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The combined effects of phosphorus and zinc on antioxidant enzyme activity and growth attributes of potato under water deficit conditions

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Abstract

This investigation wasconductingas a factorial experimentbased on randomized completeblocks design. The factors were zinc (Zn)at three levels (0,10 and20 mgZn perkgdry soil as zinc sulfate),phosphorus (P) at three levels (0,30 and60 mg P per kgdry soil as monocalcium phosphate) andsoil moisture at three levels (50-60% FC, 70-80% FC and 90-100% FC) with three replications. The growth attributes of potato such as, chlorophyll index, stomatal conductivity (SC), relative water content (RWC) and enzyme activityof superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) were measured during growth period.The results showed that the drought stress resulted in a significant decrease in POX and SOD, stomatal conductivity,chlorophyll index and RWC. Application of P increased significantly the stomatal conductivity and SOD. Application of Zn also affected significantly thechlorophyll index, stomatal conductivity and POX. The P×soil moisture and Zn×soil moisture two-way interactions effects were significant for stomatal conductivity and POX. The P×Zn×soilmoisture interactionhad significant effect for chlorophyll index, stomatalconductivity. The results showed that the two way interactions of Zn, P and soil moisture were mainly synergistic on above-mentionedattributes. In general, to achieve the optimum growth of potato in similar soils, recommended application of 20 mg Zn and 30 mg P perkgdry soil under normal irrigation conditions and applicationof 20 mg Zn and 60 mg P perkgdry soilat water deficit conditions.

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Introduction

The global water deficit dramatically affected field plants productionespecially in Asia regions because of 90% water consumption in agriculture (Huaqi*et al.*, 2002). The quality of the plants response to water deficit liaises to the stress intensity and durability, as well as the plant growth stage.

Water availability isone of the most important limiting agents in the yield and quantitative attributes of potato. It is emphasized that potato is very susceptible to drought (Ierna and Mauromicale, 2012) because of sparse and non-deeperrootsystem, and by imposing soil moisture deficit tuber yield might be reduced (Ahmadi *et al.*, 2010). Water deficit affected the formation and enlargement of potatoes different sections. The most of the potato attributes such as leaf area index stemheight and ground coverage of potatoreduced under water deficit stress conditions (Yuan *et al.*, 2003). The impact of water deficit on plants growth depends to time (Fleisher *et al.,* 2012), period and strength ofwater stress (Jefferies, 1995).

During progressive drought conditionsthe decreases in some biochemical activities corresponded fairly well todecreases in stromatal conductance, but not to decreases in relative water content ormesophyll water potential. ATP synthesis is the first biochemical stepthatimpaired during water deficit is RuBP regeneration, which related to ATP synthesis, is also susceptible to mild water deficit. Down-regulation of electron transport occursunder more severe water deficit conditions, before irreversible PSII damage (Flexes and Medrano, 2002). Water deficit may increase the formation of oxygen free radicals. These reactive oxygen species (ROS) involve superoxide (O_2•), hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), and single oxygen ($1O_2$). ROS are highly reactive to membrane lipids, protein and DNA. They are the major contributing factors for stress injuries and to cause rapid cellular damage particularly when plants was exposedto stress conditions (Gupta *et al.*, 2005). One of the stress defense mechanisms in plants is the antioxidant defense system, which includes antioxidant enzymes, such as super dismutase (SOD), peroxidase (POX), catalase (CAT) and low-molecular antioxidants. SOD converts superoxide radicals (O_2·) into hydrogen peroxide (H_2O_2), POX reduces H_2O_2 to water using various substrates as electron donors, and CAT catalyze H_2O_2 into water and oxygen too (Gaber, 2010).

The nutrients such as K, Zn and P perform important role in protecting plants from damages of water deficit stress. Zn is important micronutrient in crop production and deficiency is widespread throughout the world than those of any other micronutrient (Alloway, 2008). Zn has an important role in improvement adverse effects of water deficit in plants. Zn has functional role in maintaining the structural integrity and controlling permeability of biomembranes (Cakmak, 2000). InZn deficient conditions, plant cells loss their membrane integrity that results in higher membrane permeability to inorganic ions (Welch *et al.,* 1982). In addition to its particular role as a structural component in membranes, Zn also plays a key role in controlling the generation anddetoxification of ROS, which can damage membrane lipids and sulphydryl groups (Alloway, 2008). Zn exerts an inhibitory action on membrane damage catalyzed by ROS (Cakmak, 2000). Zn application may also affect plant water status and modify stomatalconductance (SC). Transpiration rate and SC also declined under Zn deficiencyconditions (Khan *et al.*, 2004). Zn is although required directly for SOD enzyme activity and indirectly for highactivity of the enzymes involved in H_2O_2 detoxification such as catalase, ascorbate peroxidase and glutathione reductase (Cakmak, 2000).

P deficiency is the second most yields limiting nutrients deficiency after nitrogen in plants. It is an essential component of nucleic acids, phosphorylated sugars, lipids and proteins, which control all life processes (Raghothama, 1999). P uptake by plants reduces in water deficit conditions. P deficiency affects the response of a plant to water deficit stress due to effects on stomatalaction and on the resistance to water flow within the plant. P-deficient plants close their stomata at higher leaf water potential (less negative) than those receiving an adequate P supply (Garg *et al.*, 2004).

The interactive effect of P and Zn on water relations and enzymatic activity of potato haveless evaluated especially under water deficit conditions. Therefor the aims of this study were the effects of water deficit on water relations and antioxidant enzyme activity and the ability of P and Zn on palliation of water deficit.

Materials and methods

Greenhouse and soil description

A pot experiment conducted in a naturally lighted greenhouse at Agricultural Research Station of Tabriz University, Iran during 2012. The maximum and minimum temperature of green house was measured daily and a big hydroelectric cooler was usedfor controlling greenhouse temperature. The average maximumand minimum of temperature was 32.5 and 11.5°C during plantingperiod, respectively. Relative humidity of greenhouse was maintained about 65% by periodic moisturizing of the greenhouse floor.At first a calcareous non-saline soil (EC = 0.47 ds m−1) low in available P and Zn (Olsen-P = 8.7 and DTPA-Zn = 0.5 mg kg^{-1}that werelower than the critical levels) was selected (Alloway, 2008). Thesoil was taken from Espiran village in North West of Tabriz (Iran) with the latitude of 38°15'57"N and longitude of 46°19'53"E from depth of 0–25 cm. After air-drying and sieving (2 mm in diameter), soil properties were measured as described by Motalebifard *et al.* (2013) and presentedin Table 1.

Table 1. Some physical and chemical properties of soil.

Texture	CCE (%)	SP (%)	FC (%)	OC (%)	pH(1:1)	EC(1:1) (ds m^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
Clay loam	15.25	44.4	18.5	0.5	7	0.47	8.7	556	3.98	0.52	7.01	2.2

CCE: calcium carbonate equivalent; SP: saturation percentage; FC: field capacity; EC: electrical conductivity; OC: organic carbon.

Treatments and planting description

The experiment was arranged as factorial 3 × 3 × 3 with n = 3based on randomized complete blocks design with three factors. The factors wereZn at three levels of 0, 10 and 20 mg Zn per kg of soilas $ZnSO_4 \cdot 7H_2O$, P at three levels of 0, 30 and 60 mg P per kg of soilas $Ca(H_2PO_4)$ $2H_2O$ (monocalciumphosphate) and soil moisture atthree levels of 0.5 FC–0.6 FC, 0.7 FC–0.8 FC and 0.9 FC–FC in threeapplications.All of the plots were planting at the same time (04/26/2014)with total 81 pots.

Based on soil testing 5 mg Fe from Sequestrine-138 (EDDHA-FeNa) source, 5 mg Mnfrom $MnSO_4 \cdot H_2O$ and 200 mg of N (one-third at planting andthe rest in two split until soil moisture treatments imposing) asurea ($(NH_2)_2CO$) were applied per kg of soil. Then 10 kg of abovementioned soil by bulk density of 1.1 g per $cm^3$of soil pouredineach pot (30 cm diameter and 26 cm height) and two potatoestubers (*Solanumtuberosum L.)* cv. Agria with certified seeds anduniform sizes (45–55 mm) were planted in 10 cm depth of pots soil.Two tubers were planted to avoid elimination possibility of a potbecause of plant death and need to a large number of leaves formeasuring many parameters such as LWP, SC, enzymes activity and nutrients concentrations that some of them were not presented in this manuscript. The position of the pots within each block was change once to twice per week to minimize the effects of light and temperature gradients within the greenhouse as well as border effects. Pots were irrigated equally and uniformly at 0.94 ± 0.05 FC fromsowing until flowering (64 days) and then soil moisture levels were imposed three weeks from the flowering (64th day) until harvest (85th day after planting). Throughout the experiment soil moisture was controlled by pots weighting and watering two times per day. Deionized water was added by graduated cylinder according topots weight reduction from the intended soil moisture levels (up to 0.95 FC until flowering and up to 0.6 FC, 0.8 FC and FC during soil moisture imposing period). To compensation of plant weighty increasing effect, additional pots were used for each of soil moisture levels. The whole plant fresh weight was determined every two weeks and the weight correction done.

The crude extracts of fresh and healthy leaves from adult plants prepared with separate mortars and pestles in a Tris-HCl extraction buffer pH 7.5 Tris 50 mM, sucrose 5%, ascorbic acid 50 mM, sodium metabisulfite 20 mM, PEG 2% and 2-Mercaptoethanol 0.1% before use.Valizadeh *et al.* (2011) with a ratio of 0.5 mg µl-1 (1W:2V) centrifuged at 4°C and 10,000 rpm for 10 minutes using small Eppendorf tubes.

Protein determination

Total protein content in extracts was determined spectrophotometrically at 595 nm according to the method of Bradford (1976) using bovine serum albumin as a standard.

Parameters measurements

Moisture of field capacity (FC) was determined by the pressure plate method at pressure of 300 kPa (Kirkham, 2004) and was 18.5%. For evaluating the stress intensity, SC were measured on three leaves from each pot by leaf porometer AP4 (Delta-T Devices-UK) around midday during the imposing of water deficit treatments period. Chlorophyll meter (model CL-01 manufacture by Hansatech England) was used for measuring leaves chlorophyll index. At same time, five leaves were determined from each pot for measuring of chlorophyll index with using above-mentioned device (Borzooei *et al.*, 2006). The relative content of leaf water was calculated alsoby the following formula (Riahinia*et al.*, 2006).

$$\text{Relative water content} = \frac{\text{Fresh leaf weigh} - \text{dry leaf}}{\text{Swollen leaf weight} - \text{dry leaf}} \times 100$$

The POX, CAT and SOD was stained based on protocol that was described previously by Olson and Varner (1993), Anderson *et al.* (1995) and Beauchamp and Fridovich (1971) and Sairam *et al.* (2002) methods, respectively.

Statistical analysis of data

An image analysis program (MCID1 software) was used to measure D×A (optical density×area) parameter for each isozyme (POX, CAT) band to evaluate the activity on the gels. Data were subjected to analysis of variance as factorial 3 × 3 × 3 with n= 3 based on randomized complete blocks design by using MSTATC software. Tukey's test at $p \leq 0.05$ probability levelswasapplied to compare the mean values of measured attributes. The Excel software (Excel software 2010, Microsoft Inc., WA, USA) was used to draw figures.

Result and discussion

Stomatalconductance

The analysis of variance (ANOVA) showed that the main effects of soil moisture and P, two-way interactions of P × soil moisture and Zn × P and three-way interaction of soil moisture × Zn × P weresignificant for stomatal conductance (SC) (Table 2).Water deficit stress was reduced SC so that the highest (0.87 cm / s) and the lowest (0.34 cm/s)amounts ofSC were observed under full irrigated condition (0.9 FC–FC) and under severe water deficit condition (0.5 FC–0.6FC) respectively (Table 3).

When plants suddenly encounter with water deficit stress it is important to respond as quickly as possible. A faster water deficit response means that less water is lost and the survival rate of the plants is increased. The most important quick response is stomatal closure. Stomata consist of two guard cells surrounding the stomatal pore. When the stomata are open, water transpires and CO_2 enters the leaf through the stomatal pore. During water stress, the stomatal pore closes to reduce water loss. By closing, the stomatal pore the water use efficiency increases (Zhang and Davies, 1989). Several mechanisms work together to close the stomata, such as hydro passive closure and chemical signals from the plant stress hormone ABA. Increased levels of ABA also causes increased hydraulic conductivity in the roots and xylem, enabling the plants to transport more water and thereby recover more rapidly after water stress (Kudoyarova *et al.*, 2011).

Table 2. Summary of variance analysis (mean squares) for the measured attributes of potato.

Source of variation	df	SC	LCI	RWC	SOD	CAT	POX
Replication	2	0.002^{ns}	34.87^{ns}	8.28^{ns}	525.61^{ns}	0.071^{**}	0.799^{**}
Moisture	2	1.86^{**}	1653.6^{**}	121.94^{**}	42170.23^{**}	0.018^{ns}	0.741^{**}
Zn	2	0.049^{ns}	76.27^{**}	10.25^{ns}	2993.37^{ns}	0.012^{ns}	0.168^{*}
Zn × moisture	4	0.073^{**}	1.73^{ns}	8.49^{ns}	1058.23^{ns}	0.0021^{ns}	0.132^{*}
P	2	0.157^{**}	1.92^{ns}	21.14^{ns}	3969.99^{*}	0.013^{ns}	0.041^{ns}
P × moisture	4	0.122^{**}	35.25^{*}	29.63^{*}	1058.23^{ns}	0.016^{ns}	0.055^{ns}
Zn × P	4	0.088^{**}	26.93^{*}	0.71^{ns}	77.08^{ns}	0.014^{ns}	0.091^{ns}
Zn × P × moisture	8	0.041^{*}	25.1^{*}	9.72^{ns}	1092.98^{ns}	0.012^{ns}	0.033^{ns}
Error	52	0.019	11.82	9.62	112814	0.013	0.052
Coefficient of variation (%)		22.7	10.92	3.94	28.3	29.7	27.71

RWC: relative water content; SC: stomatalconductance; LCI: leaves chlorophyll index.

ns: non-significant , * Significant at p ≤ 0.05, ** Significant at p ≤ 0.01

Table 3. Means comparison of potato measured attributes under Zn, P and soil moisture main effects.

Factors	Levels	SC (cm/s)	LCI	RWC (%)	SOD	POX
Soil moisture	0.9 FC-FC	0.87a	24.80c	87.43a	160.055a	0.558b
	0.7FC–0.8FC	0.62b	29.54b	84.88b	114.507b	0.794a
	0.5FC–0.6FC	0.34c	40.09a	83.21b	81.338c	0.875a
Zn (mg kg^{-1})	0	0.58b	29.80b	84.64a	129.974a	0.708b
	10	0.60ab	31.48ab	85.85a	125.074a	0.830a
	20	0.66a	33.16a	85.02a	127.851a	0.689b
P (mg kg^{-1})	0	0.54b	31.65a	86.13a	129.974a	0.728a
	30	0.69a	31.61a	84.39a	120.074ab	0.730a
	60	0.61ab	31.17a	85.00a	105.851b	0.729a

RWC: leaf water relative; SC: stomatalconductance; LCI: leaves chlorophyll index.
Different letters in a column of each experimental factors show significant differences in Duncan's multiple range test at p ≤ 0.05.

Reduction in the SCdue to water deficit stress was corresponded by several studies, including Westigate and Grant (1989), Samarah, and Mullen (2006). Sekiya and Yano (2008) reported that P deficiency decreased the number of stomata in the leaves of potato. Therefore, the reduction in leaf SC underwithoutP condition was due to a decrease in the number of leaves stomata. In addition, Radin (1984) reported that in P-deficient plantsthe more abscisic acid accumulated in leaves, which resulted in the closing of the stomata and reducing of stomatal conductance.

SC Means comparison for Zn ×soil moisture interaction showed that the lowest rate of SCresulted from severewater deficit conditions (without difference between Zn levels) and the highest amount of SC (1 cm/s) obtained from application of 20 mg Zn per kg of soil under full irrigated treatment (Fig. 1). Sharma *et al.* (1984) reported that Zn deficiency reduced the osmotic potential in the lettuce and water saturation deficit increased as compared withplants grown under Zn sufficient conditions.

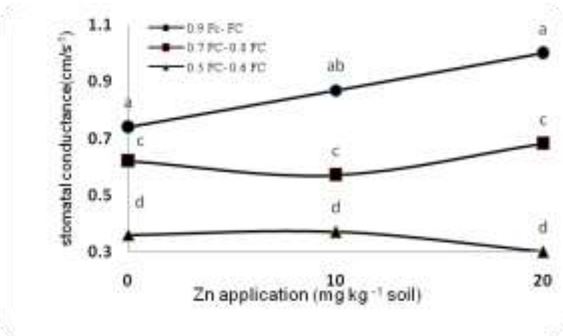

Fig. 1. Stomatal conductivity in relation to combined soil moisture and Zn treatments (n = 9) at $p \leq 0.05$.

The results showed that the interaction of Zn and soil moisture was synergistic on stomatalconductance. The means comparison of stomatal conductance under soil moisture×Pinteraction showed that the highest SC(0.97 cm/s) obtained from the application of 60 mg P per kg of soil under full irrigated treatment and the lowest SC (0.31 cm/s)resulted from thetreatment of without P application under severe water deficit condition (Fig. 2). Application of P in all levels of soil moisture were increased SCamount especiallyunder full irrigated conditions and severewater, deficit stress, which represents the ability of P was in the increased SCin normal and water deficit stress.

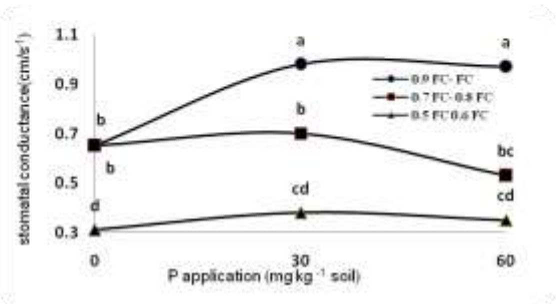

Fig. 2. Stomatal conductivity in relation to different soil moisture and P treatments (n = 9) at $p \leq 0.05$.

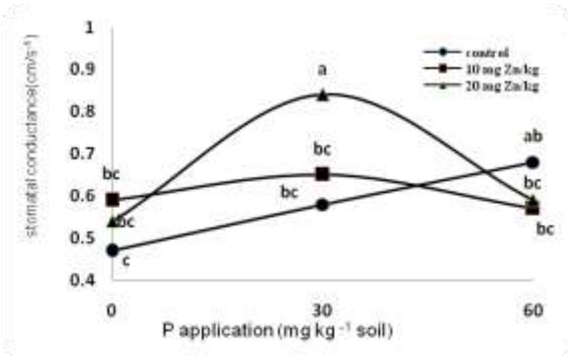

Fig. 3. Stomatal conductivity in relation to different Zn and P treatments (n = 9) at $p \leq 0.05$.

Considering to the main effect means comparison, P expanded SCby increasing the number of stomata per unit area (Sekiya and Yano, 2008), lowering accumulation of abscisic acid and regulation of the stomata opening and closing (Radin, 1984). The P × soil moisture interaction effect was synergistic on stomatal conductance. Means comparison of SC under three way interaction of Zn×P×soil moisture showed thatthe highest rate of SC were obtained underthe application of 20 mg Zn and 30 mg P per kg of dry soil under full irrigation. Althoughthe lowest amount of SC depended towithout P application and application of 20 mg Zn per kg of soil under severe stress (Table. 4). Simultaneous application of Zn and P was highly effective for increasing of SC. In this condition, the SC increased further considering the role of Zn in plant water relations, regulationstomata closure and role of Ponthe increase of stomata number, lowering of abscisic acidaccumulation and regulating of stomata closure.In addition, the role of Zn on SC was remarkable when applied together with P fertilizer and there was indeed synergistic relationship between Zn and Pin water deficit conditions in terms of the impact on SC.

Leaves chlorophyll index

The ANOVA showed that the main effects of soil moisture and Zn and two-way interactions of P×soil moisture and Zn×P and three-way interaction of Zn×P×soil moisture were significant for chlorophyll index (Table 2). Chlorophyll indexincreased from 24.8 at the full irrigation condition to 40.09 at the severe water deficit condition that this increased, process of more severe particularly in the under 0.5 FC–0.6 FC condition (Table. 3). Yasin-Ashraf *et al.* (1998) reported that the reduction in uptake and transmission of elements such as N, K and P in water deficit condition decreased chlorophyllcontent more than plant growth. Considering therelationship between chlorophyll a and b and chlorophyll index, some researchers reported that water stress decreased the concentration of chlorophyll a and b in the plant (Darvish-Boluchi *et al.*, 2010).

Table 4. Means comparisons for potato attributes under Zn × P × soil moisture interaction effects.

Soil moisture	Zn (mg/kg^{-1})	P(mg/kg^{-1})	RWC (%)	SC (cm/s^{-1})	LCI
0.9 FC -FC	0	0	86.82abc	0.48f-k	22.84jk
		30	86.88bc	0.73c-f	24.04ijk
		60	84.45b-e	1.01b	22.79jk
	10	0	92.54a	0.75cde	27.70g-k
		30	86.90bc	0.92bcd	23.87ijk
		60	86.62bc	0.95bc	21.40k
	20	0	89.51ab	0.73c-f	31.48e-h
		30	85.96b-e	1.30a	25.46h-k
		60	87.18bc	0.95bc	23.62ijk
0.7 FC0-0.8 FC	0	0	82.81cde	0.63e-i	26.97g-k
		30	84.40b-e	0.32e-i	26.70g-k
		60	85.54b-e	0.63e-i	29.25f-j
	10	0	83.60b-e	0.67d-g	25.11h-k
		30	85.13b-e	0.54e-j	35.18c-f
		60	86.18b-e	0.49e-k	29.01f-j
	20	0	84.10b-e	0.65e-h	31.34e-h
		30	85.53b-e	0.94bc	29.01f-j
		60	86.59bc	0.46g-k	32.51d-g
0.5 FC -0.6 FC	0	0	86.58bs	0.32jk	38.46a-d
		30	80.20de	0.38ijk	38.83a-d
		60	84.12b-e	0.39h-k	38.28a-d
	10	0	83.89b-e	0.36ijk	37.26b-e
		30	83.33b-e	0.48ef-k	39.10abc
		60	84.44b-e	0.27k	43.87a
	20	0	85.30b-e	0.24k	43.72ab
		30	81.14cde	0.28k	42.30ab
		60	79.87e	0.37ijk	38.99abc

RWC: relative waterc content; SC: stomatalconductance; LCI: leaves chlorophyll index. Different letters in a column of each experimental factors show significant differ-ences in Duncan's multiple range test at p ≤ 0.05.

Application of Zn increased chlorophyll index from 29.8 in without Zn to 33.16 in the application of 20 mg Zn per kg of dry soil (Table. 3). Chlorophyll index increased with application of Zn because Zn has an undeniable role in the production of proteins by activating enzymes such as RNA polymerase that involves in protein synthesis (Marschner, 1995). Lack of sufficient protein restrictsformation of protein chlorophyll complexand chlorophyll index decreased by reducing the normal activity of protein chlorophyll complex.

Means comparison of chlorophyll index under soil moisture and P interaction showed that the maximum (40.38) and minimum (22.6) of chlorophyll index obtained by application of 60 mg P per kg of soil under full-irrigated condition and application of 60 mg P per kg of soil under severe water deficit condition respectively (Fig. 4). As was observed, water deficit and P deficiency caused darker color and increased the plant's chlorophyll index.In both conditions therate ofcells and leafdilatation were slower than the formation of chlorophyll and caused higher chlorophyll index (Marschner, 1995).

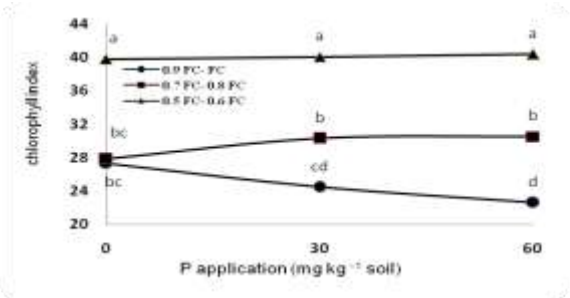

Fig. 4. Chlorophyll index in relation to different soil moisture and P treatments (n = 9) at p ≤ 0.05.

Means comparison of chlorophyll index under interaction of Zn and P showed that the minimum (29.42) and maximum (35.51) of chlorophyll index was obtained under without P and Znapplication and the without P and application of 20 mg Zn per kg of soil levels respectively (Fig. 5). The first and second levels of Zn (0 and 10 mg Zn per kg of soil) had no significant effect on chlorophyll index in all of P levels.The difference happened whenZn applied at 20 mg per kg of soil rate.This results showed that higher levels of Zn exacerbated P deficiency and this antagonistic interaction observed under higher level of Zn (20 mg Zn per kg of soil) while,when the small amounts of Zn applied, this interaction did not observed. Means comparison of chlorophyll index forthree way interaction of soil moisture Zn and P are available in Table 4. The maximum (43.87) and minimum (21.4) of chlorophyll index was obtained by application of 10 mg Zn and 60 mg P per kg of soil under severe water deficit conditionand application of 10 mg Zn and 60 mg P per kg of soil under full-irrigated condition, respectively (Table. 4). The difference between two above mentioned treatments were more than 100 percent for chlorophyll index.

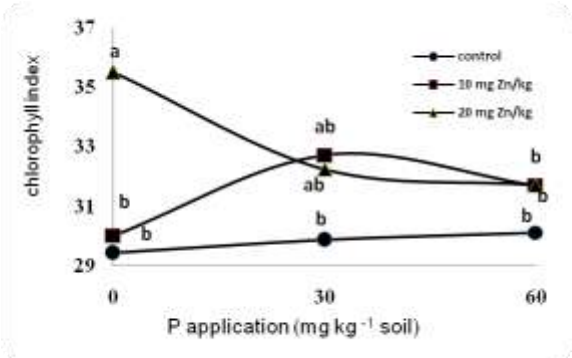

Fig. 5. Chlorophyll index in relation to different Zn and P treatments (n = 9) at p ≤ 0.05.

Leaf relative water content

Leaf relative water content (RWC) is one of theimportant indicators of the water stress induced into plants that in participation withattributes such as SC can be effective on assessment ofwater stress severitythat induced for plants. The main effect of soil moisture and soil moisture × P interaction was significant on RWC (Table 2).

Means comparison of Leaf RWC under soil moisture showed that the maximum (87.43 %) and minimum (83.21 %) of Leaf relative water content was obtained in full-irrigated condition (0.9 FC–FC)and severe water deficit condition (0.5 FC–0.6), respectively (Table 3). The results showed that water deficit (moderate to severe) caused a reductionin the leaf relative water content comparing with full irrigated condition but the difference between the moderate and severe water deficit levels were not significant. Fig. 6 shows RWC for different soil moisture and P combined treatments. The maximum (89.62 %) and minimum (81.56 %)of RWC was obtained for without P application under full-irrigated condition and application of 30 mg P per kg of soil under severe water deficitcondition, respectively. The results showed that the application of P under full-irrigated levelcaused a reduction on RWC and with opposite trend increasedthis attribute undermoderate water deficit condition. Intensification of SC due to theapplication of P (Motalebifard *et al.*, 2013) caused higher loss of leaves moisture and reduced leaf RWC. Therefore, the first level of soil moisture and Zero P application had the highest leaf RWC.

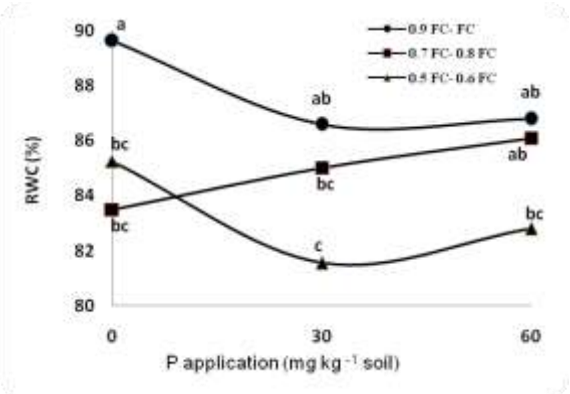

Fig. 6. Relative water content (RWC) in relation to different soil moisture and P treatments (n = 9) at $p \leq 0.05$.

Antioxidant enzyme activities

The ANOVA showed that the main effects of soil moisture, Zn and P were significant for SOD and POX activity significantly affected by soil moisture and Zn main effects and Zn×soil moisture interaction. While none of factors was not significant effect on the CAT activity (Table 2). The highest activity of POX was obtainedwiththe application of 10 mg Z per kg of soil under severe water deficit condition (Table 2). Fig. 7 shows POX activity for different soil moisture and Zn combined treatments. The highest activity of POX was obtainedwith the application of 10 mg Zn per kg of soil under severe water deficit condition (0.5 FC–0.6).Means comparison of SOD under soilmoisture levels showed that maximum activity of SOD was resulted from full-irrigated condition and the lowest activity was obtainedunder severe water deficit condition.SOD activity was decreased by application of P and the minimum activity of SOD was obtained by application of 60 mg P per kg of soil (Table 3).

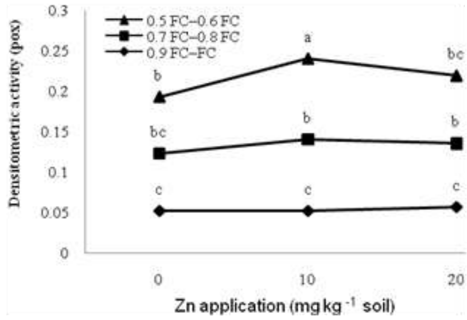

Fig. 7. Peroxidase (POX) in relation to different soil moisture and P treatments (n = 9) at $p \leq 0.05$.

Antioxidants enzymes such as SOD, POX and CAT are substantially knownto reduce the levels of superoxide and hydrogen peroxide in plants. SOD catalyses' the disputation of O_2 to molecular oxygen and H_2O_2. SOD is one of the most important enzymes that used against oxidative stress in the plant defense system and occurs ubiquitously in all types of cells. SODs areismetallic proteinsthat have different isoforms based on the metal species present at theirs active sites. Sun *et al.* (2010) showed that activity of POX and SOD followed an increase and reduction pattern in times of exposure to water deficit stress compared to normal irrigation condition.In another study, the activity of POX increased during the early stages of water deficit or heat or combination of these stresses. Therefore, it seems that water deficitstress prevented byincreasing the activity of POX to the extent possible the deleterious effects of reactive oxygen species in cell membranes (Jag tap and Bharagava, 1995). Our results on higher activity of POX under conditions of severe water deficit stress in potato were corresponded with the results of Pen *et al.* (2006), Terzi and Krdioglo (2006), Xi Yao *et al.*, (2009), and Lutfor Rahman *et al.* (2002).

Some researchers believe that Znshelters membrane proteins and lipid's against the free radicals and other products of the intracellular reduction reactions therefore causes cell membranes integrity. In addition, Zn in corporation with copper constitutes main part of superoxide dismutase enzyme as scavenging free radicals (Rion and Alloway, 2004). In the present study higher activity of POX was obtained with the application of 10 mg Z per kg of soil under severe water deficit that were corresponded with the results of Waraich *et al.* (2011), Cakmak (2000) and Morsy *et al.* (2012) that studied the role of micronutrients in plants under drought stress.

Lower SOD activity might be because of lower superoxide production by the Mahlerreaction due to keeping slight stomatal opening that resulted in resuming CO_2 fixation (Cruz de carvalho, 2008). Higher and lower activity of SOD was obtained under fullirrigated and severe water deficit condition, respectively. The results about reduction of SOD activity that obtained in this study were corresponded with the results of Benavides *et al.*, 2000 in potato, Cruz de carvalho (2008) in sunflower and Maria *et al.* (2002) in alfalfa.

Reports on CAT activity under drought stress are also heterogeneous. CAT activity has shown to increase (Mittler *et al.*, 2002; Gupta *et al.*, 2005) and also to remain unchanged or even decrease under water stress (Turkan *et al.,* 2005, Fu *et al.*, 2001). Furthermore, these authors have suggested that CAT is a less susceptible scavenging enzyme than ascorbate peroxidase (APX) regarding oxidative stress. Taken together these different reports seem to indicate that CAT activity is enhancedonly under severe water deficit stress. Whereas under moderate drought stress, H_2O_2 scavenging is made preferably by ascorbic acid through the ascorbate /glutathione cycle. In fact, CAT has a lower affinity for H_2O_2 than APX, which suggest its role in counteracting excessive H_2O_2 production (Cruz de carvalho, 2008).

Conclusion

The results showed that the drought stress resulted in a significant decrease in POX and SOD, stomatal conductivity, chlorophyll index and RWC. Application of P increased significantly the stomatal conductivity and SOD activity. Application of Zn also affected significantly thechlorophyll index, stomatal conductivity and POX activity. The P×soil moisture and Zn×soil moisture interactions were significant for stomatal conductivity and POX activity. The three-way interaction of P×Zn×soil moisture was significant for chlorophyll index, stomatal conductivity. The results showed that the two way interactions of Zn, P and soil moisture were mainly synergistic on above-mentioned attributes. The results showed that P requirement enhanced by increasing water deficit. Under deficient P condition, the higher SOD activity and lower peroxidation degree of cell membrane system play important roles in improving the photosynthesis of the potato with high-P efficiency. In general, to achieve the optimum growth of potato in similar soils, application of 20 mg Zn and 30 mg P perkgdry soilwould be recommended under normal irrigation conditions while at water deficit conditions applicationof 20 mg Zn and 60 mg P perkgdry soil could be recommended.

References

Ahmadi SH, Andersen M, Plauborg F, Poulsen RT, Jensen CR, Sepaskhah AR, Hansen S. 2010. Effects of irrigation strategies and soils on field-grown potatoes: yield and water productivity. Agricultural Water Management **97**, 1923–1930.

Anderson MD, Prasad TK, Stewart CR. 1995. Changes in isozyme profiles of catalase, peroxydase and glutathione reductase during acclimation to chilling in mesocotyls of maize seedling. Plant Physiology **109**, 1247-1257.

Beauchamp C, Fridovich I. 1971. Superoxide dismutase: Improved assays andan assay applicable to acrylamide gels. Anal Biochemistry **44**, 276-287.

Benavides MP, Marconi PL, Gallego SME, Tomaro ML. 2000. Relationship between antioxidant defence systems and salt tolerance in *Solanum tuberosum*. Australian Journal of Plant Physiology **27**, 273-278.

Borzooei A, Khazaie HR, Shariari F. 2006. The impact of post and thesis drought stress on physiologycal characteristics and antioxidant enzymes in different wheat cultivars. Agricultural Science and Technology **20(5),** 65-75.

Bradford M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72**, 248–254.

Cakmak I. 2000. Possible roles of zinc in protecting plant cells from damage byreactive oxygen species. New Physiology **146**, 185–205.

Cruz de carvalho MH. 2008. Drought stress and reactive oxygen species.Plant signaling & behavior **3**, 156-165.

Dalloway BJ. 2008. Zinc in Soils and Crop Nutrition, 2nd ed. IZA and IFA, France.

Darvish-Balochi M, Paknazhad F, Kashani A, Ardakani MR, darvish-Balochi M. 2010. Effect of Drought Stress and some Microelements on Fluorescence Chlorophyll Parameters, Chlorophyll Content, RWC, EC, and Grain Yield in Corn (SC704). Iranian Journal of Crop Science **41(3),** 541-533. (In Farsi)

FAO. 2010. FAOSTAT. Food and Agriculture Organization of the United Nations. Available in: http://faostat.fao.org/countryprofiles/

Fleisher DH, Wang Q, Timlin DJ, Chun JA, Reddy VR. 2012. Response of potato gas exchange and productivity to phosphorus deficiency and carbon dioxide enrichment. Crop Science **52**, 1803–1815.

Flexas J, Medrano H. 2002. Drought-inhibition of photosynthesis in C3 plants, stromal and non-stromal limitations revisited. Annales Botanici **89,** 183-189.

Fu J, Huang B. 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environmental and Expermental Botany **45**, 105-14.

Gaber MA. 2010. Antioxidative defense under salt stress. Plant signaling and Behavior **5**, 369-374.

Gupta KJ, Stoimenova M, Kaiser WM. 2005. In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, *in vitro* and *in situ*. Journal of experimental botany **56**, 2601–2609.

Huaqi W, Bouman BAM, Zhao D, Changgui W, Moya PF. 2002. Aerobic rice in northern China: Opportunities and challenges.Workshop on water wise rice production, 8-11 April IRRI, Los Banos, Philippines.

Ierna A, Mauromicale G. 2012. Tuber yield and irrigation water productivityin early potatoes as affected by irrigation regime. Agricultural Water Management **115**, 276–284.

Jagtap V, Bharagava S. 1995. Variation in antioxidant metabolism of drought tolerant and susceptible varieties of *Sorghum biolcolor* L. Agricultural Biotechnologe **65**, 445-454.

Jefferies RA. 1995. Physiology of crop response to drought. In: Haverkort, A.J., MacKerron, D.K.L. (Eds.), Potato Ecology and Modeling of Crops under Conditions Limiting Growth. Kluwer Academic Publishers, Dordrecht pp, 61–74.

Khan HR, McDonald GK, Rengel Z. 2004. Zinc fertilization and water stress affectsplant water relations, stomatal conductance and osmotic adjustment in chickpea (*Cicer arientinum L.*). Plant Soil **267,** 271–284.

Kirkham MB. 2004. Principles of Soil and Plant Water Relations.Elsevier Academic Press Publications, USA.

Kudoyarova G, Veselova S, Hartung W, Farhutdinov R, Veselov D, Sharipova G. 2011 Involvement of root ABA and hydraulic conductivity in the control of water relations in wheat plants exposed to increased evaporative demand. Planta **233**, 87-94

Lutfor Rahman SM, Mackay A, MesbahUddin ASM. 2002. Superoxide Dismutase Activity, Leaf Water Potential, Relative Water Content, Growth and Yield of a Drought-Tolerant and a Drought-Sensitive Tomato (*Lycopersicon esculentum* Mill.) Cultivars Plant Science **54**, 16-22.

Maria C, Esther R, González M, Minchin FR, Judith K, Arrese-Igor WC, Ramos J, Becana M. 2002. Effects of water stress on antioxidant enzymes of leaves and nodules of transgenic alfalfa overexpressing superoxide dismutases. Physiologia Plantarum **115**, 531–540.

Marschner H. 1995. Mineral nutrition of higher plants. (2[end]ed). Academic Press, USA.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science **7**, 405-410.

Morsy AA, Salama KH, Kamel HA, Mansour MM. 2012. Effect of heavy metals on lasma membrane lipids and antioxidant enzymes of Zygophyllum species. Eurasia of Biosciences **6**, 1-10.

Motalebifard R, Najafi N, Oustan S, Nyshabouri MR, Valizadeh M. 2013. Thecombined effects of phosphorus and zinc on evapotranspiration,leaf water potential, water use efficiency and tuber attributes of potato under water deficit conditions. Scientia Horticulturae **162**, 31-38.

Olson PD, Varner JE. 1993. Hydrogen peroxidase and lignification. Journal of plant **4**, 887-892.

Pan Y, Wu LJ, Yu ZL. 2006. Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of potato (*Solanum tuberosum*. L). Plant Growth Regulation **49**, 157-165.

Radin JW. 1984. Stomatal Responses to Water Stress and to Abscisic Acid in Phosphorus-Deficient Cotton Plants. Plant Physiology **76(2),** 392-394.

Raghothama KG. 1999. Phosphate acquisition. Annu. Plant Molecular Biology **50**, 665-693.

Riahinia S, Kocheki AR, Nassiri- Mahallati M. 2006. Canopy temperature in relation to plant water potential in four crop Species. Agricultural Science and Technology Journal **20(7),** 155-162. (In Farsi)

Rion B, Alloway J. 2004. Fundamental aspectsof Zinc in soils and plants. International ZincAssociation 1-128.

Sairam RK, Rao KV, Srivatava GC. 2002. Differntial response of wheat genotypes to long-term salinity stress on relation to oxidative stress, antioxidant activity and amolyte concentration. Plant Sciences **163**, 1037-1046.

Samarah NH, Mullen RE, Cianzio SR, Scott P. 2006. Dihydric-like proteins in soybean seeds in response to drought stress during seed filling. Crop Science **46**, 2141–2150.

Sekiya N, Yano K. 2008. Stomatal density of cowpea correlates with carbon isotope discrimination in different phosphorus, water and CO2 environments. New Phytologist **179(3),** 799-807.

Sun C, Du W, Chang X, Xu X, Zhang Y, Sun D, Shi J. 2010. The effects of drought stress on the activity of phosphatase and its protective enzymes in pigweed leaves. *African journal of* Biotechnology **9**, 825-833.

Terzi R, Krdioglo A. 2006. Drought stress tolerasce and the antioxidant enzyme system in Ctenanthe Setosa. Acta Biologica Botanica **48**, 89-96.

Turkan I, Bor M, Ozdemir F, Koca H. 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. Acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Science **168**, 223-31.

Valizadeh M, Mohayeji, M, Yasinzadeh N, Nasrullahzadeh S, Moghaddam M. 2011. Genetic diversity of synthetic alfalfa generates and cutivars using tetrasomic inherited allozyme markers. Journal of Agricultural *Science and Technology* **13**, 425-430.

Waraich EA, Amad R, Ashraf MY, Ahmad M. 2011.Improving agricultural water use efficiency by nutrient management. Journal of Plant Physiology **61 (4),** 291-304.

Welch R, Webb MJ, Loneragan JF. 1982. Zinc in membrane function and itsrole in phosphorus toxicity. In: Scaife, A. (Ed.), Proceedings of the Ninth Plant Nutrition Colloquium. CAB International, Warwick, UK; Wallingford, UK pp, 710–715.

Westigate ME, Grant DT. 1989. Effect of water deficits on seed developmentin soybean.I tissue water status.Plant Physiology **91**, 975-979.

XiYao W, Zou X, Wang J. 2009. Drought tolerance enhanced by phosphorus deficiency in potato plants. Zhongguo Zuo Wu XueHui **35**, 875-883.

Yasin-Ashraf M, Ala SA, Batti A. 1998. Nutritional imbalance in wheat (*Triticum aestivum* L.) genotypes grown at soil water stress. Acta Physiolologiae Plantarum **20**, 307-310.

Yuan BZ, Nishiyama S, Kang Y. 2003. Effects of different irrigation regimeson the growth and yield of drip irrigated potato. Agricultural Water Management **63**, 153–167.

Zhang J, Davies WJ. 1989. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant, Cell and Environment **12**, 73-810.