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ENHANCEMENT OF DROUGHT TOLERANCE IN RICE MEGA VARIETY (SWARNA) IN PRESENCE OF *SUB1* AND *DTY* LOCUS

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Abstract- In this study, we evaluated a mega rice variety, Swarna-*Sub1* improved for drought tolerance under submergence and drought stress condition. In submergence evaluation, rice lines having *DTY*2.1 and *DTY*3.1 locus showed intolerance response alone or in the presence of *Sub1* locus. But, Swarna-*Sub1* showed 100% survival rate in the absence of *DTY* QTLs to submergence stress. In drought evaluation, we found that rice lines with *DTY* loci showed enhanced drought tolerance in the presence of *Sub1* locus like delayed leaf rolling and the highest rate of seed setting as compared to rice lines having only *Sub1* or *DTY* locus. Furthermore, in gene expression analysis, expression of drought-inducible genes (*DREB1A*, *SalT*, *LIP9*, *LEA3*, *Rab16A*) was found strongly in rice lines having both *Sub1* and *DTY* combination rather than single locus. In protein profile, we found rice lines having both *Sub1* and *DTY* locus showed an increased amount of protein level as compared to lines having only *DTY* locus. These results support that thus improved Swarna-*Sub1* variety for drought tolerance can be used in rain-fed lowland and upland areas where incidence of submergence and drought stress occurs subsequently.

Keywords- Drought stress, Quantitative trait locus (QTL), *DTY*2.1, *DTY*3.1, Swarna-*Sub1* and gene expression

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Introduction

Drought stress is the most constraint to rice production since rice plants are adapted to aquatic environments. The biomass production of rice decreases with decreasing water availability at any growth stage depending on severity, timing and duration [1]. Particularly, during reproductive development, plants are very sensitive to water deficit than during vegetative growth [2]. According the previous report, 19 mha of upland rice and over 14 mha of rain fed lowland rice is affected by drought [3]. Another problem in rain fed lowland is flash flooding that adversely affects about 15 mha of rice-growing areas in south and southeast Asia. Generally, rice plants are unable to survive under water for more than 1 week because of anoxia [4]. Thus, rain-fed areas are prone to both flash flood and drought by sequential events (submergence followed by drought and vice versa). Therefore, improvement of rice cultivars to both submergence and drought stress would substantially increase rice productivity in rain-fed lowland areas. In this study, a mega rice variety, Swarna-*Sub1* improved for drought tolerance by introgression of *DTY*2.1 and *DTY*3.1 locus, which is associated with drought tolerance at reproductive stage in CRRI, Cuttack, was evaluated for submergence and drought tolerance under stress condition.

Materials and Method

Plant materials, submergence and drought evaluation

In this study, Swarna-*Sub1* (recipient line), improved rice lines with *Sub1* + *DTY*2.1 or *DTY*3.1 or both loci and IR 898031-B-B-195 (*DTY*2.1 and *DTY*3.1) (donor line) were used. For submergence evaluation, seeds of these lines were sowed in metal trays and seedlings were grown for 2-weeks. At 14-d-old seedling stage, the trays were shifted to submergence tank, the water level was raised to 90 cm height, and the level was maintained for 2-weeks. Then, seedlings were de-submerged and the survival rate was recorded after 10 days [5]. For drought evaluation, seeds of the above said rice lines were sowed directly in the soil in rainout shelter and seedlings were grown for 30-days under irrigated condition. In the layout, each row consisted of 20 plants. After one month, water supply to plants was withheld for creating drought stress on rice plants for 30-days. Then, rice plants were observed for tolerance to drought stress on the 15th day from stress imposed and onwards for 15 days.

Reverse transcriptase (RT) -PCR and Protein profile

Gene expression through reverse transcriptase (RT) -PCR and protein profile in Swarna-*Sub1*, improved rice lines having *Sub1* with *DTY*2.1 or *DTY*3.1 or both locus and IR 898031-B-B-195 under drought stress were analyzed. For RNA extraction, 100 mg leaf tissue was ground in liquid nitrogen using mortar and pestle and total RNA was extracted using TRIzol according to manufacturer's instructions. The RNA pellet was dissolved in 50µl RNase free water and stored at –20ºC. cDNA synthesis was carried out using SuperScript™III Reverse Transcriptase according to the manufacturer's protocol (Invitrogen, California, USA) in a reaction mixture containing 50–75 ng RNA with the final volume completed to 20µL using RNase free water. Normal PCR was done using cDNA with the primer sequence of *DREB1A*, *SalT*, *LIP9*, *LEA3* and *Rab16A* at 58ºC (annealing temperature) [6]. The primer sequence of Actin1 was used as a loading control. For protein profile, leaf materials were ground in liquid nitrogen and homoginated with phosphate buffer containing 1 mM Dithiothreitol (DTT) and phenylmethylsulfonyl fluoride (PMSF). Protein solution was collected after centrifugation for 15, 000 rpm at 4ºC for 20 minutes. The protein was estimated according to the Bradford method with bovine serum albumin (BSA) as standard [7]. Then, the protein sample (100 µg) was loaded along with protein ladder in 10% acrylamide gel and the gel was run for 6 h at 50 mA. The gel was stained with coomassive brilliant blue (CBB) solution for 2 h and destained with soultion containing

methanol and acetic acid (1:1 ratio). Gels were documented (SYNGENE, UK) and the intensive protein bands were recorded.

Results

Phenotypic evaluation

Submergence

In submergence evaluation, following the de-submergence the survival rate was recorded in the range of 0-100% among the rice lines. The highest survival rate (100%) was noted in both Swarna-*Sub1* (recipient) and improved lines having *Sub1*+*DTY* 2.1 or *DTY*3.1 or both *DTY*2.1 and *DTY*3.1. In Swarna with no *Sub1* locus, the survival rate was 30%, followed by IR 81896-B-B-195 having *DTY*2.1 and *DTY*3.1 (0%) [Fig-1].

Figure -1. Responses of Swarna-*Sub1* (SS1) having *DTY*2.1 and *DTY*3.1 loci or without it to submergence stress.

Drought

In drought evaluation, the leaf rolling incidence in rice lines varied markedly under drought condition. In Swarna-*Sub1* has *DTY*2.1 or *DTY*3.1 or both *DTY*2.1 and *DTY*3.1, the leaf rolling incidence delayed as compared to IR 81896-B-B-195 having only *DTY*2.1 and *DTY*3.1 and Swarna has no *Sub1* during the seedling stage. During reproductive stage, the rate of seed setting was higher in Swarna-*Sub1* having *DTY*2.1 or *DTY*3.1 or both *DTY*2.1 and *DTY*3.1 and IR 81896-B-B-195 than Swarna has only *Sub1* locus or without it [Fig-2].

Figure- 2. Swarna-*Sub1* showing delay panicle exertion and BC_3F_4 population of Swarna-*Sub1* having *DTY*2.1 and *DTY*3.1 loci showing panicle exertion and seed setting.

RT-PCR

In the gene expression analysis through RT-PCR, strong expression of drought-inducible genes were documented in Swarna-*Sub1* has *DTY*2.1 or *DTY*3.1 or both *DTY*2.1 or *DTY*3.1 and IR81896-B-B-195 in response to drought stress [Fig-3I]. Expression of *DREB1A* and *SalT* gene was found in Swarna-*Sub1* having *DTY*3.1 or both *DTY*2.1 and *DTY*3.1. *Rab16A* expressed in IR81896-B-B-195 and Swarna-*Sub1*having *DTY*2.1 or *DTY*3.1 or both *DTY*2.1 and *DTY*3.1. Expression of *LEA3* gene induced strongly in Swarna-*Sub1* having *DTY*2.1 or *DTY*3.1 or both *DTY*2.1 and *DTY*3.1, but not in Swarna-*Sub1* and IR 81896-B-B-195. Expression of *LIP9* gene was found strongly in all rice cultivars under drought stress condition, but not in Swarna-*Sub1* having *DTY*2.1 and *DTY*3.1.

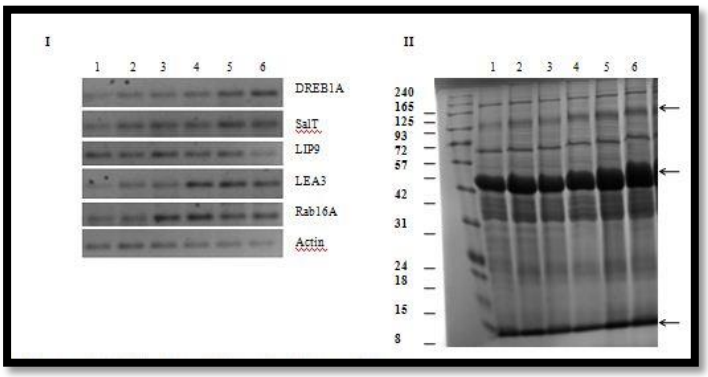

Figure-3. Gene expression (I) and protein profile (II) of 1-Swarna-*Sub1* having *DTY* loci in normal condition; 2-Swarna-*Sub1* (recipient); 3-IR 81896-B-B-195 (donor); 4-Swarna-*Sub1* having *DTY*2.1 (improved line); 5-Swarna-*Sub1* having *DTY*3.1 (improved line); 6-Swarna-*Sub1* having *DTY*2.1 and *DTY*3.1 (improved line) under drought condition.

Protein profile

In protein profile, the amount of proteins in Swarna-*Sub1* having *DTY*2.1 or *DTY*3.1 or both *DTY*2.1 and *DTY*3.1 was found to be increased at 125, 48 and 8 kDa in response to drought stress as compared to Swarna-*Sub1*, Swarna and IR 81896-B-B-195 [Fig-3].

Discussion

Swarna is an important rain-fed lowland rice variety grown on millions of hectares in Asia, but it is highly susceptible to drought and aerobic soil conditions [8]. In this study, Swarna-*Sub1* in the presence of *DTY* loci has played a significant role in drought tolerance as well as in the submergence tolerance regardless of *DTY* loci. Efficiency of *DTY* loci in submergence tolerance could not analyse in 14-days stress period since Swarna-*Sub1* having no *DTY* loci condition showed 100% survival rate. Furthermore, IR 81896-B-B-195 having *DTY* loci showed intolerant response to submergence stress. It indicates that *DTY* loci have no role in submergence tolerance even in combination with *Sub1* locus. Interestingly, *Sub1* locus in combination with *DTY* loci increased efficiency of drought tolerance in rice lines under drought condition like the delayed leaf rolling and higher seed setting rate. Moreover, we observed no difference in leaf rolling incidence between Swarna-*Sub1* having QTY loci and cultivar without it. It states that *Sub1* is more efficiency than *DTY* loci in drought tolerance. However, during reproductive stage, the role of *DTY* loci was significant than *Sub1* locus since Swarna-*Sub1* was sensitive to drought, i.e. panicle exertion was incomplete and even in complete exertion, the seed setting was poor under drought condition in rainout shelter. But, in Swarna-*Sub1* having *DTY* loci, panicle exertion and seed setting rate was noted at higher levels. Thus, *DTY* loci proved its association with drought tolerance at the reproductive stage stress [8]. Additionally, in gene expression analysis, we found the difference in expression of drought inducible genes (DREB1A, *Rab16A*, *LEA3*, *LIP9*, and *SalT*) in rice lines having *Sub1* and *DTY* locus. In a previous study, expression of these drought-inducible genes in rice line having *Sub1* locus has been demonstrated as compared to rice line with no *Sub1* under drought condition [6]. Expression of *DREB1A* associated with a significant survival rate of rice under water deficit condition [9] was noted in Swarna-*Sub1* with *DTY*3.1 or *DTY*2.1 and *DTY*3.1 combination, but it is not induced in IR 81896-B-B-195 having only *DTY* loci. Similarly, *SalT* gene induced by a high dehydration condition [10] is expressed in rice cultivar with *Sub1* and *DTY* combination. In case of *Rab16A* gene, it induced strongly only in the rice line having *DTY* locus but it was not in rice line having an only *Sub1* locus. This gene expresses in dehydrated vegetative tissues and

maturating seeds [11]. The results of *LEA3* and *LIP9* expression was contradictory each other i.e. *LEA3* expressed in rice genotype with *Sub1* and *DTY* combination and *LIP9* did not express in this combination. *LEA3* is associated with increased spikelet fertility and grain production during stress [12]. In protein profile, increased amount of protein in rice lines having *Sub1* and *DTY* combination as compared to IR 81896-B-B-195 having *DTY* loci indicates the higher amount of DNA and RNA synthesis under drought condition [13].

In conclusion, in the present study, *DTY* loci associated with drought tolerance is showed no role on submergence tolerance alone or even in the combination with *Sub1* locus. But, Swarna-*Sub1* alone showed 100% survival rate in the absence or presence of *DTY* loci to submergence stress. Perhaps, *Sub1* and *DTY* combination might resulted in submergence tolerance for longer period if the stress period extended. In case of drought, as there is no more effective QTL due to lack of effective selection criteria and low heritability of grain yield under stress, this type of QTL combination (*Sub1* and *DTY*) will support the rice plants to enhance grain yield under stress. Besides, most of the drought-inducible genes are expressed strongly in rice cultivar with *Sub1* and *DTY* combination rather than single QTL in gene expression analysis. In protein profile also, rice lines having *Sub1* and *DTY* combination showed an increased amount of protein level as compared to rice lines having only *DTY* loci. Therefore, thus improved Swarna-*Sub1* for drought tolerance too by incorporation of *DTY*2.1 and *DTY*3.1 locus will be appropriate to cultivate in rain-fed lowland and upland areas where incidence of submergence and drought stress occurs vice-versa.

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