Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 2 ~ Issue 1 (2014) pp: 01-06
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Effects of Brassinosteroid on Antioxidant System in *Salvia miltiorrhiza* Under Drought Stress

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Received 04 February, 2014; **A**ccepted 21 February, 2014

ABSTRACT:- *The roots of Salvia miltiorrhiza are widely used in traditional Chinese medicine. In our study we investigated the effects of brassinosteroid on drought resistance in S. miltiorrhiza using the method of measuring the antioxidant enzyme activity (SOD, CAT, POD) of S. miltiorrhiza seedling under drought stress after spaying different levels of brassinosteroid. The results showed that after spraying BR and proceeding with drought stress to S. miltiorrhiza seedling, the antioxidant enzyme activity (SOD, CAT, POD) and Pro content increased significantly, while the MDA content decreased significantly. Especially with the treatment of 0.1 mg/l brassinosteroid, the activity of SOD, CAT and the content of Pro raised to the highest level, while the MDA content reached its lowest point in our experiment. Our study suggested that the ameliorative effects of BR on drought resistance of S. miltiorrhiza seedlings were positive.*

Keywords:- *Drought stress, Brassinosteroid, Antioxidant System, Salvia miltiorrhiza*

I. INTRODUCE

Drought disaster are happened frequently in many areas all over the world every year, the crop production was adversely affected by frequent droughts[1]. Many studies have investigated drought stress may induce similar cellular damage, while plants have evolved complex mechanisms to perceive, respond and adapt to water deficit[2]. Various physiological practices are applied to alleviate the adverse effects of water deficit stress on the normal functioning of plants[3-5]. For example, several enzymatic systems and antioxidant molecules are responsible to counteract the deleterious effects of SOD, CAT etc.[6]. Accumulation of osmolytes, such as proline (Pro), helps to conserve tissue water and protect the proteins and cellular membranes from the osmotic and oxidative stresses[7][8].

Salvia miltiorrhiza is one of the most important and popular plants used in traditional Chinese medicine. It's mainly used to treat Cardiovascular and Cerebral vascular diseases[9]. Drought stress caused the change of the connection between membrane lipid peroxidation and the plant protection enzyme, that caused the quality reduction of *S. miltiorrhiza*[10, 11]. Yearly, the seasonal drought in late spring and early summer has restricted the growth of *S. miltiorrhiza*. Brassinosteroid (BR) is a group of plant steroidal hormones that are structurally similar to animal and insect steroids[12]. BR is ubiquitous in the plant kingdom and regulate a wide range of physiological responses, including cell elongation, photomorphogenesis, xylem differentiation, seed germination and stress responses[13,14].

In this study, after BR spraying, we investigated the change of the activity of various antioxidant systems and the change of the contents of MDA and Pro in *S. miltiorrhiza* under drought stress to reach a primary assessment of influence of BR on drought resistance capability of *S. miltiorrhiza*. The experimental studied how to use the spraying BR method to improve drought resistance of *S. miltiorrhiza*, therefore, made a preliminary understanding of the *S. miltiorrhiza* drought resistance, and provided fundamentation for further study.

II. MATERIALS AND METHODS

2.1 Plant materials and growth condition

The *S. miltiorrhiza* (seminal roots come from Zhongjiang's Agriculture bureau in Sichuan Province)

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were transplanted into plastic pots (37cm in diameter, 25cm in deep) in 2012 April, meanwhile the plant were protected by the plant house from exposing to the weather, with temperatures ranging from 20 ˚C to 30 ˚C. The BR solution spraying process was carried out at the end of June. We divided all seedlings into five groups, each included six seedlings. One group grew under normal circumstance, we named it CK; one was sprayed with water, namely proceeded with draught stress, we called it W0; and other three were first sprayed with BR solutions containing 0.1 mg/l (B1), 0.2 mg/l (B2), 0.4 mg/l (B3) pure BR separately and then 48 hours later proceeded with 20% PEG-6000(Polyethylene Glycol 6000) simulated drought treatment. Nine days after the drought stress, we started to gather experimental data. The Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD) activity and proline (Pro), Malonaldehyde (MDA) content of *S. miltiorrhiza* seedlings were measured by random sampling. CK (without BR, without drought stress) and W0 (drought stress without BR) were set as control group. Each test repeated 3 times.

2.2. Assay method

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT)[15], the activity of CAT was assayed by UV absorption method[11], POD activity was measured by Leon methods[11], MDA content was measured by 2-thiobarbituric acid reaction according to the method of Yuan and Jia[16], Pro content was determined by the method of Bates[8].

2.3 Statistical analyses

Statistical analysis was performed by using the SPSS package program version 19.0. Data was analyzed by one-way ANOVA, followed by Duncan's LSD multiple comparison test. The values are reported as means with their standard error for all results. Differences were considered significant at $p<0.01$.

III. RESULTS AND DISSCUS

3.1 The antioxidant enzymes

Fig. 1 represented the effects of BR on the activities of SOD. As is shown in Fig.1, the SOD activity at W0 treatment was increased 28.35% than CK. After spraying BR on *S. miltiorrhiza* seeding, the SOD activity with B1 and B3 solustions were increased 85.46% and 57.55% seperately than W0, but with B2 it was decreased 27.32% than W0.

The levels of BR treatment were determined and the change of POD activity was shown in Fig.2. The POD activity at W0 treatment was increased 34.48% than CK. It was 126.49% increased in B3 treatment than W0. The POD activity was unimproved at B1 and B2 treatments, decreased 53.84% and 52.99% seperately than W0.

The effects of BR on the activities of CAT were shown in Fig. 3. Our Experiments showed that CK was 211.17% lower than W0. The Maximum value of the CAT activity was experienced with B1 treatment, which was 294.1% higher than W0. With B3 treatment it was increased 63.52% than W0. The activity of CAT at B2 was the lowest compared to other BR treatments, and it decreased 27.67% than W0.

In plants, large amount of active oxygen will accumulate rapidly under drought stress, which lead to serious harm to plant cell even induce apoptosis[17, 18]. To cope with oxidative damage under extremely adverse conditions, plants have developed an antioxidant defense system that includes the antioxidant enzymes SOD, POD, and CAT[19].

The main function of antioxidant enzyme is to clear the active oxygen in plant. The main kinds of antioxidant enzyme are SOD CAT POD and etc. Usually, SOD transforms O^{-2} into H_2O_2, then CAT POD carry out the conversion of H_2O_2 to H_2O[20]. The synergies among these three enzymes above mentioned can maintain the living radical at minimum level in order to keep the cell membrane from getting harmed by active substance in plant, so to further protect plant cell. Current research proved that the activity of antioxidant enzymes in the antioxidant enzyme system of *Amorpha fruticosa* was enhanced under drought conditions[21].The levels of antioxidant enzymes are higher under drought stress than under normal environment [22]. As it can be seen in our research (Fig. 1, 2, 3) the activity of SOD, POD and CAT increased highly significantly at W0 treatment than CK treatment ($p<0.01$). Current research proved that the activity of antioxidant enzymes in the antioxidant enzyme system of *Amorpha fruticosa* was enhanced under drought conditions[21]. Brassinosteroids generate such a response because of their involvement in the modification and/or manipulation of plasma membrane structure/permeability under stress conditions [23-24]. Our study reached similar results that the brassinolide had improved on *S. miltiorrhiza* SOD, POD, and CAT activities under drought stress. The SOD and CAT activities of seedlings in the 0.1 mg/l and 0.4 mg/l brassinolide treatments were highly significantly compared to under drought stress, and the POD activity of *S. miltiorrhiza* seedlings in the 0.4mg/l were highly significantly different from CK ($p<0.01$). At present study BRs could lead to an increase of antioxidant enzymes activity in wheat, maize and Melon seedlings [20, 25].

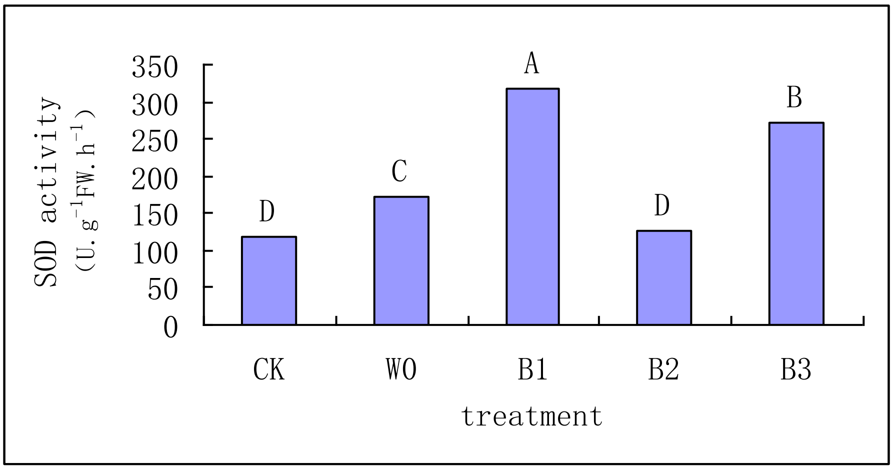

Fig. 1. Effects of brassinosteroid on the activities of superoxide dismutase (SOD)

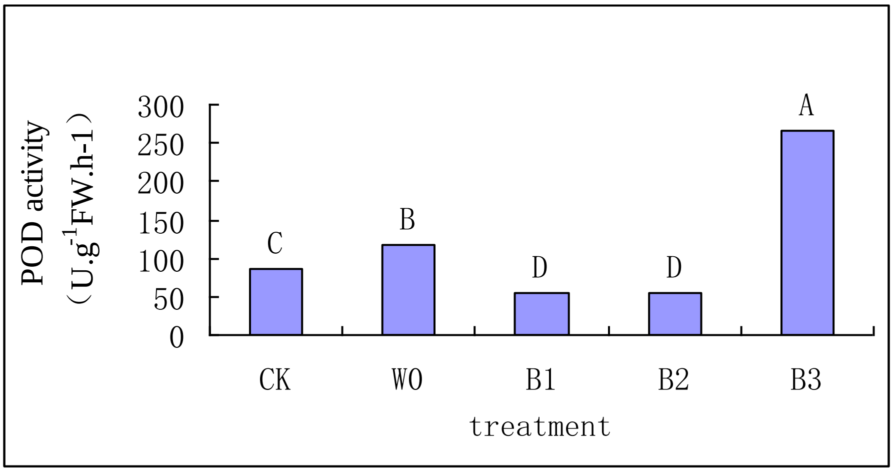

Fig. 2. Effects of brassinosteroid on the activities of peroxidase(POD)

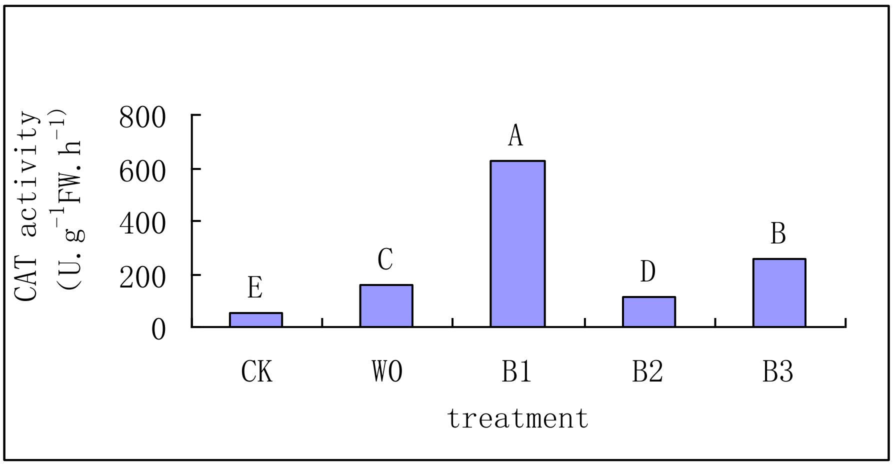

Fig.3. Effects of brassinosteroid on the activities of catalase(CAT)

Note: In Figures 1, 2, 3, different normal letters within the same line indicated significant difference among treatments at 0.01 level. BR concentration were 0 mg/l (W0), 0.1 mg/l (B1), 0.2 mg/l (B2), 0.4 mg/l (B3), CK was control group. A, B, C, D, E marks significant differences of samples.

3.2 The MDA content

The effects of BR on the content MDA were shown in Fig. 4. The *S. miltiorrhiza* seedling's MDA content at W0 was 1.59 times higher than CK. However, the MDA content with B1, B2 and B3 treatment decreased 39.71%, 21.73% and 29.56% separately than W0. It has been observed in our study (Fig.4,) the amount of the *S. miltiorrhiza* seedling's MDA contents at W0 was significantly higher than at CK treatment. The MDA content with B1, B2, B3 treatment was highly significantly lower than W0 treatment (p<0.01). The reason for this phenomenon is that the drought stress broke the balance of plant plasma membrane permeability[5]. Large amount of toxic substance including MDA will be produced in plant after drought stress. As an important index of plant plasma membrane permeability, the higher the content of MDA in plant is, the greater damage the plant suffers[11], and the MDA content levels was decline with BR spraying[26]. Present scientific research has been proved BR could reduce the content of MDA in plants therefore to limit the harm of plant plasma[19,27-29]. Take *Amorpha fruticosa* as an example, after BR spraying the content of MDA declined significantly[21].

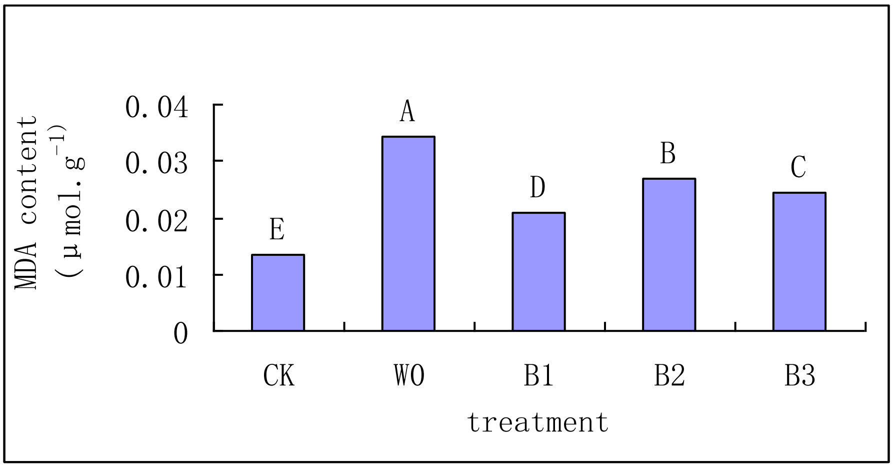

Fig. 4. Effects of brassinosteroid on the content MDA

Note: Different normal letters within the same line indicated significant difference among treatments at 0.01 level. BR concentration were 0 mg/l (W0), 0.1 mg/l (B1), 0.2 mg/l (B2), 0.4 mg/l (B3), CK was control group. A, B, C, D, E marks significant differences of samples.

3.3 The Pro content

The effects of BR on the Pro content were shown in Fig. 5; the Pro content of W0 is 33.61% less than CK. After spraying BR on *S. miltiorrhiza* seedling, the Pro content with B1, B2 and B3 treatment were separately 17.6, 17.1 and 12.9 times higher than W0. Pro can reduce the damage caused by MDA. It is a soluble in plant, the content of which will increase under adverse circumstances[2]. In our study that spraying BR on the S. miltiorrhiza seedlings, the Pro content with B1, B2, B3 treatment was all highly significant higher than W0 (p<0.01), the results show that BR spraying can increase the Pro content of *S. miltiorrhiza* seedling. Similar results showed that BR can induce the proline accumulation of *Vigna radiate.* under drought stress[29].

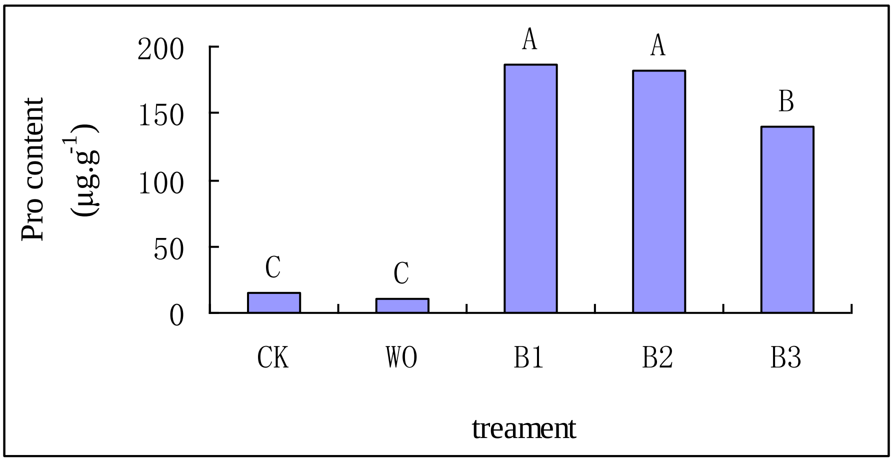

Fig.5. Effects of brassinosteroid on the content Pro

Note: Different normal letters within the same line indicate significant difference among treatments at 0.01 level. BR concentration were 0 mg/l (W0), 0.1 mg/l (B2), 0.2 mg/l (B3), 0.4 mg/l (B4), CK was control group. A, B, C, D, E marks significant differences of samples.

IV. CONCLUSION

At B1 treatment, the content of Pro increased to its peak, the activity of SOD and CAT also rose significantly, while the content of MDA hit its lowest point. Accordingly we conclude that BR at 01.mg/l can effectively enhance the resistance of *S. miltiorrhiza* under drought stress. Because of the synergies of antioxidant enzyme, we cannot just rely exclusively on a single index to evaluate the effects of BR on antioxidant system in *S. miltiorrhiza* under drought stress. Therefore, such as a plant hormone, when we undertake a comprehensive evaluation of the plant hormone, we must take account of the major of antioxidant system indicator[2, 11, 30-31]. In summary, by comprehensive consideration of above three different concentration indicators, the 0.1mg/l BR treatment is the best choice to enhance the antioxidant ability of *S. miltiorrhiza*.

The present study reveals that the level of antioxidant system (SOD, POD, CAT and Pro) increased in response to drought stress that was further improved by BR treatment. Therefore, it may be suggested that the ameliorated level of antioxidant system, at least in part, was responsible for the development of resistance against drought stress in *S. miltiorrhiza* seedlings. The increase in the degree of resistance due to the application of BR was reflected in the improvement of *S. miltiorrhiza* growth. This study provided the basic date for the in-depth research of the role of BR in the amelioration of drought stress.

ACKNOWLEDGEMENTS

This work was financially supported by the programs of agriculture science technology achievement transformation fund, China (2012GB2F000385), innovation fund for technology based firms, China (12C26215105863), the science and technology department of Sichuan province, China (2011ZO0006, 2012ZR0050), and innovation fund for technology based firms, Chengdu (11CXYB958JH-003).

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