leong SA, Iwama H, Gojobori T, Itoh T, Niimura Y, Fujii Y, Habara T, Sakai H, Sato Y, Wilson G, Kumar K, McCouch S, Juretic N, Hoen D, Wright S, Bruskiewich R, Bureau T, Miyao A, Hirochika H, Nishikawa T, Kadowaki K, Sugiura M, Project IRGS (2005) The map-based sequence of the rice genome. Nature **436:** 793-800

Matsuoka Y (2011) Evolution of Polyploid Triticum Wheats under Cultivation: The Role of Domestication, Natural Hybridization and Allopolyploid Speciation in their Diversification. Plant and Cell Physiology **52:** 750-764

Mayer KFX, Rogers J, Dolezel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P, Endo TR, Dolezel J, Kubalakova M, Cihalikova J, Dubska Z, Vrana J, Sperkova R, Simkova H, Rogers J, Febrer M, Clissold L, McLay K, Singh K, Chhuneja P, Singh NK, Khurana J, Akhunov E, Choulet F, Sourdille P, Feuillet C, Alberti A, Barbe V, Wincker P, Kanamori H, Kobayashi F, Itoh T, Matsumoto T, Sakai H, Tanaka T, Wu JZ, Ogihara Y, Handa H, Pozniak C, Maclachlan PR, Sharpe A, Klassen D, Edwards D, Batley J, Olsen OA, Sandve SR, Lien S, Steuernagel B, Wulff B, Caccamo M, Ayling S, Ramirez-Gonzalez RH, Clavijo BJ, Steuernagel B, Wright J, Pfeifer M, Spannagl M, Mayer KFX, Martis MM, Akhunov E, Choulet F, Mayer

KFX, Mascher M, Chapman J, Poland JA, Scholz U, Barry K, Waugh R, Rokhsar DS, Muehlbauer GJ, Stein N, Gundlach H, Zytnicki M, Jamilloux V, Quesneville H, Wicker T, Mayer KFX, Faccioli P, Colaiacovo M, Pfeifer M, Stanca AM, Budak H, Cattivelli L, Glover N, Martis MM, Choulet F, Feuillet C, Mayer KFX, Pfeifer M, Pingault L, Mayer KFX, Paux E, Spannagl M, Sharma S, Mayer KFX, Pozniak C, Appels R, Bellgard M, Chapman B, Pfeifer M, Pfeifer M, Sandve SR, Nussbaumer T, Bader KC, Choulet F, Feuillet C, Mayer KFX, Akhunov E, Paux E, Rimbert H, Wang SC, Poland JA, Knox R, Kilian A, Pozniak C, Alaux M, Alfama F, Couderc L, Jamilloux V, Guilhot N, Viseux C, Loaec M, Quesneville H, Rogers J, Dolezel J, Eversole K, Feuillet C, Keller B, Mayer KFX, Olsen OA, Praud S, Iwgsc (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science **345**

Mayer KFX, Waugh R, Langridge P, Close TJ, Wise RP, Graner A, Matsumoto T, Sato K, Schulman A, Muehlbauer GJ, Stein N, Ariyadasa R, Schulte D, Poursarebani N, Zhou RN, Steuernagel B, Mascher M, Scholz U, Shi BJ, Langridge P, Madishetty K, Svensson JT, Bhat P, Moscou M, Resnik J, Close TJ, Muehlbauer GJ, Hedley P, Liu H, Morris J, Waugh R, Frenkel Z, Korol A, Berges H, Graner A, Stein N, Steuernagel B, Taudien S, Groth M, Felder M, Platzer M, Brown JWS, Schulman A, Platzer M, Fincher GB, Muehlbauer GJ, Sato K, Taudien S, Sampath D, Swarbreck D, Scalabrin S, Zuccolo A, Vendramin V, Morgante M, Mayer KFX, Schulman A, Conso IBGS (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature **491:** 711-716

McGrath VB, Blakeney AB, Batten GD (1997) Fructan to nitrogen ratio as an indicator of nutrient stress in wheat crops. New Phytologist **136:** 145-152

Millet E (1986) Relationships between Grain Weight and the Size of Floret Cavity in the Wheat Spike. Annals of Botany **58:** 417-423

Miralles DJ, Calderini DF, Pomar KP, D'Ambrogio A (1998) Dwarfing genes and cell dimensions in different organs of wheat. Journal of Experimental Botany **49:** 1119-1127

Miralles DJ, Katz SD, Colloca A, Slafer GA (1998) Floret development in near isogenic wheat lines differing in plant height. Field Crops Research **59:** 21-30

Miralles DJ, Richards RA, Slafer GA (2000) Duration of the stem elongation period influences the number of fertile florets in wheat and barley. Australian Journal of Plant Physiology **27:** 931-940

Miralles DJ, Slafer GA (1995) Yield, biomass and yield components in dwarf, semi-dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breeding **114:** 392-396

Miralles DJ, Slafer GA (1997) Radiation interception and radiation use efficiency of near-isogenic wheat lines with different height. Euphytica **97:** 201-208

Mitchell JH, Chapman SC, Rebetzke GJ, Bonnett DG, Fukai S (2012) Evaluation of a reduced-tillering (tin) gene in wheat lines grown across different production environments. Crop & Pasture Science **63:** 128-141

Mitchell JH, Rebetzke GJ, Chapman SC, Fukai S (2013) Evaluation of reduced-tillering (tin) wheat lines in managed, terminal water deficit environments. Journal of Experimental Botany **64:** 3439-3451

Moeller C, Evers JB, Rebetzke G (2014) Canopy architectural and physiological characterization of near-isogenic wheat lines differing in the tiller inhibition gene tin. Frontiers in Plant Science **5**

Mohamed GES, Marshall C (1979) Physiological-Aspects of Tiller Removal in Spring Wheat. Journal of Agricultural Science **93:** 457-463

Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, Riera-Lizarazu O, Brown PJ, Acharya CB, Mitchell SE, Harriman J, Glaubitz JC, Buckler ES, Kresovich S (2013) Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proceedings of the National Academy of Sciences of the United States of America **110:** 453-458

Motzo R, Giunta F (2002) Awnedness affects grain yield and kernel weight in near-isogenic lines of durum wheat. Australian Journal of Agricultural Research **53:** 1285-1293

Muhleisen J, Piepho HP, Maurer HP, Longin CFH, Reif JC (2014) Yield stability of hybrids versus lines in wheat, barley, and triticale. Theoretical and Applied Genetics **127:** 309-316

Mullis KB, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol **155:** 335-350

Nagel M, Behrens AK, Borner A (2013) Effects of Rht dwarfing alleles on wheat seed vigour after controlled deterioration. Crop & Pasture Science **64:** 857-864

Ohto M, Onai K, Furukawa Y, Aoki E, Araki T, Nakamura K (2001) Effects of sugar on vegetative development and floral transition in arabidopsis. Plant Physiology **127:** 252-261

Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu XG, Price GD, Condon AG, Furbank RT (2011) Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. Journal of Experimental Botany **62:** 453-467

Pasam RK, Sharma R, Malosetti M, van Eeuwijk FA, Haseneyer G, Kilian B, Graner A (2012) Genome-wide association studies for agronomical traits in a world wide spring barley collection. Bmc Plant Biology **12:** 16

Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. Plos Genetics **2:** 2074-2093

Pearce S, Saville R, Vaughan SP, Chandler PM, Wilhelm EP, Sparks CA, Al-Kaff N, Korolev A, Boulton MI, Phillips AL, Hedden P, Nicholson P, Thomas SG (2011) Molecular Characterization of Rht-1 Dwarfing Genes in Hexaploid Wheat. Plant Physiology **157:** 1820-1831

Pedro A, Savin R, Parry MAJ, Slafer GA (2012) Selection for high grain number per unit stem length through four generations from mutants in a durum wheat population to increase yields of individual plants and crops. Field Crops Research **129:** 59-70

Peltonen-Sainio P, Muurinen S, Rajala A, Jauhiainen L (2008) Variation in harvest index of modern spring barley, oat and wheat cultivars adapted to northern growing conditions. Journal of Agricultural Science **146:** 35-47

Peng JR, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature **400:** 256-261

Perez SI, Diniz JAF, Bernal V, Gonzalez PN (2010) Alternatives to the partial Mantel test in the study of environmental factors shaping human morphological variation. Journal of Human Evolution **59:** 698-703

Pinthus MJ, Meiri J (1979) Effects of the Reversal of Day and Night Temperatures on Tillering and on the Elongation of Stems and Leaf Blades of Wheat. Journal of Experimental Botany **30:** 319-326

Pinthus MJ, Millet E (1978) Interactions among Number of Spikelets, Number of Grains and Grain Weight in Spikes of Wheat (Triticum-Aestivum L). Annals of Botany **42:** 839-848

Pirinen M, Donnelly P, Spencer CCA (2013) Efficient Computation with a Linear Mixed Model on Large-Scale Data Sets with Applications to Genetic Studies. Annals of Applied Statistics **7:** 369-390

Porri A, Torti S, Romera-Branchat M, Coupland G (2012) Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. Development **139:** 2198-2209

Potgieter A, Meinke H, Doherty A, Sadras VO, Hammer G, Crimp S, Rodriguez D (2013) Spatial impact of projected changes in rainfall and temperature on wheat yields in Australia. Climatic Change **117:** 163-179

Prasad PVV, Djanaguiraman M (2014) Response of floret fertility and individual grain weight of wheat to high temperature stress: sensitive stages and thresholds for temperature and duration. Functional Plant Biology **41:** 1261-1269

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics **38:** 904-909

Price AL, Zaitlen NA, Reich D, Patterson N (2010) New approaches to population stratification in genome-wide association studies. Nature Reviews Genetics **11:** 459-463

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics **155:** 945-959

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC (2007) PLINK: A tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics **81:** 559-575

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The Constans Gene of Arabidopsis Promotes Flowering and Encodes a Protein Showing Similarities to Zinc-Finger Transcription Factors. Cell **80:** 847-857

Qu HQ, Tien M, Polychronakos C (2010) Statistical significance in genetic association studies. Clinical and Investigative Medicine **33:** E266-E270

Rahman MS, Wilson JH (1977) Determination of Spikelet Number in Wheat .1. Effect of Varying Photoperiod on Ear Development. Australian Journal of Agricultural Research **28:** 565-574

Rawson HM (1970) Spikelet Number, Its Control and Relation to Yield Per Ear in Wheat. Australian Journal of Biological Sciences **23:** 1-15

Rawson HM (1971) Upper Limit for Spikelet Number Per Ear in Wheat, as Controlled by Photoperiod. Australian Journal of Agricultural Research **22:** 537-546

Rawson HM, Evans LT (1970) Pattern of Grain Growth within Ear of Wheat. Australian Journal of Biological Sciences **23:** 753-764

Rebetzke GJ, Bonnett DG, Reynolds MP (2016) Awns reduce grain number to increase grain size and harvestable yield in irrigated and rainfed spring wheat. Journal of Experimental Botany **67**:2573-2586

Rebetzke GJ, Richards RA (2000) Gibberellic acid-sensitive dwarfing genes reduce plant height to increase kernel number and grain yield of wheat. Australian Journal of Agricultural Research **51:** 235-245

Reich DE, Goldstein DB (2001) Detecting association in a case-control study while correcting for population stratification. Genetic Epidemiology **20:** 4-16

Reynolds M, Foulkes J, Furbank R, Griffiths S, King J, Murchie E, Parry M, Slafer G (2012) Achieving yield gains in wheat. Plant Cell and Environment **35:** 1799-1823

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus WJ (2009) Raising yield potential in wheat. Journal of Experimental Botany **60:** 1899-1918

Richards RA (1988) A Tiller Inhibitor Gene in Wheat and Its Effect on Plant-Growth. Australian Journal of Agricultural Research **39:** 749-757

Richards RA (1992) The Effect of Dwarfing Genes in Spring Wheat in Dry Environments .1. Agronomic Characteristics. Australian Journal of Agricultural Research **43:** 517-527

Richards RA, Rebetzke GJ, Condon AG, van Herwaarden AF (2002) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Science **42:** 111-121

Richards RA, Rebetzke GJ, Watt M, Condon AG, Spielmeyer W, Dolferus R (2010) Breeding for improved water productivity in temperate cereals: phenotyping, quantitative trait loci, markers and the selection environment. Functional Plant Biology **37:** 85-97

Riedelsheimer C, Lisec J, Czedik-Eysenberg A, Sulpice R, Flis A, Grieder C, Altmann T, Stitt M, Willmitzer L, Melchinger AE (2012) Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. Proceedings of the National Academy of Sciences of the United States of America **109:** 8872-8877

Rolland F, Moore B, Sheen J (2002) Sugar sensing and signaling in plants. Plant Cell **14:** S185-S205

Rosati A, Caporali S, Hammami SBM, Moreno-Alias I, Paoletti A, Rapoport HF (2012) Tissue size and cell number in the olive (Olea europaea) ovary determine tissue growth and partitioning in the fruit. Functional Plant Biology **39:** 580-587

Rosati A, Zipancic M, Caporali S, Padula G (2009) Fruit weight is related to ovary weight in olive (Olea europaea L.). Scientia Horticulturae **122:** 399-403

Sadras VO, Denison RF (2009) Do plant parts compete for resources? An evolutionary viewpoint. New Phytologist **183:** 565-574

Sadras VO, Richards RA (2014) Improvement of crop yield in dry environments: benchmarks, levels of organisation and the role of nitrogen. Journal of Experimental Botany **65:** 1981-1995

Sadras VO, Slafer GA (2012) Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. Field Crops Research **127:** 215-224

Saini HS, Sedgley M, Aspinall D (1983) Effect of Heat-Stress during Floral Development on Pollen-Tube Growth and Ovary Anatomy in Wheat (Triticum-Aestivum-L). Australian Journal of Plant Physiology **10:** 137-144

Saini HS, Sedgley M, Aspinall D (1984) Developmental Anatomy in Wheat of Male-Sterility Induced by Heat-Stress, Water Deficit or Abscisic-Acid. Australian Journal of Plant Physiology **11:** 243-253

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G (2000) Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science **288:** 1613-1616

Saville RJ, Gosman N, Burt CJ, Makepeace J, Steed A, Corbitt M, Chandler E, Brown JKM, Boulton MI, Nicholson P (2012) The 'Green Revolution' dwarfing genes play a role in disease resistance in Triticum aestivum and Hordeum vulgare. Journal of Experimental Botany **63:** 1271-1283

Savin R, Slafer GA (1991) Shading Effects on the Yield of an Argentinean Wheat Cultivar. Journal of Agricultural Science **116:** 1-7

Schnable PS, Ware D, Fulton RS, Stein JC, Wei FS, Pasternak S, Liang CZ, Zhang JW, Fulton L, Graves TA, Minx P, Reily AD, Courtney L, Kruchowski SS, Tomlinson C, Strong C, Delehaunty K, Fronick C, Courtney B, Rock SM, Belter E, Du FY, Kim K, Abbott RM, Cotton M, Levy A, Marchetto P, Ochoa K, Jackson SM, Gillam B, Chen WZ, Yan L, Higginbotham J, Cardenas M, Waligorski J, Applebaum E, Phelps L, Falcone J, Kanchi K, Thane T, Scimone A, Thane N, Henke J, Wang T, Ruppert J, Shah N, Rotter K, Hodges J, Ingenthron E, Cordes M, Kohlberg S, Sgro J, Delgado B, Mead K, Chinwalla A, Leonard S, Crouse K, Collura K, Kudrna D, Currie J, He RF, Angelova A, Rajasekar S, Mueller T, Lomeli R, Scara G, Ko A, Delaney K, Wissotski M, Lopez G, Campos D, Braidotti M, Ashley E, Golser W, Kim H, Lee S, Lin JK, Dujmic Z, Kim W, Talag J, Zuccolo A, Fan C, Sebastian A, Kramer M, Spiegel L, Nascimento L, Zutavern T, Miller B, Ambroise C, Muller S, Spooner W, Narechania A, Ren LY, Wei S, Kumari S, Faga B, Levy MJ, McMahan L, Van Buren P, Vaughn MW, Ying K, Yeh CT, Emrich SJ, Jia Y, Kalyanaraman A, Hsia AP, Barbazuk WB, Baucom RS, Brutnell TP, Carpita NC, Chaparro C, Chia JM, Deragon JM, Estill JC, Fu Y, Jeddeloh JA, Han YJ, Lee H, Li PH, Lisch DR, Liu SZ, Liu ZJ, Nagel DH, McCann MC, SanMiguel P, Myers AM, Nettleton D, Nguyen J, Penning BW, Ponnala L, Schneider KL, Schwartz DC, Sharma A, Soderlund C, Springer NM, Sun Q, Wang H, Waterman M, Westerman R, Wolfgruber TK, Yang LX, Yu Y, Zhang LF, Zhou SG, Zhu Q, Bennetzen JL, Dawe RK, Jiang JM, Jiang N, Presting GG, Wessler SR, Aluru S, Martienssen RA, Clifton SW, McCombie WR, Wing RA, Wilson RK (2009) The B73 Maize Genome: Complexity, Diversity, and Dynamics. Science **326:** 1112-1115

Segura V, Vilhjalmsson BJ, Platt A, Korte A, Seren U, Long Q, Nordborg M (2012) An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nature Genetics **44:** 825-U144

Semagn K, Bjornstad A, Ndjiondjop MN (2006) An overview of molecular marker methods for plants. African Journal of Biotechnology **5:** 2540-2568

Semenov MA, Stratonovitch P, Alghabari F, Gooding MJ (2014) Adapting wheat in Europe for climate change. Journal of Cereal Science **59:** 245-256

Serrago RA, Miralles DJ, Slafer GA (2008) Floret fertility in wheat as affected by photoperiod during stem elongation and removal of spikelets at booting. European Journal of Agronomy **28:** 301-308

Sharrock RA (2008) The phytochrome red/far-red photoreceptor superfamily. Genome Biology **9**

Shewry P (2009) Increasing the health benefits of wheat. Febs Journal **276:** 71-71

Siddique KHM, Belford RK, Perry MW, Tennant D (1989) Growth, Development and Light Interception of Old and Modern Wheat Cultivars in a Mediterranean-Type Environment. Australian Journal of Agricultural Research **40:** 473-487

Siddique KHM, Kirby EJM, Perry MW (1989) Ear Stem Ratio in Old and Modern Wheat-Varieties - Relationship with Improvement in Number of Grains Per Ear and Yield. Field Crops Research **21:** 59-78

Siddique KHM, Whan BR (1994) Ear-Stem Ratios in Breeding Populations of Wheat - Significance for Yield Improvement. Euphytica **73:** 241-254

Slafer GA (1996) Differences in phasic development rate amongst wheat cultivars independent of responses to photoperiod and vernalization. A viewpoint of the intrinsic earliness hypothesis. Journal of Agricultural Science **126:** 403-419

Slafer GA (2003) Genetic basis of yield as viewed from a crop physiologist's perspective. Annals of Applied Biology **142:** 117-128

Slafer GA, Abeledo LG, Miralles DJ, Gonzalez FG, Whitechurch EM (2001) Photoperiod sensitivity during stem elongation as an avenue to raise potential yield in wheat. Euphytica **119:** 191-197

Slafer GA, Andrade FH (1991) Changes in Physiological Attributes of the Dry-Matter Economy of Bread Wheat (Triticum-Aestivum) through Genetic-Improvement of Grain-Yield Potential at Different Regions of the World - a Review. Euphytica **58:** 37-49

Slafer GA, Andrade FH (1993) Physiological Attributes Related to the Generation of Grain-Yield in Bread Wheat Cultivars Released at Different Eras. Field Crops Research **31:** 351-367

Slafer GA, Andrade FH, Satorre EH (1990) Genetic-Improvement Effects on Preanthesis Physiological Attributes Related to Wheat Grain-Yield. Field Crops Research **23:** 255-263

Slafer GA, Calderini DF, Miralles DJ, Dreccer MF (1994) Preanthesis Shading Effects on the Number of Grains of 3 Bread Wheat Cultivars of Different Potential Number of Grains. Field Crops Research **36:** 31-39

Slafer GA, Elia M, Savin R, García GA, Terrile II, Ferrante A, Miralles DJ, González FG (2015) Fruiting efficiency: an alternative trait to further rise wheat yield. Food and Energy Security **4:** 92-109

Slafer GA, Kantolic AG, Appendino ML, Tranquilli G, Miralles DJ, Savin R (2015) Chapter 12 - Genetic and environmental effects on crop development determining adaptation and yield. *In* VOSF Calderini, ed, Crop Physiology (Second Edition). Academic Press, San Diego, pp 285-319

Slafer GA, Savin R (1991) Developmental Base Temperature in Different Phenological Phases of Wheat (Triticum-Aestivum). Journal of Experimental Botany **42:** 1077-1082

Slafer GA, Savin R, Sadras VO (2014) Coarse and fine regulation of wheat yield components in response to genotype and environment. Field Crops Research **157:** 71-83

Smouse PE, Long JC, Sokal RR (1986) Multiple-Regression and Correlation Extensions of the Mantel Test of Matrix Correspondence. Systematic Zoology **35:** 627-632

Snape JW, Butterworth K, Whitechurch E, Worland AJ (2001) Waiting for fine times: genetics of flowering time in wheat. Euphytica **119:** 185-190

Sokal RR (1979) Testing Statistical Significance of Geographic-Variation Patterns. Systematic Zoology **28:** 227-232

Song JC, Jiang LJ, Jameson PE (2012) Co-ordinate regulation of cytokinin gene family members during flag leaf and reproductive development in wheat. Bmc Plant Biology **12:** 7

Song XM, Ge TT, Li Y, Hou XL (2015) Genome-wide identification of SSR and SNP markers from the non-heading Chinese cabbage for comparative genomic analyses. Bmc Genomics **16:** 328

Spielmeyer W, Richards RA (2004) Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (tin) with rice chromosome 5S. Theoretical and Applied Genetics **109:** 1303-1310

Sreenivasulu N, Schnurbusch T (2012) A genetic playground for enhancing grain number in cereals. Trends in Plant Science **17:** 91-101

Stockman YM, Fischer RA, Brittain EG (1983) Assimilate Supply and Floret Development within the Spike of Wheat (Triticum-Aestivum L). Australian Journal of Plant Physiology **10:** 585-594

Su J, Hu C, Yan X, Jin Y, Chen Z, Guan Q, Wang Y, Zhong D, Jansson C, Wang F, Schnurer A, Sun C (2015) Expression of barley SUSIBA2 transcription factor yields high-starch low-methane rice. Nature **523:** 602-606

Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G (2001) CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. Nature **410:** 1116-1120

Sukumaran S, Dreisigacker S, Lopes M, Chavez P, Reynolds MP (2015) Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. Theoretical and Applied Genetics **128:** 353-363

Sun CH, Chen D, Fang J, Wang PR, Deng XJ, Chu CC (2014) Understanding the genetic and epigenetic architecture in complex network of rice flowering pathways. Protein & Cell **5:** 889-898

Sun CX, Hoglund AS, Olsson H, Mangelsen E, Jansson C (2005) Antisense oligodeoxynucleotide inhibition as a potent strategy in plant biology: identification of SUSIBA2 as a transcriptional activator in plant sugar signalling. Plant Journal **44:** 128-138

Sun CX, Palmqvist S, Olsson H, Boren M, Ahlandsberg S, Jansson C (2003) A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. Plant Cell **15:** 2076-2092

Svishcheva GR, Axenovich TI, Belonogova NM, van Duijn CM, Aulchenko YS (2012) Rapid variance components-based method for whole-genome association analysis. Nature Genetics **44:** 1166-1170

Tavakol E, Tavakol E, Verderio G, Shariati JV, Hussien A, Bilgic H, Scanlon MJ, Todt NR, Close TJ, Druka A, Waugh R, Steuernagel B, Ariyadasa R, Himmelbach A, Stein N, Muehlbauer GJ, Rossini L (2015) The Barley Uniculme4 Gene Encodes a BLADE-ON-PETIOLE-Like Protein That Controls Tillering and Leaf Patterning. Plant Physiology **168:** 164-174

Thorne GN, Wood DW (1987) Effects of Radiation and Temperature on Tiller Survival, Grain Number and Grain-Yield in Winter-Wheat. Annals of Botany **59:** 413-426

Tian F, Bradbury PJ, Brown PJ, Hung H, Sun Q, Flint-Garcia S, Rocheford TR, McMullen MD, Holland JB, Buckler ES (2011) Genome-wide association study of leaf architecture in the maize nested association mapping population. Nature Genetics **43:** 159-162

Tishkoff SA, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, Froment A, Hirbo JB, Awomoyi AA, Bodo JM, Doumbo O, Ibrahim M, Juma AT, Kotze MJ, Lema G, Moore JH, Mortensen H, Nyambo TB, Omar SA, Powell K, Pretorius GS, Smith MW, Thera MA, Wambebe C, Weber JL, Williams SM (2009) The Genetic Structure and History of Africans and African Americans. Science **324:** 1035-1044

Toyota M, Tsutsui I, Kusutani A, Asanuma K (2001) Initiation and development of spikelets and florets in wheat as influenced by shading and nitrogen supply at the spikelet phase. Plant Production Science **4:** 283-290

Trione EJ, Stockwell VO (1989) Development of Detached Wheat Spikelets in Culture. Plant Cell Tissue and Organ Culture **17:** 161-170

Tsujimoto H (2001) Production of near-isogenic lines and marked monosomic lines in common wheat (Triticum aestivum) cv. Chinese spring. Journal of Heredity **92:** 254-259

Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annual Review of Plant Biology **59:** 573-594

Ugarte C, Calderini DF, Slafer GA (2007) Grain weight and grain number responsiveness to pre-anthesis temperature in wheat, barley and triticale. Field Crops Research **100:** 240-248

Ugarte CC, Trupkin SA, Ghiglione H, Slafer G, Casal JJ (2010) Low red/far-red ratios delay spike and stem growth in wheat. Journal of Experimental Botany **61:** 3151-3162

Waddington SR, Cartwright PM, Wall PC (1983) A Quantitative Scale of Spike Initial and Pistil Development in Barley and Wheat. Annals of Botany **51:** 119-130

Wang GZ, Miyashita NT, Tsunewaki K (1997) Plasmon analyses of Triticum (wheat) and Aegilops: PCR single-strand conformational polymorphism (PCR-SSCP) analyses of organellar DNAs. Proceedings of the National Academy of Sciences of the United States of America **94:** 14570-14577

Wang MH, Jiang N, Jia TY, Leach L, Cockram J, Waugh R, Ramsay L, Thomas B, Luo ZW (2012) Genome-wide association mapping of agronomic and morphologic traits in highly structured populations of barley cultivars. Theoretical and Applied Genetics **124:** 233-246

Wang P, Zhao L, Hou HL, Zhang H, Huang Y, Wang YP, Li H, Gao F, Yan SH, Li LJ (2015) Epigenetic Changes are Associated with Programmed Cell Death Induced by Heat Stress in Seedling Leaves of Zea mays. Plant and Cell Physiology **56:** 965-976

Wang SC, Wong DB, Forrest K, Allen A, Chao SM, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plieske J, Lillemo M, Mather D, Appels R, Dolferus R, Brown-Guedira G, Korol A, Akhunova AR, Feuillet C, Salse J, Morgante M, Pozniak C, Luo MC, Dvorak J, Morell M, Dubcovsky J, Ganal M, Tuberosa R, Lawley C, Mikoulitch I, Cavanagh C, Edwards KJ, Hayden M, Akhunov E, Sequencing IWG (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal **12:** 787-796

Wang Y, Sun FL, Cao H, Peng HR, Ni ZF, Sun QX, Yao YY (2012) TamiR159 Directed Wheat TaGAMYB Cleavage and Its Involvement in Anther Development and Heat Response. Plos One **7:** e48445

Wang ZL, Cao WX, Dai TB, Zhou Q (2001) Effects of exogenous hormones on floret development and grain set in wheat. Plant Growth Regulation **35:** 225-231

Weiss KM, Long JC (2009) Non-Darwinian estimation: My ancestors, my genes' ancestors. Genome Research **19:** 703-710

Weyhrich RA, Carver BF, Martin BC (1995) Photosynthesis and Water-Use Efficiency of Awned and Awnletted near-Isogenic Lines of Hard Red Winter-Wheat. Crop Science **35:** 172-176

Whingwiri EE, Kuo J, Stern WR (1981) The Vascular System in the Rachis of a Wheat Ear. Annals of Botany **48:** 189-201

Whitford R, Fleury D, Reif JC, Garcia M, Okada T, Korzun V, Langridge P (2013) Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. Journal of Experimental Botany **64:** 5411-5428

Xie Q, Mayes S, Sparkes DL (2015) Carpel size, grain filling, and morphology determine individual grain weight in wheat. Journal of Experimental Botany **66:** 6715-6730

Yamamuro C, Ihara Y, Wu X, Noguchi T, Fujioka S, Takatsuto S, Ashikari M, Kitano H, Matsuoka M (2000) Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. Plant Cell **12:** 1591-1605

Yang ZJ, van Oosterom EJ, Jordan DR, Hammer GL (2009) Pre-anthesis ovary development determines genotypic differences in potential kernel weight in sorghum. Journal of Experimental Botany **60:** 1399-1408

Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics **38:** 203-208

Zablocki RW, Schork AJ, Levine RA, Andreassen OA, Dale AM, Thompson WK (2014) Covariate-modulated local false discovery rate for genome-wide association studies. Bioinformatics **30:** 2098-2104

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Research **14**: 415-421

Zanke C, Ling J, Plieske J, Kollers S, Ebmeyer E, Korzun V, Argillier O, Stiewe G, Hinze M, Beier S, Ganal MW, Roder MS (2014a) Genetic architecture of main effect QTL for heading date in European winter wheat. Frontiers in Plant Science **5:** 217

Zanke CD, Ling J, Plieske J, Kollers S, Ebmeyer E, Korzun V, Argillier O, Stiewe G, Hinze M, Neumann K, Ganal MW, Roder MS (2014b) Whole Genome Association Mapping of Plant Height in Winter Wheat (Triticum aestivum L.). Plos One **9:** e113287

Zhang HP, Turner NC, Poole ML (2012) Increasing the harvest index of wheat in the high rainfall zones of southern Australia. Field Crops Research **129:** 111-123

Zhao K, Tung CW, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Bustamante CD, McCouch SR (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nature Communications **2:** 467

Zheng Y, Anderson S, Zhang YF, Garavito RM (2011) The Structure of Sucrose Synthase-1 from Arabidopsis thaliana and Its Functional Implications. Journal of Biological Chemistry **286:** 36108-36118

Zhou X, Stephens M (2012) Genome-wide efficient mixed-model analysis for association studies. Nature Genetics **44:** 821-824

Zinselmeier C, Lauer MJ, Boyer JS (1995) Reversing Drought-Induced Losses in Grain-Yield - Sucrose Maintains Embryo Growth in Maize. Crop Science **35:** 1390-1400

8. Supplementary information

Table S2.1. Marker information for all 30 cultivars used.

Running number	Cultivar name	*Rht-B1* (+/-)*	*Rht-D1* (+/-)*	*Ppd-D1* (+/-)[§]
1	Bussard	-	-	-
2	Tukan	-	+	+
3	Tulsa	+	-	+
4	Türkis	-	+	+
5	Winnetou	-	-	+
6	Zentos	-	-	+
7	Zobel	-	+	+
8	Cliff	-	+	+
9	Dream	-	-	+
10	Florett	-	+	+
11	History	-	-	+
12	Lindos	-	-	+
13	Julius	-	+	+
14	Exotic	-	+	-
15	Andalou	-	+	-
16	Loch	-	+	+
17	Acienda	-	+	-
18	Aguila	-	+	-
19	Alcazar	-	+	+
20	Allister	-	+	+
21	Arack	-	+	+
22	Arobase	-	+	+
23	Astuce	-	+	+
24	Aubusson	-	+	+
25	Autan	-	+	-
26	Azimut	-	+	-
27	Azzuro	-	+	+
28	Balance	-	+	+
29	Baltimor	-	+	+
30	Bastide	-	+	+

*(+) indicates the presence of a semi-dwarfing allele

[§](+) indicates the presence of the photoperiod-sensitive allele at the *Ppd-D1* locus.

Table S3.1. Monthly average global solar radiation and temperature during the 2014 field growing season.

Climatic data\Month	April	May	June	July	August
Global solar radiation (W/m^2)	169.2	191.2	222.8	208.5	173.7
Temperature (^oC)	11.4	12.9	16.3	20.3	16.6

Table S3.2. The corresponding Waddington stages of floret1 (i.e. F1) at the seven floral developmental stages based on the phenotypic variation observed in this experiment.

Kirby scale	TS	WA	GA	YA	TP	HD	AN
Waddington scale	4-5	7-7.5	7.5-8.5	8-8.5	8.5-9	9-9.5	10

Table S3.3. Living floret (primordia) number per spikelet in apical, central and basal spikelets at seven floral developmental stages and grain number per spikelet at physiological maturity (PM) in the four selected, free-tillering genotypes (control) grown in the greenhouse.

Cultivars	Position	TS	WA	GA	YA	TP	HD	AN	PM
NOS Nordgau	Apical	3.33±1.53	7.67±1.15	**9.33±0.58**	9.00±0.00	3.00±0.00	3.67±0.58	3.00±0.00	2.17±0.41
	Central	5.00±1.00	9.33±1.15	**10.00±0.00**	9.67±0.58	4.00±0.00	4.33±0.58	4.00±0.00	3.00±0.00
	Basal	5.67±1.53	9.33±1.15	**10.00±0.00**	10.00±0.00	4.00±0.00	4.33±0.58	4.00±0.00	3.17±0.41
Adlung's Alemannen	Apical	4.33±1.15	9.00±1.00	**10.00±0.0**	9.67±0.58	4.00±0.00	4.00±0.00	3.67±0.58	2.50±0.55
	Central	5.00±1.00	8.67±0.58	**10.00±0.00**	11.00±0.00	4.00±0.00	4.67±0.58	4.67±0.58	3.67±0.52
	Basal	5.00±1.00	8.33±0.58	**9.50±0.71**	10.33±1.00	4.00±0.00	4.33±0.58	4.00±0.00	3.67±0.52
Peragis Garant	Apical	4.00±1.00	7.50±0.71	**10.00±0.00**	10.00±0.00	9.00±0.00	4.00±0.00	3.00±0.58	1.83±0.75
	Central	6.33±0.58	8.00±0.00	**11.00±0.00**	10.00±1.00	9.67±0.58	5.00±0.00	4.00±0.58	2.83±0.75
	Basal	7.33±0.58	10.00±1.41	**11.00±0.00**	10.67±0.58	10.00±0.00	5.33±0.58	4.000.00	3.17±0.75
Nandu	Apical	5.00±0.00	9.50±0.71	**9.67±0.58**	10.00±0.00	9.67±0.58	9.33±0.58	3.33±0.58	1.83±0.41
	Central	5.67±0.58	10.00±0.00	**11.00±0.00**	11.00±0.00	10.33±0.58	10.00±0.00	4.00±0.00	3.00±0.00
	Basal	6.33±1.53	10.50±0.71	**10.33±0.58**	10.33±0.58	11.33±0.58	11.33±0.58	4.00±0.00	2.50±0.84

Data are presented as the mean ±SD, n=6 for PM stage, and n=3 for the remaining stages. The bold text suggests maximum floret primordia number stage is GA stage, and the grey boxes indicate the time windows of floral degradation occrued.

Table S3.4. Living floret (primordia) number per spikelet in apical, central and basal spikelets at seven floral developmental stages and grain number per spikelet at physiological maturity (PM) in the four selected, detillered genotypes (tiller removal) grown in the greenhouse.

Cultivars	Position	TS	WA	GA	YA	TP	HD	AN	PM
NOS Nordgau	Apical	4.00±1.00	9.00±0.00	**9.67±0.58**	9.33±0.58	9.33±0.58	3.67±0.58	3.00±0.00	0.40±0.89
	Central	5.00±1.00	9.50±0.71	**10.67±0.58**	11.00±0.00	11.00±0.00	4.67±0.58	4.33±0.58	3.60±0.55
	Basal	5.00±1.00	10.00±1.41	**10.33±0.58**	10.33±0.58	11.33±0.58	5.00±0.00	4.67±0.58	3.80±0.84
Adlung's Alemannen	Apical	3.33±0.58	9.00±0.00	**10.00±0.00**	10.00±0.00	10.00±0.00	4.00±0.00	3.67±0.58	0.17±0.41
	Central	4.33±0.58	9.33±0.58	**11.00±0.00**	11.00±0.00	10.00±0.00	5.00±0.00	4.67±0.58	4.33±0.82
	Basal	4.67±0.58	9.00±0.00	**11.00±0.00**	11.00±0.00	10.67±0.58	5.00±1.00	5.00±1.00	4.67±0.52
Peragis Garant	Apical	5.00±2.00	8.33±1.15	**9.67±0.58**	10.00±0.00	10.00±0.00	10.00±0.00	3.67±0.58	1.83±1.60
	Central	6.67±1.53	9.00±0.00	**10.67±1.15**	11.67±0.58	11.00±0.00	11.33±0.58	5.33±0.58	2.83±1.72
	Basal	7.33±1.15	9.67±1.15	**10.67±1.15**	10.67±0.58	11.00±0.00	11.00±0.00	5.33±1.15	3.50±0.84
Nandu	Apical	4.33±1.15	9.67±0.58	**10.00±0.00**	9.67±1.15	10.33±0.58	10.67±0.58	4.00±0.00	1.83±0.98
	Central	5.33±0.58	10.33±1.15	**11.67±0.58**	11.33±0.58	11.67±0.58	11.33±1.53	5.33±0.58	4.50±0.55
	Basal	4.67±0.58	10.67±0.58	**11.33±0.58**	11.33±0.58	11.67±0.58	12.00±0.00	5.33±0.58	4.17±0.98

Data are presented as the mean ± SD, n=6 for PM stage, and n=3 for the remaining stages. The bold text suggests maximum floret primordia number stage is GA stage, and the grey boxes indicate the time windows of floral degradation occrued.

Table S3.5. Living floret (primordia) number per spikelet in apical, central and basal spikelets at seven floral developmental stages and grain number per spikelet at physiological maturity (PM) in twelve free-tillering genotypes (control) grown in the field.

Cultivars (control, field)	Position	TS	WA	GA	YA	TP	HD	AN	PM
01- Adlung's	Apical	2.33±0.58	9.00±1.00	**9.67±0.58**	9.67±1.15	4.33±0.58	4.33±0.58	3.00±0.00	2.50±0.55
Alemannen	Central	3.33±0.58	10.00±0.00	**10.67±0.58**	10.00±0.00	5.33±0.58	5.33±0.58	4.33±0.58	3.67±0.82
	Basal	3.33±0.58	9.33±0.58	**10.33±0.58**	11.00±0.00	5.33±0.58	5.00±1.00	4.33±0.58	3.50±0.55
2- NOS Nordgau	Apical	2.00±0.00	8.67±0.58	**10.00±0.00**	4.00±0.00	4.00±0.00	4.00±0.00	3.00±0.00	2.83±0.75
	Central	3.67±0.58	9.00±0.00	**10.33±0.58**	5.67±0.58	5.00±0.00	5.00±0.00	4.33±0.58	3.67±0.82
	Basal	3.00±0.00	9.67±0.58	**10.33±0.58**	5.67±0.58	5.00±0.00	5.00±0.00	4.33±0.58	3.67±0.52
3- Peragis Garant	Apical	4.00±0.00	8.67±1.15	**10.00±0.00**	9.33±1.15	9.67±1.15	4.00±0.00	3.67±0.58	2.67±0.52
	Central	5.33±0.58	10.33±0.58	**11.00±1.00**	11.00±1.00	10.67±1.15	5.67±0.58	5.00±1.00	3.50±0.84
	Basal	5.33±0.58	10.33±0.58	**11.33±0.58**	10.67±0.58	11.67±0.58	6.00±0.00	5.00±1.00	3.00±1.26
4- Heine's Peko	Apical	2.67±0.58	8.00±1.00	**9.33±0.58**	9.00±0.00	4.00±0.00	3.00±0.00	3.00±0.00	1.83±0.41
	Central	4.00±1.00	9.67±0.58	**10.33±0.58**	10.33±0.58	5.00±0.00	3.67±0.58	4.00±0.00	2.67±0.52
	Basal	3.67±0.58	9.67±0.58	**10.67±0.58**	10.33±0.58	5.00±0.00	3.67±0.58	4.00±0.00	2.67±0.52
5- Hohenheimer Franken II	Apical	3.33±0.58	9.00±1.00	**10.00±0.00**	4.33±0.58	4.00±0.00	3.33±0.58	3.33±0.58	2.33±0.52
	Central	4.00±1.00	9.00±0.00	**10.33±0.58**	5.00±0.00	4.67±0.58	4.00±0.00	4.00±0.00	2.50±0.55
	Basal	3.33±0.58	9.00±0.00	**10.00±0.00**	4.67±0.58	4.67±0.58	4.00±0.00	4.00±0.00	3.00±0.63
6- Probat	Apical	3.33±0.58	9.33±0.58	**10.00±0.00**	9.67±1.15	6.00±3.46	5.00±0.00	4.33±0.58	2.50±0.55
	Central	5.33±0.58	10.67±0.58	**11.00±0.00**	11.67±0.58	7.67±2.89	6.00±0.00	6.00±0.00	3.83±0.75
	Basal	4.33±0.58	11.00±0.00	**11.00±0.00**	11.00±1.00	7.33±3.21	6.00±0.00	5.33±0.58	3.33±1.03
7- Breustedt's Lera	Apical	2.67±0.58	8.33±0.58	**9.67±0.58**	9.33±0.58	4.33±0.58	4.00±0.00	4.00±0.00	2.33±1.37
	Central	5.00±1.00	9.67±0.58	**11.00±0.00**	10.67±0.58	4.67±0.58	5.33±1.15	5.33±0.58	3.83±1.47
	Basal	4.67±0.58	9.00±0.00	**10.67±0.58**	11.00±0.00	4.67±0.58	5.00±1.00	5.00±0.00	4.50±0.55
8- Arin	Apical	3.67±0.58	9.00±0.00	**9.33±0.58**	5.00±0.00	5.00±0.00	4.67±0.58	4.00±0.00	3.50±1.05
	Central	5.33±0.58	9.67±0.58	**10.67±0.58**	6.00±0.00	6.00±0.00	6.00±0.00	5.00±0.00	4.17±0.75
	Basal	5.67±0.58	10.67±0.58	**11.00±0.00**	6.00±0.00	6.00±0.00	6.00±0.00	5.00±0.00	4.50±0.84
9- Kolibri	Apical	4.00±1.00	8.50±0.71	**9.00±1.00**	4.00±0.00	3.33±0.58	4.00±0.00	3.67±0.58	2.33±0.52
	Central	5.00±1.00	9.00±0.00	**10.00±0.00**	4.67±0.58	4.33±0.58	4.67±0.58	4.67±0.58	3.17±0.75
	Basal	4.67±1.15	9.00±0.00	**10.00±0.00**	4.00±0.00	4.33±0.58	5.00±1.00	4.67±0.58	3.33±1.03
10- Ralle	Apical	4.00±1.00	8.67±0.58	**9.00±0.00**	4.33±0.58	4.00±0.00	4.00±0.00	3.67±0.58	2.17±0.41
	Central	5.67±0.58	9.67±0.58	**10.00±0.00**	5.00±0.00	5.00±0.00	5.00±0.00	4.33±1.15	3.17±0.41
	Basal	5.00±1.00	9.33±0.58	**10.00±0.00**	5.33±0.58	5.00±0.00	4.67±0.58	4.00±1.00	2.83±0.75
11- Nandu	Apical	3.33±0.58	9.00±0.00	**10.67±0.58**	10.33±0.58	10.00±0.00	10.00±0.00	3.33±0.58	2.50±0.55
	Central	5.33±0.58	10.00±0.00	**11.67±0.58**	11.33±0.58	11.00±0.00	11.00±0.00	4.33±0.58	3.67±0.52
	Basal	5.00±1.00	10.00±0.00	**11.67±0.58**	12.00±0.00	11.67±0.58	12.00±1.00	4.33±0.58	3.33±1.03
12- Fasan	Apical	2.67±0.58	8.67±0.58	**10.00±0.00**	4.67±0.58	4.33±0.58	4.00±0.00	3.67±0.58	2.83±0.41
	Central	4.33±1.15	10.33±0.58	**10.67±0.58**	6.00±0.00	5.33±0.58	5.00±0.00	5.00±0.00	3.83±1.17
	Basal	4.33±1.15	10.33±0.58	**11.00±0.00**	6.00±0.00	6.00±0.00	5.33±0.58	5.67±0.58	4.17±1.47

Data are presented as the mean ± SD, n=6 for PM stage, and n=3 for the remaining stages. The bold text suggests maximum floret primordia number stage is GA stage, and the grey boxes indicate the time windows of floral degradation occrued.

Table S3.6. Living floret (primordia) number per spikelet in apical, central and basal spikelets at seven floral developmental stages and grain number per spikelet at physiological maturity (PM) in twelve detillered genotypes (tiller removal) grown in the field.

Cultivars (detillering, field)	Position	TS	WA	GA	YA	TP	HD	AN	PM
1- Adlung's Alemannen	Apical	3.50±0.71	8.67±0.58	**10.67±0.58**	10.67±0.58	10.33±0.58	5.67±0.58	5.00±1.00	2.33±1.75
	Central	4.50±0.71	9.67±0.58	**10.67±0.58**	11.33±0.58	11.67±0.58	6.00±1.00	6.00±0.00	4.67±0.52
	Basal	4.00±0.00	9.67±0.58	**11.00±0.00**	11.33±0.58	12.00±0.00	5.67±0.58	6.00±0.00	4.33±1.21
2- NOS Nordgau	Apical	2.00±0.00	8.33±0.58	**10.67±0.58**	10.33±0.58	9.67±0.58	5.67±0.58	3.67±0.58	3.00±1.55
	Central	3.33±0.58	9.00±1.00	**10.67±0.58**	11.00±0.00	11.00±1.00	6.33±0.58	6.00±0.00	4.50±0.84
	Basal	3.33±0.58	9.67±0.58	**10.67±0.58**	10.67±0.58	11.00±0.00	6.00±0.00	6.00±0.00	4.50±0.84
3- Peragis Garant	Apical	4.00±1.73	9.67±0.58	**10.67±0.58**	10.67±0.58	10.67±0.58	9.00±3.46	3.33±0.58	2.50±1.05
	Central	5.33±0.58	10.33±0.58	**11.67±0.58**	12.00±0.00	11.33±0.58	10.00±3.46	5.00±0.00	3.33±1.03
	Basal	6.67±0.58	9.67±0.58	**11.67±0.58**	12.00±0.00	12.33±0.58	10.00±3.46	5.00±0.00	3.33±1.03
4- Heine's Peko	Apical	2.00±0.00	8.33±0.58	**9.33±0.58**	9.67±0.58	9.67±0.58	4.00±0.00	3.67±0.58	2.00±0.63
	Central	4.00±1.00	9.33±1.15	**11.00±0.00**	10.67±0.58	10.33±0.58	5.67±0.58	5.67±0.58	3.50±0.84
	Basal	4.00±1.00	9.33±0.58	**11.00±0.00**	11.33±0.58	11.33±0.58	5.67±0.58	5.67±0.58	3.33±1.03
5- Hohenheimer Franken II	Apical	2.33±0.58	9.33±1.53	**10.00±0.00**	10.00±0.00	5.00±0.00	4.00±0.00	4.33±0.58	0.50±0.55
	Central	3.00±1.00	9.67±0.58	**10.67±0.58**	11.00±0.00	5.00±0.00	5.00±0.00	5.33±0.58	3.33±0.52
	Basal	2.33±0.58	9.67±1.53	**10.00±0.00**	11.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	3.50±0.55
6- Probat	Apical	3.00±0.00	8.67±0.58	**10.33±0.58**	11.00±0.00	11.00±0.00	11.00±0.00	5.00±2.83	2.25±0.96
	Central	5.00±0.00	9.67±0.58	**11.67±0.58**	12.50±0.71	12.00±0.00	12.00±0.00	6.00±1.41	4.00±1.15
	Basal	4.00±1.41	10.67±0.58	**11.67±0.58**	12.00±0.00	12.00±0.00	12.00±0.00	6.50±0.71	3.50±1.29
7- Breustedt's Lera	Apical	2.33±0.58	8.67±0.58	**10.33±0.58**	10.00±0.00	9.50±0.71	4.50±0.71	4.00±1.41	2.00±1.73
	Central	4.00±1.00	10.33±1.15	**11.33±0.58**	11.50±0.71	11.50±0.71	5.50±0.71	6.00±0.00	4.33±0.58
	Basal	4.00±1.00	10.67±0.58	**11.33±0.58**	12.00±1.41	12.00±0.00	5.50±0.71	6.00±0.00	3.33±1.53
8- Arin	Apical	3.33±0.58	9.00±0.00	**9.67±0.58**	9.00±0.00	9.00±0.00	4.67±0.58	4.33±0.58	3.50±1.00
	Central	4.67±0.58	10.00±0.00	**10.33±0.58**	11.00±0.00	10.50±0.71	5.67±0.58	5.00±0.00	3.75±1.26
	Basal	6.00±1.00	9.67±1.15	**10.00±1.00**	10.33±1.15	11.00±0.00	6.67±0.58	5.67±0.58	3.75±0.96
9- Kolibri	Apical	3.33±0.58	9.00±0.00	**9.67±0.58**	4.67±0.58	4.33±0.58	4.00±0.00	3.33±0.58	2.83±0.41
	Central	4.67±0.58	9.67±0.58	**10.33±0.58**	5.67±0.58	5.67±0.58	5.67±0.58	5.67±0.58	4.17±1.17
	Basal	4.00±1.00	9.67±0.58	**10.33±0.58**	5.67±0.58	5.67±0.58	5.67±0.58	5.33±0.58	3.50±1.52
10- Ralle	Apical	3.33±0.58	9.00±0.00	**9.67±0.58**	9.33±0.58	5.00±0.00	4.00±0.00	3.67±0.58	1.50±1.64
	Central	5.33±0.58	9.67±0.58	**11.00±0.00**	10.33±0.58	6.00±0.00	6.00±0.00	4.67±0.58	3.33±0.82
	Basal	4.67±0.58	10.00±0.00	**11.00±1.00**	12.00±0.00	6.00±0.00	7.33±2.31	4.67±0.58	4.00±0.89
11- Nandu	Apical	3.67±1.15	8.00±0.00	**10.67±0.58**	11.00±0.00	11.00±0.00	11.00±1.00	4.67±0.58	3.29±0.00
	Central	5.67±0.58	9.00±0.00	**11.00±0.00**	12.33±0.58	12.00±0.00	12.33±0.58	6.67±0.58	4.00±0.75
	Basal	6.00±1.00	10.00±0.00	**11.33±0.58**	11.67±0.58	12.33±0.58	13.00±0.00	6.67±0.58	3.43±0.98
12- Fasan	Apical	3.00±0.00	9.00±0.00	**10.33±0.58**	10.00±0.00	4.00±0.00	4.33±0.58	3.33±0.58	1.83±1.33
	Central	5.00±0.00	10.00±0.00	**11.00±0.00**	11.00±0.00	5.00±0.00	5.67±0.58	5.00±0.00	3.00±0.63
	Basal	5.00±0.00	9.67±0.58	**11.33±0.58**	12.00±0.00	5.67±0.58	5.67±0.58	5.00±1.00	3.17±0.98

Data are presented as the mean ± SD, n=6 for PM stage, and n=3 for the remaining stages. The bold text suggests maximum floret primordia number stage is GA stage, and the grey boxes indicate the time windows of floral degradation occrued.

Table S3.7. Living floret (primordia) number per spikelet in apical, central and basal spikelets at seven floral developmental stages and grain number per spikelet at physiological maturity (PM) in twelve free-tillering genotypes (control) grown in the greenhouse.

Cultivars (control, greenhouse)	Position	TS	WA	GA	YA	TP	HD	AN	PM
1- Adlung's	Apical	4.33±1.15	9.00±1.00	**10.00±0.00**	9.67±0.58	4.00±0.00	4.00±0.00	3.67±0.58	2.50±0.55
Alemannen	Central	5.00±1.00	8.67±0.58	**10.00±0.00**	11.00±0.00	4.00±0.00	4.67±0.58	4.67±0.58	3.67±0.52
	Basal	5.00±1.00	8.33±0.58	**9.50±0.71**	10.33±1.15	4.00±0.00	4.33±0.58	4.00±0.00	3.67±0.52
2- NOS Nordgau	Apical	3.33±1.53	7.67±1.15	**9.33±0.00**	9.00±0.00	3.00±0.00	3.67±0.58	3.00±0.00	2.17±0.41
	Central	5.00±1.00	9.33±1.15	**10.00±0.00**	9.67±0.58	4.00±0.00	4.33±0.58	4.00±0.00	3.00±0.00
	Basal	5.67±1.53	9.33±1.15	**10.00±0.00**	10.00±0.00	4.00±0.00	4.33±0.58	4.00±0.00	3.17±0.41
3- Peragis Garant	Apical	4.00±1.00	7.50±0.71	**10.00±0.00**	10.00±0.00	9.00±0.00	4.00±0.00	3.00±0.00	1.83±0.75
	Central	6.33±0.58	8.00±0.00	**11.00±0.00**	10.00±1.00	9.67±0.58	5.00±0.00	4.00±0.00	2.83±0.75
	Basal	7.33±0.58	10.00±1.41	**11.00±0.00**	10.67±0.58	10.00±0.00	5.33±0.58	4.00±0.00	3.17±0.75
4- Heine's Peko	Apical	2.33±0.58	7.33±0.58	**9.00±0.00**	9.33±0.58	3.00±0.00	3.00±0.00	3.00±0.00	0.17±0.41
	Central	4.67±0.58	8.67±0.58	**10.00±0.00**	10.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	2.83±0.41
	Basal	4.33±0.58	8.33±0.58	**9.33±0.58**	9.67±0.58	3.67±0.58	4.00±0.00	4.00±0.00	3.17±0.75
5- Hohenheimer Franken II	Apical	2.00±0.00	8.33±0.58	**9.00±0.00**	9.00±0.00	3.33±0.58	3.00±0.00	3.00±0.00	1.00±1.10
	Central	2.67±0.58	8.33±0.58	**10.33±0.58**	9.00±0.00	4.00±0.00	4.00±0.00	3.67±0.58	2.00±0.63
	Basal	2.33±0.58	8.67±0.58	**9.67±0.58**	9.33±0.58	4.00±0.00	4.00±0.00	3.33±0.58	2.83±0.41
6- Probat	Apical	3.67±0.58	7.67±1.15	**10.00±0.00**	10.00±1.00	9.33±0.58	4.00±0.00	3.33±0.58	0.17±0.41
	Central	6.00±0.00	10.33±0.58	**11.00±0.00**	11.00±1.00	10.00±1.00	4.67±0.58	4.00±0.00	3.17±0.41
	Basal	5.67±0.58	9.67±0.58	**10.67±0.58**	11.00±1.00	11.33±0.58	4.67±0.58	4.00±0.00	3.17±0.41
7- Breustedt's Lera	Apical	3.00±1.00	8.00±0.00	**8.67±0.58**	8.67±0.58	3.33±0.58	3.67±0.58	3.00±0.00	0.50±0.55
	Central	5.67±1.15	8.67±0.58	**10.00±0.00**	10.00±0.00	4.67±0.58	4.67±0.58	3.67±0.58	2.33±1.21
	Basal	5.33±1.53	9.33±0.58	**9.33±0.58**	9.33±0.58	4.67±0.58	4.67±0.58	3.33±0.58	2.67±0.52
8- Arin	Apical	4.00±0.00	7.67±0.58	**8.33±0.58**	8.67±0.58	9.00±0.00	3.67±0.58	3.00±0.00	0.83±0.75
	Central	5.67±0.58	8.67±0.58	**9.00±0.00**	9.00±0.00	9.00±0.58	5.00±0.00	3.67±0.58	3.33±0.52
	Basal	5.67±1.15	9.00±0.00	**10.67±0.58**	10.33±0.58	10.00±1.00	4.33±0.58	4.00±1.00	3.00±0.63
9- Kolibri	Apical	3.00±0.00	7.67±1.15	**9.00±0.00**	9.00±0.00	4.00±0.00	3.00±0.00	3.00±0.00	1.83±0.41
	Central	5.33±0.58	9.33±0.58	**10.00±0.00**	10.00±0.00	5.00±0.00	4.00±0.00	4.00±0.00	3.00±0.00
	Basal	4.33±1.53	9.33±0.58	**9.67±0.58**	9.33±0.58	5.00±0.00	4.00±0.00	4.00±0.00	2.33±0.82
10- Ralle	Apical	4.67±1.53	8.33±0.58	**9.00±0.00**	9.33±0.58	4.00±0.00	3.33±0.58	3.00±0.00	1.80±0.45
	Central	5.67±1.53	9.67±0.58	**10.00±0.00**	10.00±0.00	4.33±0.58	4.33±0.58	3.67±0.58	2.40±0.55
	Basal	6.00±1.73	9.67±0.58	**10.00±0.00**	9.67±0.58	4.33±0.58	4.00±0.00	3.00±0.00	2.60±0.55
11- Nandu	Apical	5.00±0.00	9.50±0.71	**9.67±0.58**	10.00±0.00	9.67±0.58	9.33±0.58	3.33±0.58	1.83±0.41
	Central	5.67±0.58	10.00±0.00	**11.00±0.00**	11.00±0.00	10.33±0.58	10.00±0.00	4.00±0.00	3.00±0.00
	Basal	6.33±1.53	10.50±0.71	**10.33±0.58**	10.33±0.58	11.33±0.58	11.33±0.58	4.00±0.00	2.50±0.84
12- Fasan	Apical	4.00±1.00	8.00±1.41	**9.00±0.00**	9.00±1.00	8.67±0.58	4.00±0.00	3.00±0.00	1.50±0.84
	Central	5.67±1.53	9.50±0.71	**10.00±0.00**	10.33±0.58	9.33±0.58	4.00±0.00	4.00±0.00	2.83±0.41
	Basal	6.00±1.73	9.50±0.71	**10.67±0.58**	11.00±0.00	10.33±0.58	4.00±0.00	3.67±0.58	2.67±1.37

Data are presented as the mean ± SD, n=6 for PM stage, and n=3 for the remaining stages. The bold text suggests maximum floret primordia number stage is GA stage, and the grey boxes indicate the time windows of floral degradation occrued.

Table S3.8. Living floret (primordia) number per spikelet in apical, central and basal spikelets at seven floral developmental stages and grain number per spikelet at physiological maturity (PM) in twelve detillered genotypes (tiller removal) grown in the greenhouse.

Cultivars (detillering, greenhouse)	Position	TS	WA	GA	YA	TP	HD	AN	PM
1- Adlung's Alemannen	Apical	3.33±0.58	9.00±0.00	**10.00±0.00**	10.00±0.00	10.00±0.00	4.00±0.00	3.67±0.58	0.17±0.41
	Central	4.33±0.58	9.33±0.58	**11.00±0.00**	11.00±0.00	10.00±0.00	5.00±0.00	4.67±0.58	4.33±0.82
	Basal	4.67±0.58	9.00±0.00	**11.00±0.00**	11.00±0.00	10.67±0.58	5.00±1.00	5.00±1.00	4.67±0.52
2- NOS Nordgau	Apical	4.00±1.00	9.00±0.00	**9.67±0.58**	9.33±0.58	9.33±0.58	3.67±0.58	3.00±0.00	0.40±0.89
	Central	5.00±1.00	9.50±0.71	**10.67±0.58**	11.00±0.00	11.00±0.00	4.67±0.58	4.33±0.58	3.60±0.55
	Basal	5.00±1.00	10.00±1.41	**10.33±0.58**	10.33±0.58	11.33±0.58	5.00±0.00	4.67±0.58	3.80±0.84
3- Peragis Garant	Apical	5.00±2.00	8.33±0.00	**9.67±1.15**	10.00±0.00	10.00±0.00	10.00±0.00	3.67±0.58	1.83±1.60
	Central	6.67±1.53	9.00±1.15	**10.67±1.15**	11.67±0.58	11.00±0.00	11.33±0.58	5.33±0.58	2.83±1.72
	Basal	7.33±1.15	9.67±0.58	**10.67±0.00**	10.67±0.58	11.00±0.00	11.00±0.00	5.33±1.15	3.50±0.84
4- Heine's Peko	Apical	2.00±0.00	8.50±0.71	**9.67±0.58**	10.00±0.00	9.67±0.58	3.67±0.58	3.33±0.58	0.00±0.00
	Central	3.67±1.15	10.00±0.00	**10.33±0.58**	10.33±0.58	11.33±0.58	5.00±0.00	5.00±0.00	1.50±1.64
	Basal	3.00±1.00	10.50±0.71	**11.00±0.00**	12.00±1.00	11.00±1.00	5.00±0.00	4.67±0.58	3.83±0.41
5- Hohenheimer Franken II	Apical	2.33±0.58	7.67±0.58	**10.00±0.00**	9.67±0.58	5.67±2.89	4.00±0.00	3.33±0.58	0.00±0.00
	Central	3.33±0.58	8.67±0.58	**10.00±0.00**	10.00±0.00	8.67±2.31	5.33±0.58	4.00±0.00	1.17±1.33
	Basal	3.00±1.00	9.33±0.58	**9.67±0.58**	10.33±0.58	8.00±1.73	5.00±1.00	4.00±0.00	3.33±1.21
6- Probat	Apical	2.00±0.00	8.33±0.58	**10.33±0.58**	11.00±0.00	10.67±0.58	8.33±3.79	4.00±1.00	0.00±0.00
	Central	3.00±0.00	10.33±0.58	**11.00±1.00**	11.67±0.58	12.33±0.58	10.00±3.61	5.33±0.58	2.00±1.63
	Basal	3.33±1.15	10.33±0.58	**11.33±0.58**	12.00±1.00	12.00±1.00	10.33±4.62	5.67±0.58	3.25±0.50
7- Breustedt's Lera	Apical	3.00±0.00	8.33±0.58	**9.33±0.58**	9.67±0.58	9.33±0.58	4.00±0.00	3.00±0.00	0.00±0.00
	Central	5.33±0.58	9.33±0.58	**10.33±0.58**	10.67±0.58	12.00±0.00	6.00±0.00	5.00±0.00	2.40±2.07
	Basal	5.00±0.00	9.33±0.58	**11.00±0.00**	11.00±1.00	11.67±0.58	6.00±0.00	5.00±0.00	3.20±1.79
8- Arin	Apical	3.67±0.58	7.33±0.58	**8.67±0.58**	9.67±0.58	9.33±0.58	4.33±0.58	3.00±0.00	2.17±0.75
	Central	5.67±1.15	8.00±0.00	**10.33±1.15**	10.00±1.00	10.67±0.58	5.33±0.58	4.67±0.58	4.50±0.55
	Basal	7.00±1.00	9.67±0.58	**10.67±0.58**	11.00±0.00	10.67±0.58	5.33±0.58	4.67±0.58	4.00±1.10
9- Kolibri	Apical	4.00±1.00	8.33±0.58	**9.50±0.71**	10.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	2.83±0.41
	Central	4.67±0.58	8.67±0.58	**10.00±0.00**	10.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	4.17±0.41
	Basal	4.00±1.00	8.33±0.58	**10.00±0.00**	10.00±0.00	5.00±0.00	5.00±0.00	4.67±0.58	4.33±0.52
10- Ralle	Apical	5.00±0.00	8.67±0.58	**9.33±0.58**	9.00±0.00	9.67±1.15	4.33±0.58	4.00±0.00	1.50±0.84
	Central	5.67±0.58	10.00±0.00	**10.33±0.58**	11.00±0.00	10.00±0.00	5.00±0.00	4.67±0.58	3.50±0.55
	Basal	6.00±1.00	9.67±0.58	**10.67±0.58**	10.33±0.58	10.67±0.58	5.00±0.00	4.33±0.58	3.50±0.55
11- Nandu	Apical	4.33±1.15	9.67±0.58	**10.00±0.00**	9.67±1.15	10.33±0.58	10.67±0.58	4.00±0.00	1.83±0.98
	Central	5.33±0.58	10.33±1.15	**11.67±0.58**	11.33±0.58	11.67±0.58	11.33±1.53	5.33±0.58	4.50±0.55
	Basal	4.67±0.58	10.67±0.58	**11.33±0.58**	11.33±0.58	11.67±0.58	12.00±0.00	5.33±0.58	4.17±0.98
12- Fasan	Apical	2.00±0.00	8.00±0.00	**9.00±0.00**	9.67±0.58	10.00±0.00	10.00±0.00	3.00±0.00	1.17±0.75
	Central	4.00±0.00	9.33±0.58	**10.67±0.58**	11.00±0.00	10.50±0.71	10.67±0.58	4.33±0.58	3.83±0.98
	Basal	3.67±0.58	10.00±0.00	**11.33±1.15**	11.00±1.00	10.00±0.00	11.00±0.00	4.67±0.58	3.50±0.55

Data are presented as the mean ± SD, n=6 for PM stage, and n=3 for the remaining stages. The bold text suggests maximum floret primordia number stage is GA stage, and the grey boxes indicate the time windows of floral degradation occrued.

Table S3.9. Thermal time required for seven floral developmental stages in twelve spring wheat cultivars grown in the field (control and tiller removal).

Cultivars	TS		WA		GA		YA		TP		HD		AN	
	Control	Detilering	Control	Detilering	Control	Detilering	Control	Detilering	Control	Detilering	Control	Detilering	Control	Detilering
1- Adlung's Alemannen	761±0a	761±0a	1032±34a	1032±0a	1094±0a	1094±0a	1125±0b	1162±0a	1182±17a	1207±0a	1243±0a	1266±21a	1314±0b	1410±0a
2- NOS Nordgau	761±0a	761±0a	1012±0a	1032±0a	1094±0a	1094±0a	1162±0a	1162±0a	1172±17b	1207±0a	1207±0a	1231±41a	1314±0a	1314±0a
3- Peragis Garant	761±0a	739±0a	973±0a	973±0a	1032±0a	1032±0a	1080±0a	1094±0a	1125±21a	1137±0a	1192±0a	1182±0a	1314±0a	1314±0a
4- Heine's Peko	837±0a	837±0a	1032±0a	10730a	1094±0b	1162±0a	1162±0b	1207±0a	1219±0b	1243±21a	1290±0a	1314±17a	1352±0a	1366±0a
5- Hohenheimer Franken II	837±0a	837±0a	1032±0a	1080±0a	1094±0b	1125±0a	1172±0a	1192±0a	1231±0b	1314±0a	1267±0b	1352±0a	1335±0b	1410±0a
6- Probat	837±0a	824±0a	1032±0a	1032±0a	1080±0b	1162±0a	1146±0b	1184±0a	1231±21b	1366±0a	1314±0b	1410±0a	1366±0a	1443±0a
7- Breustedt's Lera	837±0a	824±0a	1080±0a	1105±0a	1125±0b	1192±0a	1192±0b	1243±0a	1243±21b	1314±18a	1290±0b	1366±17a	1352±0b	1388±0a
8- Arin	761±0a	739±0a	973±0a	973±0a	1032±0b	1080±0a	1080±0b	1125±0a	1125±0a	1144±0a	1162±0a	1162±0a	1266±0b	1314±0a
9- Kolibri	761±0a	761±0a	973±0a	973±0a	1032±0b	1089±0a	1080±0a	1094±0a	1137±28a	1105±0a	1182±0a	1172±0a	1290±0a	1314±0a
10- Ralle	761±0a	739±0a	973±0a	973±0a	1032±0b	1094±0a	1080±0b	1125±0a	1125±21b	1162±0a	1192±0a	1207±0a	1314±0a	1331±0a
11- Nandu	761±0a	739±0a	973±0a	955±0a	1032±0a	1048±0a	1080±0a	1080±0a	1146±21a	1125±0a	1162±0a	1162±0a	1267±0a	1267±0a
12- Fasan	761±0a	753±0a	973±0a	973±0a	1085±0a	1080±0a	1094±0a	1125±0a	1137±0a	1162±26a	1172±0b	1207±0a	1314±0a	1314±0a
Average	786±36a	776±40a	1005±37a	1014±50a	1069±33b	1104±47a	1121±43b	1149±48a	1173±47a	1207±79a	1222±54a	1252±81a	1316±32b	1349±51a

Data are presented as the mean±SD, n=3; different letters per trait indicate significant differences between control and treated plants (p<0.05).

Table S3.10. Thermal time required for seven floral developmental stages in twelve spring wheat cultivars grown in the greenhouse (control and tiller removal).

Cultivars	TS		WA		GA		YA		TP		HD		AN	
	Control	Detilering	Control	Detilering	Control	Detilering	Control	Detilering	Control	Detilering	Control	Detilering	Control	Detilering
1- Adlung's Alemannen	785±0a	785±0a	947±0b	983±0a	1037±73b	1091±0a	1073±10b	1163±0a	1145±64b	1217±10a	1145±0b	1271±0a	1253±0b	1433±21a
2- NOS Nordgau	785±0a	785±0a	983±0a	911±0b	1067±0a	1073±0a	1097±0a	1127±0a	1208±0a	1151±0b	1163±0b	1199±0a	1253±0a	1277±62a
3- Peragis Garant	749±0a	731±0a	911±0a	899±0a	947±0a	947±0a	1001±0a	983±0a	1055±0b	1085±21a	1109±0b	1145±36a	1217±0a	1217±0a
4- Heine's Peko	785±0a	785±0a	983±0a	1073±42a	1091±0b	1163±0a	1181±0b	1217±0a	1217±0b	1277±21a	1253±0b	1289±0a	1289±0b	1469±0a
5- Hohenheimer Franken II	785±0a	785±0a	1025±42a	1073±0a	1163±0a	1163±0a	1217±0a	1229±42a	1265±21a	1277±21a	1313±0a	1301±21a	1397±0b	1469±25a
6- Probat	821±0a	749±0a	1001±0a	1007±21a	1109±0a	1127±0a	1217±0a	1163±b	1241±21a	1205±10a	1271±0a	1229±21b	1301±21b	1349±21a
7- Breustedt's Lera	857±0a	857±0a	1067±0a	1163±0a	1163±0b	1217±0a	1217±0b	1265±0a	1277±10b	1337±0a	1307±0b	1373±0a	1397±0b	1487±0a
8- Arin	749±0a	749±0a	923±0a	935±21a	1073±0a	1037±0b	1145±0a	1073±0a	1181±21a	1145±0b	1217±0a	1181±0b	1289±0a	1253±0a
9- Kolibri	749±0a	731±0a	911±0a	911±21a	1001±42a	983±0a	1055±0a	1019±0b	1079±0a	1073±0a	1145±0a	1145±0a	1217±21a	1217±21a
10- Ralle	713±0a	731±0a	947±10a	923±0a	1019±0a	1019±0a	1073±0a	1073±0a	1133±21a	1145±21a	1163±42a	1181±21a	1253±0b	1289±0a
11- Nandu	749±0a	749±0a	947±0a	971±0a	1043±0a	1073±0a	1091±0a	1109±0a	1145±21a	1145±0a	1199±25a	1217±0a	1277±21a	1265±21a
12- Fasan	785±0a	773±0a	983±21a	983±0a	1019±0b	1061±0a	1103±0b	1163±0a	1229±0a	1199±0a	1235±0a	1235±0a	1277±0b	1313±0a
Average	776±37a	768±35a	969±48a	986±80a	1061±66a	1080±76a	1123±70a	1132±83a	1181±73a	1188±79a	1210±67a	1231±67a	1285±58b	1337±99a

Data are presented as the mean±SD, n=3; different letters per trait indicate significant differences between control and treated plants (p<0.05).

Table S3.11. Spikelet fertility (%) in twelve genotypes under control and tiller removal treatments in the greenhouse and field at harvest.

Genotype	Year of release	Greenhouse		Field	
		Control	Detillering	Control	Detillering
1- Adlung's Alemannen	1931	99.21±1.94a	62.65±8.27b	95.78±4.95a	80.73±12.63b
2- NOS Nordgau	1933	87.12±4.74a	70.69±8.07b	92.80±5.57a	96.01±3.73a
3- Peragis Garant	1946	84.89±11.32a	69.24±22.60a	95.03±3.17a	96.39±6.70a
4- Heine's Peko	1947	63.80±8.89a	48.83±9.77b	96.41±5.61a	88.25±9.57b
5- Hohenheimer Franken II	1951	77.91±16.77a	49.35±3.11b	90.15±13.47a	71.68±11.50b
6- Probat	1953	65.73±11.74a	50.97±12.93a	97.33±3.27a	83.49±5.35b
7- Breustedt's Lera	1959	62.44±11.44a	48.69±12.67a	94.13±4.52a	84.06±9.05b
8- Arin	1962	67.17±5.11b	86.21±8.94a	99.07±2.27a	95.74±5.90a
9- Kolibri	1966	88.39±9.39a	90.93±6.66a	95.95±5.83a	97.07±5.28a
10- Ralle	1980	92.11±4.69a	88.04±9.96a	92.73±5.31a	78.03±13.26b
11- Nandu	1988	83.71±13.98a	85.92±15.70a	92.19±5.96a	96.63±3.50a
12- Fasan	1997	71.66±12.43a	73.49±3.29a	97.83±3.64a	83.81±14.32b

Data are presented as the mean±SD, n=6; different letters per trait indicate significant differences between control and treated plants (p<0.05).

Table S4.1. Marker information for all 30 cultivars used.

Running number	Cultivar name	*Rht-B1* (+/-)*	*Rht-D1* (+/-)*	*Ppd-D1* (+/-)[§]
1	Bussard	-	-	-
2	Tukan	-	+	+
3	Tulsa	+	-	+
4	Türkis	-	+	+
5	Winnetou	-	-	+
6	Zentos	-	-	+
7	Zobel	-	+	+
8	Cliff	-	+	+
9	Dream	-	-	+
10	Florett	-	+	+
11	History	-	-	+
12	Lindos	-	-	+
13	Julius	-	+	+
14	Exotic	-	+	-
15	Andalou	-	+	-
16	Loch	-	+	+
17	Acienda	-	+	-
18	Aguila	-	+	-
19	Alcazar	-	+	+
20	Allister	-	+	+
21	Arack	-	+	+
22	Arobase	-	+	+
23	Astuce	-	+	+
24	Aubusson	-	+	+
25	Autan	-	+	-
26	Azimut	-	+	-
27	Azzuro	-	+	+
28	Balance	-	+	+
29	Baltimor	-	+	+
30	Bastide	-	+	+

*(+) indicates the presence of a semi-dwarfing allele
[§](+) indicates the presence of the photoperiod-sensitive allele at the *Ppd-D1* locus.

Table S4.2. Maximum number of floret primordia and fertile floret number in apical, central and basal spikelets for individual genotype of all the 30 cultivars in the greenhouse (mean ± standard deviation).

Genotype	Apical max	Central max	Basal max	Apical fertile	Central fertile	Basal fertile
Bussard	9.00 ± 1.00	10.00 ± 1.00	9.33 ± 0.58	3.33 ± 0.58	4.33 ± 0.58	4.00 ± 1.00
Tukan	8.33 ± 0.58	9.67 ± 0.58	9.00 ± 0.00	4.00 ± 0.00	4.67 ± 0.58	4.67 ± 0.58
Tulsa	8.50 ± 0.71	10.00 ± 0.00	10.00 ± 0.00	4.00 ± 1.00	4.67 ± 0.58	5.00 ± 1.00
Türkis	9.33 ± 1.15	9.67 ± 0.58	9.67 ± 0.58	4.00 ± 0.00	4.33 ± 0.58	4.33 ± 0.58
Winnetou	9.00 ± 1.00	10.33 ± 0.58	10.00 ± 0.00	4.33 ± 1.15	6.00 ± 0.00	5.67 ± 0.58
Zentos	8.67 ± 0.58	10.33 ± 0.58	9.67 ± 0.58	3.33 ± 0.58	5.33 ± 0.58	5.00 ± 0.00
Zobel	9.67 ± 0.58	10.67 ± 0.58	10.00 ± 1.00	4.00 ± 0.00	4.67 ± 0.58	4.00 ± 0.00
Cliff	9.00 ± 0.00	9.00 ± 0.00	11.00 ± 0.00	4.00 ± 0.00	5.33 ± 0.58	5.33 ± 0.58
Dream	8.67 ± 0.58	10.00 ± 0.00	9.67 ± 0.58	4.00 ± 1.41	5.50 ± 0.71	5.00 ± 0.00
Florett	8.33 ± 0.58	10.00 ± 1.00	9.00 ± 1.00	4.00 ± 0.00	5.33 ± 0.58	5.00 ± 0.00
History	9.33 ± 0.58	9.67 ± 0.58	9.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	5.67 ± 0.58
Lindos	8.33 ± 0.58	10.00 ± 0.00	9.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
Julius	9.00 ± 1.00	9.67 ± 0.58	10.00 ± 0.00	3.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
Exotic	7.67 ± 1.15	8.33 ± 0.58	8.00 ± 1.00	3.33 ± 0.58	3.67 ± 0.58	3.33 ± 0.58
Andalou Loch 3754	9.00 ± 0.00	10.00 ± 0.00	9.00 ± 0.00	3.00 ± 0.00	4.00 ± 0.00	3.67 ± 0.58
Adlon	8.33 ± 0.58	10.00 ± 0.00	9.33 ± 0.58	5.33 ± 0.58	6.00 ± 0.00	5.33 ± 0.58
Acienda	9.00 ± 0.00	9.33 ± 1.15	9.00 ± 1.00	4.33 ± 0.58	5.00 ± 0.00	4.33 ± 0.58
Aguila	8.33 ± 1.15	9.00 ± 0.00	9.00 ± 0.00	3.67 ± 0.58	4.67 ± 0.58	4.00 ± 0.00
Alcasar	8.67 ± 0.58	9.67 ± 0.58	9.67 ± 0.58	4.00 ± 0.00	5.00 ± 0.00	4.67 ± 0.58
Allister	9.00 ± 0.00	9.67 ± 0.58	9.00 ± 1.00	3.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
Arack	8.50 ± 0.71	9.50 ± 0.71	10.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
Arobase	9.33 ± 1.15	9.67 ± 0.58	8.67 ± 0.58	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
Astuce	8.67 ± 0.58	9.33 ± 0.58	9.67 ± 0.58	3.33 ± 0.58	4.00 ± 0.00	4.00 ± 0.00
Abusson	9.00 ± 1.00	10.00 ± 1.00	10.00 ± 0.00	4.33 ± 0.58	4.33 ± 0.58	4.33 ± 0.58
Autan	8.33 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	3.33 ± 0.58	3.67 ± 0.58	3.67 ± 0.58
Azimut	9.00 ± 0.00	9.67 ± 0.58	10.00 ± 0.00	4.00 ± 0.00	4.33 ± 0.58	4.33 ± 0.58
Azzuro	9.00 ± 0.00	9.50 ± 0.71	10.00 ± 0.00	3.67 ± 0.58	4.33 ± 0.58	4.00 ± 0.00
Balance	9.00 ± 0.00	9.00 ± 0.00	11.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
Baltimor	9.00 ± 0.00	9.67 ± 0.58	9.00 ± 0.00	3.33 ± 0.58	4.67 ± 0.58	4.33 ± 0.58
Bastide	9.33 ± 0.58	9.67 ± 0.58	9.33 ± 0.58	5.00 ± 0.00	5.33 ± 0.58	5.00 ± 0.00

Table S4.3. Maximum number of floret primordia and fertile floret number per spikelet in apical, central and basal spikelets for individual genotype of all the 30 cultivars in the field (mean ± standard deviation).

Genotype	Apical max	Central max	Basal max	Apical fertile	Central fertile	Basal fertile
Bussard	9.33 ± 0.58	10.33 ± 0.58	10.33 ± 0.58	4.00 ± 0.00	5.33 ± 0.58	5.33 ± 0.58
Tukan	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	3.67 ± 0.58	4.67 ± 0.58	4.67 ± 0.58
Tulsa	11.00 ± 0.00	11.00 ± 0.00	10.00 ± 1.00	4.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58
Türkis	9.67 ± 0.58	10.33 ± 0.58	10.00 ± 1.00	4.00 ± 0.00	4.33 ± 0.58	4.33 ± 0.58
Winnetou	9.67 ± 0.58	10.00 ± 0.00	10.00 ± 0.00	4.00 ± 0.00	4.33 ± 0.58	4.33 ± 0.58
Zentos	9.67 ± 0.58	10.33 ± 0.58	10.00 ± 0.00	3.67 ± 0.58	5.00 ± 0.00	5.00 ± 0.00
Zobel	10.50 ± 0.71	11.50 ± 0.71	10.00 ± 0.00	3.33 ± 0.58	5.00 ± 0.00	5.00 ± 0.00
Cliff	10.00 ± 1.00	11.00 ± 1.00	11.00 ± 1.00	4.00 ± 0.00	5.00 ± 0.00	4.67 ± 0.58
Dream	10.33 ± 0.58	10.33 ± 0.58	10.33 ± 0.58	3.33 ± 0.58	4.33 ± 0.58	5.00 ± 0.00
Florett	10.00 ± 0.00	10.67 ± 0.58	10.67 ± 0.58	3.67 ± 0.58	4.67 ± 0.58	4.33 ± 0.58
History	10.00 ± 0.00	11.00 ± 0.00	9.67 ± 0.58	4.33 ± 0.58	5.67 ± 0.58	5.00 ± 1.00
Lindos	10.67 ± 1.15	11.67 ± 0.58	11.33 ± 0.58	3.67 ± 0.58	5.33 ± 0.58	5.00 ± 0.00
Julius	10.00 ± 1.00	10.00 ± 1.00	10.00 ± 0.00	3.67 ± 0.58	4.33 ± 0.58	4.33 ± 0.58
Exotic	9.33 ± 0.58	9.67 ± 0.58	9.33 ± 0.58	4.67 ± 0.58	5.00 ± 0.00	5.00 ± 0.00
Andalou Loch 3754	9.67 ± 0.58	10.00 ± 1.00	9.67 ± 1.53	3.33 ± 0.58	4.00 ± 0.00	4.00 ± 0.00
Adlon	10.00 ± 0.00	10.50 ± 0.58	9.50 ± 0.58	4.00 ± 0.00	5.00 ± 0.00	4.67 ± 0.58
Acienda	9.67 ± 0.58	10.33 ± 0.58	10.33 ± 0.58	4.67 ± 0.58	5.00 ± 1.00	4.67 ± 0.58
Aguila	9.67 ± 0.58	11.00 ± 1.00	10.67 ± 0.58	4.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58
Alcasar	11.00 ± 0.00	11.00 ± 0.00	11.00 ± 0.00	3.33 ± 0.58	5.00 ± 0.00	4.33 ± 0.58
Allister	10.00 ± 0.00	11.00 ± 0.00	10.67 ± 0.58	4.33 ± 0.58	4.67 ± 0.58	4.67 ± 0.58
Arack	10.67 ± 0.58	11.00 ± 0.00	10.00 ± 0.00	5.33 ± 0.58	6.00 ± 0.00	6.00 ± 0.00
Arobase	11.00 ± 0.00	11.67 ± 0.58	11.00 ± 0.00	4.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58
Astuce	9.67 ± 0.58	10.33 ± 1.15	10.33 ± 0.58	4.33 ± 0.58	5.67 ± 0.58	5.67 ± 0.58
Abusson	9.67 ± 0.58	11.00 ± 0.00	11.00 ± 0.00	4.00 ± 0.00	4.67 ± 0.58	4.67 ± 0.58
Autan	9.33 ± 0.58	10.33 ± 0.58	9.67 ± 0.58	3.33 ± 0.58	4.33 ± 0.58	4.33 ± 0.58
Azimut	9.67 ± 0.58	10.67 ± 0.58	10.33 ± 0.58	4.33 ± 0.58	5.67 ± 0.58	5.67 ± 0.58
Azzuro	9.33 ± 0.58	10.00 ± 1.00	11.00 ± 0.00	4.67 ± 0.58	5.67 ± 0.58	5.00 ± 0.00
Balance	10.00 ± 0.00	11.00 ± 0.00	10.67 ± 0.58	4.67 ± 0.58	5.67 ± 0.58	5.67 ± 0.58
Baltimor	9.00 ± 0.00	10.33 ± 0.58	10.33 ± 0.58	4.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
Bastide	10.00 ± 0.00	10.33 ± 0.58	10.00 ± 1.00	4.33 ± 0.58	5.33 ± 0.58	5.00 ± 1.00

Table S4.4. Final grain number per spikelet in apical, central and basal spikelets and fruiting efficiency for individual genotype of all the 30 cultivars in the greenhouse (mean ± standard deviation).

Genotype	apical final grain	central final grain	basal final grain	fruiting efficiency
Bussard	3.00 ± 0.63	4.17 ± 0.75	4.17 ± 0.75	14.69 ± 2.02
Tukan	2.33 ± 0.82	3.33 ± 1.21	3.67 ± 1.51	15.74 ± 1.34
Tulsa	2.67 ± 0.52	3.17 ± 1.47	4.33 ± 1.03	25.80 ± 4.08
Türkis	2.50 ± 1.22	3.33 ± 1.51	2.50 ± 1.05	16.82 ± 0.99
Winnetou	2.33 ± 1.03	4.00 ± 0.89	4.50 ± 1.05	17.44 ± 1.25
Zentos	3.33 ± 0.52	4.00 ± 0.89	4.00 ± 0.63	15.68 ± 1.53
Zobel	3.67 ± 0.58	5.00 ± 1.00	4.33 ± 0.58	17.34 ± 1.47
Cliff	3.33 ± 0.82	4.50 ± 0.84	3.67 ± 1.51	19.74 ± 0.76
Dream	2.67 ± 0.52	3.67 ± 0.52	3.67 ± 0.52	21.60 ± 2.43
Florett	2.67 ± 0.52	3.14 ± 0.75	2.57 ± 0.82	19.03 ± 1.75
History	3.00 ± 1.22	4.20 ± 1.30	2.75 ± 1.71	17.08 ± 1.36
Lindos	2.40 ± 0.89	3.80 ± 1.10	2.20 ± 0.84	18.79 ± 0.78
Julius	2.25 ± 0.96	3.33 ± 0.58	2.50 ± 0.58	18.38 ± 1.34
Exotic	3.33 ± 0.82	4.33 ± 0.82	3.67 ± 1.03	15.33 ± 2.02
Andalou	2.86 ± 0.63	4.00 ± 0.75	3.71 ± 0.84	16.23 ± 1.23
Loch 3754				
Adlon	3.00 ± 0.89	4.00 ± 0.89	2.67 ± 1.37	20.56 ± 1.05
Acienda	3.33 ± 0.82	3.33 ± 0.82	3.17 ± 0.75	21.76 ± 2.78
Aguila	3.17 ± 0.98	3.67 ± 1.21	4.00 ± 1.41	20.38 ± 1.87
Alcasar	2.43 ± 0.52	3.43 ± 1.22	3.71 ± 1.21	20.11 ± 2.30
Allister	3.50 ± 0.55	4.00 ± 0.63	3.83 ± 1.17	18.57 ± 0.70
Arack	3.17 ± 0.75	4.50 ± 1.05	4.33 ± 1.37	15.76 ± 2.32
Arobase	4.33 ± 0.82	4.17 ± 0.98	4.50 ± 1.05	20.46 ± 1.57
Astuce	2.80 ± 0.84	3.60 ± 0.89	3.40 ± 1.14	19.05 ± 1.54
Abusson	3.00 ± 0.89	4.43 ± 1.03	4.14 ± 0.75	16.78 ± 1.02
Autan	3.57 ± 1.10	4.14 ± 0.52	3.43 ± 0.55	14.54 ± 0.78
Azimut	3.43 ± 0.84	4.00 ± 0.41	3.57 ± 0.75	22.88 ± 2.71
Azzuro	2.86 ± 1.03	3.86 ± 1.33	4.43 ± 0.84	17.10 ± 0.69
Balance	3.00 ± 1.41	3.67 ± 1.03	3.67 ± 1.37	17.83 ± 1.08
Baltimor	3.00 ± 0.89	4.00 ± 0.89	3.67 ± 0.82	18.06 ± 0.83
Bastide	3.33 ± 1.03	4.17 ± 1.17	4.00 ± 1.26	17.23 ± 0.70

Table S4.5. Final grain number per spikelet in apical, central and basal spikelets and fruiting efficiency for individual genotype of all the 30 cultivars in the field (mean ± standard deviation).

Genotype	apical final grain	central final grain	basal final grain	fruiting efficiency
Bussard	1.00 ± 1.26	3.17 ± 0.75	3.83 ± 0.41	15.10 ± 1.69
Tukan	3.33 ± 0.82	4.00 ± 0.00	4.00 ± 0.00	17.02 ± 2.60
Tulsa	3.33 ± 0.52	3.67 ± 0.52	3.50 ± 1.05	15.89 ± 5.15
Türkis	2.83 ± 1.47	4.17 ± 0.41	4.33 ± 0.52	20.18 ± 2.90
Winnetou	1.83 ± 1.72	4.67 ± 0.52	4.67 ± 0.52	20.63 ± 5.54
Zentos	1.83 ± 0.98	3.67 ± 0.52	3.83 ± 0.75	16.65 ± 5.37
Zobel	3.17 ± 0.41	3.83 ± 0.41	3.50 ± 1.22	17.58 ± 1.54
Cliff	2.67 ± 0.52	4.17 ± 0.75	3.50 ± 1.05	16.67 ± 3.46
Dream	0.50 ± 1.00	3.50 ± 0.58	3.25 ± 0.96	16.25 ± 5.42
Florett	2.67 ± 0.52	3.67 ± 0.52	3.33 ± 0.82	17.49 ± 3.67
History	3.00 ± 0.63	4.17 ± 0.41	4.17 ± 0.75	15.02 ± 5.92
Lindos	2.33 ± 0.82	4.00 ± 0.89	3.33 ± 0.82	28.93 ± 13.34
Julius	2.67 ± 0.52	3.50 ± 0.55	3.67 ± 0.52	26.80 ± 13.58
Exotic	3.17 ± 0.41	3.33 ± 0.52	3.17 ± 0.41	15.17 ± 5.59
Andalou	3.00 ± 0.63	3.83 ± 0.41	3.67 ± 0.52	15.84 ± 5.66
Loch 3754				
Adlon	3.83 ± 0.41	4.50 ± 0.55	4.33 ± 1.21	19.78 ± 9.62
Acienda	3.67 ± 0.52	4.50 ± 0.55	3.67 ± 0.52	20.03 ± 7.67
Aguila	3.17 ± 0.75	4.00 ± 0.00	3.50 ± 0.55	18.19 ± 3.97
Alcasar	3.17 ± 0.75	4.33 ± 0.82	4.33 ± 0.52	17.76 ± 3.19
Allister	2.50 ± 0.55	3.33 ± 0.52	3.50 ± 0.55	18.70 ± 5.36
Arack	3.33 ± 0.52	3.83 ± 0.75	3.83 ± 0.41	15.01 ± 3.81
Arobase	4.33 ± 0.52	4.67 ± 0.52	4.17 ± 0.41	18.95 ± 5.68
Astuce	3.00 ± 0.00	4.17 ± 0.41	4.00 ± 0.00	14.29 ± 5.78
Abusson	3.17 ± 0.41	4.17 ± 0.41	3.50 ± 0.55	17.75 ± 6.46
Autan	3.00 ± 0.00	3.33 ± 0.52	2.83 ± 0.75	14.36 ± 2.33
Azimut	3.33 ± 0.52	4.17 ± 0.41	3.83 ± 0.41	19.45 ± 7.31
Azzuro	3.17 ± 0.41	3.50 ± 0.84	3.83 ± 0.41	19.38 ± 6.73
Balance	3.33 ± 0.82	4.50 ± 0.55	4.33 ± 0.52	15.27 ± 1.07
Baltimor	2.83 ± 0.41	4.00 ± 0.00	4.00 ± 0.00	17.75 ± 2.89
Bastide	4.17 ± 1.17	5.00 ± 0.00	4.67 ± 0.52	16.93 ± 1.83

Tabel S5.1. German spring wheat cultivars studied and their years of release to the market.

Running number	Cultivar Name	Plant height	Year of release
1	Adlung's Alemannen	130.65	1931
2	NOS Nordgau	110.80	1933
3	Peragis Garant	105.88	1946
4	Heine's Peko	122.40	1947
5	Hohenheimer Franken II	114.20	1951
6	Probat	89.85	1953
7	Breustedt's Lera	121.67	1959
8	Arin	118.22	1962
9	Kolibri	106.35	1966
10	Ralle	104.53	1980
11	Nandu	94.70	1988
12	Fasan	112.93	1997

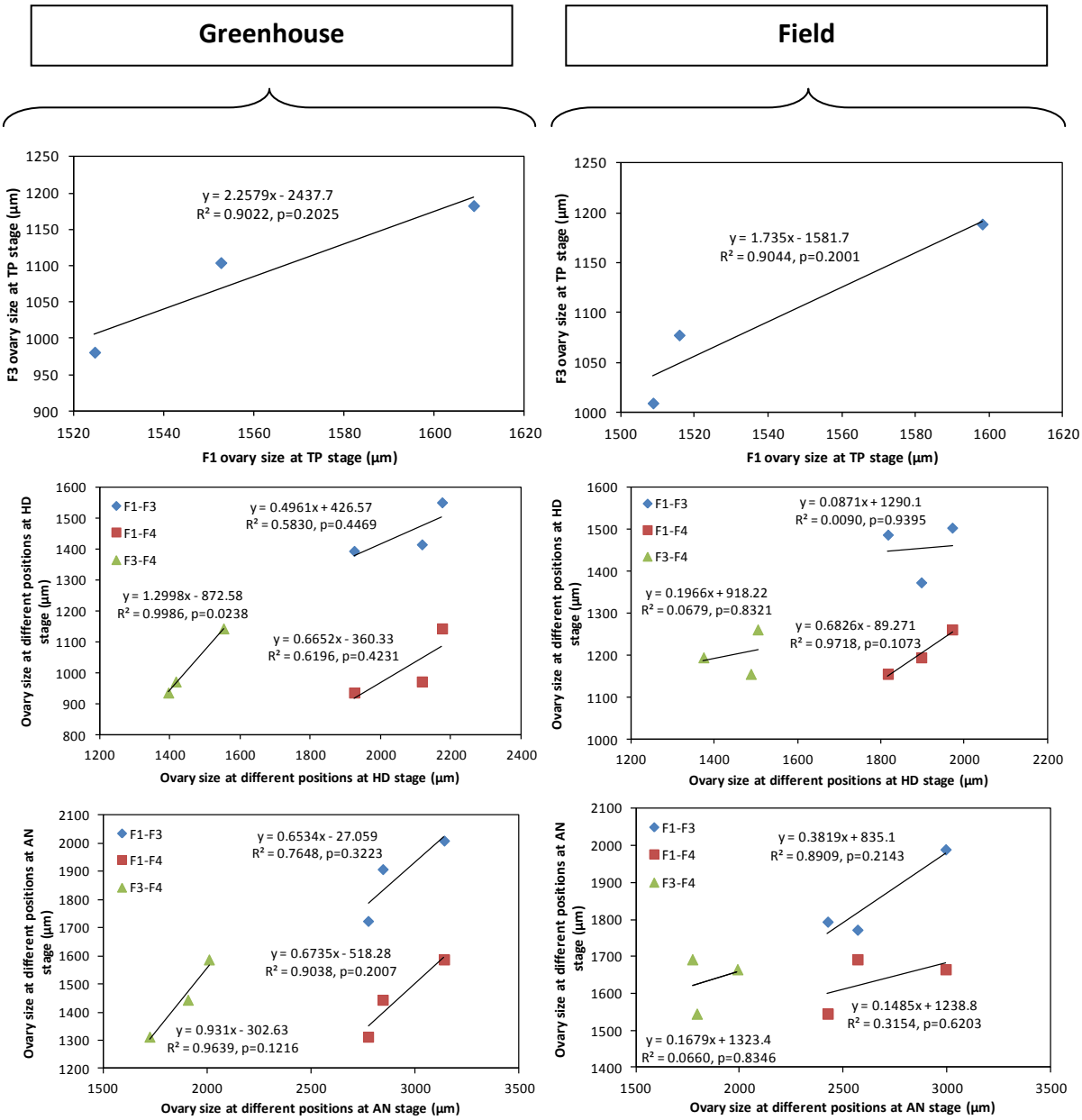


Fig. S2.1. Relationships between ovary size (µm) at different floret positions (F1, F3, F4) at different floral developmental stages under greenhouse and field conditions. The repeats for each regression line are n=3.

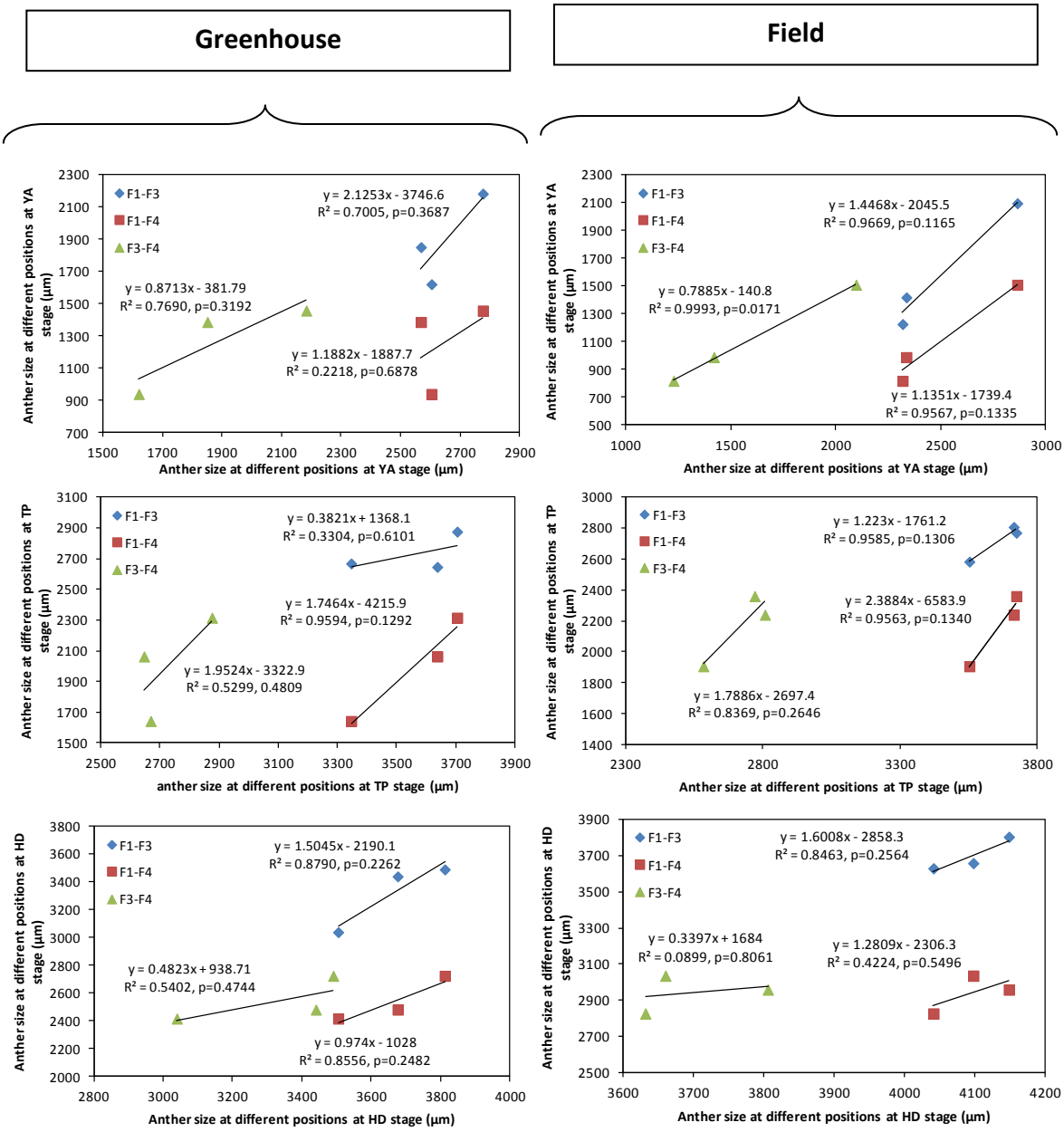


Fig. S2.2. Relationships between anther size (µm) at different floret positions (F1, F3, F4) at different floral developmental stages under greenhouse and field conditions. The repeats for each regression line are n=3.

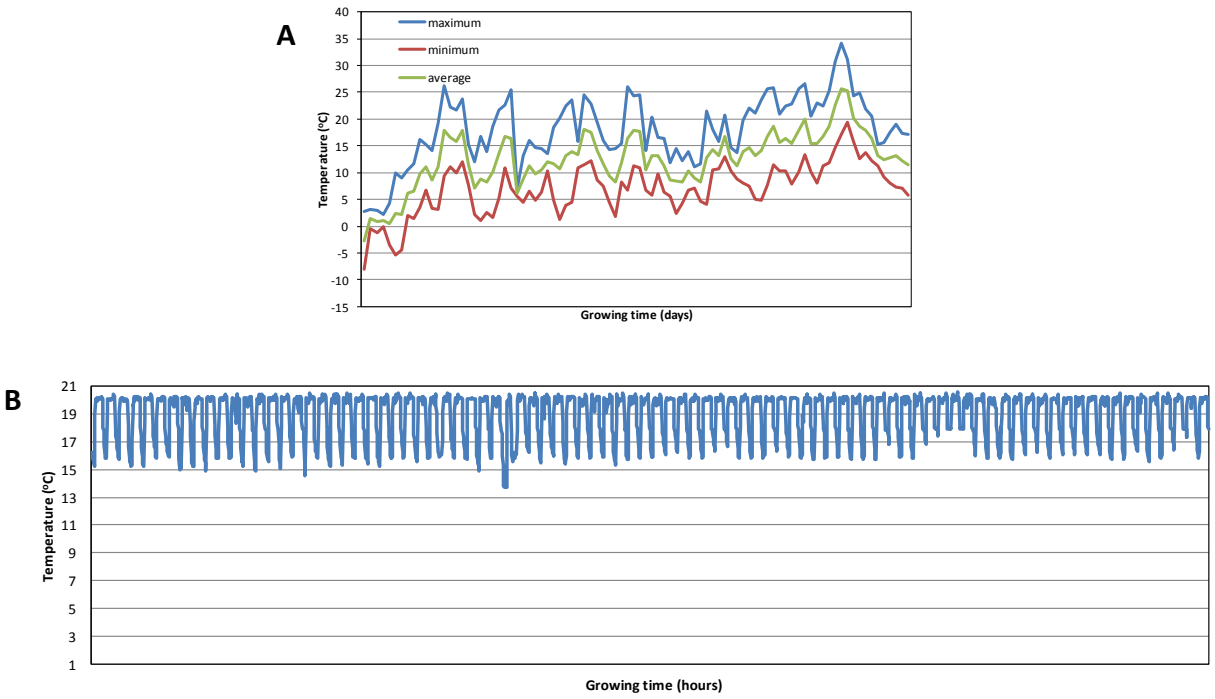


Fig. S2.3. Temperatures under field (A) and greenhouse (B) conditions. Temperatures in the field and greenhouse are shown as the maximum, minimum, and average of every day from sowing to anthesis.

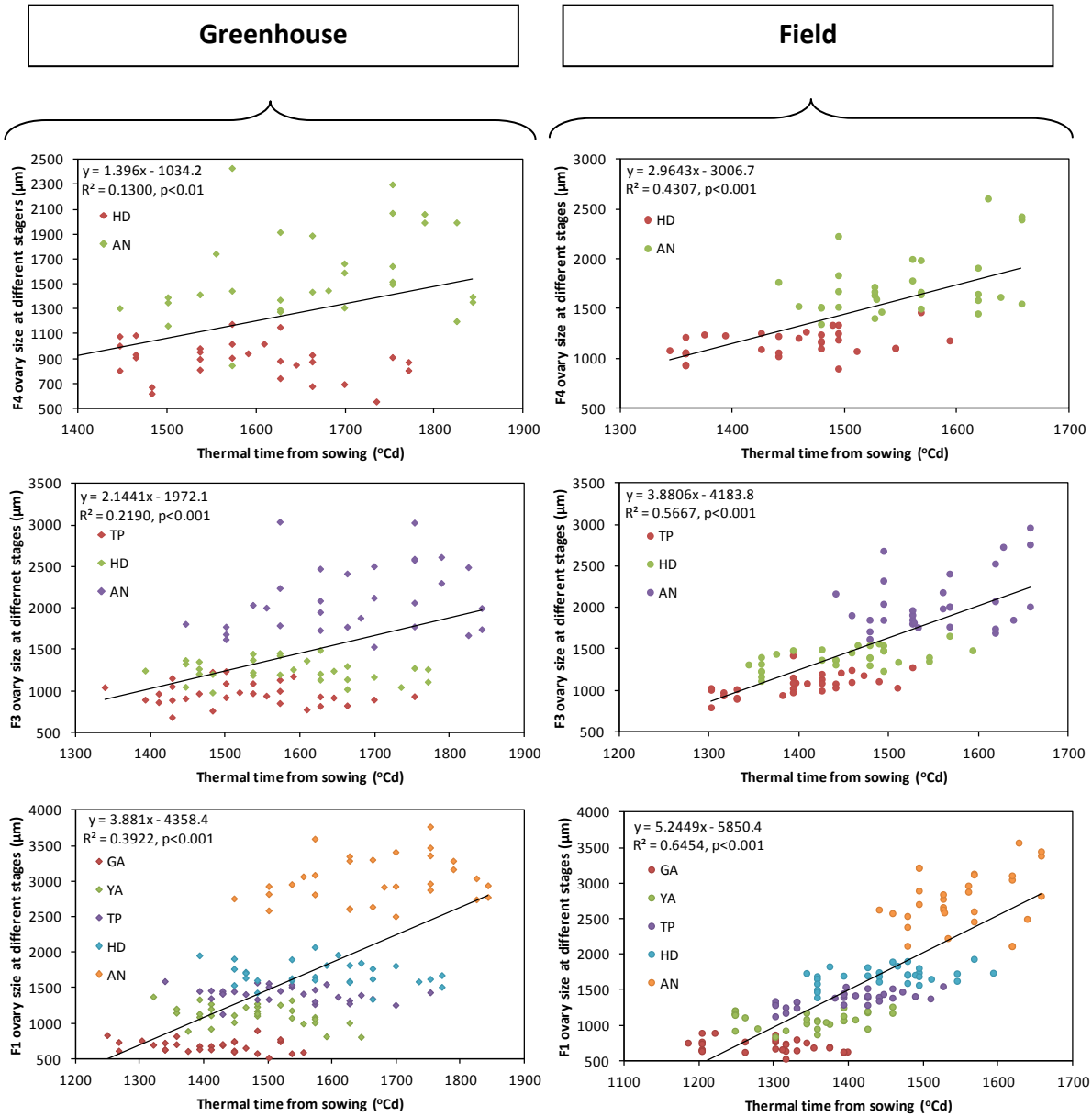


Fig. S2.4. Distribution of ovary size (μm) at seven floral developmental stages versus thermal time (^{o}Cd) and their relationships under greenhouse and field conditions. The repeats for each stage are 30, so n=60 in the first two figures, n=90 in the middle two figures, n=150 in the last two figures.

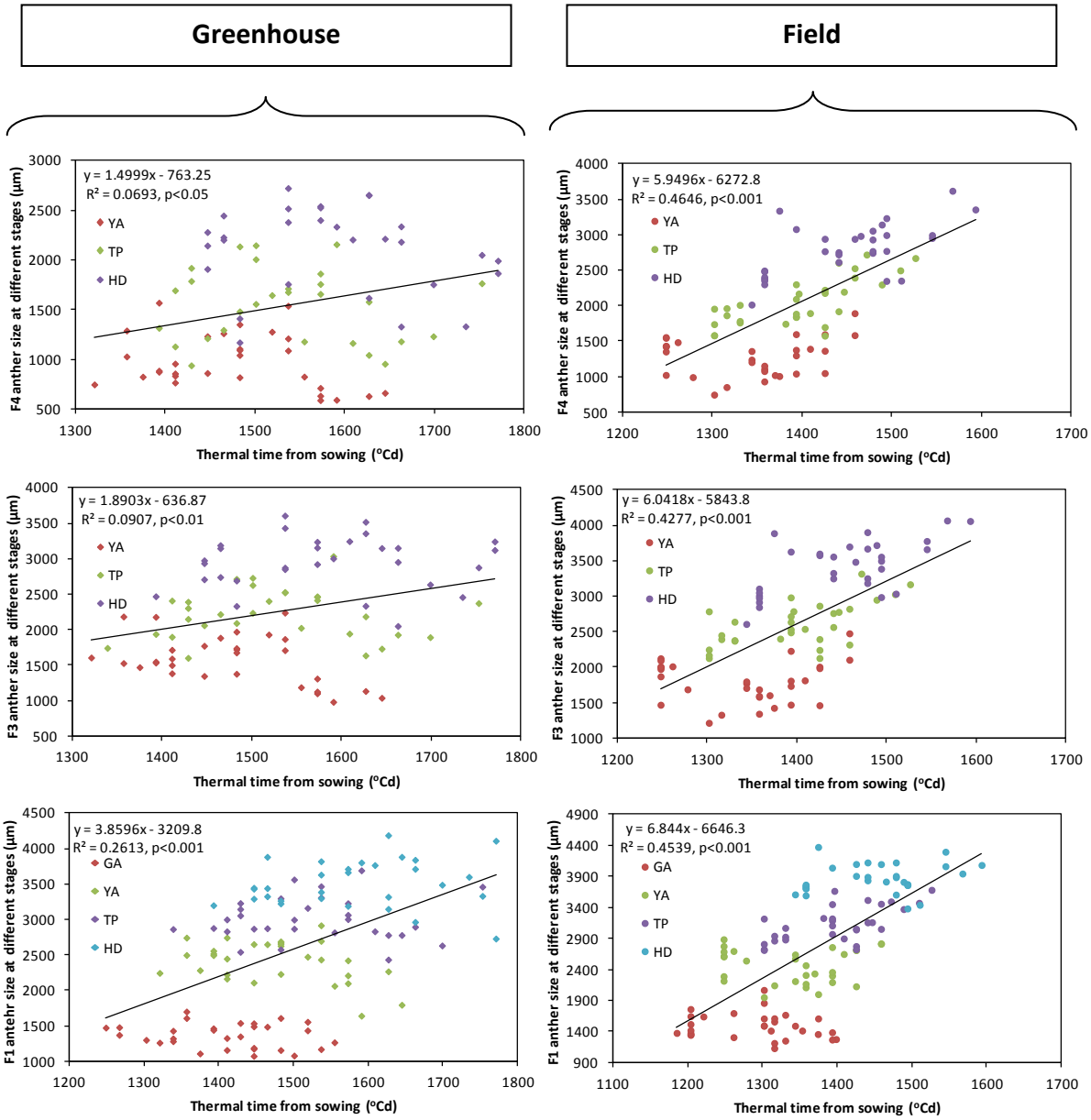
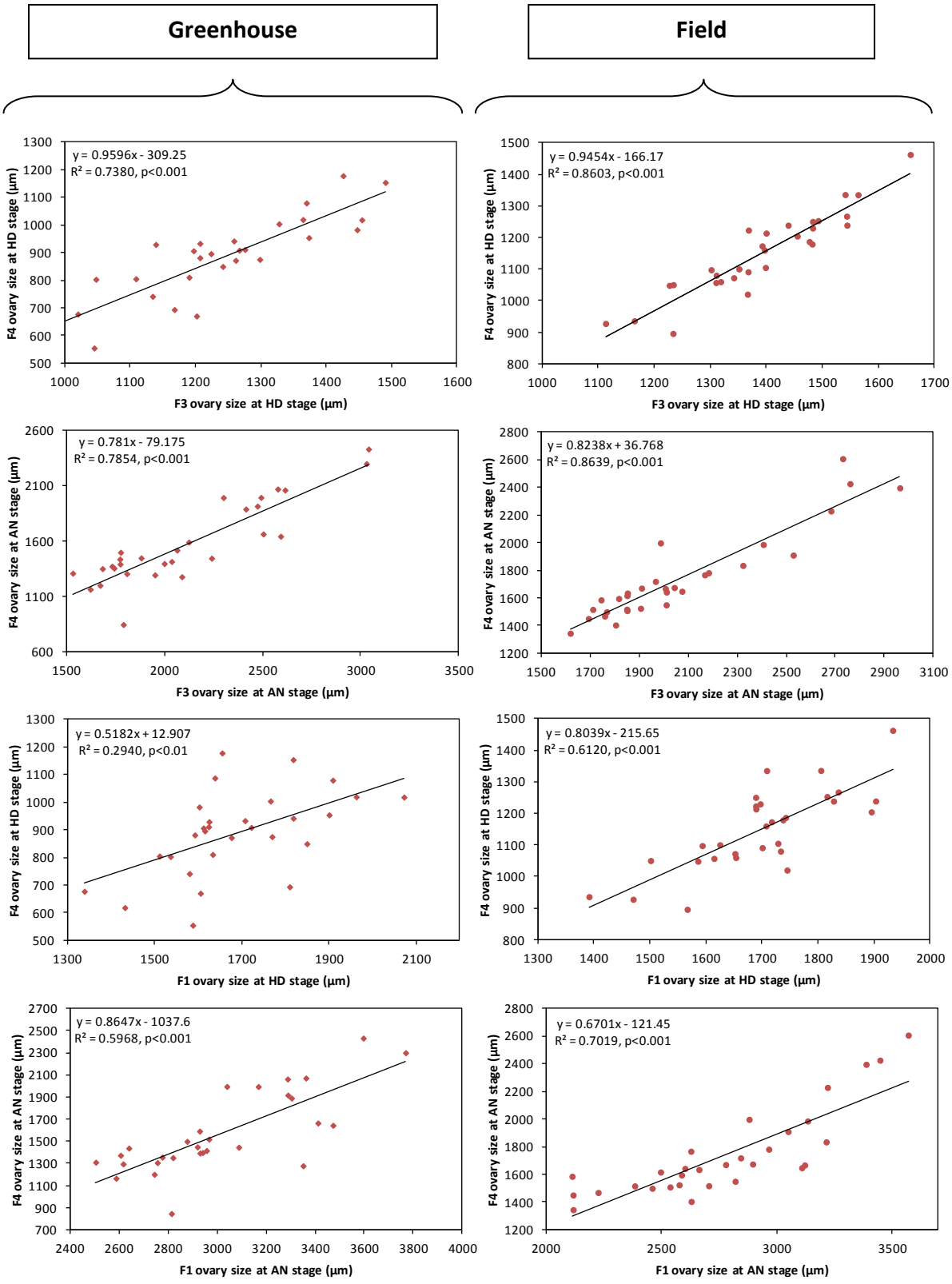


Fig. S2.5. Distribution of anther size (μm) at seven floral developmental stages versus thermal time (°Cd) and their relationships under greenhouse and field conditions. The repeats for each stage are 30, so n=90 in the first four figures, n=120 in the last two figures.


Greenhouse
Field
y = 0.9596x - 309.25
R² = 0.7380, p<0.001
F4 ovary size at HD stage (µm)
F3 ovary size at HD stage (µm)
y = 0.9454x - 166.17
R² = 0.8603, p<0.001
F4 ovary size at HD stage (µm)
F3 ovary size at HD stage (µm)
y = 0.781x - 79.175
R² = 0.7854, p<0.001
F4 ovary size at AN stage (µm)
F3 ovary size at AN stage (µm)
y = 0.8238x + 36.768
R² = 0.8639, p<0.001
F4 ovary size at AN stage (µm)
F3 ovary size at AN stage (µm)
y = 0.5182x + 12.907
R² = 0.2940, p<0.01
F4 ovary size at HD stage (µm)
F1 ovary size at HD stage (µm)
y = 0.8039x - 215.65
R² = 0.6120, p<0.001
F4 ovary size at HD stage (µm)
F1 ovary size at HD stage (µm)
y = 0.8647x - 1037.6
R² = 0.5968, p<0.001
F4 ovary size at AN stage (µm)
F1 ovary size at AN stage (µm)
y = 0.6701x - 121.45
R² = 0.7019, p<0.001
F4 ovary size at AN stage (µm)
F1 ovary size at AN stage (µm)

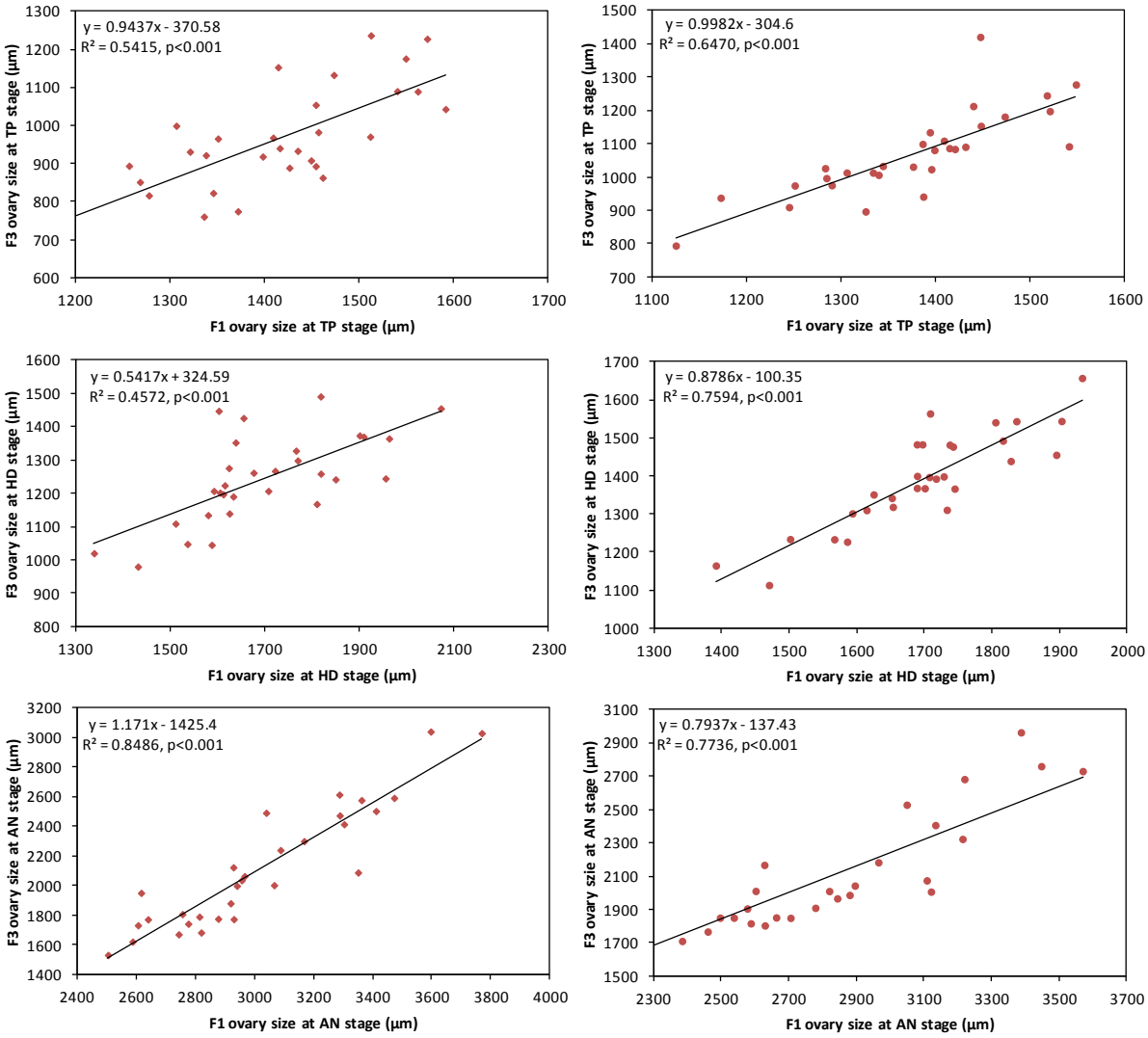
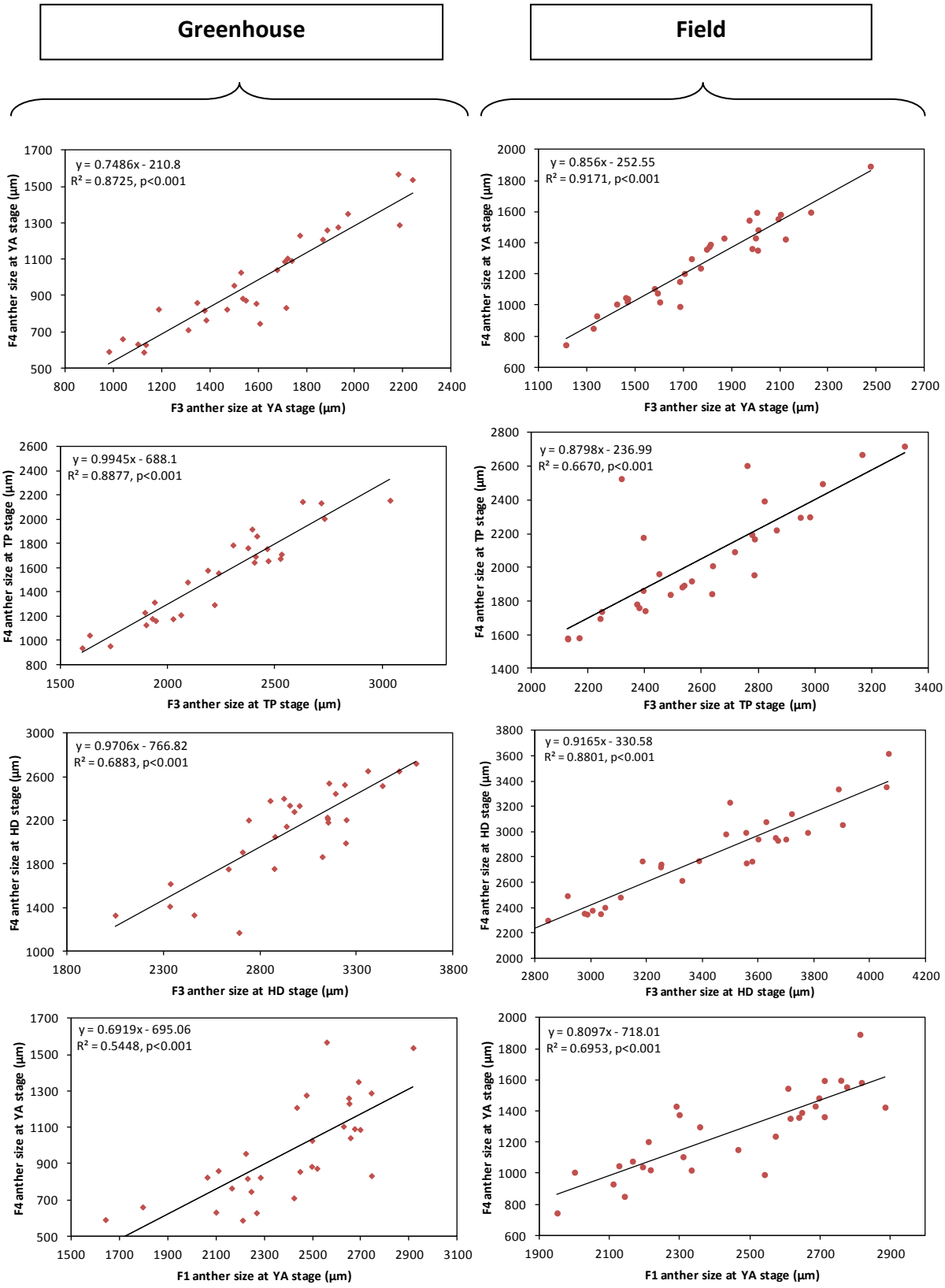
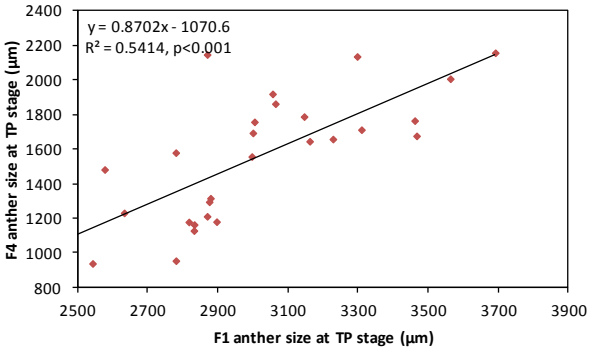
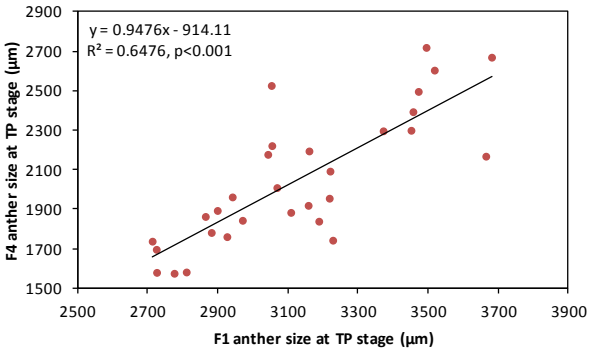
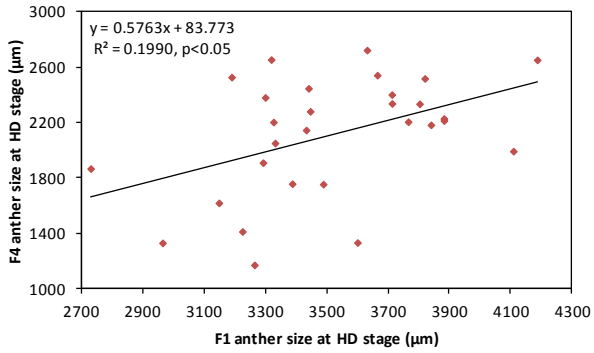


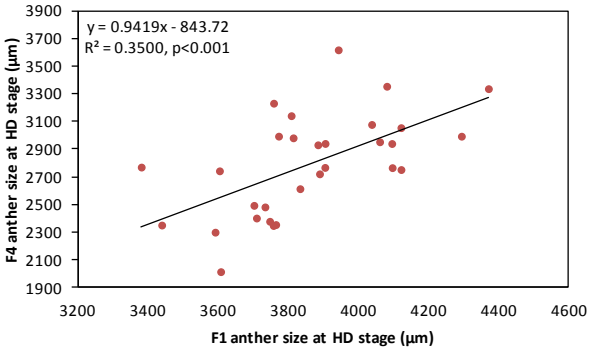
Fig. S2. 6. Relationships between ovary size (µm) at different positions at seven floral developmental stages under greenhouse and field conditions. The repeats for all the traits are n=30.

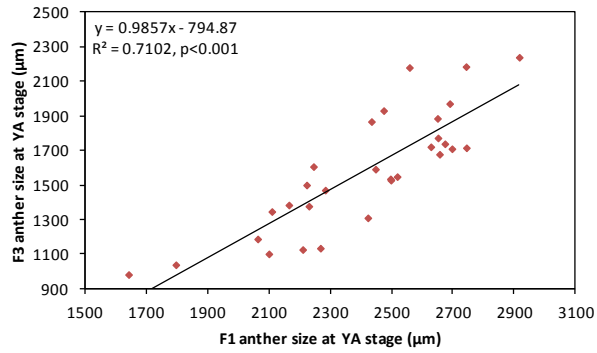

Greenhouse
Field
y = 0.7486x - 210.8
R² = 0.8725, p<0.001
F4 anther size at YA stage (µm)
F3 anther size at YA stage (µm)
y = 0.856x - 252.55
R² = 0.9171, p<0.001
F4 anther size at YA stage (µm)
F3 anther size at YA stage (µm)
y = 0.9945x - 688.1
R² = 0.8877, p<0.001
F4 anther size at TP stage (µm)
F3 anther size at TP stage (µm)
y = 0.8798x - 236.99
R² = 0.6670, p<0.001
F4 anther size at TP stage (µm)
F3 anther size at TP stage (µm)
y = 0.9706x - 766.82
R² = 0.6883, p<0.001
F4 anther size at HD stage (µm)
F3 anther size at HD stage (µm)
y = 0.9165x - 330.58
R² = 0.8801, p<0.001
F4 anther size at HD stage (µm)
F3 anther size at HD stage (µm)
y = 0.6919x - 695.06
R² = 0.5448, p<0.001
F4 anther size at YA stage (µm)
F1 anther size at YA stage (µm)
y = 0.8097x - 718.01
R² = 0.6953, p<0.001
F4 anther size at YA stage (µm)
F1 anther size at YA stage (µm)

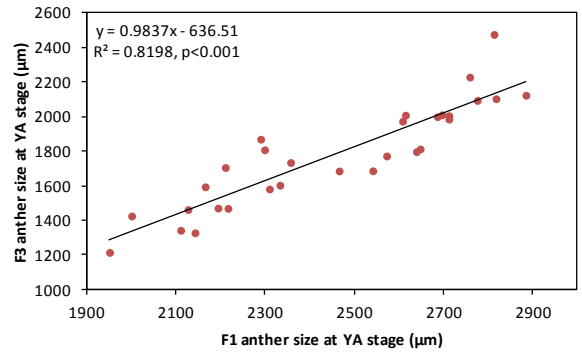

y = 0.8702x - 1070.6
R² = 0.5414, p<0.001
F4 anther size at TP stage (µm)
F1 anther size at TP stage (µm)

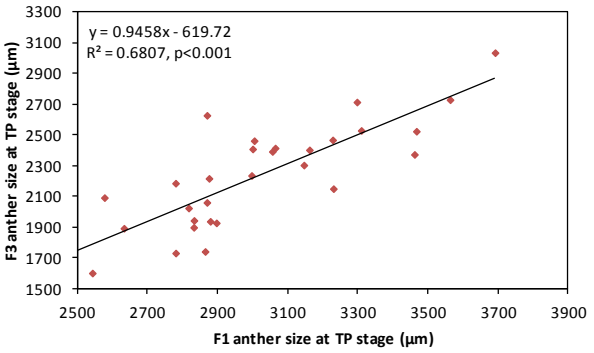

y = 0.9476x - 914.11
R² = 0.6476, p<0.001
F4 anther size at TP stage (µm)
F1 anther size at TP stage (µm)

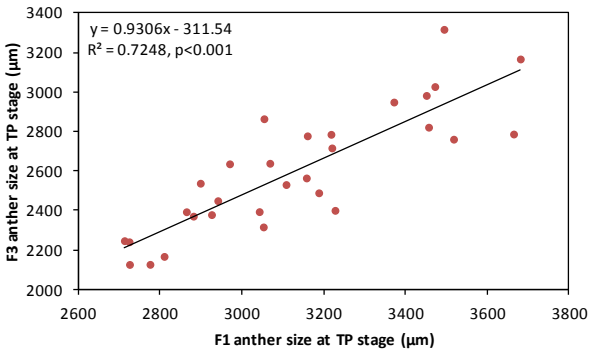

y = 0.5763x + 83.773
R² = 0.1990, p<0.05
F4 anther size at HD stage (µm)
F1 anther size at HD stage (µm)

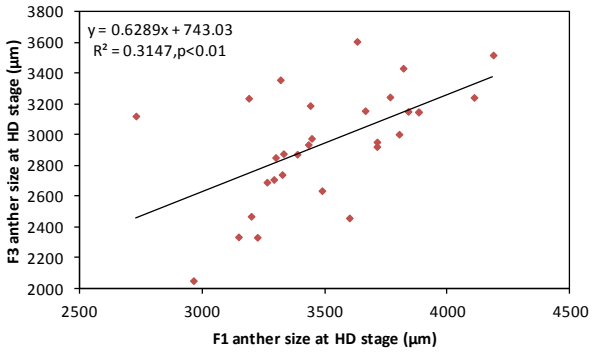

y = 0.9419x - 843.72
R² = 0.3500, p<0.001
F4 anther size at HD stage (µm)
F1 anther size at HD stage (µm)


y = 0.9857x - 794.87
R² = 0.7102, p<0.001
F3 anther size at YA stage (µm)
F1 anther size at YA stage (µm)


y = 0.9837x - 636.51
R² = 0.8198, p<0.001
F3 anther size at YA stage (µm)
F1 anther size at YA stage (µm)


y = 0.9458x - 619.72
R² = 0.6807, p<0.001
F3 anther size at TP stage (µm)
F1 anther size at TP stage (µm)


y = 0.9306x - 311.54
R² = 0.7248, p<0.001
F3 anther size at TP stage (µm)
F1 anther size at TP stage (µm)


y = 0.6289x + 743.03
R² = 0.3147,p<0.01
F3 anther size at HD stage (µm)
F1 anther size at HD stage (µm)

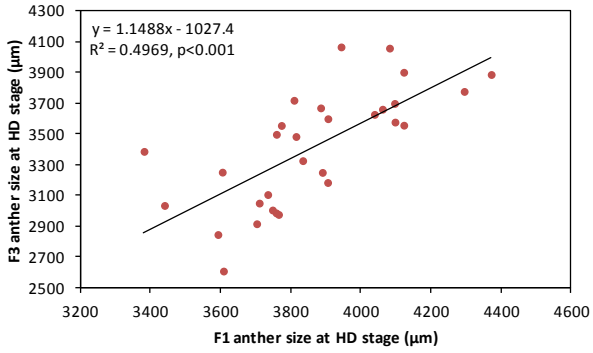

y = 1.1488x - 1027.4
R² = 0.4969, p<0.001
F3 anther size at HD stage (µm)
F1 anther size at HD stage (µm)

Fig. S2.7. Relationships between anther size (μm) at different positions at seven floral developmental stages under greenhouse and field conditions. The repeats for all the traits are n=30.

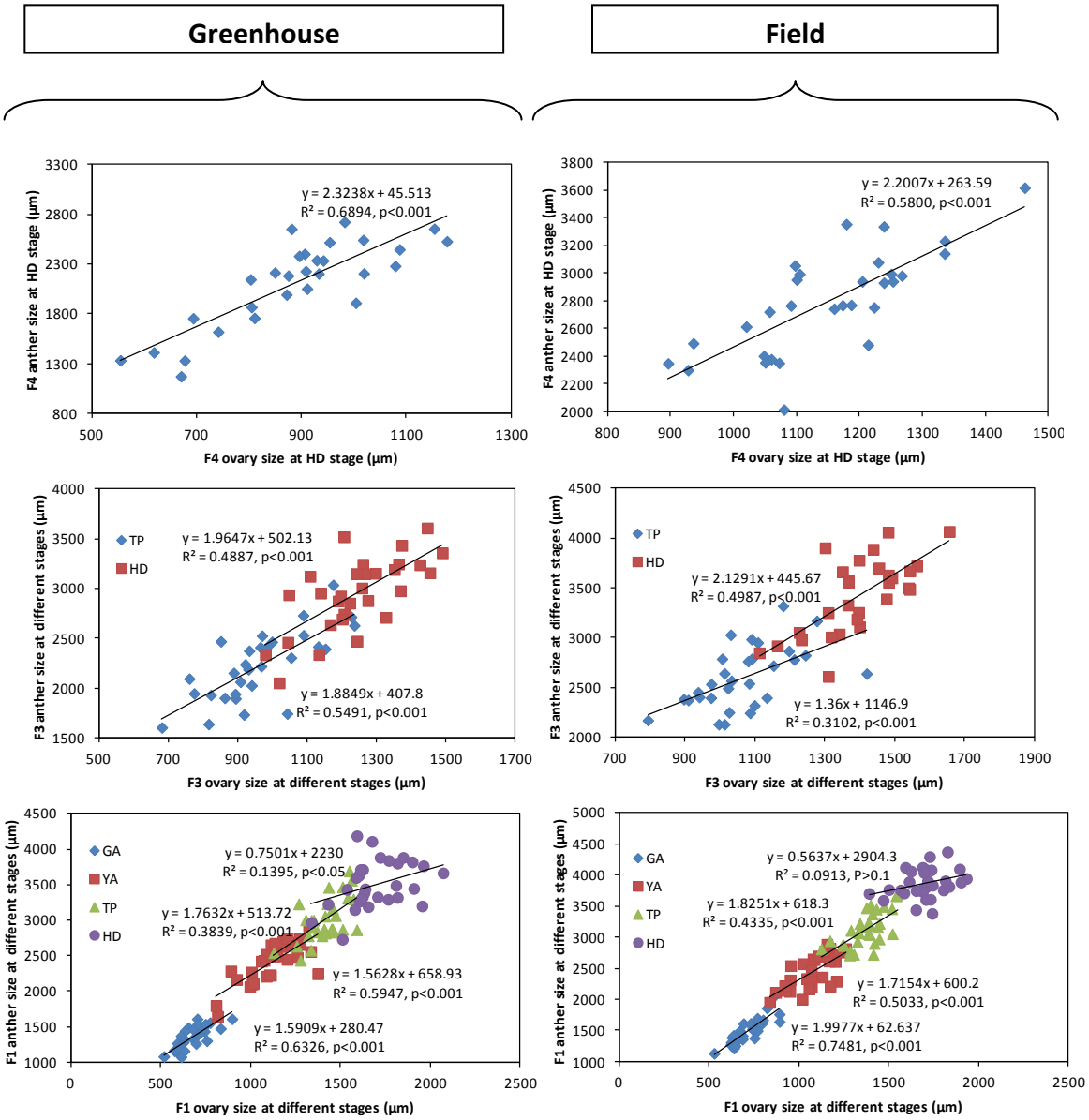


Fig. S2.8. Relationships between anther size (µm) and ovary size (µm) at the same position. The repeats for all the traits are n=30.

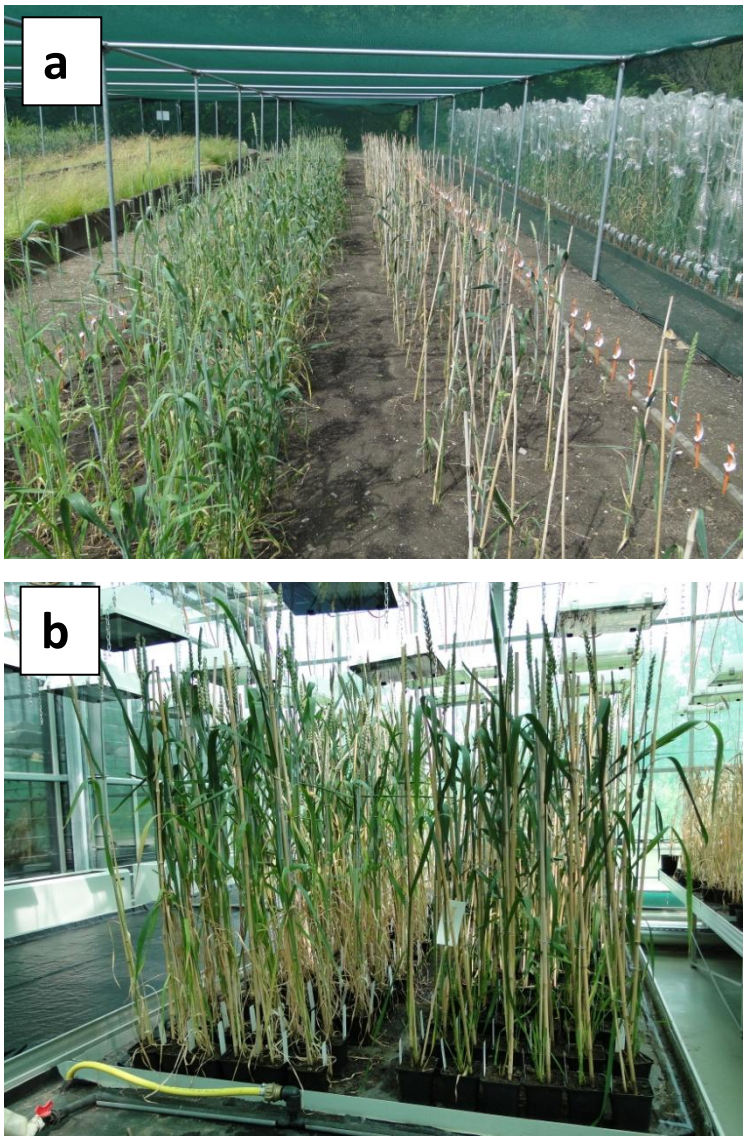


Fig. S3.1. Control and tiller removal experiments in field (a) and greenhouse (b).

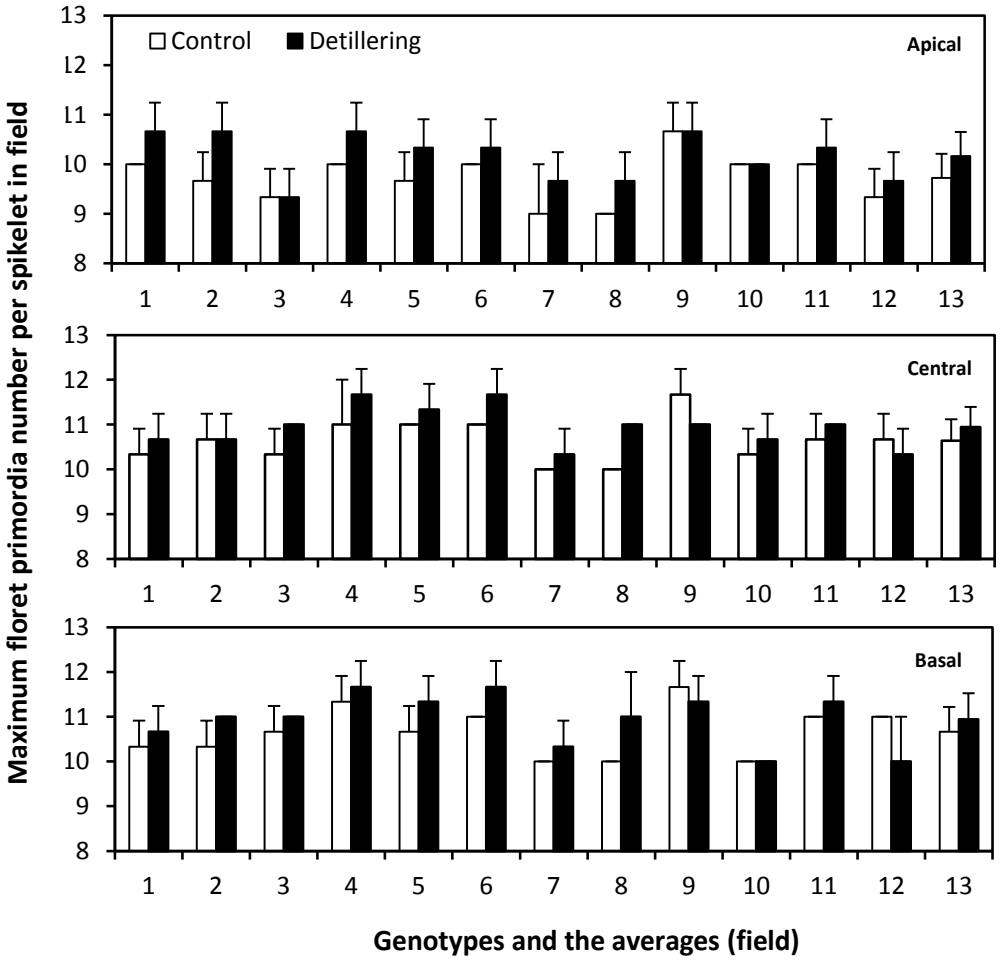


Fig. S3.2. Maximum floret primordia number per spikelet at apical, central and basal spikelets in twelve genotypes (1-12) and averages of genotypes (13) under control and detillering treatments in field (mean ± SD, n=3).

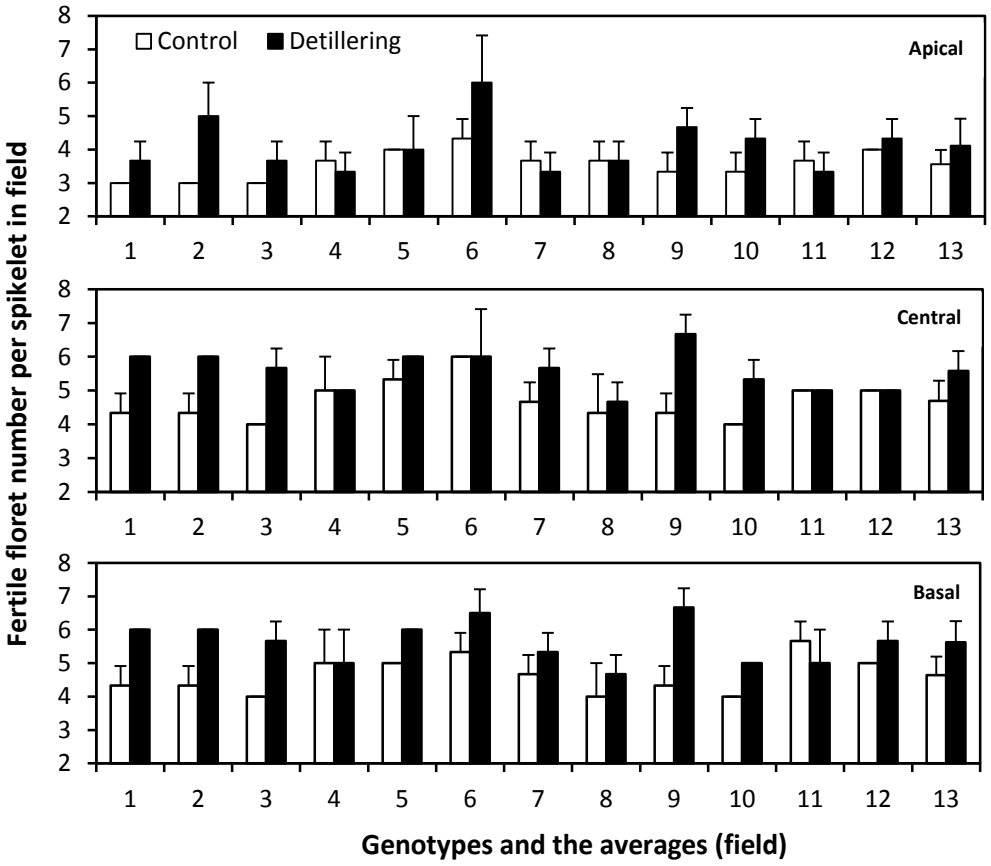


Fig. S3.3. Fertile floret number per spikelet at apical, central and basal spikelets in twelve genotypes (1-12) and averages of genotypes (13) under control and detillering treatments in field (mean ± SD, n=3).

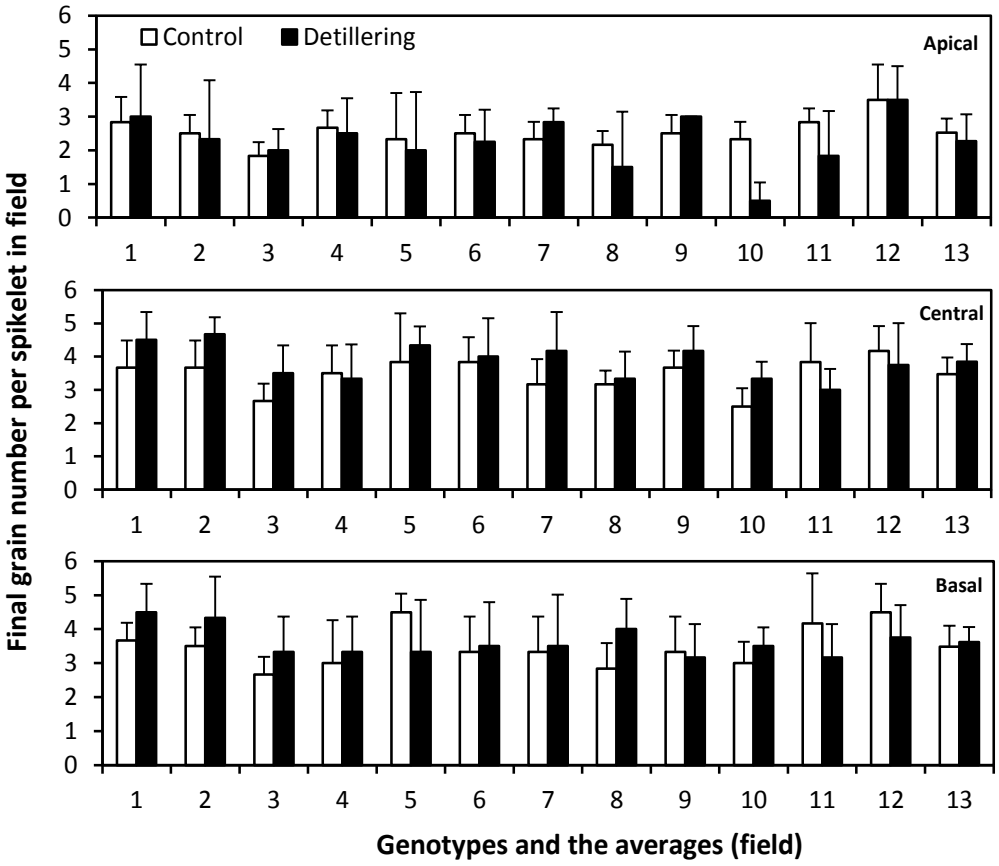


Fig. S3.4. Final grain number per spikelet at apical, central and basal spikelets in twelve genotypes (1-12) and averages of genotypes (13) under control and detillering treatments in field (mean ± SD, n=6).

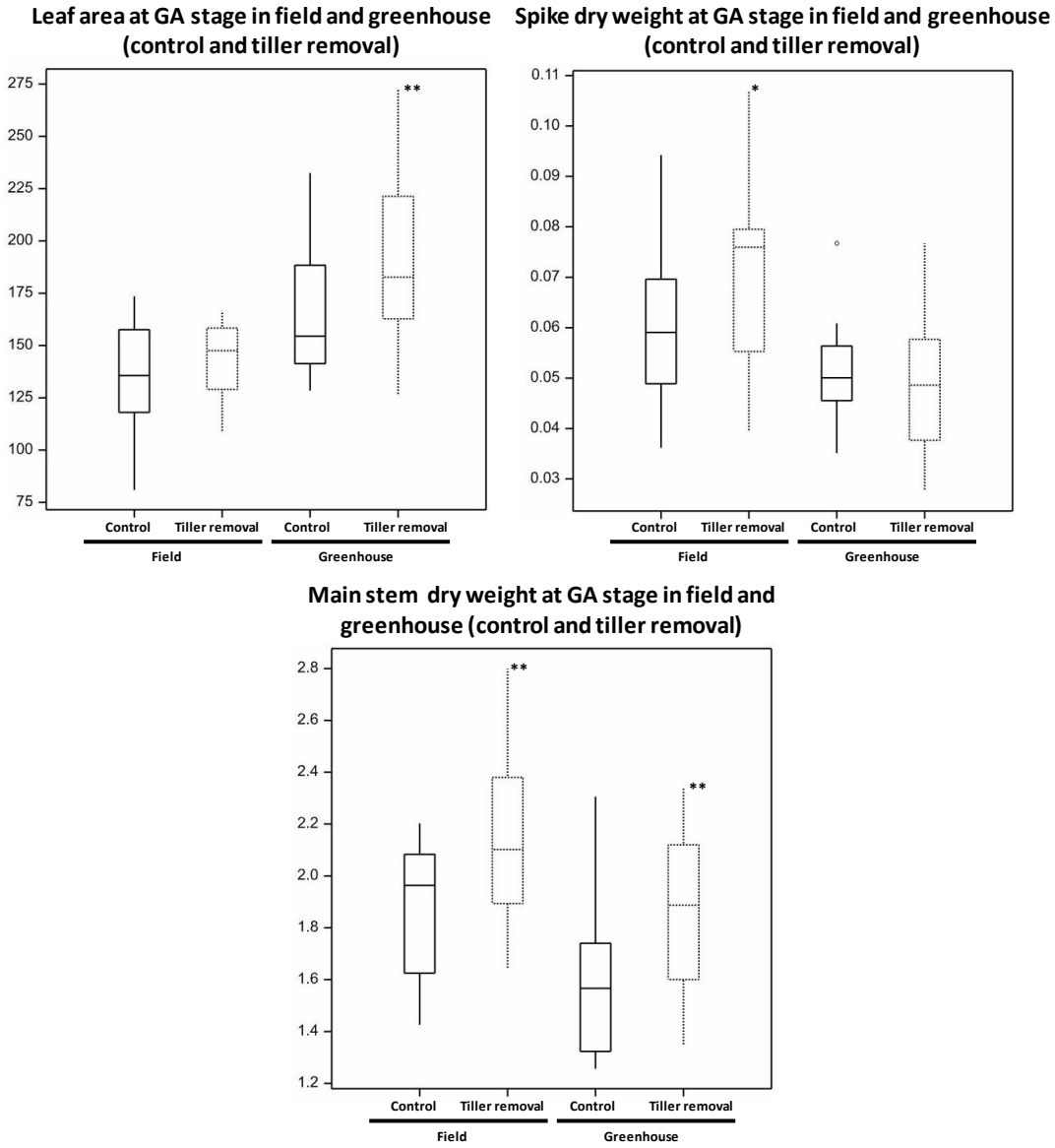


Fig. S3.5. Boxplots of control and tiller removal leaf area (cm^2), leaf dry weight (g), spike dry weight (g), main stem dry weight (g) at the GA stage and significant levels of difference between control and tiller removal. * $p < 0.05$; ** $p < 0.01$. Each boxplot displays the data of the twelve genotypes for corresponding traits at GA stage; the significances suggest comprehensive influence of detillering on different traits of the twelve genotypes.

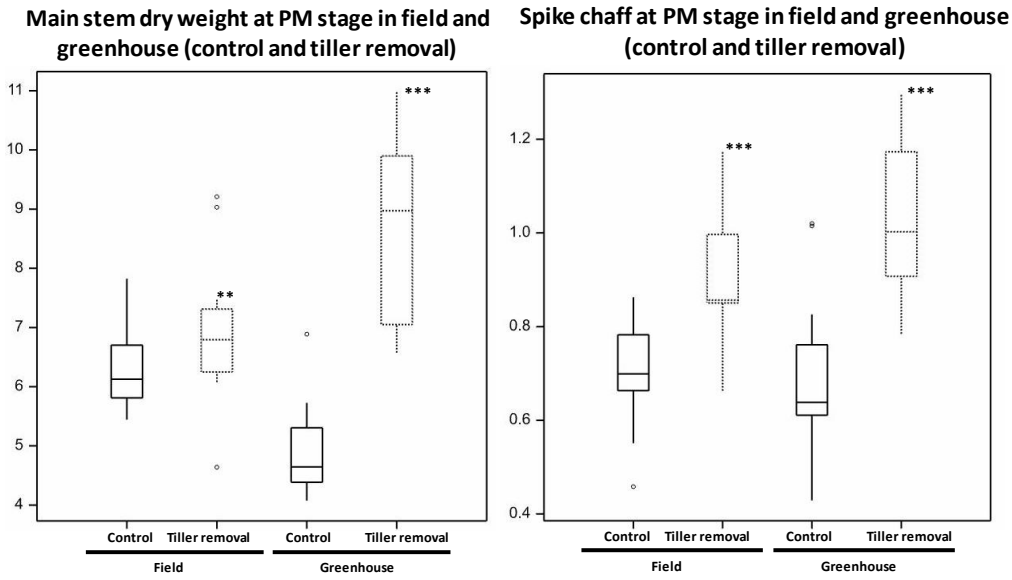


Fig. S3.6. Boxplots of main stem dry weight (g) (main shoot), spike chaff (g) (main shoot) at PM stage and significant levels of difference between control and tiller removal. **p< 0.01; ***p< 0.001. Each boxplot displays the data of the twelve genotypes for corresponding traits at PM stage, the significances suggest comprehensive influence of detillering on different traits of the twelve genotypes.

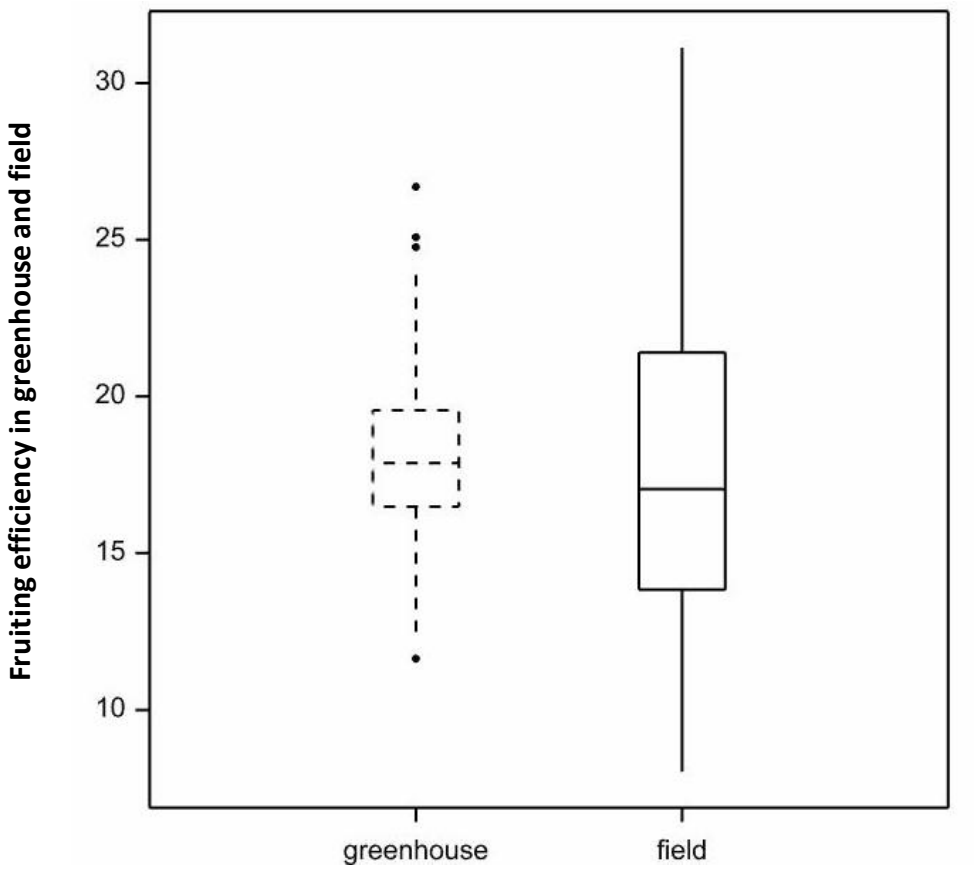


Fig. S4.1. Fruiting efficiency for the 30 cultivars in the greenhouse and field.

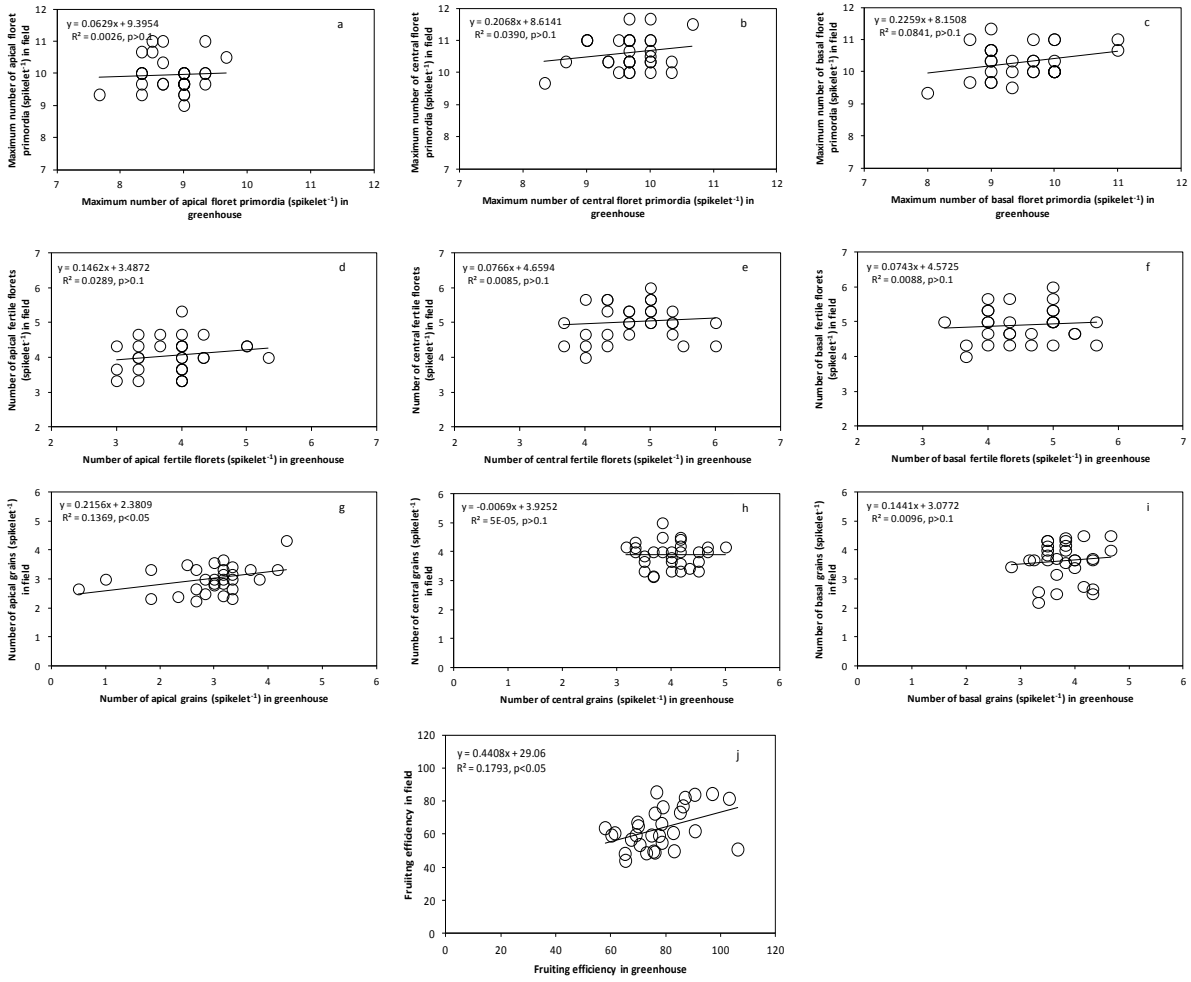


Fig. S4.2. Relationships of maximum number of floret primordia (spikelet^{-1}), number of fertile florets (spikelet^{-1}), number of grains (spikelet^{-1}) and fruiting efficiency between greenhouse and field.

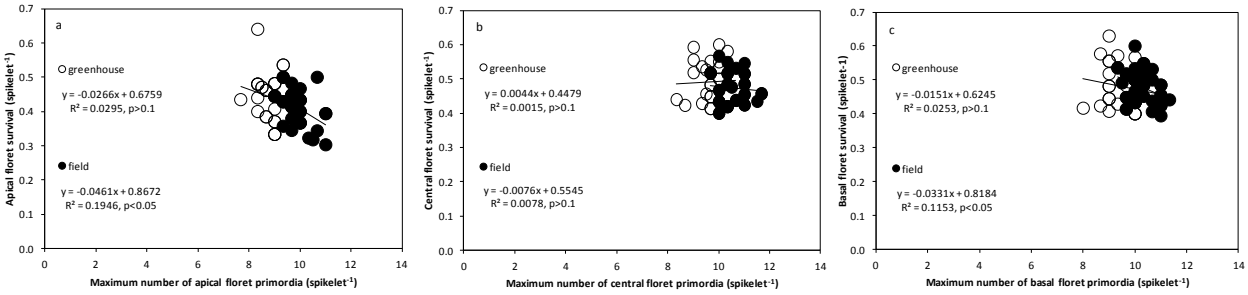


Fig. S4.3. Relationship between maximum number of floret primordia and floret survival within spikelet in apical, central and basal parts of spike under field and greenhouse conditions.

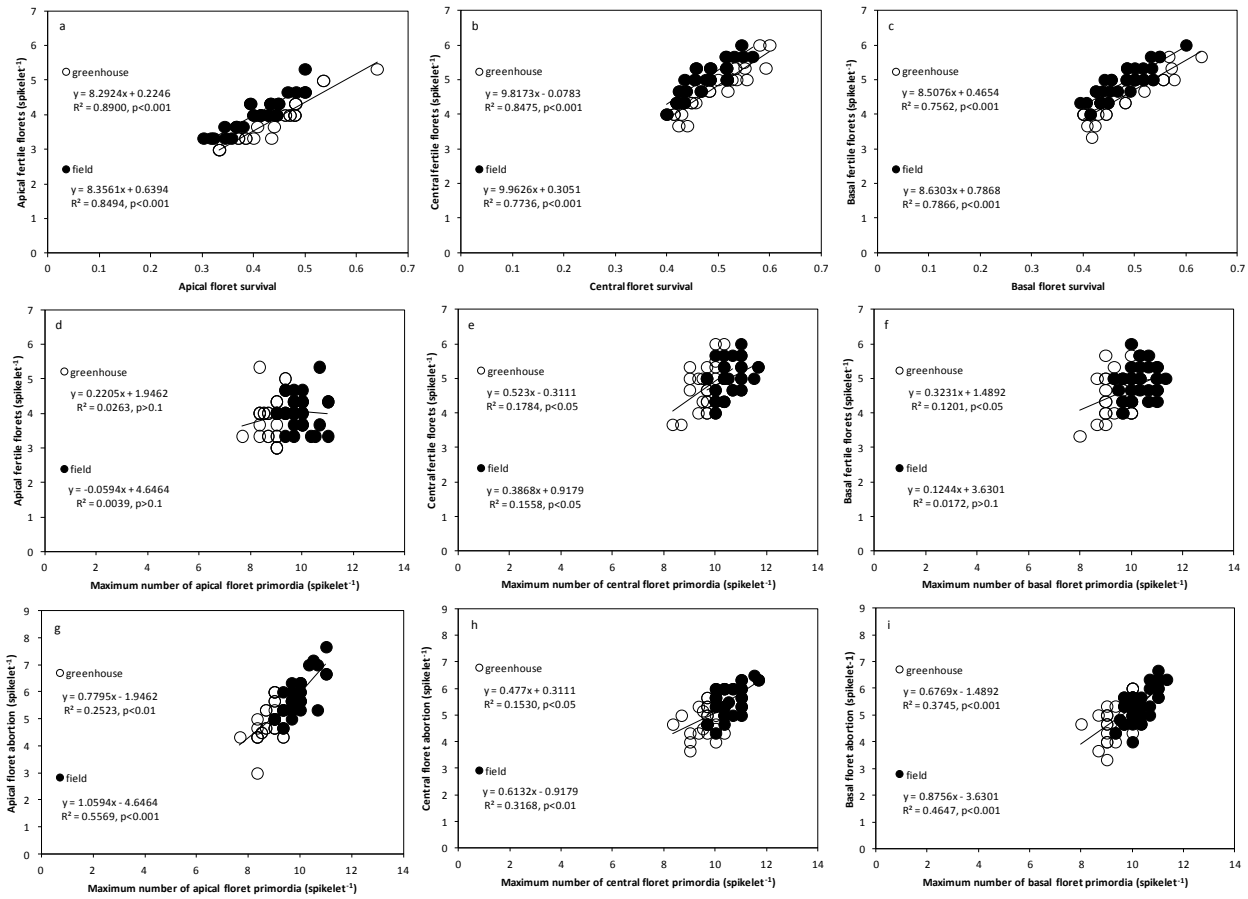


Fig. S4.4. Relationship between maximum number of floret primordia, fertile florets number and floret abortion within spikelet in apical, central and basal spikelets under field and greenhouse conditions.

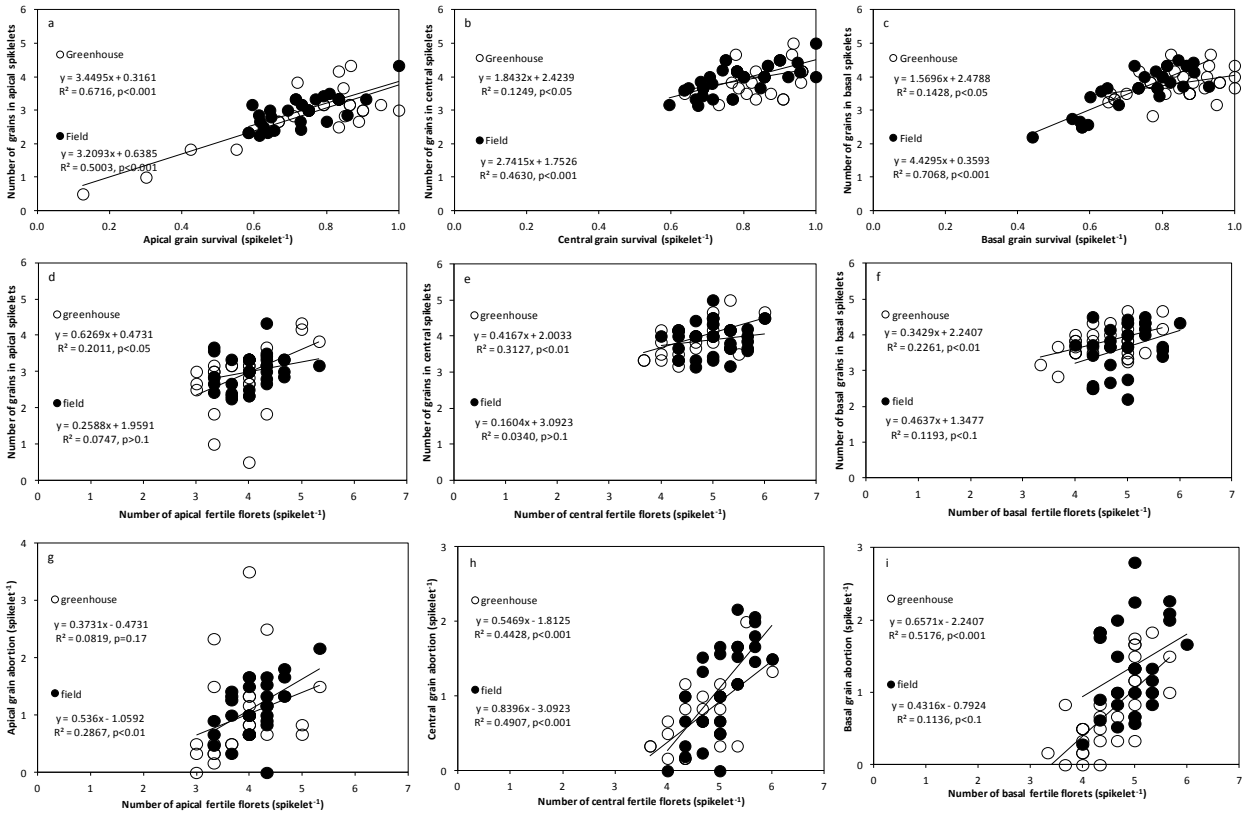


Fig. S4.5. Relationship between maximum number of fertile florets, number of grains and grain abortion per spikelet in apical, central and basal positions of spike under field and greenhouse conditions.

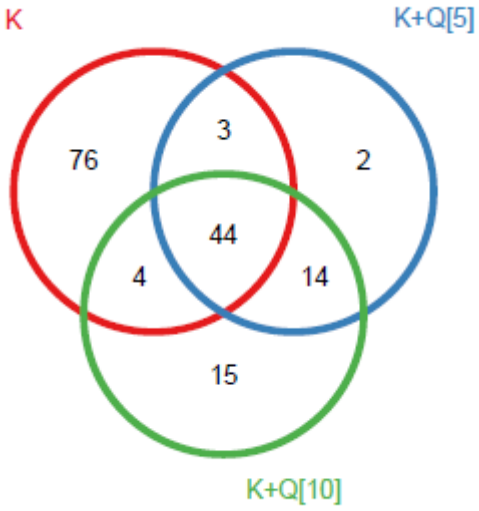
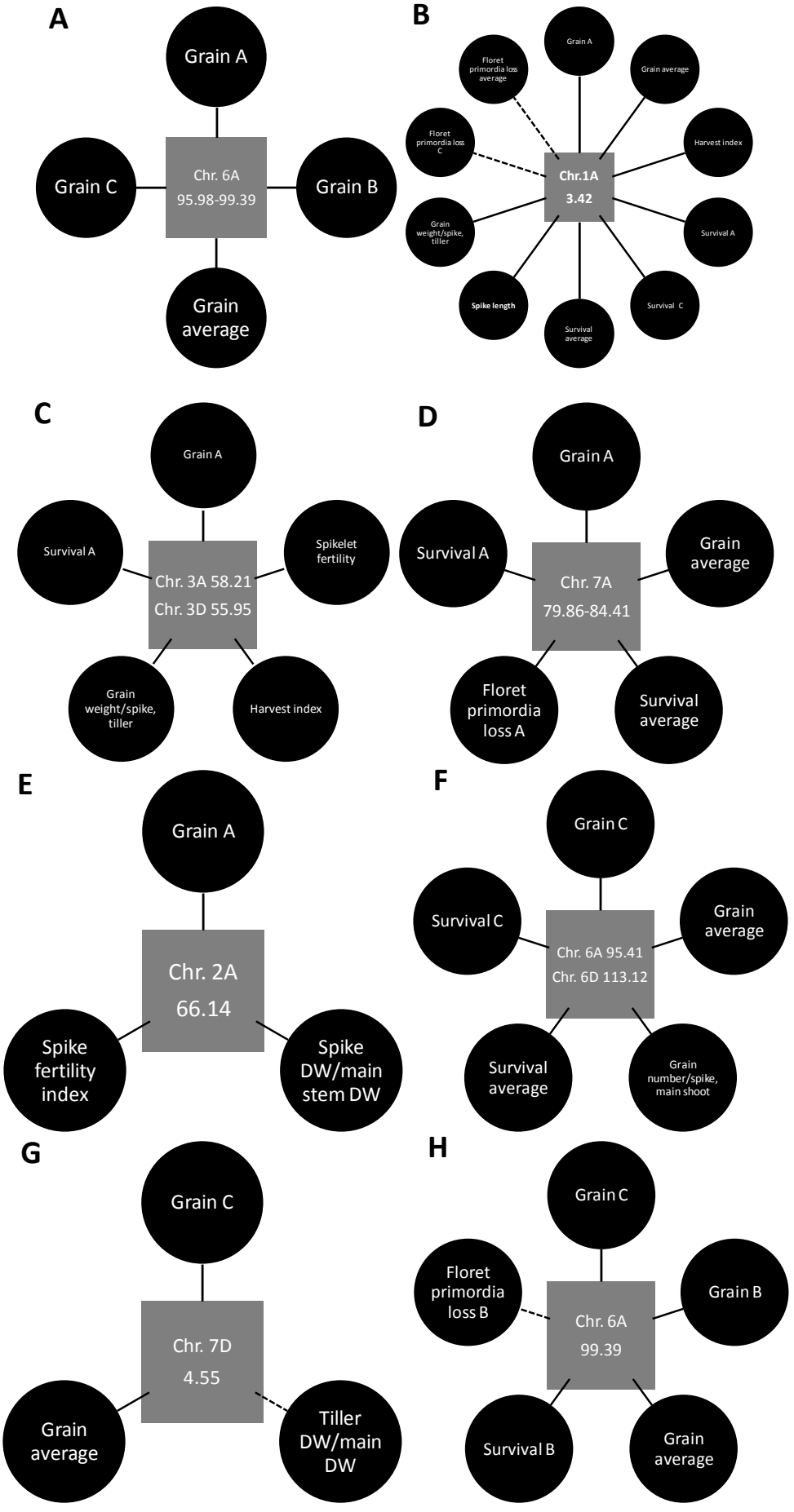


Fig. S5.2. The number of significantly associated SNP markers with all the traits detected by the three models: K, K+Q[5], K+Q[10]. Q[5] and Q[10] means the Q matrix based on the first five and ten principle components.


A
Grain A
Grain C
Chr. 6A
95.98-99.39
Grain B
Grain average
B
Grain A
Floret primordia loss average
Grain average
Floret primordia loss C
Harvest index
Chr.1A
3.42
Grain weight/spike, tiller
Survival A
Spike length
Survival average
Survival C
C
Grain A
Survival A
Spikelet fertility
Chr. 3A 58.21
Chr. 3D 55.95
Grain weight/spike, tiller
Harvest index
D
Grain A
Survival A
Grain average
Chr. 7A
79.86-84.41
Floret primordia loss A
Survival average
E
Grain A
Chr. 2A
66.14
Spike fertility index
Spike DW/main stem DW
F
Grain C
Survival C
Grain average
Chr. 6A 95.41
Chr. 6D 113.12
Survival average
Grain number/spike, main shoot
G
Grain C
Chr. 7D
4.55
Grain average
Tiller DW/main DW
H
Grain C
Floret primordia loss B
Grain B
Chr. 6A
99.39
Survival B
Grain average

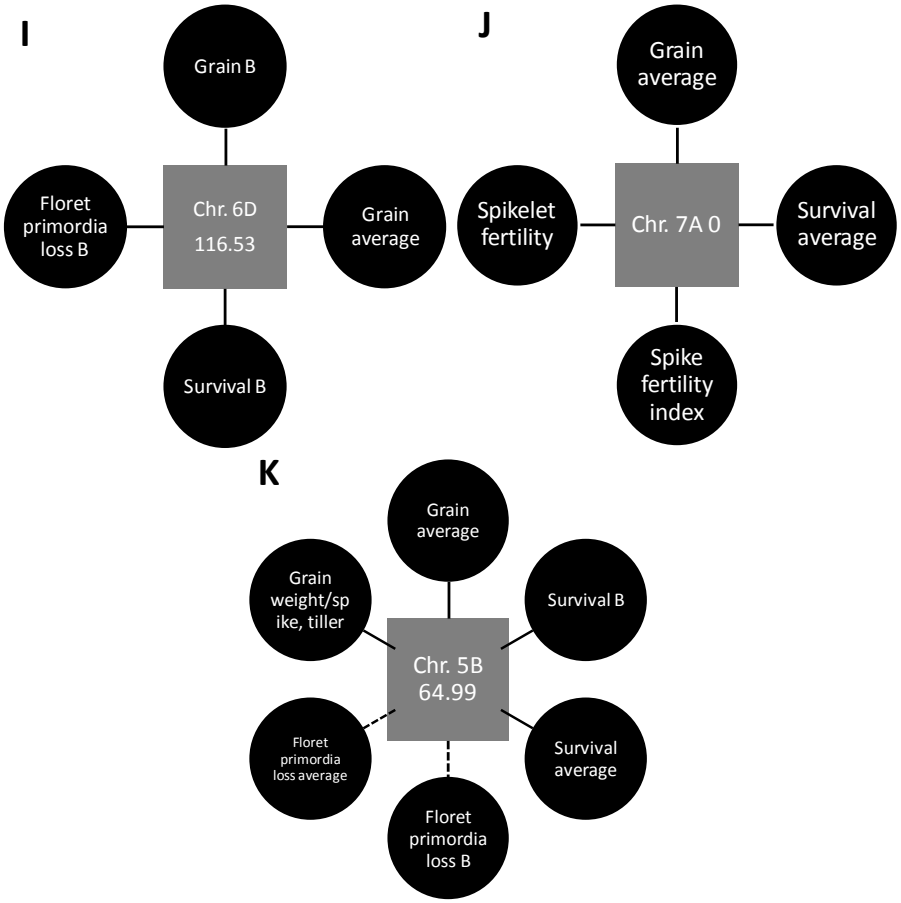


Fig. S5.3. The shared QTLs of floret fertility traits with other traits (based on the grain number-grain A, C, B, average).

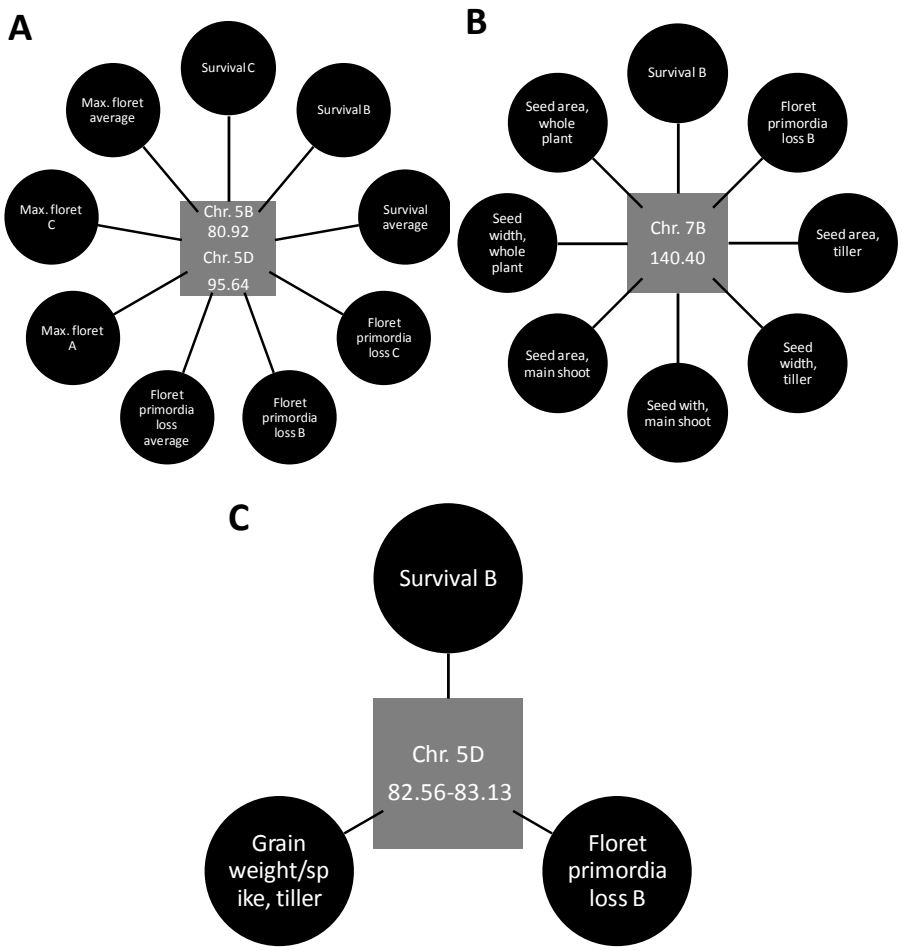


Fig. S5.4. The shared QTLs of floret fertility traits with other traits (based on the maximum number of floret primordia and floret primordia loss A, C, B, average).

9. Curriculum Vitae

Zifeng Guo *Curriculum vitae*

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■ Education and Degrees

Jul. 2012—present **PhD candidate (Agriculture)**

Research Group of Plant Architecture, Leibniz Institute of Plant Genetics and Crop Plant Research; supervision: PD Dr. Thorsten Schnurbusch.

Sep. 2009-Jul. 2012 **Master of Science (Ecology)**

Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences; supervision: Professor Zhengbing Zhang.

Sep. 2004-Jul. 2008 **Bachelor of Science (Biotechnology)**

College of Life Sciences, Northwest A&F University

■ Awards and Scholarships

07/2012—present **Scholarship from the Chinese Scholarship Council**

09/2006-06/2007 **Second Class Academic Scholarship of NWSUAF**

09/2004-08/2005 **Third Class Academic Scholarship of NWSUAF**

■ Peer-reviewed Publications

1. **Guo ZF**, Chen DJ, Alqudah AM, Röder MS, Ganal MW, Schnurbusch T (**2016**) Genome-wide association analyses of 54 traits identified multiple loci for the determination of floret fertility in wheat. *New Phytologist* 214: 257-270.
2. **Guo ZF,** Slafer GA, Schnurbusch T (**2016**) Genotypic variation in spike fertility traits and ovary size as determinants of floret and grain survival rate in wheat. *Journal of Experimental Botany* 67: 4221-4230.
3. **Guo ZF**, Schnurbusch T (**2016**) Costs and benefits of awns. *Journal of Experimental Botany* 67: 2533-2535.
4. **Guo ZF,** Schnurbusch T (**2015**) Variation of floret and spikelet fertility in wheat revealed by tiller removal. *Journal of Experimental Botany* 66: 5945-5958.
5. **Guo ZF,** Chen DJ, Schnurbusch T (**2015**) Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat. *Journal of Experimental Botany* 66:3099-3111.
6. **Guo ZF,** Zhang ZB, Xu P, Guo YN (**2013**) Analysis of Nutrient Composition of Purple Wheat. *Cereal Research Communications* 41: 293-303.
7. **Guo ZF,** Xu P, Zhang ZB, Guo YN (**2012**) Segregation ratios of colored grains in F1 hybrid wheat. *Crop Breeding and Applied Biotechnology* 12: 126-131.
8. **Guo ZF,** Xu P, Zhang ZB, Wang DW, Jin M, Teng AP (**2011**) Segregation ratios of colored grains in crossed wheat. *Australian Journal of Crop Science* 5: 589-594.

■ Oral presentations

Guo ZF. Save floret! Save yield! Save life! 5th Quedlinburger Pflanzenzüchtungstage in combination with 18th Kurt von Rümker Vorträge and the GPZ Meeting of AG Genomanalyse. Correnssstr. 3, OT Gatersleben, D-06466 Stadt Seeland, Germany, 1st-3rd, Mar., 2017.

Guo ZF. Developmental and genetic analysis of pre-anthesis phases in hexaploid winter wheat (*Triticum aestivum* L.). EU-FP7 KBBE-2011-5 'ADAPTAWHEAT' project, University of Lleida, Av. Rovira Roure 191, 25198 Lleida, Spain, 6th-7th Feb., 2014.

■ Poster contributions

Guo ZF, Röder M, Schnurbusch T. The genetic analysis of floret fertility and related traits in wheat (*Triticum aestivum* L.). Ninth Plant Science Student Conference (PSSC). Leibniz-Institute for Plant biochemistry, Halle (Saale), Germany, 26th-31th May, 2013.

Guo ZF, Röder M, Schnurbusch T. Timing and fate of floral development in wheat (*Triticum aestivum* L.). 12th International Wheat Genetics Symposium (IWGS), 1-1-1 Minato Mirai, Nishi-ku, Yokohama 220-0012, Japan, 8th -14th Sep., 2013.

Guo ZF, Schnurbusch T. Influence of de-tillering on floral degradation, maximum floret primordia and fertile floret number. Tenth Plant Science Student Conference (PSSC). Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, 2nd-5th Jun., 2014.

Guo ZF, Chen D, Ganal M, Röder M, Schnurbusch T. The genetic analysis of floret fertility and related traits in wheat. Cereals for Food, Feed and Fuel – Challenge for Global Improvement, Joint EUCARPIA Cereal Section & ITMI Conference, Wernigerode, Germany, Jun., 29th – Jul. 4th, 2014.

Guo ZF, Ganal M, Röder M, Schnurbusch T. Genome-Wide Association Study of Flowering Time in hexaploid Winter Wheat (*Triticum aestivum* L.). EU-FP7 KBBE-2011-5 'ADAPTAWHEAT' project, Centre for Agricultural Research Hungarian Academy of Sciences, Martonvásár, Hungary, 5th-7th Nov., 2014.

Guo ZF, Ganal M, Röder M, Schnurbusch T. Genome-Wide Association Study of Flowering Time in hexaploid Winter Wheat (*Triticum aestivum* L.). International Plant & Animal Genome XXIV (PAG), San Diego, CA, USA, 9th-13th Jan., 2015.

Guo ZF, Chen DJ, Ganal M, Röder M, Schnurbusch T. Genetic determinants of grain yield in wheat revealed by assimilate partitioning. International Plant & Animal Genome XXIV (PAG), San Diego, CA, USA, 9th-13th Jan., 2016.

Guo ZF, Slafer GA, Schnurbusch T. Genotypic variation in spike fertility traits and ovary size as determinants of floret and grain survival rate in wheat. 7th International Crop Science Congress, Beijing, China, 14th-19th Aug., 2016.

Eidesstattliche Erklärung / *Declaration under Oath*

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.
I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

_____ _____

Datum / Date Unterschrift des Antragstellers / *Signature of the applicant*

Erklärung über bestehende Vorstrafen und anhängige Ermittlungsverfahren /

Declaration concerning Criminal Record and Pending Investigations

Hiermit erkläre ich, dass ich weder vorbestraft bin noch dass gegen mich Ermittlungsverfahren anhängig sind. / *I hereby declare that I have no criminal record and that no preliminary investigations are pending against me.*

_____ _____

Datum / Date Unterschrift des Antragstellers / *Signature of the applicant*