

RESEARCH PAPER

Comparative assessment of sunflower (*Helianthus annuus* L.) genotypes for yield and yield attributing traits under two different temperature regimes

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Abstract: Sunflower is one of the important oil seed crops of India and it has contributed to rapid growth in oilseed production. Sunflower oil contains 70-80% poly unsaturated (PUFA) viz, Linoleic acid, oleic acid and vitamin E. On the aspect of global warming, temperature is increasing at very rapid rate. High-temperature stress affects all growth stages of the sunflower crop and ultimately yields. High temperature predominantly reduces the leaf expansion and duration of reproductive growth, since the seed filling period is very sensitive. Management options for high temperature stress are few. Hence developing intrinsically tolerant plants is essential to combat the situation. In the present study sunflower genotypes were screened at laboratory for TIR, pollen studies and evaluated for yield and its attributing traits under two different temperature locations. Its mainly because in order to study their performance under varied temperature conditions. The results revealed a significant genetic variability among the sunflower genotypes assessed and by using TIR technique genotypes like CMS-17A, CMS-234B, RHA 95-C-1 and KBSH-44 were identified as temperature tolerant. The maximum pollen germination per cent at 40°C was shown by KBSH-44,234-B and EC-734826. Wide range of variability was observed for the traits like days to 50% flowering, plant height, head diameter, stem diameter, seed yield/plant, 100 seed weight and seed set percentage. The genotypes which exhibited tolerance to temperature stress through TIR and gametophyte selection were also found tolerant under field conditions.

Key words: Genotype, Lethal, Sunflower, Temperature

Introduction

In India, sunflower is being cultivated in the area of 0.52 million hectare and production of 0.34 million tonnes with the productivity of 643 kg/ha (IIOR report 2017). Sunflower is photo insensitive crop, grown both in kharif and rabi predominantly as a rainfed crop. Apart from moisture stress, high temperature is a major constraint to attain the stable yield potential, as it is originated from sub tropics (Mamatha Reddy, 2000). Temperature stress affects plant developmental and physiological process (Amutha *et al.*, 2007). High temperature causes irreversible damage to plant function and development. The magnitude of heat stress effect rapidly increases as temperature increases above threshold level (Senthil Kumar *et al.*, 2007). A novel Temperature Induction Response (TIR) technique has been developed and standardized for the rapid assessment of cellular level tolerance in crop plants. This approach is based on the fact that, temperature stress develops gradually and the plants are normally exposed to sub lethal stress before being exposed to more severe stress. An array of response events are activated when plants experience milder level of stress. These responses would lead to the protection against a severe stress (Abdullah *et al.*, 2001). Plants overcome high-temperature stress by adapting several physiological and biochemical mechanisms. Field selection is complicated by the high variability associated with multiple interactions contributing to heat tolerance of crops, as heat stress affects and occur at different phases during the growing season, which is amplified when temperature increases gradually. This contributes to a large genotype \times environment (GE) interaction, which may explain the slow progress in developing new

cultivars of crops for heat stress conditions. In most cases, no clear cause of the GE interaction has been identified because of lack of information about the environment (such as weather or soil) or the genotypes themselves. Several indices have been proposed to describe the behavior of a given genotype under stress and non-stress conditions. In the present study sunflower genotypes were evaluated for yield and its attributing traits under two different temperature locations, its mainly because in order to study their performance under varied temperature conditions.

Material and methods

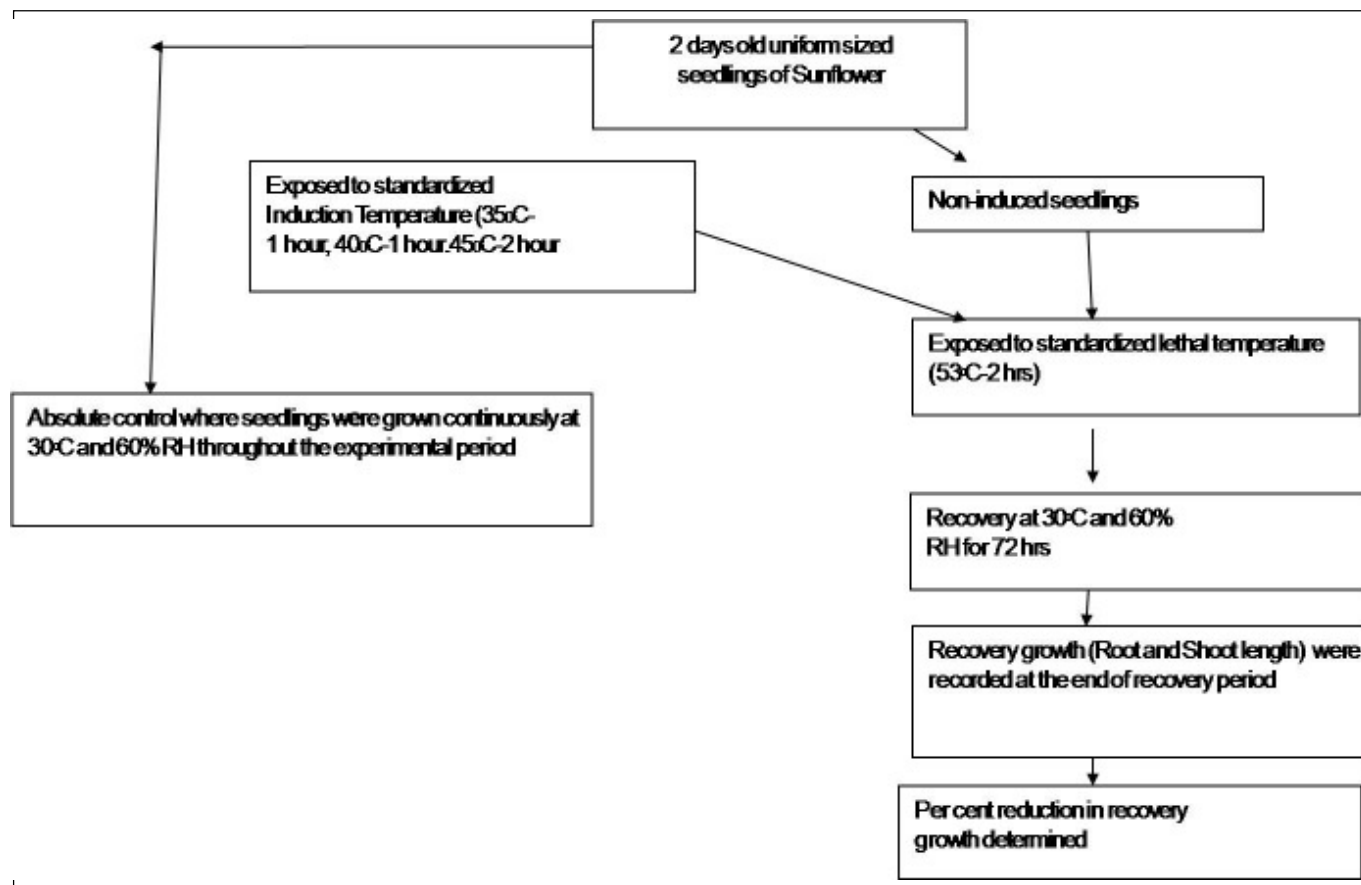
The present study envisaged evaluation of sunflower genotypes for temperature stress tolerance based on Temperature Induction Response (TIR) technique during Summer 2014. The material for the study comprised of 24 sunflower genotypes as shown in Table 1. The genotypes comprised of six parental lines (including CMS-A and B), three hybrids and fifteen Restorer (R) lines. In this technique, the young seedlings were initially exposed to a mild temperature (sub lethal stress) following which, the seedlings were again exposed to relatively a high temperature for a specific period of time. The percent survival of seedlings and recovery growth of seedlings when transferred back to normal temperature was determined as a measure of tolerance (Flow chart 1). The Pollen was collected during early in the morning around 8.00 am to 9.00 am, Pollen were placed on the slides about 30 minutes, which was placed in petri plates having moist filter paper and it was done for pre-conditioning the pollen before giving

Table 1. List of sunflower genotypes studied in the experiment

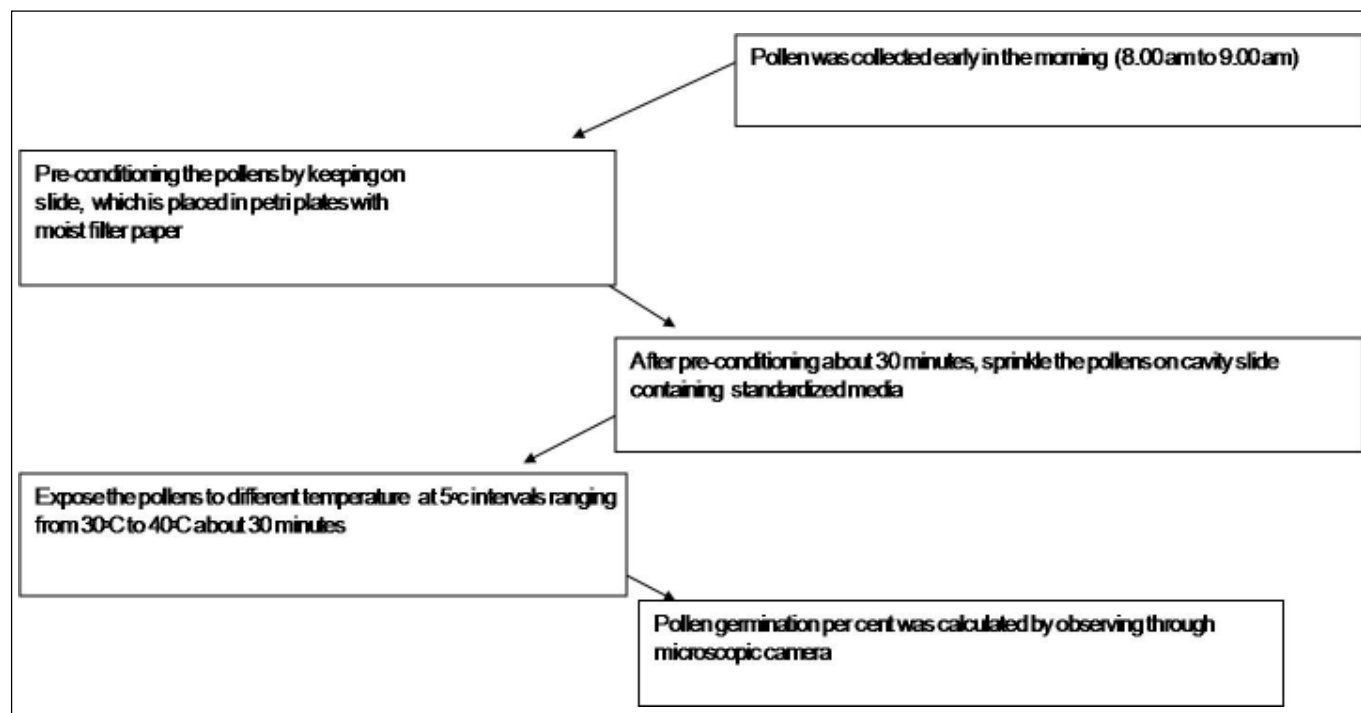
Sl. No	R-lines	Sl. No	A lines	Sl. No	B lines	Sl. No	Hybrids
1	EC-734811	16	CMS-17 A	19	17 B	22	KBSH-41
2	EC-734812	17	CMS 234 A	20	234 B	23	KBSH-44
3	EC-734819	18	CMS-335 A	21	335 B	24	KBSH-53
4	EC-734822						
5	EC-734825						
6	EC-734839						
7	EC-734840						
8	EC-734872						
9	EC-734873						
10	EC-734874						
11	EC-734876						
12	EC-734887						
13	EC-734888						
14	RHA 95-C-1						
15	RHA 6D-1						

temperature treatment. The mean temperature during pollen collection was 30°C (Flow chart 2). An experiment was conducted to determine the effects of different induced temperature on pollen germination characteristics in Sunflower genotypes. Pollen germination media for sunflower was standardized by using Calcium nitrate (CaNo₃)-300 ppm, Magnesium sulphate (MgSo₄)-200 ppm, Potassium nitrate (KNO₃)- 200 ppm, Boric acid-100 ppm and Sucrose-20%. It showed 81 percentage germination compared to other media concentrations. The observations on pollen fertility and pollen

tube growth were recorded in all the genotypes in different temperature regimes. Sowing of the selected twenty four sunflower genotypes was taken up during summer 2014 in the experimental plots of MRS, Hebbal, Bangalore, the normal annual temperature was 26°C. Same genotypes were sown during summer 2014 in the experimental plots of KVK, Gangavathi, the normal annual temperature was 34°C. Ten plants from each replication were selected for recording observations on Days to first flowering, Days to 50% flowering ,Days to complete flowering , Plant height (cm), Head diameter (cm), Stem



Flow chart 1. Temperature Induction Response method followed to evaluate the sunflower genotypes.



Flow chart 2. Gametophyte selection followed to evaluate the sunflower genotypes for temperature stress tolerance

diameter (cm), Seed yield/plant (g), Seed set per cent. Statistical analysis was carried out for genetic variability parameters.

Results and discussion

Crop growth and productivity are severely affected by different abiotic stresses causing large reduction in yield. Over the year considerable efforts have been made to ascertain the adaptive mechanism of crop plants to temperature stress and to identify traits, which would help in selection of genotypes for temperature stress tolerance. In the present study, despite the exposure of different genotypes to optimum induction temperature and lethal temperature, the recovery growth differed amongst the genotypes (Table 2). Variation in the stress adaptive mechanisms among the genotypes could be the reason for observed differences for thermo tolerance. Based on the TIR technique, genotypes CMS 17A, CMS 234 A, KBSH- 41, KBSH-44 and RHA 95-C-1 were identified as temperature tolerant lines based on the growth of the induction seedlings (0-30%) during recovery period, CMS 17B, CMS 234A, CMS 335A, CMS 335B, EC-734822, EC-734839, EC-734840, EC-734876 and KBSH-53 were identified as moderate temperature tolerant lines (31- 50%) and EC-734811, EC-734812, EC-734819, EC-734825, EC-734872, EC-734873, EC-734874, EC-734887, EC-734888 and 6D-1 were identified as high temperature susceptible lines through TIR technique. Earlier Mamatha Reddy (2000) has standardized the TIR protocol for screening the sunflower genotype for cellular level tolerance. With slight modification, the TIR protocol was again standardized and twenty four sunflower genotypes were screened in the present study for cellular level Tolerance. In the present study, despite the exposure of different genotypes to optimum induction temperature and lethal temperature, the recovery growth differed

Table 2. Genetic variability among sunflower genotypes based on the per cent reduction growth and per cent survived seedlings

Sl. No.	Genotypes	% Reduction growth	% survived Seedlings
A & B lines			
1	CMS 17 A	22.60	78.77
2	CMS 234 A	39.68	76.77
3	CMS 335 A	41.16	70.63
4	CMS 17 B	43.77	65.72
5	CMS 234 B	21.07	71.33
6	CMS 335 B	36.99	69.46
	Mean	34.21	72.11
R lines			
7	EC-734811	66.42	5.00
8	EC-734812	84.18	4.66
9	EC-734819	70.12	16.11
10	EC-734822	46.54	0.00
11	EC-734825	51.61	61.86
12	EC-734839	33.58	51.66
13	EC-734840	43.80	33.33
14	EC-734872	61.31	0.00
15	EC-734873	59.32	22.77
16	EC-734874	77.96	44.44
17	EC-734876	36.87	39.96
18	EC-734887	50.70	24.44
19	EC-734888	78.52	40.63
20	RHA 95-C-1	27.31	81.96
21	RHA 6D-1	59.97	29.93
	Mean	56.54	30.43
Hybrids			
22	KBSH-41	22.41	80.27
23	KBSH-44	19.4	89.99
24	KBSH-53	30.53	56.77
	Mean	24.11	75.67

Table 3. Effect of different induced temperature on pollen germination percentage among sunflower genotypes

Sl. No.	Genotypes	30°C	35°C	40°C
B-lines				
1	17B	61.33	54.33	44.33
2	234B	74.67	67.67	62.33
3	335B	60.00	50.33	43.33
	Mean	65.33	57.44	49.99
R-lines				
4	EC-734811	41.67	29.67	19.00
5	EC-734812	46.33	31.33	20.00
6	EC-734819	36.67	30.33	20.33
7	EC-734822	56.00	45.67	38.67
8	EC-734825	60.00	54.33	47.67
9	EC-734839	65.33	60.00	53.33
10	EC-734840	61.67	55.33	45.67
11	EC-734872	60.00	51.00	43.00
12	EC-734873	56.00	42.33	38.00
13	EC-734874	63.00	56.33	49.67
14	EC-734876	59.33	48.00	44.00
15	EC-734887	66.67	57.33	51.00
16	EC-734888	53.00	46.33	40.00
17	RHA 95-C-1	70.33	64.33	59.67
18	RHA 6D-1	57.33	43.67	35.33
	Mean	56.88	47.32	40.35
Hybrids				
19	KBSH-41	69.00	63.00	60.50
20	KBSH-44	79.00	71.00	65.00
21	KBSH-53	67.00	63.00	59.67
	Mean	71.66	65.66	59.94

amongst the genotypes. Variation in the stress adaptive mechanisms among the genotypes could be the reason for observed differences for thermo tolerance. Earlier it was reported that induction stress alters gene expression and brings greater adaptation to heat stress and that the genetic variability in thermo tolerance is only seen upon induction stress. Seedlings of parental lines including CMS 234 A, CMS 234 B and RHA 6D-1 showed considerable genetic variability for thermo tolerance and it was attributed to the expression of existing residual variability for stress responses. Thus, the existing variability forms the basis for identifying thermo tolerant lines in sunflower (Senthil Kumar *et al.*, 2003). The pollen germination of twenty one sunflower genotypes was studied under different induction temperature. The treatment temperature ranged from 30°C to 40°C at 30 minutes interval and incubated for 30 minutes. The maximum germination percentage found in 234 B (62.33%) followed by 17B (44.33 %) among B lines. In the Rlines, RHA 95-C-1 showed highest germination percentage of 59.67 %. The Hybrid KBSH-44 showed good pollen germination percentage (65) at 40°C resulted in good seed set and test weight percentage under field condition compared to EC-734811 (19 % germination) which showed less seed set and less seed yield under two different temperature locations (Table 3). The pollen parameters identified in the present study can be used as one of the parameter in the breeding programs to develop new genotypes for a niche environment. Pollen heat tolerance will be essential both in the present climate as well as in a

Table 4. Estimates of descriptive statistics for yield attributing traits of sunflower genotypes evaluated at two locations

Characteristics	Mean \pm S.E		Range				GCV %		PCV %		Heritability (Broad sense %)		GAM (%)	
			Bangalore		Gangavathi									
	Bangalore		Max	Mini	Max	Mini	Bangalore	Gangavathi	Bangalore	Gangavathi	Bangalore	Gangavathi	Bangalore	Gangavathi
	Bangalore	Gangavathi												
Days to first opening of ray florets	54.02 \pm 0.5473	54.26 \pm 0.4613	63.00	51.33	62.66	50.66	6.58	5.94	6.82	6.13	80.05	93.99	10.69	11.87
Days to 50% flowering	56.43 \pm 0.5457	58.3 \pm 0.5512	65.00	54.00	66.66	56.00	6.65	6.09	6.87	6.32	94.39	93.01	12.82	12.11
Days to complete opening of ray florets	63.41 \pm 0.7519	65.16 \pm 0.6619	70.33	59.66	72.33	61.66	5.06	4.67	5.48	5.00	88.37	87.10	9.54	8.97
Plant height (cm)	97.42 \pm 1.8269	81.86 \pm 1.751	178.00	64.90	115.96	53.93	34.40	22.45	34.56	22.77	76.34	97.24	47.74	45.62
Head diameter (cm)	12.00 \pm 0.5136	6.88 \pm 0.3300	17.70	6.63	15.63	2.73	21.57	36.14	22.86	37.12	64.67	94.78	37.40	72.48
Stem diameter (cm)	4.07 \pm 0.1055	4.07 \pm 0.3147	5.73	3.26	6.70	2.91	19.91	21.24	20.43	25.26	56.84	70.71	26.90	36.80
Seed yield / plant(g)	16.73 \pm 0.6254	13.24 \pm 0.121	52.86	2.71	48.68	2.03	70.24	68.77	70.55	68.78	94.14	99.9	136.42	141.62
100 seed weight	4.92 \pm 0.1184	3.16 \pm 0.1282	9.49	2.46	8.86	1.23	36.16	52.69	36.41	53.18	47.31	98.18	41.89	107.55
Seed set %	77.46 \pm 1.0108	73.58 \pm 0.8842	93.73	46.93	93.30	42.83	16.05	17.79	16.22	17.92	95.53	98.5	33.56	36.40

Table 5. Estimates of seed yield of sunflower genotypes across two locations during summer representing contrasting temperature

Genotypes	Seed yield /plant (g)	
	Bangalore	Gangavathi
A-lines		
17 A	19.77	13.57
234 A	13.67	13.03
335 A	14.40	12.83
Mean	15.94	13.14
B-lines		
17 B	19.80	9.90
234 B	14.40	14.57
335 B	15.27	11.07
Mean	16.49	11.86
R-lines		
EC-734811	11.50	10.17
EC-734812	4.01	3.40
EC-734819	8.83	6.30
EC-734822	11.31	9.43
EC-734825	9.3	8.77
EC-734839	17.36	14.07
EC-734840	7.55	6.37
EC-734872	12.73	12.53
EC-734873	11.20	11.07
EC-734874	2.72	2.03
EC-734876	20.57	14.23
EC-734887	13.81	11.43
EC-734888	16.55	12.37
RHA 95-C-1	13.37	13.33
RHA 6D-1	11.46	10.57
Mean	11.48	8.97
Hybrids		
KBSH-41	45.53	27.53
KBSH-44	52.86	48.60
KBSH-53	33.63	20.83
Mean	44.06	32.32

projected future warmer and variable climate. This result was in accordance with the experiment conducted by Ranasinghe *et al.*, 2010, verified that the coconut varietal differences in determining the temperature tolerance of coconut pollen. *In vitro* pollen germination and pollen tube growth severely reduced under both high and low temperature conditions.

In the present investigation an attempt was made to evaluate the relative performance of selected sunflower genotypes under two different temperature locations for growth, yield and its attributing traits. The selected twenty four sunflower genotypes were sown during summer season under two different temperature locations at Bangalore with mean temperature of 32°C and ARS, Gangavathi with mean temperature of 39°C during crop growth period respectively. The performance and genetic variability of selected sunflower genotypes for yield and its attributing traits were observed. The analysis of variance for location interaction revealed highly significant differences due to mean sum of squares among twenty four sunflower genotypes except A-lines for the traits days to first flowering and stem diameter. Genotype x location interaction showed significant differences for all traits except days to first flowering

Table 6. Effects of male sterility inducing cytoplasm on yield attributing traits of sunflower genotypes as influenced by different temperature regions prevailed in two locations

Genotype	Days to first flowering			Days to 50% flowering			Days to complete flowering			Plant height(cm)			Head diameter(cm)		
	M.S-A line			M.F-B line			M.S-A line			M.S-A line			M.S-A line		
	B	G	B	B	G	B	B	G	B	B	G	B	B	G	B
17	51.33	52.33	55.00	52.00	54.00	56.00	62.67	64.67	62.33	63.33	119.53	99.73	121.97	92.10	11.63
234	52.33	53.33	56.33	52.33	54.33	56.33	63.33	65.33	62.33	64.33	123.20	88.77	122.67	92.57	13.67
335	63.00	62.67	65.00	62.33	65.00	66.67	72.33	72.33	72.00	72.00	122.47	92.37	122.77	110.10	11.97
Seed yield/pt (g)															
100 seed weight(g)															
Seed set %															
Stem diameter(cm)															
Head diameter(cm)															
M.F-B line															
M.S-A line															
B															
G															
17	11.63	6.57	4.50	5.37	3.70	4.00	19.77	13.57	19.80	9.90	5.23	3.03	89.7	85.6	88.6
234	13.67	9.07	3.73	3.97	3.73	4.10	13.67	13.03	14.40	14.57	8.11	3.57	82.1	81.4	88.8
335	11.97	8.27	4.67	4.87	5.47	5.43	14.40	12.83	15.27	11.07	4.11	2.53	83.1	81.5	90.2
M.F-B line															
M.S-A line															
B															
G															
17	11.63	6.57	4.50	5.37	3.70	4.00	19.77	13.57	19.80	9.90	5.23	3.03	89.7	85.6	88.6
234	13.67	9.07	3.73	3.97	3.73	4.10	13.67	13.03	14.40	14.57	8.11	3.57	82.1	81.4	88.8
335	11.97	8.27	4.67	4.87	5.47	5.43	14.40	12.83	15.27	11.07	4.11	2.53	83.1	81.5	90.2

and days to complete flowering. This information indicates that variability exists for almost all the characters studied and considerable improvement can be achieved in these characters by selection (Table 4). However the analysis of variance by itself is inconclusive in explaining all the inherent genetic variability in the germplasm. This is evident by partitioning the total variability in the genotypes from the phenotypic variance. Thus it is necessary to work out the phenotypic and genotypic coefficients of variation which indicate the variability existing for various traits. Tan (2010), Reddy and Devasenamma (2004) have reported the significant differences among the genotypes and inbreds respectively, for all the characters they considered. Considerable amount of range was observed for nine characters studied. Analysis of variance also indicated highly significant differences among 24 genotypes including CMS-A and B lines, RHA lines and hybrids evaluated for the characters studied (Table 5). The wide difference observed in the traits may be attributed to their genetic makeup and evolution under different ecological niches (Jayaram Gowda, 1999). This range of variation provides a bright scope to select the superior and suitable basic material for their use in breeding programmes for further improvement. The narrow gap between phenotypic and genotypic variations suggests less influence of environment on these characters. The male sterility induced cytoplasmic effects were very low for the traits like days to first flowering, days to 50% flowering and seed set percentage and comparable differences were notified for the traits like plant height, head diameter, seed yield and test weight studied at two different

temperature locations namely Bangalore and Gangavathi (Table 6). This result indicated that locus differing for male sterility had influence on different traits performance among CMS A and CMS B lines. This result agrees with Patil *et al.*, 2003. The temperature at Bangalore and Gangavathi locations during flowering period to seed set stage was at 33°C and 38°C, respectively. The mean seed yield of CMS B lines, RHA lines and hybrids over the two locations differed significantly. Hence, rise in temperature had effects on seed set and seed yield. Overall response to higher temperature was good in the sunflower CMS lines 17 A, 234 A, 234 B, 335 A, restorer line RHA 95-C-1 and hybrids KBSH-41 and KBSH-44 (Table 7).

Conclusion

It is concluded that the above genotypes showed tolerance to higher temperature during summer season. The selected sunflower genotypes which shown tolerance to temperature stress through Temperature Induction Response technique (TIR) and gametophyte selection under laboratory condition is in consistency with their growth and yield performance when studied under two different temperature locations, indicating that selected sunflower genotypes differ in their yield performance between the two locations. This result concludes that reduction in the yield performance not only due to growth parameters and also due to reduced pollen germination and pollen viability resulting poor seed set and low yield.

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Table 7. Classification of sunflower genotypes based on TIR, gametophyte selection and field evaluation

Response to temperature	Temperature Induction Response	Gametophyte selection	Field evaluation for seed yield
Tolerant	17 A, 234 B, RHA 95-C-1, KBSH-41, KBSH-44	234 B, RHA 95-C-1, EC-734840, KBSH-41, KBSH-44, KBSH-53	17 A, 234 A, 234 B, 335 B, KBSH-41, KBSH-44, RHA 95-C-1
Susceptible	EC-734811, EC-734812, EC-734819, EC-734825, EC-734872, EC-734873, EC-734874, EC-734887, EC-734888, 6D-1	EC-734811, EC-734812, EC-734819, EC-734873	EC-734811, EC-734812, EC-734819, EC-734822, EC-734825, EC-734840, EC-734874

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