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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 mmol/L) and PEG-6000 (0%, 5%, 10%, 15%, 20% and 25%). Cotton wool (10 g) was spread at the bottom of the plastic bottles and 80 mL of the test solution (distilled water/NaCl/PEG-6000) was poured into the bottle. On the top of this soaked cotton wool, 50 seeds were placed leaving uniform distance between each other. In order to avoid loss of water, the bottles were covered with a Petri plate. This ensured that evaporation was the minimum and permitted aeration at the same time. For studying each of the parameters, three sets of such bottles were utilized.

The bottles were kept at 24 ºC ± 2 ºC, relative humidity of 55% ± 5%, under a 14 h light and 10 h dark cycle at 300 µmol/(m^2·s) provided by fluorescent tubes in a controlled growth cabinet. The growth and biochemical attributes of all the seedlings were recorded on 9 d after germination. For each experiment, there were six treatments with three replicates. The six treatments include control (unstressed condition and non-primed seeds), primed (unstressed condition and BABA primed seeds), NaCl (NaCl stress condition and non-primed seeds), NaCl + primed (NaCl stress condition and BABA primed seeds), PEG-6000 (PEG-6000 stress condition and non-primed seeds), PEG-6000 + primed (PEG-6000 stress condition and BABA primed seeds).

Morphological, physiological and biochemical studies

Shoot length was measured with the help of a student scale in cm. For fresh and dry weights, the seedlings were blotted and wrapped separately in pre-weighed labeled aluminium foils. Fresh weights were determined by weighing them immediately after wrapping. For dry weight, the samples were kept in an oven maintained at 80 ºC for 48 h, then transferred to a desiccator, cooled and weighed.

Chlorophyll estimation was carried out by the method of Arnon (1949). Total protein content was estimated using Folin-Ciocalteau reagent according to Lowry et al (1951). Total carbohydrate was estimated according to Dubois et al (1956). Proline content in the seedlings was estimated according to Bates et al (1973). The malondialdehyde (MDA) estimation was done according to Heath and Packer (1968). Nitrate reductase activity was measured according to Hageman and Reed (1980). For the estimation of superoxide dismutase (SOD) activity, the protocol of Giannoplitis and Ries (1977) was adopted. Peroxidase (POD) assay was followed after Gaspar et al (1975).

Thylakoids from leaves were isolated according to Puthur (2000) and the photochemical activities of thylakoids were assayed polarographically with a Clark-type oxygen electrode (Hansatech, Norflok, UK) as described by Jisha and Puthur (2014). The activity of PS I and PS II was calculated in terms of nmol O_2 consumed/evolved per min per mg chlorophyll.

Chlorophyll a fluorescence related parameters were measured with the Plant Efficiency Analyzer (Handy PEA, Hansatech Ltd., King's Lynn, Northfolk, UK). All measurements were performed on the upper surface of the youngest fully expanded leaves following a dark adaptation period of 30 min using the leaf clips provided by the manufacturer. The minimum dark adaptation period required for the seedlings of rice was determined in preliminary experiments according to the procedures described in the Handy PEA manual. Maximal fluorescence was induced by a 1-s pulse of white light [3000 µmol/(m^2·s)] with the gain adjusted automatically to 0.7 to avoid over scaling errors. Measurements were taken between 9:00 and 12:00 am. The vitality and efficiency of the seedlings, along with the photosynthetic activities (in terms of above mentioned parameters) were analyzed using the software Biolyzer (Strasser et al, 2004). Energy pipeline model were also deduced using the Biolyzer HP 3 software (Bioenergetics Laboratory, University of Geneva, Switzerland).

Mitochondrial isolation from the seedlings was carried out according to Kolloffel (1967). Oxygen consumption by mitochondria was measured polarographically with a Clark-type oxygen electrode (DW1/AD, Hansatech) which was connected to a digital control box (OXYG1, Hansatech) at 25 ºC as per the protocol of Schmitt and Dizengremel (1989) by using NADH as the substrate. The mitochondrial activity was calculated in terms of nmol O_2 consumed per min per mg protein.

High performance thin layer chromatography was conducted to detect and quantify the BABA content in the seeds and seedlings of rice varieties which were treated with BABA solution. Seeds and seedlings without priming treatments were used as controls. The extraction was carried out according to Giorgini and Campos (1992). BABA content in the samples was estimated by high performance thin layer chromate-graphy (HPTLC) (CAMAG. AMD2, Switzerland) on activated 20 cm × 20 cm high performance silica gel plates (60cm F_{254} TLC plate, Merck) using n-butanol : acetic acid : water (5 : 2 : 2) as solvent system, at temperature of 25 ºC ± 2 ºC and relative humidity of 40%.

Statistical analysis

Univariate analysis of variance (ANOVA) was conducted by using the software SPSS 19. Means were compared using the Duncan's test ($P < 0.05$).

RESULTS

Determination of stress imparting concentration

The stress imparting concentrations of NaCl and PEG-6000 were determined for each variety. The concentrations of NaCl and PEG-6000 which imparted 40% to 50% retardation in various growth attributes (shoot length, fresh weight and dry weight) of each variety were selected as stress imparting concentrations of NaCl and PEG-6000. Neeraja showed 50% growth retardation in 75 mmol/L NaCl or 15% PEG-6000; whereas, it was 75 mmol/L NaCl or 20% PEG-6000 for Vaisakh (drought tolerant) and 100 mmol/L NaCl or 20% PEG-6000 for Vyttila 6 (NaCl tolerant) (Supplemental Fig. 1).

Optimal concentration of BABA for seed priming

After priming the rice seeds with various concentrations of BABA, the seeds were allowed to germinate and grow in containers. The seedling growth parameters data (shoot length, fresh and dry weight) recorded revealed that the most effective concentration of BABA for bringing about the priming effect was 1 mmol/L for all the three rice varieties (Supplemental Table 1).

Seedling growth parameters

Growth retardation of seedlings was observed under NaCl and PEG-6000 stress in all the three rice varieties. Seeds after BABA priming were efficient in decreasing the extent of reduction in growth of the seedlings under NaCl/PEG-6000 stress when compared to non-primed seeds. BABA priming of the rice seeds resulted in enhancement of the seedling growth parameters under unstressed condition. Among the three varieties studied, Vaisakh showed the maximum increase in the shoot length, fresh and dry weight of the seedlings after BABA priming (Table 1).

Photosynthetic pigment composition

Irrespective of the treatments (unstressed and both stressed), BABA priming resulted in prominent increase of chlorophyll a and b content in all the three varieties. BABA priming resulted in the maximum increase of chlorophyll a and b content in Vaisakh under PEG-6000 stress (151% and 132%, respectively). In Neeraja, BABA priming resulted in an increase of 38%–52% in the carotenoid content of seedlings under stressed (NaCl/PEG-6000) and unstressed conditions. Among the tolerant varieties, Vaisakh subjected to PEG-6000 stress showed the highest increase (85%) in carotenoid content in the seedlings raised from BABA primed seeds and in Vyttila 6, the increase was to a lesser extent (21%–59%) (Table 2).

Photosystem activities

BABA priming in Neeraja, resulted in the maximum enhancement of photosystem I (PS I) activity under PEG-6000 stressed (92%) condition while under unstressed and NaCl stressed conditions, the increase of PS I activity in the seedlings was less. With regard to tolerant varieties, BABA seed priming gave the maximum enhancement in PS I activity of seedlings raised and grown under stressed conditions for which they are tolerant. Vaisakh showed the maximum increase in PS I activity under PEG-6000 stress (64%) and Vyttila 6 showed the maximum increase in the PS I activity in the seedlings under NaCl stressed (65%) (Fig. 1).

The seedlings raised from BABA primed seeds of

Table 1. Effects of beta-amino butyric acid seed priming on seedling growth parameters of Neeraja, Vaisakh and Vyttila 6.

Trait	Variety	Control	Primed	NaCl	NaCl + primed	PEG-6000	PEG-6000 + primed
Shoot length (cm)	Neeraja	9.13 ± 0.32 b	11.40 ± 0.51 a	5.57 ± 0.34 c	10.03 ± 0.54 ab	4.53 ± 0.17 c	5.17 ± 0.55 c
	Vaisakh	10.20 ± 0.43 b	10.57 ± 0.42 b	4.77 ± 0.12 d	12.53 ± 0.33 a	4.37 ± 0.12 d	8.67 ± 0.36 c
	Vyttila 6	11.77 ± 0.25 a	11.83 ± 0.19 a	4.90 ± 0.13 d	8.07 ± 0.34 bc	8.07 ± 0.34 bc	9.20 ± 0.48 b
Fresh weight (g)	Neeraja	0.0449 ± 0.0121 c	0.0590 ± 0.0133 a	0.0427 ± 0.0124 cd	0.0536 ± 0.0120 b	0.0394 ± 0.0103 d	0.0446 ± 0.0104 c
	Vaisakh	0.0569 ± 0.0123 c	0.0665 ± 0.0102 b	0.0480 ± 0.0100 d	0.0813 ± 0.0112 a	0.0463 ± 0.0114 d	0.0674 ± 0.0091 b
	Vyttila 6	0.0725 ± 0.0108 ab	0.0839 ± 0.0112 a	0.0505 ± 0.0991 d	0.0586 ± 0.0105 cd	0.0572 ± 0.0113 cd	0.0663 ± 0.0112 bc
Dry weight (g)	Neeraja	0.0136 ± 0.0099 bc	0.0156 ± 0.0104 a	0.0126 ± 0.0114 c	0.0163 ± 0.0121 a	0.0130 ± 0.0114 bc	0.0140 ± 0.0120 b
	Vaisakh	0.0131 ± 0.0113 c	0.0155 ± 0.0114 a	0.0116 ± 0.0132 d	0.0150 ± 0.0131 a	0.0116 ± 0.0112 d	0.0139 ± 0.0109 b
	Vyttila 6	0.0131 ± 0.0105 b	0.0146 ± 0.0103 a	0.0115 ± 0.0106 d	0.0129 ± 0.0134 b	0.0120 ± 0.0142 cd	0.0125 ± 0.0114 bc

The data represent mean ± standard error ($n = 9$). Values in the same line followed by different letters are significantly different at the 0.05 level (Duncan's test).

Table 2. Photosynthetic pigment content in seedling leaves under beta-amino butyric acid priming (values without parenthesis) and non-primed seeds (values in parenthesis). mg/g

Variety	Chlorophyll a			Chlorophyll b		
	Control	NaCl	PEG-6000	Control	NaCl	PEG-6000
Neeraja	2.02 ± 0.16 b	2.26 ± 0.16 a	1.70 ± 0.11 c	0.57 ± 0.05 b	0.65 ± 0.04 a	0.50 ± 0.04 c
	(1.58 ± 0.15 d)	(1.52 ± 0.13 e)	(1.15 ± 0.21 f)	(0.40 ± 0.03 e)	(0.43 ± 0.03 d)	(0.30 ± 0.03 f)
Vaisakh	2.82 ± 0.06 a	2.58 ± 0.15 c	2.76 ± 0.04 b	0.70 ± 0.03 b	0.59 ± 0.05 c	0.72 ± 0.03 a
	(2.16 ± 0.05 d)	(2.00 ± 0.14 e)	(1.10 ± 0.06 f)	(0.51 ± 0.01 d)	(0.49 ± 0.04 e)	(0.31 ± 0.02 f)
Vyttila 6	3.71 ± 0.16 c	4.04 ± 0.06 a	4.03 ± 0.13 b	1.14 ± 0.05 c	1.20 ± 0.04 b	1.29 ± 0.06 a
	(2.84 ± 0.05 d)	(2.53 ± 0.05 f)	(2.74 ± 0.18 e)	(0.83 ± 0.04 e)	(0.77 ± 0.03 f)	(0.89 ± 0.06 d)
Variety	Total chlorophyll			Carotenoid		
	Control	NaCl	PEG-6000	Control	NaCl	PEG-6000
Neeraja	2.59 ± 0.16 b	2.90 ± 0.15 a	2.19 ± 0.15 c	0.89 ± 0.02 b	0.96 ± 0.05 a	0.70 ± 0.03 c
	(1.97 ± 0.14 d)	(1.95 ± 0.13 e)	(1.45 ± 0.11 f)	(0.65 ± 0.02 d)	(0.65 ± 0.06 d)	(0.46 ± 0.06 e)
Vaisakh	3.51 ± 0.15 a	3.16 ± 0.15 c	3.48 ± 0.14 b	1.29 ± 0.05 b	1.23 ± 0.04 c	1.30 ± 0.05 a
	(2.66 ± 0.16 d)	(2.49 ± 0.11 e)	(1.41 ± 0.10 f)	(1.10 ± 0.04 d)	(1.04 ± 0.05 e)	(0.71 ± 0.06 f)
Vyttila 6	4.84 ± 0.12 c	5.26 ± 0.17 b	5.32 ± 0.15 a	1.41 ± 0.04 c	1.46 ± 0.04 b	1.61 ± 0.06 a
	(3.67 ± 0.11 d)	(3.30 ± 0.18 f)	(3.62 ± 0.14 e)	(1.17 ± 0.07 d)	(0.99 ± 0.02 f)	(1.01 ± 0.05 e)

The data represent mean ± standard error (n = 9). Values for the same variety and the same trait followed by different letters are significantly different at the 0.05 level (Duncan's test).

Vyttila 6 showed the maximum increase in PS II activity when compared to the other two varieties and this increase was particularly higher under NaCl/PEG-6000 stress (89% and 88%, respectively), while under unstressed condition, the PS II activity was less (30%). Vaisakh showed prominent increase in PS II activity under NaCl stress (66%) and Neeraja showed comparatively less increase in the PS II activity in the seedlings raised from BABA primed seeds and grown under unstressed and stressed (NaCl/PEG-6000) conditions (Fig. 1).

Chlorophyll a fluorescence related parameters

The derived parameters from chlorophyll a fluorescence measurements were visualized by means of dynamic energy pipeline leaf model of the photosynthetic apparatus and it deals with the phenomenological energy fluxes per cross section (Fig. 2). As a result of NaCl/PEG-6000 stress, the phenomenological energy flux ratios like RC/CS (density of active reaction centres), ABS/CS (photon absorption), TR/CS (trapping of photons) and ET/CS (electron transport) decreased in the rice seedlings as compared to those seedlings which were grown under unstressed conditions. Whereas the dissipation of energy (DI/CS) was found to be increased in the rice seedlings which were subjected to stress conditions as compared to the seedlings grown under unstressed conditions. BABA priming of seeds caused increase in RC/CS, ABS/CS, TR/CS and ET/CS, while decreased the DI/CS from the PS II reaction centres in all the three varieties. Among the three varieties studied, Vyttila 6 showed more positive results towards BABA priming as far as the chlorophyll a fluorescence

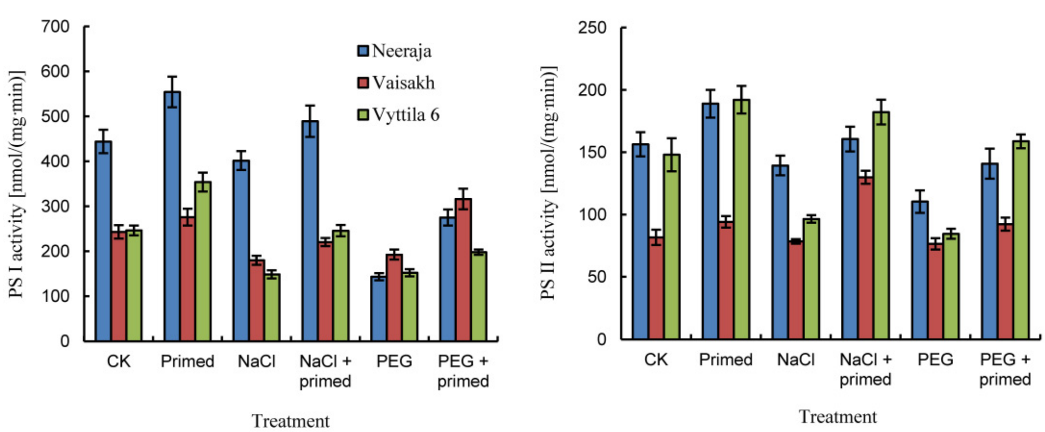

Fig. 1. Effects of beta-amino butyric acid priming on photosystem activities.

CK, Control; PEG, PEG-6000.

Bars represent *SE* of the mean values (n = 9).

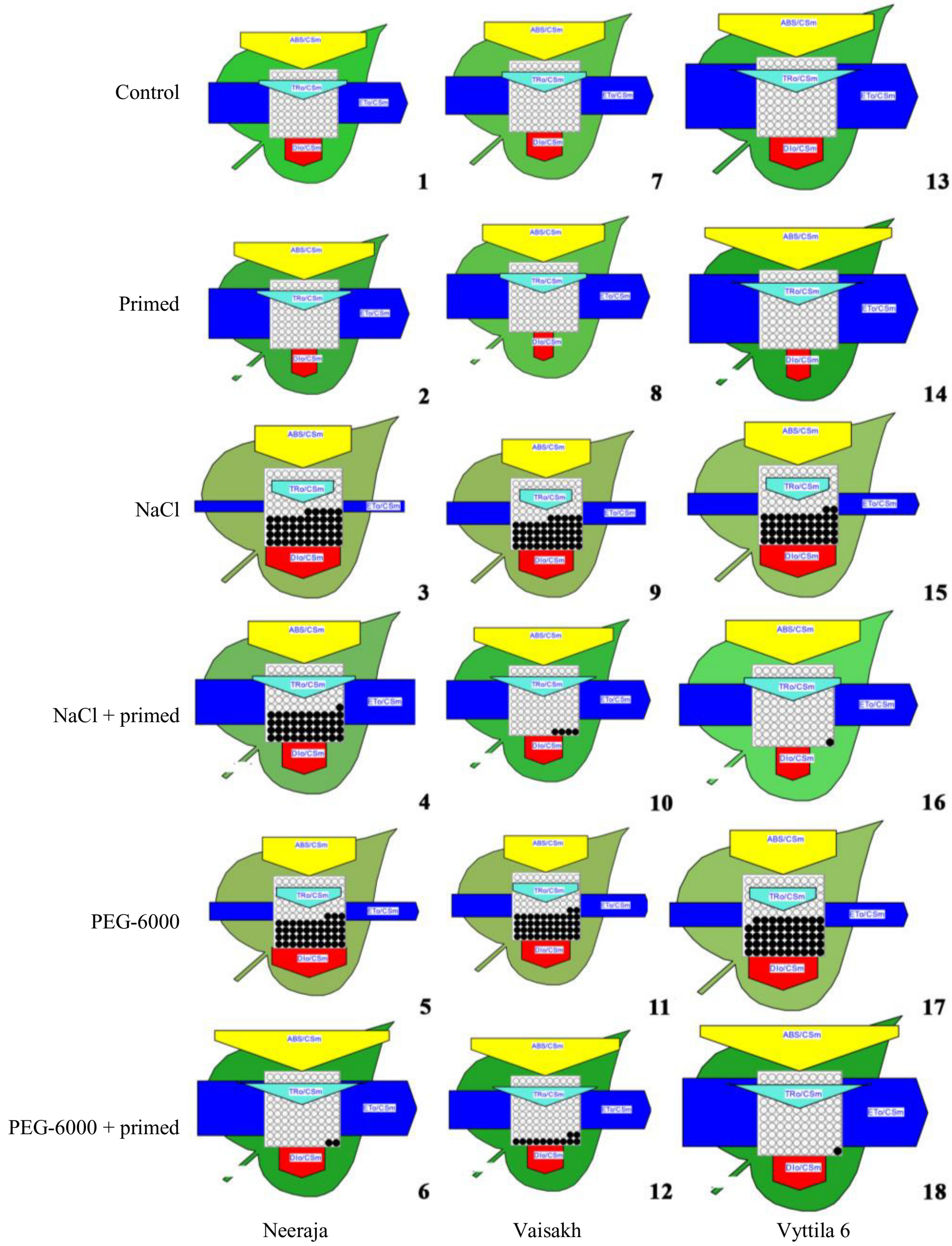

Fig. 2. Energy pipeline leaf model of phenomenological fluxes (per cross section, CS) in three rice varieties.

1–6 (Neeraja), 7–12 (Vaisakh) and 13–18 (Vyttila 6) subjected to different treatments (control, primed, NaCl, NaCl + primed, PEG-6000 and PEG-6000 + primed, respectively). The value of each parameter can be seen in relative changes in width of each arrow. Active reaction centres are shown as open circles and inactive reaction centres are shown as closed circles.

related parameters were concerned.

Mitochondrial activity

BABA priming resulted in the maximum increase of the mitochondrial activity in the tolerant varieties when compared to the abiotic sensitive variety Neeraja. In the drought tolerant variety Vaisakh and NaCl tolerant variety Vyttila 6, BABA priming caused significant increase in mitochondrial activity under NaCl (73%) and PEG-6000 stress (113%), respectively (Fig. 3).

Metabolites

The seedlings raised from BABA primed seeds of Neeraja and Vaisakh subjected to unstressed and stressed (NaCl/PEG-6000) conditions showed prominent increase (37%–60%) in total protein content. Total protein content of Vyttila 6 seedlings were enhanced at equal rates under unstressed and stressed conditions on BABA priming but the increase was not so prominent (22%–28%) as that observed in other two

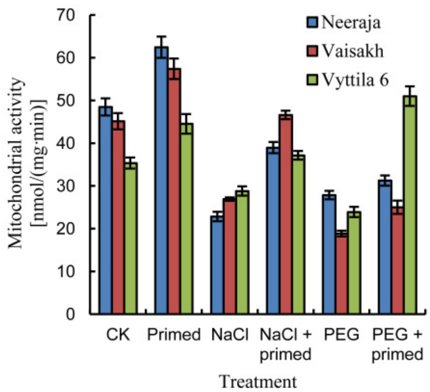

Fig. 3. Effects of beta-amino butyric acid priming on mitochondrial activity of three rice varieties.
CK, Control; PEG, PEG-6000.
Bars represent *SE* of the mean values (*n* = 9).

varieties on BABA priming (Fig. 4). BABA priming in Neeraja and Vaisakh resulted in the maximum increase of total carbohydrate content of the seedlings which were grown under NaCl stress (57% and 51%, respectively). While, in Vyttila 6, BABA priming resulted in the maximum enhancement of total carbohydrate in the seedlings subjected to PEG-6000 stress (57%) (Fig. 4).

BABA priming resulted in varied response in the case of proline accumulation in the seedlings of three rice varieties raised from BABA primed seeds and grown under unstressed and NaCl/PEG-6000 stress conditions. Neeraja after BABA priming showed significant increase in proline content under unstressed and NaCl stress conditions, but showed reduction in proline content under PEG-6000 stress. In BABA primed Vaisakh, proline accumulation occurred only under unstressed condition while reduction in proline content was observed under NaCl and PEG-6000 stress conditions. BABA priming caused accumulation of proline in Vyttila 6, especially under unstressed condition (104%) (Fig. 4).

MDA content

MDA content was found to decrease in the rice seedlings raised from BABA primed seeds as compared to seedlings raised from non-primed seeds, either in the absence or presence of stress (NaCl/PEG-6000). In Neeraja, the maximum reduction occurred in the seedlings which were grown under NaCl stress (42%), while in Vaisakh and Vyttila 6, the maximum reduction in MDA content occurred under unstressed and NaCl stressed conditions (Fig. 5).

Enzyme activities

In the varieties Neeraja and Vaisakh, BABA priming caused significant increases in the activity of nitrate reductase (NR) under PEG stress (116% and 109%, respectively). Whereas BABA priming in Vyttila 6 caused the maximum increase in the activity of NR in the seedlings which were raised and grown under unstressed condition (36%) (Fig. 5). BABA priming resulted in the maximum increase of superoxide dismutase (SOD) activity in Vaisakh seedlings when compared to the other two varieties. After BABA priming, the maximum increase in guaiacol peroxidase (POD) activity was recorded in Neeraja under unstressed condition, while in the stressed conditions, the increase in POD activity was less. BABA priming in Vaisakh resulted in the maximum increase of POD activity under PEG-6000 stress (50%) and in Vyttila 6, BABA priming caused the maximum increase in POD activity under unstressed condition (32%).

Detection and quantification of BABA

HPTLC analysis revealed the presence of BABA in the rice seeds primed with BABA solution. Among the three varieties studied, the drought tolerant variety

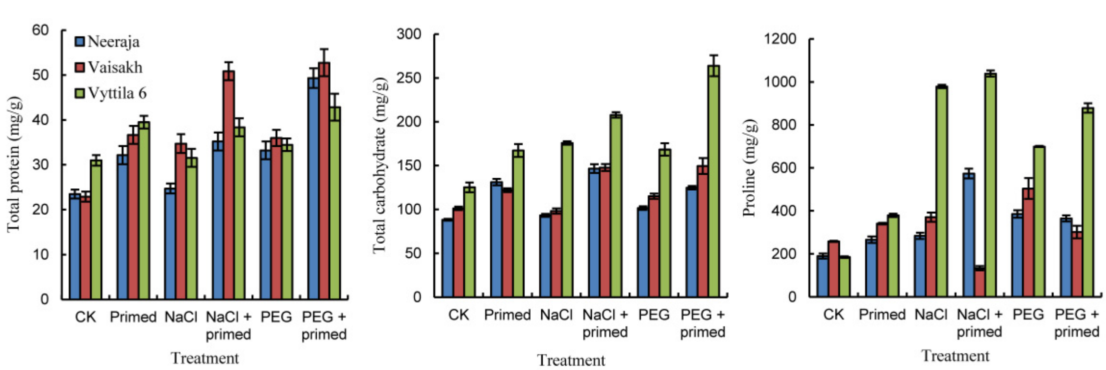

Fig. 4. Effects of beta-amino butyric acid priming on total protein, total carbohydrate and proline contents of three rice varieties.
CK, Control; PEG, PEG-6000.
Bars represent *SE* of the mean values (*n* = 9).

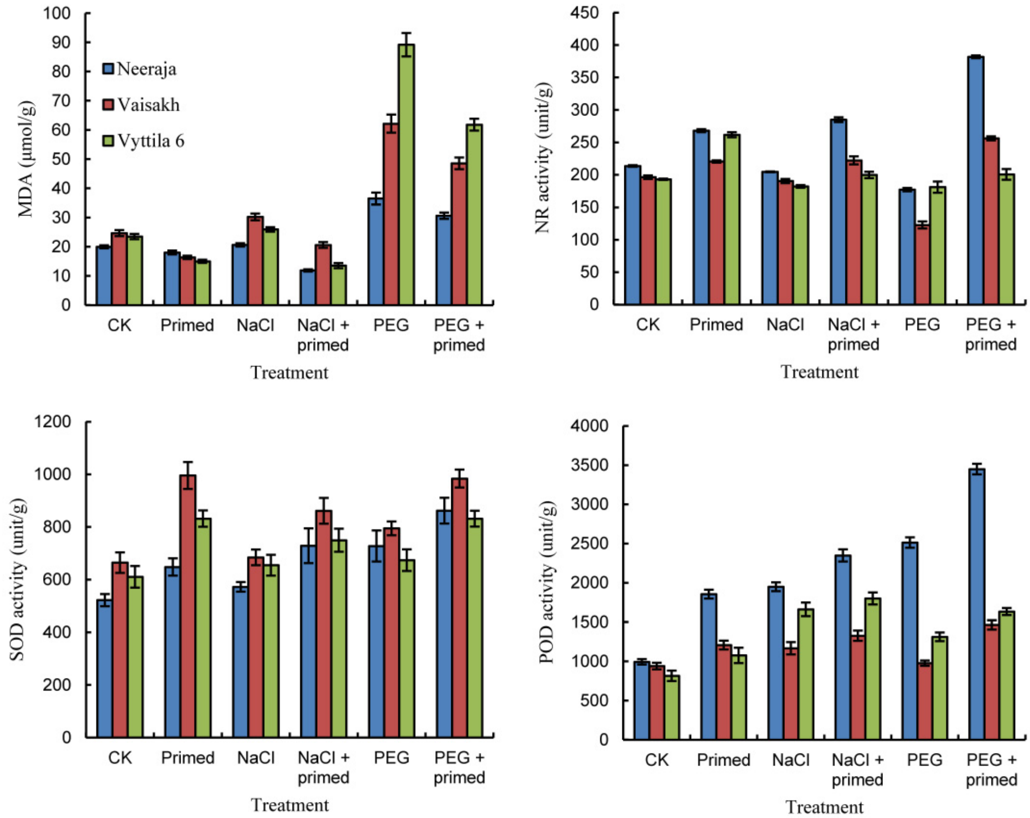

Fig. 5. Effects of beta-amino butyric acid priming on malondialdehyde (MDA) content, nitrate reductase (NR), superoxide dismutase (SOD) and guaiacol peroxidase (POD) activities of three rice varieties.
CK, Control; PEG, PEG-6000.
Bars represent *SE* of the mean values (n = 9).

Vaisakh had the maximum content of BABA (0.87 mg/g) in the seeds primed with 1 mmol/L BABA solution. Whereas 0.51 and 0.72 mg/g BABA were quantified in Neeraja and Vyttila 6, respectively. HPTLC analysis did not detect the presence of BABA in the seedlings of all the three varieties raised from BABA primed and non-primed seeds (Fig. 6).

DISCUSSION

In the present study, the rice seedlings showed reduced growth under stressed conditions. Usually, plants exposed to stressed conditions make changes in some of their physiological and biochemical features which make them to cope up with the stressed situations. The reduction in the growth attributes of rice seedlings under stressed conditions (NaCl/PEG-6000) may be an adaptive mechanism of the seedlings to cope up with the stress. In the seedlings of all the three rice varieties which were raised from BABA primed seeds, the reduction in growth attributes due to NaCl/PEG-6000 was reduced. This implies that BABA seed priming improved the capacity of seedlings to withstand the stressed conditions. Even in unstressed condition, all the rice seedlings exhibited remarkable increase in growth features as compared to non-primed ones. In rice, seed priming treatment was known to cause increase in seedling growth parameters (Goswami et al, 2013), and it was also reported that seed priming treatments reduced the time taken to initiate the germination process, improved the rate of germination and synchronization, enhanced the lengths of shoot and root and thus increased the fresh and dry weight of the seedlings (Ruan et al, 2002; Mathew and Mohanasarida, 2005; Farooq et al, 2006).

As a result of NaCl and PEG-6000 stress, the photosynthetic pigment content and the activity of photosystems reduced in all the three rice varieties studied. This reduction may be due to the degradation of chlorophyll pigments or disintegration of the complexes involved in photosynthetic machinery. There are reports on the reduction of chlorophyll content under osmotic stress in wheat varieties (Keyvan, 2010). The decrease in chlorophyll content

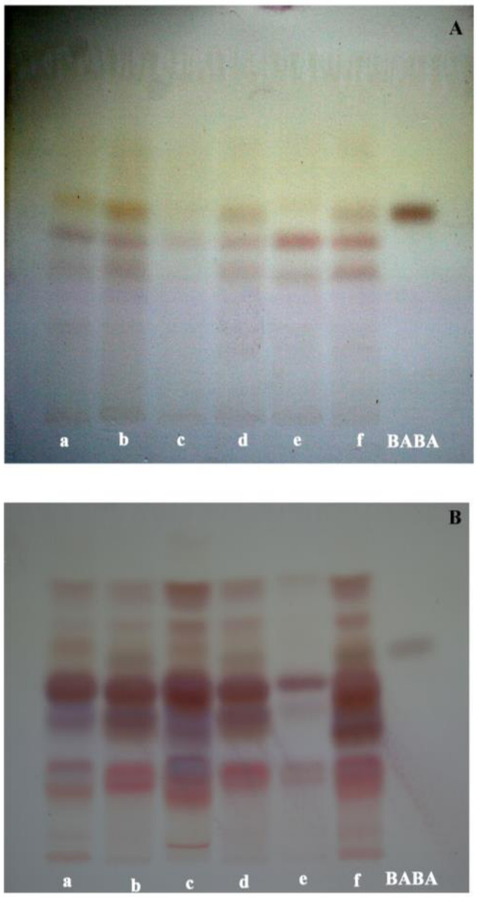

Fig. 6. Detection of beta-amino butyric acid (BABA) in seeds (A) and seedlings (B) of three rice varieties.

a, Neeraja (CK); b, BABA primed Neeraja; c, Vaisakh (CK); d, BABA primed Vaisakh; e, Vyttila 6 (CK); f, BABA primed Vyttila 6.

might be attributed to the increased degradation of chlorophyll pigments due to stress-induced metabolic imbalance (Ashraf et al, 1994). It was clear that BABA priming increased the photosynthetic pigment content including chlorophyll and carotenoid contents in all the three varieties as compared to non-primed ones. BABA priming might have a role in influencing the synthesis of cholorophylls and carotenoids in the seedlings raised from primed seeds. According to Tabrizi et al (2011), seed priming causes an increase in leaf chlorophyll content in maize seedlings.

This reduction in photosystem activities in the rice seedlings exposed to NaCl/PEG-6000 stress may be due to the inactivation of active reaction centers or the destruction of photosynthetic apparatus. A decrease in RC/CS was observed in the rice seedlings which were subjected to stressed conditions. It is due to the conversion of active centers into inactive centers and is evident from the leaf models. The decrease in ABS/CS in the rice seedlings subjected to stress conditions indicates that the energy absorption efficiency of PS II was decreased under stressed conditions. From the leaf models, it was clear that the TR/CS and ET/CS were also reduced under stressed conditions in the rice seedlings. The reduction of photosynthetic efficiency is also evident from the intensity of green colour of the leaf in the leaf models. Under stressed condition, the intensity of green colour is less, while in the seedlings raised from control and BABA primed seeds the colour intensity was found to be increased. This also implies that reduction in photosynthetic efficiency occurred under stressed conditions was significantly ameliorated on BABA seed priming. The electron transport reduction under stressed conditions is mainly due to reduced number of active reaction centers occurring under stress. In wheat leaves, the number of PS II inactive centers was found to increase under NaCl stress (Singh-Tomar et al, 2012). Moreover, drought stress is known to result in the enhanced production of superoxide on the acceptor side of PS I, ultimately reducing the PS I activity in plants (Dat et al, 2000; Mittler, 2002; Oukarroum et al, 2009). BABA priming significantly ameliorated this reduction in PS I and PS II activities under NaCl and PEG-6000 stress. Chlorophyll a fluorescence related data also confirms this result. BABA priming treatments caused an increase in the RC/CS, ABS/CS, TR/CS and ET/CS, which indicates the positive influence of BABA priming on rice seeds. It implies that the BABA priming treatments enhanced the count of open reaction centers and also increased the efficiency of absorption, trapping and transport of electrons per PS II cross section. BABA priming treatments also decreased the dissipation of energy, thus maximizing the efficiency of reaction centers by effectively trapping the energy and further utilizing it.

In this study, mitochondrial activity got reduced under stressed conditions (NaCl/PEG-6000) in the rice seedlings. This reduction may be due to the closure of stomata under stress and/or the damage of mitochondrial membrane by the production of reactive oxygen species. The seedlings raised from BABA primed seeds showed lesser reduction in the mitochondrial activity when compared to those seedlings raised from non-primed seeds. The tolerant varieties Vaisakh followed by Vyttila 6 performed better than the sensitive variety Neeraja by showing higher increase in mitochondrial activity when compared to the rice seedlings raised from non-primed seeds. BABA priming might improve the mitochondrial intactness or result in increased distribution of mitochondria per unit area. Seed priming is already known to improve the integrity of mitochondrial membrane and increase the number of mitochondria in various plant species (Ashraf and Bray, 1993; Benamar et al, 2003; Varier et al, 2010).

Rice seeds subjected to BABA priming showed higher abilities to accumulate protein and carbohydrate than that without priming treatment, when exposed to NaCl and PEG-6000 stress conditions. The increase of total protein content in the seedlings raised from primed seeds indicates that BABA priming resulted in the accumulation of additional proteins which might have a role in stress mitigation. Additional synthesis of proteins during abiotic stress such as heat-shock proteins, molecular chaperones and late embryogenesis abundant proteins are known to induce tolerance in plants (Wang et al, 2003, 2004). Seed priming (hydro and osmopriming) in amaranth cultivars caused enhancement of total protein content in the seedlings (Moosavi et al, 2009). The increase in total carbohydrate content of rice seedlings on BABA priming may be a probable mechanism to counter the stress by increasing the osmoticum. The accumulation of soluble carbohydrates was already reported to increase the drought tolerance in plants (Kameli and Losel, 1993). Halopriming is known to increase carbohydrate content of muskmelon plants (Farhoudi et al, 2011). In rice and wheat, total soluble sugar content increased in the seedlings raised from hydro and haloprimed seeds (Mondal et al, 2011; Nawaz et al, 2013).

Apart from oxidative stress, NaCl and PEG-6000 treatments also led to the accumulation of different osmolytes inside the cell to maintain a constant flow of water under stress situations (Cushman, 2001). Proline is the most important among different osmolytes and is known to interact with vital macromolecules of the cell to keep up their biological activity under stressed conditions (Gangopadhyay and Basu, 2000). Proline accumulation was reported in response to abiotic stress (Sharma and Dietz, 2006) and its accumulation has been related with stress tolerance (Kavi Kishore et al, 2005). In this study, BABA priming differentially influenced the proline biosynthesis in rice varieties. The accumulation of proline mainly occurred in the BABA primed Vyttila 6 seedlings, indicating that this variety depend more on proline accumulation for countering the stressed condition. Proline accumulation in rice seedlings raised from primed seeds has already been reported by Sun et al (2010) and Mondal et al (2011).

NaCl and PEG-6000 stress caused lipid peroxidation of biomembranes which was evident from the increased MDA content in seedlings raised from both primed and non-primed seeds under NaCl and PEG-6000 stress. The lipid peroxidation reactions could impair various metabolic functions by changing the physicochemical properties of cell membranes through disruption of lipid bilayers which further promote leakage of solutes, leading to cell death (Scandalios, 1993). BABA priming reduced MDA content in all the three varieties. This reduction in MDA content on BABA priming implies the role of BABA priming in reducing the lipid peroxidation of biomembranes. The reduction in MDA content of seedlings raised from primed seeds has already been reported in rice (Sun et al, 2010; Ella et al, 2011; Goswami et al, 2013).

After BABA priming, NR activity increases in all the three rice varieties. It has already been reported in rice seedlings that NR activity itself can be used as a measure of seedling vigor (Yang and Sung, 1980). Thus, the improved NR activity under BABA priming suggests the role of priming in influencing the nitrogen metabolism which helps in the enhancement of seedling vigour.

Antioxidant enzyme activities in rice seedlings raised from BABA primed seeds were measured under unstressed and stressed conditions to understand the mechanism of priming-mediated detoxification of ROS. Among the antioxidant enzymes, SOD constitutes the primary line of defense as it dismutates the superoxide radicals to hydrogen peroxide (Fatima and Ahmad, 2004). BABA priming caused the enhanced activities of POD and SOD in rice seedlings raised from primed seeds. There are previous reports on enhanced activities of POD and SOD under the influence of seed priming in rice cultivars (Sun et al, 2010; Goswami et al, 2013). In okra, seed priming results in higher activities of antioxidant enzymes in the seedlings (Sharma et al, 2014).

HPTLC analysis of seeds clearly indicates that BABA was imbibed by the seeds, and therefore it was detected in the seeds of all the three rice varieties which were primed with BABA, while in non-primed seeds, BABA was not detected. When the seedlings raised from BABA primed seeds were analyzed, BABA was not detected in any of the samples, indicating that BABA absorbed by seeds may not be carried over to the seedlings. There are equal chances that BABA entering into the seed may undergo structural modifications or degradation during further developmental sequences. It is very clear that BABA priming imparts many beneficial physiological and biochemical changes in the seedlings, thus it can be inferred that BABA in the seeds triggers the signaling cascades right within the seeds, which get carried over to the seedlings, so that enhanced seedling performance was observed. Thus, the seedlings emerging from BABA primed seeds can tolerate the stress conditions more effectively than those raised from non-primed seeds. From previous studies, there are clear evidence that by foliar or soil drench application of BABA, it was found to enter inside the cells and causes a cascade of signaling processes mediated through H_2O_2 (Cohen et al, 2010), salicylic acid (Jakab et al, 2005) or abscisic acid (Jakab et al, 2005; Zimmerli et al, 2008). Thus, from the results of HPTLC analysis, it can be inferred that the influence of BABA mainly occurred in seeds rather than in seedlings. However, these effects of BABA occurring in the seeds were carried over to the seedlings.

Although many researchers reported the application of BABA in the seedlings (Cohen, 2002; Jakab, 2005; Zimmerli et al, 2008), there are very less reports on the seed priming using BABA. Application of BABA at the seed stage has advantages over the application at the seedling stage. The later type of application requires large quantity of the chemical. Moreover, very less quantity of the chemical may penetrate inside the plant by traversing the cuticle and epidermis. For seed priming treatments, a very low concentration (1 mmol/L) of BABA is made use-off and positive results are obtained.

CONCLUSIONS

Among the three rice varieties studied, BABA priming improved the PEG-6000 and NaCl stress tolerance of seedlings and it was significantly evident in the abiotic stress tolerant varieties as compared to the sensitive variety. Therefore, by BABA priming of the seeds, we can evidently increase the tolerance of tolerant varieties and also make an abiotic stress sensitive variety better tolerant towards NaCl and PEG-6000 stress.

SUPPLEMENTAL DATA

The following materials are available in the online version of this article at http://www.sciencedirect.com/science/journal/16726308; http://www.ricescience.org.

Supplemental Fig. 1. Shoot length, fresh weight and dry weight of rice seedlings grown in different conditions.

Supplemental Table 1. Shoot length, fresh weight and dry weight of the seedlings of three rice varieties raised from non-primed and BABA primed seeds.

REFERENCES

Aghbolaghi M A, Sedghi M. 2014. The effect of osmo and hormone priming on germination and seed reserve utilization of millet seeds under drought stress. *J Stress Physiol Biochem*, **10**(1): 214–221.

Arnon D I. 1949. Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*, **24**(1): 1–5.

Ashraf M Y, Azmi A R, Khan A H, Ala S A. 1994. Effect of water stress on total phenols peroxidase activity and chlorophyll content in wheat (*Triticum aestivum* L.). *Acta Physiol Plant*, **16**(3): 185–191.

Ashraf M, Bray C M. 1993. DNA synthesis in osmoprimed leek (*Allium porrum* L.) seeds and evidence for repair and replication. *Seed Sci Res*, **3**: 15–23.

Ashraf M, Foolad R M. 2005. Pre-sowing seed treatment: A shotgun approach to improve germination, plant growth and crop yield under saline and non-saline conditions. *Adv Agron*, **88**: 223–271.

Bagheri M Z. 2014. The effect of maize priming on germination characteristics, catalase and peroxidase enzyme activity and total protein content under salt stress. *Int J Biosci*, **4**(2): 104–112.

Bajehbaj A A. 2010. The effects of NaCl priming on salt tolerance in sunflower germination and seedling grown under salinity conditions. *Afr J Biotechnol*, **9**: 1764–1770.

Bates L S, Waldren R P, Teare I D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, **39**: 205–207.

Benamar A, Tallon C, Macherel D. 2003. Membrane integrity and oxidative properties of mitochondria isolated from imbibing pea seeds after priming or accelerated ageing. *Seed Sci Res*, **13**: 35–45.

Cohen Y R. 2002. ß-aminobutyric acid-induced resistance against plant pathogens. *Plant Dis*, **86**(5): 448–457.

Cohen Y, Rubin A E, Kilfin G. 2010. Mechanisms of induced resistance in lettuce against *Bremia lactucae* by DL-β-amino-butyric acid (BABA). *Eur J Plant Pathol*, **126**: 553–573.

Cushman J C. 2001. Osmoregulation in plants: Implications for agriculture. *Amer Zool*, **41**: 758–769.

Dat J, Vandenabeele S, Vranova E, van Montagu M, Inzé D, van Breusegem F. 2000. Dual action of the active oxygen species during plant stress responses. *Cell Mol Life Sci*, **57**(5): 779–795.

Dubois M, Gilles K A, Hamilton J K, Rebers P A, Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem*, **28**(3): 350–356.

Ella E S, Dionisio-Sese M L, Ismail A M. 2011. Seed pre-treatment in rice reduces damage, enhances carbohydrate mobilization and improves emergence and seedling establishment under flooded conditions. *AoB Plants*: plr007.

Farhoudi R, Saeedipour S, Mohammadreza D. 2011. The effect of NaCl seed priming on salt tolerance, antioxidant enzyme activity, proline and carbohydrate accumulation of muskmelon (*Cucumis melo* L.) under saline condition. *Afr J Agric Res*, **6**(6): 1363–1370.

Farooq M, Basra S M A, Hafeez K. 2006. Seed invigoration by osmohardening in fine and course rice. *Seed Sci Technol*, **34**(1): 181–187.

Fatima R A, Ahmad M. 2004. Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in waste water. *Sci Total Environ*, **346**: 256–273.

Gangopadhyay G, Basu S. 2000. Proline as osmotic stress marker in *in vitro* system. *In*: Hemantaranjan A. Plant Physiology, Biochemistry and Plant Molecular Biology. 3rd edn. Scientific Publishers, Jodhpur, India: 283–304.

Gaspar T, Penel C, Greppin H. 1975. Peroxidase and isoperoxidase in relation to root and flower formation. *Plant Biochem J*, **2**: 33–47.

Giannopolitis C N, Reis S K. 1977. Superoxide dismutase: I. Occurance in higher plants. *Plant Physiol*, **59**(2): 309–314.

Giorgini J F, Campos C A S P. 1992. Changes in the content of soluble sugars and starch synthesis and degradation during germination and seedling growth of *Coffea arabica* L. *Rev Bras Fisiol Vegetal*, **4**(1): 11–15.

Goswami A, Banerjee R, Raha S. 2013. Drought resistance in rice seedlings conferred by seed priming: Role of the anti-oxidant defense mechanisms. *Protoplasma*, **250**: 1115–1129.

Hageman R H, Reed A J. 1980. Nitrate reductase from higher plants. *In*: Sanpietra A. Methods in Enzymology. New York, USA: Academic: 491–503.

Heath R L, Packer L. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stochiometry of fatty acid peroxidation. *Pak J Bot*, **125**(1): 189–198.

Hodge S, Thompson G A, Powell G. 2005. Application of DL-beta-aminobutyric acid (BABA) as a root drench to legumes inhibits the growth and reproduction of the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae). *Bull Entomol Res*, **95**(5): 449–455.

Jakab G, Ton J, Flors V, Zimmerli L, Metraux J P, Mauch-Mani B. 2005. Enhancing *Arabidopsis* salt and drought stress tolerance by chemical priming for its abscisic acid responses. *Plant Physiol*, **139**(1): 267–274.

Jisha K C, Vijayakumari K, Puthur J T. 2013. Seed priming for abiotic stress tolerance: An overview. *Acta Physiol Plant*, **35**(5): 1381–1396.

Jisha K C, Puthur J T. 2014. Halopriming of seeds imparts tolerance to NaCl and PEG induced stress in *Vigna radiata* (L.) Wilczek varieties. *Physiol Mol Biol Plants*, **20**(3): 303–312.

Kameli A, Losel D M. 1993. Carbohydrates and water status in wheat plants under water stress. *New Phytol*, **125**(3): 609–614.

Kavi Kishore P B, Sangam S, Amrutha R N, Sri Laxmi P, Naidu K R, Rao K R S S, Rao S, Reddy K J, Theriappan P, Sreenivasulu N. 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr Sci*, **88**: 424–438.

Keyvan S. 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *J Anim Plant Sci*, **8**(3): 1051–1060.

Kolloffel C. 1967. Respiration rate and mitochondrial activity in the cotyledons of *Pisum sativum* during germination. *Acta Bot Neerl*, **16**(3): 111–122.

Lara T S, Lira J M S, Rodrigues A C, Rakocevic M, Alvarenga A A. 2014. Potassium nitrate priming affects the activity of nitrate reductase and antioxidant enzymes in tomato germination. *J Agric Sci*, **6**(2): 72–80.

Lowry O H, Rosenbrough N J, Farr A L, Randall R J. 1951. Protein measurement with folin-phenol reagent. *J Biol Chem*, **193**: 265–275.

Macovei A, Garg B, Raikwar S, Balestrazzi A, Carbonera D, Buttafava A, Bremont J F J, Gill S S, Tuteja N. 2014. Synergistic exposure of rice seeds to different doses of γ-ray and salinity stress resulted in increased antioxidant enzyme activities and gene-specific modulation of TC-NER pathway. *Biol Med Res Int*, **2014**: 1–15.

Mathew J, Mohanasarida K. 2005. Seed priming on crop establishment and seedling vigour in semi-dry rice (*Oryza sativa*). *Res Crops*, **6**: 23–25.

Mayer A, Eskandari S, Grallath S, Rentsch D. 2006. *AtGAT1*, a high affinity transporter for γ-aminobutyric acid in *Arabidopsis thaliana*. *J Biol Chem*, **281**: 7197–7204.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*, **7**: 405–410.

Mondal S, Viji P, Bose B. 2011. Role of seed hardening in rice variety Swarna (MTU 7029). *Res J Seed Sci*, **4**(3): 157–165.

Moosavi A, Afshari R T, Sharif-Zadeh F, Aynehband A. 2009. Effect of seed priming on germination characteristics, polyphenoloxidase, and peroxidase activities of four amaranth cultivars. *J Food Agric Environ*, **7**: 353–358.

Nawaz A, Amjad M, Pervez M A, Afzal I. 2011. Effect of halopriming on germination and seedling vigor of tomato. *Afr J Agric Res*, **6**: 3551–3559.

Nawaz F, Ashraf M Y, Ahmad R, Waraich E A. 2013. Selenium (Se) seed priming induced growth and biochemical changes in wheat under water deficit conditions. *Biol Trace Element Res*, **151**(2): 284–293.

Nouman W, Siddiqui M T, Basra S M A, Afzal I, Rehman H U. 2012. Enhancement of emergence potential and stand establishment of *Moringa oleifera* Lam. by seed priming. *Turk J Agric Forest*, **36**: 227–235.

Oka Y, Cohen Y, Spiegel Y. 1999. Local and systemic induced resistance to the root-knot nematode in tomato by DL-β-amino-*n*-butyric acid. *Phytopathology*, **89**: 1138–1143.

Oukarroum A, Schansker G, Strasser R J. 2009. Drought stress effects on photosystem I content and photosystem II thermo tolerance analyzed using Chl a fluorescence kinetics in barley varieties differing in their drought tolerance. *Physiol Plant*, **137**(2): 188–199.

Patade V Y, Bhargava S, Suprasanna P. 2009. Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. *Agric Ecosyst Environ*, **134**: 24–28.

Puthur J T. 2000. Photosynthetic events in *Sesbania sesban* (L.)

Merrill in relation to osmotic stress during different developmental stages. Ph.D. Thesis, New Delhi, India.

Ruan S, Xue Q, Tylkowska R. 2002. Effects of priming on seed germination and health of rice (*Oryza sativa* L.) seeds. *Seed Sci Technol*, **30**: 451–458.

Sass R L, Cicerone R J. 2002. Photosynthate allocations in rice plants: Food production or atmospheric methane? *J Plant Physiol*, **99**: 11993–11995.

Scandalios J G. 1993. Oxygen stress and superoxide dismutases. *Plant Physiol*, **101**(1): 7–12.

Schmitt N, Dizengremel P. 1989. Effect of osmotic stress on mitochondria isolated from etiolated mung bean and sorghum seedlings. *Plant Physiol Biochem*, **27**: 17–26.

Sharma S S, Dietz K J. 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J Exp Bot*, **57**: 711–726.

Sharma A D, Rathore S V S, Srinivasana K, Tyagia R K. 2014. Comparison of various seed priming methods for seed germination, seedling vigour and fruit yield in okra (*Abelmoschus esculentus* L. Moench). *Scient Hort*, **165**: 75–81.

Singh-Tomar R, Mathur S, Allakhverdier S I, Jajoo A. 2012. Changes in PS II heterogeneity in response to osmotic and ionic stress in wheat leaves (*Triticum aestivum*). *J Bioenerg Biomembr*, **44**: 411–419.

Strasser R J, Srivastava A, Tsmilli-Michael M. 2004. Analysis of the chlorophyll a fluorescence transient. *In*: Papageorgiou G C, Govindjee. Chlorophyll a Fluorescence: A Signature of Photosynthesis. Dordrechet: Springer: 321–362.

Sudharani M, Reddy P R, Jayalakshmi V. 2012. A comprehensive review on 'genetic components of salinity tolerance in rice (*Oryza sativa* L.).' *Int J Appl Biol Pharm Technol*, **3**: 312–322.

Sun Y Y, Sun Y J, Wang M T, Li X Y, Guo X, Hu R, Ma J. 2010. Effects of seed priming on germination and seedling growth under water stress in rice. *Acta Agron Sin*, **36**: 1931–1940. (in Chinese with English abstract)

Tabrizi E F M, Yarnia M, Ahmadzadeh V, Farjzadeh N. 2011. Priming effect of different times of maize seeds with nutrient elements in water stress on corn yield. *Ann Biol Res*, **2**(3): 419–423.

Ton J, Jakab G, Toquin V, Flors V, Iavicoli A, Maeder M N, Metraux J P, Mauch-Mani B. 2005. Dissecting the β-amino butyric acid-induced priming phenomenon in *Arabidopsis*. *Plant Cell*, **17**(3): 987–999.

Varier A, Vari A K, Dadlani M. 2010. The subcellular basis of seed priming. *Curr Sci*, **99**: 450–456.

Wang W, Vinocur B, Altman A. 2003. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, **218**(1): 1–14.

Wang W, Vinocur B, Shoseyov O, Altman A. 2004. Role of plant heat shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci*, **9**(5): 244–252.

Worrall D, Holrod G H, Moore J P, Glowacz M, Croft P, Taylor J E, Paul N D, Roberts M R. 2012. Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. *New Phytol*, **193**: 770–778.

Yang C M, Sung C M. 1980. Relations between nitrate reductase activity and growth of rice seedlings. *J Agric Assoc China*, **111**: 15–23.

Zhong Y P, Wang B, Yan J H, Cheng L J, Yao L M, Xiao L, Wu T L. 2014. DL-β-amino butyric acid induced resistance in soybean against *Aphis glycines* Matsumura (Hemiptera: Aphididae). *PLoS One*, **9**(1): 1–11.

Zimmerli L, Hou B H, Tsai C H, Jakab G, Mauch-Mani B, Somerville S. 2008. The xenobiotic beta-amino butyric acid enhances *Arabidopsis* thermo-tolerance. *Plant J*, **53**(1): 144–156.

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