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Protective Roles of Brassinolide on Rice Seedlings under High Temperature Stress

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Abstract: Two indica rice (*Oryza sativa* L.) materials, Xieqingzao B (sensitive to heat stress) and 082 (tolerant to heat stress), were used to study the role of brassinolide (BR) in protection of rice seedlings from heat stress. Young seedlings were subjected to high temperature (38˚C/30˚C) and sprayed with 0.005 mg/L of BR. Analysis was conducted on the contents of chlorophyll, protein and malondialdehyde (MDA), the leakage of electrolyte, the activities of peroxidase (POD) and superoxide dismutase (SOD) and their isozymes expression levels in leaves. Under the high temperature treatment, application of BR significantly increased the contents of chlorophyll and protein, and the activities of POD and SOD, and reduced the content of MDA and the leakage of electrolyte in the leaves of the heat-sensitive material Xieqingzao B, whereas BR had less effect on those of the heat-tolerant material 082 relatively. The BR treatment enhanced the expression of POD isozymes in the leaves of both materials. Under the high temperature stress and BR treatment, the expression of four SOD isozymes reduced in 082, but the expression of two SOD isozymes increased in Xieqingzao B. This suggests that BR plays an important role in protection of rice seedlings from heat stress by enhancing the activities or expression level of protective enzymes in the leaves. The materials with various heat-tolerance might differ in the mechanism of response to heat stress with BR application.
Key words: brassinolide; rice seedlings; heat stress; antioxidase; membrane lipid peroxidation

Plant membrane system is stable and the substance metabolism is in equilibrium state under normal temperature conditions. But they will be broken when high temperature stress occurs, and the plant will be injured. In crop management, chemical regulators, as well as fertilizer and irrigation can be used to increase the tolerance to heat stress. Many reports have proved that the application of some chemical substances, such as abscisic acid, salicylic acid and jasmonic acid, and some inorganic salts, can enhance the tolerance to heat stress in crop [1-3].

Brassinolide (BR) is a new type of plant growth regulator, which is considered to be the 6th plant hormone. BR has a high bioactivity on the growth and development of plant, such as promoting growth, delaying senescence and accelerating cell redifferentiation and so on [4-5]. All these physiological effects are regulated by gene expression. Many reports indicate that BR can make the plants produce response and regulation to high temperature stress, increase the heat tolerance in upland crops or economic crops such as *Brassica napus*, tomato, orange and tobacco [6-7], however there is few reports about rice. In this experiment, two rice (*Oryza sativa* L.) materials with different heat tolerance were used to investigate the physiological regulating effects of BR on rice seedlings and the mechanism under high temperature stress.

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MATERIALS AND METHODS

Rice materials and treatments

Two indica rice materials, Xieqingzao B (sensitive to heat stress) and 082 (tolerant to heat stress), were provided by the College of Agronomy, Jiangxi Agricultural University. Rice seeds were sterilized for 12 h in the solution with 50% carbandazim being diluted to 800-fold, and rinsed with tap water. The sterilized seeds were planted on moist filter paper in culture dish under a culture temperature of 25±2˚C and a light intensity of 500 μmol/(m^2 · s) with a photoperiod of 12 h in light and 12 h in dark. Uniform rice seedlings at 1.1-leaf-age were transferred into culture pot with Kimura B nutrient solution, with 50 seedlings per pot. At the 3.1-leaf-age of the seedling, the rice seedlings were sprayed with 0.005 mg/L BR (purchased from Shandong Penglai Industry Co. Ltd) and water (CK) for seven times with once in every two days and 50 mL each time. Before spraying, a little Tween 20 was sprayed as surfactant. Each treatment was replicated three times. At 15 days after spraying, the pots with rice seedlings were moved to phytotrons for high temperature treatment (38˚C/30˚C) for three days, and then transferred to phtyotrons for normal temperature treatment (25˚C/20˚C) for three days. The rice seedlings were sampled respectively after high temperature treatment and normal temperature treatment.

Determination of chlorophyll, protein and malondialdehyde contents

One gram fresh leaves were sampled for determining the chlorophyll, protein and malondialdehyde contents. Chlorophyll content was measured with an UV755B type UV-VIS spectrophotometer based on the method of Arnon [8]. Protein content was quantified by the method of Bradford [9] with bovine serum albumin as standard protein. Malondialdehyde content was assayed by the thiobarbituric acid (TBA) method [10].

Assay of peroxidase and superoxide dismutase activities

Fresh leaves (0.5 g) of each sample were homogenized in chilled phosphate buffer (0.05 mol/L, pH 7.8) containing 10% (*W/V*) polyvinylpyrrolidone. The homogenate was transferred and diluted to 5 mL with phosphate buffer in a 5-mL scale tube and centrifuged at 13 000 r/min for 20 min. The supernatant was the crude enzyme solution for assaying peroxidase and dismutase activities and isozyme expression.

The activity of superoxide dismutase was measured by the nitroblue tetrazolium (NBT) method. The crude enzyme solution (10–50 μL) was put in a mixed solution containing 0.9 mL sodium phosphate buffer (0.05 mol/L, pH 7.8), 1.5 mL methionine sodium phosphate buffer (0.026 mol/L), 0.3 mL NBT (0.75 mmol/L), 0.3 mL EDTA (1.0 μmol/L), 50 μL riboflavin (0.02 mmol/) and illuminated for 15 min in phytotrons with a light intensity of 600 μmol/(m^2 · s) at 25˚C, then immediately placed in the dark to terminate reaction, with no crude enzyme solution as blank control. The absorbance was measured at 560 nm. One SOD unit (U) is defined as the amount (μL) of SOD necessary to inhibit 50% of NBT reduction.

The activity of peroxidase (POD) was measured by the guaiacol oxidation method [11]. The optical density (OD) change per minute was expressed as the value of enzyme activity, which is $\triangle A_{470}$/(min · g FW).

Isozyme expression

The crude enzyme solution (0.1 mL) and 0.1 mL 40% sucrose solution were put into a finger tube, and the tube was shaken thoroughly for isozyme analysis. The polyacrylamide vertical slab gel was used for electrophoresis. The stacking gel concentration was 3.5%, and the resolving gel concentrations were 10% for SOD and 7.5% for POD. The added sample was 40 μL per well. The electrophoresis was conducted in the Tris-glycine buffer in a 4˚C refrigerator for 8–9 hours, under the current intensities at 1 mA/well for stacking gel and 2 mA/well for resolving gel. After electrophoresis, the gels were stained with NBT [12] and acetic acid-benzidine [13] for SOD and POD assays, respectively.

Measurement of electrolyte leakage

Electrolyte leakage was measured according to the method described by Zhang [14] with slight modifications. The leaves of rice seedlings (0.2 g) were soaked in 20 mL deionized water and shaken for 30 min. After being stored at room temperature for 30 min, the conductivity (E_1) was measured with a conductivity meter (DDS-11A, Shanghai, China). Later, the measured samples were immersed in boiling water for 10 min, and the conductivity (E_2) were measured again after cooling. In addition, the conductivity of deionized water (E_0) was measured. The leakage of electrolyte of sample was calculated based on the formula: $(E_1–E_0)/(E_2–E_0)\times100\%$.

RESULTS

Effects of BR on chlorophyll and protein contents

Under high temperature stress, the difference in the chlorophyll contents in the leaves between BR treatment and CK (spraying water) in heat tolerant material 082 was not significant, but when seedlings