In -vitro evaluation of mung bean (*Vigna radiata* L. Wilczek) genotypes for drought tolerance and productivity

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Abstract: *Drought stress is the most important factor that limits mung bean production and productivity at large in drought-prone areas of Ethiopia. It is hence necessary to identify and verify drought-tolerant and productive varieties of major crops grown in drought areas of the country like mung bean. The present study was conducted to evaluate mung bean genotypes for drought tolerance under in-vitro conditions and to assess the performance of the in-vitro developed regenerants under greenhouse conditions. The in-vitro experiment was thus arranged in a factorial experiment using a completely randomized design with three replications. Three mung bean genotypes, NLLP-MGC-06/G6 (tolerant), VC6368 (46-40-4)/G34 (moderate), and NLLP-MGC-02/G2 (sensitive) and five polyethylene glycol (PEG) levels (0, 0.5, 1.0, 1.5, and 2.0%) were used. The analysis of variance exhibited significant differences among the genotypes for all the studied parameters except the number of roots per shoot. There were significant differences observed among PEG levels for all the studied parameters. Significant genotypes x PEG interactions were observed for all the studied traits except total roots per culture and survival percentage. Increasing polyethylene glycol concentration from 0% to 2.0% in the medium caused a gradual increase in root length from 0.49 cm at 0% PEG to 1.17 cm at 2.0% PEG, respectively. This revealed an adaptive mechanism to the decreased moisture content in the root zones of plants and enhanced increased root length to reach deeper water in the soil. Regenerant from the treatment combinations of G34 (0) exhibited the highest values for the number of primary branches per plant (4.00). Grain yield for the in-vitro regenerated plants evaluated at greenhouse conditions ranged from 552.52 kg ha^{-1} at the treatment combination of G2 (1) to 996.23 kg ha^{-1} at the treatment combinations of G6 (0). Most of the regenerants obtained from NLLP-MGC-06/G6 and VC6368 (46-40-4)/G34 showed the best performance under the greenhouse for drought-tolerance under the in-vitro condition, suggesting that the accumulated performance of the tested regenerants under in-vitro conditions was realized under greenhouse conditions. It also indicated that in-vitro culture is an important tool to identify and verify drought-tolerant genotypes and improve desirable agronomical traits. Further study is indeed required to understand the mechanism of drought tolerance for in-vitro-selected somaclones.*

Keywords: Drought tolerance, Greenhouse condition, Polyethylene glycol, Somaclonal variation

1. Introduction

Drought is a major abiotic stress that adversely affects plant production in many parts of the world and had brought a significant yield reduction. Ilker *et al*. (2011) suggested that global warming is noticeable in drought-prone areas and had significantly affected plant production, thereby leading to considerable economic and social problems because of its great importance in human nutrition. Jaleel *et al*. (2009) suggested that drought is a serious problem for crop production and food security that significantly reduces the turgor potential of plants. Water stress can result in reducing crop yield worldwide (Boyer, 1982; Smirnoff, 1993; Gonzalez *et al*., 1995). Since yield is a complex trait and is strongly influenced by the environment, severe losses can be caused by drought stress which is common in most arid and semi-arid areas. One possible way to ensure the future food needs for an increasing world

population involves the better use of water through the development of drought-tolerant crop varieties that need less water (El-Shafey *et al*., 2009; Mafakheri *et al*., 2010).

One of the screening techniques based on physiological traits is the use of various osmotica to induce stress in plant tissues. Therefore, to simulate the effect of water stress in vitro, several researchers have incorporated polyethylene glycol (PEG) in the culture medium (Handa *et al*., 1982; Bhaskaran *et al*., 1985; Newton *et al*., 1986; Newton *et al*., 1989; Ochatt *et al*., 1998; Guóth *et al*., 2010; Rai *et al*., 2011; Yang *et al*., 2012). Sané *et al*. (2005) found that the use of PEG under in-vitro culture allows quick and easy identification of genotypes tolerant to water stress. Plant cell and tissue cultures have been implemented as useful tools to study stress tolerance mechanisms under in vitro conditions (Bajji *et al*., 2000). Also, in-vitro-developed sugar cane regenerants were validated for agronomical and morphological traits (Gadakh *et al*. 2015; Rahman *et al*., 2016).

Information on drought stress's effect on the morphological aspects under in vitro conditions in mung bean is lacking. Therefore, there is a need to go to an alternative approach to field experiments related to moisture stress to induce stress using polyethylene glycol (PEG) under in-vitro conditions. Hence, the objectives of this study were to assess the effect of PEG-induced stress on plant cells of mung bean genotypes, to select surviving cell lines under different levels of PEG stress under in-vitro conditions, and to select suitable regenerants for drought tolerance.

2. Materials and Methods

2.1. Description of the study area

The study was conducted at the Plant Tissue Culture Research Laboratory of Areka Agricultural Research Center, Southern Ethiopia.

2.2. Plant material, treatments, and experimental design

Three mung bean genotypes with contrasting drought tolerance including NLLP-MGC-06/G6 (tolerant), VC6368 (46-40-4) /G34 (moderate), and NLLP-MGC-02/G2 (sensitive) were used for this experiment. Two genotypes (G34 and G6) were obtained from the Melkassa Agricultural Research Center, while the third genotype (G2) was a landrace collected from the southern region of Ethiopia. The base for selecting these genotypes was based on the moisture stress response in drought screening field experiments. The treatments comprised factorial combinations of three mung bean genotypes (G34, G6, and G2) and five polyethylene glycol (PEG_{8000}) levels of 0, 0.5, 1, 1.5, and 2% (w/v) adopted from Ferede *et al*. (2019). The experiment of the study was laid out in a completely randomized design in a factorial arrangement with three replications.

2.3. Culture media and growth conditions

Murashige and Skoog's (1962) medium (MS) was used as a basal medium with 3% sucrose and 0.75% agar added by melting in a microwave oven. The pH of all media was adjusted to 5.8 with 0.1 N NaOH before autoclaving. When the agar became clear, 50 ml medium was dispensed into culture tubes and autoclaved at 121°C for 20 minutes (Ferede *et al*., 2019).

2.4. Seed sterilization and germination

For sterilization, the seeds were first treated with 70% ethanol for 5 minutes and then washed in 8% sodium hypochlorite for 30 minutes, followed by six washes in sterile double distilled water in a laminar airflow cabinet. The sterilized seeds were cultured for two weeks under aseptic conditions containing a semisolid MS medium at 27 °C. After two weeks, young seedling leaves were excised and used for callus induction (Ferede *et al*., 2019).

2.5. Callus induction

Leaf explants (2 cm) were placed on an MS medium containing 0.75% agar and 3% sucrose for each treatment. Callus induction was initiated from the leaf explants placed on MS medium containing 2.4-D (2 mg/l), kinetin (0.2 mg/l), and 1 naphthalene acetic acid (1 mg/l). Different concentrations of PEG (0, 0.5, 1.0, 1.5, and 2.0%) were added to the callus induction medium. The culture tubes were sealed with parafilm and placed in a growth room at 27 ºC. In all experiments, three replicates were made, and 10 explants of leaf segments were placed with one replication represented by two culture tubes (Ferede *et al*., 2019).

2.6. Plant regeneration

After four weeks of incubation, the induced calli were transferred to culture tubes, sub-cultured under the same growth conditions, and in the same MS medium with various concentrations of

PEG_{8000} (0, 0.5, 1.0, 1.5, and 2.0%) adopted from Ferede *et al*. (2019). The resulting calli were excised, and transferred, into culture tubes containing MS medium supplemented with 1.5 mg/l kinetin + 0.2 mg/l NAA + 3% sucrose + 0.75% agar for shoot initiation. By doing this procedure, the efficiency of the embryogenic calli was determined and for further regeneration (shooting and rooting) in the presence of drought stress, the obtained calli were exposed to PEG_{8000} (0, 0.5, 1.0, 1.5, and 2.0%) in the plant regeneration medium. Rooting was initiated on half-strength fresh MS medium supplemented with 1.5 mg/l NAA. The incubation period was two cycles of two weeks each or two weeks for shooting and two weeks for rooting (Ferede *et al*., 2019).

2.7. Acclimatization of regenerated plants

Healthy and well-rooted plantlets were washed to remove the medium adhered and subjected to acclimatization, transplanted to the plastic tray under high humidity by covering the plant with plastic containing sterilized soils, coco peat, and compost, and placed under polythene shed with high humidity (>90% RH) for 3 weeks to harden. After acclimatization, plantlets were transplanted to pots under greenhouse conditions, and the survival percentages were taken four weeks later. Finally, the plants being survived were assessed for their agronomic, yield, and yield-related traits (Ferede *et al*., 2019).

2.8. Data collection

2.8.1. *Callus induction and plant regeneration*

Callus induction efficiency (CIE) was assessed as the number of explants induced callus/ total number of cultured explants used for each treatment x 100. Plant regeneration percent (PRP) was recorded as (number of plantlets/total number of calli) × 100 after PEG treatment. The total number of shoots per culture (TSPC) was counted at the stage of the shoot multiplication when treated by PEG. Similarly, shoot length (SL) and root length (RL) were measured using an autoclaved square paper and a well-sterilized measuring tape after two weeks of plantlet incubation. The total number of roots per culture (TRPC) and the number of roots per shoot (NRPS) were counted at the stage of the root regeneration medium. Data were also recorded for rooting percentage as the percent of rooted shoots (RP) per culture. Survival percentage (SP) was calculated as the percentage of surviving plants after four weeks of transfer to pots.

2.8.2. *Growth and yield parameters*

The selected regenerants were transferred to the pots that were labeled based on the genotype name of the original ex-plant and the PEG level at which the regenerants were grown.

Plants grown in the greenhouse were evaluated for different agronomic traits. In this study, a total of fifteen mung bean regenerants developed from in-vitro culture were evaluated for morpho-phenologic traits. The healthy and physiologically matured regenerants were selected for this study. The experiment was carried out using a completely randomized design with three replications at Areka Agricultural Research Center under greenhouse conditions in 2020. Data on days to flowering, days to maturity, peduncle length (cm), plant height (cm), the number of primary branches per plant, pod length (cm), the number of pods per cluster, the number of pods per plant, the number of seeds per pod, hundred seed weight (g), grain yield per plant (g), grain yield (kg ha^{-1}), biomass yield (kg ha^{-1}), and harvest index were recorded from five regenerants plants grown in pots.

2.9. Data analysis

Collected data were subjected to analysis of variance (ANOVA) and the means were separated using the LSD test at a 0.5% level of probability using the SAS software version 9.0.

3. Results and Discussion

3.1. Effect of genotypes on callus induction and plant regeneration

The analysis of variance result showed all the studied traits were significantly affected by genotypes except the number of roots per shoot (Table 1). This shows the existence of inherent genotypic variability. A similar result was reported by Tsago *et al*. (2014) on sorghum and Ferede *et al*. (2019) on tef. The highest callus induction efficiency (16.87%) was noted for the genotype (G34), while the genotypes G2 and G6 had relatively lower callus induction efficiency of 15.82 and 15.56%, respectively. In this study, the observed highest CIE for the genotype G34 might be due to the genetic makeup of the genotype to induce good callus as compared to the other two genotypes. This finding is supported by the previous reports of (Mekbib e*t al*., 1997; Ferede *et*

al., 2019) on tef genotypes who suggested that good callus induced in some of the genotypes was due attributed to the genetic makeup of the genotypes.

The highest plant regeneration percent (28.90%) was noted for genotype G34 while the genotypes G6 and G2 had relatively similar plant regeneration percentages of 27.82 and 27.56%, respectively. The highest rooting percent (73.07%) and the number of roots per shoot (2.00) were attributed to genotype G34 (Table 1). This could be attributed to the high-quality calli obtained from the genotype (G34), which might be due to the genetic make of the genotype. This result is supported by the previous reports of Ferede *et al*. (2019) on tef genotypes, Helaly *et al*. (2013) on wheat, who reported that callus induction was a critical phase where the regeneration of plants is highly dependent on the quality of callus. In contrast, G6 and G2 showed relatively low rooting percentages and the number of roots per shoot.

3.2. Effect of PEG stress on callus induction and plant regeneration

The analysis of variance results revealed that all the studied traits were significantly affected by PEG levels except total roots per culture and survival percentage (Table 1), signifying the existence of differential responses of genotypes to different levels of PEG. But the total shoots per culture and survival percentage were not genotype-dependent. The result showed that as the PEG level increased the values for most of the studied traits declined while the number of roots per shoot and root length increased. The highest mean values of all parameters except the number of roots per shoot and root length were observed at 0% PEG which was reduced at each subsequent higher level of PEG. On the other hand, the highest number of roots per shoot and root length of mung bean regenerants were observed at 2.0% PEG (Table 1).

The highest callus induction efficiency of 22.72% was observed at 0% PEG and the lowest 10.93% was observed at 2.0% PEG. The plant regeneration percentage of 42.11% at 0% PEG was dramatically decreased to 28.69 % at 0.5%, 25.38% at 1.0%, 23.36% at 1.5%, and reached 20.93% at 2.0% PEG concentration. The highest rooting percentage of 94.53% was observed at 0% PEG and the lowest 51.69% was observed at 2.0% PEG. On the contrary, a significant increment of root length was found at 2.0% (1.17 cm) and 1.5% (1.04 cm) PEG concentrations respectively, as compared to the control and the other PEG levels (Table 1). This reveals an adaptive mechanism to the decreased moisture content in the root zones of plants that enhances increased root length to reach deeper water in the soil. These findings are supported by the previous reports of Ahmed (2014) on rice and Ferede *et al*. (2019) on tef, who found an increase in root length associated with increasing PEG concentration and observed similar trends in the study.

The highest number of roots per culture 34.09 was observed at 0% PEG and the lowest 8.33 was observed at 2.0% PEG. The mean data of shoot length revealed that with increasing PEG stress, shoot length declined in general. The highest shoot length of 1.41 cm was observed at 0% PEG and the lowest 0.82 cm was observed at 2.0% PEG. The highest survival percentage of 90.69% was observed at 0% PEG and the lowest 55.07% was observed at 2.0% PEG (Table 1). The reduced values of regenerants in most mung bean traits at an increased concentration of PEG might be due to osmotic stress which prevents water uptake and might be attributed to the toxic effects of the increased PEG concentration. Similarly, Haruna *et al*. (2019) reported that as the concentration of PEG increased there was a decrease in callus sizes across the treatments on wheat genotypes. Likewise, Tsago *et al*. (2014) on sorghum reported that there was a decrease in shoot and root-related traits with an increase in the concentration of PEG whereas the mean root number increased with an increasing level of PEG treatment in each genotype. This exhibited that as the concentration of PEG increased; the growth of callus steadily decreased and vice versa was true. This result has confirmed the previous reports of Joshi *et al*. (2011) on rice, Farshadfar *et al*. (2012) on wheat, Tsago *et al*. (2013) on sorghum, and Ferede *et al*. (2019) on tef, who reported that the mean callus induction efficiency decreased considerably under higher PEG concentration. The adverse effect of moisture stress was stronger in higher PEG levels (2.0% PEG) and about 5.0% of the cultures induced callus and the induced calli lost their regeneration ability and further growth was inhibited. Similar results were reported by (Biswas *et al*., 2002; Sakthivelu *et al*., 2008)) who stated that the addition of high PEG-6000 in culture media lowers the water

potential of the medium and adversely affects cell division leading to reduced further callus growth.

3.3. Effects of Genotype x PEG interaction on callus induction and plant regeneration

The analysis of variance result depicted that all the studied traits were significantly affected by the interaction effects of genotype and PEG levels (Table 1). The highest number of total shoots per culture (4.2), total roots per culture (34.36), root length (1.23 cm), and survival percentage (93.66%), and the highest shoot length (1.45 cm) for genotypes (G6) were recorded in the control treatment. Also, at the PEG concentration of (0.5%), genotype (G6) showed better plant regeneration percent and rooting percentage of 30.26 and 94.36%, respectively. The significant interaction effects were observed due to genotype by PEG for some of the studied traits, indicating that the genotypes showed differential performances across the different PEG concentrations. This finding confirmed the report by Leila (2013) on six pearl millet genotypes exposed to three different (PEG_{8000}) levels, and Tsago *et al.* (2013) on sixteen sorghum genotypes exposed to five different (PEG_{8000}) levels namely (0, 0.5, 1.0, 1.5, and 2.0%) who found that significant differences among genotypes, PEG and genotype by PEG interactions for shoot length, root length, shoot number, and root number. A similar result was reported by Haruna *et al*. (2019) on sixteen wheat genotypes exposed to six different (PEG_{6000}) levels namely (0, 5 10, 15, 20, and 2, 5%) and observed that significant differences were observed among genotypes for the necrotic mass of the callus. The value of mean shoot length in control (0.0% PEG) for the genotypes (G34, G6, and G2) was 1.45, 1.45, and 1.34 cm, respectively which reduced significantly at each subsequent level of PEG stress till it reached 0.85, 0.75 and 0.85 cm, respectively at 2.0% PEG concentration. Generally, inconsistency in regenerants for most of the studied traits was observed.

Table 1: Callus induction and regeneration of mung bean as influenced by genotypes and PEG levels

Genotype		CIE (%)	PRP (%)	TSPC	RP (%)	TRPC	NRPS	SL (cm)	RL (cm)	SP (%)
G6		15.56b	27.82b	3.04a	72.41b	19.27a	1.94	1.05b	0.91a	71.18b
G34		16.88a	28.91a	2.31b	73.07a	18.50b	2.00	1.09a	0.82b	76.60a
G2		15.82b	27.56b	2.21c	72.34b	18.21b	1.98	1.02c	0.94a	72.26b
LSD (5%)		4.34	0.59	0.09	0.35	0.49	0.06	0.01	0.04	4.33
Sig. level		***	***	***	***	***	Ns	***	***	*
PEG levels										
0%		22.72a	42.11a	4.25a	94.53a	34.09a	0.59e	1.41a	0.49e	90.69a
0.5%		18.69b	28.69b	2.47b	82.54b	22.54b	1.19d	1.12b	0.83d	81.64b
1.0%		15.38c	25.38c	2.21c	72.07c	16.13c	2.10c	1.01c	0.92c	74.33c
1.5%		12.69d	23.36d	2.03d	62.21d	12.21d	2.79b	0.93d	1.04b	65.01d
2.0%		10.93e	20.93e	1.65e	51.69e	8.33e	3.18a	0.82e	1.17a	55.07e
LSD (5%)		1.03	0.90	0.14	0.53	0.75	0.10	0.02	0.06	6.59
Sig. level		***	*	***	***	Ns	***	***	***	Ns
Genotype	PEG levels									
G34	0%	25.36a	43.53a	2.01d	94.36a	33.51a	0.36g	1.45a	1.18a	89.85b
G34	0.5%	20.26b	30.26c	3.26b	83.26b	23.26b	1.26e	1.20c	1.02c	84.60c
G34	1.0%	16.02d	26.02e	2.02d	72.02d	17.02c	2.02d	1.02e	0.95d	78.69d
G34	1.5%	11.89h	23.89g	2.89c	62.89e	12.89d	2.89b	0.95f	0.78e	66.23e
G34	2.0%	10.81i	20.81i	2.81c	52.81f	8.81e	3.18a	0.85h	0.56f	59.84f
G6	0%	21.45b	41.45b	4.20a	94.85a	34.36a	0.85f	1.45a	1.23a	93.66a
G6	0.5%	17.70d	27.70d	4.18a	82.01c	22.01b	1.12e	1.03e	1.02c	81.12c
G6	1.0%	14.73e	24.73f	2.04d	72.04d	15.70c	2.13d	1.00e	0.78e	72.13e
G6	1.5%	12.88g	22.88i	1.77e	61.77e	11.77d	2.78c	0.89g	0.67f	66.11f
G6	2.0%	11.04i	21.04i	1.06f	51.06g	8.06e	3.11a	0.75i	0.45g	52.11g
G2	0%	21.34b	41.34b	4.37a	94.39a	34.39a	0.57g	1.34b	1.10b	88.57b
G2	0.5%	18.12d	28.12d	2.12d	82.35c	22.35b	1.19e	1.12d	1.07c	79.19c
G2	1.0%	15.40e	25.40f	2.03d	72.17d	15.68c	2.16d	1.00e	1.05c	72.16e
G2	1.5%	13.30f	23.30h	1.96e	61.96e	11.96d	2.70c	0.96f	1.04c	62.70f
G2	2.0%	10.94i	20.94i	1.08f	51.19g	8.11e	3.26a	0.85h	0.46g	53.26g
Sig. level		***	***	***	***	***	***	***	***	***
LSD (5%)		2.28	1.99	0.31	1.17	1.66	0.23	0.06	0.13	14.51
SE±		0.6	0.43	0.01	0.15	0.30	0.001	0.0004	0.002	23.03
CV		4.7	2.34	4.11	0.53	2.96	3.90	1.96	5.07	6.54

CIE = callus induction efficiency percent, PRP = plant regeneration percent, TSPC = total shoot per culture, RP = rooting percentage, TRPC = total roots per culture, NRPS = number of roots per shoot, SL = shoot length; RL = root length, SP = survival percentage; means followed similar letters in column are not statistically difference at $p \leq 0.05$

3.4. Evaluation of *In-Vitro* regenerated plants for validation under greenhouse conditions

3.4.1. *Flowering and vegetative growth*

The analysis of variance results revealed that the regenerants showed highly significant differences in all the measured flowering and vegetative growth traits (Table 2). Regenerants of the treatment combination of genotype G2 x 2% PEG flowered earlier, which took 33.16 days, while days to flowering for the regenerant from the treatment combination of genotype G6x1.0% PEG took longer time to flower with the value 36.67 days. In terms of maturity regeneratnts of the treatment combinations of G2x2.0% (60 days) matured earlier while those from the treatment combination of G6x0.5% matured late with a value of 76.00 days.

The in-vitro-developed mung beans having lower values for days to flowering and days to maturity were considered drought-tolerant since these genotypes had ability to escape terminal drought and could be recommended for drought-prone areas. Plaza-Wüthrich *et al*. (2013) reported that earliness for days to heading and maturity are important traits on tef for areas with low rainfall to escape terminal drought, and in high rainfall with long growing season areas, can be employed in double-cropping systems.

The highest terminal leaf length (6.86 cm) was recorded from the regenerant developed from the

treatment combination of G34x0% PEG, while the least terminal leaf length (3.53 cm) was noted from the regenerant obtained from the treatment combination of G34x2.0% PEG. The highest terminal leaf width of 11.36 and 11.05 (cm) were recorded from the regenerants from the treatment combinations of G2x0% PEG and G34x0% PEG, respectively. The least terminal leaf width (6.36 cm) was obtained from the regenerant developed from the treatment combinations of G6x2% PEG. The highest and the least peduncle length of 9.13 cm and 5.43 cm were recorded from the treatment combinations of G6x0% PEG and G2x2% PEG, respectively (Table 2). The highest plant height of 44.16 (cm) was recorded from the regenerants from the treatment combination of G6x0.5% PEG while the least (38.13 cm) was recorded from G2x0.5% PEG. The observed variations on vegetative growth parameters at different treatment combinations of PEG levels and genotypes might be attributed to the differential responses of the tested genotypes to the induced PEG levels.

Table 2: Flowering and vegetative growth of mung bean as affected by genotypes and PEG levels

Genotypes	PEG levels	DTF (50%)	DTM (90%)	TLL (cm)	TLW (cm)	PDCL (cm)	PHT (cm)	BRN	PODL (cm)
G34	0 %	36.00a	72.66a	6.86a	11.05a	8.75a	40.00b	4.00a	9.16b
G34	0.5%	36.33a	71.66a	6.36a	10.98a	8.23a	41.33a	3.26b	10.66a
G34	1.0%	36.00a	72.66a	4.92c	10.03a	8.74a	42.00a	2.00d	6.66f
G34	1.5%	35.66a	69.66b	4.60d	9.77a	8.57a	41.00a	2.00d	7.53e
G34	2.0%	35.16a	69.66b	3.53e	8.38b	6.60c	39.00b	2.00d	10.98a
G6	0 %	34.33b	70.66a	4.64d	8.60b	9.13a	42.00a	3.00c	11.26a
G6	0.5%	36.33a	76.00a	4.93d	10.25a	7.59b	44.16a	3.00c	10.00a
G6	1.0%	36.76a	74.00a	4.83d	10.76a	7.36b	40.10b	3.00c	10.00a
G6	1.5%	35.66a	70.66a	4.94d	8.36b	8.12a	39.66b	2.00d	9.03c
G6	2.0%	34.33b	69.66b	4.53d	6.36c	5.48d	38.56b	2.00d	7.60e
G2	0 %	35.83a	72.33a	6.60a	11.36a	8.66a	40.00b	3.00c	9.86b
G2	0.5%	36.00a	72.00a	5.00b	9.11a	8.17a	38.13b	2.00d	11.00a
G2	1.0%	36.00a	68.66b	5.17 b	10.22a	8.17a	39.33b	2.00d	8.96d
G2	1.5%	34.16b	68.00b	5.41b	9.17a	6.30c	40.00b	3.00c	10.96a
G2	2.0%	33.16b	60.00c	4.93d	8.96b	5.43d	39.00b	1.00e	5.50g
Sig. level		**	**	***	***	***	***	***	***
SE±		0.89	11.82	0.69	0.28	0.85	1.79	0.01	0.41
CV (%)		2.68	4.87	10.41	9.68	10.87	3.32	2.62	6.94
LSD (5%)		2.85	10.34	1.61	2.78	2.51	4.02	0.19	1.93

DTF = days to flowering, DTM = days to maturity, TLL = terminal leaf length, TLW = terminal leaf width, PDCL = peduncle length, PHT = plant height, BRN = number of primary branches per plant, PODL = pod length; means followed similar letters in column are not statistically difference at $p \leq 0.05$

3.4.2. *Yield related traits*

The analysis of variance results depicted that the regenerants showed highly significant differences in all the measured yield-related traits (Table 3). Regenerant from the treatment combinations of G34x0% PEG exhibited the highest value for the number of pods per cluster (5) and pods per plant (19.66). The highest, seeds per pod (11.36), grain yield per plant (5.22 g), grain yield (996.23 kg ha^{-1}), and harvest index (0.27) were recorded from the regenerant from the treatment combinations of G6 (0). On the other hand, regenerants obtained from the treatment combinations of G2 (1) showed poor performance for pods per cluster (2.66) and pods per plant (12.00). The highest hundred seed weight (5.49 g) was recorded from the regenerants obtained from the treatment combinations of G6 (0), while the least hundred seed weight (3.12 g) was recorded for the regenerants obtained from the treatment combinations of G2 (1.5). The highest biomass yield of 4319.80, 4219.80, and 4219.80 (kg ha^{-1}) was recorded for the regenerants obtained from the treatment combinations of G34 (1.5), G34 (2.0), and G2 (1.5), respectively (Table 3).

The result indicated that an in-vitro culture is an important tool to screen drought-tolerant genotypes and improve desirable agronomical traits. In general, most of the regenerants obtained from G34 and G6 showed the best performance under the

greenhouse and were drought-tolerant under the in-vitro condition, suggesting that the performance of the tested regenerants under in vitro conditions was realized under greenhouse conditions.

Table 3: Yield related traits of mung bean as affected by genotypes and PEG levels

Genotype	PEG levels	PPC	PPP	SPP	GYPP (g)	HSW (g)	GYLD (kg ha^{-1})	BM (kg ha^{-1})	HI
G34	0 %	5.00a	19.66a	10.63a	4.11b	4.21c	892.96b	3699.80c	0.24a
G34	0.5%	3.00c	19.00a	8.30d	4.18b	4.05c	596.12d	3819.80b	0.15c
G34	1.0%	3.00c	14.33c	10.46b	4.01b	4.12c	795.21b	3886.50b	0.20b
G34	1.5%	3.66b	14.00c	9.80c	3.97b	4.22c	822.94b	4319.80a	0.19b
G34	2.0%	3.33c	12.66d	7.40d	3.08c	4.22c	555.53e	4219.80a	0.13e
G6	0 %	4.00b	12.66d	11.36a	5.22a	5.49a	996.23a	3686.50c	0.27a
G6	0.5%	3.66b	16.66b	11.10a	3.99b	4.16c	754.74b	4119.80a	0.18c
G6	1.0%	3.00c	15.33b	10.13b	4.14b	4.18c	595.23d	3886.50b	0.15c
G6	1.5%	3.00c	15.33b	10.06b	3.96b	4.12c	729.55b	3953.10b	0.18c
G6	2.0%	3.00c	12.13e	10.16b	3.97b	4.20c	587.16d	3986.50b	0.14d
G2	0 %	3.66b	19.00a	10.20b	5.17a	5.13b	693.94c	3886.50c	0.17c
G2	0.5%	3.33b	18.66a	8.08d	4.20b	4.19c	577.27d	3786.50c	0.15c
G2	1.0%	2.66d	12.00e	10.06b	4.18b	4.08c	552.52e	4019.80b	0.13d
G2	1.5%	3.00c	17.33b	11.06a	3.95b	3.12d	589.57d	4219.80a	0.13d
G2	2.0%	3.00c	15.66b	9.60c	4.07b	4.21c	592.74d	3916.50b	0.15d
Sig. level		***	***	***	***	***	***	**	***
SE±		0.13	2.62	0.14	0.02	0.01	6136.10	29904.00	0.01
CV (%)		10.88	10.37	3.82	3.75	1.56	11.37	4.37	10.10
LSD (5%)		1.09	4.87	1.13	0.46	0.19	235.62	520.15	0.05

PPP = the number of pods per plant, SPP = number of seeds per pod, GYPP = grain yield per plant, HSW = hundred seed weight, GYLD = grain yield, BM=biomass yield and HI = harvest index; means followed similar letters in column are not statistically difference at $p \leq 0.05$

4. Conclusion

Drought is one of the most liming factors in mung bean production and productivity. Evaluating mung bean genotypes in PEG-induced drought conditions under in-vitro and greenhouse conditions is important to screen drought-tolerant genotypes. This technique is crucial because the results of the in-vitro were reproduced or realized in the greenhouse. It also indicated that an *in-vitro* culture is an important tool to develop drought-tolerant genotypes and improve desirable agronomical traits under greenhouse conditions for further field verification. Therefore, some regenerants performed better under the greenhouse conditions were became drought-tolerant under the *in-vitro* condition. In general, most of the regenerants showed the best performance under the greenhouse and were drought-tolerant under the *in-vitro* condition, suggesting that the performance of the tested regenerants under *in vitro* conditions was realized under greenhouse conditions. This suggests the accumulated performance of the tested regenerants under *in-vitro* conditions was realized under greenhouse conditions. Further study is indeed required to understand the mechanism of drought tolerance for the *in-vitro* selected somaclones and to put the recommendation on a strong basis.

Conflict of interest

The authors declare that there is no conflict ofinterest in publishing the manuscript in this journal.

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