EFFECT OF DROUGHT STRESS ON EARLY SEEDLING GROWTH OF CHICKPEA (CICER ARIETINUM L) GENOTYPES

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Abstract: The effect of drought stress on thirty genotypes of chickpea (Cicer arietinum L.) were evaluated with five osmotic stress (0,-0.2,-0.4,-0.6 and -0.8 MPa) induced by PEG 6000 on germination and seedling growth. Germination percentage, root and shoot length, vigour index and proline content were measured. The germination and seedling growth in all the genotypes stopped completely in -0.8 MPa. All factors were significantly affected by osmotic potential, results decreasing the osmotic potential led to significant reductions in germination percentage and seedling growth.

In addition to germination, the longer root and shoot length, higher vigour index with more proline accumulation can be consider for selection to drought resistant cultivar. On the basis of the result, Phule G 0511-43-2, Phule G 0752, ICC 4958, Vijay and Digvijay genotypes can be evaluated more tolerant to drought stress than other genotypes at germination and early seedling growth.

Introduction: Water stress invariably decreases several vital processes of the plant and at the same time, modifies a number of morpho-physiological characters in a manner so that at plant can thrive well under drought. Drought affecting the uppermost soil layer is responsible for poor seedling establishment and affecting the uniformity of plant density with a decrease in plant growth and consequently in crop yield. Drought can be alleviated by using drought tolerant genotypes. Thus, identifying drought tolerant mechanism is essential for measuring stress resistance in large breeding population. For drought stress, Polyethylene glycol (PEG) compound has been used to stimulate osmotic stress effect for plants maintain uniform water potential throughout the experimental period. With using this methodology, selection from a large number of breeding lines can be shortly and economically. Water stress (0,-0.2,-0.4,-0.6 and -0.8 MPa) induced by PEG 6000 affected the germination and seedling development parameters in chickpea genotypes. Seedling growth decreased with decreasing the osmotic potential (Yucel et al , 2010).

Material And Methods: Chickpea genotypes were obtained from the Principal Scientist, Pulses Improvement Project, MPKV, Rahuri. The thirty genotypes were screened for moisture stress induced by five different osmotic potential levels (0, -0.2,-0.4, -0.6 and -0.8 MPa) of polyethylene glycol 6000 (PEG-6000). Seeds of each cultivar were treated with a 5 % solution of sodium hypochlorite for 5 minutes for surface sterilization, and then residual chlorine was eliminated by washing of the seeds with distilled water. After then presoaked (16 Hr.) seeds were planted in between filter paper moistened with solution of five osmotic potential. Generation percentage, root and shoot length were measured on 16th day.

Two replications of 40 seeds from different treatment combinations were germinated using between paper methods (BP) at 25±20C in germinator for 16 days. The germination percentage was computed on the basis of normal seedlings only. Ten normal seedlings from each of replication from different treatment combinations of germination test were selected randomly for shoot and root length and were measured in centimeter. The seedlings which were used to record the seedling length were used to know the vigour index. The ten normal seedlings from each replication was selected for calculation of vigour index (Abdul-Baki and Anderson, 1973) and was calculated as under

Vigour Index = Average Seedling length (cm) X Average germination percentage

Results And Discussion: Identifying drought tolerant mechanism is essential for measuring stress resistance in large breeding population. For drought stress, polyethylene glycol (PEG) compound has been used to stimulate osmotic stress effects for plants to maintain uniform water potential throughout experimental period. With using this methodology, selection from a large number of breeding lines can be shortly and economically.

Germination percentage: The mean germination of chickpea genotype as shown in Table 1a was 86 percent in control and there was decline in germination percentage with decrease in osmotic potential in -0.2 MPa with (95%), -0.4 MPa (82.5 %), -0.6 MPa (47.5%) in respect to control in chickpea. The per cent germination at -0.6 MPa stress condition only fourteen genotypes showed resistance to stress it and ranged from 10 to 47%. The germination in all genotypes stopped completely in -0.8 MPa. The genotype Digvijay (47.5%) showed maximum germination percentage at -0.6 MPa followed by ICC 4958 (45%).

The primary action of osmotic inhibition is retardation of water uptake which is crucial for germination. The result of this study are in agreement with the observation of Yucel *et al.*(2010)

in chickpea. They observed that PEG adversely affects germination percentage.

The results of this study are in agreement with the observation of Gurbuz *et al.* (2009) and Macar *et al.* (2009) in chickpea. Gholami *et al.* (2010) and Homayoun *et al.* (2011) observed significant decrease in percentage of germination, germination rate and growth parameter. Sleimi *et al.* (2013) observed that at high PEG concentration (10 g/L), the inhibition of seed germination was highly significant for all the chickpea varieties studied.

Root length (cm): The root length (cm) was significantly decreased from 20.07 to 13.99, 11.09 and 6.91 cm under 0 to -0.2 MPa, -0.4 MPa and -0.6 MPa osmotic potentials. The inhibition of growth under stress condition is the result of inhibition of cell division, cell elongation or both. These results are in agreement with Gholami *et al.* (2010) and Alam *et al.* (2002), who proposed the root length was progressively reduced with decreasing osmotic potential. They also noted that decreasing the root length varied between cultivar as well as between osmotic potentials.

The genotype Phule G-511-43-2 was found superior in respect of root length under stress condition. Veer and Sharma (2010) reported that the length of stem, root and number of lateral roots were decreased with increase in PEG concentration in blackgram. Macar *et al.* (2009) reported that root lengths of all chickpea genotypes were found to be less than the control at -0.4 MPa potential of both PEG and NaCl. The results are in agreement with Derya *et al.* (2010).

Shoot length (cm): PEG induced water stress has been found to decrease in length and biomass of epicotyls and hypocotyls (Gupta *et al.*, 1993). It has been suggested that osmotic stress modifies the biochemical changes taking place in cell wall during growth thereby preventing extension. Under PEG stress, the genotypes ICC 4958 (11.65 and 8.05 cm) recorded highest shoot length at -0.2 and -0.4 MPa osmotic stress, Vijay (2.50 cm) recorded highest shoot length.

Macar *et al.* (2009) reported that length of epicotyls of all the chickpea cultivars and lines decreased significantly with increasing PEG potentials and there was no considerable epicotyls elongation in all genotypes at -0.8 MPa. Not only germination was inhibited but extension growth of seedlings was also observed. These results show that radical and plumule growth of the seedling was greatly adversely affected by water stress.

The root and shoot length are the most important parameter for drought stress. The responses of chickpea genotypes to osmotic potential were different. In our study , shoot length was more adversely affect than the root length. The results are in agreement with Yucel *et al.* (2010) and Okcu *et al.* (2005) who observed that water stress decreased the shoot growth rather than their root growths in chickpea and pea.

Root Shoot Ratio: The root/shoot length ratio was 1.17, 1.91, 2.33 and 3.00 under 0, -0.2, -0.4 and -0.8 MPa osmotic stress respectively (Table 2 b). The genotype Phule G-0701 recorded the higher (4.58 and 11.44) root /shoot length ratio at -0.4 and -0.6 MPa osmotic stress.

The shoot growth is more sensitive than root growth to water stress. The reason may be that root is the first organ emerged from the seed; therefore its growth is faster than shoot growth. Macar *et al.* (2009) found that drought stress brought by PEG 6000 inhibited epicotyls elongation more than the root growth and decreased the shoot/root ratio.

Vigour Index: The genotype Phule G-0752 recorded the highest vigour index (24.77 and 17.42) at -0.2 and -0.4 MPa osmotic stress while the highest vigour index at -0.6 MPa was recorded by the genotype Phule G-0511-43-2 (5.20).

Proline: The proline accumulation was increased in all genotypes in response to decreasing concentration of PEG 6000 from 0 to -0.6 MPa. The genotype Vijay (12.67 and 18.84 μ moles g-1fw) and Phule G-0752 (14.09 and 17.21 μ moles g-1fw) accumulated the highest proline at -0.4 MPa and -0.6 MPa , respectively. While the genotypes Phule G-0405-44-2 and ICCV 11117 were recorded lowest (7.34 and 7.98 μ moles g-1fw) proline content in leaves at -0.4 MPa osmotic stress.

Increase of proline causes the mediation of osmotic adjustment and thus, the plant will keep growing under drought stress. In addition to this proline has a good impact on maintaining the structure of the enzyme and removal of reactive oxygen species (Kumar *et al.* 2006). Since proline has hydrophilic property it might replace water molecules around nucleic acid, protein and membrane during water shortage. It might also prevent interaction between destabilize ions and cellular components by replacing the water molecules around these compounds, thereby, protecting against destabilizing during moisture stress. (Bayomi *et al.* 2008).

On the basis of high germination percentage, under higher osmotic potential, more root and shoot length, vigour index and proline content, Phule G 0511-43-2, Phule G 0752, ICC 4958, Vijay and Digvijay were screened as drought tolerant genotypes. The genotypes viz., Phule G-0305-3, Phule G-0405-44-2, Phule G 09103, ICCV 11 112 and ICCV 11 117 were found more sensitive to drought.

Conclusion: The genotype Phule G 0752, Digvijay , Vishal, Phule-G-511-43-2, ICC 4958 and Vijay had maintained higher amount of germination percentage under PEG 6000 induced progressive stress. Among the genotypes Phule G-511-43-2, Vishal, Digvijay,

Vijay, ICC 4958 genotypes responded better shoot and root length under decreasing osmotic potential. While, the genotype Phule G-0305-3, Phule G-0405-44-2, Phule G-0752 and ICC 4958 under PEG 6000 induced osmotic stress recorded highest vigour index. The genotypes Phule G-0511-43-2, Phule G-0752 and ICC 4958 under PEG 6000 induced osmotic stress accumulated higher proline content in their leaves. In addition to germination, the longer root and shoot length, more vigour index with high proline accumulation, Phule G 511-43-2, Phule G 0752, ICC 4958, Vijay and Digvijay were evaluated more tolerant to drought stress under PEG 6000 induced progressive stress.

References:

1. Abdul-Baki, A.A. and Anderson, J.D. 1973. Vigour determination in soybean seed by multiple criteria. Crop. Sci., 13 : 630-632.
2. Alam, M.Z., T. Stuchbury and R.E.L. Naylor, 2002. Effect of NaCl and PEG induced osmotic potentials on germination and early seedling growth of rice cultivars differing in salt tolerance. Pakistan J. Biological Sci., 5(11) : 1207-1210.
3. Bayoumi T.Y., Eid M, Metwali EM. 2008. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afr. J. Biotech.7: 2341-2352.
4. Ghomali, Mahdiyeh, Rahemi, Majidadand K.. B. .2010. Effect of drought stress induced by polyethylene glycol on seed germination of four wild Almond species. Austr. J. of Basic and Appl. Sci. 4 (5): 785-791.
5. Gurbuz, A., M. Kaya, A.D. Turkan, G. Kaya, M.D. Kaya, C.Y. Ciftci.2009. The effects of seed size and drought stress on germination characteristics of chickpea (*Cicer arietinum* L.) Akdeniz University ZIraat Fakultesi Dergisi. 22(1): 69-74.
6. Homayoun H., Mortez S.D. and Parisa Mehrabi, 2011. Study of PEG stress effects on wheat (*Triticum aestivum* L.) cultivars at germination stage,Middle-East J. Sci. Res., 9(1) : 71-74.
7. Macar T. K. , Turan O. and Ekmekcd Y.2009. Effects of water deficit induced by PEG and NAcl on Chickpea (*Cicer arietinum* L.) Cultivars and Lines at Early Seedling stages, G.U. Journal of Science 229(1):5-14.
8. Sleimi N., Bankaji I., Touchan H., Corbineau F. 2013. Effects of temperature and water stress on germination of some varieties of chickpea (*Cicer arietinum* L.) Afr. J. Biotechnol. 12(17):2201-2206.
9. Veer P. and Sharma, Y. K. 2010. Impact of osmotic stress on seed germination and seedling growth in black gram (*Phaseolus mungo* L.) J. of Environ. Biol., 31 (5): 721-726.
10. Yucel D. O., Adem E.L., Durdane M. and Celal Y. 2010. Effects of drought stress on early seedling growth of Chickpea (Cicer arietinum L.) genotypes, World Appl. Sci. J., 11(4): 478-485.

Table 1. Effect of PEG-6000-induced osmotic stress on germination %, root length and shoot length in Chickpea genotypes

SN	Genotype	Germination (%)				Root Length (cm)				Shoot Length (cm)			
		Control	-0.2 MPa	-0.4 MPa	-0.6 MPa	Control	-0.2 MPa	-0.4 MPa	-0.6 MPa	Control	-0.2 MPa	-0.4 MPa	-0.6 MPa
1.	RVSSG-10	85.0(67.2)	47.5(43.5)	25.0(30.0)	0	22.70	12.85	7.80	6.75	17.65	5.90	4.25	0
2.	JG 25	82.5(65.3)	70.0(56.7)	50.0(45.0)	0	23.90	14.70	11.00	7.10	20.75	5.65	4.20	0
3.	BG D 1069	95.0(63.6)	80.0(63.6)	60.0(50.7)	0	22.70	17.45	13.75	8.30	19.95	8.50	4.40	0
4.	JG-216	90.0(72.1)	77.5(61.7)	65.0(53.7)	0	22.15	9.15	9.00	8.80	20.05	5.80	4.45	0
5.	BGD 1070	90.0(72.1)	80.0(63.6)	67.5(55.2)	22.5(28.2)	21.65	12.60	12.25	6.85	19.50	7.90	3.75	1.40
6.	GJG 0814	95.0(77.0)	72.5(58.3)	65.0(53.7)	0	21.95	13.15	10.25	4.05	21.60	8.05	4.50	0
7.	GJG 0904	87.5(69.3)	60.0(50.8)	60.0(50.7)	0	18.75	14.40	12.25	7.30	16.25	6.10	4.65	0
8.	Phule G-0204-4	82.5(65.3)	77.5(61.7)	60.0(50.7)	0	20.65	14.85	12.50	7.20	18.30	8.35	6.85	0
9.	Phule G-0302-10	87.5(52.2)	72.5(58.3)	62.5(52.2)	0	19.65	13.60	9.90	4.30	16.60	10.15	5.75	0
10.	Phule G-0305-3	90.0(72.1)	72.5(58.3)	30.0(33.2)	0	16.05	13.25	11.50	5.40	12.85	7.25	5.85	0
11.	Phule G-0405-44-2	82.5(65.3)	67.5(55.2)	32.5(34.7)	0	21.00	13.10	6.50	5.55	18.60	6.65	2.40	0
12.	Phule G-0408-11-3	90.0(71.5)	82.5(65.3)	65.0(53.7)	15.0(22.5)	21.55	13.25	11.15	8.50	19.40	8.25	7.05	1.40
13.	Phule G-0408-14-1	95.0(77.0)	87.5 (69.3)	72.5(58.3)	32.5(34.7)	21.90	15.30	10.90	9.95	18.70	11.55	7.50	1.30
14.	Phule G-0511-43-2	95.0(77.0)	90.0(71.5)	75.0(60.1)	40.0(39.2)	21.75	16.60	15.90	11.15	18.50	8.50	6.50	2.00
15.	Phule G-0752	92.5(78.6)	92.5(74.3)	82.5(65.3)	37.5(37.7)	23.30	16.80	14.50	8.35	20.40	10.00	6.60	1.70
16.	Phule G-06102	80.0(63.4)	70.0(56.8)	45.0(42.1)	0	18.40	13.50	10.25	9.30	16.60	7.65	4.15	0
17.	Phule G-07101	82.5(65.3)	77.5(61.7)	52.5(46.4)	10.0(18.4)	15.65	13.65	13.50	6.65	13.90	8.40	2.95	0.60
18.	Phule G-07102	92.5(74.3)	85.0(67.2)	57.5(49.3)	0	17.80	14.15	12.90	6.15	16.40	5.75	3.80	0
19.	Phule G-08108	85.0(67.2)	47.5(43.5)	32.5(34.7)	0	17.65	13.75	10.50	4.00	15.50	7.00	3.50	0
20.	Phule G-09103	95.0(77.0)	60.0(50.7)	10.0(18.4)	0	19.90	14.25	6.70	0	18.50	7.75	0.75	0
21.	Phule G-09758	87.5(69.3)	70.0(56.8)	67.5(55.2)	12.5(20.6)	21.15	13.60	9.30	6.00	20.10	5.85	5.20	1.25
22	Phule G-10114	85.0(67.2)	65.0(53.7)	47.5(43.5)	17.5(26.6)	17.75	12.50	8.25	6.55	16.15	6.90	4.25	1.80
23.	Phule G-02007-10	82.5(65.3)	45.0(42.1)	37.5(37.7)	0	21.25	15.35	11.05	7.30	18.45	6.85	6.40	0
24.	ICC 4958	92.5(74.3)	82.5(65.3)	67.5(55.2)	45.0(42.1)	18.15	17.60	14.20	7.40	16.20	11.65	8.05	1.35
25.	ICCV 11112	82.5(65.3)	62.5(52.2)	32.5(34.7)	0	22.35	14.15	10.70	0.00	17.00	6.95	3.85	0
26.	ICCV 11117	82.5(65.3)	65.0(53.7)	32.5(34.7)	0	15.55	10.45	4.15	1.85	13.55	4.85	2.79	0

27.	Vijay	95.0(77.0)	85.0(67.5)	67.5(55.2)	35.0(36.2)	18.55	15.20	13.60	8.35	17.05	7.70	7.10	2.50
28.	Vishal	85.0(67.2)	82.5(65.3)	72.5(58.3)	22.5(28.2)	25.40	16.00	14.00	11.55	17.40	5.50	7.20	1.20
29.	Digvijay	97.5(83.5)	95.0(77.0)	82.5(65.3)	47.5(43.5)	15.50	13.30	12.60	10.95	14.00	10.90	7.40	1.56
30.	Virat	82.5(65.3)	67.5(55.2)	65.0(53.7)	10.0(18.4)	17.50	11.25	11.90	5.15	10.40	6.00	4.25	1.60
	GM	88.0(70.5)	72.9(59.6)	54.8(47.8)	11.4(13.0)	20.07	13.99	11.09	6.91	17.36	7.61	5.01	0.79
	SE ±	3.20	1.95	1.87	1.42	0.53	0.40	0.36	0.28	0.65	0.26	0.21	0.52
	CD at (5%)	9.24	5.63	5.39	5.55	1.52	1.17	1.04	0.80	1.89	0.75	0.61	0.34
	CV (%)	6.41	4.62	5.45	15.52	3.71	4.09	4.60	5.64	5.33	4.80	5.99	8.8

*Figures in parenthesis indicates arc sine values

Table 2 . Effect of PEG-6000-induced osmotic stress on root: shoot ratio, vigour index and proline content in Chickpea genotypes

SN	Genotype	Root : Shoot Ratio				Vigour Index				Proline (μ moles g^{-1}fw)			
		Control	-0.2 MPa	-0.4 MPa	-0.6 MPa	Control	-0.2 MPa	-0.4 MPa	-0.6 MPa	Control	-0.2 MPa	-0.4 MPa	-0.6 MPa
1.	RVSSG-10	1.29	2.18	1.84	-	34.30	8.88	3.01	-	4.66	5.56	9.43	-
2.	JG 25	1.15	2.60	2.62	-	36.83	14.25	7.60	-	3.77	6.47	10.45	-
3.	BG D 1069	1.14	2.05	3.13	-	40.52	20.78	10.89	-	4.96	8.25	9.80	-
4.	JG-216	1.10	1.58	2.02	-	37.94	11.59	8.76	-	5.85	8.13	11.89	-
5.	BGD 1070	1.25	1.83	3.27	4.93	26.02	16.40	5.21	1.86	5.11	6.27	10.00	12.69
6.	GJG 0814	1.02	1.63	2.28	-	41.37	15.39	9.58	-	3.61	4.14	10.55	-
7.	GJG 0904	1.15	2.36	2.63	-	30.60	12.31	10.14	-	4.02	4.81	8.15	-
8.	Phule G-0204-4	1.13	1.78	1.82	-	32.11	13.92	14.98	-	3.16	5.38	11.68	-
9.	Phule G-0302-10	1.18	1.34	1.72	-	31.71	14.83	11.34	-	3.56	5.73	10.73	-
10.	Phule G-0305-3	1.11	1.59	1.97	-	37.03	14.85	6.80	-	4.33	4.92	9.88	-
11.	Phule G-0405-44-2	1.10	1.81	2.71	-	28.01	13.10	5.94	-	3.54	6.72	7.34	-
12.	Phule G-0408-11-3	1.11	1.61	1.58	6.20	36.86	19.35	11.85	1.48	4.19	5.78	12.20	14.71
13.	Phule G-0408-14-1	1.14	1.32	1.45	8.04	35.52	22.17	13.36	3.54	4.28	4.77	11.61	14.57
14.	Phule G-0511-43-2	1.18	1.95	2.45	5.65	38.24	22.59	16.81	5.20	3.74	5.00	13.81	14.09
15.	Phule G-0752	1.14	1.68	2.20	4.94	40.36	24.77	17.42	4.16	4.21	4.80	14.09	17.21
16.	Phule G-06102	1.11	1.76	2.47	-	28.00	14.83	6.51	-	5.28	5.77	13.67	-
17.	Phule G-07101	1.13	1.63	4.58	11.44	24.36	17.12	8.62	0.73	3.52	5.27	8.90	11.13
18.	Phule G-07102	1.09	2.46	3.39	-	31.62	16.92	9.58	-	4.48	4.94	10.84	-
19.	Phule G-08108	1.14	1.96	3.00	-	28.18	9.86	4.55	-	4.57	5.94	11.7	-

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20.	Phule G-09103	1.08	1.84	2.93	-	36.48	13.20	0.69	-	4.55	5.20	9.15	-
21.	Phule G-09758	1.05	2.32	1.79	4.80	36.06	13.59	9.79	0.91	5.58	6.32	11.56	12.74
22	Phule G-10114	1.13	1.97	1.94	3.77	33.66	12.84	2.91	1.44	5.37	6.33	10.52	-
23.	Phule G-02007-10	1.15	2.24	1.73	-	32.74	9.99	6.55	-	5.38	5.76	11.85	-
24.	ICC 4958	1.12	1.51	1.76	5.48	31.76	24.15	15.03	3.93	5.13	9.21	13.10	17.13
25.	ICCV 11112	1.31	2.04	2.78	-	32.50	13.20	4.73	-	4.65	5.89	9.18	-
26.	ICCV 11117	1.15	2.15	1.49	-	24.00	9.95	2.25	-	4.32	4.95	7.98	-
27.	Vijay	1.09	1.97	1.92	3.34	33.82	19.44	13.98	3.80	4.57	9.26	12.67	18.84
28.	Vishal	1.46	2.91	1.94	6.42	36.38	17.73	15.37	2.46	5.30	6.75	11.73	14.71
29.	Digvijay	1.11	1.22	1.70	7.02	28.79	22.99	16.51	5.02	4.76	6.09	12.60	15.08
30.	Virat	1.68	1.88	2.80	3.22	23.04	11.63	10.50	0.68	5.38	6.16	11.69	11.97
	GM	1.17	1.91	2.33	3.00	32.96	15.75	9.37	1.18	4.52	6.02	10.96	12.41
	SE ±	0.04	0.08	0.08	0.16	1.18	0.74	0.53	0.22	0.14	0.11	0.27	0.14
	CD at (5%)	0.13	0.22	0.24	0.47	3.41	2.13	1.53	0.65	0.41	0.31	0.77	0.39
	CV (%)	5.44	5.62	4.98	7.44	5.02	6.54	7.74	17.05	4.44	2.51	3.44	3.32

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