



Correlations of Superoxide Dismutase and Catalase activities with Quantitative Traits in common Bean under Water Deficit Stress

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ABSTRACT: Two experiments based on randomized complete block design were carried out under field condition. In first experiment, plants irrigation cycle was each five days (normal condition) whereas in second experiment, once per 10 days. In both experiments, 36 genotypes of common beans: white, red, pinto as well as Akhtar, Dehghan and D81083 as improved cultivars were studied. Days to flowering and about 15 other quantitative traits were measured. Superoxide dismutase (SOD) and catalase (CAT) activities were studied via polyacrylamide gel (8%) electrophoresis. Three and one isozymes were observed for SOD and CAT, respectively. There was significant difference for quantitative traits and isozyme activities in both normal irrigation 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. A negative correlation was observed between CAT activity and days to maturation of pods, number of node in major stem and a positive correlation with weight of hundred seeds in normal irrigation system while there was correlation with number of pods per plant in water deficit condition. A negative and significant correlation was detected between SOD activity and days to maturation of pods and number of node in normal irrigation while it was positive with number of pods per plant. Likewise, there was positive correlation on length of pods, number of pods per plant, thickness of seed, weight of 100 seeds, number of seed per plant and yield per plant in water deficit condition.

Key words: Water deficit, Superoxide dismutase, Catalase, Electrophoresis, Common bean

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 (Bastiaanssen and Makin, 2003). In the arid and semi-arid regions of the world water crisis is serious (FAO, 2000). Water deficiency leads to decrease plant yield via biomass and/or distribution of dry matter in different parts of plant. This phenomenon depends on duration and tension of drought stress especially in last stages of growth (Winkel *et al.*, 1997). During the growth, plants face different kind of biotic and a-biotic stress. Among them, drought is much more harmful than other environmental stress, (Hasegawa *et al.*, 2000; Yamaguchi-Shinozaki *et al.*, 2002). Beans are sensitive to drought stress in farm, where in hot conditions high levels of water evaporation leads to closure of leaf stomata and minimize the performance of plant (Chaves *et al.*, 2002).

Number of seeds and podes in plant are most crucial traits of plant yield (Stoilova *et al.*, 2005). Seed formation is the most sensitive stage to water deficiencies (Nilsen and Nelson, 1998). As well, flowering time, duration of fully seed formation and maturation time are prominent aspects for phenologic traits. Flowering time is equilibrium of vegetative and reproductive period. Each trait has direct or indirect effect on seed yield. In addition, Sadeghipour (2008) reported lack of irrigation decrease nutrient availability to seeds and their weights, in mung bean.

Reactive oxygen species (ROS) which is known as active oxygen generates from reduction of atmospheric O₂ (Gara *et al.*, 2003; Gill and Tuteja, 2010). In metabolic pathways, oxygen receives 1, 2 and 3 electrons and turns to superoxide, hydrogen peroxide and other radicals, respectively, in stressed plants (Beak and Skinner, 2003). ROSs are oxidant molecules and harmful to plant cells. In the nature, plant scavenges with ROSs using enzymatic and non-enzymic antioxidant systems.

Enzymatic system includes peroxidase, catalase, superoxide dismutase and so on. Non enzyme pathway is glutathione, ascorbic acid, tocopherol and other antioxidant substances (Gupta *et al.*, 2005). Superoxide dismutase (SOD) is another antioxidant enzyme which reduces O_2^- . Catalase is an enzyme which scavenges hydrogen peroxide (Jiang and Huang, 2001). ROS attach to nucleotide acids, proteins and lipids and terminate to deformation, damage or mutation in DNA (Gaber, 2010). SOD and CAT are crucial enzymes against ROS-induced damage. Their levels increase during stress in beans (Jebara *et al.*, 2005). SOD is the first line against ROS in the cell. There are different reports related to SOD's role in water deficit condition. (Ashraf, 2009).

The present study was to evaluate the effect of water deficit on yield, other quantitative traits, and change in activity profile of SOD and CAT enzymes properties of common beans.

MATERIALS AND METHODS

Three kind of common bean (red, white and pinto) are used in Agricultural Research Farm of University of Tabriz, in 2012. This experiment was performed using 12 genotypes. 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 1 the plots were irrigated once in a period of five days, while in experiment 2, irrigation cycle was each 10 days. About 16 quantitative traits including days to flowering, days to pod production, days to pod maturing, shoots number in inflorescence, height of bush, number of node in major stem, number of pods per plant, length and width of pods, seed per pods, length, width and thickness of seed, seed per plant, weight of 100 seeds and seed yield of each plant were measured in at least five plants of each plot during experiments, When signs of stress

appeared in plant, green leaves were used to determine catalase (CAT) and superoxide dismutase (SOD) enzyme activity in plant.

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 $1 \text{ mg } \mu\text{l}^{-1}$ and centrifuged at 4°C and 10,000 rpm for 10 minutes using small Eppendorf 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 ($0.6 \times 15 \times 12$ cm) using TBE (Tris-Borate-EDTA) electrode buffer (Ph = 8.8). Electrophoresis was carried out at 4°C for 3 h at constant current of 30 mA and voltage of 180V (Valizadeh *et al.*, 2013).

For the statistical analysis an image analysis program (MCID software) was used to measure $D \times A$ (optical density \times area) parameter for each isozymic band to evaluate the activity onto gels. For statistical analysis and relationship estimates between isozyme markers and quantitative traits SPSS 16.0 software was used.

RESULTS AND DISCUSSION

Three and one isozymes were detected for SOD and CAT in common bean leaves, respectively (Fig. 1 and 2). SOD and CAT isozyme's activity were significantly increased in all genotypes of beans in water deficit condition compared to normal irrigation. This increment was much more for CAT activity in red bean. But, all enzymatic activity of SODs was high in white bean. Antioxidant enzyme activities were higher in water deficit condition (Fig.1 and 2). Under water deficit condition, CAT activity 54.7%, SOD1 90.6%, SOD2 80.4% and SOD3 88.8% were increased in white bean. Also, CAT activity 50.1%, SOD1 88.6%, SOD2 69.6% and SOD3 69.4% activities were increased in pinto beans.

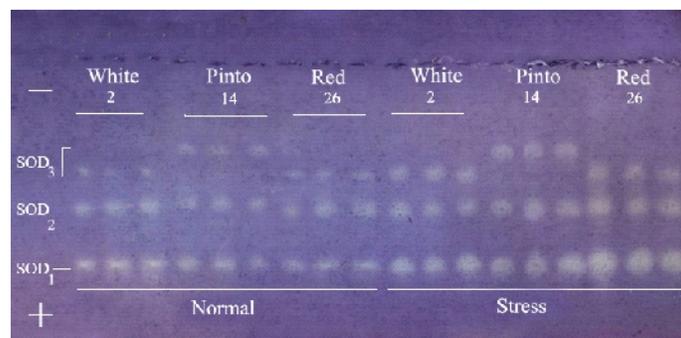


Fig 1. Activity of SOD's isozymes in common bean at normal and water deficit stressed condition.

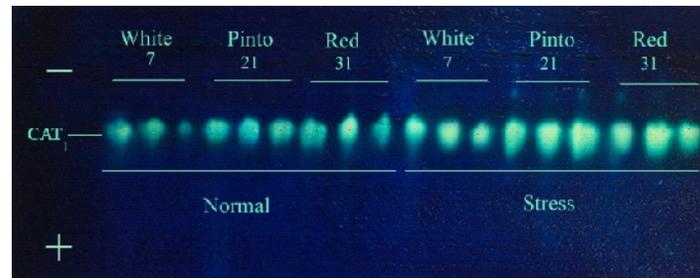


Fig. 2. Activity of CAT's isozymes in common bean at normal and water deficit stress condition.

Furthermore, CAT level 73.1%, SOD1 74.9, SOD2 76.5 and SOD3 79.6% were increased in red bean (Table 1). 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, R.K. and Srivastava, 2001, 2002). Lascano *et al.* (2005) reported that antioxidant enzyme activity increases up to 2 times in 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 (Sofa *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 stress in tomato and plantain. Controversial reports exist about catalase activity. It is reported that CAT activity increases just during severe drought stress. In 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. According to the Table 2, under water deficit condition, the following traits: seed yield of each plant, seed number per plant, number of node per stem, plant height, number of pods per plant and weight of 100 seeds were diminished by 55%, 46.4%, 38.3%, 31.3%, 30.2% and 24.3% respectively compared to normal condition. But other quantitative traits, specially pod and seed dimensions, showed very lower reductions. Previously, Rosales-sera *et al.* (2003) reported that water deficit decreases maturation days in bean. Furthermore, drought stress during flowering and seed formation stages diminished bean yield (Boutraa and Sanders, 2001). These reports are consistent with our finding. In normal irrigation condition, there was a significant and positive correlation between CAT activity and 100 seeds, weight whereas a negative one between days to maturation of pods and number of nodes. All SOD isozymes activities had significant correlation with number of node per stem. SOD3 had positive correlations with number of seed per pod and yield per plant, but had negative correlations with day to pods and maturation of pods (Table 3).

Table 1: Percent of increase in enzyme activities in three kind of common bean in water deficit condition.

Genotype	CAT	SOD1	SOD2	SOD3
White	54.7	90.6	80.4	88.8
Pinto	50.1	88.6	69.6	69.4
Red	73.1	74.9	76.5	79.6
All genotypes	59.3	84.7	75.5	79.2

Table 2: Percent of decrease in quantitative traits of three kind of common bean in water deficit condition.

	Bud Per flower	Height of plant (cm)	Node per stem	Pods per plant	Pods height (cm)	Pods width (cm)	Seeds per pod	Seed height (mm)	Seed width (mm)	Seed thickness (mm)	Seed per plant	Weight of 100 seeds (g)	Yield per plant g/plant
White bean	13.9	40	30.8	33.9	8.4	7.9	19.3	8.7	9.3	10.3	54.1	24.2	58.2
Pinto bean	16.9	27.2	48.3	23.8	10.5	6	15.3	7.8	8.1	9.9	39.2	22.3	51.2
Red bean	14.9	26.1	35.8	31.5	11.2	6.4	17.1	7.5	8.1	10.4	42.7	26.4	57.4
All genotypes	15.2	31.1	38.3	29.7	10	6.8	17.2	8	8.5	10.2	45.3	24.3	55.6

Table 3: Correlation of quantitative traits with antioxidant enzyme activities in 36 different genotypes of common bean in normal condition.

	Days to flowering	Days to pod production	Days to maturation of pods	Bud per flower	Plant height	Number of nodes	Number of pods	Pods height	Pods width	Seeds in pod	Seed height	Seed width (mm)	Seed thickness (mm)	Seed per plant	Weight of 100 seeds (g)	Yield per plant g/plant
CAT	0.366	-0.329	-0.590*	-0.312	0.521	-0.821**	-0.492	-0.492	0.426	0.308	-0.296	0.368	0.466	-0.31	0.642**	0.372
SOD ₁	0.392	-0.3	-0.248	-0.244	0.226	-0.606**	-0.346	-0.274	0.362	0.264	-0.3	0.372	0.248	-0.384	-0.164	-0.385
SOD ₂	-0.274	-0.0324	-0.214	0.218	0.234	-0.642**	0.236	-0.322	0.304	0.36	-0.26	0.328	-0.25	0.27	-0.126	-0.28
SOD ₃	-0.260	-0.576*	-0.656**	-0.214	-0.292	-0.598**	-0.246	0.284	0.308	0.612**	0.218	0.212	0.216	-0.262	-0.144	0.216

*, ** significant differences at 5 and 1% levels, respectively.

Table 4: Correlation of quantitative traits with antioxidant enzyme activities in 36 different genotypes of common bean in water deficit condition.

	Days to flowering	Days to pod production	Days to maturation of pods	Bud per flower	Plant height	Number of nodes	Number of pods	Pods height	Pods width	Seeds in pod	Seed height	Seed width (mm)	Seed thickness (mm)	Seed per plant	Weight of 100 seeds (g)	Yield per plant g/plant
CAT	-0.272	-0.258	-0.222	0.378	0.294	0.266	-0.716**	0.256	-0.218	-0.244	0.48	0.226	-0.208	-0.246	0.410	-0.282
SOD ₁	0.218	-0.244	-0.372	0.288	-0.306	-0.316	0.628*	0.306	0.354	0.202	0.316	0.426	0.606*	0.212	0.612*	0.622*
SOD ₂	0.316	-0.0220	-0.308	0.256	-0.224	0.246	0.254	0.664*	0.236	0.356	0.312	0.248	-0.288	0.308	0.374	0.418
SOD ₃	0.284	-0.278	-0.376	-0.372	-0.318	0.33	0.612*	0.570*	0.358	0.376	0.332	0.28	0.214	0.520*	0.372	0.638*

*, ** significant differences at 5 and 1% probability levels, respectively.

In water deficit condition, a negative correlation was observed between CAT and pod per plant; while a positive correlation was observed for SOD1 with pod per plant, seed thickness, 100 seeds weight and yield of each plant; SOD2 with pods height; SOD3 with pods per plant, pod height, seed

per plant and yield of each plant (Table 4). These results suggest that detected correlations between yield and yield component with antioxidant enzymes activities might be useful for further marker assisted selection studies.

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