American-Eurasian J. Agric. & Environ. Sci., 1 (3): 243-248, 2006
ISSN 1818-6769

Evaluation of Growth Characteristics of Some Citrus Rootstocks Using Protein Finger Print Technique

Omaima M. Hafez

Pomology Research Department, National Research Centre, Dokki, Cairo, Egypt

Abstract : Vegetative growth characteristics, leaf and root mineral content of six citrus rootstock seedlings namely Sour orange, Brazilian sour orange, Spanish sour orange, Troyer citrange, Rangpure lime and Volkamer lemon were evaluated. Protein finger print technique was used to differentiate between the six rootstocks. Data indicated that Troyer citrange seedlings had the highest values of vegetative growth characteristics. On the other hand, seedlings of all rootstocks showed the highest dry weight (g) compared with Sour orange and Volkamer lemon. Macronutrients (N, P and K) in both leaves and roots recorded the highest values with the Sour orange rootstock seedlings. As For micronutrients (Fe, Mn and Zn), the highest values in the leaves were recorded by the Volkamer lemon, while as the highest values in the root were obtained by Brazilian sour orange. Furthermore, protein finger print technique (SDS-PAGE) proved to be suitable for identification, discriminative and similarity among different citrus rootstocks.

Key words: Citrus rootstocks · vegetative growth characteristics · leaf and root mineral content · SDS-protein electrophoresis · DICE similarity

INTRODUCTION

Citrus rootstocks are very important factor for both quality and quantity of survival and production of citrus trees. In Egypt and other countries of the Mediterranean as well as in temperate regions, many citrus varieties are successfully used as rootstocks under different environmental conditions [1-7]. Sour orange seedlings are the main rootstock using in Egypt for citrus varieties. Recently, in the new reclaimed soils, the growth and fruiting of citrus trees are highly affected by the up normal environmental conditions specially the soil factor, which negatively affected on growth of Sour orange rootstock grown in such conditions. Nowadays, plant genetic resources are one of the most valuable assets to mankind. Protection and conservation of resources for future generation, therefore, assumes great significance. Protein electrophoresis technique is used to evaluate and differentiate between different plant varieties, species … etc. [8-10].

Therefore, the aim of the present study is to determine the physical and chemical properties of six citrus rootstock seedlings, also use protein electrophoresis technique to compare between these rootstocks.

MATERIALS AND METHODS

This study was conducted during 2004 and 2005 seasons in the National Research Centre, Dokki, Giza, Egypt. The plant material of six citrus rootstocks namely Sour orange (*Citrus aurantium* L.), Volkamer lemon (*C. volkameriana*), Rangpour lime (*C. lemonia osbeck*), Troyer citrange (Orange trifoliate X Washington orange), Brazilian sour orange (*Citrus aurantium* L.) and Spanish sour orange (*Citrus aurantium* L.) was planted as seeds. In December 2003, mature fruits of the six citrus rootstocks were collected from a single mother tree. Then seeds were extracted from the fruits, washed with tap water and submerged in hot water at 52°C for 10 min. Wet seeds were spread on the laboratory bench and exposed to a stream of air to get rid of surface moisture. Air dried seeds were treated with fungicide as recommended dose. All seeds of citrus rootstocks were put in a polyethylene bag, labeled and stored at 2-5°C till swelling. In March of both 2004 and 2005 seasons, seeds of each the rootstock chosen for this study were soaked for 24 hrs in tap water and planted in a plastic bag (18×20 cm) full with a mixture of sand and peat moss at 2: 1 ratio then transplanting after 4 weeks in April in pots (30×30 cm) with a mixture of sand, loam and peat moss at 1: 1: 1 ratio and pleased in the

Corresponding Address: Dr. Omaima M. Hafez, Pomology Department, National Research Centre, Cairo, Egypt

greenhouse of National Research Centre. Moreover, irrigation of seedlings was done at intervals as needed and fertilized three times (before spring growth cycles and at early and late summer growth cycles) by ammonium sulphate, super phosphate and potassium sulphate at the rates 6, 5 and 2 g pot^{-1}. Foliar application with micronutrients was done three times after the soil fertilization. As possible, thirty uniformed and healthy seedlings of each the previous six rootstocks were divided into three replicates and each replicate containing ten seedlings.

Measurements and determinations:

Vegetative growth: In early September of each season, seedling length (cm), stem diameter (cm), leaf numbers, leaf area (cm^2), radical root length (cm) and dry weight of both air and root systems (g) were determined.

Leaf and root mineral contents: Leaves and roots washed, dried at 70°C till constant weight, then grind and digested to determine the macro nutrients (N, P and K) according to Evenhuis & De-Waard [11] and the microelements (Fe, Mn and Zn) according to Jackson and Ulrich [12].

Protein finger print using SDS-protein electrophoresis:

Protein extraction: Total protein content of seedling leaves of each tested citrus rootstocks were prepared separately at 4°C. Fresh leaves (about 0.5 g) were homogenized in ice-cold 250 m M sucrose buffer (pH 7.2) in a chilled pestle and mortar. The homogenate was centrifuged at 12-500 rpm for 20 min. at 4°C. The supernatant was used for protein electrophoresis. Protein concentration in supernatant was quantified by the method described by Bradford [13].

Sodium Dedecyl Sulphate - Polyacrlamide Gel Electrophoresis (SDS-PAGE) method: Separation of leave proteins of citrus rootstock seedlings on the basis of molecular weight were analyzed by Sodium Dedecyl Sulphate- Polyacrlamide Gel Electrophoresis (SDS-PAGE) method as adopted by Lammli [14]. The separating gel 12% (W/v) and stacking gel 4% were used. Each protein sample (30 μg protein) was loaded in each well of the gel. High molecular weight protein marker (180-26 kDa) provided by Sigma (USA) was used in the same gel. At the end of the run, the gel was stained with coomassie blue R 250 and destined with an aqueous solution of acetic acid and methanol. When the protein bands can be visually seen, data were recorded by a photograph. Protein finger print characterizations of different citrus rootstocks were determined by scanning of the total leave protein electrophoretic gel, using computer technique. The number of discrete protein bands and its molecular weight determined by comparison with protein standard markers, Abd-El-Khair [15].

DICE similarity coefficient: The similarity coefficient was calculated according to Dice [16]. The pair wise similarity matrix based on matching co-migrating band positions between pairs of protein profiles of citrus rootstocks were evaluated from equation:

$$SD = 2a\ (2a-u) = 2a/\ (n1+n2)$$

Where:

a = Number of common bands between a pair of profiles
u = Number of unmatched bands between each pair
n1+n2 = Total number of bands in the first and the second profiles

The data were subjected to analysis of variance and the method of Duncan was used to differentiate means, Duncan [17].

RESULTS AND DISCUSSION

Vegetative growth: It is clear from Table 1 that the stem length of Ttroyer citrange rootstock seedlings was significantly increased and recorded the highest value followed by Rangpure lime and Spanish sour orange as well as Brazilian sour orange rootstock seedlings as compared with Sour orange and Volkamer lemon in a descending order in the both studied seasons. On the other hand, statistical were lacked significance when stem diameter was considered. Meanwhile, both Spanish sour orange and Rangpure lime seedling rootstocks induced the highest leaf number in comparison with the other citrus rootstocks. However, Troyer citrange rootstock seedlings gave the highest leaf area followed by Brazilian sour orange in the two seasons as compared with all other citrus rootstocks. These results are same what in harmony with those findings of Atwia [18], who reported that different rootstocks i.e. Sour orange, Volkamer lemon, Rangpure lime, Rrough lemon and Trifoliata orange varied in seedling growth. In addition, El-mady *et al.* [19] and Abou Rawash *et al.* [20] found that Rangpure lime seedlings was superior in vegetative growth parameters than Volkamer lemon and Sour orange.

It is clear from Table 2 that all citrus rootstocks gave significant highest radical root length in both seasons as

Table 1: The vegetative growth of six citrus rootstock seedlings during 2004 and 2005 seasons

	Stem length (cm)		Stem diameter (cm)		Leaf number		Leaf area (cm^2)	
Rootstocks	2004	2005	2004	2005	2004	2005	2004	2005
Sour orange	15.2c	15.2c	2.08a	2.3a	13.6c	14.8c	4.2bc	4.6d
Brazilian sour orange	21.7b	22.2b	2.05a	2.2a	27.2b	28.0b	7.6a	7.6ab
Spanish sour orange	23.6b	24.5b	2.21a	2.4a	44.3a	44.6a	5.4bc	5.9cd
Troyer citrang	31.1a	31.6a	2.16a	2.3a	24.1b	29.5b	7.8a	8.2a
Rangpure lime	25.3b	25.8b	1.84a	2.0a	44.7a	45.4a	6.2ab	6.6bc
Volkamer lemon	13.2c	13.8c	2.10a	2.2a	136.0c	14.2c	3.9c	4.6d

Table 2: Growth vigor of six citrus rootstock seedlings during 2004 and 2005 seasons

	Radical root length (cm)		Number of fine roots		Dry weight (g)							
					Shoots & stems		Leaves		Adventitious roots		Absorbed roots	
Rootstocks	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Sour orange	17.2b	17.3b	0.27bc	0.4bc	0.13b	0.14c	0.19c	0.27d	0.05c	0.27b	0.16b	0.33a
Brazilian sour orange	31.9a	32.3a	0.53abc	0.6abc	0.77a	1.13a	0.71ab	1.10a	0.13c	0.57ab	0.56a	0.77a
Spanish sour orange	27.3a	27.6a	1.00a	1.3a	0.65a	0.90b	0.73ab	0.83b	0.74a	0.87a	0.57a	0.80a
Troyer citrang	26.0a	26.3a	0.00c	0.0c	0.63a	0.77b	0.46b	0.53c	0.50b	0.53ab	0.46a	0.50a
Rangpure lime	29.9a	30.3a	0.77ab	1.7ab	0.72a	0.83b	0.91a	0.97ab	0.67ab	0.77a	0.47a	0.57a
Volkamer lemon	16.1b	16.3b	0.00c	0.0c	0.14b	0.21c	0.18c	0.23d	0.21c	0.33b	0.15b	0.23a

well as absorbed roots. Moreover, Spanish sour orange seedlings had the highest values of fine root numbers and adventitious roots in both seasons as compared with the other citrus rootstock seedlings. As for shoots and stems dry weight, in the first season all rootstock seedlings gave higher values comparing with Sour orange and Volkamer lemon rootstocks. Meanwhile, in the second season, only Brazilian sour orange seedlings recorded the highest value comparing with the other rootstocks. However, Rangpure lime followed by Brazilian and Spanish rootstocks gave higher leaf dry weight comparing with the rootstocks during the first season, while in the second season; the higher values were obtained by Brazilian sour orange followed by Rangpure lime. Regarding adventitious roots dry weight, it is clear that Spanish sour orange and Rangpure lime recorded the higher values in the first season, while in the second season, all rootstock seedlings gave high value compared with Sour orange and Volkamer lemon rootstocks. On the other hand, all citrus rootstocks recorded higher values concerning the absorbed root dry weight than Sour orange and Volkamer lemon rootstocks. This was true in the first season, while in the second one statistical differences were nil between citrus rootstocks. The previous results were similar to those obtained by El-Hammady *et al* [19] and Abou Rawash *et al.* [20], who reported that Volkamer lemon seedlings surpassed all other rootstocks (Sour orange and Rangpure lime) in total plants Fresh and dry weights.

Leaf minerals content: It is clear from Table 3 that Spanish sour orange rootstock seedlings recorded the highest nitrogen content in the leaves in the first season, meanwhile, in the second season the same rootstock followed by Brazilian sour orange rootstock gave the higher values compared with the other rootstock seedlings. As for phosphorus percentage in the leaves, Sour orange recorded the highest values in both seasons of the study. The highest significant value of K content in the leaves was recorded by Sour and Volkamer lemon rootstock seedlings. This was true in both studied seasons.

Dealing with micronutrients, it is clear that, Volkamer lemon rootstock seedlings was superior to the other rootstocks in leaf Fe, Mn and Zn content through both seasons. These results are agree with those findings by Atwia [18] who reported that leaves of Volkamer citrus were the richest in N, P and K content comparing with Sour orange and Rangpure lime seedlings.

Root mineral content: Table 4 shows that in both seasons, Rangepure lime rootstocks had higher root

Table 3: Leaf mineral content of six citrus rootstock seedlings during 2004 and 2005 seasons

	Macro elements (%)						Micro elements (ppm)					
	N		P		K		Fe		Mn		Zn	
Rootstocks	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Sour orange	3.0b	3.1b	0.53a	0.53a	4.0a	4.1a	438.0e	438.0e	41.8c	41.8c	47.5d	47.6d
Brazilian sour orange	2.9c	3.0a	0.34e	0.34d	3.2c	3.3c	458.0d	458.0d	36.7e	36.7e	45.0e	45.1f
Spanish sour orange	3.2a	3.1a	0.35d	0.35cd	2.7d	2.7d	437.0f	437.0f	47.3b	47.3b	46.0e	46.1e
Troyer citrang	2.8d	2.7b	0.36c	0.36c	3.6b	3.6b	684.0b	684.0b	40.3d	40.3d	49.5c	49.6c
Rang pure lime	2.3e	2.1b	0.26f	0.26e	1.9e	1.9e	555.0c	555.0c	35.3f	35.3f	63.3b	63.4b
Volkamer lemon	1.7f	1.7c	0.45b	0.45b	4.0a	4.1a	688.0a	688.0a	53.8a	53.8a	67.6a	67.7a

Table 4: Roots mineral content of six citrus rootstock seedlings during 2004 and 2005 seasons

	Macro elements (%)						Micro elements (ppm)					
	N		P		K		Fe		Mn		Zn	
Rootstocks	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Sour orange	1.5e	1.3e	0.40a	0.41a	1.7c	1.7c	672.0c	672.0c	68.0be	68.0be	183.0bc	143.0b
Brazilian sour orange	1.5d	1.5d	0.36b	0.37b	2.1a	2.1a	721.0a	721.0a	86.0a	88.0a	162.0a	162.0a
Spanish sour orange	1.7b	1.7b	0.35c	0.35c	2.1a	2.1a	684.0b	684.0b	70.0b	70.0b	118.0c	118.0c
Troyer citrang	1.0f	1.0f	0.09e	0.09e	0.7e	0.7e	681.0b	681.0b	39.0d	39.0d	64.0f	59.0e
Rangpure lime	2.4a	2.4a	0.31d	0.31d	1.8b	1.8b	685.0b	685.0b	65.0c	65.0c	84.0d	84.0d
Volkamer lemon	1.6c	1.6c	0.10e	0.10e	1.2e	1.2d	590.0d	590.0d	33.0e	33.0e	78.0e	78.0d

nitrogen content as compared with the other citrus rootstocks seedlings. Moreover, Sour orange seedlings had higher amounts of P content in the roots than the other citrus rootstock seedlings. Brazilian and Spanish Sour orange roots recorded the higher K content in both seasons in comparison with the other citrus rootstock seedlings.

Dealing with micronutrients, it is clear that Brazilian sour orange rootstock recorded the highest values among Fe, Mn and Zn content in the seedlings root. These results coincide with those obtained by Seyam [21] who found that Sour orange seedlings had roots with higher levels of N and P as compared with Volkamer lemon.

Protein extraction:

Protein finger print: Protein banding patterns (protein finger print and/or protein profiles) of leaf total proteins among six citrus rootstock seedlings are presented in Fig. 1. The analysis of SDS-PAGE showed that total of 27 protein bands with molecular weights ranged from 155.4 to 21.4 kDa (Table, 5). The total numbers of discrete protein bands in protein finger print were different among the six citrus rootstocks ranged from 6 to 14 bands. The protein finger print of citrus rootstocks i.e. Sour orange, Brazilian sour orange, Spanish sour orange, Troyer citrange, Rangpure lime and Volkamer lemon revealed 10, 6, 13, 12, 14 and 6 discrete protein bands, consecutively.

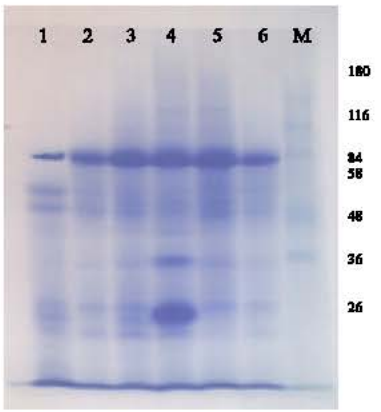

Fig. 1: SDS-PAGE of leaf total protein of six citrus rootstock seedlings
Lane 1: Sour orange, Lane 2: Brazilian sour orange, Lane 3: Spanish sour orange, Lane 4: Troyer citrange, Lane 5: Rangpure lime, Lane 6: Volkamer lemon, Lane 7: M (Protein marker with molecular weight 180-26 kDa)

Table 5: The molecular mass of protein bands and percentage of protein amount in band of citrus rootstooks protein profiles

Protein mass (kDa)	Protein amount (%) inbands					
	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6
155.4	-	-	-	-	8.31	-
153.2	-	-	8.96	-	-	-
142.1	-	-	-	3.76	5.60	-
139.8	-	-	6.31	-	-	-
134.6	-	-	-	8.65	-	-
112.1	-	-	5.96	-	-	-
109.4	-	-	-	-	7.44	-
103.9	-	-	-	7.97	-	-
79.2	8.12	12.55	8.73	12.02	8.73	11.8
61.6	8.17	13.39	5.50	6. 96	6.15	10.81
61.2	6.64	-	-	-	-	-
54.9	-	-	4.01	4.31	2.25	12.29
46.8	10.47	15.50	14.92	4.65	8.72	9.95
43.0	12.01	-	-	-	-	-
42.1	-	-	-	-	5.04	-
41.8	16.77	-	-	10.58	-	-
39.2	-	-	-	-	9.78	-
38.0	-	-	-	6.90	-	-
34.5	10.38	18.75	12.05	10.70	8.29	22.49
32.4	-	-	5.30	-	-	-
30.4	-	-	6.50	-	9.44	-
27.9	9.59	18.24	7.20	-	3.94	16.58
27.7	-	-	-	-	6.24	-
25.9	-	-	-	12.82	-	-
25.0	-	-	7.16	-	-	-
24.4	8.04	-	-	-	-	-
21.4	9.81	21.57	7.40	10.68	10.07	16.08
Total of bands	10.00	6.00	13.00	12.00	14.00	7.00

Table 6: Similarities based on matching Co-migrating band position between pairs of protein profiles of citrus rootstocks using the DICE coefficient

Citrus Rootstocks (CRS)	(CRS)					
	1	2	3	4	5	6
(CRS) 1	-					
(CRS) 2	0.85	-				
(CRS) 3	0.59	0.74	-			
(CRS) 4	0.63	0.70	0.85	-		
(CRS) 5	0.56	0.70	0.59	0.56	-	
(CRS) 6	0.82	0.96	0.78	0.74	0.74	-

In addition, it is clear from data that five of the 27 protein bands (79.2, 61.6, 46.8, 34.5 and 21.4 kDa) were the major bands in all protein profiles of tested citrus rootstocks, a tool to distinguish between the characterizations of the rootstocks, While, the other bands were differed remarkably either in presence or absence and density or intensity. Meanwhile, the twenty-two other protein bands were differed among six citrus rootstocks.

Generally, these patterns may give remarkable marker to relay the discrimination of rootstocks. In this respect, in case present protein bands with molecular weight 27.9 kDa was common in all tested citrus rootstocks except Troyer citrange rootstock. The protein of molecular 54.9 kDa was in all tested rootstocks except Sour orange and Brazilian sour orange rootstocks. Moreover, express bands in two rootstocks and disappearance in the others, i.e. the protein of molecular weight 142.1 kDa in Troyer citrange and Rangpure lime rootstocks; the protein of molecular weight 41.8 kDa which exhibited in Sour orange and Troyer citrange rootstocks: while, the protein of molecular weight 30.4 kDa was present in lanes Spanish sour orange and Rangpure lime. Also, data showed that express one band only present in one rootstock but it is absence in all the other citrus rootstocks. In this connection, Sour orange rootstock obtained the protein bands with molecular weights of 61.2, 43.0 and 24.4 kDa, While, Spanish sour orange rootstock with molecular weights of 153.2, 139.8, 112.1, 32.4 and 25.0 kDa. In contrast, Troyer citrange rootstock was distinguished with molecular weights 134.6, 103.9, 38.0 and 25.9 kDa. Also, Rangpure lime include protein bands 155.4, 109.4, 42.1, 39.2 and 27.7 kDa.

DICE similarity: The degree of similarity between pairs of citrus rootstock protein profiles was detected using the DICE coefficient as shown in Table 6. The low value of DICE coefficient was 0.56 between Sour orange x Rangpure lime rootstocks and also between Troyer citrange x Rangpure lime rootstocks. The highest value of DICE coefficient was 0.96 between Brazilian sour orange x Volkamer lemon rootstocks. Data showed that the DICE similarity was 0.59 between Sour orange x Spanish sour orange and between Spanish sour orange x Rangpure lime rootstocks. While, Sour orange x Troyer citrange rootstocks was 0.63. The electrophoretic data revealed that the DICE similarity was 0.70 between Brazilian sour orange x Troyer citrange and between Brazilian sour orange x Rangpure lime rootstocks. Meanwhile, it was 0.74 between Brazilian sour orange x Spanish sour orange rootstocks; between Troyer citrange x Volkamer lemon rootstocks and between Rangpure lime x Volkamer lemon rootstocks. The DICE similarity was 0.78 and 0.82 between Spanish sour orange x Volkamer lemon, respectively. The citrus rootstocks of Spanish sour orange x Troyer citrange and Sour orange x Brazilian sour orange showed the DICE similarity was 0.85 (Table 6). These results are somewhat in co-ordination with the finding of Mansour *et al.* [8] noticed that protein banding patterns comprise five major bands as well as a number of minor bands. The major bands are common among all the different leaf peach samples of Early-Grand peach. They were recorded at the molecular weights

approximately 98.4, 50.0, 30.0, 21.5 and 14.3 kDa. These major bands exhibited pronounced variations in their enteritis and densities among all the examined peach samples especially at the region of approximately molecular weight rang of 64.0-21.5 kDa. Also, All [22] on some citrus rootstocks, who reported that even similar proteins different rootstocks differed greatly in their concentrations, which reflect the importance in identification among different rootstocks under study. In addition, Sliva *et al.* [10] reported that the bidimensional protein electrophoresis technique was used to compare protein profiles.

ACKNOWLEDGMENTS

I would like to thank Dr. Amal Amin for helping me in protein electrophoresis analysis.

REFERENCES

1. Rodney, D.R. and D. Harris, 1973. Rootstocks for desert citrus Citrograph, 58: 124-125.
2. Ortiz, J.M., A. Garcia, J.L. Tadeo, C.D.L. Fernandez, B. Martin and A. Estelles, 1986. Comparative study of physical and chemical characteristics of four lemon cultivars. J. Hort. Sci., 61: 277-281.
3. Castle, W.S., 1987. Nursery characteristics of citrus rootstocks. In: Rootstocks for Fruit Crops. Rom, R.C. and R.F. Carlson (Eds.), John Wiley and Sons, New York, USA., pp: 361-401.
4. Monteverde, E.E., F.J. Reye, G. Laborem and J.R. Ruiz, 1988. Citrus rootstocks in Venezuela: Behaviour of Valencia orange on ten rootstocks. In Sixth Intl. Citrus Congress, Middle-East, Tel. Aviv, Israel, 1: 47-55.
5. Nieves, M., A. Garcia and A. Cerda, 1991. Effect of salinity and rootstocks on lemon fruit quality. J. Hort. Sci., 66: 127-130.
6. Azab, S.A. and A.K. Hegazy, 1995. Growth performance and plant water relations of seven citrus rootstocks under the arid environment of Qater. Arab Gulf. J. Sci. Res., 13: 605-620.
7. El-Desoky, S.A. and A.A.R. Atawia, 1998. Growth rerformance of some citrus rootstocks under saline conditions. Alex. J. Agric. Res., 43: 231-254.
8. Mansour, N.M., M.A. Fathi and H.Z. Hassan, 1998. Selection and evaluation on the horticultural and biochemical genetic bases of early peach of Sina. Proceedings of the 26th Annual Meeting of Genetics Alex., pp: 347-360.
9. El-Adawy, T.A., E.H. Rahma, A.A. EL-Bedawy and A.M. Gafar, 1999. Properties of some citrus seeds. Part 1- Physico-chemical characteristics of proteins. Nahrung. Food, 43: 373-378.
10. Silva, C.C.L.P., M.M. Antonio and L.E.G. Macdo, 2004. Differencial protein expression among zygotic and aromatic seedlings of citrus. Rev. Bras. Frutic., 26: 1-4.
11. Evenhuis, B. and W. De-Waard, 1980. Principales and practices in plant analysis. F.A.O. Soil Bull., 38: 152-163.
12. Jackson, M.L. and A. Ulrich, 1959. Analytical methods for une in plant analysis. Coll. of Agric. Exp. State Bull., 766: 35.
13. Bradford, M.M., 1976. Araped and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein - dye binding. Analytical Biochemistry, 72: 248-254.
14. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London), 227: 680-685.
15. Abd-EL-Khair, H., 1999. Genetic and serological variations between Egyptian isolates of the pear blight pathogen. Ph.D. Thesis, Faculty of Agriculture, Cairo Univ., pp: 37.
16. Dice, L.R., 1945. Measures of the amount of ecological association between species. Ecology, 26: 297-302.
17. Duncan, D.B., 1955. Multiple ranges and multiple F-Test. Biometrices, 11: 1-42.
18. Atwia, A.A.R., 1992. A comparative study on some citrus seedling rootstocks. Ann. of Agric. Sci., Moshtohor, 30: 1405-1423.
19. El-Hammady, A.M., A. Abou-Rawash, A. Abou-Aziz, A. Abdel-Hamid and E. Abdel-Moneim, 1995. Impact of irrigation with salinized water on growth and mineral content of some citrus rootstock seedlings. Ann. Agric. Sci., Cairo, 40: 327-341.
20. Abou-Rawash, M., A.M. El-Hammady, A. Abou-Aziz, N. Abdel-Hamid and E. Abdel-Moneim, 1995. Growth and mineral composition of some citrus rootstock seedlings grown under two different soil types. Ann. of Agric. Sci., Cairo, 40: 307-325.
21. Seyam, G.A.H., 2002. Study on the production of improved and standardized citrus nursery trees free of known diseases. M.Sc. Thesis Hort. Dept. Fac. of Agric. Moshtohor, Zagazig Univ.
22. All, H.M.M., 2000. Trails to improve propagation of some citrus rootstocks by using enbaryo culture techniques. Msc. Thesis Hort. Dept. Fac. of Agric. Moshtohor, Zagazig Univ.