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Evaluation of Peroxidase and Catalase Correlations with Physiological Traits in Water deficit Irrigated common Beans

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ABSTRACT: To evaluate effects of water deficit on antioxidant enzyme activities and some physiological traits, two experiments based on completely randomized design were carried out under field conditions. In experiment 1, plants irrigation cycle was each 5 days (normal condition) whereas in experiment two, once per 10 days. In both experiments, 11 genotypes of common beans: white, red, pinto as well as three improved varieties Akhtar, Dehghan and D81083 were studied. Relative water content (RWC) and stomatal resistance were studied in all experimental plots. Catalase (CAT) and peroxidase (POX) activity were determined using three plants via electrophoresis in polyacrylamide gels (8%). According to data, three isozymes were detected for POX and one monomorphic band for CAT. There was significant difference for RWC and stomatal resistance between all genotypes of bean in both irrigation conditions which is an indicator for high level of genetic variation in bean. Pinto and white beans had better performance than red during water deficit condition. There was a significant and positive correlation between CAT and POX_1 in both irrigation conditions. The highest positive and significant correlation was observed between POX_1 and POX_2 in normal irrigation, whereas POX_2 and POX_3 in water deficit condition. In both experiments, stomatal resistance had negative correlation with RWC. CAT and POX_3 activities had negative correlation with stomatal resistance in normal condition while CAT and POX_1 had negative correlation with RWC in water deficit condition.

Key words: Water deficit, Peroxidase, Catalase, Electrophoresis, Common bean

INTRODUCTION

Common bean belongs to leguminous family and contain high level of protein which is preferred and consumed worldwide. Annual production of this crop is approximately 230 million tons. It is one of the best crop products and allocated 1st place to itself among the legumes (Emeterio Payro *et al.,* 2004). Up to 60 percent of it is produced in developing countries under water deficit condition (Costa Franca et al. 2000; Turkan *et al.,* 2005). Beans are consumed in different forms like dried, pods and immature. It contains 18-22 percent protein, 55-60 percent carbohydrates and polyphenolic compounds and anthocianins which made it prominent food stuff and medicine (Beninger and Hosfield, 2003; Aparicio-Fernandez *et al.,* 2005). Plant's life depends on its stomatal activities (Kudoyarova *et al.,* 2007). Photosynthesis and transpiration are dependent to stomata. It is reported that high performance beans has much more stomata on their leaves (Yusufzai *et al.,* 2009). It is demonstrated that thick and waxy leaves has much more resistance to water lose (Singh *et al.,* 1999). Also, RWC is a factor for resistance to drought not a mechanism drought escape, so, the much more resistance the highest RWC because there is correlation between water content and water loose in leaf (Schonfeld *et al.,* 1988). When stress signals receives to the leaves, stomata closure happens and water content remains constant in plant. In this regard, plant controls water content by regulation of closure of the stomata. This phenomenon diminishes CO_2 levels and decrease photosynthesis and increase reactive oxygen species in plant subjected to water stress (Mittler, 2002). Atmosphere contains 21 pecrent oxigen and because of coupoled orbitals it exists in stabletal form. In metabolic pathways, oxigen recieves one, two and 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-enzyme antioxidant 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).

The present study was to evaluate the effect of water deficit on RWC, stomatal resistance, and change in activity profile of CAT and POX enzymes properties of common beans.

MATERIALS AND METHODS

This experiment was performed using 12 genotypes from three kinds of common bean (red, white and pinto; n = 36) in Agricultural Research Farm, University of Tabriz during 2012. Experimental design was based on randomized complete block design (RCBD) using three replications. One performed on normal condition and second on water stress condition. All experimental plots were irrigated for two months. Then to induce water deficiency in summer, in experiment one plants irrigated once in a period of five days while in experiment two, irrigation cycle was each 10 days. When signs of stress were appeared in plants, green leaves were used to determine catalase (CAT) and peroxidase (POX) enzyme activities.

A. Enzymes extraction and electrophoresis

The crude extract of fresh and healthy leaves from adult plants were prepared with separate mortar and pestle 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 (Valizadeh *et al.,* 2013).

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) electrode buffer (Ph = 8.8). Electrophoresis was carried out at 4°C for 3 h (constant current of 30 mA, and voltage of 180 V). 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. To determine relative water content of leaves, they weighted using sensitive scale (0.0001 g) and allocated into de-ionized water for 24 h. Then, they were dried with Whatman paper and weighted (saturated weight). Leaves were dried in oven at 75ºC for 24 h and weighted (dry weight) and relative water content was estimated by fallowing equation:

Relative water content (RWC, %) = (wet weight - dry weight)/(saturated weight - dry weight) *100

Also, stomatal resistance was calculated using a leaf porometer (Delta-T Devices UK) at a nearly same conditions.

B. Statistical analysis

For statistical analysis and correlation estimates between isozyme markers and physiological traits SPSS 16.0 software was used.

RESULTS AND DISCUSSION

According to the data, one isozyme for CAT and three isozymes for POX in common bean leaves have been identified (Fig. 1 and 2). According to Table 1, enzyme activities were increased in white bean for CAT (54.70%) and POX2 (28.4%), while decreased for POX1 (6.1%) and POX_3 (2.9%). Also in pinto bean, activities were increased 5.1, 17.4, 40.2 and 27.4 percent, respectively for CAT, POX1, POX_2, and POX_3. Furthermore, in red bean genotypes, CAT, POX_2 and POX_3 activity were increased 73.10, 16.1 and 56.9% respectively while POX1 activity was decreased 21.5%.

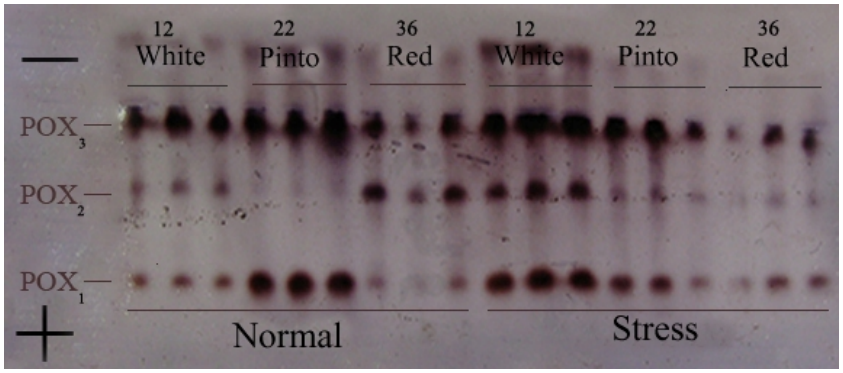

Fig. 1. Example POX banding pattern for normal and water deficit stress conditions.

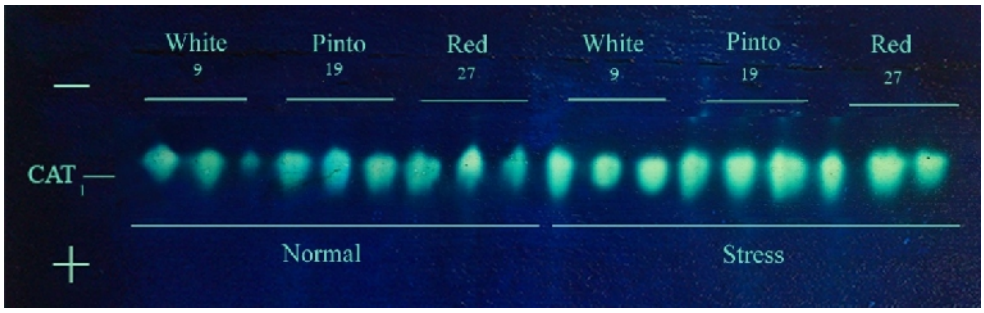

Fig. 2. Example CAT banding pattern for normal and water deficit stress conditions.

Table 1: Percent of increase or decrease in enzymes activities in different genotypes of common bean.

	CAT	POX_1	POX_2	POX_3
White bean	54.70	-6.1	28.4	-2.9
Pinto bean	5.01	17.4	40.2	27.4
Red bean	73.10	-21.5	16.1	56.9

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). Other researchers demonstrated that there is prominent correlation between oxidative resistant and environmental stress which improves antioxidant enzyme activity in photosynthetic plants (Sairam and Saxena, 2000; Sairam and Srivastava, 2001). 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).

RWC showed relative decrement in normal condition compared to stress (Table 2). The least decrement were observed in pinto bean (7.776%), and the highest in red bean (10.05%) (Table 2). In a similar study, (Kumar *et al.,* 2006) it has been revealed that RWC in first round of bean leaves was diminished from 29 to 33%, which is concordant with our findings.

Table 2: Percent of increase or decrease in physiologic traits in different genotypes of common bean.

	RWC	**Stomatal resistance**
White bean	-8.552	19.69
Pinto bean	-7.776	16.63
Red bean	-10.05	31.57

Table 3: Correlation of physiological traits with enzyme's activities in 36 genotypes of common beans in normal irrigation condition.

	RWC	**Stomatal resistance**	**CAT**	**POX_1**	**POX_2**	**POX_3**
RWC	1					
Stomatal resistance	-0.834*	1				
CAT	-0.244	-0.610*	1			
POX_1	-0.310	-0.274	0.841**	1		
POX_2	-0.263	0.110	0.294	0.865**	1	
POX_3	-0.612*	-0.625*	-0.276	0.633*	0.605*	1

*and** indicate significant differences at 0.05 and 0.01 probability levels.

In this study, stomatal resistance was increased during the stress condition. Increment levels were for red bean (31.57%), white bean (19.69%) and pinto bean (16.63%). Singh *et al.,* (1999) reported that thick and waxy leaves had much more resistance to water loose. This could confirm our results concerning stomatal resistance.

Correlation estimates between stomatal resistance and RWC showed that, there were significant and negative correlations between them in both irrigation conditions (Tables 3 and 4). In normal irrigation, POX1 and POX_2 had no correlation with physiological traits. But, CAT activity had negative and significant correlation with stomatal resistance (-0.610). Also, POX_3 activity had negative correlation with stomatal resistance (-0.625) and RWC (-0.612) (Table 3). In water deficit condition, POX_2 and POX_3 activities had no significant correlation with studied traits. Additionally, POX1 activity showed positive correlation only with RWC (0.625). CAT activity showed a significant and negative correlation (-0.605) only with RWC (Table 4).

Table 4:Correlation of physiological traits with antioxidant enzymes activities in 36 genotypes of common beans in water deficit condition.

	RWC	**Stomatal resistance**	**CAT**	**POX_1**	**POX_2**	**POX_3**
RWC	1					
Stomatal resistance	-0.810**	1				
CAT	-0.605*	-0.333	1			
POX_1	0.625*	0.373	0.881**	1		
POX_2	-0.301	-0.351	0.349	0.367	1	
POX_3	-0.315	-0.273	0.309	-0.244	0.60612*	1

*and** indicate significant differences at 0.05 and 0.01 probability levels.

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