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FLOOD TOLERANCE OF NEWLY DEVELOPED SEMI-DEEP LOWLAND RICE VARIETY (VARSHADHAN) DURING SEED GERMINATION AND SEEDLING STAGE

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ABSTRACT

In this study, newly developed rice cultivar (Varshadhan) was evaluated for flood tolerance during seed germination and seedling stage in the presence and absence of SUB1. Under flooding, the rate of seed germination was 100 per cent in both SUB1 and non-SUB1 line of Varshadhanas in the normal condition. And also, we noted that there was no significant difference in growth promotion in terms of shoot and root between SUB1 and non-SUB1 line under flooding. In the gene expression analysis, expression of *RAmy*3C, *Adh*1 and *Adh*2 genes was continuously induced up to 72 h in both rice lines under normal and flooding condition. Furthermore, in another screening for flood tolerance at seedling stage, higher survival rate was noted in Sub1 line and lower rate in non-Sub1 line. In gene expression analysis also, expression of Sub1A gene was induced strongly in SUB1 line but not in non-SUB1 line. Thus, Varshadhan rice cultivar with SUB1 has played significant role in flood tolerance during seed germination as well as seedling stage. Therefore, this cultivar will be suitable in rice growing areas where farmers facing flooding during seed germination and seedling stage.

INTRODUCTION

Rice (*Oryza sativa* L.) ecosystems are defined on the basis of hydrology and can be roughly classified into irrigated, rainfed lowland, upland and flood-prone. Among them, rainfed lowland rice occupies about 36 million ha (25% of the world rice area). But, among abiotic stresses, flooding is a serious constraint to rice plant growth and survival in rainfed lowland and deepwater areas because it results in partial or complete submergence of the plant. Out of 42 biotic and abiotic stresses surveyed in the rainfed lowlands of South and Southeast Asia, submergence stress is considered the third most important limitation to rice production frequently affecting over 15 million ha. The adverse effect of submergence is a result of various interrelated factors such as the limited gas diffusion of CO_2, O_2 and the accumulated ethylene,the low light under waterand the turbidity of floodwater. and these factors render to slow down photosynthesis during submergence (setter, *et al.*, 1988). Excess water during monsoon season adversely affects agricultural productivity in large areas of South and Southeast Asia. In deepwater areas, water stagnation can occur for more than 1 month and to a depth of more than 50 cm. Complete submergence can also occur transiently, for a period of 7–14days,commonly referred to as flash flooding or submergence and can occur at anytime and mostly more than once during the growing season. This intermittent ûooding affects more than 22 million ha (about 16% of the world rice area) of rainfed lowlands in South and Southeast Asia, of which 6.2 million ha are in India. Most of the existing rice cultivars are seriously damaged by flash flooding; however, few tolerant landraces such as 'FR13A' were identified that can withstand inundation for up to 2 weeks. Following this, a breakthrough has been achieved by tagging of a major submergence-tolerance QTL (quantitative trait locus) named SUB1, on rice chromosome 9 (Xu, *et al.*, 2006). Following water saving technology through system of rice intensification (SRI) (Riton, *et al.*, 2014), direct seeding is increasingly being practiced by rice farmers under both rainfed and irrigated conditions. The trend is the outcome of a scarcity of labour required for transplanting, simplicity and additional benefits associated with direct seeding. However, flooding the soil after direct seeding can have a benefit by improving weed control, a major constraint in direct-seeded systems (Pandey, 1995). Therefore, enhanced tolerance of flooding during germination and early seedling growth could help improve crop establishment. In the present study, a newly developed rice variety, Varshadhan was found for flood tolerance in order to manage the submergence stress during seed germination and seedlings stage.

MATERIALS AND METHODS

Screening for flood tolerance during seed germination

For screening of seed germination under normal and flooded condition, 25 grains of Varshadhan with SUB1 and without it were dry seeded at about 1cm depth in plastic tray containing finely ground field soil, followed by either normal watering (control) or flooding with about 10cm of tap water up to 21-days. Then, the percentage of seed germination and plant growth was calculated (Ismail, *et al.,* 2009).

Gene expression analysis during seed germination through reverse transcriptase (RT)-PCR analysis

For gene expression analysis, RNA of seed embryogenic tissue of Varshadhan-Sub1 and cultivar without it was isolated at 24, 48 and 72h after seed incubation under normal and flooding condition using Trizol reagent according to the manufacturer's protocol. Then, cDNAs were synthesized using reverse transcriptase (RT) enzyme according to the manufacturer's protocol. Using cDNAs, normal PCR was done using the primer sequence of *RAmy*3C, *RAmy*3D, *RAmy*3E, *Sus*1, *Sus*3, *Pdc*1, *Pdc*2, *Adh*1 and *Adh*2 at 56ºC, annealing temperature. In this analysis, primer sequence of Actin was used as loading control (Fukao, *et al.,* 2006).

Screening for flood tolerance during seedling stage

For evaluating flood tolerance at seedling stage, 14-days old seedlings of Varshadhan-Sub1 and cultivar without it grown in metal tray were submerged in water tank for 2-weeks. Then, the survival rate of seedlings was calculated after 10-days followed by de-submergence (Septiningsih, *et al.,* 2009).

Gene expression analysis during seedling stage through reverse transcriptase (RT)-PCR analysis

For gene expression analysis, RNA of Varshadhan-Sub1 and cultivar without it was isolated at 0 h and 24h under submergence condition using Trizol reagent according to the manufacturer's protocol. Then, cDNAs were synthesized using reverse transcriptase (RT) enzyme according to the manufacturer's protocol. Using cDNAs, normal PCR was done using the primer sequence of SUB1A and SUB1C at 59ºC, annealing temperature. In this analysis, primer sequence of Rubisco was used as loading control (Septiningsih *et al.,* 2009).

RESULTS AND DISCUSSION

Seed germination under normal and flooding condition

In normal condition, all sowed seeds were completely germinated in both Varshadhan-Sub1 and cultivar having no-Sub1. Similarly, we found the complete seed germination (100%) in both cultivars under flooded condition also (Fig.1). Higher percentage of seed germination in flooding condition is associated with the higher rate of starch consumption in flood tolerant genotypes (Ismail *et al.,* 2009). Significantly, in this screening, there were no significant difference in seed germination between Varshadhan having SUB1 and cultivar with no SUB1 under flooding. In previous studies, it has been reported that submergence tolerant genotypes (having SUB1) *i.e.* FR13A, M202 and Nipponbare were showed slow seed germination under flooding (Magneschi and Perata, 2009; El-Hardawy, *et al.,* 2011). In growth of seedlings, we found no significant difference between SUB1 and non-SUB1 cultivars also in shoot and root length under both conditions. But, less difference was found between normal and flooding condition. In normal condition, the growth rate was slightly higher compare to growth of seedlings under flooding. In normal condition, the mean value of shoot length of SUB1-cultivar was 35cm and 34cm in non-SUB1 cultivar. The root length of SUB1 cultivar was 15cm and 14cm in non-SUB1 cultivar. But, under flooding condition, the mean value of shoot length of Varshadhan with SUB1 was 24cm, whereas, in Varshadhan with no SUB1, the length of shoot was 23cm. In case of root, the length was higher in non-SUB1 cultivar (12cm) and lower in SUB1 cultivar (11cm) (Fig. 2). Thus, it reveals that the elongation-suppression effect of SUB1 is not reflected in Varshadhan-SUB1 during seed germination under flooding condition. Therefore, there was no significant difference in growth promotion between SUB1 and non-SUB1 cultivar in this study. Hence, this phenomenon will support the seedlings not to be affected in cultivar with SUB1 by flooding during seed germination.

Gene expression analysis during seed germination

In the gene expression analysis, there was induce of *RAmy*3C, *RAmy*3D, *RAmy*3E, *Sus*1, *Sus*3, *Pdc*1, *Pdc*2, *Adh*1 and *Adh*2 gene expression in both SUB1 and non-SUB1 cultivars under normal and flooding condition during seed germination (Fig. 3). Expression of these genes was gradually increased from 24h to 72h under normal condition in both cultivars. But, under flooding condition, gene expression commenced to decline after 48h. However, in this analysis, we found that the expression of RAmy3C, Adh1 and Adh2 genes was expressed continuously up to 72h under both conditions. Hence, in this study, the result of phenotypic screening mimicked in gene expression analysis also, since, there was no significant difference between both cultivars as well as both conditions. Here, the activity of amylase and the alcohol fermentation pathway might have associated with faster rate of starch breakdown and the higher soluble sugar concentration for generating the energy needed for the growth and maintenance processes in Varshadhan-Sub1 and cultivar without it (Ismail, *et al.,* 2009). In this analysis, expression of genes associated with fermentation pathway has induced even under normal condition in both SUB1 and non-SUB1 cultivars. Supportively, in a previous study also, the expression of these genes has been reported under aerobic condition (Guglielminelti, *et al.* 2001). Generally, the expression of Pdc and Adh genes are to be expressed under anaerobic condition.

Submergence tolerance during seedling stage

In submergence screening at seedling stage, there was significant difference between Varshadhan with SUB1 and cultivar without it. In SUB1 cultivar, the survival rate was higher (90%), whereas, non-SUB1 cultivar, the survival rate was lower (10%) (Fig. 4). Here, the shoot-elongation suppression of SUB1 is activated at seedling stage during submergence.

During submergence, leaf elongation was observed in non-SUB1 line (30.0%) and its rate was noted to be reduced in SUB1 line (11.0%). The leaf elongation is associated with consumption of carbohydrate energy reserves more slowly in SUB1 line and it is maintained for the growth after flood recedes. But, this process is contrary in non-Sub1 line and the stored food is completely consumed before water recedes and it renders the seedlings to die (Fukao, *et al.,* 2006).

Gene expression analysis at seedling stage

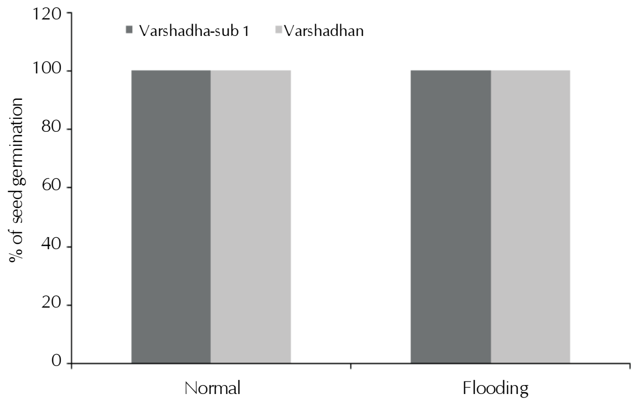

Figure 1: Percentage of seed germination of Varshadhan-Sub1 and Varshadhan under normal and flooding condition

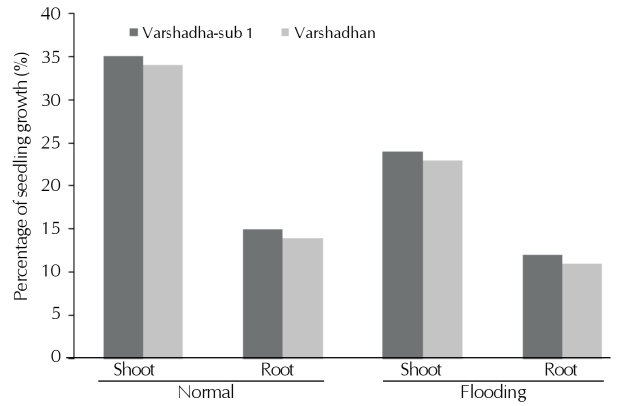

Figure 2: Seedling growth percentage of Varshadhan-Sub1 and Varshadhan under normal and flooding condition

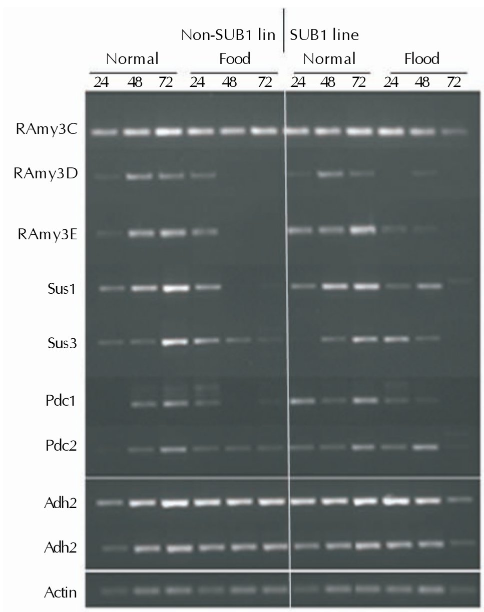

Figure 3: Gene expression analysis through reverse-transcript (RT)-PCR Gene expression of *RAmy*3C, *RAmy*3D, *RAmy*3E, *Sus*1, *Sus*3, *Pdc*1, *Pdc*2, *Adh*1 and *Adh*2 in Varshadhan-Sub1 and Varshadhan at 24, 48 and 72 h during seed germination under normal and flood condition

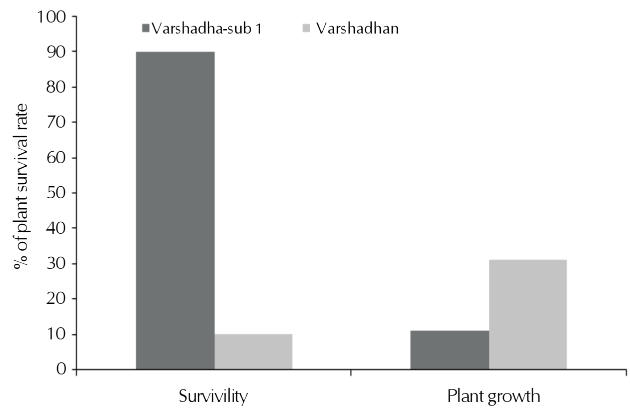

Figure 4: Seedling survive rate and leaf elongation in Varshadhan-Sub1 and Varshadhanunder submergence condition

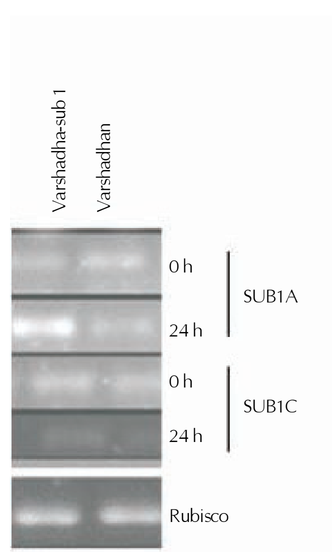

Figure 5: Gene expression analysis through RT-PCR. Expression of Sub1A and Sub1C in SUB1 and non-SUB1 line of Varshadhan at 0 h and 24 h under submergence condition. *Rubisco* was used as loading control

In this analysis, the expression of SUB1A gene was induced at 24h in Varshadhan-Sub1 under submerged condition, whereas, there was no expression in Varshadhan with no SUB1 (Fig. 5). There was no significant expression of Sub1C gene in both cultivars under submerged condition at 24h. Generally, the expression of Sub1A gene is associated with the activity of gibberellene (GA) response repressors, SLENDER RICE 1(SLR1) and SLR1-LIKE1 (SLR1) and controls the consumption of carbohydrate through pyruvate decarboxylase(Pdc) and Alcohol dehydrogenase(Adh) gene expression under submerged condition (Fukao and Bailey-Seres, 2008). Thus, the higher survival rate in Varshadha-sub1 cultivar followed by de-submergence is associated with the expression of Sub1A gene.

Thus, in the evaluation of Varshadhan, the rice line without

SUB1 has showed flood tolerance only for seed germination but line with SUB1 has associated with tolerance to both seed germination and seedling stage submergence stress. Very recently also, a new rice line with adaptation to both anaerobic germination (AG) and submergence tolerance (Sub1) conditions developed by the International Rice Research Institute (IRRI) has showed more fast seed germination rate than other rice genotypes without it under both aerobic and anaerobic conditions (Mackill *et al.,* 2010). Similarly, in the present study also, Varshadhan with SUB1 has proved its high efficiency of flood tolerance during seed germination and seedling stage and this variety will be suitable for semi-deep lowland areas in eastern India.

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