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Physiological Evaluation of Pistachio Frost Damage Resistant Rootstocks

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Abstract: On 16th of March 2008, temperature decreased to -6ºC in some parts of the Kerman pistachio plantation area that was caused the heavy damage to pistachio growers. After the damage, some rootstocks were resistance to this temperature and no damage was interned to them. In the present study five rootstocks of resistant and five rootstocks of susceptible to frost damage were selected and evaluated from physiological point of view in order to found physiological parameter that affected on resistance. In May 2010 some samples were got from leaves of both resistant and susceptible rootstocks and were measured in laboratory condition proline, soluble sugars, proteins, nutrient elements including potassium, magnesium, calcium, phosphorus, iron, zinc, copper, manganese. The results showed that no significant differences were observed in content of soluble sugars, proline among resistant and susceptible rootstocks. However, rootstock No. 4 of resistant rootstocks had greatest Magnesium uptake in compare to susceptible rootstocks and rootstock No. 3 of resistant rootstocks had greatest protein and potassium content in compare to susceptible rootstocks. Rootstocks in amount of other elements did not show much difference. As a conclusion the possibility frost damage resistance back to the soluble protein, potassium and magnesium and had a role in resistance to frost damage in pistachio.

Keywords: Resistance; Proline; Frost Damage; Nutrient Elements; Soluble Sugars; Protein

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INTRODUCTION

Cold stress is a major environmental factor that limits the agricultural productivity of plants. Low temperature has a huge impact on the survival and geographical distribution of plants. Cold stress often affects plant growth and crop productivity, which causes significant crop losses [35]. Over the past years, pistachio has been not immune from frost damage in Iran and a large percentage of the product was destroyed in some years. Observational experiences and reports from various areas have emphasized that much of the damage caused by the spring frost [7]. Increase in leaf protein is one of the traits of adaptation to cold stress. Protein in plant tissue has a role in reducing cold and frost damage [15]. Levitt stated that the amount of soluble carbohydrates is associated with increased resistance to cold stress [16]. Proline accumulates in many plant species under a broad range of stress condition [13]. Activity and stability of Ca^{+2}-ATPase under 2℃ low temperature are the key factors in the development of cold resistance of winter wheat. It was reported that when exposed to cold conditions, the Ca^{+2} concentration in cold-insensitive plants has been transiently increased, suggesting that Ca^{+2} acts as a second messenger during cold acclimation [31]. Mg^{2+} and K^{+} are also involved in plant stress response [6]. Levitt stated that phosphorus increased cold acclimation [16]. Reactive oxygen species (ROS) formation in plants

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grown at low temperatures can be additionally exacerbated under deficiencies of K and Zn [1]. Mn, Cu, Fe are involved in the MnSOD, CuSOD and FeSOD enzymes. These enzymes are a group of the metalloenzymes that protect cells from superoxide radicals by catalyzing the dismutation of the superoxide radicals by catalyzing the dismutation of the superoxide radicals to molecular O_2 and H_2O_2 [8]. Climate change induced in recent years show that the pistachio products are susceptible to the cold spring and high probability of frost will not be unexpected in the next few year. So doing the research is essential to reduce crop loss caused by spring frost. Due to the frost damage has been entered during the several years; we should explore and expand resistant rootstocks. Mavali [21] studied on electrolyte leakage of pistachio that they showed resistance to the spring frost in 16th of March 2008. Results showed that four rootstocks of five selected rootstocks as resistant rootstocks that exhibited lowest electrolyte leakage. The objective of this study was determining the cause of resistance of these rootstocks to the spring frost damage.

MATERIALS AND METHODS

On 16th of March 2008, temperature decreased to -6ºC in some parts of the Kerman pistachio plantation area that was caused the heavy damage to pistachio growers. After the damage, some rootstocks were resistance to this temperature and no damage was interned to them. In the present study, five rootstocks of resistant and five rootstocks of susceptible to frost damage in Sirjan pistachio plantation area were selected in May 2010. Five species that are resistant and five susceptible rootstocks to a control sample were prepared. Of each tree, four branches one- year-old were selected in around of trees. Sampling was done randomly. All of samples were transferred to laboratory in Iran's Pistachio Research Institute (IPRI) to be examined. For measuring proline, sugars and proteins were used of leaves. For measuring nutrient element was used from dried leaves of the second and third in each shoot.

Proline and soluble sugars Analysis

To determine the proline content, 0.5 g of dry leaves was homogenized with 5 ml of 95% ethanol. Above phase of filtrate was separated and its sediments were washed by 5 ml of 70% ethanol for two times and its above phase added to the previous over compartment. The mixture was centrifuged at 3500 g for 10 min at 4ºC and the supernatant was recovered and alcoholic extract was kept in refrigerator at 4ºC [18]. One ml of alcoholic extract was diluted with 10 ml of distilled water and 5 ml of ninhydrin (0.125 g ninhydrin, 2 ml of 6 mM NH3PO4, 3 ml of glacial acetic acid) and 5 ml of glacial acetic acid added then mixture placed in boiling water bath for 45 min at 100ºC. The reaction was stopped by placing the test tubes in cold water. The samples were rigorously mixed with 10 ml benzene. The light absorption of benzene phase was estimated at 515 nm. The proline concentration was determined using a standard curve. Free proline content was expressed as μmol g-1 DW of leaves [14]. To measuring the content of soluble sugars, 0.5 g of dry leaves was homogenized with 5 ml of 95% ethanol. One-tenth ml of alcoholic extract preserved in refrigerator mixed with 3 ml anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid,W/W). The samples were placed in boiling water bath for 10 minutes. The light absorption of the samples was estimated at 625 nm. Contents of soluble sugar were determined using glucose standard and expressed as mg. g-1 DW of leaves.

Protein extraction

The method described by Guy [9] was modified to obtain better results. This method consisted of homogenizing the tissue in a chilled mortar and pestle, using a buffer containing ice-cold 50 mM Tris-HCl, pH 7.5; 2 mM EDTA and a 0.04% (v/v) 2-mercaptoethanol. The homogenate was centrifuged at 4000 rpm for 30 min at room temperature [20]. The supernatant was re-centrifuged for 20 min and stored at –20°C for later analysis [11].

Quantification of proteins using the Bradford assay

Protein extracts were thawed and their concentration was determined by a colorimetric method based on the method described by Bradford [3], using a commercially available reagent (Bio-Rad protein assay dye reagent). The protein concentration of the extracts was determined from the standard curve, using a Spectrophotometer at 595 nm.

Nutrient Analysis

The samples were placed in the oven for 24 hours and then were ground. For each sample 1 g, was weighed and they were placed in the furnace for 12 hours at 540°C. 10 cc (HCL) hydrochloric acid was added to each sample after take from the furnace. After 30 minutes, they were purred in 100 cc balloon and water was added to them. Nutrient concentrations were measured by atomic absorption spectroscopy (Ca, Mg, Mn, Zn, Cu) and flame photometry (K and Na) in Soil testing Laboratory at the Pistachio Research Institute of Iran.

Data analyses

Descriptive statistics (mean, standard error, and coefficient of variation) were determined with MSTATC (Michigan State University 1995). The effects of rootstock, harvest time and salinity on measured parameters were assessed using analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

In this research, there were no significant differences between amount of proline in two susceptible and resistant rootstocks (Table 1). Proline is considered to be a compatible solute. It protects folded protein structures against denaturation, stabilises cell membranes by interacting with phospholipids, functions as a hydroxyl radical scavenger, or serves as an energy and nitrogen source [27]. In some plant species, proline plays a major role in osmotic adjustment such as in potato [4], while in others such as in tomato proline accounts for only a small fraction of the total concentration of osmotically active solutes [22]. Therefore, its contribution to osmotic adjustment and tolerance of plants exposed to unfavorable environmental conditions is still controversial [12].

In this research, there were no significant differences between amount of slobale sugar in two susceptible and resistant rootstocks (Table 1). Carbohydrates were associated with cold resistance. It seems that this feature was connected to protect components of cell and ability to delay formation of ice crystals. Accumulation of soluble carbohydrates reduced the freezing point of cell sap. So freezing temperatures was reduced [26]. Mansouri [19] showed that in pistachio, greatest content of soluble sugars in pistachio branches in autumn. So it seems that role of sugars in cold tolerance was less in spring.

Synthesis of specific proteins is an important mechanism involved in increasing freezing tolerance during cold acclimation [29]. The low temperature induced accumulation of proteins [29]. In this research, greatest amounts of protein were observed in No. 2, 4, 5 resistant rootstocks (Fig. 1). Mansouri [19] showed that in pistachio, greatest amount of protein accumulate in leaves in spring [19]. It seems that increase of protein was related to cold resistance in spring.

Calcium is as regulator of growth and metabolism and it is as messenger in plants [28]. It is commonly held that Ca^{2+} channels play tremendous roles in the growth of root hairs and the low temperature acclimation of chilling resistant plants. It is suggested that the cold-resistant agent CR-4 plays a momentous role in stabilizing plasma membrane Ca^{2+}-ATPase under low temperature stress [32]. Our results showed greatest amounts of calcium were observed in No. 2 susceptible rootstock and No. 4 resistant rootstock (Fig. 2). Mansouri [19] showed that in pistachio, amount of calcium increase in the period after the leaves fall to bud swell and begin to decrease. Minimum amount of Ca^{2+} there was in at the time of opening of flowers [19]. So it seems that role of calcium in cold tolerance is less in spring.

In this study maximum levels of phosphorus were observed in No. 3 resistant rootstock and No. 4 susceptible rootstock (Fig. 3). It seems that the phosphorus is less involved in resistance to cold stress of pistachio rootstocks. Applications of phosphorus is generally associated with increased cold hardiness [23], but Tyler in 1980 showed that maximum level of cold hardiness was obtained with wheat plants grown in solution low in K and low in P [33].

Applying high rates of K^+ can effectively increase the frost resistance of some of the frost-susceptible genotypes [24]. Our results revealed No. 2, 3, 4 resistant rootstocks had greatest amount of potassium (Fig. 4). It seems that potassium is involved in pistachio rootstocks resistance to cold stress. Maximum growth response and chilling resistance in tomato, eggplant and pepper plants with the addition of K^+ were associated with increase in phospholipids, membrane permeability and improvement in biophysical and biochemical properties of cell [10]. The effects of the high K^+ content of the cell in increasing frost tolerance have also been related to regulation of osmotic and water potential of the cell sap and reduction of electrolyte leakage caused by chilling temperature [2, 30].

Our results also showed that No. 4 of susceptible rootstock and No. 5 of resistant rootstock had greatest amount of iron (Fig. 5). It seems that the iron is less involved in pistachio rootstocks resistance to cold stress.

Formation of ROS by NADPH oxidase and weakening of the antioxidative defensive systems are also important in chilling-induced cell damage [34]. Insufficient supplies K and Zn lead to significantly increased NADPH oxidase activity [5]. In this study maximum levels of Zinc there were in No. 2 of resistant rootstock and No. 3 of susceptible rootstock (Fig. 6). It seems that the Zinc is less involved in resistance to cold stress in pistachio rootstocks. Zn is a necessary element for pistachio and amount of Zn increase in time of flowers open. However, Zn storage form is not useful for plant in resistance to cold stress [19].

Table 1: Mean squares of analysis of variance

Source of Variation	**df**	**(M.S) : Mean squares**										
		Sugar (mg/g)	Protein (mg/g)	Proline (mlmol/g)	K (%)	P (%)	Mg (%)	Ca (%)	Cu (ppm)	Fe (ppm)	Mg (ppm)	Zn (ppm)
Replication	2	ns 55.906	ns 0.006	ns 0.001	ns 0.0001	ns 0.0001	ns 0.0001	ns 0.008	ns 0.364	ns 3.63	0.433 ns	0.233 ns
Rootstocks	9	ns 124.722	**7.355	ns 0.001	**0.096	**0.001	**0.077	**0.660	**12.659	**257.144	**27.570	**67.170
Error	18	167.466	0.0747	0.001	0.005	0.000	0.003	0.006	0.174	2.411	0.470	0.270
CV%	—	17.43	14.33	15.25	11.75	3.17	19.10	3.43	4.26	2.48	2.84	2.29

ns , * and ** = Non significant, significant at 0.05 and 0.01, respectively

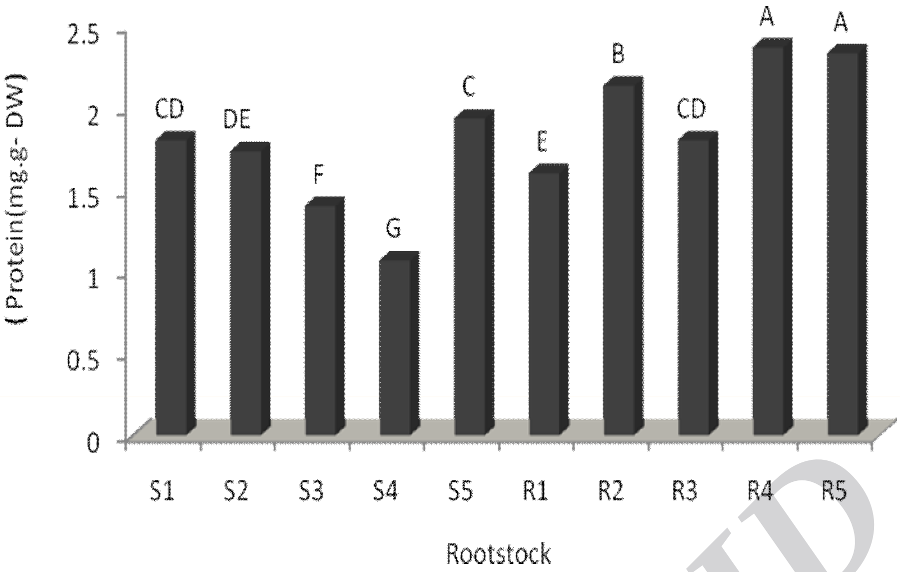

Fig 1. Changes in amount of protein between R (resistant) and S (susceptible) rootstocks

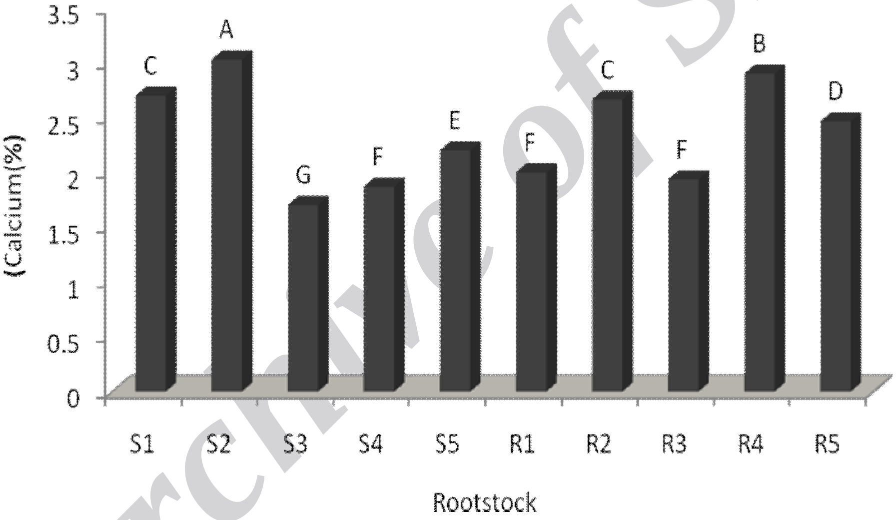

Fig 2. Changes in amount of calcium between R (resistant) and S (susceptible) rootstocks

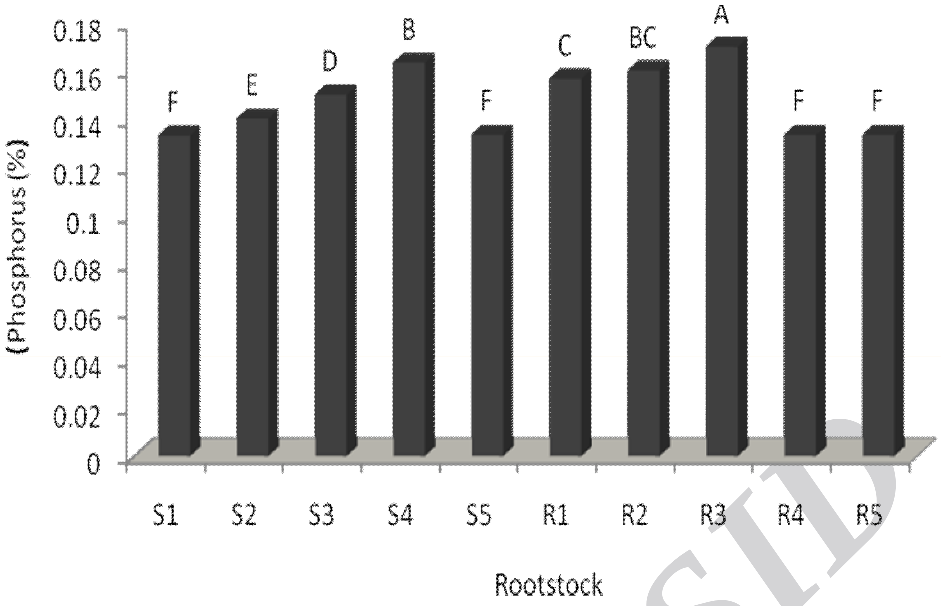

Fig 3. Changes in amount of phosphorus between R (resistant) and S (susceptible) rootstocks

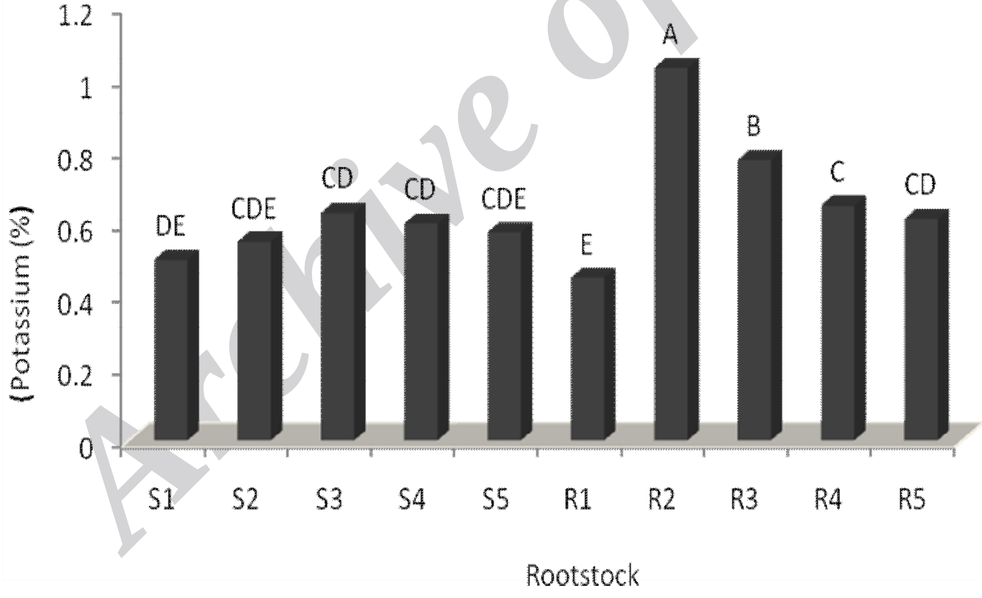

Fig 4. Changes in amount of potassium between R (resistant) and S (susceptible) rootstocks

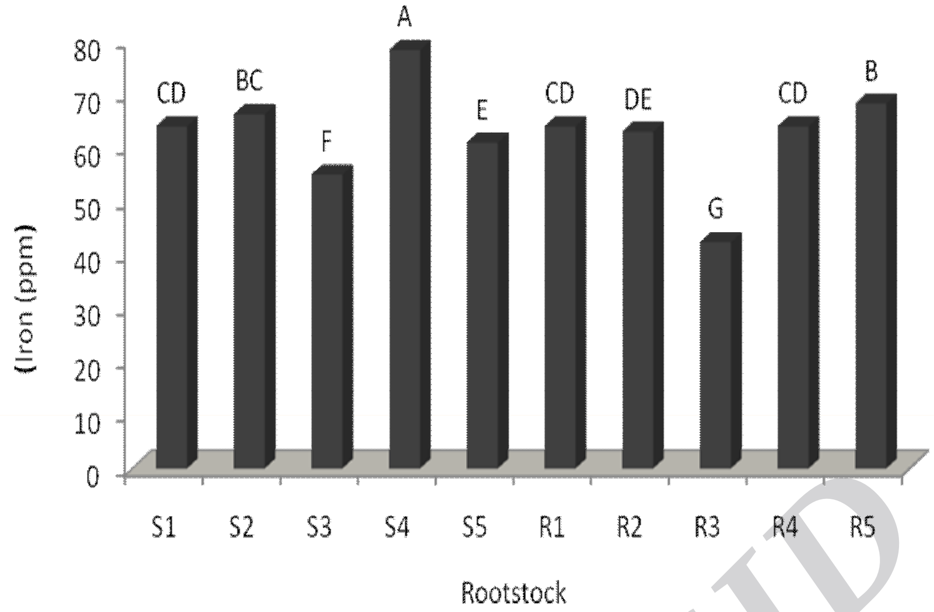

Fig 5. Changes in amount of iron between R (resistant) and S (susceptible) rootstocks

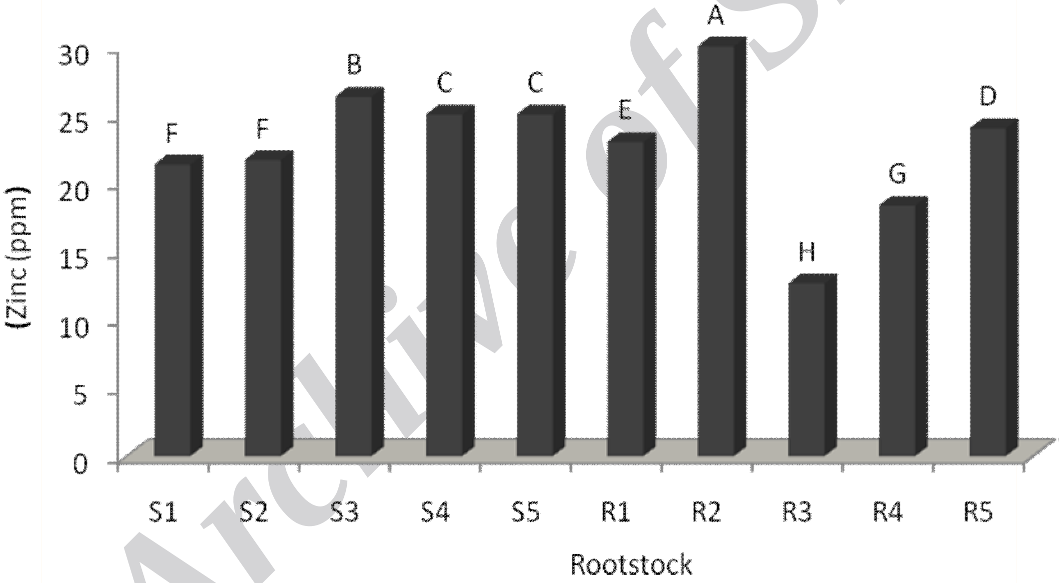

Fig 6. Changes in amount of zinc between R (resistant) and S (susceptible) rootstocks

Magnesium, like many metals, assists in enzyme activity; two conflicting papers looked specifically at interactions between cold hardiness and magnesium in coniferous species: in one, cold damage is associated with high soil magnesium and the other reports the opposite relationship. It is not clear why magnesium has been associated with cold hardiness; it certainly is not based on available science [17]. In this research, greatest amounts of magnesium were observed in No. 2, 3, 4, 5 of resistant rootstocks (Fig. 7). It seems that magnesium is involved in pistachio rootstocks resistance to cold stress.

Mn there is in MnSOD enzyme which protect mitochondrial of ROS in during stress like chilling and freezing stress [8]. In this study maximum levels of Mn were in No. 2 of resistant rootstock and No. 4 of susceptible rootstock (Fig. 8). It seems that the Mn is less involved in resistance to cold stress in pistachio rootstocks.

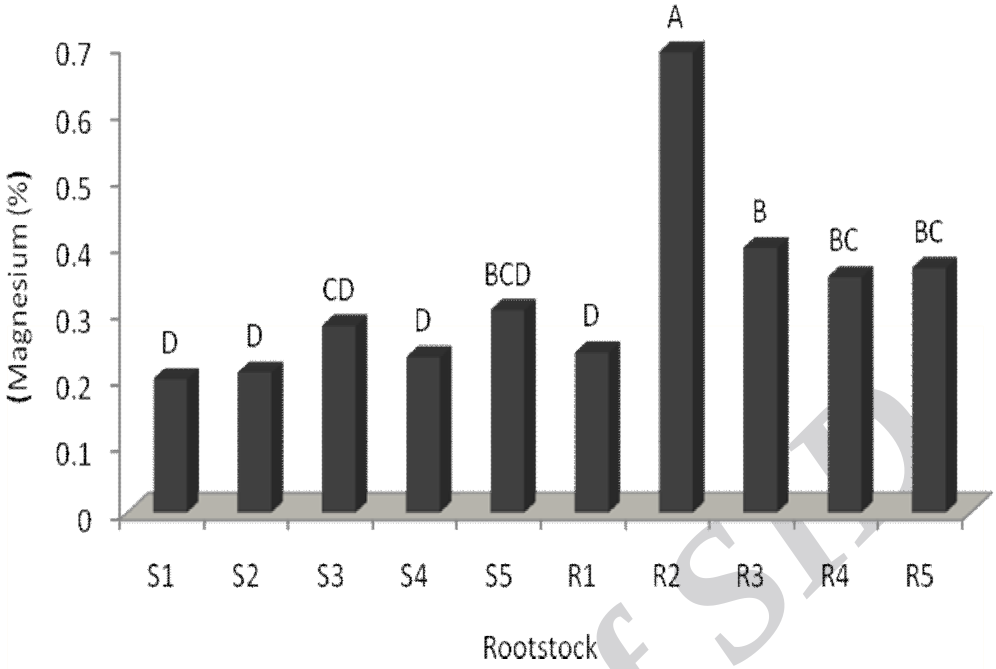

Fig 7. Changes in amount of magnesium between R (resistant) and S (susceptible) rootstocks

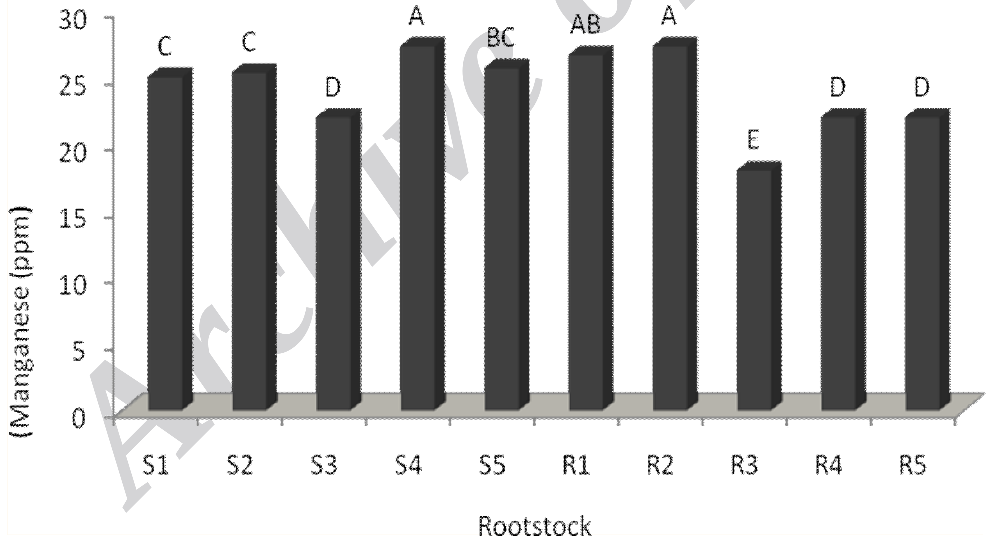

Fig 8. Changes in amount of manganese between R (resistant) and S (susceptible) rootstocks

Cu there is in Cu/ZnSOD enzyme, which protect chloroplastic of ROS in during stress like chilling and freezing stress [8]. In this research, greatest amounts of Cu were in No. 3 of susceptible rootstock (Fig. 9). It seems that the Cu is less involved in resistance to cold tress of pistachio rootstocks.

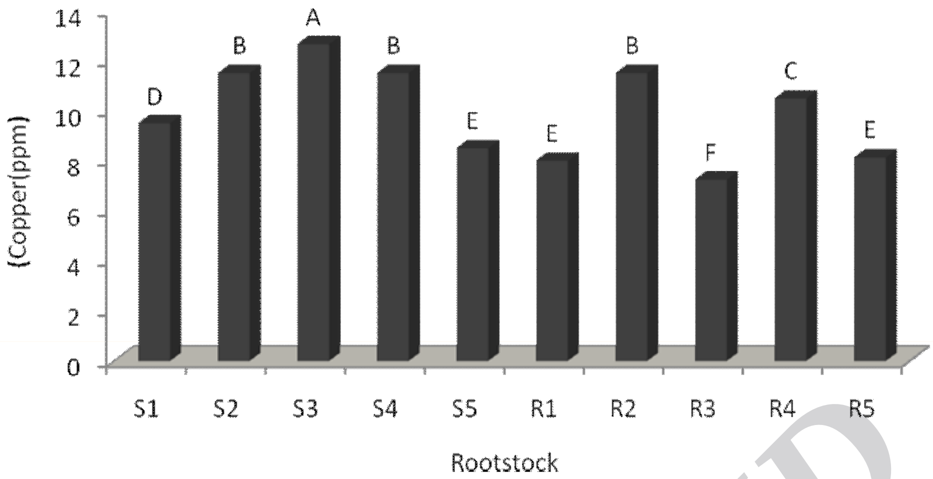

Fig 9. Changes in amount of copper between R (resistant) and S (susceptible) rootstocks

As conclusion we found that No. 2 of resistant rootstocks had more tolerance to spring frost damage and the factors affecting in resistance of pistachio rootstocks to frost damage were proteins content, potassium and magnesium.

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