Environmental and Experimental Botany 137 (2017) 58–72

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Seed polyamines metabolism induced by seed priming with spermidine and 5-aminolevulinic acid for chilling tolerance improvement in rice (Oryza sativa L.) seedlings

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ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 19 January 2017
Accepted 3 February 2017
Available online 6 February 2017

Keywords:
Chilling stress
Oryza sativa
Polyamines
Gene expression
Antioxidant enzymes
ALA
Spd
Priming

ABSTRACT

Polyamines (PAs) have been demonstrated to be involved in plant in response to abiotic stresses including chilling stress. The present study was designed to investigate the effects of seed priming with 5 mM of spermidine (Spd) and 8.5 mM of 5-Aminolevulinic acid (ALA) on seed polyamines metabolism associated with the improvement of chilling tolerance in two rice cultivars, Zhu Liang You 06 (ZY) and Qian You No.1 (QY). Germination percentage, seedling growth and seedling vigor index was decreased under chilling stress, but this physiological parameters was improved by Spd and ALA priming in both studied cultivars as compared with unprimed seeds. As well, total phenolics, flavonoids and glycine-betaine were improved by priming treatment. Contrarily, significant decrease of α-amylase activity, soluble sugars and soluble protein contents of both cultivars was observed in chilling stressed plants as compared with normal growth condition (25 °C). However, priming with Spd and ALA significantly increased α-amylase activity, soluble sugars and soluble protein contents with more prominent increase in QY cultivar. Results showed that chilling stress significantly improved superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione peroxidase (GPX), and further enhancement was observed by Spd and ALA-primed seeds. Spd and putrescine (Put) were decreased under chilling stress, while a reverse tendency was observed in case of spermine (Spm) content. The enzymes involved in the PAs biosynthesis, arginine decarboxylase (ADC), ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC) was improved by priming treatment. The relative expressions of genes encoding enzymes involved in PAs biosynthesis increased by Spd and ALA priming. Additionally, priming treatment improved leaf cell and grain structure as compared with the unprimed seeds.

1. Introduction

Chilling stress is one of the most abiotic stresses limiting the productivity, distribution, and the quality of many important strategy crops including rice. Most of the rice cultivars are extremely sensitive to chilling stress during emergence and early seedling development stages (Hussain et al., 2016). It is grown under wide range of environments covering approximately 11% of world arable lands (Seck et al., 2012). The kinetics of many physiological and metabolic processes of plants can be repressed by chilling stress (Ruelland et al., 2009). Earlier studies have reported that chilling stress severely inhibited the germination percentage, seed vigor, and can also delay the seedling growth stages (Cheng et al., 2007; Kang and Saltveit, 2002).

Seed priming is a technique that improves seed performance by uniform and rapid germination with vigorous and normal seedlings. PAs, particularly Put, Spd and Spm, are aliphatic amines with low-molecular-weight involved in various physiological and biochemical processes related to the regulation of plant growth and development under different abiotic stresses (Roychoudhury et al., 2011). Put can be synthesized directly by decarboxylation of ornithine (catalyzed by ODC), or indirectly by decarboxylation of arginine (catalyzed by ADC) via agmatine and N-carbamoylputrescine intermediates. Whereas, Spd and Spm are synthesized directly from Put by successive addition of aminopropyl groups from decarboxylated S-adenosylmethionine that is derived from

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http://dx.doi.org/10.1016/j.envexpbot.2017.02.007
0098-8472/

S-adenosylmethionine (SAM) by the action of SAMDC (Duan et al., 2008). Recent studies have focused on the involvement of PAs in the defense reaction of plants to various environmental stresses (Bouchereau et al., 1999). Some studies have shown the beneficial effects of priming with PAs on seed germination percentage, seed vigor, seedling growth and development of wheat (Farooq et al., 2011), sunflower (Farooq et al., 2007), rice (Farooq et al., 2008) and tomato (Afzal et al., 2009).

Recently, several studies reported that priming with Spd successfully alleviated various abiotic stresses, and protected cell structure of the plants against salinity (Shu et al., 2012), heat (Mostofa et al., 2014) and chilling (Yamamoto et al., 2012) stresses. Furthermore, it has been suggested that priming with Spd plays an important role for improvements of stress tolerance of plants (Kasukabe et al., 2004). Previous studies have reported the beneficial effects of seed priming under chilling stress in different crops. In this regard, Xu et al. (2011) found that chilling tolerance of tobacco seed was improved by priming treatment during seed germination and seedling growth by activation of antioxidant system in the plant tissues during chilling stress. Likewise, Guan et al. (2009) observed that seed germination and seedling growth of maize were enhanced by seed priming under chilling stress. Earlier study demonstrated that exogenous Spd protected rice seedlings from chilling-induced injuries in terms of lower MDA and proline levels, as well as significant increase in SOD, POD, CAT and APX activities coupled with increased endogenous hormones metabolism was observed in Spd-primed plants (Zeng et al., 2016). Furthermore, Spd pretreatment enhanced chloroplast of rice seedlings under chilling stress as compared with untreated plants (Zeng et al., 2016). Similarly, it has reported that seeds primed with salicylic acid solutions produced a higher root and shoot length, final emergence percentage, and relative water content and ultimately induced higher antioxidant activity under chilling stress as compared with untreated seeds (Pouramir-Dashtmian et al., 2014). Our previous study reported that Spd treatment significantly improved seed germination as well as enhanced seed vigor which was indicated by higher germination index, vigor index, shoot heights and dry weights of shoot and root of sweet corn compared with the untreated seeds (Huang et al., 2017). Moreover, exogenous application of Spd significantly increased endogenous Spd content, gibberellins and ethylene contents and simultaneously reduced ABA concentration in embryos of sweet corn during seed imbibition (Huang et al., 2017).

Exogenous applications of ALA have been found to regulate plant growth and development and to enhance chlorophyll biosynthesis and photosynthesis resulting in increasing of seed germination and crop yield (Hotta et al., 1997). It has been stated that treating rice, barley, potato and garlic plants at early growth stages with ALA promoted plant growth and photosynthetic rates resulting in significant increase of crop yield (Tanaka et al., 1992). Furthermore, ALA at low concentrations enhanced the tolerance of plant to chilling (Wang et al., 2004) and salinity stresses (Nishihara et al., 2003). It is also observed that ALA at low concentration regulated the physiological processes associated with plant growth under abiotic stresses, including low temperature (Zhang et al., 2012), salinity (Naeem et al., 2012), drought (Li et al., 2011) and heavy metals (Ali et al., 2013) stresses. Moreover, further studies are required to elucidate the mechanism underlying regulation of specific PAs reactions by Spd and ALA priming to increase the tolerance of crop seeds to low temperature stress. Therefore, the present study provides an interesting findings regarding Spd and ALA priming-induced tolerance to chilling stress in *Oryza sativa* seedlings.

The present study aimed to elucidate the mechanism of Spd and ALA priming to regulate PAs metabolism at physiological levels under chilling stress for improving rice seed chilling-tolerance. In addition, this study also investigated the biochemical changes in rice seed induced by seed priming with Spd and ALA in response to chilling stress. The regulation of PAs metabolism at the transcription level during exposure to chilling stress was analyzed for genes encoding enzymes involved in biosynthesis of PAs to obtain a better understanding of priming-induced mechanisms for enhancements the chilling tolerance and to provide insights for further analyses.

2. Materials and methods

2.1. Seed materials and priming treatments

The seeds of ZY and QY cultivars used in this study were obtained from the Seed Science Center of Zhejiang University. Seeds were surface sterilized according to the method of Sheteiwy et al. (2015). Thereafter, one part of sterilized seeds was primed with Spd (5 mM) and ALA (8.5 mM) according to the method of Sheteiwy et al. (2015). Thereafter, the primed seeds were dried at room temperature for 24 h until they reach their original moisture content. Another part of sterilized seeds without both ALA and Spd priming was used as control (Ck) treatment. The primed and unprimed seeds were stored at room temperature for 24 h prior to germination or analyses.

2.2. Seed germination and seedling growth measurements

After priming treatment, the germination tests and seedlings characters measurements were conducted according to the method of Hu et al. (2016). Three replicates for each treatment, and fifty seeds for each replicate were germinated in covered germination box ($12 \times 18 \times 6$ cm) containing 3 layers of moistened filter paper and placed in germination chambers under a diurnal cycle of 8 h of light at 30 °C, and 16 h of darkness at 20 °C for 14 days (Hu et al., 2016). The number of germinated seeds was recorded after 14 days and the final percentage of seed germination was calculated (ISTA, 2004). The length of shoots and roots of randomly selected ten seedlings for each treatment were manually measured. The seedling fresh weights of ten seedlings for each treatment were immediately weighed after harvesting, and used for dry weights measurement. Seedling vigor index was calculated according to the method of Sheteiwy et al. (2016). For estimation of total phenolics, α-amylase activity, PAs contents, enzymes involved in PAs biosynthesis and their related genes expression in rice seeds, the seeds were imbibed with Spd and ALA in three replications and exposed to chilling stress for only 7 days, thereafter the seeds were separated from the seedlings and stored at -80 °C for their respective analysis.

2.3. Analysis of total phenolics content and α-amylase activity

The content of total phenolic was determined using the Folin-Ciocalteu method as described by Shohag et al. (2012) with slight modification. The total phenolics were quantified by external calibration using gallic acid (Sigma-Aldrich) as a standard. The seeds samples (0.5 g) were independently analyzed in triplicate. For the α-amylase activity measurement, seeds after imbibition in Spd and ALA for 7 days were frozen in liquid nitrogen and then stored at -80 °C. The seed sample (0.5 g) were then hulled and ground into fine powder, followed by fine homogenization with 10 mL distilled water, after which the mixtures were centrifuged at $5000 \times g$ for 10 min. Supernatant was collected in 10 mL-centrifuge tube for chromogenic reaction. The enzyme activity was measured by 3, 5-dinitrosalicylic acid colorimetric method as described by Sheteiwy et al. (2016).