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RESEARCH ARTICLE

5-Aminolevulinic acid alleviates herbicide-induced physiological and ultrastructural changes in *Brassica napus*

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Abstract

It is well known that application of 5-aminolevulinic acid (ALA) could promote the plant growth under abiotic stress in oilseed rape (*Brassica napus* L.). However, the specifics of its physiological and ultrastructural regulation under herbicide stress conditions are poorly understood. In the present study, alleviating role of ALA in *B. napus* was investigated under four levels of herbicide propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy) benzylamino) benzoate (ZJ0273) (0, 100, 200 and 500 mg L^{-1}) with or without 1 mg L^{-1} ALA treated for 48 or 72 h. Results showed that after 48 h of herbicide stress, the growth of rape seedlings was significantly inhibited with the successive increases of the ZJ0273 concentrations from 0 to 500 mg L^{-1}, but this inhibition was obviously alleviated by exogenous application of ALA. However, when treatment time prolonged to 72 h, the recovery effects of ALA could not be evaluated due to the death of plants treated with the highest concentration of ZJ0273 (500 mg L^{-1}). Further, the root oxidizability and activities of antioxidant enzymes (superoxide dismutase, peroxidase and ascorbate peroxidase) were dramatically enhanced by the application of 1 mg L^{-1} ALA under herbicide stress. Therefore, plants treated with ALA dynamically modulated their antioxidant defenses to reduce reactive oxygen species (ROS) accumulation and malondialdehyde (MDA) content induced by herbicide stress. Additionally, exogenously applied ALA improved the ultrastructure's of chloroplast, mitochondria and nucleus, and induced the production of stress proteins. Our results suggest that ALA could be considered as a potential plant growth regulator for the improvement of herbicide tolerance through alleviation of the physiological and ultrastructural changes induced by the herbicide in crop production.

Keywords: *Brassica napus* L., ALA, ZJ0273, plant growth, antioxidant enzyme activities, ultrastructure, two-dimensional gel electrophoresis (2-DE)

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1. Introduction

Oilseed rape (*Brassica napus* L.) is one of the main oilseed crops in the word and is the major source of edible oil in China (Zhou 2001; Momoh *et al*. 2002). However, the cruciferous weeds are hard to control and have become a serious threat to winter oilseed rape production in the field (Diepenbrock 2000). They compete with crop plants for light, water, nutrients and space (Song *et al*. 2005). Chemical weed management is the mostly used practice to control weeds. Among these herbicides, a new effective herbicide propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy) benzylamino) benzoate (ZJ0273) is being widely used in the oilseed rape fields with the advantages of low dosage, low mammalian toxicity, broad weeding spectrum and environmental compatibility (Tang *et al*. 2005). This original herbicide is a derivative of 2-pyrimidinyloxy-N-aryl benzoate with novel structure and efficient biological activity (Wu *et al*. 2003), and it also was supposed to be one of those herbicides which inhibit biosynthesis of amino acids, protein and so on in the plants (Zhang *et al*. 2009; Jin *et al*. 2010). Moreover, ZJ0273 has similar phytotoxic symptoms and biological activities as the acetolactate synthase (ALS, EC 4.1.3.18) inhibiting herbicides (Zhou *et al*. 2007; Xu *et al*. 2015). More precisely, this new herbicide ZJ0273 shows pro-herbicide characters as it has shown only a weak inhibiting effect on ALS *in vitro* (Zhang *et al*. 2009).

However, application of herbicide produces stress responses in crop plants, which results in cell damage because of peroxidation of membrane lipids, protein oxidation, enzyme inhibition, DNA and RNA damage (Islam *et al*. 2016; Kaya and Doganlar 2016). In order to adapt the stress conditions, plants have developed different molecular and bio-physiological responses through hormones dependent signaling pathways (Yang *et al*. 2012; Xia *et al*. 2015, 2016). Previous studies revealed that 5-aminolevulinic acid (ALA) application can enhance chlorophyll biosynthesis and photosynthesis, and improve antioxidant capacity as well as reduce membrane lipid peroxidation damage of plants (Hotta *et al*. 1997). Similarly, ALA is considered as one of most important plant growth regulators, which is known as essential precursor for the biosynthesis of tetrapyrrols such as heme and chlorophyll (Akram and Ashraf 2013). Recently, Ali *et al*. (2014b) reported that ALA improved the plant biomass, uptake of nutrients in the leaves and roots of *B. napus* plants, and enhanced the performance of antioxidant and some non-antioxidant enzyme activities due to its ameliorative potential under Pb stress conditions. ALA has been demonstrated to alleviate the water stress in *B. napus* (Liu *et al*. 2011). Moreover, ALA has been found to promote the plant growth through alleviation of antioxidant systems under cadmium stress in *B. napus* (Ali *et al*. 2013a, b, 2014b). Thus, wealth of knowledge indicated that exogenous application of ALA could modulate antioxidant defense, and thereby increasing the resistance of plants to the abiotic stresses (Naeem *et al*. 2010, 2011, 2012; Akram *et al*. 2012). Although, the previous study showed that ALA alleviated toxicity of new herbicide ZJ0273 (Zhang *et al*. 2008a, b), the information regarding the optimal treatment interval, ultrastructural and proteomic changes induced by exogenous ALA under herbicide stress is poorly understood. Keeping in view the importance of oilseed rape and the alleviating effects of ALA under abiotic stress, the present study was conducted to prove the hypothesis that ALA has the ameliorating capacity to improve the plant growth under herbicide ZJ0273 stress by recovering antioxidant enzyme activities, mitigating ultrastructural and proteomic attributes.

2. Materials and methods

2.1. Chemicals

The ALA was purchased from Cosmo Oil Co., Ltd. (Japan) and the herbicide propyl ZJ0273 (10%, emulsion) was provided by the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences.

2.2. Seed germination experiments

Seeds of oilseed rape (*B. napus cv*. ZS758, popular commercial cultivars in China) were selected for the experiments. Seeds were washed with distilled water and air-dried before use. The concentrations of ALA and herbicide ZJ0273 treated on the seeds were determined according to the results of our previous study (Zhang *et al*. 2008b). The details of the experiments were conducted as the following procedures:

1) ALA pre-treatment: 6 mL 0 and 1 mg L^{-1} ALA solutions were added to two pieces of filter paper in Petri dishes previously, respectively, and then 50 seeds were sown for different intervals (48 or 72 h). Later the excess solutions were removed and 6 mL 0, 100, 200, and 500 mg L^{-1} ZJ0273 solutions were added to the Petri dishes, respectivley, for another 48 or 72 h, respectively.

2) ALA post-treatment: 6 mL 0, 100, 200, and 500 mg L^{-1} ZJ0273 solutions were added firstly, then 6 mL 0 and 1 mg L^{-1} ALA solutions was added, respectively, other procedures were the same as the ALA pre-treatment procedure 1).

3) Culture conditions: The excess solutions were discarded; seeds and filter paper were transferred onto a sponge floating in a high-wall white porcelain plate filled with half-strength Hoagland solution. Seeds were cultured in the incubator at a temperature of 20°C under a 12-h photoperiod (light intensity of 140 μmol m^{-2} s^{-1}) and high relative humidity (85–90%).

2.3. Determination of biomass and physiological characters

Fresh weights of shoot (10 plants) and root (50 plants), and shoot lengths of *B. napus* seedlings were measured after