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Utilization of leaf temperature for the selection of leaf gas-exchange traits to induce heat resistance in sunflower (*Helianthus annuus* L.)

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Abstract

Heat stress is a major production constraint of sunflower worldwide. Therefore, various populations (parental, F_1, F_2, F_3, and plant progenies) of sunflower were screened for leaf gas-exchange traits with the objectives to formulate selection criteria of heat resistance and development of heat-resistant lines. Initial screening and F_2 seeds exposed to heat stress (45°C) resulted in the development of an adapted F_2 population that showed leaf gas-exchange and morphological traits better than the unadapted population. Correlation coefficients of traits were partitioned into direct and indirect effects *via* a path analysis technique to determine the cause of their relationship with a basic parameter such as a reproductive head mass (HM). Path analysis showed a positive direct effect of leaf temperature (T_{leaf}) (0.32) on HM and also an indirect effect (0.77) of the transpiration rate (E) on HM. Moreover, T_{leaf} showed high heritability estimates. T_{leaf} was used to select superior plants within the F_2 population. This selection brought about an improvement in the net photosynthetic rate (P_N) and E as it was indicated from progeny performance and realized heritability. Progenies selected on the basis of T_{leaf} also showed an increase in achene yield and heat resistance over unselected F_3 progenies and a commercial hybrid.

Additional key words: gametes; genetic variability; germplasm; heat stress.

Introduction

Sunflower is a heat-sensitive crop. Qadir *et al.* (2007) found that 25–30°C is the optimal temperature for growth and germination, while temperatures exceeding 30°C pose a stress on the plant. Sunflower is more sensitive to abiotic stresses, such as moisture, salinity, and heat, than other plant species (Dekov *et al.* 2001, Rauf 2008, Rauf and Sadaqat 2008, Rauf *et al.* 2008, 2012).

Among various growth stages, a plant reproductive phase experiences the most deleterious effects under heat stress (Martiniello and Teixeira da Silva 2011). Various studies are available indicating the repressive effect of heat stress on sunflower yield and quality in which high temperature has been correlated with critical growth stages, such as anthesis or grain filling (Rondanini *et al.* 2003, 2006). Rondanini *et al.* (2006) quantified the effects of day/night high temperature regimes on sunflower grain yield and quality and they showed that high temperature (35°C) stress lasting 4 or more days caused significant reductions in grain yield and quality. Similarly, Rondanini *et al.* (2003) exposed the capitulae of sunflower plants to a range of high temperatures (35, 37, and 40°C) for 7 consecutive days during grain filling. Heat stress resulted in 40% reduction in grain mass at temperatures >35°C during early grain development because of the reduction of grain growth, while at the same temperature oil grain percentage was reduced by 30%. Lower number of grains/cob may be attributed to the abortive effect of heat stress on the susceptible gametes of maize (Petolino *et al.* 1990). Thus, selection for heat-resistant gametes is an index of heat-resistant genotypes and improves the performance of the genotype under heat stress in the next generation. *In vitro* germination of gametes or pollination under heat stress has been proposed to develop heat-resistant genotypes (Acar and Kakani 2010). Furthermore, exposing the reproductive stage (anthesis) of the plants to heat stress in the field could also help

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Abbreviations: C×B – CM-207 × BF-88 cross; C×C – CM-207 × CM-631 cross; DPH – droopy plant height; E – transpiration rate; g_s – stomatal conductance; HM – head mass; LM – leaf mass; NL – number of leaves; P_N – net photosynthetic rate; SD – stem diameter; SM – stem mass; T_{air} – ambient air temperature around leaf; T_{leaf} – leaf temperature; WUE – water-use efficiency; σ^2E – environmental variance; σ^2G – genotypic variance; σ^2P – phenotypic variance.

select for heat-tolerant gametes and finally seeds (Rahman 2004).

Despite significant losses in the yield caused by heat stress in sunflower, very few studies have been conducted to enhance heat tolerance of this crop. Regarding breeding, efforts have been carried out only to screen germplasm and to identify tolerant inbred lines for the development of hybrids. However, genetic variability for thermotolerance exists within cultivated germplasm of sunflower (Senthil-Kumar *et al.* 2003). Despite this known fact, specific breeding programmes to enhance the heat tolerance of breeding material are rare. Furthermore, information regarding the applicability of specific leaf gas-exchange or morphological traits as selection criteria and their relationships with the yield in sunflower breeding programmes is still unknown.

On these grounds, a research project was undertaken to study the usability of leaf gas-exchange traits as selection criteria for the development of heat-resistant and productive sunflower populations, to understand the contribution of various leaf gas-exchange and morphological traits on reproductive biomass, and to evaluate the outcome of selection in terms of: (*1*) improvement in the mean values of leaf gas-exchange traits as a result of selection of genotypes having these superior leaf gas-exchange traits; (*2*) correlated response of selection to achene yield and heat resistance.

This information could also be useful to understand the heat tolerance and the sustainability of sunflower yield under heat stress.

Materials and methods

Plant material: The study was carried out in large plastic bags and in experimental research field of the University College of Agriculture, University of Sargodha, for two consecutive years (2011–2012). Sunflower germplasm was introduced from the USDA germplasm collection during 2009. In the same year, germplasm was evaluated for various morphological and biochemical traits and crosses were attempted among various inbred lines. In 2010, crosses were evaluated to maturity at multiple locations under various temperature regimes, *i.e.* optimal temperature (25–30°C) and heat stress (45°C). Two crosses, CM-207 × CM-631 (C×C) and CM-207 × BF-88 (C×B), were selected on the basis of their performance under heat stress. These crosses were the source of F_2 seeds. Two types of F_2 seeds were obtained: one from F_1 crosses under an optimal regime and the other from the heat stress regime. F_2 seeds obtained from crosses grown under the heat stress were designated as the heat stress-adapted seeds, while F_1 grown in the optimal regime resulted in unadapted F_2 seeds. Parents (CM-207, CM-631, BF-88) involved in the F_1 crosses and in a subsequent F_2 generation were again used to produce fresh F_1 seeds for comparing the parents, F_1, and F_2 generations (adapted and unadapted) under a heat stress (45°C) regime.

Cultivation of plant material: Parental lines, F_1 crosses, and F_2 seeds were grown in large plastic bags containing 17 kg of soil (an equal amount of sand, silt, and farmyard manure) to have a homogenous fertility gradient in all plant populations. In total, 15 bags were used for F_1 and parental lines, while 80 bags were used for the F_2 population. Three seeds were sown per a bag for all populations at a uniform depth of 2.5 cm to grow seedlings. They were thinned out to a single plant after the germination 10 d after sowing (DAS).

Development of plant progenies and F_3 population: Superior plants (on the basis of high mean values of measured physiological traits) were selected in the F_2 adapted populations for a progeny test. For this purpose, the first few rows of selected sunflower head (capitula) were manually emasculated early in the morning to remove immature anthers with the help of forceps. Emasculated rows within head were pollinated with the pollen from a heat-susceptible F_2 plant showing inferior performance under the heat stress. The plant was considered as a tester since it was used to test the performance of the selected F_2 plants. The seeds, which developed from emasculated rows and were pollinated subsequently by the tester, were harvested separately from other rows within the same head. F_3 seeds were developed by bagging the heads of F_2 plants.

Raising plant progenies in a replicated trial: Plant progenies along with the F_3 population of crosses (C×B, C×C) were grown in a replicated trial under a randomized complete block design with three replications and at two locations during 2012. There were three rows per progeny within each replication, while the F_3 population was represented by 6 rows per replication. Each row was 450 cm in length, row-to-row distance was 60 cm, and plant-to-plant distance was 22 cm. All weeds were controlled manually. Any leaf disease was scored as absent or present and insects were controlled by recommended insecticide (pyriproxyfen 620 ml ha^{-1} and lufeneron 500 ml ha^{-1}) before they could cause any significant damage to the crop. Populations were raised according to the production package adapted in the region.

The heat stress regime: During the vegetative phase, plants were grown under optimal conditions, then the temperature was gradually raised to a maximum of 45°C. Photosynthetically active radiation was 1,900 µmol m^{-2} s^{-1} during the vegetative phase just before the start of the reproductive phase and the temperature was held until maturity (Fig. 1). During the growth cycle, all plants were

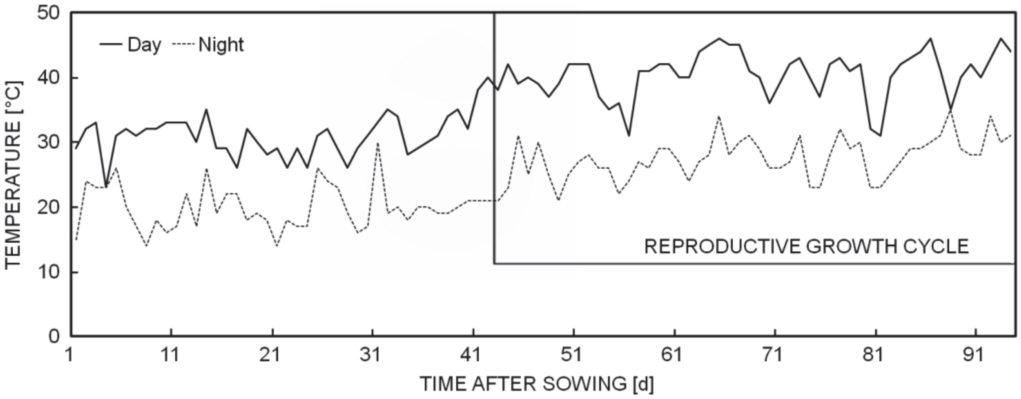

Fig. 1. Mean minimum and maximum temperature (°C) throughout the growth cycle of sunflower (*Helianthus annuus* L.).

irrigated with an equal quantity of water according to the plant demand to avoid drought stress. Leaf gas-exchange traits were measured at anthesis (70 DAS), while morphological traits were measured at maturity (100 DAS).

Plant traits: Leaf gas-exchange characteristics (P_N, E, and g_s) as well as T_{leaf} and temperature of ambient air around the leaf (T_{air}) were measured with *Photosystem CI-340* (*Camas*, WA, USA) between 10:00 and 12:00 h. T_{air} was determined for each plant with an infrared thermometer installed on *Photosystem CI-340*. Average T_{air} for plants was 40.4°C ± 1.0. Leaf to air temperature difference (Δ) was calculated by subtracting T_{air} from T_{leaf} (Royo *et al.* 2002), while water use efficiency (WUE) was calculated as the ratio of P_N to E.

Data were obtained from 50 F_2 plants and 15 F_1 plants and of the parental generation within each cross. At maturity (100 DAS), morphological traits, *i.e.* stem diameter (SD), head mass (HM), leaf mass (LM), stem mass (SM), head diameter (HD), plant height (PH), droopy plant height (DPH), and number of leaves (NL) were measured. For this purpose, plants were cut from the base and dissected into three parts: head, stem, and leaves. The masses of all three organs were measured on a digital balance after drying at 60°C until dry mass became constant. PH, SD, and HD were measured with a measuring tape in cm. DPH was measured from the base to the bend in the drooping stem.

Seed yield per plant of 30 plants within each progeny was measured by threshing seed from the capitula and measuring their mass on a digital balance.

Seedling survival (%) was estimated by growing sunflower seeds in germinating trays filled with a loam type of soil. Within each tray, 100 seeds were sown at 25°C, 60% relative humidity, and in a growth chamber until the first true leaves emerged. At this stage, temperature in the growth chamber was raised to 50°C for 5 h. After the heat stress treatment, seedlings were allowed to recover for 72 h and final seedling mortality was assessed.

Biometric and statistical procedures: Generation means were estimated by averaging the total number of measurements in each generation. Variation among the values within each generation was computed by measuring the variance of each generation. Phenotypic variance (σ^2P) was estimated by measuring the variance of the F_2 population as it included both environmental (σ^2E) and genotypic variation (σ^2G). On the other hand, σ^2E was estimated by averaging the variance of F_1 and the parental generation, while σ^2G was estimated by subtracting σ^2E from σ^2P. Estimates of σ^2P, σ^2E, and σ^2G were summarized in the following equations:

$$\sigma^2P = \sigma^2F_2;$$
$$\sigma^2E = (\sigma^2F_1 + \sigma^2P_1 + \sigma^2P_2)/3;$$
$$\sigma^2G = \sigma^2P - \sigma^2E.$$

Heritability was estimated as σ^2G/σ^2P. Realized heritability was estimated as the ratio of selection response to the selection differential (X_SF_3 – XF_3/X_SF_2 – XF_2), in which X_SF_3, XF_3, X_SF_2, XF_2 are means for the F_3 progenies for the selected F_2 plant, F_3 progenies from the whole F_2 population (including selected plants), selected F_2 plants, and the whole F_2 population, respectively. The selection response was the difference between the selected plants and the total mean of the F_2 population. 10 plants from each of the F_2 populations were drawn to formulate 3 classes on the basis of the leaf temperature differences, *i.e.* the lowest T_{leaf}, medium T_{leaf}, and high T_{leaf}. Averages of these plants were used to estimate the selection differential since this was the difference between the averages of a selected plant and the original F_2-adapted population. Correlations were computed with computer-based software *MiniTab 15*, while the path diagram was constructed with *AMOS-5* software (*Arbuckle 2000*, Bethlehem Pike, USA). Correlation coefficients showed the magnitude of association between two traits. The intensity of the relationship ranged from 0–1, where "1" was the maximal degree of association. Path coefficient analysis is a technique used to partition the correlation coefficient into direct and indirect effects and it measures the cause of the relationship between two traits. Values ranging from 0.30–0.99 are considered to be high, while it is also possible that values may exceed 1. The direct effect shows the individual effect, while the indirect effect shows the effect of a trait *via* other traits (Singh and Narayanam 2007). Parents, F_1, F_2, and progeny means were compared using *Fisher'*s LSD test at $p<0.05$.

Results

The mean performance of various generations for leaf gas-exchange traits is provided in Table 1. The mean of the F_1 population derived from the C×C cross was significantly higher than its parents and the F_2 population for traits, such as P_N and E. The mean value for T_{leaf} in the F_1 population derived from the C×C cross was significantly lower than one of the parents (CM-631) but higher than the other parent, CM-207. The mean of Δ in F_1 (C×C) was significantly lower than the parent CM-207, but higher than the other parent, CM-631. The F_2 mean for P_N, E, and T_{leaf} was lower than that of F_1, while the mean for Δ and WUE in F_2 was higher than that of F_1 in the adapted population derived from C×C (Table 1). However, the F_2 mean for g_s showed an insignificant difference in the adapted population compared with the F_1 population and the unadapted F_2 population. The adapted F_2 population showed a higher mean for P_N, E, WUE, and Δ than the unadapted F_2 population (*i.e.* F_2 seed obtained under optimal regimes) derived from C×C.

The mean for P_N in the F_1 population derived from C×B was significantly higher than the mean of the F_1 population derived from the C×C cross but it was insignificant for other traits. The F_1 mean for P_N and E was higher than that of both parents in the cross of C×B. The F_1 (C×B) means for WUE and T_{leaf} were significantly higher than the means of its parent CM-207. Parents (CM-207 and BF-88) showed significant differences for all traits except WUE. The F_2 means for E and T_{leaf} of the adapted population derived from the cross C×B were significantly lower than $F_{1,}$ while the mean was higher than that of F_1 for Δ. The adapted F_2 population showed higher or similar mean values for all traits except for T_{leaf} and g_s when compared with the unadapted F_2 population, showing positive selection for P_N, E, and Δ in the cross derived from C×B.

On the basis of T_{leaf}, the plants were divided into three major groups: low (28°C), medium (37°C), and high (42°C) within the F_2 population. The plants maintaining T_{leaf} of approximately 37°C or a Δ of 1.6 showed higher values for all leaf gas-exchange traits, followed by plants which maintained the low T_{leaf} or high Δ. The plants showing the high T_{leaf} (42°C) with low Δ showed lower estimates for P_N, g_s, and WUE.

Sunflower populations were also evaluated according to several morphological traits listed in Table 2. The F_1 population showed higher mean than F_2 or parental generations for all traits except for SM and PH in population derived from cross C×C and for DPH in F_1 derived from C×B. Parents of both crosses showed significant differences for all traits except for HD in parents (CM-207 and BF-88). Adapted F_2 plants showed higher mean value for all traits than unadapted F_2 plants except for PH and DPH in population derived from C×C. The F_2 population had also lower values than F_1 in both crosses except for SD, SM, and PH in cross C×C. The adapted F_2 plants were further divided into plants that maintained medium or low T_{leaf}. The plants possessing lower T_{leaf} showed lower means for all traits except SD, SM, and NL compared with those that maintained medium T_{leaf}. All traits showed low to medium heritability reflecting a significant impact of the environment on the phenotypic expression of these traits. However, traits such as NL and SM showed high heritability estimates only in cross C×C. Among the traits, the highest broad sense heritability was shown by NL, followed by SD in the cross C×C. In the cross C×B, SD and DPH showed the highest broad sense heritability. Selection on the basis of T_{leaf} brought also the highest selection differential for HM in both crosses.

Among all traits, P_N and E showed the highest realized heritability (Table 3), while heritability in a broad sense was high for all leaf gas-exchange traits except for g_s in the populations derived from both crosses. The cross C×B showed higher estimate of realized heritability than C×C (Table 3). Within this cross, P_N followed by E showed the highest estimates of realized heritability.

Progenies derived from both crosses were evaluated along with the F_3 population as a standard check in replicated trials over two locations. Analyses of variance showed significant variation within progenies for all traits of the crosses. Furthermore, the progeny also differed significantly over all means from the F_3 mean as indicated from contrast of F_3 *vs.* progenies showing that selection was effective in F_2 population over leaf gas-exchange traits. The locations or progenies *vs.* location was insignificant for most of the traits. Therefore progenies and F_3 mean performance was averaged over the location. The mean performance of progenies and F_3 over three replications is displayed in Table 3. Overall mean performance of the progenies surpassed that of the F_3 population in P_N, E, WUE, and T_{leaf} in the cross derived from C×C, while progenies obtained from the cross of C×B showed significantly higher values for P_N, E, and T_{leaf}. However, progenies within crosses also showed significant differences and showed higher mean performance than overall mean of the progeny for specific trait (Table 3).

Table 1. Population means, selection responses, selection differential, and heritability estimates of photosynthesis rate (P_N, [µmol m^{-2} s^{-1}]), transpiration (E, [mmol m^{-2} s^{-1}]), stomatal conductance (g_s, [mmol m^{-2} s^{-1}]), water-use efficiency (WUE, [µmol(CO_2) mmol(H_2O)$^{-1}$]), leaf temperature (T_{leaf}, [°C]) and leaf-to-air temperature difference (Δ [°C]) of various populations of sunflower (*Helianthus annuus* L.) under heat stress. Data are expressed as mean values (parental and F_1 population $n = 15$ plants while F_2 population $n = 50$ plants), ± SD is shown for selected plants, F_1, and parents. Values within columns followed by *different letters* showed difference among genotypes at $p<0.05$ using *Fisher'*s LSD test.

Population	P_N	E	g_s	WUE	T_{leaf}	Δ
CM-207	3.93 ± 1.61^{C}	3.13 ± 0.98^{C}	0.90 ± 0.44^{A}	1.25 ± 0.52^{Bc}	31.76 ± 0.59^{E}	10.26 ± 1.37^{A}
CM-631	0.63 ± 0.21^{E}	0.55 ± 0.18^{D}	0.19 ± 0.09^{C}	1.14 ± 0.55^{C}	42.21 ± 0.61^{A}	-1.21 ± 0.42^{E}
CM-207 × CM-631 (F_1)	8.58 ± 2.41^{A}	6.74 ± 2.06^{A}	0.55 ± 0.26^{B}	1.27 ± 0.58^{B}	36.73 ± 0.61^{C}	2.37 ± 0.81^{C}
CM-207 × CM-631 (F_2) (adapted)	5.92^{B}	4.63^{B}	0.59^{B}	2.57^{A}	32.64^{D}	7.30^{B}
CM-207 × CM-631 (F_2) (unadapted)	2.44^{D}	3.61^{C}	0.58^{B}	0.61^{D}	37.29^{B}	1.04^{D}
Selected plants (high T_{leaf})	0.76 ± 0.17	0.85 ± 0.23	0.17 ± 0.02	0.89 ± 0.21	42.08 ± 1.01	-1.31 ± 0.15
Selected plants (medium T_{leaf})	9.56 ± 1.37	7.78 ± 0.51	0.60 ± 0.27	1.24 ± 0.17	37.47 ± 0.45	1.63 ± 0.61
Selected plants (low T_{leaf})	5.59 ± 2.58	5.48 ± 1.89	0.46 ± 0.91	1.25 ± 0.67	28.37 ± 1.37	12.39 ± 0.80
σGenotype	10.34	7.66	527.05	7.88	18.38	18.36
σPhenotype	13.15	8.81	936.15	10.11	18.76	19.12
σEnvironment	2.81	1.15	409.02	2.31	0.38	0.76
Heritability in a broad sense	0.78	0.87	0.56	0.78	0.98	0.96
CM-207	3.93 ± 1.61^{D}	3.13 ± 0.98^{E}	0.90 ± 0.44^{A}	1.25 ± 0.52^{C}	31.76 ± 0.59^{D}	10.26 ± 1.37^{A}
BF-88	6.91 ± 2.98^{C}	6.84 ± 0.22^{C}	0.57 ± 0.11^{D}	1.41 ± 1.11^{Bc}	37.21 ± 0.71^{B}	2.88 ± 1.06^{C}
CM-207 × BF-88 (F_1)	11.48 ± 2.51^{A}	7.71 ± 0.24^{A}	0.64 ± 98.37^{Cd}	1.61 ± 1.06^{Ab}	36.96 ± 0.73^{B}	3.38 ± 1.08^{C}
CM-207 × BF-88 (F_2) (adapted)	11.09^{A}	7.26^{B}	0.70^{Bc}	1.72^{A}	33.57^{C}	5.29^{B}
CM-207 × BF-88 (F_2) (unadapted)	8.21^{B}	6.31^{D}	0.80^{Ab}	1.54^{AB}	39.55^{A}	0.78^{D}
Selected plants (medium T_{leaf})	12.44 ± 3.53	7.48 ± 0.60	0.68 ± 0.14	1.66 ± 0.64	37.46 ± 0.48	1.80 ± 0.37
σGenotype	31.38	1.29	71.32	9.78	3.76	7.98
σPhenotype	36.76	1.52	118.64	10.66	4.22	9.35
σEnvironment	5.60	0.23	47.37	0.88	0.46	1.37
Heritability in a broad sense	0.84	0.85	0.40	0.92	0.89	0.85

Table 2. Population means, selection responses, selection differential, heterosis, inbreeding depression, and heritability estimates of morphological traits, *i.e.* stem diameter (SD, [cm]), head mass (HM, [g]), leaf mass (LM, [g]), stem mass (SM, [g]), head diameter (HD, [cm]), plant height (PH, [cm]), droopy plant height (DPH, [cm]), and number of leaves (NL) of various populations of sunflower (*Helianthus annuus* L.) under heat stress. Data are expressed as mean values (parental and F_1 population: $n = 15$ plants, while F_2 population: $n = 50$ plants), ± SD is shown for selected plants, F_1, and parents. Values within columns followed by *different letters* showed difference among genotypes at $p<0.05$ using *Fisher's* LSD test.

Population	SD	HM	LM	SM	HD	PH	DPH	NL
CM-207	4.00 ± 0.29^{C}	28.63 ± 2.58^{B}	7.13 ± 1.88^{D}	20.00 ± 1.05^{B}	9.90 ± 0.61^{B}	93.63 ± 5.10^{B}	81.13 ± 6.89^{C}	20.75 ± 1.81^{C}
CM-631	4.11 ± 0.36^{B}	9.11 ± 1.77^{D}	5.12 ± 1.29^{E}	13.00 ± 1.31^{D}	6.43 ± 0.88^{D}	77.89 ± 3.89^{C}	69.89 ± 7.73^{E}	12.89 ± 0.61^{E}
CM-207 × CM-631 (F_1)	4.55 ± 0.15^{A}	34.17 ± 2.33^{A}	14.83 ± 1.69^{A}	20.50 ± 1.11^{B}	10.63 ± 0.51^{A}	104.17 ± 6.11^{A}	89.33 ± 8.22^{A}	25.17 ± 1.93^{A}
CM-207 × CM-631 (F_2) (adapted)	4.48^{A}	28.71^{B}	12.25^{B}	22.53^{A}	9.32^{C}	103.83^{A}	75.21^{D}	24.43^{B}
CM-207 × CM-631 (F_2) (unadapted)	3.65^{D}	21.35^{C}	8.82^{C}	16.33^{C}	6.39^{D}	104.88^{A}	82.33^{B}	19.22^{D}
Selection differential	0.83	7.36	3.43	6.2	2.93	0.00	0.00	5.21
Selected plants (medium T_{leaf})	4.38 ± 0.61	37.30 ± 11.60	16.70 ± 3.89	21.60 ± 7.04	10.06 ± 1.79	106.70 ± 16.14	73.30 ± 25.87	23.20 ± 3.99
Selected plants (low T_{leaf})	4.46 ± 0.98	26.56 ± 5.46	8.06 ± 3.17	18.67 ± 5.92	8.84 ± 1.04	105.22 ± 17.84	86.56 ± 17.09	24.78 ± 2.54
Heritability in a broad sense	0.71	0.38	0.32	0.88	0.42	0.69	0.69	0.91
CM-207	4.00 ± 0.29^{D}	28.63 ± 2.58^{B}	7.13 ± 1.88^{E}	20.00 ± 1.05^{C}	9.90 ± 0.61^{B}	93.63 ± 2.10^{C}	81.13 ± 2.89^{A}	20.75 ± 1.81^{D}
BF-88	4.20 ± 0.31^{C}	22.33 ± 2.11^{C}	15.00 ± 2.22^{B}	21.00 ± 0.87^{B}	9.80 ± 0.88^{B}	85.00 ± 3.33^{E}	79.00 ± 3.44^{B}	27.00 ± 0.73^{B}
CM-207 × BF-88 (F_1)	4.88 ± 0.41^{A}	37.88 ± 2.92^{A}	18.35 ± 1.91^{A}	23.33 ± 1.01^{A}	12.21 ± 0.90^{A}	103.33 ± 4.17^{A}	81.22 ± 2.22^{A}	31.13 ± 1.58^{A}
CM-207 × BF-88 (F_2) (adapted)	4.48^{B}	28.43^{B}	14.07^{C}	20.47^{D}	9.71^{B}	95.83^{B}	63.60^{C}	23.09^{C}
CM-207 × BF-88 (F_2) (unadapted)	4.12^{Cd}	20.55^{D}	7.90^{D}	19.50^{E}	6.50^{C}	91.35^{D}	60.25^{D}	18.55^{E}
Selection differential	0.36	7.88	6.17	0.97	3.21	4.48	3.35	4.54
Selected plant (optimum T_{leaf})	4.39 ± 0.38	35.33 ± 10.08	15.26 ± 3.85	18.35 ± 7.03	11.35 ± 1.69	91.80 ± 12.44	52.35 ± 17.56	25.50 ± 3.40
Heritability in a broad sense	0.67	0.37	0.52	0.59	0.52	0.46	0.65	0.48

Table 3. Mean performance of plant progenies averaged over locations in replicated trial during 2012. Data are expressed as mean values (each plant progeny *n* = 30 plants per replication), ± SD is shown for progeny mean. Values within columns followed by *different letters* showed difference among genotypes at $p<0.05$ using *Fisher's* LSD test. P_N – net photosynthetic rate, [μmol m^{-2} s^{-1}]; *E* – transpiration rate, [mmol m^{-2} s^{-1}]; g_s – stomatal conductance, [mol m^{-2} s^{-1}]; WUE – water-use efficiency, [μmol(CO_2) mmol(H_2O)$^{-1}$]; T_{leaf} – leaf temperature, [°C]; Δ – leaf-to-air temperature difference, [°C].

Progeny	Cross	P_N	*E*	WUE	Δ	g_s	T_{leaf}
UCA-0	CM-207 × CM-631 (F_3)	12.27^B	9.11^B	1.29^B	3.23^A	0.83^A	34.91^B
UCA-1	(CM-207 × CM-631; F_2) × Tester	15.01 ± 0.92	8.28 ± 1.22	1.81 ± 0.54	2.63 ± 0.78	0.86 ± 0.16	36.00 ± 0.38
UCA-2	(CM-207 × CM-631; F_2) × Tester	13.33 ± 0.78	10.10 ± 0.95	1.32 ± 0.42	3.82 ± 0.58	0.76 ± 0.15	36.38 ± 0.42
UCA-3	(CM-207 × CM-631; F_2) × Tester	10.57 ± 0.69	11.47 ± 0.81	0.93 ± 0.38	3.08 ± 0.61	0.78 ± 0.15	37.30 ± 0.57
UCA-4	(CM-207 × CM-631; F_2) × Tester)	17.16 ± 1.05	9.08 ± 0.79	1.89 ± 0.36	4.31 ± 0.88	1.17 ± 0.12	36.76 ± 0.58
Progeny average		14.02^A	9.73^A	1.49^A	3.46^A	0.89^A	0.89^A
Selection differential		3.64	3.15	0.00	0.00	4.7	4.7
Selection response		1.75	0.62	0.20	0.23	57.51	57.51
Realized heritability		0.48	0.20	0.00	0.00	0.00	0.00
UCA-2-1	(CM-207 × BF-88) × Tester	15.44 ± 0.54	9.66 ± 0.56	1.59^B ± 0.42	2.91 ± 0.54	0.67 ± 0.14	37.88 ± 0.19
UCA-2-2	(CM-207 × BF-88) × Tester	21.80 ± 0.72	9.46 ± 0.48	2.32^A ± 0.39	2.95 ± 0.68	0.67 ± 0.14	38.70 ± 0.29
UCA-2-3	(CM-207 × BF-88) × Tester	13.29 ± 0.59	9.01 ± 0.21	1.48^B ± 0.44	6.16 ± 0.93	1.02 ± 0.16	34.38 ± 0.36
UCA-2-4	(CM-207 × BF-88) × Tester	14.37 ± 0.61	8.43 ± 0.34	1.70^B ± 0.36	3.60 ± 0.81	0.68 ± 0.16	36.16 ± 0.41
UCA-2-0	CM-207 × BF-88 (F_3)	15.34^B	8.51^B	1.69^A	3.73^A	0.75^A	35.12^B
Progeny average		16.23^A	9.14^A	1.77^A	3.91^A	0.76^A	0.76^A
Selection differential		4.42	0.72	0.41	0.9	26.43	26.43
Selection response		1.35	0.22	0.00	0.00	0.00	0.00
Realized heritability		0.66	0.65	0.00	0.00	0.00	0.00

Table 4. Mean performance of plant progenies over locations for seed yield per plant (SYP) and seedling survival (SS) in a replicated trial during 2012. Data are expressed as means ± SD of $n = 30$ for SYP and $n = 100$ for SS. Values within columns followed by *different letters* showed difference among genotypes at $p<0.05$ using *Fisher's* LSD test.

Progeny	Cross	SYP [g]	SS [%]
UCA-0	CM-207 × CM-631 (F_3)	93.80^{B}	71.34^{B}
UCA-1	(CM-207 × CM-631; F_2) × Tester	98.34 ± 17.83	85.00
UCA-2	(CM-207 × CM-631; F_2) × Tester	109.00 ± 26.51	90.16
UCA-3	(CM-207 × CM-631; F_2) × Tester	158.34 ± 14.14	94.12
UCA-4	(CM-207 × CM-631; F_2) × Tester	126.22 ± 8.18	95.15
Progeny average		122.98^{A}	91.10^{A}
UCA-2-1	(CM-207 × BF-88) × Tester	125.22 ± 7.62	96.88
UCA-2-2	(CM-207 × BF-88) × Tester	116.34 ± 8.51	93.85
UCA-2-3	(CM-207 × BF-88) × Tester	109.33 ± 9.35	85.11
UCA-2-4	(CM-207 × BF-88) × Tester	105.41 ± 10.31	86.35
UCA-2-0	CM-207 × BF-88 (F_3)	91.25^{B}	69.22^{B}
Progeny average		114.33^{A}	90.55^{A}
S-278			56.33^{C}

Table 5. Correlation coefficients for photosynthesis rate (P_N), transpiration rate (E), stomatal conductance (g_s), water use efficiency (WUE), leaf temperature (T_{leaf}), and number of leaves (NL) on head mass (HM) in the F_2 population under heat stress. ^{NS} – nonsignificant at $p<0.05$; * – significant at $p<0.05$; ** – highly significant at $p<0.01$.

Traits	P_N	E	g_s	T_{leaf}	Δ	NL	HM
P_N							0.49**
E	0.91**						0.57**
g_s	0.03^{NS}	0.07^{NS}					0.14^{NS}
T_{leaf}	0.75**	0.83**	–0.13^{NS}				0.47**
Δ	–0.53**	–0.58**	0.18^{NS}	–0.87**			–0.31*
NL	0.12^{NS}	0.02^{NS}	0.08^{NS}	–0.09^{NS}	–0.04^{NS}		0.24^{NS}
WUE	–0.31*	–0.44*	–0.15^{NS}	–0.06^{NS}	–0.11^{NS}	–0.05^{NS}	–0.16^{NS}

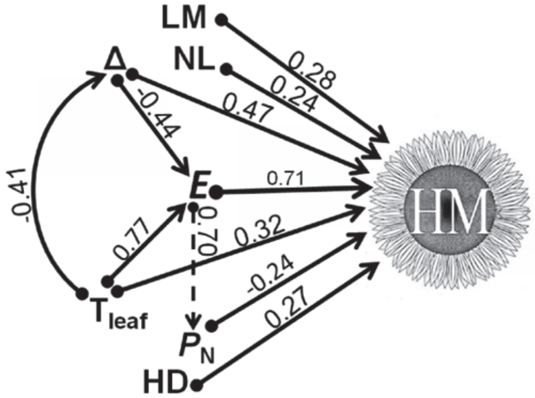

Fig. 2. Path diagram showing direct and indirect effects of transpiration rate (E), leaf-to-air temperature difference (Δ), leaf temperature (T_{leaf}), photosynthesis rate (P_N), head diameter (HD), and leaf mass (LM) on head mass (HM).

Mean performance of the F_3 population was also better than the F_2 population showing improved plant attributes related to their physiology and resistance to heat stress. Improvement in mean performance of the populations due to the selection could be implied from the realized heritability, which was > 0.6, regarded as a high value. The realized heritability for P_N and E was high, while that for T_{leaf} was moderate, reflecting the benefits of the selection in the F_2 population (Table 3). Among the two populations, the progenies obtained from the C×B cross showed greater estimates for the realized heritability. The realized heritability estimate for P_N and E were high in the progenies derived from the cross of C×B (Table 3).

Seed yield per plant and heat stress resistance in terms of seedling survival (SS [%]) of selected progenies was also estimated (Table 4). The progenies derived from C×C and C×B crosses showed 31 and 26% higher achene yield, respectively, than their F_3 progenies. Furthermore, the progenies derived from these crosses showed 28 and 31% better survival under heat stress indicating the superiority of progenies in the yield and resistance as a result of the selection for T_{leaf}.

Correlation analysis of various physiological and morphological traits was estimated within the F_2 population of the cross of C×C, which showed a positive relationship between P_N and E and also between P_N and T_{leaf} (Table 5). P_N also showed a positive and significant relationship with HM. A negative relationship was observed between P_N and Δ and between P_N and WUE. Δ showed a negative and significant relationship with HM. E showed a significant positive relationship with T_{leaf} and a negative relationship with Δ and WUE.

Path analysis was carried out to determine the direct and indirect effects of various leaf gas-exchange traits on HM. E showed the highest direct effect on HM (Fig. 2). T_{leaf} also showed a positive direct effect on HM, although it showed the highest indirect effect on T_{leaf} *via* E. The indirect effect of T_{leaf} *via* E was greater than the direct effect of E on HM. P_N showed a negative direct effect on HM despite having a positive relationship with HM. This positive relationship might be explained by its positive indirect effect on HM *via* E. The Δ direct effect on HM was positive, but it was negative on HM *via* E. Morphological traits (LM, HD, and NL) showed positive and moderate direct effects on HM. However, the direct effects were lower than T_{leaf}, E, and Δ. None of the morphological traits were able to impose a high indirect effect on HM *via* leaf gas-exchange traits.

Discussion

Initial screening and low F_2 seed set under heat stress increased the fitness of the F_2 plant population. This may be due to discrimination of heat-susceptible gametes under the heat stress regime (Jóhannsson and Stephenson 1998, Kalyar *et al.* 2013). Kalyar *et al.* (2013) found a significant variation within F_1 heads of sunflower in pollen fertility indicating that the haploid genotype of gametes was also involved in determining its resistance to heat stress and eliminating heat-susceptible gametes or genotypes. This resulted in the development of an adapted F_2 population, which showed higher leaf gas-exchange and some morphological traits than the un-adapted population. The heat-induced selection responses and further selection within the adapted F_2 population resulted in high realized heritability for P_N, E, and T_{leaf}. There was also a high selection differential for HM, HD, and NL among the studied morphological traits. Thus, heat-induced selection could enhance the fitness of the population in a direction relevant to heat stress tolerance.

Path analysis was carried out to determine the criterion of the selection for heat-resistant genotypes in the adapted F_2 population. The correlation analysis was used to find an association between two traits, while the path analysis showed the cause of this relationship. This could also be utilized for understanding the mechanism of heat resistance. The information obtained could be further utilized in discriminating the resistant from the susceptible genotypes. Path analyses were used to partition the correlation into direct and indirect effects on the dependent trait, *i.e.* the yield (Rauf *et al.* 2004, 2007). The path analysis showed that T_{leaf} had a positive direct effect and the highest indirect effect *via* E on reproductive HM. E also showed the highest positive direct effect on reproductive HM. Thus, a high E was important for the productivity under heat stress. High E was also indicated from T_{leaf} due to a high positive correlation between the traits and the highest indirect effect of T_{leaf} on HM *via* E indicating that T_{leaf} regulates E. Previous literature also showed the importance of canopy temperature since it regulates E (Royo *et al.* 2002). T_{leaf} also showed the highest heritability (Table 1). Moreover, genotypes were easily characterized into various classes on the basis of T_{leaf}. Therefore, T_{leaf} was used to select superior plants within the F_2 population. This selection brought about an improvement in P_N and E as indicated from the progeny performance and the realized heritability. Progenies selected on the basis of T_{leaf} also showed an increase in achene yield and heat resistance compared with unselected F_3 progenies and the commercial hybrid. Blum *et al.* (1989) and Saint Pierre *et al.* (2010) showed a significant negative correlation between canopy temperature and wheat yield under drought stress. Under heat stress, we found this correlation to be positive, showing the difference under different stresses. The positive correlation between T_{leaf} and reproductive HM was indicative of introgression of heat resistance in the sunflower population and it showed an overall positive response of various leaf gas-exchange traits (E, P_N) due to an increase in T_{leaf}. Bahar *et al.* (2008) showed changes in the direction of correlation between canopy temperature and yield in wheat when measured at various growth stages. The correlation was negative and insignificant at the spike emergence stage and positive and significant at the anthesis stage.

On the basis of T_{leaf}, different groups of plants were selected in the adapted F_2 population, *i.e.* the plants showing low (28°C), medium (36–37°C), or high (42°C) leaf temperature. The group showing the highest T_{leaf} showed the worst (the most reduced) leaf gas-exchange and morphological traits, while the plants with the medium T_{leaf} showed the highest values for the same traits, indicating that selection for the high T_{leaf} was not effective due to diminished P_N, E, and g_s. On the other hand, the plant group with the lowest T_{leaf} showed the highest Δ. However, these plants had a slightly higher WUE and lower E and P_N even though they had a similar NL and high SD (Table 1). This group showed very low mean LM relative to the group which maintained medium T_{leaf} (Table 2). This showed that canopy cooling was achieved by producing smaller leaves, which might intercept less radiation and thus had a low leaf temperature. Leaf area is important for high yield due to interception of (Leite *et al.* 2006). Therefore, plants that maintained a cooler canopy also demonstrated low dry matter accumulation and productivity. Thus, a cool canopy showed no obvious benefit in sunflower.

In conclusion, in this study, the plants selected and considered to be the heat resistant were those that maintained the medium T_{leaf} (36–37°C), the low Δ, and the high P_N and E. They were able to accumulate higher dry matter in the leaves, stem, and head. The P_N, E, and T_{leaf} could be useful traits for improving the resistance to heat stress and to increase dry matter (*i.e.* the yield) under such stressful conditions.

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