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Evaluation the Efficiency of Some Egyptian Wheat *Triticum aestivum* L. Cultivars to Zn Deficiency through Peroxidase Activity and Protein Profile Techniques

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Abstract

Zinc (Zn) is an essential micronutrient for plants. The ability of plants to maintain significant yields under low zinc is called Zn efficiency (ZE). Eleven wheat (*Triticum aestivum* L.) (Banisuef 1, Gemiza 10, Gemiza 7, Giza 170, Giza 168, Sohag 3, Sids 7, Sids 5, Sids 1, Sakha 94, and Sakha 93) cultivars were grown under controlled environmental conditions in nutrient solution for 30 days to study the effect of varied supply of Zn (0 and 1 μM) on shoots, root dry production, shoot and root Zn efficiency, peroxidase activity. Also the peroxidase isozyme and protein on gel electrophoresis were investigated. Among all wheat cultivars Banisuef 1, Sohag 3 and Sakha 93 were less affected by Zn deficiency. Banisuef 1 showed the lowest value for shoot/root ratio under Zn deficiency. Peroxidase activity increased in Sohage 3 and Sakha 93. A new polypeptide with molecular weight 34 Kda was expressed in Banisuef 1, Gemiza 10, Gemiza 7 and Giza -170 under Zn deficiency. It can be concluded based on the experimental results that, for assessing wheat cultivars for resistance to Zn deficiency the POD enzyme activity together with protein profile could be considered as a better reliable techniques to select the resistance cultivars for zinc deficiency stress.

Keywords: Zn efficiency, isozyme protein, profile wheat, *Triticum aestivum* L., Zn deficiency

Introduction

Extremely micro-concentrations of heavy metals affect different cellular compounds, inter-fearing with the normal metabolic functions (Prasad, 2004). Zinc deficiency is recognized as one of the most critical micronutrient deficiency in plants grown on calcareous soils with high pH values. This is the reason for classification of the Zn deficienty as one of the limiting factors for crops yield production. There is significant genetic variation both within and among plant species in their ability to maintain significant growth and yield under Zn deficiency conditions; this has been called Zn efficiency (Graham and Rengel, 1993). Zinc efficiency (ZE) has been attributed to the efficiency of acquisition of Zn under low zinc availability conditions

It is well known that zinc is an important component of many vital enzymes, a structural stabilizer for proteins, membrane and DNA-binding proteins (Vallee and Auld, 1990). Deletion effects induced by Zn deficiency include the disturbances of metabolic processes such as change in the levels of several metaloenzymes activity was established (Erenoglu et al., 1999). Moreover, the lack of zinc proved to cause an overproduction of oxy-radicals (Zago and Oteiza, 2001). The generation of reactive oxygen species (ROS) such as superoxide anion radical (O_2), singlet oxygen (O_2), hydrogen peroxide (H_2O_2) and hydrogen radical (OH) can damage many cellular components including protein, membrane lipids so, plant cells response to the formation of reactive oxygen species (ROS) by increasing the production of metaloenzymes such as super oxide dismutase (SOD); EC 1.15.1.1, peroxidase (POD); EC 1.11.1.7. A close positive correlation between (POD) activity and tolerance of cultivars to Zn deficiency has been described by (Yu et al., 1999). Recently, it has been shown that zinc efficiency (ZE) in wheat is correlated with enhanced expression and activity of zinc - requiring enzymes (Singh et al., 2005).

The aim of present work was to evaluate the efficiency of wheat cultivars under zinc deficiency stress by using the changes in zinc efficiency, antioxidant enzyme activity POD and isozyme profile by using biochemical techniques.

Materials and method

Plant growth conditions and Zn treatments

Eleven cultivars of wheat (*Triticum aestivum* L.) abbreviated as Banisuef 1, Gemiza 10, Gemiza 7, Giza1 70, Giza 168, Sohag 3, Sids 7, Sids 5, Sids 1 Sakha 94, and Sakha 93 were sieved and 15 gram of each cultivar were used for germination in sand moistened with CaSO4 in dark at 18°C solution for 5 days as described by (Rengel et al., 1998). Ten uniformly sized seedlings were transferred into 1 litre polyethylene vessels containing an aerated nutrient solution. Seedlings were grown under controlled conditions, 16/8 h light /dark period at 20/22°C, relative humidity 50-60%, and light intensity 300 µmolm-2. Plants were grown in a

half - strength Hoagland and Arnon, (1950) control solution for the first 5 days. Seedlings were supplied with two levels of zinc as ZnSO4 (0 - 1 μM) for 30 days.

Plant analysis

Plant tissues (shoots and roots) were harvested after 30 days. Samples of each treatment were collected from all pots, mixed and reprehensive samples of shoots and roots of different plant cultivars were taken, separated and washed by bidistilled water, and then dried at 70°C. The shoot and root zinc efficiency were calculated according to the method of Grewal et al., (1997):

Zn efficiency = organ (shoot or root)*[Dry weight of (- Zn)/Dry weight of (+Zn)]*100

Values of ZE above 100% reveal that the cultivar is more efficient compared with values of ZE below 100%.

Detrmination of antioxoidant enzyme activity

Assay of POD activity

Cytosolic fractions were separated according to the method used by Polar, (1976), and the supernatant was used for enzymatic analysis.

Leaf tissues of 0.5 gm were ground with 50 mM phosphate buffer. The homogenate was centrifuged at 13,000 g for 20 min and the supernatant was used for the determination of POD activity. Peroxidase activity was determined spectrophotometrically by increasing in the absorbance at 430 nm (Σ = 2.47mM−1 cm−1) using the method of (Amako et al., 1994). The reaction mixture contained 100 mM potassium phosphate buffer pH 6.8, 60 mM pyrogallol, 60 mM H2O2 and enzyme extract. Activity was measured by following the change of absorption at 430 nm due to pyrogalol oxidation.

POD isoenzyme detection

Native PAGE was performed for isoenzyme in vertical polyacrylamide gels with a discontinuous buffer system. Peroxidase (POD) was stained according to Stegmann et al. (1987).

Statistical analyses

The experimental determinations were completed three times. Statistical analysis were done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values < 0.05 were considered significant.

Electrophoresis SDS –PAGE- protein profile

Half gram of fresh leaf tissues was homogenized in 1 ml of sodium phosphate buffer (pH6.8), and centrifuged for 10 min at 10,000 g. The supernatant was used for the protein electrophoresis profiles according to Laemmli (1970) by using of 10% acryl amide in the separating gel and 3% in the stacking gel. The separation was carried out using EC mingle unit at 60 v/ 4hr. The gel was stained with Coommassie[1] brilliant blue R-250 and destained with 40% methanol in 10% acetic acid. Low molecular weight standard protein of Pharmacia 2 was used with samples for the determination of molecular weights of the polypeptides.

Results and discussion

Plant growth and Zn efficiency

The data presented in table (1) showed remarkable differences in Zn efficiency based on shoot and root dry weights in eleven wheat cultivars. It was found that the value of Zn efficiency ratio dependent on shoot dry weight (varied from 52% to 182%). Zn efficiency of ohag 3 cul-

Table 1 Dry weight and Zn efficiency for eleven wheat cultivars grown in nutrient solution in presence of zinc (+Zn) or absence of zinc (-Zn) as Zn SO4 for 30 days old

Cultivar	Shoot D.W g/pot		Shoot Zn efficiency %	Root D.W g/pot		Root Zn efficiency %	Shoot/Root ratio
	+Zn	- Zn		+Zn	-Zn		
Banisuef 1	4.60	5.05	110	11.33	14.79	131	0.34
Gemiza 10	4.87	6.26	129	4.80	6.20	129	1.01
Gemiza 7	3.73	3.81	102	4.30	5.30	123	0.72
Giza 170	5.86	6.48	123	7.28	8.31	114	0.78
Giza 168	6.60	6.40	97	11.1	8.50	77	0.75
Sohag 3	2.90	5.28	182	6.86	9.78	142	0.54
Sids7	11.97	6.24	52	4.40	5.30	120	1.17
Sids 5	4.83	4.77	99	4.83	4.77	99	1.00
Sids 1	4.55	4.28	94	15.40	7.63	50	0.56
Sakha 94	4.29	4.54	106	8.58	9.02	107	0.50
Sakha 93	4.40	4.55	103	15.08	9.67	64	0.47

1 Dye for staining proteins in sodium dodecyl sulphate poly acrylamide, gel electrophoresis.

tivar showed the highest value (182% and 142%). Whereas the lowest value for shoot ZE was detected in Sids 7 (52%). Sids 1, Sakha 93 and Giza 168 revealed the lowest value (50%- 64%-77%, respectively). The lowest value for S/R ratio (0.34) was obtained by Banisuef 1 cultivar. These results confirmed the results obtained by Schlegel et al., (1998) who concluded that the high value of Zn efficiency attributed to the tolerance to Zn deficiency in different wheat cultivars and can be used as a useful parameter for recognizing the efficiency of wheat cultivars differing in their response to zinc deficiency. Cakmak and Marschner, (1988) concluded that the high values for Zn efficiency is associated to the enhanced root uptake and shoots translocation.

2-Peroxidase activity (POD)

Since zinc deficiency caused oxidative stress in wheat leaves, the behaviour of the antioxidant enzyme activity (POD) was examined. Activity of POD was measured in leaves of eleven cultivars differing in ZE. Under zinc deficiency POD activity reduced by about 50% and 56 % for (Gemiza 10 and Giza 170) cultivars respectively (Table 2). POD, an important enzyme involved in antioxidation processes and present in different organelles in plants, catalyzes the superoxide radical conversion to H2O2. The decrease of the reduction percentage of POD activity for Sohag 3 and Giza 168 cultivars may be account for the accumulation of superoxide radicals (O2-) in zinc-deficient cultivars (Yu et al., 1999, and Wu et al., 1999).

Overproduction of ROS can lead to oxidative injury such as membrane lipid peroxidation, protein oxidation, enzyme inhibition. DNA and RNA damage (Mittler, 2002). POD is an important H2O2 detoxifying enzyme in plants. Our results showed that the activities of POD were reduced under zinc deficiency (Table 2). The decrease of the enzyme activity of POD in the leaves of Gemiza 10 and Giza 170 indicate that they may not able for detoxifying ROS under zinc deficiency (Sun et al., 2007). Therefore, POD activity might be used as a good tool for screening the efficient cultivars under Zn stress conditions.

4-POD isozyme

The response of POD activity for Zn deficiency was detected in leaves of eleven wheat cultivars using native polyacrylamide gel electrophoresis.

Peroxidase isoforms, which were detected in the cytosolic extract of leaves in wheat cultivars grown under zinc stress condition, were photographed and presented in (Figure 2).

The separation of POD isoforms has confirmed the data obtained through spectrometric analysis on the generalized reduction in POD activity in wheat cultivars. It is also interesting to note that under Zn-deficiency the electrophoretic POD isoenzyme bands showed differences in its mobility and intensity compared to Zn -sufficient treatment.

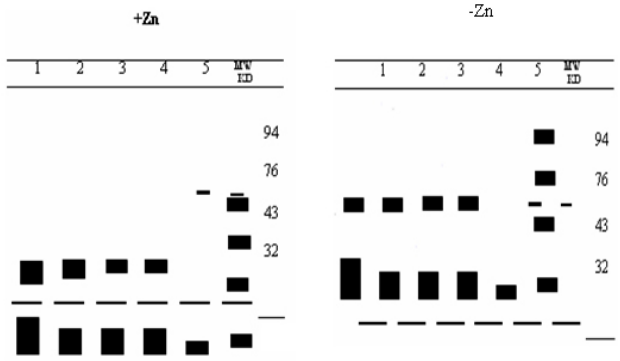

Figure 2 Protein patterns of wheat cultivars grown in nutrient solution in presence or absence of Zn for 30 days old.

Lane 1: Banisuef 1, Lane 2: Gemiza 10, Lane 3: Gemiza 7, Lane 4: Giza 170, Lane 5: Giza 168

Table 2 Peroxidase activity in leaves extract for eleven wheat Cultivars grown in nutrient solution in presence of zinc (+Zn) or absence of zinc (-Zn) as Zn SO4 for 30 days old

Wheat cultivars t	POD activity (Unit/mg protein/min) (- Zn)	%	POD activity (Unit/mg protein/min) (+ Zn)	Reduction %
Banisuef 1	30 ± 0.87a		35 ± 0.38b	14.28
Gemiza 10	40 ± 0.66b		80 ± 0.45a	50.00
Gemiza 7	45 ± 0.66b		75 ± 0.45a	40.00
Giza 170	35 ± 1.09a		9 ± 0.31b	56.00
Giza 168	75 ± 1.80b		78 ±2.50a	3.85
Sahag 3	75 ± 1.80b		77±2.50a	2.59
Sids 7	30 ± 1.34a		35± 0.60b	14.29
Sids 5	72 ± 2.08b		80± 0.40a	10.00
Sids 1	45 ± 1.08b		72± 0.63a	37.50
Sakha 94	45 ± 3.08b		80± 0.33a	43.75
Sakha 93	39 ± 1.60[b]		69± 0.40[a]	43.48

Data represent the mean ± S.E. of three experimental replicates; All values with the same letters are not significantly different at p< 0.05

It was observed that the bands of isoperoxidase of some cultivars were limited when grown under Zn deficiency when compared to control treatment 1μM zinc as Zn SO_4. Results of POD isozymes showed that, two fast bands appeared with (Rf 0.42 and Rf 0.68) in leaves of Banisuef 1cultivar not present in the other cultivars.

Under Zn deficiency a new slow band with (Rf 0.29, Rf 0.30, and Rf 0.32) was appeared in Gimiza 10, Sakha 93 and Gemiza 7. Sakha 94 showed a new slow band with Rf 0.24 with high intensity which disappeared under Zn sufficient treatment (1 µM).

The induction of new isozymes and the change in the isozyme profile in efficient cultivars Sohag 3 and Banisuef 1 may be considered to play an important role in the cellular defence against oxidative stress. Under zinc sufficient level Gemiza 10 represented one slow band with Rf 0.37. It is worth to note that Sohag 3 expressed one band with high intensity with Rf 0.24 not present in the other cultivars. It is important to emphasize that under zinc deficiency stress the induction of the new isozyme bands could be considered as a leaves reaction to metal-caused oxidative damage (Gokhan et al., 2003). Similar observation could be assumed for Banisuef 1 and Sakha 94 cultivars, the pattern indicating that the high activity of POD (distinct bands) could be related to Zn stress adoption of these cultivars.

Protein profile

Wheat cultivars responded to Zn stress by induction

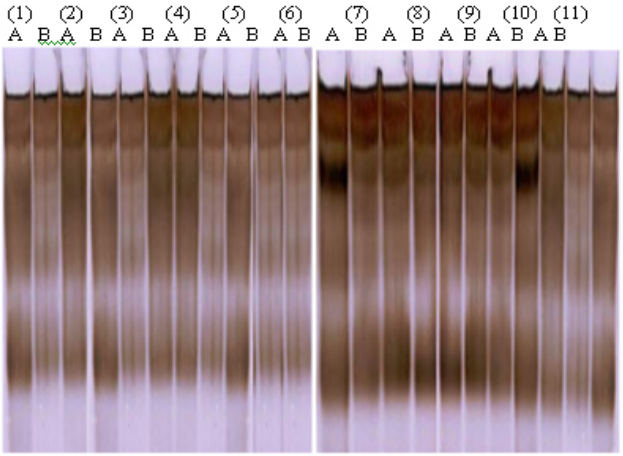

Figure1 Zymogram of POD activity staining of leaves extracts of eleven wheat cultivars A: without Zn (zero); B: with Zn 1 µM, 1: Banisuef 1; 2: Gemiza 10; 3: Gemiza 7; 4: Giza 170; 5: Giza 168, 6: Sohag 3; 7: Sids 7; 8: Sids 5; 9: Sids 1; 10: Sakha 94; 11: Sakha 93

and repression in synthesis of few polypeptides. The polypeptide profile of leaves protein showed slight differences between control and Zn deficiency plants. As a whole the inhibition of protein synthesis was reduced under Zn deficiency for all cultivars .Banisuef 1 cultivar showed the same protein patterns in the presence or absence of zinc. Two bands with high molecular weight (93 and 66 KDa) were expressed in Banisuef 1.Under zinc deficiency one band was appeared in Sohag 3 cultivar with molecular weight 91 KDa with high polymorphic bands and marked variation level of intensity compared to (+Zn) treatment (Figures 2 and 3).

One possible explanation for completely disappearance of some proteins is that the gene responsible for cer-

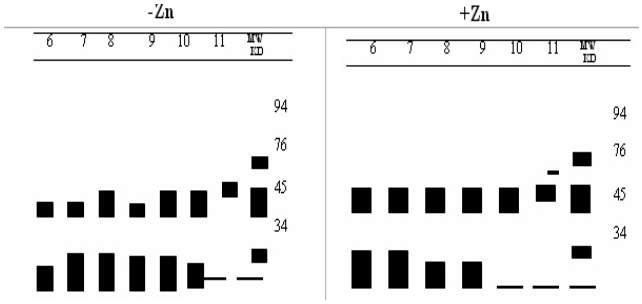

Figure 3 Protein patterns of wheat cultivars grown in nutrient solution in presence or absence of Zn for 30 days old.

Lane 6: Sohag 3, Lane 7: Sids 7, Lane 8: Sids5, Lane 9: Sids1, Lane 10: Sakha 94, Lane 11: Sakha 93

tain proteins had been completely suppressed as a result of zinc stress (Wu et al., 1999&Yu et al., 1999).

Several authors demonstrated that oxidatively modified proteins are selectively used as target substances for proteases and have proposed the use of proteins degradation as an index of oxidative stress (Yu et al., 1999). Another possible reason for the decrease of protein biosynthesis may be as a result of binding the metals to sulphyhdryl groups in proteins leading to an inhibition of activity or disruption of its structure (Wu et al., 1999). Under Zn deficiency one band was detected in Banisuef 1, Gemiza 10, Gemiza7 and Giza 170 with molecular weight 34 Kda not presence in other cultivars. The synthesis of the 34 Kda polypeptide in plasma membrane occurs only under Zn deficiency in Zn efficient wheat cultivars but not in Zn inefficient under Zn deficiency in Zn-efficient wheat cultivars (Rengel and Hawkesford, 1997) .They suggested that this polypeptide might be a structural or regulatory unit of a putative plasma membrane Zn transporter and may therefore be connected with the Zn uptake process. There is a promise in the use of such specific polypeptides for screening genotypes for Zn efficiency.

Conclusions

Based on the results obtained in this study it might be concluded that the changes in the POD activity, the induction of new POD isoformes and the protein profile appear useful reliable biochemical techniques for evaluation the efficiency of wheat cultivars under zinc deficiency stress.

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