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# SELECTION OF SALINITY TOLERANCT CITRUS ROOTSTOCK SPECIES

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## ABSTRACT

The present investigation was carried out to study the effects of two mutagenic agents (Sodium azid and gamma radiation) on salt tolerance of Cleopatra Mandarin (*Citrus reticulate* L.) (CM), Troyer Citrange (*C. sinensis* (L.), C. Osbeck X *C. trifiolata* (L.) (TC), Volkamer lemon (*C. volkameriana*) (VL) and Sour orange (*Citrus aurantium* L.) (SO) grown at the Agricultural Experimental Station, Faculty of Agriculture (El-Shatby), Alexandria University. Seedlings were treated with saline water 6000 ppm for Sour orange, Volkamer lemon and Cleopatra mandarin and 4000 ppm for Troyer Citrange, in addition to and normal controls Alterations were recorded for morphological characters (salt tolerance %, stem height, leaf number, leaf area and leaf burning %), leaf chemical constituents (proline content) and leaf element content (N, Mg, Ca, Na and Cl), the differences between each citrus rootstock and molecular analysis RAPD were also examined to detect polymorphic variants associated with responses under saline treatments. According to these results, it was observed that gamma rays surpassed sodium azid in increasing leaf number, leaf area, leaf praline content and leaf contents of Ca and Na. Meanwhile, sodium azid caused a pronounced effect in Cl content. Moreover, no significant differences were found in respect to tolerance to salt percentage, stem height, burning percentage and leaf N, Mg and Cl contents due to the mutagenic materials. Salinity had negative effects on, leaf defoliation, leaf injury, vegetative growth and leaf and root mineral contents. Cleopatra mandarin can be considered as a salt tolerant rootstock, meanwhile sour orange and Volkamer lemon can be considered as moderate tolerant rootstock and Troyer Citrange was a salt sensitive rootstock. RAPD markers can cover a high proportion of the genome because of the high number of bands scored in each analysis, due its neutral origin, there is no guarantee that such bands fall in coding regions of the genome involved in morphological and agronomic traits. Morphological and RAPD analysis turned to be good tools to identify desired plants.

## INTRODUCTION

The genus *Citrus* is considered as the pioneer economic fruit crop in Egypt. The total *citrus* cultivated area in Egypt reached 364,798 feddans, producing were 3,030,244 tons of fruit according to the statistics of the Ministry of Agriculture, Cairo, 2005. However, the expansion in its production is limited due to several biotic and abiotic stresses, especially salinity. Salinity was found to be the major limiting factor in citrus production not in Egypt only, but

worldwide, causing severe reduction in growth and physiological disorders in citrus plants (Awtar *et al.*, 2002; Atmane, *et al.*, 2005). Cleopatra mandarin and Rangpur lime are relatively salt tolerant rootstocks. Meanwhil trifoliate orange and its hybrids Carrizo and Troyer are very sensitive to salinity (Patil and Bhambota 1978).

Progress in genetic improvement of citrus rootstock by conventional breeding methods is difficult, mainly because of the reproductive biology and heterozygosty of genitors. Mutation breeding techniques have shown some promise for the induction of salt tolerance in citrus. Matsumoto, and Yamaguchi, (1984) obtained lines with the highest salt tolerance derived from *Poncirus trifoliata* material which had undergone the longest exposure to ethyl methane sulphonate (EMS). Garcia-Agustin and Primo-Millo (1995) selected three NaCl-resistant planlets of troyer citrange, regenerated from ovules treated with ethyl methane sulphonate (EMS). To increase mutation rate, Wan *et al.*, (1992) reported that, a mutation frequency 300 times greater than natural frequency was seen in citrus when callus was exposed to ethyl methanesulfonate. Deng *et al.*, (1993) obtained NaCl tolerant lines from orange (*Citrus sinensis*) cultivars Jincheng and Taoyecheng by subjecting nucellar calli to gamma ray treatments, followed by 10 generations of in vitro selection for salt tolerance. Zahed *et al.*, (2006) reported that, a stable NaCl-tolerant mutant (R1) of *Chrysanthemum morifolium* Ramat has been developed by in vitro mutagenesis with gamma radiation (5 gray; Gy). Luan-YuShi *et al.*, (2007) studied salt tolerant cultivars of sweet potato (*Ipomoea batatas* L.) that were obtained by using 0.5%.

Traditionally, morphological traits are used to detect mutations in fruit trees. In the present study attempts were made to detect induced primary genotypes under saline treatment in some citrus rootstocks, i.e. Sour orange, Troyer citrange, Volkamer lemon and Cleopatra mandarin. Based was on both morphological and molecular markers using Random Amplified Polymorphism DNA (RAPD) technique.

## MATERIALS AND METHODS

### Materials:

Seeds of rootstocks Cleopatra Mandarin (*Citrus reticulate* L.) abbreviated as (CM), Troyer Citrange (*C. sinensis* L.), C. osbeck X *C. trifiolata* (L.) (TC), Volkamer lemon (*C. volkameriana*) (VL) and Sour orange (*Citrus aurantium* L.) (SO) were soaked in tap water for 24 h before germinated in pots filled with pure sand. Seedlings were irrigated with nutrient solution with commercial fertilizer (christalon 19:19:19) as well as a micro-nutrient solution for 6 months.

### Methods:

**Mutagenesis:** Sodium azid, as a chemical mutagenic agents, and gamma radiation, as a physical mutagenic agent, were used in the present study to induce mutations in the given rootstocks according to Wan *et al.*, (1992). Table (1) summarizes the mutagenesis protocols used.

**Table-1:** mutagenic treatments (T).

| | | | |
|---|---|---|---|
| T0 | Control | | |
| T1 | Sodium azid at 0.1% for 10 min | T6 | Sodium azid at 0.3% for 30 min |
| T2 | Sodium azid at 0.1% for 20 min | T7 | 5 Kr gamma radiation |
| T3 | Sodium azid at 0.1% for 30 min | T8 | 6 Kr gamma radiation |
| T4 | Sodium azid at 0.3% for 10 min | T9 | 7 Kr gamma radiation |
| T5 | Sodium azid at 0.3% for 20 min | T10 | 8 Kr gamma radiation |

**Saline treatment:** Seedlings (6-month old) were treated for three months with saline water at 6000 ppm for Sour orange, Volkamer lemon and Cleopatra mandarin and 4000ppm for Troyer Citrange accorreding to Shiyab *et al.*, (2003). The

control group was irrigated with non-saline solution (tap water) for the same period. Saline solution used consisted of a mixture of 3NaCl: $CaCl_2$: $MgCl_2$) (3:1:1). Each treatment contained 3 replicates (5 plants / replicate). Seedlings were irrigated with saline solution 2 times and one time with tap water per week to avoid salt accumulation. Seedlings were fertilized with nutrient solution (christalon 19:19:19) as needed.

**Chemical analysis:** All plant parts were oven dried at 56°C to a constant weight, and their dry weights were recorded. The dried materials of leaves and roots for each replicate were divided into two groups; the first group was subjected to proline analysis. Free proline content was determined, using 0.1gm of dried leaf and root materials, and tolune as a blank. The proline was determined from standard curve according to Singh *et al.,* (1973). The data was expressed as percentages of dry weight. While the second group was ground digested with concentrated sulfuric acid + 30% Hydrogen peroxide for mineral analysis. In the extract, the sodium concentrations were determined using a flam photometer Jenway, PFP-7 (Chapman and Pratt., 1978). $Ca^{2+}$ and $Mg^{2+}$ concentrations were carried out by atomic absorption spectrometry (Perkin-Elmer 5500). Nitrogen was determined by the micro-Kjeldahl method of Ma and Zauzage, (1942). Chloride concentrations were measured using a sliver ion titration chloridometer (Cotlove, 1965).

**Morphological traits assessment:** Leaf samples from surviving plants were used to determine the survival percentage; leaf burning percentages, Stem height, leaf number, and leaf area were recorded.

**RAPD analysis:** Leaf samples were washed several times with distilled water and then used for DNA extraction. PCR analysis was carried out by using the genomic DNA from different seeds. Five primers obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, Ebgland HP79 NA), were tested in this experiment to amplify the template DNA according to Williams, *et al.,* (1990), in table (2). Amplification reaction volumes were 25µl, each containing 1 x PCR buffer with $MgCl_2$ (50mM KCl, 10mM Tris-HCl (pH=9.0), 2mM $MgCl_2$ and 1% trition x-100), 200µM each of dATP, dCTP, dGTP and dTTP, 50PM primer, 50ng template DNA and 1.5µl of Taq polymerase. Reaction mixtures were overlaid with 15 µl mineral oil and exposed to the following conditions: 94°C for 3min, followed by 30 cycles of 1 min. at 94°C, 1 min. at 36° C, 2 min. at 72°C, 2min and a final 7 min. extension at 72°C.

**Table-2:** Primer sequences used in the study**.**

| No. of Primer | Nucleotide Sequence 5` to 3` |
|---|---|
| Primer 1 | CTG AGA CGG A |
| Primer 2 | GGA CGG CGT T |
| Primer 3 | GAG TCA GCA G |
| Primer 4 | CAT TCG AGC C |
| Primers5 | TCA ACG GGA C |

**Statistical anaylysis:** The data collected through out the course of the present study were statistically analyzed according to Snedecor and Cochran (1990) and L.S.D test was used for comparison between citrus rootstocks.

## RESULTS AND DISCUSSION

### Detection of morphological alterations:

The data presented in table (3) indicate that sodium azid at 0.1% for 10 min and 0.3% for 10, 20 and 30 min slightly increased salt-tolerance percentage as compared with the control. Meanwhile, 0.1 sodium azid for 20 min. slightly decreased survival percentage. Moreover, 0.1 sodium azid for 30 min. markedly decreased survival percentage as compared with the control. The data also, revealed that gamma rays at all doses increased salt-tolerance percentage as compared with control, but the differences were not significant. Generally the highest salt-tolerance percentage was obtained with TC, followed by SO, CM and VL, respectively. The interaction between rootstocks and mutagenic agents, regarding salt-tolerance percentages was significant. The highest salt-tolerance percentage was found for TC rootstock treated with gamma rays at 7Kr; meanwhile the lowest survival percentage was obtained with VL received gamma rays at 8 Kr.

The response of these rootstocks to mutagenic agents was variable, where VL, TC and SO had high values for stem height (without significant differences). Meanwhile, CM had the least values for stem height. The data also revealed that for SO, all treatments increased stem height except T6 and T10. For TC the stem height was observed but at T1. For VL all treatments increased stem height. Also, for CM all treatments increased stem height except at T2 and T5 (Table 3). The data showed that, in general, gamma treatments were better than sodium azid in increasing rootstocks stem height. The highest stem height was observed with VL treated with sodium azid at 0.3 for 30 min. Meanwhile, the lowest stem height was obtained with SO treated with sodium azid at 0.3 for 30 min.

As for the effect of the mutagenic agents on leaf number, the data in Table (3) show that TC has the highest leaf number (7.35) followed by VL (6.49), CM (6.20) and SO (5.09) than control seedlings. It can be noticed also that the highest leaf number was observed in VL seedlings treated with 6Kr gamma rays. Meanwhile the lowest leaf number was obtained with VL control seedlings. Generally gamma rays treatments were better than sodium azid in respect to seedling leaf number, especially with TC and VL rootstocks. In general gamma rays treatments gave the high leaf area in comparison to sodium azid treatments. Moreover, the differences between gamma rates were not significant. The interaction between rootstocks and mutagenic agents was significant. The highest leaf area was found with VL seedlings treated with sodium azid at 0.3 for 30 min. Meanwhile the lowest value was observed with VL control seedlings.

The data of the effect of mutagenic treatments on burning percentage of rootstock leaves are shown in Table (3). The burning percentages were markedly high as compared with the control. The data of the interaction between mutagenic agents and rootstocks showed that the differences between treatments were significant. The highest leaf burning percentage was observed with SO control seedlings; meanwhile the lowest burning percentage was obtained with CM treated with gamma at 8Kr.

Similarly, Shiyab *et al*, (2003) studied the growth and nutrient uptake by sour orange under salt stress *in vitro*. A decrease in growth (shoot length, shoot number, leaf number and dry weight) with elevated salinity level was detected. Awtar *et al*, (2004) reported that the response of *C.*

*jambhiri, C. limonia* and *Poncirus trifoliata* rootstocks to various levels of soil salinity (0, 2, 4, 6 or 8 dS Cl-/m) under greenhouse conditions reduced plant height, stem diameter, number of leaves per seedling, fresh top, root biomass, dry top and root biomass. Atmane *et al.* (2005) studied the effect of salinity (NaCl at 0, 35 or 70 mM for 30 or 60 days) on growth of citrus rootstocks they concluded that an imbalance of essential nutrients may contribute to the growth reduction in the rootstocks under saline conditions. Zahed *et al.*, (2006) reported that, a stable NaCl-tolerant mutant (R1) of *Chrysanthemum morifolium* Ramat has been developed by *in vitro* mutagenesis with gamma radiation (5 gray; Gy). Salt tolerance was evaluated by the capacity of the plant to maintain both flower quality and yield under NaCl stress. The R1 mutant developed by gamma ray treatment was considered a salt-tolerant mutant showing all the positive characteristics of tolerance to NaCl stress.

**Chemical constituents**

**Proline content:** The effects of mutagenic agents on leaf proline content are presented in Table-4. The data did not show a specific trend, where treatments T6 and T7 increased leaf proline content as compared with control, meanwhile treatments T1, T2, T3, T4, T5, T8, T9 and T10 decreased it as compared with control. With respect to the interaction the data revealed significant differences between treatments. Generally the lowest value was obtained with CM treated with sodium azid (0.1 for 10 min).

Other studies indicated clear cut results for proline involvement with increased salinity tolerance in many plants. According to Deng *et al.*, (1993), NaCl tolerant lines obtained from orange (*Citrus sinensis*) cultivars Jincheng and Taoyecheng by subjecting nucellar calluses to gamma rays and ethyl methansulfonate (EMS) treatments, followed by 10 generations of *in vitro* selection for salt tolerance, accumulated more proline, maintained higher levels of K+ and absorbed less Cl- and Na+ than the original calluses. Woodward and Bennett (2005) investigated a number of roles that had been proposed for the involvement of proline in salinity tolerance. They indicated that the increase in proline concentration is correlated with reducing the physiologically detrimental effects of salinity. Ferreira and Lima-Costa (2006) reported that, the Citrus hybrid cv. 'Carvalhal' cell line displayed a salt resistant behavior, even at high salt concentrations. This salt-resistance behavior operated primarily by impeding the uptake of Na+ and Cl- ions combined with intracellular proline accumulation and a high level of scavenging of reactive oxygen species (ROS).

**Leaf elements contents:** The data concerning the effect of the mutagenic agents on leaf elements content (N, Ca, Mg, Na and Cl) are shown in Table-4. The data revealed that, generally, mutagenic treatments increased leaf N, Ca and Mg contents as compared with the control. The data of the interaction between rootstocks and mutagenic agents revealed significant differences between treatments, generally the highest leaf N content was found with TC treated with gamma (7Kr), meanwhile, CM treated with sodium azid at 0.3 for 10 min showed the lowest N content. As for leaf Na content as influenced by mutagenic treatment, the data in Table (4) showed that highest leaf Na content was found with TC followed by VL, CM and SO, respectively. Regarding to the interaction, the data showed significant differences between treatments. Generally, the highest leaf Na content found with TC treated with gamma rays at 6kr, meanwhile the lowest leaf Na

content was achieved with TC treated with sodium azid at 0.1% for 10 min.

The effect of mutagenic treatments on leaf Cl content is shown in Table (4). The data revealed that all mutagenic agents significantly decreased leaf Cl content as compared to control. As for interaction between rootstocks and mutagenic materials, the data showed significant differences between treatments, generally the highest leaf Cl content was obtained with TC control seedlings. Meanwhile the lowest leaf Cl content was found with VL treated with sodium azid at 0.3 for 30 min.

This results agreements with Deng *et al.* (1993) obtained callus cell lines treated with various mutagens (gamma radiation, EMS [ethyl methanesulfonate] and sodium azide. By continuous selection for 5-7 passages, mutant cell lines able to survive in 0.8% NaCl were obtained in 4 rootstock varieties. The mutants also exhibited altered Na+ and Cl- absorption. Storey and Walker (1999) inducted that saline ions can affect nutrient uptake through competitive interactions or affecting the ions selectivity of membranes. The effects include Na+, Ca+2 and K+ deficiencies, Ca2+ and Mg2+. Camara-Zapata *et al.* (2004) investigated the effects of salinity stress-relief on mineral composition (leaf and root) of one-year-old seedlings of Cleopatra mandarin and sour orange. Salinity induced a decrease in K+, Ca2+ and total N in Cleopatra mandarin leaves and an increase in K+ in sour orange leaves.

**DNA Marker Analysis:**

**RAPD analysis:** According to the results of morphological characters, proline content and leaf element contents, three markedly altered seedlings resulting from different mutagenic treatments, were chosen for each rootstock as promising tolerant genotypes, i.e. for Sour orange (SO5, SO7, SO8), for Troyer citrange (TC6, TC7, TC9), for Volkamer lemon (VL4, VL6, VL8), and for Cleopatra mandarin (CM1, CM9 and CM10) were used for RAPD analysis, in addition to the untreated controls.

During RAPD - PCR amplification using five decameric primers succeeded to produce polymorphic bands. Concerning for DNA polymorphism in relation to volka genotype was using primer 1, which exhibited in Figure (1). The bands ranged from 1200 bp to 252 bp and showed that four bands were found in the control. Showed 7 bands in the treatment with VL4 and VL6 dosage,, the band has 1000 bp was found in VL6 dosage, the band has 961 bp was present in VL4 dosage and absent in all treatments.

Regarding to DNA polymorphism was relation to Tryoer, Cleopatra and Sour Orange genotype using primer 1, which exhibited in Figure (3). The band with 1000 bp was found in the control and all treatment for Sour Orange only. The band has Mw 844 was found in TC7 dosage in Tryoer, CM1 dosage in Cleopatra and in control and all treatment for Sour Orange. The bands have 517 and 423 bp were present in the control and all treatment in Tryoer.

Concerning for DNA polymorphism in relation to volka genotype using primer 2 showed in Figure (1). For the bands have 1066 and 910 were present in VL6 and VL8 dosage, the band has 961, 879, 400, 321 and 275 were present in the control only. The bands with 800 bp, 626, 500 and 432 were found in VL4 dosage only. The band was size 558 bp found in control and VL8 dosage. The band has 381 bp present in all treatment except control.

For DNA polymorphism in relation to Tryoer, Cleopatra and Sour Orange genotype using primer 2 was exhibited in Figure (1). The bands with 1220 and 1024

were present in control and CM9 dosage in Cleopatra. The band has 689 bp was present in control for Tryoer , control, CM9 and CM10 dosage Cleopatra and SO5 dosage for Sour Orange. The band has 570 bp was found in CM10 dosage for Cleopatra only. The band with 239 bp was present in TC6, TC7 and TC9 for Tryoer, CM9 and CM10 dosage for Cleopatra and control and SO8 dosage in Sour Orange. The band with 218 was present only in control for Tryoer.

For DNA polymorphism in relation to volka genotype using primer 3, which exhibited in Figure-1. The band with 1000, 879 and 800 bp were found in VL4 and VL6 dosage. The bands with 961 and 754 were found in control. The bands 910, 500 and 400bp were found in VL4 dosage.

Regarding to DNA polymorphism in relation were Tryoer, Cleopatra and Sour Orange genotype using primer 3, which exhibited in Figure-1. The band with 1020 bp was present in SO7 dosage for Sour orange, and the band has 918 bp was found in SO8 dosage for the same genotype. The band with 275 bp was present in TC7 dosage for Tryoer only. The band with 231 bp was present in CM10 dosage for Cleopatra and SO7 dosage for Sour Orange.

Concerning for DNA polymorphism was relation to volka genotype using primer 4, which exhibited in Figure-1. The band with 1000 bp was present in VL4 dosage only; the bands with 952, 828 and 720 bp were present in Control, VL6 and VL8 dosage respectively. The band has 790 bp was found in control and VL4 dosage. The common band with 558 bp was present in the control and all treatments. On other hand the band has 315 bp was found in VL8 dosage only.

For DNA polymorphism in relation to Tryoer, Cleopatra and Sour Orange genotype using primer 4, this exhibited in Figure-1. The band with 1275 bp was present in control for Tryoer and Cleopatra, on other hand the band with 1195 bp was found in control for Cleopatra only. The band with 871 bp was present in CM1 and CM9 dosage for Cleopatra. The band with 405 bp was present in control for Tryoer and SO5 dosage for Sour Orange. The band with 363 bp was present in TC9 dosage for Tryoer and control for Cleopatra.

For DNA polymorphism with relation to volka genotype using primer 5, which exhibited in Figure-1. The bands have 790, 600, 315 and 168 bp was found in control and VL4 dosage. The bands have 753, 292 and 141 bp was found in VL6 and VL8 dosage. The band with 457 was present in control only. On other hand the band with 236 bp was present in VL6 dosage only.

Concerning for DNA polymorphism in relation were Tryoer, Cleopatra and Sour Orange genotype using primer 5, which exhibited in Figure-1. The bands with 800 and 339 bp were present in CM1 dosage for Cleopatra and control and all treatment for Sour Orange. The band with 771 bp was found in control, TC6 and TC9 dosage for Tryoer and control, CM9 and CM10 dosage for Cleopatra. The band with 366 bp was present in SO7 dosage for Sour Orange.

RAPD analysis has been used in citrus breeding to evaluate polymorphism between 39 Mediterranean mandarin genotypes (Coletta *et al.*, 1998). In addition was assessment of genetic variability in grapefruit and pummelos (Corazza-Nunes *et al.*, 2002). Vilarinhos *et al*., (2000) studied molecular markers have become a useful tool to analyze DNA directly, without the influence from the environment or other factors. The technique RAPD has been used to study the genetic origin of Carvo lemon plants which had been visually selected as possible hybrids to study the genetic diversity and identify

inter specific crosses. Recently, Pongtongkam *et al*., (2005) cultured seeds of *Panicum maximum* TD58 on MS medium to induce multiple shoots. The gamma-irradiated shoots grown in 0-2.0% NaCl gave 58 clones of purple guinea grass. Ten clones of grass with good morphological properties were selected and subsequently grown in the salt-stressed environment. However, there was no difference in AFLP fingerprinting patterns found as compared with the controlled non-irradiated guinea grass. The salt tolerance character might be due to the mutation at a certain location or on a specific gene which could not be distinctly detected by the available AFLP primers.

**Screening for polymorphic primers in citrus rootstocks cultivars and their mutations:** Five primers were screened for their ability to amplify the genomic DNA of four citrus rootstocks (SO, TC, VL and CM) and their mutant (Table 5). The number of DNA fragment amplified ranged from 2 to 21 depending on the primer and DNA sample with a mean value of 76.5 bands per primer. The size of fragment ranged from 100 to 1200bp. A total of 373 fragments were produced by five primers all of them were polymorphic. The critical level of salinity was found to be about 5000ppm for SO, VL and CM, meanwhile, it was about 3000ppm for TC. Sodium azid and gamma rays can be used to increase salt tolerate genetic variation. Previously similar fragment size has been obtained among Citrus species (Corazza-Nunes *et al*., 2002). These results are considered rather high for RAPD amplification, compared to the average numbers of amplified bands recorded among mandarins (2-8) (Coletta *et al*., 1998), (1-3) among accessions of grapefruits and pummelos (4-15) and among zygotic and nucellar seedlings of *C. reshni* (Rodriguez *et al.*, 2005).

**Table-5:** Sequences primers and number of their generated amplification products of the chosen rootstock seedlings.

| No of primer | Primer sequence 5\……3\ | No of amplification (a) | No of polymorphisms (b) | Polymorphism b/a (%) | Total number of bands |
|---|---|---|---|---|---|
| 1 | CTG AGA CGG A | 13 | 13 | 100 | 74 |
| 2 | GGA CGG CGT T | 14 | 14 | 100 | 83 |
| 3 | GAG TCA GCA G | 15 | 15 | 100 | 74 |
| 4 | CAT TCG AGC C | 12 | 11 | 92 | 74 |
| 5 | TCA ACG GGA C | 13 | 12 | 92 | 68 |
| Total | | | | | 373 |

In conclusion, these results could be regarded as a preliminary data, for the identification of salt tolerant genotypes. Nevertheless, the results were encouraging, it provided information on the molecular genetic level, and brought new prospective for the use of such marker in the breeding program for improving salinity tolerance in citrus rootstocks. Both morphological and molecular analysis showed a high degree of variation among analyzed genotypes, which can be considered as an important source of genetic variation that can be used in future breeding programs.

Comparison between RAPD analysis and morphological assessment, showed differences; although both of them proved that (SO5, SO7, SO8, TC6, TC7, TC9, VL4, VL6, VL8, CM1, CM9 and CM10) differed genetically. Although RAPD markers can cover a high proportion of the genome because of the high number of bands scored in each analysis, due its neutral origin, there is no guarantee that such bands fall in coding regions of the genome involved in morphological and agronomic traits.

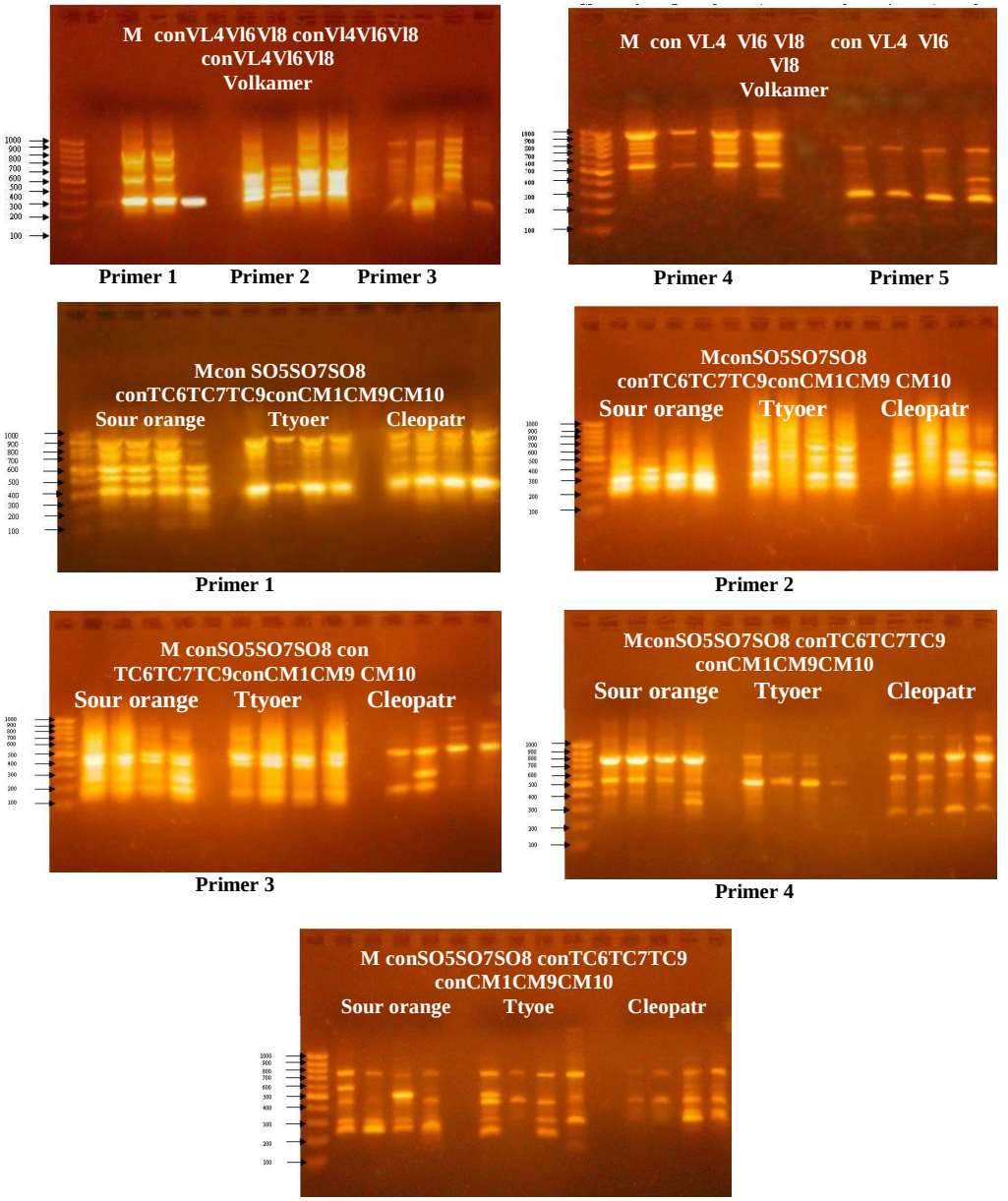


**Figure-1**: RAPD fragments amplified from four rootstock species (Volkamer lemon(VL), Sour orange(SO), Troyer citrange(TC) and Cleopatra mandarin(CM)) by five primers

Table- 2: **The mean performance for characters studied under mutagenic agents on salinity tolerance of some citrus rootstock species**

| codes | Character phenotypes | Survival % | | | | Stem height | | | | Leaf Number | | | | Leaf Area ($cm^2$) | | | | Leaf burning % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SO | TC | VL | C | SO | TC | VL | CM | SO | TC | VL | CM | SO | TC | VL | CM | SO | TC | VL | CM |
| **T0** | **Control** | **33.3** | **49.6** | **40.0** | **49.0** | **9.7** | **9.2** | **5.2** | **6.8** | **2.3** | **3.4** | 2.1 | **2.2** | **3.8** | **3.6** | 2.5 | **3.8** | 78.3 | **66.6** | **68.3** | **53.3** |
| **T1** | **Sodium azid at 0.1% 10 mins** | **64.4** | **33.3** | **21.1** | **80.0** | **11.8** | **8.5** | **11.6** | **9.9** | **5.9** | **6.8** | **3.3** | **7.1** | **12.1** | **3.3** | **42.3** | **28.3** | **16.5** | **14.3** | **26.6** | **2.0** |
| **T2** | **Sodium azid at 0.1% 20mins** | **51.1** | **38.0** | **16.6** | **31.6** | **12.0** | **12.0** | **12.3** | **5.9** | **6.5** | **4.5** | **5.1** | **4.9** | **11.8** | **3.5** | **40.3** | **11.0** | **19.1** | **6.1** | **13.4** | **10.0** |
| **T3** | **Sodium azid at 0.1% 30 mins** | **6.6** | **44.3** | **17.7** | **32.2** | **9.8** | **12.4** | **14.8** | **9.6** | **8.0** | **6.3** | **5.3** | **7.2** | **10.3** | **3.3** | **61.6** | **25.6** | **1.0** | **4.9** | **18.8** | **4.1** |
| **T4** | **Sodium azid at 0.3% 10 mins** | **59.9** | **39.6** | **22.2** | **56.6** | **13.2** | **10.9** | **14.4** | **7.4** | **5.5** | **7.4** | **6.3** | **6.7** | **11.6** | **3.0** | **67.3** | **22.6** | **28.6** | **2.6** | **14.1** | **2.2** |

| T10 | T9 | T8 | T7 | T6 | T5 |
|---|---|---|---|---|---|
| **Gamma radiation 8 Kr** | **Gamma radiation 7 Kr** | **Gamma radiation 6 Kr** | **Gamma radiation 5Kr** | **Sodium azid at 0.3% 30 mins** | **Sodium azid at 0.3% 20 mins** |
| **17.7** | **48.8** | **68.8** | **82.2** | **37.7** | **75.5** |
| **86.0** | 95.0 | **86.0** | **87.6** | **62.0** | **43.0** |
| 6.6 | **8.3** | **11.1** | **11.1** | **52.4** | **17.7** |
| **80.0** | **64.4** | **6.6** | **11.1** | **65.5** | **53.3** |
| **9.2** | **10.8** | **12.4** | **13.1** | 4.8 | **18.0** |
| **12.1** | **12.6** | **12.6** | **13.1** | **12.3** | **12.2** |
| **11.9** | **10.0** | **12.4** | **11.6** | 15.4 | **9.5** |
| **10.4** | **8.9** | **9.0** | **9.0** | **7.4** | **5.7** |
| **3.6** | **2.9** | **5.4** | **4.3** | **2.4** | **8.8** |
| **8.6** | **9.8** | **9.0** | **9.6** | **8.9** | **6.1** |
| **10.2** | **7.7** | 11.3 | **5.6** | **9.0** | **5.2** |
| **7.4** | **8.5** | **6.8** | **6.1** | **5.5** | **5.4** |
| **11.3** | **11.2** | **13.6** | **13.4** | **4.2** | **14.1** |
| **3.0** | **4.3** | **3.8** | **4.3** | **3.5** | **3.4** |
| **54.0** | **60.3** | **64.0** | **58.3** | 72.3 | **41.6** |
| **36.6** | **32.0** | **30.0** | **26.0** | **21.6** | **17.3** |
| **21.0** | **18.3** | **17.7** | **15.1** | **22.3** | **10.2** |
| **8.7** | **2.7** | **4.1** | **1.1** | **2.0** | **9.4** |
| **51.1** | **37.1** | **22.6** | **8.5** | **10.1** | **33.3** |
| 1.93 | **2.6** | **3.0** | **20.8** | **7.2** | **2.6** |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **LSD** | **Mutagenic substances** | **15.1** | **15.1** | **15.1** | **15.1** | **2.26** | **2.26** | **2.26** | **2.26** | **1.29** | **1.29** | **1.29** | **1.29** | **4.66** | **4.66** | **4.66** | **4.66** | **9.76** | **9.76** | **9.76** | **9.76** |
| **LSD** | **rootstocks** | **9.1** | **9.1** | **9.1** | **9.1** | **1.36** | **1.36** | **1.36** | **1.36** | **0.78** | **0.78** | **0.78** | **0.78** | **2.82** | **2.82** | **2.82** | **2.82** | **5.88** | **5.88** | **5.88** | **5.88** |
| **LSD** | **interaction** | **10.3** | **10.3** | **10.3** | **10.3** | **1.54** | **1.54** | **1.54** | **1.54** | **0.88** | **0.88** | **0.88** | **0.88** | **3.18** | **3.18** | **3.18** | **3.18** | **5.13** | **5.13** | **5.13** | **5.13** |

**SO** = Sour orange **TC** = Tryoer **VL** = Volka **CM** = Cleopatra

**Significant at 5% and 1% respectively.**

**Table-3:** The mean performance for leaf elemental content studied under mutagenic agents on salinity tolerance of some citrus rootstock species**.**

| codes | Leaf elemental content | Proline content | | | | Nitrogen (N) % | | | | % Magnesium (Mg) | | | | calcium (Ca) % | | | | Sodium (Na) % | | | | Colored (Cl) % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SO | TC | VL | CM | SO | TC | VL | CM | SO | TC | VL | CM | SO | TC | VL | CM | SO | TC | VL | CM | SO | TC | VL | CM |
| **T0** | **Control** | **2.8** | **2.2** | **2.79** | **2.69** | **1.48** | **1.44** | **0.74** | **2.23** | **0.26** | 0.18 | 0.18 | **0.26** | **2.70** | **2.53** | 1.73 | **2.53** | 0.28 | **0.16** | **0.26** | **0.31** | **1.54** | **0.54** | **0.64** | **0.23** |
| **T1** | **Sodium azid at 0.1% / 10 mins** | **5.6** | **0.11** | **1.21** | 2.31 | **2.07** | **0.82** | **1.54** | **2.66** | **0.33** | **0.27** | **0.67** | **0.35** | **4.04** | **1.87** | **3.40** | **3.08** | **0.30** | **0.06** | **0.33** | **0.09** | **0.07** | **0.06** | **0.04** | 0.03 |
| **T2** | **Sodium azid at 0.1% 20mins** | **1.9** | **2.1** | **1.21** | **1.35** | **1.01** | **2.37** | **1.27** | **1.43** | **0.37** | **0.36** | **0.26** | **0.38** | **3.64** | **2.05** | **3.73** | **1.94** | **0.20** | **0.34** | **0.29** | **0.13** | **0.06** | **0.11** | **0.04** | **0.07** |
| **T3** | **Sodium azid at 0.1% 30 mins** | **1.9** | **2.01** | **0.81** | **0.49** | **2.31** | **2.34** | **1.70** | **0.88** | **0.38** | **0.33** | **0.27** | **0.25** | **4.24** | **2.62** | **2.38** | **0.92** | **0.16** | **0.22** | **0.29** | **0.24** | **0.05** | **0.11** | **0.02** | **0.05** |

| T9 | T8 | T7 | T6 | T5 | T4 |
|---|---|---|---|---|---|
| **Gamma radiation 7 Kr** | **Gamma radiation 6 Kr** | **Gamma radiation 5Kr** | **Sodium azid at 0.3% 30 mins** | **Sodium azid at 0.3% 20 mins** | **Sodium azid at 0.3% 10 mins** |
| **2.6** | **4.2** | **5.1** | **3.4** | 6.2 | **3.3** |
| **2.98** | **0.05** | **3.10** | **4.43** | **1.94** | **2.08** |
| **1.85** | **2.49** | **2.10** | **2.36** | **1.17** | **2.30** |
| **2.68** | **2.18** | **2.10** | **1.73** | **1.21** | **2.12** |
| **1.07** | **2.47** | **2.76** | **1.79** | 3.26 | **2.13** |
| 5.09 | **0.61** | **2.46** | **2.54** | **2.52** | **2.37** |
| **1.45** | **2.58** | **0.68** | **2.47** | **1.67** | **2.48** |
| **2.42** | **0.60** | 0.51 | **2.60** | **0.81** | **0.50** |
| **0.25** | **0.33** | **0.40** | **0.32** | **0.43** | **0.33** |
| **0.45** | **0.27** | **0.36** | **0.48** | **0.36** | **0.24** |
| **0.44** | **0.52** | **0.40** | **0.42** | **0.31** | **0.33** |
| **0.39** | **0.23** | **0.22** | **0.36** | **0.38** | **0.42** |
| **1.38** | **3.98** | **4.72** | **3.48** | **4.84** | **3.44** |
| **3.88** | **2.09** | **4.90** | **4.16** | **3.00** | **3.61** |
| **1.44** | **4.04** | **1.30** | 4.30 | **3.36** | **3.92** |
| **4.46** | **0.80** | **0.91** | **2.81** | **2.42** | **4.61** |
| **0.13** | **0.05** | **0.11** | **0.21** | **0.13** | **0.20** |
| **0.21** | **0.83** | **0.19** | **0.12** | **0.12** | **0.11** |
| **0.20** | **0.14** | **0.21** | **0.17** | **0.29** | **0.06** |
| **0.11** | **0.24** | **0.22** | **0.33** | **0.18** | **0.34** |
| **0.08** | **0.07** | **0.04** | **0.06** | 0.04 | **0.04** |
| **0.04** | **0.05** | **0.05** | **0.05** | **0.08** | **0.07** |
| **0.07** | **0.05** | **0.07** | **0.01** | **0.04** | **0.03** |
| **0.04** | **0.06** | **0.06** | **0.08** | **0.05** | **0.06** |

| T10 | Gamm radiatio 8 Kr | 3.3 | 1.19 | 0.95 | 2.62 | 1.10 | 0.70 | 1.70 | 2.27 | 0.21 | 0.27 | 0.26 | 0.39 | 2.64 | 1.15 | 4.08 | 3.40 | 0.21 | 0.33 | 0.42 | 0.12 | 0.07 | 0.07 | 0.07 | 0.03 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LSD | Mutagenic substances | 0.14 | 0.14 | 0.14 | 0.14 | 0.19 | 0.19 | 0.19 | 0.19 | 0.052 | 0.052 | 0.052 | 0.052 | 0.306 | 0.306 | 0.306 | 0.306 | 0.009 | 0.009 | 0.009 | 0.009 | 0.041 | 0.041 | 0.041 | 0.041 |
| LSD | rootstocks | 0.09 | 0.09 | 0.09 | 0.09 | 0.11 | 0.11 | 0.11 | 0.11 | 0.036 | 0.036 | 0.036 | 0.036 | 0.184 | 0.184 | 0.184 | 0.184 | 0.006 | 0.006 | 0.006 | 0.006 | 0.025 | 0.025 | 0.025 | 0.025 |
| LSD | interaction | 0.10 | 0.10 | 0.10 | 0.10 | 0.13 | 0.13 | 0.13 | 0.13 | 0.036 | 0.036 | 0.036 | 0.036 | 0.209 | 0.209 | 0.209 | 0.209 | 0.007 | 0.007 | 0.007 | 0.007 | 0.028 | 0.028 | 0.028 | 0.028 |

SO = **Sour orange** TC = **Tryoer** VL = **Volka** CM = **Cleopatra**

**Significant at 5% and 1% respectively.**

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