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Selection of potato *(Solanum tuberosum* L. cv. Cardinal) plantlets tolerant to *in vitro* salt and drought stress

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Abstract: The results of present investigation revealed that for micropropagation of potato (*Solanum tuberosum* L.) cv. Cardinal agar solidified medium gave the best results. As far as stress studies were concerned three different concentrations of PEG and NaCl were used for providing water and salt stress to *in vitro* potato plantlets respectively. In MS basal medium amended with PEG (6000) potato plantlets showed positive growth in all parameters in 5% PEG concentration except the shoot and root lengths while the plantlets were sensitive to 10% and 20% PEG concentrations and gave poor results for almost all parameters. In MS basal medium supplemented with NaCl all the parameters showed a sharp decrease in growth except number of branches in 0.1 and $0.5gL^{-1}$ NaCl concentrations where number of branches remained the same though greatly reduced in length while $1gL^{-1}$ NaCl concentration gave negative results for all parameters. In conclusion, we can say that this cultivar is very sensitve to salinity but is moderately tolerant to water stress.

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INTRODUCTION

One of the most important abiotic factors limiting plant productivity is water stress brought about by drought and salinity[1-3] which are widespread problems around the world[4]. Salinity and drought affect the plants in a similar way[5]. Reduced water potential is a common consequence of both salinity and drought[6]. Saline water occupies 71 % of the earth area[7]. Nearly 20 % of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity[8]. At present, there are nearly 954 million hectares of saline soils on the earth's surface. In Pakistan, about 6.30 million hectares of land are affected by salinity[9].

Furthermore, about a half of all the existing irrigation systems of the world ($3x10^8$ ha) are under the influence of secondary salinization, alkalization and waterlogging, and about $10x10^6$ ha of irrigated land are abandoned each year because of the unfavorable effects of secondary salinization and alkalization[10]. Because of the increased need for food production, decrease in availability of water and subsequent increase in distribution of soils affected by salinity, research on plant responses to salinity and drought stress has rapidly expanded in recent years[11]. Simulation of water and salt stress under *in vitro* conditions during the regeneration process constitutes a convenient way to study the effects of these stresses on the morphogenic responses[12].

Cardinal is very successful potato cultivar in Pakistan and is of great economic importance. In Pakistan, there is a lot of waste land which is under utilized because of twin problem of water logging and salinity. If we can find any variant of any economically important plant that can endure these conditions, it will help to utilize those marginalized parcels of land. The purpose of present study was to evaluate tolerance level of potato plantlets in response to *in vitro* PEG and NaCl stresses and to select the most hardy *in vitro* variant of cv. Cardinal.

MATERIALS AND METHODS

Healthy and disease-free plantlets of potato (*Solanum tuberosum* L.) cv. Cardinal were procured from the Tissue Culture Laboratory, Botany Department, University of the Punjab, Lahore. Nodal explants were excised and inoculated on MS basal solid medium (amended with 8 g L^{-1} agar), MS basal liquid medium (with no agar added) and MS basal cotton based liquid medium (having cotton at the base) for *in vitro* multiplication. The optimal medium was selected for further multiplication that was MS basal solid medium. The process was continued till large number of complete plantlets were obtained. The four-week old plantlets were excised to get nodal cuttings. These excised nodal cuttings were again used as explants for further multiplication. For the study of salt and water stresses on potato plantlets, three types of MS basal media were used. MS basal media (solid) supplemented with $0.1gL^{-1}$, $0.5gL^{-1}$, $1gL^{-1}$ concentrations of NaCl were used for providing salt stress to nodal explants of potato. NaCl was added to MS basal medium prior to adjustment of pH. Approximately,10 cm^3 of each category of medium was poured in a culture tube. These culture tubes were secured with rubber bands and sterilized. MS basal media (liquid) supplemented with 5%, 10% and 20% concentrations of PEG (6000) with the water potential of –0.35, -0.5 and –1.15MPa, respectively were used for providing water stress to nodal explants of potato. For providing water stress with PEG, the use of agar solidified medium was abandoned because of precipitation with PEG. Polyethylene glycol was added to MS basal culture medium prior to

adjustment of pH. For preparing PEG liquid medium an aliquot measuring 2ml of liquid medium amended with PEG, for each concentration was transferred separately to a small culture tube (10ml). These culture tubes (were secured with rubber bands and sterilized. The polypropylene covers from each culture tube containing sterilized medium were removed and the mouth of each culture tube was heated on the flame by holding it at an angled position. Nodal explants ranging 1.5-2.3cm in length were inoculated on the surface of solid and liquid medium, respectively in each culture tube. Mouth of each culture tube was quickly covered with polypropylene sheet and secured with rubber band. After inoculation, culture tubes were kept in the culture room where temperature was maintained at 26°C±2. Fluorescent tube lights were used to maintain 12 h photoperiod with 269µmol m^{-2} s^{-1} irradiance. After 4 weeks of incubation plantlets were harvested and growth parameters such as fresh weight, dry weight, shoot length, root length, number of roots, number of leaves and number of branches were studied. For measuring fresh weight, plantlets were taken out from culture tubes and quickly washed, gently blotted and weighed. Also recorded the number of roots, leaves and branches. Shoot length was measured with the help of a millimeter (mm) ruler. Roots were removed from the main axis and the length of each root was measured separately. Subsequetly, mean value for root length was calculated. These plantlets were dissected into component parts (shoot, root) for further study. The root and shoot samples were placed in labelled brown envelops in oven at 70 °C for 72 h for dry weight determination.

Statistical analysis

The results obtained in the present study were statistically analyzed with One Way Analysis of Variance in completely randomized design[16]. The means were separated by Student-Newman-Keuls Test at 5% level of significance using Co-stat [version 6.3].

RESULTS AND DISCUSSION

Effect of physical nature of medium on regeneration of potato plantlets

The physical nature of the medium can influence growth and multiplication rate of plants *in vitro*[13-15]. Effect of MS basal solid (MS1), MS basal liquid (MS2) and MS basal cotton based liquid medium (MS3) on regeneration of potato plantlets was also evaluated after 28 days. The data revealed that solid medium was best for regeneration of potato plantlets (Table 1, Figure 1) while cotton based liquid medium gave poorest results. These results are in conformity with the findings of Bhagyalakshmi and Singh (1988) and contrary to the reports of Kanwal *et al*., (2006) who found that cotton based liquid medium is better for induction of microtuberization in potato as compared to solid and liquid medium.

Table 1: Effect of physical nature of medium on the growth of potato plantlets after 28 days. The data represent an average of three replicates for each medium**.**

Growth parameters	MS1	MS2	MS3
Fresh weight (mg)	435.0±0.5	106.0±1.1 (-75.6 %)	98.0±6.1 (-77.4 %)
Dry weight (mg)	42.0±1.1	10.0±0.5 (-76.1 %)	8.0±0.5 (-80.9 %)
Shoot length (cm)	14.0±1.1	7.3±0.1 (-47.8 %)	5.3±0.8 (-62.1 %)
Root Length (cm)	3.78±0.1	3.04±0.1 (-19.5 %)	1.9±0.5 (-49.7 %)
No. of roots	33.0±1.7	8.0±1.1 (-75.7 %)	6.0±0.5 (-81.8 %)
No. of leaves	22.0±1.5	11.3±1.7 (-48.6 %)	10.0±1.1 (-54.5 %)
No. of branches	3.0±0.5	1.3±0.3 (-56.6 %)	3.6±0.8 (+20.0 %)

MS1= MS basal solid medium, MS2= MS basal liquid medium, MS3= MS basal cotton based liquid medium

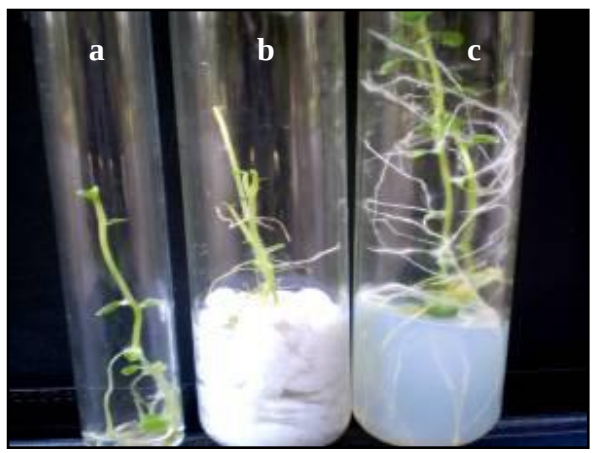

Figure 1: Differential growth of potato plantlets cv. Cardinal after 28 days on MS basal (a) liquid, (b) cotton based liquid and (c) agar - solidified medium, respectively (1x).

Effect of different concentrations of PEG in MS liquid medium on explants

The effect of different concentrations [5% (-0.35MPa), 10 % (-0.5MPa) and 20% (-1.15 MPa)] of PEG on different growth parameters of four weeks old plantlets were studied and data are given in Table 2. The response of potato plantlets cv. Cardinal in terms of overall growth and development is shown in Figure 2. It was observed that 5% PEG (-0.35MPa) in MS liquid medium was stimulatory for the growth of potato plantlets which was manifested by a significant increase in fresh and dry weight of the plantlets as compared to control plantlets (Table 2). These results corroborate the previous findings[17] which reported an increase in dry weight of tomato cells under *in vitro* drought conditions. However, there was sharp

decrease in fresh weight in 10% (0.5MPa) and 20% (-1.15MPa) PEG concentrations. Similar results have been reported[18] for soybean plants. It was also noted that in 10% PEG concentration dry weight was slightly higher than control. However, there was a sharp decrease in dry weight in plantlets growing in 20% PEG concentration.

Table 2: Effect of different concentrations of PEG in MS liquid medium on growth parameters of potato plantlets. The data represent an average of three replicates for each concentration.

Growth Parameters	PEG (6000) Concentrations (%)			
	Control	5	10	20
Fresh weight (mg)	106.0±1.1	136.0±1.1 (+28.0 %)	75.0±0.5 (-29.0 %)	45.0±2.9 (-85.0%)
Dry weight (mg)	10.0±0.5	17.0±2.9 (+70.0 %)	12.0±1.1 (+20.0 %)	6.0±0.5 (-40.0 %)
Shoot length (cm)	7.3±0.1	5.5±0.1 (-24.6 %)	4.7±0.3 (-35.6 %)	2.3±0.1 (-68.4 %)
Root Length (cm)	3.04±0.1	2.03±0.04 (-33.2 %)	0.61±0.01 (-79.9%)	0.53±0.05 (-82.0%)
No. of roots	8.0±1.1	16.0±2.3 (+100.0 %)	6.0±0.8 (-25.0 %)	2.0±0.5 (-75.0%)
No. of leaves	11.3±1.7	10.0±0.5 (-11.5 %)	8.0±1.1 (-29.0 %)	5.3±0.6 (-53.0 %)
No. of branches	1.3±0.3	2.0±0.5 (+53.8 %)	4.0±0.5 (+207.0 %)	1.0±0.0 (-23.0 %)

Figure 2: Response of potato plantlets cv. Cardinal after 28 days of incubation on MS basal medium supplemented with (a) 0%, (b) 5%, (c) 10% and (d) 20% PEG in liquid medium, respectively (1x).

There was gradual decrease in shoot length of plantlets in 5 and 20% PEG concentrations. These results are similar to the previous findings[19,20]. The decrease in shoot length and increase in dry weight indicated that plantlets had thick strong internodes and were highly condensed. It has also been that stolon numbers was enhanced by drought but total length of stolons was reduced in some cultivars of potato[20].

In the present study, there was a gradual decrease in the root length of plantlets in 5, 10 and 20% PEG concentrations as compared to root length in control plantlets. It is noted that number of roots in 5% PEG medium were 100% higher as compared to control plantlets. Analysis of data revealed that while root length in 5% PEG medium decreased as compare to control, the number of roots were highest in this medium. It appears that water stress is instrumental in proliferation of root primordia for efficient absorption of water.

In the present study, number of leaves also decreased gradually with the increase in concentration of PEG in media[21]. The number of branches were almost double in 5 % and four times in 10 % PEG liquid medium as compared to control plantlets. These results were contrary to the findings of Tourneux *et al.*, (2003) who have reported that stem number was not significantly affected by drought.

The growth parameters given in Table 3 indicate that at 5% PEG concentration, fresh weight, dry weight, number of roots and number of branches showed a significant increase. It appears that this cultivar of potato can easily adapt to moderate drought conditions (-0.35MPa). The plantlets were healthy and appeared to be very hardy with well established branches in 5 and 10% PEG medium (Figure 2). It is recommended that more trials be conducted for assessement of drought resistance of potato (*Solanum tuberosum* L.) cv. Cardinal.

Effect of different concentrations of NaCl in MS solid medium on explants

The effects of different concentrations (0.1, 0.5, 1gL^{-1}) of NaCl on different growth parameters of four weeks old plantlets were studied and data are given in Table III. The response of potato plantlets cv. Cardinal in terms of overall growth and development is shown in Figure 3. The cultivar Cardinal was very sensitive to the effect of *in vitro* salinity. All the parameters investigated except the number of branches showed sharp decrease in 0.1 g L^{-1} NaCl concentration.

The decrease in subsequent concentrations (0.5 and 1gL^{-1} NaCl) showed gradual decrease in all the growth criteria. The number of branches remained the same though greatly reduced in length at 0.1 and 0.5gL^{-1} NaCl. A slight decrease in number of branches in medium with 1 g L^{-1} NaCl was recorded. Our results are in conformity with previous studies[22-24] and tested different cultivars of potato for salt tolerance at concentrations of 30, 60, 90, 120 mM. They found that cv. White Desiree, Russet Burbank, Maradona and Diamant are moderately salt tolerant while cv. Agria, Marfona and Marfona are sensitive cultivars.

Table 3: Effect of different concentrations of NaCl in MS solid medium on growth parameters of potato plantlets. The data represent an average of three replicates for each concentration.

Growth Parameters	NaCl concentrations (gL^{-1})			
	Control	0.1	0.5	1
Fresh weight (mg)	435.0 ±0.5	200.0±1.1 (-54.0 %)	121.0±0.8 (-72.1%)	41.0±1.1 (-90.5 %)
Dry weight (mg)	42.0 ±1.1	18.0±1.7 (-57.1 %)	10.0±1.1 (-76.1 %)	5.0±0.5 (-88.0 %)
Shoot length (cm)	14.0 ±1.1	6.0±0.5 (-57.0 %)	4.2±0.1 (-70.0 %)	2.5±0.2 (-82.1 %)
Root Length (cm)	3.78 ±0.1	2.48±0.01 (-34.3 %)	1.35±0.08 (-62.2 %)	1.05±0.02 (-72.2 %)
No. of roots	33.0 ±1.7	16.0±0.5 (-51.0 %)	10.0±0.3 (-69.6 %)	8.0±1.1 (-75.7 %)
No. of leaves	22.0 ±1.5	19.0±1.1 (-13.6 %)	14.0±0.5 (-36.3 %)	11.0±1.1 (-50.0 %)
No. of branches	3.0 ±0.5	3.0±0.5 (0.0 %)	3.0±0.0 (0.0 %)	2.6±0.3 (-1.3 %)

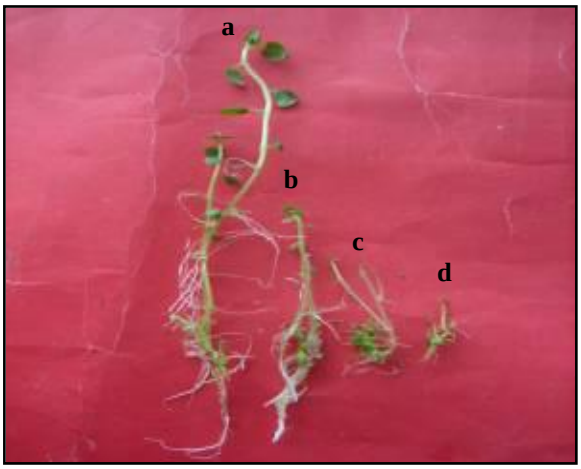

Figure 3: Response of potato plantlets cv. Cardinal after 28 days of incubation on MS basal medium supplemented with (a) $0gL^{-1}$, (b) $0.1gL^{-1}$, (c) $0.5gL^{-1}$ and (d) $1gL^{-1}$ NaCl in solid medium, respectively (1x).

CONCLUSION

In conclusion, it is recommended that further studies on somaclonal variants with regard to drought resistance in cv. Cardinal be carried out. Although resistance to drought and salinity is a genotypic character, chances of finding a hardy and drought resistant variant are fairly good, if this cultivar is multiplied by *in vitro* micropropagation and subjected to increasing levels of water stress and scored for resistant variant in a large population of *in vitro* plantlets. In the present investigation this plant showed fairly good viability at –0.35 MPa.

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