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Seed Priming with Beta-Amino Butyric Acid Improves Abiotic Stress Tolerance in Rice Seedlings

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Abstract: We studied the influence of seed priming with beta-amino butyric acid (BABA) on the growth, physiological and biochemical parameters of seedlings with varied abiotic stress tolerance, which were raised and grown under unstressed and stressed (NaCl/PEG-6000) conditions. Under stressed conditions, the growth of rice seedlings was less when compared to control plants. After BABA priming, the seedling growth increased both under unstressed and stressed conditions as compared to the respective controls. BABA priming of rice seeds caused increase in the photosynthetic pigment content of the leaves, modified the chlorophyll a fluorescence related parameters and also enhanced the photosystem activities of seedlings when compared to their respective non-primed controls. BABA priming also caused increased mitochondrial activities of the rice seedlings. Moreover, BABA priming significantly reduced malondialdehyde content in the seedlings and also resulted in accumulation of proline especially in the NaCl tolerant variety Vyttila 6. BABA seed priming also enhanced the activity of nitrate reductase enzyme and activities of antioxidant enzymes like guaiacol peroxidase and superoxide dismutase. The presence of BABA was detected by high performance thin layer chromatography analysis in the rice seeds whereas in the seedlings it was not detected. Thus, it can be inferred that the seed priming effect of BABA mainly occurred within the seeds, which was further carried to the seedlings. It is concluded that BABA priming of seeds improved the drought and salinity stress tolerance of all the three rice varieties and it was significantly evident in the drought tolerant variety Vaisakh and NaCl tolerant variety Vyttila 6, when compared to the stress sensitive variety Neeraja.

Key words: abiotic stress; drought; mitochondrial activity; photochemical activity; seed priming; rice; beta-amino butyric acid

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and it forms the staple food of more than 50% of the world's population. Rice contributes 43% of total food grain production and 46% of total cereal production in India. Thus, rice plays a vital role in the national food supply (Mondal et al, 2011). Due to its importance, an adequate and stable supply of rice is essential for the financial growth, food security and poverty reduction in Asia, particularly in India. According to Macovei et al (2014), increasing rice production is expected to play a significant role in the advancement of the economic status of developing countries in Asia and Africa. The traditional agricultural practices are not adequate to produce rice grains according to the needs of ever increasing world population. It has been postulated that the world's annual paddy production have to increase up to 7.8×10^8 t by 2020 and over 1.0×10^9 t by the next century (Sass and Ciecerone, 2002). Abiotic stress alone results in 50% of the total yield loss in rice crop, and salinity, drought and extreme temperatures are major obstacles which limit global rice production (Sudharani et al, 2012). Abiotic stress causes many physiological and biochemical changes in the seedlings, which includes the generation of reactive oxygen species (ROS), leading to membrane

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damage and cell leakage, destruction of photosynthetic components (Jisha and Puthur, 2014).

Several ways and means for enhancing the plant tolerance towards abiotic stress have been experimented like breeding of plants and developing transgenics (Jisha et al, 2013). Seed priming is an easy, low cost and low risk method for improving growth and development of plants especially under adverse environmental conditions. It is the induction of a particular physiological state in plants by the treatment of natural and/or synthetic compounds to the seeds before germination. Primed seeds germinate faster and more uniformly than the non-primed ones. Several seed priming methods were successfully used in agriculture for seed conditioning to accelerate the germination rate and improve the seedling uniformity (Nouman et al, 2012; Aghbolaghi and Sedghi, 2014; Bagheri, 2014; Lara et al, 2014). Moreover, seed priming is reported in many crops, which helps them to neutralize the adverse effects of abiotic stress (Ashraf and Foolad, 2005; Patade et al, 2009; Jisha et al, 2013).

Various chemicals, like NaCl, KNO_3, KCl and $CaCl_2$, are usually employed for the seed priming techniques (Farooq et al, 2006; Bajehbaj, 2010; Nawaz et al, 2011). Recently, non-protein amino acids like β-amino butyric acid (BABA) were employed in seed priming of various crops against biotic and abiotic stress (Worrall et al, 2012). GABA (γ-amino butyric acid) is an isomer of naturally occurring non-protein amino acid and is a xenobiotic compound (Jakab et al, 2005; Mayer et al, 2006), whose natural occurrence is very rare. BABA is known as a potent inducer of resistance in plants against nematodes (Oka et al, 1999), microbial pathogens (Cohen, 2002), insects (Hodge et al, 2005) and abiotic stress (Jakab et al, 2005; Zimmerli et al, 2008). BABA exerts its action by priming plants to respond faster and stronger to future stress. According to Zhong et al (2014), BABA can bring plants into a sensitization state in which defenses are not expressed, but are able to react more rapidly and/or more strongly to various stress. BABA-induced priming functions were probably by the interaction of several hormones like salicylic acid (SA), abscisic acid (ABA) and ethylene (Jakab et al, 2005; Ton et al, 2005).

Although BABA priming effects on abiotic stress tolerance of various other crops have been studied, there are no reports with regard to rice, Moreover, most of the earlier reports with regard to other crops have not made an in depth study of the physiological and biochemical changes associated with abiotic stress tolerance of the particular crop subjected to BABA priming. This study was carried out to study the seed priming effects of BABA on abiotic stress tolerance of three rice varieties with varied tolerances to NaCl and drought, by analyzing the morphological, physiological and biochemical changes of primed and non-primed plants subjected to unstressed and stressed conditions (NaCl/PEG-6000).

MATERIALS AND METHODS

Rice materials

Three rice varieties were selected based on their varied levels of drought/NaCl tolerance. Neeraja is abiotic stress sensitive, whereas Vaisakh is drought tolerant and Vyttila 6 is NaCl tolerant. The seeds of Neeraja and Vaisakh were procured from Regional Rice Research Station, Pattambi, Kerala, India, and the seeds of Vyttila 6 were procured from Rice Research Station, Vyttila, Kerala, India.

Methods

Seed priming techniques

Uniform sized seeds were selected for the seed priming treatments and were pre-washed for 1 min with 0.25% Triton X-100 (Boehringer Mannheim Gmbh) to remove dirt. The washed seeds were immersed in different concentrations of BABA solution (0, 0.5, 1.0, 1.5, 2.0 and 2.5 mmol/L) for the seed priming treatments. The volume of BABA solution in which the seeds were immersed was three times more than the seed volume. After 12 h priming treatment in a bottle, the seeds were washed with distilled water for 2 min and surface dried on absorbent paper. During the priming treatment, the container carrying the seeds was swirled intermittently to ensure proper aeration. Surface dried seeds were placed on a piece of clean filter paper, allowing dehydration under shade at 25 ºC for 48 h to retrieve the original seed moisture before priming treatment. The untreated seeds were used as the control. Primed as well as non-primed seeds were germinated in light transparent plastic bottles (19 cm × 11 cm) containing absorbent cotton soaked with distilled water (control), different concentrations of NaCl and polyethylene glycol-6000 (PEG-6000) solution as the case may be. To select stress imparting concentrations of NaCl and PEG-6000, the rice seeds were germinated in various concentrations of NaCl (0, 25, 50, 75, 100 and 125