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In vitro Influence of Salinity Stress on Callus and Plantlets Regeneration of Apple Rootstocks

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IN this investigation a screening of two apple rootstocks (Balady and MM106) to salinity, stress was achieved for an attempt to introduce more tolerant apple rootstocks. Calluses of the two rootstocks were cultured on MS medium supplemented with NaCl as a source of salinity at five concentrations (0.00, 500, 1000, 2000, 3000 mg/l). Morphological characteristics of both apple rootstocks Balady and MM106, negatively responded to the raising of NaCl concentrations *in vitro* such as callus survival and regeneration percentages, plantlets length (cm), number of multiple shoots, number of leaves/plant, number of roots/plant and root length (cm). The higher salinity concentration (3000 mg/l NaCl) induced the lowest significant percentage of callus survival, especially with MM106 (32.0 %) if compared with control (60.0 %). The mean of regeneration percentage reached the maximum number with control (38.67 %), and decreased gradually with higher NaCl concentrations until it was (23.67%) with 3000 mg/l. It was feasible that salinity did not reduce the average length of shoots. Number of multiple shoots was affected by raising salinity level into medium. The highest significant value was obtained with control (0.00 NaCl) (14.00) while, the lowest significant Value was (8.50) with 3000 mg/l. Moreover, number of leaves per plant, were reduced under salinity stress, which (0.00 and 500 mg/l NaCl) treatments achieved the highest significant number of leaves Values (8.83 and 7.83), respectively, followed by 1000 mg/l (7.33). Balady was more tolerant than MM106 to salinity stress and the average number of roots was (6.27 and 4.80), respectively. The proline concentration in rootstock increased with the raising of salt concentration, meanwhile, total chlorophyll content decreased by the increasing of salt concentration in the culture medium. On the other hand, peroxidase, poly phenyl oxidase, alcohol dehydrogenase and malate dehydrogenase isozymes banding patterns represent differences in some bands density or absent bands with different NaCl concentrations if compared with control treatment.

Keywords: Apple rootstock, Callus, *In vitro*, Isozyme, NaCl, Regeneration, Salt stress.

Apple is one of the major recommended fruit crops for new reclaimed soils in Egypt. Salinity is a critical problem facing agriculture, especially in irrigated lands located in semiarid zones. Soil salinity considered one of the most important abiotic stresses that limits plant growth and yield of most crops. (Zhu, 2001 and Chinnusamy *et al.*, 2005).

Resistance to abiotic stresses such as drought, salinity and alkalinity is an important trait for selection of fruit trees rootstocks, which affect the nutritional status of the scion and appreciably influence scion tolerance to salinity (Sotiropoulos *et al*., 2006).

Producing sustainable and profitable crops under these conditions needs technological and biological approaches, including selection of new and more salt tolerant cultivars of named plants using conventional breeding programs or tissue culture techniques (Ashraf & Akram, 2009 and Ashraf *et al*., 2012). Therefore, developing rootstocks that tolerate different biotic and abiotic stresses is one of the major goals of breeding programs of apple rootstocks to insure rootstocks suitable for different environmental conditions (Ryugo, 1988).

Tissue culture technique is an ideal tool for obtaining salt tolerant plants offering potential for quick screening of germplasm against salt stress (Vijayan *et al*., 2003 and Molassiotis *et al*., 2006). Screening or evaluation methods involving *in vitro* shoot culture have proven to be better applicable system for testing salt tolerance and were used in several plants such as grape (Sivritepe and Eris, 1999), "Gisela 5" (*Prunus cerasus* × *Prunus canescens*) sweet cherry rootstock (Erturk *et al*., 2007), quince (Vitaglino *et al*., 1992), Nemagard (*Prunus perisca*) and GF677 (*Prunus persica* × *Prunus amygdalus*) rootstocks (Sotiropoulos *et al*., 2006) and apple rootstocks (Therios & Misopolinos, 1989 and Sotiropoulos *et al*., 2005).

Isozyme markers provide a convenient method for detecting genetic changes and offer a possible method for cultivars and rootstocks identification. In apple, isozymes have been used as biochemical markers for the identification of clonal apple rootstocks (Batlle and Aleston, 1994).

Thus, the objective of this study was to investigate the growth under salt stress at callus and regeneration of two apple rootstocks (Balady and MM106), and attempt to introduce more tolerant apple rootstocks.

Materials and Methods

This work was conducted in the laboratory of Fruit and Ornamentals Breeding Dept., Hort. Res. Inst., Agric. Res. Center, Giza, Egypt. All tissue culture experiments were carried out during 2013 and 2014 seasons.

Plant materials

Two apple rootstocks (*Malus domestica* Borkh) MM106 and Balady which are the most common rootstocks in Egypt were used. There leaves were used as source of explants for callus initiation, regeneration, multiplication and rooting. Leaves were washed with tap water and sterilized by immersion in 70 % ethanol for 5 min, followed by immersion in 20 % sodium hypochlorite supplemented with 150 mg/l of ascorbic acid for 20 min., then rinsed four times each for ten min., in sterile distilled water supplemented with 150 mg/l of ascorbic acid.

Mode of excision

Margins of expanded leaves (10-12 mm long) were removed and the remaining part was cut transversely to the midrib into two portions. Then the leaf portions were dissected into small pieces (0.3 cm) and placed with the adaxial surface in contact with different MS media (Murashige and Skoog, 1962).

Culture conditions, Callus induction, regeneration and rooting

Leaf segments were placed with the adaxial surface in contact with MS medium supplemented with 3% sucrose, 0.7% agar, 1.0 mg/l BA, 0.5 mg/l IBA and the pH was adjusted to 5.6. Explants were grown at 25°C using 16 h light photoperiod with a light intensity of 2000 lux provided by cool white fluorescent tubes. For *in vitro* salt stress, Callus were then cultured in MS medium supplemented with 0.00, 500, 1000, 2000 and 3000 mg/l NaCl as a source of salinity. The subculture was performed every four weeks using the same medium. Then the maintained calluses were transferred to the same previous MS medium supplemented with 2.0 mg/l BA and 0.5 mg/l IBA for plant regeneration. Number of shoots per callus pieces was recorded after two months from transferring the callus to the regeneration medium. Multiple shoots were separated and transferred vertically on ½ MS medium supplemented with 2% sucrose, 0.7% agar, 0.5 mg/l IBA and the pH was adjusted to 5.6 as a rooting medium for another 4 weeks.

The following data were recorded

Vegetative growth/plant

Percentage of survival and regeneration of callus, Plantlet length (cm), number of multiple shoots, number of leaves/plant, number of roots/plant, root length (cm).

Chemical analysis

Total chlorophyll: Total chlorophyll was determined using chlorophyll meter (Model SPAD-502). Total chlorophyll was estimated as µg/cm^2.

Proline determination: The proline content was determined according to the method described by Bates *et al*. (1973).

Isozymes electrophoresis

Extraction of isozymes was done as described by Jonathan and Weeden (1990). Native–polyacrylamide gel electrophoresis (Native-PAGE) was performed on 12% (W/V) slab gels, then, gels were stained according to Wendel and Weeden (1989) for isozymes of peroxidase (Px), poly phenyl oxidase (PPO), alcohol dehydrogenase (Adh) and malate dehydrogenase (Mdh). The stained gels were incubated at 37°C in dark conditions for complete staining after adding the appropriate substrates and staining solutions. Gel bands were scanned and analyzed using Gel Doc., Bio-Rad system.

Statistical Analysis

Experiments were set in completely randomized design. Each treatment was performed in ten jars containing five explants and each experiment was replicated three times. Data were subjected to analysis of variance. Duncan's

multiple range test at 5% level of significance (p=0.05), was used for means comparisons according to (Snedecor and Cochran, 1980).

Results and Discussions

Effect of salinity stress on callus and vegetative growth characteristics of Balady and MM106 apple rootstocks

Data in Table 1 and Fig. 1 showed that salinity greatly affected survival percentages of callus in both rootstocks. At (3000 mg/l NaCl), the mean of callus significantly decreased (37.83 %) if compared with control (60.00 %). The salinity was more harmful to MM106 rootstock (44.47 %) than Balady rootstock (51.40 %). Interactions between salinity and rootstocks showed that, the higher salinity concentration (3000 mg/l) induced the lowest significant percentage of callus especially with MM106 (32.0 %) if compared with control (60.00 %). The ability of Balady and MM106 rootstocks to renew growth was affected under the stress conditions of different salinity levels as NaCl. The mean of regeneration percentage reached the maximum number with control (38.67 %), and decreased gradually with higher NaCl concentrations until it was (23.67 %) with 3000 mg/l. MM106 rootstock was affected by salinity treatments (25.27 %) more than Balady (35.73 %). Concerning interactions between salinity and rootstocks it was prevailed that, the regeneration percentage was high significantly with Balady rootstock cultured on 0.00 NaCl (42.00 %), while, the lowest significant mean was obtained with MM106 cultured on 3000 mg/l (18.00 %).

It was feasible that salinity did not reduce the average length of shoots. Concentration of 500 mg/l gave the highest value of shoot length (5.43 cm). However, significant difference detected between rootstocks, Balady rootstock gave the higher significant value (4.88 cm) if compared with MM106 (4.07 cm). Regarding the interactions between rootstocks, Balady achieved the highest significant mean (6.46 cm) at 500 mg/l, if compared with other treatments, which resulted in insignificant differences.

In addition, Table 1 and Fig. 1 showed that the number of multiple shoots was affected by raising salinity level into medium. The highest significant mean was obtained with control (0.00 NaCl) (14.00) while, the lowest significant mean was (8.50) with 3000 mg/l. Salinity was more effective on MM106 than Balady rootstock. Concerning the interaction between both rootstocks and Balady, the control gave the highest significant mean (16.00) if compared with other treatments. While, the lowest significant mean achieved with MM106 rootstock at 3000 mg/l (6.67).

Salinity affected the number of leaves per plant, which (0.00 and 500 mg/l NaCl) treatments achieved the highest significant number of leaves means (8.83 and 7.83), respectively, followed by 1000 mg/l (7.33). Whereas, the lowest significant number was recorded at 2000 and 3000 mg/l with insignificant differences (6.16 and 5.83), respectively. Balady rootstock gave the highest significant mean (7.67) compared with MM106 (6.73).

As shown in Table 1 and Fig. 2 salinity had a clear significant effect on the average number of roots per plantlet. The highest significant value was achieved with control (0.00 NaCl) (6.83), while the lowest significant value was recorded with 3000 mg/l (4.33). Rootstocks showed the same trend, Balady was more tolerant than MM106 to salinity stress and the mean number of roots was (6.27 and 4.80), respectively. The interactions between studied variables revealed significant differences in number of roots of both rootstocks grown in (0.00 NaCl) (7.00 and 6.67), respectively. The lowest significant (3.66 and 3.66) was recorded with MM106 rootstock cultured on 2000 and 3000 mg/l, respectively.

Data in Table 1 and Fig. 2 showed the effect of exposure to salinity *in vitro* on the root length, control (0.00 NaCl) and 500 mg/l treatments were recorded the highest significant mean of root length (7.02 and 6.83 cm), respectively. While, the lowest significant mean were recorded with 2000 mg/l (5.73 cm) followed by 3000 mg/l (5.53 cm). The rootstocks means cleared that Balady produced significant length of roots (6.67 cm) compared with MM106 (5.92 cm). The interactions cleared that the highest significant mean of root length was achieved with Balady rootstock cultured on (0.00 NaCl). While, the lowest significant means (5.30 and 5.20 cm) were recorded with MM106 cultured on 2000 mg/l followed by 3000 mg/l NaCl, respectively. MM106 rootstock showed more sensitivity to salt stress. These results are agree with those found by Naeniei *et al*. (2006) who, reported that increasing salinity caused reduction of stem length, leaf surface, number of internodes and its length of pomegranate "Malas Torsh" and "Alak Torsh" cultivars when its rooted cuttings were planted in pots under levels of salinity up to 120 mM. While, the growth rate of pomegranate "Malas Shirin" cv. increased with raising salinity level up to 40 mM., and El-Agamy *et al*. (2010), who found that, survival percentage, plantlet height, average leaves number, average number of nodes, average internode length and fresh and dry weight were significantly decreased using 6.0 and 8.0 g/l NaCl if compared with control treatment. Moreover, Rayan and Awad (2013) stated that no survival calluses were obtained at 5000 mg/l of NaCl of *in vitro* pear (*Pyrus Communis* var. *Betulifolia*) explants. Regenerated plants were also decreased with the increasing of salt concentration. NaCl at 1000 mg/l concentration revealed high morphological measurements if compared with control treatment, while, the opposite was true for the 2000, 3000 and 4000 mg/l concentrations.

Effect of salinity stress on proline accumulation and total chlorophyll of Balady and MM106 rootstocks' growth characteristics

Figure 3 showed the effect of salinity stress on proline accumulation and total chlorophyll of Balady and MM106 rootstocks. The root analyses of Balady and MM106 plantlets under increasing NaCl concentrations showed that proline concentration was increased with raising NaCl concentration in the culture medium. At the highest concentration (3000 mg/l) of NaCl, the proline was 11.66 and 10.95 if compared with control (0.00 NaCl) which was 5.33 and 5.12 for Balady and MM106 rootstocks, respectively. On the other hand, total chlorophyll was decreased when salt concentration increased as shown in Fig. 3.

TABLE 1. *In vitro* salinity effect as NaCl (mg/l) at different concentrations on callus and plantlets vegetative growth characteristics of Balady and MM106 rootstock.

Traits in tissue culture	NaCl mg/l	Balady	MM 106	Means
Percentage of callus survival	Control	60.00 a	60.00 a	60.00 A
	500	54.00 b	50.67 bc	52.33 B
	1000	51.67 b	44.00 d	47.83 C
	2000	47.67 c	35.67 e	41.67 D
	3000	43.67 d	32.00 f	37.83 E
	Means	51.40 A″	44.47 B″	
Percentage of regeneration	Control	42.00 a	35.33 bc	38.67 A
	500	36.33 ab	29.67 b-d	33.00 B
	1000	36.33 ab	23.33 de	29.83 BC
	2000	34.67 bc	20.00 e	27.33 CD
	3000	29.33 cd	18.00 e	23.67 D
	Means	35.73 A″	25.27 B″	
Plantlet length (cm)	Control	4.83 b	4.80 b	4.817 AB
	500	6.46 a	4.40 b	5.43 A
	1000	4.63 b	4.13 b	4.38 AB
	2000	4.46 b	3.70 b	4.08 B
	3000	4.00 b	3.33 b	3.66 B
	Means	4.88 A″	4.07 B″	
No. of multiple shoots	Control	16.00 a	12.00 b-d	14.00 A
	500	14.00 ab	10.00 d-f	12.00 B
	1000	13.67 ab	8.66 e-g	11.17 BC
	2000	12.67 bc	7.66 fg	10.17 C
	3000	10.33 c-e	6.67 g	8.50 D
	Means	13.33 A″	9.00 B″	
No. of leaves/plantlets	Control	9.00 a	8.66 ab	8.83 A
	500	8.33 ab	7.33 a-c	7.83 AB
	1000	7.66 a-c	7.00 b-d	7.33 B
	2000	7.00 b-d	5.33 d	6.16 C
	3000	6.33 cd	5.33 d	5.83 C
	Means	7.67 A″	6.73 B″	
No. of roots/plantlets	Control	7.00 a	6.67 ab	6.83 A
	500	7.33 a	5.00 c	6.16 AB
	1000	6.33 ab	5.00 c	5.67 B
	2000	5.66 bc	3.66 d	4.67 C
	3000	5.00 c	3.66 d	4.33 C
	Means	6.27 A″	4.80 B″	
Root length (cm)	Control	7.50 a	6.53 cd	7.02 A
	500	7.13 ab	6.53 cd	6.83 A
	1000	6.70 bc	6.03 de	6.36 B
	2000	6.17 c-e	5.30 f	5.73 C
	3000	5.86 e	5.20 f	5.53 C
	Means	6.67 A″	5.92 B″	

Means followed by the same letters in each column, row or interaction are not significantly different at 5% level.

Fig. 1. Effect of NaCl on growth and development of apple rootstocks (B) Balady (M) MM106 in vitro.

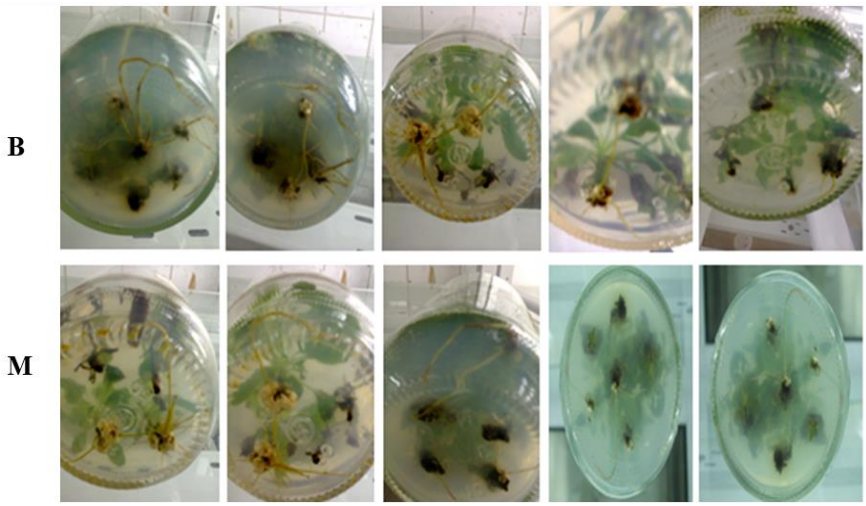

Fig. 2. Development of apple rootstocks (B) Balady (M) MM106 plants *in vitro* rooting in culture vessels.

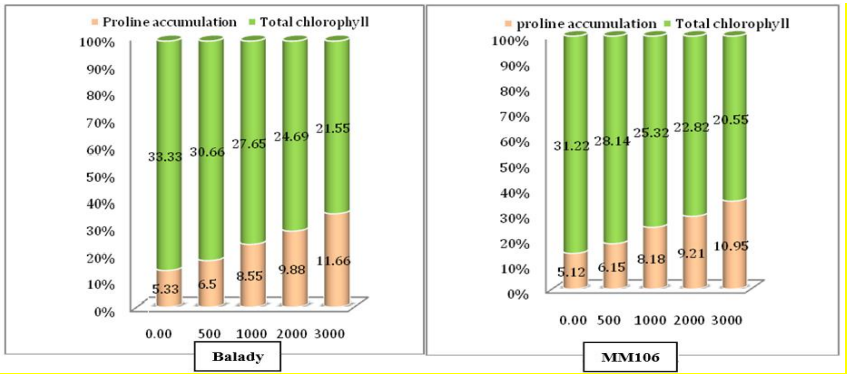

Fig. 3. Effect of salinity stress on proline accumulation and total chlorophyll of rootstock Balady and MM106.

There were differences between the control treatment and the tested concentrations of NaCl (500, 1000, 2000 and 3000 mg/l). At the highest concentration 3000 mg/l, NaCl the total chlorophyll was 21.55 and 20.55, if compared with control (0.00 NaCl) which was 33.33 and 31.22 for Balady and MM106 rootstocks, respectively. Numerous studies have linked the accumulation of proline to salt stress such as, Erturk *et al*. (2007), who found that increasing NaCl concentration *in vitro* up to 150 mM caused reduction of growth and chlorophyll content of sweet cherry rootstock Gisela 5 (*Prunus cerasus* × *Prunus canescens*) shoots, but had no effect on water content. Similar results were achieved by Sotiropoulos (2007) who found that increasing NaCl and $CaCl_2$ concentrations in the culture medium of apple rootstock 'M4' under *in vitro* conditions, increased concentrations of proline and soluble sugars in plantlets, whereas, chlorophyll concentrations decreased in comparison to the control treatment. Moreover, Rayan and Awad (2013) stated that the pear plantlets content of Na, Cl and proline were gradually increased with the raising of sodium chloride concentrations. Meanwhile, total chlorophyll content was decreased by the increasing of salt concentration *in vitro*.

In addition El-Sabrout (2003) mentioned that N, Na, Cl and proline contents showed a tendency of positive responses to salinity treatment, while P, K, Ca, Mg and total chlorophyll contents showed negative responses of *in vitro*, shoot cultures of Early Superior, Flame Seedless and Thompson Seedless grape cultivars.

Isozymes electrophoresis:

Peroxidase banding patterns

Table 2 and Fig. 4 represent peroxidase electrophoresis banding patterns from fresh leaves of Balady and MM106 apple rootstocks and their treatments with different concentrations of NaCl (T1, T2, T3 and T4). In Balady rootstock, there were differences in bands density between control and some treatments that cause reduction in banding density. In Px1 Px2, Px7 and Px9 with relative mobility 0.30, 0.43, 0.67 and 0.80, respectively there were reducing in band density to moderate density in treatments if compared with control, which had

high density of bands. In addition, in each of Px3 Px4, Px5 and Px11 there were some treatments manifesting high density of bands if compared with control and other treatments that appeared moderate and low density bands with relative mobilities at 0.46, 0.50, 0.60 and 0.90, respectively. While, in each of Px6, Px8 and Px10 there were no differences in banding pattern density between control and all treatments. In Px1 Px7, Px9, Px10 and Px11 with relative mobility 0.3, 0.67, 0.80, 0.85 and 0.90, respectively, there were differences in band density in some treatments if compared with control and other treatments.

Results of MM106 rootstock obviously showed similarity in band density in control and all treatments at Px8 only with relative mobility 0.70. While, each of Px3 Px4 and Px5 in treatment (T4) manifesting low density of bands if compared with control and other treatments which appeared moderate density bands with relative mobilities at 0.46, 0.50 and 0.60, respectively. On the other hand, there were differences in banding pattern density in some treatments which appeared density increasing in, T1, T2, T3, and T4 at Px1, Px2, Px6, Px7, Px9, Px10 and Px11 with relative mobilities 0.30,0.43, 0.63, 0.67, 0.80, 0.85 and 0.90, respectively, if compared with control. The results of isozyme are in harmony with Rayan *et al*. (2010) and Rayan *et al*. (2014).

TABLE 2. Densitometric analysis for leaf peroxidase isozyme of two apple rootstocks treated with four concentrations of sodium chloride.

Peroxidase	Relative	Balady					MM106				
Groups	Mobility	C	T1	T2	T3	T4	C	T1	T2	T3	T4
Px1	0.30	1^{++}	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{+}	1^{++}	1^{++}	1^{-}
Px2	0.43	1^{++}	1^{+}	1^{+}	1^{++}	1^{+}	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}
Px3	0.46	1^{+}	1^{++}	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{-}
Px4	0.50	1^{+}	1^{++}	1^{+}	1^{++}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{-}
Px5	0.60	1^{+}	1^{++}	1^{+}	1^{++}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{-}
Px6	0.63	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}
PX7	0.67	1^{++}	1^{++}	1^{++}	1^{++}	1^{-}	1^{+}	1^{+}	1^{++}	1^{++}	1^{-}
PX8	0.70	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}
PX9	0.80	1^{++}	1^{++}	1^{++}	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}	1^{++}	1^{++}
Px10	0.85	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{+}
Px11	0.90	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1	1^{+}	1^{+}	1^{+}	1^{-}

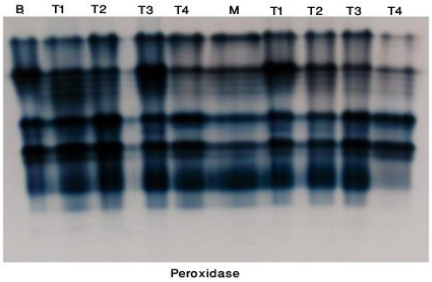

Fig. 4. Leaf peroxidase isozyme banding patterns for two apple rootstocks treated with four concentrations of sodium chloride.

Poly phenyl oxidase banding pattern

Table 3 and Fig. 5 represent poly phenyl oxidase electrophoresis banding patterns among fresh leaves of Balady and MM106 apple rootstocks and their treatments with different concentrations of NaCl (T1, T2, T3 and T4). In Balady rootstock, there were differences in banding patterns density between control and all treatments that have increasing in banding density in T1, T2, T3 and T4 at PPO1, PPO7 and PPO8. On the other hand, there were a reduction in banding density in each of T1, T2 and T3 treatments with relative mobilities 0.10, 0.70 and 0.80, respectively at PPO2, PPO3,PPO4, PPO5 and PPO6 in compared to control. Results of MM106 rootstock obviously showed similarity in band density in control and all treatments at PPO5, PPO7 and PPO8 with relative mobility 0.45, 0.70 and 0.80, respectively. While, there were a reduction in banding density in each of T1 and T4 treatments at PPO1, PPO4 and PPO6 with relative mobilities 0.10, 0.40 and 0.50, respectively in compared to control. On the other hand, there were differences in banding pattern density in some treatments, which appeared density increasing in: T1, T2, T3, and T4 at PPO2 and PPO3 with relative mobilities 0.20 and 0.30, respectively, if compared with control. These results obtained herein are in harmony with Rayan *et al*. (2010) and Rayan *et al*. (2014), who clarified that peroxidase and poly phenyl oxidase banding patterns represent differences in density of bands with increase or decrease and absent of bands in treatments in comparison with control in plum and pear cultivars.

TABLE 3. Densitometric analysis for leaf poly phenol oxidase isozyme of two apple rootstocks treated with four concentrations of sodium chloride.

PolyPhenyl Oxidase Groups	Relative Mobility	Balady					MM106				
		C	T1	T2	T3	T4	C	T1	T2	T3	T4
PPO1	0.10	1^{+}	1^{++}	1^{+}	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{+}
PPO2	0.20	1^{++}	1^{+}	1^{++}	1^{++}	1^{++}	1^{+}	1^{++}	1^{+}	1^{++}	1^{++}
PPO3	0.30	1^{+}	1^{-}	1^{+}	1^{++}	1^{++}	1^{+}	1^{-}	1^{++}	1^{+}	1^{++}
PPO4	0.40	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}	1^{++}	1^{+}	1^{++}	1^{++}	1^{+}
PPO5	0.45	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}
PPO6	0.50	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}	1^{++}	1^{+}	1^{++}	1^{++}	1^{++}
PPO7	0.70	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}
PPO8	0.80	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}

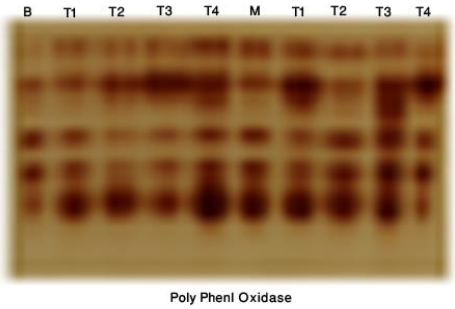

Fig. 5. Leaf Poly rhenol Oxidase isozyme banding patterns for two apple Rootstocks treated with four concentrations of sodium chloride.

Alcohol dehydrogenase banding patterns

Table 4 and Fig. 6 illustrated alcohol dehydrogenase (ADH) electrophoresis banding patterns from fresh leaves of Balady and MM106 apple rootstocks and their treatments with different concentrations of NaCl (T1, T2, T3 and T4). In Balady rootstock, data demonstrated similarity in band density in control and all treatments at Adh3, Adh4 and Adh5 with relative mobilities 0.50, 0.56 and 0.60, respectively. Meanwhile, there were differences in bands density between control and some treatments in banding density in each of T1, T2 and T4 at Adh6 and Adh7 with relative mobilities 0.80 and 0.90, respectively, if compared with control. On the other hand, there was decreasing in banding density in T1 to low density in compared with moderate density in each of control and other treatments at Adh1 and Adh2 with relative mobilities 0.30 and 0.40, respectively. Results of MM106 rootstock obviously showed similarity in band density in control and all treatments at Adh2, Adh3, Adh4, Adh5 and Adh6 with relative mobilities 0.40, 0.50, 0.56, 0.60 and 80, respectively. While there were differences in banding pattern density in some treatments and control at Adh1 and Adh7 with relative mobilities 0.30 and 0.90, respectively. These results are in harmony with Marquard and Chan (1995) who found that ADH produced a fast monomorphic region and a slower polymorphic region with seven different banding patterns.

TABLE 4. Densitometric analysis for leaf alcohol dehydrogenase isozyme of two apple rootstocks treated with four concentrations of sodium chloride.

Alkohol Dehydroogenase Groups	Relative Mobility	Balady					MM106				
		C	T1	T2	T3	T4	C	T1	T2	T3	T4
Adh1	0.30	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{++}	1^{-}	1^{+}	1^{-}
Adh2	0.40	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1	1^{+}
Adh3	0.50	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
Adh4	0.56	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
Adh5	0.60	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
Adh6	0.80	1^{++}	1^{++}	1^{++}	1^{++}	1^{-}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}
Adh7	0.90	1^{++}	1^{+}	1^{-}	1^{++}	1^{-}	1^{++}	1^{++}	1^{+}	1	1^{-}

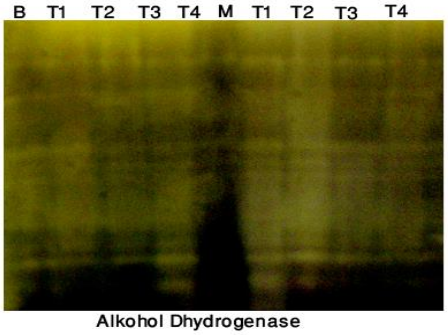

Fig. 6. Leaf alcohol dehydrogenase isozyme banding patterns for two apple rootstocks treated with four concentrations of sodium chloride

Malate dehydrogenase banding pattern

Table 5 and Fig. 7 represent malate dehydrogenase (MDH) electrophoresis banding atterns among fresh leaves of Balady and MM106 apple rootstocks and their treatments with different concentrations of NaCl (T1, T2, T3 and T4). In Balady rootstock, data showed similarity in band density in control and all treatments at Mdh2 and Mdh4 with relative mobilities 0.40 and 0.60, respectively. Meanwhile, there were differences in bands density between control and some treatments in banding density in each of T1, T2 and T4 at Mdh3 and Mdh6 with relative mobilities 0.50 and 0.90, respectively, if compared with control. On the other hand, there were increasing in band density to moderate density in all treatments if compared with control, which had low density of bands.

TABLE 5. Densitometric analysis for leaf malate dehydrogenase isozyme of two apple rootstocks treated with four concentrations of sodium chloride.

Malate Dehydroogenase Groups	Relative Mobility	Balady					MM106				
		C	T1	T2	T3	T4	C	T1	T2	T3	T4
Mdh1	0.30	1^-	1^+	1^+	1^+	1^+	1^+	1^+	1^+	1^+	1^+
Mdh2	0.40	1^+	1^+	1^+	1^+	1^+	1^+	1^+	1^+	1^+	1^+
Mdh3	0.50	1^{++}	1^+	1^+	1^{++}	1^-	1^{++}	1^-	1^+	1^+	1^+
Mdh4	0.60	1^+	1^+	1^+	1^+	1^+	1^+	1^+	1^+	1^+	1^+
Mdh5	0.80	1^-	1^+	1^+	1^+	1^+	1^-	1^+	1^+	1^+	1^+
Mdh6	0.90	1^+	1^-	1^-	1^{++}	1^-	1^+	1^+	1^-	1	1^+

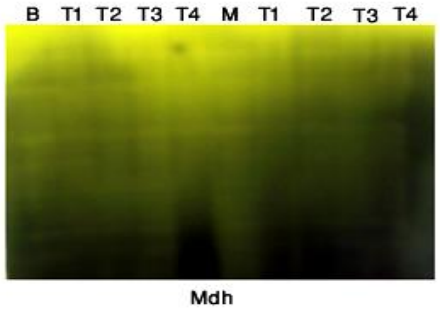

Fig. 7. Leaf malate dehydrogenase isozyme banding patterns for two apple rootstocks treated with four concentrations of sodium chloride.

Results of MM106 rootstock obviously showed similarity in band density in control and all treatments at Mdh1, Mdh2 and Mdh4 with relative mobilities 0.30, 0.40 and 0.60, respectively. While, there were a reduction in banding density in control with low density of band compared with all treatments at Mdh5 with relative mobility 0.80. On the other hand, data illustrated differences in banding density between control and treatments at Mdh3 and Mdh6 with

relative mobilities 0.50 and 0.90, respectively. These results are in agreement with Biruk and Kazlovskaya (2008) who found that MDH gave two monomorphic areas, but only some bands were variable between the studied apple cultivars.

In conclusion, it can be concluded that the high concentration of NaCl caused a negative influence on all studied parameters. Increasing NaCl concentrations were reflected on the behavior of different explants under *in vitro* conditions.

Finally, a specific advantage of salinity stress is to develop a range of tolerant lines. All variant regenerated plantlets were acclimatized to be transplanting into the permanent field for further studies and to be used in breeding programs and selection procedures.

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تأثير الإجهاد الملحي على الكلس والنبتات الناتجة من اصول التفاح في المعمل

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تمثل ملوحة التربة قلق للباحثين وهي من الامور الهامة فى برامج تربية الفاكهة للتغلب على المشاكل التي تواجه مربى النباتات. لذلك أجريت هذه الدراسة علي أصلين من اصول التفاح الاكثر انتشارا تحت ظروف جمهورية مصر العربية هما (البلدي وMM106) من خلال زراعة الانسجة ولقد شملت الدراسة اربعة تركيزات من كلوريد الصوديوم (500-1000-2000-3000 mg/l) ولقد أظهرت الدراسة.

تأثر جميع الصفات تحت الدراسة (نسبة بقاء الكلس - نسبة تكون النباتات - طول النباتات - عدد الأوراق - عدد الجذور طول الجذر) حيث كانت أقل من المقارنة وان الاصل البلدي كان اكثر تحملا من MM106. حيث بلغت نسبة بقاء الكالوسات حوالي ٣٢٪ مقارنة بالكنترول ٦٠٪ كذلك بلغت النسبة المئوية لتكوين النبتات حوالي ٣٨,٦٧٪ وتناقصت تدريجيا حيث بلغت ٣٢,٦٧٪ غند تركيز ٣٠٠٠مليجرام/لتر. وبلغ عدد النبتات المتضاعفة ١٤٪ في المقارنة ثم تناقصت تدريجيا ختي وصل ٨,٥٠٪ عند تركيز ٣٠٠٠ مليجرام/لتر.

كذلك كان عدد الاوراق المتكونة على النبتات قليل في المقارنة وبلغ عدد الاوراق ٨,٨٣و٧,٨٣ عند تركيز ٢٠٠٠و٣٠٠٠ مليجرام/لتر. كان الاصل البلدي أفضل في تكوين الجذور حيث كان متوسط تكوين الجذور ٦,٢٦ وفي MM106 ٤,٨٠

ومن الناحية الكيميائية ادي زيادة تركيزات الملوحة الى زيادة تركيز البرولين ونقص حاد في المحتوى الكلورفيلى كذلك دلت نتائج التفريد الكهربي للمشابهات الإنزيمية peroxidase, poly phenyl oxidase, alcohol dehydrogenase and malate dehydrogenase لأصلي التفاح أن هناك تشابه وأحيانا تباين بين الحزم المحددة وكذلك كثافة الصبغ فمثلا صبغة الأنزيم لورقة المقارنة تختلف عن باقى المعاملات الاحري وهذة الاختلافات الوراثية ترجع الي الاختلاف في التعبير الجيني تحت تأثير المستويات المختلفة من الملوحة.

نستخلص من هذه الدراسة انة كلما ارتفع مستوي تركيز الملوحة زادت نسبة تراكم البرولين فى جذور النباتات وان البرولين يحمى النباتات من أضرار ارتفاع الملوحة الي حد معين وأن هناك اختلاف وتشابه فى عدد الحزم وكثافة الصبغ يمكن على اساسة التفرقة وراثيا بينهم. كل هذه النتائج قد تساعد مربى النباتات في استنباط اصول وأصناف تتحمل مستويات اعلى من الملوحة.

كذلك كان الأصل البلدي أفضل في جميع الصفات تحت الدراسة ولذلك يمكن التوصية بإكثاره وزيادة التوسع في استخدامه كأصل للتطعيم عليه.