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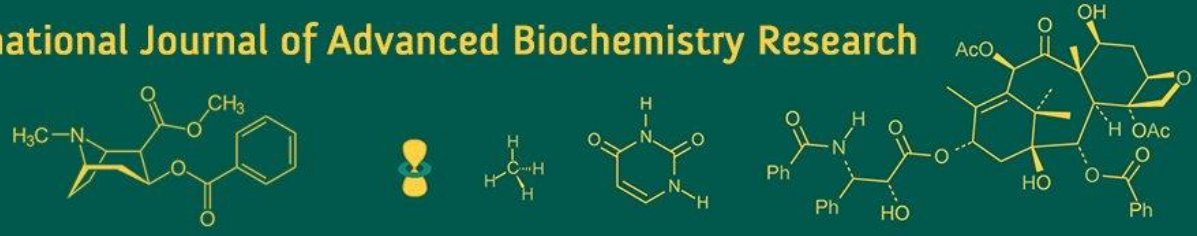

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In vitro screening of chickpea genotypes for drought tolerance using peg-induced osmotic stress

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Abstract
Drought is a major constraint affecting chickpea production, especially in semi-arid regions. This study evaluated five chickpea genotypes for drought tolerance using polyethylene glycol (PEG 6000) to simulate osmotic stress under *in vitro* conditions. Seed germination, shoot length, and root length were assessed under 0%, 5%, 7%, and 10% PEG treatments. Analysis of variance showed significant effects of PEG on all traits. Germination and shoot length decreased with increasing stress, with Phule Vishwaraj and BG-10216 showing higher tolerance. Interestingly, certain genotypes, particularly Phule Vishwaraj and BG-10216, maintained or increased root length under stress, indicating adaptive responses. These findings highlight genetic variation in drought tolerance and suggest that root growth could serve as an important selection trait in breeding programs. The study confirms the usefulness of *in vitro* screening as a fast and economical approach for identifying drought-tolerant chickpea genotypes and reports, increased root length under PEG stress in chickpea.

Keywords: Chickpea, drought tolerance, peg 6000, root length, *ın vitro* screening

Introduction
Chickpea (*Cicer arietinum* L.) is the third most important food legume after the common bean and field pea. It is a diploid, self-pollinated, cool-season crop with a 738 Mb genome encoding ~28,000 genes (Varshney *et al.*, 2013) [24]. Two main cultivar groups are recognized: Desi, characterized by small angular seeds with thick coloured seed coats, and Kabuli, with larger, smooth, beige/white seeds (Moreno *et al.*, 1978; Frimpong *et al.*, 2009; Zhang *et al.*, 2024) [5, 18, 26]. Desi types are widely grown in Asia and Africa, whereas Kabuli is prevalent in West Asia, North Africa, and developed countries. Chickpea is nutritionally dense, supplying carbohydrates, protein (~80% of seed dry weight), fibre, vitamins, and minerals, and is often referred to as "poor man's meat" (Barman *et al.*, 2012; Jukanti *et al.*, 2012) [3, 7].

In semi-arid regions, chickpea is mainly cultivated on marginal lands where limited and irregular rainfall exposes it to severe drought and high temperatures during flowering and maturity. Two types of drought affect chickpea: terminal drought, where soil moisture progressively declines at the end of the season, and intermittent drought, resulting from erratic and insufficient rainfall (Talebi *et al.*, 2013) [23]. Water is essential for seed metabolism, aiding the breakdown and transport of nutrients from stored reserves during germination and seedling growth (Macar *et al.*, 2009) [14]. Drought stress negatively impacts all growth stages, particularly germination and seedling emergence, which rely on rapid and uniform germination under low water availability (Arjenaki *et al.*, 2011) [1]. Limited water leads to reduced growth rates and impaired seedling development, underscoring the need for breeding drought-tolerant varieties. Conventional breeding is hindered by long cycles, restricted gene pools, and biological barriers. *In vitro* screening using polyethylene glycol (PEG) offers a cost-effective and efficient alternative to simulate drought stress, allowing the testing of multiple genotypes in a short period (Mishra *et al.*, 2021; Hamayun *et al.*, 2010) [6, 17]. High molecular weight PEG-6000 effectively mimics drought by lowering water potential without disrupting plant metabolism, making it a suitable compound for plant tissue culture studies (Khodarahmpour *et al.*, 2011) [11].

Considering these factors, the present study evaluated five chickpea genotypes under varying concentrations of PEG 6000 to identify potential drought-tolerant genotypes.

Material and Methods: The experiment was carried out at the Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Five chickpea genotypes BG-10216 (IARI, New Delhi), Phule Vishwaraj (MPKV, Rahuri), AKGK-1801, Gulak-1, and PDKV-Kabuli-4 (Dr. PDKV, Akola) were used as experimental material.

Uniform seeds from each genotype were selected and surface sterilized using 1% sodium hypochlorite for 5 minutes, followed by 70% alcohol for a few seconds, and rinsed three times with distilled water. PEG 6000 solutions of 0%, 5%, 7% and 10% were prepared by dissolving 0 g, 5 g, 7 g, and 10 g of PEG in distilled water and making up the volume to 100 ml. Each experimental unit was treated with 15 ml of the respective solution. Four seeds per genotype were placed on blotting paper in a petri dish in a circular arrangement. The experiment was laid out in a completely randomized design with three replications per treatment. Petri dishes were sealed with parafilm to prevent evaporation before incubation. Seeds were incubated at 25°C, kept in the dark for the first four days, and then exposed to 16 hours of white light for the next eight days. Germination percentage was recorded on day 4, while seedling traits shoot length, root length were measured on day 12. The data were analyzed using ANOVA, and genotypes were ranked based on their performance under control and stress conditions to identify superior ones.

Results and Discussion: *In vitro* screening was conducted to assess variability in drought-related traits at the seedling stage. Mean data for different traits shoot length, root length, and germination percentage were analyzed using ANOVA. Significant variability among genotypes was observed, and the interaction between PEG levels and genotypes indicated that drought responses varied across treatments is shown in Plate.1.

Germination Percentage: The effect of PEG-induced osmotic stress on seed germination percentage is shown in Table.1 and Fig.1. ANOVA revealed that PEG treatment significantly reduced germination across all tested genotypes. Higher osmotic stress led to a greater decline in germination. BG-10216 and Phule Vishwaraj recorded the highest germination rates under both control (98.33% and 96.67%) and 10% stress (93.33% and 91.25%), while PDKV Kabuli-4 (66.67%), AKGK-1801 (70%), and Gulak-1 (74.33%) had the lowest rates. The overall mean germination decreased from 91.87% (unstressed) to 79.20% (10% stress). These results align with previous studies reporting reduced chickpea germination under osmotic stress (Mbarek *et al.*, 2013; Awari & Mate, 2015; Dharanguttiker *et al.*, 2015; Koskosidis *et al.*, 2020; Vus, 2020; Masomi *et al.*, 2023) [2, 12, 15, 16, 25], mainly due to restricted water uptake and delayed metabolic activation, along with hormonal imbalances such as increased ABA and decreased GA activity.

Shoot length

Shoot length is a sensitive indicator of drought stress in chickpea and decreases as osmotic stress increases. Measured after 12 days of growth, shoot length varied among genotypes in response to PEG-induced stress (Table.2, Fig.2). Analysis showed that PEG had a significant effect on shoot length, which declined from an average of 3.59 cm (control) to 2.04 cm at 10% PEG. Under control conditions, shoot length ranged from 2.33 to 4.93 cm, with all genotypes showing reductions under stress. Phule Vishwaraj maintained the highest shoot length under both conditions, indicating better tolerance, while AKGK-1801 and PKV-K-4 showed the largest declines, suggesting higher sensitivity. These results confirm that osmotic stress hampers seedling growth, consistent with earlier studies (Romo *et al.*, 2001; Kandil *et al.*, 2012; Mbarek *et al.*, 2013; Dharanguttikar *et al.*, 2015; Awari & Mate, 2015) [2, 4, 8, 16, 21], likely due to reduced cell expansion, turgor loss, hormonal imbalance, and oxidative damage.

Root length

Root traits are important for drought adaptation in chickpea. Enhanced root growth helps in extracting water and improving yield under stress (Kashiwagi *et al.*, 2006) [9]. Root length is a key trait used to assess drought tolerance (Kumar *et al.*, 2012) [13]. Typically, osmotic stress reduces root length by limiting cell expansion (Mujtaba *et al.*, 2016) [19], but in this study, root length increased with stress (Table.2, Fig.2). After 12 days, root length varied among genotypes. Under control conditions, it ranged from 2.20 cm (BG-10216) to 7.03 cm (AKGK-1801). Under 10% PEG stress, Phule Vishwaraj (5.80 cm) and BG-10216 (4.10 cm) increased root length, while others showed declines. This shows differences in drought tolerance, with some genotypes maintaining or enhancing growth under stress. This is the first report of increased root length under PEG stress in chickpea, though similar results were seen in lentil (Kaur *et al.*, 2011; Swathi *et al.*, 2017). Increased root length is an indicator of drought tolerance potential (Rohit *et al.*, 2020) [10, 20, 22].

Conclusions

In vitro screening is an effective and cost-efficient method for initially assessing variability. It saves time and creates uniform drought-like conditions, which are difficult to achieve consistently in field trials. The present study demonstrated that PEG-induced osmotic stress significantly affected seed germination, shoot length, and root length in chickpea. Germination and shoot growth declined with increasing stress, highlighting the sensitivity of these traits to water deficit. In contrast, certain genotypes, such as Phule Vishwaraj and BG-10216, were able to maintain or even enhance root growth under stress, indicating adaptive responses to drought. These results reveal genotypic variation in drought tolerance and suggest that root traits, in particular, may serve as important selection criteria in breeding programs aimed at improving drought resilience in chickpea. The findings align with previous research and contribute new insights, including the report of increased root length in chickpea under *in vitro* osmotic stress.

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Table 1: Effect of *in vitro* screening of chickpea germplasms using a discriminating dose of PEG 6000 on seed germination percentage

Sr.No.	Genotypes	Unstressed (0% PEG)	Stressed (10% PEG)	Percentage decrease in germination
1	BG-10216	98.33	93.33	5.0
2	Phule Vishwaraj	96.67	91.67	5.0
3	AKGK-1801	82.70	70.00	12.7
4	Gulak-1	93.33	74.33	19.0
5	PDKV-Kabuli-4	88.33	66.67	21.6
AVERAGE		91.87	79.20	-
SE(M)±		2.97	4.72	-
CD 5%		9.67	15.40	-
CD 1%		14.08	22.41	-

Table 2: Effect of *in vitro* screening of chickpea germplasms using a discriminating dose of PEG 6000 on shoot length and root length

		Shoot length (cm)			Root length (cm)		
Sr. No.	Genotypes	Unstressed (0%) PEG	Stressed (10%) PEG	Decrease in the shoot length over stressed	Unstressed (0%) PEG	Stressed (10%) PEG	Increase in root length over stress
1	BG-10216	2.33	1.83	0.5	2.20	4.1	5.3
2	Phule Vishwaraj	4.93	4.07	0.86	5.43	5.8	4
3	AKGK-1801	4.3	1.3	3.0	7.03	1.5	1.8
4	Gulak-1	3.40	1.73	1.67	4.17	1.3	2
5	PDKV-Kabuli-4	2.93	1.32	1.61	4.57	1.40	1.15
AVERAGE		3.59	2.04	-	4.68	2.81	-
SE(M)±		0.43	0.30	-	0.79	0.53	-
CD 5%		1.39	0.97	-	2.57	1.74	-
CD 1%		2.03	1.41	-	3.74	2.53	-

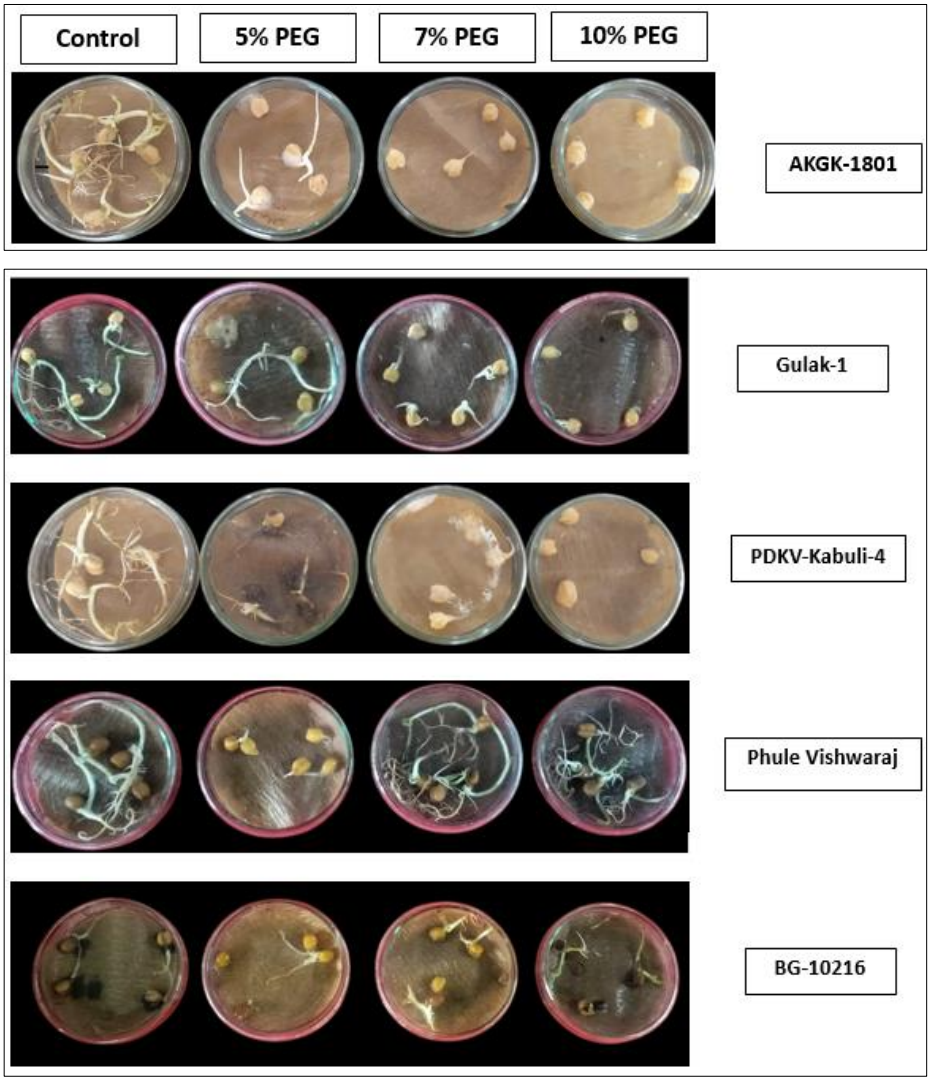

Plate 1: *In-vitro* screening of chickpea germplasm for drought tolerance using PEG-6000

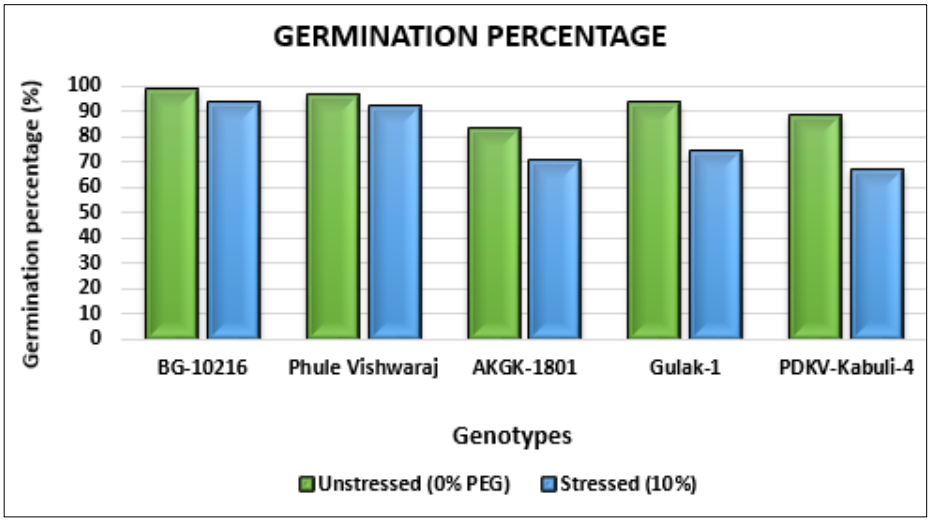

Fig 1: Effect of discriminating dose of PEG-6000 on the Germination percentage of chickpea germplasm

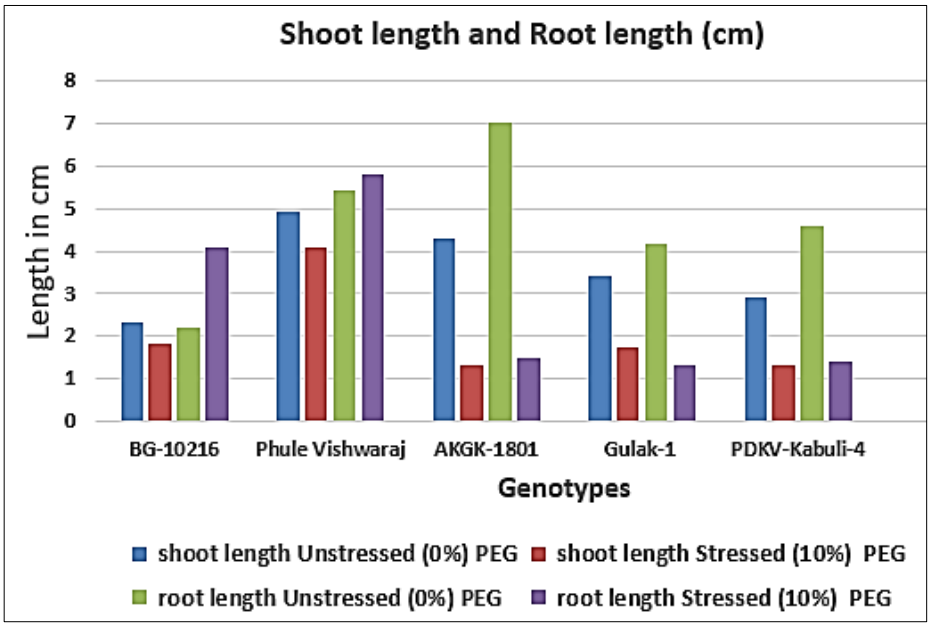

Fig 2: Effect of discriminating dose of PEG-6000 on shoot and root lengths of chickpea germplasm

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