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Role of total sugar and starch content of rice seedlings at different ages in variable submergence tolerance

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ABSTRACT

Rate of decreasing levels of total sugar and starch content in rice plants during complete submergence could be considered as an indicator of submergence tolerance as revealed through repeated experiments with two submergence tolerant and two susceptible varieties. Younger the seedlings (7 days), quicker are depletion than in older seedlings of 10 and 15 days. Drastic reduction is thus prominent under prolonged submergence period (15 days) than 7 or 10 days of complete submergence. Susceptible varieties show maximum decrease in starch and sugar content than resistant varieties.

Key words: *rice, submergence, total sugar, starch*

Flooding is one of the most important environmental stresses worldwide. It has been suggested that survival is a balancing act that results from, the management of carbohydrate consumption and the avoidance of oxidative stress. Flooding of croplands is a frequent natural disaster in many regions of the world. Flooding at early growth stage that persists for several days usually reduces plant survival and productivity of rice in rainfed lowlands. More than 20 million hectare is annually affected in South and Southeast Asia. (Ella and Ismail, 2006). Rice grows under flooded condition. The flooding of root systems and partial to complete submergence of aerial organs can dramatically reduce crop productivity.

The adverse effects of flooding constitute a complex phenomenon that varies with genotype and carbohydrate status of the plant before and after submergence, developmental stage of the plant when flooding occurs, duration and severity of flooding and degree of turbidity of floodwater (Setter *et al*., 1995; Ramakrishnayya *et al*., 1999; Jackson and Ram, 2003; Das *et al*., 2005).

A broad range of metabolic and morphological adaptations characterizes tolerant species. Flood-tolerant plants have the capacity to generate ATP without the presence of oxygen (fermentative metabolism) and/or to develop specific morphological entities like air channels and enhanced shoot elongation, that improve the entrance of oxygen (Fukao and Bailey-Serres,2004,Voesenek *et al*., 2006; Lasanthi-Kudahettige.*et.al.*, 2007).

The success of the crop can be partially explained by its ability to grow in diverse tropical environments, including the extensive and complex rainfed lowlands of south and south-east Asia. Such lowlands occupy 25 % of the world's rice-producing lands. In these areas, rice plants often get completely submerged for days. Traits found to be associated with selection for submergence tolerance used thus far include extent of underwater shoot elongation, shoot carbohydrate storage and extent of chlorophyll retention, significantly correlated with seedling survival. Post-submergence nonstructural carbohydrates have also been used as an indicator of submergence tolerance (Das *et al*., 2005, Agarwal and Grover, 2006). Therefore, long-term submergence causes extensive carbohydrate and energy (nucleotide triphosphate) starvation. *Oryza sativa* ssp. *indica*, cultivar FR13A, is highly tolerant and survive up to two weeks of complete submergence owing to a major quantitative trait locus designated *Submergence 1* (*Sub1*),containing two alleles, near the centromere of chromosome 9. These two are: a tolerance-specific allele named *Sub1A-1* and an intolerance-specific allele named *Sub1A-2*. Over expression of *Sub1A-1* in a submergence-intolerant *cultivar* confers enhanced tolerance to the plants, down regulation of *Sub1C* and up regulation of *Alcohol dehydrogenase 1* (*Adh1*), indicating that *Sub1A-1* is a primary determinant of submergence tolerance. The FR13A *Sub1* locus was introgressed into a widely grown Asian rice cultivar using marker-assisted selection (Xu *et al*.2006).

The *Sub*1 haplotype controls the regulation of the transcript levels of genes encoding enzymes like á-amylase and sucrose synthase, required for carbohydrate catabolism during submergence (Fukao *et al*.2006). *Sub1A* is confirmed as the primary contributor to tolerance, while *Sub1C* alleles do not seem important. Lack of dominance of *Sub1* suggests that the *Sub1A-1* allele should be carried by both parents for developing tolerant rice hybrids (Septiningsih *et al*.2009).

MATERIALS AND METHODS

The experiment was conducted at the Rice Research station of the Orissa University of Agriculture and Technology, Bhubaneswar in green house tanks and in pot culture. The materials taken for the experiment were two semi-dwarf high-yielding varieties Jagannath and Manika and two improved varieties FR13A and FR 43B.In spite of their low yield potential and undesirable characters FR 13A and FR 43B were selected for having proven resistance for flash flood submergence. After surface sterilization with 0.1 per cent $HgCl_2$ solution, presoaked seeds were sown in rows in enamel trays (40x25x5cm), each containing 4 kg of fine soil. After 5 days, the plants were thinned out leaving only ten uniform and normal healthy seedlings in each row. They were subjected to submergence in three separate tanks at three different ages, i.e.7 days, 10 days and 15days.Water level was maintained at 60cm above the soil surface. Trays were taken out successively after 7 days, 10 days and 15 days of submergence after the completion of treatment. Water was drained off and plant samples were collected for the determination of total sugar content. Experiment was conducted with three replications containing four sets of trays in each, where the first three were subjected to submergence and fourth was the control.

Fresh samples (excluding roots) were immediately utilized for the determination of the total sugar as well as starch content of the seedlings following the procedure of Yoshida *et al.* (1976).The absorbance of the plant extract was measured at 630 nm and the total sugar and starch content was calculated by the help of a standard curve for glucose and starch respectively. Another set of sample was kept for dry weight estimation.

RESULTS AND DISCUSSION

Total sugar content and starch content of the rice seedlings of three different ages (7-day Old, 10-day old and 15-day old) of four cultivars estimated before and after submergence have been presented in (Table 1) for 7, 10 and15 days of submergence. The experiment was conducted in a split plot design having seedling age in main-plot and period of submergence in sub-plots. The analysis of variance exhibited significance difference between the genotypes both for total sugar and starch content (Table 2).

Before subjecting to complete submergence, there were no apparent difference between the tolerant and susceptible varieties in terms of total sugar and starch content. However, a gradual decrease in total sugar and starch content was noticed in post submergence periods. During submergence, the available carbohydrates get exhausted rapidly because of low photosynthetic rate (Palada and Vergara, 1972). Maintenance of high levels of stored carbohydrates in the seedlings prior to submergence coupled with minimum shoot elongation and retention of chlorophyll are all desirable traits for submergence tolerance (Sarkar et al.2006). The initial high starch content with the advancement of age is probably an important factor that affects the resistance of the plants to submergence (Palada and Vergara, 1972). The analysis of the enzymes involved in carbohydrate metabolism indicates that anoxic rice seedlings possess a set of enzymes that allow the efficient metabolism of starch and sucrose to fructose-6-phosphate (Guglielminetti *et. al*, 1995. Lasanthi-Kudahettige.et.al. 2007).

In seven day old seedlings, complete submergence for 7 days and 10 days, marked decline in total sugar content was observed. However, percentage reduction was always found to be higher in susceptible varieties in Comparison to that of tolerant varieties. When these seedlings were subjected to complete submergence for fifteen days, further decline

Table 1. Effect of submergence on starch and total sugar content (mg /g. dry wt.) of rice seedlings

Varieties	Age of seedlings before submergence	Before submergence		Duration of submergence in days					
				7		10		15	
		Starch	Total sugar	Starch	Total sugar	Starch	Total sugar	Starch	Total sugar
Jagannath		66.877	14.167	56.25 (15.89)	7.713 (45.56)	50.63 (24.29)	4.167 (70.59)	44.377 (33.64)	1.25 (91.18)
Manika		61.25	11.88	50.213 (18.02)	7.297 (38.58)	45.63 (25.5)	3.75 (68.43)	42.71 (30.27)	1.25 (89.48)
FR 13A	7	67.503	14.167	63.963 (5.24)	11.253 (20.57)	61.25 (9.26)	10 (29.41)	58.753 (12.96)	7.713 (45.56)
FR 43B		64.38	12.503	61.25 (4.86)	10.627 (11.41)	58.753 (8.74)	7.297 (41.64)	57.5 (10.69)	6.88 (44.97)
Susceptible		64.063	13.023	53.231 (16.91)	7.505 (42.37)	48.13 (24.87)	3.958 (69.61)	43.543 (32.03)	1.25 (90.4)
Tolerant		65.941	13.335	62.606 (5.06)	10.94 (17.96)	60 (9)	8.648 (35.15)	58.126 (11.85)	7.296 (45.29)
Jagannath		70.627	17.917	64.38 (8.84)	14.583 (18.6)	56.25 (20.36)	8.13 (54.62)	48.127 (31.86)	4.583 (74.42)
Manika		66.25	15.417	59.38 (10.37)	11.25 (27.03)	51.253 (22.64)	7.297 (52.67)	45.63 (31.12)	3.75 (75.67)
FR 13A	10	75	20	73.13 (2.49)	18.75 (6.25)	71.25 (6)	15.833 (20.83)	68.963 (8.05)	15.5 (22.5)
FR 43B		73.13	19.167	71.25 (2.57)	17.083 (10.87)	70.627 (3.42)	15 (21.74)	68.567 (6.24)	13.75 (28.26)
Susceptible		68.438	16.667	61.88 (9.58)	12.916 (22.5)	53.75 (21.46)	7.713 (53.72)	46.88 (31.5)	4.166 (75)
Tolerant		74.065	19.583	72.19 (2.53)	17.916 (8.51)	70.94 (4.22)	15.416 (21.28)	68.76 (7.16)	14.625 (25.32)
Jagannath		1.8 75	20.837	66.877 (10.83)	15.417 (26.01)	58.753 (21.66)	9.377 (54.99)	52.5 (30)	4.167 (80)
Manika		71.877	18.75	61.25 (14.78)	13.127 (29.99)	50.63 (29.56)	7.297 (61.08)	51.253 (28.690	2.917 (84.44)
FR 13A	15	78.13	24.377	77.503 (0.8)	22.92 (5.98)	76.25 (2.41)	22.297 (8.53)	76.25 (2.41)	20.627 (15.38)
FR 43B		76.25	22.92	75.417 (1.09)	20 (12.74)	75 (1.64)	17.917 (21.83)	75 (1.64)	17.038 (25.47)
Susceptible		73.44	19.793	64.06 (12.77)	14.272 (27.89)	54.691 (25.53)	8.337 (57.88)	51.88 (29.36)	3.452 (82.1)
Tolerant		77.19	23.648	76.46 (0.94)	21.46 (9.25)	75.62 (2.03)	20.107 (14.97)	75.62 (2.03)	18.855 (20.27)

Values in parentheses indicate percentage decrease in total sugar and starch content

in total sugar content (90.40%) was estimated in susceptible varieties. The decline in total sugar content in two tolerant varieties FR13A (45.56%) and FR43B (44.97%) was comparatively much lower than that in two susceptible varieties like Jagannath (91.18%) and Manika (89.48%).In all the three periods of submergence, 7-day old seedlings of FR13A(9.15%) and FR43B(8.10%) had shown very low rate of decrease in starch in comparison to Jagannath (24.61%) and Manika (24.60%)., possibly a stage for genetic differentiation of susceptible and tolerant genotypes. Reduced starch content during submergence was found to be higher in 7-day old seedlings in comparison to 10-day old and 15-day old seedlings; which may be apparently due to low tolerance of these seedlings related to lower starch content as was established by Emes et al.(1988).

Percentage decrease in total sugar content of ten-day-old seedlings of tall varieties treated with seven days of complete submergence was observed to be very low (8.51%) compared to that of two semi-dwarf

Table 2. Effect of submergence on starch and total sugar content (mg /g. dry wt.) of rice seedlings

Sources of variation	Degree of freedom (df)	Mean Square (MS)	
		Starch	Total sugar
Rep.	2	0.791	2.6 37
Var.	3	2101.284**	55 2.428**
Err.a	6	1.435	1. 281
As	2	1686.326**	799.721**
Var.x As	6	36.984**	37.751**
Err.b	16	0.179	0.906
Ds	3	1163.771**	604.503**
Var.x Ds	9	160.109**	39.371**
As x Ds	6	9.907**	2.968**
Var. x As x Ds	18	5.601**	2.75**
Err.c	72	0.437	0.899
Total	143		

**Significant at 0.05 and 0.01 level of probability
Rep.-Replication Var.-Varieties Err.-Error
As-Age of the seedlings Var.x As-Interaction of varieties and age of the seedlings
Ds-Duration of submergence Var.xDs-Interaction of varieties and duration of submergence

varieties, where it was 22.50%. With enhanced duration of submergence to ten and fifteen days, percentage of loss of total sugar was further hastened. The rate of decrease in the total sugar content was found to be 75.00% in susceptible cultivars and 25.32% in tolerant cultivars. As far as starch content was concerned, the difference between the percentages of reduction in starch content was very negligible in FR13A and FR43B, but it was remarkable in Jagannath and Manika. Fifteen days of complete submergence led to the retardation in starch content by 31.50% in Comparison to that of tolerant varieties. When these seedlings were subjected to complete submergence for fifteen days, further decline in total sugar content (90.40%) was estimated in susceptible varieties. The decline in total sugar content in two tolerant varieties FR13A (45.56%) and FR43B(44.97%) was comparatively much lower than that in two susceptible varieties like Jagannath (91.18%) and Manika (89.48%).In all the three periods of submergence, 7-day old seedlings of FR13A(9.15%) and FR43B(8.10%) had shown very low rate of decrease in starch in comparison to Jagannath (24.61%) and Manika (24.60%)., possibly a stage for genetic differentiation of susceptible and tolerant genotypes. Reduced starch content during submergence was found to be higher in 7-day old seedlings in comparison to 10-day old and 15-day old seedlings; which may be apparently due to low tolerance of these seedlings related to lower starch content as was established by Emes et al.(1988).

Percentage decrease in total sugar content of ten-day-old seedlings of tall varieties treated with seven days of complete submergence was observed to be very low (8.51%) compared to that of two semi-dwarf varieties, where it was 22.50%. With enhanced duration of submergence to ten and fifteen days, percentage of loss of total sugar was further hastened. The rate of decrease in the total sugar content was found to be 75.00% in susceptible cultivars and 25.32% in tolerant cultivars. As far as starch content was concerned, the difference between the percentages of reduction in starch content was very negligible in FR13A and FR43B, but it was remarkable in Jagannath and Manika. Fifteen days of complete submergence led to the retardation in starch content by 31.50% in Jagannath (56.30 %) and tolerant cultivar FR 43B (23.29 %). Minimum decrease was observed in FR 13A (17.50 %).The superior tolerance of deepwater rice genotypes to prolonged complete submergence may be due to their greater photosynthetic capacity developed by leaves newly emerged above the floodwater. Vigorous upward leaf elongation during continuous submergence is therefore critical for ensuring shoot emergence from water, leaf area extension above the water surface and a subsequent strong increase in shoot biomass (Sakagami *et al*.2009). Drastic reduction in sugar content was observed in 7-day old (younger) seedlings which might be due to low rate of carbohydrate synthesis under low light intensities and rapid exhaustion of carbohydrate content because of high catabolic activity. It was noticed that with the increase in submergence duration, all the three ages of seedlings had shown a successive reduction in the starch content quantitatively more in susceptible than in tolerant varieties. This also confirms the study of Mohanty and Ong (2003).As suggested by Palada and Vergara(1972), Emes *et al.(*1988), Chaturvedi *et al*.(1996) and Roy *et al*.(2007),the higher starch(carbohydrate) content in plants correlate positively with the submergence tolerance. A low oxygen level selectively induces the synthesis of proteins known as the anaerobic polypeptides, most of which are enzymes involved in sugar metabolism, glycolysis and fermentation pathways (Perata *et al*. 2007, Sarkar *et al*. 2006, Agarwal and Grover,2006).Another possible reason behind the percentage decrease in starch content in rice seedlings during submergence might be due to the low light intensity and lower chlorophyll content causing lower photosynthesis leading to lower starch content, which confirms the result of Janardan *et al*. (1980), and Singh(1994).

It has become apparently clear that total sugar and starch content is a key factor in deciding a cultivar to be susceptible or tolerant to submergence in addition to chlorophyll content. The initial higher carbohydrate content and slow rate of depletion of total sugar and starch content in the submerged seedlings may be considered as one of the adaptive traits of submergence tolerance in both the tolerant varieties. This is in consistence with the results of Ghosh and Chatterjee (1979),Emes *et al*.(1988) and Adak *et al (1998).*

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