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RESEARCH ARTICLE

Exogenous application of a low concentration of melatonin enhances salt tolerance in rapeseed (*Brassica napus* L.) seedlings

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Abstract

Melatonin is a naturally occurring compound in plants. Here, we tested the effect of exogenous melatonin on rapeseed (*Brassica napus* L.) grown under salt stress. Application of 30 μmol L^{-1} melatonin alleviated salt-induced growth inhibition, and the shoot fresh weight, the shoot dry weight, the root fresh weight, and the root dry weight of seedlings treated with exogenous melatonin increased by 128.2, 142.9, 122.2, and 124.2%, respectively, compared to those under salt stress. In addition, several physiological parameters were evaluated. The activities of antioxidant enzymes including peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) were enhanced by 16.5, 19.3, and 14.2% compared to their activities in plants without exogenous melatonin application under salt stress, while the H_2O_2 content was decreased by 11.2% by exogenous melatonin. Furthermore, melatonin treatment promoted solute accumulation by increasing the contents of proline (26.8%), soluble sugars (15.1%) and proteins (58.8%). The results also suggested that higher concentrations (>50 μmol L^{-1}) of melatonin could attenuate or even prevent the beneficial effects on seedling development. In conclusion, application of a low concentration of exogenous melatonin to rapeseed plants under salt stress can improve the H_2O_2-scavenging capacity by enhancing the activities of antioxidant enzymes such as POD, CAT and APX, and can also alleviate osmotic stress by promoting the accumulation of osmoregulatory substances such as soluble proteins, proline, and water soluble glucan. Ultimately, exogenous melatonin facilitates root development and improves the biomass of rapeseed seedlings grown under salt stress, thereby effectively alleviating the damage of salt stress in rapeseed seedlings.

Keywords: melatonin, rapeseed (*Brassica napus* L.), salt, seedlings

1. Introduction

Salinity is one of the major abiotic factors limiting crop yield and threatening food security worldwide (Sah *et al*. 2016). In plants, salt stress can result in the production of excessive reactive oxygen species (ROS), and can also cause the peroxidation of membrane lipids or proteins and destroy the

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normal structure of cell membranes, possibly leading to cell death. Additionally, high concentrations of salt can cause osmotic stress with a reduction of water potential in plant roots, subsequently impedes water and nutrient uptake, and severely inhibits plant growth and development, possibly resulting in the wilting and death of plants (Julkowska and Testerink 2015).

Developing crops to grow successfully under salt stress has been a concern for a long time (Munns 2002). Plant growth regulators are extensively used to regulate plant growth and to enhance plant stress tolerance. Therefore, exploring potential growth regulators and their mechanisms is highly important for improving salt tolerance in crops. Melatonin (N-acetyl-5-methoxytryptamine) is an indole hormone widely presenting in plants and animals (Barratt *et al*. 1977; Dubbels *et al*. 1995; Reiter *et al*. 2011; Nawaz *et al*. 2015; Shi *et al*. 2016). Exogenous melatonin has been reported to improve salt tolerance effectively in certain plants. Li *et al*. (2012) found that pretreatment with melatonin attenuated the inhibitory effects of salt stress on plant growth signifcantly, including retarding the degradation and loss of chlorophyll, maintaining relatively high photosynthetic efficiency, and reducing the oxidative damage caused by salt stress in *Malus hupehensis*. Under salt stress, the expression of the ferredoxin gene *PetF* was decreased in soybean seedlings and could be effectively increased by exogenous melatonin through modulating the ascorbate content and inhibiting chlorophyll degradation (Zhang *et al*. 2014; Wei *et al*. 2015). Zhang *et al*. (2014) also found that pretreatment with exogenous melatonin enhanced the expression of genes encoding antioxidant enzymes and significantly improved the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in cucumber seeds, thereby attenuating the oxidative damage and improving the germination rate of cucumber seeds under salt stress. SOD, POD, CAT, and APX are important antioxidant enzymes in plant, as they can help maintain the stability and integrity of the cell membrane by scavenging hydroxyl peroxide and hydrogen peroxide, which reduce the damage caused by ROS (Li *et al*. 2012; Kostopoulou *et al*. 2015).

Rapeseed (*Brassica napus* L.) as a major resource for oil production, is moderately sensitive to salt stress, and the yield is affected by salt stress, especially in arid and semi-arid regions (Musgrave 2000). Despite previous reports on melatonin regarding salt stress, to the best of our knowledge, no relevant study has been conducted on salt stress in rapeseed. Therefore, in the present study, we examined the adaptability of rapeseed seedlings in salt stress *via* the exogenous application of melatonin by evaluating several phenotypic and physiological indices, aiming to exploring the possible mechanism of salt tolerance.

2. Materials and methods

2.1. Plant materials

The rapeseed variety ZS11 was supplied by the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences.

2.2. Methods

Healthy seeds were selected and disinfected by soaking in 3% NaOCl solution for 10 min. After being rinsed with distilled water, the seeds were sown on fine gauze and cultured in a 24°C culture chamber with a 16 h/8 h light-dark photoperiod for 7 d.

Preliminary test The uniform seedlings with two leaves were transferred into modified Hoagland's nutrient solution (Dun *et al*. 2016) with different concentrations for selecting the optimal NaCl salt stress. The concentrations of the Hoagland's nutrient solution were as follows: 0 NaCl (CK), 0.25% NaCl (T1), 0.5% NaCl (T2), 0.75% NaCl (T3), 1.0% NaCl (T4), and 1.25% NaCl (T5). Each treatment consisted of eight seedlings, and three replications were performed. After 7 d treatment, the dry weight and fresh weight of shoot and root for each seedling was determined.

Main test After the optimal NaCl concentration was decided, the main experiment was continued. Seedlings were prepared as the preliminary test. Then, uniform seedlings were transferred into solutions with the optimal NaCl and different melatonin concentrations. The treatments were as follows: 0 NaCl and 0 melatonin (CK1), optimal NaCl and 0 melatonin (CK2), optimal NaCl and 30 μmol L^{-1} melatonin (30MT), optimal NaCl and 45 μmol L^{-1} melatonin (45MT), optimal NaCl and 60 μmol L^{-1} melatonin (60MT), optimal NaCl and 75 μmol L^{-1} (75MT), and optimal NaCl and 100 μmol L^{-1} melatonin (100MT). After 9 d treatment, root length, stem length (height from cotyledons to the growing point), dry weight and fresh weight of shoot and root, and leaf area for each seedling was determined.

Data collection methods The dry weight was measured at 105°C for 30 min and kept at 80°C to a constant weight.

Additionally, fresh samples of the third leaf were collected from each seedling and stored at –80°C until analysis. The leaf samples were used for the determination of biochemical indices, including POD, APX, CAT, H_2O_2, proline, water soluble protein (WSP), and water soluble glucan (WSG). All biochemical indices of leaves were determined using commercial kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).