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Genetic variation for tolerance to extreme temperatures in wild and cultivated sunflower (*Helianthus annuus*) during early vegetative phases

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Abstract. The increased incidence of extreme temperature events due to global climate change poses a major challenge for crop production. Ability to increase temperature tolerance through genetic improvement requires understanding of how crops and their wild relatives respond to extreme temperatures. We developed a high-throughput technique to evaluate tolerance to freezing stress (FS) and heat stress (HS) in wild, crop–wild hybrid and cultivated sunflower (*Helianthus annuus* L.). We also investigated whether trade-offs exist between stress tolerance and growth under benign conditions. Eleven experiments were performed under a combination of growth-chamber and field conditions. In growth-chamber experiments, FS and HS consisted of exposing acclimated plants at the 2–4-leaf stage to temperatures ranging from to –2.5°C to –4°C for 2–4 h and from 52°C to 54°C for 2–3 h. In the field, plants were grown for 32 days during midwinter (FS: average T_{mean} = 9.9°C and T_{min} = 3.8°C) or for 10 days in a heat tent (HS: average T_{mean} = 30.1°C and T_{max} = 43.3°C). We observed large differences in tolerance to FS and HS between wild and cultivated sunflower. Wild sunflower showed higher FS tolerance than cultivated in both growth-chamber and field experiments, whereas cultivated sunflower showed higher HS tolerance in growth-chamber experiments. No differences in HS tolerance were observed in the field. Crop–wild hybrids generally showed intermediate HS and FS tolerance. We found no evidence of a growth-tolerance trade-off, which suggests that tolerance might be introgressed into elite germplasm without growth penalties. The study reveals that wide genetic variation for the tolerance to extreme temperatures exists in the primary gene pool of sunflower.

Additional keywords: crop wild relatives, extreme temperatures, freezing stress, global change.

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Introduction

Conditions of abiotic stress such as soil salinity, drought and extreme temperatures are the major causes of crop yield losses worldwide (IPCC 2014). Abiotic stress can reduce crop yields through multiple mechanisms: by reducing biomass accumulation throughout the life cycle, by reducing biomass partitioning to harvestable organs, and, in extreme cases, by causing plant death (Witcombe *et al*. 2008). However, climate predictions generally agree that we can expect an increase not only in mean temperatures but also in climatic variability (Battisti and Naylor 2009; Gornall *et al*. 2010). We will experience more extreme weather events such as extreme temperatures, extended periods of drought, and larger precipitation events. Extreme events are statistically rare climatic episodes (e.g. temperatures higher or lower than 95% of the temperatures historically recorded), which can irreversibly affect the normal function of an organism (Diez *et al*. 2012). In plants, extreme temperatures may affect establishment, growth rate, crop yields and survival (Deryng *et al*. 2014; Niu *et al*. 2014; Vara Prasad *et al*. 2017). In this era of climate change, then, it becomes important to discern responses of crops to extreme conditions.

Stress tolerance is the plant's ability to maintain growth or yield despite the presence of an abiotic stress (Mickelbart *et al*. 2015). Plant tolerance to extreme temperatures is commonly increased after exposure to stressful, but non-lethal, temperatures, a process called acclimation (Thomashow 2010; Mickelbart *et al*. 2015) or acquired thermotolerance (Senthil-Kumar *et al*. 2003; Yeh *et al*. 2012). The duration of the acclimation process might vary from hours for heat stress (HS) (Senthil-Kumar *et al*. 2003; Rampino *et al*. 2009) to days for freezing stress (FS) (Byun *et al*. 2014; Robison *et al*. 2017). During acclimation, the expression level of many stress-responsive genes is altered in

order to deal with the ongoing stress (Hua 2009; Rampino *et al*. 2009; Thomashow 2010; Jia *et al*. 2016).

Acclimated or not, annual crops may have to experience extreme temperatures. For annual crops, the last frost date in spring determines both the flowering date of winter–spring crops (e.g. wheat) and the planting date of summer crops (e.g. maize, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.; and sunflower, *Helianthus annuus* L.). Although many assume that summer crop species cannot tolerate frosts, the increased incidence of extreme climatic events (including late frosts) has renewed the interest in breeding FS tolerance in summer crops (Li *et al*. 2016; Robison *et al*. 2017; Miladinović *et al*. 2019). Thus, it is essential to improve understanding of the tolerance of our crops to abiotic stresses, how tolerance changes through the life cycle, and what the impacts on productivity tend to be. Wild populations may prove to be a useful source of this FS tolerance for crop improvement. In wild populations, seed dormancy and germination timing have been selected to maximise fitness, in part by matching the reproductive phases to the most favourable period of growth (Donohue 2014; Hernández *et al*. 2017), sometimes exposing early vegetative phases to stressful conditions.

In cultivated populations, it is the management of planting date by farmers that, in part, determines the environmental conditions accompanying various life stages. With summer crops, farmers reduce the chance of stressful conditions during the reproductive phase (especially heat and drought stress) by tending to sow crops as soon possible after the last frost date (Andrade 1995; Parker *et al*. 2017) or during early summer (Bonelli *et al*. 2016; Gambin *et al*. 2016). However, these planting windows can expose the early life stages of crops (i.e. seeds and seedlings) to FS in early planting dates or HS in late planting dates. Nevertheless, changing the planting date is one of the easiest and most effective ways to mitigate climatic risks associated with global change (Dobor *et al*. 2016; Zhang *et al*. 2019), although the constraints imposed on that strategy by extreme temperatures have already been recognised. For example, Acharjee *et al*. (2019) pointed out that delaying planting of Boro rice in Bangladesh reduces water requirements of the crop, thereby increasing both water-use efficiency and yields; however, this strategy requires currently non-existent, heat-tolerant cultivars. Similarly, Parker *et al*. (2017) showed that earlier planting dates of maize in Germany can be associated with higher yields, but also with higher risk of cold stress during early vegetative phases. Therefore, improving extreme-temperature tolerance during early plant phases will facilitate the use of different sowing dates in the new cultivars.

Sunflower is the fourth most important oilseed crop, with Ukraine, Russia, the European Union and Argentina as the four most important producers (FAOSTAT 2020). Recognised as a drought-tolerant crop, sunflower production is often restricted to marginal, rainfed areas where water scarcity makes soybean and maize production less attractive, and heat stress during growing season is common (Debaeke *et al*. 2017). This is true in Argentina where, in the last 25 years, soybean has supplanted sunflower in the central and most productive regions (Hall *et al*. 2013; de la Vega *et al*. 2007). Argentina's now-marginal sunflower production areas vary from subtropical environments in the north (24–26°S) to cold-temperate environments in the south (36–38°S). These areas are characterised by both lower suitability for agriculture and higher climatic variation (de la Vega *et al*. 2007; Castaño 2018). In the north, winter sowings avoid extreme heat events during crop establishment, whereas in the south, owing to the narrow frost-free period, establishing sunflower often faces extreme heat events.

Although there may be variation in existing cultivars for tolerance to FS and HS, it is important seek other sources of genetic variation for extreme-temperature tolerance. The process of domestication derived crop phenotypes from the wild (Hancock 2012; Meyer and Purugganan 2013), generally increasing seed size, reducing seed dormancy, reducing shattering of seeds, increasing the rate of early growth, and maximising reproductive allocation (Mercer *et al*. 2007; Hancock 2012; Presotto *et al*. 2012; Hernández *et al*. 2017). Increased resource allocation to growth may have reduced resource allocation to biotic stress tolerance (Agrawal *et al*. 2015) and/or abiotic stress tolerance (Koziol *et al*. 2012). Thus, wild progenitors and relatives of crops may be an excellent source of genetic variation for abiotic stress tolerance. In fact, trade-offs between growth and stress tolerance have been suggested as a major factor in distribution of wild species (Koehler *et al*. 2012; Oakley *et al*. 2014; Wos and Willi 2015). For example, populations from higher latitudes may exhibit high freezing tolerance accompanied by growth penalties, whereas relaxed selection on freezing tolerance at lower latitudes may have resulted in populations that allocate more resources to growth (Koehler *et al*. 2012; Oakley *et al*. 2014). Such trade-offs have been recently suggested as important for domestication and evolution of weedy sunflower (Mayrose *et al*. 2011; Koziol *et al*. 2012; Presotto *et al*. 2017). Understanding these trade-offs between growth and stress tolerance could help in the prediction of likely constraints on improvement of genetic stress tolerance.

We sought to improve understanding of heat and freezing tolerance of an annual crop and its wild relative by performing multiple laboratory and field experiments with sunflower (cultivated and wild), as well as with various generations of crop–wild hybrids. Our aims were: (*i*) to develop a high-throughput technique for evaluating the tolerance of wild, crop–wild hybrid and cultivated sunflower to extreme temperatures (HS and FS) during early plant life stages; and (*ii*) to determine the degree to which early growth under benign conditions trades off with the tolerance to extreme temperatures. Understanding how the crop and its wild relative respond to extreme temperatures will help breeders to improve use of wild genetic resources in crop breeding.

Materials and methods

We performed 11 experiments testing various combinations of genetic materials with FS and HS under growth-chamber and field conditions.

Plant material

Wild and cultivated sunflower achenes (hereafter referred to as seeds) were used in all 11 experiments, and crop–wild hybrids of various generations in five (Expts 3, 4, 9, 10, 11; Table 1). In

Table 1. Detailed information of treatments and biotypes included in each experiment

Among wild biotypes, AAL (Adolfo Alsina), BAR (Colonia Barón), BRW (Barrow), DIA (Diamante), LMA (Las Malvinas) and RCU (Río Cuarto) are invasive populations collected in central Argentina (Cantamutto *et al*. 2010; Casquero *et al*. 2013); KS (Kansas) is a mixture of 10 populations collected around Lawrence, KS, USA; and IN and IL are populations from the USA provided by the USDA (PI 468633 and 435540, respectively). Among cultivated biotypes, HS03 and VDH487 are commercial cultivars used in Argentina, and HA89 and B59 are public inbred lines from the USA and Argentina, respectively. BC_W, F_1 backcrossed with the wild parent; BC_C, F_1 backcrossed with the crop parent; NH, natural hybrid collected near the BAR population, identified as a natural crop–wild hybrid according to its morphological characterisation. For field experiments, provided temperature is the average of maximum and minimum temperatures (for HS and FS, respectively) during the experiments

Stress type	Expt	Temperature	Duration	Environment	Wild biotypes	Cultivated biotypes	Crop–wild hybrids
Heat stress (HS)	1	52°C, 54°C	2 h, 3 h	Growth chamber	AAL, BRW, DIA, LMA, RCU	HS03, VDH487	–
	2	52°C	3 h	Growth chamber	AAL, BRW, DIA, LMA, RCU	HS03, VDH487	–
	3	54°C	3 h	Growth chamber	BAR, DIA, BRW	HS03, VDH487	F_{1DIA}(HS03 × DIA), F_{1BAR}(HS03 × BAR), $BC_W(F_{1DIA} \times DIA)$, $BC_W(F_{1BAR} \times BAR)$, NH
	4	43.5°C	10 days	Field	DIA	HS03	F_1(HS03 × DIA), F_1(DIA × HS03)
Freezing stress (FS)	5	–2°C, –4°C	2 h, 3 h, 4 h	Growth chamber	AAL, DIA	HA89	–
	6	–4°C	4 h	Growth chamber	AAL, BRW, DIA, IL, IN	HS03, VDH487	–
	7	–4°C	4 h	Growth chamber	DIA, LMA	HS03, VDH487	–
	8	–4°C	4 h	Growth chamber	BRW, DIA, LMA, RCU	B59	–
	9	–4°C	4 h	Growth chamber	BAR, DIA, BRW	HS03, VDH487	F_{1DIA}(HS03 × DIA), F_{1BAR}(HS03 × BAR), $BC_C(F_{1DIA} \times HS03)$, $BC_C(F_{1BAR} \times HS03)$, $BC_W(F_{1DIA} \times DIA)$, $BC_W(F_{1BAR} \times BAR)$, NH
	10	3.8	32 days	Field	DIA	HS03	F_1(HS03 × DIA), F_1(DIA × HS03)
	11	–2.5	3 h	Growth chamber	KS	HA89	F_1(KS × HA89), $BC_C(F_{1KS} \times HA89)$, $BC_W(F_1 \times KS)$

10 of the experiments (not Expt 11), wild seeds were sourced from six invasive populations collected from contrasting environments in central Argentina: Adolfo Alsina (AAL), Colonia Barón (BAR), Barrow (BRW), Diamante (DIA), Las Malvinas (LMA), and Rio Cuarto (RCU) (Cantamutto *et al*. 2010; Casquero *et al*. 2013). In Expts 6 and 11, wild populations were sourced from the USA: Indiana (IN) and Illinois (IL) populations were provided by the USDA (PI 468633 and 435540, respectively); Kansas (KS) seeds were a mixture of 10 populations collected around Lawrence. Cultivated biotypes included HS03 and VDH487, which are commercial cultivars used in Argentina, as well as HA89 and B59, which are public inbred lines from the USDA Sunflower Breeding Program (Fargo, ND, USA) and the INTA Germplasm Bank (Manfredi, Córdoba, Argentina), respectively. For crop–wild hybrids, we used naturally occurring hybrids, as well as hybrids produced by hand-pollination as described below. Hand-pollinated hybrids were from various backgrounds and of four cross types: F_1 hybrids produced on wild and cultivated plants, and backcrosses of the F_1 with the wild (BC_W) and cultivated (BC_C) parents.

For Expts 1–10 (Table 1), wild seeds were produced in a common garden (Hernández *et al*. 2017) at the Departamento de Agronomía, Universidad Nacional del Sur, Bahia Blanca (38°41′38″S, 62°14′53″W), Argentina. For Expts 1–3 and 5–9, wild seeds were produced during the 2014–15 growing season, and for Expts 4 and 10, wild seeds were produced during the 2016–17 growing season. The populations included the five from Argentina and the Illinois and Indiana populations from the USA. Wild seeds were produced under controlled pollination of the heads of at least 15 plants and were covered with paper bags at the pre-flowering stage, following Presotto *et al*. (2014). At the flowering stage, heads were hand-pollinated with pollen from sibling plants on days 3, 5 and 7 after the beginning of flowering. Two inbred lines (HA89 and B59) and two commercial cultivars (HS03 and VDH487) represented the cultivated panel. Cultivated seeds used in Expts 1–10 were obtained from seed suppliers (commercial cultivars, HS03 and VDH487) or produced by self-pollination (inbred lines, HA89 and B59) in the same common garden as wild seeds by covering the heads with paper bags at the pre-flowering stage.

In Expts 3 and 9, the panel of crop–wild hybrids was represented by F_1 hybrids (HS03 as ♀ and both BAR and DIA as ♂) and backcrosses of the F_1 (♀) with the cultivated parent (BC_C, HS03 as ♂, only in Expt 9) or the wild parent (BC_W, BAR and DIA as ♂). In addition, one natural hybrid collected near the BAR population was included, identified as a natural crop–wild hybrid according to its morphological characterisation. All seeds were the result of hand-pollinations as described in Hernández *et al*. (2017). Briefly, at flowering, we emasculated the heads in the morning and pollinated during late afternoon with the corresponding pollen source to produce crop–wild seeds.

For Expt 11, the KS wild population and cultivated seeds from HA89 were used, as well as F_1 hybrids (wild ♀) and backcrosses of the F_1 (♂) with the wild (BC_W) and cultivated (BC_C) parents. All seeds were the result of hand-pollinations

as described in Pace *et al*. (2015). Details of cross types and biotypes included in each experiment are provided in the Table 1.

Experimental design

With this array of genetic material, we performed a number of experiments: four testing for HS tolerance, and seven testing for FS tolerance. Most experiments were completed in growth chambers, but one experiment was performed in the field for each stress: Expt 4 for HS, and Expt 10 for FS. Experiments under controlled conditions were done in three growth chambers. The HS experiments (Expts 1–3) used a growth chamber of 700 L volume (160 cm high, 80 cm wide, 55 cm deep), with four removable racks provided with two fluorescent tubes (36–40 W) on each rack and a temperature range of 4°C–56°C (±0.5°C). Six of the FS experiments (Expts 5–9) used a growth chamber of 450 L volume (210 cm high, 69 cm wide, 66 cm deep), with four to six removable racks, eight fluorescent tubes (36–40 W), and a temperature range of –5°C–40°C (±0.5°C). One FS experiment (Expt 11) was performed in a growth chamber of 800 L volume (196 cm high, 85 wide, 85 cm deep), four to six removable racks, one tier of lighted shelving lit by LEDs, and a temperature range of –10°C–44°C (±0.5°C).

The optimum treatments for screening HS and FS tolerance under controlled conditions (i.e. in the growth chamber) were determined in Expts 1 and 5, respectively, and the performance of wild and cultivated sunflower under controlled conditions was then evaluated in Expts 1 and 2 (against HS) and Expts 5–8 (against FS). The performance of wild, cultivated and crop–wild hybrid sunflower against HS and FS under field conditions was evaluated in Expts 4 and 10, respectively. Finally, Expts 3 and 9 and 11 were performed to evaluate the trade-off between early growth under benign conditions and tolerance to extreme temperatures for wild, cultivated and crop–wild hybrid sunflower.

In growth-chamber experiments, the biotypes noted in Table 1 were grown in groups of 20 pre-germinated seeds (experimental unit) planted into 20 adjacent cells (15 cm^2) within 200-cell plastic trays (54 by 28 cm; Fig. 1*a*). Experimental units were arranged in a completely randomised design with three or four replicates. In order to avoid the uncontrolled effect of non-uniform temperatures within the growth chamber, plastic trays were rotated to different racks

Fig. 1. Overview of plots used in different experiments: (*a*) plants growing in plastic trays used in growth chamber experiments, (*b*) plants growing in 2.5-L pots used to evaluate heat stress in the field, (*c*) plants growing in 10-L pots to evaluate freezing stress in the field, (*d*) manual thermo-hygrometer used to record temperature and relative humidity.

during experiments. Seedlings were grown in the greenhouse at 20–25°C until the 4-leaf stage, at which time, plants were subjected to an acclimation treatment. For HS experiments, this consisted of increasing temperatures from 28°C to 42°C over 4 h (28°C for 1 h, 40°C for 1 h, 42°C for2 h; adapted from Senthil-Kumar *et al*. 2003), and for FS experiments, exposing plants for 4 days to cold, but not freezing, temperatures (constant 4°C with 12 h light and 12 h dark). After acclimation, plants were watered in HS but not in FS experiments, and the number of plants per biotype and replicate was recorded. The plants were then transferred to the target temperature. After treatment, plants were transferred back to the greenhouse. After 5 days of recovery, the number of plants per biotype and replicate was recorded. Plant survival was estimated as the proportion of plants surviving in an experimental unit and ranged from 0 (no plant survival) to 1 (no effect of HS or FS on plant survival).

Other response variables were measured in the growth-chamber experiments. Biomass was collected by cutting entire plants at ground level, drying the tissue at 60°C for 7 days, and weighing. Leaf width was measured on the newest fully expanded leaf, and plant height was measured from ground level to the uppermost leaf scar. We also predicted biomass of each biotype from leaf width and plant height with multiple linear regression models. This reduced the number of plants required for destructive sampling. Morphometric variables were recorded on at least 30 plants per biotype (10 plants per replicate), and biomass was recorded in at least six of these 30 plants (two per replicate). Multiple linear regression models were run using these six plants per biotype:

$$y = b0 + b1 \times x1 + b2 \times x2$$

where y is aboveground biomass; $b0$ is the intercept; $b1$ and $b2$ are slopes of the multiple linear regression; and $x1$ and $x2$ are leaf width and plant height, respectively. Multiple regression models were estimated for each experiment by pooling all biotypes (full model) or pooling biotypes within each cross type (three cross-type models: cultivated, wild and crop–wild). The R^2 values from multiple regressions were 0.85 for the full model and 0.81, 0.9, and 0.76 for cultivated, wild and crop–wild models, respectively. Biomass was estimated independently by using parameters from full and cross-type models. Afterward, we evaluated the accuracy of biomass estimation with plants used for constructing models by estimating Pearson correlation coefficients between actual and estimated biomass. Biomass estimated using the full model showed a higher correlation (r = 0.92) with actual biomass than did biomass estimated using cross-type models (r = 0.66). Therefore, we used mean values of leaf width and plant height of each experimental unit (averaged from eight plants, excluding plants used for model construction) and full-model parameters ($b0$, $b1$, and $b2$) for estimating aboveground biomass.

Application of heat stress in growth-chamber experiments

In Expts 1–3, we evaluated the performance of wild, cultivated and crop–wild hybrid sunflower against HS. In Expts 1 and 2, wild and cultivated cross types were represented by five wild populations and two commercial cultivars. In Expt 3, wild and cultivated cross types were represented by three wild populations and two commercial cultivars, and crop–wild hybrids were represented by five biotypes including F_1s, BCs with wild parents (BC_W) and a natural hybrid (Table 1). In Expt 1, after plants were acclimated, they received one of four HS treatments: HS1 (52°C for 2h), HS2 (52°C for 3h), HS3 (54°C for 2h), and HS4 (54°C for 3h). Expts 2 and 3 each had only one treatment: HS2 in Expt 2 and HS4 in Expt 3. In Expt 1, treatments were applied in the same growth chamber during two successive days (two treatments per day). In all experiments, all of the replicates of the same treatment were simultaneously acclimated and evaluated in the same growth chamber.

Application of freezing stress in growth chamber experiments

In Expts 5–9 and 11, we evaluated the performance of wild, cultivated and crop–wild sunflower against FS. Wild and cultivated sunflowers were represented by two to five wild populations and one or two cultivars, and crop–wild hybrids were represented by seven biotypes, including F_1s, backcrosses with cultivated (BC_C) and wild (BC_W) parents, and a natural hybrid (Table 1). In Expt 5, after plants were acclimated, they received one of four treatments: FS1 (–2°C for 2 h), FS2 (–2°C for 3 h), FS3 (–4°C for 3 h), and FS4 (–4°C for 4 h). Expts 6–9 and 11 each had only one treatment. In Expt 5, treatments were applied in the same growth chamber during two successive days (two treatments by day). In all experiments, all of the replicates of the same treatment were simultaneously acclimated and evaluated in the same growth chamber.

Heat-stress field experiment

In Expt 4, DIA (wild), HS03 (cultivated) and their reciprocal crosses (crop–wild hybrids) were randomly assigned in six replicates of 12 plants per treatment (control and HS). Plants were established by sowing 12 pre-germinated seeds in 15-cm-diameter plastic pots (2.5 L) in a greenhouse up to the 4-leaf stage (Fig. 1*b*). At the 4-leaf stage, six replicates per biotype were transferred to a field-based heat tent (Bergkamp *et al*. 2018) for 10 days (Fig. 1*b*), and six replicates were kept in the greenhouse. Experimental units were arranged in a completely randomised design. Pots were manually watered every second day in the greenhouse and daily in the heat tent to avoid soil water deficit. The heat tent used was a fixed structure (18 m long, 10 m wide, 4 m high) covered with clear polyethylene film that transmits ~85% of incoming solar radiation (Bergkamp *et al*. 2018). Daily maximum and minimum values of temperature and humidity were recorded in the greenhouse and in the heat tent during 10 consecutive days. After treatment, plants exposed to HS in the heat tent were transferred back to the greenhouse, and after 6 days of recovery, biomass and plant survival was recorded in both treatments. Biomass was collected by cutting entire plants at ground level, drying the tissue at 60°C for 7 days, and weighing. Temperature and relative humidity were measured with a manual thermo-hygrometer (TA298; Fig. 1*d*) located in the middle of the experiment, at the same height as surrounding plants. Daily mean temperature and relative humidity were estimated from the minimum and maximum values. The temperature of the HS treatment was significantly higher than that of the control:

T_{mean} = 23.6°C (control) and 30.1°C (HS), *t*-value = 5.3, $P < 0.0001$; T_{max} = 28.8°C (control) and 43.5°C (HS), *t*-value = 13.9, $P < 0.0001$.

Freezing-stress field experiment

Experiment 10 was part of a larger study of natural selection during winter of wild, cultivated and crop–wild hybrid sunflower (not reported here), from which we took advantage of the low seed dormancy of one wild population (DIA), one commercial cultivar (HS03) and their reciprocal-cross hybrids (Hernández *et al*. 2017) to explore differences in freezing tolerance under natural conditions during winter. Plants of each type were randomly assigned to six replicates of 50 seeds. Plants were established by sowing fresh seeds in groups of 50 in 23-cm-diameter plastic pots (10 L) in the field (Fig. 1*c*). Seeds were collected in late March from a common garden, with biotypes produced by hand-pollination as explained above. Seeds were sown on 20 April (early autumn to simulate natural dispersal). The number of established plants was recorded on 12 June (t1) for each replicate as 26 ± 4 plants, on average, per replicate and biotype; the remaining seeds were ungerminated (dead or dormant). At this time, all of the pots were at the 2-leaf stage and there were no plants with visible damage; therefore, 12 June (t1) was considered the beginning of the experiment. On 14 August (t2), the number of plants was recorded, and the ratio between plant numbers at t2 and t1 (i.e. plant survival) was calculated for each replicate and biotype. Temperatures between t2 and t1 were T_{mean} = 9.9°C ± 0.6°C, T_{max} = 16.9°C ± 0.7°C, and T_{min} = 3.9°C ± 0.8°C. During this period, sub-zero T_{min} was recorded on 3 days: –2.4°C on 31 July, –0.2°C on 1 August, and –1.2°C on 3 August.

Statistical analyses

Generalised linear models (GLM) or generalised linear mixed models (GLMM) were fitted with PROC GLM and PROC GLIMMIX, respectively (SAS University Edition; SAS Institute, Cary, NC, USA). GLMM were used to incorporate random effects when necessary. When input data were proportions (e.g. plant survival), data were arcsine-transformed to improve homoscedasticity.

Selection of optimum treatment for screening extreme temperatures tolerance

We used the first model to select the optimum temperatures for use in subsequent experiments. Expts 1 and 5, consisting of four treatments (HS1–HS4 and FS1–FS4, respectively), were used (Fig. 2*a*, *b*). GLM were run that included treatment, biotype and treatment × biotype as fixed factors in the model. Significant differences between treatments were evaluated, using Tukey–Kramer adjustment for multiple comparisons, and

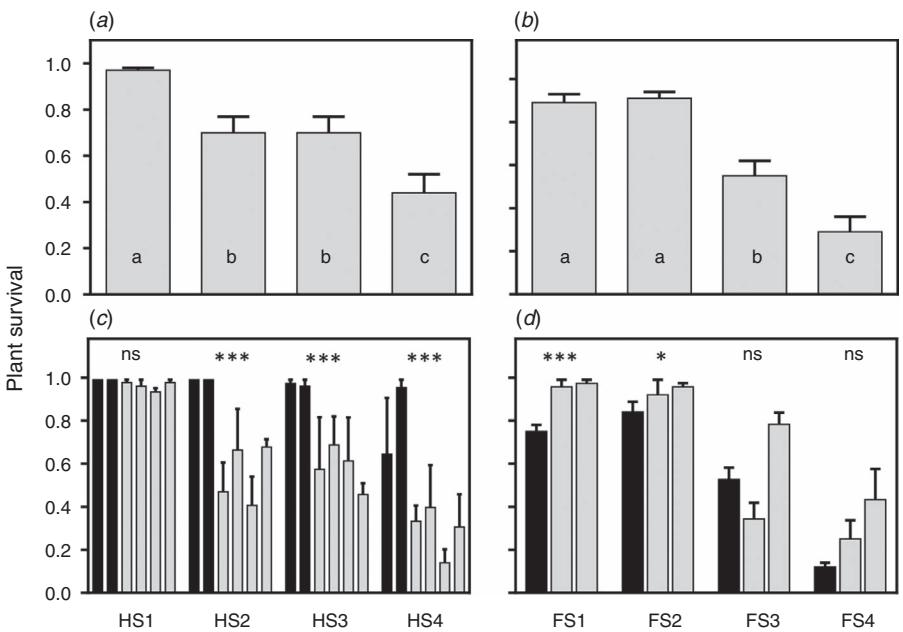

Fig. 2. Selection of the optimum treatment for screening tolerance to extreme temperatures. Main effect of (*a*) heat stress (HS) and (*b*) freezing stress (FS) treatment on mean plant survival; capped vertical lines are standard errors; means with the same letter are not significantly different, using Tukey–Kramer adjustment from multiple comparisons. Differential biotype responses to (*c*) HS (biotypes from left to right: HS03, VDH487, AAL, BRW, DIA, LMA), and (*d*) FS (biotypes from left to right: HA89, AAL, DIA). Black bars represent cultivated biotypes and grey bars wild biotypes. *$P < 0.05$; ***$P < 0.001$; n.s., not significant: for significance of differences between these two cross types, after contrasts for each treatment. Treatments are: HS1, 52°C for 2 h; HS2, 52°C for 3 h; HS3, 54°C for 2 h; HS4, 54°C for 3 h; FS1, –2°C for 2 h; FS2, –2°C for 3 h; FS3, –4°C for 3 h; FS4, –4°C for 4 h. All treatments were applied after acclimation (28°C for 1 h, 40°C for 1 h, 42°C for 4 h for HS, and 4 days at constant 4°C for FS).

differences between cultivated and wild cross types were evaluated by using contrasts for each treatment.

Divergence among cross types in response to extreme temperatures

With our second model, we aimed to discern the difference among cross types (wild vs cultivated) in their response to extreme temperatures, analysing Expts 1 and 2 for HS and Expts 5–8 for FS (Table 1). The biotype was considered fixed, and in the two experiments with different temperature treatments (Expts 1 and 5), temperature treatment was considered random, using GLMM instead of GLM. Differences between cultivated and wild types were evaluated by using contrasts. The presence of a trade-off between growth and stress tolerance (as in Presotto *et al*. 2017) was evaluated through analyses of covariance (ANCOVA) including (or not) biomass before stress as a continuous covariate. If the effect of the covariate was significant and explained differences between cross types (by reducing the sum of squares of the contrast between wild and cultivated), we interpreted these results to suggest a trade-off. With regard to the field experiments, treatment, biotype (DIA, HS03 and their reciprocal hybrids) and treatment × biotype effects were considered as fixed in the HS experiment (Expt 4), whereas in the FS experiment (Expt 10), biotype was the only source of variation and it was considered as fixed.

Relationship between the percent of crop alleles and stress tolerance

With our third model, we inquired into the relationship between the percentage of crop alleles in the biotypes and their resulting stress tolerance, through one experiment for HS (Expt 3) and two experiments for FS (Expts 9 and 11, using biotypes from Argentina and USA, respectively). In Expts 3 and 9, respectively, biotypes were pooled within four cross types (wild, cultivated, F_1, and BC_W) and five cross types (wild, cultivated, F_1, BC_C and BC_W), and the natural hybrid was considered as F_1. In Expt 11, each cross type (cultivated, F_1, BC_C and BC_W) was represented by a pooled sample of biotypes. Thus, in Expts 3 and 9, cross-type effect was considered as fixed. In addition, the continuous predictor variable of percentage of crop alleles typical of each biotype was included to evaluate the relationship between the percentage of crop alleles and biomass before the application of stress, as well as subsequent plant survival. Percentages of crop alleles were as follows: 0%, wild populations; 25%, F_1 crop–wild hybrids backcrossed with their wild parent; 50%, F_1 crop–wild hybrids; 75%, F_1s backcrossed with their crop parent; 100%, cultivated varieties (commercial cultivars or inbred lines).

Results

Selection of the optimum treatment for screening tolerance to extreme temperatures

Heat stress

Significant differences in plant survival occurred among treatments ($F = 7.84$, $P = 0.0002$). Plant survival decreased with HS treatment intensity (Fig. 2*a*) from 0.97 ± 0.01 (HS1) to 0.44 ± 0.08 (HS4). Treatments of intermediate intensity (HS2 and HS3) produced similar and intermediate plant-survival values (0.70 ± 0.07, Fig. 2*a*). Biotype effect was significant ($F = 12.63$, $P < 0.0001$), but not biotype × treatment interaction ($F = 1.42$, $P = 0.1759$), indicating similar response to temperature among biotypes. Genetic effect was mainly explained by differences between wild and cultivated cross types; plant survival was much higher in cultivated than wild types in three of four treatments (Fig. 2*c*). Therefore, three of four treatments (52°C for 3 h and 54°C for 2 h and 3 h) were useful for evaluating genetic differences in HS tolerance.

Freezing stress

Significant differences in plant survival occurred among treatments ($F = 51.35$, $P < 0.0001$). Plant survival was similar for treatments at –2°C (~0.90 for FS1 and FS2, Fig. 2*b*), but decreased with treatment intensity (0.55 ± 0.07 for FS3 and 0.29 ± 0.07 for FS4, Fig. 2*b*). In addition, biotype effect was significant ($F = 11.86$, $P = 0.0003$), but not biotype × treatment interaction ($F = 2.29$, $P = 0.1011$), indicating similar temperature response among the three biotypes evaluated. Plant survival was higher in wild than cultivated types in two of four treatments (FS1 and FS2, Fig. 2*d*), but these treatments produced high levels of plant survival. Therefore, the most intense treatments (FS3 and FS4, Fig. 2*b*, *d*), by inducing lower plant survival, were the most accurate for evaluating genetic differences in FS tolerance (Fig. 2*d*).

Evolutionary divergence in the response to extreme temperatures

Heat stress

Significant differences in plant survival were found among biotypes, mostly explained by differences between cultivated and wild cross types (Fig. 3*a*, *b*). The cultivated cross types exhibited greater HS tolerance than the wild cross types (survival means 0.94 ± 0.18 vs 0.60 ± 0.32 in Expt 1 and 0.64 ± 0.35 vs 0.29 ± 0.26 in Expt 2). In addition, generally there were no differences between wild biotypes or between cultivars (Fig. 3*a*, *b*). In Expt 1, both cultivars significantly outperformed wild biotypes, although in Expt 2, only HS03 significantly outperformed wild biotypes (Fig. 3*a*, *b*). Inclusion of biomass as a covariate explained differences between cross types observed in Expt 2 (contrast wild vs crop: $F = 0.13$, $P = 0.7262$), but not in Expt 1 (contrast wild vs crop: $F = 15.11$, $P = 0.0002$).

Freezing stress

Significant differences in plant survival were found among biotypes in two of four experiments (Expts 5 and 8, Fig. 4*a*, *d*); such differences were mostly explained by differences between cultivated and wild cross types. Plant survival was very low in Expts 6 and 7 (Fig. 4*b*, *c*) and no significant biotype effects were found. Despite this, wild biotypes tended to exhibit greater mean FS tolerance than cultivated types in all experiments (Fig. 4). DIA showed higher FS tolerance in

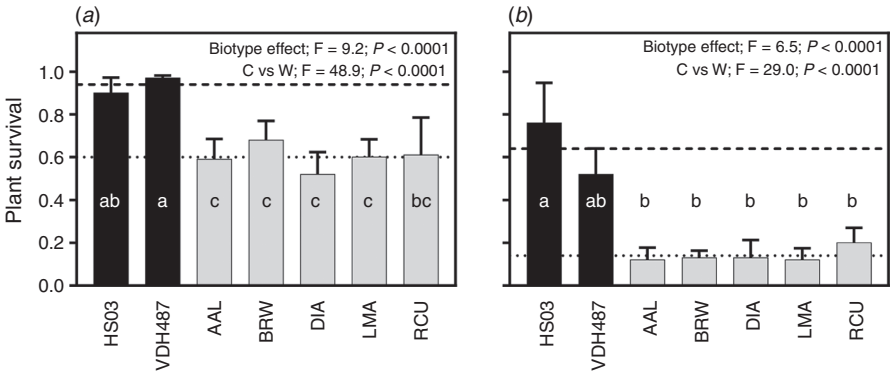

Fig. 3. Genetic variation for heat stress tolerance as measured by plant survival in (*a*) Expt 1 and (*b*) Expt 2. Black bars represent cultivated biotypes and grey bars wild biotypes. Capped vertical lines are standard errors. Biotype means with the same letter are not significantly different, using Tukey–Kramer adjustment from multiple comparisons. Dashed lines indicate mean plant survival of cultivated types, and dotted lines wild types; differences between cultivated and wild types were evaluated using contrasts (C vs W).

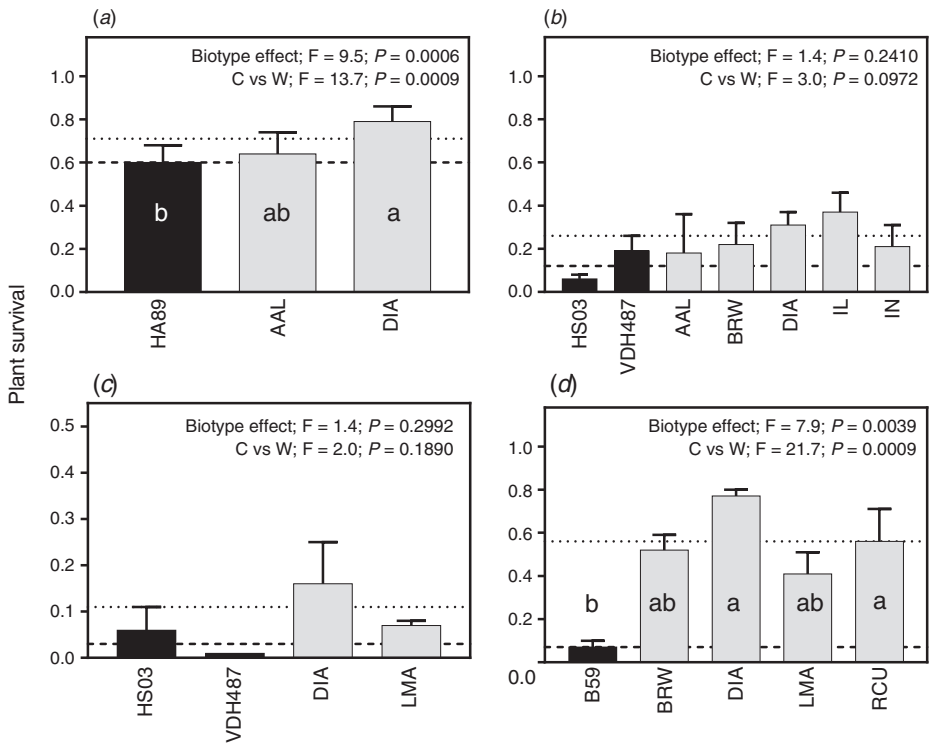

Fig. 4. Genetic variation for freezing stress tolerance as measured by plant survival in (*a*) Expt 5, (*b*) Expt 6, (*c*) Expt 7, (*d*) Expt 8. Black bars represent cultivated biotypes and grey wild biotypes. Capped vertical lines are standard errors. Biotype means with the same letter are not significantly different, using Tukey–Kramer adjustment from multiple comparisons. Dashed indicate mean plant survival of cultivated types and dotted lines wild types; differences between cultivated and wild types were evaluated using contrasts (C vs W).

three of four experiments (Fig. 4). When biomass was included as a covariate, a significant cross-type effect remained in two of four experiments ($F = 9.76$, $P = 0.0261$ and $F = 5.61$, $P = 0.042$ for Expts 6 and 8, respectively), indicating that differences between wild and cultivated types in FS tolerance are not explained by differences in biomass.

Relationship between percentage of crop alleles and stress tolerance

Heat stress

In the growth-chamber experiment (Expt 3), we pooled all biotypes within the four cross types (wild, cultivated, F_1 and

BC_W) and analysed the cross-type effect. There was a significant cross-type effect for biomass accumulated before stress (F = 23.13, P < 0.0001), but not for plant survival (F = 1.25, P = 0.3106). However, plant survival was intermediate in crop–wild hybrids with respect to the parents. The cultivated cross type exhibited higher HS tolerance than crop–wild hybrids and wild cross types (survival: cultivated 0.92 ± 0.03, hybrid 0.79 ± 0.05, wild 0.72 ± 0.07). When biomass and plant survival were separately regressed on the percentage of crop alleles, there was a significant and positive relationship between percentage of crop alleles and both biomass (Fig. 5*a*) and plant survival (Fig. 5*b*). In the field (Expt 4), the biotype effect on biomass was significant (F = 8.29; P = 0.0011), especially due to the higher biomass of the cultivated biotype (Fig. 6*a*), but temperature treatment and treatment × biotype effects were not significant (treatment: F = 0.00, P = 0.9514; treatment × biotype: F = 0.18, P = 0.9114). Therefore, HS in the field did not affect plant survival or biomass accumulation.

Freezing stress

In the two growth-chamber experiments, we pooled biotypes within four cross types (Expt 11: cultivated, F_1, BC_C and BC_W) and five cross types (Expt 9: wild, cultivated, F_1, BC_C and BC_W). A significant cross type effect was found in Expt 11, with biotypes from the USA (F = 5.39, P = 0.0158), but not in Expt 9, with biotypes from Argentina (F = 0.23, P = 0.9195). However, the wild cross type exhibited higher FS tolerance than both cultivated and crop–wild cross types (mean survival: wild 0.49 ± 0.02, cultivated: 0.39 ± 0.04, hybrid: 0.39 ± 0.05). The percentage of crop alleles explained differences in plant survival in Expt 11 (Fig. 5*c*) but not in Expt 9 (Fig. 5*d*). In the field (Expt 10), plant survival was significantly (F = 21.99; P < 0.0001) higher in the wild than the cultivated type (0.85 ± 0.09 and 0.13 ± 0.02). We also found maternal effects on plant survival: F_1 hybrids produced on wild mother plants exhibited higher FS tolerance than F_1 hybrids produced on cultivated mother plants (0.91 ± 0.03 vs 0.44 ± 0.10, Fig. 6*b*).

Discussion

Development of a high-throughput technique for screening tolerance to extreme temperatures

With the dramatic decreases in genotyping costs and the availability of commercial single-nucleotide polymorphism (SNP) arrays for most crops, high-throughput phenotypic techniques appear as a major bottleneck in current plant-breeding programs for stress tolerance (Zhu *et al*. 2008; Anderson *et al*. 2016; Fan *et al*. 2016). In the present work, we developed a high-throughput technique for evaluating tolerance to extreme temperatures. With this technique,

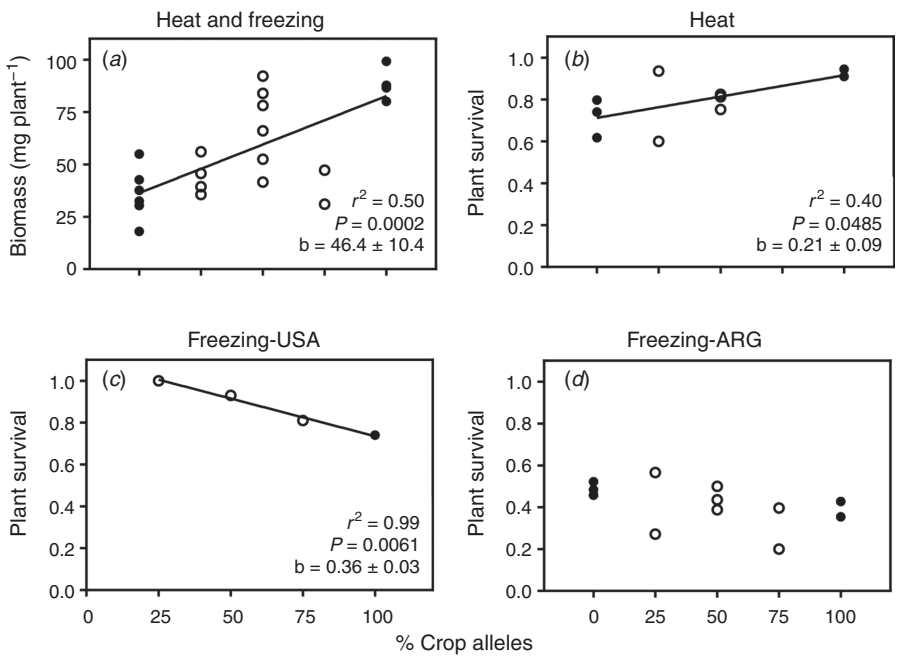

Fig. 5. Relationship between the percentage of crop alleles and (*a*) plant biomass before stress and (*b–d*) plant survival in growth-chamber experiments: (*b*) Expt 3 (heat stress with biotypes from Argentina), (*c*) Expt 11 (biotypes from USA), (*d*) Expt 9 (biotypes from Argentina). Each point represents the mean value of a biotype; filled circles are wild and cultivated biotypes, open circles are crop–wild hybrids. Percentage crop alleles: 0%, wild populations; 25%, F_1 crop–wild hybrids backcrossed with their wild parent; 50%, F_1 and F_2 crop–wild hybrids; 75%, F_1 crop–wild hybrids backcrossed with their crop parent; 100%, cultivated varieties. Values of r^2, P, and b (slope) are presented for significant linear regressions. Note that no wild biotypes were included in (*c*).

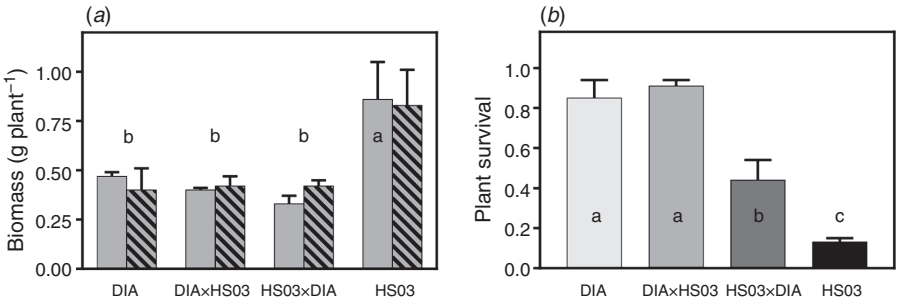

Fig. 6. Genetic variation in (*a*) heat-stress (Expt 4) and (*b*) freezing-stress (Expt 10) tolerance. In (*a*), treatments are greenhouse (shaded bars) and heat tent (hatched bars). Means with the same letter are not significantly different for (*a*) average biomass and (*b*) plant survival between biotypes using Tukey–Kramer adjustment from multiple comparisons. Note that in (*a*), treatment and treatment × biotype effects were non-significant.

400–1200 plants (depending on the size of growth chamber) may be grown up to the 4-leaf stage in just 25–30 days; thus, running one experiment takes ~1 month. Compared with field-based experiments, those in a growth chamber can be performed throughout the year, regardless of the climatic conditions, further increasing throughput (Yeh *et al*. 2012; Chopra *et al*. 2017). Furthermore, owing to the unpredictable occurrence of extreme events, assessment of crop responses to natural extreme events in the field requires breeders and researchers to evaluate genotypes in many environments and years (Chen *et al*. 2017), increasing costs and duration of stress-tolerance breeding programs. For HS, we adapted the temperature induction-response technique (Senthil-Kumar *et al*. 2003, 2007) previously used for evaluating HS tolerance at the seedling stage (2–3 days old seedlings) for use at the 2–4 leaf stages (Fig. 1*a*). For FS, we developed a similar approach (acclimation treatment before stress).

In plant breeding, optimal environments for selection must have at least two basic features. First, they must allow the expression of genetic diversity in order to ensure that genetically superior genotypes are distinguishable from genetically inferior genotypes. Second, the performance of genotypes within selection and target environments should correlate. These two basic features are also necessary in research experiments when extrapolating results from controlled conditions to natural environments. With our assays, we were able to control the intensity of the stress, and we uncovered genetic differences in HS and FS tolerance. In particular, under HS, three of four treatments allowed the expression of genetic diversity, and therefore, they were useful for discriminating biotypes with genetic differences in HS tolerance. Under FS, the lowest intensity treatments allowed differentiation between cultivated and wild sunflower; however, the highest intensity treatments induced high plant mortality, which might be desirable for uncovering genetic differences in larger collections. Of course, with different genetic materials or with another species, the temperatures used might need to be revised.

Here, extreme temperatures were imposed on plants in a single phenological phase, with a few combinations of intensity and duration of stressful temperature. However, in the field, crops may be exposed to multiple extreme temperature events, varying in intensity and duration and occurring at different parts of the life cycle (Barlow *et al*. 2015). In addition, such events may occur in combination with other stresses, in particular with drought stress (Killi *et al*. 2017). Therefore, we propose that high-throughput seedling phenotyping be used in combination with more varied evaluation that combines exposure of plants to different stress intensities and durations at various life stages and after a range of growth conditions. In this way, we may use multiple kinds of experiments to improve prediction of the response of relevant germplasm to current and future climates. Despite the genetic differences we observed for each stress, both wild and cultivated sunflower showed an ability to cope with stress after an acclimation treatment; that is, they showed acquired thermotolerance (Senthil-Kumar *et al*. 2003; Yeh *et al*. 2012). Acquired thermotolerance has previously been reported for summer crops in response to HS (reviewed in Senthil-Kumar *et al*. 2007; Yeh *et al*. 2012), and in response to FS, for example in rice (Baruah *et al*. 2011), soybean (Robison *et al*. 2017) and sunflower (Cabello *et al*. 2012). Although stress-responsive genes have mostly been identified in model species, many orthologous genes have been identified and described in crop species, suggesting that the function of these genes is well conserved in higher plants. For instance, Cabello *et al*. (2012) found evidence of a conserved mechanism of response to freezing stress between *Arabidopsis* and two summer crops (soybean and sunflower), mediated by HD-Zip I transcription factors. Conserved mechanisms in cold-responsive pathways have also been reported in maize (Lu *et al*. 2017) and rice (Ito *et al*. 2006), suggesting that summer crop species have potential for improved freezing tolerance.

Implications for crop breeding

Here, we found genetic variation for FS tolerance under both controlled and field conditions. In the lowest intensity treatments, both cultivated and wild sunflower showed survival values close to 90%, indicating high tolerance to light freezing events. Similarly, Li *et al*. (2016) reported

high variability among cultivated maize germplasm, with up to 97% plant survival after a light simulated frost in the most tolerant line. However, when treatment intensity increased, we observed high variability among experiments, although wild plants clearly outperformed the cultivated plants. In addition, we found variability within the wild germplasm, with DIA generally showing the highest levels of FS tolerance. Similarly, Robison *et al*. (2017) found that wild soybean generally outperformed cultivated varieties under freezing conditions, but that considerable genetic diversity existed within cultivated and wild germplasm. In the present study, we used a limited number of biotypes within each cross type; therefore, further experiments including a larger number of biotypes (both cultivated and wild) are necessary to explore genetic variation for FS tolerance within the primary gene pool of sunflower.

In addition to the differences in FS tolerance between cultivated and wild sunflower, in the field experiment, we observed large maternal genetic effects in FS tolerance (Fig. 6*b*), whereby hybrids produced on wild plants outperformed hybrids produced on cultivated plants. Maternal control of seed traits such as seed size, weight and dormancy has been previously reported (Roach and Wulff 1987; Weiss *et al*. 2013); however, studies of maternal genetic effects on later life-history traits (e.g. seedling traits) are less common (Alexander *et al*. 2014; Singh *et al*. 2017). In the biotypes used here, we showed that reciprocal hybrids differed in seed morphological, anatomical and physiological traits (Hernández *et al*. 2017) and also in seedling traits such as leaf size and seedling height (measured here, data not shown), which can affect plant survival against FS. Maternal genetic effects on FS tolerance have direct implications in plant breeding. Sunflower cultivars are mostly single-cross hybrids, with maintainer (B) and restorer (R) lines being the two major heterotic groups, used as female and male parents, respectively (Mandel *et al*. 2011; Filippi *et al*. 2015). Therefore, if the maternal effect on FS tolerance is confirmed in single-cross cultivars, maintainer lines should be the target of improvements to FS tolerance.

On the other hand, the higher FS tolerance of wild germplasm observed here was not explained by differences in biomass. This absence of a trade-off between growth and stress tolerance is in line with recent findings for flooding tolerance in cultivated sunflower (Gao *et al*. 2019), and may facilitate the introgression of tolerance within elite inbred lines without growth penalties. Despite this, the use of recombinant populations (e.g. crop–wild RILs) or a wider collection of wild populations could unmask growth–stress tolerance trade-offs. In addition, trade-offs that emerged during crop diversification (Meyer and Purugganan 2013) would be observed only by evaluating many cultivated accessions. The sunflower association mapping population (Mandel *et al*. 2011, 2013) may be a valuable resource for investigating genetic variation for FS tolerance and identifying resistant alleles with and without negative effects on growth (Gao *et al*. 2019).

We observed extreme HS tolerance within cultivated sunflower under both controlled and field conditions. Under controlled conditions, temperatures up to 54°C were needed to reduce plant survival, whereas in the field, not even 10 consecutively warm days (T_{mean} = 30.1 ± 0.7°C and T_{max} = 43.5 ± 0.9°C) negatively affected plant survival or biomass accumulation. Within the cultivated germplasm, we observed slight but inconsistent differences between the two cultivars. The increased HS tolerance of cultivated sunflower is probably a byproduct of domestication and modern breeding. During domestication, by planting many seeds, early farmers likely selected for high seedling vigour and rapid crop establishment (Hancock 2012; Meyer and Purugganan 2013; Singh *et al*. 2018). In addition, modern breeding has selected for rapid crop establishment across very different environments (localities and sowing dates), which may lead to stress-tolerant plants, at least during early vegetative phases.

The nature of the differences among the experiments reported jointly here results in a few necessary caveats. First, our HS field experiment represented a longer term but more moderate HS than the shorter term, more extreme HS imposed in our growth chambers (Yeh *et al*. 2012). Differences between field and growth-chamber studies may have resulted, in part, from this discrepancy. Second, under natural conditions, HS is often accompanied by several other stressors, especially drought stress, which, when combined, may trigger additional responses (Killi *et al*. 2017). Further studies that apply HS in the field in combination with other stressors are needed to improve understanding of crop responses to current and future extreme heat events. Summer planting dates in multi-location trials (Chopra *et al*. 2017) and the use of heat tents with infrared lamps (Smith 2011) in the field may be useful for exploring crop responses to natural heat waves and artificial extreme heat events.

Hernández *et al*. (2018) evaluated the HS tolerance of cultivated and wild sunflower during reproduction. In contrast to our observations, wild populations showed higher HS tolerance than cultivated sunflower, suggesting independent genetic control of HS tolerance during early vegetative and reproductive phases. Xu *et al*. (2017) described similar results for cultivated tomato, finding genetic variation for tolerance to HS during both vegetative and reproductive phases but no correlation between phenological phases; that is, performance of cultivars under HS during reproductive phases could not be predicted from performance under HS during vegetative phases, and vice versa. This variation between phenological phases should be addressed in future studies investigating the molecular basis of HS tolerance, because HS-responsive genes identified during early vegetative phases may not necessarily improve HS tolerance during reproduction (Yeh *et al*. 2012). The natural stress tolerance of cultivated sunflower during vegetative phases may explain their successful movement to marginal crop areas in Argentina (de la Vega *et al*. 2007; Hall *et al*. 2013), and the use of sunflower as a source of stress tolerance genes or alleles for biotechnological improvements (Cabello *et al*. 2014, 2017; González *et al*. 2019).

Implications for crop allele introgression

The low relative fitness of crop–wild hybrids is a major barrier to crop–wild gene flow (Mercer *et al*. 2007; Presotto *et al*.

2012). However, the fitness gap between crop–wild hybrids and wild types can be reduced and may even disappear under some stressful conditions (e.g. herbicide application or interspecific competition; Mercer *et al*. 2007, 2014), making crop gene introgression highly dependent on environment. Here, we showed that plant survival was explained by the percentage of crop alleles under both HS and FS. In wild sunflower, overwinter survival occurs mostly as dormant seeds, at least in the native area (Snow *et al*. 1998; Weiss *et al*. 2013; Alexander *et al*. 2014; Pace *et al*. 2015). However, the increased FS tolerance observed in wild populations (here mostly invasive populations) suggests that this trait may play a role in local adaptation outside the native area. Recently, Presotto *et al*. (2020) reported that the emergence peak from the seedbank of invasive sunflower occurred well before the onset of spring (during July and August in Argentina), exposing plants to prolonged cold and freezing stress. Although early germination and exposure to cold can cause mortality, it can also enhance fitness of remaining plants in wild populations by reducing competition and increasing the probability of surviving and reproducing (Mercer *et al*. 2011; Presotto *et al*. 2020). Further experiments comparing native and invasive populations under natural and controlled FS conditions are necessary to improve understanding of the natural variation and adaptive importance of FS tolerance in wild sunflower.

Finally, the increased HS tolerance of cultivated varieties could increase the fitness of crop–wild hybrids in heat-prone environments, thereby increasing the chances of introgression of alleles from crop to wild sunflower in such environments. The increased HS tolerance of crop–wild hybrids could also facilitate the spread of invasive sunflower over warmer areas. Despite this, the increased HS tolerance of cultivated and crop–wild hybrid sunflower over wild sunflower must be further validated under field conditions. Therefore, in the face of warmer and more erratic environments due to climate change, the effects of extreme temperatures and the differential tolerance to extreme temperatures between cultivated and wild populations should be addressed for better predicting the risk of crop–wild gene flow along with the evolutionary implications.

Conclusions

We found large differences between wild and cultivated sunflower for tolerance to extreme temperatures. Both wild and cultivated sunflower showed acclimation ability against FS and HS. As expected, wild sunflower tolerated FS much better than crop sunflower in controlled and field conditions. By contrast, cultivated sunflower exhibited higher HS tolerance than wild sunflower. We found no evidence of trade-offs between growth under benign conditions and tolerance to extreme temperatures, suggesting that stress-tolerance traits might be introgressed into the elite inbred lines without growth penalties and that extreme temperatures should be included in crop–wild gene flow studies. Finally, the ability to increase both FS and HS tolerance after an acclimation period in cultivated sunflower encourages us to explore the genetic variability for tolerance to extreme temperatures within elite germplasm.

Conflict of interest

The authors declare no conflicts of interest.

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