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Some Antioxidant enzymes banding patterns and their correlation in common bean genotypes under water deficit stress

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ABSTRACT: To evaluate effects of water deficit on some antioxidant enzyme activities two experiments based on randomized complete block design were carried out under field conditions. In experiment one, plants irrigation cycle was each five days (normal condition) whereas in experiment two, once per 10 days (stress condition). In both experiments, 23 genotypes of common beans were studied. Catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) activities were studied via polyacrylamide gel (8%) electrophoresis. Three isozymes for POX and SOD and one isozymes for CAT were observed. There was significant difference for isozyme activities in both normal and water deficit conditions, indicating high genetic variations in common beans. According to the results, SOD and CAT activity levels were significantly increased in all genotypes in water deficit condition compared to normal irrigation, but POX has an increase and decrease pattern during stress. There was a significant and positive correlation between CAT and all isozymes of SOD and POX1 in both irrigation conditions. Furthermore significant correlations were observed between between some isozymes of SOD and POX in both irrigation conditions.

Keywords: Catalase, Common bean, Electrophoresis, Peroxidase, Superoxide dismutase, Water deficit.

INTRODUCTION

Annual production of common bean is approximately 230 million tons. It is one of the best crop product and allocated 1st place among the legumes (Emeterio Payro *et al.,* 2004). One of the greatest challenges of 21 century is to use lesser water for crop products (Bastiiaanssen and Makin, 2003). During the growth, plants face different kind of biotic and abiotic stresses. Among them, drought is much more harmful than other environmental stress, (Hasegawa *et al.,* 2000; Yamaguchi-Shinozaki *et al.,* 2002). Atmosphere contains 21% oxygen and because of coupoled orbitals it exists in stabletal form. In metabolic pathways, oxigen recieves one, two or three electrons and turns to supreoxide (O_2^-), hydrogen peroxide (H_2O_2) and other radicals, respectively in stressed plants (Beak and Skinner, 2003). Reactive oxigens are oxidant molcules and harmfull to plant cells In the nature, plant scavenge reactive oxygen species (ROS) using enzymatic and non-enzymetic systems. Enzyme system includes peroxidase (POX), catalase (CAT), supraoxide dismutase (SD) and a few others. Non enzyme pathway could be glutathione, ascorbic acid, tocopherol and other antioxidant substances (Gupta *et al.,* 2005). Catalase is an enzyme which scavenges hydrogen peroxides (Jiang and Huang, 2001).

POX using phenolic compounds act as donor of electron and scavenges H_2O_2 (Noctor and Foyer, 1998). Antioxidant enzymes especially CAT and POX have key role in reduction of ROS activities (Kuk *et al.,* 2003). The balance and cooperation between enzymes is important to control ROS in the plant while the insufficient levels of antioxidant enzymes leads to accumulation of hydrogen peroxide and changes to hydroxyl radicals (Blokhina *et al.,* 2003).

MATERIALS AND METHODS

This experiment was performed using 23 genotypes of common bean in Agricultural Research Farm of University of Tabriz, in 2012 (Table 1). Experimental design was based on randomized complete block design (RCBD) using three replications. One experiment performed under normal condition and second under water stress condition. All experimental plots were irrigated for two months. Then, to induce water deficiency in summer, in normal condition, the plots were irrigated once in a period of five days, while in stress condition, irrigation cycle was each 10 days period. When signs of stress were appeared in plants, green leaves were used to determine superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) enzyme activities.

Table 1. Numbers and codes of common bean genotypes.

Code	Number of genotype
1	41128
2	41158
3	41136
4	41164
5	41165
6	41116
7	41176
8	41214
9	41150
10	41154
11	41159
12	41396
13	21471
14	21177
15	21528
16	21249
17	21366
18	21158
19	21170
20	21538
21	21154
22	21153
23	21152

Enzymes extraction and electrophoresis

The fresh and healthy leaves from adult plants were squashed into mortar 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) with a ratio of 1 mg/µl-1 and centrifuged at 4ºC and 10,000 rpm for 10 minutes using small Eppendof tubes. Enzyme extracts were immediately absorbed onto 3*5 mm wicks cut from Whatman 3 mm filter paper and loaded onto 8% horizontal slab polyacrylamide gel (0.6*15*12 cm) using TBE (Tris-Borate-EDTA) electrod buffer (pH = 8.8). Electrophoresis was carried out at 4ºC for 3 h at constant current of 30 mA and voltage of 180V (Valizadeh *et al.*, 2013). An image analysis program (MCID software) was used to measure D*A (Optical density*Area) parameter for each isozymic band to evaluate the activity onto gels. For statistical analysis and relationship estimates between isozyme markers SPSS 20.0 software was used, after testing normal distribution of data.

RESULTS AND DISCUSSION

According to results, one isozyme for CAT and three isozymes for POX and SOD in common bean leaves have been identified (Fig. 1, 2 and 3). SOD and CAT isozyme's and POX2 activity were significantly increased in all genotypes of beans in water deficit condition compared to normal irrigation.

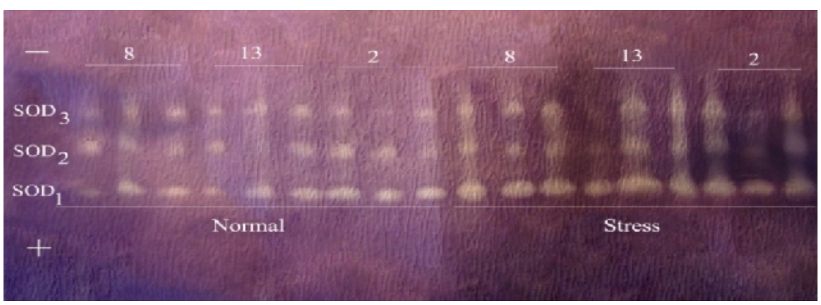

Fig.1. Example of leaf's SOD activities in different common beans at normal and stressed water deficit condition.

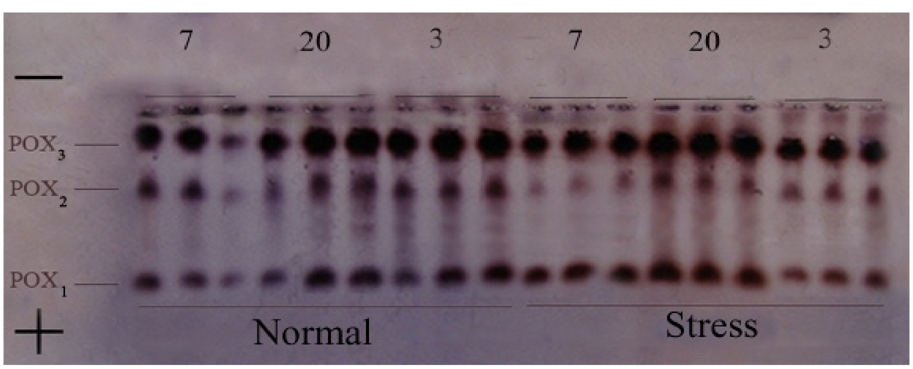

Fig. 2. Example of leaf's POX activities in different common beans at normal and stressed water deficit conditions.

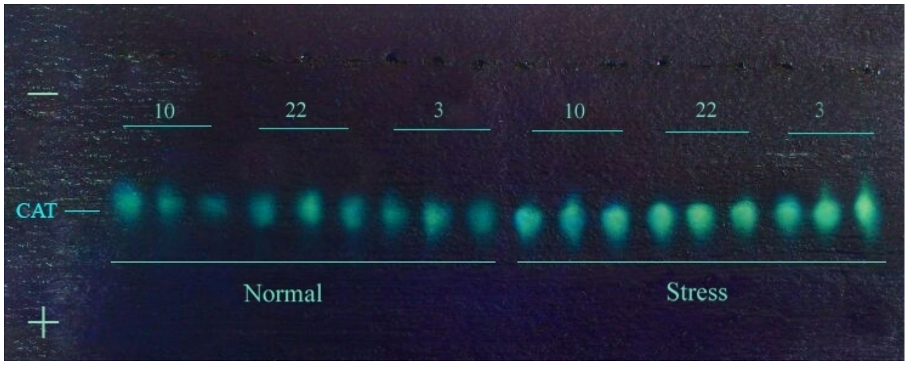

Fig. 3. Example of leaf's Catalase activities in different common beans at normal and stressed water deficit condition.

This increment was much more for CAT activity in 1th genotype. But, all enzymatic activity of SODs was high in 11th and 18th genotypes. These Antioxidant enzyme activities were higher in water deficit condition (Fig.1 and 3), While POX has an increase and decrease pattern during stress (Table 2). Previous studies demonstrated that there is a strong correlation between stress resistance and increase in antioxidant levels in photosynthetic plants (Sairam and Saxena, 2000; Sairam, and Srivastava, 2001, 2002). Lascano *et al.* (2005) reported that antioxidant enzyme activity increases up to 2 Fold under stress period in wheat cultivars. Also, it has been reported that drought induced-stress increases SOD activity in wheat (Badiani *et al.*, 1990), pea (Mittler and Zilinskas, 1994), bean (Turkan *et al.*, 2005), rice (Sharma and Dubey, 2005), olive tree (Sofo *et al.*, 2005). SOD in transgenic plants was able to increase resistance to stress in plant (Cruz de Carvalho, 2008). Sekmen *et al*, (2007) reported that CAT activity increases during salt stress in tomato and plantain. Controversial reports exist about catalase activity, it is reported that CAT activity increases just during severe drought stress.In other study mild drought stress, scavenging of H_2O_2 is performed by ascorbate-glutathione cycle (Cruz de Carvalho, 2008). However, depending on plant genotype and stress severity and duration, some decrements can be observed in antioxidant enzyme activities.

Table 2: Percent of increase or decrease in antioxidant enzymes in different genotypes of common bean.

POX3	POX2	POX1	SOD3	SOD2	SOD1	CAT	Genotypes
-21	64	23	64	37	80	153	1
48	26	23	86	45	37	61	2
14	10	-34	101	126	140	57	3
20	-12	41	54	53	81	43	4
17	-6	8	103	70	61	72	5
17	49	-20	72	90	89	32	6
-10	91	-10	91	94	109	91	7
-83	15	-17	40	27	26	27	8
398	-31	-31	33	14	20	25	9
12	40	13	164	164	236	57	10
-18	22	-47	275	281	221	60	11
21	49	39	84	122	71	14	12
175	258	42	42	38	89	60	13
-16	-64	-52	20	70	76	31	14
0	82	34	66	46	76	57	15
29	48	-8	41	37	41	36	16
46	181	145	50	45	58	69	17
5	18	-38	150	255	314	47	18
10	-46	24	270	138	256	92	19
33	10	-20	103	82	87	71	20
18	87	131	25	19	21	61	21
77	70	78	203	104	166	66	22
51	34	56	37	18	23	11	23

There are reports which claim there is correlation between resistance to oxidative stress and antioxidant enzymes activity during drought induced-stress (Zhang, and Davies, 1987). Researchers reported antioxidant enzymes levels 2 fold increased in stress and improved plant resistant against stress (Lascano *et al.*, 2005). Formerly, Zhang *et al.*, (2004) controversial response was observed in POX response to water deficit stress. During this condition, POX levels increases (Pan *et al.*, 2006; Terzi *et al.*, 2006; Fazeli *et al.*, 2006; Abedi and Pakniyat, 2010). POX has an increase and decrease pattern during stress which by increase in water deficit stress, POX activity decreases (Sun *et al.*, 2010).

Correlation estimates between CAT with isozymes of SOD and POX showed that, there were a significant and positive correlations between them in both irrigation conditions. A significant correlation observed between SOD's isozymes with isozymes of POX in both irrigation conditions (Tables 3 and 4).

Table 3: Correlation of antioxidant enzymes in 23 genotypes of common beans in normal irrigation condition.

	POX_1	POX_2	POX_3	CAT
SOD_1	-0.308	-0.602*	0.329	0.763**
SOD_2	0.403	-0.317	0.762**	0.762**
SOD_3	0.317	-0.361	-0.738**	0.602**
CAT	0.841**	0.294	-0.276	1

Table 4: Correlation of antioxidant enzymes in 23 genotypes of common beans in water deficit condition.

	POX_1	POX_2	POX_3	CAT
SOD_1	0.325	-0.762*	0.410	0.705**
SOD_2	-0.768**	-0.473	0.772**	0.718**
SOD_3	0.328	-0.498	-0.475	0.425
CAT	0.881**	0.349	0.309	1

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