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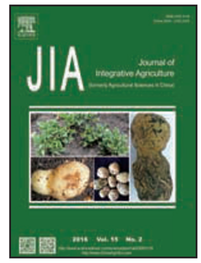

RESEARCH ARTICLE

Effects of chilling tolerance induced by spermidine pretreatment on antioxidative activity, endogenous hormones and ultrastructure of *indica-japonica* hybrid rice seedlings

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Abstract

Spermidine (Spd) is known to be involved in the regulation of plant responses to chilling stress and counteract the adverse effect of stress conditions. Antioxidant activities, endogenous hormones and ultrastructure change under chilling stress were investigated in *indica-japonica* hybrid rice seedlings. 12-d-old seedlings were subjected to exogenous Spd (1 mmol L^{-1}) and then a chilling stress (6°C, 4 d) was induced, followed by a subsequent recovery (25°C, 4 d). Results showed that malondialdehyde (MDA) and proline content were enhanced significantly, whereas shoot fresh and dry weights decreased during chilling stress and after recovery; chlorophyll content of chilling-stressed seedlings increased slightly but declined after recovery; additionally, total soluble sugar, sucrose, fructose and starch contents increased significantly during chilling stress, and only soluble sugar and fructose contents were observed in increase after recovery; chilling stress-induced increases in superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities, but declined after recovery, and the level of ascorbate peroxidase was lower during chilling stress and after recovery; however, endogenous indole-3-acetic acid (IAA), zeatin riboside (ZR), gibberellic acid (GA_3), and abscisic acid (ABA) levels were induced decreased compared with Spd pretreatment. The microscopic analysis revealed that chilling stress-induced destruction of the chloroplast envelope during chilling stress and increased the number of plastoglobuli along with aberrations in thylakoid membranes after recovery. In contrast, exogenous Spd protected rice seedlings from chilling-induced injuries in terms of lower malondialdehyde, proline and carbohydrates accumulation coupled with increased endogenous hormones metabolism. After recovery, Spd pretreatment chilling-exposed seedlings showed higher activities of antioxidant enzymes and normal physiological function of chloroplasts. These results suggest that Spd could promote effectively chilling tolerance which might be largely attributable to the integrity of cell structure and normal metabolism of endogenous hormones in *indica-japonica* hybrid rice seedlings.

Keywords: polyamines, chilling stress, antioxidative activity, endogenous hormones, ultrastructure, *indica-japonica* hybrid rice (*Oryza sativa* L.)

1. Introduction

Low temperature is a limiting environmental factor on plants that can cause significant growth and yield reductions in many agronomic crops, including rice (Boyer 1982). Gen-

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erally, the mean lethal temperature for rice is ~4.7°C, and when the ambient temperature goes below 5–10°C, the plant seedlings' growth and development is impeded (Sanchez *et al*. 2014). During chilling stress, cellular membranes can be attacked by free radicals, resulting in membrane lipid peroxidation and accumulating malondialdehyde (MDA) and proline (Dai *et al*. 2012). The generation of reactive oxygen species (ROS) has been associated with oxidases in plasma membrane and the electron transport of chloroplast (Laloi *et al*. 2004) and will affect membranes of cell ultrastructure (Taylor and Craig 1971). Plants exhibit several antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), which have been reported to be correlated with increased stress tolerance, engaged in protection of the cellular membranes and alleviation of ROS effects (Xu *et al*. 2011; Mostofa *et al*. 2014). Jouve *et al*. (2004) reported that carbohydrates are a major category of compatible solutes that include hexoses (mostly fructose and glucose), disaccharides (sucrose), sugar alcohols, all of which accumulate during stress. In previous studies, carbohydrates, such as sugars (glucose, fructose, sucrose and fructans) and starch, were reported to accumulate in plants under salt stress (Parida *et al*. 2002), drought stress (Sheoran and Saini 1996) and chilling stress (Morsy *et al*. 2007), yet data on polyamines application is limited under chilling stress of rice (Morsy *et al*. 2007). Chilling condition causes rapid changes in membrane structure of plant leaves (Xu *et al*. 2008), resulting from closure of stomata induced by osmotic stress. Moreover, endogenous hormones, such as cytokinins (ZR), abscisic acid (ABA), gibberellin (GA_3), and indole-3-acetic acid (IAA), regulate the growth and development of plant leaves by affecting cell division, elongation and differentiation within apical meristems (Luomala *et al*. 2005), are vitally sensitive to ambient environment although they are much little concentration *in vivo* of plant. When plants were encountered to chilling stress, it could adjust the changes of ambient environment in the regulation of the content of endogenous hormones (Wang *et al*. 2006a).

Polyamines (PAs), including putrescine, spermidine (Spd) and spermine, are small aliphatic, low molecular weight polycations nitrogenous compounds that are ubiquitous in prokaryotic and eukaryotic organisms and participate in the regulation of physiological and developmental processes. Pretreatment with PAs significantly enhanced the levels of antioxidant capacity and alleviated osmotic damage caused by membrane lipid peroxidation (Mostofa *et al*. 2014). Meanwhile, PAs mediated in ABA inducing in stomata closure by protecting cell structure to avoid swollen chloroplast (Konstrantinos *et al*. 2010). It has been demonstrated that exogenously applied PAs can rapidly enter the intact chloroplast (He *et al*. 2002) and play a role in protecting the photosynthetic apparatus from adverse effects of environmental stresses. There is a close relationship between PA and hormones in the regulation of plant growth (Liu *et al*. 2005). The function of PAs and hormones (CTK) was superposition, because of chloroplast being rich in PAs, but when it was lacked, it might affect the chloroplast structure and function (Shu *et al*. 2012). In recent studies, exogenous Spd applications successfully alleviated various abiotic stresses, and protected cell structure such as salinity (He *et al*. 2008; Shu *et al*. 2012), drought (Kubis 2008), heat (Mostofa *et al*. 2014), and chilling (Yamamoto *et al*. 2012).

Plants are subject to changes in temperature, both during changes in season and more rapidly within individual days. *indica-japonica* hybrid rice had been widely applied in the field practice owing to the powerful heterosis and super-high yield potential, but it is severely affected by aberrant changes in temperature (Li *et al*. 1997). This fluctuation in temperature might affect the physiology of rice plants and can cause severe damage, especially during the seedling stage. Mostofa *et al*. (2014) reported that foliar application of Spd enhanced heat tolerance during heat stress and after recovery of rice. However, the effect of Spd application on rice seedlings during chilling stress and after recovery is unclear. Moreover, exogenous Spd applications were seldom tested as a way to alleviate chilling stress, particularly in oxidative damage and carbohydrate accumulation, endogenous hormones and ultrastructure of chloroplasts in *indica-japonica* hybrid rice (Yamamoto *et al*. 2012). We hypothesize that low temperature may cause carbohydrate, endogenous hormones and ultrastructure changes in leaves of rice and higher activities of antioxidants with Spd application may alleviate those changes and protect cell membranes from lipid peroxidation. Therefore, in this study, we examined the effect of chilling stress with or without application of exogenous Spd on the carbohydrate content, endogenous hormones, ultrastructure of chloroplasts, and the activity of the antioxidant system in rice plants. The results will be helpful to understanding the roles of Spd applications on enhancing chilling tolerance in *indica-japonica* hybrid rice.

2. Results

2.1. Plant growth

In the present study, the plant height, shoot fresh weight (FW), shoot dry weight (DW), and relative water content decreased significantly during chilling stress and after recovery in the non-Spd-pretreated chilling-stressed seedlings (6°C) as compared with the control, while the Spd pretreatment seedlings showed significantly lower reductions in these