

Screening of sunflower genotypes for drought tolerance with PEG 6000

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ABSTRACT A study was conducted at Regional Agricultural Research Station (RARS), Nandyal during Kharif 2010 to screen the sunflower entries LDM 02, ARM 239 B, CMS 852 B, IB-5, 136-R, NDR-71, CMS 23 B, CMS 40 B, CMS 17 B, ARM 242 B, 150-R for drought tolerance with PEG 6000. The results showed that CMS 17 B recorded higher germination (93.33%), seedling length (11.56 cm) and seedling dry weight (7.525 g). High Promptness Index (PI = 4.096) and Root Length Stress Tolerance Index (RLSI = 79.53) were recorded by LDM 02 and Germination Stress Tolerance Index (GSI = 255.9) and Dry Matter Stress Tolerance Index (DMSI = 380.94) were by CMS 23 B. With increase in PEG concentration from 5 to 15% there was decrease in germination, seedling length, seedling dry weight and other parameters. In the analyses of interaction effects it was clearly shown that genetic variation is useful to develop cultivars suitable to arid and semi-arid zones. From this study, on the basis of DMSI values, which are considered as reliable indicator of drought tolerance in sunflower, the inbred lines CMS 23 B and CMS 40 B and CMS 17 B were grouped as best lines, IB-5 and LDM 02 as medium lines and remaining as sensitive to drought condition.

Key words: Sunflower seeds, PEG 6000, drought tolerance, germination, inbred lines

Sunflower is the fourth important oilseed crop in the world, next to soybean, groundnut and rapeseed. Optimal plant growth and successful crop production is affected by inadequate water and nutrient supply. Water stress is one of the most severe limitations for crop growth in semi-arid and arid region of the world as it plays a vital role in plant metabolism at all growth stages. Water stress affects protein synthesis, photosynthesis, respiration and nucleic acid synthesis which may result in yield reduction. However, limited rainfall during growing season constraints seed yield of sunflower if grown without irrigation. Though sunflower is tolerant to drought compared to other crops, 50% of yield reduction was recorded due to water stress at critical growth stages, like germination, seedling growth and flowering. Therefore development of drought tolerant cultivars/hybrids is needed to have a more stable sunflower production.

A study was conducted with the objectives of screening genotypes against drought stress at

germination and seedling growth using PEG 6000. PEG 6000 is a higher molecular weight compound that creates stress in the outer layer of sunflower seeds by restricting the entry of water into the seeds during imbibition. In the present experiment, PEG 6000 was used to create osmotic stress as most of the researchers utilized it for the development of water deficit in growth chamber studies [1, 2].

MATERIALS AND METHODS

The experiment was conducted during Kharif 2010 at RARS, Nandyal with the genotypes obtained from Directorate of Oilseed Research, Hyderabad, for conducting drought tolerant studies. They are LDM 02, ARM 239 B, CMS 852 B, IB-5, 136-R, NDR-71, CMS 23 B, CMS 40 B, CMS 17 B, ARM 242 B, 150-R which were evaluated at field level during two Rabi seasons.

PEG 6000 solutions at different concentrations were prepared using distilled water as solvent.

Received: August 2011

Revised: May 2012

Accepted: June 2012

Table 1. Treatments applied

T ₁	: Control (distilled water)
T ₂	: 5% PEG 6000 solution (5g/100ml)
T ₃	: 10% PEG 6000 solution (10g/100ml)
T ₄	: 15% PEG 6000 solution (15g/100ml)
T ₅	: 20% PEG 6000 solution (20g/100ml)

The experiment was laid out in a completely randomized design in which each treatment was kept in three replications. Seeds were first surface sterilized with 10% sodium hypochlorite solution for five min and then washed three times with distilled water. Ten seeds of each sunflower genotype were placed in each Petri plate containing filter paper. 10 ml of designated treatment solution was applied daily in each Petri plate after washing out the previous solution. The data on following were recorded:

(1) germination (%), (2) seedling length (cm), (3) seedling dry weight (g). From this data, the following indices were calculated.

1. *Promptness Index (PI)*

$$PI = nd_2 (1.00) + nd_4 (0.75) + nd_6 (0.5) + nd_8 (0.25)$$

Where "n" is the number of seeds germinated at day "d"

2. *Germination Stress Tolerance Index (GSI)*

$$GSI = (PI \text{ of stressed seeds} / PI \text{ of control seeds}) \times 100$$

3. *Root Length Stress Tolerance Index (RLSI)*

$$RLSI = (\text{Root length of stressed plants} / \text{root length of control plant}) \times 100$$

4. *Dry Matter Stress Tolerance Index (DMSI)*

$$DMSI = (\text{Dry matter of stressed plants} / \text{Dry matter of control plant}) \times 100$$

RESULTS AND DISCUSSION

Genotype reaction to PEG concentration

The data presented in table 2 showed that among the genotypes except 150 R (48%), all others

recorded significantly higher germination values *i.e.* above 70% (as per seed certification standards). Higher germination percentage was recorded by CMS 17 B (93%) followed by LDM 02, ARM 239 B (87.5%), CMS 40 B and IB 5 (85%). Reduction in germination percentage on PEG treatment, due to decrease in the water potential gradient between seeds and their surrounding media had been reported earlier [3]. Variable cultivar response clearly shows that genetic variation among cultivars could be utilized to develop cultivars suitable for arid and semi-arid zones. Similar results were reported in wheat cultivars [4, 5, 6]. CMS 17 B recorded significantly higher seedling length (11.5 cm) followed by IB 5 (9.97 cm), CMS 40 B (9.63 cm).

However, significantly lower seedling length was recorded by 136 R (6.42 cm) and 150 R (6.12 cm). This might be due to an impediment of cell division and elongation leading to tuberization of the root system [7]. In seedling dry weight, significantly higher values were recorded by CMS 17 B (7.53 g) followed by CMS 23 B (7.0 g) and CMS 40 B (6.40 g) and lowest was recorded by 150 R (1.09 g). This was attributed to development of smaller leaves with increased PEG concentration of growth media and drought resistance is characterized by reduction of shoot growth under drought stressed conditions [8, 9]. Similar reports [10] mentioning shoot length and shoot fresh weight as the most affected traits under *in vitro* conditions.

No. of seeds germinated per day (PI) were significantly more in LDM 02 (4.09) followed by IB 5 (3.88), CMS 23 B (3.49), CMS 17 B (3.33) and CMS 40 B (3.24), whereas significantly lower value was recorded by 150 R (1.41) which is due to different genotypic response to PEG concentration. GSI was significantly more in CMS 23 B (255.90) followed by CMS 40 B (142.7), CMS 17 B (128.77) and LDM 02 (105.33), whereas significantly lower value was recorded by ARM 239 B (61.0). Difference in GSI among inbred lines was due to genetic variation and osmotic stress created by PEG [11].

RLSI was recorded significantly more in LDM 02 (79.53) followed by ARM 239 B (60.62), IB 5 (46.56) and CMS 17 B (42.0), whereas lowest RLSI

was recorded by NDR-71 (20.67). Dry Matter Stress Tolerance Index was significantly more in CMS 23 B (380.94) and CMS 40 B (364.77), whereas significantly lower value was recorded in 150 R (13.33).

Effect of PEG concentrations

With increase in PEG concentration, there was a decrease in germination percentage. Significantly higher germination was recorded in control followed by PEG 5% (89.39%) and 10% (87.57%), whereas lowest germination was recorded with 15% PEG (47.72%). This decline in germination might be due to inability of seed to imbibe water at low osmotic potential [12]. Similar trend was followed for seedling length in which significantly higher values were recorded in control (12.98 cm) followed by 5% PEG (11.74 cm) and 10% PEG (8.19 cm). This was due to fall in mobilization of reserves to plumule, thus preventing growth under stress.

In seedling dry weight, PEG at 5% recorded significantly higher value (7.203 g) followed by PEG 10% (5.885 g) and control (5.606 g). Different genotypes responding differently to water stress in terms of seedling length has been reported earlier [14, 15]. Promptness Index recorded was significantly more at 5% PEG (4.00) followed by 10% PEG (3.74) and control (3.35). In Germination Stress Tolerance Index, Root Length Stress Tolerance Index and Dry Matter Stress Tolerance Index similar trend was followed *i.e.* significantly more values were recorded at 5% PEG (153.99, 72.73 and 233.49, respectively) followed by 10% PEG (138.7, 49.27 and 168.71, respectively) and 15% PEG (36.78, 10.99 and 28.44, respectively).

In interactions for germination and PEG concentration, the data recorded clearly showed the response of genotypes to different concentrations of PEG. The germination values in control were significantly more (90%) for all the genotypes except NDR 71 (86.67%) and 150 R (60%). With increase in concentration of PEG, germination percent was decreased. Decrease in germination percentage with increase in osmotic stress in germinating media was also reported in sunflower [16, 17]. The entries CMS 23 B (90%), CMS 17 B (90%), IB-5 (80%), CMS 40 B (80%)

and LDM 02 (70%) recorded higher germination values even at 15% PEG concentration. Similar results were reported in sunflower [11]. All genotypes recorded significantly higher germination above 90% at all concentrations except ARM 242 B and 150 R which recorded 86% with 5 and 10% PEG concentration and 60% germination in control.

In seedling length, CMS 17 B with 5% PEG (16.83 cm) followed by CMS 852 B in control (14.6 cm) and IB 5 at 5% (14.57 cm) recorded significantly higher values and almost all genotypes in general recorded lower values at 15% PEG. In seedling dry weight CMS 17 B and CMS 23 B in control recorded significantly higher values (14 g) followed by CMS 17 B with 5% (12.67 g), ARM 239 B (11.9 g) and NDR 71 (11.33 g) with 10% PEG (Table 2).

In Promptness Index with 5% PEG, LDM 02 (6.33), ARM 239 B (5.67) and CMS 852 B (5.0) and with 10% PEG, CMS 23 B (5.0) and IB 5 (5.33) recorded significantly higher values and all genotypes in general recorded lower values with 15% PEG. In Germination Stress Index, CMS 23 B with 5% recorded significantly higher values (316.63) followed by with 10% (240.53) and CMS 40 B with 5% (244.4). Lowest value was recorded by IB 5 with 15% PEG (69.33). In Root Length Stress Tolerance Index, ARM 239 B recorded significantly higher values (101.43) followed by LDM 02 with 5% PEG (98.7), whereas the lowest value by CMS 17 B (14.0). PEG exposure causes osmotic stress, reduction of turgor pressure, limitation of nutrient uptake and inhibition of photosynthetic carbon dioxide uptake in plant growth [18]. For Dry Matter Stress Tolerance Index, CMS 40 B recorded significantly higher value with 5% PEG (677.67) followed by CMS 23 B at 5% PEG (550), whereas the lowest value with 15% by all the genotypes except CMS 23 B (172) and CMS 17 B (77).

In conclusion, on the basis of Dry Matter Stress Tolerance Index values, which are considered as reliable indicator of drought tolerance in sunflower, the inbred lines CMS 23 B, CMS 40 B and CMS 17 B were grouped as best lines (Table 3), IB-5 and LDM 02 as medium lines and remaining were sensitive to drought condition.

Table 3. Effect of PEG 6000 on Promptness Index, Germination Stress Tolerance Index, Root Length Stress Tolerance Index and Dry Matter Stress Tolerance Index in sunflower genotypes

SNo	Genotype	Promptness Index			Germination Stress Tolerance Index			Root Length Stress Tolerance Index			Dry Matter Stress Tolerance Index							
		Control	5%	10%	15%	Mean	5%	10%	15%	Mean	5%	10%	15%	Mean				
1	LDM 02	3.63	6.33	4.33	2.08	4.09	166.87	149.13	0.00	105.33	98.70	88.87	51.03	79.53	188.90	174.33	0.00	121.08
2	ARM 239B	3.50	5.67	2.83	0.00	3.00	88.87	55.53	38.60	61.00	80.43	101.43	0.00	60.62	91.50	37.33	10.07	46.03
3	CMS 852 B	3.75	5.00	4.67	0.00	3.35	90.23	139.56	0.00	76.60	71.17	46.87	0.00	39.34	52.27	21.90	47.50	40.56
4	IB-5	3.58	4.33	5.33	2.27	3.88	133.33	177.76	69.33	126.81	80.73	40.60	18.63	46.56	208.33	112.23	5.543	108.70
5	I36-R	3.67	3.67	3.00	0.00	2.58	139.23	133.10	0.00	90.78	52.60	16.67	0.00	23.09	150.00	100.00	0.00	83.33
6	NDR-71	2.25	3.17	3.33	0.00	2.19	76.30	110.06	89.77	92.04	45.70	16.30	0.00	20.67	252.67	66.67	0.00	106.44
7	CMS 23 B	3.00	3.67	5.00	2.31	3.49	316.63	240.53	120.53	255.90	70.13	69.07	20.83	53.34	550.00	420.83	172.00	380.94
8	CMS 40 B	5.00	4.00	2.33	1.62	3.23	244.43	183.66	0.00	142.70	82.77	55.33	16.47	51.52	677.67	416.67	0.00	364.78
9	CMS 17 B	3.63	6.33	4.33	2.08	4.09	166.87	149.13	0.00	105.33	98.70	88.87	51.03	79.53	188.90	174.33	0.00	121.08
10	ARM 242 B	2.76	3.60	3.67	0.00	2.51	215.83	83.53	0.00	99.79	70.03	53.33	0.00	41.12	84.80	92.80	0.00	13.33
11	150-R	1.73	1.60	2.30	0.00	1.40	94.43	80.57	0.00	58.33	74.80	14.47	0.00	29.76	23.33	16.67	0.00	13.33
	Mean	3.35	4.00	3.74	0.94	3.00	153.99	138.70	36.78	109.82	72.73	49.27	10.99	44.32	233.49	168.71	28.44	143.55
	SEd	0.45	0.30	0.99			45.76	23.89	79.26			7.74	4.04	13.40	73.39	38.33	127.12	
	CD (5%)	0.99	0.59	1.98			91.41	47.74	NS			15.45	8.07	26.77	146.60	76.56	NS	

REFERENCES

1. SMOK MA, CHOJNOWSKI M, CORBINEAU F AND COME D (1993). Effects of osmotic treatments on sunflower seed germination in relation with temperature and oxygen. In: Come D and Corbineau F (eds) *Proc 4th Intl Workshop on Seed: Basic and Applied Aspects of Seed Biology*, Angers, France, pp 1033-38
2. HU FD AND JONES RJ (2004). Effects of plant extracts of *Bothriocola pertusa* and *Urochloa mosambicensis* on seed germination and seedling growth of *Stylosanthes hamata* cv. Verano and *Stylosanthes scarab* cv. Seca. *Aust J Agric Res* **48**: 1257-64
3. DODD GL AND DONOVAN LA (1999). Water potential and ionic effects on germination and seedling growth of two cold deserts scrubs. *Am J Bot* **86**: 1146-53
4. ALAEI M, ZAEFIZADEH M, KHAYATNEZHAD M, ALAEI Z AND ALAEI Y (2010). Evaluation of germination properties of different durum wheat genotypes under osmotic stress. *Middle-East Journal of Scientific Research* **6**: 642-46
5. JAJARMI V (2009). Effect of water stress on germination indices in seven wheat cultivar. *World Academy of Science Eng Technol* **49**: 105-06
6. METWALI MR, EHAB, MANAL HE AND TAREK YB (2011). Agronomical traits and biochemical genetic markers associated with salt tolerance in wheat cultivars (*Triticum aestivum* L). *Australian J of Basic and Applied Science* **5**(5): 174-83
7. FRASER T, SILK W AND ROSR T (1990). Effect of flow water potential on cortical cell length in growing region on maize roots. *Plant Physiology* **93**: 648-51
8. MING DF, PEI ZF, NAEEM MS, GONG HJ AND ZHAN WJ (2012). Silicon alleviates PEG induced-deficit stress in upland rice seedling by enhancing osmotic adjustment. *Journal of Agronomy and Crop Science* **198**: 14-26
9. MOUCHESHI A, HEIDARI B AND ASSAD MT (2012). Alleviation of drought stress effects on wheat using arbuscular mycorrhizal symbiosis. *International Journal of Agriscience* **291**: 35-47
10. TURHAN H AND BASER I (2004). *In vitro* and *in vivo* water stress in sunflower (*Helianthus annuus* L). *Helia* **27**(40): 227-36
11. AHMAD SHAMIM, AHMAD RASHID, ASHRAF MY, ASHRAF M AND WARAICH EH (2009). Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pak J Bot* **41**(2): 647-54
12. SINGH KP AND AFRICA BS (1985). Evaluation of moisture stress tolerance in castor cultivars during germination, seedling growth and emergence. *Proc Indian Nat Sci Acad* **51**: 364-68
13. SINGH KP AND SINGH K (1983). Water uptake and germination of wheat seeds under different external water potentials in osmotic solutions. *Seed Res* **11**: 13-19
14. ASHRAF MY, NAQVI MH AND KHAN AH (1996). Evaluation of four screening techniques for drought tolerance in wheat (*Triticum aestivum* L.). *Acta Agronomica* **44**: 213-20
15. MANJULA K, SHARMA PS, RAMESH TATIKUNTA AND NAGESHWARA RAO T (2003). Evaluation of castor (*Ricinus communis* L) genotypes for moisture potential stress. *Indian J Plant Physiol* **8**(3): 319-22
16. SAJJAN AS, BADANUR VP AND SAJJANAR GM (1999). Effect of external water potential on seed germination, seedling growth and vigor index in some genotypes of sunflower. In: Faroda SA, Joshi NL, Kathuja S and Kar A (eds) *Proc Symp Recent Advances in Management of Arid Ecosystem* pp 215-18
17. MOHAMMAD M EI, BENBELLA M AND TALOUIZETE A (2002). Effect of sodium chloride on sunflower (*Helianthus annuus* L) seed germination. *Helia* **37**: 51-58
18. PUGNARIE FI, ENDOLZ LS AND PARDOS J (1994). Constraints by water stress on plant growth. In: Pessaraki M (ed), *Plant and Crop Stress*, Marcel Dekker Inc pp 247-59.