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Micropropagation and Biochemical Genetic Markers detection for Drought and Salt Tolerance of Pear Rootstock

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Abstract: An efficient *in vitro* rooting of shoots obtained from meristem tip culture was regarded as a key issue in a program aimed at virus elimination from traditional pears. The present study reports the trials undertaken to optimize the *in vitro* proliferation, rooting, drought and salinity stresses of pear rootstock (*Pyrus communis L.*). A reduced relative humidity inside the culture vessels proved to enhance water loss control from leaves of micropropagated pear was studied. Procedures leading to an *in vitro* hardening during the last micropropagation phase generally facilitate plantlet acclimatization. Individual shoots from *in vitro* pear multiple shoots were cultured on Murashige and Skoog (MS) medium, supplemented with 0, 1, 2 or 3mg/l 6-benzylaminopurine (BA) or kinetin (Kin) and thidiazuron (TDZ). Increasing BA concentration from 0 to 2mg/l increased multiplication. They were then transferred to MS, with 2mg/l BA under aerated or non-aerated conditions, for shoot development. Development of shoots cultured for 6 weeks in aerated was highly compared with in non-aerated. Successful rooting was achieved using 2 mg/l IBA/liter and led to the highest number of roots per rooted shoot. Thus, root induction under aerated conditions results in efficient rooting of *in vitro* pear shoots. The produced shoots were used to evaluate the genetic response of pear rootstock under polyethylene glycol (PEG) concentrations (0, 10, 15, 20 and 25%). These levels of PEG progressively inhibited shoot growth, and decreased shoot formation. When the salinity (NaCl) level was increased to 0, 0.5, 1, 2 and 4%, all variables increased drastically. Plantlets were rooted *in vitro*, acclimatized and cultivated in glasshouse in soil mixture of peatmoss: perlite 2:1 v/v was successfully. Biochemical genetic markers such as SDS-PAGE for water soluble proteins (w.s.p) of pear plantlets was substantially involved in drought and salinity stresses. Esterase (EST) isozyme were used to determine the genetic effect of drought and salinity stresses on pear. SDS-PAGE analysis of w.s.p showed 14 protein bands, among them three new bands (with molecular weight 65, 12 and 6 KDs) were appeared and involved commonly in salt and/ or drought tolerance, other bands showed higher intensities than control. EST isozymes showed 12 bands among them; only 2 bands were common, the others exhibited new appearance, absence of other bands or higher intensity as a response to drought and /or salinity stresses.

Keywords: *In vitro*, Shoot proliferation, Pear rootstock (*Pyrus communis*L.), Aeration, Root formation, Osmotic stress, Protein and Esterase isozymes

INTRODUCTION

Commercial micropropagation of rootstocks for pear was undertaken at the same time as for apple. Rootstocks of *Pyrus communis* L those were difficult to root formation conventional cuttings could be micropropagated, although a differential rooting between clones was observed (Jones and Webster, 1989). Micropropagated plants were then used to establish stock plants in the field and cuttings from these stock plants rooted more readily than those from conventionally propagated stock plants. Salinity is one of the major environmental stresses that challenge plant growth and crop productivity world wide (Sen and Mohammed, 1994). Producing sustainable and profitable crops under these conditions needs technological and biological approaches, including selection of new, more salt tolerant cultivars of named plants using conventional breeding programs or tissue culture techniques (Al Mansoori *et al.,* 2007). Tissue culture is a

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useful method for increasing the stress tolerance of plants chiefly because of totpotency, which refers to the fact that most plant cells contain a complete genome of the species and thus are at least potentially capable of becoming entire plants. Providing that individual cells can be convinced to produce entire plants or at lest shoots, large population of cell can be maintained and in many cases selected to obtain agriculturally useful mutant (Chandler *et al*., 1987). Multibuds of papaya grown in culture flasks after one-week without aeration followed by a two-week aeration treatment caused a 41% increase in the number of shoots $\geq$0.5 cm, 42% increase in leaf expansion, and 17% increase in leaf numbers in comparison with unaerated materials. The multishoots grown for 3 weeks without aeration showed growth retardation on leaves and epinasty on petioles (Lai *et al.,*1998). Drought and salinity tolerant cultivars have been identified in many crops (Cherian and Reddy, 2003; Badiane *et al.*, 2004) and in few trees (Tahir *et al., 2003*) by developing strategies based on using polyethylene glycol (PEG) or NaCl. PEG widely used to induce water stress, is a nonionic water polymer, which is not expected to penetrate into plant tissue rapidly**.** In the contrast, ions Na^+ and Cl^- penetrate into plant cells and can be accumulated in the vacuole for the tolerant plants or in the cytoplasm for sensitive cultivars (Kefu *et al.,* 2003). Djibril *et al*., (2005) showed that some date palm varieties can be drought tolerant or salinity tolerant, suggesting that these two factors are independant. The *in vitro* response of sweet cherry rootstock Gisela 5 (*Prunus cerasus* × *Prunus canescens*) to increasing concentrations of NaCl (0, 50, 100 and 150 mM) in MS culture medium was studied and found that inducing salinity reduced growth and chlorophyll content in shoots but had no effect on water content (Erturk *et al.,* 2007). The regeneration frequency in salt stressed callus of rice was lower as compared to control. 15 d and 30 d after inoculation proline content increased several fold whereas total protein content decreased markedly with increase in salt concentration (Shankhdhar *et al.,* 2000).The present investigation was carried out on proliferation at different concentrations of BA, Kin and TDZ, rooting at different concentrations of NAA and IBA and comparing the ventilation of vessels used for pear culture *in vitro*. Also focus on the utility of pear rootstock tissue culture for *in vitro* selection of drought and salinity tolerant. In addition, to study the expression of tolerance to drought and salt stresses of the *in vitro* selected of pear rootstock by using protein fingerprinting and esterase isozymes.

MATERIALS AND METHODS

This work was conducted in the Department of Genetics and Cytology, National Research Center. Dokky. Giza, Egypt and Tissue Culture Laboratory, Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia University, Sadat City, Egypt during the years of 2006 and 2007.

Explants Material and Preparation:

Aseptic cultures were established from meristem tips of pear rootstocks Pyrus communis L from branches of field-grown trees were obtained from the farm of Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia University, Sadat City, Egypt. Explants were disinfected and stabilized shoot cultures of clonal pear were subcultured and maintained for three months on proliferation medium Murashige and Skoog (1962) (MS) supplemented with 1mg/l 6-benzylaminopurine (BAP), 0.5 mg/l naphthalene- acetic acid (NAA), 30g/l sucrose and 7g/l agar, pH5.7 (Bahri-Sahloul *et al.,* 2005). The Explants were grown at 25°C during a 16 h light photoperiod with a light intensity of 2000 lux provided by cool white fluorescent tubes. In order to obtain sufficient number of explants, the produced shoots were subcultured four times.

Proliferation Stage:

Effect of Cytokinin at Different Concentrations on Shoot Proliferation of Pear:

In order to achieve shoot proliferation of pear (shoots of about 2cm long with 2-5 leaves) obtained from the previous stage were cultured in tissue culture jars (350 ml) containing 60 ml MS medium supplemented with different concentrations of BA, Kin and TDZ (0.0, 1.0, 2.0 and 3.0 mg/l) each alone, leading to 10 treatments. After six weeks data were recorded as number of shoots, number of leaves and shoot length (cm)/ culture (jar).

Effect of Aeration on Shoot Proliferation of Pear:

This experiment was carried out to study the effect of aeration on the growth and development of pear *in vitro*. Four explants each contained three shoots at length of 2 cm produced from previous experiment were cultured in tissue culture jars (350 ml) containing 60 ml of MS medium supplemented with 2mg/l BA and divided two parts, the first part covered with plastic closure (6.5 cm diameter, normal aeration) and the second

part covered with plastic closure containing a pore (0.5cm diameter) and the pore covered with filter membrane 0.25µm. After six weeks of incubation, data were recorded as shoot number, leaves number, shoot length (cm).

Rooting Stage:

Effect of Auxin at Different Concentrations on Growth and Development of Pear Roots:

To investigate the effect of auxin (NAA or IBA), it added to MS medium at 0, 0.5, 1, 1.5 and 2mg/l. After six weeks of culture, plantlets growth and development were measured as length of plantlets (cm), roots number, root length (cm) and leaves number,

Effect of Aeration on Root Formation of Pear:

To determine the effect of aeration on the growth and development of pear *in vitro*. Six shoots (2cm length) produced from previous experiment were cultured in tissue culture jars (350 ml) containing 60 ml of MS medium supplemented with 2mg/l IBA and divided two parts, the first part covered with plastic closure (6.5 cm diameter, normal aeration) and the second part covered with plastic closure containing a pore (0.5cm diameter) and the pore covered with filter membrane 0.25µm. After six weeks of incubation, data were recorded as roots number, root length (cm) leaves number and plantlets length (cm).

Effect of PEG on Growth and Development of Pear:

To determine the lethal dose of PEG for pear, PEG was added to proliferation MS medium at 0, 10, 15, 20 and 25%. The produced shoots were cultured for four weeks on the medium and the survival percentage, shoot number, shoot length (cm) and leaves number were measured.

Effect of Salinity on Shoot Formation of Pear:

Shoots were obtained from proliferation stage cultivated in MS medium supplemented with 2mg/l BA, and different concentrations of NaCl (0, 0. 5, 1, 2 and 4%). Data estimated as shoot number, shoot length (cm), leaves number and survival of shoots % after four weeks from cultured. All previous experiments were incubated at 25ºC day and night, light was provided by white fluorescent tubes giving light intensity 2000 lux for 16 hours per day.

Transfer to Greenhouse:

After proliferation success the shoots were transferred on rooting MS medium supplemented with 2 mg/l IBA and maintained under *in vitro* conditions (ventilation of vessels). The plants were transplanted into small plastic pots containing of peatmoos and perlite 2:1 (v/v) in greenhouse**.** The randomized factorial design was used and data were subjected to analysis of variance. Separation of means among treatments was determined using LSD test at 5% (Steel and Torrie, 1980).

Water Soluble Proteins and Esterase Isozymes Electrophoretic Analysis:

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to (Laemmli, 1970). Leaves of pear explants were ground in the liquid nitrogen and 1 ml of extraction buffer was added. After centrifugation for 10 min at 12,000 rpm under 4°C the supernatant was collected**.** Electrophoresis was carried out at 4°C until the bromophenol blue front passed completely through the gel. The gel was stained for 12 hr in 0.1% coomassie brilliant blue and distained until the bands were clearly observed. Also EST enzyme was extracted from plants under same treatments. Samples centrifugated for 10 min at 12,000 rpm at 4°C. Polyacrylamide gel electrophoresis was performed according to (Stegemann 1979). Gel preparation and enzyme staining solutions were performed according to (Wendel and Weeden 1989). Gel bands were scanned and analyzed using Gel Doc Bio-Rad system.

RESULTS AND DISCUSSIONS

Shoot Proliferation:

Effect of Cytokinin at Different Concentrations on Shoot Proliferation of Pear:

Adding different concentrations of growth regulators to proliferating medium plays an important role on growth and development of pear. The mean values revealed that adding growth regulator (BA) at 2mg/l in MS medium gave the best multiplication rate (highest shoots and leaves number) than other treatments as shown in Table (1). Among tested concentrations of growth regulators, kin had the least efficiency on shoot proliferation. Regarding the effect of MS medium with kin at 1 and 2mg/l clearly apparent longest shoots than other treatments as shown in Fig. (1). Shoots produced on TDZ treatments had a poor appearance, with some

Table 1: Effect of cytokinin at different concentrations on growth and development of pear rootstock.

Cytokinin type	Con. mg/l	Number of shoots	Number of leaves	Shoot length (cm)
BA	0.0	1.00e	10.67e	1.83 cd
	1.0	16.00b	96.67b	2.06 c
	2.0	22.33a	200.00a	2.86b
	3.0	16.67b	90.00b	2.10c
Kin	1.0	2.66de	25.33cde	3.50a
	2.0	1.33e	16.67e	3.56a
	3.0	2.00e	17.33De	2.23c
TDZ	1.0	8.33c	65.00bc	2.20c
	2.0	7.33cd	60.00bcd	1.50d
	3.0	7.00cd	46.67 cde	1.40d
LSD at 5% level		4.731	42.85	0.5532

Fig. 1: Effect of cytokinin at different concentrations on pear shoot proliferation *in vitro*.

discoloration of the basal portion of the shoots produced. Evaluating the response of the shoots and quality of the shoots produced 2mg/l BA was chosen as the preferred treatment because it yielded the maximal number of shoots with the best quality and used the least amount of BA to produce the desired result. This result is in agreement with Haw and Keng (2003) reveled that the aseptic axillary buds formed multiple shoots within five weeks when cultured on MS medium supplemented with 2.0 mg/l N 6 -Benzyl adenine (BA). Developed procedures for the regeneration of *Bambusa vulgaris* using nodal segments from adult plants. Optimal shoot growth was after 16 days cultivation on modified MS medium supplemented with 2 mg/l of BAP (Ndiaye *et al.*2006). Mng'omba *et al.,* (2007) they found that MS media supplemented with 2.0 mg/L benzylaminopurine was superior in the number of micro-shoots produced for jacket plum (*Pappea capensis*)

Effect of Aeration on Shoot Proliferation of Pear:

Data presented in Table (2) and Fig. (2) show the effect of aeration on shoot proliferation estimated as number of shoots, shoot length (cm) and number of leaves. The results indicate that with aeration was more effective than without aeration for all estimated number of shoots, shoot length and number of leaves. Statistically analysis showed significant difference among the treatments at 5% level. This result is

Table 2: Effect of aeration on shoot proliferation of pear *in vitro.*

Aeration	Shoot number	Shoot length(cm)	Leaves number
Without aeration	29.60 b	2.80 b	140.00b
With aeration	50.20a	3.90 a	179.60 a
LSD at 5%	12.79	0.5179	38.94

Fig. 2: Development of shoot proliferation of pear with aeration.

similar to Campostrini and Otoni, (1996) who reported that, utilization of tightly closed vessels that reduce gas exchange may affect negatively the normal growth and development of plants during cultivation *in vitro*. Several studies showed the advantages of using closures with filters or vented vessels, which allow gas exchange, increasing the photosynthetic capacity, the multiplication rate, and the survival of plants after transfer to *ex vitro* conditions (Chuo-Chun *et al*., 1998; Murphy *et al.,* 1998; Zobayed *et al.,* 2000; Benzioni *et al.,* 2003; Gribaudo *et al.,* 2003; Lucchesini and Mensuali-Sodi, 2004; Park *et al.,* 2004). The hyperhydricity of the shoot cultures could be decreased by progressively ventilating the vessels (Lai *et al.,*2005).

Rooting Stage:

Effect of Auxin at Different Concentrations on Growth and Development of Pear Roots:

Preliminary experiment with IBA resulted in 100% rooting. However, a decline in rooting ability obtained with NAA. The poor rooting (10%) in shoots harvested from the control (free uxin). The highest number of roots per shoot of pear was 14 on MS medium supplemented with 2mg/l IBA Table (3) and Fig. (3). The morphological parameters of rooted plantlets which reached approximately 4.30, 3.50 or 4.6 cm long was obtained on MS with 2mg/l NAA or 1.5 and 2mg/l IBA respectively after six weeks in root initiation medium are presented in Table 3. Using this treatment, pear were rooted, and then transferred to pots consists of peatmoss and perlite (2:1, v/v) and acclimatized, 95% of the plants surviving in the greenhouse Fig. (4). The highest number of roots per shoot from *in vitro* rooting of pear (*Pyrus communis* L.) was 16.67 on ½MS medium gelled with a mixture of agar plus guar galactomannan supplemented with 4.90 µM IBA (Lucyszyn *et al.,* 2006).

Table 3: Effect of auxin concentrations on growth and development of roots *in vitro.*

Auxin type	Auxin conc. (mg/l)	Number of roots	Root length (cm)	Rooting%
NAA	0.0	2.00 d	2.00 b	10
	0.5	3.00 cd	0.50 c	40
	1.0	2.00 d	1.40b	70
	1.5	2.00 cd	2.00 b	55
	2.0	4.00 c	4.30 a	64
IBA	0.5	7.00 b	1.70 b	100
	1.0	7.00 b	2.10 b	100
	1.5	9.00 b	3.50a	100
	2.0	14.00 a	4.60 a	100
L.S.D at level 5%		1.878	1.172	

Fig. 3: Development of pear roots *in vitro* at rooting stage.

Fig. 4: Pear plant grown after one month in soil mixture consists of peatmoss and perlite (2:1, v/v) in greenhouse.

Effect of Aeration on Root Formation of Pear:

Aeration plays an important role in growth and development of pear *in vitro*. Root formation was induced in the *in vitro* regenerated shoots by culturing them on MS medium with 2 mg/l IBA with and without aeration of culture jars. Aeration was found to be most effective in producing roots Fig. (5). Using ventilated of culture jars enhanced plant length, leaves number, roots number and root length. The utilization of tightly closed vessels that reduce the gas exchange may affect negatively the normal growth and development of plants during cultivation *in vitro*. Using closures with filters or vented vessels, which allow gas exchange, increasing the photosynthetic capacity, multiplication rate, and survival of plants after transfer to *ex vitro* conditions. This result is in agreement with During rooting of *Phillyrea latifolia* L, the use of ventilated vessels in comparison with the closed ones

Fig. 5: Development of pear plants *in vitro* rooting as affected by the aeration in culture vessels.

enhanced development of roots, and doubled the dry weight of plantlets (Lucchesini and Mensuali-Sodi 2004). Medium aeration at the proximal end of the microcutting is more important than shoot orientation for *in vitro* rooting of lentil microcuttings (Newell *et al.,*2006).

Effect of PEG on Shoot Proliferation of Pear:

Polyethylene glycol (PEG) is low toxic hydrophilic alcohol polymer with high water solubility. It has been used as an inert non-penetrating osmoticum. It forms hydrogen bonds with water, decreasing the water potential of culture medium and finally inhibits both water and mineral uptake (PEG problems). Shoot survival was affected by PEG levels Table (5) and Fig. (6). PEG treatments reduced shoot multiplication and inhibited growth; they were influenced by increasing PEG concentration from 10 to 25%. The effect of PEG may be due to reducing ability uptaking water and minerals. Similar correlations had been reported for different plants Tahir *et al.,*(2003) reported that polyethylene glycol (PEG) or NaCl. PEG widely used to induce water stress, is a nonionic water polymer, which is not expected to penetrate into plant tissue rapidly. The effect of PEG level may be relate to the adverse effect of PEG on both mobilization of nutrients (eg. sucrose) and the structural organization or synthesis of metabolites in the cultured tissues (Almansouri *et al.*,2001).

Effect of Salinity on Shoot Proliferation of Pear:

Salt tolerance assessed on the basis of shoot development and survival % showed a strong, significant positive. Salinity markedly affected shoots number, shoot length, leaves number and survival %. However, a

Table 4: Effect of aeration on pear roots growth and development.

Aeration	Plant length (cm)	Leaves number	Root number	Root length (cm)
Without aeration	6.56b	12.07 a	2.26b	5.03a
With aeration	7.56a	13.87 a	7.73a	5.03a
LSD at 5%	0.6191	1.932	1.386	1.587

Fig. 6: Effect of PEG on growth and development of pear *in vitro*.

significant difference between NaCl concentrations was noticed on shoot formation. Growth and development of pear shoots increased by decreasing the salinity NaCl levels. Best shoot survival % was obtained from control medium followed by 0.5% NaCl as shown in Table (6) and Fig.(7). The concentrations of P, K, Ca, Mg, Na, Fe, Mn and Zn of pear (*Pyrus communis* L.) rootstock plants were affected by B and NaCl concentration of the medium (Sotiropoulos *et al.,* 2006). Induced salinity reduced growth of cherry rootstock and chlorophyll content in shoots but had no effect on water content (Erturk *et al.,*2007).

Table 5: Influence of PEG on growth and development of proliferation pear *in vitro*.

PEG conc. %	Shoots number	Shoot length	Leaves number	Survival %
Control	10.00 a	8.33 a	78.33a	100
10	6.00 b	5.66 b	45.00b	100
15	6.00b	5.00 bc	50.00b	100
20	6.00 b	4.33c	31.67 c	100
25	6.00b	4.00c	30.00 c	100
LSD at 5%	0.8420	1.045	9.250	

Table 6: Influence of NaCl concentration on growth and development of pear proliferation *in vitro*.

NaCl conc.%	Shoots number	Shoot length(cm)	Leaves number	Survival %
Control	9.66a	7.66a	80.00a	100.00a
0.5	8.33b	6.33b	65.00b	94.33a
1	7.66b	6.33b	63.33b	83.00 ab
2	5.33c	6.66b	41.67c	66.33b
4	1.66d	2.33c	3.66d	12.70 c
LSD at 5%	1.114	0.8420	8.625	23.14

Fig. 7: Effect of NaCl on growth and development of pear *in vitro*.

Biochemical Genetic Markers:

The SDS-PAGE of water soluble protein fraction of *in vitro* shoot tip culture of pear grown at four levels of two stresses (drought and salinity) with control for one month, showed genetic response in different degrees by appearance of new bands or higher intensity Fig. (8). A total of 14 bands were detected in different molecular weights ranging from 296 to 6 KDs Table (7), among them three new bands appeared. The first band (65 KDs) appeared under the highest concentration of both drought (25%PEG) and salinity (4% NaCl), second band (12 KDs) appeared as an effect of all treatments except of 10%PEG. The third band (6 KDs) appeared as a unique band for drought tolerance only. Other common bands exhibited higher intensities

Table 7: Influence of drought and salinity tolerance on variable protein bands of pear.

M.W (KDs)	C	Drought %				Salinity %			
		10	15	20	25	0.5	1	2	4
296	+	+++	+++	+++	+++	0.5	++	+++	+++
190	+	+	+	+	+	+	+	+	+
123	+	+	+	+	+	+	+	+	+
83	+	+	+	+	+	+	+	+	+
73	+	+	+	+	+	+	+	+	+
65					++				++
54	+	+	+	+	+	+	+	+	+
47	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+
23	+	+++	+++	+++	+++	+	+	+	+ +
17	+	+	++	++	+	+	+	++	+
15	+	+	+	+ +	++	+	+	+	+
12		+	+	+	+	+	+	+	+
6			+	+	+				

+: little intensity; ++: moderate intensity and +++: high intensity

Table 8: Effect of drought and salinity stresses on variable EST bands of pear.

EST band number	C	Drought %				Salinity %			
		10	15	20	25	0.5	1	2	4
1	0	10	15	+++	+++	0.5	+	+	+++
2	0	+	+	++	+++	+	+	+	+++
3	0	+	+	+	+				
4						+	+	+	+++
5								+	4
6						0			
7				+	+				
8		20	0	+++	+++				
9	0	+	+	+	+				
10								2	0
11						+	+		
12	0	+	+	+	+				

comparing to control, such as band with molecular weight 296 KDs, showed moderate intensity under 0.5 and 1% NaCl, while it was with higher intensity under the other treatments. Also, band with molecular weight 23 KDs, showed higher intensity under all PEG treatments, and moderate under 4% NaCl, while band with molecular weight 15 KDs exhibited moderate intensity under 20 and 25%PEG. These results showed that, there are common pathways of tolerance to drought and/or salinity, these is in agreement with (Taji *etal.*,1999).

Genetic Effects of Drought and Salinity Stresses on Est Isozymes of Pear:

EST isozymes analysis of drought and salinity stresses (Fig. 10 and Table 8) can be classified in three types, appearance of new bands or inhibition of other bands or higher intensity comparing to control. The first type was appearance of band number 4 that uniquily appeared as a genetic response to tolerate salinity stress at the four levels (0.5, 1, 2, and 4% NaCl), while band number 8 appeared as a unique band to drought stress at the four levels (10, 15, 20 and 25% PEG), other bands appeared under the higher two concentration of salinity such as band 5 and 10 under 2 and 4% NaCl, or under the lowest two concentrations (0.5 and 1% NaCl) or like bands number 6 which appeared only under the first concentration of NaCl 0.5%. While band number 7 appeared under the highest two concentrations of drought salinity. The second type was inhibition of bands number 3,9 and 12 under salinity concentrations (0.5-4%NaCl). The third type was the appearance of moderate or high intensities of some bands under one or more concentrations of salinity and/or drought (i.e bands number 1, 2, 4 and 8).

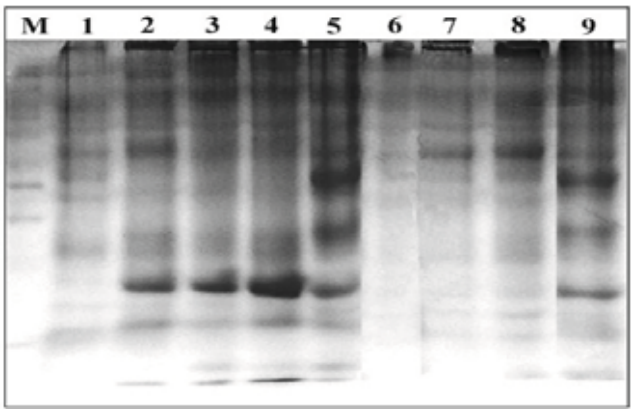

Fig. 8: SDS-PAGE proteins of pear at different PEG and NaCl concentrations.
M: Marker 1-Control 2-10% PEG 3-15% PEG 4-20% PEG 5-25%PEG
6- 0.5% NaCl 7- 1% NaCl 8- 2% NaCl 9- 4% NaCl

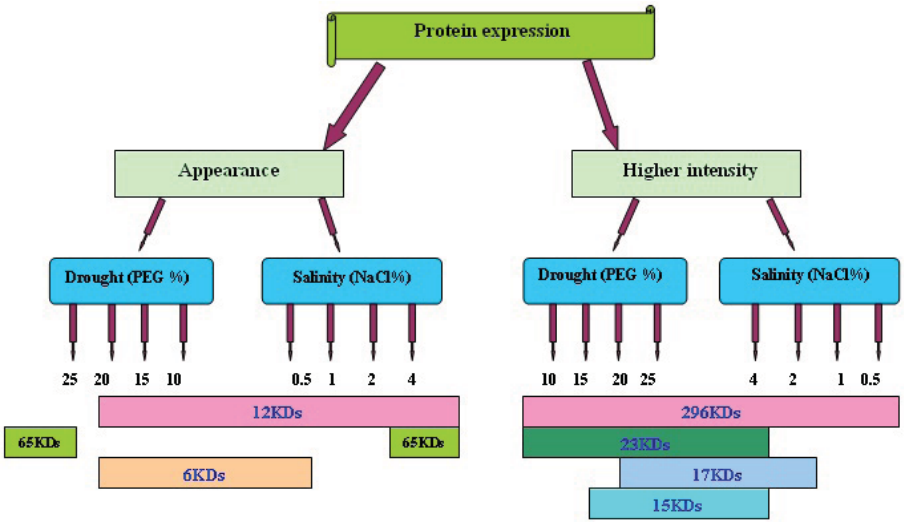

Fig. 9: Appearance and different intensities of pear under drought and salinity stresses.

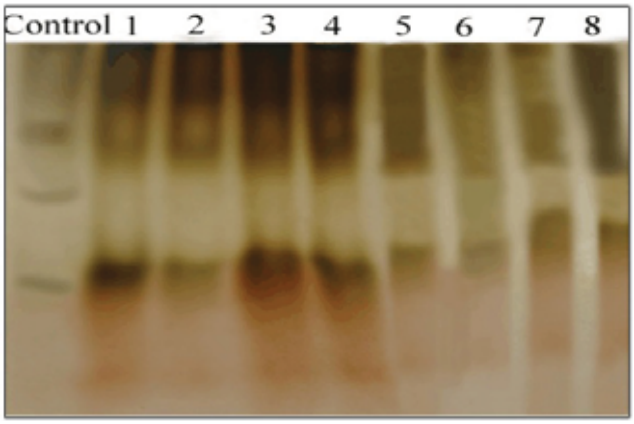

Fig. 10: Esterase enzyme (EST) of pear at different PEG and NaCl concentrations.
Control: untreated 1-10% PEG 2-15% PEG 3-20% PEG 4-25%PEG
5- 0.5% NaCl 6- 1% NaCl 7- 2% NaCl 8- 4% NaCl

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