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In vitro screening of chickpea genotypes for drought related traits using PEG 6000

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Abstract
Chickpea is adversely affected by drought stress at all the growth stages including germination and seedling emergence thereby seedling establishment. An *in vitro* screening method was used to screen the genotypes for drought tolerance using polyethylene glycol (PEG) 6000. Six drought related seedling traits *viz.* germination percentage, shoot length (cm), root length (cm), shoot-root ratio, number of secondary roots and relative water content were studied in fifteen genotypes including thirteen MAGIC lines and two varieties JG 11 and HC 5. PEG solutions of 3%, 5% and 7% were used to induce different levels of water stress to compare with control (0%). Analysis of variance revealed significant variability among genotypes, different levels of PEG as well as between genotypes and stress levels. In general a decrease in the mean values under subsequent water induced stress was observed as compared to control in all the six traits under study with few exceptions like root length and shoot root ratio. The root of few genotypes *viz.* M 31, JG 11, HC 5, M 30 and M 27 increased with increase in stress level. Similarly, shoot root ratio also increased in two genotypes namely M 37 and M 32. Considering complex and quantitative nature of drought tolerance, drought related traits were assigned rank score given to each genotype in order of showing increase or minimum decrease in that seedling trait at control and 7% PEG induced stress levels. Total sum of scores over six seedling parameters revealed that JG 11 was most drought tolerant among all fifteen genotypes under study with total score of seventy six, followed by HC 5 whereas M33 was the most susceptible genotype for drought tolerance.

Keywords: Polyethylene glycol (PEG), shoot root ratio, MAGIC (multiparent advanced generation inter-cross), rank score

Introduction
Chickpea (*Cicer arietinum* L.) is one of the major pulse crop grown throughout the world. Globally, it is leading legume crop after dry beans and peas. Chickpea is a good source of protein (20–22%), and is rich in carbohydrates (around 60%), dietary fiber, minerals and vitamins (Jukanti *et al*., 2012) [4]. As more and more people are becoming aware towards health benefits of vegetarian sources of protein the global demand for chickpea and other pulses has increased. Chickpea is cultivated all over the world in about 57 countries under varied environmental conditions (Merga and Haji 2019) [11]. India is the largest producer and consumer of chickpea accounting for 60-65% of global chickpea production. Chickpea and pigeonpea are the two most preferred pulses in India. As India is targeting to become self sufficient in pulses productivity there is a need to increase the overall yield of chickpea and other pulses, under which short duration varieties and increase in the area under pulses is projected. To increase the area under chickpea, non- irrigated areas are focused which possess the challenge of unpredictable water deficit conditions. As everyone knows water is essential for the solubilisation and transportation of reserved food in a seed being the part of enzymatic hydrolytic breakdown of polypeptides, lipids and carbohydrates. Seedling establishment depends not only upon the potential of the genotype to absorb water and hydrolyze the reserved food but also to transport the food efficiently from cotyledons to epicotyls and root (Macar *et al*. 2009) [9]. Therefore, drought is recognized as major abiotic stress which globally causes annual yield losses upto 50% (Sabaghpour *et al.*, 2006, Varshney *et al.*, 2010) [14, 17]. Drought stress adversely affects this crop at all the growth stages including germination and seedling emergence. Successful crop establishment depends on the rapid and uniform seed germination, which is strictly associated with the ability of seeds to germinate under low water availability (Arjenaki *et al.*, 2011) [1]. Limited water availability results in impaired seedling growth and impeded growth rate. This situation emphasizes the urgent need to screen the variability for drought tolerance among chickpea genotypes and select the tolerant genotypes for an increased productivity in drought affected areas. Unpredictability of drought stress in

natural field conditions makes the selection procedure of tolerant genotypes difficult. The *in vitro* screening method using polyethylene glycol (PEG) can overcome these difficulties and large number of genotypes can be screened economically in short period of time. PEG -6000 has high molecular weight, in solution it mimics drought stress and unlike other low molecular weight osmolytes it does not interfere with metabolism of plants. In the light of above, different conditions of water availability were created *in vitro* using PEG at different concentrations and controlled physiological drought was imposed on chickpea genotypes to select for drought tolerance.

Material and Methods

The present experiment was conducted in Pulses laboratory, Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Experiment material comprised of fifteen chickpea genotypes including thirteen multiparent advanced generation inter-cross (MAGIC) lines and two chickpea varieties HC 5 and JG 11. The MAGIC lines were developed by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Hyderabad, using intercrosses of eight parents *viz.* ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105 and ICCV 00108. Some of the high yielding MAGIC lines were selected from advanced generations and evaluated in station trial against check. The MAGIC lines selected for *in vitro* screening to assess drought related traits were numbered as M-27 to M-39.

Seeds were selected precisely for uniform shape and size from each genotype. These were surface sterilized with one percent sodium hypochlorite solution for five minutes followed by treatment with 70% alcohol for few seconds and then washed three times using distilled water. PEG 6000 solutions of different concentrations *viz.* 3%, 5% and 7% were made by dissolving 3, 5 and 7 gram of PEG in distilled water and the volume made up to 100 ml. Treatment with 15 ml of respective solution was used in each experimental unit. Ten seeds of each genotype were placed on blotting paper in petridish in circular order and the experiment was conducted in completely randomized design with three replications for each experimental unit. Petridishes were sealed with parafilm in order to prevent evaporation before transferring to seed incubator. The seeds were allowed to grow in seed incubator at 25 ºC temperature and were kept in dark for first four days followed by their transfer to 16 hours of white light for next eight days. Germination percentage was recorded on fourth day and other seedling parameters *viz.* shoot length (cm), root length (cm), shoot-root ratio, number of secondary roots and relative water content were recorded on twelth day. The data generated was subjected to a two factorial ANOVA and performance of genotypes under control and stress was ranked to elucidate superior genotypes.

Results and Discussion

In vitro screening was performed to initially screen for variability for drought related traits at seedling stage. The mean data obtained for six seedling parameters *viz.* shoot length, root length, shoot-root ratio, secondary roots, germination percentage and relative water content (RWC) was subjected to analysis of variance (ANOVA). Significant variability among the genotypes for drought related traits was present. The significant interaction component between different levels of PEG and genotypes indicated that the expression of genotypes differed significantly at different levels of PEG for drought related seedling traits.

Shoot length

The mean shoot length decreased significantly with increase in PEG concentration. Mean value over all the genotypes was 9.43 cm without PEG which decreased to 7.64 cm, 6.72 cm and 5.91 cm at 3%, 5% and 7% PEG, respectively. In Figure 1 the reduction at highest PEG level (7%) was compared with the control, it was lowest for M 31 (-10.10%), followed by JG 11 (-11.26%), M 32 (-12.59%) and HC 5 (-16.68%), suggesting that these genotypes had potential to resist change in shoot length at water deficit condition. The rate of decrease in their epicotyl growth was also less with increase in concentration of PEG. This may be attributable to improved mobilization of reserved food and polypeptides from cotyledons to epicotyls as suggested by Macar *et al.* (2009) [9]. Decline in shoot length under drought *in vitro* stress at seedling stage was also reported by Romo *et al.* (2001) [13], kandil *et al.* (2012) [5], Mbarek *et al.* (2013) [10], Dharanguttiker *et al.* (2015) [3] and Awari and Mate (2015) [2] in chickpea genotypes.

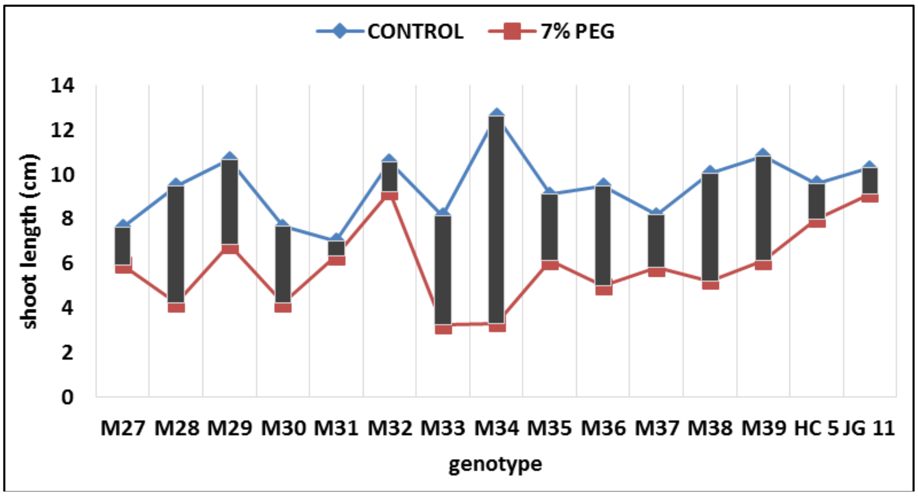

Fig 1: Comparison of shoot length between control and 7% PEG

Root length

The mean root length over the genotypes decreased with increase in PEG concentration *viz.* 20.69 cm (control), 17.97 cm (3% PEG), 17.79 cm (5% PEG) and 17.17 cm at 7% PEG. Though the overall mean root length decreased with gradual increase in water stress but some genotypes showed the potential to increase the root length which is an interesting finding of the present investigation. Genotypes which possessed an increment in root length at 7% PEG level to that of control were M31 (+11.83%), JG 11 (+8.25%), HC 5 (+3.50%), M 30 (+2.48) and M 27 (+0.96%) as presented in Figure 1. Although root length did increased in some genotypes at higher stress level of PEG but the volume of root decreased, these were thin and thread like. The increment in root length in chickpea is reported first time under *in vitro* PEG stress, though it has been reported by Kaur *et al.* (2011) [6] and Swathi *et al.* (2017) [16] in lentil genotypes. Increase in root length under stress is a good indicator of putative genetic potential of drought stress tolerance in the genotype.

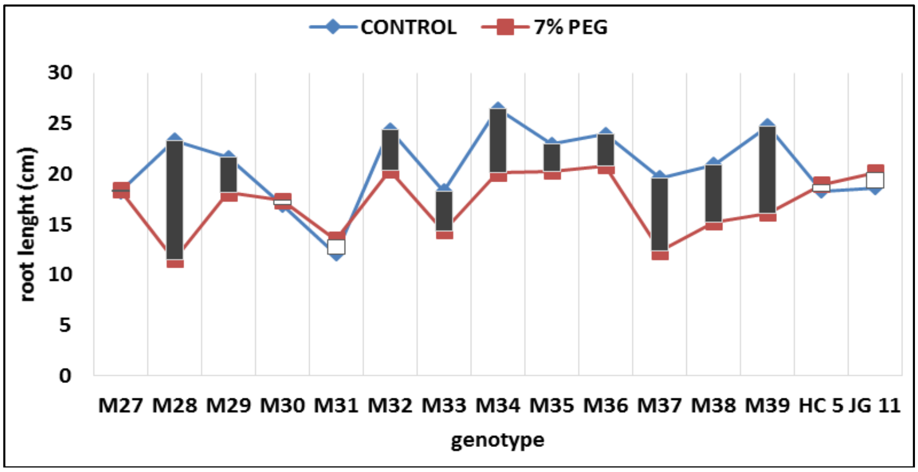

Fig 2: Comparison of root length between control and 7% PEG

Shoot length decreased more drastically in comparison to root length as water stress increased. Therefore shoot length is more adversely affected trait under drought stress in chickpea which is in agreement to findings of Romo *et al.* (2001) [13], Yucel *et al.* (2010) [19] and Awari and Mate (2015) [2] in chickpea.

Shoot root ratio

The mean values over the genotypes at 0%, 3%, 5% and 7% PEG levels were 0.46, 0.43, 0.39 and 0.35, respectively, indicating a gradual decrease with increase in concentration of PEG. However, some genotypes showed the potential to show increase in this trait. In Figure 2, comparison of shoot and root ratio between control and 7% PEG is made. Genotypes M 37 (+0.05) and M 32 (+0.02), showed an increment in the values. Rest all the genotypes showed reduction in shoot root ratio. Macar *et al.* (2009) [9] also reported decrease in shoot and root ratio in chickpea seedling genotypes under water stress which is attributed to more reduction in shoot elongation relative to root elongation. Similar findings were reported by Muscolo *et al.* (2014) [12] and Awari and Mate (2015) [2]. Swathi *et al.* (2017) [16] reported the same in mungbean cultivars in *in vitro* study.

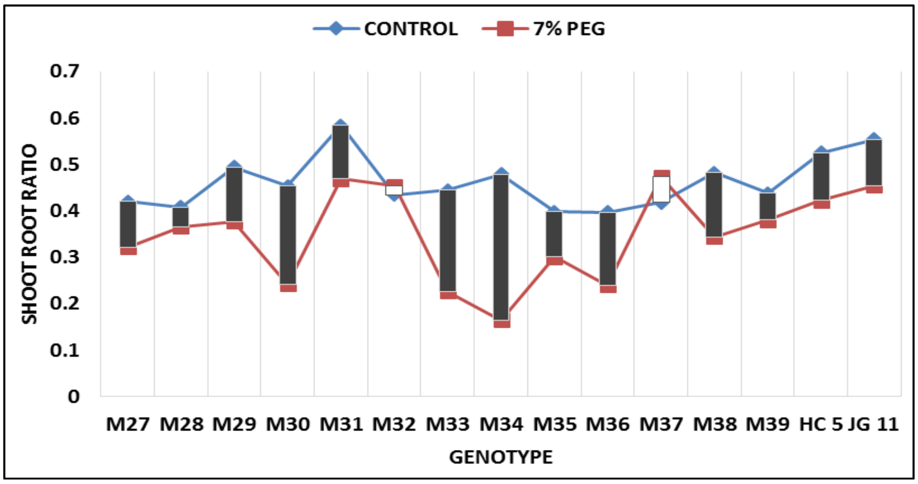

Fig 3: Comparison of shoot and root ratio between control and 7% PEG.

Number of secondary roots

Mean values for number of secondary roots over the genotypes under study were 9.43, 3.10, 1.78 and 1.20 at 0%, 3%, 5% and 7% level of PEG, respectively. A perusal of Table 1 indicated that number of secondary roots was highly affected trait by water stress. On comparing 7% PEG stress with control minimum reduction was found in HC 5 (-60.18%), followed by M 38 (-79.12%), M 36 (-80.93), M 39 (-81.25) and JG 11 (-81.34). However M 33 showed maximum reduction of 97.98%. Secondary or lateral roots may give an increased ability to these plants for early establishment of seedlings and increase the area of water absorption that imparts increased vigor. Veer and Sharma (2010) [18] reported decrease in number of lateral roots with increase in PEG concentration in blackgram. Similar results were reported in tomato by Kulkarni and Deshpande (2007) [8].

Germination percentage

Table 1 indicated that the mean values over the genotypes at 0, 3%, 5% and 7% PEG were 99.82, 97.82, 89.43 and 84.46, respectively. Genotypes that showed highest germination percentage at 7% PEG level were M 31 (93.16%), M 29 (90.83%), JG 11 (90.76%) and HC 5 (90.12%). At control germination ranged between 99.16% to 100% while at 7% PEG, it varied from 74.16 to 93.16%. Thus, availability of water affects the germination in chickpea and therefore a negative relation can be established between germination percentage and increased water stress. Germination percentage in chickpea was also studied by Mbarek *et al.* (2013) [10], Awari and Mate (2015) [2] and Dharanguttiker *et al.* (2015) [3] using PEG induced water stress.

Relative water content

Mean relative water content (RWC) over all the genotypes decreased gradually with increase in PEG induced stress. It was 78.37, 75.20, 72.38 and 67.80 at 0, 3, 5 and 7% PEG, respectively (Table 1). Individual RWC values were highest for each genotype before treatment while it gradually decreased with increase in stress level. Since tolerant genotypes must have potency to resist or minimise decrease in the RWC values under stress. Here, deviations between control and 7% PEG level were also estimated. Genotypes which showed the minimum reduction were JG 11 (-3.94), M 37 (-5.08), HC 5 (-5.81), M 38 (-6.67) and M 36 (-6.88). Similar studies for *in vitro* drought tolerance were conducted by Khakwani *et al.* (2011) [7] in wheat varieties, Muscolo *et al.* (2014) [12] in lentil genotypes and Salma *et al.* (2016) [15] in chickpea genotypes.

Rank scores

Together all the seedling parameters may help us to screen drought tolerant genotype. Therefore, each genotype was given a rank score for each of the seedling parameter studied. The basis of ranking was the least reduction or positive increase between the performance of genotype at control and 7% PEG stress, for all the seedling traits. As the total genotypes used in the experiment are 15 therefore the highest score was 15 and subsequently as the rank decreased in the seedling trait, the score gradually decreased from 15 to 1. Higher scores corresponded to higher tolerance and lower scores corresponded to susceptibility of that genotype to water stress for respective seedling parameter.

To elucidate the most tolerant genotype all the rank scores were summed up over each seedling trait. In Table 2 overall sum of rank scores over six seedling parameters revealed that JG 11 was most drought tolerant genotype with a total score of 76 out of 90. JG 11 had highest value for RWC among all the genotypes and second highest for shoot length and root length which shows the importance and relatedness of these seedling traits in imparting drought tolerance at seedling stage in chickpea. HC 5 was the second most tolerant genotype with overall score of 73 which showed best performance for secondary roots. Among the M-series M 31 was most tolerant to drought and ranked third with overall score of 64. M 34 was the most susceptible genotype with minimum score of 23.

Table 1: Effect of increased levels of PEG on secondary roots, germination percentage and relative water content in chickpea genotypes

Genotype	Secondary roots						Germination Percentage					Relative Water Content (RWC)					
	Control	PEG level			mean	∞	Control	PEG level			Mean	Control	PEG level			Mean	∞
		3%	5%	7%				3%	5%	7%			3%	5%	7%		
M27	11.3	6.45	5.35	1.9	6.25	-83.19	99.16	98.33	85	74.16	89.17	76.03	74.275	72.08	64.155	71.635	-11.88
	(100)	(57.08)	(47.35)	(16.81)													
M28	5.65	5.4	0.5	0.9	3.113	-84.07	100	96.67	91	86.33	93.5	79.06	72.41	68.025	65.355	71.212	-13.71
	(100)	(95.58)	(8.85)	(15.93)													
M29	11.24	3.85	1.565	0.25	4.226	-97.78	100	98.16	93.5	90.83	95.62	76.42	71.875	72.63	66.9	71.956	-9.52
	(100)	(34.25)	(13.92)	(2.22)													
M30	11.125	2.05	1.615	0.25	3.76	-97.75	100	95	86	78.16	89.79	74.425	71.44	61.38	53.28	65.131	-21.15
	(100)	(18.43)	(14.52)	(2.25)													
M31	9.3	1.15	0.94	1.1	3.123	-88.17	100	98.16	96.16	93.16	96.87	76.115	76.495	72.285	65.89	72.696	-10.23
	(100)	(12.37)	(10.11)	(11.83)													
M32	8.84	2.885	1.65	1.15	3.631	-86.99	100	100	92.6	89.5	95.52	81.215	77.235	75.715	74.035	77.05	-7.18
	(100)	(32.64)	(18.67)	(13.01)													
M33	12.35	2.2	0.6	0.25	3.85	-97.98	100	96.67	83	81.33	90.25	84.15	78.085	73.35	55.475	72.765	-28.68
	(100)	(17.81)	(4.86)	(2.02)													
M34	6.315	1.055	0.765	0.515	2.163	-91.84	100	98.16	87	80.83	91.5	74.2	71.185	64.085	64.23	68.425	-9.97
	(100)	(16.71)	(12.11)	(8.16)													
M35	8.33	1.32	1.105	0.35	2.776	-95.8	100	96.73	91.5	86.66	93.72	82.385	77.38	78.005	74.435	78.051	-7.95
	(100)	(15.85)	(13.27)	(4.2)													
M36	6.845	2.15	0.815	1.305	2.779	-80.93	98.16	94.16	85.5	76.66	88.62	77.285	77.37	70.1	70.4	73.789	-6.88
	(100)	(31.41)	(11.91)	(19.07)													
M37	14.05	2.565	1.835	1.245	4.924	-91.14	100	100	86	83.16	92.29	80.485	77.315	77.45	75.405	77.664	-5.08
	(100)	(18.26)	(13.06)	(8.86)													
M38	7.47	2.25	1.525	1.56	3.201	-79.12	100	95.83	82.66	75.83	88.58	86.06	80.175	82.215	79.39	81.96	-6.67
	(100)	(30.12)	(20.41)	(20.88)													
M39	8.345	2.885	1.75	1.565	3.636	-81.25	100	100	92.2	89.5	95.42	70.54	68.36	64.195	60.575	65.918	-9.97
	(100)	(34.57)	(20.97)	(18.75)													
HC 5	8.84	3.935	3.57	3.52	4.966	-60.18	100	100	95.93	90.12	96.51	79.205	78.15	78.145	73.395	77.224	-5.81
	(100)	(44.51)	(40.38)	(39.82)													
JG 11	11.525	6.42	3.105	2.15	5.8	-81.34	100	99.33	93.33	90.76	95.85	78.005	76.325	76.045	74.07	76.111	-3.94
	(100)	(55.7)	(26.94)	(18.66)													
Mean	9.435	3.104	1.779	1.201			99.822	97.816	89.426	84.46		78.372	75.205	72.38	67.799		

∞ = deviation between control and 7% PEG stress (+ve sign indicates increase, -ve sign indicates decrease)

Table 2: Rank scores of chickpea genotypes for six seedling parameters

Genotype	Relative Ranking Score						Total
	Shoot length	Root length	Shoot root ratio	Secondary roots	Germination %	RWC	
M 27	11	11	10	10	1	4	47
M 28	3	1	13	9	8	3	37
M 29	8	8	6	2	14	8	46
M 30	6	12	3	3	4	2	30
M 31	15	15	7	7	15	5	64
M 32	13	7	14	8	10	10	62
M 33	2	6	2	1	6	1	18
M 34	1	5	1	5	5	6	23
M 35	9	10	11	4	9	9	52
M 36	5	9	4	14	3	11	46
M 37	10	2	15	6	7	14	54
M 38	4	4	5	13	2	12	40
M 39	7	3	12	12	11	7	52
HC 5	12	13	8	15	12	13	73
JG 11	14	14	9	11	13	15	76

Conclusion

In vitro screening is very useful and economical way to initially screen for variability, it saves time and also provides uniform drought like water deficit conditions which is hard to obtain at field. Germination percentage is the least affected seedling trait in chickpea under water stress. Above all, findings suggest that seed germination, shoot length, root length, shoot root ratio, number of secondary roots and RWC can be used as early seedling traits for selection of drought tolerant genotypes in chickpea. JG 11 and HC 5 can be used in breeding program for drought tolerance.

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