

*Full Length Research Paper*

# Screening for drought tolerance in cotton (*Gossypium hirsutum* L.) using *in vitro* technique

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Drought is an important abiotic factor that is affecting crop production worldwide. Cotton (*Gossypium hirsutum* L.) is an important fibre affected by drought. Identifying cotton lines that are drought tolerant is crucial for breeding for drought tolerance in cotton, hence this experiment was undertaken to evaluate 12 lines of cotton for drought tolerance, viz: SAMCOT 8, SAMCOT 9, SAMCOT 10, SAMCOT 11, SAMCOT 12, SAMCOT 13, CL-07, LINE 17, LINE 18, LINE 30, MA-1 and MA-15 under *in vitro* condition using polyethylene glycol (PEG) 6000 at 0, 5, 10 and 15 g/L PEG concentrations. These were arranged in a 4 × 12 factorial experiment with three replications in a completely randomized design. Data were collected on shoot length (cm), shoot fresh weight (g), seedling fresh weight, root length (cm), root fresh weight (g) and number of roots. Results indicated that the PEG simulated drought stress adversely affected all the traits measured. Significant ( $P \leq 0.01$ ) reduction was observed in all the traits as the PEG concentration increases from 0 to 15 g/L PEG. Result showed that CL-07, SAMCOT 8, SAMCOT 9 and LINE 30 recorded the maximum values for most of the shoot and root traits. Thus, these lines could be considered, having exhibited drought tolerance *in vitro*.

**Key words:** Cotton, drought tolerance, *in vitro*, PEG 6000.

## INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the world's most important natural textile fibre and an important source of feed, foodstuff and oil (Chen et al., 2007). About 44.43 million metric tons of cotton are produced globally from 34.16 million hectares with average productivity of 1.35 metric tons per hectare. India is the largest producer of cotton, producing about 12.52 million metric tonnes from 13.30 million hectares with an average productivity of 1.06 metric tonnes and yield of about 0.94 million tonnes per hectare. (USDA,

2019); while Nigerian is ranked the 29th producer of cotton in the World. The world average production 43.11 million metric tons is higher than that obtained in Nigeria 0.40 million metric tons and this gap may be due to several factors such as biotic and abiotic stress like diseases, heat stress, drought stress, etc. (USDA, 2019).

Drought and dry spell are important abiotic stress limiting crop productivity around the world and this is projected to worsen with climate change. Dry spell is

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becoming prevalent as an annual constraint to crop productivity in Nigeria especially in Northern Nigeria in cotton lint (Maqbool, 2009; Basal et al., 2009). Establishing an efficient drought screening technique and identifying cotton lines that are drought tolerant is crucial for breeding for drought tolerance in cotton. Polyethylene glycol (PEG) is a chemical commonly used in physiological experiments to induce controlled drought stress in nutrient solution cultures and *in vitro* cultures. One of the benefits of PEG-simulated drought is that most often there is a positive correlation between drought tolerance of genotypes in the laboratory experiments and in the field (Kosturkova et al., 2014). Through *in vitro* techniques it is possible to rapidly screen large numbers of plants using small amounts of plant material within a relatively short period of time. *In vitro* screening for salinity has been reported in cotton (Sagar and Vamadevaiah, 2017) but there is little or no information on *in vitro* screening for drought tolerance in cotton. The present study aimed at establishing a reliable screening technique for cotton using *in vitro* technique and to identify drought tolerant cotton line(s).

## MATERIALS AND METHODS

### Study area

The experiment was carried out in the Biotechnology Laboratory of the Department of Plant Science, Ahmadu Bello University, Zaria.

### Experimental materials, design and procedures

Treatment comprises of 12 lines of cotton: SAMCOT 8, SAMCOT 9, SAMCOT 10, SAMCOT 11, SAMCOT 12, SAMCOT 13, CL-07, LINE 17, LINE 18, LINE 30, MA-1 and MA-15 and four stress levels (0, 5, 10 and 15 g/L PEG). These were arranged in a 4 × 12 factorial experiment in a completely randomized design replicated three times. Seeds of the lines were obtained from the cotton breeding unit of the Institute for Agricultural Research (IAR). The seeds were surface sterilized with 80% ethanol for 3 min, after which 25% sodium hypochloride (commercial bleach) was added with 2 drops of tween 20 for 20 min with occasional stirring under the laminar hood. The seeds were then rinsed thrice with sterilized distilled water. Embryonic axes were excised from sterile seeds and used as explants. Embryonic axes were cultured on Murashige and Skoog (MS) media (Murashige and Skoog, 1962) supplemented with varying concentrations (0, 5, 10 and 15 g/L) of Polyethylene Glycol (PEG 6000) solidified with 8 g/L agar. The pH of the media was adjusted to 5.8 before autoclaving for 15 min at 121°C and 15 psi. Cultures were maintained in the growth room at 26±2°C under 16/8 h light/dark photoperiod provided by white cool fluorescence for two weeks. Five test tubes were used per treatment and these were laid out in a completely randomized design replicated three times.

### Data collection and analysis

Data was collected on seedling fresh weight (g), shoot length (cm), shoot fresh weight (g), root length (cm), root fresh weight (g) and

number of roots. Seedling fresh weight was measured using a weighing balance.

The shoot length of seedlings was measured from the collar region to the tip of the shoot by using a metre rule. Shoot fresh weight was measured by separating the shoot from the seedlings and weighed by using a weighing balance. Root lengths of seedlings were measured from the collar region to the tip of the longest root by using a meter rule. Roots were separated from the seedling and weighed by using a weighing balance and number of root was counted directly from the root. Data recorded were subjected to Analysis of Variance (ANOVA) using factorial ANOVA procedure of SAS 9.0 (SAS Institute Inc. 2002). Means with significant differences at 0.05 level of probability were separated using Least Significant Difference (LSD). Data recorded were subjected to Analysis of Variance (ANOVA) using factorial ANOVA procedure of SAS 9.0 (SAS Institute Inc. 2002). Means were separated using Least Significant Difference (LSD).

## RESULTS

### Effect of PEG concentrations on shoot traits of cotton lines

Significant ( $P \leq 0.05$ ) differences were observed for all the shoot traits evaluated (Table 1). Shoot fresh weight, seedling fresh weight and shoot length significantly ( $P \leq 0.05$ ) decreases with increase in PEG concentration from 0 to 15 g/L. Maximum (0.99 g) shoot fresh weight was observed under control condition while the least (0.43 g) shoot fresh weight was observed in the medium supplemented with 15 g/L PEG.

Highest and longest (1.24 g and 12.61 cm) seedling fresh weight and shoot length were also observed under control condition, while the least (0.62 g) and shortest (7.28 cm) seedling fresh weight and shoot length were observed in medium supplemented with 15 g/L PEG.

Significant ( $P \leq 0.05$ ) variation was observed among the lines for shoot traits. SAMCOT 11 recorded the highest shoot fresh weights followed by SAMCOT 8, MA-1 and SAMCOT 10 with shoot fresh weight of 0.81, 0.80, 0.71 and 0.69 g, respectively, while LINE 17 recorded the least shoot fresh weight of 0.47 g.

SAMCOT 9 recorded the highest seedling fresh weight followed by SAMCOT 8, CL-07 and MA-1 with seedling fresh weight of 1.06, 1.00, 0.95 and 0.95 g, respectively, while SAMCOT 13 recorded the least seedling fresh weight (0.66 g). CL-07 recorded the longest shoot length followed by SAMCOT 8, LINE 30 and LINE 18 with shoot length of 12.04, 11.53, 11.21 and 10.96 g, respectively.

### Effects of PEG concentrations on root traits of 12 cotton lines

Highly significantly ( $P \leq 0.01$ ) decreases with increase in PEG concentration from 0 to 15 g/L was observed for root traits (Table 2 and Figure 1). Highest (0.41 g) root fresh weight was observed under control condition, while

**Table 1.** Effect of PEG and lines on shoot traits of cotton.

Treatment (PEG)	SFW (g)	SDFW (g)	SL (cm)
0	0.99 <sup>a</sup>	1.27 <sup>a</sup>	12.61 <sup>a</sup>
5	0.68 <sup>b</sup>	0.93 <sup>b</sup>	11.19 <sup>b</sup>
10	0.56 <sup>c</sup>	0.72 <sup>c</sup>	9.80 <sup>c</sup>
15	0.43 <sup>d</sup>	0.60 <sup>d</sup>	7.28 <sup>d</sup>
SE±LINES (L)	0.03	0.03	0.13
SAMCOT 8	0.80 <sup>ab</sup>	1.00 <sup>ab</sup>	11.53 <sup>ab</sup>
SAMCOT 9	0.68 <sup>bcd</sup>	1.06 <sup>a</sup>	10.56 <sup>cd</sup>
SAMCOT 10	0.69 <sup>abcd</sup>	0.99 <sup>abc</sup>	8.95 <sup>e</sup>
SAMCOT 11	0.81 <sup>a</sup>	0.78 <sup>efg</sup>	10.04 <sup>d</sup>
SAMCOT 12	0.60 <sup>d</sup>	0.87 <sup>cdef</sup>	10.20 <sup>d</sup>
SAMCOT 13	0.61 <sup>d</sup>	0.66 <sup>g</sup>	10.09 <sup>d</sup>
CL-07	0.64 <sup>d</sup>	0.95 <sup>abcd</sup>	12.04 <sup>a</sup>
LINE 17	0.47 <sup>e</sup>	0.74 <sup>fg</sup>	9.25 <sup>e</sup>
LINE 18	0.68 <sup>cd</sup>	0.82 <sup>def</sup>	10.96 <sup>bc</sup>
LINE 30	0.25 <sup>cd</sup>	0.82 <sup>def</sup>	11.21 <sup>bc</sup>
MA-1	0.71 <sup>abc</sup>	0.95 <sup>abcd</sup>	8.70 <sup>e</sup>
MA-15	0.56 <sup>de</sup>	0.91 <sup>bcd</sup>	9.11 <sup>e</sup>
PEG × L	NS	*	*
SE±	0.04	0.05	0.23
CV (%)	19.15	15.17	6.38

Mean with the same letter (s) are not significantly different at  $P < 0.05$  level of significance. SFW=Shoot fresh weight, SDFW=Seedling fresh weight, SL=Shoot.

the least value (0.16 g) for root fresh weight was observed in medium supplemented with 15 g/L of PEG-6000. Root length and number of roots per plantlet were also highly significantly ( $P \leq 0.01$ ) influenced by the PEG concentration with the control recording the longest and highest (16.59 cm and 38) root length and number of roots, respectively, while the shortest and least (8.76 cm and 15) root length and number of roots were observed in the medium supplemented with 15 g/L of PEG-6000.

Highly significant ( $P \leq 0.01$ ) differences were observed among the cotton lines evaluated for root fresh weight, root length and number of roots per plantlet (Table 2). MA-1 recorded statistically similar root fresh weight with LINE 17 but higher (0.37 and 0.35 g) root fresh weight than the other lines, while the least value (0.19 g) for root fresh weight was observed in MA-15. CL-07 recorded the longest (19.21 cm) root length, followed by Line 17 (17.01 cm), while MA-15 recorded the shortest (3.88 cm) root length. The highest (42) number of roots per plantlet was observed in SAMCOT 8, while the least (13) was recorded in MA-15.

#### PEG concentrations and Lines Interaction on shoot and root traits of cotton

There was significant ( $P \leq 0.05$ ) interaction between PEG concentration and lines for seedling fresh weight (Table

3). All the lines consistently recorded highest values for seedling fresh weight under control condition, and the least values in 15 g/L PEG with the exception of SAMCOT 8 which recorded highest value in 5 g/L PEG and SAMCOT 13 which recorded least value on 5 g/L PEG.

CL-07 recorded the longest shoot length followed by SAMCOT 8 with shoot length of 14.55 and 13.35 cm, respectively, while SAMCOT 10 recorded the shortest (11.25 cm) shoot length under control condition (Table 4). When the medium was supplemented with 15 g/L of PEG SAMCOT 8 recorded the longest shoot length followed by CL-07 and LINE 30 with 10.45, 9.45, and 9.35 cm shoot length, respectively, while MA-15 (3.00 cm) recorded the shortest shoot length.

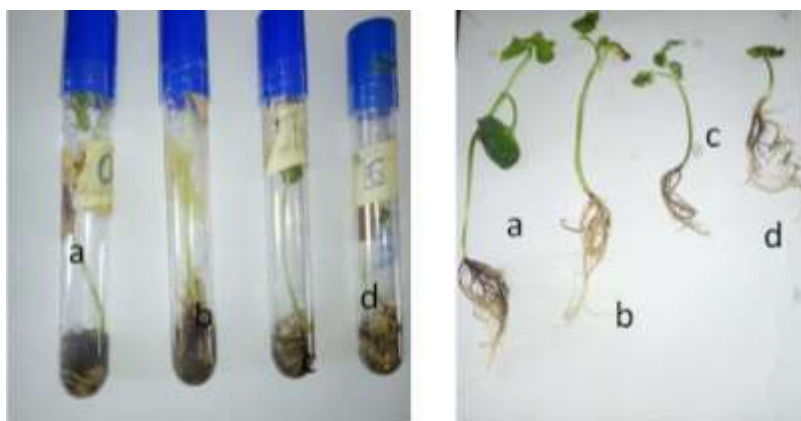
In the control treatment, highest (0.55 g) value for root fresh weight was recorded in MA-1 followed by CL-07, while MA-15 recorded the least root fresh weight (Table 5). When the medium was supplemented with 15 g/L PEG, LINE 17 followed by LINE 30 and SAMCOT 9 recorded the highest root fresh weight of 0.25, 0.25 and 0.23 g, respectively.

CL-07 recorded longer root length followed by LINE 17, SAMCOT 9, SAMCOT 13 and SAMCOT 10 with 21.40 and 21.00 cm, 19.55, 19.55 and 19.00 cm root lengths, respectively, while MA-15 recorded the least value (7.50 cm) under control condition (Table 6). In 15 g/L PEG medium CL-07 recorded longer (17.20 cm) root length,

**Table 2.** Effect of PEG and lines on root traits of cotton.

Treatment (PEG)	RFW (g)	RL (cm)	NR
0	0.41 <sup>a</sup>	16.59 <sup>a</sup>	37.89 <sup>a</sup>
5	0.30 <sup>b</sup>	11.86 <sup>b</sup>	29.42 <sup>b</sup>
10	0.25 <sup>c</sup>	10.07 <sup>c</sup>	22.33 <sup>c</sup>
15	0.14 <sup>c</sup>	7.19 <sup>c</sup>	14.78 <sup>c</sup>
SE±LINES (L)	0.01 <sup>c</sup>	0.08 <sup>c</sup>	0.51 <sup>c</sup>
SAMCOT 8	0.24 <sup>bc</sup>	13.34 <sup>c</sup>	42.13 <sup>a</sup>
SAMCOT 9	0.34 <sup>a</sup>	12.99 <sup>c</sup>	39.00 <sup>b</sup>
SAMCOT 10	0.33 <sup>a</sup>	11.09 <sup>e</sup>	32.75 <sup>c</sup>
SAMCOT 11	0.21 <sup>de</sup>	10.29 <sup>f</sup>	23.63 <sup>e</sup>
SAMCOT 12	0.22 <sup>cd</sup>	9.90 <sup>fg</sup>	22.75 <sup>e</sup>
SAMCOT 13	0.25 <sup>b</sup>	9.95 <sup>fg</sup>	13.38 <sup>g</sup>
CL-07	0.33 <sup>a</sup>	19.21 <sup>a</sup>	33.88 <sup>c</sup>
LINE 17	0.35 <sup>a</sup>	17.01 <sup>b</sup>	28.88 <sup>d</sup>
LINE 18	0.24 <sup>bc</sup>	9.61 <sup>g</sup>	23.25 <sup>e</sup>
LINE 30	0.25 <sup>b</sup>	12.53 <sup>d</sup>	23.00 <sup>e</sup>
MA-1	0.33 <sup>a</sup>	7.34 <sup>h</sup>	14.75 <sup>f</sup>
MA-15	0.19 <sup>e</sup>	3.88 <sup>i</sup>	16.00 <sup>fg</sup>
MEAN	0.27	11.43	26.11
PEG × L	**	**	**
SE±	0.01	0.15	0.88
CV (%)	9.11	3.71	10.06

Mean with the same letter (s) are not significantly different at P<0.05 level of significance. RFW=Root fresh weight, RL=Root length, NR=Number of roots.



**Figure 1.** Effect of PEG on Rooting in cotton plantlets; a) control, b) plantlet on 5g/L PEG, c) plantlet on 10g/L PEG and d) plantlet on 15g/L PEG

while MA-15 and MA-1 recorded the least (1.80 and 0.00 cm) root lengths, respectively.

SAMCOT 9 recorded higher roots number followed by SAMCOT 10 and SAMCOT 8 with 55, 53 and 51 root number, respectively, while SAMCOT 13 recorded the least (20) in the control (Table 7). When the medium was supplemented with 15 g/L of PEG, SAMCOT 8 recorded the highest (33) number of root, while MA-15

recorded the least (4) number of root. No root was observed in MA-1.

## DISCUSSION

Drought stress minimizes nutrient uptake and distribution within the body of cotton plant, resulting in reduction

**Table 3.** Interaction between PEG concentrations and Lines on shoot length (cm) under *in vitro* condition

Line	0	5	10	15
SAMCOT 8	1.22	1.35	0.76	0.76
SAMCOT 9	1.40	1.02	0.95	0.89
SAMCOT 10	1.47	0.97	0.77	0.76
SAMCOT 11	1.14	0.72	0.63	0.62
SAMCOT 12	1.16	0.60	0.85	0.86
SAMCOT 13	1.70	0.29	0.81	0.65
CL-0 7	1.29	0.96	0.99	0.58
LINE 17	1.03	0.82	0.67	0.42
LINE 18	1.01	0.87	0.76	0.65
LINE 30	1.27	0.83	0.63	0.55
MA-1	1.67	1.30	0.49	0.35
MA-15	1.53	1.16	0.52	0.44
SE±0.22				

**Table 4.** Interaction between PEG concentrations and Lines on shoot length (cm) under *in vitro* condition.

Line	0	5	10	15
SAMCOT 8	13.35	11.70	10.60	10.45
SAMCOT 9	12.20	10.70	10.35	9.00
SAMCOT 10	11.25	10.35	9.80	4.40
SAMCOT 11	12.20	11.30	9.30	7.35
SAMCOT 12	12.40	9.95	9.55	8.90
SAMCOT 13	12.55	12.25	8.75	6.80
CL-0 7	14.55	13.50	10.65	9.45
LINE 17	11.90	10.25	8.85	6.00
LINE 18	12.50	11.55	10.75	9.05
LINE 30	12.35	11.80	11.35	9.35
MA-1	12.35	10.40	8.45	3.60
MA-15	13.75	10.55	9.15	3.00
SE±1.21				

of growth and yield (Zhang et al., 2017). In the present study, seedling fresh weight, shoot fresh weight and shoot length decrease with increase in PEG concentration from 0 to 15 g/L. Megha et al. (2017) screened 21 cotton genotypes and observed that shoot length decreases with increase in PEG concentration from 0 to 20%. The decreased shoot length and leaves help in reducing transpiration water loss from shoot surfaces (Megha et al., 2017). Genotypic variation was observed among the lines evaluated for shoot traits with SAMCOT 8 consistently recording the highest seedling fresh weight, shoot fresh weight and shoot length. The observed performance when compared with the other lines for the shoot traits could be attributed to genotypic influence. Genotypic variation has also been

reported in wheat and rice cultures (Amandeep and Rashpal, 2020; Shahriar et al., 2019). Interaction between PEG concentrations and lines were found to be significant where SAMCOT 8 recorded higher seedling fresh weight at 5 g/L but recorded statistically similar seedling fresh weight at 10 and 15 g/L, while SAMCOT 12 and SAMCOT 13 recorded lesser seedling fresh weight at 5 g/L compared to the control but increases at 10 and 15 g/L PEG. SAMCOT 8, CL-07 and LINE 30 recorded longer shoot length compared to the other lines. The increase in shoot length might be as a result of cell division in the shoot apical meristem. Reduction in shoot length was similar to the findings of Amandeep and Rashpal (2020) who reported shoot length decreases as the PEG concentration increases from 0

**Table 5.** Interaction between PEG concentrations and Lines on root fresh weight (g) under *in vitro* condition

LINE	0	5	10	15
SAMCOT 8	13.35	11.70	10.60	10.45
SAMCOT 9	12.20	10.70	10.35	9.00
SAMCOT 10	11.25	10.35	9.80	4.40
SAMCOT 11	12.20	11.30	9.30	7.35
SAMCOT 12	12.40	9.95	9.55	8.90
SAMCOT 13	12.55	12.25	8.75	6.80
CL-0 7	14.55	13.50	10.65	9.45
LINE 17	11.90	10.25	8.85	6.00
LINE 18	12.50	11.55	10.75	9.05
LINE 30	12.35	11.80	11.35	9.35
MA-1	12.35	10.40	8.45	3.60
MA-15	13.75	10.55	9.15	3.00
SE±1.21				

**Table 6.** Interaction between PEG concentrations and Lines on root length (cm) of cotton under *in vitro* condition

LINE	0	5	10	15
SAMCOT 8	18.55	15.54	10.20	9.15
SAMCOT 9	19.55	12.75	12.20	7.45
SAMCOT 10	19.00	10.70	7.60	7.05
SAMCOT 11	18.35	10.75	8.45	3.60
SAMCOT 12	15.05	12.60	8.39	3.50
SAMCOT 13	19.55	9.25	7.00	2.45
CL-0 7	21.40	18.80	19.35	17.30
LINE 17	21.00	18.30	14.95	13.80
LINE 18	11.60	10.05	9.60	7.20
LINE 30	14.45	12.50	11.75	11.40
MA-1	13.10	7.55	8.70	0.00
MA-15	7.50	3.60	2.60	1.80
SE±3.15				

18 g/L PEG in wheat.

Root traits such as root length, fresh root weight, and dry root weight contribute more to drought tolerance as compared to the shoot related traits (Iftikhar et al., 2019). As good root growth is necessary for establishment of crop stand and root helps in uptake of water and mineral elements necessary for plant growth and plays an important role in drought tolerance (Brunner et al., 2015). In the present investigation, significant differences were observed for all the root traits evaluated. Root length, root fresh weight and number of roots decrease as the PEG concentration increases from 0 to 15 g/L PEG. Medium supplemented with 15 g/L of PEG recorded the least root length, root fresh weight and number of roots, while the control

recorded the highest values. This is similar to the findings of Amandeep and Rashpal (2020) who reported that root length decreased significantly with increasing moisture stress in wheat. Also Nadir et al. (2019) studied the effects of PEG on *Indigofera* seeds and reported that seed that were not treated with PEG showed longer roots than those treated with PEG at 10, 20, 30 and 40 g/L. This is contrary to the work of Babu et al. (2014) who reported that root length was increased with the increasing PEG-6000 concentrations up to 10% PEG-6000 concentrations, it declined thereafter. Root plays an important role in the uptake of nutrients and water in cotton (Hulugalle et al., 2015). This accounts for the increase in root length with increase in PEG concentration as observed in the study. Among the cotton

**Table 7.** Interaction between PEG concentrations and Lines on number of root under *in vitro* condition

Line	0	5	10	15
SAMCOT 8	51	48	37	34
SAMCOT 9	55	39	38	25
SAMCOT 10	53	44	22	12
SAMCOT 11	37	27	21	11
SAMCOT 12	38	26	18	10
SAMCOT 13	20	14	13	8
CL-0 7	48	34	30	24
LINE 17	35	33	28	21
LINE 18	36	26	19	13
LINE 30	31	24	20	18
MA-1	26	22	12	0
MA-15	28	19	13	4
SE±4.27				

lines evaluated, LINE 17 recorded the highest root fresh weight which was statistically similar with SAMCOT 9, SAMCOT 10, CL-07 and MA-1, while CL-07 recorded the longest root length and SAMCOT 8 the highest number of roots per plantlet. MA-15 recorded the least value for most of the root traits. The observed variation could be attributed to genotypic differences. Water stress sensitive genotypes show more reduction in root growth, whereas drought stress tolerant genotypes appeared to be relatively less affected under moisture stress condition in cotton. The longer root length of tolerant varieties might be due to higher osmotic adjustment and retention of more water content in cotton plant (Hassan et al., 2015).

The results of the study also showed that PEG concentration × Line interaction was significant for root fresh weight, root length and number of roots. These interactions revealed LINE 17, LINE 30, SAMCOT 9 and CL-07 recorded higher root fresh weight, CL-07 recorded longest root length, while SAMCOT 8 recorded higher number of root compared to the other lines under 15 g/L PEG. Among the drought related morphological traits, root length has been reported to be one of the most important trait for drought tolerance and thus, may be used to measure the level of tolerance of cotton varieties at seedling phase (Basal et al., 2005; Hassan et al., 2015). Amandeep and Rashpal (2020) also reported interactions for root fresh weight and root length in wheat. Under water stress, plants partitioned more photosynthates for the growth of roots rather than shoots to help in the absorption of more water from deeper surfaces (Babu et al., 2014). The findings of the present study is also in line with earlier studies where seedling root length, root surface area, root volume, number of tips and root dry weight were significantly impacted by drought stress and genotype in kenaf at 0,

10 and 20% PEG concentration (Tang et al., 2019).

## Conclusion

Results from this study revealed that there was a reduction in all the traits evaluated with increasing PEG concentration and MS medium supplemented with 15 g/L PEG 6000 can be used to screen cotton for drought tolerance at seedling stage under *in vitro* condition. Among the cotton lines evaluated, CL-07, SAMCOT 8, SAMCOT 9 and LINE 30 recorded the maximum values for most of the shoot and root traits. Thus, these lines could be considered, having exhibited drought tolerance *in vitro*.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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