RESPONSE OF SUB1 INTROGRESSION LINES OF RICE TO VARIOUS FLOODING CONDITIONS

Respon Galur Padi Introgresi SUB1 terhadap Berbagai Kondisi Banjir

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ABSTRACT

Two types of floods can be happen in rice crops, i.e. flash floods and stagnant floods. Flash floods cause complete submergence for up to 2 weeks, while stagnant floods (SF) could partially submerge part of rice plant. To overcome yield loss due to the floods, introgression of \textit{SUB1} gene, known as a gene suppressing cell elongation and carbohydrate metabolism, to rice genotype can increase plant tolerance to complete submergence for 10 days or more. The study aimed to evaluate the response of 18 rice genotypes, including the recently developed sixth pair \textit{SUB1} near isogenic lines (NILs) of mega-rice varieties (Swarna, Sambha Mahsuri, IR64, TDK1, BR11, and CR1009), to various flooding conditions. The rice genotypes were planted at field ponds at Los Banos, Philippines, in the wet season (WS) of 2009. The treatments were 15 days submergence, SF, SF follows submergence and normal conditions. Each treatment was arranged in completely randomized block design with three replications. The results showed that the \textit{SUB1} introgression rice lines had higher survival compared to the non-\textit{SUB1} and did not much elongate their shoots during submergence. Nevertheless, under SF the rice genotypes should elongates their shoots to allow restoring contact with the air. SF and SF follows submergence decreased the panicle number, grain number per panicle and panicle fertility. Consequently, the yield declined. It suggests that sensitive genotypes are mostly source-limited during grain filling. The \textit{SUB1} introgression lines had higher chlorophyll concentration and less depletion in soluble sugar and starch after submergence. Under SF, soluble sugar and starch contents between the \textit{SUB1} NILs and non-\textit{SUB1} lines were not significantly different. Introggression of the \textit{SUB1} into high-yielding varieties improved submergence tolerance without affecting yield potential. The study indicates that introgression of the \textit{SUB1} into taller type rice varieties should be done to compensate the effect of suppressed elongation.

[Keywords: Rice, flooding, introgression, \textit{SUB1} gene, adaptation]

INTRODUCTION

Flood is a major abiotic stress in most lowland and rainfed ecosystem. The flooded rice field areas increased due to the rising earth atmosphere temperature (global warming) that induces heavy precipitation and tropical cyclone in the most part of Asia (Easterling et al. 2007; Zeigler and Barclay 2008). In
Indonesia, total damaged rice field area and rice production loss due to floods were estimated at 268,823 ha and 1.344 million tons of rice, respectively, worth USD353.7 million per year (Manikmas 2008).

Based on the depth and duration, floods in rice ecosystem can be differentiated into short-term complete submergence and stagnant floods (Ismail et al. 2008). Complete submergence can be found any time during the season and for various durations, whereas stagnant flood occurred when a height of water head (20-60 cm) remained in the field.

In Indonesia, stagnant flood is mostly found in basin swampy area of about 4.7 million ha exposed to shallow floods of ~50 cm depth regularly during the season (Widjaja-Adhi 2000). Rice yield in swampy areas of Sumatra and Kalimantan on less than 1 million ha is quite low due to subsistent cultivation system. The limited high-yielding flood tolerant variety is one of the main problems in increasing rice yield in this area. Flood-tolerant rice varieties for swampy ecosystem are characterized by their ability to escape from flood condition by increasing plant height through stem elongation.

Under complete submergence during vegetative stage for 10 days or more, tolerant rice genotypes require quiescent strategies where SUB1-A1 gene suppresses cell elongation and carbohydrate metabolism (Fukao et al. 2006; Xu et al. 2006; Septiningsih et al. 2008). Therefore, introgression of SUB1 gene to rice varieties would increase survival rate under complete submergence without causing significant grain yield loss compared to that under non-submerged conditions (Singh et al. 2009). However, if the inundation is prolonged to stagnant flood (20-50 cm level) the plant adaptation will be different.

Prolonged partial stagnant floods decrease rice production in vast areas of rainfed lowlands, and sometimes occur following short-term complete submergence. Screening of a large set of genotypes showed that partial flood of 50 cm water depth until maturity affects plant phenology such as plant height, panicle number, spikelet fertility, yield and survival rate (Ismail et al. 2008). Recent study showed that short stature type variety of submergence tolerance, Swarna Sub1, did not perform well under stagnant flooding treatment (Singh et al. 2009). Here, we studied phenology and physiology responses of selected rice genotypes to flash flood, stagnant flood, and combination of flash flood and stagnant flood until maturity. The recently developed six mega-varieties of SUB1 NILs with their respective recurrent parent and other lines carrying SUB1 A-1 gene were evaluated under various flooding treatments.

**MATERIALS AND METHODS**

**Experimental Design and Management**

The experiments were carried out at the Experimental Station of the International Rice Research Institute (IRRI), Los Baños the Philippines, in the wet season of July-November 2009. The soil was an Aquandic Epiaquoll with pH 6.0, 16.2 g kg⁻¹ organic C, 1.50 g kg⁻¹ total N, and 32.9 cmol kg⁻¹ cation exchange capacities. Eighteen rice genotypes consisted the sixth pair of mega-variety with their near isogenic lines (NILs) of SUB1 A-1 gene, five IRRI breeding materials which possess SUB1 A1 gene, and one of the stagnant flooding tolerant genotypes were used in this experiment. The genotypes were planted at four sets of field experiment with different flooding conditions, e.g.: (1) normal shallow flood/irrigated conditions (2-5 cm water level); (2) complete submergence from 21 days after transplanting (DAT) for 10-15 days and if the sensitive genotypes showed 50% extend damage then continued with normal irrigated conditions; (3) complete submergence from 21 DAT for 10-15 days and if the sensitive genotypes were seen 50% extend damage then continued with ~25 cm stagnant flood until most of genotypes flowered, and (4) stagnant flooding (SF) with ~50 cm water depth from 21 DAT until most of genotypes flowered.

Crop management followed the standard rice cultural practices. Pre-germinated seeds for all genotypes were sown in the seedling bed of 50 g m⁻². At 21 days after sowing, the seedlings were uprooted and transplanted to the field, one plant per hill. The rice genotypes were planted in each plot using randomized completely block design with three replications at 9 rows x 20 plants per plot or a total of 7.2 m². Nitrogen, phosphorus, potash and zinc are applied at 90:30:30:5 kg ha⁻¹ as basal fertilizers. Forty-five kg of N was applied at 40 and 60 DAT.

Submergence treatment was started at noon to give plants enough time to accumulate carbohydrate through photosynthesis in the morning. Water depth was maintained by adding water regularly to the ponds. After seven days submergence, 10 border plants of sensitive genotypes (IR42) were randomly uprooted daily from the extra row to observe extend damage. If the sensitive check genotypes were rotten and its basal stems were soft as much 60% of the samples, the submergence treatment (10-15 days) was applied. Algae populations were minimized by partially removing the plant from the water surface using small fish net filter.
On the same day with submergence treatment, the stagnant flooding (SF) treatment was given at 30 cm water level, then the water level was gradually increased to ~40 cm and ~50 cm at one week (36 DAT) and two weeks (43 DAT) after initial flooding, respectively. The water level was then maintained at ~50 cm until the genotypes were flowering. The ~25 cm SF followed with 12 days submergence treatment was applied followed the procedure as described above, but the extent damage of sensitive variety (IR42) was 40% of the total samples randomly uprooted. The water was then receded until it reached ~25 cm and maintained until the plants were mature.

The cluster analysis or Scott-Knott method (Scott and Knott 1976) as described by Bhering et al. (2008) was used to compare genotypes in one environment. The data were tabulated and analyzed using Microsoft Excel 2007, while analysis of variance was using SAS 9.0 PROC GLM (SAS 2002).

**Characterization of Flood Water and Environment**

Floodwater conditions in the submerged ponds were monitored daily at 08.30 am and 2.00 pm by measuring water levels at 25, 50 and 100 cm during submergence and stagnant flooding. Dissolved O$_2$ was measured below the water surface by an O$_2$ meter (YSI EcoSense DO200, YSI Environmental, Inc., OH, USA). Temperature of floodwater was measured by using a digital temperature meter (Omega, HH 64A Thermometer, Omega Engineering, Inc., Stamford, CT, USA) and the pH was by using a pH-meter (250A, Orion Research Inc., Boston, USA). The climatic data were obtained from the IRRI agrometeorological station located within 200 m from the experimental site.

**Assessment of Plant Physiological Status**

Leaves of main tiller were sampled at 21 DAT (before submergence) and 36 DAT (after desubmergence or during stagnant flooding) as described by Bruinsma (1963). The leaves were soaked in liquid nitrogen and put into paper envelopes, then freeze-dried by using air ballast freeze-dried machine at -40°C to get rid of excess moisture for 3 days. The dried leaves were then grinded to fine powder and extracted by putting homogen freeze-dried material in 80% acetone for 24 hour in a fridge (5°C). Readings were carried out using a spectrophotometer and the optical density was recorded accordingly at 663, 652 and 645 nm after overnight incubation. Based on optical density recorded, the concentration of chlorophylls a and b were adjusted by using equations described by Bruinsma (1963).

Soluble sugar and starch concentration of the stem were estimated before submergence and just after water was receded from complete submergence (36 DAT) and 15 days during stagnant flooding (36 DAT). The soluble sugar was analyzed using a method described by Fales (1951). Briefly, for each measurement, the stem samples were freeze-dried and grounded to get fine powder. As much as 200 mg of powdered samples were placed into a 15-ml centrifuge tube and added with 10 ml of 80% ethanol. A glass ball was placed on top of the tube and boiled in water bath at 80-85°C for 20 minutes. Final extraction was done by adding anthrone reagent and sulfuric acid. Optical density of absorbance was measured using spectro-photometer at 630 nm wave length.

The residues of soluble sugar were used to determine starch concentration as described by Kunts (1988). Starch hydrolysis was done by adding the samples with 2 ml acetate buffer and 1 ml amyloglucosidase solution. All tubes were incubated for 24 hours at 37°C and every 10 minutes the samples were centrifuged. Then, the samples were decanted in a 25 ml volumetric flask. Peroxidase Glucose Oxidase
(PGO) enzyme-color reagents were added to the 3 ml samples and then incubated in the dark at room temperature for 30 minutes. Absorbance at 450 nm was used for reading against a sample blank (reference). Readings of all sample absorbance were done within 30 minutes.

RESULTS AND DISCUSSION

General Conditions of Flooding Experiment

The concentration of O$_2$ in the field pond was lower in the morning and slightly increased in the afternoon. At 25 and 50 cm water depths, O$_2$ concentrations were slightly different, but decreased by two fold from 7 to 3 at 100 cm water depth. The pH within the water depths was relatively stable, but slightly increased at afternoon. Continuous rainfall and cloudiness were experienced during whole crop life, which reduced the average daily sunshine hours. Moreover, the temperatures were relatively stable at 27-28°C.

The Effect of SUB1 on Plant Height under Various Flooding Conditions

Plant heights among rice genotypes varied, ranged from 91 to 129 cm under submergence. Although there was an excessive elongation on non-SUB1 lines during submerged conditions, after recovery the final plant heights were not significantly different (Table 1). The variations were obviously appeared on SUB1 lines and non-SUB1 lines compared to normal condition. Under submergence, most of the genotypes had lower plant height compared to that at normal condition (Fig.1), as shown by the negative value of the plant height difference under submergence and normal conditions. This is because after desubmergence, the plants required energy to recovery resulted in compensation on biomass and plant height. The effect of SUB1 on plant height under submergence was shown by the difference between the recurrent parents and its NILs compared with that at normal condition (Fig. 2), where the SUB1 contributed positively to the plant height of NILs as much as 1.5 cm in average.

Following ~50 cm SF increased plant height by 20 cm compared to normal conditions (Fig 1). The NILs had significantly shorter plant heights compared to their respective recurrent parents, for example IR64 Sub1 vs IR64 (117cm vs 123cm), Swarna Sub1 vs Swarna (117cm vs 126 cm) and CR1009 Sub1 vs CR1009 (123cm vs 126 cm) (Table 1). The effect of SUB1 on suppressing plant height under SF conditions can be seen in Figure 2, where in average the plant height of SUB1 reduced by 4.3 cm. Stagnant flooding at ~25 cm follows submergence also increased plant height but lower compared to that of ~50 cm SF.

Performance of Other Agronomy Traits of SUB1 NILs under Various Flooding Conditions

Under submergence conditions, days to flowering of the SUB1 lines were similar compared to their respective recurrent parents in the normal flooding condition. However, days to flowering were varied among genotypes under submergence, in which the non-SUB1 were 2-5 days longer compared to that of SUB1 lines. The same results were obtained when submergence followed shallow stagnant flooding (~25 cm). Under ~50 cm SF condition, plant flowering was delayed, but it was not different between the SUB1 and non-SUB1 lines.

Delaying in the days to flowering occurred after submergence or during SF in all genotypes as the plants needed additional time to recovery and resume normal vegetative growth, and to overcome damage during and after submergence. However, under submergence the SUB1 lines were last delayed in flowering because it had least damage due to submergence.

The SUB1 lines had significantly higher number of panicles compared to their respective recurrent parents under submerged condition (Table 2). Lower survival of sensitive lines was compensated with increasing panicle number per hill, but reduced competitions among plants allowed the plants had more tiller during recovery. However, the loss in panicle number per unit area could not be compensated for non-SUB1 lines due to high mortality (up to 90%) and low number of effective tillers. Singh et al. (2009) reported the reduction in the number of tillers per area under submergence.

Following ~50 cm SF reduced the number of panicles of most genotypes compared to the normal conditions (Table 2). IR49830-7, IR70181-32, IR67440-M and PSBRc68 produced high number of panicles under SF although under normal condition they produced small panicles. This meant that high panicle
Table 1. Survival, plant height and days to flowering of rice genotypes under 15 days submergence, ~50 cm stagnant flooding (SF), ~25 cm SF follows 12 days submergence and normal condition, IRRI experimental farm, Los Banos, WS 2009.

| Genotypes   | Survival (%) | Plant height (cm) | Day to flowering (d) |
|-------------|--------------|-------------------|----------------------|
|             | Sub SF Sub + SF Normal | Sub SF Sub + SF Normal | Sub SF Sub + SF Normal |
| IR64 Sub1   | + 91a 59b 62b 100a | 97e 117e 98c 98d | 102h 93d 108d 85f |
|             | - 56c 76a 32c 100a | 91e 123d 100c 98d | 110g 91d 110d 89e |
| Swarna Sub1 | + 84a 39c 46c 99a | 96d 117e 99c 101d | 122c 115b 124b 107c |
|             | - 25d 44c 8d 100a | 90e 126d 102c 100d | 123c 116b 130a 108c |
| S. Mahsuri Sub1 | + 85a 62b 61b 99a | 96d 123d 101c 99d | 120d 114b 120b 107c |
|             | - 51c 51b 11d 99a | 92d 126d 101c 99d | 122c 114b 123b 108c |
| BR11 Sub1   | + 78b 50b 31c 100a | 113b 134c 116b 115b | 119d 106c 117c 103d |
|             | - 22d 59b 20d 99a | 116b 132c 121b 115b | 120d 109c 123b 103d |
| CR1009 Sub1 | + 84a 54b 42c 98a | 109b 126d 117b 111b | 119b 109c 128a 119a |
|             | - 43c 37b 15d 99a | 106c 128c 115b 108c | 130a 123a 131a 120a |
| TDK1 Sub1   | + 81b 78a 66b 99a | 113b 135c 118b 116b | 117e 114b 122b 108c |
|             | - 50c 87a 15d 99a | 114b 137c 119b 117b | 122c 114b 125b 109c |
| Inpara 3    | + 92a 87a 54b 99a | 116b 144b 127a 115b | 113f 109c 114c 102d |
|             | - 67c 40a 40b 100a | 118b 146b 127a 121a | 114f 101c 113c 100d |
| PSBRc68     | + 91a 88a 76a 100a | 111c 135c 118b 115b | 122c 115b 124b 112b |
| IR49830-7   | + 93a 80a 59b 99a | 114b 144b 117b 116b | 110g 105c 114c 92e |
| IR70181-5   | + 81b 87a 56b 99a | 103c 127d 113b 108c | 96i 87e 95e 81f |
| IR70181-32  | + 92a 87a 80a 99a | 129a 161a 135a 127a | 128a 117b 127a 111b |
| IR67440-M   | - 57c 88a 34c 98a | 107b 132 114 110 | 118 109 119 104 |
| Means       | 70 67 43 100 | 107 132 114 110 | 118 109 119 104 |

| F Genotypes | 7.8* 4.6* 7.1* 0.2 | 10.2* 15.5* 9.7* 15.0* | 7.0* 49.1* 30.9* 118.4* |
|-------------|-------------------|------------------------|------------------------|
| F Sub1 vs Non Sub1 | 10.8* 0.2NS 5.2* 0.1NS | 0.1NS 4.1* 2.0* 0.1NS | 10.9* 1.6NS 3.1NS 0.9NS |
| CV (%)       | 13.2 17.8 16.9 1.8 | 5.0 7.5 10.2 6.5 | 1.2 2.3 2.5 1.67 |

Sub = 15 day submergence; SF = -50 cm stagnant flooding until maturity; Sub + SF = -25 cm stagnant flooding follows 12 days submergence; + = genotypes with SUB1; - = genotypes without SUB1.

Small letter following a value in a column is a mean separation by Scott-Knott at 5% level. * and ** are significantly different at 5% and 1% level, respectively.
Fig. 1. Plant height difference of SUB1 NILs and rice varieties and their respective parents under submergence, ~50 cm stagnant flooding (SF) and ~25 cm SF follows submergence, IRRI experimental farm, Los Banos, WS 2009.

Fig. 2. Plant height difference as a result of the effect of SUB1 on recurrent parents with its NILs rice varieties under submergence, ~50 cm SF and ~25 cm stagnant flooding (SF) follows submergence, IRRI experimental farm, Los Banos, WS 2009.
number under normal conditions was not always expressed under prolonged flooding stress. The low number of panicles was also probably due to the compensation for increase in plant height. The limited biomass produced under stressed condition caused the plant to choose whether to produce more tillers or to elongate the stem. Because the elongation was more important for survival, then the number of tillers or panicles were reduced. Vergara and Ismail (2006) proposed the criteria for genotype tolerance under SF conditions that should include the ability to produce more panicles, as much as if they were grown in normal conditions.

**Grain Yield and Yield Components**

Most of genotypes had reduced filled grain per panicle, increased unfilled grain and reduced fertility under SF conditions, but the numbers of filled and unfilled grains per panicle under submergence were similar to normal conditions (Table 2). Sambha Mahsuri and its NILs had the highest number of filled grains under submergence (189 and 187 grains, respectively) similar with that under normal conditions (184 and 189). Meanwhile, IR64 and its NILs had lower unfilled grains resulted in higher panicle fertility. Most of genotypes had low panicle fertility when exposed to flooding and more severe when it was subjected to SF. Under this condition, the panicle fertility ranged from 44 to 81%.

Stagnant flooding at 50 cm reduced the number of filled grains per panicle, increased unfilled grain and reduced panicle fertility. Improper grain filling under SF conditions was also reported by Amante (1986) and Singh et al. (2008). This is because the prolonged partial submergence would reduce translocations of assimilates to sink. Photosynthetic ability also declined and respiratory rate decreased due to reducing photosynthetic active leaves under water which only received diffused light. Further-more, photosynthesis became weak under reduced light conditions, thus decreased translocation of assimilate as a result of photosynthesis activity to grains.

The ~50 cm SF and ~25 cm SF follows submergence reduced the means of seed weight. However, there was no significantly different between SUB1 and non-SUB1 for all flooding conditions. Lower 1,000-grain weight under SF conditions was due to improper grain filling and uneven filling stage, therefore, at harvest the grains had different maturity stages thus lowered seed weight.

Most of genotypes had lower grain yields under flooding treatments compared to normal conditions (Table 3), but reduction in grain yields of SUB1 lines was smaller compared to non-SUB1 lines under submerged conditions. The average of grain yield difference reached 4 fold between SUB1 lines and non-SUB1 lines. Meanwhile, following ~50 cm SF condition, most of the SUB1 lines had lower grain yield compared to that of non-SUB1 lines. But, some SUB1 lines like PSBRc68 and IR70181-32 had higher grain yield under this condition. SF followed by submerged condition resulted in poor grain yield on all genotypes. But again, the SUB1 lines had better grain yield compared to respective recurrent parents under this condition, ranged from 0.89 to 3.36 t ha⁻¹.

Reduction in grain yield under submergence and SF conditions could be attributed to the degree of injury experienced by each genotype, depending on the level of tolerance. The higher the genotypes tolerance to flooding conditions, the higher the yield can be produced. The sensitive genotypes would lose their biomass, leaves and tillers and take much longer time to recover and develop new organs. These will affect production of assimilate to be translocated to the sink. Our result suggests that introgression of SUB1 gene does not always give negative effect on grain yield when it is exposure to SF. The SUB1 A1 gene also supports the plants to increase grain yield under SF when it was combined after complete submergence. Under this condition, the non-SUB1 genotypes or recurrent parents had greater reduction in grain yield compared to SUB1 lines.

**Above Ground Dry Matter Weight and Harvest Index**

Flooding reduced above ground dry matter weight (AGDMW) on most genotypes (Table 3). The fast recovery and more survivors of SUB1 lines produced high biomass compared to the non-SUB1 lines. Reduction in AGDMW due to submergence did not lower harvest index (HI) and even it was higher than that in normal condition. This is because the stressed conditions reduced plant survival and plant population per plot, hence decreased plant competition after water recede resulting in favorable growth for individual plant to produce more grains. The significant difference in HI between SUB1 and non-SUB1 lines can be observed in the lowest plant survival among all treatments, e.g. ~25 cm SF follows 12 day submergence.
Table 2. Panicle number, filled grains per panicle and panicle fertility of rice genotypes under 15 days submergence, ~50 cm SF, ~25 cm SF follows 12 days submergence and normal condition, IRRI experimental farm, Los Banos, WS 2009.

| Genotypes       | SUB1  | Panicle number m⁻² (no) | Filled grain panicle⁻¹ (no) | panicle fertility (%) |
|-----------------|-------|-------------------------|-----------------------------|-----------------------|
|                 |       | Sub | SF | Sub + SF | Normal | Sub | SF | Sub + SF | Normal | Sub | SF | Sub + SF | Normal |
| IR64 Sub1       | +     | 317a | 119c | 186a | 344a | 98c | 96b | 90c | 98d | 88a | 80a | 80a | 89a |
| IR64 -          | -     | 215b | 167b | 88c | 325a | 99c | 95b | 80c | 98d | 86a | 81a | 81a | 89a |
| Swarna Sub1     | +     | 255a | 75d | 123b | 327a | 137b | 91b | 132b | 140b | 76b | 45d | 69b | 83a |
| Swarna -        | -     | 90c | 120c | 25d | 329a | 144b | 92b | 131b | 148b | 83a | 49c | 76b | 82b |
| S. Mahsuri Sub1| +     | 270a | 142c | 162b | 309a | 189a | 113a | 161a | 184a | 72b | 48c | 68b | 81b |
| S. Mahsuri -    | -     | 173b | 114c | 31d | 320a | 197a | 113a | 161a | 184a | 72b | 46c | 63c | 79b |
| BR11 Sub1       | -     | 243a | 124c | 102c | 276b | 139b | 59c | 123b | 147b | 71b | 46c | 63c | 74c |
| BR11 -          | -     | 103c | 174b | 69c | 325a | 143b | 62c | 123b | 147b | 71b | 46c | 58c | 67d |
| CR1009 Sub1     | +     | 224b | 122c | 128b | 318a | 142b | 85c | 122b | 130b | 71b | 46c | 58c | 67d |
| CR1009 -        | -     | 163b | 132c | 47c | 326a | 128b | 79c | 108b | 131b | 67b | 44d | 59c | 68d |
| TDK1 Sub1       | +     | 236b | 186b | 180a | 296a | 110c | 73c | 106c | 107c | 70b | 50c | 64b | 80b |
| TDK1 -          | -     | 164b | 239a | 71c | 307a | 101c | 81c | 103c | 110c | 70b | 61b | 65b | 78b |
| Inpara 3        | +     | 292a | 191b | 155b | 255b | 131b | 70d | 119b | 125c | 75b | 47c | 68b | 75c |
| Inpara 3 -      | -     | 167a | 191b | 155b | 255b | 131b | 70d | 119b | 125c | 75b | 47c | 68b | 75c |
| PSBRc68 +       | +     | 245a | 218a | 224a | 272b | 121b | 102a | 118b | 129b | 75b | 71b | 69b | 75c |
| PSBRc68 -       | -     | 164b | 217a | 224a | 272b | 121b | 102a | 118b | 129b | 75b | 71b | 69b | 75c |
| IR49830-7 +     | +     | 288a | 226a | 193a | 281b | 107c | 88c | 106c | 107c | 70b | 59c | 65c | 76b |
| IR49830-7 -     | -     | 164b | 217a | 193a | 281b | 107c | 88c | 106c | 107c | 70b | 59c | 65c | 76b |
| IR70181-5 +     | +     | 273a | 150b | 173a | 258b | 113c | 88c | 106c | 122c | 74b | 62b | 65c | 74c |
| IR70181-5 -     | -     | 164b | 173a | 150b | 258b | 113c | 88c | 106c | 122c | 74b | 62b | 65c | 74c |
| IR70181-32 +    | +     | 249a | 197a | 229a | 254b | 109c | 83c | 107b | 122c | 75b | 67b | 69b | 73c |
| IR70181-32 -    | -     | 147c | 238a | 96c | 329a | 129b | 107a | 118b | 132b | 68b | 58c | 61c | 74c |
| Inpara 3        | +     | 292a | 191b | 155b | 255b | 131b | 70d | 119b | 125c | 75b | 47c | 68b | 75c |
| Inpara 3 -      | -     | 164b | 217a | 193a | 281b | 107c | 88c | 106c | 107c | 70b | 59c | 65c | 76b |
| IR67440-M       | -     | 147c | 238a | 96c | 329a | 129b | 107a | 118b | 132b | 68b | 58c | 61c | 74c |
| Means           |       | 219  | 163  | 127  | 303  | 130  | 88  | 117  | 131  | 74  | 55  | 67  | 77  |

**F**<sub>Genotypes</sub> = 9.1** 12.3** 10.3** 3.1** 7.8** 4.3** 4.3** 13.6** 4.0** 29.0* 2.2** 8.3**

**F**<sub>Sub1 vs Non Sub1</sub> = 2.0* 2.2* 2.0* 0.1 NS 0.1 NS 2.3* 2.0* 0.1 NS 0.0 NS 2.4* 2.0* 0.0 NS

CV% = 17.0 21.1 16.6 13.1 13.2 12.9 10.6 10.0 8.3 11.1 4.5

Sub = 15 day submergence; SF = ~50 cm stagnant flooding until maturity; Sub + SF = ~25 cm stagnant flooding follows 12 day submergence; + = genotypes with SUB1; - = genotypes without SUB1.

Small letter following a value in a column is a mean separation by Scott-Knott at 5% level. * and ** are significantly different at 5% and 1% level, respectively.
Table 3. Above ground dry matter weight, grain yield and harvest index of rice genotypes under 15 day submergence, ~50 cm stagnant flooding (SF), ~25 cm SF follows 12 day submergence, and normal condition, IRRI experimental farm, Los Banos, WS 2009.

| Genotypes   | SUB1     | Above ground dry matter weight (g m⁻²) | Grain yield (t ha⁻¹) | Harvest index |
|-------------|----------|----------------------------------------|----------------------|---------------|
|             | Sub SF   | Sub + SF Normal                        | Sub SF   | Sub + SF Normal | Sub SF   | Sub + SF Normal |
| IR64 Sub1   | + 637a   | 415c 360b 946c                         | 4.05b    | 2.87b 2.03b 4.62a | 0.40b    | 0.41a 0.37b 0.33b |
| IR64        | - 401b   | 563b 219c 907c                         | 2.69d    | 3.44a 1.37c 4.85a | 0.41b    | 0.38a 0.41b 0.35b |
| Swarna Sub1 | + 687a   | 294d 319c 1068b                        | 4.43b    | 0.89e 2.09b 5.45a | 0.39b    | 0.23d 0.39b 0.34a |
| Swarna      | - 107c   | 331c 55e 1050b                        | 1.02e    | 1.20d 0.89c 5.36a | 0.35c    | 0.27b 0.62a 0.34a |
| S. Mahsuri Sub1 | + 773a | 472c 383b 1015c                      | 3.87b    | 1.32d 1.89b 4.36b | 0.34c    | 0.22c 0.33b 0.30b |
| S. Mahsuri  | - 488b   | 426c 70e 1052b                        | 1.70e    | 1.44d 0.8c 4.36b  | 0.26d    | 0.25c 0.58a 0.29c |
| BR11 Sub1   | + 438b   | 398c 269c 986c                        | 5.16a    | 1.00d 2.01b 5.44a | 0.54a    | 0.22c 0.43b 0.35a |
| BR11        | - 133c   | 449c 144d 1064b                       | 1.49f    | 1.43d 1.2c 5.39a  | 0.52a    | 0.25c 0.46a 0.34b |
| CR1009 Sub1 | + 790a   | 413c 280c 1180b                       | 5.10a    | 1.33d 2.14b 4.90a | 0.39b    | 0.24d 0.44b 0.29c |
| CR1009      | - 373b   | 311d 119d 1108b                       | 1.94e    | 0.89e 1.27c 4.98a | 0.34b    | 0.22c 0.50a 0.31b |
| TDK1 Sub1   | + 777a   | 661b 485a 1326a                       | 4.53a    | 2.13c 2.13b 4.86a | 0.37c    | 0.25c 0.31b 0.27c |
| TDK1        | - 513b   | 759a 121d 1252a                       | 2.33d    | 2.86b 1.72b 4.84a | 0.33c    | 0.27b 0.60a 0.28c |
| Inpara 3    | + 809a   | 863a 419b 1371a                       | 4.37b    | 2.22c 1.88b 4.11b | 0.36c    | 0.20d 0.31b 0.23c |
| PSBRC68     | + 849a   | 801a 630a 1335a                       | 5.21a    | 3.47a 3.36a 5.51a | 0.38c    | 0.28b 0.35b 0.29c |
| IR49830-7   | + 878a   | 660b 471b 1226a                       | 4.36b    | 2.56b 2.08b 4.92a | 0.33c    | 0.28b 0.31b 0.28c |
| IR70181-5   | + 746a   | 841a 1362a                           | 4.94a    | 2.82b 3.04a 5.04a | 0.40b    | 0.25c 0.40b 0.27c |
| IR70181-32  | + 810a   | 688b 570a 934c                       | 3.18c    | 3.15a 2.23b 4.71a | 0.29d    | 0.31b 0.28b 0.34a |
| IR67440-M   | - 695a   | 807a 266c 1379a                       | 2.36d    | 2.5b 1.88b 4.84a | 0.25d    | 0.24c 0.45b 0.26c |
| Means       | 610      | 564 312 1142                           | 3.53     | 2.08 1.89 4.92 | 0.38     | 0.27 0.42 0.30 |

| F₆₉₉ Genotypes | 17.8** 8.6** 7.0** 1.5** 24.1** 23.7** 4.5** 1.4** 3.92* 7.75* 2.1* 4.3* |
| F₆₉₉ SUB1 vs Non SUB1 | 2.9* 2.2* 3.5* 0.1 13.2* 2.1* 2.2* 0.00* 0.16* 0.30* 22.2* 0.00* |
| CV(%)         | 24.8 18.2 29.1 8.4 23.6 16.3 18.9 11.8 18.1 12.97 28.9 9.5 |

Sub = 15 day submergence; SF = ~50 cm stagnant flooding until maturity; Sub + SF = ~25 cm stagnant flooding follows 12 day submergence; + = genotypes with SUB1; - = genotypes without SUB1.

Small letter following a value in a column is a mean separation by Scott-Knott at 5% level. * and ** are significantly different at 5% and 1% level, respectively.
Leaf Chlorophylls and Stem Carbohydrates

Total chlorophyll (a and b) concentration and ratio of chlorophyll a/b at 21 DAT were not determinant characters because they were not significantly different among genotypes and no interaction of genotypes by environments (GxE). But, these characters became important when measured after submergence or during stagnant flooding at 36 DAT. All of the genotypes showed reductions in the total chlorophyll concentration and ratio of chlorophyll a/b under submergence and SF follows submergence (Fig. 2). Although the total chlorophyll reduced, the SUB1 lines had a higher concentration of chlorophyll and ratio of chlorophyll a/b compared to non-SUB1 lines. Under SF conditions both total chlorophyll and ratio of chlorophyll a/b were similar compared to those in normal conditions. Small ratio of chlorophyll a/b was also found under submerged conditions and this much greater than that under SF, especially for non-SUB1 lines.

There was no variation in carbohydrate (soluble sugar and starch) concentrations in stem based on dry weight at 21 DAT (Fig. 3). However, inherent variations of carbohydrate contents were observed at 36 DAT or just after de-submergence and during stagnant floods. All of the genotypes showed reduction in stem soluble sugar and starch from the last measured at pre-submergence at 36 DAT under submergence and submergence followed ~25 cm SF. Reduction in carbohydrates of SUB1 lines was significantly lower compared to that of non-SUB1 lines. Under ~50 cm SF, all of the genotypes also had slightly loss of stem soluble sugar concentration, but it was not significantly different between SUB1 and non-SUB1 lines.

Submerged condition enhanced anaerobic respirations, resulting in increasing consumption of accumulated carbohydrates and decreasing photosynthetic rate which lowered plant growth (Mazerado and Vergara 1982; Setter et al. 1987, Ram et al. 1999; Das et al. 2005). Reduction in shoot and leaf dry weights under submergence due to death or decay of living tissues also reduced the supply of additional carbohydrates through concurrent under-water photosynthesis. Reduction in soluble sugars and starch during submergence is probably one of the crucial biochemical processes that affects plant survival and growth during submergence and recovery.

At 36 DAT (post-submergence), total chlorophyll concentration under submerged condition in all genotypes decreased due to chlorosis in which the respective recurrent parents and other non-SUB1 lines suffered as much 10 fold compared to the SUB1 lines. Increasing ethylene concentrations during submergence is a possible reason for chlorophyll degradation (Fukao and Serres 2008). This has been confirmed in studies that chlorophyll degradation was prevented by blocking the action of ethylene during submergence (Sarkar et al. 2001). Submergence also increases the transcript level and activity.

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**Fig. 2.** Total chlorophyll concentration based on leaf dry weight (A) and ratio of chlorophyll a/b (B) of SUB1 and non-SUB1 lines under various flooding conditions at 21 and 36 days after transplanting (DAT), IRRI experimental farm, Los Banos, WS 2009.
Response of SUB1 introgression lines of rice (Yudhistira Nugraha et al.)

suggesting that sensitive genotypes are mostly source-limited during grain filling.

The SUB1 introgression lines had low chlorophyll degradation and soluble sugar and starch depletion after submergence, but soluble sugar and starch were not significantly different between SUB1 NILs and their respective recurrent parents. Introgression of SUB1 into high-yielding popular varieties will improve submergence tolerance without affecting yield potential. To improve rice variety adaptation to SF and following submergence, then introgression of SUB1 gene into taller type varieties is important to compensate the effect of suppressed elongation.

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