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Phenotypic and genome-wide association analysis of spike ethylene in diverse wheat genotypes under heat stress

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Summary

- The gaseous phytohormone ethylene plays an important role in spike development in wheat (*Triticum aestivum*). However, the genotypic variation and the genomic regions governing spike ethylene (SET) production in wheat under long-term heat stress remain unexplored.
- We investigated genotypic variation in the production of SET and its relationship with spike dry weight (SDW) in 130 diverse wheat elite lines and landraces under heat-stressed field conditions. We employed an Illumina iSelect 90K single nucleotide polymorphism (SNP) genotyping array to identify the genetic loci for SET and SDW through a genome-wide association study (GWAS) in a subset of the Wheat Association Mapping Initiative (WAMI) panel.
- The SET and SDW exhibited appreciable genotypic variation among wheat genotypes at the anthesis stage. There was a strong negative correlation between SET and SDW. The GWAS uncovered five and 32 significant SNPs for SET, and 22 and 142 significant SNPs for SDW, in glasshouse and field conditions, respectively. Some of these SNPs closely localized to the SNPs for plant height, suggesting close associations between plant height and spike-related traits.
- The phenotypic and genetic elucidation of SET and its relationship with SDW supports future efforts toward gene discovery and breeding wheat cultivars with reduced ethylene effects on yield under heat stress.

Introduction

Ambient temperatures, on a global scale (IPCC, 2007) as well as at the on-farm level (Battisti & Naylor, 2009), have been increasing since the beginning of the century and this trend is predicted to continue into the future (IPCC, 2013). Field studies (White *et al.*, 2011) and controlled environment experiments (Prasad & Djanaguiraman, 2014) have reported a substantial negative impact of heat stress (HT) on wheat (*Triticum aestivum*) yield. Further, short episodes of extreme heat are expected to become more frequent, which could have an adverse impact on yield components, and thus grain yield (Prasad *et al.*, 2006; Jagadish *et al.*, 2007). Such negative impacts are more likely to occur at lower latitudes, where *c.* 100 million ha of wheat is cultivated producing *c.* 280 million tons of grain, although some positive benefits at higher latitudes can be expected (Cossani & Reynolds, 2012). Intensified efforts employing a diverse set of wheat genotypes can help to better understand the physiological and genetic responses to HT and this understanding potentially can aid in enhancing future wheat yields.

Under heat stress, many plants produce extra ethylene (ET) (Hays *et al.*, 2007), a colorless gas that has been extensively used in commercial agriculture to force the ripening of fruits. Ethylene has been linked to yield penalty under heat stress in various crops including wheat (Hays *et al.*, 2007; Huberman *et al.*, 2013), which mainly is attributed to a reduction in spike fertility (Cheng & Lur, 1996) and final grain weight (Yang *et al.*, 2004). These trait responses are attributable partly to reduced carbohydrate export to pollen grains, accompanied by poor pollen germination and accelerated senescence (Torre *et al.*, 2006; Feng *et al.*, 2011). Further, accelerated stigma and ovule development and abnormal ovary development result in reduced pollen tube growth and grain set when heat stress-induced ET coincides with meiosis (Barnabás *et al.*, 2008). From these studies, it is apparent that ET physiology plays a crucial role in the regulation of spike fertility and kernel abortion under heat stress, although ET-induced kernel abortion and premature maturation can be seen as a strategic survival trait in order to maintain optimal progeny load in warm, dry climates.

The effects of heat stress vary among species and between individuals in the same species and are highly dependent on the timing of the high-temperature challenge (Prasad *et al.*, 2006). Previous studies have followed various approaches to reduce the negative impact of heat-induced ET production on crop yield. Typically, the application of various chemicals that act as antagonists of ET biosynthesis or sensitivity of response has been used in practice, which has significantly increased grain yield in wheat (Hays *et al.*, 2007; Huberman *et al.*, 2013) and rice (*Oryza*

sativa) under conditions where ET might otherwise be limited by heat stress (Tamaki *et al.*, 2015). At the molecular level, biotechnological interventions have been employed to reduce ET biosynthesis and its sensitivity in crop plants. Down-regulation of one or more metabolic components of ET signaling (e.g. *ACS6* (*1-Aminocyclopropane-1-carboxylic acid Synthase 6*) (Young *et al.*, 2004; Habben *et al.*, 2014) and *ETR2* (*Ethylene Response 2*) (Wuriyanghan *et al.*, 2009)) has been shown to decrease ET biosynthesis, and sensitivity to this hormone in maize (*Zea mays*) (Young *et al.*, 2004; Habben *et al.*, 2014) and rice (Wuriyanghan *et al.*, 2009). Such manipulations contributed to an increased grain yield under both optimal growing conditions (Wuriyanghan *et al.*, 2009) and field-drought conditions (Habben *et al.*, 2014). These studies suggest that manipulating ET signaling may have a positive impact on crop yield under stress.

A longer term, robust and cost-effective approach to reduce the negative effect of ET on crop yield could be through exploiting natural diversity in ET production. A substantial genetic diversity in pollen viability and spike fertility under heat stress has been identified in rice (Prasad *et al.*, 2006; Jagadish *et al.*, 2007), cotton (*Gossypium hirsutum*) (Burke & Chen, 2015), maize (Herrero & Johnson, 1980), and *Brachypodium* (Harsant *et al.*, 2013), while in wheat, variation in spikelet fertility in response to various agronomic treatments has been reported (Cantrell & Haro-Arias, 1986; Guo & Schnurbusch, 2015). Further, wheat genotypes differ in heat stress tolerance (Fu *et al.*, 2015) and exhibit large intraspecific variation in grain yield (Lopes *et al.*, 2015). However, there is a significant lack of understanding of genotypic variation in spike dry weight (SDW), a proxy for grain number and grain weight (Fischer, 1985; González *et al.*, 2011), and its association with spike ethylene (SET), in a diverse set of wheat genotypes on exposure to heat stress under field conditions.

Plant height is an important agronomic trait closely linked to yield and biomass. In heat- and drought-stressed environments, a considerable yield variation between taller and shorter phenotypes has been reported (Richards, 1992; Buttler *et al.*, 2005). Further, taller genotypes have been shown to maintain cooler canopies, although only when surrounded by shorter plots, in heat-stressed environments (Miller *et al.*, 1981; Pask *et al.*, 2014) as a result partly of a greater coupling of the plant boundary layer with the environment. We hypothesize that taller genotypes may produce lower SET compared with their shorter counterparts, particularly in a heat-stressed environment. Nevertheless, this hypothesis has never been explicitly tested.

To elucidate the impact of heat stress and other environmental stresses, proteomic and transcriptomic analyses routinely have been performed in various crops including wheat (Li *et al.*, 2013; Wang *et al.*, 2015a,b). However, there have been few studies, limited to only a few crop species, dissecting the genetic basis of fertility dynamics under heat stress, for instance, in rice (Ye *et al.*, 2015), cotton and tobacco (*Nicotiana tabacum*) (Burke & Chen, 2015). Genome-wide association analysis offers the high-resolution genetic capacity to capture insights into the genetic architecture of complex traits such as SET. To our knowledge, there are no reports of the use of genome-wide association mapping to elucidate the basis of heat stress responses of SET and SDW in wheat.

In this study, we first (Exp 1) examined a diverse set of 130 spring wheat genotypes that consisted of elite material (EM) and landraces (LR) to examine the genotypic variation of SET and SDW in the presence of an ET response inhibitor under heat-stressed field conditions. We hypothesized that different wheat genotypes differ for SDW sensitivity to heat stress particularly through their response to SET (Hays *et al.*, 2007). Second, a random subset of 30 genotypes (Exps 2 and 3) from the initial 130 genotypes was used to dissect the responses of, and the tradeoffs between, spike-related traits, plant height, yield and yield attributes in response to SET. Finally, in order to dissect the genetic basis of SET and SDW, a genome-wide association study (GWAS) was performed on a separate subset of the Wheat Association Mapping Initiative (WAMI; Lopez *et al.*, 2012) panel grown under controlled environment (Exp 4) and field conditions (Exp 5).

Materials and Methods

Plant material

A diverse set of spring wheat (*Triticum aestivum* L.) genotypes (130 genotypes; Supporting Information Table S1) consisting of EM (85) and LR (45) was chosen for the initial genetic diversity study (Exp 1). Subsequently, a random subset (30 genotypes: EM, 13; LR, 17; Exps 2 and 3; Table S2) was chosen for further studies. In addition, a separate diverse set of the spring wheat elite WAMI panel (190 genotypes; Exps 4 and 5) was used for genetic studies.

Experimental design

Four field experiments (Exps 1–3 and 5) were carried out during 2013–2015 in the Yaqui Valley at CIMMYT's Experimental Station in Obregon (20°27′N, 109°54′W; 38 m above sea level), Mexico. One glasshouse study (Exp 4) was carried out during 2014–2015 at the Lancaster Environment Center, Lancaster University, UK.

All field experiments (Exps 1–3 and 5) were sown during mid-to-late March in each year (24, 29 and 19 March in 2013, 2014 and 2015, respectively). The plots were arranged in a randomized lattice design with two replications in 2-m-long and 0.8-m-wide plots consisting of one raised bed with two rows per bed (0.3 m between rows). The soil type is a sandy-clay, low in organic matter (< 1%) and slightly alkaline (pH 7.5), with a plant available water-holding capacity of *c.* 200 mm. Gravity-based flooding irrigation was used with a frequency of 2–3 wk to avoid water deficit. Similar fertilizer regimes and appropriate cultural practices such as weed, disease, and pest control were implemented to avoid any yield limitations.

Sowing in the month of March generally exposes the crop to a heat-stress environment compared with the normal planting time (November) of yield potential trials. Hence, the crop plants encountered a natural high-radiation environment. The average air temperatures during the preanthesis period were 32.8°C,

33.8°C and 34°C (maximum: 37.5°C, 41.0°C and 40.4°C; minimum: 28.1°C, 26.8°C and 27.4°C) with an average air relative humidity of 65%, 59% and 60% in 2013, 2014 and 2015, respectively. The average temperatures of the crop canopy throughout the preanthesis period, however, were 29.5°C, 31.2°C and 31.9°C in 2013, 2014 and 2015, respectively. Thus, the crop experienced heat stress (average maximum temperature (T_{max}) > 32°C; total crop water supply > 700 mm) during the preanthesis period.

For genetic analysis, in addition to one field study (Exp 5, sown on 19 March 2015), one controlled glasshouse study (Exp 4) was carried out using the same subset of the WAMI panel. Briefly, seeds of a randomly selected subset of WAMI lines (190) were germinated on wet filter paper placed in Petri dishes at room temperature. Seven-day-old seedlings were transplanted into the plastic pots (20 × 15 cm) containing a well-prepared mixture of soil-based compost (John Innes No. 2; John Innes Manufacturers Association, Berkshire, UK). The plants were grown in the glasshouse which was set to a 14-h light regime (*c.* 900 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR)) with day : night temperatures of 35°C : 23°C, respectively, at a relative humidity of 70–75%. All plants were well watered daily and a half-strength Hoagland solution was supplied as a source of nutrients on alternate days. Two pots per genotype, and two plants per pot were maintained. Samples for SET and SDW were collected at the appropriate developmental stage.

Silver nitrate spray in field experiments

In Exps 1–3, two treatment conditions were maintained: heat stress without silver nitrate spray (control; HT) and heat stress with silver nitrate spray (HSN). Both the HT and HSN conditions were imposed in an area of a 1-m length of plot containing two rows. These plots were specifically marked at an early seedling stage to eliminate border effects. When the plants reached an early booting stage (ranging from 50 to 70 d after sowing (DAS) with a range of heading dates of 61–78 DAS), leaves of all plants in the premarked area were sprayed with either silver nitrate (Sigma-Aldrich, Toluca, Mexico) as described previously (Kumar *et al.*, 2009) or water (control plots). As a consequence of its high water solubility and a lack of phytotoxicity at effective concentrations (Beyer, 1976), silver nitrate has been used extensively for both agronomic and research purposes to inhibit the ET response (Rodríguez *et al.*, 1999). Silver nitrate was dissolved in water (0.05 mM) containing 0.01% (v/v) Tween 20 (Sigma-Aldrich) and ethanol (0.1%), and the solution was foliar-sprayed onto the leaves of whole plants until runoff. The spraying always took place between 06:00 and 08:00 h and spraying of each plant was repeated on each of three successive days. The control plots were sprayed with water containing 0.01% (v/v) Tween 20 and ethanol (0.1%) only. During spraying, a specially made wind protector was used to cover the spray area. As the genotypes reached an early booting stage at different time-points, a staggered spray schedule was followed to avoid the confounding effects of spraying at different developmental stages.

Spike ethylene measurement

In all the experiments, spike sampling was performed at the anthesis stage. Endogenous ET production from spikes was measured using a laser-based ET detector (ETD-300; Sensor Sense BV, Nijmegen, the Netherlands) in combination with a gas handling system (VC-6; Sensor Sense BV) (Valluru *et al.*, 2016). Five tagged spikes (2–2.5 g FW) on the main tillers (in Exp 4, only four spikes) were sampled and placed in paper bags, and the bags were closed and transported to the lab with similar transport times (< 10 min). Each spike was then weighed and placed in a glass tube. Previous studies have shown that ET evolution from excised leaves and other shoot tissues can be steady for *c.* 15 min and then increase, presumably as a consequence of wounding (Sharp *et al.*, 2000; Yang *et al.*, 2006). We therefore left the glass tubes open for 60 min to allow any wound-induced ET to subside (Yang *et al.*, 2006). Spikes were then transferred into 50-ml glass vials containing moist filter paper, which were tightly capped with a double-bent rubber stopper, and were further incubated for 2 h under an artificial light setting in the lab. A thermometer was used to maintain a constant temperature regime of *c.* 35°C (± 1°C) during incubation for all spikes. Using a 5-ml syringe, 4 ml of gas was extracted through the rubber stopper and stored in 4-ml sealed glass vials. These vials were connected to inlet and outlet cuvettes of the VC-6 system, which allowed measurement of six cuvettes at once. Vials were continuously flushed with air at a constant flow of 4 l h^{-1}. ET released from each vial was successively connected to the ET detector in sample mode for 10 min. To remove any traces of external ET or other hydrocarbons, the air flow was passed through a platinum-based catalyzer before entering the cuvettes. A scrubber with KOH and $CaCl_2$ was placed before the ET detector to reduce the CO_2 and water content in the gas flow, respectively. The SET production was corrected for incubation period and SDW to determine ET production rate. After the SET measurement, SDW was determined after oven drying at 70°C for 72 h.

Plant height and grain yield sampling

Plant height (cm) was measured as the distance from the soil surface to the tip of the spike excluding the awns at the anthesis stage. At harvest, the HT and HSN plots were harvested separately for yield analysis whereby grain yield (GY; m^{-2}) and thousand-grain weight (TGW; g) were estimated.

Statistical analyses

All statistical analyses were performed using the software R v.3.1.3 (R Core Team, 2015). The SDW and SET data were subjected to one-way and two-way ANOVA, and a two-sample *t*-test with Bonferroni-adjustment alpha level was used for post hoc comparison of treatments, genotype categories and their interaction. In order to analyze the response of SDW to changes in SET, an exponential model was fitted between SDW and SET. Pearson's correlations were performed between SET, SDW and plant height. Hierarchical clustering (HCLUST function in R) was used to

categorize all genotypes into two SET groups, the low-ET group (LETG) and the high-ET group (HETG), while a quantile approach was used to categorize genotypes into two extreme plant height groups (short plant height group (SPHT), < 10% quantile value; and taller plant height group (TPHT), > 90% quantile value).

DNA extraction and genotyping

The methods of DNA isolation and genotyping are reported elsewhere (Sukumaran *et al.*, 2015a,b). Briefly, each genomic DNA sample of WAMI lines was isolated using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Saghai-Maroof *et al.*, 1984). The DNA samples were SNP genotyped at the United States Department of Agriculture, Agricultural Research Service Small Grain Genotyping Center, Fargo (http://wheat.pw.usda.gov/GenotypingLabs) using an Illumina iSelect 90K SNP array (Wang *et al.*, 2014). SNP allele clustering and genotype calling were performed with GENOME STUDIO software v.2011.1 (Illumina Inc., San Diego, CA, USA). We used the default clustering algorithm implemented in GENOME STUDIO to identify distinct clusters corresponding to the AA, AB and BB genotypes. The accuracy of SNP clustering was validated visually.

Genotype selection, linkage disequilibrium and population structure

In order to detect genotype outliers between field and glasshouse data sets, we used a Bonferroni *outlierTest* (CAR package in R; Williams, 1987). The Studentized genotype residuals with significant Bonferroni *P*-values (< 0.05) were identified (also confirmed using Cook's distance measure; Cook & Weisberg, 1982) and eliminated, and all subsequent analyses were then performed on the remaining 163 lines.

Linkage disequilibrium (LD) among SNPs was calculated using the full matrix and sliding window options in TASSEL 4.0 (Bradbury *et al.*, 2007) using 18 704 SNPs. Only SNPs with minor allele frequency > 5% were considered. Using the squared allele frequency correlations, pair-wise LD was measured according to Weir (1996). LD decay and the percentage of SNP pairs below and above the critical LD were determined (Sukumaran *et al.*, 2015a,b).

Patterns of population structure detected using the principal component analysis (PCA) approach were also confirmed using the model-based clustering approach implemented in STRUCTURE (Pritchard *et al.*, 2000). Briefly, we ran STRUCTURE using the default model parameters and varying the assumed number of genetic groups (*K*) from 1 to 15, using an admixture model consisting of 20 000 burn-in iterations and 5000 replicates. Finally, we used the SPAGEDI 1.3 program (Hardy & Vekemans, 2002) to calculate the genetic relationship matrix among the 163 lines. The broad-sense heritability for SET, SDW and their responses to HSN was calculated as $H^2 = \delta_g^2/(\delta_g^2 + \delta_{g\times e}^2 + \delta_e^2)$, where δ_g^2 is the genetic variance, $\delta_{g\times e}^2$ is the genotype by environment (G × E) variance, and δ_e^2 is the residual variance. These parameters were obtained using the linear mixed model, treating genotype and G × E as random effects.

GWAS

We used the TASSEL algorithm population parameters previously determined (P3D; Zhang *et al.*, 2010) to perform GWAS. To control for the familial relatedness and population structure, we incorporated a kinship matrix as the random component and the principal components (PCs) as fixed effects. We compared different models to select the best model based on the deviation of the observed *P*-values from the expected *P*-values for each trait (Q–Q plots) for detecting SNP–trait associations following unified mixed model association mapping (Sukumaran *et al.*, 2012). The threshold for defining an SNP to be significant was taken as 10^{-03}, considering the number of SNPs and the deviation of the observed *F*-test statistics from the expected *F*-test distribution in the quantile–quantile (QQ) plots (Sukumaran *et al.*, 2012).

Results

Genotypic variation for spike dry weight at anthesis stage among 130 wheat lines

SDW under HT conditions ranged from 0.31 to 1.68 g, with a mean of 0.94 g, and a broad-sense heritability of 61% (Fig. 1a). The SDW increased after the plants were sprayed with silver nitrate solution (HSN) with values ranging from 0.36 to 1.67 g, with a mean of 1.18 g (Fig. 1a), that is, an average *c.* 25% higher than without HSN ($P < 0.05$; Fig. 1b). The response of SDW to HSN among lines varied by up to 3-fold with an average SDW response of 1.2-fold, and with a broad-sense heritability of 57%. Genotypes ($P < 0.001$), treatments ($P < 0.001$) and their interaction ($P < 0.001$) had a significant effect on SDW (Table S3a). It is noticeable that, in a few genotypes, particularly those that had higher SDW under HT, SDW decreased in response to HSN.

The EM and LR differed significantly for SDW (Fig. 1c,d). EM had a significantly higher SDW compared with LR across the two conditions, the HT conditions (+ 14%) and the HSN conditions (+ 11%). Two-way ANOVA indicated a significant main effect of genotype category on SDW ($P < 0.001$); however, its interaction with treatment was not significant (Table S3a). These results suggest that there exists large genotypic variation for SDW among the wheat genotypes studied and silver nitrate spray at the early booting stage contributed to an increased SDW at the anthesis stage.

Genotypic variation for spike ethylene at anthesis stage among 130 wheat lines

SET under HT conditions (Fig. 2a) ranged from 0.017 to 0.155 nl g DW^{-1} h^{-1}, with a mean of 0.077 nl g DW^{-1} h^{-1}, and a broad-sense heritability of 25%. In response to HSN, SET decreased noticeably among genotypes, ranging from 0.01 to 0.145 nl g DW^{-1} h^{-1}, with a mean of 0.063 nl g DW^{-1} h^{-1}, that is, an average *c.* 18% lower than without HSN (Fig. 2b). The response of SET to HSN had a broad-sense heritability of 39%. Genotypes that had high SET under HT exhibited more reduction in SET compared with low-SET genotypes. There were significant main effects of genotype ($P < 0.001$), treatment

($P < 0.001$), and their interaction ($P < 0.001$) on SET (Table S4b).

In response to HSN, both EM and LR exhibited a reduction in SET, by 18% and 17%, respectively (Fig. 2c,d). Across both HT and HSN conditions, EM genotypes had a significantly lower SET (−13%; $P < 0.001$; Fig. 2c) compared with LR. Genotype category had a significant ($P < 0.001$) effect on SET, but its interaction with treatment was not significant (Table S3b). Overall, these findings suggest that there exists large genotypic variation for SET among the wheat genotypes studied and SET decreased significantly in response to HSN.

Spike ethylene was negatively correlated with spike dry weight

We used an exponential model to fit the relationship between SDW and SET. SDW decreased exponentially with increasing SET across all the genotypes (Fig. 3a). The relationship between SDW and SET was not altered by HT and HSN conditions (for HT: $r^2 = 0.73$; $P < 0.001$; for HSN: $r^2 = 0.62$; $P < 0.001$; Fig. 3a) or by the genotype category (EM HT: $r^2 = 0.79$; $P < 0.001$; EM HSN: $r^2 = 0.67$; $P < 0.001$; LR HT: $r^2 = 0.71$; $P < 0.001$; LR HSN: $r^2 = 0.56$; $P < 0.001$) (Fig. 3b,c). On average, 68 ± 3% of the variance of the SDW decay rate across HT and HSN conditions can be explained by an exponential regression of the mean rate on the SET.

HT and HSN condition-specific exponential models between SDW and SET were similar to the global model of HT+HSN conditions across all the genotypes ($r^2 = 0.50$; $P < 0.001$; Fig. S1a), within the EM genotypes ($r^2 = 0.56$; $P < 0.001$; Fig. S1b) or within LR ($r^2 = 0.45$; $P < 0.001$; Fig. S1c). We also checked the relationship between SDW and SET in a relatively small subset of genotypes (30 genotypes; Exps 2 and 3), which also revealed a significant negative relationship between SET and SDW (Fig. S2a–c).

We further examined the tradeoffs between SET and SDW using a hierarchical clustering by establishing two ET groups: LETG and HETG. The LETG had a significantly lower SET

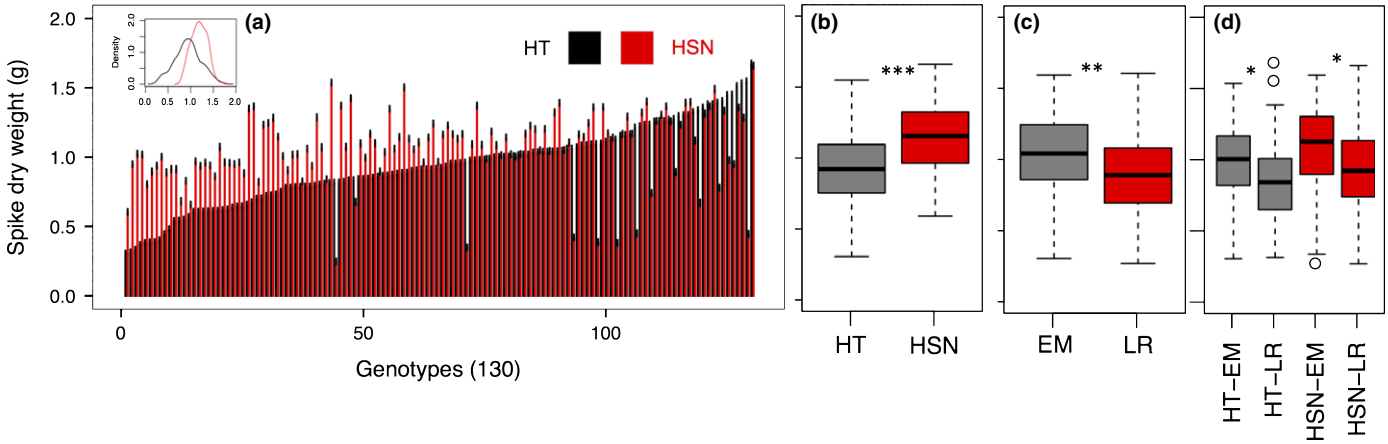

Fig. 1 Spike dry weight (SDW) of 130 wheat genotypes grown under heat stress (HT) or HT plus ethylene (ET) response inhibitor silver nitrate spray (HSN) conditions. (a) Natural variation in SDW under two treatments (HT and HSN). SDW under HT was arranged in an ascending order. (b, c, d) Average mean values of SDW in (b) HT and HSN conditions, (c) two genotype categories (EM, elite material; LR, landraces) and (d) their combination. The inset in (a) is a density plot of SDW. The vertical bars represent ± SE. *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$.

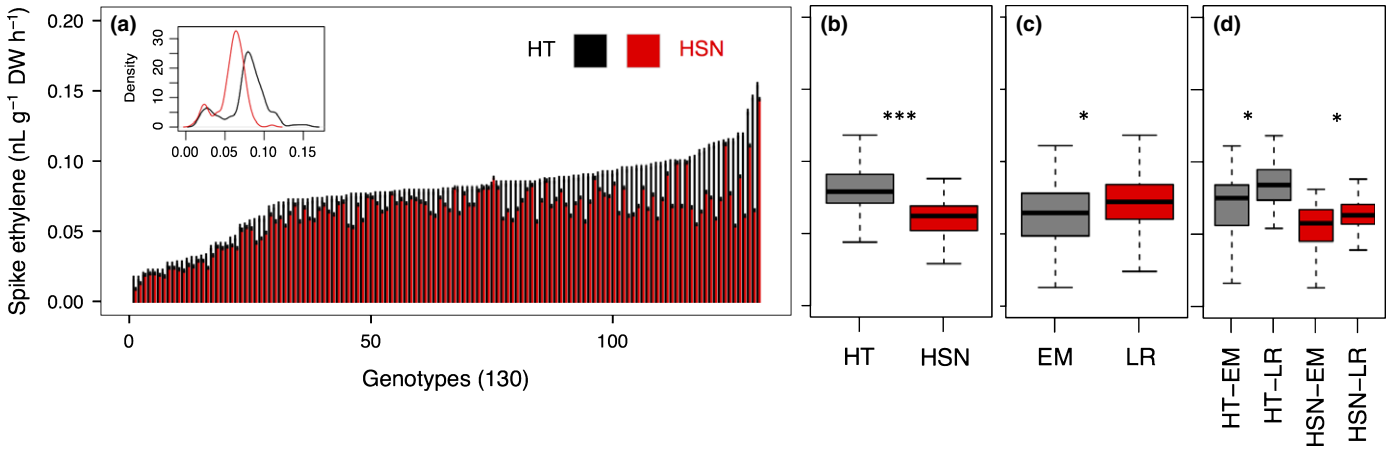

Fig. 2 Spike ethylene (SET) of 130 wheat genotypes grown under heat stress (HT) or HT plus ethylene (ET) response inhibitor silver nitrate spray (HSN) conditions. (a) Natural variation in SET in two treatments (HT and HSN). SET under HT was arranged in an ascending order. (b, c, d) Average mean values of SET in (b) two treatments (HT and HSN), (c) two genotype categories (EM, elite material; LR, landraces), and (d) their combination. The inset in (a) is a density plot of SET. The vertical bars represent ± SE. *, $P = 0.05$; ***, $P = 0.001$.

(−41.5%; $P < 0.001$; Fig. S3a) compared with the HETG. By contrast, the LETG had a significantly higher SDW (+41%; $P < 0.001$; Fig. S3b) compared with its counterpart. Taken together, these results suggest that there was a consistent negative relationship between SET and SDW, exhibiting differential SDW sensitivity to increasing levels of SET, whereby higher SET profiles appeared to be detrimental to SDW.

Correlations between spike-related traits and plant height

We generated Pearson's correlations between SDW, SET and plant height using phenology as a covariate (Fig. 4). Plant height had a negative correlation with SET under HT conditions ($r^2 = 0.66$; $P < 0.001$) and HSN conditions ($r^2 = 0.10$) (Fig. 4a). By contrast, plant height showed a positive correlation with SDW ($r^2 = 0.36$; $P < 0.05$; and $r^2 = 0.01$) under both HT and HSN conditions, respectively (Fig. 4b). However, these correlations were not significant under HSN conditions. These correlations were present across all genotypes (Fig. 4a,b), within EM genotypes (Fig. S4a,e), and within LR (Fig. S4c,g).

To further dissect the association between SET, SDW and plant height, we used a quantile approach to develop two extreme plant height groups based on plant height (genotypes below the 10% quantile were assigned to the shorter plant height group (SPHT) while genotypes above the 90% quantile were assigned to the taller plant height group (TPHT)). The taller group had a significantly lower SET across genotypes (−53%; $P < 0.001$; Fig. 4b), within EM genotypes (−50%; $P < 0.05$; Fig. S4b) and within LR (−70%; $P < 0.001$; Fig. S4d). By contrast, the taller group had a significantly higher SDW across all genotypes (+19%; $P < 0.001$; Fig. 4d), within EM genotypes (+36%; $P < 0.05$; Fig. S4f) and within LR (+42%; $P < 0.05$; Fig. S4h) compared with the shorter group. These results indicate that the SET and SDW changes identified by this arbitrarily chosen limit of 10% reveal close associations of these traits with plant height.

Lower spike-ethylene dynamics were strongly associated with higher grain yield

In order to examine the effects of SET on grain yield, grain yield was regressed against SET, which revealed negative correlations

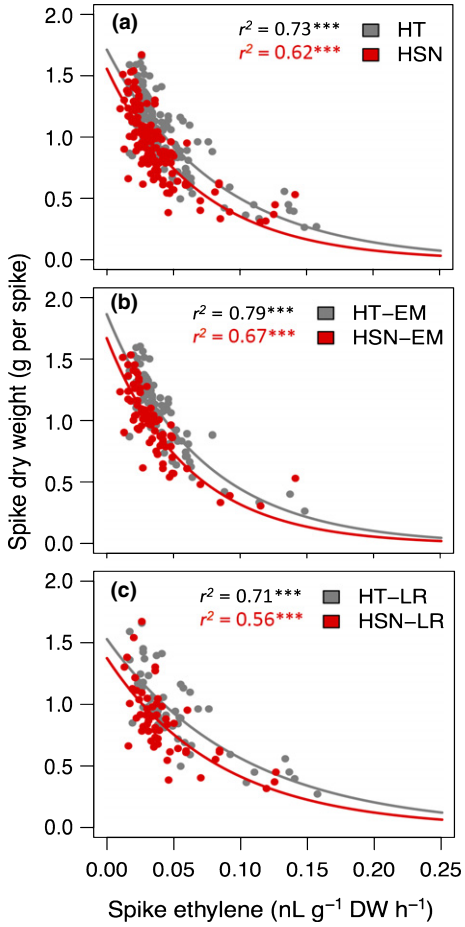

Fig. 3 The relationship between spike ethylene (SET) and spike dry weight (SDW) in 130 wheat genotypes grown under (a) heat stress (HT) or HT plus ethylene (ET) response inhibitor silver nitrate spray (HSN) conditions, (b) in elite material (EM), and (c) in landraces (LR). An exponential model was used to fit the relationship between SET and SDW. Each data point represents one individual genotype in both HT (gray circles) and HSN (red circles) conditions. ***, $P < 0.001$.

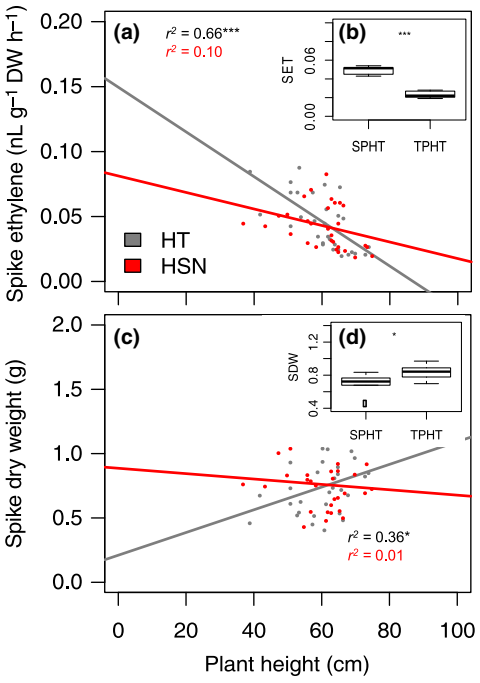

Fig. 4 Pearson's correlations between spike-related traits and plant height in 30 wheat genotypes grown under heat stress (HT) and HT plus ethylene inhibitor silver nitrate spray (HSN) conditions. (a) Pearson's correlations between plant height and spike ethylene under HT and HSN conditions. (c) Pearson's correlations between plant height and spike dry weight under HT and HSN conditions. Insets: spike ethylene (b) and spike dry weight (d) in two extreme plant height phenotypes, short plant height (SPHT) and tall plant height (TPHT), which contained genotypes falling within the 10% quantile and genotypes falling beyond the 90% quantile, respectively. The vertical bars represent ± SE. *, $P < 0.05$; ***, $P < 0.001$.

between SET and grain yield under HT conditions ($r^2 = 0.37$; $P < 0.001$) and HSN conditions ($r^2 = 0.17$; $P < 0.05$) (Fig. 5a) as well as among ET groups (low-ET group: $r^2 = 0.42$; $P < 0.001$; high-ET group: $r^2 = 0.23$; $P < 0.01$; Fig. 5b). These results suggest that there is a yield penalty associated with higher SET profiles. Similar results were observed between SET and TGW, although they were not significant (Fig. S5).

Further yield analyses indicated that, across all genotypes, HSN conditions had a yield gain of 23% over HT conditions ($P < 0.05$; Fig. 6b). A similar yield gain (22%) was observed in the low-ET group compared with the high-ET group ($P < 0.01$; Fig. 6a). When different combinations of ET groups, treatments, and genotype categories were compared for yield gain (Fig. S6), in all cases, the low-ET group had a higher yield gain compared with the high-ET group, ranging from 12% to 45%, with an average yield gain of 26% (Fig. S6). When only significant comparisons, that is, five out of eight combinations ($P < 0.05$), were considered, the mean yield gain rose to 34%. The yield gain of the low-ET group over the high-ET group was higher for EM genotypes (42%) compared with LR (12%; $P < 0.001$).

TGW differed significantly between ET groups ($P < 0.01$; Fig. 6d), treatments ($P < 0.05$; Fig. 6e) and different combinations of ET groups and treatments (Fig. 6c–e) but not between genotype categories. HSN conditions as well as the low-ET group had a TGW gain of 5.5% and 9%, respectively, while their combination varied from 3% to 7% (Fig. S6c,d). Taken together, these results suggested that changes in SET were closely but negatively associated with changes in grain yield and TGW.

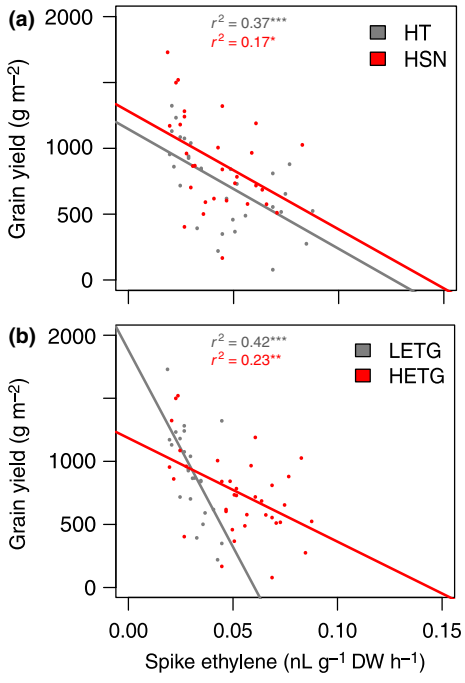

Fig. 5 The relationship between grain yield and spike ethylene under (a) heat stress (HT) or HT plus ethylene response inhibitor silver nitrate spray (HSN) conditions and (b) in two ethylene groups (LETG, low ethylene group; HETG, high ethylene group), for 30 wheat genotypes. *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$.

Genome-wide association for spike ethylene and spike dry weight

We performed a GWAS on SET and SDW for the glasshouse and field data separately using 18 704 SNPs. We detected a number of candidate SNPs (five and 32 SNPs in the glasshouse and field, respectively, with P-values $< 10^{-3}$). These SNPs are located on chromosomes 1A, 1B, 1D, 3B, 5B and 7B (Fig. 7; Table 1) and explained 5.7–10.5% of the variation in SET. A major SNP (*kukri-c15603-1116*) at 69 cM on chromosome 3B explained 10.5% while a major SNP (*wsnp-JD-c38123-27754848*) localized at 40 cM on chromosome 5B explained 8.8% of total SET variation under field conditions. Interestingly, two distinct SNPs (*kukri-c29668-338* and *BS00009866-51* in glasshouse and field conditions, respectively) are localized at the same position at 70 cM on chromosome 1A (Fig. 7a,b; Table 1). The SNP *BS00009866-51* is predicted to be a putative membrane steroid-binding protein (MSBP1; Balmer *et al.*, 2006). For the SNP *kukri-c29668-338*, 149 accessions had genotype AA and 10

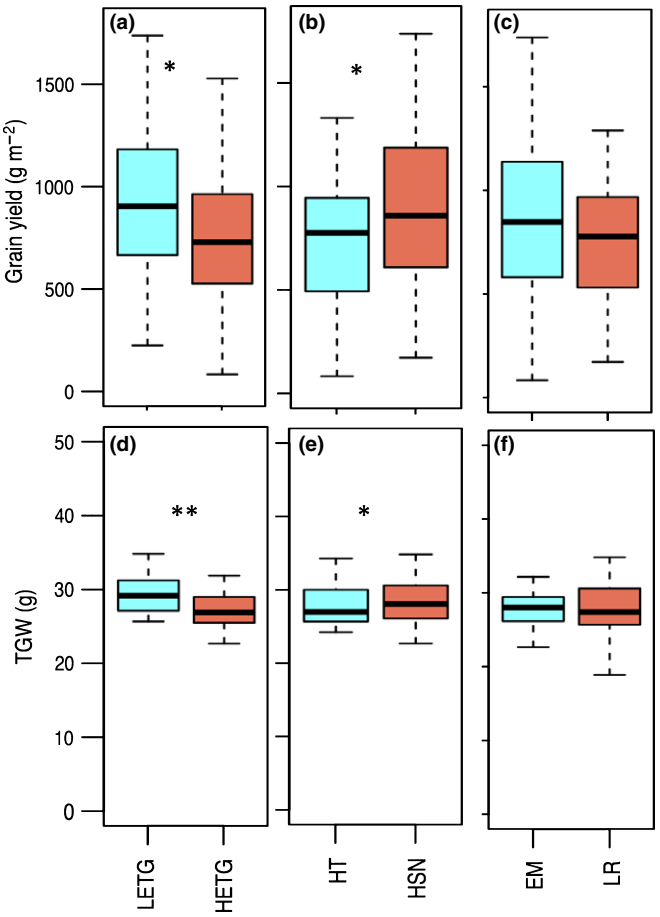

Fig. 6 (a–c) Grain yield and (d–f) thousand-grain weight (TGW) in (a, d) two ethylene groups (LETG, low ethylene group; HETG, high ethylene group), (b, e) two treatments (HT, heat stress; HSN, HT plus ethylene response inhibitor silver nitrate spray), and (c, f) two genotype categories (EM, elite material; LR, landraces) of 30 wheat genotypes grown under HT or HSN conditions. The vertical bars represent ± SE. *, $P = 0.05$; **, $P = 0.01$.

accessions had genotype GG at the locus, while based on the SNP *BS00009866-51*, 10 accessions had genotype AA and 148 accessions had genotype GG at the locus. The allelic diversity for these two SNPs indicates that the AA genotype had significantly lower and higher SET (38% and 25% for SNPs *kukri-c29668-338* and *BS00009866-51*, respectively) compared with the GG genotype (Fig. 8a,b).

The association mapping for SDW identified a number of candidate SNPs in glasshouse and field conditions (22 and 142 SNPs, respectively, with *P*-values $< 10^{-3}$). These SNPs were localized on chromosomes 2A, 2B, 3A, 3B, 3D, 4B, 5A, 5B, 6A, 6B, 7A and 7B, and explained 6.8% to 14.9% of SDW variation (Fig. 7c,d; Table 1). A major SNP, *BS00055584-51*, which explained *c.* 15% of total SDW variation, was detected at 140 cM on chromosome 7B under field conditions (Fig. 7c,d; Table 1). Further, we detected six SNPs (*wsnp_Ku_c5693_10079278*, *RAC875_rep_c109658_211*, *Tdurum_contig49576_75*, *RAC875_c31299_1302*, *Kukri_c32958_390* and *Kukri_c657_1139*) on chromosomes 7A, 5B, 5A, 6B, 4B, and 2B, respectively, that were common across environments. Among 163 accessions, two groups could be established based on two SNPs (*wsnp_Ku_c5693_10079278* and *Kukri_c657_1139*) whose allelic diversity (AA vs CC and CC vs TT, respectively) exhibited a significant effect on SDW. For the SNP *wsnp_Ku_c5693_10079278*, eight accessions had the AA genotype, and this had a significantly higher SDW (15%) compared with the CC genotype, which was found in 150 accessions, while for the SNP *Kukri_c657_1139*, 77 accessions had the CC genotype, and this had a significantly higher SDW (10%) compared with the TT genotype, which was found in 80 accessions (Fig. 8c,d). The SNP *Kukri_c657_1139* is predicted to be an E3 ubiquitin-protein ligase (He *et al.*, 2013).

Discussion

Plants synthesize ET in response to numerous environmental stimuli. ET has long been thought to act mainly as a growth-inhibiting phytohormone, although positive organ growth at lower concentrations has been widely reported in several species (Ku *et al.*, 1970; Valluru *et al.*, 2016). To date, only a limited

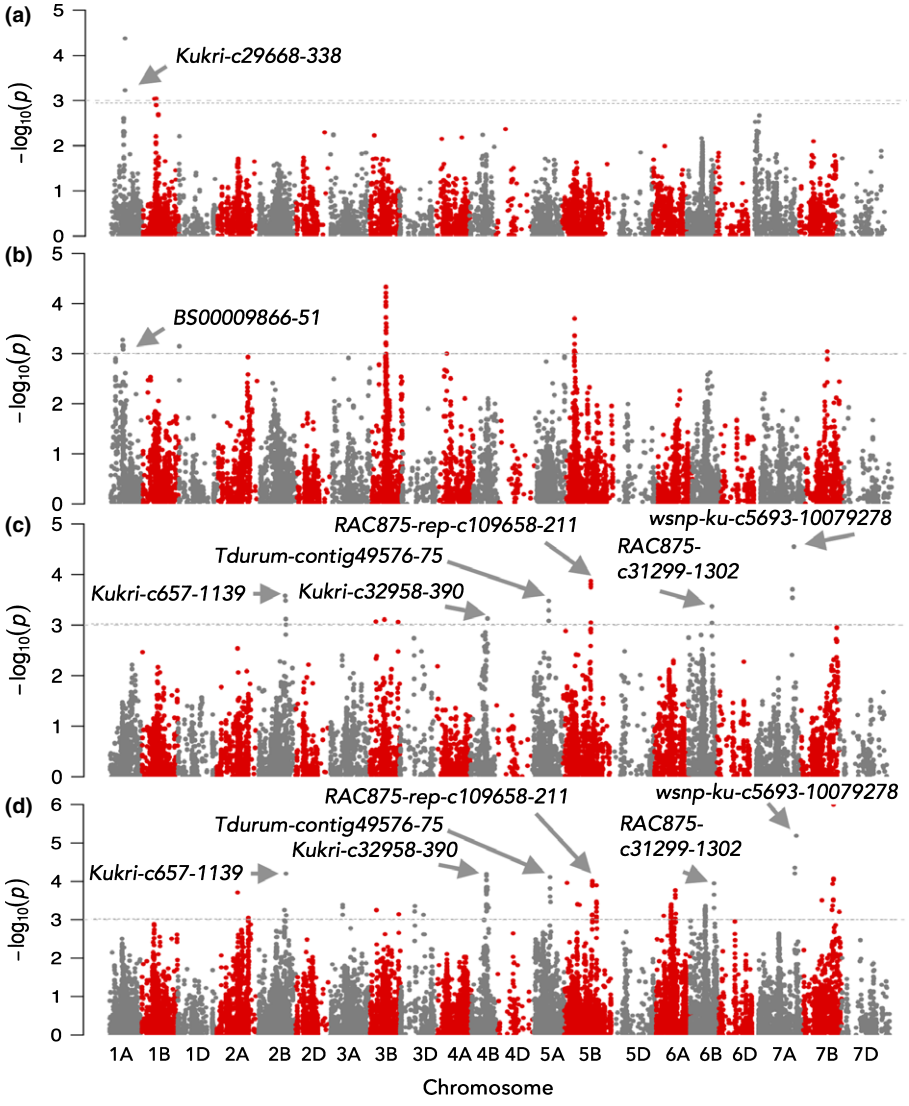

Fig. 7 Manhattan plots of genome-wide association (GWA) mapping results for ethylene production and dry weight of the spike at the anthesis stage of wheat. GWA mapping was performed for (a, b) spike ethylene production and (c, d) spike dry weight of 163 lines from the wheat association mapping initiative panel grown in (a, c) the glasshouse or (b, d) field conditions.

Table 1 Markers that were significantly ($P < 0.01$) associated with spike ethylene (SET) production and spike dry weight (SDW) in wheat in laboratory and field conditions

Trait	Marker	Chromosome	cM	*P*	PVE (%)	Allele	MAF
SET, lab	*Tdurum-contig32033-125*	1B	68	9.00E-04	6.8	A/C	A (0.05)
	Kukri-c29668-338	1A	70	2.44E-03	5.7	A/G	G (0.05)
SET, field	*Kukri-c15603-1116*	3B	69	4.63E-05	10.5	C/T	C (0.34)
	wsnp-JD-c38123-27754848	5B	40	1.98E-04	8.8	G/T	G (0.32)
	BS00009866-51	1A	70	6.85E-04	7.1	A/G	A (0.07)
	RAC875-c37183-331	1D	3	7.10E-04	7.0	A/G	G (0.92)
	wsnp-CAP11-rep-c4076-1926235	7B	102	9.01E-04	6.8	A/G	A (0.19)
SDW, lab	*wsnp-Ku-c5693-10079278*	7A	208	2.80E-05	10.3	A/C	A (0.06)
	RAC875-rep-c109658-211	5B	131	1.33E-04	8.7	C/T	T (0.82)
	Tdurum-contig49576-75	5A	82	3.30E-04	7.7	A/C	A (0.11)
	RAC875-c31299-1302	6B	109	4.27E-04	7.6	A/G	A (0.29)
	Kukri-c32958-390	4B	81	7.38E-04	6.8	C/T	C (0.06)
	Kukri-c657-1139	2B	141	7.57E-04	6.8	C/T	C (0.49)
	TA003922-1105	3B	72	7.76E-04	6.8	A/G	A (0.08)
SDW, field	*BS00055584-51*	7B	140	1.01E-04	14.9	C/T	T (0.54)
	wsnp-Ku-c5693-10079278	7A	208	6.51E-06	12.3	A/C	A (0.06)
	Kukri-c657-1139	2B	141	6.27E-05	9.9	C/T	C (0.49)
	Ku-c24961-1176	4B	71	6.43E-05	9.8	A/G	A (0.26)
	Tdurum-contig49576-75	5A	82	7.81E-05	9.5	A/C	A (0.11)
	RAC875-rep-c109658-211	5B	131	9.90E-05	9.3	C/T	T (0.82)
	RAC875-c31299-1302	6B	109	1.12E-04	9.3	A/G	A (0.29)
	Kukri-c32958-390	4B	81	1.41E-04	8.8	C/T	C (0.06)
	wsnp-Ra-c12086-19452422	6A	91	1.72E-04	8.9	C/T	C (0.46)
	Kukri-c11327-977	2A	101	1.94E-04	8.9	G/T	T (0.79)
	wsnp-Ku-rep-c102901-89769309	6A	91	2.24E-04	8.7	C/T	C (0.45)
	Ku-c14907-456	6A	72	3.95E-04	7.9	A/C	A (0.42)
	wsnp-Ex-rep-c69816-68774416	3A	68	4.08E-04	7.9	A/C	A (0.45)
	Kukri-c43208-335	3D	67	4.39E-04	8.0	A/G	G (0.65)

A subset (163) of the wheat association mapping initiate (WAMI) panel accessions were grown in experimental glasshouse conditions (2014) and field conditions (2015) for scoring SET and SDW of wheat, and 18 704 single nucleotide polymorphism (SNP) markers (MAF > 5%) of the wheat Illumina iSelect 90K SNP array were applied for genotyping. PVE, the percentage of phenotypic variation explained by each marker. *P*-values indicate significance levels. MAF, minor allele frequency.

number of studies have explored the possibility of the 'ET trait' being used as a means of crop improvement under stress. Further, previous studies focused only on a few individual genotypes (Yang *et al.*, 2006; Hays *et al.*, 2007). This study used as a base a population showing wide genetic diversity, and found substantial genotypic variation for SET and SDW at the anthesis stage (Figs 1, 2). There was a strong negative correlation between SET and SDW (Figs 3, S1, S2). An application of an ET response inhibitor significantly decreased SET (Fig. 2; Hays *et al.*, 2007; Huberman *et al.*, 2013), and subsequently contributed to increased SDW and grain yield under heat stress (Figs 1, 6, S6). Finally, we detected numerous putative SNPs explaining genotypic variation of SET and SDW (Fig. 7; Table 1), suggesting that both SET and SDW are accessible for genetic manipulation in wheat.

Conditions without silver nitrate spray during an entire vegetative period caused a significant decline in SDW compared with silver nitrate spray conditions (Fig. 1), with the result being a genotype-dependent decrease in grain yield (Fig. 6). This decrease in SDW and grain yield was partly rescued by silver nitrate spray (Figs 1, 6), a phenomenon previously observed (Hays *et al.*, 2007; Huberman *et al.*, 2013). Taking this into account, our results suggest that an 18% decline in SET contributed to increases in SDW and grain yield of 14% and 22%, respectively, which are not inconsistent with increases in yield gains (1–45%) reported previously in various crops in response to the use of inhibitors of the biosynthesis of, or the response to, ET (Young *et al.*, 2004; Hays *et al.*, 2007; Wuriyanghan *et al.*, 2009; Huberman *et al.*, 2013; Tamaki *et al.*, 2015). Notably, a moderate heritability of the responses of SET and SDW to silver nitrate spray (39% and 57%, respectively) further suggests the possibility of leverage of decent genetic gains through these traits. These results support the view that SET is an invaluable 'candidate' for partial mitigation of heat stress effects on yield. In this regard, silver nitrate spray can be seen as a cost-effective field approach to enhancing on-farm yields under heat stress. Importantly, silver nitrate spray decreased SDW in a few genotypes that already had lower SET profiles, warranting a genotype-specific tailoring of SET, as a drastic reduction of ET signaling may not be beneficial to maximizing grain yields (Guo *et al.*, 2014). Such responses may, however, vary depending on the stress conditions.

In an agricultural context, an exploration of the potential natural variation for SET seemed to be warranted. We found large genotypic variation for SET with a low broad-sense heritability, suggesting that SET may be conditioned by a large number of

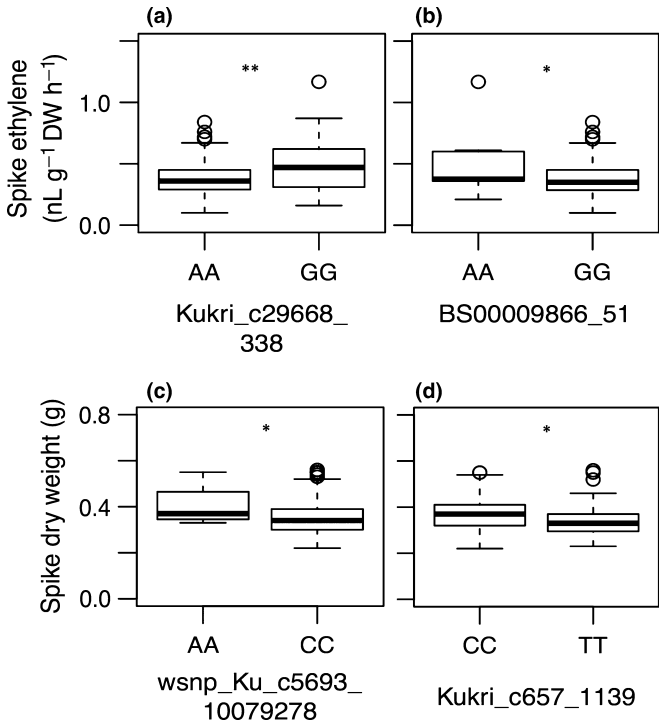

Fig. 8 Boxplots of the relationship of ethylene production and dry weight of the spike with polymorphism of the markers *Kukri-c29668-338*, *BS00009866-51*, *wsnp-Ku-c5693-10079278* and *Kukri-c657_1139*. (a, b) Ethylene production in the spike and (c, d) dry weight of the spike in 163 lines from the wheat association mapping initiative panel grouped according to their base calls for the markers *Kukri-c29668-338*, *BS00009866-51*, *wsnp-Ku-c5693-10079278* and *Kukri-c657_1139*. Boxes represent the median and the first and third quartiles, respectively. All outliers are represented as open circles. *, $P = 0.05$; **, $P = 0.01$.

genes of small effect, reflecting a high nonadditive genetic variance and strong genotype by environment crosstalk. Further, EM lines had a significantly lower mean SET profile compared with LR (Fig. 2), indicating that SET might have been inadvertently selected against in EM lines, probably as a consequence of targeted selection for higher yield potential. Substantial genotypic variation, albeit in a small set of genotypes, has been previously reported for numerous phytohormones such as ET in sorghum (*Sorghum bicolor*) (Zhang & Kirkham, 1990), and abscisic acid (ABA), gibberellic acid (GA) and indole acetic acid in wheat (Sridhar, 2003). Further, ABA concentrations differ markedly among different ploidy levels of wheat (Iehisa & Takumi, 2012), and also between landraces and hybrids of tomato (*Solanum lycopersicum*) (Ruiz *et al.*, 2006). Such a wide genetic diversity provides an excellent base for uncovering the physiological and genetic underpinning of ET insensitivity. For instance, SDW seemed to be less sensitive to SET in higher SET profile genotypes (Fig. 3). It is attractive to speculate that these genotypes or spikes may have metabolic signals that rendered them less sensitive to ET. These genotypes would therefore be useful for developing new cultivars with enhanced yield, although breeding for favorable SET expression would be very challenging, particularly in field settings.

ET-based strategies with the aim of improving crop yields have so far been limited, partly as a consequence of the fact that comprehensive understanding of ET regulation at the whole-plant level is limited (Sharp *et al.*, 2000; Sharp & LeNoble, 2002), as most studies have focused largely on the cell and tissue levels (Takatsuka & Umeda, 2014). Moreover, an inconsistent pattern of gene expression and protein activity has been reported for certain ET biosynthetic genes (e.g. *ACO1* (*1-Aminocyclopropane-1-Carboxylate Oxidase 1*); Van de Poel *et al.*, 2014), reflecting the complex nature of ET regulation in plants. The lack of a high-throughput phenotyping tool to quantify ET production, especially in the field setting, is another bottleneck. In view of this, SDW can serve as a high-throughput proxy with which to rapidly estimate SET, as it is strongly negatively correlated with SET (Fig. 3); it represents the degree of spike fertility at the anthesis stage (González *et al.*, 2011), and also determines grain weight and grain number at maturity (Fischer, 1985; González *et al.*, 2011). Notably, SDW can easily be rapidly measured and hence crops can be screened for SDW on a large scale in the field. Therefore, use of SDW can be widely applied to high-yield breeding in wheat.

Genetic knowledge of ET in crops has been lacking partly because of the fact that ET is a complex trait. Through a GWAS, we detected five and 32 SNPs for SET in glasshouse and field conditions, respectively (Fig. 7; Table 1), suggesting that some are environment-specific. A limited SNP overlap and a lower heritability (25%) suggest that SET may indeed be governed by a large number of genes of small effect that may not be possible to detect consistently across environments. However, two distinct SNPs, *kukri-c29668-338* and *BS00009866-51*, were consistently detected at the same position on chromosome 1, indicating that this genomic region may be an important regulator of SET. Further, many SNPs are localized close to previously detected markers controlling several agronomic traits such as peduncle length, maturity and plant density adaptation (Sukumaran *et al.*, 2015a,b).

Two distinct SNPs (*kukri-c29668-338* and *BS00009866-51*) of SET were detected at the same position (at 70 cM on chromosome 1A). The latter SNP, a homolog of MSBP1 in Arabidopsis, is implicated in diverse hormone signaling (Balmer *et al.*, 2006) and negatively regulates brassinosteroid (BR) signaling (Song *et al.*, 2009). In young panicles of rice, a putative serine carboxypeptidase-like protein, *OsGS5* (Oryzae sativa *Grain Size 5*), which is implicated in natural variation of grain size (Li *et al.*, 2011), has been shown to competitively inhibit MSBP1, and thereby promote BR signaling and grain size (Xu *et al.*, 2015). The *OsGS5* homolog in wheat, *TaGS5*, has been recently cloned and shown to be associated with grain weight (Wang *et al.*, 2015a,b). Whether *TaGS5* and its promoter polymorphism cause MSBP1 inhibition and promote BR signaling and spike growth in wheat remains to be investigated.

The spike-related traits are important components of wheat yield that are under complex genetic control and are more highly heritable (61% in this study) than yield *per se* (Ma *et al.*, 2006). We detected 22 and 142 SNPs for SDW in glasshouse and field conditions, respectively (Fig. 7; Table 1). Of these, six SNPs were common across the two conditions. The SNP *wsnp_Ku_c5693_10079278* on chromosome 7A controls the stem water-

soluble carbohydrate (WSC) concentration, while another SNP, *RAC875_rep_c109658_211* on chromosome 5B, is localized at 31 cM, close to the marker that controls stem WSC content, as reported in the double-haploid population of wheat (Rebetzke *et al.*, 2008). The allelic diversity of the former SNP also revealed a significant effect on SDW (Fig. 8c). In addition, an SNP on chromosome 6B, related to stem WSC remobilization efficiency, is localized at 82 cM, close to a fructan catabolic gene, 1-fructan exohydrolase (*1-FEH w3*), which triggers stem fructan-based WSC release that is indeed used to buffer grain-filling in wheat, and its expression is regulated by hormones including ET (Zhang *et al.*, 2014). These findings emphasize a key role for the contribution of stem WSC to SDW. A spatiotemporal analysis of WSC and hormones (Valluru, 2015) in different spike organs is therefore required if we are to track spike development under heat stress.

In addition, a vernalization gene, *vrn-A1* on chromosome 5A, controlling days to heading, has been detected across two conditions (Lopes *et al.*, 2015). This indicates a key role for phenological phases in modifying SDW. It has long been known that an extended stem elongation period greatly improves SDW in wheat (Miralles *et al.*, 2000). An SNP associated with maturity on chromosome 2B (Sukumaran *et al.*, 2015a,b) is 4 cM from the SNP detected in this study, whose allelic diversity (CC vs TT) had a significant effect on SDW (Fig. 8d). This SNP is predicted to be an E3-ubiquitin ligase (He *et al.*, 2013), which is involved in the regulation of ET action by targeting ubiquitination of ACS proteins, particularly types 2 and 3 (Lee & Seo, 2015). Of the 873 E3-ligase genes identified so far in wheat, 173 genes are expressed differentially during grain development (Capron *et al.*, 2012). Some of them are closely linked to ET signaling, and may underlie the process of balancing growth and stress tolerance (Lee & Seo, 2015). Previously, E3 APC/C ligases have been shown to be positively associated with grain yield in maize (Fu *et al.*, 2010). These findings emphasize a crucial role for ET signaling in the regulation of SDW.

Interestingly, two of the SET SNPs, *kukri-c29668-338* and *BS00009866-51* on chromosome 1A, and one of the SDW SNPs, *Kukri-c32958-390* on chromosome 4B (Table 1), are localized close to the previously detected markers of plant height (Lopes *et al.*, 2015; Sukumaran *et al.*, 2015b), establishing a causal genetic link between SET and plant height. Subsequent analysis suggests that allelic variation in SET SNPs (AA vs GG) had appreciable effects on SET and height, with the effect on SET being opposite to that on height. By contrast, allelic variation (CC vs TT) in an SDW SNP had effects on SDW and height in the same direction. The height difference between two distinct alleles of these SNPs was, however, not significant (data not shown), as expected, because the WAMI panel is corrected for height variation (Lopes *et al.*, 2015). Nevertheless, the observed directionality of the allelic effects suggests that genetic pleiotropy may act on these traits. In support of this conclusion, physiological data show significant negative and positive correlations of SET and SDW with plant height, respectively (Figs 4b–e, S4a–h). These findings call into question the physiological benefits of using dwarf plants to enhance yield in future high-temperature scenarios, although a stem-specific ET manipulation would be useful to alter plant height, as shown recently in poplar (*Populus alba*) (Plett *et al.*, 2014).

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Author contributions

R.V., M.P.R. and W.J.D. devised the experimental design; R.V. carried out the experimental work and data analysis; R.V., and S.S. performed the GWAS analysis; R.V. wrote the manuscript and all authors reviewed and commented on the manuscript.

References

Balmer Y, Vensel WH, DuPont FM, Buchanan BB, Hurkman WJ. 2006. Proteome of amyloplasts isolated from developing wheat endosperm presents evidence of broad metabolic capability. *Journal of Experimental Botany* **57**: 1591–1602.

Barnabás B, Jäger K, Fehér A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell & Environment* **31**: 11–38.

Battisti DS, Naylor RL. 2009. Historical warnings of future food insecurity with unprecedented seasonal heat. *Science* **323**: 240–244.

Beyer EM. 1976. A potent inhibitor of ethylene action in plants. *Plant Physiology* **58**: 268–271.

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.

Burke JJ, Chen J. 2015. Enhancement of reproductive heat tolerance in plants. *PLoS ONE* **10**: e0122933.

Buttler JD, Byrne PF, Mohammadi V, Haley S. 2005. Agronomic performance of alleles in a spring wheat population across a range of moisture levels. *Crop Science* **45**: 939–947.

Cantrell RG, Haro-Arias ES. 1986. Selection for spikelet fertility in a semidwarf durum wheat population. *Crop Science* **26**: 691.

Capron D, Mouzeyar S, Boulaflous A, Girousse C, Rustenholz C, Laugier C, Paux E, Bouzidi MF. 2012. Transcriptional profile analysis of E3 ligase and hormone-related genes expressed during wheat grain development. *BMC Plant Biology* **12**: 1–18.

Cheng C-Y, Lur H-S. 1996. Ethylene may be involved in abortion of the maize caryopsis. *Physiologia Plantarum* **98**: 245–252.

Cook RD, Weisberg S. 1982. *Residuals and influence in regression.* New York, NY, USA: Chapman and Hall.

Cossani CM, Reynolds MP. 2012. Physiological traits for improving heat tolerance in wheat. *Plant Physiology* **160**: 1710–1718.

Feng H-Y, Wang Z-M, Kong F-N, Zhang M-J, Zhou S-L. 2011. Roles of carbohydrate supply and ethylene, polyamines in maize kernel set. *Journal of Integrative Plant Biology* **53**: 388–398.

Fischer RA. 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science* **105**: 447–461.

Fu J, Bowden RL, Prasad PVV, Ibrahim AMH. 2015. Genetic variation for heat tolerance in primitive cultivated subspecies of *Triticum turgidum* L. *Journal of Crop Improvement* **29**: 565–580.

Fu J, Thiemann A, Schrag TA, Melchinger AE, Scholten S, Frisch M. 2010. Dissecting grain yield pathways and their interactions with grain dry matter content by a two-step correlation approach with maize seedling transcriptome. *BMC Plant Biology* **10**: 1–15.

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

Guo M, Rupe MA, Wei J, Winker C, Goncalves-Butruille M, Weers BP, Cerwick SF, Dieter JA, Duncun KE, Howard RJ *et al.* 2014. Maize *ARGOS1* (*ZAR1*) transgenic alleles increase hybrid maize yield. *Journal of Experimental Botany* **65**: 249–260.

Guo Z, Schnurbusch T. 2015. Variation of floret fertility in hexaploid wheat revealed by tiller removal. *Journal of Experimental Botany* **66**: 5945–5959.

Habben JE, Bao X, Bate NJ, DeBruin JL, Dolan D, Hasegawa D, Helentjaris TG, Lafitte RH, Lovan N, Mo H *et al.* 2014. Transgenic alteration of ethylene biosynthesis increases grain yield in maize under field drought-stress conditions. *Plant Biotechnology Journal* **12**: 685–693.

Hardy OJ, Vekemans X. 2002. spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618–620.

Harsant J, Pavlovic L, Chiu G, Sultmanis S, Sage TL. 2013. High temperature stress and its effect on pollen development and morphological components of harvest index in the C_3 model grass *Brachypodium distachyon*. *Journal of Experimental Botany* **64**: 2971–2983.

Hays DB, Do JH, Mason RE, Morgan G, Finlayson SA. 2007. Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. *Plant Science* **172**: 1113–1123.

He C, Cui K, Zhang J, Duan A, Zeng Y. 2013. Next-generation sequencing-based mRNA and microRNA expression profiling analysis revealed pathways involved in the rapid growth of developing culms in Moso bamboo. *BMC Plant Biology* **13**: 1–14.

Herrero MP, Johnson RR. 1980. High temperature stress and pollen viability of maize. *Crop Science* **20**: 796.

Huberman M, Riov J, Goldschmidt EE, Apelbaum A, Goren R. 2013. The novel ethylene antagonist, 3-cyclopropyl-1-enyl-propanoic acid sodium salt (CPAS), increases grain yield in wheat by delaying leaf senescence. *Plant Growth Regulation* **73**: 249–255.

Iehisa JCM, Takumi S. 2012. Variation in abscisic acid responsiveness of *Aegilops tauschii* and hexaploid wheat synthetics due to the D-genome diversity. *Genes & Genetic Systems* **87**: 9–18.

IPCC. 2007. Climate change 2007: synthesis report. In: The core writing team, Pachauri RK, Reisinger A, eds. *Climate change report 7: synthesis report*. Geneva, Switzerland: IPCC, 26–73.

IPCC. 2013. Climate change 2013: The physical science basis. In: Pachauri RK, Reisinger A, eds. *The core writing team*. IPCC: Geneva, Switzerland, 1–29.

Jagadish SVK, Craufurd PQ, Wheeler TR. 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany* **58**: 1627–1635.

Ku HS, Suge H, Rappaport L, Pratt HK. 1970. Stimulation of rice coleoptile growth by ethylene. *Planta* **90**: 333–339.

Kumar V, Giridhar P, Ravishankar GA. 2009. AgNO3 – a potential regulator of ethylene activity and plant growth modulator. *Electronic Journal of Biotechnology* **12**: 1–15.

Lee K, Seo PJ. 2015. The E3 ubiquitin ligase HOS1 is involved in ethylene regulation of leaf expansion in Arabidopsis. *Plant Signaling & Behavior* **10**: e1003755.

Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Xu C, Li X, Xiao J *et al.* 2011. Natural variation in GS5 plays an important role in regulating grain size and yield in rice. *Nature Genetics* **43**: 1266–1269.

Li Y-F, Wang Y, Tang Y, Kakani VG, Mahalingam R. 2013. Transcriptome analysis of heat stress response in switchgrass (*Panicum virgatum* L.). *BMC Plant Biology* **13**: 153.

Lopes MS, Dreisigacker S, Peña RJ, Sukumaran S, Reynolds MP. 2015. Genetic characterization of the wheat association mapping initiative (WAMI) panel for dissection of complex traits in spring wheat. *Theoretical and Applied Genetics* **128**: 453–464.

Lopes MS, Reynolds MP, Jalal-Kamali MR, Moussa M, Feltaous Y, Tahir ISA, Barma N, Vargas M, Mannes Y, Baum M. 2012. The yield correlations of selectable physiological traits in a population of advanced spring wheat lines grown in warm and drought environments. *Field Crops Research* **128**: 129–136.

Ma Z, Zhao D, Zhang C, Zhang Z, Xue S, Lin F, Kong Z, Tian D, Luo Q. 2006. Molecular genetic analysis of five spike-related traits in wheat using RIL and immortalized F_2 populations. *Molecular Genetics and Genomics* **277**: 31–42.

Miller PC, Hajek E, Poole DK, Roberts SW. 1981. Microclimate and energy exchange. In: Miller PC, ed. *Ecological studies. Resource use by Chaparral and Matorral*. New York, NY, USA: Springer, 97–121.

Miralles DJ, Richards RA, Slafer GA. 2000. Duration of the stem elongation period influences the number of fertile florets in wheat and barley. *Functional Plant Biology* **27**: 931–940.

Pask A, Joshi AK, Manes Y, Sharma I, Chatrath R, Singh GP, Sohu VS, Mavi GS, Sakuru VSP, Kalappanavar IK *et al.* 2014. A wheat phenotyping network to incorporate physiological traits for climate change in South Asia. *Field Crops Research* **168**: 156–167.

Plett JM, Williams M, LeClair G, Regan S, Beardmore T. 2014. Heterologous over-expression of ACC SYNTHASE8 (ACS8) in *Populus tremula* x *P. alba* clone 717-1B4 results in elevated levels of ethylene and induces stem dwarfism and reduced leaf size through separate genetic pathways. *Frontiers in Plant Science* **5**: 514.

Prasad PVV, Boote KJ, Allen LH Jr, Sheehy JE, Thomas JMG. 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research* **95**: 398–411.

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.

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

R Development Core Team. 2015. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL http://www.R-project.org.

Rebetzke GJ, van Herwaarden AF, Jenkins C, Weiss M, Lewis D, Ruuska S, Tabe L, Fettell NA, Richards RA. 2008. Quantitative trait loci for water-soluble carbohydrates and associations with agronomic traits in wheat. *Australian Journal of Agricultural Research* **59**: 891–905.

Richards RA. 1992. The effect of dwarfing genes in spring wheat in dry environments. I. agronomic characteristics. *Australian Journal of Agricultural Research* **43**: 517–527.

Rodríguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB. 1999. A Copper cofactor for the ethylene receptor ETR1 from Arabidopsis. *Science* **283**: 996–998.

Ruiz J, Valero M, García-Martínez S, Serrano M, Moral R. 2006. Effect of recent genetic improvement on some analytical parameters of tomato fruit quality. *Communications in Soil Science and Plant Analysis* **37**: 2647–2658.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences, USA* **81**: 8014–8018.

Sharp RE, LeNoble ME. 2002. ABA, ethylene and the control of shoot and root growth under water stress. *Journal of Experimental Botany* **53**: 33–37.

Sharp RE, LeNoble ME, Else MA, Thorne ET, Gherardi F. 2000. Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene. *Journal of Experimental Botany* **51**: 1575–1584.

Song L, Shi Q-M, Yang X-H, Xu Z-H, Xue H-W. 2009. Membrane steroid-binding protein 1 (MSBP1) negatively regulates brassinosteroid signaling by enhancing the endocytosis of BAK1. *Cell Research* **19**: 864–876.

Sridhar G. 2003. *Studies on endogenous hormonal changes during grain development in wheat genotypes*. PhD thesis, Indian Agricultural Research Institute (IARI), New Delhi, India.

Sukumaran S, Dreisigacker S, Lopes M, Chavez P, Reynolds MP. 2015a. 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.

Sukumaran S, Reynolds MP, Lopes MS, Crossa J. 2015b. Genome-wide study for adaptation to agronomic plant density: a component of high-yield potential in spring wheat. *Crop Science* **55**: 2609–2619.

Sukumaran S, Xiang W, Bean SR, Pedersen JF, Kresovich S, Tuinstra MR, Tesso TT, Hamblin MT, Yu J. 2012. Association mapping for grain quality in a diverse sorghum collection. *Plant Genome Journal* **5**: 126.

Takatsuka H, Umeda M. 2014. Hormonal control of cell division and elongation along differentiation trajectories in roots. *Journal of Experimental Botany* **65**: 2633–2643.

Tamaki H, Reguera M, Abdel-Tawab YM, Takebayashi Y, Kasahara H, Blumwald E. 2015. Targeting hormone-related pathways to improve grain yield in rice: a chemical approach. *PLoS ONE* **10**: e0131213.

Torre F, del Carmen Rodríguez-Gacio M, Matilla AJ. 2006. How ethylene works in the reproductive organs of higher plants. *Plant Signaling & Behavior* **1**: 231–242.

Valluru R. 2015. Fructan and hormone connections. *Frontiers in Plant Science* **6**: 180.

Valluru R, Davies WJ, Reynolds MP, Dodd IC. 2016. Foliar abscisic acid-to-ethylene accumulation and response regulate shoot growth sensitivity to mild drought in wheat. *Frontiers in Plant Science* **7**: 461.

Van de Poel B, Bulens I, Hertog MLATM, Nicolai BM, Geeraerd AH. 2014. A transcriptomics-based kinetic model for ethylene biosynthesis in tomato (*Solanum lycopersicum*) fruit: development, validation and exploration of novel regulatory mechanisms. *New Phytologist* **202**: 952–963.

Wang X, Dinler BS, Vignjevic M, Jacobsen S, Wollenweber B. 2015b. Physiological and proteome studies of responses to heat stress during grain filling in contrasting wheat cultivars. *Plant Science* **230**: 33–50.

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L *et al.* 2014. Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. *Plant Biotechnology Journal* **12**: 787–796.

Wang S, Zhang X, Chen F, Cui D. 2015a. A Single-nucleotide polymorphism of TaGS5 gene revealed its association with kernel weight in Chinese bread wheat. *Crop Science and Horticulture* **6**: 1166.

Weir BS. 1996. *Genetic data analysis II: methods for discrete population genetic data.* Sunderland, MA, USA: Sinauer Associates.

White JW, Kimball BA, Wall GW, Ottman MJ, Hunt LA. 2011. Responses of time of anthesis and maturity to sowing dates and infrared warming in spring wheat. *Field Crops Research* **124**: 213–222.

Williams DA. 1987. Generalized linear model diagnostics using the deviance and single case deletions. *Applied Statistics* **36**: 181.

Wuriyanghan H, Zhang B, Cao W-H, Ma B, Lei G, Liu Y-F, Wei W, Wu H-J, Chen L-J, Chen H-W *et al.* 2009. The ethylene receptor ETR2 delays floral transition and affects starch accumulation in rice. *Plant Cell* **21**: 1473–1494.

Xu C, Liu Y, Li Y, Xu X, Xu C, Li X, Xiao J, Zhang Q. 2015. Differential expression of GS5 regulates grain size in rice. *Journal of Experimental Botany* **66**: 2611–2623.

Yang J, Zhang J, Liu K, Wang Z, Liu L. 2006. Abscisic acid and ethylene interact in wheat grains in response to soil drying during grain filling. *New Phytologist* **171**: 293–303.

Yang JC, Zhang JH, Ye YX, Wang ZQ, Zhu QS, Liu LJ. 2004. Involvement of abscisic acid and ethylene in the responses of rice grains to water stress during filling. *Plant, Cell & Environment* **27**: 1055–1064.

Ye C, Tenorio FA, Argayoso MA, Laza MA, Koh H-J, Redoña ED, Jagadish KS, Gregorio GB. 2015. Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genetics* **16**: 41.

Young TE, Meeley RB, Gallie DR. 2004. ACC synthase expression regulates leaf performance and drought tolerance in maize. *Plant Journal* **40**: 813–825.

Zhang Z, Ersoz E, Lai C-Q, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM *et al.* 2010. Mixed linear model approach adapted for genome-wide association studies. *Nature Genetics* **42**: 355–360.

Zhang J, Kirkham MB. 1990. Variation in ethylene production by sorghum. *Euphytica* **46**: 109–117.

Zhang J, Xu Y, Chen W, Dell B, Vergauwen R, Biddulph B, Khan N, Luo H, Appels R, Van den Ende W. 2014. A wheat 1-FEH w3 variant underlies enzyme activity for stem WSC remobilization to grain under drought. *New Phytologist* **205**: 293–305.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1 The relationship between spike ethylene and spike dry weight in 130 wheat genotypes.

Fig. S2 The relationship between spike ethylene and spike dry weight in 30 wheat genotypes.

Fig. S3 Spike ethylene and spike dry weight in two SET groups.

Fig. S4 Pearson's correlations between spike ethylene, spike dry weight and plant height.

Fig. S5 The relationship between thousand-grain weight and spike ethylene in 30 wheat genotypes.

Fig. S6 Grain yield and thousand-grain weight in different combinations of experimental factors.

Table S1 The full set of genotypes (130) used in the initial genetic diversity study under field conditions

Table S2 The subset of genotypes (30) used in the field experiments

Table S3 Individual two-way ANOVAs for spike dry weight and spike ethylene in 130 wheat genotypes grown under heat-stressed field conditions

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