EVALUATION OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) SINGLE CROSS HYBRIDS UNDER HEAT STRESS CONDITION

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ABSTRACT

Sunflower is an important oilseed crop which shows susceptibility to heat stress. In present study, 63 single cross hybrids were evaluated under heat stress condition for two years and compared with the two commercial hybrids. Genotype and genotype × environment (GGE) was used to differentiate single cross hybrids on the basis of multiple traits. GGE biplot showed that several single cross hybrids had higher seed yield potential than standard checks. Moreover, SYP was related with pollen viability showing that achene yield was product of high gametophytic fertility under heat stress. Hybrids having high seed yield potential under heat stress had lower cell membrane injury (CMI) showing that potential hybrids could be selected on the basis of CMI during seedling stages. GGE biplot for SYP and its components showed that single cross hybrids were characterized into two major groups. Group I was further characterized into two sub group. Group Ia included hybrids with high 100-SW, while group Ib had the hybrids with high number of achenes head^{-1} and head diameter. Group II had the hybrids with high kernel weight and kernel to achene ratio. The hybrids could be recommended according to their potential utilization in the seed industry.

Key words: Achene, gametes, kernel, cell membrane injury, heterosis, biplot

INTRODUCTION

Heat stress is a major production constraint in summer crops which causes significant repressing effects on grain yield and quality (Kalyar et al. 2014; Niazi et al. 2014). Sunflower reproductive cycle has also been subjected to heat stress which reduces the achene and oil yield in various parts of the world (Rondanini et al. 2003; Rondanini et al. 2006; Kalyar et al. 2014; Van der Merwe et al. 2015). High temperature induced fine tuning of canopy architecture such as leaf and petioles angles (Kalyar et al. 2013b). Moreover heat stress reduces the pollen viability, seed germination, leaf area, reproductive biomass and increased cell membrane injuries when sunflower breeding populations were subjected to the reproductive heat stress (Kalyar et al. 2014). It was observed that heat stress accelerate the heat unit accumulation and thus reduce the growth period (Rondanini et al. 2003). The brief exposures of capitula to the heat stress > 35°C decreased the grain weight and oil contents by 40% and 30% respectively due to reduction in growth period (Rondanini et al. 2003). Exposure of temperature greater than 29°C at 10-12 days after anthesis for period of 7 days reduced grain yield by 6% (Rondanini et al. 2006). Corbineau et al. (2002) noted that exposure of the sunflower seed to high temperature of 45°C inhibits its germination and it also increased the electrolyte leakage in the incubation medium. High temperature was also known to modify oil quality and oleic acid has been shown to increase at the expense of linoleic acid (Flagella et al. 2002; Rondanini et al. 2006).

Sunflower segregating and advanced germplasm has been screened with various selection criteria for the introgression of heat resistance in inbred lines. Kalyar et al. (2013a) selected segregating plants maintaining medium leaf temperature (T_{leaf}) under heat stress. The plants which maintained medium T_{leaf} had high values of leaf gas exchange traits. The usefulness of this trait was also depicted from high heritability, selection gains and its positive relationship with reproductive biomass. Similarly, Kalyar et al. (2013b) identified differences within F_2 population with respect to leaf inclination and upward leaf inclination was found useful to avoid high post noon temperature and cell membrane injury. Differences between pre and post noon leaf temperature (Δ) was useful criteria for selection of heat resistant plants. Δ was also found useful due to presence of high realized heritability and provided sustainability to achene and oil yield under heat stress when plants differing for Δ were compared in F_4 generation (Kalyar 2015). On the basis of these grounds, progenies obtained from the selection of high Δ were advanced to derive 19 CMS lines which were crossed with 5 different restorer lines to develop 63 single cross hybrids. These single cross hybrids were then tested under heat stress condition to select prominent hybrids under prevailing conditions.

MATERIAL AND METHODS

The studies were carried out in the research field of the Department of Plant Breeding & Genetics, University College of Agriculture, Sargodha during the year 2014-15.

DEVELOPMENT OF PLANT MATERIAL

Development of heat stress resistant plant material was started in 2008. Heat resistant breeding material was selected in promising F_2 crosses. Selection was carried out on the basis of plant ability to maintain low post noon temperature as compared to pre noon leaf temperature (Δ). Effectiveness of the selection was further tested in selected progenies on the basis of genetic gain and realized heritability. Promising plants were evaluated for general combining ability. The superior lines were then converted to cytoplasmic lines in back cross scheme. Sixty three single cross hybrids were developed by using 19 cytoplasmic male sterile line and 5 male restorer lines in all possible combinations i.e. each of female line with each of male sterile lines. The lines were selected on the basis of their relative heat tolerance. 63 combinations were obtained from the controlled mating of these lines. In order to obtain cross combinations, male and female lines were planted in experimental field during the crop season February 2013 and 2014. Each line was grown in single row of 4.5 meter containing 14 plants. All female lines floral heads were covered with net bags to avoid pollen contaminations due to insect pollinators. Pollen from each male restorer lines was collected in early morning and dusted over the female line with the help of the camel brush. Pollination was continued until all the stigmas withered on floral head. The developed seed from each cross combination was harvested separately from mature heads, dried and stored in cool place for cultivation in next crop season.

EVALUATION OF PLANT MATERIALS

63 single cross hybrids along with check variety HYSUN-33 and S-278 were evaluated for heat stress tolerance in randomized complete block design with three replications at field research area of University College of Agriculture, University of Sargodha during the cropping season 2014-2015. Sowing was done on raised bed of 75 cm consecutively for two years on March 15, 2014 and March 18, 2015 to expose the hybrids reproductive growth cycle to the high temperature of May-June (Figure 1).

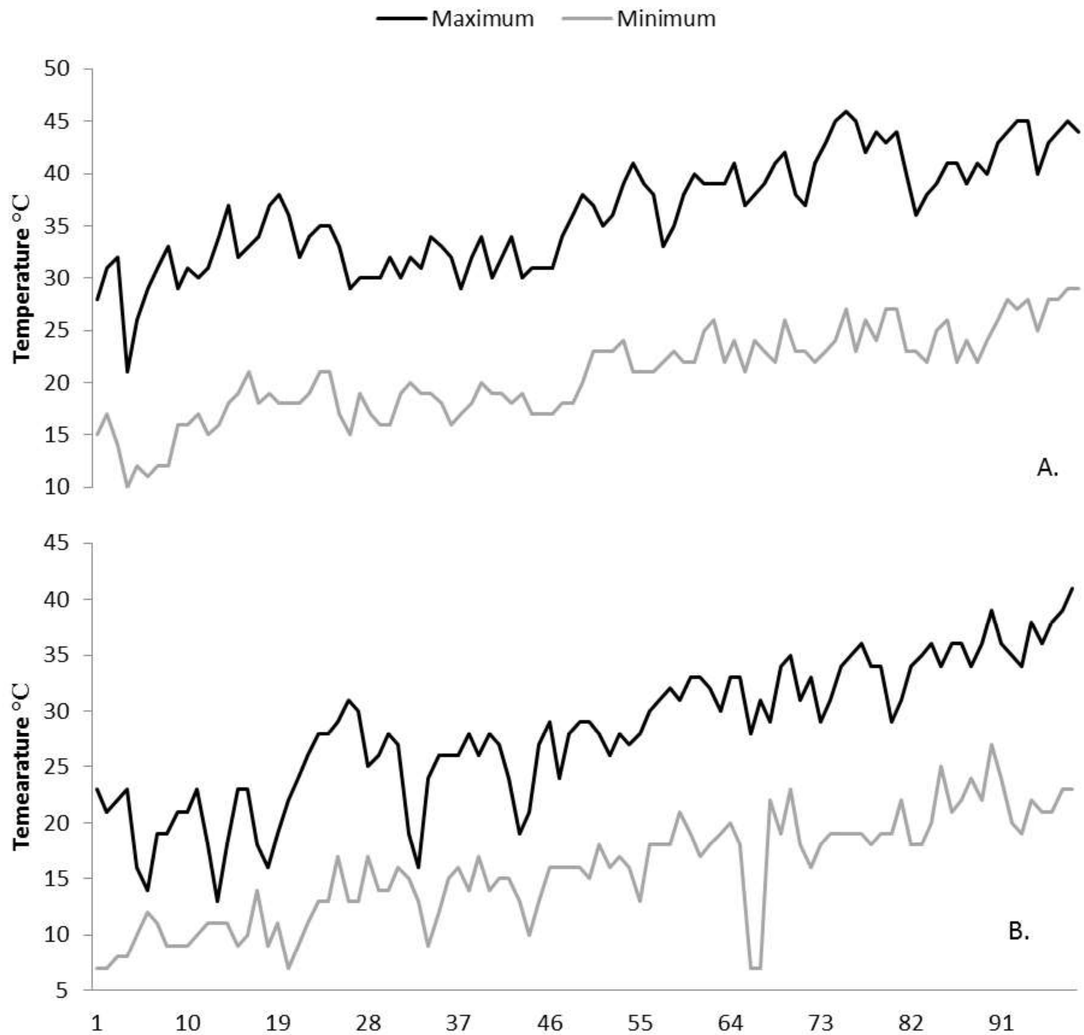


Figure 1. Mean maximum and minimum temperature during the crop season year 2014 and 2015

Soil texture and minerals analysis showed a sandy loam type of soil with EC=2.91±0.19; EC=2.84±0.33; pH=7.16±0.11 pH=7.07±0.14; available potassium was 226±10.34 and 187 ±7.52 and available phosphorous was 17.34±3.69 and 21.58±5.92 during the year 2014-15 at the time of sowing. Each hybrid was sown in three rows each of 4.5 meter with plant to plant distance of 30 cm. The crop was irrigated from canal water to avoid the water stress condition when soil moisture contents were below the field capacity. The field capacity of the soil was 14% by weight measured through gravimetric method. The fertility of soil was raised by using inorganic urea and diammonium phosphorus fertilizer at the rate of 70 kg acre^{-1} of nitrogen and 45 kg acre^{-1} of phosphorous. Pest scouting was carried out at regular interval and insecticide was sprayed to control the attack of armyworm (200 mL lufenuron, Match ® Syngenta) and red pumpkin beetles (250 / 100 mL dichlorovos, Diptrex) before they exceed threshold level during the year 2014 and 2015 respectively. Herbicide S-metachlor, Dual gold ® Syngenta was used to control pre-emergent weeds after sowing. The crop was evaluated for various plant traits. Pollen viability was measured at the time of anthesis while yield and yield components were determined at the time of maturity.

POLLEN VIABILITY

Pollen viability was estimated by 2% tri-phenyl tetrazolium chloride stain (Prasad et al. 2006). Disc florets were obtained from each head rows during early in the morning (08:00 h) from each plant. Anthers were sequezzed to obtain pollen grains. The sequzed pollen were collected on clean slides. Pollen were stained by adding a drop of stain. The stained pollen with reddish purple color were considered alive due to formation of insoluble red formazan. The reading was noted 30 min 30 min after staining under (10x) light microscope. Pollen viability was noted as the ratio of stained redish pollen to the total pollen.

MORPHOLOGICAL TRAITS

Seed yield was determined by harvesting five heads from each middle row of each hybrid from each block. Heads were manually threshed and seed was dried to uniform moisture content (12%). Dried seed harvested from each head in a row was weight over digital balance to calculate seed yield head^{-1}. Kernel mass was measured by removing the seed coat of 100 seeds. Kernels mass were measured over the analytical balance. Dead seed was determined by carefully examining 100-kernels and all the black kernels or achenes without any kernels were considered as dead. Dead seed (%) was calculated by dividing no. of dead seed to the total no. of seeds observed. Random sample of 100 seeds was counted manually and mass was measured over digital balance. No. of achene head-^{1} was counted through seed counter. Plant height was measured with measuring tape from stem base to the attachment of head. Head diameter was also measured with scale. Oil contents were estimated through petroleum ether extraction on Soxhlet apparatus. Achene size was determined through vernier caliper.

CELL MEMBRANE INJURY

Cell membrane injuries (CMI) were determined through electrolyte leakage in the leaf disc in the department of Agronomy, University of Agriculture, Faisalabad. In order to determine the cell membrane injuries in sunflower single cross hybrids, seedlings were raised in small pots of 15 × 4 cm. Two seeds were sown in each pot which was thinned to single seedling after germination (DAE). First true leaves were tagged on seedling and leaves of similar age (15 DAE) were used to determine the cell membrane injury. Temperature was maintained at 25±2 and humidity was 45%. Photon flux density was 650 µmol m^{-2} s^{-1}. Experiment was laid out in completely randomized design with six replications. Four leaf discs of 5mm in size were dissected from each leaf. Leaf disc were put in glass vial. Two set of leaf disc having three vials for each hybrid was created by treating the leaf disc with two temperature regime. One set was kept at room temperature 25 °C and other set received a treatment of high temperature 42-50°C for one hour with 2 °C increment after every 10 minutes. 20 mL of deionized water was added to each vial after two temperature treatment. Vials were incubated at 10°C for 12 hours, afterward electric conductivity of both treatments was determined. All vials were autoclaved at 121 °C and final electric conductivity was measured. Cell membrane injury was determined using following formula:

CMI%=(1-(T1/T2))/(1-(C1/C2)) ×100

%injury= (100-CMS)

where T1 and T2 are treatment conductivities before and after autoclaving and C1 and C2 are the respective control conductivities.

BIOMETRICAL PROCEDURES

Data obtained was subjected to the analyses of variance under factorial arrangement where hybrids and years were considered as factors. Biometrical parameters such as genotypic, phenotypic, GCV% and heritability over year were measured as outlined by Allard (1960) where $\sigma^2 g$ (genotypic) = (MSg-MSgl)/rY where MSg is the mean sum of square due to hybrids, MSgl is mean sum of square due to interaction of hybrids × year and r= replication and Y was year). $\sigma^2 p$ (phenotypic)= $\sigma^2 g + \sigma^2 gl + \sigma^2 e$. Heriatbility = ($\sigma^2 g / \sigma^2 p$) x 100 ($\sigma^2 g$ = genotypic variance/σ^2 p=phenotypic variance). Genotypic coefficient of variation (GC%) = ($\sigma^2 g$ /X) × 100. A genotype plus genotype by environment (GGE) analysis (Yan & Kang 2003) was carried out to analyze the heat tolerance traits and yield in order to select promising single cross hybrids under heat stress. Another biplot was developed to study the relationship of yield components and select promising hybrids on the basis of multiple yield contributing traits. The traits were standardized before the analysis in accordance with different scales of the chosen variables. The biplot calculations were made using the 'scale' and 'svd' procedures of the R software (R 2013).

RESULTS

Analysis of variance showed significant variation ($P \leq 0.01$) for single cross hybrids and years for all traits under study. However, interaction due to single cross hybrids and year was significant ($P \leq 0.05$) for traits such as seed yield plant-^{1} (SYP), head diameter (HD), leaf area (LA), dead seed (DS%), kernel weight (KW) and kernel to seed ratio (K to S). Significant ($P \geq 0.05$) interaction showed that single cross hybrids changed their relative ranking for these traits. Hertiability estimates for various yield and its components was moderate to low. Heritability estimates for SYP was moderate which showed that high yielding hybrids may be directly selected through SYP per se under heat stress environment. Traits such as 100-SW, PH, DS%, KW and K to S had moderate heritability while traits such as HD, PV and LA had low heritability (Table 1-2). Among the traits, the highest heritability was shown by SPH. Thus, SPH could also be used along with SYP to determine the yield potential of hybrids under heat stress environment. Among the traits the highest phenotypic variation was shown by leaf area and seed yield while SYP showed the highest genotypic variation among single cross hybrids.

GGE biplot analysis was carried out to characterize single cross hybrids on the basis of multiple traits relevant to SYP and adaptability under heat stress. GGE biplot analysis characterized the germplasm into two groups on the basis of four selected traits (Fig. 2.). Group 1 included the hybrids with high yield and pollen viability while group II included single cross hybrids with high CMI and DS%. The traits such as PV and SYP were close to each other showing positive relationship between the two traits during the year 2015. It was also concluded that SYP could also be dependent over high PV under heat stress. Therefore, simultaneous selection for PV and SYP could also be practiced. Hybrids such as H-32 and H-21 had high SYP with good PV during the year 2014 (Fig. 2a) while H-35 had high SYP and PV during the year 2015 (Fig. 2b). Hysun-33 had low SYP with high DS% under heat stress. There was also relationship between CMI and DS%. Hybrid H-51, H-48 and H-32 had the highest DS (ratio) while H-7 and H-26 had high CMI and DS during the year 2014 and respectively 2015.

GGE-biplot yield and its components have been shown in Fig. 4a and 4b. GGE-biplot showed that all chosen traits had positive relationship with yield. Traits characterized the single cross hybrids into two groups. The single cross hybrids were grouped on the basis of HD, SYP, SPH and 100-SW in group I. The yield components such as HD, SPH and 100-SW were found close to SYP and had positive relationship with SYP. H-35 showed the highest HD and 100-SW during the year 2014, and thus seed yield was dependent over seed size in this hybrid (Fig. 4a). H-27 and H-29 had high SYP and NSH during the year 2014 while the same hybrid had the highest SYP, NSH, 100-SW and HD during the year 2015 (Fig. 4b). During the year 2015, H-35 was also characterized as high yielder but its yield was not dependent over higher values of morophological traits. Group II included hybrids with high KW% and K to S ratio. Hybrids with high KW and K to S ratio were good for industrial exploitation. Hybrids such as H-58 and H-40 had the highest KW and K to S ratio in both years (Fig. 4a and 4b).

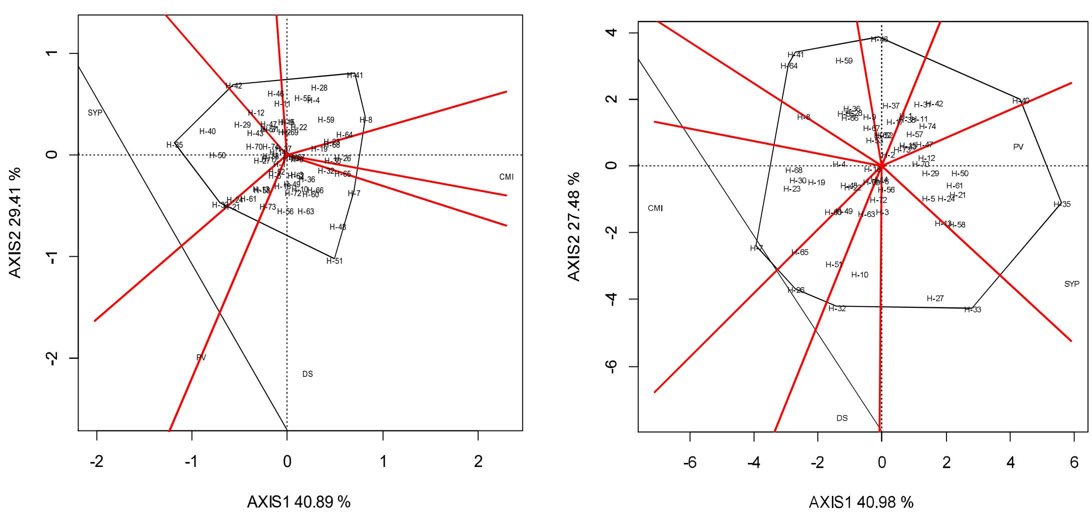
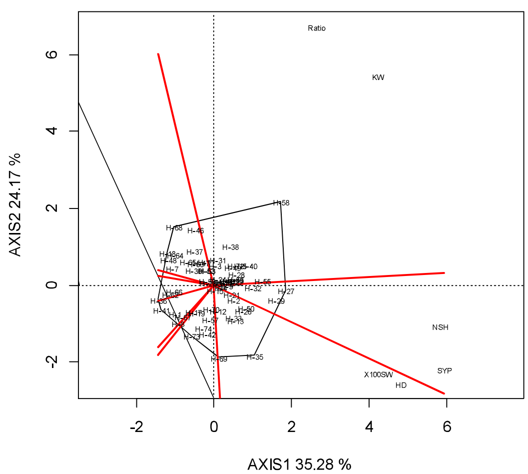


Figure 2. GGE biplot for standardized seed yield plant^{-1}(SYP), pollen viability (PV), cell membrane injury (CMI) and dead seed ratio (DS) for sixty five single cross hybrids under heat stress for year 2014 (a) and 2015 (b). All traits were averaged across replications for each combination of Genotype-by-Trait. The first principal component axis (PC1) retains 49% and the second 29% and 27% of sum of squares for year 2014 and 2015 respectively.

GGE-biplot yield and its components have been shown in Fig. 3a and 3b. GGE-biplot showed that all chosen traits had positive relationship with yield. Traits characterized the single cross hybrids into two groups. The single cross hybrids were grouped on the basis of HD, SYP, SPH and 100-SW in group I. The yield components such as HD, SPH and 100-SW were found close to SYP and had positive relationship with SYP. H-35 showed the highest HD and 100-SW during the year 2014, and thus seed yield was dependent over seed size in this hybrid (Fig. 4a). H-27 and H-29 had high SYP and NSH during the year 2014 while the same hybrid had the highest SYP, NSH, 100-SW and HD during the year 2015 (Fig. 4b). During the year 2015, H-35 was also characterized as high yielder but its yield was not dependent over higher values of morophological traits. Group II included hybrids with high KW% and K to S ratio. Hybrids with high KW and K to S ratio were good for industrial exploitation. Hybrids such as H-58 and H-40 had the highest KW and K to S ratio in both years (Fig. 3a and 3b).

Mean values of promising hybrids have been shown in Table 1. Results showed that commercial hybrid S-278 had the highest oil contents% followed by the H-58 and H-29. Other commercial hybrid HYSUN-33 had significant lower oil content% than promising hybrid H-58 and H-29. Commercial hybrid S-278 had the lowest oil yield (Table 3). Hybrid H-35 also had higher oil yield than commercial hybrids but had very low oil contents and could be considered as non-oil seed type. On the other hand hybrids H-58 and H-29 could be regarded as oilseed type hybrids for cultivation under heat stress condition. Achene size was estimated on the basis of achene length, width and area. H-35 had the highest while commercial hybrids S-278 had the lowest achene size (Table 1). H-58 had lower achene width than commercial hybrids HYSUN-33.



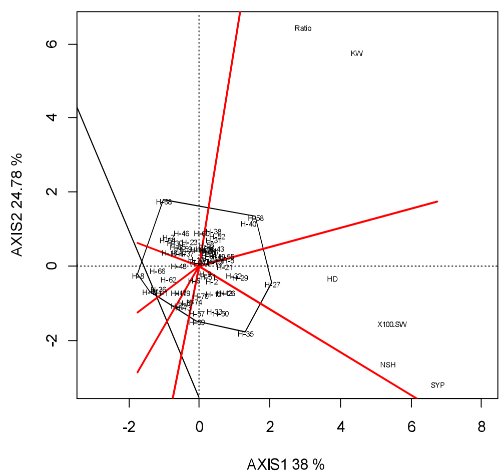


Figure 3. GGE biplot for standardized head diameter (HD), seed head^{-1} (SPH), seed yield (SY) and 100-seed weight (100-SW), kernel weight (KW), kernel to seed ratio (ratio) for sixty five single cross hybrids under heat stress for year 2014 (a) and 2015(b). HD, SPH, SY, KW and ratio were averaged across replications and years for each combination of Genotype-by-Trait. The first principal component axis (PC1) retains 35%, 39% and the second 24% and 25% of sum of squares for year 2014 and 2015 respectively.

Table 1. Variation in mean performance of promising single cross hybrids along with standard hybrids for traits related achene and oil contents.

Hybrid	Year	Oil Content%	Oil Yield (g)	Achene		
				Length	Width	Area
H-58	1	35.32±1.15	27.53±3.47	10.81±0.07	5.27±0.10	56.97±2.38
	2	38.27±2.19	34.83±2.91	9.59±0.05	5.53±0.06	53.03±3.16
	Average	36.79b	31.18a	10.20d	5.21e	53.14d
H-29	1	35.71±2.08	29.85±1.98	11.01±0.05	5.85±0.04	64.41±2.64
	2	36.12±1.52	34.31±2.07	10.52±0.06	5.19±0.08	54.60±3.18
	Average	35.92b	32.08a	10.77c	5.52d	59.42c
H-35	1	24.37±2.65	30.46±3.42	12.85±0.08	7.27±0.05	93.42±1.89
	2	26.48±1.69	35.22±2.77	13.68±0.04	7.85±0.02	107.39±2.38
	Average	25.43d	32.84a	13.27a	7.56a	100.28a
H-27	1	25.29±2.39	29.84±3.41	10.84±0.05	6.48±0.06	70.24±1.93
	2	27.12±1.71	26.04±2.82	11.15±0.06	7.10±0.08	79.17±2.12
	Average	26.21d	27.94b	11.00b	6.79b	74.66b
S-278	1	42.13±1.92	10.95±3.19	9.65±0.08	4.47±0.09	43.14±2.26
	2	39.48±2.19	9.08±2.64	10.52±0.03	5.16±0.06	54.28±2.67
	Average	40.81a	10.02d	10.09e	4.82f	48.56e
Hysun-33	1	32.72±1.09	19.30±1.97	10.70±0.09	5.82±0.08	62.27±3.42
	2	34.16±1.28	19.81±2.14	9.83±0.06	5.63±0.03	55.34±2.34
	Average	33.44c	19.56c	10.27d	5.73c	58.77c

DISCUSSION

Heat stress was one of major production constraints in sunflower which caused significant yield losses of sunflower (Kalyar et al. 2014; Rondanini et al. 2006; Kalyar et al. 2014; Van der Merwe et al. 2015). It was observed that heat stress accelerate the heat unit accumulation and thus reduce the growth period (Rondanini et al. 2003). The brief exposures of capitula to the heat stress > 35°C decreased the grain weight and oil contents by 40% and 30% respectively due to reduction in growth period (Rondanini et al. 2003). Exposure of temperature greater than 29°C at 10-12 days after anthesis for period of 7 days reduced grain yield by 6% (Rondanini et al. 2006). Corbineau et al. (2002) noted that exposure of the sunflower seed to high temperature of 45°C inhibits its germination and it also increased the electrolyte leakage in the incubation medium.

In this study, plant material was originally selected for heat resistance on the basis of (Δ) in the initial segregating generation (Kalyar 2015). Δ was also found useful due to presence of high realized heritability and provided sustainability to achene and oil yield under heat stress when plants differing for Δ were compared in F_4 generation (Kalyar 2015). In comparison to the commercial hybrids, selected progenies showed an advantage of 5%, 47%, 5% and 45% for oil contents (OC%), 100-SW, HD and seedling survival % respectively while 62% and 75% lower unfilled grain% and pollen sterility% over commercial hybrids. Promising progenies were converted to the cytoplasm male sterile lines and mated to the diverse restorer lines to generate single cross hybrids. These hybrids showed substantial genetic variation and moderate heritability for seed yield under heat stress. The variation for seed yield appears due to differences in pollen viability, cell membrane injury and dead seed%. High yielding hybrids had higher pollen viability showing high seed yield was function of higher gametophytic tolerance (Coast et al. 2015; Das et al. 2014). It has been noticed earlier that heat stress reduces the pollen viability in various species and pollen viability was used as marker to differentiate heat tolerant genotypes (Coast et al. 2015; Das et al. 2014; Kalyar 2015). Hybrids showing high pollen viability were negatively related with cell membrane injury (r^2=-0.43). Therefore, Cell membrane injury could be used to discriminate sunflower hybrids for heat resistance during seedling. Presence of relationship between the pollen viability show that seedling stage heat resistance was also depicted in adult phase heat resistance (Fokar et al. 1998). Lowered cell membrane injuries also tend to reduce the dead seed% (r^2=0.35). High dead seed% affects the oil content to greater extent and thus selection for lower CMI tends to reduce oil yield losses under heat stress (Kalyar et al. 2014).

GGE biplot analysis also partitioned the single crosses hybrids on the basis of various yield components. Generally, GGE biplot partitioned hybrids into two major groups on the basis of yield component. Group I included hybrid on the basis of high 100-SW, seed head^{-1} and head diameter. These traits were significant contributor for seed yield. This group contained two type of hybrids i.e. high seed yield due to higher 100-SW. Thus 100-SW was an important yield component and indicated the importance of greater grain filling or greater seed size for high seed yield. Increased grain filling% has been an important contributor of seed yield under heat stress and indicative of better photosynthates mobilization and food reserve mobilization (Kalyar et al. 2014). However, high 100-SW also tends to reduce seed per head and increase seed size which may reduce the oil yield potential of hybrids. However, our study indicated in-significant ($P \geq 0.05$) relationship between seed per head and 100-SW (r^2=0.15) showing mixed response of hybrids to the increased 100-SW. Another sub group included hybrids with high seed yield head^{-1} and head diameter. Thus photosynthetic process was used to maximize the number of seed per head through high head diameter (Rauf & Sadaqat 2008). Group II included hybrids with high kernel weight and kernel to seed ratio. Both traits are positive contributors to oil yield extraction and thus hybrids with these traits were considered superior for oil yield potential.

It is concluded from the above results that hybrid H-29 and H-58 could be regarded as potential high oil yielding hybrid with good achene yield potential and moderately high heat tolerance. On the other hand, H-35 was promising non-oil seed hybrid with high heat resistance.

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