Journal of Experimental Botany, Vol. 66, No. 11 pp. 3099–3111, 2015
doi:10.1093/jxb/erv117 Advance Access publication 28 March 2015
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

RESEARCH PAPER

Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat

Zifeng Guo[1], Dijun Chen[2] and Thorsten Schnurbusch[1,*]

[1] DFG-HEISENBERG Research Group Plant Architecture, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Stadt Seeland, OT Gatersleben, Germany
[2] Research Group Image Analysis, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Stadt Seeland, OT Gatersleben, Germany

* To whom correspondence should be addressed. E-mail: thor@ipk-gatersleben.de

Received 25 November 2014; Revised 12 January 2015; Accepted 12 February 2015

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: Anther size, heritability, ovary size, sensitivity, spike dry weight, wheat.

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, 2011; Ferrante *et al.*, 2010; 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. 1); floral development and differentiation is an important part of the pre-anthesis stage (Fig. 1). Anther and ovary growth is a vital factor determining grain number per spike and grain size, and further affects wheat

Abbreviations: F1, F3, F4 anther (ovary) means anther (ovary) in the first (F1), third (F3), fourth (F4) floret from the base; Z, Zadoks scale; TS, terminal spikelet; WA, white anther; GA, green anther; YA, yellow anther; TP, tipping; HD, heading; AN, anthesis; DW, dry weight.

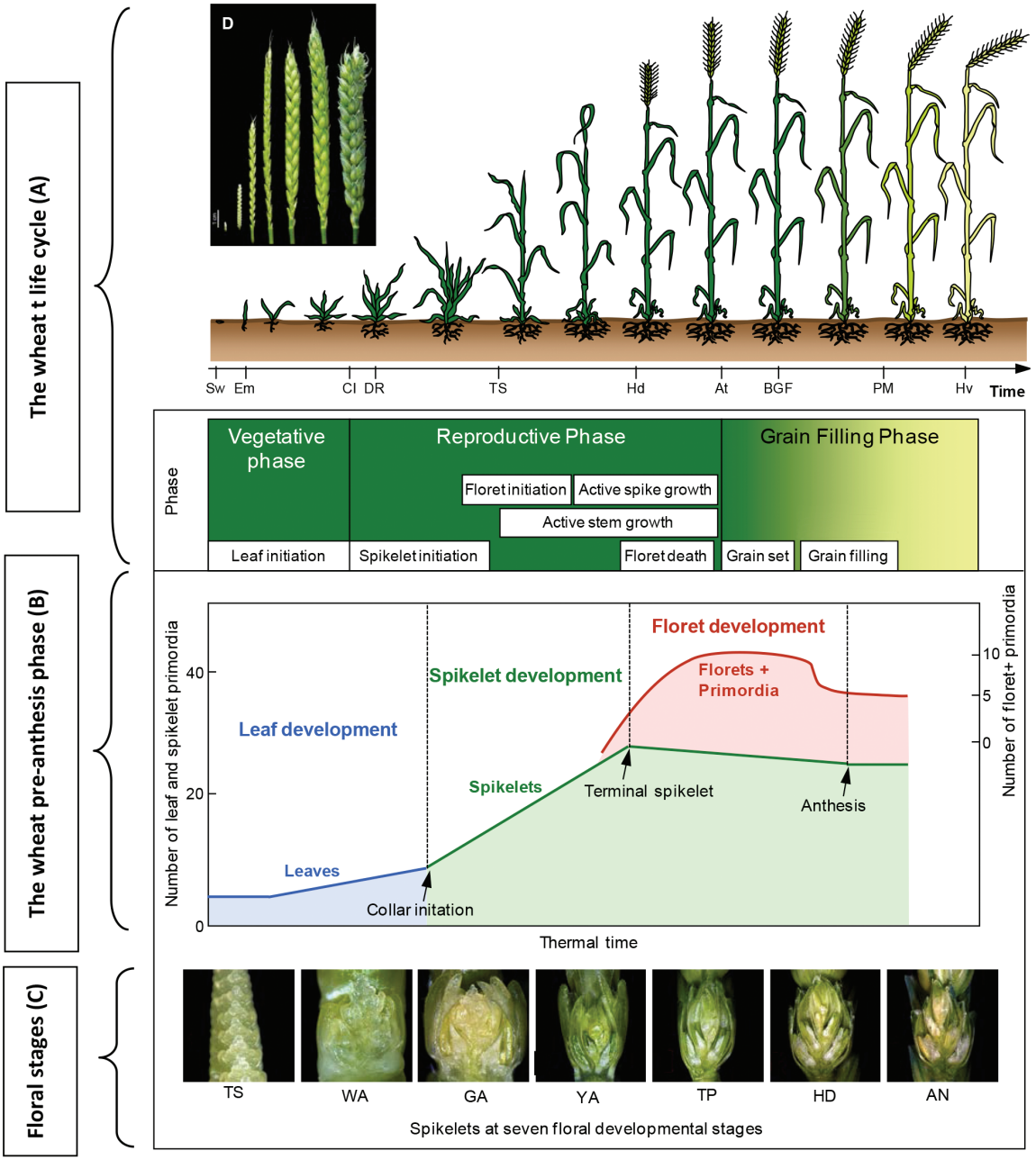

Fig. 1. Different developmental stages in the wheat life cycle. (A) The wheat life cycle. (B) The wheat pre-anthesis phase, including leaf, spikelet, and floret initiation. (C) The spikelets at the seven floral developmental stages (after Kirby and Appleyard, 1987): TS (terminal spikelet stage), WA (white anther stage), GA (green anther stage), YA (yellow anther stage), TP (tipping stage), HD (heading stage), and AN (anthesis stage) (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).

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, 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.

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 night-time temperature or a high source-sink ratio, and that the influence of low night-time temperature 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°C compared with 20°C night-time temperatures, indicating that low night-time 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 favouring 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 suggest 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, 2007; Abbate *et al.*, 1995, 1997; Demotes-Mainard and Jeuffroy, 2001, 2004; 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 of spike dry weight and of each other; and (iii) anther and ovary size at F1 (i.e. Floret 1 from the spikelet base) are less sensitive to the environment compared with florets at F3/4 positions.

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 2012/3 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 given in Supplementary Table S1.

For both greenhouse and field experiments, seeds were sown in 96-well trays on the same day (1 January 2013) and germinated under greenhouse conditions (16/8 h day/night; ~20/~16°C) for 14 d. Seedlings at the two- to three-leaf stage were transferred to 4°C to vernalize for 63 d. Vernalized seedlings were transferred to a hardening stage (12/12 h day/night, and ~15 °C) for 7 d to gradually acclimatize. Finally, half of the plants were transplanted (15 April 2013) into 0.5 litre pots (one plant per pot; 9 cm×9 cm×9 cm) under greenhouse conditions (16/8 h day/night; ~20/~16°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. The other half of the plants was directly transplanted into the field (15 April 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 *et al.*, 1974); heading time (HD) stage (Z55), 50% of spikes visible (Zadoks *et al.*, 1974); and anthesis (AN) stage (Z65), 50% of spikes with anthers (Zadoks *et al.*, 1974). The spikes and spikelets at the seven floral developmental stages can be found in Fig. 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°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°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 were not measured on spikes of tillers. From GA to AN stages (Fig. 2A), spikelets from the centre of the main culm spike were dissected to obtain digital images of anthers and ovaries for three plants in each stage (Fig. 2B). When the anther or ovary was 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 1.

Statistical analysis

We performed an additive main-effects and multiplicative interaction (AMMI) model analysis to determine differences among genotype (G), environment (E), and the interaction between G×E for each measured trait. The AMMI analysis combines the analysis of variance (ANOVA) 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 $i-\mu$; e_j is the mean in environment $j-\mu$; 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-centred 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 analysed using the following full linear mixed model:

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

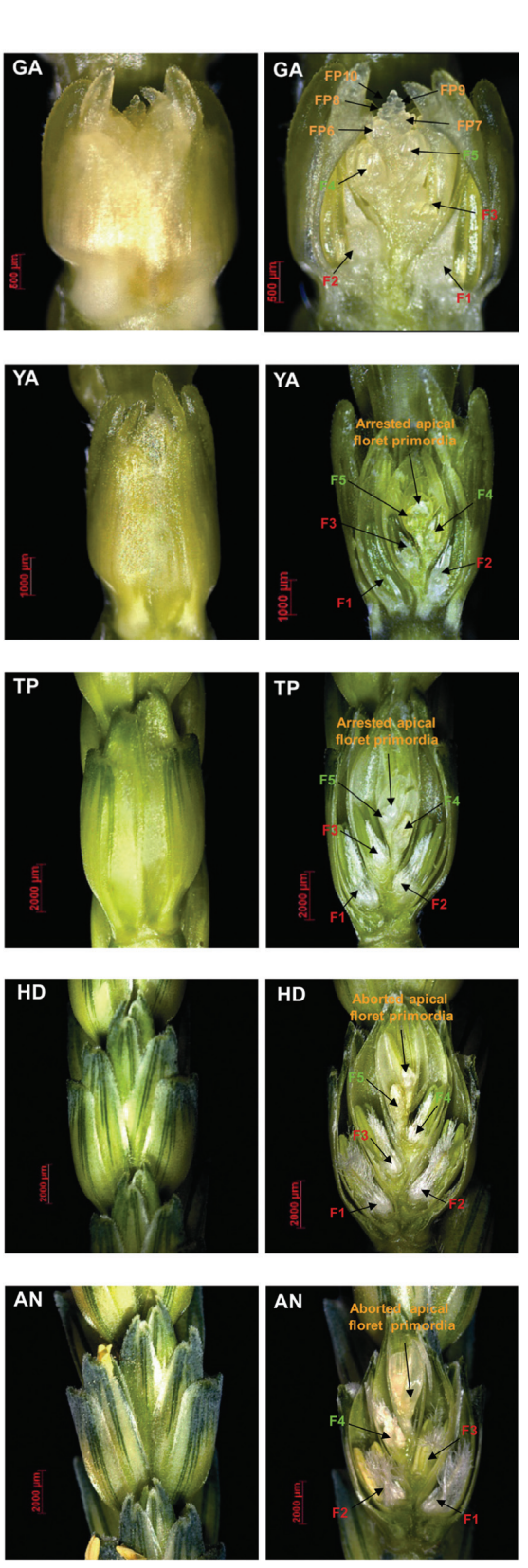

Fig. 2. (A) Details of spikelet structure at GA, YA, TP, HD and AN developmental stages in wheat after Kirby and Appleyard (1987); (B) the four examples display the measurements of ovary and anther size in this experiment, the two top images show the anther and ovary at F1 positions at GA stage, the bottom two images show the anther and ovary at F1 positions at YA stage.

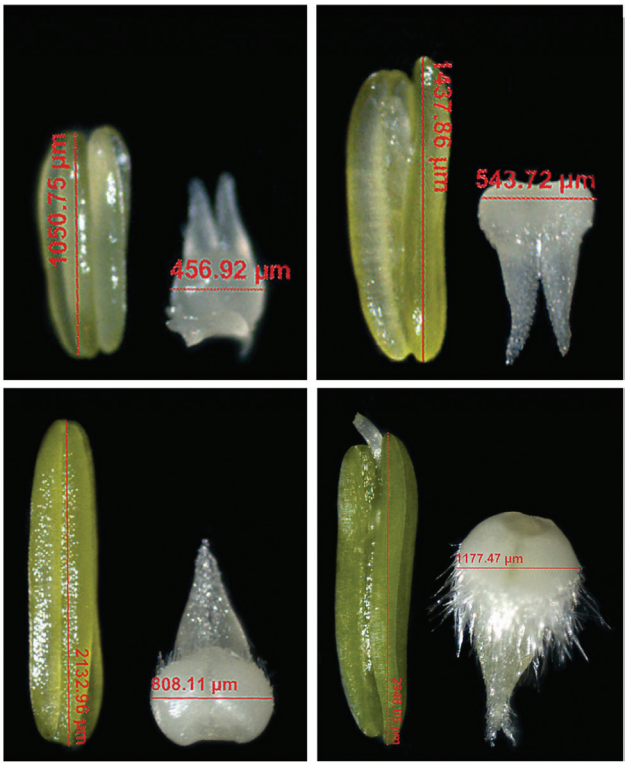

Fig. 2. Continued

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 $g \sim (0, \mathrm{I}\sigma_G^2)$, where I is the identity matrix], l [E effect, $l \sim (0, \mathrm{I}\sigma_E^2)$], gl (G×E effect, $gl \sim (0, \mathrm{I}\sigma_{GE}^2)$], 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:

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

where $\log\left(likelihood_{alt}\right)$ is the log-likelihood of the alternative model (with more parameters) and $\log\left(likelihood_{null}\right)$ 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.

Table 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

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. 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. 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. 3B). We assumed that if heritability was high, the increased anther/ovary size would be reflected across all floret positions within individual genotypes. We selected genotype 'Tukan' to calculate the correlations between anther/ovary size at different floret positions across different floral developmental stages under field and greenhouse conditions (Supplementary Figs S1, S2). Clearly, anther/ovary at different positions display close relationships in most cases, but there are no strong correlations in some instances due to the variation within genotypes. Environment (i.e. σ^2_E green bar; Fig. 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 grey bar; Fig. 3B). On the contrary, ovary size at position F1 was generally more stable than F3 and F4 at most 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 grey bars, GA, 46.7%; YA, 30.4%; TP, 36.5%; HD, 27.8%; Fig. 3B) indicated that within-genotype variation was rather high.

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. 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. 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

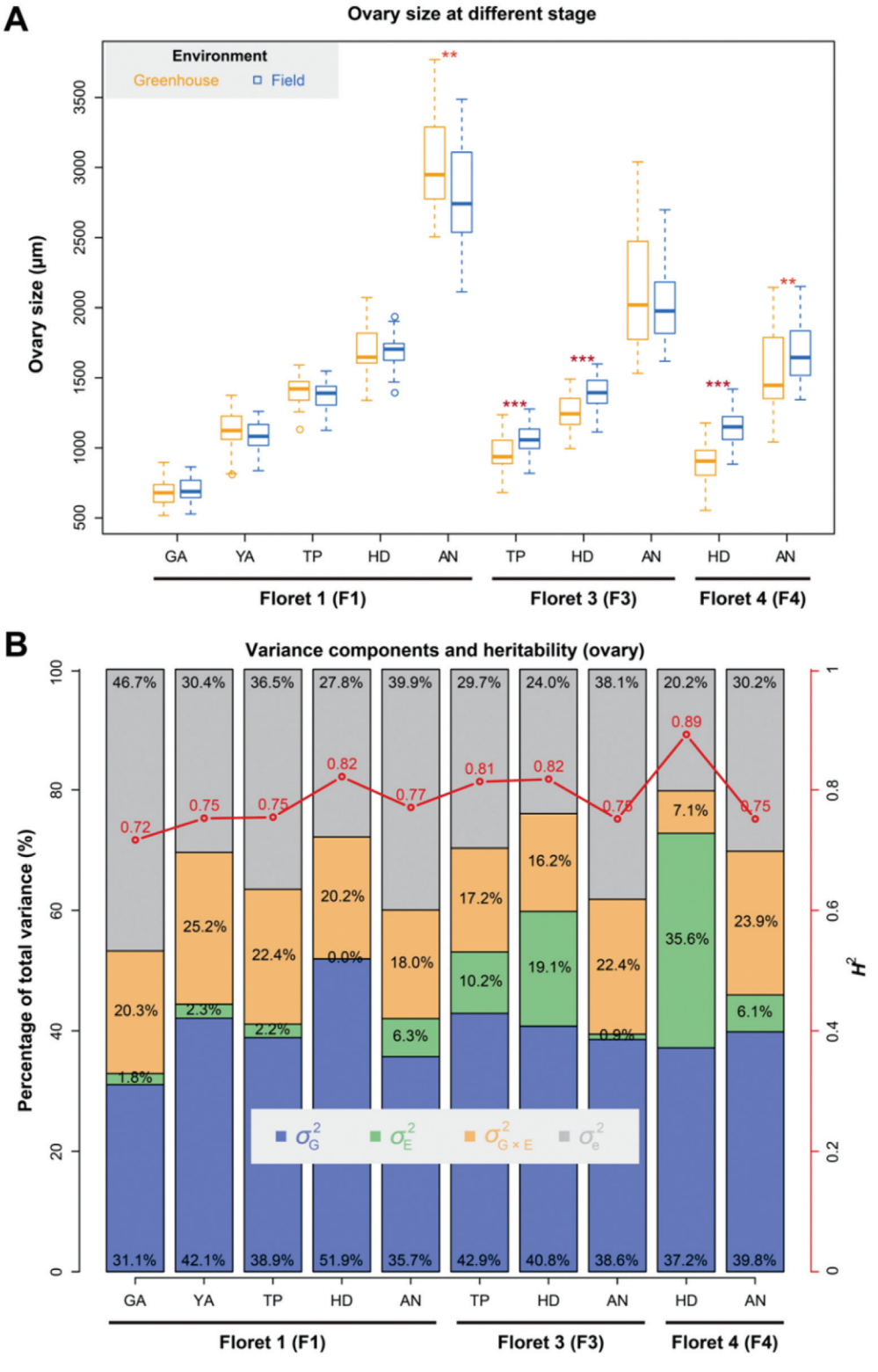

Fig. 3. (A) Ovary size (μm) at different positions at floral developmental stages under greenhouse and field conditions; (B) variance components and heritability (red point) of ovary size. **p<0.01; ***p<0.001.

(0.92; Fig. 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. 4B). Generally, the residual proportions for anther size (i.e. σ^2_e grey bars; Fig. 4B), ranging from 14.7% to 40.5%, were not as big as those for ovary size, ranging from 20.2% to 46.7% (Fig. 3B), which indicates that within-genotype variation of anther size is more stable compared with ovary size. The proportion of genotypic influence on anther size, ranging from 30.0% to 53.5%, and ovary size, ranging from 31.1% to 51.9%, was relatively large at most stages.

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. 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. 5). Large temperature fluctuations were observed in the field, whereas greenhouse temperatures were more stable for plant growth (Supplementary Fig. S3). Clearly, there was a significant difference in temperature amplitude between the greenhouse and field, indicating different environmental influences

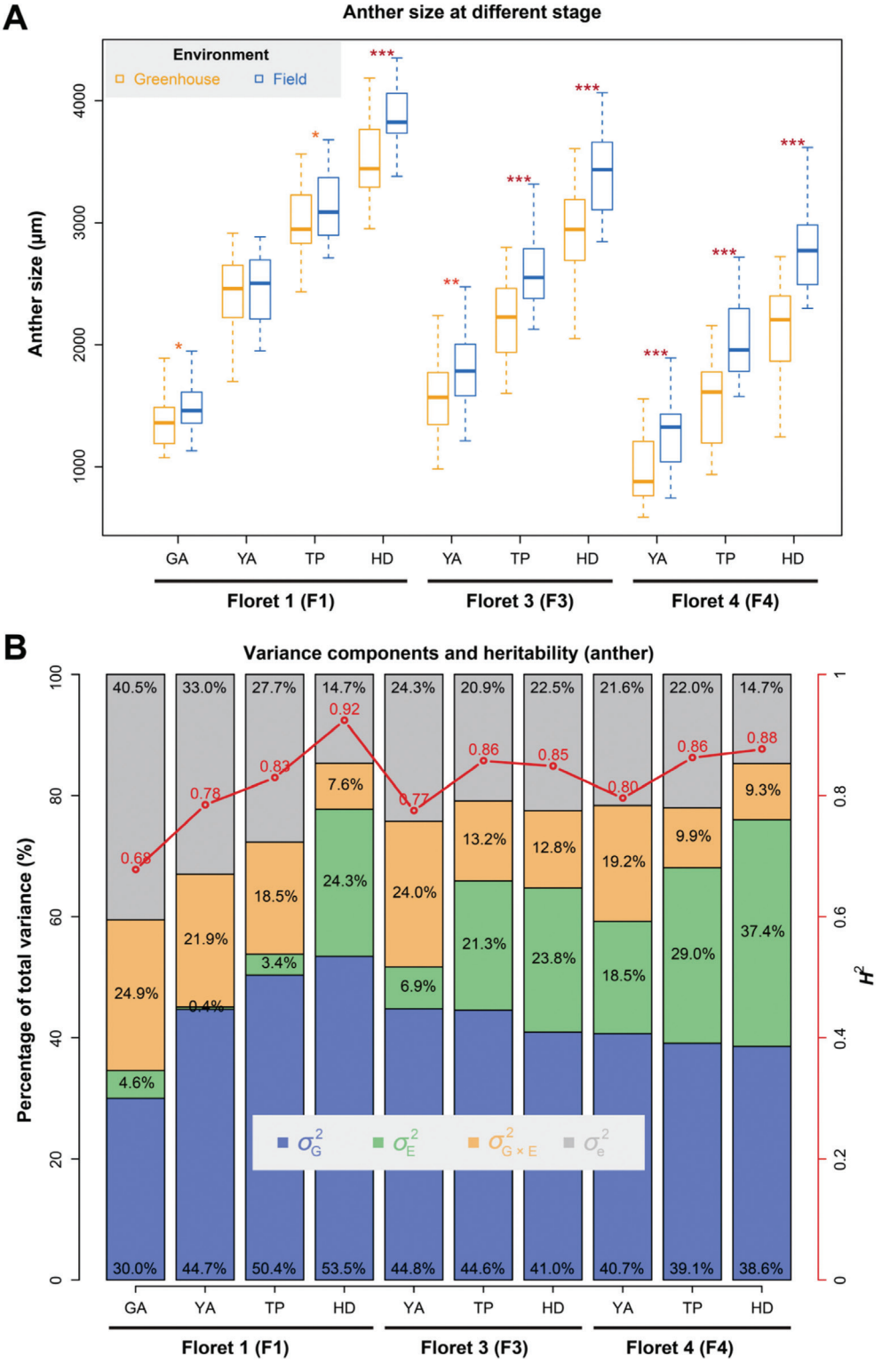

Fig. 4. (A) Anther size (μm) at different positions at floral stages under greenhouse and field conditions; (B) variance components and heritability of anther size. *p<0.05; **p<0.01; ***p<0.001.

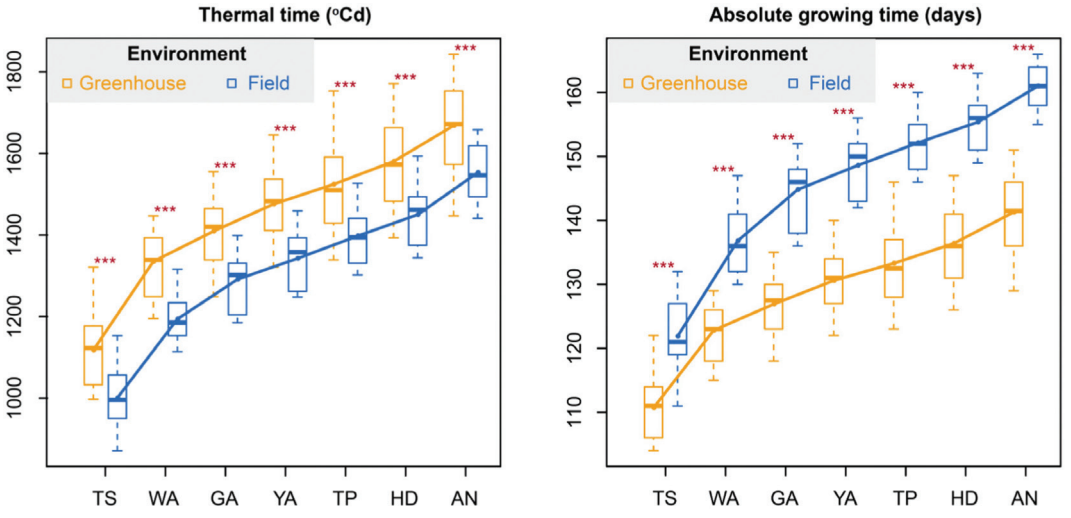

Fig. 5. The range and difference of thermal time (°Cd) and absolute growing time (days) between greenhouse and field conditions at seven floral developmental stages. ***p<0.001.

on ovary and anther growth during floral development. Supplementary Figs S4 and S5 show that most of the correlations were strong between thermal time and ovary and anther size 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 was markedly higher than in the greenhouse.

As shown in Figs 6 and 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 (Supplementary Figs S6, S7). Moreover, anther and ovary size at the same position also strongly correlated (Supplementary Fig. S8), 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.

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 (Supplementary Table S1). Broad-sense heritability of anther and ovary size was 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, 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 (Figs 3B, 4B) and close relationships between anther/ovary at different floret positions within genotypes (Supplementary Figs S1, S2).

We also found 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

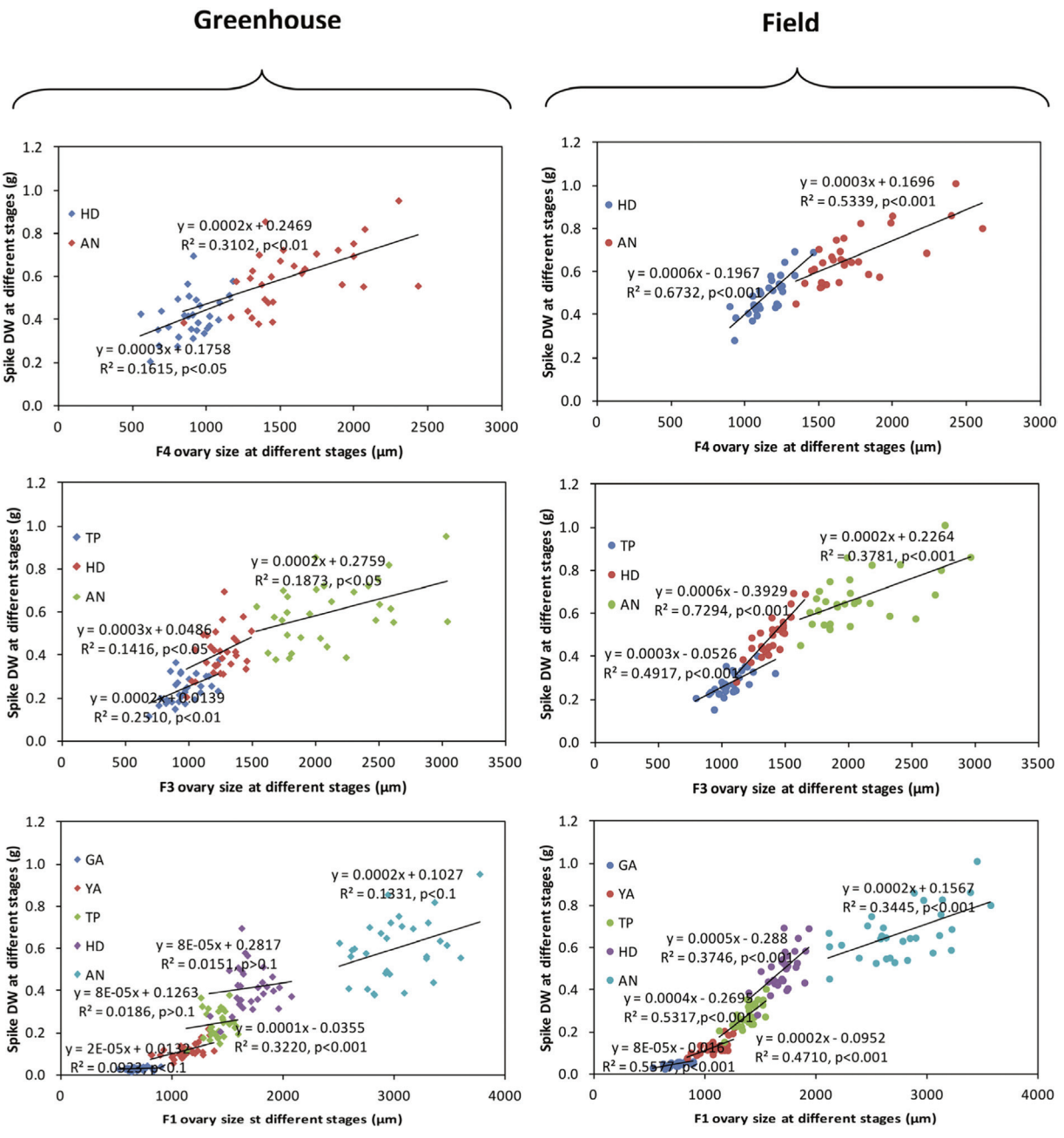

Fig. 6. Relationship between ovary size (μm) and spike dry weight (g) at different floral developmental stages under greenhouse and field conditions. Solid lines were fitted by linear regression.

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 (Supplementary Fig. S3). 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. 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 favoured 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

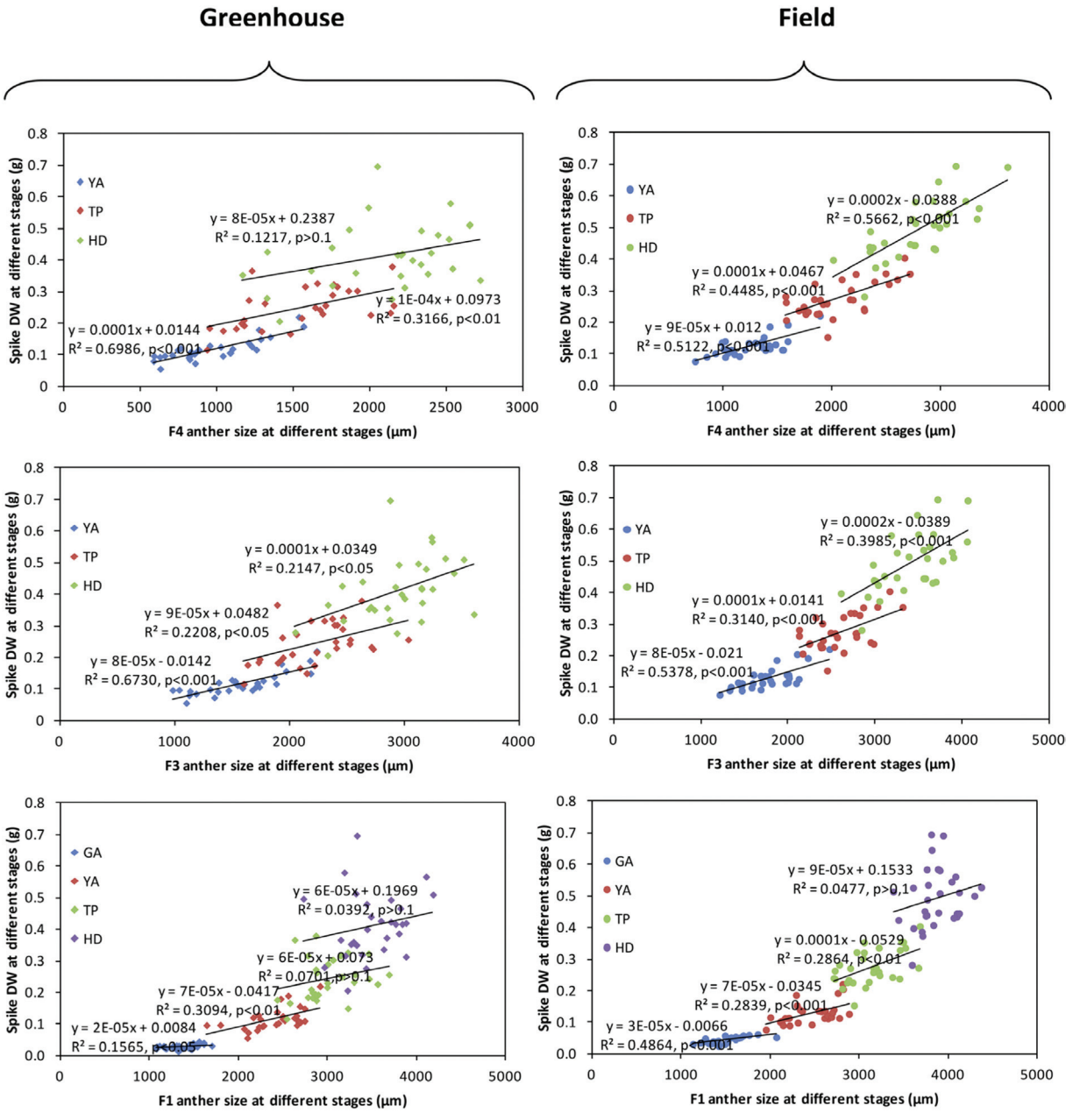

Fig. 7. Relationship between anther size (μm) and spike dry weight (g) at different floral developmental stages under greenhouse and field conditions.

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.

Stem dry weight is also considered a crucial factor affecting anther and ovary growth. Fig. 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.

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

Correlation analyses in this study revealed that (i) anther and ovary size are closely associated with spike dry weight, indicating that anther and ovary size are good predictors of spike dry weight (Figs 6, 7); (ii) 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

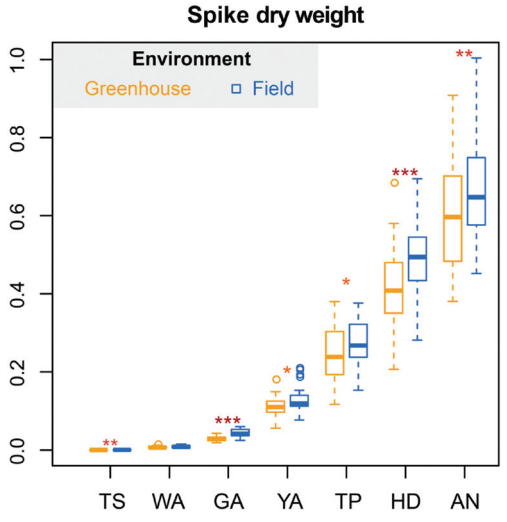

Fig. 8. Spike dry weight (g) at the seven floral developmental stages under greenhouse and field conditions. *p<0.05. **p<0.01. ***p<0.001.

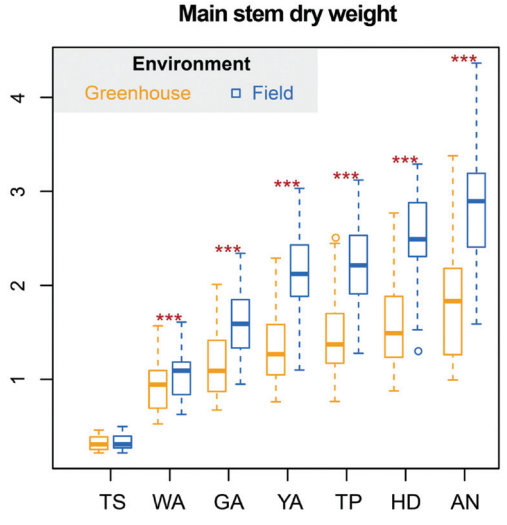

Fig. 9. Main-stem dry weight (g) at the seven floral developmental stages under greenhouse and field conditions. *p<0.05; **p<0.01; ***p<0.001.

are good predictors of each other within the germplasm studied. However, correlations were substantially different within the two environments (Supplementary Figs S6–S8). As shown in Fig. 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. 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. 10), whereas F1 and F3 ovary size benefited from greenhouse conditions (i.e. red line below dashed line and closer to greenhouse; Fig. 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. 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.

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 G×E 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 night-time temperature, large temperature fluctuations, more spike dry-mass and stem reserve accumulation in the field are thought to be responsible for the detected difference in anther and ovary size.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Table S1. Marker information for all 30 cultivars used.

Supplementary Fig. S1. Relationships between ovary size (μm) at different floret positions (F1, F3, F4) at different floral developmental stages under greenhouse and field conditions.

Supplementary Fig. S2. Relationships between anther size (μm) at different floret positions (F1, F3, F4) at different floral developmental stages under greenhouse and field conditions

Supplementary Fig. S3. Temperatures under field (A) and greenhouse (B) conditions.

Supplementary Fig. S4. Distribution of ovary (μm) at seven floral developmental stages *versus* thermal time (°Cd) and their relationships under greenhouse and field conditions.

Supplementary Fig. S5. Distribution of anther size (μm) at seven floral developmental stages *versus* thermal time (°Cd) and their relationships under greenhouse and field conditions.

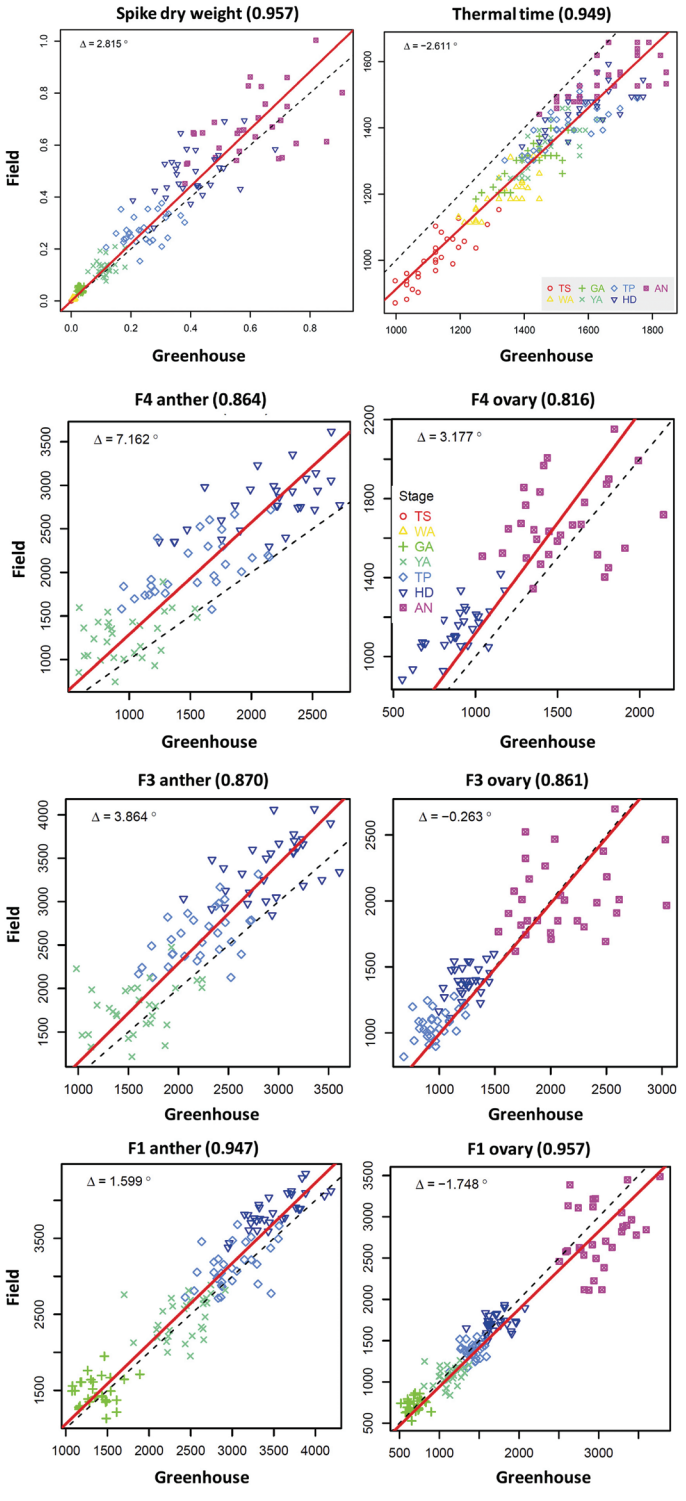

Fig. 10. Sensitivity and correlation (i.e. values in brackets) analysis of spike dry weight (g), thermal time (°Cd), and anther and ovary size (μm) at the seven floral developmental stages between greenhouse and field conditions. Red line, trend line between the greenhouse and field; the traits are equivalent 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.

Supplementary Fig. S6. Relationships between ovary size (μm) at different positions at seven floral developmental stages under greenhouse and field conditions.

Supplementary Fig. S7. Relationships between anther size (μm) at different positions at seven floral developmental stages under greenhouse and field conditions.

Supplementary Fig. S8. Relationships between anther size (μm) and ovary size (μm) at the same position.

Acknowledgements

We acknowledge the contribution of germplasm from the GABI-WHEAT/VALID Consortium and Marion Röder from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. We thank Karin Lipfert and Heike Ernst for help in figure design, and Mechthild Pürschel for excellent technical assistance. This study received financial support from a scholarship from the Chinese Scholarship Council to ZG and from the EU-FP7 KBBE-2011–5 'ADAPTAWHEAT' project number 289842 to TS.

References

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.

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.

Bidinger F, Musgrave RB, Fischer RA. 1977. Contribution of stored pre-anthesis assimilate to grain-yield in wheat and barley. *Nature* **270,** 431–433.

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.

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.

Cruz-Huerta N, Williamson JG, Darnell RL. 2011. Low night temperature increases ovary size in sweet pepper cultivars. *Hortscience* **46,** 396–401.

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

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.

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, 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.

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.

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.

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. II. 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, Stockman YM. 1980. Kernel number per spike in wheat (*Triticum aestivum* L)—responses to preanthesis shading. *Australian Journal of Plant Physiology* **7,** 169–180.

Giles BE, Bengtsson BO. 1988. Variation in anther size in wild barley (*Hordeum vulgare* ssp *spontaneum*). *Hereditas* **108,** 199–205.

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. 2005. Floret development and survival in wheat plants exposed to contrasting photoperiod and radiation environments during stem elongation. *Functional Plant Biology* **32,** 189–197.

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.

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

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.

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.

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, Appleyard M. 1987. *Cereal Development Guide* , 2nd ed. UK, Stoneleigh: Arable Unit.

Komaki MK, Tsunewaki K. 1981. Genetic studies on the difference of anther length among common wheat cultivars. *Euphytica* **30,** 45–53.

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.

Langer RHM, Hanif M. 1973. Study of floret development in wheat (*Triticum aestivum* L). *Annals of Botany* **37,** 743–751.

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

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.

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.

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.

Savin R, Slafer GA. 1991. Shading effects on the yield of an Argentinean wheat cultivar. *Journal of Agricultural Science* **116,** 1–7.

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.

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

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.

Trione EJ, Stockwell VO. 1989. Development of detached wheat spikelets in culture. *Plant Cell, Tissue and Organ Culture* **17,** 161–170.

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.

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.

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

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.