

Physiological studies on salinity tolerance of pomegranate seedlings

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The present study was carried out during two consecutive seasons of 2000 and 2001 in Institute Horticultural Research Nursery Giza, Egypt. Uniform and Healthy 3 month old transplants (rooted cuttings) of three pomegranate cultivars namely: Manfalouty; Nab El-Gamal and Wardy were the plant material used in this study, on February 1st during both seasons plastic pots of 35 cm. diameter that had been filled with about 10 kg clay and sand (1: 1) by volume. Soil type were taken from a depth of 0-30 cm; from ground surface was chemically and mechanically analyzed before period of equilibration. Then irrigation was done at the rate of one liter/ pot at two days intervals till 1st June, whereas irrigation with different investigated saline solutions were started during both seasons of study. Saline solutions were prepared for irrigation at the concentration of tap water (0), 2000, 4000 and 6000 ppm of NaCl, Na₂SO₄, CaCl₂, MgSO₄, KCl and K₂SO₄, as well as each concentration was prepared with two levels of sodium adsorption ratios (S.A.R.) i.e., 3 and 6 and each level of sodium adsorption ratio have two Cl: SO₄ levels i.e., (low and high). To prevent salts accumulation irrigation with tap water was done for all experimental pots every 21 days, then followed by rewatering with the next day with the corresponding saline solution. It was aimed to investigate salt tolerance of these pomegranate cultivars. Summary and Conclusion Thus factorial experiment was conducted using the complete randomized block design with three replications for arranging the differential investigated treatments (combinations between four investigated factors i.e. 4 concentrations x 3 cultivars x 2 SAR x 2 Cl: SO₄ ratios. Each replicate was represented by 4 plants (grown individually in 35.0 cm. diameter plastic pots). Effect of salt concentration; SAR and Cl: SO₄ ratio, on three pomegranate cultivars transplants: Thirty nine treatments represented the different possible combinations between four investigated factors namely; a) 3 pomegranate cultivars (Manfalouty; Nab El-Gamal and Wardy); b) saline concentration (2000; 4000 and 6000 ppm, beside tap water as control); c) SAR (3 & 6) and d) Cl: SO₄ ratios (low & high), were investigated. Specific and interaction effects of the investigated factors and their combinations included in the experiment was studied through the response of the following measurements: V.I. Growth measurements: Average length of (stem and root); number of (shoots & leaves per plant); (leaf & total assimilation area); fresh and dry weights of various transplants organs (stem, leaves, roots and total plant) and net increase in stem length, No. of shoots and No. of leaves were the investigated growth measurements. V.H. Some physiological properties: Leaf relative turgidity (L.R.T), leaf succulence grade (L.S.G.), hard leaf character. (H.L.C.), leaf osmotic pressure (O.P.) in bar, leaf transpiration rate (L.T.R.) and leaf water potential (L.W.P.) in response to investigated treatments were studied. VIII. Chemical analysis: In this regard, foliar photosynthetic pigments (chlorophyll a & b) and carotenin; stem total carbohydrates and soluble sugars; leaf total free amino acids and proline contents, some enzymes activity (Catalase & peroxidase) and nutritional status (Leaf, Cl, Na, N, P, K, Ca, Mg, Fe, Mn and Zn contents) in response to the included treatments of experiment were investigated. V.IV. Anatomical structure: Leaf and root structures of 3 pomegranate cultivars transplants (Manfalouty; Nab El-Gamal and Wardy) irrigated with saline water were investigated regarding the effect of salt concentration, SAR, chloride levels. Besides, (upper & lower) epidermis; (palisade & spongy) layers of blade. Besides, thickness of the main vein and thickness of vascular bundle were the leaf anatomical characteristics investigated through microscopic examination of

leaf cross section. However, periderm layer, phloem, cambium, xylem vessels and transverse section diameter of root were the investigated root anatomical characteristics. Obtained results during both 2000 & 2001 experimental seasons regarding the response of differential measurements to specific and interaction effects of various investigated factors and their combinations could be summarized as follows:

Effect of salt concentrations, SAR and chloride levels (Cl: SO₄ ratio) on three pomegranate cultivars transplants:

V.I. Vegetative growth measurements: In this regard 18 growth parameters namely: average length of (stem and root); number of (shoots & leaves per plant); (leaf & total assimilation area); fresh and dry weights of various transplants organs (stem, leaves, roots and total plant) and net increase in stem length, No. of shoots and No. of leaves in response to specific effect of four studied factors i.e., pomegranate cultivars; salt concentrations, sodium adsorption ratio and chloride levels (Cl: SO₄ ratio) of saline solutions used for irrigation as well as interaction effects of their possible combinations were investigated during both 2000 and 2001 experimental seasons.

A- Specific effect:

1. Regarding the specific effect of pomegranate cultivars, data obtained during both seasons revealed that, Manfalouty cultivar was the superior descendingly followed by Nab El-Gamal and Wardy. Such trend was true for all the 18 investigated growth measurements during two seasons.
2. As for the specific effect of salt concentrations, results also declared a significant decrease in lengths of (root and stem); average area of either individual leaf or total assimilation area /plant and fresh & dry weights of plant organs (leaves, stem, roots and total plant) all being progressively depressed by raising salinity concentrations, where the depressive effect was more pronounced with the highest salts concentration during two seasons of study.
3. Results also declared a significant decrease in all vegetative growth parameters previously mentioned with increasing sodium adsorption ratio (SAR) from 3 to 6 during the 2000 and 2001 experimental seasons.
4. With respect to the specific effect of chloride level (Cl: SO₄ ratio), data obtained displayed that increasing chloride levels (Cl: SO₄ ratio) in irrigation water resulted in an obvious reduction of all investigated growth measurements during the two seasons of study.

B. Interaction effect:

1. Data obtained revealed that specific effect of each investigated factor was directly reflected on its own combinations. Herein, the Manfalouty cultivar rooted cuttings irrigated with 2000 ppm X SAR 3 X lower chloride level (Cl: SO₄ ratio) exhibited statistically the least decrease in various vegetative growth parameters below the continuously tap water irrigated ones.
2. On the contrary, the least values of the investigated vegetative growth measurements were always in concomitant to those pomegranate transplants representing the combination between Wardy cultivar X highest salt concentration (6000 ppm) X SAR 6 X higher Cl: SO₄ ratio whereas, the severest decrease was detected during two seasons of study. However, other combinations were in between the aforesaid two extremes.

V.II. Some physiological properties of leaf: Leaf water potential (L.W.P); leaf osmotic pressure (L.O.P); leaf relative turgidity (L.R.T), transpiration rate (T.R.); leaf succulence grade (L.S.G.) and hard leaf character (H.L.C.) in response to specific and interaction effects of pomegranate cultivars; salt concentration; SAR and chloride levels (Cl: SO₄ ratio) in saline solution, as well as their combinations were the investigated leaf physiological properties in this respect.

A. Specific effect:

1. Regarding the specific effect of pomegranate cultivars, data obtained revealed that the highest values of leaf water potential, leaf osmotic pressure, leaf relative turgidity and leaf succulence grade were always in concomitant to Manfalouty cultivar transplants while the reverse was true with Wardy cultivar. Differences between three pomegranate cultivars were significant with the aforesaid four physiological leaf characteristics. On the other hand, Wardy cultivar exhibited the highest values of both transpiration rate and hard leaf character while the opposite was found with Manfalouty cultivar transplants. In addition, Nab El-Gamal cultivar was intermediate as compared to two other pomegranate cultivars under study regarding the response of all investigated leaf physiological properties.
2. Concerning the specific effect of salt concentration, obtained results revealed that the values of (L.W.P.); (L.R.T.); (T.R.) and (L.S.G.) were significantly decreased gradually by increasing salt concentration in irrigation water as three concentrations were compared either each other or to control. On the contrary, increasing salt concentration in irrigation water, increased significantly values of both leaf osmotic pressure and hard leaf character during two seasons of study.
3. Results also declared that increasing both SAR (from 3 to 6) and Cl: SO₄ ratio decreased significantly leaf water potential (L.W.P.) and leaf relative turgidity (L.R.T.);

transpiration rate (T.R.) while leaf succulence grade (L.S.G.) was slightly decreased by increasing both sodium adsorption ratio and chloride level in irrigation water. However, with both leaf osmotic pressure (L.O.P.) and hard leaf character (H.L.C.) the trend took the other way around during two seasons of study.

B. Interaction effect:

1. Data obtained regarding the interaction effect of (pomegranate cultivars X salt concentration X SAR X chloride levels) pointed out that the highest values of leaf water potential (L.W.P.); leaf relative turgidity (L.R.T.); transpiration rate (T.R.); and leaf succulence grade (L.S.G.) were statistically in concomitant to Manfalouty cultivar transplants irrigated with 2000 ppm saline solution of SAR 3 and lower Cl: SO₄ ratio. However the least value were coupled with irrigated transplants of Wardy cultivar with 6000 ppm saline solution of SAR 6 and higher Cl: SO₄ ratio during the study. Other combinations were in between regarding the response of the aforesaid 4 leaf physiological properties.
2. On the other hand, for both leaf osmotic pressure and handle of character the interaction effect between the 4 investigated factors i.e., pomegranate cultivars; salinity concentration; SAR and Cl: SO₄ ratio in the irrigation water caused noticeable variances. Herein, the highest decrease in both leaf osmotic pressure and hard leaf character was detected by pomegranate transplants irrigated with 2000 ppm, regardless of (cultivars; SAR and Cl: SO₄ ratio), however the highest values were coupled with those transplants irrigated with 2000 ppm solution of SAR 6 X higher Cl: SO₄ ratio. In addition, other combinations were in between regarding such leaf physiological properties.

VIII. Chemical composition:

- 1- Photosynthetic pigments (foliar pigments): Data obtained regarding pomegranate leaf photosynthetic pigments contents (Chlorophyll A; B and carotenoids compounds) as influenced by the specific and interaction effects of pomegranate cultivars; salt concentration; SAR and chloride levels (Cl: SO₄ ratio), as well as their possible combinations revealed the following:
 - A. Specific effect: 1. Manfalouty cultivar leaves were statistically the richest followed in a descending order by Nab El-Gamal cultivar while Wardy cultivar was the poorest.
 2. The obtained results revealed that leaf photosynthetic pigments i.e., Chlorophyll A & B and carotenoids compounds, all progressively decreased by rising salinity concentrations during the two seasons of study.
 3. Increasing level of either sodium adsorption ratio (SAR) or Cl: SO₄ ratio in irrigation water significantly decreased leaf chlorophyll A, B and carotenoids compounds during the study.
- B. Interaction effect: 1. The interaction between pomegranate cultivars; salinity levels; sodium adsorption ratio and chloride level (Cl: SO₄ ratio) was obviously detected. Herein, irrigated transplants of Wardy cultivar with 6000 ppm saline solution of SAR 6 and higher Cl: SO₄ had the severest decrease in their leaf chlorophyll A, B and carotenoids compounds, however the least decrease was in leaves of Manfalouty cultivar transplants irrigated with 2000 ppm saline solution. of SAR 6 and lower Cl: SO₄ ratio. In addition, other combinations were in between the aforesaid 2 extremes.

- 2- Shoots total soluble sugars and total carbohydrates contents:
 1. Data obtained during both seasons revealed that total soluble sugars and total carbohydrates of pomegranate cultivars stem respond specifically to the 4 investigated factors. Hence, Manfalouty cultivar was statistically the richest followed in descending order by Nab El-Gamal and Wardy during two seasons of study.
 2. However, both components followed two conflicted trends in response to either salt concentration; sodium adsorption ratio (SAR) or chloride levels (Cl: SO₄ ratio). Hence, rising any of salinity (concentration); SAR or Cl: SO₄ ratio resulted in increasing shoot total soluble sugars, while the reverse was true with total carbohydrates content.
- B. Interaction effect: Obtained results revealed that total soluble sugars and total carbohydrates each followed its own trend regarding the response to interaction effect of four investigated factors. Herein, the highest level of total soluble sugars associated with the lowest total carbohydrates from one hand was always in concomitant to the Manfalouty transplants irrigated with 6000 ppm salinized water of SAR 6 and higher Cl: SO₄ ratio. The reverse (least total soluble sugars and highest total carbohydrates levels) was markedly coupled with Wardy transplants irrigated with 2000 ppm saline solution of SAR 3 and lower Cl: SO₄ ratio during the two seasons of study.

- 3- Effect on leaf total free amino acids and proline contents:
 - A. Specific effect: 1. Data obtained displayed that total free amino acids and proline contents responded specifically to each of the four investigated factors (pomegranate cultivars; salt concentration; SAR and Cl: SO₄ ratio), where both parameters followed the same trend. Wardy cultivar had statistically the richest leaves followed in a descending order by Nab El-Gamal and Manfalouty cultivars, where the later was the inferior in this concern.
 2. The obtained results revealed

that, total free amino acids and proline contents being progressively increased with salinity concentrations. 3. Results also declared increase in both total free amino acids and proline contents with increasing either SAR or chloride levels (Cl: SO₄ ratio), however differences were more pronounced with SAR during two seasons of study. B. Interaction effect: The interaction effect of combinations between the four investigated factors i.e., pomegranate cultivars; salinity concentration; SAR and Cl: SO₄ ratio data obtained revealed that the highest value of total free amino acids and proline contents were detected by .Wardy cultivar X highest salinity concentration (6000 ppm) X SAR 6 X higher Cl: SO₄ ratio while the reverse was true with the tap water irrigated transplants followed in an increasing order by those of Manfalouty cultivar irrigated with saline solution of the lowest salinity concentration (2000 ppm); SAR 3 and lower Cl: SO₄ ratio. V.IV. Catalase and peroxides enzyme activities: Wardy cultivar showed the least level of activity for both enzymes. However, both Catalase and peroxides followed two conflicted trends regarding their response to either salinity concentration or chloride level (Cl: SO₄ ratio) in irrigation water. Herein, peroxidase activity was obviously increased in leaves of transplants with rising either concentration or sodium adsorption ratio (SAR) but negatively responded to chloride level. Meanwhile, the trend took the other way around for Catalase enzyme especially as the response the Cl: SO₄ was concerned. V.V. Leaf mineral composition: Data obtained during both seasons regarding the leaf Cl, Na, N, P, K, Ca, Mg, Fe, Mn and Zn contents of 3 pomegranate cultivars in response to specific and interaction effects of pomegranate cultivars; salt concentration; SAR and chloride level (Cl: SO₄ ratio) and their possible combinations revealed that the results could be summarized as follows: A- Specific effect: 1-Referring the specific effect of pomegranate cultivars, data obtained during both season revealed that Manfalouty pomegranate cultivar exceeded statistically the two other ones i.e., Nab El-Gamal and Wardy cultivars regarding their leaves N, P, K, Mn, Zn and Fe contents. However, Manfalouty cultivar leaves had the least Cl; Na and Ca levels. Meanwhile, Wardy cultivar leaves had the highest level of Cl, Na, and Ca that associated with the least values of N; P and Fe contents. Meanwhile, Nab El-Gamal cultivar in most cases was intermediate as compared to two other pomegranate cultivars. 2-As for the specific effect of salt concentrations, the leaves N, P, K, Mg, Zn, Fe and Mn contents decreased significantly and gradually with increasing salt concentration in irrigation water while the reverse was found with leaf Cl, Na and Ca contents during two seasons of study. 3-Increasing either sodium adsorption ratio (SAR) from 3 to 6 and /or chloride level (Cl: SO₄ ratio) in irrigation water significantly decreased leaf N, P, K, Mg, Mn, Fe and Zn contents. The reverse was true with leaf Cl; Na and Ca contents, where raising either SAR or Cl: SO₄ ratio increased them significantly during two seasons of study. B- Interaction effect: Regarding the interaction effect of various combinations between four investigated factors (pomegranate cultivars; salt concentration; sodium adsorption ratio and Cl: SO₄ ratio), data obtained during 2000 & 2001 experimental seasons revealed that specific effect of each investigated factor was directly reflected on its own combinations. Herein, the irrigated pomegranate transplants (especially Nab El-Gamal and Wardy) with the highest concentrated saline solution (6000 ppm) of higher SAR (6) and Cl: SO₄ ratio had the richest leaves in their Cl; Na; and Ca contents associated with the least levels of N; P; K; Mg; Mn; Fe and Zn. The trend took the other way around with control (continuously irrigated transplants with tap water) followed by those especially of Manfalouty cv. supplied with 2000 ppm saline solution of lower SAR (3) and lower Cl: SO₄ ratio where the least values of leaf Cl; Na; and Ca contents with the higher N; P; K; Mg; Mn; Fe and Zn contents were recorded. Anatomical Structure: Concerning the effect of irrigation with saline solution (6000 ppm) with SAR (3 & 6) and chloride (high & low) on the anatomical structure of three pomegranate cultivars during 2001 growing seasons, data obtained revealed the following: A- Leaf structure: 1-The upper and lower epiderm were increased in Manfalouty and Nab El-Gamal compared to Wardy. In addition, blade thickness was increased in Wardy as the sodium adsorption ratio increased. The opposite was true for Nab El-Gamal and Manfalouty pomegranate cultivars. 2-The Palisade layer was reduced when irrigated with salt solutions. Reversly, the spongy layer was increased in low concentration, but increased with the higher one. In Wardy cultivar both the palisade and spongy layer didn't take definite trend. 3-Midrib thickness decreased with salt solutions as SAR and chloride increased. 4-The vascular bundles were increased in Manfalouty and Nab El-Gamal, whereas Wardy wasn't affected

especially at higher SAR and chloride. B- Root structure: 1- The periderm layer was decreased in root of 3 pomegranate cultivars. 2- The thickness of phloem and cambium was decreased, especially Manfalouty and Nab El-Gamal cvs. at the higher SAR and chloride, while Wardy didn't respond. Moreover, cambium thickness did not influence in 3 pomegranate cvs. 3- The thickness of xylem layer and root were decreased in 3 cultivars. Eventually, pomegranate seedlings showed different anatomical structure when irrigated with salt solutions regardless to SAR or chloride. In the same time, Manfalouty cv. exhibited the anatomical structure which enable it to avoid salinity disorders. Followed by Nab El-Gamal and Wardy. from the obtained results of the present dissertation it could be recommended for nursery men that under shortage of available fresh water that saline solution of relatively lower level of salt concentration (4000 ppm); SAR 3 and Cl: SO₄ could be safely used for irrigation purposes.