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ASSESSMENT OF DROUGHT TOLERANCE IN ERECT AND SEMI-ERECT CHICKPEA (*CICER ARIETINUM* L.) GENOTYPES UNDER POLYETHYLENE GLYCOL INDUCED OSMOTIC STRESS

B.S. Chaithra[1]*, H.Y. Patil[1], B.S. Patil[1], U.V. Mummigatti[2], P. Nethra[2] and S.R. Salakinkop[3]

[1]Department of Crop Physiology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India.

[2]ICAR-IARI, Regional Research Centre, Dharwad, Karnataka, India.

[3]AICRP on Maize, MARS, University of Agricultural Sciences, Dharwad, Karnataka, India.

*Corresponding author E-mail : chaithrareddyagri@gmail.com

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important legumes crops globally, valued for its high protein content. However, its productivity is significantly impacted by abiotic stresses, with drought being the most critical factor, leading to reduced growth and substantial yield losses. An experiment was conducted to evaluate seed germination and seedling growth performance under water-stressed conditions. This study examines the effects of osmotic stress induced by polyethylene glycol (PEG 6000) on the germination, seedling growth and vigor of selected chickpea genotypes. Five levels of osmotic stress (0%, 5%, 10%, 15% and 20% PEG) were applied and the genotypes were evaluated for germination percentage, root and shoot length, seedling dry weight and seedling vigor indices. Among the genotypes evaluated, the highest germination percentage was observed in ICCV 4958 (93.80%) under control conditions, while the lowest was in NBeG 47 (46.17%) under 20% PEG. Shoot and root lengths decreased with increasing PEG concentration, with ICCV 4958 and BGD 111-1 consistently showing superior growth over other genotypes under stress. Seedling dry weight and vigor indices also varied significantly, with ICCV 4958 and BGD 111-1 exhibiting higher values compared to other genotypes under osmotic stress. Drought stress had a significant negative impact on all growth parameters, with the severity of the effects corresponding to the intensity of the applied stress. Seedling vigor index proved to be a reliable selection criterion for drought tolerance. Notably, ICCV 4958 and BGD 111-1 exhibited high seedling vigor indices, reflecting their enhanced tolerance to high levels of drought stress. In contrast, DBGV 204 and NBeG 47 showed low seedling vigor indices.

Key words : Chickpea, PEG, Osmotic stress, Seedling length, Vigour indices.

Introduction

Chickpea (*Cicer arietinum* L.) is the most prominent pulse crop in India, leading in terms of area, production, and productivity. Globally, it ranks as the third most important pulse crop. As a protein-rich legume, chickpea plays a vital role in human nutrition and is cultivated across 13.56 million hectares in more than 50 countries, including major producers such as Australia and Canada (FAOSTAT, 2020). However, chickpea production is predominantly concentrated in Asia, which accounts for over 90% of the global cultivation area and 86% of total production. According to the fourth advance estimates for 2021–22, India produced 13.75 million tonnes of chickpea, cultivated over an area of 10.91 million hectares, with an average productivity of 12.6 quintals per hectare (DES, 2023; Ministry of Agriculture & Farmers' Welfare, Government of India). Among Indian states, Madhya Pradesh leads in chickpea production, contributing 36.96 lakh tonnes, followed by Maharashtra with 23.05 lakh tonnes from an area of 21.47 lakh hectares and a productivity of 1,074 kg/ha (Anonymous, 2022). The widespread cultivation and popularity of chickpea in India are largely due to its nutrient-rich seeds, which serve as a critical source of protein and essential minerals for the largely vegetarian population, particularly in regions with limited access to alternative protein sources.

Chickpea, often cultivated on marginal land, is particularly susceptible to terminal drought stress. Drought, as a complex abiotic stress, can adversely affect all growth stages of chickpea crops. The negative impacts can be evaluated through various indicators, including seed emergence and seedling establishment. Low moisture availability at the time of sowing has a detrimental impact on seed germination and early seedling growth in chickpea (*Cicer arietinum* L.) (Hassan *et al*., 2023). With ongoing climate change and the progressive depletion of water resources, the development of drought-tolerant germplasm has become increasingly important (Khalil and Ahmed, 2022). However, breeding for drought tolerance based on yield performance under field conditions poses significant challenges. These include the difficulty of creating uniform drought stress environments and the slow and labor-intensive nature of identifying drought-tolerant genotypes. As an alternative approach, Richards highlighted the germination stage as the most sensitive phase in the chickpea life cycle and proposed that screening at this stage could effectively identify drought-tolerant genotypes. In this context, the seedling vigor index, which combines germination rate with seedling length, has been recognized as a useful selection criterion for drought tolerance during germination.

In vitro screening methods offer a promising alternative to traditional field-based approaches, providing greater reliability and time efficiency. Among various techniques, the use of polyethylene glycol (PEG) has proven effective in simulating drought stress. PEG induces osmotic stress by lowering the water potential of the medium without penetrating plant cells (Hassan *et al*., 2023). This makes it a suitable agent for assessing drought responses during germination. The objective of this study was to evaluate the impact of PEG-induced drought stress on seed germination and seedling vigor index as key indicators for screening chickpea genotypes for drought tolerance. The findings are intended to support the identification of promising genotypes for use in breeding programs or commercial cultivation under water-limited conditions.

Materials and Methods

Experimental details

This study explored osmotic stress tolerance mechanisms in chickpea genotypes. The experiment was conducted at seed unit laboratory, Department of seed science and technology, UAS, Dharwad during 2023. Selected chickpea genotypes (ICCV 4958, BGD 111-1, BGD-103, DBGV 204, GBM-2 and NBeG 47) were subjected for comparison of germination and seedling growth in Polyethylene glycol 6000 solutions of five osmotic stress regimes by subjecting the seeds of chickpea genotypes to polyethylene glycol treatments along with control Viz., 0% (Control) , 5% (-0.05 MPa), 10% (-0.148 MPa), 15% (-0.295 MPa) and 20% (-0.491 MPa). Polyethylene glycol (PEG) is a natural polymer with a molecular weight of 6000 that is both water-soluble and non-ionic. The water potential is lowered by PEG 6000 in a way that is similar to drought due to osmotic stress. In a completely randomized design (CRD), three replicated tests were conducted.

Chickpea seeds were surface-sterilized by treating them with 2% sodium hypochlorite solution for 5 minutes. Following sterilization, the seeds were subjected to various concentrations of polyethylene glycol 6000, while distilled water served as the control treatment. Three batches of 50 seeds from each genotype were placed evenly on two Germitest® germination paper sheets. These sheets were moistened with PEG solutions at a volume equal to 2.5 times the dry weight of the paper, then rolled up. The rolls were sealed in plastic containers to minimize evaporation and maintain near 100% humidity. Germination trials were conducted in a germinator set to a constant temperature of 25˚C (±1˚C) under suitable lighting conditions. The data was collected 14 days after the chickpea seeds were germinated after being treated. The following is a detailed description of the observations that were recorded.

Seed germination (%) : Seeds were considered as germinated once the radicle extended beyond 1.0 mm, following the criteria described by Koskosidis *et al*. (2022).

$$\text{Germination percentage (\%)} = \frac{\text{Number of normal seedlings}}{\text{Number of seeds put for germination}} \times 100$$

Root length (cm) : On the fifteenth day of the germination test, ten healthy seedlings were randomly selected from all replications within each treatment group. Root length was measured using a ruler, from the tip of the primary root to the base of the hypocotyl, and the average root length was recorded in centimetres (cm).

Shoot length (cm) : Shoot length was measured using the same ten healthy seedlings previously selected for root length assessment. The measurement was taken from the tip of the primary leaf to the base of the hypocotyl and expressed in centimetres (cm).

Seedling vigour indices : Seedling vigour indices were calculated following the method described by Abdul Baki and Anderson (1973). Seedling Vigour Index I (SVI

I) was determined by multiplying the germination percentage by the average seedling length (cm), and expressed as a numerical value:

SVI I = Germination (%) × Seedling length (cm)

Seedling Vigour Index II (SVI II) was obtained by multiplying the germination percentage by the average dry weight of the seedlings (in grams), with the result presented as a whole number:

SVI II = Germination (%) × Seedling dry weight (g)

Seedling dry weight (g) : To determine seedling dry weight, ten healthy seedlings still attached to their cotyledons and previously used for root and shoot length measurements were first air-dried. They were then placed in butter paper pouches and dried in a hot air oven at 70/ °C for 24 hours. After drying, the seedlings were weighed and the dry weight was recorded in grams (g).

Results and discussion

The study revealed that different levels of osmotic stress had a significant impact on several key parameters, including germination percentage, seedling length, seedling dry weight, and vigour indices I and II. The detailed results of these effects are presented below.

Germination percentage : Germination represents one of the most importat and foundational stages in a plant's life cycle, as it plays a pivotal role in determining successful seedling establishment and robust early growth. This phase not only initiates the plant's development but also significantly influences overall crop productivity. Moreover, germination performance under stress conditions, particularly moisture stress, serves as an effective and widely used indicator for evaluating drought tolerance. Table 1 displays the germination rates observed across different chickpea genotypes, osmotic concentrations and their interactions. The mean germination percentage was observed to be highest in case of ICCV 4958 at 0% PEG (93.80%) and lowest was exhibited by NBeG 47 at 20% PEG (46.17%). As osmotic stress conditions intensified, a corresponding decline in germination percentage was observed. However, among all the genotypes tested, ICCV 4958 consistently exhibited the highest germination percentage across all polyethylene glycol (PEG) concentrations, followed by BGD 111-1. In contrast, NBeG 47 and DBGV 204 recorded the lowest germination percentage under the same conditions. Similar results were obtained by (Soumya *et al*., 2024), who studied response of chickpea to drought stress by PEG and also recorded reduced germination at 20% for chickpea and it was proposed as the threshold osmotic potential.

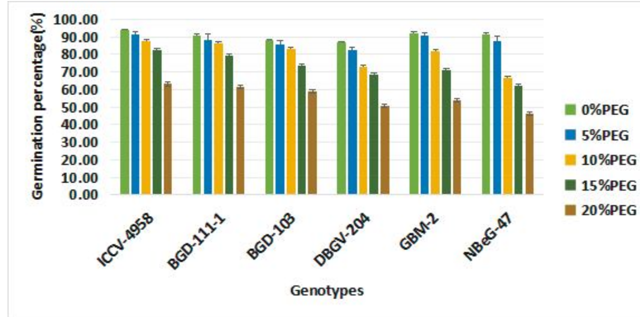

Fig. 1 : Effect of osmotic stress on germination percentage of chickpea genotypes.

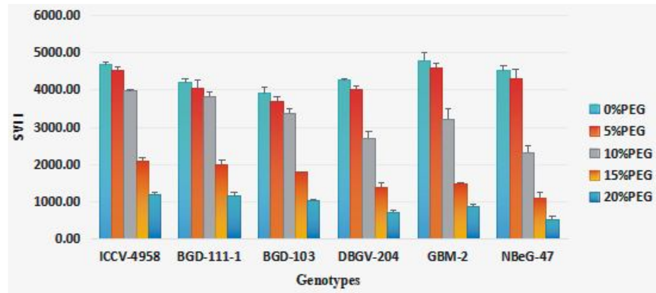

Fig. 2 : Effect of osmotic stress on vigour index I of chickpea genotypes.

Root length : Significant variation in root length was observed among the different genotypes and across varying levels of osmotic stress, as presented in Table 1. Compared to control treatment, the highest mean root length was recorded under 10% PEG concentration (17.05 cm), with a progressive decline observed at higher PEG levels. Among all the genotypes tested, ICCV 4958 and BGD 111-1 consistently demonstrated superior root lengths across all PEG concentrations, indicating better tolerance to osmotic stress. Conversely, NBeG 47 exhibited the lowest root length under the same conditions. Greatest reduction in root length was observed in NBeG 47 (6.46 cm) followed by DBGV 204 (8.78 cm) at 20% PEG. This trend underscores the inhibitory effect of osmotic stress on root development, consistent with the patterns observed in the present study. Similar findings were observed by Soumya *et al*. (2024), who worked on chickpea with five different concentration and high root length was recorded in 10% PEG (- 0.148 MPa). The increased root length might be due to the factor that under water stress, the plant partitioned more photosynthates for the growth of roots rather than shoots which is a drought adaptive mechanism.

Shoot length : Table 1 made it evident that genotypes, osmotic pressures and their interactions had a considerable impact on shoot length. The highest shoot length was observed under control conditions (0% PEG), with a gradual decline as PEG concentration increased. The average shoot length at 0% PEG was 33.00 cm, which decreased progressively to 31.76 cm, 23.23 cm, 10.33 cm and 6.60 cm under 5%, 10%, 15% and 20% PEG concentrations, respectively. Among the genotypes, NBeG 47 recorded the highest mean shoot length of 36.59 cm under control conditions, while the shortest shoot length was noted in the same genotype (4.95 cm) at 20% PEG. This reduction in shoot elongation under increased osmotic stress suggests that decreased osmotic potential leads to a more drastic inhibition of shoot tissue elongation and the similar findings were also reported by Jamaati *et al.* (2011).

Seedling dry weight : Polyethylene glycol (PEG) affects seedling dry weight by reducing water availability, which is essential for various metabolic activities. This limitation hampers normal physiological functions, ultimately leading to decreased biomass accumulation. Table 2 presents a summary of the effects of genotype, osmotic pressure, and their interactions on seedling dry weight. Significant variations in mean seedling dry weight were observed across the different levels of osmotic stress, highlighting the sensitivity of seedling biomass to water deficit conditions. The highest mean seedling dry weight of 1.60 g was recorded under control conditions (0% PEG), followed by 1.48 g at 5% PEG and 1.36 g at 10% PEG. Among the genotypes, ICCV 4958 had the highest mean dry weight of 1.42 g, followed by BGD 111-1 (1.38 g). In contrast, BGD-103 and NBeG 47 had the lowest mean dry weight at 1.16 g. Significant differences were observed in genotypic interactions with osmotic pressure. Under 20% PEG stress, ICCV 4958 (0.95 g) and BGD 111-1 (0.89 g) followed by BGD 103 (0.88 g) maintained the highest seedling dry weights, whereas NBeG 47 had the lowest seedling dry weight of 0.51 g.The observed decrease may be attributed to damage inflicted on the meristematic cells of both root and shoot due to drought stress, which disrupts essential processes such as cell division and elongation. Additionally, reduced water absorption under drought conditions likely leads to a decline in cellular turgor pressure, further contributing to growth inhibition. This reduction in turgor limits the cell's ability to expand, thereby accelerating overall growth retardation.

Vigour Index I : Significant variations in vigour index I were observed across different genotypes, osmotic stress levels and their interactions (Fig. 2). The vigour index I decreased with increasing osmotic pressure. The highest mean vigour index I was recorded at 0% PEG, with a value of 4391.00, which was significantly greater than the 10% PEG level (3239.88). The vigour index I values were lowest under 20% PEG, reaching a mean value of 920.56. Among the genotypes, GBM 2 recorded

Table 1 : Effect of osmotic stress on germination percentage (%) in chickpea genotypes.

Genotypes	Germination percentage (%)						Shoot length (cm)						Root length (cm)					
	0% PEG (control)	5 % PEG	10 % PEG	15 % PEG	20% PEG	Mean	0% PEG (control)	5 % PEG	10 % PEG	15 % PEG	20% PEG	Mean	0% PEG (control)	5 % PEG	10 % PEG	15 % PEG	20% PEG	Mean PEG
ICCV 4958	93.80	91.29	87.59	82.27	63.21	**83.63**	32.08	31.72	26.33	10.91	7.58	**21.72**	17.75	17.83	19.03	14.71	11.47	**16.16**
BGD 111-1	90.50	88.29	86.43	79.40	61.68	**81.26**	29.73	29.00	25.82	11.16	7.51	**20.65**	16.62	16.76	18.59	13.82	11.20	**15.40**
BGD -103	87.99	85.88	83.20	73.50	59.00	**77.91**	29.20	26.42	22.54	10.77	7.34	**19.26**	15.19	16.77	18.04	13.65	10.23	**14.78**
DBGV 204	86.77	82.23	73.07	68.30	50.60	**72.19**	34.11	33.49	21.69	9.82	5.37	**20.90**	14.97	15.17	15.48	10.60	8.78	**13.00**
GBM-2	92.25	90.95	81.70	71.27	53.92	**78.02**	36.26	34.33	22.01	10.08	6.86	**21.91**	15.73	16.17	17.30	10.69	9.58	**13.89**
NBeG 47	91.26	87.49	66.70	61.99	46.17	**70.72**	36.59	35.61	20.97	9.23	4.95	**21.47**	12.92	13.57	13.87	8.55	6.46	**11.07**
Mean	**90.43**	**87.69**	**79.78**	**72.79**	**55.76**		**33.00**	**31.76**	**23.23**	**10.33**	**6.60**		**15.53**	**16.04**	**17.05**	**12.00**	**9.62**	
Factors	**S.Em ±**			**CD @ 1%**			**S.Em ±**			**CD @ 1%**			**S.Em ±**			**CD @ 1%**		
Genotypes	0.712			2.018			0.12			0.35			0.28			0.80		
Treatments	0.65			1.842			0.11			0.32			0.26			0.73		
G×T	1.591			4.512			0.27			0.78			0.63			1.79		

Table 2 : Effect of osmotic stress on Seedling vigour indices in chickpea genotypes.

Genotypes	Seedling dry weight (g)					
	0% PEG (control)	5 % PEG	10 % PEG	15 % PEG	20% PEG	Mean
ICCV 4958	1.59	1.58	1.53	1.42	0.95	**1.42**
BGD 111-1	1.56	1.55	1.51	1.36	0.89	**1.38**
BGD -103	1.31	1.23	1.20	1.17	0.88	**1.16**
DBGV 204	1.65	1.51	1.28	1.05	0.54	**1.21**
GBM-2	1.69	1.53	1.48	1.11	0.77	**1.32**
NBeG 47	1.76	1.47	1.11	0.94	0.51	**1.16**
Mean	**1.60**	**1.48**	**1.36**	**1.18**	**0.76**	
Factors		**S.Em ±**			**CD @ 1%**	
Genotypes		0.01			0.02	
Treatments		0.01			0.02	
G×T		0.02			0.05	

Genotypes	Seedling vigour index I					
	0% PEG (control)	5 % PEG	10 % PEG	15 % PEG	20% PEG	Mean
ICCV 4958	4673.36	4523.82	3972.15	2107.11	1203.05	**3295.90**
BGD 111-1	4195.12	4041.84	3837.34	1984.64	1153.87	**3042.56**
BGD -103	3906.47	3708.62	3375.59	1794.09	1036.71	**2764.30**
DBGV 204	4258.40	4001.39	2716.57	1396.09	714.52	**2617.39**
GBM-2	4795.36	4593.12	3214.40	1479.31	887.21	**2993.88**
NBeG 47	4517.30	4305.02	2323.22	1104.80	528.03	**2555.67**
Mean	**4391.00**	**4195.63**	**3239.88**	**1644.34**	**920.56**	
Factors		**S.Em ±**			**CD @ 1%**	
Genotypes		35.09			99.51	
Treatments		32.03			90.84	
G×T		78.47			222.52	

Genotypes	Seedling vigour index II					
	0% PEG (control)	5 % PEG	10 % PEG	15 % PEG	20% PEG	Mean PEG
ICCV 4958	149.45	144.51	134.31	117.06	60.27	**121.12**
BGD 111-1	140.91	137.08	130.85	108.22	55.09	**114.43**
BGD -103	115.55	105.88	100.13	86.27	52.13	**91.99**
DBGV 204	143.25	124.41	93.81	71.91	27.47	**92.17**
GBM-2	156.20	139.43	121.22	78.86	41.68	**107.48**
NBeG 47	160.76	128.92	74.27	58.44	23.71	**89.22**
Mean	**144.35**	**130.04**	**109.10**	**86.79**	**43.39**	
Factors		**S.Em ±**			**CD @ 1%**	
Genotypes		0.92			2.62	
Treatments		0.84			2.39	
G×T		2.07			5.86	

the highest seedling vigor index under control conditions, with a value of 4795.36. In contrast, the lowest vigor index under control was observed in BGD 103, which recorded 3906.47. At the highest level of osmotic stress (20% PEG), ICCV 4958 exhibited the least reduction in vigor index, maintaining a relatively higher value of 1203.05. On the other hand, NBeG 47 showed the greatest decline in seedling vigor, with the lowest value of 528.03, indicating its higher sensitivity to osmotic stress.

Vigour Index II : Table 2 illustrates the effects of genotype, osmotic concentration, and their interactions on Vigour Index II. The data reveal significant variations in Vigour Index II across the different levels of osmotic stress, indicating that both genetic factors and increasing osmotic pressure notably influence seedling vigor under drought-simulated conditions. The highest mean Vigour Index II was observed under control conditions (0% PEG), with a value of 144.35, followed by 5% PEG at 130.04. A marked decline was noted at 20% PEG, where the mean Vigour Index II dropped significantly to 43.39, highlighting the adverse effect of increased osmotic stress. Among the genotypes, ICCV 4958 recorded the highest mean Vigour Index II (121.12), closely followed by BGD 111-1 (114.43) and GBM 2 (107.48), indicating their better tolerance to osmotic stress. In contrast, NBeG 47 showed the lowest Vigour Index II (89.22), followed by BGD 103 (91.99) and DBGV 204(92.17), reflecting their greater sensitivity to drought-like conditions. According to Macar *et al.* (2009) evaluating drought tolerance during germination and seedling establishment relies on key traits such as germination percentage, root length, and vigor index. In particular, the seed vigor index serves as an effective indicator, capturing both drought resistance and the overall quality of germination under stress conditions.

Conclusion

The study evaluated the effects of osmotic stress induced by polyethylene glycol (PEG 6000) on germination, seedling growth and seedling vigor across six chickpea genotypes. The findings revealed significant genotype-by-stress interactions, underscoring the genetic variability in drought tolerance among the tested genotypes. This variation highlights the potential for identifying and selecting drought-resilient genotypes for breeding programs aimed at improving crop performance under water-limited conditions.

Based on the research findings, it can be concluded that the chickpea genotypes ICCV 4958 and BGD 111-1 exhibited superior performance under drought-induced osmotic stress and can thus be classified as drought-tolerant. BGD 103 demonstrated moderate tolerance, while NBeG 47 and DBGV 204 showed poor seedling vigor and performance under higher PEG concentrations, indicating their sensitivity to drought conditions. These observations suggest that key traits such as germination percentage, root and shoot length, seedling dry weight, and vigor index can serve as reliable indicators for early-stage drought screening. Selecting genotypes based on these traits at the seedling stage allows for efficient, cost-effective, and less labor-intensive screening of large germplasm collections, thereby accelerating the identification and development of drought-resilient chickpea varieties.

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