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EFFECTS OF DROUGHT STRESS ON ANTIOXIDANT ENZYMES IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) SEEDLINGS

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Keywords: Common bean, Drought stress, Antioxidant Enzymes

Abstract

Drought is one of the most severe environmental constraints which reduces common bean production worldwide. Exploration of the physiological mechanism of common bean under drought stress is important for the efficient production and variety selection of common beans. In the present study, non-drought-resistant variety (Longyundou10) and drought-resistant variety (Longyundou17) were identified to elucidate the effects of drought stress on antioxidant system of common beans at seedling stage. Under drought stress, APX and SOD activities showed a single peak curve that first increased and then decreased, and the dynamic changes of CAT and POD activities were more complicated. Under different levels of drought treatment, the average values of APX, SOD, CAT and POD activities of common bean were found to be higher than those of normal water conditions, and the average values were the highest under severe drought stress, indicating that these antioxidant enzymes were stimulated under drought stress.

Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important crops for micronutrients and protein in the diets of people and is cultivated in many countries of the world (Mekbib *et al*. 2003, Wu *et al*. 2020). For years, common bean have been a valuable commodity which was exported to worldwide and the yield reached 3.3×10^7 tons in 2019 (http://www.fao.org/). However, as the global drought condition is getting worse, the production of common bean is usually limited by drought which is one of the most severe environmental constraints and devastating on a global scale (Dipp *et al*. 2017). Most of the planting areas of common bean suffer from drought in the world, and the yield reduction of South Africa and Kenya is as high as 80% (Beebe *et al*. 2008).

The complex physiological changes could take place in the process of responding to adversity. Previous researches suggested that antioxidant system mediate plant physiological activities to adapt to drought stress (Deeba *et al*. 2012, Laxa *et al*. 2019). Drought-induced deregulation of metabolism enhances generation of reactive oxygen species (ROS) which are able to lead to plant cell death. (Gill and Tuteja 2010, Choudhury *et al*. 2016). ROS-scavenging antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POD), play a key role in drought resistance of plant (Ashraf. 2009, Singh *et al*. 2012). The APX activity of pea increased significantly under drought conditions (Mittler *et al*. 1994), SOD is important antioxidant enzymes which scavenge H_2O_2 and $\cdot O_2^-$, thus prevent the formation of the highly toxic $\cdot OH^-$ radical (Scandalios. 1993, Foyer *et al*. 1994). CAT plays a key role when the plant is exposed to a severe drought stress (Sofo *et al*. 2015). POD is a plant oxidoreductase that can catalyze multiple physiological reactions and has the dual role of eliminating H_2O_2 in antioxidant system of plant (Foyer and Shigeoka 2011). The ability of drought resistance is an

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important index of production and breeding (Sica *et al*. 2021). In the present study, the fluctuations of antioxidative enzymes were identified in order to shed new light on technical guidance for efficient breeding and production of drought-resistant common bean varieties.

Materials and Methods

A drought-resistant variety named Longyundou17 and a non-drought-resistant variety named Longyundou10 were obtained from germplasm resources center of Heilongjiang Academy of Agricultural Sciences. The experimental design was followed by Wang (2019). LS and SS stands for mild drought stress and severe drought stress, respectively. The relative water content of soil of control, LS and SS were 75+5, 60+5 and 45+5%, respectively. The activity of SOD, CAT, APX and POD were identified by ELISA.

The kits required for the experiment were provided by Shanghai Enzyme-linked Biotechnology Co., Ltd. SPSS 18.0 and Microsoft Excel 2016 software were used to process the data.

Results and Discussion

SOD, CAT, APX and POD are key antioxidant enzymes that are involved in regulating plant drought resistance (Lascano *et al*. 2003, Askari and Ehsanzadeh 2015). A correlation between these enzymes and drought tolerance was displayed by comparing the sensitive cultivars with tolerant cultivars in a number of plants (Azooz 2009, Kadkhodaie *et al*. 2014). The SOD, CAT, APX and POD activities of soybean leaves increased firstly and then decreased with the increase of drought stress (Ma *et al*. 2018). In this study, under drought treatment, a trend of SOD activity first increasing and then decreasing was observed with the time (Fig. 1). Hence, the present results are consistent with previous studies. Under normal water treatment, the average value of SOD activity of Longyundou10 and Longyundou17 was 1.14 and 1.25 U/l, respectively. Under mild drought stress, the SOD activity peaks of Longyundou10 and Longyundou17 both appeared on the 8th days after treatment, and the peak values were 1.55 and 1.67 U/l, respectively, compared with

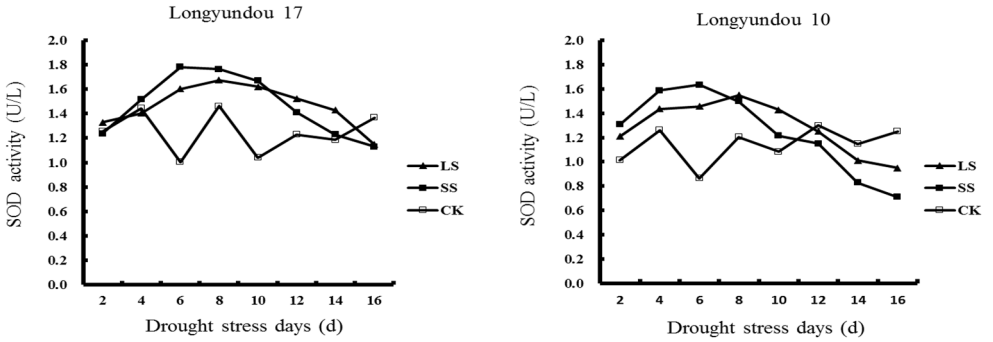

Fig.1. Changes of SOD activity in common bean leaves under drought stress (LS: mild drought stress; SS: severe drought stress; CK: normal water condition).

normal water conditions; the SOD activity increased by 29.2 and 12.6%, respectively. After the peak appeared, the SOD activity of Longyundou10 decreased rapidly, while the drought-tolerant variety Longyundou17 decreased slowly, it can maintain a relatively high level of SOD. The SOD peaks of the two cultivars under severe drought stress appeared on the 6th days after treatment, which was earlier than the time under mild drought stress. The SOD of Longyundou10 dropped

sharply at the peak, while the SOD of Longyundou17 showed a sharp decline trend after the 10 days of treatment. On the 8 days after treatment of Longyundou10, the SOD activity under mild drought stress was higher than that under severe drought stress, while this phenomenon appeared on the 10 days after treatment of Longyundou17, indicating that the drought-tolerant variety maintained the SOD activity compared with non-drought-tolerant variety and it can resist severe drought stress for a longer time.

Under normal water conditions, the CAT in the leaves of common bean seedlings fluctuated within a certain range. The CAT activity of Longyundou10 fluctuated in the range of 461.51～572.81 U/ml, and the average value was 528.95 U/ml, the CAT activity of Longyundou17 ranged from 483.52 to 597.90 U/ml, with an average value of 557.49 U/ml (Fig. 2). The CAT activity of Longyundou17 was higher than that of Longyundou10. Under drought stress, CAT activity varies with different resistant varieties. With the extension of drought stress, CAT activity first increased and then decreased in a single-peak curve. The results of this study are more or less similar to those in the common bean (Wang *et al.* 2014). Under LS and SS treatments, the peaks value of CAT were 726.64 and 697.02 U/ml, respectively. The peaks appeared on the 6th and 8th days after treatment of drought stress. In the late drought stress period, the CAT activity decreased and was significantly lower than the control. The changes of the activity of CAT of Longyundou17 was more complicated. Under mild drought stress, it showed a change process of first increasing, then decreasing and then increasing. The maximum CAT activity appeared on the 10th days after drought stress, and its value was 684.77 U/ml and CAT activity can be increased in the later stage of mild drought stress. Under severe drought stress, the CAT activity of Longyundou17 basically maintained a single-peak curve change, the peak appeared on the 8th days after treatment, which was 744.20 U/ml, and dropped to a level similar to normal water during 12-16 days of treatment. The average values of CAT activity of Longyundou10 under LS and SS treatments were 547.29 and 552.27 U/ml, respectively. The average value of CAT activity of Longyundou17 under LS and SS treatments was 580.49 and 618.46 U/ml, respectively, the CAT activity of Longyundou17 was higher than that of Longyundou10 under drought stress. The average CAT activity of the two common bean varieties was between the treatments.

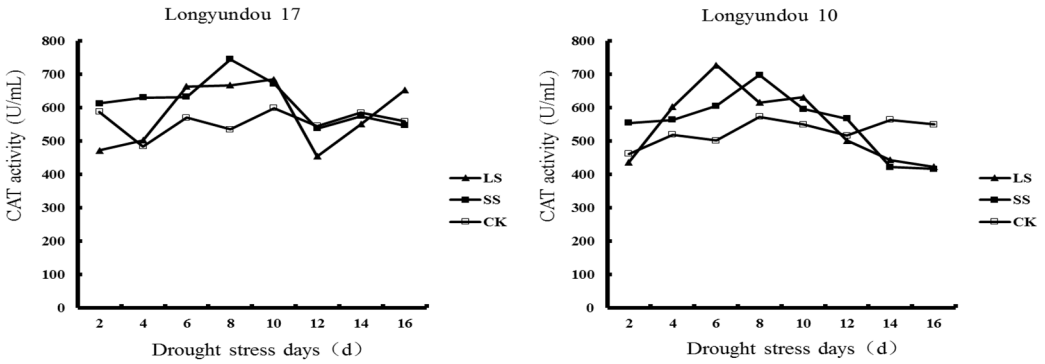

Fig. 2. Changes of CAT activity in common bean leaves under drought stress.

Under normal water treatment, the APX activity fluctuated with great variation range. The APX activity of Longyundou10 varied from 1.09 to 1.63 IU/l, with an average value of 1.37 IU/l, APX activity of Longyundou17 fluctuated from 1.10 ～ 1.76 IU/L, with an average value of 1.54 IU/l, APX activity of Longyundou17 was 11.0% higher than Longyundou10 (Fig. 3). In the present study, under drought stress, the APX activity of two cultivars showed a single-peak curve

on day 8 and day 6, respectively. The reasons for this could be that the APX activity of drought-tolerant variety of common bean increased more than that of non-drought-tolerant variety. The peaks level of Longyundou10 under LS and SS appeared on the 6th days of treatment and the peaks were 2.26 and 2.39 IU/l, respectively, after the peak appears, APX activity decreased linearly and is lower than normal water conditions after 12 days of treatment. The APX activity peaks of Longyundou17 under LS and SS were 2.28 and 2.64 IU/L respectively, and all the peaks appeared on the 8th days of treatment, which was later than Longyundou10, and continued to be higher after the peak level. The average APX activity was between treatments, indicating that severe drought stress can stimulate APX to respond positively to alleviate the damage of drought stress.

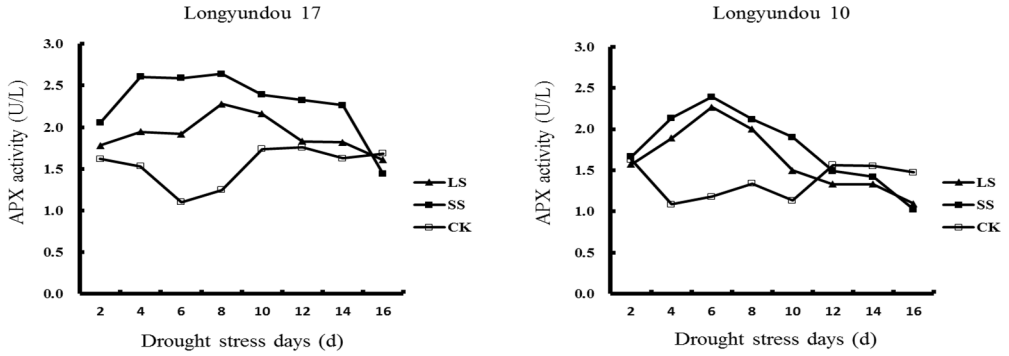

Fig. 3. Changes of APX activity in common bean leaves under drought stress.

The POD activity in sugarcane leaves was significantly increased under drought stress (Do *et al.* 2018). In the present study, under drought stress, POD activity of Longyundou17 and Longyundou10 increased, and the mean values of POD activity under LS and SS were 2.78 and 2.83 U/l, respectively (Fig. 4). There was no significant difference between the two treatments, which were 22.5 and 24.7% higher than those under CK, respectively. The POD activity of Longyundou17 was in other treatments except 2d, and the mean values of POD activity under mild and severe drought stress were 2.80 and 3.32 U/l, respectively, which were 15.2 and 36.6% higher than those under normal water stress, respectively. Therefore, POD can be used as a physiological index for the identification of drought resistance in different crops.

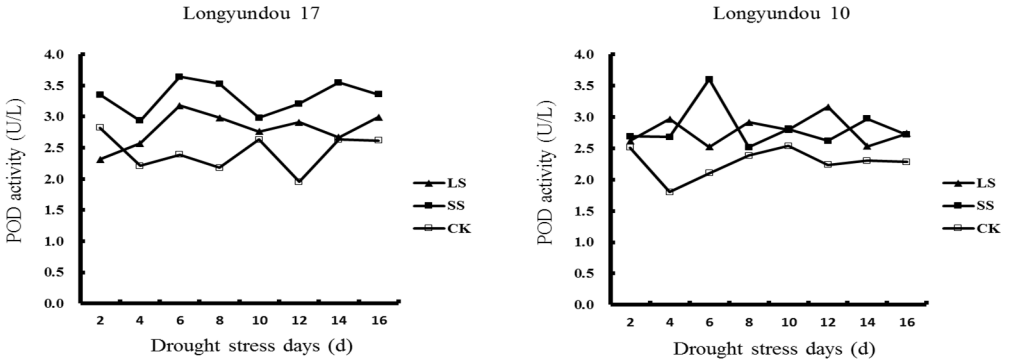

Fig. 4. Changes of POD activity in common bean leaves under drought stress.

In the present study, under different degrees of drought stress, the average values of APX, SOD, CAT and POD activities of common bean were all higher than those under normal water conditions. Under severe drought stress, the average values were the highest, indicating that the activities of these antioxidant enzymes are stimulated by drought stress and play a positive role in protecting common beans from damage. Under drought stress, the average value of antioxidant enzymes activities of Longyundou17 was higher than that of Longyundou10. According to the different response of antioxidant enzymes activity between varieties and treatments, the present results suggest that POD, CAT, APX and SOD can be used as reference indicators for drought resistance identification of common bean seedlings. In future research, it is necessary to perform gene mining and utilization of enzymes related to the common bean antioxidant system to provide technical support for common bean drought-resistant breeding.

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