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Identification of Drought Tolerant Chickpea Genotypes under Simulated Drought

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important legume crops worldwide, with high protein content. Chickpea productivity is highly threatened by abiotic stresses, of which drought exerts the most crucial role in terms of growth inhibition and yield losses encountered. Since germination is a critical stage that is negatively affected by drought, an experiment was conducted to estimate the genotypic variability among 27 chickpea genotypes and to determine the seed germination and seedling growth ability under water stress conditions. Seeds were subjected to water stress by using polyethylene glycol (PEG-6000) at five stress levels (0, 5, 10, 15 and 20 % PEG). Among the genotype DIBG 205 (94.50%) recorded highest germination percentage

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compared to other genotypes. Root length recorded was highest in ICCV 4958 (18.48 cm) under 10% PEG -6000 and decreased with increase in osmotic stress. Drought significantly affected germination as well as all other associated traits with the effects of stress being analogous to the stress level applied. Seedling vigour index is a suitable selection criterion for drought tolerance, high seedling vigour index was observed in DIBG 205 and ICCV 4958 showing increased drought tolerance at high stress level, whereas low seedling vigour index was recorded in ICCV 201217 and ICCV 201116 indicating their possible exploitation as valuable genetic material for further breeding programs for drought tolerance.

Keywords: Chickpea; genotypes; cool season leguminous crop.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a self-pollinated, cool season leguminous crop and originated from south east turkey. Belongs to the Fabaceae family. It is also known as gram, Chhana or Egyptian pea and is one of the earliest cultivated legumes. Chickpea is the third most important pulse crop globally, after common bean and pea. Globally it is grown on a 159.66 lakh ha area, with a total production of 182.25 lakh tonnes [1] and an average productivity of 1356 kg/ha. According to India (MOAFW), in the year 2021–22 Madhya Pradesh stands first place in area production productivity in chickpea followed by Maharashtra [2]. Chickpea is a good source of protein with significant amounts of all the essential amino acids. Starch is the major storage carbohydrate for simple sugars like glucose and sucrose. Though chickpea has a low lipid content, it is rich in nutritionally important unsaturated fatty acids. Chickpea is also a good source of nutrients like calcium, magnesium, phosphorus, and especially potassium.

Chickpea productivity is restricted by a number of abiotic and biotic variables. Drought is the most significant abiotic constraint on chickpea production. The availability of soil moisture decreases over time during a terminal drought, potentially resulting in severe drought stress later in crop development. Chickpeas are suffering from terminal drought stress due to its cultivation on marginal terrain [3].

Low moisture availability at sowing stage adversely affects germination and seedling Hassan et al, [4]. Considering the ongoing climate change as well as the gradual decline of available water resources, research efforts towards developing of drought tolerant germplasm is of paramount Khalil and Ahmed, [5] Breeding for drought tolerance by evaluation of yield performance under water-deficit stress conditions in the field is a difficult procedure due to the difficulties both in terms of obtaining homogeneous stress conditions and identifying drought tolerant genotypes [6] is rather slow and laborious. As an alternative, Richards [7] marked germination phase as the most sensitive stage in chickpea lifecycle and proposed that screening at this phase brings suitable genotype for drought tolerance. Further, seedling vigour index, which combines seed germination rate with seedling length, is a suitable index for selecting drought tolerance at germination [8]. *In vitro* screening finds an alternative method that is useful over the traditional approaches as it is reliable and time-effective. In this method, stress is induced through the use of osmotic solutions, among which Polyethylene glycol (PEG) has proven as potential Hassan et al. [9]. PEG is capable of imposing water stress by decreasing the osmotic potential without entering into the plant cells [10]. The objective of this experiment was to study the effect of PEG-induced drought stress in germination and seedling vigour index as parameters for screening chickpea genotypes in selecting drought tolerance which could to be employed for breeding purposes or for commercial use.

2. MATERIALS AND METHODOLOGY

The germination study conducted in seed unit laboratory, Department of seed science and technology, UAS, Dharwad. During (2021). The experimental application of poly ethylene glycol (PEG) has proven its ability to mimic drought stress conditions by generating osmotic stress, resulting in a decrease in plant water potential. In order to assess the response of chickpea genotypes across different PEG concentrations, twenty seven chickpea genotypes were characterized under four osmotic stress condition 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). This experiment aids in analyzing the germination percentage and seedling growth behaviour of all the examined chickpea

genotypes across various osmotic stress levels. Based on the performance displayed by these genotypes, fifteen specific chickpea genotypes were chosen for further field evaluation. The concentrations of PEG-6000 required to obtaining these values were determined by using the equation of Michel and Kaufmann [11]. The experiment was laid out in a completely randomized design (CRD) with two replications.

$\Psi s = - (1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C_2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C_2T$,

Where Ψs = osmotic potential (MPa)
C = concentration (g L^{-1} PEG in water)
T = temperature (ºC).

JG-11, BGD 111-1, BGD 103, BGD 133, BGD 138, BGD 1536, BGD 225, BGD 7050, BGD 163, ICCV 191608, DBGV 213, ICCV 191102, DBGV 210, ICCV 19113,ICCV 21204, ICCV 19110,JAKI 9218,ICCV 4958,ICCV 20121,A-1, DBGV 204, SA 1, NBeG 506,DIBG 205, DBGV 206, ICCV 201111 and ICCV 201116.

Chickpea seeds were surface sterilized using sodium hypochlorite solution (2%, v/v) for 5 minutes. After that, seeds were treated with various concentrations of polyethylene glycol 6000 (PEG 6000). Distilled water was employed as a control. Two sets of 50 seeds from each genotype were evenly distributed across two Germitest® germination paper sheets. These sheets were soaked with different PEG solutions in an amount equal to 2.5 times the paper's dried mass, and then rolled. These rolls were subsequently sealed in plastic containers to prevent evaporation and maintain a humidity level close to 100 per cent. The germination trials were carried out in a germinator, maintaining a constant temperature of 25 °C (within the range of 24-26 °C) with adequate lighting. Seeds were considered germinated when the radicle length exceeded 1.0 mm, in accordance with Koskosidis et al. [12]. The data were recorded 15 days after germination of treated chickpea seeds. The following section provides a detailed description of the observations that were recorded during germination study.

Observation recorded is seed germination, Shoot length, Root length and Seedling dry weight on the 15 th day after the germination test

Seed germination (%):

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

Seedling vigour indices: The seedling vigour indices *viz*, seedling vigour index I and seedling vigour index II was calculated by adopting the method as suggested by Abdul and Anderson [13] and expressed in number by using following formula.

Seedling vigour index I (SVI I) = Germination percentage (%) × Seedling length (cm)

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

3. RESULTS AND DISCUSSION

3.1 Germination Percentage (%)

Germination is one of the most critical and important stage, as it is directly correlated with seedling establishment and early growth [14] and also a suitable criterion for screening drought tolerance. Among PEG concentrations, the maximum mean germination percentage (85.36 %) was recorded in control 0% PEG. And least germination was recorded at 20% PEG concentration. The maximum germination percentage was recorded in DIBG 205 (94.50 %) in control. And lowest germination percentage was recorded in ICCV 201217 (79.25 %), with an increase in osmotic stress condition, there was a decrease in germination percentage. However, Zero germination recorded occurred with increasing osmotic stress under 20 % PEG in ICCV 201217 and ICCV 201204 genotypes. DIBG 205 recorded the highest germination percentage across all the PEG concentrations among all the genotypes. Similar results were obtained by Yucel et al. [15] who studied response of chickpea to drought stress by PEG and also recorded no germination at -0.8 Mpa for chickpea and it was proposed as the threshold osmotic potential.

3.2 Shoot Length (cm)

The highest shoot length was recorded in control compared to other PEG concentrations. Shoot length decreased as the concentration of PEG increased and the mean shoot length under 0% PEG was 16.85cm and decreased under 5, 10, 15 and 20 % PEG (15.69, 13.11, 10.38 and 7.84 cm respectively). Among the genotypes, the mean highest shoot length was recorded in DBGV 206 (22.26 cm) in control (0% PEG). And lowest shoot length was recorded in BGD 163(11.90 cm). Decreased osmotic potential leads to a more drastic inhibition of shoot tissue elongation and the similar findings were

observed by Jamaati et al. [16]. Shoot length was major factor which was affected more under osmotic stress, decreased osmotic potential leads to a more drastic inhibition of shoot tissue elongation and the similar findings were observed by Jamaati et al. [16].

3.3 Root Length (cm)

Mean root length was recorded highest in 10% PEG treatment (12.91 cm) compare to the control 0% PEG (10.54 cm). Among the genotypes under 10% PEG treatment, the highest root length was recorded in ICCV 4958 (18.48 cm) had recorded highest root length and the mean lowest root length recorded under 10% PEG treatment are BGD 7050 (9.48 cm), Among all the PEG concentration mean highest root length recorded in ICCV 4958 and DIBG 205 (15.79 and 15.23 cm) ICCV 201116 and ICCV 201217 (6.59 and 6.67 cm) recorded lowest root length. In the highest osmotic stress condition at 20% PEG, genotypes ICCV 4958 had showed less reduction in root length. Similar findings were observed by Babu et al. (2014) who worked on cotton with five different concentration and high root length was recorded in 10% PEG (-0.148). 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.

3.4 Seedling Dry Weight

Mean highest seedling dry weight recorded highest in control 0% PEG 1.38 g and the lowest recorded in 20% PEG treatment 0.60g. Among the treatments highest seedling dry weight recorded in DIBG 205 (1.82 g) and lowest was recorded in ICCV 201217 (1.06g) similarly high level of PEG concentration DIBG 205 recorded highest seedling dry weight lowest seedling dry weight recorded in NBeG 506. The decrease could be due to the damage caused to meristem cells of root and shoot by drought which disrupted the cell division and elongation process. Another possible reason might be that lowered water absorption by cells under drought conditions decreased the turgor pressure of cells which accelerated the growth retardation [17].

Table 1. Effect of osmotic stress on germination percentage (%) under different PEG concentration in chickpea genotypes

Genotypes	Germination percentage (%)					
	0%PEG (Control)	5 % Peg	10 % Peg	15 % Peg	20% Peg	Mean
JG-11	90.65	88.25	86.50	79.50	63.25	**81.63**
BGD 111-1	87.25	83.75	83.00	75.50	57.25	**77.35**
BGD 103	85.75	85.75	81.00	72.75	54.25	**75.90**
BGD 133	84.50	81.00	79.25	72.75	53.00	**74.10**
BGD 138	83.25	78.00	75.50	72.25	48.00	**71.40**
BGD 1536	82.25	78.75	75.00	70.25	45.00	**70.25**
BGD 225	86.75	83.75	80.25	73.00	54.50	**75.65**
BGD 7050	82.25	77.50	75.50	68.00	36.00	**67.85**
BGD 163	81.25	77.75	70.60	52.25	0.00	**56.37**
ICCV 191608	89.25	85.25	84.00	76.25	56.50	**78.25**
DBGV 213	81.25	78.50	78.25	69.00	52.25	**71.85**
ICCV 191102	82.25	78.75	76.25	65.25	49.25	**70.35**
DBGV 210	82.50	80.75	77.75	69.25	51.25	**72.30**
ICCV 19113	82.25	78.75	79.25	71.75	46.50	**71.70**
ICCV 201204	80.25	76.00	70.50	51.50	0.00	**55.65**
ICCV 191106	85.25	80.25	79.25	69.75	52.50	**73.40**
JAKI-9218	86.25	84.75	82.50	75.50	64.50	**78.70**
ICCV 4958	92.18	91.75	90.25	83.00	61.50	**83.75**
ICCV 201217	79.25	75.75	67.50	47.00	0.00	**53.90**
A-1	92.25	90.75	87.75	78.50	58.50	**81.55**
DBGV 204	84.25	79.75	70.50	63.00	44.50	**68.40**
SA 1	85.40	82.25	80.25	71.00	52.50	**74.28**
NBeG 506	91.70	90.50	90.25	81.00	59.50	**82.59**
DIBG 205	94.50	92.75	91.25	82.25	63.55	**84.85**
DBGV 206	90.15	88.25	86.50	79.00	63.50	**81.48**
ICCV 201111	82.30	80.23	78.53	70.24	64.32	**75.12**
ICCV 201116	79.50	76.00	69.60	47.50	0.00	**54.52**
Mean	**85.36**	**82.36**	**79.24**	**69.60**	**45.96**	

Table 2. Effect of osmotic stress on shoot length under different PEG concentration in chickpea genotypes

Genotypes	Shoot length (cm)					
	0% Peg (Control)	5 % Peg	10 % Peg	15 % Peg	20% Peg	Mean
JG-11	20.00	19.50	16.70	13.25	11.26	**16.14**
BGD 111-1	20.65	19.05	16.85	12.45	10.60	**15.92**
BGD 103	18.75	17.60	14.30	11.50	10.02	**14.43**
BGD 133	17.40	16.65	13.65	9.75	9.50	**13.39**
BGD 138	13.15	12.65	9.85	7.40	7.50	**10.11**
BGD 1536	13.40	12.65	9.25	7.05	6.65	**9.80**
BGD 225	18.65	16.75	13.35	11.70	10.45	**14.18**
BGD 7050	13.10	11.80	9.45	6.45	6.12	**9.38**
BGD 163	11.90	11.55	8.75	6.20	0.00	**7.68**
ICCV 191608	18.90	16.55	14.05	10.85	9.50	**13.97**
DBGV 213	13.05	11.80	11.60	9.05	7.55	**10.61**
ICCV 191102	12.35	11.35	9.35	6.80	6.71	**9.31**
DBGV 210	13.75	11.80	9.35	8.25	6.95	**10.02**
ICCV 19113	11.95	10.86	8.80	8.10	7.12	**9.37**
ICCV 201204	13.85	11.85	9.30	6.40	0.00	**8.28**
ICCV 191106	19.35	18.15	14.80	12.15	9.23	**14.74**
JAKI-9218	18.75	17.60	15.15	11.10	10.65	**14.65**
ICCV 4958	22.10	20.65	17.80	14.23	10.23	**17.00**
ICCV 201217	11.92	11.60	8.95	6.90	0.00	**7.87**
A-1	20.15	20.15	17.45	16.30	10.65	**16.94**
DBGV 204	14.95	13.10	10.35	8.90	7.00	**10.86**
SA 1	21.10	20.80	18.65	14.80	11.35	**17.34**
NBeG 506	20.85	19.45	16.10	12.60	9.75	**15.75**
DIBG 205	21.00	19.45	18.35	15.40	11.45	**17.13**
DBGV 206	22.26	22.25	18.50	14.55	11.95	**17.90**
ICCV 201111	19.63	17.23	15.21	12.32	9.51	**14.78**
ICCV 201116	12.05	10.70	7.95	5.90	0.00	**7.32**
Mean	**16.85**	**15.69**	**13.11**	**10.38**	**7.84**	

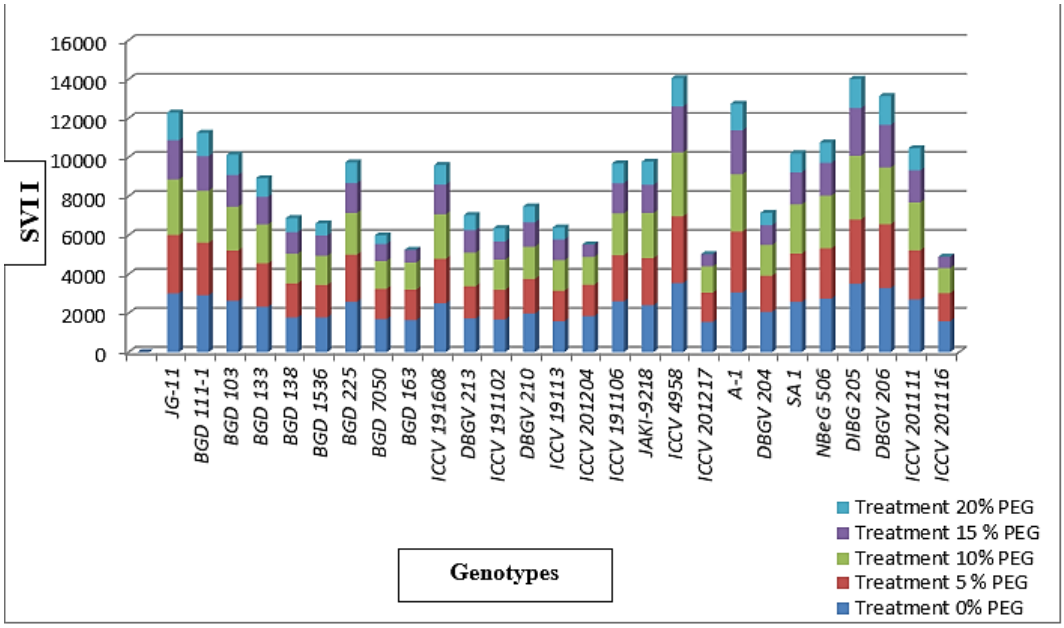

Fig. 1. Graphical representation of effect of osmotic stress on seedling vigour index I in Chickpea genotypes

Table 3. Effect of osmotic stress on root length under different PEG concentration in chickpea genotypes

Genotypes	Root length (cm)					
	0% Peg (Control)	5 % Peg	10 % Peg	15 % Peg	20% Peg	Mean
JG-11	13.30	14.41	16.25	12.00	11.20	**13.43**
BGD 111-1	12.85	13.07	15.30	11.05	10.30	**12.51**
BGD 103	12.00	12.45	13.46	10.85	9.12	**11.58**
BGD 133	10.13	10.89	11.51	9.75	8.52	**10.16**
BGD 138	8.28	9.72	10.30	7.99	7.36	**8.73**
BGD 1536	8.25	8.51	10.65	7.85	7.13	**8.48**
BGD 225	11.15	12.07	13.37	9.25	9.02	**10.97**
BGD 7050	7.50	8.24	9.48	6.45	6.00	**7.53**
BGD 163	8.38	8.54	10.95	6.20	0.00	**6.81**
ICCV 191608	9.30	10.09	13.18	9.10	8.23	**9.98**
DBGV 213	8.27	9.15	10.48	7.70	7.20	**8.56**
ICCV 191102	8.00	8.02	11.00	7.40	7.12	**8.31**
DBGV 210	10.25	10.24	11.87	9.75	9.03	**10.23**
ICCV 19113	7.33	9.05	11.01	6.65	6.15	**8.04**
ICCV 201204	9.22	9.30	10.98	5.95	0.00	**7.09**
ICCV 191106	11.30	11.30	12.23	10.05	10.02	**10.98**
JAKI-9218	9.11	11.05	12.81	8.05	7.80	**9.76**
ICCV 4958	16.37	16.65	18.48	14.23	13.24	**15.79**
ICCV 201217	7.67	8.26	11.00	6.42	0.00	**6.67**
A-1	13.04	14.29	16.02	12.42	12.63	**13.68**
DBGV 204	9.62	10.08	12.12	7.23	7.05	**9.22**
SA 1	9.15	9.26	12.90	8.30	7.15	**9.35**
NBeG 506	9.11	9.02	13.89	8.20	7.65	**9.57**
DIBG 205	16.07	16.13	17.51	14.32	12.14	**15.23**
DBGV 206	14.23	14.79	15.20	13.15	11.25	**13.72**
ICCV 201111	13.21	14.23	16.00	11.13	8.21	**12.56**
ICCV 201116	7.95	8.17	10.60	6.21	0.00	**6.59**
Mean	**10.54**	**11.04**	**12.91**	**9.17**	**7.54**	

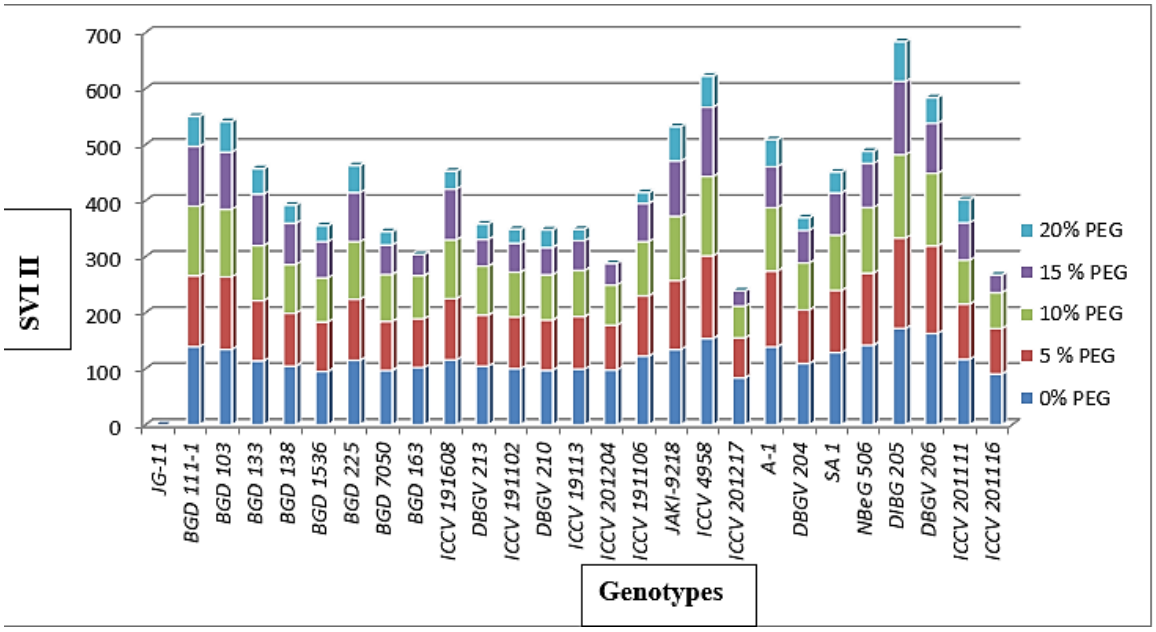

Fig. 2. Graphical representation of effect of osmotic stress on seedling vigour index II in Chickpea genotypes

Table 4. Effect of osmotic stress on seedling dry weight under PEG concentration in chickpea genotypes

Genotypes	Seedling dry weight (g)					
	0%Peg (Control)	5 % Peg	10 % Peg	15 % Peg	20% Peg	Mean
JG-11	1.63	1.59	1.47	1.41	0.75	**1.37**
BGD 111-1	1.60	1.51	1.50	1.41	0.94	**1.39**
BGD 103	1.57	1.51	1.49	1.40	1.05	**1.40**
BGD 133	1.35	1.33	1.24	1.26	0.86	**1.21**
BGD 138	1.26	1.21	1.15	1.02	0.68	**1.06**
BGD 1536	1.16	1.12	1.05	0.92	0.64	**0.98**
BGD 225	1.33	1.30	1.28	1.20	0.89	**1.20**
BGD 7050	1.18	1.13	1.11	0.77	0.67	**0.97**
BGD 163	1.26	1.12	1.09	0.72	0.00	**0.84**
ICCV 191608	1.30	1.28	1.25	1.19	0.57	**1.12**
DBGV 213	1.29	1.16	1.12	0.69	0.53	**0.96**
ICCV 191102	1.22	1.17	1.05	0.79	0.51	**0.95**
DBGV 210	1.18	1.11	1.04	0.70	0.62	**0.93**
ICCV 19113	1.21	1.19	1.04	0.74	0.44	**0.92**
ICCV 201204	1.22	1.05	1.02	0.75	0.00	**0.81**
ICCV 191106	1.44	1.34	1.22	0.98	0.37	**1.07**
JAKI-9218	1.56	1.45	1.39	1.31	0.82	**1.31**
ICCV 4958	1.67	1.61	1.57	1.49	0.90	**1.45**
ICCV 201217	1.06	0.94	0.84	0.58	0.00	**0.68**
A-1	1.51	1.49	1.29	0.93	0.83	**1.21**
DBGV 204	1.30	1.20	1.19	0.92	0.51	**1.02**
SA 1	1.51	1.35	1.23	1.06	0.71	**1.17**
NBeG 506	1.55	1.42	1.30	0.97	0.37	**1.12**
DIBG 205	1.82	1.74	1.63	1.59	1.12	**1.58**
DBGV 206	1.81	1.77	1.50	1.13	0.72	**1.39**
ICCV 201111	1.42	1.23	1.00	0.95	0.64	**1.05**
ICCV 201116	1.14	1.07	0.92	0.66	0.00	**0.76**
Mean	**1.38**	**1.31**	**1.22**	**1.02**	**0.60**	

3.5 Seedling Vigour Index I and II

Mean seedling vigour index I was recorded highest in control, 0% PEG (2369.84), and the lowest (847.70) recorded in high osmotic stress (20% PEG). Seedling vigour index I showed less reduction in 5% PEG concentration (2239.39) compared to control and seedling vigour index I decreased with increase in osmotic stress concentration. Among the genotypes, in control (0% PEG) ICCV 4958 (3548.86), recorded the highest seedling vigour index I. And lowest recorded in ICCV 201217 (1550.92). Seedling vigour index II was recorded in ICCV 201116 and ICCV 201217 (978.09 and 1005.60). The vigour index decreased with the increase in osmotic levels. Significantly higher mean vigour index II was exhibited under control 0% PEG (118.91) and lowest in 20% (32.95). Among the genotypes, the highest were recorded in control (0% PEG) DIBG 205 (171.99). The lowest seedling vigour index was recorded in ICCV 201217 (84.01).

4. CONCLUSION

In conclusion, keeping in view the above stated research finding it can be concluded that the two chickpea genotypes DIBG 205 and ICCV 4958 performed better under drought conditions and hence can be declared as drought tolerant while ICCV201117 and ICCV201116 genotypes of chickpea were regarded drought sensitive. Selection can be made on the basis of these characters at early growth stage to screen a large population for drought stress. It would be cost effective, less time consuming and less laborious to screen the germplasm at early growth stage.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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