Genetics and Plant Physiology – 2011, Volume 1 (3–4), pp. 155–162

Available online at http://www.ifrg-bg.com

NON-STRUCTURAL CARBOHYDRATE METABOLISM ASSOCIATED WITH SUBMERGENCE TOLERANCE IN RICE

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Received: *04 September 2011* Accepted: *12 March 2012*

Summary. The role of non-structural carbohydrate (NSC) status and metabolism in three *Indica* rice (*Oryza sativa* L.) cultivars, FR 13A, IR 42 and Sabita, with varying degrees of tolerance to submergence was studied 8 days after submergence and subsequent re-aeration for 24 h. Difference in shoot non-structural carbohydrate (NSC) content (soluble sugar and starch) were observed among the cultivars. The tolerant cultivar FR 13A maintained significantly higher contents of NSC compared to the susceptible IR 42 and elongating Sabita cultivars before and after submergence. ADP glucose pyrophosphorylase (AGPPase) activity was found to be higher in FR 13A followed by Sabita and IR 42 under control conditions, during submergence and 24 h after re-aeration. Total and α-amylase activities were significantly increased under submergence, especially in FR 13A, whereas in IR 42 and Sabita the activities decreased during the submergence and re-aeration periods as compared to the control plants. Sucrose synthase (SSyn), sucrose phosphate synthase (SPSyn) and invertase activities were not significantly different among the varieties studied. It is concluded that the submergence tolerance of the tolerant cultivar FR 13A was due to the relatively higher accumulation of non-structural carbohydrates compared to the susceptible cultivars together with increased synthesis and degradation of carbohydrates with the participation of the enzymes AGPPase and amylase, respectively.

***Keywords*:** Non-structural carbohydrate; rice; submergence tolerance.

INTRODUCTION

One forth of the global rice crops is growing in rain-fed lowland plots which are subjected to seasonal flooding. The submergence is due to water-logging conditions *inter alia* various biotic and abiotic constraints resulting in partial to near complete crop damage and poor grain yield (Sarkar et al., 2006). Submergence

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tolerance is a metabolic adaptation in response to anaerobiosis that enables cells to maintain their integrity, and plants to survive without major damage. Plants that survive or succumb to transient submergence differ in the timing and duration of carbohydrate consumption and anaerobic metabolism (Fukao and Bailey-Serres, 2004). The better understanding of the physiological and biochemical mechanisms of submergence tolerance may facilitate future breeding programs. The pre-submergence stored non-structural carbohydrates play an important role in supplying the required energy for survival during and after withdrawal of submergence (Das et al., 2005; Panda et al., 2008).

The tolerant rice variety has the ability to store more non-structural carbohydrates before submergence and can withstand submergence efficiently. However, the mechanism underlying this phenomenon remains still unclear. Identification of an enzyme or a set of enzymes which could help in accumulation of non-structural carbohydrates would fill up the gaps in scientific knowledge and could help in the identification of gene/s responsible for higher accumulation of non-structural carbohydrates in lowland tolerant cultivars. So far, to withstand submergence emphasis was given to the proper utilization of carbohydrates. The present investigation was undertaken to study the role of non-structural carbohydrates in relation to key enzymes of carbohydrate metabolism such as ADP-glucose-pyrophosphorylase, sucrose synthase and sucrose phosphate synthase along with invertase and amylase, which could provide evidences for unraveling the submergence tolerance mechanism behind.

MATERIALS AND METHODS

Plant materials

Three *Indica* rice cultivars, FR 13A, IR 42 and Sabita having differential response to flooding were used in the present study. FR 13A, which is known to be tolerant to complete submergence and IR 42, which is susceptible have been used in many physiological studies (Panda et al., 2006). Sabita is a local cultivar that elongates fast under complete submergence and survives if leaf tips remain above the water surface (Sarkar et al., 2001).

Growth conditions

The three cultivars were sown directly in pots containing 2 kg of farm soil and farmyard manure in a 3:1 ratio (Panda et al., 2006). Each pot was supplied with 88 mg urea, 190 mg single super phosphate (P_2O_5) and 50 mg murate of potash (K_2O). Ten days after germination, the seedlings were thinned and five plants per pot were maintained. The pots containing twenty one-days-old seedlings were completely submerged in concrete tanks filled with water to a height of 110 cm. The plants were subjected to three treatments: (1) complete submergence for 8 d; (2) complete submergence for 8 d followed by aeration for 24 h; (3) control growth conditions (without submergence treatment). The experiments were carried out in three replications and were statistically analyzed.

The characteristics of the floodwater in terms of light transmission (%) were measured at 12:00 h (LI-COR, Lincoln, USA), and water temperature and oxygen concentration were determined at 06:00 and 17:00 h (Syland, Heppenheim, Germany) every alternate day. Light intensity at 60 cm water depth or in the vicinity of canopy level ranged from 215 to 319 μmol $m^{-2}s^{-1}$ whereas above the water surface it was 1743 to 1812 μmol $m^{-2}s^{-1}$. The oxygen concentration at the same water depth was 2.5 - 3.1 mg L^{-1} at 06:00 h and 4.6 - 5.8 mg L^{-1} at 17:00 h. The temperatures varied between 26.2°C and 30.3°C throughout the experiment.

Measurement of non-structural carbohydrates

The content of non-structural carbohydrates (soluble sugar and starch) in the shoot was estimated following the procedure of Yoshida et al. (1976) in three replications. Briefly, shoot samples were oven-dried, ground to a fine powder and homogenized with 80% ethanol (v/v). The extract was then used for soluble sugar analysis after the addition of anthrone reagent, followed by measurement of the absorbance at 630 nm using a spectrophotometer (model SL 164 double beam, ELICO, Hyderabad, India). The residue remaining after soluble sugars extraction was dried, extracted using perchloric acid and analyzed for starch (as glucose equivalent) using the anthrone reagent.

Assay of carbohydrate metabolic enzymes

ADP glucose pyrophosphorylase (AGPPase, EC 2.7.7.27) activity was measured according to the method of Rocher et al. (1989). The activity was calculated based on the time dependent increase at A_{340} and expressed as μmol NAD^+ reduced min^{-1} mg^{-1} protein by using the extinction coefficient of 6.22 μmol cm^{-1} s^{-1}. Sucrose phosphate synthase (SPSyn, EC 2.4.1.14) and sucrose synthase (SSyn, EC 2.4.1.13) activities were determined according to Huber (1983). Results were obtained from a standard curve of different sucrose concentrations at 520 nm. The enzyme activity was expressed in mM sucrose synthesized h^{-1} mg^{-1} protein. The activity of invertase (EC 3.2.1.26) was assayed according to Sadasivum and Manicum (1997) using a standard curve of different glucose concentrations and the enzyme activity was expressed as mg glucose released h^{-1} mg^{-1} protein. Total and α-amylase (EC. 3.2.1.1) activity was measured according to Guglielminetti et al. (1995) by measuring the rate of generation of reducing sugars from soluble starch and the enzyme activity was expressed as mg maltose liberated 30 min^{-1} mg^{-1} protein.

Statistical Analysis

Differences between the enzyme activities were compared by ANOVA using IRRISTAT (International Rice Research Institute, Philippines) software's least significant difference (LSD* $P<0.05$) to determine significant differences between means.

RESULTS AND DISCUSSION

Submergence imposes complex abiotic stress (Sarkar et al., 2006) and the extent of injury caused by complete submergence is largely dependent on floodwater conditions, particularly its temperature, turbidity and the extent of light penetration (Das et al., 2009). The extent of visible injury caused by flooding was used as an indicator of plant sensitivity to submergence. In the present experiment, the three cultivars gave distinctly different responses to submergence in terms of survival. The tolerant cv. FR 13A showed

100 % survival after 8 d of submergence, whereas it was less than 15 % in IR 42 and Sabita (data not shown).

Non-structural carbohydrate (sugar and starch) content in the shoot before submergence was significantly different among the cultivars (Fig. 1). The tolerant cv. FR 13A had significantly higher non-structural carbohydrate content (sugar and starch) compared to the susceptible cv. IR 42 and cv. Sabita (Fig. 1). Similar results were also reported by Sarkar (1998). A critical evaluation of submergence tolerant and non-tolerant rice cultivars revealed that seedlings of the tolerant species had 30-50% more non-structural carbohydrates compared to the susceptible cultivars. Our results showed that after 8 days of complete submergence, the reduction in sugar content was by 25% in cv. FR 13A and by 45% in cvrs. IR 42 and Sabita compared to the non-submerged control plants whereas the reduction of starch content was by 32, 59 and 64% in cvrs. FR 13A, IR 42 and Sabita, respectively. Fukao et al. (2006) have demonstrated that genotypes lacking submergence-induced Sub 1A rapidly consume leaf starch and soluble sugars to maintain elongation growth during submergence. By contrast, genotypes with the Sub 1 haplotype introgressed from FR13A consume carbohydrate energy reserves more slowly during submergence, maintaining growth at a rate similar to plants in air. Non-structural carbohydrate contents before

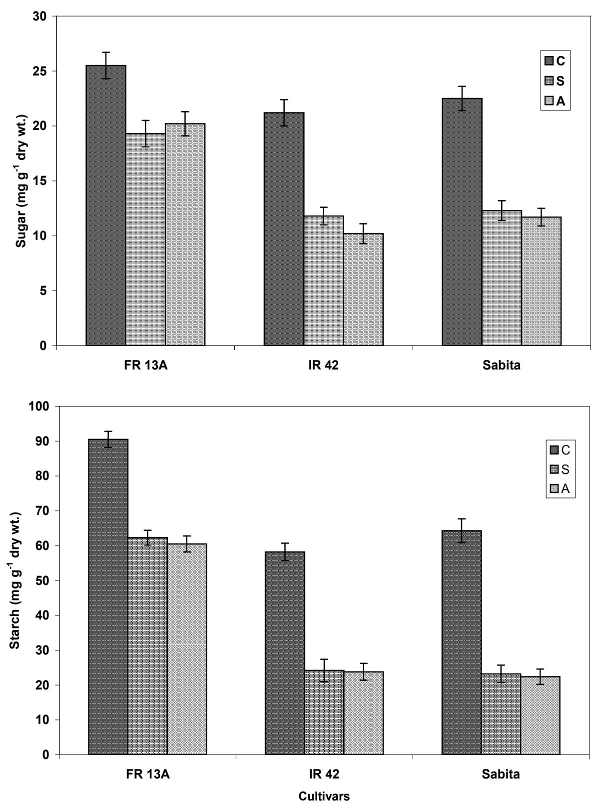

Fig.1. Changes of non-structural carbohydrate content in shoot tissues of different rice cultivars. Data are means of three replications. Vertical bars represent the standard deviation. C – control; S – submergence for 8 d; A – submergence for 8 d followed by 24 h re-aeration.

and after submergence are important for providing energy needed for maintenance metabolism during submergence and for regeneration and recovery of seedlings after submergence (Sarkar, 1998; Das et al., 2005)

Further, we studied the behavior of different enzymes related to carbohydrate metabolism during submergence and subsequent re-aeration. Under control conditions the AGPPase activity was found to be higher in cv. FR 13A followed by cvrs. Sabita and IR 42. Submergence decreased AGPPase activity in all genotypes at a lesser degree in cv. FR 13A compared to cvrs. Sabita and IR 42. After 24 h of re-aeration the AGPPase activity increased only in cv. FR 13A, whereas in cvrs. IR 42 and Sabita its activity decreased further (Fig. 2). AGPPase is a key enzyme in starch synthesis (Zeeman et al., 2007; Rosti et al., 2007) and the

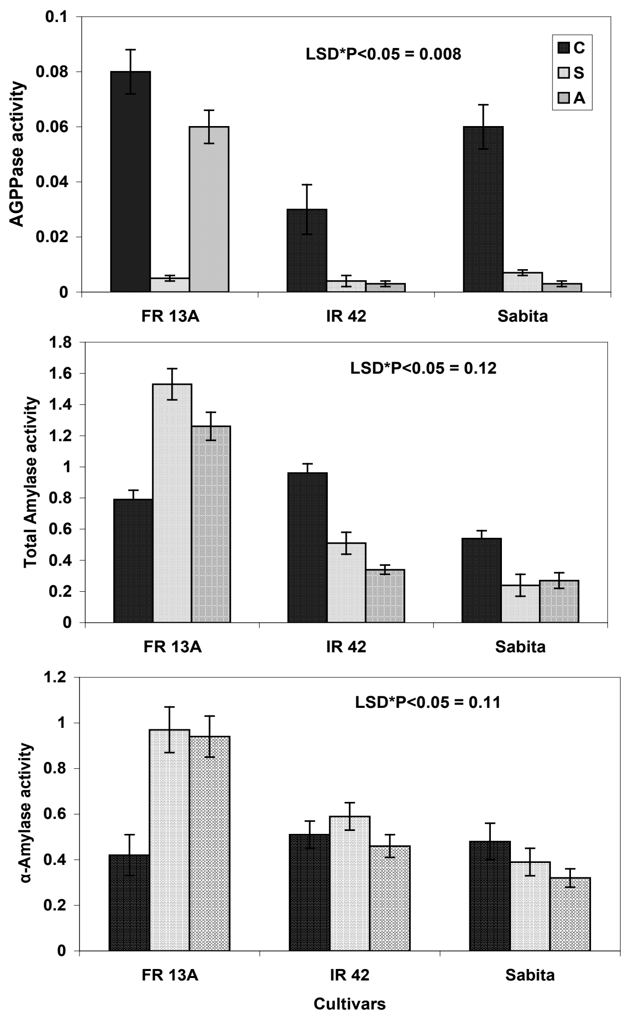

Fig. 2. Changes in the activities of AGPPase, [μmol NAD^+ reduced min^{-1} mg^{-1} protein], total and α-amylase [mg maltose released 30 min^{-1} mg^{-1} protein] in rice seedlings during submergence and subsequent re-aeration. Data are means of three replications. Vertical bars represent the standard deviation. C – control; S – submergence for 8 d; A – submergence for 8 d followed by 24 h re-aeration.

tolerant cultivar might be synthesizing more starch in the leaf than the susceptible and avoiding type of cultivar. Under control conditions the activities of sucrose synthase and sucrose phosphate synthase were higher in cv. IR 42 followed by cvrs. FR 13A and Sabita. Under submergence a stronger decrease of sucrose synthase activity was observed in the tolerant cv. FR 13A, whereas after 24 h of re-aeration the enzyme activity decreased in all cultivars with greater magnitude in the susceptible cv. IR 42. There was no varietal difference in the activity of SPS during submergence and the subsequent period of re-aeration. Invertase activity increased in cvrs. FR 13A and IR 42 under submergence and 24 h after re-aeration compared to the control plants. The change in the activity of invertase was non-significant in cv. Sabita (Table 1). Photosynthetically fixed carbon is partitioned into sucrose and starch: sucrose is exported to sink organs, whereas starch is stored transiently in the chloroplast. Carbon partitioning between starch and sucrose appears to be genetically controlled (Huber, 1983). Sucrose phosphate synthase (SPS) is a key enzyme in the partitioning of photoassimilates to either sucrose or starch in the leaves (Huber, 1983). Rice accumulates sucrose in response to high photosynthetic rate (Gesch et al., 2002). Sucrose is the transport form of carbohydrate between plant organs. In many cases sucrose is hydrolyzed by invertase yielding glucose and fructose, which enters the glycolytic pathway. Sucrose is also metabolized by sucrose synthase yielding UDP glucose and fructose (Sauter, 2000).

Total and α-amylase activity significantly increased under submergence especially in cv. FR 13A, whereas in cvrs. IR 42 and Sabita the activity was decreased during submergence and the re-aeration period compared to control plants (Fig. 2). The decrease in starch content coincides with a marked increase in amylolytic activities under submergence. Besides, submergence greatly enhances the export of newly fixed carbon from the leaves to the stem that ultimately satisfies

Table 1. Changes in the activities of invertase [mg glucose released h^{-1} mg^{-1} protein], sucrose synthase [mmol sucrose released h^{-1} mg^{-1} protein] and sucrose phosphate synthase [mmol sucrose released h^{-1} mg^{-1} protein] in rice seedlings during submergence and 24 h subsequent re-aeration. C – control; S – submergence for 8 d; A – submergence for 8 d followed by 24 h re-aeration.

Cultivar	Invertase			Sucrose Synthase			Sucrose phosphate synthease		
	C	S	A	C	S	A	C	S	A
FR13A	0.75	1.01	1.02	0.96	0.48	0.35	0.89	0.98	0.53
IR 42	0.72	1.08	1.23	1.21	1.06	0.42	1.06	0.81	0.73
Sabita	0.96	0.95	0.94	0.72	0.81	0.51	0.58	0.88	0.64
LSD*$P<0.05$		0.15			0.18			0.20	

the increase demand for energy (Raskin et al., 1984). Starch breakdown under submergence is the result of the activity of hydrolytic enzymes and through the concerted action of α-amylase, β-amylase, debranching enzyme and α-glucosidase (Mohanty et al., 1993; Guglielminetti et al., 1995).

From the present study, it can be concluded that the submergence tolerance conferred by the tolerant cultivar FR 13A was due to the relatively higher accumulation of non-structural carbohydrates compared to the susceptible cultivars along with increased synthesis and degradation of carbohydrates with the participation of the enzymes AGPPase and amylase, respectively.

ACKNOWLEDGEMENT

Authors are grateful to Director, Central Rice Research Institute, Cuttack for providing the necessary facilities for this work.

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