



Drought tolerance in popular sunflower hybrids at seedling stage

H G PRAVEEN^{1*}, T K NAGARATHNA² and Y A NANJA REDDY¹

University of Agricultural Sciences, GKVK, Bengaluru 560 065, India

Received: 17 March 2020; Accepted: 18 December 2020

ABSTRACT

Drought is a major abiotic stress coupled with high temperature that limits the crop productivity in arid and semi-arid regions. Productivity of sunflower under moisture stress is primarily determined by seed germination and the seedling vigour for appropriate crop stand. Screening hybrids for moisture stress and high-temperature tolerance under field condition has uncertainties of uncontrolled environmental conditions and interaction of biotic and other abiotic stresses. Hence, in the present experiment, 17 popular hybrids were evaluated for moisture stress tolerance at the seedling stage using PEG-induced osmotic stress and; for high-temperature tolerance using temperature induction response (TIR) technique. The study revealed that root growth was affected higher as compared to the shoot growth in both the experiments. The shoot growth was affected higher due to high temperature as compared to the PEG-induced stress. The hybrids NDSH-1012, PSH-1962, RSFH-130, and GK-202 were tolerant to PEG induced moisture stress while, the hybrids KBSH-41, KBSH-44, KBSH-53, and SB-207 were tolerant to high temperature-induced stress. The hybrids, DRSH-1, KBSH-1 and GK-2009 were moderately tolerant for both the stresses. These hybrids are suitable for drought tolerance at seedling level. This study concludes that the evaluation of hybrids for combined stresses (drought and high temperature) would be appropriate than the individual stress in sunflower. Furthermore, hybrids tolerant to stresses at seedling level should be evaluated at later growth stages for confirmation of drought/heat tolerance and suitable for rainfed cultivation.

Keywords: Osmotic stress, Recovery growth, Seedling survival, TIR approach

In the global climate change scenario, limited availability of water, adversely affects the growth and productivity of crops. In India, sunflower is cultivated in an area of 0.28 million ha with a production of 0.17 million metric tonnes and productivity of 0.61 metric tonnes per ha and; 80% of the area is under rainfed condition (USDA 2020). Globally, sunflower contributes about 87% of vegetable oil production (Razzaq *et al.* 2015). Several high-yielding hybrids of sunflower have already been developed and are extensively being cultivated. However, most of these hybrids were developed for resource-rich environments and not for stressful environments.

Germinating the seeds in media with different water potentials is a convenient method for studying the germination response of seeds to low water potential or moisture stress. Polyethylene glycol (PEG) was used to model the *in-vitro* osmotic stress effects and; PEG 6000 was found to be a convenient solute to create osmotic stress without causing any toxicity to the plant cells. The

PEG, withdraws water from the cell without entering into the apoplast and therefore, PEG stress mimics the dry soil (Ivanova *et al.* 2014). Hence, different PEG concentrations can be effectively used for screening genotypes to drought tolerance under laboratory condition.

High temperature causes irreversible damage to plant function and development. Different screening techniques at field-level were used to identify drought/high temperature tolerant genotypes, but field screening is season bound, laborious and time consuming, especially with large number of genotypes (Razzaq *et al.* 2017). Therefore, screening at the seedling stage under laboratory conditions would provide a rapid assessment of the relative performance of a genotypes/hybrids for drought tolerance/high temperature tolerance. In this direction, a novel Temperature Induction Response (TIR) technique has been standardized for the rapid assessment of cellular level tolerance to high temperature/drought tolerance in sunflower (Senthilkumar *et al.* 2003). Therefore, in the current study, PEG induced osmotic stress and TIR approaches were used to screen 17 popular hybrids of sunflower to identify the hybrids tolerant to osmotic and or high-temperature tolerance.

MATERIALS AND METHODS

An experiment was conducted at the AICRP on Sunflower, the University of Agricultural Sciences, GKVK,

Present address: ¹University of Agricultural Sciences, GKVK, Bengaluru; ²Protection of Plant Varieties and Farmers Rights Authority, Ministry of Agriculture and Farmers Welfare, Government of India, NASC Complex, New Delhi. *Corresponding author e-mail: praveenhg.agril@gmail.com

Bengaluru during 2017-18. Seventeen popular sunflower hybrids were evaluated for their response to PEG-induced osmotic stress and high temperature (TIR) conditions to identify tolerant hybrids to moisture stress and or high temperature tolerance. Popular sunflower hybrids released from State Agricultural Universities, research stations of different states, viz. Karnataka, Andhra Pradesh, Punjab, Indian Institute of Oilseeds Research (IIOR), Hyderabad and private sector, viz. Syngenta, Kaveri, and Ganga Kaveri were selected for the study.

Osmotic stress (PEG treatment): The experiment was conducted under laboratory conditions with 17 hybrids under six PEG concentrations (0, -2, -4, -6, -8, and -10 bars solute potential using PEG 6000 MW) with three replications in completely randomised design for each PEG concentration or water. Aluminium plates (30 cm × 30 cm × 2.5 cm, LBH respectively) lined with germination paper was used in the experiment. The germination paper was completely soaked in water for control and respective PEG solutions as per the treatments and placed in aluminium plates. Twenty uniform-sized dry seeds were placed in each aluminium plate and kept at room temperature for 6 days (water or respective solutions were added on 4th day to avoid the dryness of germination paper). On the 7th day, seed germination percentage and seedling length (root and shoot) were measured and; seedling vigour index was calculated using the formula (Germination % × Seedling length; ISTA 1996).

Cellular level tolerance (Temperature induction response, TIR): The TIR technique works based on the fact that temperature stress develops gradually. Therefore seedlings were exposed to sub-lethal stress before being exposed to severe stress. The percentage of seedlings that

survived and maintained better recovery growth under induced temperature was considered tolerant (Senthilkumar *et al.* 2003). Two-day-old (48 h) germinated seeds (20 No's) of uniform length (0.5 cm) were placed in aluminium tray lined with wet germination paper. For the control treatment, the plates were maintained at room temperature. For induction treatment, the temperature was gradually increased in the growth chamber from 35-45°C for 4 h (35°C-2 h, 40°C-1 h, and 45°C-1 h) followed by lethal temperature (50°C-3 h; Fig 1).

For lethal temperature, seedlings were directly exposed to the lethal temperature of 50°C for 3 h. After the treatment period, the seedlings were allowed to recover at room temperature for 72 h. At the end of the recovery period, the percent seedling survival and seedling recovery growth were measured. Based on the growth in control and induction treatments, the percent reduction in recovery growth (% RRG) was computed. Each treatment had three replications with 17 hybrids. The formula used for calculating seedling survival and percent recovery growth is given below.

$$\text{Seedling survival (\%)} = \frac{\text{Number of seedlings survived at the end of a recovery period}}{\text{Total number of seedlings placed in the tray}} \times 100$$

$$\text{Percent reduction in recovery growth (\% RRG)} = \frac{\text{Seedling growth in control} - \text{Seedling growth in induction}}{\text{Seedling growth in control}} \times 100$$

Statistical analysis: The data collected from each experiment were subjected to analysis of variance (ANOVA) using OPSTAT statistical package in CRD for each treatment considering the hybrids and replications (Sheoran *et al.* 1998).

RESULTS AND DISCUSSION

Performance of popular sunflower hybrids under PEG induced osmotic stress: Seed germination is considered as the first and foremost fundamental life stages of a plant, which influences the final crop stand, growth and yield, especially under rainfed conditions. Laboratory screening for moisture stress using polyethylene glycol (PEG) was found to be appropriate (Geetha *et al.* 2012) and; therefore, in the present experiment, PEG-6000 was used to create the osmotic stress condition. Increased stress levels (PEG concentration) resulted in decreased seed germination percentage, root length, shoot length, and seedling vigour index. The effect of PEG stress was severe on shoot length (68.1%) as compared to the shoot

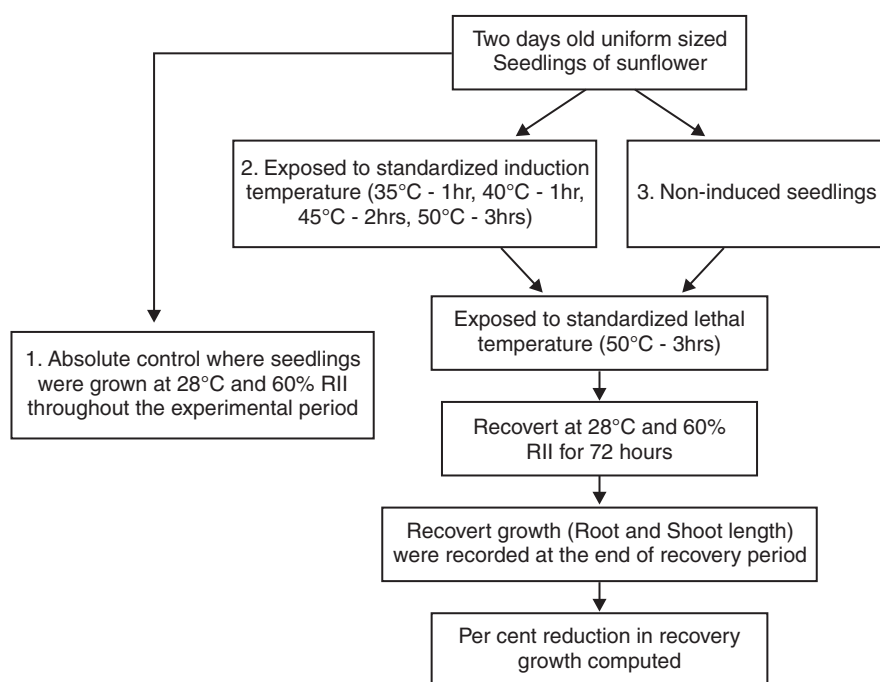


Fig 1 Protocol followed in temperature induction response (TIR) technique.

Table 1 Effect of PEG induced moisture stress on seed germination, root length, shoot length, and SVI in sunflower hybrids

Hybrid	Germination (%)			Root length (cm)			Shoot length (cm)			Seedling vigour index		
	Control	-2 bars	% red	Control	-2 bars	% red	Control	-2 bars	% red	Control	-2 bars	% red
DRSH-1	98.3	83.3	15.3	15.5	12.1	22.1	16.0	2.8	82.5	3097	1236	60
KBSH-1	93.3	81.7	12.5	10.6	7.4	30.4	12.7	5.5	56.7	2554	1441	51
KBSH-41	98.3	86.7	11.9	11.5	10.7	7.6	14.5	6.0	58.6	3334	1854	44
KBSH-44	93.3	66.7	28.6	9.7	8.0	17.1	10.5	4.7	55.2	2435	1090	55
KBSH-53	95.0	63.3	33.3	10.8	5.1	52.6	14.8	4.0	73.0	2582	1272	76
NDSH-1012	98.3	85.0	13.6	18.3	15.9	13.2	15.6	5.9	62.2	1209	1055	44
PSH-1962	98.3	90.0	8.5	11.6	11.2	3.2	13.2	2.5	81.1	2171	1054	55
RSFH-130	96.7	90.0	6.9	7.0	5.7	19.0	19.7	8.4	57.4	2426	573	51
RSFH-1887	96.7	81.7	15.5	5.9	5.6	6.3	7.3	6.6	9.6	1883	849	13
K-618	95.0	83.3	12.3	8.2	6.0	26.2	7.5	1.4	81.3	1489	623	58
GK-202	90.0	83.3	7.4	6.8	6.2	8.3	6.7	1.3	80.6	1218	632	48
GK-2002	93.3	70.0	25.0	11.2	9.5	14.9	13.3	4.3	67.7	1399	397	58
GK-2008	93.3	83.3	10.7	6.6	5.8	12.7	7.0	2.0	71.4	2281	963	49
GK-2009	93.3	58.3	37.5	6.6	6.1	7.4	8.4	0.7	91.7	1637	1184	72
PAC-3794	98.3	88.3	10.2	8.0	7.7	3.3	8.7	5.7	34.5	1174	463	28
SB-207	93.3	81.7	12.5	6.4	5.0	22.4	6.2	0.7	88.7	1999	601	61
SH-416	91.7	56.7	38.2	6.7	4.1	39.2	15.1	6.5	57.0	1278	646	70
Mean	95.1	78.4	17.6	9.5	7.8	18.0	11.6	3.7	65.2	2010	937	53
SEm (±)	3.4	1.5		0.4	0.4		0.5	0.4		68.1	46.0	
CD @ 5%	9.8	4.3		1.2	1.1		1.4	1.1		196.6	132.8	
CV (%)	7.4	4.1		7.4	5.6		10.9	20.2		5.9	8.5	

% red, % reduction

length (17.9%) at -2 bars of PEG and; suggests that the root tries to grow deeper under stress conditions. The deep root growth under stress could be due to the diversion of the shoot mass towards the root growth (Praveen *et al.* 2020) enabling higher osmotic adjustment for cell division and cell enlargement of root system (Rauf and Sadaqat 2008). In addition, decreased shoot growth might be an avoidance mechanism to reduce water loss by restricting the cell division and proliferation in the shoot system (Anjum *et al.* 2011, Claeys and Inze 2013).

PEG solution of -4 bars and above resulted in a drastic reduction in all the parameters, Hence, a PEG concentration of -2 bars was considered for interpretation of response of hybrids to PEG stress. The hybrids with significantly higher value for a given trait as compared to the mean plus a lesser reduction as compared to mean percent reduction were considered as tolerant. When such tolerant nature was observed for more than two traits, such hybrids were selected as tolerant. Accordingly, KBSH-1, KBSH-41, DRSH-1, NDSH-1012, PSH-1962, PAC-3794, and RSFH-130 displayed superior performance for germination percent, root length, shoot length, and SVI and were termed as tolerant (Table 1). Previously, Kaya *et al.* (2006) have

also used PEG-induced moisture stress for the selection of drought tolerant hybrids in sunflower.

Cellular level tolerance of popular sunflower hybrids (TIR approach): One of the approaches extensively used in recent years in selection of genotypes for high-temperature tolerance was the TIR technique (Senthilkumar *et al.* 2003). However, the technique was used only in a few released varieties/hybrids or in germplasm evaluation but not for all the released hybrids at a time. In the present study, we have attempted in 17 popular hybrids of sunflower cultivated in India at a time for their differential response to temperature tolerance through the TIR approach.

The temperature-induced stress treatment reduced the seedling survival percentage and seedling length significantly as compared to control (non-stress) seedlings. Lethal temperature decreased the mean seedling survival to a higher extent (71.5%) compared to the induction treatment (34.3%). Hence, the selection of genotypes at the level of induction treatment would be a better option. Under induction temperature, shoot length decreased to a higher extent (74.0%) to avoid water loss as observed in the case of PEG-induced stress. The reduction in root length under stress (59.4%) could be due to experience of root

Table 2 Effect of induction temperature on seedling characters of sunflower hybrids

Hybrid	Seedling survival (%)			Root length (cm)			Shoot length (cm)			Seedling length (cm) (Shoot + Root)		
	Control	Induction	% red	Control	Induction	% red	Control	Induction	% red	Control	Induction	% red
DRSH-1	98.3	70.0	28.8	6.8	3.4	50.7	5.3	2.1	59.6	12.1	4.4	63.9
KBSH-1	96.7	73.3	24.2	7.5	3.7	50.8	5.0	1.7	66.5	12.5	4.3	65.9
KBSH-41	98.3	81.7	16.9	6.4	1.5	75.9	4.2	0.5	88.2	10.6	1.5	85.4
KBSH-44	98.3	81.7	16.9	7.8	5.2	33.2	6.4	4.2	35.4	14.2	5.7	59.9
KBSH-53	91.7	78.3	14.6	2.2	1.7	22.3	7.5	2.5	66.1	9.7	2.6	73.0
NDSH-1012	96.7	73.3	24.2	11.0	1.0	91.1	7.7	1.5	80.4	18.7	1.8	90.6
PSH-1962	98.3	78.3	20.3	5.5	3.0	46.6	4.6	1.9	57.7	10.1	3.5	65.2
RSFH-130	98.3	71.7	27.1	6.7	0.8	88.2	5.2	1.6	68.5	11.9	1.3	88.8
RSFH-1887	98.3	56.7	42.3	6.6	2.4	63.2	6.7	0.5	92.5	13.3	2.7	80.0
K-618	95.0	56.7	40.3	6.6	3.1	53.3	7.9	1.2	84.4	14.5	3.3	76.9
GK-202	96.7	61.7	36.2	8.5	2.5	70.1	7.1	0.8	88.5	15.6	2.8	81.7
GK-2002	91.7	31.7	65.4	7.9	0.7	90.6	6.1	1.0	83.5	14.0	0.7	94.7
GK-2008	96.7	63.3	34.5	6.6	2.2	66.5	7.7	0.9	88.4	14.4	2.2	84.5
GK-2009	96.7	63.3	34.5	7.7	4.3	44.0	5.0	1.4	71.5	12.7	5.0	60.7
PAC-3794	96.7	58.3	39.7	5.1	0.0	100.0	5.8	0.8	86.8	10.9	0.9	91.4
SB-207	98.3	61.7	37.2	5.9	4.3	26.0	5.3	2.3	57.0	11.1	5.3	52.1
SH-416	95.0	55.0	42.1	4.5	2.8	37.4	6.3	1.1	82.6	10.9	2.8	73.8
Mean	96.6	65.7	32.1	6.7	2.5	59.4	6.1	1.5	74.0	12.8	3.0	75.8
SEm (±)	4.3	1.2		0.4	0.2		0.2	0.3		0.5	0.2	
CD @ 5%	NS	3.5		1.2	0.4		0.7	1.0		1.4	0.5	
CV (%)	6.0	1.7		10.6	10.4		6.8	8.4		6.6	9.8	

Note: Lethal temperature: 50°C for 3 h; Induction temperature: 35°C (2 h) - 40°C (1 h) - 45°C (1 h) - 50°C (3 h); % red – % reduction

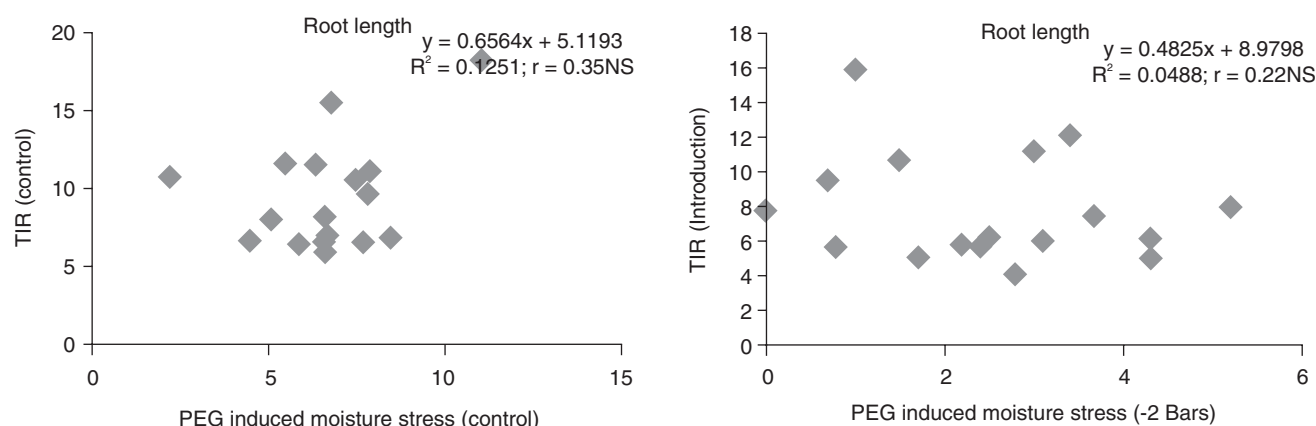


Fig 2 Relationship between PEG induced moisture stress and high temperature-induced stress for root length.

towards moisture stress (Anjum *et al.* 2011, Claeys and Inze 2013) and hence, the root acclimates rapidly to the gradual increase in stress condition. In addition, the root length is also important for the uptake of water and nutrients under stress conditions (Kaya *et al.* 2006, Kulkarni and Deshpande 2007). Accordingly, hybrids DRSH-1, KBSH-1, KBSH-44, KBSH-53, PSH-1962, GK-2009, and SB-207 performed superior considering seedling survival percent, root length, shoot length, and seedling length (Table 2) under temperature-induced conditions. Interestingly, hybrids KBSH-41 and KBSH-44 showed higher acclimation capacity with 81.7% seedling survival.

Comparison of PEG and high-temperature acclimation treatments: When the PEG-induced and high temperature-induced stress were compared, the relationship of root length between PEG-induced and high temperature-induced treatments was not related both under control and stress conditions (Fig 2).

The reduction in shoot length remained more or less similar in both the experiments, but the reduction in mean root length was higher (59.4%) in the case of temperature induction treatment as compared to the PEG mediated water stress (18.0%). The higher effect on root growth in high-temperature stress was due to the combined effect of high temperature and moisture stress as against only water stress in PEG induced stress. The less growth of shoot or root could be due to damage to the organelle membranes, electrolyte leakage, and loss of chlorophyll in a shoot during the recovery period (Amrutha *et al.* 2007). Based on the germination percent, seedling growth, and seedling vigour under -2 and -4 bars of PEG, 17 sunflower hybrids were classified into three groups (Tolerant, moderately tolerant and sensitive hybrids). Hybrids NDSH-1012, PSH-1962, RSFH-130, and GK-202 as best performing hybrids (with <8.8 and <23 % reduction in seed germination at -2 bars and -4 bars respectively and with <26.7 and <39.4 % reduction in SVI at -2 bars and -4 bars respectively). Hybrids DRSH-1, KBSH-1, KBSH-41, KBSH-44, RSFH-1887, GK-2009, PAC-3794, and SH-416 have medium tolerance to water stress (between 8.8 to 17.6% and 23 to 46.1% reduction of seed germination at -2 bars and

-4 bars respectively and; with 26.7 to 53.4 % and 39.4 to 78.8 % reduction in seedling vigour at -2 bars and -4 bars respectively). Hybrids KBSH-53, K-618, GK-2002, GK-2008, and SB-207 were sensitive (with >17.6 and >46.1 % reduction of seed germination and with >53.4 and >78.8 % reduction in seedling vigour at -2 bars and -4 bars respectively; Table 1).

Based on the mean reduction in seedling survival and total seedling length after TIR screening, 17 sunflower hybrids were classified into three groups (Tolerant, moderately tolerant and sensitive hybrids). Hybrids KBSH-41, KBSH-44, KBSH-53, and SB-207 were the best performing hybrids (with <20 and <60 % reduction in seedling survival and total seedling length respectively). Hybrids, DRSH-1, KBSH-1, NDSH-1012, PSH-1962, RSFH-130, and GK-2009 were medium tolerant (between 20 to 32.1 % and 60 to 75.8 % reduction in seedling survival and total seedling length respectively). Hybrids RSFH-1887, K-618, GK-202, GK-2002, GK-2008, PAC-3794 and SH-416 were highly sensitive (with >32.1 and >75.8 % reduction in seedling survival and total seedling length). Following the selection for both drought and high-temperature stresses, 3 hybrids out of 14 hybrids were found moderately tolerant for both drought and high-temperature stress (DRSH-1, KBSH-1, GK-2009). Similarly, the common sensitive hybrids were 2 out of 9 susceptible hybrids separately for both PEG-induced and high temperature-induced stresses (K-618, GK-2008). This suggests the cross-tolerance of nearly 40-60% between these two stresses. Hence it would be appropriate to screen for combined stresses to achieve stable selection either for drought or high-temperature tolerance in sunflower. The identified popular hybrids (DRSH-1, KBSH-1, GK-2009) may be advocated for rainfed sowing for better crop stand and productivity.

ACKNOWLEDGEMENTS

The authors are grateful to All India Coordinated Research Project on Sunflower, Zonal Agricultural Research Station, University of Agricultural Sciences, Bengaluru for providing the necessary facilities during the study period.

REFERENCES

- Amrutha R, Muthulaksmi S, Baby Rani W, Indira and Mareeswari P. 2007. Physiological studies on evaluation of sunflower (*Helianthus annuus* L.) genotypes for high-temperature stress. *Research Journal of Agriculture and Biological Sciences* 3(4): 245-51.
- Anjum S A, Xie X Y, Wang L C, Saleem M F, Man C and Lei W. 2011. Morphological, physiological, and biochemical responses of plants to drought stress. *African Journal of Agricultural Research* 6(9): 2026-32.
- Claeys H and Inze D. 2013. The agony of choice: How plants balance growth and survival under water-limiting conditions. *Plant Physiology* 162(4): 1768-79.
- Geetha A, Sivasankar A, Lakshmi Prayaga, Suresh J and Saidaiah P. 2012. Screening of sunflower genotypes for drought tolerance under laboratory conditions using PEG. *Sabara Journal of Breeding Genetics* 44(1): 28-4.
- ISTA 1996. International rules for seed testing. *Seed Science and Technology* 13: 299-513
- Ivanova R V, Shtereva L, Kraptchev B and Karceva T. 2014. Response of sunflower (*Helianthus annuus* L) genotypes to PEG-mediated water stress. *Central European Journal of Biology* 9(12): 1206-14
- Kaya M D, Okcu G, Atak M, Cakil Y and Kolsarici O. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *European Journal of Agronomy* 24: 291-95.
- Kulkarni M and Deshpande U. 2007. *In vitro* screening of tomato genotypes for drought resistance using polyethylene glycol. *African Journal of Biotechnology* 6: 691-96.
- Praveen H G, Nagarathna T K and Nanja Reddy Y A. 2020. Root length and leaf cuticular wax: The traits associated with drought avoidance in sunflower hybrids. *International Journal of Chemical Studies* 8(4): 2588-93.
- Rauf S and Sadaqat H A. 2008. Identification of physiological traits and genotypes combined to high achene yield in sunflower (*Helianthus annuus* L.) under contrasting water regimes. *Australian Journal of Crop Science* 1(1): 23-30.
- Razzaq H, Tahir H N, Sadaqat H A and Sadia B. 2015. Genetic variability in sunflower accessions for achene yield and its related traits in sunflower. *International Journal of Science and Nature* 5: 669-76.
- Razzaq H, Tahir H N, Sadaqat H A and Sadia B. 2017. Screening of sunflower (*Helianthus annuus* L.) accessions under drought stress conditions, an experimental assay. *Journal of Soil Science and Plant Nutrition* 17(3): 662-71.
- Senthilkumar M, Srikanthbabu V, Mohan Raju B, Ganeshkumar, Shivaprakash N and Udayakumar M. 2003. Screening of inbred lines to develop a thermotolerant sunflower hybrid using the temperature induction response (TIR) technique: a novel approach by exploiting residual variability. *Journal of Experimental Botany* 392(54): 2569-78.
- Sheoran O P, Tonk D S, Kaushik L S, Hasija R C and Pannu R S. 1998. Statistical software package for agricultural research workers. Recent advances in information theory, statistics & computer applications, Department of Mathematics & Statistics, CCS HAU, Hisar, 139-43.
- USDA. 2020. United States Department of Agriculture, Foreign Agricultural Service Circular Series WAP, 2020. World Agricultural Production, pp 1-20.