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Physiological dissection of submergence tolerance in rice under island ecosystem

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ABSTRACT

Rice cultivation in the islands encounters submergence at various growth stages leading to substantial yield and quality loss. Continuous and heavy rainfall during a short-span leads to water logging and continuous submergence even up to five days. Developing submergence/flooding tolerant versions of adapted elite rice genotypes helps to reduce the yield loss in these areas. The present study was undertaken to evaluate the physiological response of rice genotypes adapted to island conditions under submergence stress. The outcome of the study will help to identify promising donor for breeding submergence stress tolerance cultivars with the background of popular rice varieties of A&N islands. The tolerant genotypes like Black Burma, IR64 Sub1, revealed that they posses many of the adaptive traits like slower elongation during submergence, chlorophyll retention, higher stem reserves post submergence, higher survival per centage required for the flood-prone island ecosystem.

Key words: *Submergence stress, SUB 1 gene, Island ecosystem, Rice, Tolerance, Adaptive traits*

Introduction

Paddy is a principal and staple food crop of the Andaman and Nicobar Islands and occupies an area of 5340.25 ha (India Stat, 2019). Among the districts of A & N islands, the North and Middle Andaman contribute a major share under rice cultivation followed by South Andaman and Nicobar. The current annual rice productivity is about 2.4 tons while the estimated demand is about 6 tons. Several abiotic and biotic stress factors further impose threat to meet this additional yield gap of 3.6 tons. One of the major stresses in the islands is the flooding/water logging, especially in the low lying and coastal areas. It is quite alarming to note that A & N, post-tsunami about 4206.64 ha of the total geographical is under permanent submergence. This represents 9 per cent of the total agriculture land of the islands and the climate change projections imply a further expansion of the same.

In A & N islands, Rice is cultivated as a monocrop, only during kharif season (covering 17% of the total available land). Rice cultivation in these islands, especially in coastal regions is constantly exposed to soil salinity coupled with acid sulphate soils and water logging (Gautam *et al.*, 2013; Velmurugan *et al.*, 2014). Rice cultivation in the islands although conducive with the high monsoon (May-October; 3100 mm), encounters water logging/submergence at various growth stages leading to substantial yield and quality loss. Continuous, heavy rainfall during a short span led to water logging and even complete submergence even up to ten days in these areas. In certain areas of the A & N is-

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lands, where rice is cultivated along the coast, high tides cause water stagnation/flooding coupled with salinity for more than fifteen days. Above causes of waterlogging infer that submergence under the island ecosystem differs from that of the other ecosystems. Therefore, rainfed lowlands constitute highly fragile ecosystem often prone to flash floods (submergence) along with high tides in the A & N islands. Overcoming the effects of water logging on rice production is essential for meeting the additional demand gap in the future. Although rice crop is predominantly grown under stagnant water conditions, prolonged periods of flooding severely affect the crop growth and yield (Voesenek and Bailey-Serres, 2013) because of poor seedling establishment, high seedling mortality, poor recovery (Ismail *et al.,* 2008; Singh *et al.,* 2013).

In the rice growing belts of island, most of the time the seedlings face a complete submergence leading to poor seedling establishment. The other crop stages (vegetative, reproductive and harvest) are posed with flash floods where water level reaches up to 50 cm causing substantial yield loss. Although information on floodwater characteristics is available, the surface water depth in paddy changes continuously during the crop growth period. The initial step towards this objective is to understand and draw inferences on the major physiological processes and biochemical basis conferring submergence tolerance in rice genotypes. Further, this would aid in identifying donors or improve the existing elite CIARI varieties for flooding/submergence tolerance to the island ecosystem through Market Assisted Breeding as it is necessary that high-yielding cultivars have tolerance to complete submergence like the submergence tolerance gene SUBMERGENCE1 (SUB1) introgressed into background of popular varieties like IR64 and Swarna (Ismail *et al.,* 2013; Rahman *et al.,* 2018, Sarkar and Bhattacharjee, 2011). But studies pertaining to water logging and submergence in rice under the island ecosystem are much limited. The current status depicts those studies pertaining to water logging tolerance among the existing traditional rice (khusbuyya, Black Burma, Red Burma, White Burma, Mushley, Nayawin, Jaya)/ improved CARI varieties of the islands is limited. Thus, the varieties need to be further improved for water logging and submergence conditions in the Islands. Hence, the present investigation was planned to understand the physiological response of rice cultivars to submergence stress that are being cultivated in the islands.

Materials and Methods

Seed materials

The genotypes taken for the study included released cultivars of CARI namely CARI 5, CARI 6, CARI 8; Green Super Rice varieties GSR 4, 7, 11, 18; landraces Black Burma and Khusbuya along with checks IR64 and IR64 *Sub1*. The seeds were tested for proper germination and viability prior to conduct of experiments.

Screening for submergence tolerance

Screening for submergence tolerance was performed in an artificial pond created for submergence screening constructed in the Bloomsdale Farm, CIARI, Garacharma, Port Blair. Seeds were grown in pots of dimension: 38.5 cm diameter (top), 30 cm diameter (bottom) and 35 cm height. Two seedlings were maintained per pot, three replications (five pots) for control and stress were maintained for experimentation. Seedlings were grown up to 21 days under well irrigated condition. After 21 days of growth which coincides with peak tillering stage in most of the genotypes taken, the stress pots were shifted to the artificial submergence pond and submerged completely for 14 days. The control set of pots were maintained outside under well irrigated conditions. After 14 days of submergence period, pots were taken out and recovery was scored after 2 weeks. Survival percentage of plants was scored 14 days after de-submergence.

Percentage of plants survived were calculated based on the submergence tolerance scale developed by IRRI, Philippines, where, Scale 1 indicates more than 80 % of plant survival, 3 indicates 61-80 % of plant survival, 5 indicates 41-40 % of plant survival, 7 indicates 11-40 % of plant survival, 9 indicates less than 10 % of plant survival. After de-submergence, physiological parameters plant height and chlorophyll content (Yoshida *et al.,* 1976) were recorded to investigate the elongation of plants during submergence, total leaf chlorophyll loss after submergence period in the control and stress pots; biochemical analysis on total carbohydrate levels in stem was estimated after submergence. The total carbohydrate content of the stem samples was estimated by following the method of Hedge and Hofreiter, (1962), expressed in mg g^{-1} of dry weight basis.

Results and Discussion

Phenotypic evaluation of rice plants

The data on survival percentage and plant height (cm) of plants after submergence period is presented as below (Table 1). The percent plant population before and after stress showed that most of the island varieties and landraces had a survival percentage in the range of 68-86, including GSR lines and the check IR64 *Sub1* depicted the highest survival percentage of 89. The genotypes CARI 5, GSR 7, Black Burma along with the Check IR64 *Sub1* exhibited greater level of tolerance (Scale 1) while the susceptible genotype IR64 had the lowest tolerance scale of 9. Almost 90 per cent of the plant had died in IR 64 post the submergence stress.

There was a significant increase in the plant height of rice plants after the stress period, ranging from 32.1 to 38.6 cm. The elongation rate of plants ranged from 11.7 to 29.5 per cent and above 20 % in almost many plants immediately after the de-submergence period, which is highly significant. The elongation rate was lowest in IR64 *Sub1* followed by GSR 4, which shows that the plants have adapted a quiescence strategy showing limited underwater elongation which is an important trait for submergence tolerance (Luo *et al.*, 2011, Sarkar and Bhattacharjee 2012; Vergara *et al.*, 2014). All the genotypes that recorded less than 14 per cent elongation rate had tolerance scale of 1 to submergence stress post the recovery period when compared with the sensitive cultivars. The IR 64 plants were wilted and unable to recover to measure the plant height (Table 1). This is reported to be because of the decrease in hydraulic conductivity in leaves of the sensitive cultivar IR64 which causes the subsequent wilting and desiccation of the leaves after submergence period (Setter *et al.*, 2010).

The impact of submergence stress on leaf chlorophyll and total carbohydrate content in the stem is discussed below (Table 2). Data was recorded before submergence and 14 days after de-submergence. Due to the mortality of IR 64 plants, data was not obtained. There was a reduction in the chlorophyll content post submergence stress in almost all the genotypes evaluated except CIARI 6. Total chlorophyll content is an important parameter because, limited light transmission alters the pigment function in impairing photosynthesis as well excess energy of the submerged plants (Panda *et al.*, 2006, Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009; Ella and Ismail, 2006). Also, the non-structural carbohydrate reserve in the stem was analyzed, which showed that during the submergence period most of the genotypes had a greater utilization of carbohydrate reserves which led to reduction in stem reserves post the stress period. Maintenance of stem carbohydrate reserves post-stress is ideally associated to re-generation ability referred to as an adaptive trait for submergence tolerance (Sarkar and Bhattacharjee, 2011; Fukao *et al.*, 2006; Panda *et al.*, 2008).

There was a greater reduction of carbohydrate

Table 1. Impact of submergence stress on survival percentage, plant height & elongation rate

Genotypes	Survival percentage (%)		Tolerance scale of stress plants	Plant height (cm)		Shoot elongation rate (%)
	Before Stress	After Stress		Before Stress	After Stress	
CARI 5	100	86	1	32.4	36.2	11.73
CARI 6	99	74	3	28.1	35.7	27.05
CARI 8	100	68	3	30.5	37.6	23.28
GSR 4	98	71	3	28.8	32.1	11.46
GSR 7	100	82	1	29.8	33.5	12.42
GSR 11	100	79	3	31.3	35.8	14.38
GSR 18	100	80	3	28.4	33.9	19.37
Black Burma	100	86	1	30.2	34.2	13.36
Khusbuya	100	75	3	29.8	38.6	29.53
IR64	98	13	9	30.4	NS	NS
IR64 Sub 1	100	89	1	29.9	31.8	6.35
SE.D.	0.0457			0.1511		
P (0.001)	**			**		

*NS: Not Survived; Before Stress: 0 day before submergence; After stress: 14 days after de-submergence reserves in CIARI 8 while the genotype Black Burma had higher non-structural carbohydrate retention capacity (there was only 3 per cent reduction over control as compared to 39.3 per cent in CIARI 8).

The recovery dynamics of the rice plants to submergence stress is correlated to the expression of several traits, while some of them have been investigated in this study. The stress tolerance ability of plants to prolonged period of flooding conditions requires to have a coordinated trait expression viz., faster regeneration or recovery post stress, which is enabled by stored carbohydrate partitioning from the stem, better photosynthesis activity facilitated by the chlorophyll pigments even if there has been higher elongation of internodes mediated by GA hormone (Winkel *et al.*, 2013; Singh *et al.*, 2001; Fukao and Bailey-Serres 2008; Bailey-Serres *et al.* 2010). All these responses contribute for the survival percentage and stress tolerance either via quiescence or escape mechanisms (Luo *et al.*, 2011; Sarkar and Bhattacharjee, 2011; Vergara *et al.*, 2014). The Fig. 1. below shows that traits like survival percent is significantly correlated to non-structural carbohydrate reserves and plant height (r= 0.9 indicating lesser energy use during the submergence period) as it helps the plant for quicker regeneration following the stress; similarly, elongation rate and plant height are significantly correlated, where plant height is highly influenced by the carbohydrate metabolism (r= 0.82). Hence, the results obtained above also show that rice genotypes which could survive and facilitate rapid regeneration of growth that exhibited lesser elongation rate, higher retention of chlorophyll pigments and higher stored reserves of carbohydrates in the stem post the submergence period. Genotype Black Burma was having the highest tolerance to submergence stress compared to other genotypes while genotype CIARI 8 had stress tolerance score on 3 with poor survival percentage (68), higher elongation rate (27.05), higher chlorophyll degradation and low stem reserves (76.7) post the stress period although it is a high yielding variety under optimum conditions. IR 64 was observed to

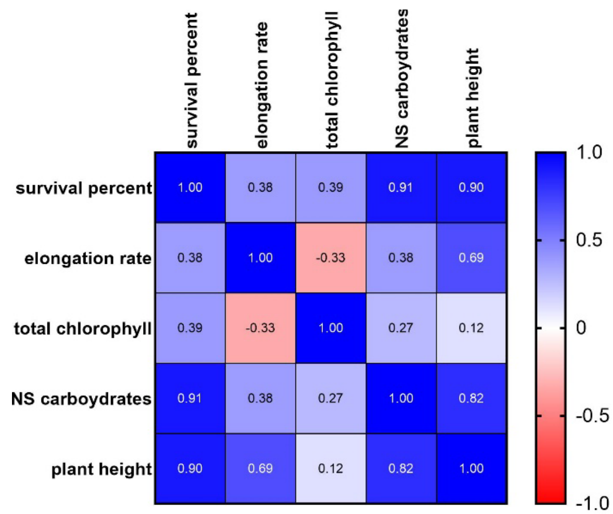

Fig. 1. Correlation matrix of traits evaluated for submergence tolerance of the island cultivars

Table 2. Evaluation of rice plants for total chlorophyll content and stem carbohydrate under submergence

Genotypes	Total chlorophyll content (mg g^{-1} of fresh weight)		Total stem carbohydrate (mg g^{-1} of dry weight)	
	Before Stress	After Stress	Before Stress	After Stress
CARI 5	1.89	1.76	120.3	88.5
CARI 6	1.73	1.74	130.6	90.4
CARI 8	1.98	1.25	126.4	76.7
GSR 4	2.37	2.28	110.7	80.0
GSR 7	2.03	1.88	137.9	104.2
GSR 11	1.84	1.73	128.5	88.3
GSR 18	1.91	1.79	112.3	75.5
Black Burma	1.87	1.70	142.1	136.7
Khusbuya	1.98	1.87	133.7	92.8
IR64	2.51	1.54	118.8	-
IR64 *Sub 1*	2.61	2.40	119.2	97.9
SE.D.	0.0116		0.7712	
F (0.001)	**		**	

*-: could not measure as plants were wilted except few green leaves, BS: 0 day before submergence stress; AS: 14 days after de-submergence

be the most susceptible genotype that could not survive and regenerate, except for few green leaves visible in plants which failed to regenerate even during the recovery period or displayed poorer emergence of new tillers and leaves. The findings from this study were similar to Singh *et al.* (2014) and Winkel *et al.* (2013) where the physiological basis of submergence tolerance is reported in detail. While, the *Sub1* introgressed IR 64 check was highly tolerant highlighting the important role of *Sub 1* gene in imparting submergence tolerance especially via restricted elongation which in turn helps to conserve energy during stress and utilization of energy for regeneration post stress. In fact, the *Sub1* introgressed IR 64 plants were observed to be taller than some of the sensitive cultivars during the early recover stages. Several studies have reported the effectiveness of MAS breeding for SUB 1 gene in popular rice cultivars in conferring tolerance to submergence stress and higher yields (Iftekharuddaula *et al.,* 2011; Singh *et al.,* 2013; Dar et al., 2013; Bailey-Serres *et al.,* 2010; Nagai *et al.,* 2010; Sarkar and Bhattacharjee, 2012).

Conclusion

The present investigation on performance of rice genotypes to submergence stress tolerance to the frequent flooding conditions in the islands has revealed interesting physiological responses. The present study has also depicted the effectiveness of *Sub 1* introgression to high yielding genotypes that exhibit tolerance to submergence stress through quiescence survival strategy and post stress recovery. The variation among the tolerant and susceptible genotypes clearly indicates the necessity to identify more donors that can be exploited for breeding programmes to improve the high yielding cultivars. Results observed in this study provide clear evidence of the interrelation among the potential physiological traits that are useful for genetic variation in the genotypes studied as well in other plants.

Contribution by the authors

SS conceived the study, both SS and SPK designed the experiments. Both the authors executed the experiments where SPK provided the materials and inputs for the study. SS drafted the manuscript. Both the authors checked and approved the final manuscript.

Conflicts of interest

No conflict of interest among the authors

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