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The relationship between some physiological traits and salt tolerance in Pistachio genotypes

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Abstract

To determine the relative salt tolerance and/or resistance in seedlings of pistachio genotypes, four pot experiments were conducted. In experiments 1 and 2, the effects of salinity on growth indices were studid. Experiments were done in the form of split plot based on RCBD. In each experiment, mainplots consisted of different electrical conductivities of irrigation water (EC_{iw}s). In experiment 1, the seedlings of seven cultivars (cvs.) of Pistacia vera L. (Momtaz, Akbari, Kale-ghoochi, Owhadi, Ahmad-aghai, Sarakhs, Badami-riz) and also *P. atlantica* sub. Mutica (Baneh) were allocated in subplots. In the second experiment, three cvs. of P. vera L. (Badami-riz,Momtaz and Owhadi) with different salt tolerances were used. In order to determine the resistance of pistachio cvs. in respect to seedlings survival, experiment 3 was done. In experiment 3, salinity treatments continued upto 70% of leaf damaging in seedlings. In experiment 4, the effects of salinity on pistachio seedlings in regards to chlorophyll-meter figures and proline contents studied. Nevertheless, the ratio of damaged leaves, K/Na ratio and total dry weight was determined. In experiment 4, the relationship between some physiological traits and salt tolerance in pistachio genotypes determined. We observed that not only salt tolerance varies cv. to cv., but also it changes in different sources of a given cv. In order to determine the salt tolerance of pistachio genotypes, the sensible traits (relative percent of total dry weight and seedlings survival) were more reliable than traits such as K/Na ratio, chlorophyll meter figure, and/or a single physiological character as same as leaf proline content.

Keywords: Chlorophyll meter figure; Growth indices; Pistachio; Proline content; Salt tolerance and resistance; Seedlings survival

1. Introduction

The cultivalted areas of pistachio in Iran are often in arid regions and of the most important problems for economic crop production in these areas is high concentration of ions specially NaCl either in soil or in irrigation water. According to the problems, the survey and recongnition of tolerant seedling rootstocks of pistachio to salinity and induced changes particularly NaCl in them have a specific importance (Alkhani and Ghorbani, 1992). Although several researchers (Parsa and Karimian, 1975; Sepaskhah and Maftoun, 1981)

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have reported the relative salt tolerance of pistachio seedlings; there are no enough informations regarding the effects of salinity on seedlings survival, K/Na ratio, proline contents and chlorophyll-meter figures in seedlings. The effects of different salts (NaCl, CaCl2 and MgCl₂) and EC_{iw}s on seedlings of different cvs. were not studied, considerably. There are some contradictions in reports, including some researchers (Moeinrad, 2005 a,b; Parsa and Karimian, 1975; Sepaskhah and Maftoun, 1985) have reported the higher relative salt tolerance of seedlings of cv. Badami (-riz) whereas others (Sepaskhah and Maftoun, 1981); reffered to the tolerance of cv. Fandoghi (Owhadi). Some researchers (Barone and Di.1996; Chao et al.,2004; Kafkas et al.,2006; Mirzae et al., 2005; Vargass et al., 1995)have recently studied partly

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the genetical traits of pistachio plants. Nevertheless, information on relative salt tolerance and/or resistance on seedlings of different genotypes in respect to the characters cited above; is still scarce. The present study was therefore undertaken to determine the relative salt tolerance and/or resistance in pistachio seedlings for a variety of cvs. and in response to different salinities. Nevertheless, the trait of salt tolerance is studied in different sources of a given cv. Finally, in order to judge salt tolerance of pistachios; we have been intended to determine the most reliable character.

2. Materials and methods

In order to determine the relative salt tolerance and/or resistance in seedlings of pistachio genotypes four pot experiments were conducted. The type of experimental designs which used were split plot based on RCBD. In experiments, based on the traits which studied, the number of replications were 4 upto 14. In experiment 1, main plots were ECiws included 0.5, 2.5, 4.5, 8.5 and 16.5 dSm¹. With the respect of results which obtained from experiment 1, EC_{iw}s of 0.5, 4.5 and 8.5 dSm⁻¹ with significant differences selected for experiment 2. The source of salinity for experiments 1 and 2 were NaCl, CaCl2 and MgCl₂. In experiments 1 and 2, three split plots were used; such as in splits I, II and III the ratios of Na:Ca:Mg were 5:1:1, 1:5:1 and 1:1:5; respectively. In experiment 1, the seedlings of seven cvs. of Pistacia vera L. including Momtaz, Akbari, Kale-ghoochi, Owhadi. Ahmad-aghai, Sarakhs, Badami-riz and also Baneh (P.atlantica sub. mutica) allocated in subplots. In experiment 2 the source of seeds changed, such as the seedlings of cvs. of Badami-riz, Owhadi and Momtaz with different salinity tolerances (which had been screened from experiment 1) were allocated as main plots. The germinated seeds were transferred into plastic container at size of 15 centemeter (in diameter). Two and half kilogram (kg) of soil was put in each pot. In experiments, the physical and chemical characteristics of soil for control plants determined. Sodium, potassium, calcium and magnesium were 10.4-15.2, 8.9-9.5, 3-6 and 6-2.2 milliequivallent/liter (mel⁻¹), respectively. pH ranged 6.8 upto 7.5. The electerical conductivity of soil saturation extract (ECe) was 4.5 upto 5.9 dSm⁻¹. Sodium absorption ratio (SAR) ranged 6.5 upto 10.1. CaCO₃ and gypsum 12.5-14 and 0.1-0.6 percent, respectively. Sand, silt and clay were 60-65, 21-30 and 10-14 percent, respectively. Soil texture in experiments was sandy loam. The medium culture of each pot was irrigated with saline water to near field capacity, 250 cubic centimeter in two day intervals; upto 20 and 15 times in experiments 1 and 2, respectively. In experiment 1, we observed leaf marginal necrosis in some plants; partly. Consequently, In experiment 2; the times of irrigation with saline water was reduced.

In order to determine the tolerance of cvs. to NaCl salinity (5:1:1 ratio), the mean of TDW of each cv.; in each level of ECiws compare to control (0.5 dSm⁻¹). In comparison with control, where in each level of ECiws the growth of a given cv. was not decreased; meaningfully, considered as high tolerant cv. Where growth decreased in EC_{iw} of 16.5 dSm⁻¹, considered as tolerant cv. Decreasing of growth in $EC_{iw}s$ of 8.5 and 16.5 dSm^{-1} was considered as moderately sensitive cv. Where growth decreased in EC_{iw}s of 4.5, 8.5 and 16.5 dSm⁻¹ considered as sensitive cv. Finally, decreasing of growth in each level of ECiws considered as high sensitive cv. In the cases of the two other ratios, in each experiment; similar comparisons were done.

To find out the effects of salinity on pistachio cvs. in regard to seedlings survival, experiment 3 was conducted. The materials and methods were the same to the first experiment but salinity treatments continued upto 70% of leaves damaging in seedlings. The number of days from the first necrosis which have been observed in each pot (seedlings) upto 70% of leaves damaging were used as data for statistical analysis. In lower salinity treatments(ECiws of 0.5 and 2.5 dSm⁻¹), we did not observe leaf necrosis; markedly. In order to analysis the data, we assumed that the seedlings of all cvs. in each level of salinity (even control) reach to 70% of leaves damaging upto 100 days. To classify the seedlings of pistachio cvs. in different salinity resistance classes used table 1, arbitrarily. For example, where a given cv.; just in the highest level of salinity (EC_{iw} of 16.5 dSm⁻¹) reached to 70% of leaves damaging upto 100 days, considered as resistant cv. In each ratio of salts, similar comparisons were done.

In experiment 4, the ratio of damaged leaves, K/Na ratio, TDW, chlorophyll meter figure and proline content were determind. The materials and methods were similar to experiment 2. In order to determine leaf chlorophyll meter used Minolta chlorophyll meter (SPAD-502), which has been introduced by Peterson (1993). The fourth leaf from the surface of medium culture selected for chlorophyll meter readings.

After selecting the leaf to be sampled, reading on about the same location on each leaf was recorded. We collected the readings from a point one half the distance from the leaf tip to the leaf petiole, and halfway between the leaf margin or edge and the leaf midrib. Leaf proline contents determined by the method which were used by Bates et al. (1973). To meassure these characters, pots were irrigated with saline water upto 15 times.

Table 1. Classification of pistachio cultivars in different salinity resistance classes

| | EC _{iw} s (dSm ⁻¹) ¹ | Highly resistant | Resistant | Moderately sensitive | Sensitive | Highly sensitive |
|---|--|------------------|------------------|----------------------|-----------|------------------|
| , | 0.5 | ns ² | ns | ns | ns | ns |
| | 2.5 | ns | ns | ns | ns | dec |
| | 4.5 | ns | ns | ns | dec | dec |
| | 8.5 | ns | ns | dec | dec | dec |
| | 16.5 | ns | dec ³ | dec | dec | dec |

In order to judge the tolerance of pistachio cvs. to salinity, the relative percent of TDW; proline content and chlorophyll meter figure in seedlings determined (used formula: Relative percent of a given triat = mean of treatment/ mean of control × 100). Analysis of variance were performed on the data which obtained in each experiment. Treatment means were compared by using Duncan's new multiple range test (DNMRT). For some characters,

combined analysis of experiments was determined.

3. Results and discussion

3.1. Results of experiment 1

The results of experiment 1 are presented in Tables 3, 4 and 5. TDW of seedlings in control plants presented in table 2.

Table 2. Effects of different pistachio cultivars on total dry weight of seedlings (experiment 1) in control plants. Mean comparisons by DNMPT at 1% level

| Cultivars | Total Dry | Weight (g.) |
|--------------|-----------|-------------|
| Akbari | 3.33 | a |
| Badami-riz | 2.84 | a |
| Kale-ghoochi | 2.64 | a |
| Owhadi | 2.58 | a |
| Ahmad-aghai | 2.18 | b |
| Momtaz | 2.06 | b |
| Sarakhs | 1.24 | c |
| Baneh | 0.99 | c |

Table 3. Effects of different levels of electrical conductivities of irrigation water (EC_{iw}s) on total dry weight of pistachio seedlings (experiment 1), mean comparisons by DNMRT at 1% level

| EC _{iw} s (dSm ⁻¹) | | | Total Dry W | /eight (g.) | | |
|---|------------------|---|------------------|-------------|------------------|-----|
| EC _{iw} S (uSIII) | Na:Ca:Mg (5:1:1) | | Na:Ca:Mg (1:5:1) | | Na:Ca:Mg (1:1:5) | |
| 0.5 | 1.95 | a | 2.24 | a | 2.02 | a |
| 2.5 | 2.16 | a | 1.84 | abc | 1.78 | ab |
| 4.6 | 2.11 | a | 1.95 | ab | 1.66 | abc |
| 8.6 | 1.26 | b | 1.48 | bc | 1.45 | bc |
| 16.5 | 1.18 | b | 1.35 | c | 1.25 | c |

Table 4. Effects of different pistachio cultivars on total dry weight of seedlings (experiment 1), mean comparisons by DNMRT at 1% level.

| 11100 | in companionis | ej Branita | C 1 / O 10 / O | 11 | | | | | |
|-------|------------------|------------|----------------|-------------------|---------------|---|------------------|------|---|
| | | | Tota | l Dry Weight of C | ultivars (g.) | | | | |
| | Na:Ca:Mg (5:1:1) | | | Na:Ca:Mg (1:5:1) | | | Na:Ca:Mg (1:1:5) | | |
| | Owhadi | 2.37 | a | Badami-riz | 2.27 | a | Akbari | 1.97 | a |
| | Badami-riz | 2.32 | a | Kale -ghoochi | 2.24 | a | Ahmad-aghai | 1.94 | a |
| | Akbari | 2.01 | a | Owhadi | 2.01 | a | Momtaz | 1.93 | a |
| | Ahmad-aghai | 1.89 | a | Ahmad –Aghai | 2.08 | a | Kale-ghoochi | 1.90 | a |
| | Kale-ghoochi | 1.79 | a | Akbari | 1.97 | a | Badami-riz | 1.89 | a |
| | Momtaz | 1.78 | a | Momtaz | 1.90 | a | Owhadi | 1.67 | a |
| | Sarakhs | 1.04 | b | Sarakhs | 1.01 | b | Sarakhs | 0.93 | b |
| | Baneh | 0.68 | b | Baneh | 0.61 | b | Baneh | 0.60 | b |

Table 5. Effects of different salt ratios (Na:Ca:Mg) on relative salt tolerance of pistachio cultivars with

the respect of seedlings total dry weight (experiment 1)

| Ratio of salt | Cultivars | | | | | | | | | |
|------------------|-------------------|----------|----------|----------|----------|---------------------|----------|-----------------------|--|--|
| Katio of Sait | $(Mo)^1$ | $(AK)^2$ | $(Ka)^3$ | $(Ba)^4$ | $(Ow)^5$ | $(Ah)^6$ | $(Sa)^7$ | (Ba-riz) ⁸ | | |
| Na:Ca:Mg (5:1:1) | M.S. ⁹ | S. 10 | T.11 | T. | M.S. | H.T. ¹² | H.T. | H.T. | | |
| Na:Ca:Mg (1:5:1) | T. | H.T. | H.T. | T. | T. | H.S ¹³ . | M.S | H.T. | | |
| Na:Ca:Mg (1:1:5) | H.T. | T. | H.T. | T. | T. | H.T. | T. | H.T. | | |

1- Mo= Momtaz

6- Ah= Ahmad-aghai

11- T=Tolerant

2- Ak= Akbari

7- Sa= Sarakhs 8- Ba-riz= Badami-riz 12- H.T=High Tolerant 13- H.S= High Sensitive

3- Ka= Kale-ghoochi 4- Ba= Baneh

9- MS=Moderately Sensitive

10- S=Sensitive

5- Ow=Owhadi

In lower salinity levels (5:1:1 ratio), a nonsignificant increase in TDW was observed (Table 3). The positive effects of low Na ion concentration in permeability of root cell walls in absorbance of nutritional elements and also the positive effects of Ca and Mg ions in growth may be responsible. This finding supported by Pessarakli (2003) who showed that the low levels of salts stimulate growth and increase the yield of cotton. In each ratio of salts, in respect to TDW; we observed important differences among cvs. and as well as ECiws (Tables 3, 4 and 5).

3.2. Results of experiment 2

In tables 6 and 7 the effects of EC_{iw}s and also different salt ratios (Na:Ca:Mg) on relative salt tolerance of pistachio cvs. in respect to TDW of seedlings presented (experiment 2).

Table 6. Effects of different levels of electrical conductivities of irrigation water (ECiws) on total dry weight of pistachio seedlings (experiment 2), mean comparisons by DNMRT at 1% level

| I | | | | | | | | | | | |
|---|--------------|------------|--------|-------------|-------|-----------------------|--|--|--|--|--|
| | ECiws | | | | 7 | Γotal Dry Weight (g.) | | | | | |
| | (dSm^{-1}) | Na:Ca:Mg (| 5:1:1) | Na:Ca:Mg (1 | :5:1) | Na:Ca:Mg (1:1:5) | Combined analysis experiments of salts | | | | |
| | 0.5 | 1.44 | a | 1.48 | a | 1.54 a | 1.50 a | | | | |
| | 4.5 | 1.06 | b | 1.20 | b | 1.16 b | 1.14 b | | | | |
| | 8.5 | 1.06 | b | 1.16 | b | 0.96 c | 0.06 b | | | | |

Table 7. Effects of different salt ratios (Na:Ca:Mg) on relative salt tolerance of pistachio cultivars with the respect of seedlings total dry weight (experiment 2)

| Ratio of salts | Cultivars | | | | | |
|------------------|-----------------------|-----------|----------|--|--|--|
| Ratio of saits | (Ba-riz) ¹ | $(Mo)^2$ | $(Ow)^3$ | | | |
| Na:Ca:Mg (5:1:1) | S. ⁴ | $H.S^5$. | T^6 . | | | |
| Na:Ca:Mg (1:5:1) | T. | H.S. | T. | | | |
| Na:Ca:Mg (1:1:5) | S. | H.S. | S. | | | |

¹⁻ Ba-riz= Badami-riz 2. Mo= Momtaz

In experiment 1, cv. Owhadi whereas; in experiment 2, cv. Badami-riz were less tolerant to salinity (especially NaCl). As expected, we observed that not only salt tolerance varies from cv. to cv., but also it changes in different sources of a given cv. In compatible, Walker et al. (1987) observed a considerable plant to plant variation; with some plants suffering growth inhibition and some plants having growth rates similar to control plants, suggesting that it would be possible to select from within a population of seedlings for tolerance of high salinities. According to Pessarakli (2003) salinity tolerance traits are controlled by a number of genes located throught the chromosome complement.

3.3. Results of experiment 3

Results of experiment 3 presented in Tables 8 and 9. In regard to seedlings survival of pistachios under salt stress conditions (experiment 3), results showed significant differences among cvs., ECiws and also in different ratios of salts. The effects of salt ratios (Na:Ca:Mg) on relative salt resistance of pistachio cvs. with the respect of seedlings survival presented in Table 8.

³⁻ Ow=Owhadi 4- S= Sensitive

⁵⁻ H.S=High Sensitive 6- T= Tolerant

Table 8. Effects of salts ratios (Na:Ca:Mg) on relative resistance of pistachio cvs. with the respect of seedlings survival (experiment 3)

| sui vivai (experiment 3) | | | | | | | | | |
|--------------------------|-----------------------|-----------|----------|------------------------------|-------------------|--------------------|----------|-----------------------|--|
| Ratio of salt | Cultivars | | | | | | | | |
| Ratio of Sait | $(Mo)^1$ | $(AK)^2$ | $(Ka)^3$ | (Ba) ⁴ | (Ow) ⁵ | (Ah) ⁶ | $(Sa)^7$ | (Ba-riz) ⁸ | |
| Na:Ca:Mg (5:1:1) | M.S. ⁹ | S. 10 | R.11 | M.S. | R. | H.R. ¹² | R. | R. | |
| Na:Ca:Mg (1:5:1) | H.R. | R. | R. | R. | H.R. | R. | H.R. | H.R. | |
| Na:Ca:Mg (1:1:5) | R. | M.S. | R. | R. | R. | M.S. | R. | R. | |
| 1- Mo= Momtaz | 5- Ow=O | whadi | | 9- M.S= Moderately Sensitive | | | | | |
| 2- Ak= Akbari | 6- Ah=Al | hmad-agha | i | 10- S= Sensitive | | | | | |
| 3- Ka= Kale-ghoochi | 7- Sa=Sarakhs | | | 11- R=Resistant | | | | | |
| 4- Ba= Baneh | 8- Ba-riz= Badami-riz | | | 12- H.R=High Resistant | | | | | |

Badami-riz and Owhadi were screened as resistant cvs. to NaCl, CaCl₂ and MgCl₂ (all ratios). Momtaz was also selected as moderately sensitive cv. to salinity of NaCl (5:1:1 ratio). With considering that in high salinity areas, NaCl is mostly dominant salt; therefore screening of resistant genotype to NaCl is of prime importance. The seedlings survival of

pistachios decreased, significantly; in $EC_{iw}s$ of $4.5dSm^{-1}$ and more where the ratio of Na:Ca:Mg was 5:1:1. Detrimental effect of salinity observed with $EC_{iw}s$ of $8.5~dSm^{-1}$ and more in 1:5:1 ratio, whereas; in 1:1:5 ratio, in each salinity level; seedlings survival decreased, significantly (Table 9).

Table 9. Effects of different levels of electrical conductivities of irrigation water ($EC_{iw}s$) on the number of days From irst necrosis (pistachio seedlings survival), mean comparisons by DNMRT at 1% level (experiment 3)

| FG (1g -l) | Number of days from the first necrosis (days) | | | | | | | | | |
|---------------------------|---|--------------|-------|------------------|-------|---|--|--|--|--|
| $EC_{iw}s$ (dSm^{-1}) | Na:Ca:Mg (5: | Na:Ca:Mg (1: | 5:1) | Na:Ca:Mg (1:1:5) | | | | | | |
| 0.5 | 80.94 | a | 81.16 | a | 88.50 | a | | | | |
| 2.5 | 70.03 | ab | 64.34 | ab | 60.41 | b | | | | |
| 4.6 | 59.03 | b | 59.63 | ab | 52.66 | b | | | | |
| 8.6 | 35.22 | c | 43.34 | bc | 46.22 | b | | | | |
| 16.5 | 27.69 | c | 30.16 | c | 27.22 | c | | | | |

None of cvs. were not sensitive to 1:5:1 ratio (table 8). Toxic symptoms in all ratios of salts were similar. We observed leaf marginal necrosis, firstly in adult leaves which would be extended to the middle of them.

The reduction of growth in a given cv. may be higher, but probably could survive salinity in a longer period. This characteristic may consider as a type of resistance, whereas; the 50% reduction in dry weight can be considerd as the threshold value of tolerance. According to Sepaskhah and Maftoun (1988), the 50% reduction in the top and root dry weights were obtained; respectively, at an EC of the saturation extract (ECe) of 9.3 and 10.0 dSm⁻¹ for Badami-(riz), 7.9 and 7.9dSm⁻¹ for Fandoghi (Owhadi). They concluded that the growth of tops and roots was stoped, respectively; at ECe values of 18.7 and 20.6 dSm⁻¹ for Badami (-riz), 15.9 and 15.5 dSm⁻¹ for Owhadi. Similarly, in experiment which conducted by Walker et al. (1987) salinity level of 100 mol m⁻³ Cl⁻, known to cause a significant (50%) reduction in dry weight; whereas, salinity level of 175 mol m⁻³ Cl shown as a threshold value over which growth of Pistacia vera plants ceases. In this experiment, we concluded in each ratio of salts; the 50% reduction in TDW of pistachio seedlings occurred at EC_{iw} of 4.5 dSm $^{\text{-}1}$. In such level of salinity of irrigation water; ECes were 17, 15.72 and 14.56 dSm⁻¹ in 5:1:1, 1:5:1 and 1:1:5 ratios; respectively. EC_{iw} of 8.5 dSm⁻¹ was considered as the threshold value over which the growth of pistachio seedlings ceases. In such level of salinity of irrigation water; ECes were 24.5, 23.6 and 23.7 dSm⁻¹ in 5:1:1, 1:5:1 and 1:1:5 ratios; respectively. These levels of EC_es were partly greater than values which reported by Sepaskhah and Maftoun (1985). The difference is probably concerned to the method of salt increasing. We imposed salinity by adding salts to medium culture as aqueous solutions at two day intervals gradually, whereas; they added salinity to soil at the beginning of experiment. Indeed, we considered acclimatation. According to Walker et al. (1988), the rate of acclimatation to salinity and duration of salt treatment are clearly the key factors influencing the plants response.

Results of experiment 4

Results of experiment 4 presented in Tables 10, 11 and 12. In experiment 4, with increasing of EC_{iw} in conditions of 5:1:1 and 1:1:5 ratios and in combined analysis experiments of salts (ratios), chlorophyll-metter figures reduced, significantly (Table 10).

Table 10. Effects of different levels of electrical conductivities of irrigation water (EC_{iw}s) on chlorophyll- meter figures, mean comparisons by DNMRT at 1% level (experiment 4)

| EC _{iw} s (dSm ⁻¹) | | | Chloro | phyll m | eter figure | |
|---|------------------|---|------------------|---------|--|---|
| EC _{iw} s (dSIII) | Na:Ca:Mg (5:1:1) | | Na:Ca:Mg (1:1:5) | | Combined analysis experiments of salts | |
| 0.5 | 44.20 | a | 45.62 | a | 43.59 | a |
| 4.5 | 37.56 | b | 43.00 | b | 40.50 | b |
| 8.5 | 35.27 | b | 42.49 | b | 39.70 | b |

In contrast, in studies of Walker et al. (1988); chlorophyll content did not decrease by NaCl salinity (4:1:1 ratio). They used the method of Krik (1968) and chlorophyll content of pistachio leaves was determined by the technique of Bruinsma (1961). In fact, they determined the chlorophyll content of leaf tissue; quantitatively, whereas; we used chlorophyll-meter. In compatible to Walker et al. (1988), Behboudian et al. (1986) reported the same result. This finding was not supported by Pessarakli (2003) who reported that leaf chlorophyll content decreases in proportion to increased salt concentration.

In 5:1:1 ratio; the relative percent of TDW in cvs. Owhadi, Badami-riz and Momtaz were 70.68, 53.14 and 41.78; respectively (table 12). Consequently; salt tolerance in cv. Owhadi was higher than the two other cvs. whereas the relative percent of chlorophyll meter figure in cvs. were 81.33, 90.26 and 86.70, respectively (table 12). There was no clear correlation between the traits of TDW and chlorophyll meter figures.

In the highest salinity level (EC $_{\rm iw}$ s=8.5 dSm $^{-1}$) of NaCl (5:1:1 ratio) leaf proline content increased, significantly (Table 11).

Table 11. Effects of different levels of electrical conductivities of irrigation water ($EC_{iw}s$) on proline content, mean comparisons by DNMRT at 1% level (experiment 4)

| | | | Leaf pro | line conte | ent (micro-mol L | 1) | | |
|---------------------------|------------------|---|------------------|------------|------------------|----|--|---|
| $EC_{iw}s$ (dSm^{-1}) | Na:Ca:Mg (5:1:1) | | Na:Ca:Mg (1:5:1) | | Na:Ca:Mg (1:1:5) | | Combined analysis experiments of salts | |
| 8.5 | 8.25 | a | 19.80 | a | 36.30 | a | 17.21 | a |
| 4.5 | 6.01 | b | 17.09 | a | 22.38 | a | 16.06 | a |
| 0.5 | 5.04 | b | 7.45 | b | 9.00 | b | 7.16 | b |

Some researchers (Bates et al., 1973; Orcutt and Nilsen, 2000; Pessarakli, 2003; Pitman, 1984; Walker et al., 1988) have been cited the mechanism of compatible solutes accumulation (e.g. proline) in response to Na absorption, whereas information on mechanism of Ca and Mg salts is partly scarce. According to Orcutt and Nilsen (2000) and also based on the results of experiment 4, it seems that the increasing of Ca and Mg ions in medium culture is effective on proline leaf content. According to Orcutt and Nilsen (2000), accumulation of organic solutes in hypersaline conditions occurs in response to the osmotic effects of the soil solution and is not a response to specific ions (e.g. Na). Based on their report, the ionic composition of the soil environment does not influence the types or amount of compatible solutes accumulated. Nevertheless, in regard to different salts(ratios); probably the trait of proline accumulation is qualitatively and quantitatively different which needs to be surveyed.

In 5:1:1 ratio, the relative percent of proline content in cvs. Owhadi, Badami-riz and Momtaz were 146.46, 130.91and 150.79 (Table 12). Therefore, we observed no clear correlation between the triats of TDW and proline content. As Orcutt and Nilsen (2000) have reported, in

many cases; the concentration of proline alone cannot account for the required osmotic adjustement needed to maintain turgor under hypersaline conditions. Proline may have significance to metabolism other than just osmotic regulation. Plants have been found to accumulate a number of different solutes in the cytoplasm when they experience conditions. Accumulation of cytoplasmically compatible solutes can not be the only method of osmotic adjustment for plants in long-term hypersaline conditions because much of the total plant photosynthate would be required for osmotic adjustment. The use of nonprotein aminoacids such as proline for adjusting osmotic potential is relatively expensive compared to the other osmotic moieties, because an induced biosynthetic pathway is required to produce the osmotica. Nevertheless, the results which have been obtained by some researchers (Orcutt and Nilsen, 2000; Walker et al., 1988) suggest that the accumulation of proline in tissues confers some salinity tolerance.

In Table 12 the effects of 5:1:1 ratio on relative salt tolerance of pistachio cvs. in respect to different traits presented (experiment 4).

| Table 12. Relative | percent of traits | in seedlings in | 5:1:1 ratio | (experiment 4) |
|--------------------|-------------------|-----------------|-------------|----------------|
| | | | | |

| Traits | Cultivars | | |
|---|------------|----------------|----------------------|
| | Badami-riz | Momtaz | Owhadi |
| Ratio of Damaged Leaves | 310.71 | 195.24 | 160.87 |
| K/Na Ratio | 0.76 | 0.72 | 0.93 |
| Relative Percent of Chlorophyll meter figure | 90.26 | 86.70 | 81.33 |
| Relative Percent of Proline Content | 130.91 | 150.79 | 146.46 |
| Relative Percent of Total Dry Weight | 53.14 | 41.78 | 70.68 |
| Salt Tolerance in Regard to Percent of Total Dry Weight | Sensitive | High Sensitive | Relatively Resistant |

While cv. Owhadi was more tolerant to salinity (table 12) could maintain partly a wider K/Na ratio than cvs. Badami-riz and Momtaz (0.93 in comparison to 0.76 and 0.72). Nevertheless, in respect to the trait of K/Na ratio, the differences between cvs. Badami-riz and Momtaz was not considerable (0.76 in comparison to 0.72). Whereas the former was more tolerant to salinity. In compatible to the first comparison but in contrast to the second comparison, Mohammadkhani and Lessani (1993) observed while cv. Badami was more tolerant to salinity; could maintain a wider K/Na ratio than sensitive cv. of Sarakhs. Simillarly, Sapaskhah and Maftoun (1985) observed the higher K/Na ratio in more tolerant cv. of Badami (-riz) in comparison with cv. Owhadi. A wide K/Na ratio is recommended by Rao et al. (1981) as a sensible criterion of salt tolerance in higher plant species. As a matter of fact, the level of salinity tolerance of a given crop species or genotype is the collective expression of a number of processes. It is not surprising that no single physiological mechanism and/or trait could show a clear-cut direct relationship to salnity tolerance (Orcutt and Nilsen, 2000). Genotypes may differ in one or many processes which regulate entry of Na, K or other ions into the plant or qualitative or quantitative differences in the organic solutes. These processes interact at the organism level to determine the ultimate level of tolerance (Pessarakli, 2003). It is not feasible to select a single parameter which could isolate tolerant varieties from others. Salinity tolerance is the collective expression of a number of physiological traits. Some differences can be observed among genotypes within a crop species, which is reflected in contradictory reports for various crop species either confirming or disputing a direct correlation between K/Na selectivity and level of salinity tolerance (Pitman, 1984).

4. Conclusions

Regard to TDW and seedlings survival of pistachios under salt stress condition, results

showed significant differences in cvs., ECiws and also in different ratios of salts. The 50% reduction in TDW of pistachio seedlings occurred at ECiw of 4.5 dSm-1 whereas, ECiw of 8.5 dSm-1 was considered as the threshold value over which the growth of pistachio seedlings ceases.

In researches which have been done previously, researchers have not been considered genetical diversities of pistachio seedlings (genotypes); considerably. If they had been changed the source of seeds (of a given cv.), probably their results would be changed; differently.

The seedling rootstocks of cvs. Sarakhs and Bench, probably may be considered as low vigor; In

but their resistance to salinity in compare to cvs. Badami-riz and Kale-ghoochi was lower. It would be possible to select from within some populations of seedlings (mostly different sources of cv. Badami-riz) for salt resistance of high salinities, when they experience saline conditions.

To investigate the effects of salinity on plant; the sensible traits (relative percent of TDW and seedlings survival) were more reliable than characteristics such as K/Na ratio; chlorophyll meter figure and/or a single physiological character as same as leaf proline content.

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