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Effects of soil moisture, phosphorus and zinc on isoenzymes activity and banding patterns of peroxidase in potato plant

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Abstract

Water deficit stress is a major abiotic factor that limits crop production. Hence plant Nutrition can have play-determining role in moderating the adverse effects of water deficit stress. This research was conducted as a factorial experiment based on randomized complete blocks design with zinc (Zn) at three levels (0, 10 and 20 mg Zn per kg dries soil as $ZnSO_4{\cdot}7H_2O$), phosphorus (P) at three levels (0, 30 and 60 mg P per kg dry soil as Calcium (Ca) $(H_2PO_4)_2{\cdot}H_2O$) and soil moisture at three levels (0.5FC-0.6FC, 0.7FC-0.8FC and 0.9FC-FC) using three replications under greenhouse conditions. The results showed that the moderate (0.7FC-0.8FC) and severe water deficit conditions (0.5FC-0.6FC) decreased significantly activity of peroxidase isozymes (POX) than to the enzyme activity in full irrigated (0.9FC-FC) conditions ($P < 0.01$).The higher activity of peroxidase isozymes appeared in POX_1 under the moderate water deficit condition and the lowest related to POX_5 isozyme under severe water deficit condition. In addition, the main effect of Zn and two way interaction of Zn × soil moisture were significant on the enzymatic activity of POX_2, POX_3 and POX_4 isozymes. The highest activity of peroxidase isozymes resulted for POX_2 at application of10 mg Zn per kg of soil. The two ways interaction of soil moisture × Zn for POX_3, POX_2 and POX_4 showed that the effect of Zn application on these esozymes were significant only under severe water deficit condition the highest activity of POX2 and POX3 were obtained at application of 10 mg Zn per kg dried soil and for POX_4 under using of 20 mg Zn per kg soil condition.

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Introduction

Water deficit is one of the most prevalent manifestations of abiotic stress in plants. Water deficit is the major yield-limiting factor of crop plants and it's actively and continuously determines the natural distribution of plant species. Water deficit exacerbates the effect of the other abiotic or biotic stresses which plants are submitted and effect of several different abiotic stresses (like salt and cold stresses) tense in water stress condition (Cruz de carvalho, 2008). Among these the water deficit is a major abiotic factor that limits crop production (Reddy *et al.*, 2004). Water deficit may increase the formation of free radicals of oxygen. These reactive oxygen species (ROS) involve superoxide ($O_2^•$), hydroxyl radical ($OH^•$), hydrogen peroxide (H_2O_2), and single oxygen ($O_{1/2}$). ROS are highly reactive to membrane lipids, protein and DNA. It is believed that ROS are the major contributing factors to stress injuries and to cause rapid cellular damage, particularly when plants are exposed to stress conditions (Gupta *et al.*, 2005). In order to protect cell membranes and organelles from ROS damaging effects, plants are equipped with an antioxidant system. This system consists of low molecular weight antioxidants such as ascorbate, glutathione, α _tocopherol and carotenoids, as well as several enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), and glutathione reductase (GR) (Gaber, 2010).

Peroxidase isoenzymes are hem groups contain (iron), which catalyzes the conversion of hydrogen peroxide to water and oxygen and have Important role in clearing the toxic effects of hydrogen peroxide in cells (Gaber, 2010). There is POX on all cellular components producing ROS, s and more stretch are to it (at the micro-molar) and with specific delicacy to a sustainable level ROS regulates, purposes signal transduction (Cruz de carvalho, 2008).

Zinc (Zn) deficiencies is one of the most widespread deficiencies of nutrients in plant at all part of the world, especially in arid and semi-arid climate because of alkaline soils and higher phosphorus availability (Cakmak, 2000). Zinc has structural role inside the important enzymes like Carbonic anhydrase, alcohol dehydrogenase, superoxide dismutase, alkaline phosphatase, phospholipase and carboxyl peptidase and activates a number of enzymes such as dehydrogenases, aldolases and isomerizes. Zinc plays an exclusive role in DNA replication, transcription and regulation of gene expression (Coleman, 1992; Vallee and Falchuk, 1993). Due to its main role in the activation of gene expression, perhaps zinc in gene expression induced by oxidative stress and production of anti-oxidant enzymes such as superoxide dismutase, peroxidase, glutathione reductase is involved in detoxification of hydrogen peroxide (Cakmak, 2000).

Phosphorus is the second important nutrient that required by plants. It is an essential component of nucleic acids, phosphorylated sugars, lipids and proteins, which control all life processes (Raghothama, 1999). Plants phosphorus uptake reduces in water deficit condition. Phosphorus Consumption or utilization of methods with higher phosphorus uptake can be useful in reducing the destructive effects of water deficit (Yuncai and Schmidhalter, 2006).

Limited studies have been conducted on the combined effects of Zn and P on peroxidase activity especially under water deficit condition. The objectives of this study were to evaluate (a) effect of soil moisture on peroxidase activity and banding pattern, (b) ability of phosphorus and zinc on prevention of oxidative stress in potato under with or without water stress conditions.

Materials and methods

Plant material and stress of treatment

A pot experiment was conducted in a naturally lighted greenhouse during 2012 at Agricultural Research Station of Tabriz University, Iran. The experiment was arranged as factorial 3 × 3 × 3 with n = 3 based on randomized complete block's design with three factors, including Zn at three levels of 0, 10 and 20 mg Zn per kg of soil as $ZnSO_4•7H_2O$, P at three levels of 0, 30 and 60 mg P per kg of soil as Ca $(H_2PO_4)_2•H_2O$ (mono calcium phosphate), and soil moisture at three levels of 0.5FC-0.6-FC, 0.7-FC-0.8-FC and 0.9-FC-FC in three replications that all of these factors plants cultivated at same time (04/26/2013) with total 81 pots. Soil moisture levels were applied at flowering period for 21 days. The physic-chemical properties of used soil were described previously by Motalebifard *et al.* (2013).

Enzyme extraction and electrophoresis

The crude extracts of fresh and healthy leaves from adult plants were prepared with separate mortars and pestles in a Tris-HCl extraction buffer with pH 7.5 (Tris 50 mM, sucrose 5%, ascorbic acid 50 mM, sodium metabisulfite 20 mM, PEG 2% and 2-Mercaptoethanol 0.1%) as described by Valizadeh *et al.*, (2011) with a ratio of 0.5 mg µl-1 (1W:2V) and centrifuged at 10,000 rpm under 4ºC and for 10 minutes using small Eppendorf tubes. Enzyme extracts were immediately absorbed onto 3×5 mm wicks cut from What man 3 mm filter paper and loaded onto 7.5% horizontal slab polyacrylamide gels (0.6×15×12 cm) using TBE (Tris-Borate-EDTA) electrode buffer (pH= 8.8). Electrophoresis was carried out at 4ºC for 3 hours (constant current of 30mA, and voltage of 180V). Peroxidase isoenzymes were analyzed in this study. The gel slabs rifted horizontally and stained based on protocol that was performed for POX by Olson and Varner (1993). The gels were fixed and scanned immediately after staining.

Statistical analysis

A factorial experiment on the basis of completely randomized design was carried out. An image analysis program (MCID1 software) was used to measure D×A (optical density × area) parameter for each isozymic band to evaluate the activity on to 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≤0.05* probability level was applied to compare the mean values of measured attributes. The Excel software (Excel software 2007, Microsoft Inc., WA, USA) was used to draw Figures.

Results

Considering to Fig. 1 five isozymes was observed for the POX enzyme. Under water deficit stress conditions (0.5FC-0.6FC, 0.7FC-0.8FC), POX isozymes generaly had further activity than what was observed under full irrigated (0.9-FC-FC) conditions (Fig. 1). Fig. 2 shows an example of the two pots with cultivated potato that growth under different conditions of soil moisture, Zn, P. Based on this figure, the growth of plant reduced by increasing water deficit (Fig. 2).

The analysis of variance (ANOVA) showed that the main effect of soil moisture were significant for POX_1, POX_2, POX_3, POX_4, and POX_5 at 0.01 probability level. The main effect of zinc and two way interactions of Zn × soil moisture were significant for POX_2, POX_3 and POX_4 at mostly 0.05 probability level (Table 1). The POX isozymes activity were not affected significantly by the main effect of P, two way interaction of P × soil moisture and three way interaction of factors (Table 1).

The means Comparison of POX isozyme activity under different Zn, P and moisture levels are summarized in Table 2. According to Table 2 the higher peroxidase activity related to POX_1 under the moderate water deficit conditions (0.7FC-0.8FC), whereas, POX_5 showed the Minimum activity at severe water deficit conditions (0.5FC-0.6FC). The activity of POX_1, POX_2, POX_3 and POX_4 isoenzymes increased 48, 43, 29, 17, and 43, 40, 17, 22 percent under moderate and severe water deficit conditions compared to full irrigated condition, respectively. The activity of POX_5 had totally opposite trend under stress conditions. The activity of POX_5 increased 14 percent under moderate water deficit condition but surprisingly decreased 21 percent at severe water deficit condition compared to full irrigation.

The effect of soil moisture × Zn interaction on POX_2 is shown in Fig. 3. Zn had no significant effect on POX_2 isozymes activity under full irrigated and moderate water deficit conditions. While under severe water deficit condition Zn usage affected significantly the enzymatic activity of POX_2 and the most enzymatic activity is obtained when zinc applied in 10 mg Zn per soil kg of dried soil.

Table 1. Summary analysis of variance (mean squares) densitometric activities of peroxidase isozymes in potato.

Source of variation	DF	POX_1	POX_2	POX_3	POX_4	POX_5
Replication	2	0.147^{**}	0.791^{**}	0.252^{**}	0.195^{**}	0.065^{**}
Moisture	2	0.301^{**}	0.721^{**}	0.126^{**}	0.112^{**}	0.376^{**}
Zn	2	0.02^{ns}	0.185^{*}	0.051^{*}	0.198^{**}	0.004^{ns}
P	2	0.009^{ns}	0.031^{ns}	0.007^{ns}	0.031^{ns}	0.01^{ns}
Zn × moisture	4	0.014^{ns}	0.122^{*}	0.034^{*}	0.057^{*}	0.008^{ns}
P × moisture	4	0.042^{*}	0.045^{ns}	0.024^{ns}	0.039^{ns}	0.012^{ns}
Zn × P	4	0.01^{ns}	0.081^{ns}	0.026^{ns}	0.005^{ns}	0.005^{ns}
Zn × P × moisture	8	0.034^{ns}	0.023^{ns}	0.018^{ns}	0.008^{ns}	0.029^{ns}
Error	52	0.017	0.042	0.013	0.018	0.012
Coefficient of variation (%)		24.97	27.71	21.05	20.42	24.99

Zn: Zinc; P: phosphorus; ns, non-significant; * Significant at $p \le 0.05$.; ** Significant at $p \le 0.01$.

The quality of soil moisture × Zn interaction for POX_3 was also almost like the POX2 (Fig. 4). This means that only in severe water deficit condition, the application of 10 mg Zn per kg soils caused a significant increase in POX_3 enzyme activity. The means Comparison for POX_4 in relation to combined soil moisture and Zn treatments are shown in Figure 5. The interaction was same as the POX2 and POX3 variations under soil moisture × Zn two ways interaction. Again it can be seen which the difference between POX_4 means under the three levels of Zn effects were significant only in severe water deficit condition but Unlike POX_2 and POX_3 the highest amount of POX_4 isoenzyme obtained with application of 20 mg Zn per kg of soil.

Table 2. The mean densitometric activity of isozymes POX (Gram per of fresh weight) in conditions levels of water stress.

Soil moisture	POX_1	POX_2	POX_3	POX_4	POX_5
0.9 FC–FC	0.029b	0. 055b	0. 047b	0. 057b	0. 04b
0.7 FC–0.8 FC	.0.058a	0. 079a	0. 061a	0. 067a	0. 056a
0.5 FC–0.6 FC	0.056a	0. 077a	0. 055a	0. 069a	0. 033a
The rate of increase or decrease in the average stress conditions than to normal mode (%)	48+	**+ 43**	**+ 29**	**+ 17.5**	+14
The rate of increase or decrease in the severe stress conditions than to normal mode (%)	+43	+40	+17	+22	-21

Two ways Interaction of P × soil moisture for densitometric activity of is shown in Figure 6. Application of phosphorus showed significant difference in activity of POX_1 isozyme under full irrigated conditions and enzymatic activity of POX_1 under without P application was significantly higher than the values of 30 and 60 mg P per kg of soil. However, the application of phosphorus had no significant effect on POX_1 isozyme activity under severe and moderate water deficit conditions.

Discussion

Sun *et al.* (2010) showed that activity of isoenzymes of peroxidase (POX) conform from an increase and reduction pattern when exposed to water stress and under severe and prolonged water deficit stress conditions occurs reduction of mentioned enzyme activity. In present study, study was observed coincides with the increase in activity, isozymes POX_1, POX_2, POX_3 and POX_4 than normal water stress conditions and reduction of activity of isozyme POX_5 than the normal stress conditions. In another study, it was observed that POX activity increased during the early stages of water deficit, heat, or combination of these imposing. However when stress was prolonged, the POX enzyme activity was reduced.

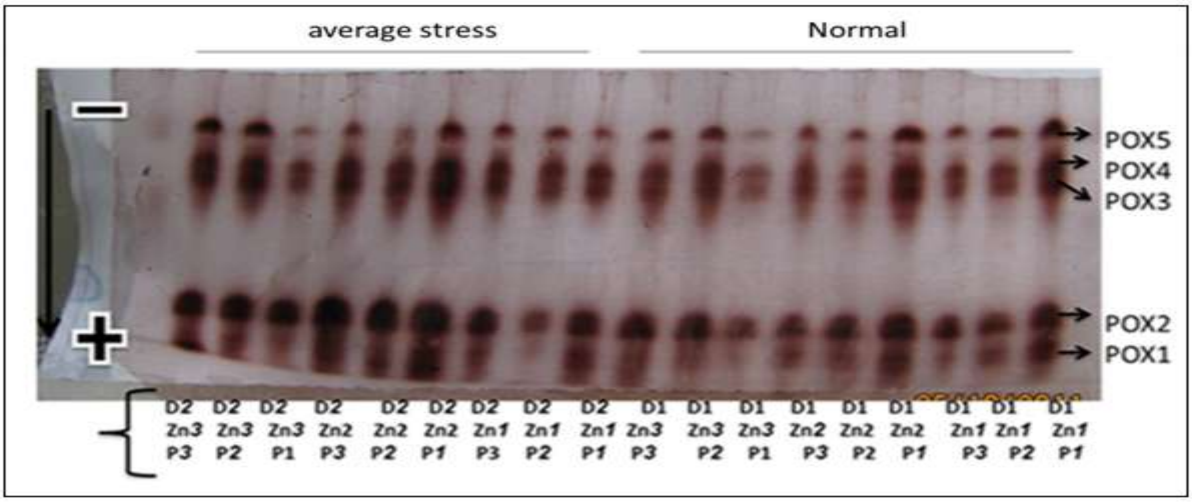

Fig. 1. The naming of peroxidase isozymes in plants potatoes.

Fig. 2. A sample of the potato plants growth under stress of Zn, P and drought.

Therefore it seems that the adverse effects of water deficit such as cell membrane injuries slaked by increasing the activity of POX (Jagtap and Bharagava, 1995). Contradictory responses have been reported to water deficit stress by the POX isoenzymes (Zhang *et al.*, 2004). The results (increasing activity of POX isozyme under water deficit conditions) corresponded with the results of Pen *et al.* (2006), Terzi and Krdioglo (2006), XiYao *et al.* (2009), and Lutfor Rahman *et al.* (2002). Conflicting result is obtained over the years that this difference could be reason's age or developmental stage of the plant duration and severity of treatment water deficit stress be attributed against the water deficit tolerance strategies.

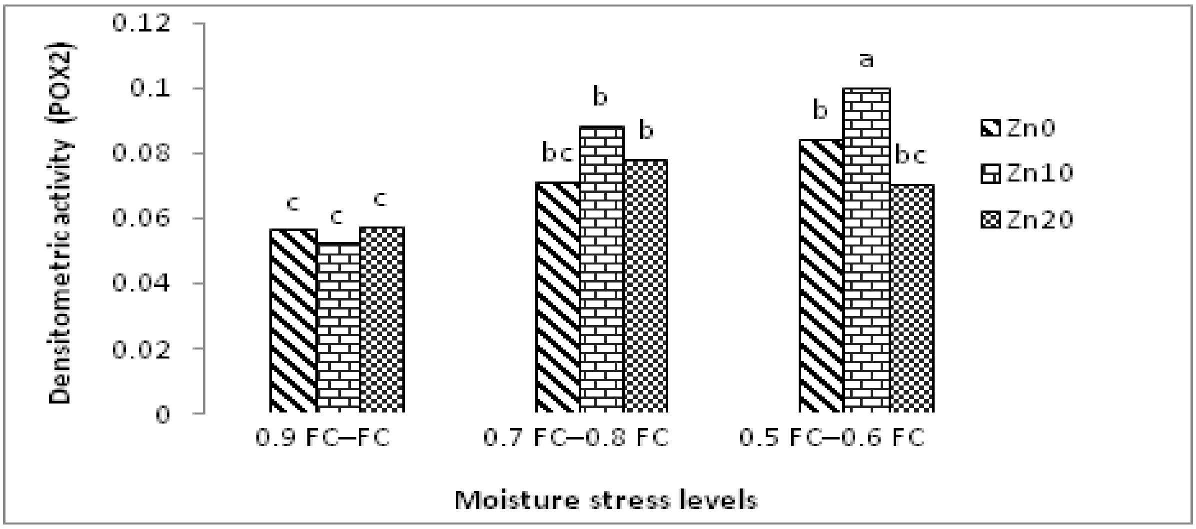

Fig. 3. The mean of densitometric activity of POX_2 under water stress treatment combination and Zn at probability level of 5%.

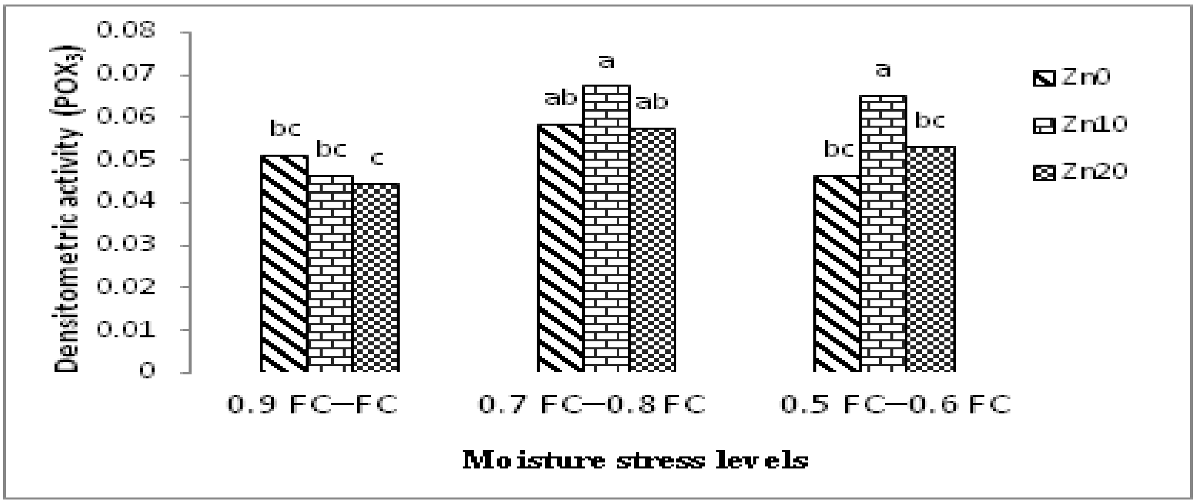

Fig. 4. The mean of densitometric activity of POX_3 under water stress treatment combination and Zn at probability level of 5%.

Despite this some authors have reported a significant correlation between the degree of water deficit tolerance and induction of antioxidant system in some species or genus (Cruz de carvalho, 2008). Some researchers believed that zinc protected membrane proteins and lipid's against the adverse effects of free radicals and other products of the intracellular reduction reactions that resulted in maintain cell membranes integrity In addition zinc in corporate with copper had main role in superoxide dismutase enzyme structure as scavenging free oxygen radicals (Rion, and Alloway, 2004). In the present study addition of 10 Zn mg per kg of dry soil increased the activity of POX_3 and POX_2 isozymes under severe water stress conditions. While for POX_4 this affluence was occurred by application of 20 mg Zn per kg of soil. The results showed that the higher amounts of antioxidant enzymes induced by Zn may as a secondary defense mechanism against oxidative stress. The results of this study was in agreement with results of Waraich *et al.* (2011), Cakmak (2000) and Morsy *et al.* (2012) that examined the role of micronutrients in plants under drought stress. Phosphorus is essential for storage and transfer processes of energy, photosynthesis, regulating the activity of enzymes and transport of carbohydrates. The higher availability of phosphorus in soil could be useful under water deficit stress. Garg *et al.* (2004) noted that the phosphorus deficiency was one of the first effects of mild to moderate water deficit stress in plants. So, the application of phosphorus fertilizer could markedly improve plant growth in such conditions. In the present study, the phosphorus deficiency (P_0) was ability for upraising of peroxidase isozymes activity (POX_1) that by application of phosphorus (30 or 60 mg P per kg of dry soil) reduced POX activity. However, the significant deference in peroxidase (POX_1) activity was not observed by P levels imposing in moderate and severe water deficit conditions.

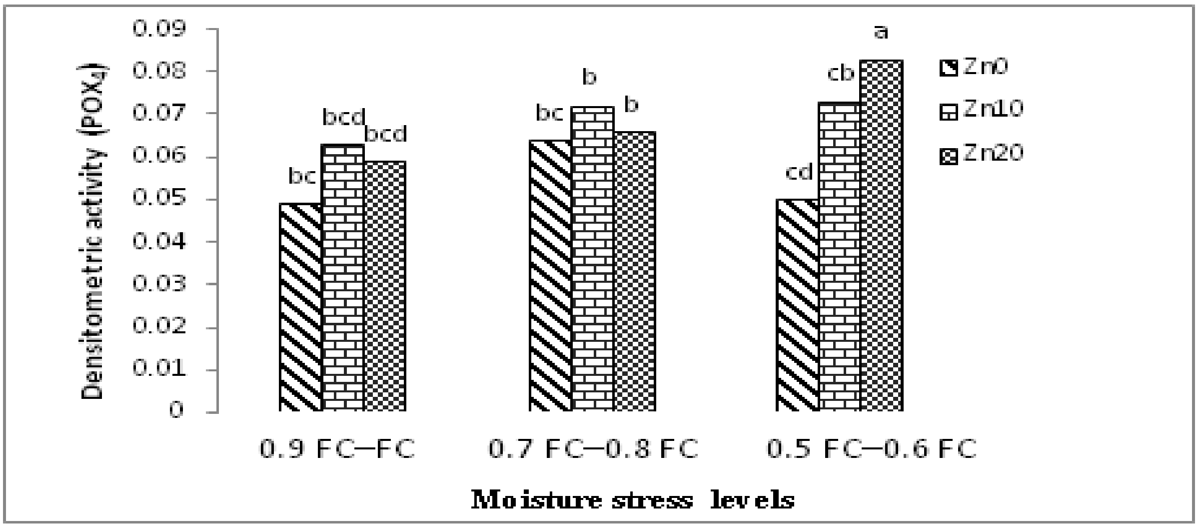

Fig. 5. The mean of densitometric activity of POX_3 under water stress treatment combination and Zn at probability level of 5%.

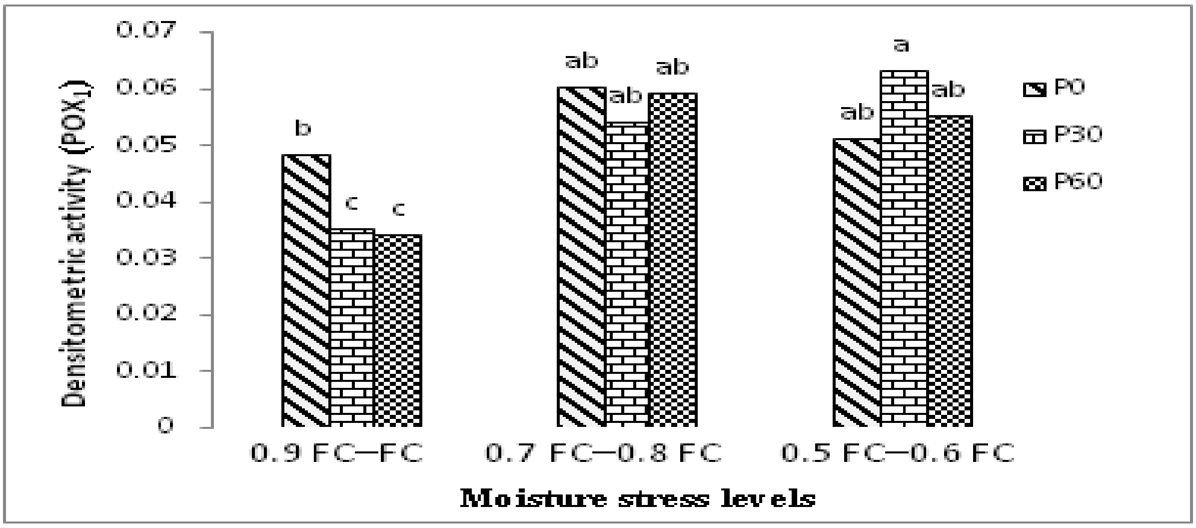

Fig. 6. The mean of densitometric activity of POX_1 under water stress treatment combination and P at probability level of 5%.

Conclusion

XiYao *et al.* (2009) in review on the potato plant were reported that phosphorus requirement enriched in water stress conditions to two times than normal conditions. significant increase in POX isozymes activity under phosphorus deficiency have been reported by Ahn *et al.* (2005) in tomatoes, Han *et al.* (2007) in rice, Jin *et al.* (2010) in soybean which were in agreement with present studies results.

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