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Heat stress effects on reproductive traits in cultivated and wild sunflower (*Helianthus annuus* L.): evidence for local adaptation within the wild germplasm

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Abstract Heat stress (HS) is a major threat to current and future crop production. Crop improvement for HS tolerance is a major tool for dealing with HS and crop wild relatives (CWR) offer the greatest variability for such improvement. Here, we evaluated the HS tolerance on four reproductive traits in cultivated and wild sunflower and tested for local adaptation to HS within the wild germplasm. Three cultivars and 23 wild populations (from native and invasive ranges) were grown in field experiments for 2 years. Flowering heads were covered with white (control) and black (HS) paper bags during seven consecutive days. Additionally, biogeographic tools were used to test for local adaptation. HS increased air temperature on black bags compared to the white ones by 9.4 °C on average and strongly decreased seed number and yield with smaller effects on head diameter and seed weight. We found large variability for HS tolerance, mainly in seed number and yield. The invasive group outperformed the cultivated and native groups in both years. Biogeographic analysis reveals a clinal variation in HS tolerance, populations from wetter (but not from warmer) environments were more tolerant to HS. In addition, the positive correlation observed between reproductive traits under control conditions and HS tolerance helps to explain the better performance of the invasive populations. We proposed the use of invasive populations for future sunflower improvements in HS tolerance and the adoption of biogeographic tools in another CWR species to identify HS tolerant populations.

Keywords Heat stress · Crop wild relatives · Biogeographic analysis · Native · Invasive

Introduction

A general agreement among climate model predictions exists about the increase, not only in the mean temperatures, but also in the climatic variability for the next 50–100 years (Battisti and Naylor 2009; Gornall et al. 2010). It is likely that by the end of this century growing season temperatures will exceed even the most extreme seasonal temperatures recorded in the last century for most agricultural regions (Battisti and

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Naylor 2009) resulting in more common and more severe heat stress (HS). HS will probably negatively affect crop yields directly, e.g. by exposing crops to temperatures beyond their optimum range for growth, and indirectly, e.g. by increasing the chance of a water deficit due to a higher evapotranspiration rate and by increasing the aggressiveness of pests and diseases (Debaeke et al. 2017; Fuhrer 2003). Conversely, CO_2 fertilization effects, the expansion of production to higher latitudes and increases in the growing season length may alleviate such negative effects (Ainsworth and Long 2005; Debaeke et al. 2017; Lobell et al. 2015). HS affects crops during the vegetative phase by decreasing photosynthetic rates and water and nutrient use efficiencies, increasing evapotranspiration rates and speeding up the developmental rates. However, crops are particularly sensitive to HS during the reproductive phases (Jha et al. 2014; Prasad et al. 2017; Wahid et al. 2007).

Among the reproductive phases, flowering is the most sensitive phase to brief periods of HS (Hatfield and Prueger 2015; Prasad et al. 2017). It has been observed that pollen viability is especially sensitive to HS across most cultivated species, being female tissues less sensitive (Driedonks et al. 2016; Hatfield and Prueger 2015; Mesihovic et al. 2016). Reductions in seed set by decreases in pollen viability has been reported in many crops, including legumes, summer and winter cereals and oilseed crops (Barnabás et al. 2008; Devasirvatham et al. 2012; Jha et al. 2014; Kalyar et al. 2014; van der Merwe et al. 2015; Rattalino-Edreira et al. 2011). Negative effects of HS on crop yields are usually well captured in crop simulation models, which are used to assess climate change impacts (Gourdji et al. 2013). Despite this, the impact of brief periods of HS on crop yields has been rather underexplored and it is often not well quantified in current simulation models, underestimating future crop yield losses (Gourdji et al. 2013; Lobell et al. 2015). The ongoing adaptation to HS should combine stress avoidance strategies, such as changes in the sowing date, adjustments in the crop phenology, and the genetic stress tolerance of the new cultivars. Several genes were discovered for the HS response in model and cultivated species (Barah et al. 2013; Hu et al. 2009; Yeh et al. 2012) increasing our understanding of the genetics of the HS response. However, most of the studies were made on early vegetative phases (Yeh et al. 2012) and therefore they need to be validated at the reproductive phases. In this sense, the lack of field phenotyping techniques emerges as a major bottleneck (Jha et al. 2014).

Owing to selection and genetic drift during domestication and modern breeding, cultivars have suffered a reduction in genetic diversity, hindering future genetic gains in stress tolerance (Dempewolf et al. 2014; Hajjar and Hodgkin 2007). Crop wild relatives (CWR) are the main reservoirs of genetic variability for future improvements in crop yields (Guarino and Lobell 2011; Warschefsky et al. 2014; Zhang et al. 2017). These CWR have been successfully used to improve tolerance to biotic stress and they have the potential to improve the abiotic stress tolerance of cultivars (Hajjar and Hodgkin 2007; Warschefsky et al. 2014). In addition, wild populations offer local adaptation to stress. Local adaptation to stress has previously been reported, mainly in the model species *Arabidopsis thaliana* but also in CWR (Barah et al. 2013; Baruah et al. 2009; Kang et al. 2013; Wolfe and Tonsor 2014; Zuther et al. 2012).

In sunflower (*Helianthus annuus* L.), previous research has demonstrated that brief periods of HS during grain filling negatively impacts on many yield components, such as seed number and weight, oil yield and fatty acid composition (Rondanini et al. 2003; van der Merwe et al. 2015). However, the effect of brief periods of HS during flowering, the most critical period of the sunflower lifecycle (Andrade 1995; Andrade et al. 2005) is less understood. Moriondo et al. (2011) using two future climate scenarios (a warmer climate for the period 2071–2100 with and without the inclusion of brief periods of HS at flowering), estimated yield losses of $\sim$ 13, and $\sim$ 7% probability of low yield occurrence for sunflower in the Mediterranean region. However, when brief periods of HS were included in the models, both yield losses and the probability of low yield occurrence increased up to $\sim$ 34 and $\sim$ 23%, respectively. The wide diversity in wild germplasm, including native and invasive populations along with recurrent crop-wild gene flow, makes sunflower an ideal model system for studying local adaptation to HS.

Recently, a detailed survey of the distribution of 36 native taxa closely related to sunflower (Kantar et al. 2015) identified populations adapted to wide extreme environments and those populations may possess valuable traits for crop improvement. However, more importantly for the crop improvement is the fact that

much of the primary gene pool (GP1; wild *H. annuus*) occurs in extreme environments, overlapping most adapted taxa and indicating that wild *H. annuus* may possess useful traits for abiotic stress tolerance improvement (Kantar et al. 2015). However, as in most wild populations, wild sunflowers possess many strategies for adapting to stressful conditions and some of these are useless for crop improvement (e.g. increased seed dormancy, more investment in branching and higher production of small heads; Whitney et al. 2010). Therefore, the HS tolerance of wild populations needs to be tested together with the cultivars using common techniques for identifying useful sources for crop improvement.

The aims of this work were: (1) to develop a high-throughput technique to test for HS tolerance under field conditions during flowering (R5); (2) to explore the genotypic differences in HS tolerance between cultivated and wild sunflower germplasm; and (3) to investigate for local adaptation to HS within wild sunflower.

Materials and methods

Plant material

Cultivated sunflower has a typical domestication phenotype, i.e. single large heads, decreased seed dormancy, no branching, high oil content of achenes (hereafter referred to as seeds) whereas the wild sunflower is branched, with several small heads, small seeds and lower oil content in the seeds (Harter et al. 2004; Presotto et al. 2011). The distribution of the wild sunflower can be split into two distribution ranges, the native and the invasive ranges. Native populations are distributed across North America, from southern Canada to northern Mexico (Harter et al. 2004; Kantar et al. 2015) whereas the invasive populations are distributed mainly in central Argentina, southern Australia and southern Europe (Muller et al. 2009; Poverene et al. 2008; Seiler and Gulya 2016).

The cultivated group was represented by three current commercial cultivars (HS503, VDH487 and PAR1000) in both years for the current experiments. The chosen commercial cultivars are widely sown in Argentina and each one comes from a different pedigree. In year 1 (2015/16) 11 populations represented the wild group: six from the native range (AR, IL, IN, ND, NM and TX) and five from the invasive one (BRW, DIA and LMA from Argentina and SAU and WAU from Australia). In year 2 (2016/17) the wild group was more broadly represented: 12 populations from the native range (AK, CA, CO, IN, IO, MI, ND, MO, OH, OK, SA and TX) and eight from the invasive one (AAL, BRW, DIA, LMA and RCU from Argentina and EAU, SAU, and WAU from Australia). Table 1 summarizes the information of each population used in the present study. Cultivars were obtained from seed suppliers in Argentina. Wild populations from Argentina were collected in central Argentina (Cantamutto et al. 2010) whereas native and Australian populations were obtained from the USDA´s North Central Regional Plant Introduction Station.

Experimental design

Experiments were carried out in a common garden at the Agronomy Department, Universidad Nacional del Sur, Bahia Blanca, Argentina (38°14′10″S; 62°11′40″W). Cultivars were arranged in three randomized complete blocks. Due to the low availability of seeds from most wild populations, only one plot per wild population was established and each plot was randomly arranged across the experiment. Thus, field experiments consisted of 20 (9 and 11 cultivated and wild plots, respectively) and 29 (9 and 20 cultivated and wild plots, respectively) plots at year 1 and 2, respectively. Control and HS treatments were randomly applied within each plot. To test whether populations could be biased by their position in the experimental field, we used the yield of cultivated materials at control conditions to estimate the block effect. As there was no significant block effect, each plant was considered as a replicate, with low risk of pseudo-replication effects. Each plot comprised three rows 2 m long and plants were spaced at 1 m and 0.3 m (between and within rows, respectively; stand density = 3.3 plants m^{-2}). Seed dormancy, present in most of the wild populations, was overcome by stratification, incubating seeds in a wet chamber at 5 °C for 1 week (ISTA 2004). Seedlings were grown for 30 days in the greenhouse at 20–25 °C in 28 × 54 cm^2 200-cell plastic trays and then transplanted. Cultivars were sown directly in the common garden and thinned manually at the V4–V6 leaf stage (Schneiter and Miller 1981). Planting dates were 23 November 2015 and 14 November 2016. Both

Table 1 Wild populations used in the present study

Population	State/city	ID/USDA PI	Range	Latitude	Longitude	Altitude
SA[1,2]	Saskatchewan	592321	Native	49.4	− 104.3	671
MO[1,2]	Montana	531035	Native	46.6	− 108.5	1058
ND[1,2]	North Dakota	586888	Native	46.0	− 98.4	428
IO[2]	Iowa	613779	Native	41.7	− 96.0	305
IL[1]	Illinois	435540	Native	41.5	− 88.1	178
MI[2]	Missouri	613789	Native	40.0	− 95.3	291
OH[2]	Ohio	649853	Native	39.2	− 84.5	213
CO[2]	Colorado	468621	Native	39.1	− 108.6	1405
CA[2]	California	413131	Native	38.7	− 121.8	17
IN[1,2]	Indiana	468633	Native	38.5	− 87.3	144
BRW[1,2]	Barrow	–	Invasive	− 38.2	− 60.1	161
AAL[2]	Alsina	839	Invasive	− 37.2	− 62.6	134
AK[2]	Arkansas	613727	Native	36.4	− 93.7	433
OK[2]	Oklahoma	468483	Native	35.3	− 99.6	561
LMA[1,2]	Las Malvinas	835	Invasive	− 34.5	− 68.2	611
SAU[1,2]	South Australia	653586	Invasive	− 34.2	140.6	37
WAU[1,2]	West Australia	664685	Invasive	− 33.8	121.9	20
NM[1]	New Mexico	468470	Native	33.3	− 104.5	1096
RCU[2]	Rio Cuarto	832	Invasive	− 33.1	− 64.2	389
AR[1,2]	Arizona	613731	Native	32.7	− 114.6	39
DIA[1,2]	Diamante	834	Invasive	− 32.0	− 60.4	86
TX[1,2]	Texas	613728	Native	27.4	− 97.8	13
EAU[2]	East Australia	653582	Invasive	− 26.6	148.9	340

Superscript number in the population column indicates the year at which the population was evaluated. Argentine populations were collected by the group (AAL, BRW, DIA, LMA, and RCU; Cantamutto et al. 2010). All the Argentine populations except for BRW are deposited in the INTA´s sunflower germplasm bank and their register number (ID) is provided. On the other hand, the USDA supplied Australian (SAU, WAU, and EAU) and all the native populations and their passport number is provided (USDA PI)

experiments were drip irrigated and fertilized with 80 kg ha^{-1} of diammonium phosphate at pre-sowing and 100 kg ha^{-1} of urea at the V4–V6 leaf stage (Schneiter and Miller 1981) for optimal plant growth. Data were collected from 220 and 230 heads (individuals in cultivated plants) in years 1 and 2, respectively.

Treatment description

HS during flowering was imposed by covering the heads with black paper bags (6 × 19 cm and 12 × 43 cm, for wild and cultivated heads, respectively), which increased the temperature through higher radiation absorption. White and black paper bags were used for the control and heat stressed-heads, respectively (Fig. 1a). Treatments were applied to random plants (cultivated) or heads (wild) within each plot (Fig. 1a, b). Heads were covered at the R4 stage (when the inflorescence begins to open and immature ray flowers are visible; Schneiter and Miller 1981; Fig. 1b) during seven consecutive days to cover the entire flowering period (R5; Schneiter and Miller 1981). The time and duration of the HS treatment were chosen according to the critical period of yield determination in sunflower (flowering, from R5 up to R6; Andrade 1995) and the minimum number of days with HS considered as a heat wave (De Boeck et al. 2010). Thus, heads were heat-stressed during flowering when the head diameter, seed number per head and potential seed size are defined.

Heads of wild plants were pollinated by hand with sister plants from the same treatment (control and heat stressed-heads) because of the natural self-incompatibility of wild sunflower. Pollination was carried out on the 3rd, 5th and 7th day after the beginning of the treatment to ensure the pollination of every flower. After 7 days, all the black paper bags were replaced by white paper bags of the same size until harvest. To measure the extent of HS, the temperature of the control and heat stressed-bags was measured by using a hand-held infrared thermometer (Scout 1, INF155) at

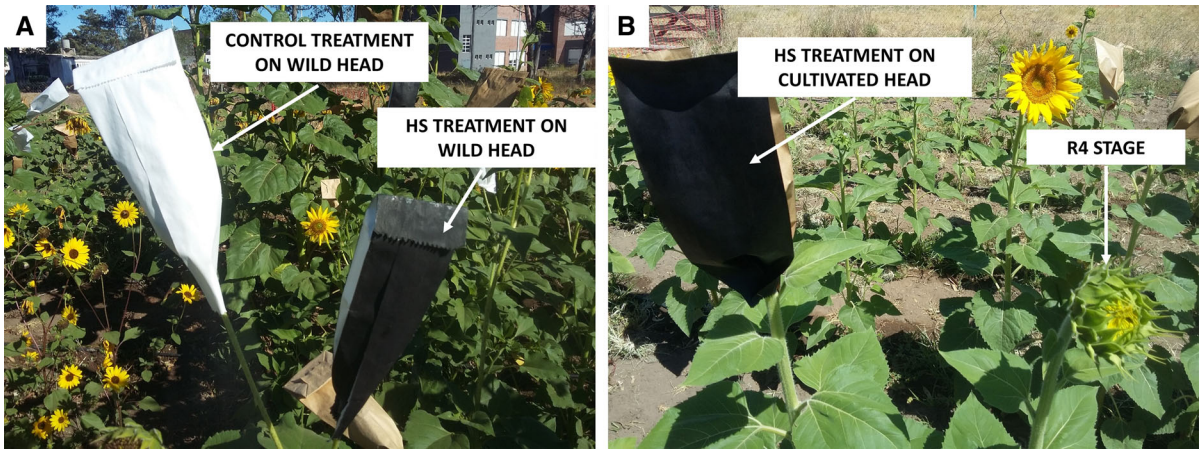


Fig. 1 Paper bags used for control and heat stress (HS) treatments. Heads were covered at R4 stage (**b**) with white and black paper bags for control and HS treatments, respectively (**a**)

random times during the day within the growing season. Thirty-six and 39 pairwise readings were made on the control and heat stressed-bags in year 1 and 2, respectively. Each reading was the average of two readings on each side of the bag. In addition, temperature and relative humidity (RH) of the control and heat stressed-bags were measured by using a manual thermo-hygrometer (TA298) at random times during the day. Eighteen readings were simultaneously made on one control and one heat-stressed bags during nine days (Fig. S1). For each reading, temperature and RH were recorded. All four reproductive traits: head diameter, seed number and weight, and yield were measured at harvest on each of the air-dried heads. Head diameter (cm) is the mean of two opposite measurements, seed weight is the mean weight (mg) of 50 seeds, yield is the total seed weight per head (mg $head^{-1}$), and seed number was estimated as the ratio between yield and seed weight. When the seed number was lower than 50, seeds were counted by eye and the seed weight was estimated as the ratio between the yield and the seed number. In addition, when the seed number was lower than 10, the estimated seed weight was not included in the analyses. All the seeds considered for seed number, seed weight and yield estimations were filled seeds.

Statistical analysis

Generalized linear mixed models (GLMM) were fitted using PROC GLIMMIX (SAS University edition; SAS Institute Inc., Cary, NC), unless otherwise specified. GLMM were chosen to incorporate random effects when necessary (Bolker et al. 2009). Significance of fixed effects was tested using a quasi-Newton pseudo-likelihood estimation and reported by the F and p values. Meanwhile, the significance of random effects was tested using Wald tests. All the input data were square root-transformed to improved homoscedasticity and all the means in the text are the untransformed data, reported with their corresponding standard error.

Heat stress effect on yield and yield components

One GLMM was fitted for each trait with square root-transformed data as input. Year (1 and 2), treatment (control and HS) and group (cultivated, invasive and native) along with their interactions were considered as fixed. Biotype nested within the group effect was considered as random. Because each plant was considered as a replicate we could not test for replicate nor interaction by replicate effects. Percentage of variance explained by each effect was calculated using the F values for each effect.

Heat tolerance in cultivated and wild germplasm

Owing to the large differences in all four traits associated with domestication (Table S1), we standardized the HS response as the ratio between each replicate under HS and the biotype mean value under the control conditions. To obtain the mean value of each biotype under controlled conditions we ran two

separate GLMM, for each cultivated and wild (invasive plus native groups) with raw data as input. Year, biotype and year by biotype interaction were considered as fixed. When neither year nor year by biotype interaction effects were statistically significant ($p > 0.05$), the grand mean of each biotype was used as the control value. When either year or year by biotype effects were statistically significant, the control value of each biotype was calculated for each of the 2 years. Therefore, HS tolerance varied commonly, although not restricted to, from zero (complete failure in seed set) to one (no effect of HS). In the GLMM, year, group and year by group interaction were considered as fixed. When significant, the year by group interaction was broken down using four a priori orthogonal contrasts for each year, contrasting cultivated versus wild (native plus invasive groups), cultivated versus native, cultivated versus invasive and invasive versus native.

Climate data and PCA of climatic variables

We obtained 19 biologically relevant climatic variables from the BIOCLIM dataset (Hijmans et al. 2005) for each of the 23 wild populations included in this study. In addition, four variables related to temperature (mean and maximum temperatures during spring and summer) and two related to precipitation (amount of precipitation during spring and summer) were calculated and added to better reflect the environmental conditions during the growing season of the sunflower. Because of the high correlation between variables, we ran pairwise correlation analyses for all the variables. To avoid redundancy and facilitate the interpretation, for all the pairwise comparisons with $r > 0.90$, one variable of each pair was removed for the analysis. After this removal, 13 BIOCLIM variables represented the environmental input data (Table 2). We used principal component analysis (PCA), based on a correlation matrix to avoid scale effects, for producing the principal components (PCs) that explained the multivariate variation in the environmental variables. In order to split the effects of temperature and precipitation, we ran two separated PCAs, one for the seven temperature-related variables and one for the six precipitation-related ones (Table 2).

Table 2 BIOCLIM variables used in our principal component analyses (PCAs)

Variable	Description
BIOCLIM *temperature*	
BIO1	Annual mean temperature
BIO2	Maximum temperature of warmest month
BIO3	Minimum temperature of coldest month
BIO4	Mean temperature of Spring
BIO5	Mean temperature of Summer
BIO6	Maximum temperature of Spring
BIO7	Maximum temperature of Summer
BIOCLIM *water*	
BIO8	Annual precipitation
BIO9	Precipitation of wettest month
BIO10	Precipitation of driest month
BIO11	Precipitation seasonality (CV)
BIO12	Precipitation of Spring
BIO13	Precipitation of Summer

Variables for each population were extracted from the DIVA-GIS 7.5 software (Hijmans et al. 2005)

Local adaptation within wild sunflower

Native and invasive groups were compared for traits with significant group effect. To test for local adaptation, we compared a null model (without covariates) with a full model (with environmental PCs as covariates). Year, group and year by group interaction effects were included as fixed in both null and full models. Significance effects of first and second PCs of each temperature and precipitation were tested but only significant PCs were included in the full model. If differences in HS tolerance observed in the null model are reduced or disappear in the full model such differences may be attributable to differences in the environment where populations come from (Colautti et al. 2009). In such cases, a correlation between the HS response and significant PCs will determine the strength and direction of the cline. In addition, if any environmental PC is significant in the full model but differences between native and invasive ranges persist, this indicates that although the cline in HS tolerance exists (significant PC effect) the differences between ranges respond to factors others than this cline.

Results

Growing and experimental conditions

Cultivated plots reached the flowering stage (R5) around January 25 in year 1 and January 19 in year 2. At those data, all the heads of the control and HS treatments were bagged with white and black paper bags, respectively. Thus, the environmental conditions during flowering of the cultivated group were defined by the mean values of the environmental variables on the seven consecutive days after these unique flowering data (Table 3). On the other hand, flowering of wild plants, typically scattered, began in December 14 in year 1 and December 22 in year 2 and it was extended to February 17 and February 8 in the earliest populations in years 1 and 2, respectively. However, treatments were applied from January 14 to February 9 in year 1 and from January 25 to February 27 in year 2. Thus, the environmental conditions during treatment in the wild group were defined as the mean of these periods (Table 3). The mean temperature during flowering was similar for the wild and cultivated groups in year 1 (22.7 ± 0.5 and 20.6 ± 0.8, respectively; t = − 1.63, p = 0.1122) and year 2 (24.0 ± 0.6 and 24.1 ± 1.7, respectively; t = 0.07, p = 0.9466). During both the growing cycle and flowering, year 2 was slightly warmer and drier than year 1 (Table 3). However, the mean temperature of the hand-held infrared thermometer readings was significantly higher in year 1 than in year 2 for the control (30.7 ± 0.6 °C vs. 25.5 ± 1.0 °C; t = 4.11, p = 0.0001) and HS (40.0 ± 0.9 °C vs. 35.0 ± 1.2 °C; t = 2.72, p = 0.0084) treatments. Nevertheless, the increase in the air temperature imposed by the HS treatment did not vary significantly between the years (9.3 ± 0.6 and 9.5 ± 0.8 °C for years 1 and 2, respectively; t = − 0.96; p = 0.3406), thus the differences (consisting in 75 values) were combined and the temperature during HS was estimated by adding 9.4 °C to the recorded temperature (Table 3). On the other hand, RH during the day was slightly lower in heat-stressed than in control bags (24.4 ± 2.1 and 29.2 ± 2.9%, respectively) but there was no significant variation between treatments (t = 1.34; p = 0.1907). Note that the values of measured RH correspond to daytime hours. Mean RH during days when RH was measured was 54.7 ± 3.5% and significantly correlated with measured values in control and heat-stressed bags (n = 9, p = 0.0476, r = 0.67 and n = 9, p = 0.0304, r = 0.71, respectively).

Table 3 Mean and standard errors of the environmental variables recorded in each of the 2 years for the entire growing season and the flowering period

Variable	Year	
	1	2
Growing season		
N	160	137
Mean T°	**20.4 ± 0.3**	**22.3 ± 0.3**
Maximum T°	**29.2 ± 0.3**	**31.3 ± 0.4**
RH	**60.6 ± 0.9**	**52.4 ± 1.4**
Precipitation amount	297.2	323.8
Flowering period		
N	27	34
Mean T°	22.7 ± 0.6	23.8 ± 0.6
Maximum T°	**31.2 ± 0.7**	**33.2 ± 0.7**
Mean T° treatment	32.1	33.2
Maximum T° treatment	**40.6**	**42.6**
RH	**63.5 ± 2.4**	**56.0 ± 2.6**
Precipitation amount	104.14	149.09

Values in bold indicate significant differences ($p < 0.05$) between years after unpaired t test. Mean and maximum temperatures of the treatment were obtained by adding 9.4 °C to the control values. Daily data were obtained from the Bahía Blanca Aerodrome (S 38°44′48″; W 62°9′36″)

Heat stress effect on yield and yield components

When non-standardized, root squared transformed data were used for the analysis, HS reduced the head diameter, seed number, and yield (F = 77.4, $p < 0.0001$; F = 312.6, $p < 0.0001$; F = 464.3; $p < 0.0001$, respectively) whereas it increased the mean seed weight (F = 7.24, p = 0.0076). Despite the large differences in all the four traits between the groups under the control conditions (Table S1), the treatment effect explained most of the variation in seed number and yield (64 and 50% of the total variation, respectively). The group effect explained most of the variation observed in seed weight (∼ 80%) and the group and treatment effects altogether explained most of the variation in head diameter (41 and 49.7%, respectively). In addition,

significant treatment by group effect was found in head diameter, seed number and yield (F = 6.74, $p = 0.0013$; F = 66.07, $p < 0.0001$; F = 174.4, $p < 0.0001$, respectively) suggesting a differential response to HS between the groups. Significant year by treatment and year by treatment by group interactions in seed number (F = 18.5, $p < 0.0001$; F = 21.6, $p < 0.0001$, respectively) and yield (F = 64.0, $p < 0.0001$; F = 40.1, $p < 0.0001$, respectively) indicate that HS-imposed reductions in these traits varied between years and this variation was not similar in the groups. On the other hand, no significant year by treatment, or year by treatment by group interactions were found either in seed weight (F = 0.6, $p = 0.4324$; F = 2.5, $p = 0.0794$, respectively) or head diameter (F = 0.1, $p = 0.7744$; F = 0.2, $p = 0.8257$, respectively).

Heat tolerance in cultivated and wild germplasm

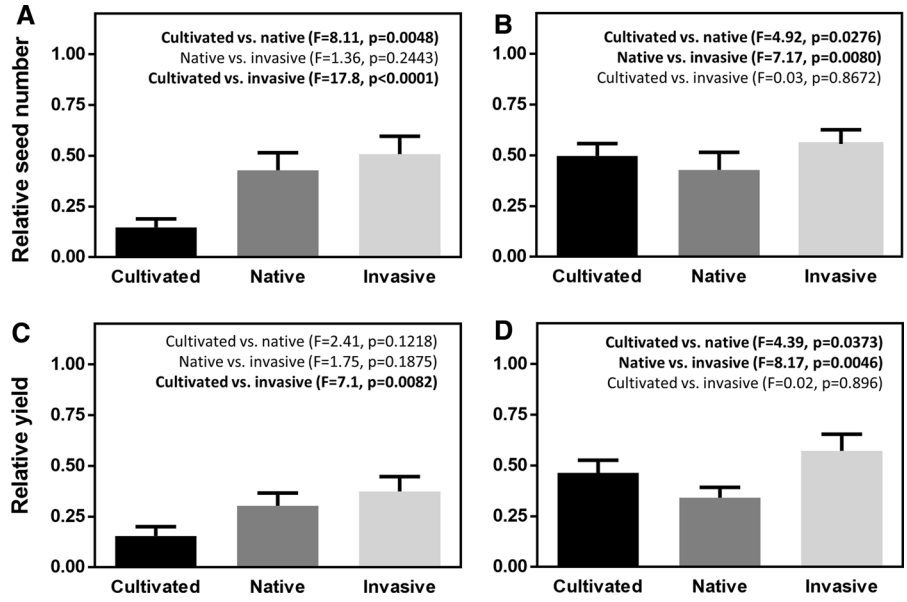
When comparing standardized traits, we found significant year, group and year by group interaction effects for seed number (F = 6.37, $p = 0.0124$; F = 5.1, $p = 0.0070$; and F = 7.1, $p = 0.0010$, respectively) and yield (F = 15.6, $p = 0.0001$; F = 4.4, $p = 0.0133$; and F = 3.3, $p < 0.0383$, respectively). No significant effects were found for either seed weight or head diameter (all $p > 0.1$), indicating no differences in HS response between groups for these traits. In year 1, a higher HS tolerance was observed in the wild group than in the cultivated group, for seed number (0.47 ± 0.06 vs. 0.15 ± 0.04, respectively) and yield (0.34 ± 0.05 vs. 0.15 ± 0.05, respectively). Most of the difference was explained by the difference between the cultivated and invasive groups, the native group being intermediate (Fig. 2). In year 2, a much improved performance of cultivated group was observed for both seed number and yield (Fig. 2). As in year 1, the invasive group outperformed the cultivated and native groups but significant differences were only observed between the invasive and native groups and the cultivated and native groups for seed number and yield (Fig. 2).

PCA of the environmental variables

The PCA of the temperature variables resulted in two composite variables designated as PC1*temp* and PC2*temp*, which captured 79.8 and 17.5% of the total variation, respectively (Fig. 3a). Likewise, PCA of the precipitation variables resulted in two composite variables designated as PC1*water* and PC2*water*, which captured 74.4 and 18.8% of the total variation, respectively (Fig. 3b). PC1*temp* was represented by positive values of all the temperature-related variables (Table 2) with eigenvector coefficients ranging from 0.33 to 0.41 (Table S3), i.e. the higher the PC1*temp* the warmer the environment. PC2*temp* was represented by positive values of BIO2, BIO5 and BIO 7, associated with maximum temperatures of the warmest month, and mean and maximum temperatures of the summer, respectively (Table S3), and negative values of BIO1, BIO3, BIO4, and BIO6 (Table S3). PC1*water* was represented by positive values of all the variables describing the amount of precipitation (Table 2) with eigenvector coefficients ranging from 0.41 to 0.47 (Table S3) whereas PC2*water* was mostly represented by BIO11 (eigenvector coefficient of 0.76), describing the precipitation seasonality (Table 2). That is, the higher the PC1*water* the wetter the environment and the higher the PC2*water*, the higher the precipitation seasonality, especially due to drier summers.

Comparison between native and invasive populations reveals local adaptation to heat stress

Native and invasive groups were compared for the two traits with significant group effect (seed number and yield). In the null model, we found no significant year or year by group effect for either seed number (F = 0.08, $p = 0.7779$ and F = 0.20, $p = 0.5949$, respectively) or yield (F = 3.46, $p = 0.0645$ and F = 0.69, $p = 0.4080$, respectively). The invasive group exhibited higher HS tolerance than the native one for seed number and yield (Fig. 4). In the full model, the PC1*water* was the only significant effect for both yield (F = 21.12, $p < 0.0001$) and seed number (F = 14.14, $p = 0.0002$), and differences between invasive and native groups were enlarged (Table S3). That is, the wetter the environment where the population comes from the higher the HS tolerance for seed number and yield (Fig. 5). In addition, the higher HS tolerance of the invasive populations, even when controlled by PC*water*, suggests that factors others than environmental clines are involved in the higher HS tolerance of the invasive group.



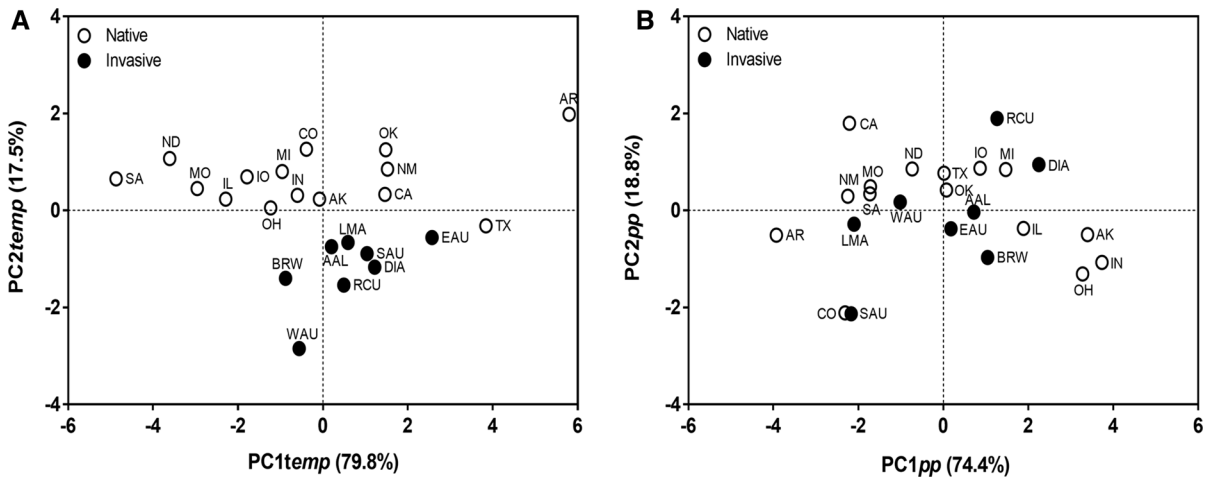


Fig. 2 Relative seed number (**a**, **b**) and yield (**c**, **d**) in cultivated, native and invasive groups for years 1 (**a**, **c**) and 2 (**b**, **d**). The bars represent mean values of each group. Standard error bars indicate variability within each group. Within each year and variable, groups were compared by using three a priori orthogonal contrasts. Significant contrasts are ($p < 0.05$) are in bold

Fig. 3 Environmental variability in native and invasive populations of wild sunflower. Both axes represent the first and second axes of PCA of the seven and six variables related to temperature (**a**) and precipitation (**b**), respectively (Table 2). Higher values of PC1*temp* and PC1*water* represent warmer and wetter environments, respectively. Higher values of PC2*temp* and PC2*water* represent higher temperature seasonality (warmer summers and lower annual temperature) and precipitation seasonality, respectively

Higher values of reproductive traits help to explain the higher heat stress tolerance of invasive populations

To test whether HS tolerance could be predicted by reproductive values under control conditions we compared the head diameter, seed number and weight, and the yield in native and invasive groups and we correlated variables with significant differences between groups against HS tolerance. Invasive populations showed higher values of head diameter (F = 9.29, $p = 0.0063$), seed number (F = 14.3, $p = 0.0013$), seed weight (F = 6.84, $p = 0.0166$), and yield (F = 15.1, $p = 0.0009$) than native populations. In addition, when environmental PCs were included as covariates, PC1*water* was the only significant covariate for three of the four variables and differences between the native and invasive groups

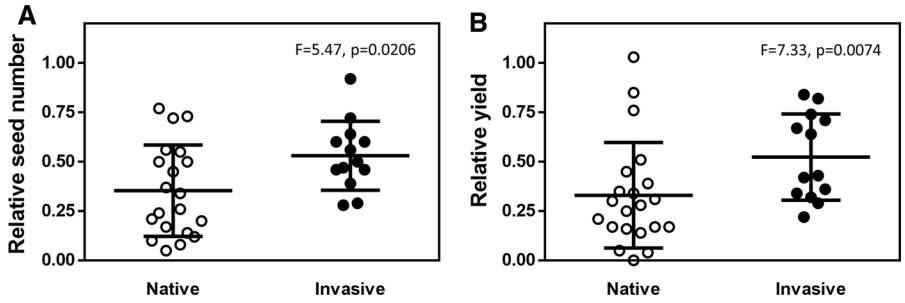


Fig. 4 Relative seed number and yield for native and invasive populations. Each point corresponds to mean heat stress tolerance of the population within each year. Data from both years were included. The larger horizontal line represents the mean value of the group. Error bars represent the standard deviation. *F* and *p* statistics indicate the group effect in the null model (GLMM without environmental PCs as covariates)

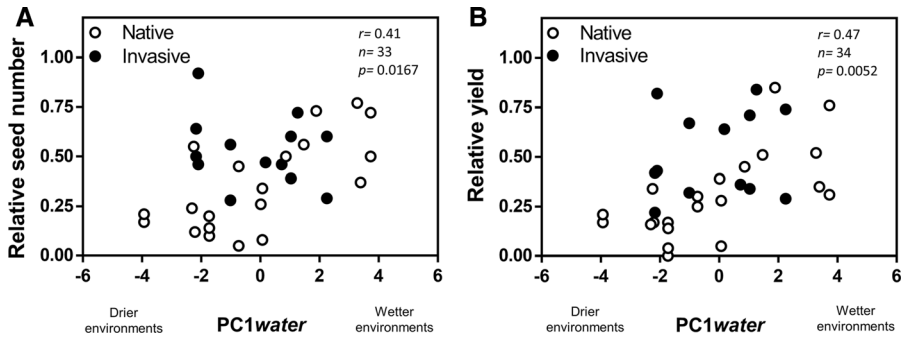


Fig. 5 Relationship between precipitation-related variables and relative seed number (**a**) and yield (**b**). PC1*water* represents the first axes of PCA of the six precipitation variables (Table 2). Higher values of PC1*water* represent wetter environments. Each point is a population mean within each year. Statistics from Pearson correlation (*n*, *p* and *r*) are shown

were enlarged. Since the four variables differed significantly between groups in both the null and the full models, we ran a PCA for the four traits that resulted in one composite variable designated as PC1*rep*, which explained 84.2% of the total variation. PC1*rep* was represented by positive values of head diameter, seed weight and number, and yield (with eigenvectors coefficients of 0.53, 0.47, 0.46, and 0.54, respectively). We found a strong correlation between PC1*rep* and HS tolerance for seed number (r = 0.64, n = 23, $p = 0.0012$) and yield (r = 0.62, n = 23, $p = 0.0017$) within the native range. However, no correlation was found within the invasive range (r = −0.08, $p = 0.8221$ and r = −0.04, $p = 0.9134$ for yield and seed number, respectively).

Discussion

In the present study, we developed a simple, rapid and high-throughput technique to test for HS tolerance during the most sensitive phase (flowering) under field conditions. The use of heat tents and infrared heaters are promising field-based approaches for evaluating HS tolerance of crops during reproductive phases (Prasad and Djanaguiraman 2015; Rattalino-Edreira et al. 2011; Siebers et al. 2015, 2017). However, the main limitation of these approaches is the scale of the experiments, allowing the simultaneous evaluation of a few genotypes. Contrariwise, with our technique, we were able to test a wide spectrum of wild germplasm of sunflower and we identified candidate populations for future HS tolerance improvements. In addition, this technique allowed us to compare highly divergent groups, such as cultivated and wild sunflower. However, one major limitation arise from this approach: the increased humidity produced by paper bags may

limit their use out of arid and semiarid regions. It is known that sunflower pollen viability decreases upon high RH conditions (e.g. > 85–90%). In humid regions, dewy mornings and rainy days during flowering may increase humidity around the reproductive organs affecting the efficiency of the present approach. To overcome this limitation, for humid regions we recommend covering bags only around noon and afternoon when humidity is low and radiation high avoiding the more humid times of the day (night and early morning). Although this modification may decrease the scale of the experiments, it does not affect the whole efficiency of the technique.

Heat tolerance in cultivated and wild germplasm

Cultivated HS tolerance for yield varied from 13 to 17% in year 1 and 39 to 57% in year 2. Since year 2 was slightly warmer than year 1 during both the growing cycle and the flowering period (Table 3), factors other than temperature were involved in HS tolerance variation observed in the cultivated group. For instance, the higher RH in year 1 (Table 3) plus the wetter condition imposed by the paper bags might increase the severity of the HS treatment. Warmer temperatures along with high RH could have affected our results by worsening the performance in year 1, at least in the cultivars. Despite this, RH during the day was similar between control and heat-stressed bags and much lower than RH used in controlled heat stress evaluations (65–85%; Pradhan et al. 2012; Pradhan and Prasad 2015; Prasad and Djanaguiraman 2015; Valluru et al. 2016). In addition, we did not observe any symptom of excessive humidity in cover heads (rotting or pathogens).

As in our study, severe reductions in crop yields (> 60%) under HS in the reproductive phase were previously reported for many crops, such as chickpea (Devasirvatham et al. 2012), maize (Rattalino-Edreira et al. 2011) and wheat (Prasad and Djanaguiraman 2015). In contrast, Siebers et al. (2015, 2017) observed slight decreases (∼ 10%) for maize and soybean, respectively. Such differences are likely to reside in the temperatures used for the HS treatment: ∼ 30 °C in the Siebers´ experiments and ∼ 40 °C in the rest.

We identified at least five (BRW, DIA, LMA, IL, and IN) and two (LMA and RCU) wild populations in years 1 and 2, respectively, which clearly outperformed cultivars, most of them from the invasive range (Fig. S2). Interestingly, all of these populations (including the native ones) showed strong evidence of crop allele introgression (Cantamutto et al. 2010; Casquero et al. 2013), which suggests a role of the admixture between wild and cultivated taxa in the observed HS tolerance. Of the three yield-related traits evaluated, head diameter and seed weight were slightly affected by HS or not, whereas the difference between the HS susceptible and tolerant populations was the greater ability to set seeds (higher seed number) under HS conditions of the tolerant populations ($r_{SEED\ NUMBER\text{-}YIELD} = 0.86$, $n = 33$, $p < 0.0001$). The strong correlation between seed number and yield is commonly associated with empirical rather than functional processes (Sinclair and Jamieson 2006), i.e. the available resources for plant (e.g. nitrogen, carbon, water and light) determine their yield and the number of setting seeds is just a consequent adjustment.

Despite this, brief periods of HS may result in poor seed set, with no effects on the total biomass (Jha et al. 2014). In our study, the HS was applied to heads simulating a brief period of HS, thus the final seed number was not a consequence of the plant resource availability but the ability of reproductive tissues to survive and complete the seed set. Whether HS tolerance resides in lower pollen/ovule sterility and/or lower seed abortion cannot be answered from our study. For instance, HS tolerant genotypes of chickpea showed higher pod set under HS conditions throughout higher levels of pollen viability (Devasirvatham et al. 2012). In sunflower, the availability of high-throughput techniques for evaluating pollen viability (Atlagić et al. 2012) may help to identify populations with higher levels of pollen viability under HS. On the other hand, in wild rice, an early morning flowering trait, which shifted the peak of flowering to the cooler hours of the morning, was found and successfully introgressed into elite inbred lines (Bheemanahalli et al. 2017; Hirabayashi et al. 2015). This trait is an exciting example of one wild trait successfully introgressed into elite cultivars to deal with HS and needs to be explored in wild sunflower.

Comparison between native and invasive populations reveals local adaptation to heat stress

Invasive populations usually exhibited larger size, higher reproductive allocation, and higher fitness than

their native counterparts (Felker-Quinn et al. 2013; Turner et al. 2013; van Kleunen et al. 2010). By using biogeographic tools, we found that invasive populations exhibited higher HS tolerance than the native ones for seed number and yield (Fig. 4a, b) even when environmental covariates were included in the model. In addition, we found a strong positive correlation between precipitation PCs (Fig. 5), showing local adaptation to HS but no correlation with the temperature PCs. The absence of any correlation between the temperature PCs and HS tolerance may be explained by the different strategies used by wild sunflower to deal with stress. For instance, a decreased seed dormancy and earlier and extended flowering to avoid exposure to the warmest months, and a higher production of small heads to scatter the seed production, may be beneficial in the warmest and driest habitats, such as Arizona, Texas and New Mexico. Evidence of selection of the above mentioned traits was found in the colonization of extreme habitats by sunflower species (Ludwig et al. 2004; Whitney et al. 2010) supporting this idea. Besides, in the present study we did not evaluate the performance in warmer conditions (e.g. 2–4 °C above the control temperature) but against brief periods of HS (seven consecutive days with maximum temperatures $\sim$ 40 °C). Therefore, although brief periods of HS are more likely to occur in warmer environments, whether the frequency of such periods is accounted for in our temperature PCs, or whether they act as natural selection agents, remains unclear.

The positive correlation observed between reproductive traits under control conditions and HS tolerance helps to explain why the invasive populations outperformed the native ones and why the increased HS tolerance is correlated with PC1*water* (Fig. 5). Firstly, invasive populations exhibited larger reproductive traits and these differences could not be explained by the environmental differences between ranges. Admixture between previously isolated wild populations and more likely between wild populations and cultivars during the introduction in the invasive range are the probable causes of the increased values in reproductive traits (Mesgaran et al. 2016; Mondon et al. 2018; van Kleunen et al. 2015). Large molecular and morphological variability found in invasive populations from Argentina (Cantamutto et al. 2010; Garayalde et al., 2011) makes the admixture a probable scenario. Secondly, because PC1*water* explained the variation in both the reproductive traits and HS tolerance, the correlation observed between PC1*water* and HS tolerance may be empirical rather than functional. In this context, a trait-based approach rather than an environment-based approach would be recommended for future pre-breeding activities.

Finally, the increased HS tolerance found in invasive populations widens the genetic variability within sunflower germplasm and turns them into a valuable source for current and future breeding programs, and demands their ex situ and in situ conservation, regardless of the monitoring efforts due to their invasive condition. In addition, we need to focus on pre-breeding activities, e.g. construction of segregating populations, molecular and physiological characterization of HS tolerance and advanced backcrosses of HS tolerant populations with elite inbred lines, to improve the usefulness of wild germplasm.

Conclusions

In the present study, we developed a simple, rapid and high-throughput technique to test for HS tolerance during the most sensitive phase, flowering, under field conditions. With this technique, we found large variability for HS tolerance within wild sunflower germplasm. Invasive populations outperformed cultivars and native populations, turning the former a valuable source for current and future breeding programs. We also found a clinal variation in HS tolerance. Populations from wetter environments (but not from warmer) were more tolerant to HS. Finally, the increased performance of invasive populations could not be explained by environmental differences between native and invasive ranges suggesting that factors other than the observed cline are involved in the increased HS tolerance of invasive populations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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