

Evaluation of Genotypes for Drought Stress Resistance in Soybean (*Glycine max* (L.) Merrill) Under Induced Water Stress

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Abstract: In present investigation under induced water stress in soybean (*Glycine max*. (L.) Merrill) investigated that, soybean genotypes viz., AMS-99-33, TAMS-98-21 and JS-335 was maintained better in stress environment for the RWC and total chlorophyll content resulting from better drought tolerance capacity. The genotype viz., AMS-99-33 (7.22), TAMS-98-21 (7.85) and JS-335 (7.95) recorded the less stomatal index in stress condition at 30 and 50 DAS showing its better drought tolerance capacity. The genotype AMS-99-33 recorded highest RWC (52.44%) in stress condition followed by TAMS-98-21 (51.89%) and JS-335 (51.10%). The genotype JS-335 recorded the highest drought intensity followed by TAMS-38 and TAMS-98-21. The study of different physiological parameters concluded that, the genotype JS-335 recorded the highest drought stress resistance for various drought stress parameters followed by the genotype TAMS-38 and TAMS-98-21.

Keywords: Water stress, Drought, Physiological parameters and Soybean.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the important oilseed crops having higher protein content. India is the fifth largest producer of soybean in the world after USA, Brazil, China and Argentina. Soybean grows well in warm and moist climate. Soybean is a diploid species having chromosome number $2n=40$, it is a legume crop which belongs to family Leguminosae and sub family Papilionaceae and genus *Glycine*.

Water is main constituent of cell protoplasm and by altering various metabolic processes and it become limiting factor for crop growth under receding soil water. It plays the additional role of providing the energy necessary to drive photosynthesis. So, growth and yield performance of crop is totally depends and decided by moisture regime and drought. It is unrealistic consideration that there is a single gene for drought tolerance and hence the need in understanding of the physiological response of plant under water limited conditions is of critical importance.

Thus, aim is to identify physiological component causing performance difference in stressful condition and evaluate different genotypes of soybean which

are more productive and tolerant to stress condition with utilizing existing resources on basis of physiological parameter.

MATERIAL AND METHODS

The investigation studies on drought tolerance in soybean (*Glycine max* (L.) Merrill) was carried out during *kharif*, 2013–2014 in Department of Agriculture Botany, Dr. Pajrabrao Deshmukh Krishi Vidyapeeth, Akola (MS). The experiment was conducted under control as a well watered field condition.

Experiment was conducted in two sets, each consisting of seven soybean genotypes with four replications. One set was supplied with water as per requirement up to harvest, whereas second set was supplied with water up to flowering and further there was no supply of water for creating stress condition. Both the sets were supplemented with water up to flowering. The second set was placed under control condition (polyhouse) for creating drought (stress). The genotypes undertaken in this study are AMS-MB-5-19, AMS-MB-5-1, AMS-353, AMS-99-33, TAMS- 98-21, TAMS-38 and JS-335.

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Table 1
Effect of different physiological parameters in Soybean genotypes at 30 and 50 days after sowing. (DAS)

Sr. No.	Genotype(s)	Stomatal Index				Stomatal Frequency				Relative Water Content (RWC%)				Total Chlorophyll content (mg g ⁻¹)			
		30 DAS		50 DAS		30 DAS		50 DAS		30 DAS		50 DAS		30 DAS		50 DAS	
		Stress	N.S.	Stress	N.S.	Stress	N.S.	Stress	N.S.	Stress	N.S.	Stress	N.S.	Stress	N.S.	Stress	N.S.
1	AMS-MB-5-19	10.92	10.52	8.48	18.85	28.98	29.10	25.08	38.63	47.66	50.20	46.82	63.09	0.609	0.660	0.363	0.497
						(43.62)	(45.11)	(43.17)	(52.53)								
2	AMS-MB-5-1	8.55	8.69	8.46	17.87	29.75	29.95	28.03	40.17	43.42	52.39	39.71	62.07	0.538	0.529	0.357	0.352
						(41.21)	(46.32)	(39.06)	(51.94)								
3	AMS-353	8.30	9.90	8.60	19.81	30.25	28.81	27.66	43.99	50.16	45.59	40.21	58.77	0.468	0.473	0.387	0.480
						(42.42)	(39.35)	(50.01)									
4	AMS-99-33	7.22	8.45	7.36	15.02	27.00	25.24	24.04	34.77	55.65	59.81	52.44	65.34	0.807	0.731	0.911	0.921
						(48.22)	(50.65)	(53.91)									
5	TAMS-98-21	7.85	8.61	7.99	16.26	27.13	26.80	24.52	35.48	54.40	58.65	51.89	64.39	0.697	0.713	0.800	0.863
						(47.52)	(49.95)	(46.03)	(53.31)								
6	TAMS-38	10.70	11.62	8.66	17.11	29.96	29.47	27.30	44.48	48.33	49.64	44.65	57.77	0.470	0.493	0.404	0.465
						(44.03)	(44.77)	(41.90)	(49.43)								
7	JS-335	7.95	8.62	8.43	16.56	27.66	28.45	24.77	37.30	51.67	55.59	51.10	63.99	0.667	0.688	0.691	0.512
						(45.92)	(48.16)	(53.07)									
	F Test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE(m) ±	0.433	0.314	0.250	0.651	0.385	0.492	0.939	0.688	0.862	0.987	0.873	0.798	0.149	0.148	0.166	0.155
	C.D. at 5%	1.336	0.833	0.743	1.637	0.922	1.423	-	1.975	2.475	2.834	2.506	2.291	0.427	0.424	0.476	0.445

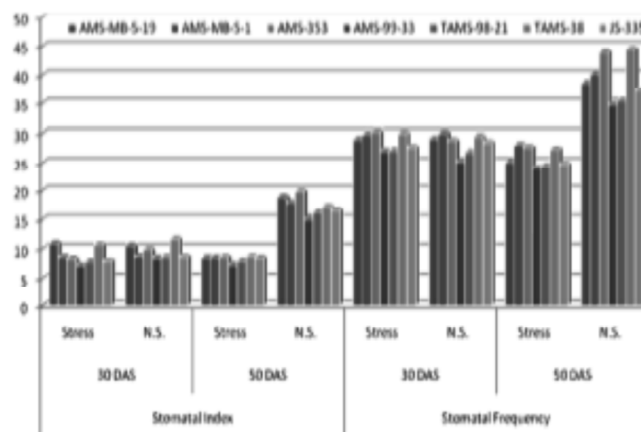


Figure 1:

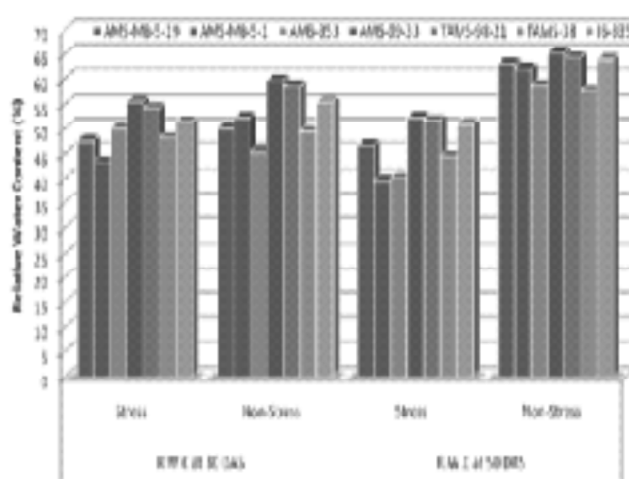


Figure 2: Relative water content (%) at 30 and 50DAS in different Soybean enotypes

The stomatal index was calculated by using method developed by Wolf *et al.* (1979). Also, computed the stomatal frequency in mm² per unit of area with the help of scanned area and Stomatal number as per Dhopte and Livera (1989). The chlorophyll content in leaf was estimated by adopting the procedure given by Hiscox and Israelstan (1979) and extraction of chlorophyll was done in DMSO (dimethyl sulphoxides) method. The chlorophyll stability index method was determined by Dhopte and Livera (1989) based on leaf pigment changes induce by heating and non heating method. The different physiological drought parameters studied were drought tolerance test, drought intensity, drought index and drought tolerance efficiency. The statistical analysis were carried out by following standard statistical procedure by analysis of variance (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

Stomatal Index (%) and Stomatal Frequency

Table 1 indicates estimation of stomatal index (%) and stomatal frequency (mm^2) of different soybean genotypes. It was worked out at 30 and 50 DAS, the important plant growth stages. The stomatal index (Table 1 and Fig. 1) showed significant differences in both stress and non stress conditions. The genotype AMS-MB-5-19 recorded significantly highest (10.92%) stomatal index than all the genotypes. However, it remained at par with TAMS-38 (10.70%) in stress condition. In non-stress condition the genotype AMS-99-33 (8.48%), followed by TAMS-98-21 (8.61%) and JS-335 (8.62%) found statistically lowest whereas TAMS-38 (11.62%) and AMS-MB-5-19 (10.52%) had highest stomatal index. At 50 DAS both conditions showed significant differences. The genotypes TAMS-38 recorded the highest stomatal index (8.66%), while the lowest stomatal index was found to be in AMS-99-33 (7.36%) and TAMS-98-21 (7.99%). In non-stressed condition the genotypes AMS-99-33 (15.02%) recorded significantly lowest stomatal index over the rest of genotypes. The other genotypes remained at par among themselves. In respect of genotype ranking, the stomatal index at 50 DAS in stress condition observed that, AMS-99-33 remained at Ist level, TAMS-98-21 IInd, JS-335 IIIrd and AMS-MB-5-1 at IVth level. The differences in stress and non stressed plants in respect of stomatal frequency found significant except in stress condition at 50 DAS. The stomatal frequency (mm^2) at 30 DAS in stress pots though found significant, but all genotypes remained at par among themselves, However, AMS-99-33 recorded lowest stomatal frequency (27mm) over the highest AMS-353(30.25 mm). In non stress pots the stomatal frequency was significantly more in all the genotypes. The genotype AMS-MB-5-1(29.95 mm) recorded highest stomatal frequency and found at par with AMS-MB-5-19 (29.10 mm) and AMS-353 (28.81mm), while the lowest stomatal frequency found to be by the genotype AMS-99-33(25.24 mm), TAMS-98-21(26.8 mm) and JS-335 (28.45 mm). At 50 DAS all genotypes produced statistically lower stomatal frequency in stress pot AMS-99-33 (24.04mm) and TAMS-98-21 (24.52mm). However the genotype AMS-MB-5-1 (28.03mm) recorded highest stomatal frequency and found at par among them. In respect of their ranking AMS-99-33 remained at Ist ranking, TAMS-98-21 IInd, JS-335 at IIIrd and AMS-MB-5-19

remained at IVth level. In non stress condition the genotype AMS-99-33 (34.77 mm) followed by TAMS-98-21 (35.18mm) and JS-335 (37.03 mm) produced significantly lowest stomatal frequency except TAMS-38 (44.48 mm).

The drought tolerance was associated with reduced stomatal index (Dongre, 1997) under water stress condition. The genotypes with less stomatal index had better drought tolerance capacity. In present investigation, the genotype AMS-99-33 (7.22), TAMS-98-21(7.85) and JS-335 (7.95) recorded less stomatal index in stress condition at 30 and 50 DAS showing its better drought tolerance capacity.

Relative Water Content (RWC %)

The relative water content (Table 1 and Fig. 2) was estimated at 30 and 50 DAS, in both stress and non stress conditions. The RWC in stress as well as non stress at 30 DAS showed significant difference. In stress conditions at 30 DAS all genotypes recorded significantly maximum relative water content, *i.e.* in AMS-99-33 (55.65%), TAMS-98-21(54.40%) and JS-335 (51.67%), While the lowest AMS-MB-5-1 (43.42%) in stress condition. In non stress condition varietal differences found significant. All genotypes maintained statistically higher relative water content *viz.* in AMS-99-33 (59.81%), TAMS-98-21 (58.65%), JS-335 (55.59%) and AMS-MB-5-1 (52.39%). While AMS-353 (45.59%) had lowest relative water content.

At 50 DAS in both stress and non stress conditions. The genotype AMS-99-33 recorded highest RWC (52.44%) in stress condition followed by TAMS-98-21 (51.89%) and JS-335 (51.10%). In non stress condition, the relative water content was found to AMS-99-33 (65.34%), TAMS-98-21 (64.39%) and JS-335 (63.99%). It is more as compared to stress condition in all the genotypes

The drought tolerance was associated with high value of relative water content under water stress condition. While it has the great significance for drought tolerance (Chopra and Mukhopandhya, 1991). In the present investigation, the genotype AMS-99-33 recorded high relative water content (55.65%) in stress condition at 30 DAS and also at 50 DAS recorded high relative water content (52.44%) in stress condition showing its better drought tolerance capacity. There is a built up of nutrients in the root and shoot during the stress free early period which later used to fill up the grains in the season when stress developed. Mederski *et al.*, (1973) studied that a low

or non stress soil moisture level permits greater genotypic expression, thereby increasing genotypic variance among the varieties. A low soil moisture stress environment for selecting soybean yield attributes is desirable.

Total Chlorophyll Content (mg g⁻¹)

The table 2 and fig. 3 (A) indicates total chlorophyll content of different soybean genotypes and was worked out at 30 and 60 DAS in both stress and non stress conditions. At 30 DAS, the statistical differences were found significant in both the conditions. The variation in the stress condition for total chlorophyll content at 30 DAS ranged from 0.468 mg g⁻¹ to 0.807 mg g⁻¹. Higher total chlorophyll content at 30 DAS was observed in AMS-99-33 (0.807 mg g⁻¹) (Table 3). However the lowest chlorophyll content at 30 DAS was observed in AMS-353 (0.468 mg g⁻¹). While among the non stress condition the total chlorophyll content

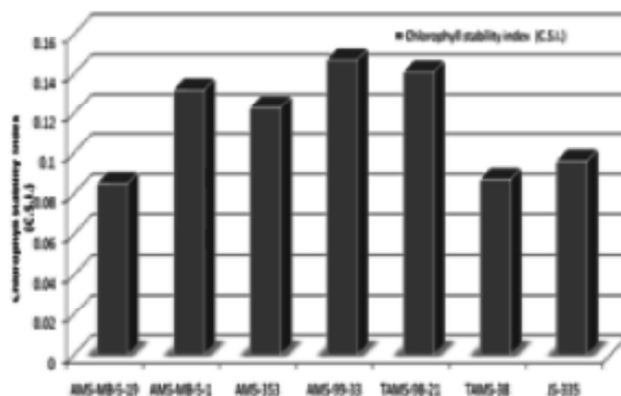


Figure 3B: Chlorophyll stability index (C.S.I.)

at 30 DAS ranged from 0.473 mg g⁻¹ to 0.731 mg g⁻¹, the genotype, AMS-99-33 (0.731 mg g⁻¹) was numerically higher in total chlorophyll content at 30 DAS followed by TAMS-98-21 (0.713 mg g⁻¹), JS-335 (0.688 mg g⁻¹) and AMS-MB-5-19 (0.660 mg g⁻¹). Numerically lowest total chlorophyll content at 30 DAS was observed at AMS-353 (0.473 mg g⁻¹).

The total chlorophyll content at 60 DAS in stress condition ranged from 0.352 mg g⁻¹ to 0.921 mg g⁻¹. The genotype AMS-99-33 (0.921 mg g⁻¹) was numerically higher in total chlorophyll content at 60 DAS followed by TAMS-98-21 (0.863 mg g⁻¹) in non stress condition. However under stress condition the total chlorophyll content at 60 DAS ranged from 0.357 mg g⁻¹ to 0.911 mg g⁻¹. The genotype AMS-99-33 (0.911 mg g⁻¹) was numerically higher in total chlorophyll content at 60 DAS followed by TAMS-98-21 (0.800 mg g⁻¹). Numerically lowest total chlorophyll content was found in AMS-MB-5-1 (0.357 mg g⁻¹).

It was observed from Table 2 and fig. 3 (A) that total chlorophyll content was progressively increased from 30 days after sowing to 60 days after sowing and decreased onward. Highest value indicating higher metabolic turnover and photosynthetic rate during this period (Sharma and Shantakumari, 1996).

Results also revealed that total chlorophyll content was found to be significantly affected by drought condition. Similar results were observed by Satbhai *et al.* (1997) who found that decreased in reducing sugar and free amino acid content of plant due to water stress resulted in reduction in chlorophyll content. The variation in total chlorophyll content in different genotypes at all the stages of growth was observed in present study. Increased chlorophyll content of the plant might have helped for higher photosynthetic rate reflecting into good

Table 2
The chlorophyll content at 75 DAS and chlorophyll stability index in Soybean genotypes.

Sr. No.	Genotype(s)	75 DAS Chlorophyll content (mg g ⁻¹)		Chlorophyll Stability Index
		Heated	Non- heated	
1.	AMS-MB-5-19	0.177	0.262	0.085
2.	AMS-MB-5-1	0.125	0.257	0.132
3.	AMS-353	0.138	0.261	0.123
4.	AMS-99-33	0.321	0.468	0.147
5.	TAMS-98-21	0.308	0.449	0.141
6.	TAMS-38	0.173	0.260	0.087
7.	JS-335	0.309	0.405	0.096
	F Test	Sig.	Sig.	Sig.
	SE(m) ±	0.108	0.151	0.057
	C.D. at 5%	0.311	0.435	0.164

DAS: Days after sowing, N.S.: Non Stress

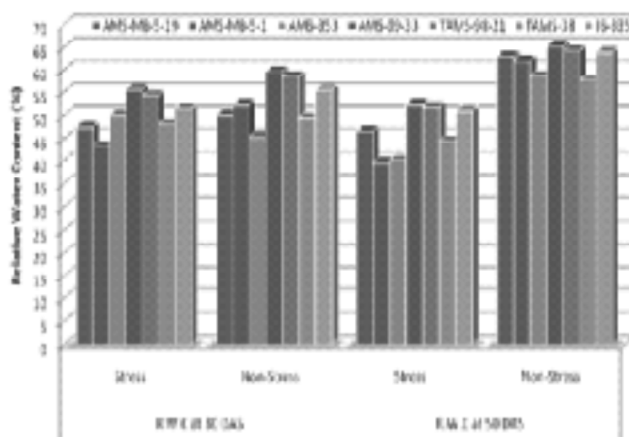


Figure 3A: The chlorophyll content (mg/g) in Soybean genotypes at different growth stages

amount of dry matter production and yield under water stress condition in different genotypes. Dhopte *et al.* (1995) concluded that drought tolerant genotypes had more chlorophyll content and drought tolerance could be obtained by increasing leaf chlorophyll content in plants. Further, Krishnamurthy *et al.* (1974 a) stated that higher total chlorophyll content in leaves were helpful in increasing photosynthetic efficiency and in turn helped in osmotic regulation in plants under water stress condition.

Chlorophyll Stability Index (CSI)

Table 2 and Fig. 3 (B) indicates chlorophyll stability index and was worked out at 75 DAS. The statistical differences remained significant. In respect of the genotypes ranking, the chlorophyll stability index at 75 DAS, AMS-MB-5-19 remained at Ist rank, TAMS-98-21 at IInd rank, JS-335 at IIIrd rank and AMS-353 at IVth rank. The drought tolerance was associated with small values of CSI. Wright *et al.*, (1983) reported that, the drought tolerant genotypes had significantly less chlorophyll stability index. In the present investigation, the genotype AMS-MB-5-19 recorded low CSI (0.085), TAMS-38 (0.087) and JS-335 (0.096).

Drought intensity, drought index and drought tolerance efficiency (%)

Table 3 and Fig. 4 indicate estimation of drought intensity, drought tolerance efficiency % (DTE) and drought index. The statistical difference was found significant. Though the differences found significant, the genotype JS-335 recorded highest drought intensity (0.175) followed by TAMS-38 (0.163) and TAMS-98-21 (0.139). However, all genotypes remained at par among themselves. The data presented in table 3 revealed that drought intensity was negatively associated with drought tolerance (Premchandra *et al.*, 1996). Among drought intensity the genotypes which recorded significantly lower drought intensity was AMS-MB-5-19 (0.058), AMS-353 (0.096) and AMS-99-33 (0.115).

Drought intensity is also known as drought susceptibility index and is inversely proportional to drought tolerance capacity of plant. It is absolutely ability of plant to with stand water deficit and intensity of water deficit could be measured by degree and duration of low plant water potential. (Dhopte and Livera, 1989). Present results indicated that genotypes varied among drought intensity. Investigations result were in argument with observation of Dhopte and Livera (1989 a) who reported that higher drought intensity of soybean

Table 3
Effect of drought intensity, drought tolerance efficiency (DTE) and drought index of different soybean genotype at harvest

Sr. No.	Genotype(s)	Drought Intensity	DTE (%)	Drought Index
1	AMS-MB-5-19	0.058	94.23 (76.06)	0.942
2	AMS-MB-5-1	0.126	87.45 (69.21)	0.874
3	AMS-353	0.096	90.43 (71.95)	0.904
4	AMS-99-33	0.115	88.50 (70.18)	0.885
5	TAMS-98-21	0.139	86.19 (68.11)	0.861
6	TAMS-38	0.163	83.73 (66.19)	0.837
7	JS-335	0.175	82.57 (65.27)	0.825
F Test		Sig.	Sig.	Sig.
SE (m) ±		0.080	0.822	0.081
C.D. at 5%		0.231	2.360	0.234

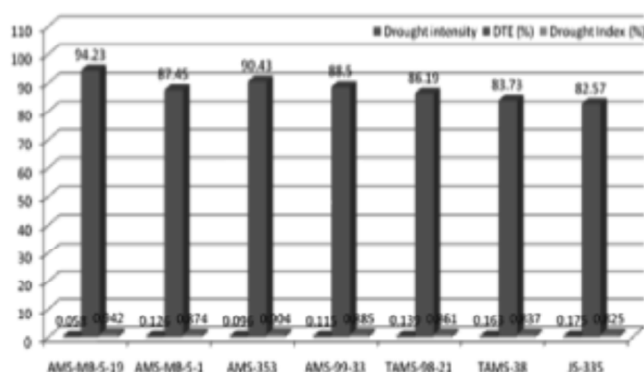


Figure 4: Drought intensity, drought index and D.T.E. of different soybean genotype

were due to higher harvest index and higher biological yield.

In respect of DTE (%) (Table 3) the drought tolerance efficiency varied from 82.57% to 94.23% the genotype AMS-MB-5-19 (94.23%) followed by AMS-353 (90.43%) and AMS-99-33 (88.50%) found in significantly higher DTE. These genotypes *i.e.*, AMS-MB-5-19, AMS-353, AMS-99-33 and JS-335 may found drought tolerant efficient genotypes in present investigation, however study needs further confirmation. Whereas, lowest drought tolerance efficiency was observed in the genotype JS-335. Drought tolerance efficiency is the difference between yields under moisture stress condition and non stress condition (Dhopte and Livera, 1989 a). Genotypes significantly differ among themselves in drought tolerance efficiency in present investigation. Similar findings were observed by Wenzel (1997) who reported the drought tolerance efficiency (%) was important for higher harvest index and higher yield.

The statistical differences for drought index (Table 3) were significant. The range of genotypes was

observed 0.825 to 0.942. Drought index was significantly more in AMS-MB-5-19 (0.942), AMS-353 (0.904) and AMS-99-33 (0.885). While the lowest drought index was found in JS-335 (0.825) and TAMS-38 (0.837), respectively.

Drought index also called as yield stability index where comparison of seed yield per plant at stress and non stress level was estimated. Drought resistance was determined from relative yield loss due to moisture stress (Dhopte and Livera, 1989 b). The results from present investigation revealed that genotypes showed significant difference among drought index.

Thus, study of different physiological parameters concluded that, the genotype JS-335 recorded the highest drought stress resistance for various drought stress parameters followed by the genotype TAMS-38 and TAMS-98-21.

REFERENCES

- Chopra, R.K. and Mukhopadhyaya, A. (1991), Osmotic adjustment a mechanism for maintaining yield stability in drought environment. National Symposium on Recent Advances in Drought Resistance, Kottayam, Kerala. PP: 45-51.
- Dhopte, A. M. and M. Livera (1989a), Chlorophyll stability index for drought tolerance in plants. *Useful Techniques for Plant Scientists (Ed.)* PP: 102-103.
- Dhopte, A. M. and M. Livera. (1989b), Estimation of stomatal index and stomatal frequency with replica method. *Useful Techniques for Plant Scientists (Ed.)* PP: 278 -279.
- Dhopte, A. M., V. B. Shekar., R. Pandrangi., S. G. Wankhade and S. L. Rahangdale. (1995). Variation in leaf amino acid content in drought tolerance and susceptible genotypes and relationship of physiological traits with yield stability in grain sorghum. *Ann. Plant Physiol.* **9**(1): 28-33.
- Dongre, Y. Y. (1997), Response of sorghum hybrid to potassium and soil moisture regimes under rainfed condition. MSc. Thesis, Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola, Maharashtra, India.
- Hiscox, J. D. and G. F. Israelstan. (1979), A method for extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57**: 1332-1334.
- Krishnamurthy, K., B. G. Rajashekara., G. Raghunatha., M. K. Jagannath., N. Venugopal and A. Bommegowda. (1974a), Investigation on the varietals differences in the growth component of sorghum. *Mysore J. Agric. Sci.* **8**: 52-59.
- Mederski, H. J. and D. L. Jeffers. (1973), Yield response of soybean varieties grown at two soil moisture stress levels. *Agron. J.* **65**: 410-412.
- Panse, V. G. and B. V. Sukhatme. (1967), Statistical methods for agriculture workers. ICAR Pub., New Delhi.
- Premchandra, G. S., K. Saneoka., K. Fujita and S. Ogata. 1996. Leaf water relation, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. *J. Experimental Bot.* **43**: 1569-1576.
- Satbhai, R. D., R. M. Naik, A. A. Kale and B. B. Desai. (1997), Effect of water stress on metabolic alteration in rabi sorghum. *J. Maharashtra Agric. Univ.* **22**(2): 158-160.
- Sharma, A. D. and D. Shantha Kumari. (1996), Effect on water stress variety and potassium on sorghum in vertisole. *J. Potassium Res.* **12**(1): 96-99.
- Wenzel, W. G. (1997), Drought resistance of sorghum inbred lines and varieties. *Angewandte Botanic.* **71**(5/6): 201-204.
- Wolf, D. D., W. Carson and D. J. Parrish. (1979), A replica method of determining stomatal and epidermal cell intensity. *J. Agro. Educ.* **8** : 52-54.
- Wright, G. C., R. C. G. Smith and J. M. Morghun. (1983), Difference between two grain sorghum genotypes in adoption to drought stress. II Physiological response. *Aust. J. Agric. Res.* **34**(6): 637-651.

