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Saccharide content and growth parameters in relation with flooding tolerance in rice

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Abstract

Pre-submergence reserve saccharides was found adequate to substantiate the survival of tolerant cultivar under flooding. Survival percentage declined in both tolerant and susceptible cultivars with less saccharide content. However, it was more apparent in susceptible cultivar. Plant height, fresh and dry mass of shoots, leaf mass/area ratio and starch content per plant before flooding showed significant positive association with submergence tolerance. Hence, the improved seedling vigour could be used to increase submergence tolerance.

Additional key words: chlorophyll, *Oryza sativa*, starch, soluble sugars.

Introduction

Complete submergence of lowland rice crops occurs during flash flood in wide areas of Southeast Asia when plants may be completely under water for up to 1 - 2 weeks, resulting in an increased mortality of plants and low grain yield. Rice cultivars differ in their tolerance to flooding. At the early seedling stage under complete submergence some cultivars survive better due to elongation of the leaf sheath and leaf blade. As photosynthesis decreases due to complete submergence (Setter *et al.* 1989), it is assumed that the pre-submergence stored saccharides may play an important role in supplying the required energy and as osmoticum to sustain rapid elongation (Raskin and Kende 1984, Smith *et al.* 1987, Sarkar *et al.* 1996). Still, most of the reports to the relation between saccharide status and submergence tolerance are mainly with floating rice and studies under suspension culture comparing rice with other crops or comparison among different cultivars differ in saccharide content and submergence tolerance (Setter *et al.* 1987, Mohanty *et al.* 1993, Gomosta and Vergara 1996, Sarkar *et al.* 1996).

Therefore, this investigation reports variation in saccharide status within the same cultivar and its ability to survive under submergence. In addition, the association between flooding tolerance and growth parameters was examined.

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Materials and methods

Cultivars of rice (*Oryza sativa* L.) were selected per their ability to tolerate flooding: Panidhan and Tulasi as susceptible and Hatipanjari as elongating tolerant type. Three different experiments were conducted during the wet season of 1993 and 1994.

In the first experiment plants of cv. Tulasi were raised by direct sowing in plastic pots. Ten days after germination thinning was done and two seedlings per pot were maintained. After 17 d to induce variation in pre-submergence saccharide level, 50 % of seedlings were predisposed to complete darkness for 8 d under normal ambient temperature and humidity. Twenty five-d-old seedlings were submerged for 2 - 10 d under 75 cm of water. Survival count was taken visually immediately after submergence.

In the second experiment cv. Hatipanjari was used. Growth conditions were similar, however, to create a difference in pre-submergence saccharide status, seedlings were predisposed to complete darkness for 0, 2, 4, 6, and 8 d. The seedlings were submerged continuously for 10 d.

The third experiment was conducted with cvs. Panidhan and Tulasi. Seedlings of these two cultivars were raised in beds by sowing lesser seed rate (30 g m^{-2}) than usual practices (50 g m^{-2}) in order to get more number of robust seedlings (taller and thicker culm). Two such seed beds were raised: one without N application and another with N application when the seedlings were 15-d-old corresponding 32.6 kg urea ha^{-1}. 25-d-old seedlings were uprooted and sorted out into three categories depending on the fresh mass of the seedlings. A bunch of 300 seedlings was made taking into account of the best type of seedlings available for, and designated as Grade 1 type. For each cultivar with each N treatment separate bunches of Grade 1 type of seedlings were made. The mass of the other bunches was approximately 2/3 and 1/3 of grade 1 type of seedlings and were marked by Grade 2 and 3, respectively. The seedlings of different grades were planted by two seedlings/hill with a spacing of 20 × 10 cm in a improvised field tank with four replications with 125 hills/replication. After 15 d of planting submergence treatment was provided likely as described in the first experiment.

Observations were recorded at the time of transplanting and just before submergence on seedling height, comprising leaf sheath and leaf blade of the most elongated leaf (2^{nd} from the top), as well as leaf mass/area ratio (Yoshida *et al.* 1976), nitrogen and chlorophyll content of the same leaf. For chlorophyll estimation, 0.1 g finely chopped fresh leaves were put in a 25 cm^3 capped measuring tube containing 80 % acetone, and kept inside a refrigerator (4 °C) for 28 h. The chlorophyll was measured spectrophotometrically following Arnon (1949).

The soluble sugar and starch contents and nitrogen percentage was estimated following the methods of Somogyi (1945), Yoshida *et al.* (1976) and micro-Kjeldahl, respectively, from the stem portion comprised mainly of the leaf sheath.

Results and discussion

Dark treatment imposed to the seedlings reduced the non-structural saccharide (soluble sugar and starch) content in both the susceptible (*e.g.* Tulasi) and tolerant (*e.g.* Hatipanjari) cultivars (Table 1 and 3). The plant height, leaf mass/area ratio (LM/LA), seedling dry mass and chlorophyll content was less in dark-treated seedlings as compared to the control plants. Hence, seedlings became weak due to the dark treatment.

Table 1. Plant height, leaf mass/area ratio (LM/LA), total dry mass, and chlorophyll, soluble sugar and starch contents in rice seedlings cv. Tulasi after 8-d dark treatment in comparison to control; Mean of three replications ± S.E.

Treatment	Height [cm]	LM/LA [mg cm^{-2}]	Dry mass [mg seedling^{-1}]	Chlorophyll [mg g^{-1}(f.m.)]	Soluble sugars [%]	Starch [%]
control	35 ± 0.9	3.52 ± 0.08	191 ± 5.7	2.79 ± 0.02	7.6 ± 0.09	17.5 ± 0.6
8-d dark	23 ± 0.6	2.07 ± 0.07	92 ± 0.9	1.57 ± 0.01	2.7 ± 0.06	5.9 ± 0.2

Complete submergence with longer duration decreased the percentage of survival in both the treated and normal grown seedlings of cv.Tulasi (Table 2) and after 6 d of submergence survival percentage was 0 % and 78 %, respectively. It was possibly that accumulation of non-structural saccharide (NSS) content was not sufficient in treated seedings to sustain greater longevity.

Table 2. Survival percentage of rice seedlings (cv. Tulasi) after submergence.

Time of submergence [d]	Control	Dark-treated
2	100	97
4	100	38
6	78	0
8	47	0
10	8	0

The tolerant cv. Hatipanjari at NSS level of 15.4 % could show 80 % of survival after 10 d of submergence, whereas survival percentage was only 8 % for the same period in cv. Tulasi with higher NSS content (25.1 %). This consequence is not in conflict with the contention that higher saccharide status increases the possibility of greater survival under submergence. It was possibly that under submergence saccharide utilization was comparatively better in tolerant cultivar, because the acetaldehyde, an intermediary of saccharide metabolism, formed by pyruvate decarboxylase during anaerobic respiration (Cobb and Kennedy 1987, Andreev and Vartapetian 1992), is accumulated more in susceptible cultivars as compared to tolerant cultivars (Kundu *et al.* 1993). We have shown that tolerant cultivars consume more saccharides during the initial 6 d of submergence and could utilize that for better elongation (Sarkar *et al.* 1996).

Table 3. Plant height, leaf mass/area ratio (LM/LA), total dry mass, chlorophyll, soluble sugar, and starch contents and survival percentage of rice seedlings cv. Hatipanjari after 0-, 2-, 4-, 6- and 8-d dark treatment. Mean of three replications ± S.E.

Dark [d]	Height [cm]	LM/LA [mg cm^{-2}]	Dry mass [mg seedling^{-1}]	Chlorophyll [mg g^{-1}(f.m.)]	Sugars [%]	Starch [%]	Survival [%]
0	44 ± 0.3	2.71 ± 0.07	340 ± 8.1	4.08 ± 0.11	8.6 ± 0.1	27.5 ± 0.8	100
2	46 ± 0.2	2.42 ± 0.03	280 ± 8.3	3.11 ± 0.17	7.9 ± 0.3	18.5 ± 0.7	100
4	47 ± 0.5	1.87 ± 0.02	214 ± 5.3	2.58 ± 0.11	5.4 ± 0.2	10.0 ± 0.4	80
6	45 ± 0.6	1.75 ± 0.03	135 ± 4.5	1.80 ± 0.11	3.0 ± 0.1	5.8 ± 0.3	12
8	39 ± 0.5	1.62 ± 0.01	107 ± 3.8	1.59 ± 0.13	3.0 ± 0.1	3.9 ± 0.3	0

In both cvs. Panidhan and Tulasi, wherever the nitrogen application was there, the plant height and dry mass of the seedlings was greater (Table 4). Immediately after

Table 4. Characteristics of seedlings cvs. Panidhan and Tulasi at the time of transplanting under two nitrogen treatments (N_0 and N_1 corresponding to 0 and 32.6 kg urea ha^{-1}); 3 grades of seedling as sorted out on the basis of fresh mass.

Cultivar	Treatment	Grade	Fresh mass [g seedling^{-1}]	Height [cm]	LM/LA [mg cm^{-2}]	Dry mass [g seedling^{-1}]	Starch [%]
Panidhan	N_0	1	0.779	38	3.20	0.113	10.7
		2	0.479	35	2.02	0.064	10.6
		3	0.283	32	1.95	0.048	13.2
	N_1	1	1.517	52	2.84	0.142	8.4
		2	0.850	51	2.10	0.096	8.2
		3	0.450	44	1.98	0.082	10.3
Tulasi	N_0	1	0.771	30	2.44	0.091	11.0
		2	0.500	26	2.35	0.043	11.2
		3	0.283	23	2.01	0.029	10.9
	N_1	1	1.046	43	2.99	0.162	9.3
		2	0.755	36	2.54	0.089	7.5
		3	0.342	30	2.07	0.046	6.6
F value	treatment		870.74**	45.83**	13.41**	28.38**	30.95**
	grade		3166.13**	39.43**	56.23**	103.63**	10.24**
	nitrogen		1921.26**	223.37**	NS	73.74**	212.41**
	cultivar		243.72**	184.46**	NS	10.60**	24.76**

** - significant at 1 % level, NS - not significant.

transplanting, starch percentage of the stem was found to be lower in those seedlings that received N. After 15 d of transplanting, starch percentage was little higher in those types of seedlings. The fact that there is a negative relationship between nitrogen and starch content (on dry mass basis) in the plants (Wada 1995) also confirmed that a low nitrogen level in a plant contributed to an accumulation of starch in the shoot (Table 4). After transplanting approximately 5 - 7 d is required for establishment of rice plants. As there was no application of N at the time of transplanting, the seedlings received certain amount of N at seed bed showed greater efficiency. Hence, after 15 d of transplanting starch percentage increased in the plants that received N at seed bed. (Table 5). The percentage of nitrogen, in general,

Table 5. Characteristics of seedlings cvs. Panidhan and Tulasi 15 d after transplanting under two nitrogen treatments (N_0 and N_1 corresponding to 0 and 32.6 kg urea ha^{-1}); 3 grades of seedling as sorted out on the basis of fresh mass.

Cultivar	Treatment	Grade	Height [cm]	LM/LA [mg cm^{-2}]	Dry mass [g $seedling^{-1}$]	Leaf N [%]	Stem N [%]	Starch [%]	Survival [%]
Panidhan	N_0	1	47	4.37	0.273	2.42	0.90	15.4	34
		2	74	3.95	0.149	2.32	1.01	17.5	18
		3	36	3.49	0.075	2.36	1.02	12.2	7
	N_1	1	54	4.49	0.521	2.38	1.02	17.2	80
		2	47	4.12	0.274	2.42	1.09	16.9	53
		3	42	3.83	0.150	2.40	1.14	16.5	29
Tulasi	N_0	1	35	4.32	0.195	2.41	1.08	15.3	50
		2	34	3.99	0.149	2.35	1.01	14.3	42
		3	29	3.62	0.073	2.40	1.04	11.0	25
	N_1	1	40	3.98	0.383	2.62	1.14	14.3	68
		2	35	3.69	0.234	2.60	1.10	13.7	52
		3	33	3.59	0.103	2.65	1.11	14.9	28
F value	treatment		31.86**	7.63**	40.02**	8.60**	7.50**	9.43**	31.37**
	grade		52.95**	31.03**	134.23**	NS	NS	11.74**	90.22**
	nitrogen		42.49**	NS	106.58**	37.14**	38.48**	12.05**	105.96**
	cultivar		189.19**	6.69**	17.40**	30.17**	11.88**	29.47**	11.17**

** - significant at 1 % level, NS - not significant.

was more in the seedlings that received nitrogen earlier. LM/LA was more in Grade 1 type of seedlings, irrespective of cultivar and nitrogen application. LM/LA has direct relationship with N content and photosynthesis (Dingkuhn *et al.* 1992). Nitrogen content greater than 2 % (on dry mass basis) is considered optimum for rice (Schnier *et al.* 1990). Hence, the influence of N on LM/LA was insignificant (Sarkar 1995), because in all types of seedlings irrespective of cultivar nitrogen percentage of leaf was always higher than 2 % (Table 5). The seeds in a lot do not germinate together. Then, there was possibility that the Grade 1 type of seedlings could develop from the seeds that germinated early and could grow fast and therefore, had higher LM/LA as compared to other.

Plant height just before submergence did not show any significant association with survival percentage. The percentage of nitrogen in leaf and stem also did not show any significant association. Other growth characteristics as fresh mass, plant height, LM/LA and shoot dry mass at the time of transplanting and LM/LA and shoot dry mass just before submergence, however showed significant positive association. The correlation coefficients (r) were 0.901**, 0.588*, 0.647*, 0.788** and 0.642* and 0.891**, respectively. Hence, for selecting the seedlings that can tolerate submergence, greater emphasis may be given on the mass of the shoots. Though starch percentage did not increase in robust seedlings, yet due to the accumulation of more total saccharide (on single plant basis), good type of seedlings could withstand complete submergence better as compared to other. The correlation coefficient between survival percentage and total starch content showed a highly significant positive correlation (r = 0.839**).

Finally, it must be admitted that it is somewhat difficult to increase the saccharide percentage (on dry mass basis) than total saccharide content (on plant basis) and submergence tolerance might be improved in seedlings with more vigour.

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