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In Vitro Screening of Cactus [*Opuntia ficus-indicia* (L.) Mill] Genotypes for Drought Tolerance

Berhan Mengesha[1*], Firew Mekbib[2], Eyasu Abraha[3]

[1]Department of Plant Science, College of Agriculture, Axum University, Axum, Ethiopia
[2]Department of Plant Sciences, College of Agriculture and Environmental Science, Haramaya University, Dire Dawa, Ethiopia
[3]Tigray Agricultural Research Institute, Mekelle, Ethiopia
Email: *mengesha.berhan@yahoo.com

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Abstract

Drought is one of the complex environmental factors affecting growth and yield of crops in arid and semi-arid areas of the world. In this context, this investigation was carried out to select drought tolerance cactus genotypes under *in vitro* condition. An experiment was carried out at Laboratory of Mekelle Agricultural Research Center, Northern Ethiopia. Six cactus pear genotypes namely, Gerao, Keyih Beles, Shenkor, Limo, Lemats Beles and Suluhna were used. Areoles were used as explants in tissue culture. The non-ionic water soluble polymer polyethylene glycol (PEG) of molecular weight 6000 was used as osmoticum to simulate water stress. In the first culture, the MS medium was supplemented with (2, 4-D (4 mg/l) and BA (0.5 mg/l) for callus induction. In all cultures MS medium was supplemented with 0, 10, 20 and 40 g/l polyethylene glycol (PEG) and was solidified with 0.8% agar and 30 g of sucrose. Significant differences were observed among the genotypes, PEG levels. In the first culture highest number of explants initiated callus on medium supplemented with no PEG but had not shown significant difference with 10 g/l PEG. At 10 g/l PEG, the callus induction frequency, callus fresh weight and plantlet regeneration were recorded highest for Suluhna (83.3%, 5.5 g and 63.3%), respectively. At 40 g/l PEG, callus induction frequency, callus fresh weight and plantlet regeneration were produced highest for Suluhna (41.7%, 2.75 g and 45%), respectively but no significant difference with Gerao, Limo and Lemats Beles. However, Shenkor and Keyih Beles were induced callus but became reddish black within 35 days supplemented with 40 g/l PEG. Both shoot and root production decreased with increased PEG level in the medium. At 40 g/l PEG in MS medium, the highest shoot number was in Suluhna genotype (4.33) followed by Gerao (3.67). The highest shoot length was in Suluhna (2.11 cm) with no significant difference with Gerao (2.02 cm). Root number (5.00 and root length (1.41 cm) were in the genotype Suluhna. Survival percentage of *in*

vitro regenerated plantlets was 100% during hardening. By taking into consideration, all the growth parameter tested revealed that Suluhna, Gerao, Limo and Lemats Beles showed better drought stress tolerance at the highest level of PEG while Keyih Beles and Shenkor appeared to be drought sensitive at the highest level of PEG.

Keywords

Callus Induction, Polyethylene Glycol (PEG 6000)

1. Introduction

Drought is the main limiting factor of products in agricultural systems in the arid and semi-arid region [1]. Despite the large range of commodities cactus pear could provide in areas with little available resources, it has received scant attention by horticultural research [2] [3]. Most of the research in plant physiology and ecology originates in the USA, while Israeli, Italian, and South African researchers produce most of the horticultural research. Advancing desertification, coupled with the urgent need for appropriate technologies and crops capable of developing sustainable and valuable production in arid and semiarid areas, has raised new interest for the environmental role and the potential production of cactus pear. There is, therefore, a need for increased farmer interest and knowledge of production and adaptability of cactus pear through published information and more research. Increasing knowledge of environmental influences on cactus-pear productivity and quality will also allow more profitable production [4] [5]. Although relevant information on the above-ground growth and development of the cactus-pear plant is available [6] [7], very limited studies have been done on *Cactaceae* roots. They certainly differ from that of other plants, as they develop xeromorphic characteristics [8].

Drought severely disturbs water balance of the plant body and causes alterations in water uptake patterns of plant [9] [10]. Water availability is the most important environmental factor limiting plant growth and survival in arid areas [11] [12]. Deleterious effects of water stress have been reported in different crops such as tomato [13] soybean [14] [15] corn [16] and citrus [17]. Root systems of these plants can be subjected to prolonged droughts that are interrupted by sporadic and often light rainfall [18] [19]. The root system of the succulent cactus pear occurs predominantly in the upper layers of the soil, where the soil water content is both temporally and spatially heterogeneous [20] [21]. As for other species, seasonally high temperatures limit root proliferation near the soil surface [22].

For *in vitro* drought stress induction, one of the most popular approaches is to use high molecular weight osmotic substances, like polyethylene glycol (PEG). Polyethylene glycol PEG (6000) is a non penetrable and nontoxic osmotic substance which is used to lower the water potential of the culture medium and it has been used to simulate drought stress in cultured plant tissues [23]. Polyethylene glycol (PEG) has long been used in research programs to induce water deficit stress in plants [24]-[26]. PEG nei-

ther enters nor substantially degrades and is not absorbed by cells in culture. The cells are left under the stress of water deficits in a manner similar to that they would be under actual desiccation condition [27]. *In vitro* selected plants with a significant improvement for drought tolerance were reported for maize [24] sorghum [28] wheat [29] rice [25] and Tagetes [30]. However, report on cactus pear grown *in vitro* for plantlets production to select ambient genotypes is little. The present experiment was undertaken to provide information on tissue culture and *in vitro* regeneration of cactus genotypes for selection of drought tolerant plantlet under *in vitro* stress media.

The specific objectives were:

- To evaluate cactus genotype/s for *in vitro* drought tolerance.
- To determine the response of cactus genotypes for different polyethylene glycol (PEG) concentrations.

2. Materials and Methods

2.1. Plant Material

Six cactus pear genotypes namely: Gerao. Keyih Beles, Shenkor, Limo, Lemats Beles and Suluhna were used. Explants were obtained from the mother plants present at the cactus nursery site of Mekelle Agricultural Research Center.

2.2. Sterilization and Inoculation of Explants

Young cactus cladodes were cut into pieces and surface disinfected by washing under running tap water for 30 min and for a laundry bleach during 20 min. The cladodes were sprayed with 70% ethanol and cleaned with a clean towel and then rinsed in distilled water for three times Under laminar flow hood in sterile conditions, the cladodes were soaked in ethanol 70% for 1 min and rinsed three times with double distilled water followed by immersion in 5% sodium hypochlorite plus 3 drops of Tween-20 for 25 min, and then rinsed five times with double sterile distilled water. Equipments like forceps, blades and other working materials were sterilized using a Glass Bid Sterilizer under the laminar flow hood during inoculation. Finally, disinfected explants were cut to 1 cm^2 pieces each and cultured in a MS [31]. In the first culture the MS Medium supplemented with 30 g/l sucrose, 0.8% agar, 4 mg/l 2, 4-D and BA 0.5 mg/l for callus induction. In the second culture callus was sub-cultured on fresh MS with BAP (0.5 mg/l) for plantlet initiation and 1mg/l for shoot multiplication. In the third culture, initiated plantlets were separated as healthy and unhealthy groups and sub-cultured again on fresh MS containing NAA (1.5 mg/l) for root development. In all cultures, MS was supplemented with 0, 10, 20 and 40g/l poly ethylene glycol (PEG) as treatment. The pH of the media was adjusted to 5.7 and was maintained by 0.1 N NaOH before autoclaving. The medium was then transferred into the test tube (40 ml each) after that it was heated at 100˚C for proper mixing prior to autoclaving at 121˚C for 20 min. After inoculation, each culture vessel was sealed with a parafilm, labeled with the date of inoculation and name of the genotypes. Finally they were transferred into the growth room and arranged randomly on the shelves.

2.3. Culture Conditions

The bottles were then kept in a growth chamber under 16:8 h (light: dark) photoperiod regime with light intensity of 2000 - 2500 lux provided by Mahtab, Iran, 40 W white bulbs at 25˚C ± 2˚C and 70% relative humidity (RH). The explants were sub cultured with the same media every three weeks interval.

2.4. Data Collection and Analysis

After the end of callus induction period callus induction frequency (CIF) was recorded as the number of calli induced divided by total number of explants cultured × 100. After four weeks of culture, callus was excised and callus fresh weight (CFW) was determined. Finally, at the end of plant regeneration, data for plant regeneration percent (PRP) was recorded as the number of plantlets obtained divided by number of calli planted for regeneration cultured × 100, number of shoot and root determined by counting shoot and root length measured using ruler. Analysis of variance (ANOVA) was performed to test the significance of the difference between treatments in CRD in factorial arrangement in three replications. When significant differences were found ($P \leq 0.05$), a multiple comparison of means was done by Duncan Multiple Range Test.

2.5. Acclimatization of Plantlets

In vitro healthy and rooted plantlets were washed to remove medium adhered with roots and the plants were planted in polystyrene tray which filled with sand, coco peat, compost and their mixture at the respective ratio of 2:1:1 and covered by transparent polyethylene sheets. The acclimatized *in vitro* plants were kept in acclimatized green house for four weeks before transplanting out doors and finally transferred to pots.

3. Results and Discussion

3.1. Days to Callus Induction

Days required for callusing was recorded by careful observation of the explants every day. Number of days required for callus induction varied depending on the different concentration of PEG whereas the genotypes had not shown significant difference for days to callus induction among them (Table 1). Days required for callus induction was significantly influenced by the concentrations of PEG. The control treatment responded earlier towards callusing and required least number of days (22 days) for Suluhna and Keyih Beles genotypes. However, MS medium supplemented with 40 g/l PEG responded later for all genotypes. The results of this experiment showed that, increasing PEG (6000) was an effect on days to callus induction frequency.

3.2. Callus Induction Frequency (%)

Callus induction was observed following 4 - 5 weeks of culture. Cactus pear genotypes exhibited a significant interaction with PEG stress levels for callus induction frequency (Table 1). Explants produced callus varied depending on PEG concentration of me-

Table 1. Interaction effect of different peg concentrations on days to callus initiation, callus induction frequency and fresh weight (g).

Genotype	PEG concentration (g)	Days to callus induction	Callus induction frequency (%)	Callus fresh weight (%)
Gerao	0	22.3^{gh}	97.1^{a}	5.60^{ab}
	10	24.3^{fgh}	83.3^{ab}	4.87^{bcd}
	20	24.6^{efgh}	58.3^{bcd}	3.18^{ef}
	40	31.6^{a}	38.7^{efg}	2.40^{fg}
Keyih Beles	0	22^{h}	75^{abc}	4.85^{bcd}
	10	25^{defgh}	66.7^{abcd}	4.42^{d}
	20	27.6^{bcde}	41.7^{def}	2.78^{efg}
	40	32.3^{a}	21.7^{f}	1.33^{h}
Shenkor	0	23^{gh}	66.7^{abcd}	4.92^{bcd}
	10	25.3^{defg}	41.7^{def}	4.38^{d}
	20	27^{cdef}	33.3^{ef}	2.92^{efg}
	40	30.3^{ab}	16.7^{f}	1.26^{h}
Suluhna	0	22^{h}	91.7^{a}	6.08^{a}
	10	23.3^{gh}	83.3^{ab}	5.5^{abc}
	20	27.3^{cdef}	70.0^{abc}	3.42^{e}
	40	29.6^{abc}	41.7^{def}	2.75^{efg}
Lemats Beles	0	22^{h}	83.3^{ab}	4.67^{cd}
	10	23.3^{gh}	60.3^{abcd}	4.25^{d}
	20	27^{cdef}	50^{cde}	2.95^{efg}
	40	31^{a}	36.0^{ef}	2.11^{g}
Limo	0	22.3^{gh}	83.3^{ab}	5.42^{abc}
	10	25.3^{defg}	75^{abc}	4.88^{bcd}
	20	28^{bcd}	58.3^{bcde}	3.12^{ef}
	40	31^{a}	33.3^{fg}	2.15^{g}

Mean values of the same column followed by the same letter are not significantly different at 0.05 level using Duncan's Multiple Range Test.

dium. In control (0 g PEG), almost all explants initiated callus. With the increasing level of PEG, the mean percentage of callus induction reduced significantly. Among the genotypes callus induction percentage were varied distinctly. The highest number of explants induced callus in the genotype Gerao and Suluhna (91.7%) followed by Limo and Lemats Beles. (83.3%), Keyih Beles (75%) and the lowest was recorded at Shenkor 66.7% at control Figure 1 and Figure 2(a) and Figure 2(b). With increasing level of PEG supplement into medium callus induction decreased gradually. However, explants of all genotypes induced callus vigorously in medium with no PEG but had not shown significant difference with MS medium supplemented 10 g/l PEG. The result indicated

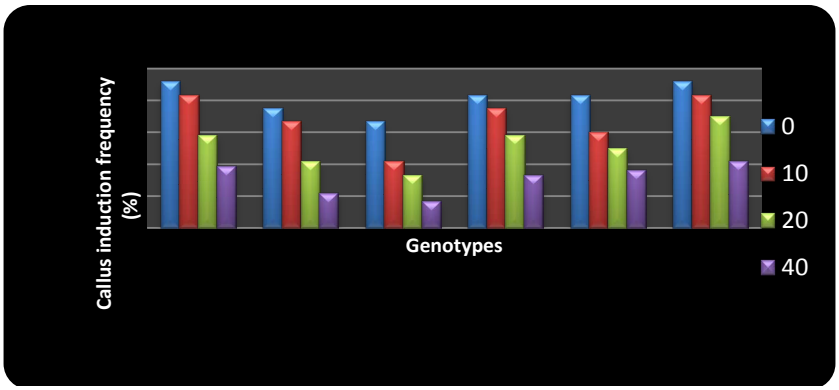
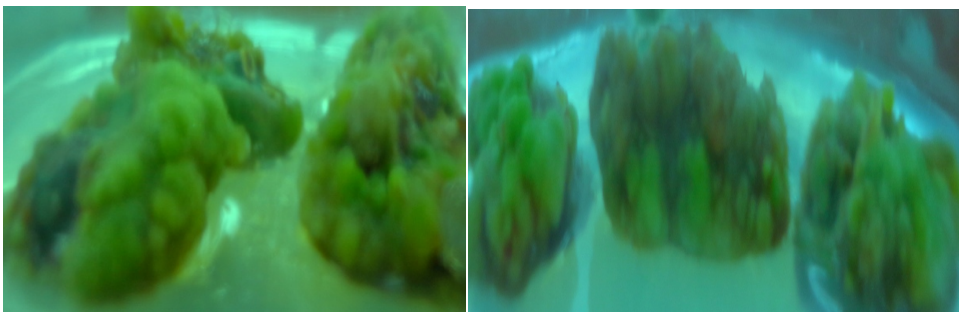


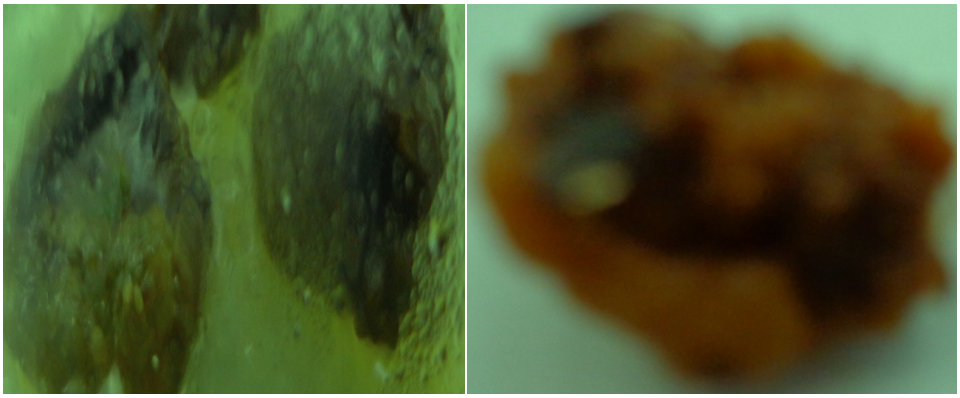
Figure 1. Mean callus induction frequency for each PEG level.



(a) (b)

Figure 2. Cactus pear treated without PEG (control). (a) Gerao genotype; (b) Suluhna genotype.

that increasing level of PEG from 0 to 10 g/l PEG had lowest effect in mean callus induction for the tested genotypes. At 20 g/l PEG explants of Suluhna initiated the highest of 70% callus followed by Gerao and Limo 58.3%. The lowest callus was initiated at genotype of Shenkor 33.3%. However, the genotypes Lemats and Keyih Beles had not shown significant difference between them for callus induction. At 40 g/l PEG Suluhna gave highest value 41.7% followed by Gerao genotype (38.7%) **Figure 3(a)**. The lowest was at Shenkor genotypes 16.7% **Figure 3(b)** Suluhna, Gerao, Lemats Beles and Limo indicating their relative tolerance for the highest PEG compare with Shenkor and Keyih. [32] reported that reduction in callus induction ability and plant regeneration efficiency with increased levels of PEG stress. Likewise, [33]-[35] repotted that decreasing in callus induction is a typical response of explants of crop genotypes when subjected to PEG stress. However, the response of callus induction for PEG treatment was genotype dependent. [36] also reported that the difference in decreasing trend in callus induction of the genotypes might further explain difference in osmotic regulation among genotypes, which enables them to maintain osmotic balance to assist initiation of callus cells under severe stress conditions or might be due to either water shortage which led to profuse mutation in cellular metabolism including protein functioning and alteration in amount of proteins. Cells grown under stress may have to spend more metabolic energy than those grown in the absence of stress. The extra energy is probably used up in regulating osmotic adjustment resulting in declined callus growth [37].



(a) (b)

Figure 3. Cactus pear treated with 40 g/l PEG. (a) Gerao genotype; (b) Shenkor genotype.

Generally the mean callus induction frequency decreased drastically in genotypes under higher PEG level than lower PEG level.

3.3. Callus Fresh Weight (CFW)

Calli were sub-cultured for 4 - 5 weeks period on MS media with different levels of PEG (**Table 1**). The amount of callus varied with genotypes and fresh weights were determined. Total callus fresh weight varied among genotypes, as indicated on **Table 1** and in all genotypes, effect of the PEG treatments resulted in decrease of the mean CFW with increasing level of the PEG from 0 to 40 g/l. Genotype Suluhna produced the highest callus fresh weight (2.75 g) whereas the lowest was recorded in Shenkor (1.26 g) at PEG 40 g/l (**Figure 4**). Such a decrease in callus fresh weight in response to PEG stress might be due to water shortage which affects development and growth of cells. The result was indicated that the lowest callus fresh weight in the latter genotype was due to PEG stress. The major effect of PEG stress in callus growth is mainly observed in the form of decreasing the callus fresh weight which is a typical response in callus tissue of many crop plants [14] [32] [38] [39]. Addition of PEG-6000 in solid media lowers water potential of the medium that adversely affect cell division leading to reduced callus growth [14] [40]. Cell division and cell growth are the two primary processes involved in increase of fresh weight. In general, cell division is considered to be less sensitive to drought when compared with cell enlargement or growth [14]. However, both cell expansion and cell division can be influenced by relatively mild osmotic stress. Generally, the results of this experiment showed that genotypic differences in callus proliferation are genetically.

3.4. Plant Regeneration Percent (PRP)

Plant regeneration percent decreased with increase in PEG concentration irrespective of the genotypes tested (**Table 2**). The calli sub-cultured on new fresh MS supplemented with different levels of PEG and PGR regenerated plantlets 4 - 5 weeks culture. Shoot regeneration after sub-culturing on fresh MS media supplemented with different levels of PEG started after four weeks of callus growth. The medium was changed in 15

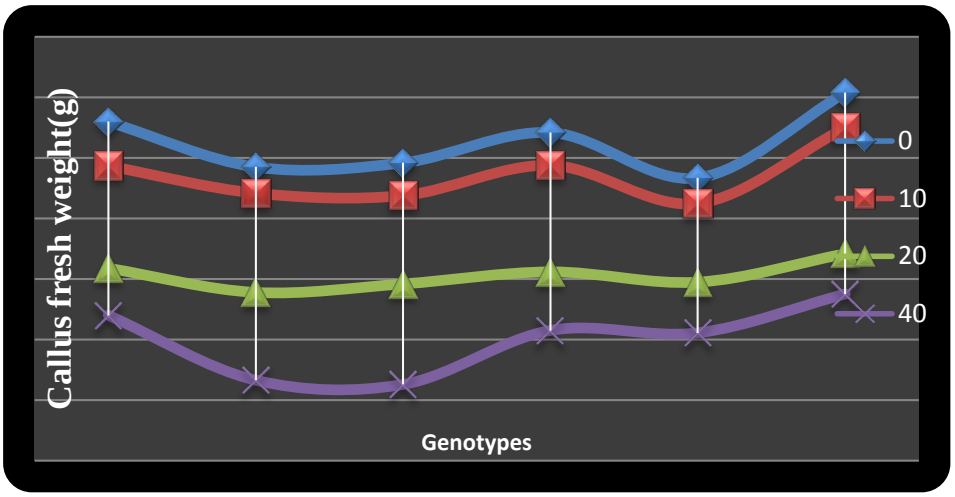


Figure 4. Mean callus fresh weight for each PEG level.

Table 2. Interaction effect of different PEG concentrations on plant regeneration (%), shoot number and shoot length (cm).

Genotype	PEG concentration (g)	Plant regeneration (%)	Shoot number	Shoot length (cm)
Gerao	0	66.7^{ab}	9.67^{ab}	2.91^{abc}
	10	53.3^{abcde}	8.67^{abcd}	2.65^{cdef}
	20	41.7^{bcdef}	6.0^{fgh}	2.25^{hij}
	40	35.0^{cdef}	3.67^{ijk}	2.02^{jkl}
Keyih Beles	0	50.0^{abcde}	7.33^{def}	2.88^{abc}
	10	33.3^{cdef}	5.00^{ghi}	2.45^{fgh}
	20	25.0^{efg}	2.33^{kl}	2.1^{ijk}
	40	0.00^{g}	0.00^{m}	0.00^{n}
Shenkor	0	41.7^{cdef}	7.67^{cdef}	2.83^{abcd}
	10	31.6^{cdef}	4.67^{ghi}	2.15^{ijk}
	20	16.7^{fg}	1.67l^{m}	1.9^{klm}
	40	0.00^{g}	0.00^{m}	0.00^{n}
Suluhna	0	75^{a}	10.33a	3.07^{abc}
	10	63.3^{abc}	9.33^{abc}	2.59^{def}
	20	56.7^{abcd}	6.00^{fgh}	2.32^{ghi}
	40	45.0^{bcdef}	4.33^{hij}	2.11^{ijk}
Lemats Beles	0	61.7^{abc}	10.00^{a}	2.75^{bcde}
	10	41.7^{bcdef}	8.67^{abcd}	2.71^{cde}
	20	38.3^{bcdef}	6.33^{efg}	1.9^{klm}
	40	33.3^{cdef}	2.67^{jkl}	1.82^{lm}
Limo	0	58.3^{abcd}	9.00^{abcd}	2.99^{ab}
	10	50.0^{abcde}	7.33^{def}	2.55^{efg}
	20	33.3^{cdef}	4.00^{ijk}	2.10^{jkl}
	40	28.3^{defg}	2.33^{kl}	1.7^{m}

Mean values of the same column followed by the same letter are not significantly different at 0.05 level using Duncan's Multiple Range Test.

days and after four weeks, calli with clearly differentiated shoots were scored as regenerating callus, regardless of the number of shoots. Calli regenerating plantlets were revealed a highly significant variation among the tested genotypes. At control (0 PEG) the highest value was recorded in genotype Suluhna (75%) followed by Gerao (66.7% (**Figure 5(a)** and **Figure 5(b)**. But no significant difference among the other tested genotypes. At 10 g/l PEG treatment imposed the lowest reduction in PRP indicating that the genotypes are the least affected by PEG treatment in terms of plant regeneration (**Figure 6**). However, MS medium supplemented with 20 g/l and 40 g/l PEG had shown highest effect for most genotypes. At 20 g/l PEG, Suluhna genotype was recorded the highest value (56.7%) followed by Gerao and Lemats Beles (41.7%) and Keyih Beles (25%) whereas the lowest was recorded in Shenkor genotype (16.7%). At 40 g/l PEG, the highest concentration in the experiment had showed highest effect in the genotypes tested for plant regeneration percent. The highest plant regeneration was recorded in Suluhna (45%) genotype followed by Gerao (35%), Lemats Beles 33.3% and Limo (28.3%). However, there was no regeneration in Shenkor and Keyih Beles. [41] reported that decrease in callus growth, proliferation and calli volume (in first two sub-cultures in callus culture medium) and reduced plantlet regeneration (in follow-up regeneration

(a) (b)

Figure 5. Shoot regeneration from callus. (a) At control for suluhna genotype; (b) At 10 g/l PEG for gerao genotype.

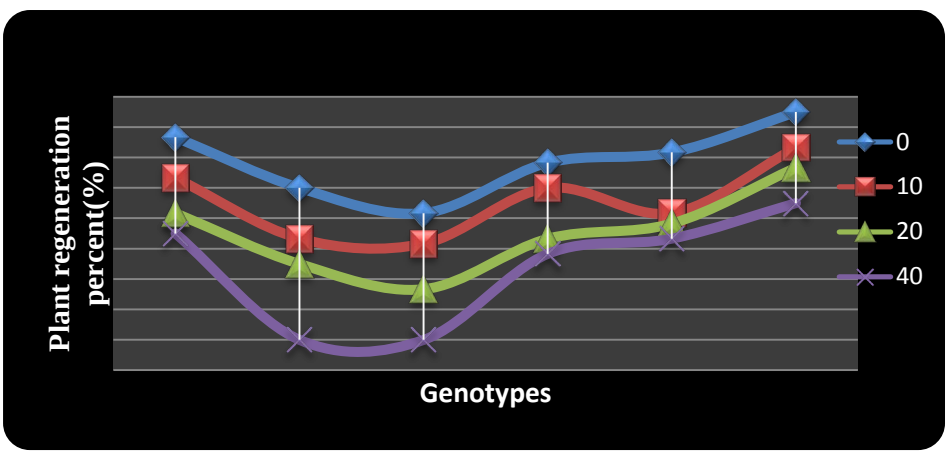


Figure 6. Means of plant regeneration percent for each PEG level.

medium) to a certain level to acquire tolerance to PEG-induced drought. The typical decrease in plant regeneration in callus cells of crop plants in response to water stress is due to water shortage in the cells which leads to a decrease in cell turgor and eventually cell growth. Addition of PEG-6000 in culture media lowers water. Similar to this findings [32] [42] [43] reported that an increment in PEG stress level caused reduction of plant regeneration percent (PRP) in sorghum genotypes which is a typical response of callus cells of many plants. Similarly, [44] reported that the regeneration potential of calli decreased significantly with increasing stress duration and osmotic stress in culture media in wheat genotypes. At the cellular level, the effect of water stress on the slowdown of cell divisions and elongation by the loss of turgor has been widely reported [45]. The addition of PEG in the medium causes cell dehydration by reducing water availability to cells, which leads to a loss of cell turgor and hence a loss of growth [46].

3.5. Shoot Number

The total number of shoot significantly varied among different genotypes (**Table 2**). Number of shoot was decreased significantly with increasing level of PEG (6000) supplemented into medium. The interaction effects of genotypes to PEG concentrations on number of shoot varied significantly. All genotypes regenerated the highest number of shoots in control compared to all PEG levels. The highest number of shoots (10.33 per culture) was produced in Suluhna followed by Lemats Beles, Gerao and Limo (10, 9.67 and 9), respectively (**Figure 7**) whereas at 10 g/l PEG, all genotypes had not varying as compared to control except Keyih Beles, Shenkor and Limo genotypes. At 40 g/l PEG level, the highest number of shoots was produced in Suluhna (4.3) followed by Gerao and Lemats Beles (3.67 and 2.67) respectively. 0.0 was recorded in Keyih Beles and Shenkor. The reasons could be attributed to the presence of non-viable cells in the callus, which later on affected the formation of shoots. This finding is in agreement with the findings of [47]. Different growth performances of genotypes under increasing PEG level in the present experiment might be due to genetic variability of the genotypes.

3.6. Shoot Length

Regarding the shoot length no significant differences were found among the genotypes

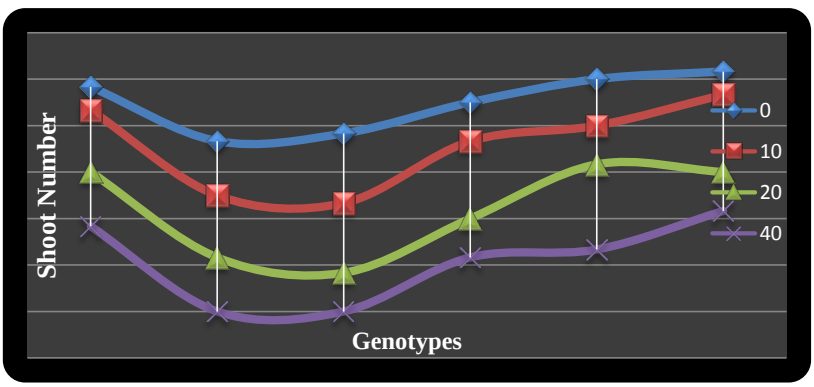


Figure 7. Means of shoot number for each PEG level.

at free PEG (Table 2). At 10 g/l the longest shoot length was found in Lemats Beles 2.71 with no significant difference with Gerao, Suluhna and Limo (2.65 cm, 2.59 cm and 2.55 cm) respectively whilst the shortest was exhibited by Shenkor (2.15 cm) genotype. On the contrary, at 20 g/l PEG (6000) the highest shoot length was in genotype Suluhna (2.32 cm) followed by (2.25 cm) Gerao. In case of the highest concentration (40 g/l PEG), root length decreased in all tested genotypes (Figure 8). The highest was recorded in Suluhna followed by Gerao and Lemats (2.11 cm, 2.02 cm and 1.82 cm), respectively. Similarly, [48] reported that remarkable decrease in shoot length of tomato has been observed with increasing PEG concentrations.

3.7. Root Number

Root initiation in mini shoots sub-cultured on fresh MS medium supplemented with different level of PEG started after 2 - 4 weeks (Table 3). After three weeks the number and length of root varied significantly depending upon PEG supplement into medium. The number of roots per shoot was the highest in Suluhna (10.6) followed by Lemats Beles, Limo, Gerao and Shenkor (10.1, 9.9, 9.00 and 9.00), respectively. The lowest was recorded in Keyih Beles (8.17). The number of roots per plantlet gradually decreased with increase level of PEG. At 10 g/l PEG, the highest number of roots was recorded in genotypes of Suluhna (8.67) and Lemats Beles (8.67). But when the PEG concentration increase from 0 to 40 g the number of roots produced per culture should decreased. At 20 g/l PEG the highest number of roots were produced in the genotypes Suluhna (6.00 per shoot) followed by Lemats Beles and Limo (5.67 per shoot). The lowest was at Shenkor (4.00). At the highest PEG concentration (40 g/l) Suluhna recorded highest root number followed by Gerao, Limo and Lemats Beles (5.00, 4.00, 3.67 and 3.67), respectively (Figure 9). Under drought stress, roots are generally affected first than other plant parts [49]-[51] also reported that, roots of cacti die back when subjected to drought.

3.8. Root Length

Significant differences were found among genotypes under investigation for root length

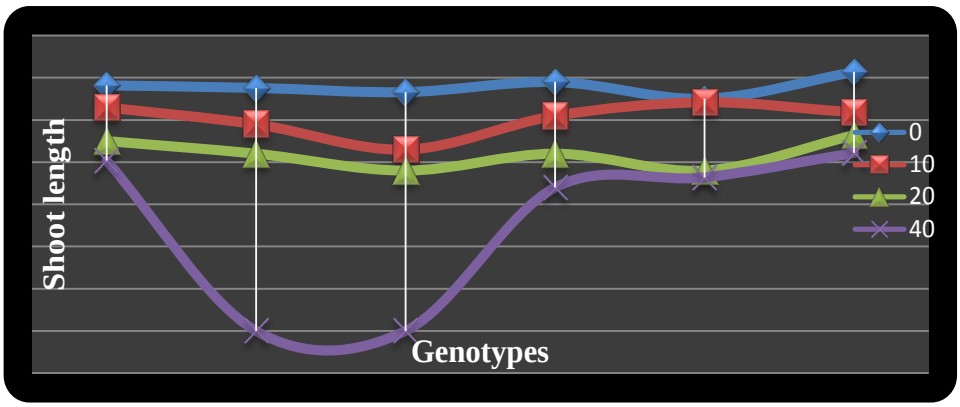


Figure 8. Means of shoot length for each PEG.

Table 3. Interaction effect of different PEG concentrations on root number and root length (cm).

Genotype	PEG concentration (g)	Root number	Root length (cm)
Gerao	0	9.00^{abcd}	1.93^{b}
	10	7.16^{def}	1.73^{bcde}
	20	5.16^{ghi}	1.54^{efg}
	40	4.00^{hi}	1.32^{ghi}
Keyih Beles	0	8.17^{cd}	1.78^{bcd}
	10	7.33^{def}	$1.43f^{ghi}$
	20	5.00^{ghi}	1.24^{hi}
	40	0.00^{hi}	0.00^{j}
Shenkor	0	9.00^{abc}	1.9^{b}
	10	5.5^{ghi}	1.53^{efg}
	20	4.00^{hi}	1.27^{hi}
	40	0.00^{i}	0.00^{j}
Suluhna	0	10.6^{a}	2.2^{a}
	10	8.67^{bcd}	1.83^{bc}
	20	6.00^{efg}	1.61^{defg}
	40	5.00^{ghi}	1.41^{ghi}
Lemats Beles	0	10.1^{ab}	1.73^{bcde}
	10	8.67^{bcd}	1.65^{def}
	20	5.62^{fgh}	1.42^{fghi}
	40	3.67^{i}	1.27^{hi}
Limo	0	9.9^{abc}	1.75^{bcde}
	10	7.53^{dc}	1.53^{efg}
	20	5.67^{fgh}	1.47^{fgh}
	40	3.67^{i}	1.23^{i}

Mean values of the same column followed by the same letter are not significantly different at 0.05 level using Duncan's Multiple Range Test.

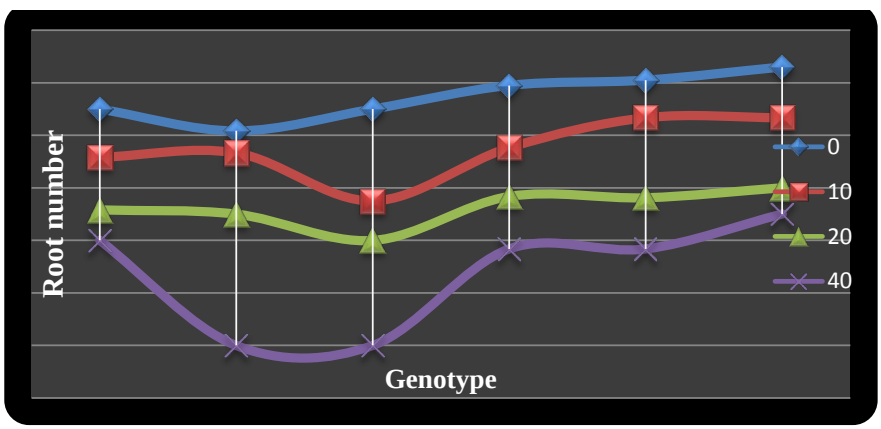


Figure 9. Mean root number for PEG level.

Root length ranged between 1.73 cm to 2.2 cm for genotypes of Suluhna and Lemats Beles at PEG free (control), respectively (**Table 3**, **Figure 10**). At 10 g all cactus genotypes, except Shenkor and Keyih Beles had not varying root length as compared to control (free PEG). But at 20 g/l PEG the root length decreased. Moreover, PEG 40 g/l also declined in root length in all genotypes. The highest was recorded in Suluhna (1.41 cm) followed by Gerao (1.37 cm) but no significant difference with Limo and Lemats Beles (1.23) and (1.27) respectively. The result of this studies indicated that the root length of the genotypes gradually decreased with increase level of PEG. Reduced root lengths under osmotic stress conditions have been reported in safflower [52] and pea [53] [54] also reported that effect of PEG on root length displaces significance difference on tomato varieties and the genotypic differences have also reminded significant among tomatoes genotypes. Similarly, [55] reported that root length of pear millet was significantly declined with increase external water potential and consequently, all treatment caused a decrease in root elongation in all genotypes compared to their controls.

3.9. Acclimatization

In the present study, the acclimatization procedures applied was successful. *In vitro* regenerated (from stressed and non stressed plants) were planted on a mixture of sand, coco peat and compost at the rate of 2:1:1 and showed the highest survival capacity (100%) when transferred to the soil **Figure 11(a)** and **Figure 11(b)**.

4. Conclusion

In conclusion, we investigated the capacity of cactus genotypes, culture in *vitro,* to grow under drought stress using polyethylene glycol as osmotic agent in the medium (PEG 6000). There was normal plant regeneration in the no-stress medium, but increased PEG concentration in medium decreased percent plant regeneration in the tested genotypes. The control treatment responded earlier towards callusing and required least number of days (22) for Suluhna and Keyih Beles genotypes. However, MS medium supplemented with 40 g/l PEG responded later for all genotypes. The callus induction frequency (CIF), Callus fresh weight and plant regeneration percent decreased with increasing PEG concentration. At the highest level of 40 g/l of PEG Suluhna followed by

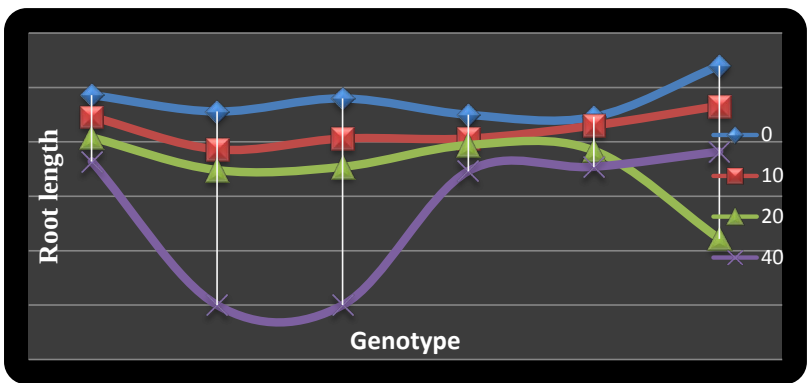


Figure 10. Means of root length for each PEG level.

(a) (b)

Figure 11. Acclimatization of plantlets. (a) In plastic trays; (b) Plantlets in pot.

Gerao, Lemats Beles and Limo were recorded better performance for CIF, for callus fresh weight, Suluhna produced the highest weight (2.75 g). However, Shenkor and Keyih Beles induced callus but became reddish black and showed poor regeneration within 35 days. At 40 g/l PEG in MS medium, the highest shoot number was in Suluhna genotype (4.33) followed by Gerao (3.67). The highest shoot length was in Suluhna (2.11 cm) with no significant difference with Gerao (2.02 cm). Root number (5.00) and root length (1.41 cm) were recorded for Suluhna genotype. From this study, it revealed that Suluhna followed by Gerao, Lemats Beles and Limo genotypes were better drought tolerant comparing with other genotypes tested in the experiment. Survival percentage of *in vitro* regenerated plantlets was 100% during hardening. By taking into consideration, all the growth parameter tested was revealed that Suluhna, Gerao, Limo and Lemats Beles showed better drought tolerant at the highest level of PEG compare with Keyih Beles and Shenkor.

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