



Evaluation of low temperature tolerance indices in seedlings of *Citrus aurantium* under potassium nitrate nutrition

Zeinab Rafie Rad^{1*}, Esmail Dordipour¹, and Yahya Tajvar²

1. Department of Soil Science, Gorgan University of Agricultural Sciences and Natural Resources, Iran.

2. Horticultural Science Research Institute, Citrus and Subtropical Fruits Research Center, Agricultural Research Education and Extension Organization (AREEO), Ramsar, Iran.

Abstract

In order to evaluate the effects of potassium nitrate nutrition on some low temperature tolerance indices in *Citrus aurantium* seedlings a factorial experiment was conducted based on a completely randomized design with four replications at the Institute of Citrus Research in Ramsar. Treatments were potassium nitrate nutrition at four levels (0, 2.5, 5, and 10 mM) and temperature at four levels (25, 0, -3, and -6 °C). Results showed that increasing the concentration of KNO₃ reduced superoxide dismutase activity and carotenoid. Concentration of 10 mM potassium nitrate also increased water content, leaf color, proline, phenol, and total chlorophyll. Maximum leaf damage, electrolyte leakage, and carotenoid were observed at -6 °C, while the highest antioxidant capacity and superoxide dismutase activity with means 37.7% and 24.4 IU. mg⁻¹ FW, respectively were observed in *Citrus aurantium* leaves at -3 °C. Highest electrolyte leakage was observed at concentration 0 mM potassium nitrate and temperature of -6 °C while highest superoxide dismutase was observed at -3 °C with the same concentration of potassium nitrate. Results revealed that concentration of 10 mM potassium nitrate increases *Citrus aurantium* tolerance under low temperature stress.

Key words: *Citrus aurantium* potassium nitrate; low temperature stress; proline

Rafie Rad, Z., E. Dordipour and Y. Tajvar. 2017. 'Evaluation of low temperature tolerance indices in seedling of *Citrus aurantium* under potassium nitrate nutrition'. *Iranian Journal of Plant Physiology* 7 (2), 2007-2015.

Introduction

Citrus is a genus of flowering plants in the family Rutaceae and a tropical and subtropical plant which is sensitive to cold stress (Clark and Prakash, 2001). *Citrus aurantium* is one of commercial citrus varieties that is considered as a biotic and abiotic stress tolerant rootstock (Kaanane et al., 1988). Low temperature is the major environmental stress that limits plant growth, productivity, and distribution (Campos et

al., 2003). Citrus crops which are among the tropical and subtropical fruits are generally classified as cold tender plants so these crops are vulnerable to freezing stress (Fotouhi et al., 2008). The first place that reacts to low temperature stress, is cell membrane (Chen et al., 2006). Overall, chilling results in loss in membrane integrity and increase in active oxygen radical productions, which leads to leaf damage and electrolyte leakage. The integrity of

*Corresponding author
E-mail address: z_raffii@yahoo.com
Received: May, 2015
Accepted: January, 2016

intracellular organelles is also disrupted leading to the loss of compartmentalization, reduction and impairing of photosynthesis, chlorophyll pigments, protein assembly, and general metabolic processes (Mahajan and Tuteja, 2005; Saneoka et al., 2004). Low temperature stress may lead to the formation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl, and single oxygen (Ruth, 2002). Hatamnia et al. (2013) reported that reactive oxygen species (ROS) production and antioxidant enzyme activity increased in abiotic stress condition. ROS, which is produced in chloroplast during metabolic processes, can destroy cellular components such as proteins, pigments, membrane, lipids, and nucleic acids (Hernandez et al., 2001). The participation of low temperature and increasing antioxidant capacity of citrus fruit have been demonstrated (Rapisarda et al., 2008). Generally, low temperature stress is considered as a damage factor particularly in Iranian north and south citrus trees. To avoid this phenomenon, in addition to mechanical methods can be used effective security counsels to increase plant resistance against cold and frost damage. One of the strategies to increase tolerance of plants under low temperature and one of the important activities of plant compatibility to cold stress, is osmotic balance reaction to maintain plant water content. These activities are affected by osmotic pressure regulatory concentration compounds such as secondary metabolites including proline and some inorganic ions such as potassium.

Potassium increases cells' tolerance against cold stress because it affects freezing point of liquid inside the vacuoles (Obreza, 2003). Potassium is one of macronutrient elements in plants which composes about 3-5% of their dry weight. Potassium can affect plant survival under different environmental stress by creating osmotic balance and protection against oxidative damage (Liu and Zhu, 1997). Potassium influences many physiological processes such as starch and sugar formation, protein synthesis, cell division, and plants growth (Obreza, 2003). Gong et al. (2011) reported that proline aggregated in corn starch under potassium deficiency and low temperature stress. Research results on ginseng plants under low temperature stress and

potassium nitrate showed that potassium can increase effective genes expression on reaction of secondary metabolites and improve antioxidant reactions in decreasing reactive oxygen species (ROS) and so plant amplification survival under low temperature stress (Devi et al., 2012). In fact, the optimal concentration of potassium nitrate associated with water content maintain can affected on survival of plants under low temperature (Lindhauer et al., 1986). It is reported that potassium fertilizer increased inflationary turgor pressure and reduced the osmotic potential in cotton (Pervez et al., 2004).

The current experiment was an attempt to determine the effect of potassium nitrate on plant responses of *Citrus aurantium* in low temperature stress.

Materials and Methods

To study low temperature tolerance indices in seedlings of *Citrus aurantium* under potassium nitrate nutrition, an experiment was conducted in a factorial plan based on completely randomized design. Treatments included temperature at four levels, namely, 25, 0, -3 and -6 °C and four levels of potassium nitrate, namely, 0, 2.5, 5 and 10 mM. Seedlings were grown in soilless culture and foliar feeding with Hoagland solution (Hogland and Arnon, 1950). At the beginning of incubation, the temperature was lowered by 1 °C/day and cold acclimation was set up from 23 to 10 °C after 2 weeks. The plants were kept in each temperature for 24h (Pietrini et al., 2005). Then, leaf damage, electrolyte leakage (by fresh leaf), leaf color, leaf water content (five weeks after transfer from the incubator), total chlorophyll, carotenoid, antioxidant capacity, proline, phenol, and superoxide dismutase were assessed. Electrolyte leakage was assessed as described by Lutts et al. (1996).

Leaf discs were placed in closed vials containing 10 ml of deionized water and incubated at 25 °C on a rotary shaker for 24 h; subsequently, electrical conductivity of the solution (*Lt*) was determined. Samples were then autoclaved at 120 °C for 20 min and the last electrical conductivity (*LO*) was obtained after

equilibration at 25 °C. The electrolyte leakage was defined as follows:

$$\text{Electrolyte leakage (\%)} = \left(\frac{L_t}{L_0} \right) \times 100$$

Leaf color was determined by a colorimeter device (Minolta CR 400, Japan). Then numbers were calculated based on green leaves percentage (Sanchez et al., 2003). Also, leaf water content was calculated based on the following formula proposed by Versleous et al. (2006):

$$\text{Leaf water content} = \left[\left(\frac{\text{fresh weight of leaf} - \text{dry weight of leaf}}{\text{fresh weight of leaf}} \right) \right] \times 100$$

To measure total chlorophyll and carotenoid content, one g leaf sample was ground in 90% acetone. The absorbance was measured with a UV/visible spectrophotometer (Pye Unicam SP6-550, UK) and total chlorophyll and carotenoid concentrations were calculated using the equations proposed by Strain and Svec (1966):

Total Chl = Chla + Chl b, and

Total carotenoid = (1000 A 470 -1.8 Chla -85.02 Chl b)/198

Where A470 represents absorbance values read at 470 nm wavelengths.

DPPH free-radical scavenging of the methanolic extracts was measured according to the method by Kim et al. (2003). Briefly, methanolic extract (0.1 ml) was incubated in 0.4 ml of 0.1 M HCl (pH 7.5 ± 0.1) and 0.5 ml of 0.3 mM DPPH in the dark at room temperature for 20 min. The absorbance of the solution (as sample) was measured at 517 nm and compared to the absorbance of a control (as control). The free-radical scavenging was calculated according to the following equation:

$$\% \text{ DPPH free-radical scavenging} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Proline was determined according to the method described by Bates et al. (1973). Approximately, 0.5 g of fresh leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. Two milliliter of the filtrate was mixed with 2 ml of acid- ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluene and the absorbance was measured at 520 nm with a Shimadzu UV 1601 spectrophotometer.

The total phenolic concentration of each sample was determined by the modified Folin-Ciocalteu method (Singleton and Rossi, 1965). Methanolic extract (50 µl) was incubated in 450 µl of deionized water, 250 µl of the Folin-Ciocalteu phenol reagent, and 1.25 ml of 0.2 M Na₂CO₃ in the dark at room temperature for 20 min. The absorbance of the solution was measured at 735 nm and compared to a gallic acid standard curve. Superoxide dismutase (SOD) activity was measured by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. The reaction mixture (1 ml) consist of 75 µM NBT, 13 mM L-methionine, 0.1 mM EDTA, and 2 µM riboflavin in 50 mM potassium phosphate buffer (pH 7). The reaction and control mixture were incubated for 15 min in 300 µmol.m⁻¹. s⁻¹ irradiance at 25 °C. A non-irradiated reaction mixture was used as blank. One unit of SOD activity was expressed as the quantity of SOD required to produce a 50% inhibition of NBT photochemical reduction. The specific enzyme activity was measured as units per mg of leaf fresh wet (Sheng et al., 2006).

Statistical analysis was carried out using SAS 9.1 by analysis of variance and mean comparison was performed using Duncan's Multiple Range Test (p≤0.05).

Results

Table 1
Analysis of variance of potassium nitrate nutrition on low temperature tolerance indices in seedling of *Citrus aurantium*

SOV	df	Mean of squares									
		Leaf Damage	Electrolyte Leakage	Water Content	Leaf Color	Total Chlorophyll	Carotenoid	Antioxidant Capacity	Proline	Total Free Phenolic	Superoxide Dismutase Activity
Temperature (T)	3	9423**	6063**	4656**	14078.6**	19.9**	2.184**	2432**	0.1**	6.93**	792.1**
KNO ₃ (K)	3	289 ^{ns}	54.27 ^{ns}	45.3*	283.5*	5.9**	2.749**	157.5**	0.04 ^{ns}	2.69*	454.4**
T×K	9	122.6 ^{ns}	70.2*	28.1 ^{ns}	77.3 ^{ns}	0.68 ^{ns}	0.44**	182.2**	0.06**	3.16**	125.3**
Error	48	182.9	28.17	16	83.2	0.77	0.102	14.14	0.01	0.71	3.16
CV (%)	-	21.72	22.45	8.13	11.9	12.14	11.03	18.99	11.01	21.83	11.18

*, **: significant at p≤0.05 and p≤0.01, respectively; ns: not significant

Table 2

Means values of low temperature stress for some plant indices of *Citrus aurantium* seedling.

Temperature (°C)	Leaf damage (%)	electrolyte leakage (%)	water content (%)	Leaf color (%)	Total chlorophyll (mg g ⁻¹ FW)	Carotenoid (mg g ⁻¹ FW)	total free phenolic (mg g ⁻¹ FW)	Proline (mg g ⁻¹ FW)	Antioxidant capacity (%)
25	43 ^c	5.6 ^d	65.4 ^a	100 ^a	8.7 ^a	2.4 ^d	3.21 ^c	1.11 ^c	11.9 ^c
0	48.7 ^c	9.7 ^c	55.5 ^b	92.4 ^b	7.5 ^b	2.8 ^c	4.29 ^c	1.16 ^{bc}	11.6 ^c
-3	60.4 ^b	32.4 ^b	50.6 ^c	80.5 ^c	7 ^b	3.0 ^b	5.01 ^b	1.22 ^{ab}	37.7 ^a
-6	97 ^a	46.9 ^a	25.3 ^d	33.9 ^d	6 ^c	3.3 ^a	7.2 ^a	1.29 ^a	18 ^b

Means in each column followed by similar letters are not significantly different at the 5% probability Level using Duncan Test.

Table 3

Mean values of potassium nitrate nutrition for some indices of *Citrus aurantium* seedling

KNO3(mM)	Water content (%)	Leaf color (%)	Antioxidant capacity (%)	total free phenolic (mg g ⁻¹ FW)	Superoxide dismutase activity (IU mg ⁻¹ FW)
0	47.44 ^b	71.32 ^b	16.61 ^b	2.51 ^c	10.1 ^d
2.5	48.66 ^{ab}	77.20 ^{ab}	17.7 ^b	3.47 ^{bc}	13.73 ^c
5	49.25 ^{ab}	77.61 ^{ab}	23.15 ^a	4.75 ^{ab}	22.63 ^a
10	51.46 ^a	80.54 ^a	21.73 ^a	5.09 ^a	17.13 ^b

Means in each column followed by similar letters are not significantly different at the 5% probability level using Duncan Test.

Analysis of variance revealed the significant effect of low temperature on all indices except for proline ($p \leq 1\%$). Effect of potassium nitrate on characteristics of leaf water content and color was significant at 5 % probability level and on total chlorophyll, carotenoid, antioxidant capacity, proline, phenol, and superoxide dismutase this effect was significant at 1% probability level. The interaction between the two treatments on antioxidant capacity, proline, total phenol, and superoxide dismutase was also significant at $p \leq 0.01$ (Table 1). Mean comparison of the main effect of temperature on studied indices showed that leaf damage percentage, electrolyte leakage, total phenol, and carotenoid content increased significantly with reduced temperature.

Antioxidant capacity and superoxide dismutase activity increased up to -3° C and the maximum amount of these indices with mean 37.7 % and 24.4 (IU/mgr FW) were observed in this temperature, respectively. Maximum leaf water content, leaf color, and total chlorophyll were assessed in control plants (Table 2 and Fig. I).

Mean comparison of the simple effect on studied factors showed that concentration of 10 mM potassium nitrate resulted in the highest

values of leaf water content, leaf color, proline, total chlorophyll, and phenol. Also in 5 mM potassium nitrate, antioxidant capacity was about 28.2 % more than control treatment. The lowest

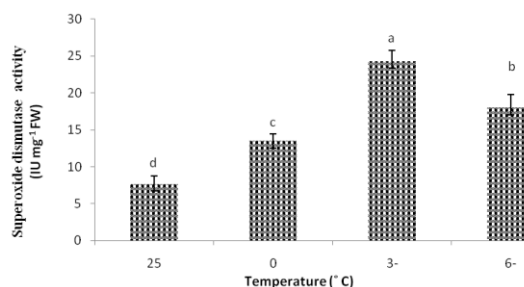


Fig. I. Effect of different temperatures on superoxide dismutase activity

superoxide dismutase activity and total chlorophyll were observed in control treatment (Table 3 and Fig. II). Results showed that

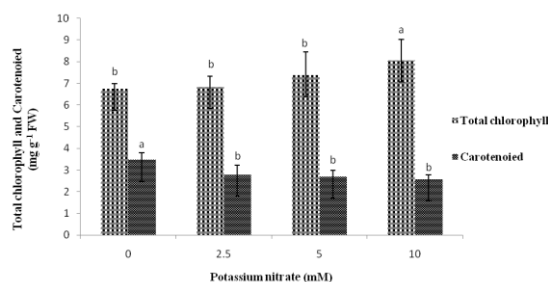


Fig. II. Effect of potassium nitrate concentrations on total chlorophyll and carotenoid content

Table 4

Means comparison of potassium nitrate × temperature effect on some indices of *Citrus aurantium* seedling in low temperature

KNO ₃ (mM)	Temperature (°C)	electrolyte leakage (%)	Antioxidant capacity (%)	Proline (mg g ⁻¹ FW)	total free phenolic (mg g ⁻¹ FW)	Superoxide dismutase activity (IU mg ⁻¹ FW)	Carotenoid (mg g ⁻¹ FW)
0	25	8.37 ^{de}	21.81 ^d	1.15 ^{bc}	2.47 ^d	20.13 ^d	3.11 ^c
	0	13.18 ^d	19.3 ^{def}	1.17 ^{bc}	3 ^{bcd}	23.07 ^{bc}	2.46 ^{ef}
	-3	33 ^{b^c}	31.04 ^c	1.24 ^{bc}	2.51 ^{cd}	26.4 ^a	3.24 ^{bc}
	-6	49.34 ^a	20.47 ^{de}	1.3 ^{ab}	4.72 ^{bcd}	20.93 ^{cd}	3.71 ^a
2.5	25	6.12 ^{de}	7.88 ^{ij}	1.19 ^{bc}	4.39 ^{bcd}	7.2 ^f	2.21 ^{efg}
	0	10.75 ^{de}	8.9 ^{hi}	1.23 ^{bc}	4.39 ^{bcd}	21.87 ^{bcd}	3.36 ^{abc}
	-3	29.64 ^c	32.55 ^c	1.2 ^{bc}	4.29 ^{bcd}	24.13 ^{ab}	3.63 ^{ab}
	-6	48.96 ^a	21.48 ^d	1.26 ^{bc}	5.09 ^b	15.33 ^e	3.29 ^{abc}
5	25	4.04 ^e	2.68 ^j	1.07 ^c	2.47 ^d	1.73 ^h	2.62 ^{de}
	0	7.03 ^{de}	9.39 ^{ghi}	1.14 ^{bc}	3.47 ^{bcd}	4.53 ^g	2.61 ^{de}
	-3	26.37 ^{de}	49.16 ^a	1.21 ^{bc}	4.21	26.53 ^a	3.27 ^{abc}
	-6	48.62 ^a	16.11 ^{def}	1.21 ^{bc}	4.75 ^{bcd}	22.13 ^{bcd}	3.18 ^{bc}
10	25	3.7 ^e	14.9 ^{efg}	0.85 ^d	2.84 ^{cd}	1.73 ^h	1.79 ^g
	0	7.97 ^{de}	8.9 ^{hi}	1.2 ^{bc}	3.99 ^{bcd}	4.66 ^g	2.11 ^{fg}
	-3	40.45 ^b	38.26 ^b	1.25 ^{bc}	4.67 ^{bcd}	20.27 ^{cd}	3.03 ^{cd}
	-6	40.65 ^b	13.9 ^{fghi}	1.48 ^a	7.41 ^a	13.73 ^e	2.65 ^{de}

Means in each column followed by similar letters are not significantly different at 5% probability level using Duncan Test.

electrolyte leakage had a significant upward trend in all of potassium nitrate concentrations with decreasing temperature and the lowest value of electrolyte leakage observed in concentration of 10 mM potassium nitrate and control temperature (table 4). According to the results of the experiment, it was observed that the treatment with 5 mM potassium nitrate increased antioxidant capacity in -3° C temperature (Table 4). As shown in Table 4, with increasing potassium nitrate concentration an ascending trend was observed in proline content in leaves. Table 4 shows the interaction effect between potassium nitrate and temperature. According to the Table, the highest amount of proline was observed in *C. aurantium* leaves at -6° C temperature and the highest potassium nitrate concentration (10 mM).

The effect of different concentrations of potassium nitrate and temperature levels was also examined on the total phenol. The results showed that seedlings grown with 10 mM potassium nitrate and -6° C led to a higher phenol content (7.41 mg/gr FW) production. The results also showed that reducing temperature to -3° C led to a significant increase in superoxide dismutase activity in all potassium nitrate

concentrations and maximum activity of this enzyme was observed at -3 °C and 0 mM potassium nitrate concentration (Table 4).

Discussion

Generally, results of the study demonstrated that among different temperature treatments, -6° C led to the highest leaf damage. In fact, low temperature affects cell membrane as it increases reactive oxygen species (ROS) production and damages lipids and membrane fatty acids causing loss of membrane integrity. It leads to activation of oxidation reactions and continuation of this process brings to complete leaf cell membrane damage (Allen, 1995). Similar increase in leaf damage percentage has been reported in coffee leaves under low temperature stress (Compose et al., 2003) and olive tree (Azzarello et al., 2009).

Lipid peroxidation and decrease in membrane fluidity indicators representing cell membrane injury and electrolyte leakage increased by freezing stress (Azzarello et al., 2009). Electrolyte leakage is an effective parameter to assess membrane permeability and therefore it is used as an indicator of membrane

integrity (Marangoni et al., 1996). Potassium causes leakage and maintenance of water in plant tissues with increasing osmotic pressure and cell membrane fluidity and thus it prevents cell membrane rupture, plant tissue damage, and increases electrolyte leakage (Wagner, 1967). Increase in electrolyte leakage under low temperature stress in wheat (Zhang et al., 2008), coffee seedlings (Azzarello et al., 2009), and tomato (Ghiasi and Razavi, 2013) confirm the results of this study. Webster and Ebdon (2005) found that electrolyte leakage decreased in dandelion leaves (*Lolium temulentum*) under potassium nitrate nutrition and low temperature stress.

Potassium has substantial effects on stomata movement and water-relation (turgor regulation and osmotic adjustment) in plants under cold stress condition (Marschner, 1995). Numerous research studies indicated that application of high concentrations of potassium nitrate may increase leaf water content under low temperature stress (Pervez et al., 2004). The present results are in agreement with those reported on Valencia leaves (Yelenosky and Guy, 1989) and walnut shoots (Poirier et al., 2010) who found that potassium deficiency reduced water content in low temperature stress. Reactive oxygen species (ROS) interferes with the balance of photosynthesis reaction and destroys cell tissues and chlorophyll pigment in chloroplast membrane and thylakoids. Therefore, leaf color reduces while carotenoid increases (Pietrini et al., 2005). Potassium affects transport of sucrose from the leaves, carbon dioxide fixation, and chlorophyll production, eliminating negative effects of low temperature (Cackmak, 2005). There is a bulk of studies on some indices in *C. aurantium* including maize and rice for leaf color (Kaya et al., 2013; Yu-Chuan et al., 2008), Valencia and wheat for chlorophyll (Ribeiro et al., 2009; Berova et al., 2002) and *Ilex paraguariensis* for carotenoid (Varone and Gratani, 2007).

Results of the study showed that optimum potassium concentration affected osmotic balance through accumulation of metabolites such as proline that influence osmotic pressure so it due to increase proline production that is one of secondary metabolites (Morgan, 1984). Proline, as a multifunctional

amino acid, plays key roles in the osmotic regulation between cytoplasm and vacuole, the redox regulation of the NAD⁺/NADH ratio, membrane stabilization, and finally promoting ROS scavenging systems (Bohnert and Jensen, 1996). Studies conducted on alfalfa (Antolin and Sanchez, 1993), maize (Gong et al., 2011), and melon (Kaya et al., 2007) showed that proline was increased under potassium nutrition.

Phenolic compound is an antioxidant metabolite that impacts on neutralization of reactive oxygen species increasing plants adaptability to low temperature and increases its amount in cold stress condition. When plant growth increases and there is more photosynthesis at higher potassium rates, increased phenolic concentrations may correspondingly occur due to allocation of excess fixed carbon to the shikimic pathway (Crozier et al., 2006; Shaw et al., 1998). Low temperature stress and potassium nutrition led to an increase in total phenol as reported by Sweet Thai basil (Nguyen et al., 2010), maize (Rengel and Damon, 2008), and *Pinus banksiana* (Teklemariam and Blake, 2004). Potassium deficiency reduces photosynthetic activity because carbohydrates accumulate in leaves and absorption process of carbon dioxide is conducted incompletely. Indeed, electrons which are produced by light reactions of photosynthesis tend toward superoxide radical production increasing superoxide dismutase activity under cold stress because they do not consume carbon dioxide. There is also evidence for increased superoxide dismutase activity and antioxidant capacity in peach sapling (Leng and Qi, 2003), maize (Farooq et al., 2008; Gong et al., 2011), and *Crataegus aronia* (Kirakosyan et al., 2003).

Conclusion

As a conclusion, this study demonstrated the positive effects of potassium nitrate on some low temperature tolerance indices. It seems that 10 mM potassium nitrate caused maintaining membrane integrity by increasing leaf water content, proline, and phenol and decreasing superoxide dismutase activity and so it due to tolerance to -6 °C in this experiment.

Acknowledgements

We would like to acknowledge the support of Institute of Citrus Research in Ramsar for utilizing its laboratory facilities to execute this study.

References

- Allen, D.J and D.R. Ort.** 2001. 'Impacts of chilling temperatures on photosynthesis in warm-climate plants'. *Trends Plant Sci*, 6: 36–42.
- Antolin, M.C and M. Sanchez –Diaz.** 1993. 'Photosynthetic nutrient use efficiency, nodule activity and solute accumulation in drought stressed alfalfa plants'. *Photosynthetica*, 27: 595 -604
- Azzarello, E., S. Mugnai, C. Pandolfi, E. Masi, E. Marone and S. Mancuso.** 2009. 'Comparing image (fractal analysis) and electrochemical (impedance spectroscopy and electrolyte leakage) techniques for the assessment of the freezing tolerance in olive'. *Trees*, 23: 159-167.
- Bates, L.S., R.P. Waldren and I.D. Tears.** 1973. 'Rapid determination of free proline for water stress studies'. *Plant Soil*, 39: 205-207.
- Berova, M., Z. Zlatev and N. Stoeva.** 2002. 'Effect of paclobutrazol on wheat seedling under low temperature stress'. *Plant Physiol*, 28: 75-84.
- Bohnert, H. J. and R. G. Jensen.** 1996. 'Strategies for engineering water-stress tolerance in plants'. *Trends in Biotechnology*, 14(3):89–97.
- Cakmak, I.** 2005. 'The role of potassium in alleviating detrimental effects of abiotic stresses in plants'. *J. Plant Nutr. Soil Sci*, 168: 521–530.
- Chen, Y., M. Zhang, T. Chen, Y. Zhang and L. An.** 2006. 'The relationship between seasonal changes in anti –oxidative system and freezing tolerance in the leaves of evergreen woody plants of Sabina'. *South Afr. J. Bot*, 72: 272 – 279.
- Clark, k. and N. Prakash.** 2001. 'Floral morphology and embryology of two Australian species of citrus (Rutaceae)'. *Australian Journal of Botany*, 49:199-207.
- Compose, P.S., V. Quartin, J.C. Ramalho and M.A. Nunes.** 2003. 'Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. Plant'. *J. Plant Physiol*, 160: 283-292.
- Crozier, A., I.B. Jaganath and M.N. Clifford.** 2006. 'Phenols, polyphenols and tannins: An overview'. In A. Crozier, M. N. Clifford, & H. Ashihara (Eds.), *Plant secondary metabolites: Occurrence, structure and role in the human diet*. Oxford: Blackwell Publishing, pp, 1–24.
- Devi, B.S.R., J.Y. Kim, S.K. Selvi, S. Lee and D.C. Yang.** 2012. 'Influence of potassium nitrate on antioxidant level and secondary metabolite genes under cold stress in Panax ginseng'. *Rus. J. Plant Physiol*, 59: 318–325.
- Farooq, M., T. Aziz, Z.A. Chemma, M. Hussian and A. Khaliq.** 2008. 'Activation of antioxidant system by KCl improves the chilling tolerance in hybrid maize'. *J. Agron. Crop Sci*, 194:438–448.
- Fotouhi Ghazvini, R., M.R. Baghbanha, A. Hatamzadeh and M. Heidari.** 2008. 'Effect of water stress on freezing tolerance of Mexican lime (*Citrus aurantifolia* L.) seedling'. *Hort. Environ. Biotechnol.* 49 (5): 267-280.
- Ghiasi, N and F. Razavi.** 2013. 'Impact of postharvest prohexadione calcium treatment on PAL activity in tomato fruit in response to chilling stress'. *Iranian Journal of Plant Physiology*, 4(1): 865-871
- Gong, X., L. Chao, M. Zhou, M.Hong, L. Luo, L.Wang, W. Ying, J. Cai, G. Songjie and F. Hong.** 2011. 'Oxidative damages of maize seedlings caused by exposure to a combination of potassium deficiency and salt stress'. *Plant Soil*, 340:443–452.
- Hatamnia, A.A., N. Abbaspour, R. Darvishzadeh, F. Rahmani and R. Heidari.** 2013. 'Tobacco responds to salt stress by increased activity of antioxidant enzymes. *Iranian Journal of Plant Physiology*, 3(4): 801- 807
- Hernandez, J.Z., M.A. Ferrer, A. Jimenez, A.R. Barelo and F. Sevilla.** 2001. 'Antioxidant systems and O²/H₂O₂ production in the apoplast of pea leaves. Its relation with salt induced necrotic lesions in minor veins'. *Plant Physiol*, 127:817-831.

- Hoagland, D.R and D.I. Arnon.** 1950. 'The water-culture method for growing plants without soil'. *California Agricultural Experiment Station Circular*, p. 337.
- Kaanane, A., D. Kane and T.P. Labuza.** 1988. 'Time and temperature effect on stability of Moroccan processed orange juice during storage'. *Journal of food Science*, 53:1470-1473.
- Kaya, C., M. Ashraf, M. Dikilitas and L. Atilla.** 2013. 'Alleviation of salt stress-induced adverse effects on maize plants by exogenous application of indole acetic acid (IAA) and inorganic nutrients – A field trial'. *AJCS*, 72: 249-254.
- Kim, D.O., O.K. Chun, YJ. Kim, H.Y. Moon and C.Y. Lee.** 2003. 'Quantification of polyphenolics and their antioxidant capacity in fresh plums'. *J. Agric. Food Chem*, 516:509-6515.
- Kirakosyan, A., E. Seymour, P.B. Kaufman, S. Warber, S. Bolling and S.C. Chang.** 2003. 'Antioxidant Capacity of Polyphenolic Extracts from Leaves of *Crataegus laevigata* and *Crataegus monogyna*(Hawthorn) Subjected to Drought and Cold Stress'. *J. Agric. Food Chem*, 51(14): 3973–3976.
- Leng, P and J.X. Qi.** 2003. 'Effect of anthocyanin on David peach (*Prunus davidiana* Franch) under low temperature stress'. *Sci. Horticulture Amsterdam*, 97:27-39.
- Lindhauer, M.G., H. Beringer and K. Koch.** 1986. 'Sucrose accumulation and osmotic potentials in sugar beet at increasing levels of potassium nutrition'. *J. Sci. Food Agric*, 37: 211–218.
- Liu, J and J. Zhu.** 1997. 'An Arabidopsis mutant that requires increased calcium for potassium nutrition and salt tolerance'. *Proc. Nat. Acad. Sci*, 94: 14960–14964.
- Lutts, S., J.M. Kinet and J. Bouharmont.** 1996. 'Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance'. *Plant Growth Reg*, 19: 207–218.
- Mahajan, S and N. Tuteja.** 2005. 'Cold, salinity and drought stresses: An overview'. *Arch. Biochem. Biophys*, 444:139–158.
- Marangoni, A.G., T. Palma and D. W. Stanley.** 1996. 'Membrane effects in postharvest physiology'. *Postharvest Biology and Technology*, 7(3): 193-217.
- Marschner H.** 1995. 'Mineral nutrition of higher plants'. Academic, San Diego.
- Morgan, J.M.** 1984. 'Osmoregulation and water stress in higher plants'. *Ann. Rev. Plant Phys*, 35: 299–319.
- Nguyen, P. M and E. D. Niemeyer.** 2008. 'Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.)'. *Journal of Agricultural and Food Chemistry*, 56, 8685–8691.
- Obreza, T.A.** 2003. 'Importance of potassium in a florida citrus nutrition program'. *Better crops*, 87: 19-22.
- Pervez, H., M. Ashraf and M.I. Makhdum.** 2004. 'Influence of potassium nutrition on gas exchange characteristics and water relations in cotton (*Gossypium hirsutum* L.)'. *Photo synthetic*, 42: 251–255.
- Pietrini, F., D. Chaudhuri, A.P. Thapliyal and A. Massacci.** 2005. 'Analysis of chlorophyll fluorescents in mandarin leaves during photo-oxidative cold shock and recovery'. *Agr. Eco. Environ*, 106: 189-198.
- Poirier, M., A. Lacoite, T. Ameglio and M. Ball.** 2010. 'Semi physiological model of cold hardening and dehardening in walnut stem'. *Tree Physiol*, 30: 12.1555-1569.
- Rapisarda, P., M.L. Bianco, P. Pannuzzo and N. Timpanaro.** 2008. 'Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [*Citrus sinensis* (L.) Osbeck]'. *Postharvest Biol. Tec*, 49: 348–354.
- Rengel, Z and P. M. Damon.** 2008. 'Crops and genotypes differ in efficiency of potassium uptake and use'. *Physiologia Plantarum*, 133, 624–636.
- Ribeiro, R.V., E.C. Machado, M.G. Santos and R.F. Oliveira.** 2009. 'Seasonal and diurnal changes in photosynthetic limitation of young sweet orange trees'. *Environ. Exp. Bot*, 66: 203–211.
- Ruth, G.** 2002. 'Oxidative stress and Acclimation Mechanisms in plants'. *American society of plant biologists*, 17: 1-20.

- Sanchez, F.G., M. Carvajal, I. Porras, P. Botia and V. Martinez.** 2003. 'Effects of salinity and rate of irrigation on yield, fruit quality and mineral composition of Fino 49, lemon'. *Euro. J. Agr.*, 19: 427-437.
- Saneoka, H., R.E.A. Moghaieb, G.S. Premachandra and K. Fujita.** 2004. 'Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relation'. *Environmental and Experimental Botany*, 17: 123-131.
- Shaw, T. M., J. A. Moore and J. D. Marshall.** 1998. 'Root chemistry of Douglas-fir seedlings grown under different nitrogen and potassium regimes'. *Canadian Journal of Forest Research*, 28, 1566–1573.
- Sheng Wu, Q., Y. Ning Zou and R. Xue Xia.** 2006. 'Effects of water stress and arbuscular mycorrhizal fungi on reactive oxygen metabolism and antioxidant production by citrus (*Citrus tangerine*) roots'. *Eur. J. Soil Biol.*, 42:166–172.
- Singleton, V.L and J.A. Rossi.** 1965. 'Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents'. *American Journal of Enology and Viticulture*, 16: 144-158.
- Strain, H.H and W.A. Svec.** 1966. 'Extraction, separation, estimation and isolation of chlorophylls'. In: Vernon, L. P. and Seely, G. R., Eds., *The Chlorophylls*, Academic Press, New York, 21-66.
- Teklemariam, T and T.J. Blake.** 2004. 'Phenylalanine ammonia-lyase-induced freezing tolerance in jack pine (*Pinus banksiana*) seedlings treated with low, ambient levels of Ultraviolet-B radiation'. *Physiol. Plantarum*, 122: 244–253.
- Varone, L and L. Gratani.** 2007. 'Physiological response of eight Mediterranean marquis species to low air temperatures during winter'. *Photosynthetica*, 45: 3385-391.
- Verslues, P.E., M. Agrawal, S. Katiyar –Agrwal, J. Zhu and J.K. Zhu.** 2006. 'Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status'. *The Plant of Journal*, 45: 523-539.
- Wagner, R.E.** 1967. 'The role of potassium in the metabolism of turfgrasses,. In: *Gronomy abstracts*'. Amer. Soc. Agron, Madison, Wis, 55.
- Webster, D.E and J.S. Ebdon.** 2005. 'Fertilization on perennial ryegrass cold tolerance during deacclimation in late winter and early spring'. *Hort Science*, 40: 842 -849.
- Yelenosky, G and C.L. Guy.** 1989. 'Freezing tolerance of Citrus, Spinach, and Petunia leaf tissue osmotic adjustment and sensitivity to freeze induced cellular dehydration'. *Plant Physiol*, 89: 444-451.
- Yu-Chuan, Ding., Chun-Ron, CHANG., Wen, LUO., Yan-Shou, WU., Xiao-Li, REN., Ping, WANG and XU Guo-Hua.** 2008. 'High Potassium Aggravates the Oxidative Stress Induced by Magnesium Deficiency in Rice Leaves'. *Soil Science Society of China. Pedosphere*, 18: 316–327.
- Zhang, P., C.K. Ebel, R.C. Dane and W.A. Dozier.** 2005. 'Identification of cold acclimated genes in leaves of *Citrus unshiu* by mRNA differential display'. *Gene*, 359: 111-118.



ارزیابی شاخص‌های تحمل‌پذیری به دمای پایین در دانهال نارنج (*Citrus Aurantium*) تحت تغذیه نیترات پتاسیم

زینب رفیعی راد*^۱، اسماعیل دردی پور^۲، یحیی تاجور^۳

^۱ دانش‌آموخته کارشناسی ارشد، گروه علوم خاک، دانشگاه علوم کشاورزی منابع طبیعی گرگان

^۲ دانشیار گروه علوم خاک، دانشگاه علوم کشاورزی منابع طبیعی گرگان

^۳ استادیار موسسه تحقیقات علوم باغبانی، پژوهشکده مرکبات و میوه‌های نیمه‌گرمسیری،

سازمان تحقیقات، آموزش و ترویج کشاورزی، رامسر

* عهده دار مکاتبات: z.raffii@yahoo.com

چکیده فارسی

این پژوهش، اثرات تغذیه نیترات پتاسیم را بر تعدادی از شاخص‌های تحمل‌پذیری به دمای پایین در دانهال‌های نارنج مورد بررسی قرار می‌دهد. یک آزمایش فاکتوریل بر پایه طرح کامل تصادفی با چهار تکرار در پژوهشکده مرکبات و میوه‌های نیمه‌گرمسیری رامسر انجام گرفت. تیمارها شامل، نیترات پتاسیم با چهار سطح (صفر، ۲/۵، ۵ و ۱۰ میلی‌مولار) و دما با چهار سطح (۲۵، ۰، ۳- و ۶- درجه سانتی‌گراد) بودند. نتایج نشان داد که حداکثر میزان تخریب برگ، نشت یونی، پرولین و کارتنوئید در دمای ۶- درجه سانتی‌گراد مشاهده گردید، درحالی‌که بیشترین میزان ظرفیت آنتی‌اکسیدانی (۳۷/۷ درصد) و فعالیت سوپراکسید دیسموتاز (۲۴/۴ واحد آنزیمی در گرم وزن تازه برگ) در دمای ۳- درجه سانتی‌گراد در برگ‌های نارنج مشاهده شد. بالاترین غلظت نیترات پتاسیم، محتوای کارتنوئید را در برگ‌ها کاهش داد. غلظت ۱۰ میلی‌مولار نیترات پتاسیم نیز مقدار محتوای نسبی آب، رنگ برگ، فنول آزاد و کلروفیل کل را افزایش داد. اثر متقابل دمای پایین و نیترات پتاسیم بر میزان نشت یونی، ظرفیت آنتی‌اکسیدانی، پرولین، فنول آزاد کل و فعالیت آنزیم سوپراکسید دیسموتاز (SOD) معنی‌دار بود. در غلظت صفر میلی‌مولار نیترات پتاسیم (شاهد)، حداکثر مقدار نشت یونی در دمای ۶- درجه سانتی‌گراد ایجاد شد. در حالی‌که در دمای ۳- درجه سانتی‌گراد، غلظت ۵ میلی‌مولار نیترات پتاسیم باعث ایجاد بیشترین فعالیت آنزیم سوپراکسید دیسموتاز و ظرفیت آنتی‌اکسیدانی شد. کاربرد بالاترین غلظت نیترات پتاسیم در دمای ۶- درجه سانتی‌گراد منجر به تولید پرولین و فنول آزاد کل بیشتری در مقایسه با سایر تیمارها گردید. به طور کلی، نیترات پتاسیم باعث افزایش محتوای نسبی آب و مقدار پرولین گردید، همچنین افزایش فعالیت سوپراکسید دیسموتاز و ظرفیت آنتی‌اکسیدانی منجر به افزایش پایداری غشاء سلول در تنش دمای پایین گردید.

کلمات کلیدی: نارنج، نیترات پتاسیم، تنش دمای پایین، پرولین، فعالیت سوپراکسید دیسموتاز