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 *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 *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 *SUB1* introgression rice lines had higher survival compared to the non-*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 *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 *SUB1* NILs and non-*SUB1* lines were not significantly different. Introgression of the *SUB1* into high-yielding varieties improved submergence tolerance without affecting yield potential. The study indicates that introgression of the *SUB1* into taller type rice varieties should be done to compensate the effect of suppressed elongation.

[***Keywords:*** Rice, flooding, introgression, *SUB1* gene, adaptation]

ABSTRAK

Dua jenis banjir dapat terjadi pada pertanaman padi, yaitu banjir bandang dan banjir stagnan. Banjir bandang menyebabkan tanaman padi terendam penuh sampai 2 minggu, sementara banjir stagnan dapat merendam sebagian dari tanaman padi. Untuk mengatasi penurunan hasil akibat banjir, introgresi gene SUB1 *yang dikenal sebagai gen yang menekan pemanjangan sel dan metabolisme karbohidrat, pada genotipe padi dapat meningkatkan toleransi tanaman terhadap rendaman lebih dari 10 hari. Penelitian ini bertujuan untuk mengevaluasi toleransi 18 genotipe padi, termasuk padi* near isogenic lines *(NILs)* SUB1 *dan megavarietas, yaitu Swarna, Sambha Mahsuri, IR64, TDK1, BR11, dan CR1009. Genotipe padi ditanam di kolam pembesaran di Los Banos, Filipina pada musim hujan 2009. Perlakuan yang diuji yaitu 15 hari perendaman, banjir stagnan (SF), SF berikut perendaman, dan kondisi normal. Setiap perlakuan disusun dalam rancangan blok acak lengkap dengan tiga ulangan. Hasil penelitian menunjukkan bahwa galur padi introgresi* SUB1 *memiliki kelangsungan hidup yang lebih tinggi dibandingkan dengan non-*SUB1 *serta pemanjangan tunas lebih pendek selama perendaman. Namun, pada kondisi banjir stagnan, genotipe padi perlu memiliki tunas yang lebih cepat memanjang untuk memungkinkan kontak dengan udara. SF dan SF berikut perendaman menurunkan jumlah malai, jumlah gabah per malai, dan malai isi sehingga hasil menurun. Ini menunjukkan bahwa genotipe yang sensitif umumnya memiliki lumbung yang terbatas selama pengisian biji. Galur introgresi* SUB1 *memiliki kandungan klorofil yang lebih tinggi serta penurunan kandungan gula larut dan pati yang lebih rendah setelah perendaman. Pada kondisi banjir stagnan, tidak ada perbedaan yang signifikan dalam gula terlarut dan pati antara* SUB1 *NILs dan non-*SUB1*. Introgresi* SUB1 *pada varietas unggul padi meningkatkan toleransi tanaman terhadap rendaman tanpa memengaruhi hasil. Studi ini menunjukkan bahwa introgresi* SUB1 *perlu dilakukan pada varietas padi tipe tinggi untuk mengompensasi pengharuh penekanan pemanjangan tanaman.*

[***Kata kunci:*** *Padi, rendaman, intogresi, gen* SUB1*, adaptasi*]

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 dan 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 *SUB1A-1* 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 submer-gence 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 m^2 and plant spacing of 20 cm x 20 cm. 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 Survival and Phenology

Plant survival percentage was measured by counting the number of survived plants per total number of plants before flooding. Days to maturity was counted when 80% of panicles were mature or yellowing. Shoot elongation was measured by substracting plant height at 21 DAT (before submergence) with the plant height at 36 DAT (after submergence or during 15 days SF). Plant height was measured from soil surface to the tip of the tallest panicle (awns excluded) during maturity stage. The number of fertile grains were counted and divided to the total number of grains per main panicle from five plant samples. The grains were measured in grams of 1,000 well-developed whole grains, dried to 13% moisture content, and weighed on a precision balance. Grain yield was the total of all grains harvested from sample area of 6 rows x 0.2 m x 4 m or 4.8 m^2, including two border plants along side of plot. Grain yield per hectare was counted at 14% moisture content.

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 conditions (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	*SUB1*	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	85 f
IR64	-	56c	76a	32c	100a	91e	123d	100c	98d	110g	91d	110d	89 e
Swarna Sub1	+	84a	39c	46c	99a	96d	117e	99c	101d	122c	115b	124b	107 c
Swarna	-	25d	44c	8d	100a	90e	126d	102c	100d	123c	116b	130a	108 c
S. Mahsuri Sub1	+	85a	62b	61b	99a	96d	123d	101c	99d	120d	114b	120b	107 c
S. Mahsuri	-	51c	51b	11d	99a	92d	126d	101c	96d	122c	114b	123b	108 c
BR11 Sub1	+	78b	50b	31c	100a	113b	134c	116b	115b	119d	106c	117c	103 d
BR11	-	22d	59b	20d	99a	116b	132c	121b	115b	120d	109c	123b	103 d
CR1009 Sub1	+	84a	54b	42c	98a	109b	126d	117b	111b	126b	118b	128a	119 a
CR1009	-	43c	37b	15d	99a	106c	128c	115b	108c	130a	123a	131a	120 a
TDK1 Sub1	+	81b	78a	66b	99a	113b	135c	118b	116b	117e	114b	122b	108 c
TDK1	-	50c	87a	15d	99a	114b	137c	119b	117b	122c	114b	125b	109 c
Inpara 3	+	92a	87a	54b	99a	116b	144b	127a	115b	113f	109c	114c	102 d
PSBRc68	+	91a	88a	76a	100a	118b	146b	127a	121a	114f	101c	113c	100 d
IR49830-7	+	93a	80a	59b	99a	111c	135c	118b	115b	122c	115b	124b	112 b
IR70181-5	+	81b	87a	56b	99a	114b	144b	117b	116b	110g	105c	114c	92 e
IR70181-32	+	92a	87a	80a	99a	103c	127d	113b	108c	96i	87e	95e	81 f
IR67440-M	-	57c	88a	34c	98a	129a	161a	135a	127a	128a	117b	127a	111 b
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*	0.2^{NS}	5.2*	0.1^{NS}	0.1^{NS}	4.1*	2.0*	0.1^{NS}	10.9*	1.6^{NS}	3.1^{NS}	0.9^{NS}
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 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.

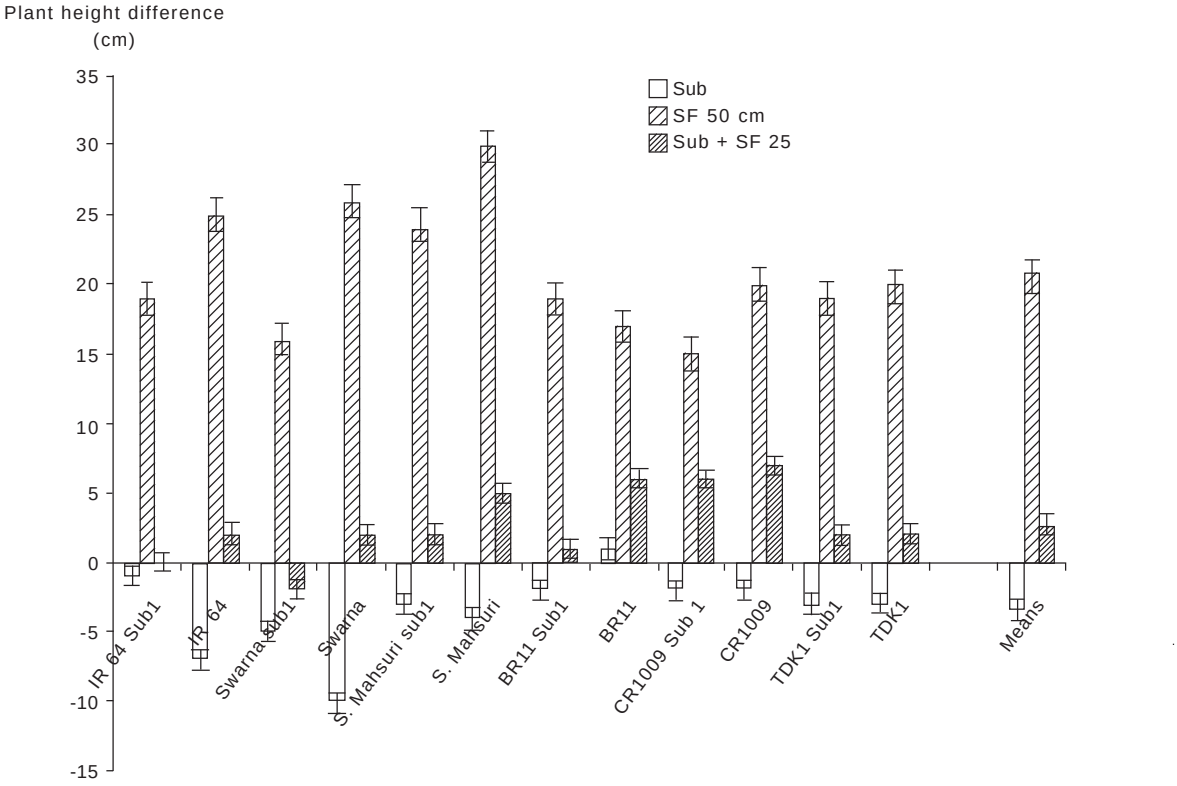


Fig. 1. Plant height difference of *SUB1* NILs and rice varieties and their respective parents under submergence, ~50 cm stagnant flooding (SF) and ~25 cm SF follows submergence, IRRI experimental farm, Los Banos, WS 2009.

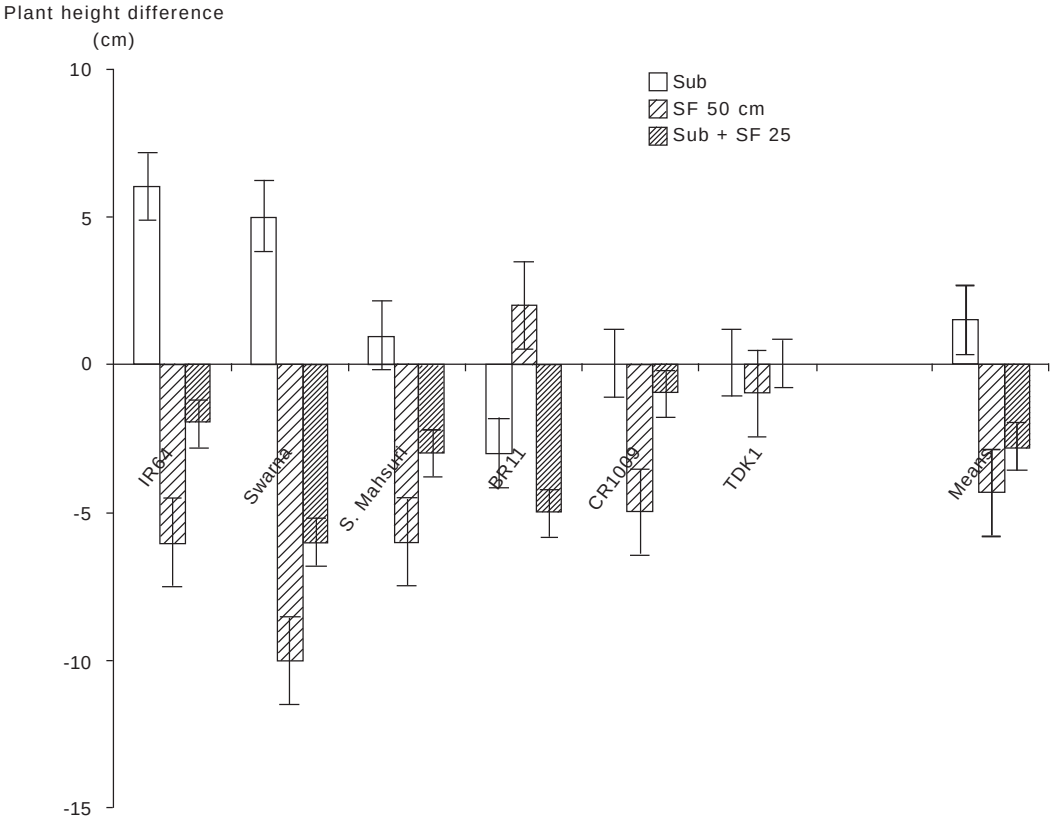


Fig. 2. Plant height difference as a result of the effect of *SUB1* on recurrent parents with its NILs rice varieties under submergence, ~50 cm SF and ~25 cm stagnant flooding (SF) follows submergence, IRRI experimental farm, Los Banos, WS 2009.

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 grains 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	116a	148a	184a	72b	48c	68b	81b
S. Mahsuri	-	173b	114c	31d	320a	197a	113a	161a	188a	71b	46c	63c	79b
BR11 Sub1	+	243a	124c	102c	276b	139b	59c	123b	147b	75b	37e	67b	77b
BR11	-	103c	174b	69c	325a	143b	62c	123b	147b	71b	41d	63c	74c
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
PSBRc68	+	245a	218a	224a	272b	121b	102a	118b	129b	75b	71b	69b	75c
IR49830-7	+	288a	226a	193a	281b	107c	88c	106c	107c	70b	59c	65c	76b
IR70181-5	+	273a	150b	173a	258b	113c	88c	106c	122c	74b	62b	65c	74c
IR70181-32	+	249a	197a	229a	254b	109c	83c	107b	122c	75b	67b	69b	73c
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
$F_{Genotypes}$		9.1*	12.3**	10.3**	3.1**	7.8**	4.3**	4.3**	13.6**	4.0**	29.0*	2.2**	8.3**
$F_{Sub1\ vs\ Non\ Sub1}$		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	17.0	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	444b	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	0.45a	0.26c
Means		610	564	312	1142	3.53	2.08	1.89	4.92	0.38	0.27	0.42	0.30
$F_{Genotypes}$		17.8**	8.6**	7.0**	1.5 $^{n.s}$	24.1**	23.7**	4.5**	1.4^{ns}	3.92*	7.75*	2.1*	4.3*
$F_{Sub1\ vs\ Non\ Sub1}$		2.9*	2.2*	3.5*	0.1	13.2*	2.1*	2.2*	0.00	0.16^{ns}	0.30^{ns}	22.2*	0.00^{ns}
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.

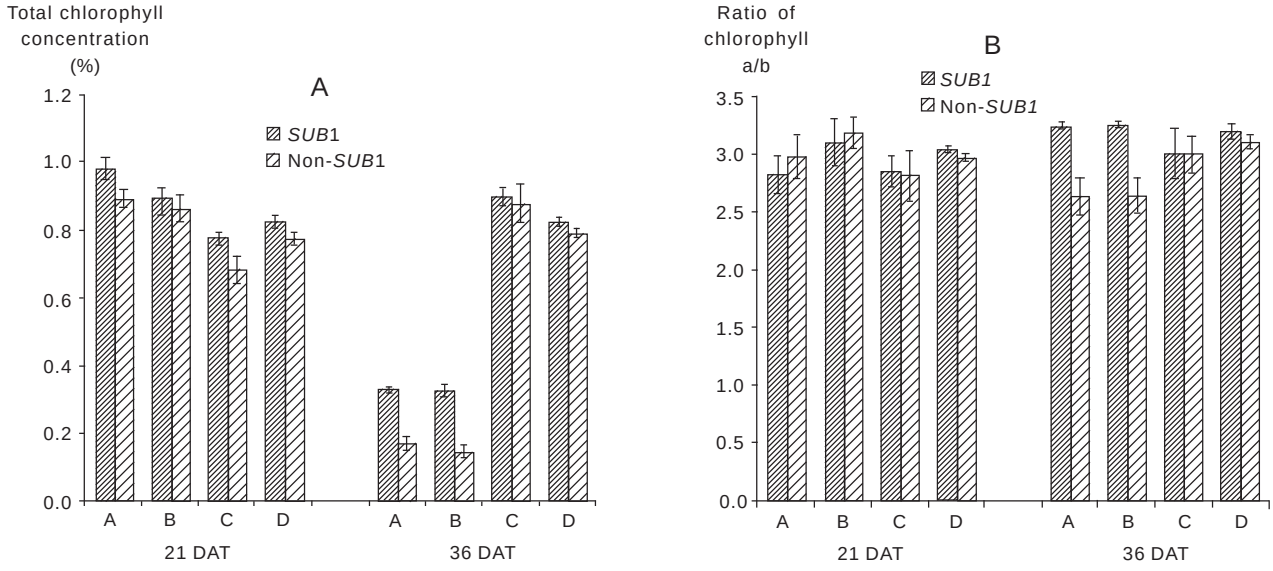
Leaf Chlorophylls and Stem Carbohydrates

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.

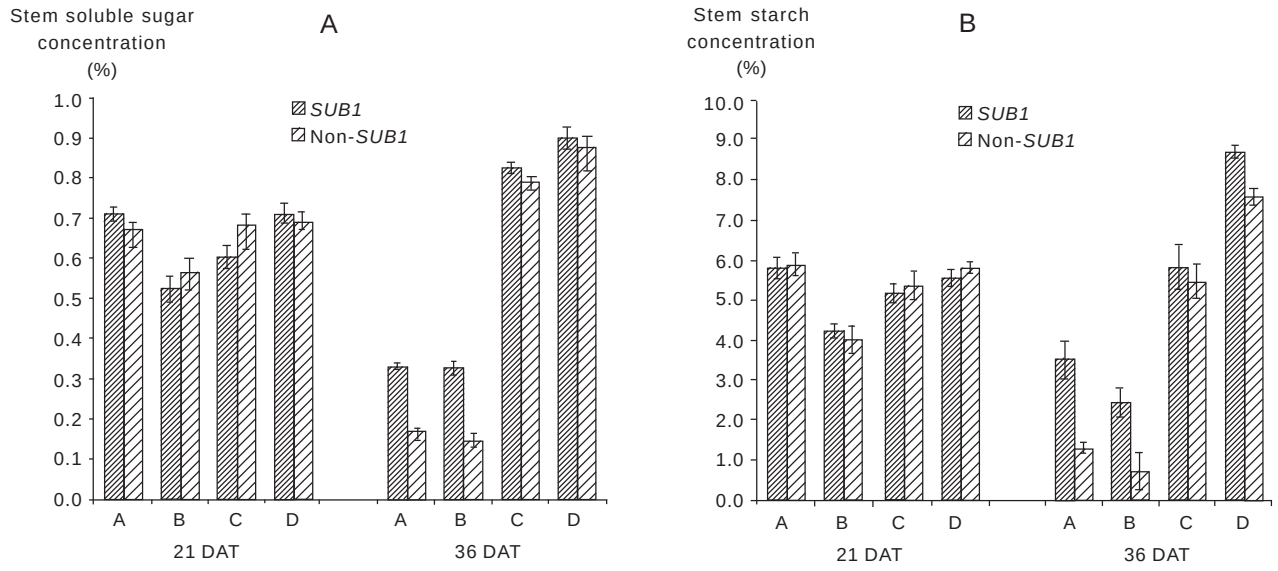
Submerged condition enhanced anaerobic respirations, resulting in increasing consumption of accumulated carbohydrates and decreasing photosynthetic rate which lowered plant growth (Mazerado and Vergara 1982; Setter *et al.* 1987, Ram *et al.* 1999; Das *et al.* 2005). Reduction in shoot and leaf dry 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



A = 15 d submergence, B = 25 cm SF folows submergence C = SF follows 12d submergence, D = normal conditions

Fig. 2. Total cholorophyll 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.



A = 15 day submergence, B = 25 cm SF folows submergence, C = SF follows 12 day submergence, D = normal conditions

Fig. 3. Stem soluble sugar concentration on dry weight basis (A) and stem starch concentration on dry weight basis (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.

of chloro-phyllase enzym, the key enzyme in degradation pathway of chlorophyll (Ella *et al.* 2003). This meant that higher ability of *SUB1* lines to retain chlorophyll content during and after submergence increased plant survival and allowed the plants to generate more energy during submergence and recovery.

Small ratios of chlorophylls a and b were also found in all flooding conditions and the values were greater under SF, especially for the non-*SUB1* lines. Reduction in chlorophyll a/b molar ratio was also observed by Ismail and Ella (2006) in senescing leaves of rice. This results suggest that chlorophyll a was degraded more than chlorophyll b, resulting in a significant decrease in chlorophyll a/b molar ratio after submergence.

CONCLUSION

Introgression of *SUB1*gene on rice varieties did not have any negative impact under normal conditions with respect to phenology. However, the *SUB1* lines showed substantially higher yields after submergence, flowered earlier, had higher panicle number and above ground dry matter yield. A significant decrease in panicles number, grain number per panicle, panicle fertility and 1,000-grain weight was the main cause of yield decline under SF and SF follows submergence, 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 significanttly 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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