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IDENTIFICATION OF DROUGHT-TOLERANT KABULI CHICKPEA (*CICER ARIETINUM* L.) GENOTYPES AT THE EARLY SEEDLING STAGE

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SUMMARY

Chickpea (*Cicer arietinum* L.) is negatively affected by drought stress at all the growth stages, including germination and seedling emergence. The recent study aimed to investigate the drought-tolerant Kabuli chickpea genotypes at the seedling stage under osmotic stress conditions. An *in vitro* screening technique evaluated the 120 different genotypes of Kabuli chickpea for drought tolerance by using different concentrations of polyethylene glycol (8000) solution, viz., T_0: Control, T_1: -0.19 MPa, and T_2: -0.47 MPa, arranged in a completely randomized design. Data were recorded on germination percentage, root length, shoot length, seedling length, root/shoot ratio, dry matter, seedling vigor index, and proline content. The results of a pooled analysis of variance revealed significant variability among genotypes, different polyethylene glycol treatments, as well as, between genotypes and treatments. The increase of polyethylene glycol levels negatively affected most of the parameters under study, except for proline content, which increased with an increase in polyethylene glycol concentration. The study results indicated that the advanced lines, 15KCC-106, 13KCC-114, 6KCC-103, GP-37, FS-10, and 12KCC-106, performed better under different osmotic stress conditions and gained selection as drought-tolerant advanced lines at early seedling stage. These advanced lines suitably combine with their desirable traits to cope with the drought condition and can serve as a baseline for the improvement of Kabuli chickpea breeding material for drought tolerance.

Keywords: Kabuli chickpea, drought tolerance, polyethylene glycol, proline

Key Findings: The breeding lines, 15KCC-106, 13KCC-114, 6KCC-103, GP-37, FS-10, and 12KCC-106 were identified as drought tolerant at the seedling stage and will be used in future breeding programs for the development of drought-tolerant Kabuli chickpea genotypes.

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INTRODUCTION

Currently, chickpea (*Cicer arietinum* L.) places the third most essential pulses crop in terms of worldwide production, after *Phaseolus vulgaris* L. and *Pisum sativum* L., with a yield of above 14.7 million mt. Its global production has varied over the last five years due to

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climatic changes. Pakistan ranked third among chickpea-producing countries worldwide, with an annual production of 319,000 t obtained from 867,000 ha of total cultivated area (GOP, 2021 2022). Although chickpea is considered as drought-tolerant crop, some researchers have reported significant harmful effects during its production due to drought stress (Rani *et al*., 2020; Shah *et al*., 2020). Thus, the need to develop climate-resilient varieties that perform better under the prevailing climatic conditions (Massawe *et al*., 2015; Korres *et al*., 2016; Tripathi *et al*., 2016). As current change in climate resulted to spatio temporal unpredictability in the amount and distribution of rainfall, hence, it is the major source of drought stress across the world (Azam *et al*., 2020; Mengistu *et al*., 2020; Yang *et al*., 2021).

Drought can be mitigated by the development of drought-tolerant genotypes. Therefore, a viable and practical approach requires the screening of drought-resistant genotypes from existing available germplasm. For drought-stress measurement at the seedling stage, polyethylene glycol (PEG) chemical of higher molecular weight has been used to arouse osmotic stress effect for plants to sustain consistent water potential during the investigational period (Salma *et al*., 2016). With the help of this technique, the drought-tolerant genotypes can be selected easily and economically from large germplasms at the seedling stage. Eight Kabuli chickpea (*C. arietinum* L.) genotypes were evaluated under lab conditions with four different osmotic pressures treatments: -0.33, -4, -6, and -8 bars created by polyethylene glycol 8000. Osmotic water stress negatively affected seedling characters and enhanced proline accumulation (Mbarek *et al*., 2013). Varshini *et al*. (2018) standardized the different concentrations of PEG 6000 on different genotypes of chickpeas. The report described that the concentration of PEG showed significant effects on germination, seedling length, and seedling vigor index. Koskosidis *et al*. (2020) also studied the effect of different concentrations of the PEG solution and found most of the characters got adversely affected by the increase in PEG concentration.

The correlation coefficient demonstrates linear association among various characters. The main objective of the correlation studies serves to identify mainly the relationship between yield and yield components. The determination of the relationship between different traits assists to achieve optimum combinations of characters in chickpea for attaining higher yield (Singh *et al*., 2016; Astereki *et al*., 2017 Hegde *et al*., 2018). Hence, selection practiced for one character may simultaneously bring changes in the other trait. Thus, to effect change in a character in the desired direction, it is almost mandatory to have an in-depth understanding of the association among contributing traits (Sudhanshu and Devendra, 2014; Jamil *et al*., 2022).

Therefore, the latest work focused to identify different drought-tolerant chickpea advanced lines at the seedling stage under PEG-created drought conditions. The finest Kabuli chickpea advanced lines selected based on the seedling characters for drought tolerance will be used in future breeding programs for the development of drought-tolerant Kabuli chickpea genotypes.

MATERIALS AND METHODS

The study set up the experiment in the laboratory of the Department of Plant Breeding and Genetics, PMAS Arid Agriculture University Rawalpindi, Pakistan, in 2018. The seeds of 120 different Kabuli chickpea (*C. arietinum* L.) genotypes were collected from Barani Agricultural Research Institute, Chakwal; Nuclear Institute for Agriculture and Biology, Faisalabad; Arid Zone Research Institute, Bakkhar; and the Pulses Research Institute (PRI), AARI, Faisalabad (Table 1). The seeds of all the cultivars were grown in petri dishes to observe drought tolerance under the different concentration levels of PEG (8000) solution, viz., T_0: Control, T_1: -0.19 MPa, and T_2: -0.47 MPa. Chickpea seeds received sterilization in 6% sodium hypochlorite solution for 5 min and 75% ethanol for 3 min. The seeds were then rinsed with distilled water. Five mature seeds per genotype were grown in each petri dish by using the completely randomized design with two replications and kept in the growth chamber under controlled conditions like temperature, relative humidity, and light intensity as described by Mbarek *et al.* (2013). The germination count got noted after every eight days at intervals from sowing and expressed in percent.

Data recorded

The recorded data consisted of the following characters after 25 days. Germination percentage calculation**:** the mean values of the germinated seeds from each replication and each treatment used the following formula

Table 1. The Kabuli chickpea genotypes collected from various research institutes in Pakistan.

Sr. Code	Genotype No.	Origin	Sr. Code	Genotype No.	Origin	Sr. Code	Genotype. No	Origin
G1	17KCC-101	BARI	G41	13KCC-114	BARI	G81	6KCC-103	BARI
G2	17KCC-105	BARI	G42	13KCC-115	BARI	G82	6KCC-121	BARI
G3	17KCC-106	BARI	G43	13KCC-116	BARI	G83	6KCC-124	BARI
G4	17KCC-107	BARI	G44	12KCC-101	BARI	G84	6KCC-126	BARI
G5	17KCC-108	BARI	G45	12KCC-103	BARI	G85	09AG-15	AZRI
G6	17KCC-109	BARI	G46	12KCC-104	BARI	G86	09AG-37	AZRI
G7	17KCC-114	BARI	G47	12KCC-105	BARI	G87	11AG-38	AZRI
G8	17KCC-115	BARI	G48	12KCC-106	BARI	G88	11AG-41	AZRI
G9	17KCC-116	BARI	G49	12KCC-108	BARI	G89	11AG-43	AZRI
G10	17KCC-117	BARI	G50	12KCC-109	BARI	G90	11AG-48	AZRI
G11	17KCC-118	BARI	G51	12KCC-110	BARI	G91	Aus Sel-100	BARI
G12	16KCC-101	BARI	G52	12KCC-111	BARI	G92	Aus Sel-101	BARI
G13	16KCC-105	BARI	G53	12KCC-112	BARI	G93	Aus Sel-102	BARI
G14	16KCC_106	BARI	G54	12KCC-119	BARI	G94	12AG-56	AZRI
G15	16KCC-107	BARI	G55	12KCC-120	BARI	G95	12AG-60	AZRI
G16	15KCC-101	BARI	G56	11KCC-112	BARI	G96	12AG-61	AZRI
G17	15KCC-106	BARI	G57	11KCC-113	BARI	G97	12AG-129	AZRI
G18	15KCC-107	BARI	G58	11KCC-114	BARI	G98	12AG-133	AZRI
G19	15KCC-110	BARI	G59	11KCC-115	BARI	G99	12AG-230	AZRI
G20	15KCC-112	BARI	G60	11KCC-119	BARI	G100	12AG-235	AZRI
G21	15KCC-113	BARI	G61	11KCC-127	BARI	G101	12AG-247	AZRI
G22	14KCC-102	BARI	G62	11KCC-129	BARI	G102	12AG-248	AZRI
G23	14KCC-103	BARI	G63	11KCC-130	BARI	G103	CM/731/06	NIAB
G24	14KCC-104	BARI	G64	10KCC-101	BARI	G104	CM/736/06	NIAB
G25	14KCC-107	BARI	G65	10KCC-102	BARI	G105	CM/742/06	NIAB
G26	14KCC-108	BARI	G66	10KCC-111	BARI	G106	CM/762/06	NIAB
G27	14KCC-109	BARI	G67	10KCC-112	BARI	G107	CM/771/06	NIAB
G28	14KCC-110	BARI	G68	10KCC-113	BARI	G108	CM/792/06	NIAB
G29	14KCC-111	BARI	G69	10KCC-114	BARI	G109	CM/813/06	NIAB
G30	14KCC-114	BARI	G70	9KCC-160	BARI	G110	FS-4	PRI
G31	14KCC-115	BARI	G71	9KCC-163	BARI	G111	FS-5	PRI
G32	13KCC-101	BARI	G72	9KCC-163	BARI	G112	FS-6	PRI
G33	13KCC-102	BARI	G73	9KCC-164	BARI	G113	FS-7	PRI
G34	13KCC-103	BARI	G74	9KCC-172	BARI	G114	FS-8	PRI
G35	13KCC-105	BARI	G75	8KCC-151	BARI	G115	FS-9	PRI
G36	13KCC-108	BARI	G76	8KCC-152	BARI	G116	FS-10	PRI
G37	13KCC-110	BARI	G77	8KCC-153	BARI	G117	FS-13	PRI
G38	13KCC-111	BARI	G78	8KCC-154	BARI	G118	CM-2008 (C)	NIAB
G39	13KCC-112	BARI	G79	7KCC-154	BARI	G119	TAMMAN (C)	BARI
G40	13KCC-113	BARI	G80	7KCC-156	BARI	G120	NOOR-2013 (C)	NIAB

BARI: Barani Agricultural Research Institute, Chakwal, NIAB: Nuclear Institute for Agriculture and Biology Faisalabad, AZRI: Arid Zone Research Institute, Bakkhar; PRI: Pulses Research Institute, AARI Faisalabad

(Percent germination = No. of seeds germinated/Total number of seeds sown × 100) given by Salma *et al*. (2016). The random selection of three seedlings of each genotype from each treatment determined the seedling shoot length. Attaining shoot length began from the position of the attachment of the cotyledon to the tip of the seedling, expressed in centimeters. Similarly, determining the seedlings' root length comprised the random selection of three seedlings of each genotype from each treatment. The root length measurement, in centimeters, starts from the position of the attachment of the cotyledon to the tip of the root. Seedling length determination was calculated by adding the length of the root and shoots from three different seedlings from each replication and each treatment and then averaged. The root/shoot ratio of the three seedlings in each replication and each treatment was computed, and the mean was expressed as the root/shoot ratio. The formula given by Dharanguttikar *et al*. (2015) measured the seedling vigor index of each genotype from each replication and treatment. After drying the seedlings in the oven at 70°C for 24 h, the dry matter determination used the method according to Raza (2012). Proline accumulation in fresh leaves used the method of Bates *et al*. (1973).

Percent germination = No. of seeds
germinated/Total No. of seeds sown ×100

SVI = TG (%) × seedlings length (cm)/100

Where TG is the total germination percentage.

Statistical analysis

The collected data were compiled and analyzed statistically using the analysis of variance (Steel et al., 1997). The magnitude of phenotypic association was computed by using the equation given by Snedecor and Cochran (1989).

$$r_p = COVp_{xy} / (\text{б}^2p_x \times \text{б}^2p_y)^{1/2}$$

Where r_p: phenotypic correlation coefficient among attributes x and y; $COVp_{xy}$: phenotypic covariance among x and y variables, respectively; б^2p_x: phenotypic variances for x variable; and б^2p_y: phenotypic variances for y variable

RESULTS

Seedling characters

The results clearly showed differences among the characters for all the genotypes under study because the means squares for the genotype were highly significant, representing different behavior of genotypes and the presence of genetic variability among them (Table 2). In addition, the response of the genotypes to the treatments was also highly significant, which is interpreted as the change of treatment concentration from control to -0.19 MPa and -0.47 MPa stress treatments notably impacts the growth of Kabuli chickpea seedlings as revealed from the significant treatment mean squares. The performance of 120 Kabuli chickpea genotypes was tested for significance, and the results showed to be highly relevant for all seedling traits (Table 3). The data showed that increases in water stress caused a considerable decrease in seedling traits, except proline content, which increased as the Kabuli chickpea genotypes were subjected to an increase in osmotic stress. However, the performance of genotypes showed certain differences, the most reflective reduction recorded at a transition from -0.19 MPa to -0.47 MPa treatments.

The mean germination percentage ranged from 88.83% in the control to 63.17% plants subjected to high-stress level (-0.47 MPa). The samples, 15KCC106 and GP-37, showed excellent germination percentage (100%) at all levels of stress, followed by 17KCC117, 15KCC112, 14KCC111, 13KCC114, and 6KCC103 (93%), whereas the sample 11KCC112 displayed the minimum germination percentage (63%) for all genotypes. Drought stress adversely affects germination. Maximum germination percentages (100 and 100) were recorded in 15KCC106 and GP-37 at the osmotic potential of -0.19 MPa and -0.47 Mpa, respectively, followed by 17KCC117, 15KCC112, 14KCC111, 13KCC114, and 6KCC103. In contrast, the genotype, 11KCC112 demonstrated the most severe effect in germination (60% and 50%) at the osmotic potential of -0.19 MPa and -0.47 Mpa, respectively (Figure 1).

Table 2. Pooled analysis of variance for seedling traits of Kabuli chickpea at early growth stages.

Characters	Genotype (df=119)	Treatment (df=2)	G×E (df=238)	CV
Germination %	322.2**	39845.0**	97.7**	6.52
Shoot length	6.146**	423.697**	0.117**	0.61
Root length	9.350**	922.829**	0.261**	1.01
Seedling length	30.65**	2582.29**	0.72**	0.66
Root-shoot ratio	0.00036**	1.96638**	0.00018**	1.18
Seedling vigor index	4205.2**	3911.54**	672.9**	6.17
Dry matter	0.02200**	5.13841**	0.00119**	0.81
Proline content	1.93446**	3956.82**	0.09886**	4.53

Table 3. Ranges, means, mean squares, and coefficient of variation under control (T_0), -0.19 MPa (T_1) and -0.47 MPa (T_2) osmotic potential in Kabuli chickpea.

Characters	Treatments	Ranges	Means	Mean Squares	CV
Germination %	T_0	80.00-100.00	88.833±0.6424	182.129**	4.55
	T_1	60.00-100.00	75.083±0.7208	208.389**	8.30
	T_2	40.00-100.00	63.083±0.5495	127.045**	6.69
Shoot length	T_0	4.100-10.600	6.8679±0.0807	3.12629**	0.56
	T_1	3.400-8.6000	5.5925±0.0658	2.0751**	0.58
	T_2	2.500-6.5000	3.2113±0.0496	1.1797**	0.69
Root length	T_0	5.200-13.300	8.5837±0.1010	4.87842**	0.55
	T_1	4.300-11.300	6.6021±0.0860	3.52348**	1021
	T_2	2.800-7.3000	4.7233±0.0556	1.46999**	1.41
Seedling length	T_0	9.300-23.900	15.452±0.1817	15.8099**	0.46
	T_1	7.700-19.900	12.845±0.1516	10.9993**	0.74
	T_2	5.300-13.800	8.9346±0.1051	5.27956**	0.86
Root-shoot ratio	T_0	1.212-1.2860	1.2599±0.0007	0.00016*	0.66
	T_1	1.211-1.3520	1.2975±0.0003	0.00031*	1.27
	T_2	1.057-1.1670	1.4917±0.0001	0.00025*	1.50
Seedling vigor index	T_0	7.440-23.900	13.770±0.2088	20.5458**	4.16
	T_1	4.620-19.900	9.7299±0.1804	14.9115**	8.03
	T_2	3.180-13.800	5.7056±0.1090	5.53574**	6.67
Dry matter	T_0	0.253-0.7660	0.5131±0.0004	0.01483**	0.61
	T_1	0.170-0.5230	0.3457±0.0003	0.00676**	0.73
	T_2	0.106-0.3470	0.2215±0.0001	0.0028**	1.37
Proline content	T_0	2.880-5.6000	4.3312±0.0374	0.65817**	0.38
	T_1	6.880-9.6000	8.2485±0.0388	0.71187**	0.16
	T_2	10.78-14.000	12.450±0.0406	0.76213**	0.07

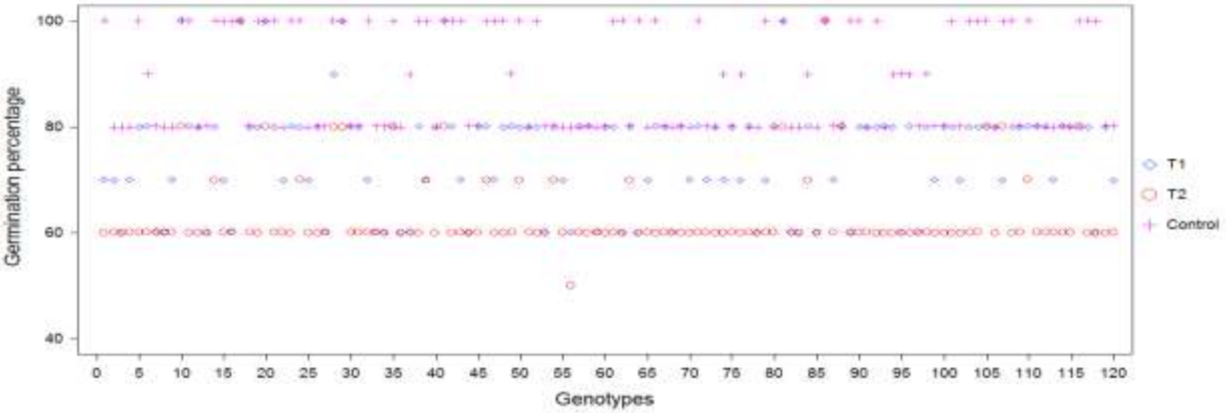

Figure 1. The distribution of mean values of 120 Kabuli chickpea genotypes on scatter plot for germination (%) under T_0 (Control), T_1 (-0.19 MPa) and T_2 (-0.47 MPa).

Results revealed that mean shoot length ranged from 6.86 cm (control) to 3.21 cm in plants subjected to a high-stress level (-0.47 MPa) (Figure 2). In the control treatment, the maximum shoot length (10.40 cm) resulted from GP-37, followed by 13KCC114 (10.30 cm) and 6KCC103 (10.20 cm). The minimum shoot length (4.20 cm) resulted from 9KCC160. Shoot length is inversely proportional to drought stress. Sample GP-37 gave excellent shoot length (8.10 and 5.40 cm), followed by 13KCC114 (8.00 and 5.35 cm) and 6KCC103 (8.00 and 5.30 cm) at the osmotic potential of -0.19 and -0.47 Mpa, respectively. The average root length data predicted that under the control treatment, the maximum root length measured 8.59 cm, while the lowest root length (4.72 cm) resulted when plants were subjected to a high-stress level (-0.47 MPa) (Figure 3). In the control treatment, the maximum root length (13.10 cm) came from GP-37, followed by 13KCC114 (12.9 cm) and 6KCC103 (12.8 cm), whereas the minimum root length (5.30 cm) resulted from

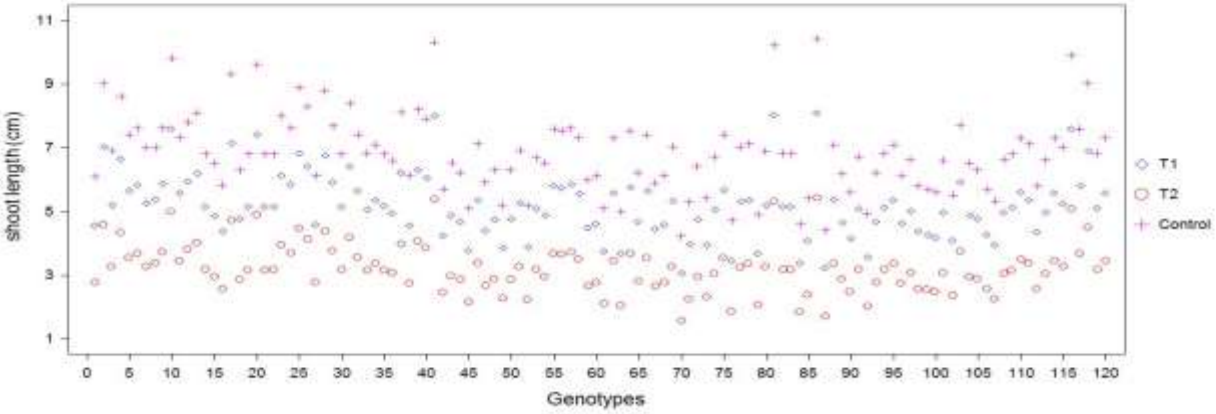

Figure 2. The distribution of mean values of 120 Kabuli chickpea genotypes on scatter plot for shoot length under T_0 (Control), T_1 (-0.19 MPa) and T_2 (-0.47 MPa).

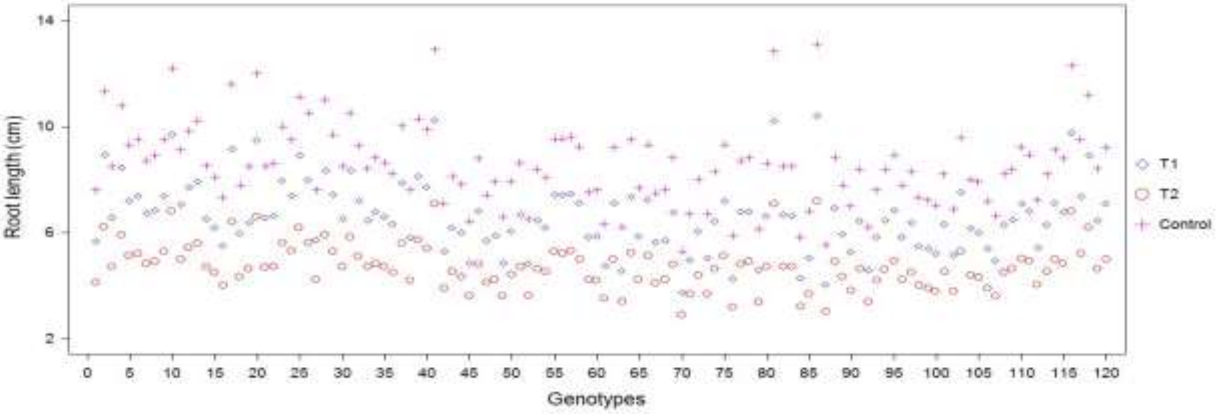

Figure 3. The distribution of mean values of 120 Kabuli chickpea genotypes on scatter plot for root length under T_0 (Control), T_1 (-0.19 MPa) and T_2 (-0.47 MPa).

9KCC160. Root length showed a sharp decline with an increase in osmotic potential in the studied Kabuli chickpea genotypes. Sample GP-37 gave an improved root length (10.40 and 7.20 cm), followed by 13KCC114 (10.25 and 7.10 cm) and 6KCC103 (10.20 and 7.10 cm) at osmotic potentials of -0.19 and -0.47 Mpa, respectively. Concerning seedling length, the GP-37 also gave enhanced seedling length (19.55 and 13.55 cm), followed by 13KCC114 (19.30 and 13.40 cm) and 6KCC103 (19.25 and 13.40 cm) at osmotic potentials of -0.19 and -0.47 Mpa, respectively (Figure 4). The most severe effect on seedling length resulted from 9KCC160 (5.40 cm) at the osmotic potential of 0.47Mpa.

The average root/shoot ratio ranged from 1.250 in the control to 1.492 in plants at the highest stress level (-0.47 MPa). The root/shoot ratio increased gradually with the increase in osmotic potential in Kabuli chickpea genotypes under study. Sample 6KCC103 gave an excellent root/shoot ratio (1.310), followed by 14KCC103 (1.305) at the osmotic potential of -0.19 Mpa, and GP-37 exhibited the highest root/shoot ratio (1.840), followed by GP-38 (1.794) at the osmotic potential -0.47 Mpa (Figure 5).

The most reflective reduction in the mean seedling vigor index was recorded when the plants received a high concentration of PEG solution (-0.47 MPa). The data demonstrated that as the PEG concentration increased, the seedling vigor index considerably decreased. At osmotic potentials of -0.19 and -0.47 MPa, GP-37 gave excellent seedling vigor index (19.55

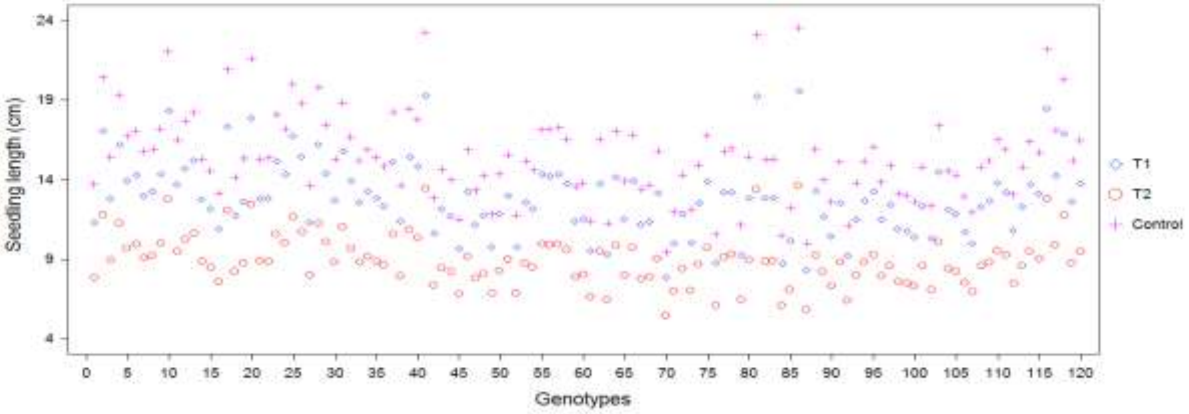

Figure 4. The distribution of mean values of 120 Kabuli chickpea genotypes on scatter plot for seedling length under T_0 (Control), T_1 (-0.19 MPa) and T_2 (-0.47 MPa).

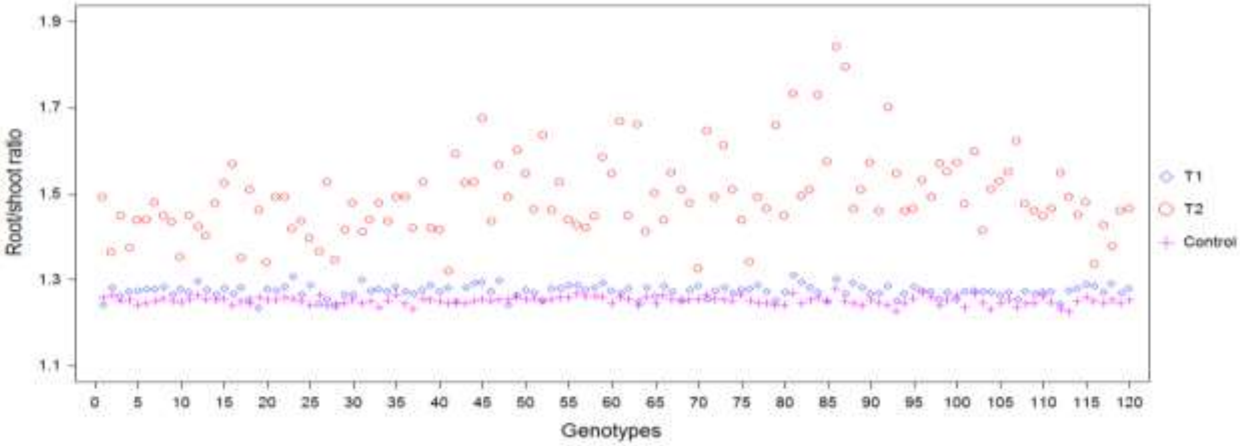

Figure 5. The distribution of mean values of 120 Kabuli chickpea genotypes on scatter plot for root-shoot ratio under T_0 (Control), T_1 (-0.19 MPa) and T_2 (-0.47 MPa).

and 13.55 cm), followed by 13KCC114 (19.30 and 10.72 cm), respectively (Figure 6). Dry matter accumulation decreased rapidly with an enhanced osmotic potential concentrationon Kabuli chickpea genotypes. The results confirmed that GP-37 displayed higher dry matter (0.327), followed by FS-10 (0.315) at an osmotic potential of -0.47 MPa compared with all the other studied genotypes. However, the most prominent effect on dry matter accumulation was observed in 13KCC116 (0.175 and 0.112) at osmotic potentials of -0.19Mpa and -0.47Mpa, respectively (Figure 7).

The results further showed that the average proline content ranged from 4.33 in control to 12.45 in plants subjected to the highest stress level (-0.47 MPa) (Figure 8). Data showed that an increase in osmotic stress caused a considerable increase in proline content. In the control treatment, the maximum proline content (5.49) was recorded in 12KCC106, followed by 13KCC105, 14KCC104, and 14KCC111 (5.29), whereas the minimum proline content (2.94) came from 13KCC103. Samples 12KCC106 and 6KCC103 gave more proline content (9.50), followed by 13KCC105 (9.30) at an osmotic potential of -0.19 Mpa, and 6KCC103 exhibited the highest proline content (13.86), followed by GP-37 (13.76) at an osmotic potential of -0.47 MPa. The overall results demonstrated that under stress conditions, proline accumulation increased significantly.

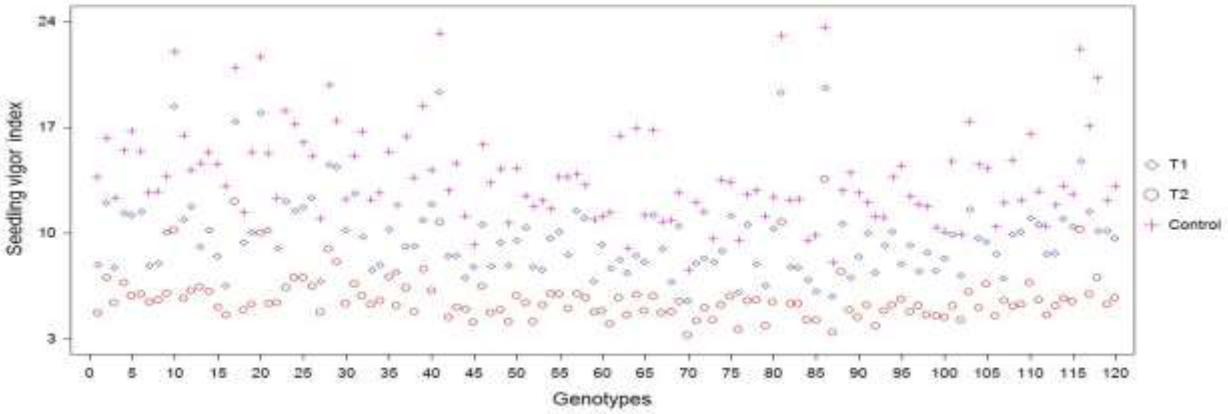

Figure 6. The distribution of mean values of 120 Kabuli chickpea genotypes on scatter plot for seedling vigor index under T_0 (Control), T_1 (-0.19 MPa) and T_2 (-0.47 MPa).

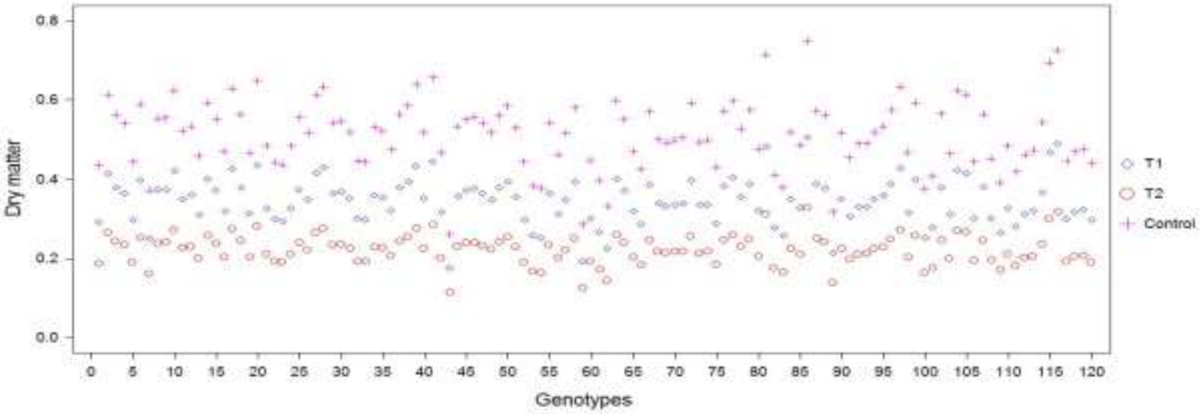

Figure 7. The distribution of mean values of 120 Kabuli chickpea genotypes on scatter plot for dry matter under T_0 (Control), T_1 (-0.19 MPa) and T_2 (-0.47 MPa).

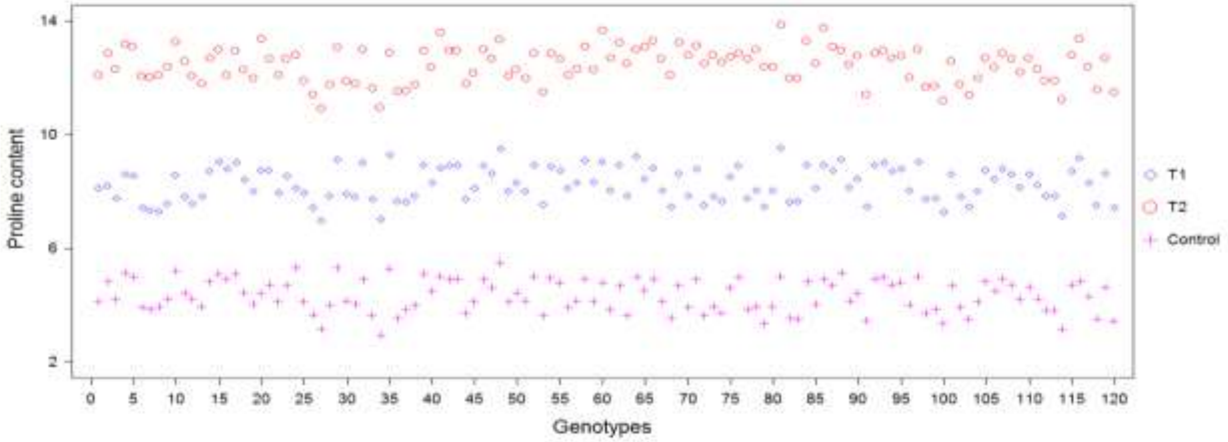

Figure 8. The distribution of mean values of 120 Kabuli chickpea genotypes on scatter plot for proline content under T_0 (Control), T_1 (-0.19 MPa) and T_2 (-0.47 MPa).

Correlation analysis

A correlation analysis was performed to ascertain the type and magnitude of relationship among the 120 Kabuli chickpea genotypes for seedling traits under control (T_0), -0.19 MPa (T_1), and -0.47 MPa (T_2) osmotic potential in Kabuli chickpea (Figures 4, 5, and 6). Correlation analysis results under the control treatment showed that a positive and vital link was found among root and shoot lengths, whereas root length showed a positive and significant correlation with the dry matter, but the correlation coefficient with the root/shoot ratio resulted in highly significant showing positive effects under control conditions. The shoot length showed a positive and highly significant association coefficient with the dry matter with a positive direction of effect. The root/shoot had a significant association with the root length but had a negative linked coefficient for shoot length (Table 4). Under -0.19 MPa and -0.47 MPa osmotic potential conditions, the correlation matrix showed almost in the same direction (Tables 5 and 6).

Table 4. Correlation coefficient among various seedling traits under control osmotic potential (T_0) in Kabuli chickpea.

Characters	DM	GP	RL	RSR	SL	SLT	SVI
GP	0.1007*						
RL	0.3411**	0.1555*					
RSR	0.0655*	-0.0201*	0.0162*				
SL	0.3383**	0.1567*	0.9989**	-0.0304			
SLT	0.3399**	0.1560*	0.9998**	-0.0045	0.9997**		
SVI	0.3375**	0.5992**	0.8795**	-0.0141	0.8802**	0.8800**	
Proline	0.3120**	0.2147**	0.6318**	-0.0161	0.7381**	0.8102**	0.5124**

*, ** Significant at *P* ≤0.05 and *P* ≤0.01, respectively, DM: Dry matter, GP: Germination percentage, RL: Root length, SL: Shoot length, RSR: Root/shoot ratio, SLT: Seedling length, SVI: Seedling vigor index

Table 5. Correlation coefficient among various seedling traits under -0.19 MPa osmotic potential (T_1) in Kabuli chickpea.

Characters	DM	GP	RL	RSR	SL	SLT	SVI
GP	0.3475**						
RL	0.3397**	0.2856*					
RSR	0.0231*	-0.0610*	0.1540*				
SL	0.3412**	0.2954*	0.9967**	-0.0748			
SLT	0.3406**	0.2901*	0.9994**	-0.1198	0.9989**		
SVI	0.4436**	0.7520 **	0.8365**	-0.0298	0.8430**	0.8400**	
Proline	0.4031**	0.6271**	0.8923**	-0.0342	0.8349**	0.8265**	0.5821**

*, ** Significant at *P* ≤0.05 and *P* ≤0.01, respectively, DM: Dry matter, GP: Germination percentage, RL: Root length, SL: Shoot length, RSR: Root/shoot ratio, SLT: Seedling length, SVI: Seedling vigor index

Table 6. Correlation coefficient among various seedling traits under -0.47 MPa osmotic potential (T2) in Kabuli chickpea.

Characters	DM	GP	RL	RSR	SL	SLT	SVI
GP	0.4803**						
RL	0.3492**	0.4270**					
RSR	0.0595	-0.0260*	0.0514*				
SL	0.3451**	0.4321**	0.9962**	-0.0347			
SLT	0.3476**	0.4298**	0.9991**	0.0108	0.9989**		
SVI	0.4892**	0.8053*	0.8708**	-0.0193	0.8745**	0.8734**	
Proline	0.3976**	0.8427**	0.8192**	-0.0303	0.8623**	0.8843**	0.6142**

*, ** Significant at *P* ≤0.05 and *P*≤0.01, respectively, DM: Dry matter, GP: Germination percentage, RL: Root length, SL: Shoot length, RSR: Root/shoot ratio, SLT: Seedling length, SVI: Seedling vigor index

DISCUSSION

Drought stress is believed as one of the crucial crop performance restrictive aspects and a threat for the successful production of crops. The yield reduction under drought conditions in the chickpea is the foremost concern of plant breeders. Therefore, breeders emphasized the use of various techniques to assess genetic differences in drought tolerance to minimize these production losses. Drought can be mitigated by the development of drought-tolerant genotypes. Researchers used different screening techniques to estimate genotypic differences for drought tolerance among the chickpea genotypes. Consequently, a viable and practical approach needs consideration for the screening of drought-resistant genotypes of existing available germplasm. For drought stress measurement at the seedling stage, the PEG chemical served to arouse osmotic stress effects for plants to sustain consistent water potential during the investigational period. The selection of drought-tolerant genotypes from large germplasm at the seedling stage, with the help of these techniques, can be done quickly and economically.

Germination is the most crucial stage and is considered one of the primary factors in the vegetative growth, as well as, seed yield of chickpea. The effect of PEG solution reduces the water potential among seeds and their adjacent media, which seriously influences the germination of the seed (Mbarek *et al.*, 2013). The Kabuli chickpea genotypes showed a genetic difference from each other concerning germination, hence, a considerable decrease in germination in all the studied Kabuli chickpea genotypes. These results are in agreement with the outcome of Mbarek *et al.* (2013), Awari and Mate (2015), and Dharanguttikar *et al.* (2015). They explained that with the increase in drought stress, germination percentage decreases. Excellent germination under drought stress is an essential parameter to screen various chickpea genotypes tolerant to drought. Such genotypes having good germination percentages should be selected for better final grain yield.

The shoot length is inversely proportional to drought stress. Thus, shoot length continues to decrease when concentrations of osmotic stress increase. In the recent research, the highest reduction in shoot length was noted under stress conditions at the osmotic potential of -0.47 Mpa. Mbarek *et al.* (2013), Awari and Mate (2015), and Dharanguttikar *et al.* (2015) presented that drought stress depresses shoot growth. These conclusions agree with the findings of Koskosidis *et al.* (2020), who reported that shoot growth decreases more compared with root growth under drought-stress conditions. Healthy root growth is an essential factor for plants to cope with drought conditions as roots are the key plant part for fulfilling transpiration needs and play an imperative role in making water available to plants (Koskosidis *et al.,* 2020). In the study, the root lengths of all Kabuli chickpea genotypes notably shortened at the different levels of osmotic stress. Similar effects were noticed by Rohit *et al.* (2020). This investigation reports root length is affected less than shoot length under induced drought stress. The arrest of cell division and elongation caused tuberization, ultimately resulting in a decrease in shoot and root length. The study results are in line with the investigations of Rohit *et al.* (2020) and Koskosidis *et al.* (2020) who examined drought stress reducing root growth.

The seedlings' length is a principal character measured by adding shoot and root length. Therefore, it exhibited similar effects when induced with drought stress that was also observed for both these characters. Seed priming play an important role to raise the amylase activity and total soluble sugar contents under drought and normal conditions, resulting in excellent germination and more rapid growth as compared with control (Zheng *et al.,* 2015). The study results are in line with the investigations of Varshini *et al.* (2018) who documented that induced drought stress decreases the seedling length as compared with control. In the recent research, gradual increase in root/shoot ratio was recorded as the concentration of stress conditions enhanced. Awari and Mate (2015) and Dharanguttikar *et al.* (2015) explained that root/shoot ratio increases gradually when levels of stress concentration increase. This effect of drought stress hindered epicotyls' growth more than root development and increased the root/shoot ratio. Study findings also concur with the conclusion of Koskosidis *et al.* (2020), who gave similar results. The seedling vigor was calculated by using the germination and seedling length traits, which explains why this character displayed a similar variation trend noted in both these characters. The outcomes of the research study are in concord with the findings of Varshini *et al.* (2018), who explained that the seedling vigor index decreases with an increased level of PEG osmotic potential.

Biological yield reduced with the increased drought stress and the highest effect was observed at -0.47 MPa treatments. The tolerant genotypes were observed with higher dry matter accumulation as compared to the sensitive ones under PEG induced drought stress. The recent research study outcome are in concord with the conclusions of Dharanguttikar *et al.* (2015) who stated that high levels of induced drought stress through PEG reduces the dry matter accumulation than the control one and the drought tolerant genotypes produced higher dry matter as compared to sensitive one.

Proline content causes the mediation of osmotic adjustment, and as a result, the plant can tolerate and grow well under drought stress. Proline content also plays a vital and essential role in maintaining the structure of the enzyme and in the removal of reactive oxygen species (Awari and Mate, 2015). Given that proline has hydrophilic properties, and during drought stress conditions, it might replace water molecules around protein, nucleic acid, and membrane (Bayomi *et al.,* 2008; Awari and Mate, 2015). Study results align with that of Salma *et al.* (2016), who reported that when concentration of drought stress induced by PEG increased, the proline content notably increased. Shah *et al.* (2020) also suggested that proline content could be used as a diagnostic criterion for the selection of drought-tolerant Kabuli chickpea genotype.

Dry matter accumulation is the chief objective of most plant breeding experiments; therefore, more emphasis should be given to the traits which exhibited highly significant positive interrelationship with the dry matter. Accordingly, to enhance dry matter production, the shoot length, root length, and seedling length need more focus in future research. Under drought stress condition, dry matter also displayed a strong positive and significant association with root length, shoot length, and seedling length. Ultimately, it was confirmed that these characters play a vital role in dry matter production, which is associated with a drought-tolerant mechanism. Thus, attention should be given to the traits where a highly significant positive association is observed between dry matter production and other interrelated characters. Therefore, to screen drought-tolerant Kabuli chickpea genotypes at the seedling stage under drought-stress conditions, shoot length, root length, and seedling length need more focus.

CONCLUSIONS

The study results present most of the characteristics resulted in a negative effect as polyethylene glycol concentration increases, except for the proline content, which increased with an increase in PEG concentration. Hence, the more effective treatment for the screening of drought-tolerant Kabuli chickpea breeding lines was T_2 (-0.47 Mpa) as compared with T_0 and T_1 because, in this treatment, most of the genotypes did not perform well, but the drought-tolerant genotypes perform excellently under the T_2 (-0.47 Mpa) treatment. The results indicated the advanced lines 15KCC-106, 13KCC-114, 6KCC-103, GP-37, FS-10, and 12KCC-106, which performed better under T_2 (-0.47 Mpa) treatment attained selection as drought tolerant, and these six advanced lines can serve as a baseline for the improvement of Kabuli chickpea breeding materials. For *in vitro culture*, the selection of drought-tolerant chickpea genotypes, based on germination parameters and proline accumulation proves very informative and helpful for plant breeders for the development of drought-tolerant Kabuli chickpea genotypes.

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