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Genetic variability in high temperature effects on seed-set in sorghum

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Abstract. Sorghum (*Sorghum bicolor* (L.) Moench) is grown as a dryland crop in semiarid subtropical and tropical environments where it is often exposed to high temperatures around flowering. Projected climate change is likely to increase the incidence of exposure to high temperature, with potential adverse effects on growth, development and grain yield. The objectives of this study were to explore genetic variability for the effects of high temperature on crop growth and development, *in vitro* pollen germination and seed-set. Eighteen diverse sorghum genotypes were grown at day : night temperatures of 32 : 21°C (optimum temperature, OT) and 38 : 21°C (high temperature, HT during the middle of the day) in controlled environment chambers. HT significantly accelerated development, and reduced plant height and individual leaf size. However, there was no consistent effect on leaf area per plant. HT significantly reduced pollen germination and seed-set percentage of all genotypes; under HT, genotypes differed significantly in pollen viability percentage (17–63%) and seed-set percentage (7–65%). The two traits were strongly and positively associated ($R^2 = 0.93$, $n = 36$, $P < 0.001$), suggesting a causal association. The observed genetic variation in pollen and seed-set traits should be able to be exploited through breeding to develop heat-tolerant varieties for future climates.

Additional keywords: heat tolerance, pollen germination, seed set percentage.

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the major dryland summer grain crop grown in semiarid subtropical areas where grain yield is often limited by variable and unreliable precipitation (Chapman *et al.* 2002; Bandaru *et al.* 2006). In addition, in many of these environments, average maximum temperatures during the growing season often reach 32−35°C (Muchow *et al.* 1994; Prasad *et al.* 2006*a*, 2008), which exceeds the optimum temperature of 30°C for growth and development of sorghum (Hammer *et al.* 1993). Associated with these maximum temperatures is a substantial frequency of occurrence of extreme high temperature (>35°C) conditions (Hennessy *et al.* 2010). Climate change is causing an increase in temperature, with estimates for Australia of an increase of 0.5–2.5°C by 2030 (Intergovernmental Panel on Climate Change 2007). Consequently, the frequency of occurrence of extremely high (>35°C) temperatures is likely to increase (Hennessy *et al.* 2010). This could adversely affect the grain yield of cereals (Barnabas *et al.* 2008), potentially destabilising farm income. Large investments in production infrastructure make it difficult to shift production of field crops to new areas. Therefore, genetic or agronomic solutions are appropriate adaptation responses for the industry to manage current or future high temperature challenges.

As a major modulator of biochemical processes, temperature influences the growth, development and yield of cereals. High temperatures (heat stress) during vegetative growth stages can affect many crop physiological and metabolic processes (Barnabas *et al.* 2008). At temperatures up to a threshold temperature for the maximum rate of development, an increase in daily temperature will advance the development rate and shorten phenological phases (Ravi Kumar *et al.* 2009). The resulting reduction in intercepted radiation can reduce pre-anthesis biomass accumulation and, potentially, grain yield. Supraoptimal temperatures, however, can delay phenology (Prasad *et al.* 2006*b*, 2008; Craufurd and Wheeler 2009). This

does not necessarily increase biomass production, as metabolic processes are also affected by such high temperatures (Barnabas *et al.* 2008). In sorghum, the overall effect of high temperature (36–40°C) on leaf area per plant appears to be minor, as a reduction in leaf size is compensated by an increased leaf number per axis (Jain *et al.* 2007; Prasad *et al.* 2008, 2009; van Oosterom *et al.* 2011). Similarly, the effect on photosynthesis of sorghum seems to be minor (Jain *et al.* 2007; Prasad *et al.* 2008, 2009), although high night temperature alone has been reported to reduce photosynthesis in sorghum (Prasad and Djanaguiraman 2011). As a consequence of these contrasting results, moderately high temperatures have been reported to either have little impact on plant mass (Prasad *et al.* 2006*b* for rice (*Oryza sativa* and *Oryza glaberrima*); Jain *et al.* 2007 for sorghum) or to reduce plant mass (Koti *et al.* 2007 for soybean (*Glycine max* L.); Prasad *et al.* 2008, 2009 for sorghum), mainly through a reduction in stem mass.

The impact of high temperatures on reproductive processes is more pronounced than on vegetative processes. *In vitro* pollen germination, pollen viability and seed-set were significantly reduced by temperatures above 32 : 22°C (day : night) around anthesis in sorghum, particularly by temperatures above 36 : 26°C (Prasad *et al.* 2006*a*, 2008). Genotypic variations in the response of pollen characteristics and seed-set to supraoptimal temperatures have been reported for rice (Prasad *et al*. 2006*b*; Chakrabarti *et al*. 2010) and soybean (Koti *et al.* 2005). For cotton (*Gossypium hirsutum* and *G. barbadense*, Kakani *et al.* 2005) and groundnut (*Arachis hypogea* L., Kakani *et al.* 2002), genotypic differences in the temperature response of pollen germination and pollen tube length have been reported. Little information is available on genotypic variation in sorghum for the sensitivity of reproductive processes to high temperature, as most studies conducted for this species included only one or two genotypes. We hypothesise the presence of genetic variation in response to high temperature for reproductive processes in sorghum. Thus the objective of this study was to explore the extent of variation among sorghum genotypes for the effects of high temperature on *in vitro* pollen germination and seed-set and to identify any association with effects on growth and development. This provides the underlying basis to develop a breeding-based adaptation to high temperature conditions in production environments.

Materials and methods

Genetic material

The genotypes comprised 15 inbred sorghum (*Sorghum bicolor* (L.) Moench) lines and three hybrids (Table 1). Inbred lines were selected from a diverse gene pool, based on observed differences in grain yield response to heat stress in breeding trials (D Jordan, pers. comm.). These lines represent diverse germplasm originating from the United States, Australia, Africa and Asia, and included parents of mapping populations and elite lines that are parents of hybrids used by the sorghum

Table 1. The 15 inbred lines and three hybrids of sorghum used in the controlled environment experiments, listed by country of origin

DAFF, Department of Agriculture, Fisheries and Forestry

Genotype name	Characteristics
USA	
R9188	Partially converted derivative of the sweet sorghum cultivar Rio
Tx7000	Early hybrid parent lacking in stay-green drought resistance
BTx623	Elite female parent line
Australia	
CCH1 (hybrid)	Commercial check hybrid with high tillering capability
CCH2 (hybrid)	Commercial check hybrid with high levels of stay-green
AQL33/QL36 (hybrid)	Check hybrid
KS4	Ancestral female parent line
R931945–2-2	Elite low-tillering stay-green parent line; DAFF breeding program
B923296	Elite stay-green, heat sensitive parent line; DAFF breeding program
R9403463–2-1	Elite moderately senescent parent line; DAFF breeding program
QL33	Elite moderately senescent female parent line; DAFF breeding program
B963676	Elite stay-green female parent line
Africa	
Ethiopia	
B35	Highly stay-green, partially converted Durra landrace
IS 8525	Early flowering parent of mapping population for ergot resistance
SC170–6-8	High tillering, heat-sensitive, wide root angle, partly converted Caudatum line
Mali	
PI609489	Two-dwarf,breeding line
PI563516	Two-dwarf, breeding line
Asia	
China	
Ai4	Two-dwarf, photoperiod-insensitive, possible cold tolerance

breeding program of the Department of Agriculture, Fisheries and Forestry (Queensland) in south-east Queensland, Australia (Jordan *et al.* 2011).

Experimental details

The research was conducted in two growth chambers at the Controlled Environment Facility, Queensland Bioscience Precinct, St Lucia, Queensland. Each chamber was 3 m long, 2.7 m wide and 3.6 m high, and had fully automated control of temperature, photoperiod and relative humidity. The photoperiod was set at 14 h and the light level (350–850 nm) to 600 µmol m^{-2} s^{-1} at canopy height. One chamber was set to operate at a maximum : minimum temperature of 32 : 21°C (optimum temperature, OT) and the other at 38 : 21°C (high temperature, HT) (Fig. 1). During the 10-h dark period, the temperature was kept constant at 21°C in both chambers. Around 1 h after the onset of the light period, the temperature was gradually increased at a rate of around 3°C per hour until the preset maximum temperature was reached in each chamber (Fig. 1). After 7 h (OT) and 3 h (HT) at their respective maximum temperatures, temperature declined at a rate of 3°C per hour until the minimum temperature was reached around the end of the light period. Hence treatments differed in temperature only during the few hours in the middle of the light period, when the temperature in the HT treatment exceeded the maximum temperature of the OT treatment. This eliminated any potential effect on phenology of the treatment differences in temperature at the time of light–dark transition (Morgan *et al.* 1987; Ellis *et al.* 1997). Relative humidity was programmed at 94–54% for the OT treatment and 98–57% for the HT treatment, such that the vapour pressure deficit (VPD) was around 0.16 kPa during the night and reached similar values during the day (2.19 kPa for OT and 2.39 kPa for HT) in both treatments (Fig. 1) to minimise any confounding effects of differences in VPD between temperature treatments. The temperature and relative humidity settings in each room were tested for several days before the start of the experiment and the settings were maintained from sowing until the final harvest. Hence differences in results between the two chambers could be attributed to differences in maximum temperature during the middle of the light period.

Plants were grown in Twinpot ANOVApots (Anova Solutions, www.anovapot.com) where a smaller pot is placed inside a larger one, such that the space between the two pots serves as a reservoir for water. This reservoir was automatically watered several times per day. Capillary tape provided a pathway for water to flow from the reservoir into the base of the inner pot, ensuring a constant watertable for the inner pot. The water supply per pot was increased gradually from 1 L per day at the seedling stage to ~3 L per day from flowering onwards. Each inner pot (20 cm high and 20 cm in diameter) contained ~3 kg of a soil mixture that consisted of 90% sterilised fine potting mix and 10% coir. Each pot was fertilised with 18 g of Osmocote (Scotts Australia, Baulkham Hills, NSW, Australia) for trees and shrubs (16% N, 3.5% P and 10% K), 15 g gypsum, 15 g dolomite and 5 g micromix (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo and 1% Zn; (Micromax, The Scotts Company, Marysville, OH, USA). Fertiliser was mixed thoroughly into each 3 kg of the soil before filling.

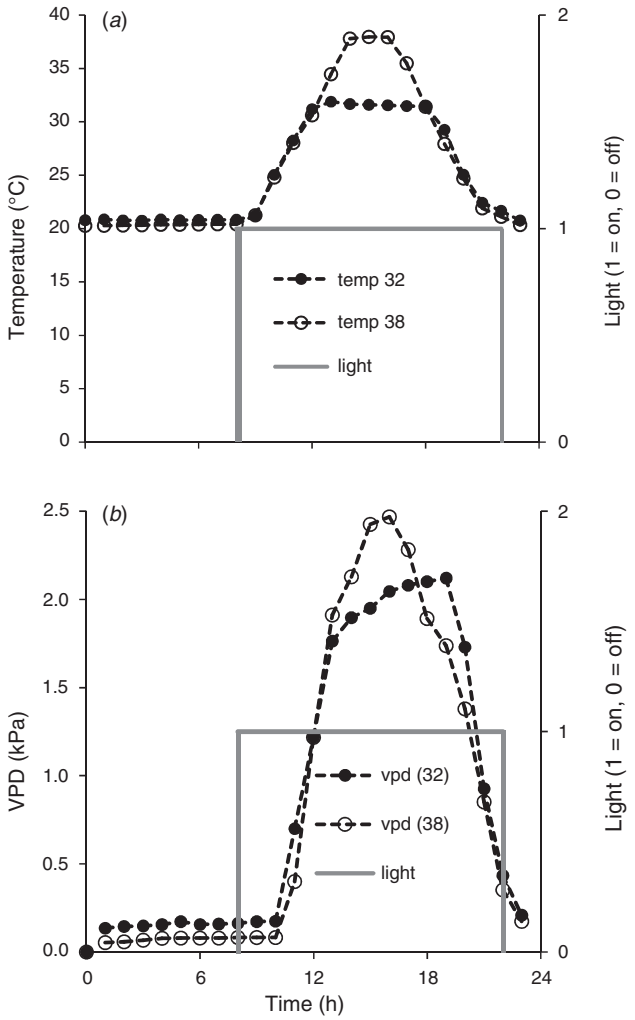

Fig. 1. (*a*) Temperature and (*b*) vapour pressure deficit (VPD) throughout the day for the controlled environment experiment (for details, see Materials and methods).

Pots were watered to field capacity before sowing and five seeds, treated with fungicide for soilborne diseases (Thiram WP Fungicide, Kendon Chemical and MNFG. Co. Pty. Ltd, Thornbury, Vic., Australia), were sown at 3 cm depth in each pot. Germinated seedlings were gradually thinned until one plant per pot remained 10 days after sowing. To minimise Ca deficiency symptoms, 0.3% $Ca(NO_3)_2$ was sprayed into the whorl of each axis at regular intervals during the vegetative growth period.

In each of the two temperature treatments, the experiment was laid out as a randomised complete block design with three replications.

Phenology and leaf size

Data on phenology (days from sowing to emergence, full flag leaf appearance and anthesis) were recorded for all plants. The height at leaf ligules 4, 8, 12 and 16 was measured *in situ*. The area of all individual main shoot leaves of each plant was determined *in situ* by measuring their length and width at full emergence, multiplied by a shape factor of 0.69 (Lafarge and Hammer 2002).

In vitro *pollen germination*

Pollen germination was measured *in vitro* on a growth medium that contained 150 mg H_3BO_3, 500 mg $Ca(NO_3)_2\_4H_2O$, 200 mg $MgSO_4\_7H_2O$, 100 mg KNO_3 and 300 g sucrose, dissolved in 1 L of deionised water to which 15 g of agar per litre was added. The medium was slowly heated on a hotplate until the agar was completely dissolved (Tuinstra and Wedel 2000; Prasad *et al.* 2006*a*). The medium was then poured into Petri dishes and cooled until the agar solidified. Petri dishes were stored in the dark at 28°C until use. At the opening of the first set of florets of each main shoot panicle, when the lights had just switched on, pollen grains were dusted onto a Petri dish. After incubating in the dark for 45 min at 28°C, pollen grains were photographed at 100× magnification with a digital camera (Nikon FX-35DX, Nikon, Tokyo, Japan) mounted on an Olympus stereo microscope (Nikon SMZ-D AFX-11A Photo microscopic system). Digital images were modified to enhance contrast using Adobe Photoshop Elements ver. 2.0 software (Adobe Systems Incorporated). The percentage of pollen germination was estimated by manual counts of the total number of pollen grains and number of germinated pollen grains on images taken at random from three sections of each Petri dish. A pollen grain was considered to have germinated when the pollen tube had started to emerge from the pollen grain (Fig. 2). This *in vitro* pollen germination measure tends to be slightly lower than the pollen viability of sorghum using a vital stain technique, but pollen germination and viability respond similarly to temperature (Prasad *et al.* 2006*a*; Prasad and Djanaguiraman 2011).

Seed-set

Panicles were harvested at physiological maturity and three branches from each of three sections of the panicle (top, middle and bottom sections) were collected. For each branch, the total number of florets, and the number of viable (seed filled) and nonviable (unfilled) florets was counted. Seed-set percentage was calculated as the number of viable florets as a percentage of the total number of florets.

Statistical analyses

ANOVA was performed using Genstat ver. 12 (Payne *et al.* 2009). The data for the various attributes measured were analysed across experiments as a randomised complete block design (18 genotypes) within experiments (two temperature treatments) with three replications, after checking the homogeneity of variance. Genotype (G) effects were considered random, whereas experiment (temperature, T) effects and the G × T interactions were considered fixed effects. Differences among G and T effects were evaluated for significance by the LSD ($P = 0.05$).

Results

High temperatures accelerated phenology and reduced plant height, but had no consistent effect on leaf area per plant

High temperatures in general slightly accelerated phenological development (Table 2). On average, the time from sowing to flag leaf appearance and anthesis was shorter under HT (Table 2) but leaf appearance rate (LAR) was also more rapid under HT so that the number of leaves was significantly greater. There were, however, significant G × T interactions for phenological traits, which highlighted that the temperature effect on phenology was genotype-dependent. For example, the effect of HT on days to anthesis ranged from an acceleration of 12 days for Tx7000 to a delay of 2 days for CCH1 (data not shown). This G × T interaction for days to anthesis appeared to be associated with a G × T interaction for LAR and leaf number, as genotypes with a greater increase in LAR under HT tended to be those with a greater reduction in days to flag leaf ($R^2 = 0.53$, $n = 18$, $P < 0.001$), although they also tended to have a greater increase in leaf number under HT ($R^2 = 0.52$, $n = 18$, $P < 0.001$).

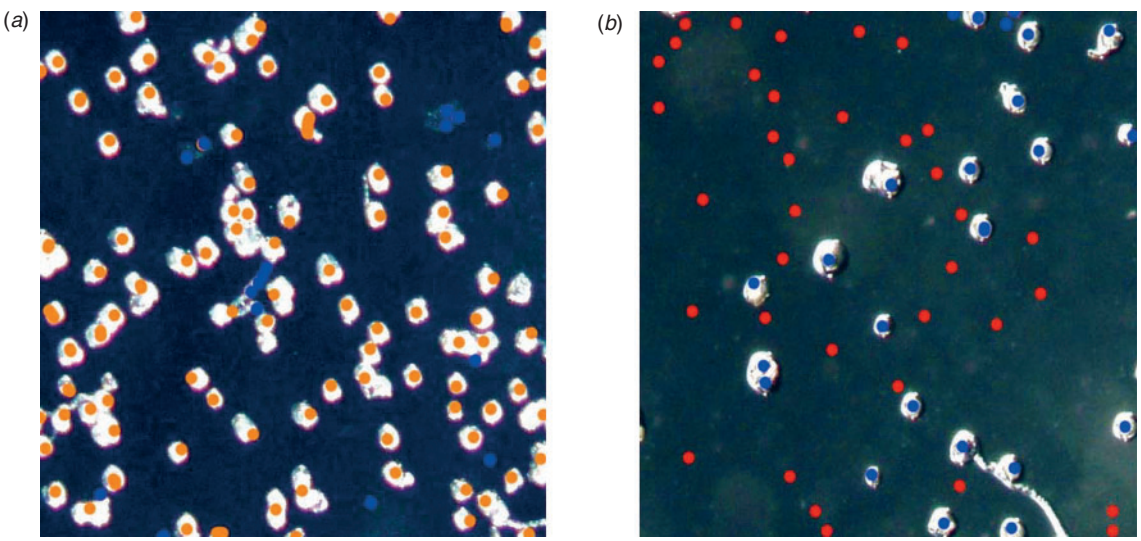

Fig. 2. Images showing the extent of pollen germination for genotype B35 for pollen collected from either (*a*) the optimum temperature (32 : 21°C) or (*b*) the high temperature (38 : 21°C) treatment. Pollen grains that have germinated are enhanced with a white surround.

Table 2. Mean values of the phenological and morphological growth traits of diverse sorghum genotypes for the high temperature (HT, 38 : 21°C) and optimum temperature (OT, 32 : 21°C) treatments and significance levels of effects from ANOVA

NS, not significant ($P > 0.05$); *, $P <= 0.05$; **, $P <= 0.01$; ***, $P <= 0.001$; G, genotype; T, temperature

Trait	HT	OT	Significance level		
			G	T	G × T
Time to emergence (days)	3.4	5.4	*	***	NS
Time to flag leaf (days)	85.0	112.0	***	***	*
Time to anthesis (days)	100.3	129.4	***	***	*
Time to maturity (days)	126.6	155.4	***	***	**
Leaf appearance rate (leaves per day)	0.29	0.21	***	NS	NS
Leaf number (per plant)	24.6	23.8	***	*	NS
Leaf area (cm^2 per plant)	6525	6732	***	NS	**
Plant height at Leaf 16 (cm)	51.96	60.76	***	***	**

The faster LAR under HT was associated with reduced leaf length. The increase in the maximum length of successive main shoot leaves from Leaf 2 to Leaf 12 was, on average, around 7.54 cm under HT compared with 8.45 cm under OT. The effect on leaf width was generally smaller (data not shown). As the reduced leaf size under HT was partly compensated by increased leaf number, the overall effect of temperature on leaf area per plant was not significant (Table 2). However, a highly significant ($P < 0.01$) G × T interaction for leaf area per plant indicated significant genotypic differences in the response of leaf area to temperature.

Plant height at the ligule of Leaf 16 was significantly lower under HT than under OT (Table 2). Hence stem internodes were generally shorter under HT than OT, as illustrated in Fig. 3 for B923296.

Genotypes differed in the extent of reduction in pollen germination and seed-set under high temperature

There were significant effects of temperature ($P < 0.001$) and genotype ($P = 0.03$) on pollen germination. In general, pollen germination was ~70–80% under OT for most genotypes, but this was significantly decreased across most genotypes under HT (Fig. 4). However, there were differential genotypic responses, with pollen germination under HT ranging from 17.1% (B35) and 18.8% (SC170–6-8) to 59.1% (R9403463–2-1) and 62.9% (IS8525).

The effect of HT on pollen germination was also reflected in seed-set (Fig. 5), which was significantly reduced for most genotypes under HT (Fig. 6). Under OT, seed-set ranged from just over 60% (SC170–6-8) to nearly 90% (B923296), but under HT, the range was greater, with seed-set percentage varying from 7.3% (B923296) to 61.5% (R9403463–2-1). The greatest decrease in seed-set percentage under HT compared with that under OT was for genotype B923296 with a decrease of 80% (relative to the OT treatment), whereas the seed-set of R9403463–2-1 and IS8525 were the least sensitive, with a relative decline of only 23% and 28% respectively. As a consequence, there was a significant ($P < 0.001$) G × T interaction for seed-set (Fig. 6).

Fig. 3. Photograph of genotype B923296 grown under either optimum temperature (control, 32 : 21°C) or high temperature (38 : 21°C) conditions, showing the treatment effect on plant stature.

Pollen germination and seed-set were positively related

Under HT conditions, the seed-set percentage of genotypes was linearly related to pollen germination percentage, and the extrapolation of this relationship was consistent with data under OT conditions (Fig. 7). The regression fitted to all data ($R^2 = 0.93$, $n = 36$, $P < 0.001$) indicated that a pollen germination of around 10% was required before any seed-set occurred, whereas 100% pollen germination would, in theory, result in 100% seed-set.

Discussion

The effects of high temperature on seed-set are likely to have operated through an effect on reproductive processes

High temperature reduced pollen germination (Fig. 4) and seed-set (Fig. 6) for all sorghum genotypes in this experiment. Similar effects of HT on pollen germination (or pollen viability), seed-set or both have been reported previously for a range of crops, including sorghum (Prasad *et al.* 2006*a*, 2008; Jain *et al.* 2007), *Brassica napus* (L.) (Young *et al.* 2004), rice (Prasad *et al.* 2006*b*), wheat (*Triticum aestivum* L., Prasad *et al.* 2011) and soybean (Koti *et al.* 2005). However, in most of these published experiments, both the minimum and maximum temperatures varied. In our experiments, only the maximum temperature was changed, with VPD comparable

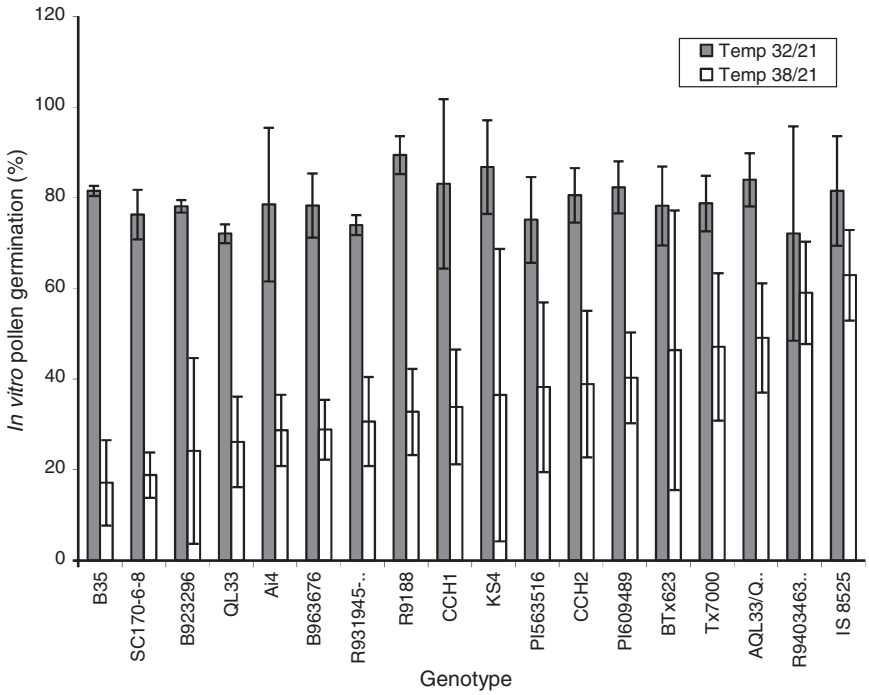

Fig. 4. *In vitro* pollen germination (%) for various genotypes grown under optimum temperature (OT, 32 : 21°C, shaded bars) or high temperature (HT, 38 : 21°C, open bars) conditions. Vertical lines indicate the s.e. of the mean for each genotype × temperature combination.

throughout the day, and therefore the observed declines in pollen germination and seed-set under HT were a consequence of increased maximum temperature *per se*. Prasad and Djanaguiraman (2011) reported a decline in pollen viability and *in vitro* pollen germination of sorghum even if only the night temperature was increased from 22°C to 28°C. Although this is well below the optimum temperature of around 30–32°C for sorghum, the deterioration of pollen in their experiment was associated with decreased phospholipids and increased reactive oxygen species in the pollen grains. As these effects on pollen germination and seed-set by high night temperatures were similar to those observed by high day temperatures in our experiments, it is possible that the oxidative damage to pollen grains during their formation observed by Prasad and Djanaguiraman (2011) also occurs under elevated day temperatures, albeit at higher temperatures than during the night.

The decrease in pollen germination and seed-set under HT were not due to decreased photosynthetic area, because leaf area per plant was similar under both temperature treatments (Table 2). The reduction in leaf size and increase in leaf number under HT, resulting in an absence of a clear effect of HT on leaf area per plant, is consistent with previous reports for sorghum (Jain *et al.* 2007; Prasad *et al.* 2008, 2009; van Oosterom *et al.* 2011). In addition, for both sorghum (Jain *et al.* 2007) and rice (Prasad *et al.* 2006*b*), HT does not seem to affect photosynthetic rates. It is therefore unlikely that assimilate availability *per se* accounted for the reduction in pollen germination and seed-set. Rather, the lower pollen germination at HT could be the result of impaired carbohydrate metabolism in the anthers, possibly associated with degeneration of the tapetum cells (Jain *et al.* 2007). Pressman *et al.* (2002) observed a decrease in the concentration of soluble carbohydrates in the anther wall and pollen of tomato (*Lycopersicon esculentum* Mill.) after continued exposure to high temperatures, whereas Karni and Aloni (2002) observed a decline in the fructokinase activity of the anthers and pollen of bell pepper (*Capsicum anuum* L.) under high temperatures that was associated with reduced pollen germination. The potential effect of damage to the tapetum cells and the changes in the carbohydrate metabolism under HT on pollen germination (Karni and Aloni 2002) are consistent with the observation of Prasad *et al.* (2008) that sorghum is most sensitive to HT during the period immediately before and during anthesis. These results support the hypothesis that HT effects on grain yield are predominantly associated with effects on reproductive rather than vegetative organs.

If an effect of temperature on pollen germination occurred around anthesis, it would be possible that these genotypic differences in heat tolerance could simply be a consequence of differences in the timing of flowering within a day, as earlier flowering would avoid higher temperatures and VPD later in the day. For rice, such differences in the timing of flowering have been observed, although the correlation with pollen viability was weak (Prasad *et al.* 2006*b*). For sorghum, Herde *et al.* (2005) observed that CCH1 flowered earlier in the day than the inbred line R931945–2-2. Although this observation would be consistent with the better seed-set of CCH1 (Fig. 6), the two genotypes differed little in pollen germination (Fig. 4). In our experiments, pollen was always collected at the same time of

Fig. 5. Photographs of panicles of a susceptible (B923296) and a tolerant (CCH2) genotype, showing differences in the effect of high temperature (HT, 38 : 21°C) on seed-set relative to optimum temperatures (control, 32 : 21°C). (*a*) Contrast between panicles of B923296 for the two temperature conditions. (*b*) Contrast between panicles of CCH2 and B923296 under HT conditions.

the day, so it is unlikely that differences in the timing of flowering would have affected our results. Rather, sorghum seems to be most sensitive to high temperatures during pollen development (microsporogenesis) and ovule development (macrosporogenesis) (Prasad *et al.* 2008). Similarly, low temperatures have been reported to affect developing pollen around meiosis of the pollen mother cells (Downes and Marshall 1971). Hence it is likely that the effects of high temperature observed in our experiments occurred before flowering and were unlikely to be confounded by genotypic differences in the timing of anthesis within the day.

Genetic variability in seed-set is related to pollen attributes

Significant genotypic differences in the response to high temperature were observed for both pollen germination (Fig. 4) and seed-set (Fig. 6). The observed genotypic range in pollen germination under HT (17–63%) was similar to the range of 7–47% reported for soybean (Koti *et al.* 2005) and 62–90% reported for rice (Prasad *et al.* 2006*b*), suggesting that ample genetic variation is available in sorghum that could be exploited in breeding programs.

Seed-set in our experiments was strongly associated with pollen germination (Fig. 7). This concurs with previous findings for sorghum (Prasad *et al.* 2006*a*, 2008) and rice (Prasad *et al.* 2006*b*), although the association in our experiments was much stronger than that reported for rice by Prasad *et al.* (2006*b*), one of the few studies that also included a range of genotypes. However, the large number of pollen grains produced still far exceeded the number of stigmas. The tight correlation of Fig. 7 suggests a possible mechanism in which the stigma becomes unreceptive once it receives a pollen grain (i.e. only the first pollen grain that hits a stigma has an opportunity to produce a pollen tube), so pollen germination becomes critical. This mechanism is unlikely, though, as multiple pollen tubes have been reported in the stigma and style, and even near the ovary of sorghum (Cisneros-López *et al.* 2010). Alternatively, it is possible that pollen production and, consequently, pollen reception per stigma were reduced. A decline in pollen production per anther under HT has been documented for a range of crops, including rice (Prasad *et al.* 2006*b*), soybean (Koti *et al.* 2005) and sorghum (Prasad *et al.* 2006*a*). In rice, reduced pollen production resulted in lower pollen reception per stigma and, consequently, lower spikelet fertility, with five pollen grains on average being required to increase spikelet fertility by 1% (Prasad *et al.* 2006*b*). Similar reductions in pollen reception under HT have been reported for flax (*Linum usitatissimum* L.) (Cross *et al.* 2003). For sorghum grown under cold stress, Cisneros-López *et al.* (2010, 2012) observed that less than 50% on average of pollen deposited on a stigma produced a pollen tube in the stigma, and on average less than two pollen tubes reached the ovary, representing ~10% of pollen reception. In concurrence with the results of Dupuis and Dumas (1990), who observed for maize (*Zea mays* L.) that mature pollen is more sensitive to high temperature than female tissue, the strong relationship between pollen germination and seed-set (Fig. 7) suggests that pollen attributes rather than ovule characteristics are a major determinant of seed-set in sorghum (Prasad *et al.* 2008) and that genetic variability in seed-set under HT is most likely to be related to differences in pollen attributes.

Implications for adaptation to future climates

Sorghum is often grown in environments where abiotic stress, particularly drought (Chapman *et al.* 2002; Bandaru *et al.* 2006) and high temperature (Muchow *et al.* 1994; Prasad *et al.* 2006*a*) are prevalent. Both stresses often occur concurrently, as HT is often associated with high VPD and inefficient water use (Kholova *et al.* 2010). Hence adaptation to both drought and high temperature is potentially important for sorghum. Stay-green, the

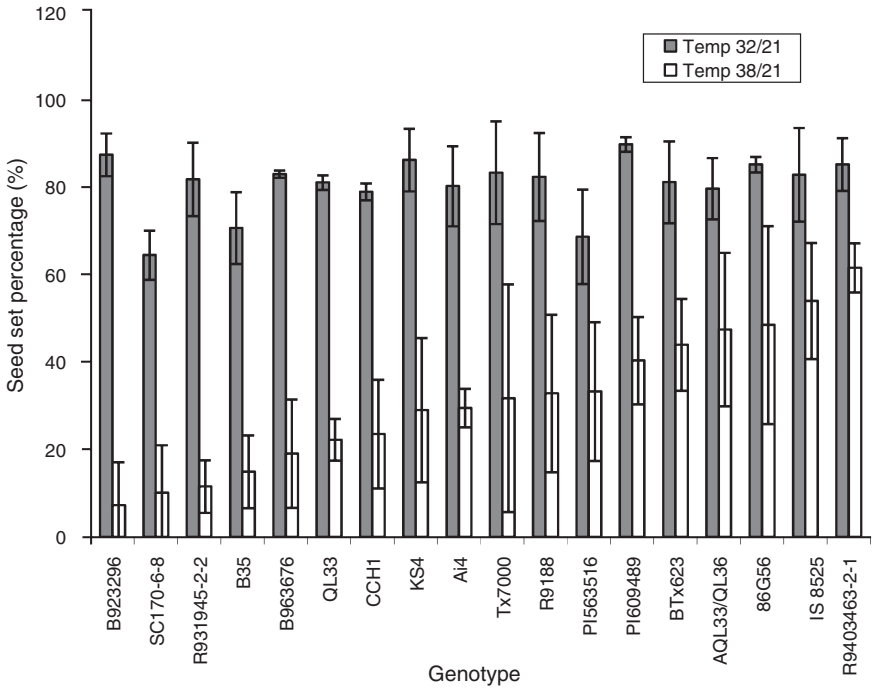

Fig. 6. Seed-set (%) for various genotypes, grown under optimum temperature (32 : 21°C, shaded bars) and high temperature (38 : 21°C, open bars) conditions. Vertical lines indicate the s.e. of the mean for each genotype × temperature combination.

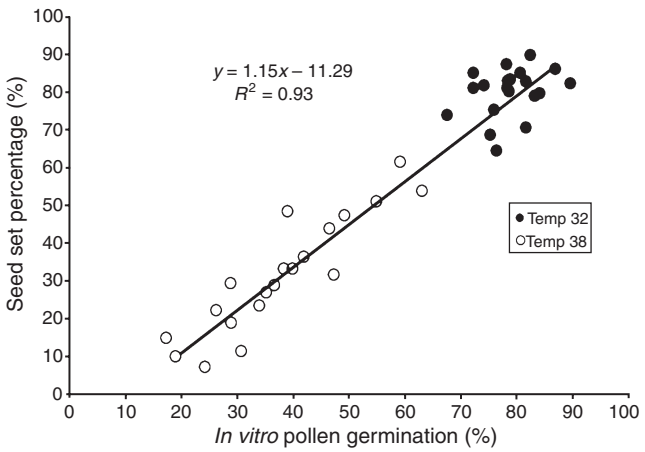

Fig. 7. Seed-set percentage versus pollen germination percentage for various grain sorghum genotypes grown under optimum temperature (●, 32 : 21°C) and high temperature (○, 38 : 21°C) conditions.

ability of a crop to retain green leaf area during grain filling, has been associated with grain yield under drought stress after anthesis in sorghum (Borrell *et al.* 2000). In our experiments, there was no clear association between stay-green and heat tolerance for seed-set. Whereas the stay-green hybrid CCH2 had medium to high levels of heat tolerance (Figs 4, 6), some of the stay-green inbred lines (B923296, R931945–2-2, B35; Table 1) were amongst the most heat-sensitive genotypes (Fig. 6). Similarly, senescent inbred lines ranged from highly tolerant (R9403463–2-1) to relatively susceptible (QL33) to heat stress (Figs 4, 6). This poor association between stay-green and heat tolerance can be explained through the difference in the physiological processes associated with these adaptation mechanisms. In sorghum, adaptation to drought after anthesis is, to a large extent, associated with water use, and therefore plant size before anthesis (van Oosterom *et al.* 2011). Whereas drought adaptation is associated with vegetative processes, adaptation to high temperature instead appears to be determined by the response of reproductive processes and pollen attributes. The independence of these two adaptation mechanisms would allow potential pyramiding into a single genotype through plant breeding to allow adaptation to multiple abiotic stresses.

Maximum temperatures in many sorghum growing areas around the world are close to or can exceed 32°C (Prasad *et al.* 2006*a*). This is particularly the case in the sorghum belt of eastern Australia (Muchow *et al.* 1994). Climate predictions indicate that Australia is likely to get warmer and drier, with the number of days with maximum temperatures exceeding 35°C increasing (Hennessy *et al.* 2010). Our results indicate such an increase in temperature may have serious implications for sorghum productivity. The presence of extensive genotypic variation for pollen germination and seed-set supports the view that plant breeding can potentially mitigate some of these adverse effects. However, further research is required to identify whether these differences in heat tolerance are associated with genotypic differences in the threshold temperature at which pollen germination and seed-set are affected, differences in sensitivity to high temperature *per se* or a combination of these effects.

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