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Effects of exogenous ABA on the physiological characteristics and chlorophyll fluorescence of *Gynura cusimbua* seedlings under drought stress

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Abstract: This paper is to investigate the physiological mechanism of exogenous abscisic acid (ABA) on alleviating drought stress in Gynura cusimbua The effects of exogenous ABA on physiological characteristics and chlorophyll fluorescence parameters of the leaves of Gynura cusimbua under drought stress were studied with 10% polyethylene glycol 6000 (PEG 6000) in simulated drought stress. The results showed that the activities of antioxidant enzymes and some fluorescence parameters increased slightly after spraying exogenous ABA. Under drought stress, exogenous ABA can increase chlorophyll content, enhance antioxidant enzyme activity, reduce MDA content, increase maximum photochemical efficiency Fv / FM, regulatory energy dissipation Y (NPQ), photochemical quenching QL, actual quantum efficiency Y (II), non-photochemical quenching NPQ fluorescence Fo parameters, so as to reduce the damage to seedlings under drought stress. Among them, the ABA concentration of 200 μ mol/L was the best to alleviate 10% drought stress.

1. Introduction

According to statistics, about 43% of the cultivated land in the world is located in arid and semi-arid areas, which seriously affects the growth and development of plants, resulting in crop yield reduction(YAN H.,et al,2008). Therefore, how to improve the drought resistance of crops has become one of the key problems that modern plant researchers need to solve urgently.

Abscisic acid (ABA) is an important regulator in response to drought stress in plants. It can promote plant bud dormancy, inhibit plant growth and promote plant senescence(RUAN Y. H.,et al,2012). In recent years, with the gradual deepening of research, ABA plays an important role in the process of plant abiotic stress, and it has been proved that exogenous ABA can improve stress resistance in many plants(CAO F.Y.,et al,2011). Previous studies only focused on cold resistance^(LI G. H.,et al,2015), uranium stress, Sr, Cs stress(TANG Y.J.,et al,2013)and other stresses, but there was no relevant report on drought stress and exogenous ABA to alleviate it. Under stress, ABA can improve the activities of related antioxidant enzymes to a certain extent, thus reducing the accumulation of reactive oxygen species (ROS) in plants(LI C.N.,et al,2010).

Gynura cusimbua is a plant of the genus Gynura cusimbua. The whole plant can be used for medicine, which has the functions of smoothing intestine, cooling heat and promoting defecation. The leaves contain a variety of vitamins, as well as calcium, iron, phosphorus and other nutrients. They are mostly distributed in Shanghai and Jiangsu, and are not drought tolerant. Therefore, it is important to study the

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physiological response and fluorescence characteristics of ABA under drought stress for understanding the drought tolerance mechanism of *Gynura cusimbua*.

The effects of exogenous ABA on some physiological and biochemical mechanisms and chlorophyll fluorescence parameters in the process of a certain drought stress were preliminarily studied by PEG 6000 simulation of drought stress, combined with exogenous ABA treatment, so as to provide reference for further exploring the physiological and biochemical mechanism of drought resistance and the mechanism of ABA improving the ability of anti-drought stress of *Gynura cusimbua*.

2. Materials and methods

2.1. Material sources

The seeds of Gynura cusimbua are provided by Shouguang Xinxinran Horticulture Co., Ltd.

2.2. Material handling

Select full seeds, soak them in warm water for 1-2 days, and then evenly plant them in a tray containing quartz sand, and put them into the incubator under the condition of 25 °C and light/dark cycle for 24h/0h. Water was poured every 3 days until it reached the stage of growing three complete leaves and one lobus cardiacus. Drought stress was simulated with 10% polyethylene glycol 6000 (PEG 6000). The following treatments will be done to the *Gynura cusimbua* seedlings: 1) CK1: clear water treatment (control 1); 2) CK2: drought treatment, spraying water (control 2); 3) T1: drought treatment, spraying 50 mol/L of ABA; 4) T2: drought treatment, spraying 100 mol/L of ABA; 5) T3: drought treatment, spraying 150 mol/L of ABA; 6) T4: drought treatment, spraying 200 mol/L of ABA; 7) T5: drought treatment, spraying 250 mol/L of ABA; 4 plants in each treatment, repeated three times. The measurements were taken nine days later.

2.3. Parameter determination

2.3.1. Physiological indexes

Superoxide dismutase (SOD) activity was measured by nitroblue tetrazole method(LI H.S,1999). Peroxidase (POD) activity was measured by guaiacol method(LI H.S,1999). Catalase (CAT) activity was measured by titration method(LI H.S,1999). Malondialdehyde (MDA) content was determined by thiobarbituric acid method(LI H.S,1999). Chlorophyll content was determined by ethanol (95%) extraction method(LI H.S,1999).

2.3.2. Chlorophyll fluorescence parameters

After one hour of dark adaptation, the third leaf from the top to the bottom was selected, and the chlorophyll fluorescence parameters were determined by PAM-2100 portable pulse modulation fluorometer, including maximum photochemical efficiency Fv/Fm, actual photochemical efficiency $Y(\Pi)$, photochemical quenching coefficient qL, non-photochemical quenching coefficient NPQ, regulatory energy dissipation Y(NPQ), non-regulatory energy dissipation Y(NO), and minimum fluorescence Fo. These parameters are automatically generated in set mode.

2.4. Data analysis

Excel 2016 software was used for drawing, and SPSS software was used for significance analysis (P < 0.05).

3. Results and analysis

3.1.Effects of Exogenous ABA on SOD, pod and cat activities of Gynura cusimbua seedlings under drought stress

As shown in Fig. 1, compared with CK1 treatment, the activities of SOD, POD and CAT were significantly increased in CK2 treatment. After treatment with different concentrations of ABA, the activities of SOD, POD and CAT increased first and then decreased, and the differences among the treatments were significant, and were higher than those of CK2 treatment. Among them, T4 (200 μ mol/L) treatment, SOD, pod, CAT activities reached the highest, increased by about 3.25 times, 3. 32 times and 3.04 times.



Fig. 1 Effect of Exogenous ABA on SOD, POD and CAT in leaves of *Gynura cusimbua* seedlings under drought stress

3.2. Effects of Exogenous ABA on malondialdehyde (MDA) content of Gynura cusimbua seedlings under drought stress

As shown in Fig. 2, compared with CK1 treatment, MDA content of CK2 treatment was significantly increased under drought stress treatment, which indicated that drought stress caused membrane lipid peroxidation and promoted the production of MDA. Under different concentrations of ABA solution treatment, the content of MDA decreased first and then increased, but it was lower than that of CK2 treatment. There were significant differences among the treatments, among which T4 (200 μ mol/L) had the largest decrease.

3.3.Effect of Exogenous ABA on chlorophyll content of Gynura cusimbua seedlings under drought stress. As shown in Fig. 2, the chlorophyll content of CK1 was significantly lower than that of CK2 under drought stress. After treatment with different concentrations of ABA, the content of chlorophyll first increased and then decreased, and the content was higher than that of CK2. Under T4 (200 μ mol / L) treatment, the chlorophyll content reached the highest, which was the best to alleviate drought stress.



Fig. 2 Effect of Exogenous ABA on MDA and green content in leaves of *Gynura cusimbua* seedlings under drought stress

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3.4.Effects of Exogenous ABA on Fv/Fm and Fo of Gynura cusimbua seedlings under drought stress As shown in Fig. 3, compared with CK1 treatment, the Fv/Fm decreased significantly and Fo increased significantly in CK2 treatment compared with CK1 treatment under drought stress. Under different concentrations of ABA treatment, Fv/Fm showed a significant first rise and then decline trend, and Fo showed a first decline and then rise trend, and were higher than CK2 treatment, the difference was significant among the treatments. Among them, under T4 (200 μ mol/L), Fv/Fm increased the most, and Fo decreased the most, which had the best effect on relieving stress.



Fig. 3 Effects of Exogenous ABA on Fv / Fm and Fo of *Gynura cusimbua* seedlings under drought stress

3.5.Effects of exogenous ABA on NPQ and qL of Gynura cusimbua seedlings under drought stress As shown in Fig. 4, under drought stress treatment, NPQ and qL decreased significantly with the increase of stress days in CK2 treatment compared with CK1 treatment. After analysis of variance it is found that NPQ and qL showed a significant first rising and then decreasing trend under different concentrations of ABA treatment, and were higher than CK2 treatment. Among them, under T4 treatment (200µmol/L), NPQ and qL increased the most, which had the best stress relief effect on Gynura cusimbua seedlings.



Fig. 4 Effects of exogenous ABA on NPQ and qL of Gynura cusimbua seedlings under drought stress

3.6.Effects of exogenous ABA on Y(NPQ), Y(II), Y(NO) of Gynura cusimbua seedlings under drought stress

Fig. 5 shows that under drought stress treatment, Y (NPQ), Y (II) increased significantly and Y (NO) decreased significantly with the increase of stress days in CK2 treatment compared with CK1 treatment. The analysis of variance showed that Y (NPQ) and Y (II) showed an upward and downward trend under different concentrations of ABA treatment, which were higher than CK2 treatment, and Y (NO) showed an upward and downward trend at first, and were higher than CK2 treatment, with obvious differences among treatments. Among them, under T4 treatment (200µmol/L), Y (NPQ), Y (II) increased the most and Y (NO) decreased the most.



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Fig. 5 Effects of exogenous ABA on Y (NO), Y (II), Y (NPQ) of *Gynura cusimbua* seedlings under drought stress

4. Conclusion

When plants are subjected to adversity stress, excessive free radicals will be produced in the cells, which will destroy the balance of free radical metabolism, causing or accelerating lipid peroxidation in the cell membrane, and damaging the cell membrane system(LIANG X.H,et al,2006). The main function of SOD is to scavenge superoxide ion groups in organisms and prevent the damage of reactive oxygen species or other oxidative free radicals to cell membranes. POD can scavenge harmful free radicals in cells and catalyze toxic substances to protect plant cell membrane system. CAT can decompose the H2O2 produced in the process of stress and reduce the damage of plant cells caused by drought stress. Compared with those without sprayed ABA under drought stress, exogenous ABA significantly increased the activities of SOD, POD and CAT in Gynura cusimbua seedlings under drought stress, alleviated the peroxidation of membrane lipids, alleviated the degree of cell membrane damage, reduced the damage of reactive oxygen species to cells, reduced membrane lipid peroxidation, stabilized membrane permeability, and scavenged free radicals, thus reducing the damage of drought to sunflower seedlings. This indicates that exogenous ABA can alleviate the damage caused by plant adversity. MDA is a product of membrane lipid peroxidation, and its content to some extent reflects the level of membrane lipid peroxidation and the degree of damage to membrane structure in plant cells(CAO Q.H., et al,2016). It can be seen from the above figure that MDA content will increase significantly when plants are stressed, but after spraying exogenous substance ABA, it can effectively alleviate the accumulation of MDA in sunflower seedlings under drought stress, indicating that ABA significantly inhibits the generation of MDA and protects the cell membrane structure to a certain extent. Chlorophyll content is the material basis of plant photosynthesis, and its content is an important physiological index to evaluate the photosynthetic capacity of plants(LI Z.Z., et al,2007). Fo represents the fluorescence yield when the PS II reaction center is fully open, and it is related to the chlorophyll concentration. Fv/Fm represents the maximum photochemical efficiency and reflects the intrinsic light energy conversion efficiency of plant PS II photosynthetic center(LI H.M., et al, 2009), which is the most important index to evaluate the stress of plants. Y(II) reflects the proportion of energy consumed by photochemical reactions, embodying of the actual photosynthetic efficiency of plants(SONG X.M.,et al,2013), and also is a positive indicator of plant photosynthesis. With the occurrence of stress, plant chlorophyll content decreased significantly, Fo increased significantly, Fv/Fm and Y(II) decreased significantly, indicating that the photosynthetic system was damaged, plant photosynthesis was affected, and photosynthesis was reduced, which was not conducive to plant growth and affected the accumulation of plant biomass. Exogenous ABA can significantly increase chlorophyll content, reduce Fo, increase Fv/Fm, Y(II), alleviate the degree of stress on plants, improve plant photosynthesis, and reduce the loss of biomass caused by plants. QL is the fluorescence quenching caused by photosynthesis, representing the part of photosynthetic energy used for fixed energy of dark reaction. The higher the value, the more energy transformed into active chemical energy in light energy, the higher the utilization efficiency of light energy of plants. NPQ and Y (NPQ) are redundant light energy that reflects the energy dissipated by plants in the form of heat and cannot be used for photosynthetic electron transport. Thermal dissipation of redundant light energy is an important form of plant protection(ZHANG S.B., et al, 2016). Experiments showed that qL, NPQ and Y (NPQ) were significantly decreased under drought stress, which reduced their ability to convert light energy, decreased their ability to protect light, and caused periphery damage to plants. However, under the application of exogenous ABA, the indicators showed IOP Conf. Series: Earth and Environmental Science 615 (2020) 012089 doi:10.1088/1755-1315/615/1/012089

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an upward trend, indicating that exogenous ABA played a mitigating role in drought stress. Y(NO) reflects the non-regulated quantum yield of plant photosynthetic system caused by photopassivation and is a negative evaluation index of light damage(LIU L.Y.,et al,2018). Y(NO) increased significantly, indicating that drought stress caused damage to sunflower photosystem, and spraying of ABA reduced Y(NO), again indicating that exogenous ABA can alleviate the damage to plants in adversity.

However, this experiment only studied the effects of ABA on some physiological characteristics and chlorophyll fluorescence physiology of *Gynura cusimbua* seedlings under drought stress. The research on *Gynura cusimbua* is not comprehensive, and only further physiological and molecular studies on *Gynura cusimbua* can better explain this physiological process.

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