



***In-vitro* Screening of Tomato Genotypes for Drought Tolerance**

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A study was carried out to assess the drought tolerance ability of 32 tomato genotypes using PEG - 6000 induced moisture stress, on the basis of seedling parameters such as germination percentage, root length, shoot length, seedling dry weight and vigour index. *In vitro* screening revealed that seedling characters were reduced significantly at -0.2 MPa compared to control. Among the genotypes LE 18, LE 57, LE 27, LE 13 and LE 118 performed better under stress in terms of germination percentage, root length, shoot length, seedling dry weight and vigour index. Many of the genotypes did not germinate at -0.35 MPa. Varieties like CO 3, PKM 1, TNAU TH CO 3 and COTH 2 showed poor performance in terms of germination percentage, root and shoot length. This result can be used for pot and field level screening and manipulations in tomato cultivars for improvement of drought tolerance.

Key words: Drought tolerance, PEG – 6000, Tomato, Germination, Vigour index.

Vegetable production under drought is essential to cope up with increasing demand for vegetable crops considering increasing world population. Water is a major constraint in tomato production under rainfed condition. It is one of the important environmental factors that influence germination and plant growth. Reduced water potential in the environment can either delay or reduce the germination rate of many plant species because it interferes with the imbibition process and cell lengthening within the embryo axis (Schuab *et al.*, 2007).

Tomato (*Solanum lycopersicon*) is one of the most important vegetable crops grown in India. The estimated area and production of tomato for India are about 6.34 lakh hectares and 124 lakh tons respectively and the country is the sixth largest tomato producer in the world. The average productivity of tomato in India is merely 158 q/ha while its productivity in USA is 588 q/ha. This low yield may be either due to lack of high yielding varieties, poor management practices, and abiotic and biotic stresses. High yielding variety is an important factor for maximizing the yield of tomato and to fulfill the increasing demand for this nutritionally important vegetable, tomato cultivation under drought is also advocated.

Germination of seeds under water deficit should be assessed in the laboratory under controlled temperature and humidity conditions, because, according to Lopes and Takaki (1988) the sensitivity of seeds to water stress can be influenced by different factors such as light, temperature, humidity and oxygen availability. Low water potential in the substrate can be obtained using water solutions containing saccharine, sodium chloride, manitol and

polyethylene glycol (Santos *et al.*, 1996). However, the product used as an osmotic agent should not be absorbed by the seeds nor metabolized by the seedlings (Slavik, 1974). Polyethylene glycol 6000 can be used without restrictions as an osmotic agent (Hasegawa *et al.*, 1984), because it does not penetrate the cells, cannot be degraded and does not cause toxicity due to its high molecular weight. Polyethylene glycol (PEG) of high molecular weight has been long used to stimulate drought stress in plants as non-penetrating osmotic agents lowering the water potential in a way similar to soil drying (Larher *et al.*, 1993). The use of high molecular weight osmotic substances, like polyethylene glycol is one of the most popular approaches for drought induction (Landjeva *et al.*, 2008).

Drought is among the most detrimental environmental factors limiting plant productivity through osmotic stress. Drought creates elevated osmotic pressure in the root zone and decreases the water potential, limiting plant growth. Osmotic stress leads to decrease in plant water potential, which in turn has a negative effect on water availability and almost all plant functions (Yamaguchi-Shinozaki *et al.*, 2002). Performance of seed germination, crop growth and yield are the result of genotypic expression as modulated by continuous interaction with environment. Among the various traits which help to assess drought tolerance, seed germination percentage and root traits are more reliable on account of their high correlation with drought tolerance mechanism (Chang *et al.*, 1982) and considerable genotypic variations for root traits existing in tomato. Hence, understanding the changes that take place in tomato roots under water deficit would help in developing cultivars better suited to the drought

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situation. The present study was undertaken to assess the effect of PEG-6000 induced short term moisture stress on drought tolerance of genotypes on the basis of changes in some important seedling parameters.

Materials and Methods

A laboratory experiment was undertaken in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. Thirty two genotypes, *i.e.* LE 1, LE 2, LE 3, LE 5, LE 6, LE 10, LE 11, LE 13, LE 14, LE 15, LE 18, LE 20, LE 22, LE 23, LE 27, LE 57, LE 85, LE 87, LE 89, LE 100, LE 104, LE 114, LE 115, LE 116, LE 118, LE 125, LE 150, LE 355, CO 3, PKM 1, TNAU TH CO 3 and COTH 2 obtained from Department of Vegetable Science, Horticultural College and Research Institute, TNAU, Coimbatore were used for the present study. The experiment was laid out in factorial randomized block design with three replications. Polyethylene Glycol (PEG) 6000 has been used to stimulate the drought stress with two treatments *viz.*, -0.2 MPa and -0.35 MPa. PEG 6000 solutions were prepared by using the formula described by Michel Kaufmann (1973) as follows.

$$-(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C_2 + (2.67 \times 10^{-4}) + (8.39 \times 10^{-7}) C_2 T$$

where C = Weight of PEG (g l⁻¹), T = Room Temperature (°C)

Based on above formula, 123 and 169 g of PEG - 6000 were dissolved in 1000 ml of distilled water to develop different osmoticum solutions having water potential of -0.2 and -0.35 MPa. Distilled water was used as control. Surface sterilized seeds were placed on moistened filter paper in each petridish separately. Filter paper was moistened in regular interval with respective solutions for all observations. The petridishes were kept in laboratory under room temperature. The germination percentage was recorded at every 24 h interval up to 15 days. Seeds were considered germinated when the radical was at least 2 mm long. Five seedlings were chosen randomly and seedling growth was measured by dry weight of root and shoot of the seedling. Dry weight was determined after kept in hot air oven at 80°C for 48 hrs. The length of root and shoot was measured with a ruler. The vigour index was calculated by the procedure described by Abdul Baki and Anderson (1973) as vigour index = germination percentage X (Root length + Shoot length). The data were analyzed statistically and the treatment means were compared using LSD at 5 % probability (Panse and Sukhatme, 1985).

Results and Discussion

Effect of PEG on germination percentage

The cumulative germination of all the genotypes significantly decreased with increasing intensity of water deficit levels (Table 1). Most of the genotypes had 100% seed germination except LE 20, LE 3, LE

85 and LE 100 under control condition. Among the level of stress, control recorded the highest germination percentage than other two stresses (-0.2 and -0.35 MPa). The progressive fall in the germination percentage with decreasing water potential of the environment, observed in this experiment, was caused probably by the low hydraulic conductivity of the environment, where PEG 6000 makes water unavailable to seeds, affecting the imbibition process of the seed, which is fundamental for germination (Lobato *et al.*, 2008). Among the different genotypes, LE 18 recorded the highest germination percentage (100, 96.7 and 60.0) followed by LE 27 (100.00, 93.30 and 36.70) and LE 57 (100.00, 90.00 and 56.7 at control, -0.2 and -0.35 MPa respectively) (Fig 1).

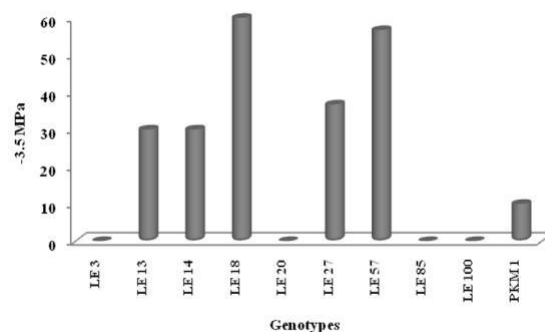


Fig. 1. Effect of PEG induced stress on the germination percentage of tomato genotypes at -0.35 MPa.

The lowest reduction of germination percentage was recorded in LE 18 (3.30 %) followed by LE 27 (6.70 %) and LE 57 (10 %) at -0.2 MPa. The least germination percentage was observed in LE 20 (56.60 %) followed by LE 3, LE 85, LE 100, PKM 1 (60.0 %) at -0.2 MPa.

Water stress due to drought is probably the most significant abiotic factor limiting germination and also crop growth and development (Hartmann *et al.*, 2005). Drought stress is physiologically related, because it induces osmotic stress and affects most of the metabolic responses of plants (Djibril *et al.*, 2005). Water deficit affects the germination of seed and the growth of seedlings negatively (Van Den Berg and Zeng, 2006).

The addition of PEG decreased the water potential and induces water stress that adversely affects the callus growth and *in vitro* regeneration capacity of tomato cultivars (Gopal and Iwama, 2007). This largest reduction with PEG solution could be attributed to high viscosity, where solubility and diffusion of oxygen were reduced compared to water (Delachieve and De Pinho, 2003). The reason for decreasing germination with increasing level of stress may be due to water potential and osmotic potential as mediated by solute developed additive effect on the inhibition of seed germination (Bernstein, 1961). Water deficit conditions induced with PEG, decreased germination percentage and

Table 1. Effect of PEG 6000 on germination percentage (%), root length (cm) and shoot length (cm) of tomato genotypes

Genotypes	Germination %			Root length (cm)			Shoot length (cm)		
	Control	-0.2 MPa	-0.35 MPa	Control	-0.2 MPa	-0.35 MPa	Control	-0.2 MPa	-0.35 MPa
LE 1	100.0	63.3	0.0	8.40	2.80	0.00	6.20	4.20	00
LE 2	93.3	73.3	0.0	7.30	2.20	0.00	7.90	4.70	00
LE 3	100.0	60.0	0.0	7.20	2.70	0.00	5.70	2.60	00
LE 5	100.0	66.7	0.0	6.00	1.70	0.00	5.60	2.20	00
LE 6	100.0	63.3	0.0	4.80	1.00	0.00	5.80	3.10	00
LE 10	100.0	73.3	0.0	7.50	3.30	0.00	8.60	4.10	00
LE 11	93.3	70.0	0.0	7.70	3.50	0.00	7.10	4.20	00
LE 13	100.0	86.7	30.0	9.20	6.40	3.10	7.20	4.80	2.10
LE 14	100.0	83.3	30.0	7.20	5.10	2.80	7.40	5.30	3.20
LE 15	100.0	80.0	16.7	8.60	5.80	2.90	6.70	5.20	3.10
LE 18	100.0	96.7	60.0	12.20	8.70	4.30	7.70	5.50	4.20
LE 20	96.7	56.6	0.0	6.60	1.70	0.00	4.60	3.40	00
LE 22	100.0	70.0	20.0	6.00	2.90	1.30	6.80	4.40	2.80
LE 23	100.0	80.0	0.0	5.80	1.90	0.00	5.10	3.90	00
LE 27	100.0	93.3	36.7	8.80	6.40	3.50	7.40	5.00	3.40
LE 57	100.0	90.0	56.7	10.50	7.50	4.00	7.60	5.30	3.80
LE 85	100.0	60.0	0.0	6.70	2.80	0.00	5.20	3.80	00
LE 87	96.7	73.3	0.0	6.50	2.50	0.00	5.70	3.60	00
LE 89	100.0	73.3	0.0	7.20	3.10	0.00	5.50	4.10	00
LE 100	93.3	60.0	0.0	7.10	2.90	0.00	6.20	4.10	00
LE 104	100.0	70.0	0.0	7.00	3.70	0.00	5.80	4.50	00
LE 114	100.0	86.7	10.0	5.80	4.10	2.50	5.50	4.10	3.20
LE 115	100.0	76.7	10.0	6.10	4.30	2.70	6.30	4.90	3.30
LE 116	100.0	63.3	0.0	5.20	3.20	0.00	6.10	4.40	00
LE 118	100.0	86.7	16.7	6.30	4.40	1.80	5.10	4.20	2.80
LE 125	100.0	66.7	10.0	6.00	3.50	1.90	6.30	4.80	3.20
LE 150	100.0	76.7	06.7	5.80	2.90	1.90	5.50	4.20	2.90
LE 355	100.0	73.3	0.0	5.20	1.70	0.00	6.70	4.50	00
CO 3	100.0	66.6	06.7	6.80	3.50	1.70	6.50	4.40	3.30
PKM 1	100.0	60.0	10.0	6.60	3.00	1.40	6.10	4.30	3.10
THCO3	100.0	70.0	03.3	7.10	3.60	1.60	6.60	4.60	3.30
COTH 2	100.0	66.7	10.0	6.70	3.20	1.70	6.70	4.50	3.50
Mean	99.17	73.64	10.42	7.06	3.62	1.22	6.35	4.28	1.60
SEd	1.13	0.57	0.85	0.08	0.09	0.07	0.05	0.04	0.09
CD (0.05)	2.26	1.14	1.70	0.16	0.18	0.15	0.10	0.08	0.17

also delayed germination time at higher concentrations. This was due to the lower water uptake by seed resulting in decreased germination under increased concentration of PEG. Therefore, the decrease in water potential gradient between seeds and their surrounding media by the effects of PEG 6000 adversely affects seed germination (Digdem Kaydan and Mehmet Yagmur, 2008). The results showed that germination of most of the genotypes arrested completely at -0.35 MPa and genotypes varied significantly in their germination capability under reduced water potential.

Effect of PEG on root and shoot length

Increasing moisture deficit resulted in the reduction of root and shoot length in all the genotypes (Table 1). Among the different levels, control recorded the highest root length and shoot length than other two stresses (-0.2 and -0.35 MPa). The genotype, LE 18 recorded maximum root length (12.20, 8.70 and 4.30 cm) followed by LE 57 (10.50, 7.50 and 4.00 cm) LE 27 (8.80, 6.40 and 3.50 cm) at control, -0.2 and -0.35 MPa respectively. The highest shoot length was recorded in LE 18 (7.70, 5.50 and 4.20

cm) followed by LE 15 (7.60, 5.30 and 3.80 cm) and LE 27 (7.40, 5.00 and 3.40). The highest reduction in root length was observed in LE 6 (79.17 %) followed by LE 5 (71.67%) and LE 2 (69.86 %) at -0.2 MPa. The lowest reduction in shoot length was observed in LE 118 (17.65 %), LE 27 (20.37 %) and LE 18 (21.54 %) at -0.2 MPa (Fig 2).

In general, plants with longer root growth have drought resistance (Leishman and Westoby, 1994). In the present study, decreases in the external osmotic potential caused a reduction in seedling growth of tomato. The increasing concentration of PEG induced water deficit leading to decrease in root and shoot length was observed by Al-Karaki (1998). The percentage reduction in root length was higher than shoot length and this might be due to the direct contact of root with PEG solution.

Effect of PEG on root and shoot dry weight

Root dry weight and shoot dry weight significantly decreased with increasing intensity of water deficit (Table 2). The maximum root and shoot dry weight was observed in LE 18 (10.01, 7.50 and 4.5 mg for

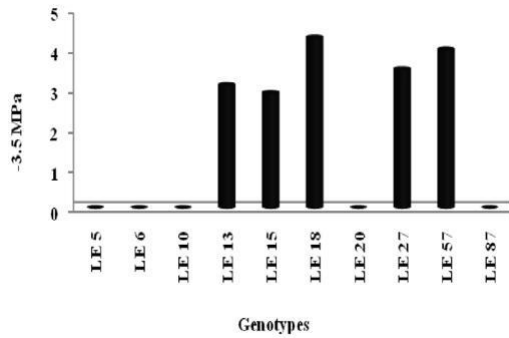


Fig. 2. Effect of PEG induced stress on the root length of tomato genotypes at -0.35 MPa.

root dry weight and 22.17, 16.60 and 4.9 mg for shoot dry weight) followed by LE 57 (9.95, 7.45 and 4.4 mg root dry weight and 22.05, 16.49 and 9.7 mg shoot dry weight) and LE 27 (9.81, 7.47 and 3.9 mg root dry weight and 21.73, 16.56 and 9.6 mg shoot dry weight) at control, -0.2 MPa and -0.35 MPa respectively. Therefore, it may be concluded that genotypes which have good seed germination and seedling growth (root/shoot length and its dry weight) under higher moisture deficit condition are superior to ensure good seedling establishment and further crop growth. The result of this study also showed

that the genotypes display distinct response to drought stress. In this regard genotypic variability within species offer variable tool for studying mechanism of drought. Genotypic variations in germination and seedling growth under lower water potential were critical for quick establishment ability under water stress condition (Redona and Mackill, 1996). Seedling growth in terms of seedling dry weight, shoot and root length was also completely inhibited in most of the genotypes at -0.35 MPa. The germination percentage, shoot and root length and seedling dry weight under reduced water potential indicating their ability to tolerate drought. This was in confirmation with study of Goswami and Baruah (1994) and Jha and Singh (1997).

Effect of PEG on vigour index

Vigour index is an important parameter to assess drought tolerant capacity of crop plants. Vigour index is a combination of germination percentage with root and shoot length. PEG induced water deficit decreases the vigour index and thus might be due to decreased germination percentage and reduction of root and shoot length. Among the genotypes, the highest vigour index was recorded in LE 18 (1373.14) followed by LE 57 (1152.00) and

Table 2. Effect of PEG 6000 on root and shoot dry weight of tomato genotypes

Genotypes	Root dry weight (mg)			Shoot dry weight (mg)			Vigour Index at (-0.2 MPa)
	Control	-0.2 MPa	-0.35MPa	Control	-0.2 MPa	-0.35MPa	
LE 1	7.01	2.92	0.00	15.52	6.46	0.00	443
LE 2	7.40	3.31	0.00	16.38	7.34	0.00	506
LE 3	6.62	2.76	0.00	14.68	6.10	0.00	318
LE 5	6.45	2.18	0.00	14.30	4.83	0.00	260
LE 6	6.61	2.46	0.00	14.64	5.45	0.00	260
LE 10	7.40	3.39	0.00	16.38	7.50	0.00	542
LE 11	7.70	4.03	0.00	17.06	8.92	0.00	539
LE 13	8.35	5.90	2.9	18.50	13.06	6.3	1064
LE 14	8.57	5.99	2.7	18.98	13.27	6.1	866
LE 15	7.88	5.72	2.3	17.47	12.66	5.3	880
LE 18	10.01	7.50	4.5	22.17	16.60	4.9	1373
LE 20	7.28	3.00	0.00	16.13	6.66	0.00	289
LE 22	7.46	4.77	2.0	16.54	10.56	4.8	581
LE 23	8.37	4.57	0.00	18.54	10.12	0.00	464
LE 27	9.81	7.47	3.9	21.73	16.56	9.6	998
LE 57	9.95	7.45	4.4	22.05	16.49	9.7	1152
LE 85	7.08	4.07	0.00	15.67	9.01	0.00	396
LE 87	8.60	4.37	0.00	19.05	9.68	0.00	447
LE 89	8.55	5.04	0.00	18.95	11.16	0.00	528
LE 100	6.69	3.58	0.00	14.83	7.93	0.00	420
LE 104	7.18	4.68	0.00	15.90	10.37	0.00	574
LE 114	8.66	6.30	2.7	19.19	13.95	7.5	711
LE 115	8.33	6.19	2.8	18.47	13.70	7.2	706
LE 116	8.10	5.42	0.00	17.95	12.01	0.00	481
LE 118	9.17	6.98	2.0	20.33	15.47	7.7	746
LE 125	8.50	3.23	2.0	18.83	7.16	4.6	720
LE 150	7.93	5.01	2.2	17.57	11.11	4.9	545
LE 355	7.56	3.78	0.00	16.75	8.38	0.00	454
CO 3	8.23	4.90	2.4	18.22	10.86	5.1	526
PKM 1	7.96	4.62	2.6	17.62	10.22	5.3	438
THCO3	7.90	4.76	1.7	17.50	10.53	3.8	574
COTH 2	8.44	4.85	1.8	18.71	10.76	3.9	514
Mean	7.99	4.73	1.34	17.71	10.47	3.02	601
SEd	0.020	0.031	0.031	0.043	0.067	0.068	5.270
CD (0.05)	0.039	0.062	0.062	0.086	0.134	0.136	10.535

LE 13 (1063.62) and the lowest index were observed in LE 6 (259.53) and LE 5 (260.13) at -0.2 MPa. The highest vigour index of LE 18 might be due to highest germination percentage (96.70), root length (8.70 cm) and shoot length (5.50 cm) at -0.2 MPa (Table 2). The reduction in the water entry speed in the cells during the seed imbibition process (Peske and Delouche, 1985) causes reduced vigour index. The disarrangements in the cell membranes that favor faster water absorption and solute loss, can result in tissue death (Powell and Matthews, 1979).

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Conclusion

Based on the present investigation, 32 tomato genotypes are grouped into three categories viz., tolerant, moderately tolerant and susceptible to the drought condition. Among the genotypes, LE 18, LE 57, LE 27, LE 14 and LE 13 are characterized as tolerant and LE 3, LE 20, LE 85, LE 100 and PKM 1 are susceptible to drought. The identified genotypes shall be taken into the field for confirmation through the physiological and biochemical analysis and including molecular approaches.

References

- Abdul-Baki, A.A. and Anderson, J.D. 1973. Vigour determination in soybean and multiple criteria. *Crop Sci.*, **13**: 630.
- Al-Karaki, G.N. 1998. Seed size and water potential effects on water uptake, germination and growth of lentil. *J. Agron. Crop Sci.*, **181**(4): 237-242.
- Bernstein, L. 1961. Osmotic adjustment of plants to saline media. 1. Steady state. *Am. J. Bot.* **48**: 909-918.
- Chang, T.T., Loresto, G.C., Toole, J.C. and Armenta-Soto, J.L. 1982. Strategy and methodology of breeding rice for drought prone areas. In: Drought Resistance in Crops with Emphasis on Rice, IRRI, Philippines, pp. 217-244.
- Delachieve, M.E.A. and De pinho, S.Z. 2003. Germination of *Senna occidentalis* Link: seed at different osmotic potential levels. Brazil. *Arch. Biol. and Tech.*, **46**(2): 163-166.
- Digdem Kaydan and Mehmet Yagmur. 2008. Germination, seedling growth and relative water content of shoot in different seed sizes of *Triticale* under osmotic stress of water and NaCl. *Afr. J. Biotechnol.*, **7**(16): 2862-2868.
- Djibril, S., Mohamed, O.K., Diaga, D., Diegane, D., Abaye, B.F., Maurice, S. and Alain, B. 2005. Growth and development of date palm (*Phoenix dactylifera* L.) seedlings under drought and salinity stresses. *Afr. J. Biotechnol.*, **4**(9): 968-972.
- Gopal, J. and Iwama, K. 2007. *In vitro* screening of potato against water stress mediated through sorbitol and polyethylene glycol. *Plant Cell Rep.*, **26**: 693-700.
- Goswami, R.K. and Baruah, K.K. 1994. Effect of water potential treatments on germination and seedling growth of some upland rice cultivars. *Indian J. Pl. Physiol.*, **37**: 61-63.
- Hartmann, T., Colledge, M. and Lumsden, P. 2005. Responses of different varieties of *Lolium perenne* to salinity. Annual Conference of the Society for Experimental Biology, Lancashire.
- Hasegawa, P.M., Bressan, R.A., Handa, S. and Handa, A.K. 1984. Cellular mechanisms of tolerance to water stress. *Hort Sci.*, **19**: 371-377.
- Jha, B.N. and Singh, R.A. 1997. Physiological responses of rice varieties to different levels of moisture stress. *Indian J. Pl. Physiol.*, **2**: 81-84.
- Landjeva, S., Neumann, K., Lohwasser, U. and Borner, A. 2008. Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. *Biologia Pl.*, **52**: 259-266.
- Larher, F., Lepout, L., Petrivalsky, M. and Chappart, M. 1993. Effectors for the osmoinduced proline response in higher plants. *Pl. Physiol. Biochem.*, **31**(6): 911-922.
- Leishman, M.R. and Westoby, M. 1994. The role of seed size in seedling establishment in dry soil conditions-experimental evidence from semi-arid species. *J. Ecol.*, **82**(2): 249-258.
- Lobato, A.K.S., Oliverira Neto, C.F., Costa, R.C.L., Santos Filho, B.G., Silva, F.K.S., Cruz, F.J.R., Abboud, A.C.S. and Laughinghouse, H.D. 2008. Germination and seedling growth of winter wheat. *Crop Sci.*, **34**: 169-171.
- Lopes, V.B. and Takaki, M. 1988. Seed germination in *Phaseolus vulgaris* L. II. Effects of waterpotential and photoperiod. *Arquivos de Biologia e Tecnologia*. **31**: 307-312.
- Michel, B.E. and Kaufmann, M.R. 1973. The osmotic potential of polyethylene glycol 6000. *Pl. Physiol.* **51**: 914-917.
- Pansee, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers*, ICAR Publications, New Delhi. pp. 1-21.
- Peske, S.T. and Delouche, J.C. 1985. Planting soybean seeds under low soil moisture condition. *Pesquisa Agropecuaria Brasileira*. **20**: 69-85.
- Powell, A.A. and Matthews, S. 1979. Deteriorative changes in pea seeds (*Pisum sativum* L.) stored in humid or dry conditions. *J. Experimental Botany.*, **28**: 225-234.
- Redona, E.G. and Mackill D.J. 1996. Genetic variation for seedling vigour traits in rice. *CropSci.*, **36**: 285-290.
- Santos, V.L.M., Silva, R.F., Sedyama, T. and Cardoso, A.A. 1996. Utilization of osmotic stress in the evaluation of vigor of soybean seeds (*Glycine max* (L.) Merrill). *Revista Brasileira de Sementes*. **18**: 83-87.
- Schuab, S.R.P., Braccini, A.L., Scapim, C.A., Franca-Neto, J.B., Meschede, D.K. and Avila, M.R. 2007. *Seed Sci. and Technol.*, **35**: 187-199.
- Slavik, B. 1974. Methods of studying plant water relations. Springer-Verlag, New York. p. 449.
- Van Den Berg, L. and Zeng, Y.J. 2006. Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. *Afr. J. Bot.*, **72**: 284-286.
- Yamaguchi-Shinozaki, K., Kasuga, M., Liu, Q., Nakashima, K., Sakuma, Y., Abe, H., Shinwaki, Z.K., Seki, M. and Shinozaki, K. 2002. Biological mechanisms of drought stress response. *Japan international Res. Centre for Agricultural Sci.*, **23**: 1-28.