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RESEARCH ARTICLE

GERMINATION INDICES IN RESPONSE TO WATER DEFICIT INDUCED THROUGH PEG AND MANNITOL IN CHICKPEA (CICER ARIETINUM L.) GENOTYPES

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Abstract

In vitro screening method is an ideal method for evaluation of large set of germplasm accurately and cost effectively. The experiment was carried out with twenty chickpea genotypes in completely randomized design. Polyethylene glycol 6000 (0, -0.2, -0.4MPa) and mannitol (2% and 4%) were used for creating different moisture stress levels. Germination percentage, seedling length, seedling fresh weight and dry weight, vigour index were considerably lowered while membrane permeability index raised with the rise of osmotic stress levels**.** More considerable reduction was obtained under polyethylene glycol than under mannitol, due to greater water stress caused by former substrate. The highest values of germination parameters were obtained with no osmotic potential and least values were found with -0.4 MPa PEG concentration. Genotypes GL22044 and GNG1861 were found to be sensitive and RSG 963 and PDG3 emerged as tolerant genotypes.

Introduction

Water is an important environmental factor and a major limitation for plant growth, development and yield. Water deficit causes limited growth and productivity of the crops in a large part of the agricultural areas in the world. Chickpea (Cicer arietinum L.) is the fourth largest grain legume crop in the world belonging to genus Cicer, tribe Cicereae, family Fabaceae, and subfamily Papilionaceae. Moisture stress conditions prior to and during germination play a dominant role in regulating germination which represents the beginning of the life cycle of plants and is critical for establishment of plant populations. One technique for studying the effect of water stress on germination is to stimulate stress conditions using artificial solutions to provide variable water potentials (Falusi et al 1983). PEG is frequently used to simulate drought stress (Chen et al. 2010) as an inert osmoticum in germination tests which results in osmotic stress that inhibits seed germination through the prevention of water uptake. Similarly, mannitol, a member of sugar alcohols, is an osmotic adjustment chemical to control osmotic potential in the culture media in order to induce water deficit conditions (Zang and Komatsu 2007). The present study was conducted by using twenty genotypes of chickpea and have discussed the validity of seed germination experiment with respect to water stress tolerant and susceptible genotypes.

Materials and Methods

Seeds of Cicer arietinum L., equal in size and weight were surface sterilized with 0.1% mercuric chloride, followed by thorough washing with distilled water. To simulate drought stress, polyethylene glycol solutions (PEG-6000) equivalent to the following water potential -0.2,-0.4 MPa were prepared according to method given by (Michael and Kaufman 1973) and used for screening of twenty chickpea genotypes. Along with PEG, simultaneously another experiment with 2% and 4% of mannitol was conducted. Twenty seeds of each genotype

were germinated in sterilized petri dishes (15 cm diameter) lined with sterilized germination paper moistened with 7-8 ml solution of PEG-6000 or mannitol having an appropriate osmotic pressure and kept in BOD incubator at 25±2°C. Seeds germinated using only distilled water served as control. The design was set up as a factorial experiment, using a completely randomized design (CRD) with three replications. Parameters were recorded at the tenth day of experiment.

Germination Assay**:** Germination counts were made at 24 hour intervals till tenth day in controls, PEG and mannitol treated seeds. The seeds were considered to have germinated when the radical obtained a length of 2mm or more. Vigour index: Vigour index (VI) defined as the product of length of the seedling and the percentage germination, was calculated as: VI = Seedling length X Germination. percentage. Seedling length, fresh and dry weight**:** Seedling length of randomly chosen five seedlings was measured on tenth day after germination using centimeter scale. These seedlings were weighed to obtain their fresh weight and were dried at 70°C for 72 hrs in an oven and weighed to determine their dry weight. Membrane permeability index: The Percent leakiness was determined by following the method given by Fletcher and Drexlure (1980). Triphenyl tetrazolium chloride (TTC) test: Viability test was conducted by Triphenyl tetrazolium chloride test (TTC) following the method by Steponkus and Lanphear (1967).

Results

Germination Response**:** Germination percentage decreased under induced low water potential levels over control in all genotypes (Table 1). Noticeable reduction was observed under -0.2MPa PEG concentration, where maximum decline was observed in RVSSG 4 (57.7%) and minimum in RSG 963 (2.29%). Significant lowering in germination was witnessed on further lowering the water potential to -0.4 MPa PEG concentrations. GL22044 did not germinate depicting its sensitivity to water deficit conditions, followed by GNG1861, where 90.9% reduction over control occurred. Mannitol concentration 2% had less affect on germination. Under 4% mannitol, visible alteration was observed, highest decline in genotype RVSSG 4 (50.7%) and least change in genotype RSG 963 (7.1%) occurred.

Vigour Index**:** Emergence rate is an important criterion in breeding for high yield. Vigour index decreased as water potential was lowered (Table 2). In -0.2MPa, -0.4MPa PEG, 2% and 4% mannitol level of concentration, minimum value was recorded in genotype GL22044, while maximum value in concentrations -0.2MPa PEG and 4% mannitol was observed in genotype RSG 963. At -0.4MPa PEG and 2% mannitol, highest and next to highest values were present in genotype PDG3 respectively. Cumulative mean was recorded least in GL22044 and highest in RSG963 followed by PDG3.

Seedling Length**:** Under -0.2 MPa concentrations, overall decrease was observed (Table 3). Longest seedling was observed in genotype RSG963 (12.36 cm) and shortest in GL22044 (3.63 cm). Under -0.4 MPa concentration, GL22044 growth was completely inhibited, while longest seedling of (6.51 cm) was observed in genotype PDG3. Under 2% mannitol concentration level, seedling length varied between (4.66 cm) in genotype GL22044 to maximum (15.33 cm) in genotype GL28137, followed by (13.33 cm) in genotype PDG3. Under 4% level of mannitol, significant reduction in length was observed. Minimum length of (2.31 cm) was recorded in GL22044, while maximum length (7.67 cm) was observed in RSG 963.

Seedling Fresh And Dry Weight: Seedling fresh (Table 4) and dry weight (Table 5) reduced under lower water potential levels. At -0.2MPa PEG concentration, maximum reduction in fresh weight (61.6%) was observed in RVSSG 4 while minimum (37.7%) was noticed in genotype GL28151. Higher alteration was observed in -0.4MPa PEG concentration level, where GL22044 showed no growth followed by GNG1861 with 77.5% reduction over control. Minimum alteration (55.7%) was observed in genotype PDG3. Mannitol (2% and 4%) reduced fresh weight in all genotypes, later being more effective than former, but genotypes showed no specific trend. Dry weight reduced under low water potential levels, at -0.2MPa concentration of PEG, minimum weight was observed in GL22044 while maximum was recorded in GL28186, RSG 963 and BGM 547. -0.4 MPa PEG had marked difference imposed on all genotypes, GL22044 ceased growth. Mannitol (2%) had very less affect, showing minimal difference over control conditions. Under 4% mannitol level, dry weight decreased highly in comparison to control, though no specific trend was followed among genotypes.

Membrane Permeability Index: Membrane permeability index increased under low water potential levels in all genotypes studied (Fig. 1). In genotype GNG1861, under -0.2MPa and -0.4MPa concentration, significant increase 46.4% and 61.6% was noticed respectively, showing its susceptible character. Maximum values of electrolyte leakage were observed in GL22044 under both concentrations of PEG and 4% mannitol. Under 2% mannitol concentration, values increased over control without following any specific trend regarding genotypic variation. Under all studied concentrations, average membrane permeability index was recorded least in genotype GNG1594, followed by RSG 963 and PDG3 showing their tolerant behavior under imposed water deficit conditions.

Discussion: Slower transition of decomposed materials to seedlings reduces percentage germination in the water stressed conditions. Reduction in seed's water content due to low media water potential decrease the activity of hydrolytic enzymes such as α amylase, proteases and lipases responsible for hydrolyzing cotyledons reserves

required for providing energy in the early stages of seed growth by respiration (Dahal et al. 1996). Similar results were observed in PEG treated chickpea seedlings under laboratory conditions (Macar et al. 2009). Decline in germination percentage by various concentrations of mannitol over control in Glycine max (L.) Merrill was also observed (Machado Neto et al. 2004). Seedling vigour index decreased in response to osmotic stress imposed by PEG solution in wheat (Khodarahmpour 2012). Decreasing seed vigour index is probably due to decreasing moisture availability, which causes enzyme activity by some problems in the transfer of endosperm reserves in the form used for the growth of embryonic axis and synthesis compounds of seed (Van Gastel et al. 1996). Seedling growth was reduced by PEG-6000 solutions as observed in chickpea (Kalefetogllu et al. 2009). Best results in case of hypocotyls length in control, while reduction in water deficit induced by mannitol was observed in Glycine max (L.) Merrill seedling (Machado Neto et al. 2004). The cellular elongation process and carbohydrates wall synthesis were very susceptible to water deficit and the growing decrease was a consequence of the turgescence laying down of those cells (Wenkert et al. 1978). Cell division, which is a post germination phenomenon responsible for seedling elongation and development is more sensitive to PEG than mannitol (Hosseini et al. 2002).

Decline in seedling dry weight in response to drought may be a consequence of decline in weight of mobilized seed reserve due to lower water uptake by germinating seeds (Soltani et al. 2006). Seedling fresh and dry weight decreased with lowering of water potential by PEG in Phaseolus mungo (Garg 2010). Shoot dry weight gradually decreased in Glycine max (L.) Merrill with water deficit increase in mannitol concentration (Machado Neto et al. 2004). Similar results were obtained in Solanum tuberosum cultivars (Arvin and Donnelly 2008), where significant genotypic differences were noticed in electrolyte leakage when drought stress was imposed by using 35% PEG. Electrolyte leakage was significantly greater in mannitol stressed Solanum melongona L. (Sekara et al. 2012).

In conclusion ,-0.4 MPa concentration of PEG proved advantageous in order to distinguish between tolerant and sensitive chickpea genotypes. Among PEG and mannitol, PEG was observed to be more detrimental than mannitol to germination and early germination stage parameters studied.

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Table 1. Effect of water deficit induced through PEG and Mannitol on germination percentage (%) in chickpea (Cicer arietinum L.) genotypes.

Gl22044	GL26054	GL26074	GL28137	GL28151	GL28186	GNG1594	GNG1861	DCP 92-3	GG1362	RSG 811	RVSSG 4	RSG 963	RSG 957	BGM 547	PDG3
85.63± 0.87	95.55± 0.29	93± 0.63	82.21± 0.81	90.82± 0.35	84.44± 1.90	97.9± 0.63	97.77± 0.54	93.33± 0.46	90.79± 0.11	89± 0.86	97.78± 1.14	90.89± 0.22	91.11± 0.40	95.55± 1.07	95.55± 1.35
62.01± 1.02	88.88± 0.23	75.22± 1.11	66.66± 0.39	51.1± 0.22	70.77± 0.40	73.33± 0.46	68.8± 0.41	86.55± 0.23	71.21± 0.73	41.66± 1.66	41.32± 1.67	88.8± 0.90	53.33± 1.92	73.1± 1.75	77.75± 2.58
Nil	28.88± 1.44	28.87± 1.13	26.66± 1.92	42.22± 1.11	59.99± 0.38	28.88± 2.22	8.86± 0.29	22.22± 1.13	28.88± 1.28	46.05± 1.43	40.66± 0.66	57.77± 1.28	37.77± 2.45	32.68± 1.40	73.33± 1.92
81.95± 0.72	88.6± 1.55	84.35± 2.48	80.79±. 0.33	88.82± 0.26	82.92± 0.42	86.19± 3.12	87.17± 0.59	89.28± 2.30	90.57± 0.30	86.24± 2.39	80.45± 1.49	85.6± 2.38	88.92± 0.96	85.46± 0.46	85.5± 1.33
42.68± 1.92	77.44± 2.70	82.44± 1.47	78.02± 1.80	73.95± 1.96	52.33± 2.31	61.3± 1.91	71.8± 1.89	68.94± 1.69	72.3± 2.01	82.6± 3.31	48.2± 1.58	84.42± 2.24	72.99± 2.79	78.89± 1.30	80.27± 2.23

LSD (0.05) Treatment=0.951; LSD Genotype=1.902; LSD (Treatment×Genotye)=4.254; Each value is the mean of three replicates ± Standard Error**.**

Table 2. Effect of water deficit induced through PEG and Mannitol on vigour index in chickpea (Cicer arietinum L.) genotypes. Otherwise as Table 1.

2044	GL26054	GL26074	GL28137	GL28151	GL28186	GNG1594	GNG1861	DCP 92-3	GG1362	RSG 811	RVSSG 4	RSG 963	RSG 957	BGM 547	PDG3	
.16± .78	896.09± 24.10	1290.19± 47.30	1548.79± 74.93	1154.21± 34.19	806.91± 29.84	1418.80± 72.33	1794.71± 29.22	1493.80± 61.04	1319.12± 31.11	972.94± 28.50	916.50± 24.42	1514.51± 83.51	1720.74± 87.60	1770.28± 9.93	1811.72± 33.97	1
.26± .72	456.85± 10.44	583.66± 17.39	583.85± 11.93	504.46± 15.30	464.39± 22.39	391.57± 13.31	466.77± 38.90	863.92± 38.46	546.73± 13.96	312.73± 16.25	283.75± 22.10	1096.54± 52.12	483.83± 11.26	643.84± 60.73	661.98± 38.80	6
il	101.69± 5.08	121.25± 4.75	49.22± 7.13	147.72± 3.10	209.93± 9.00	122.84± 8.36	32.33± 1.90	70.14± 3.02	99.41± 2.43	206.71± 15.56	203.30± 12.01	318.34± 23.12	205.99± 17.44	113.52± 13.03	462.94± 16.86	2
.34± .07	779.26± 24.52	872.83± 52.96	1238.61± 29.93	977.12± 52.50	718.02± 27.73	823.91± 46.70	623.98± 11.85	1038.14± 34.71	996.04± 50.10	762.99± 33.18	523.41± 16.73	1006.64± 59.05	977.65± 86.60	612.50± 64.82	1140.07± 81.10	7
25± 17	427.12± 36.57	356.89± 9.09	311.79± 15.55	518.74± 34.45	246.29± 4.80	316.80± 9.06	405.74± 8.67	494.13± 37.97	325.57± 16.62	395.69± 28.75	186.21± 9.05	646.60± 8.67	326.44± 19.56	258.15± 10.26	428.19± 19.02	4

LSD (0.05) Treatment= 24.514; LSD (0.05) Genotype= 49.029; LSD (0.05) (Treatment×Genotye)= 109.633.

Table 3. Effect of water deficit induced through PEG and Mannitol on seedling length (cm) in chickpea (Cicer arietinum L.) genotypes. Otherwise as Table 1.

Gl22044	GL26054	GL26074	GL28137	GL28151	GL28186	GNG1594	GNG1861	DCP 92-3	GG1362	RSG 811	RVSSG 4	RSG 963	RSG 957	BGM 547	PDG3
11.27± 0.52	9.38± 0.28	13.87± 0.45	18.83± 0.79	12.71± 0.39	9.55± 0.15	14.69± 0.70	18.37± 0.38	16.00± 0.58	14.53± 0.35	10.95± 0.43	9.38± 0.15	11.74± 0.89	24.33± 0.89	18.87± 0.13	19.30± 0.18
3.63± 0.15	5.14± 0.11	7.77± 0.21	3.06± 0.20	4.15± 0.26	4.73± 0.29	5.35± 0.18	4.87± 0.54	8.07± 0.42	4.80± 0.22	7.50± 0.10	6.85± 0.29	12.37± 0.68	8.11± 0.44	8.22± 0.62	5.95± 0.36
Nil	3.53± 0.17	4.20± 0.00	1.83± 0.17	2.50± 0.02	3.50± 0.15	4.27± 0.07	6.66± 0.27	3.17± 0.06	3.47± 0.21	6.52± 0.20	5.00± 0.29	5.50± 0.29	5.44± 0.15	3.22± 0.31	7.51± 0.07
4.67± 0.33	17± 0.42	10.33± 0.33	18.33± 0.42	11.00± 0.33	9.00± 0.34	5.00± 0.42	7.17± 0.17	11.67± 0.67	11.00± 0.58	4.33± 0.15	4.50± 0.10	16.67± 0.36	11.00± 1.00	7.17± 0.72	13.33± 0.88
2.31± 0.13	5.50± 0.31	4.33± 0.10	4.00± 0.21	7.00± 0.29	4.73± 0.27	5.17± 0.08	7.17± 0.22	7.27± 0.40	4.50± 0.16	3.60± 0.17	4.50± 0.21	7.67± 0.23	4.47± 0.18	3.27± 0.08	5.33± 0.11

LSD (0.05) Treatment= 0.274; LSD (0.05) Genotype=0.549; LSD (0.05) (Treatment×Genotye)=1.228.

Table 4. Effect of water deficit induced through PEG and Mannitol on fresh weight (g) in chickpea (Cicer arietinum L.) genotypes. Otherwise as Table 1.

Gl22044	GL26054	GL26074	GL28137	GL28151	GL28186	GNG1594	GNG1861	DCP 92-3	GG1362	RSG 811	RVSSG 4	RSG 963	RSG 957	BGM 547	PDG3
0.48± 0.015	0.60± 0.006	0.56± 0.012	0.48± 0.015	0.53± 0.006	0.52± 0.012	0.62± 0.015	0.58± 0.010	0.55± 0.023	0.53± 0.025	0.52± 0.017	0.60± 0.012	0.58± 0.012	0.49± 0.012	0.57± 0.012	0.52± 0.012
0.25± 0.020	0.27± 0.030	0.28± 0.035	0.26± 0.010	0.33± 0.019	0.31± 0.021	0.27± 0.034	0.26± 0.010	0.30± 0.046	0.31± 0.011	0.22± 0.035	0.23± 0.017	0.31± 0.030	0.29± 0.010	0.25± 0.046	0.26± 0.020
Nil	0.15± 0.006	0.18± 0.012	0.20± 0.012	0.22± 0.015	0.22± 0.010	0.20± 0.08	0.13± 0.06	0.16± 0.010	0.22± 0.010	0.18± 0.003	0.17± 0.007	0.22± 0.015	0.19± 0.006	0.14± 0.004	0.23± 0.012
0.43± 0.010	0.55± 0.006	0.43± 0.012	0.47± 0.026	0.44± 0.025	0.52± 0.012	0.58± 0.017	0.46± 0.021	0.49± 0.023	0.49± 0.006	0.46± 0.010	0.49± 0.015	0.52± 0.010	0.39± 0.020	0.47± 0.012	0.46± 0.012
0.28± 0.010	0.26± 0.012	0.22± 0.015	0.20± 0.012	0.25± 0.006	0.23± 0.006	0.19± 0.012	0.21± 0.010	0.22± 0.006	0.25± 0.010	0.24± 0.006	0.22± 0.010	0.29± 0.006	0.22± 0.010	0.26± 0.006	0.22± 0.006

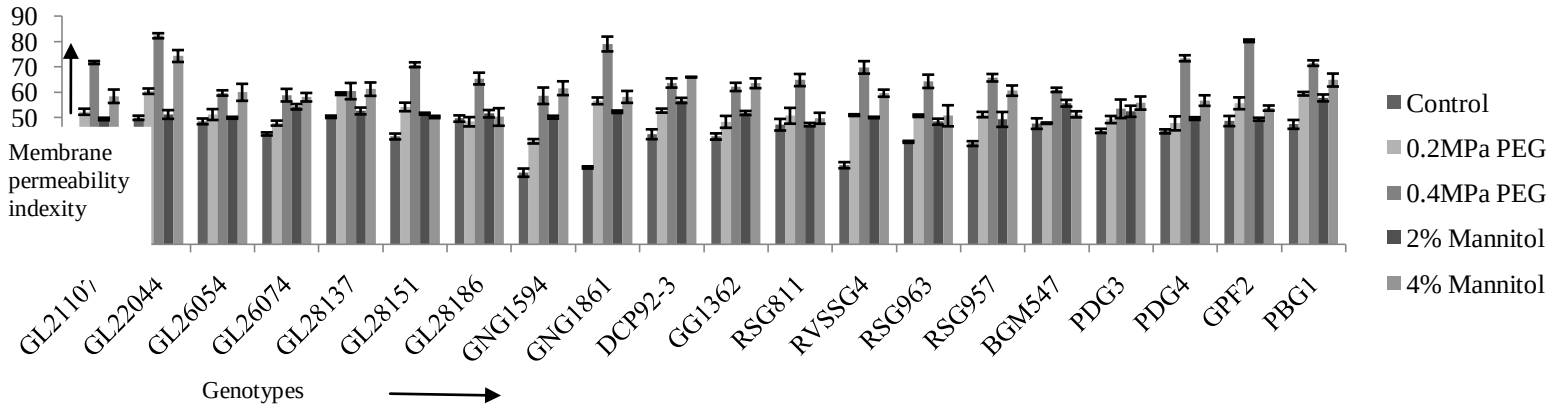
LSD Treatment (0.05)=0.008; LSD Genotype (0.05)=0.017; LSD (0.05) (Treatment×Genotye)=0.038

Table 5. Effect of water deficit induced through PEG and Mannitol on dry weight (g) in chickpea (Cicer arietinum L.) genotypes. Otherwise as Table 1.

l22044	GL26054	GL26074	GL28137	GL28151	GL28186	GNG1594	GNG1861	DCP 92-3	GG1362	RSG 811	RVSSG 4	RSG 963	RSG 957	BGM 547	PDG3	P
.026± 0.001	0.032± 0.002	0.029± 0.001	0.026± 0.001	0.028± 0.001	0.032± 0.001	0.033± 0.002	0.034± 0.002	0.320± 0.002	0.041± 0.001	0.028± 0.001	0.037± 0.001	0.034± 0.001	0.029± 0.001	0.027± 0.001	0.030± 0.004	0. 0
.016± 0.0001	0.024± 0.002	0.019± 0.001	0.017± 0.001	0.025± 0.002	0.027± 0.001	0.031± 0.001	0.019± 0.002	0.022± 0.002	0.018± 0.001	0.038± 0.002	0.037± 0.0001	0.023± 0.001	0.022± 0.0006	0.028± 0.0003	0.024± 0.0035	0. 0.
Nil	0.018± 0.001	0.019± 0.001	0.018± 0.002	0.020± 0.001	0.020± 0.003	0.016± 0.001	0.014± 0.001	0.016± 0.002	0.017± 0.002	0.021± 0.001	0.018± 0.001	0.019± 0.001	0.018± 0.001	0.012± 0.002	0.021± 0.001	0. 0
.021± 0.003	0.026± 0.002	0.024± 0.001	0.027± 0.001	0.032± 0.001	0.023± 0.001	0.028± 0.002	0.020± 0.001	0.028± 0.001	0.029± 0.001	0.031± 0.001	0.029± 0.001	0.027± 0.002	0.030± 0.001	0.025± 0.002	0.027± 0.001	0. 0
.012± 0.002	0.017± 0.002	0.021± 0.001	0.020± 0.001	0.015± 0.001	0.016± 0.001	0.016± 0.002	0.017± 0.001	0.016± 0.002	0.020± 0.001	0.023± 0.000	0.015± 0.001	0.013± 0.002	0.020± 0.002	0.018± 0.001	0.019± 0.001	0. 0

LSD (0.05) Treatment= 0.000; LSD (0.05) Genotype= 0.001; LSD (0.05) (Treatment×Genotye)= 0.003

Fig.1. Effect of water deficit induced through PEG and Mannitol on membrane permeability index in chickpea (Cicer arietinum L.) genotypes.



LSDTreatment (0.05)=1.117; LSD Genotype(0.05)=2.235; LSD (Treatment×Genotye)= (0.05)=4.998