

Impact of Variety and Seed Priming on Anaerobic Germination-Tolerance of Rice (*Oryza sativa* L.) Varieties in Sri Lanka

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ABSTRACT: Ability of some rice (*Oryza sativa* L.) genotypes to germinate and grow under submerged conditions where oxygen supply is limited provides an efficient mean for weed control in direct seeded rice. This study was conducted to screen 38 rice varieties to anaerobic germination (AG) tolerance and to identify the influence of hydro-priming on survival and physiological responses of germinated seeds. Seed paddy was sown on saturated soil and soil inundated with 10 cm of stagnant water (submerged) to estimate the survival scores at 21 days after seeding. Six varieties having high survival scores under submerged condition were selected and their seeds were hydro-primed for 24 hrs in the dark at room temperature (25 °C) and were sown in saturated and submerged (10 cm water level) conditions. Soluble sugars, starch and α -amylase of germinating seeds were extracted and a separate set was kept to count the survival score. Varieties including Bg 300, Bg 310 and At 308 had moderate level of tolerance to AG. Hydro-priming improved the survival of rice seeds to about 40%. The synthesis of soluble sugars, starch degradation and α -amylase activity increased by hydro-priming compared to non-primed seeds germinated under submerged condition. Survival rate correlated positively with soluble sugar ($r = 0.74$) and α -amylase ($r = 0.79$) contents and negatively with starch content ($r = -0.51$) at $p < 0.05$. These results emphasized the importance of hydro-priming as a seed treatment to enhance anaerobic germination tolerance in rice.

Keywords: Anaerobic germination, physiological responses, seed hydro-priming, submergence

INTRODUCTION

In Sri Lanka, a significant proportion of rice lands is abandoned or fallowed long term due to flooded conditions and other soil problems associated with poor drainage (Bentota *et al.*, 2010). Transplanting has been practiced to minimize the risk of early season flooding; however, it is costly due to high labor usage and scarcity. Thus, about 95% of the rice grown extent in Sri Lanka at present is direct seeded rice (DSR). Apart from the irregular stand establishment, the most disastrous constraint in DSR across all rice growing agro-ecologies is the invasion of weeds and weedy rice (Marambe 2009; Gunawardena *et al.*, 2013). The yield loss caused by uncontrolled weeds ranges from 30-40% (Herath Banda *et al.*, 1998).

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Impact of water management on weed control in rice is well-recognized and has long been practiced as an effective cultural practice under lowland transplanted rice (Rao *et al.*, 2007). However, in DSR systems, fields are drained off before sowing and standing water is introduced 7-10 days after sowing (DAS), as many of the cultivated rice varieties are extremely sensitive to flooding during germination (Ismail *et al.*, 2012). Rice varieties that can tolerate flooding during germination and seedling development would help to reduce the hazards of early floods and provide an efficient means for weed control in DSR (Ismail *et al.*, 2012).

Among cereal seeds studied to-date, only rice can germinate and elongate coleoptiles under low O₂ (hypoxia) or very low/absence of O₂ (anoxia). The ability of some rice varieties to germinate, grow and survive under oxygen-limiting conditions is known as anaerobic germination tolerance or AG tolerance (Ella and Setter, 1999). They develop different strategies to cope with O₂ limited conditions, including anaerobic respiration to sustain energy supply, initiation and maintenance of carbohydrate catabolism in germinating seeds, maintenance of cellular extensibility of the growing embryo (Ismail *et al.*, 2009) and maintenance of higher activity of α -amylases (Yamaguchi *et al.*, 1994, Hung *et al.*, 1999). Tolerant rice genotypes store relatively more soluble sugars in their endosperm than the sensitive genotypes and have greater ability to break down starch into soluble sugars during germination (Ismail *et al.*, 2012).

Combining genetic tolerance to AG with appropriate management options such as seed pre-treatment (priming, pre-soaking, etc.) and seed bed management improves seedling establishment of rice sown in flooded soils (Ella *et al.*, 2011). Seed priming is a physiological seed enhancement method and involves soaking with water (hydro-priming) or with an organic solvent (osmo-priming) followed by partial dehydration and further drying before radical emergence (Ella *et al.*, 2011). It improves seed vigor and longevity by increasing the activities of α amylase, superoxide dismutase, catalase, scavenge reactive oxygen species and carbohydrate mobilization and decreasing lipid peroxidation activity compared to non-primed seeds, particularly in flood-sensitive rice entries (Ella *et al.*, 2011). Higher and synchronized emergence of primed seeds ensure vigorous crop stand with rapid canopy development giving rice a preliminary advantage over weeds (Anwar *et al.*, 2012).

In Sri Lanka, only one reported attempt has been made to screen and identify the ability of local rice germplasm to AG tolerance. Magneschi *et al.*, (2008) screened 23 Sri Lankan rice entries under simulated anaerobic condition and found that Bg 94-1 had the longest coleoptiles (13.8 ± 4.5 mm) while Bg 250 and Bg 745 had the shortest (1.3 ± 0.5 mm and 2.3 ± 1.6 mm, respectively) after four days of submergence. Therefore, screening of existing rice germplasm, identification of AG tolerant varieties, testing their adaptabilities and validation of management options to enhance the crop establishment is a need to make use of this technology under DSR of Sri Lanka. This study was conducted to screen selected rice varieties for AG tolerance and to identify the effect of hydro-priming on survival, soluble sugars, starch and α -amylase activity of rice seeds germinated under submerged conditions.

MATERIALS AND METHODS

The experiments were conducted at the International Rice Research Institute (IRRI) at Los Banos, Philippines in 2015.

Experiment I: Screening of Rice Varieties to AG Tolerance

The experiment was laid down in a RCBD with 3 replicates at ambient temperatures (25 – 34 °C) under greenhouse conditions. Thirty seven Sri Lankan rice varieties and two check entries (Mazhan Red – AG tolerant, FR 13A – AG susceptible) were used (Table 1). The dormancy of the seed paddy was removed by keeping the seeds at 50 °C for 5 days. Thereafter, the healthy seeds with > 85 % germination were sown dry at about 0.5 cm depth on sieved garden soil filled in plastic seedling trays with grids of 34 columns. Each column had 17 rows with holes at dimensions of 1 cm × 1 cm × 1.5 cm (length × width × depth). A total of 102 seeds of each entry were sown in six columns in each replicate. Immediately after sowing, the trays were dipped in 10 cm below water surface in concrete tanks filled with tap water. The water level was maintained by adding tap water to the tank daily. The mean water temperature during the day time was 32.3 °C with a range of 28.9-35.0 °C during the period while pH was varied from 8.8 to 9.7 by an average of 9.2. A control set up was maintained in an adjacent area under saturated conditions. Survival score *i.e.* % of seedling survived after 21 days (Ismail *et al.*, 2009) was recorded by counting those seedlings that emerge above water surface.

Table 1. The rice varieties used for the screening of AG tolerance

<i>Entry No.</i>	<i>Variety</i>	<i>Entry No.</i>	<i>Variety</i>
1	Bg 11-11	21	Bg 300
2	Bg 379-2	22	Bg 304
3	Bw 451	23	At 308
4	Bw 452	24	Bg 310
5	Bg 454	25	Bg 251
6	Bg 403	26	Suwadal
7	At 405	27	Pokkali
8	Ld 408	28	Goda Heenati
9	Bg 455	29	Kuruluthuda
10	Bg 380	30	Devaraddiri
11	Bg 94-1	31	Kaharamana
12	Bg 352	32	Kalumadaal
13	Bg 366	33	Madaal
14	Bg 357	34	Pachchaperumal
15	Bg 358	35	Kahatawee
16	Bg 359	36	Kalu wee
17	Bg 360	37	Rathu Heenati
18	Bg 369	38	Mazhan Red
19	Bw 351	39	FR 13A
20	Bg 250		

Experiment II: Testing the Effect of Hydro-Priming on Survival, Carbohydrate Mobilization and α -Amylase Activity

Four rice varieties, which showed higher survival scores among all entries *i.e.* Bg 300, Bg 305, Bg 310 and At 308 from the Experiment 1 were further evaluated with Mazhan Red and

FR 13A in Experiment II. The initial moisture contents of seeds were recorded and 50% of the seeds of each variety was hydro-primed for 24 hours (1 g of seeds: 5 mL of tap water) in dark (set up was wrapped with Aluminum foils) at room temperature (25 °C). The set up was aerated to ensure a slow hydration. The water was decanted after the specified duration and seeds were placed on paper towels with frequent changes of wet with dry papers. They were kept two hours at room temperature and dried for seven days under a supply of air current with regular mixing, until the seeds regained its original moisture content recorded before priming. The seeds were then kept in room temperature (25 °C) for three weeks in nylon bags before sowing.

The experiment was laid down as a split plot design with 3 replications in both submerged (10 cm of water depth) and saturated conditions in the greenhouse. Main plots were allocated to seed treatments i.e. primed and non-primed, and sub plots were allocated to varieties. Seeds were sown in seedling trays filled with sieved garden soils. A total of 25 germinating seeds of each variety/treatment were harvested on day 0, 3, 5, 7 and 9 for soluble sugar and starch assays, while 40 seeds were harvested on day 0, 2 and 4 for α -amylase activity assay. All seed samples were frozen in liquid N₂ immediately after harvesting and kept at -70 °C until analysis. A separate set of trays with 51 seeds of each variety/treatment was maintained to record the survival score after 21 days.

Soluble Sugars and Starch Analysis

Soluble sugars and starch contents of germinated seeds were assayed by enzymatic methods described by Ismail *et al.* (2009). A freeze-dried ground seed sample was extracted in 80% ethanol (v/v) and used for soluble sugar analysis with colorimetric determination by anthrone reagent (Fales, 1951). The absorbance reading was taken at 620 nm of wave length. The remaining residues after soluble sugar extraction were dried at 70 °C for 24 hrs and used for starch solubilization using acetate buffer. They were subsequently boiled in water for 3 hours and hydrolyzed by incubating in amyloglucosidase enzyme for 24 hrs at 37 °C. The absorbance was taken at 450 nm using PGO enzyme+0-dianisidine dihydrochloride enzyme as the color reagent.

α -amylase Activity Assay

Alpha-amylase activity was measured in four selected varieties (Bg 300, Bg 310, Mazhan Red and FR 13A) according to Bernfed (1951). Crude extracts of the germinating seeds harvested on day 0, 2 and 4 were obtained using 0.02 M NaH₂PO₄ buffer at pH 6.9 (200 μ g ground sample: 2 mL buffer). Total protein concentration of the crude extract was determined following the method of Bradford (1976). The samples were added Coomassie Brilliant Blue G-250 dye reagent and the absorbance at 595 nm was taken to determine the protein concentration against Bovine serum albumin (BSA). All samples were then normalized into 10 μ g protein/mL by adding the same 0.02 M NaH_xPO₄ buffer and used for the assay. The extract was heated to 70 \pm 0.05 °C for 60 minutes to deactivate β -amylase since β -amylase degrades at > 60 °C while α -amylase is stable at even higher temperatures until 80 °C. Five ml of starch (0.1%) was added to 250 μ L of the heated sample at a timely interval and they were incubated exactly 3 minutes to release maltose. They were added di nitrosalicylic acid color reagent and the absorbance reading at 540 nm was taken to determine the amount of maltose produced per unit time against a maltose standard curve. The α -amylase activity was expressed as 1 μ mole of maltose released per minute per mg protein.

Statistical analysis

Data from each experiment were subjected to analysis of variance (ANOVA) and mean separation was performed using STAR (Statistical Analysis for Agricultural Research; IRRI, 2013) computer package. Correlation coefficients (r) among different attributes were studied using Pearson correlation analysis.

RESULTS AND DISCUSSION

Experiment I: Seedling Survival Among Varieties When Normal (non-primed) Seeds are Used

Approximately 64% of the test varieties had survival score < 2% under submergence compared to the saturated condition indicating that submergence during germination has decreased the survival of rice. However, ten varieties had higher survival scores than the susceptible check under submergence, but less than the tolerant check (Table 2). Mazhan Red, the tolerant check, recorded the highest survival score (53.2%). Among the tested varieties, Bg 300 had the highest survival score under flooded condition (23.5 %) followed by Bg 310 and At 308. Green shoots of all varieties under flooded condition appeared 2-3 days later than those under saturated condition. Mazhan Red and Bg 300 started to emerge within 5th and 6th days after flooding (DAF). Greening of shoots of Bg 310 and At 308 were observed at 8 DAF. Mazhan Red and Bg 300 emerged above the water (10 cm) at the 10 and 12 DAF, respectively, while the shoot emergence of other varieties was delayed up to 13-15 DAF.

Table 2. Survival (%) of rice varieties germinated under submerged and saturated conditions

Variety	Survival score	
	submerged	saturated
Mazhan Red (Tolerant check)	53.2 ^a	82.6 ^{ab}
Bg 300	23.5 ^b	80.6 ^{ab}
Bg 310	17.6 ^{bc}	80.8 ^{ab}
At 308	16.0 ^{bc}	78.8 ^{ab}
Bg 305	11.8 ^{cd}	79.0 ^{ab}
Kahata wee	11.1 ^{cde}	70.6 ^c
Bg 94-1	7.2 ^{def}	77.4 ^{ab}
Bg 360	6.9 ^{def}	77.6 ^{ab}
Bg 455	6.9 ^{def}	83.3 ^{ab}
Bg 251	5.6 ^{def}	81.4 ^{ab}
Goda Heenati	4.6 ^{def}	74.7 ^{bc}
FR 13 A (susceptible check)	3.9 ^{def}	82.6 ^{ab}
Pokkali	3.3 ^{ef}	72.4 ^{bc}
Bg 369	2.3 ^f	89.6 ^a
CV %	49.9	17.7

Means within a column followed by same letter are not significantly different at $p=0.05$

Experiment II: Influence of Hydro-priming on Survival Score

Seed priming, variety and their interactions with respect to survival score were found to be significant under submergence while none of them were found to be significant under saturated condition (Table 3). Priming significantly enhanced the germination of all varieties (except that of Mazhan Red, standard AG-tolerant check (Table 4)) suggesting the importance of seed treatment to enhance the stand establishment of rice under submergence stress. This was comparatively more evident in FR 13 A, where the survival rate increased by approximately 9-folds with priming while Bg 300 increased the survival rate by 63 %. In contrast, hydro-priming decreased the survival of Mazhan Red compared to non-priming. According to Ella *et al.*, (2011), priming is done to activate enzyme in germinating seeds before radical emergence. The poor survival rate of Mazhan Red may be due to early radical emergence during 24 hours of soaking and becoming dead during the drying process causing detrimental effect to the rate of survival under submergence. This emphasizes the importance of determining the right time of duration for priming depending on varieties if this technique was used to enhance the survival under submerged condition. However, Ella *et al.*, (2011) have observed an enhancement of survival of AG-tolerant entries 'Kaiyan' and 'Khao Hlan On' under submerged condition when primed seeds were submerged for 24 hours.

Table 3. Effect of seed treatment and variety on survival score under submerged and saturated conditions

Factor	Submerged	Saturated
Priming (a)	*	ns
Variety (b)	*	ns
Priming × variety	*	ns
Mean	22.9	93.4
CV % (a)	24.3	9.4
CV % (b)	18.4	4.8

ns – non significant

Table 4. Survival (%) between primed and non-primed treatments in submerged condition in different varieties

Treatment	Variety					
	Bg 300	Bg 310	At 308	Bg 305	Mazhan Red	FR 13 A
Primed	34.1 ^a	26.1 ^a	22.9 ^a	21.6 ^a	26.1 ^b	29.1 ^a
Non-primed	20.9 ^b	17.7 ^b	15.0 ^b	8.5 ^b	49.0 ^a	3.3 ^b
Control						
CV %	24.3					

Means within a column with the same letter are not significantly different at $p=0.05$

Effect of hydro-priming on soluble sugar content

The soluble sugar content significantly varied between submerged and saturated conditions, primed and non-primed treatments and among varieties from day 3-9 after imposing submergence treatment on sowing. The interaction effect of variety × days after sowing varied between treatments (primed and non-primed) under both submerged and saturated

conditions. Concentration of soluble sugars of all varieties increased with time (Figure 1). The rate of increasing soluble sugars from 3 to 5 days was comparatively higher with priming under saturated conditions but not under submerged condition (Figure 1A). In submerged non-primed treatment, the rate of increasing sugar concentration in FR 13A, the susceptible check was considerably lower than that of other varieties but almost similar to other varieties under submerged primed condition from 5 to 9 days (Figure 1B). Conversely, Mazhan Red (the AG-tolerant check) increased sugar content significantly ($p < 0.05$) than the rest from 3 to 7 days under submerged non-primed condition, confirming its ability to AG tolerance without priming. Under saturated condition, all the varieties except Mazhan Red performed similarly for both with priming and non-priming conditions (Figures 1 C & D). However, Mazhan Red showed a higher increasing rate of sugar content with priming from 3 to 5 days after sowing than that of the rest under non-primed condition. These results indicate the positive effect of hydro-priming in increasing soluble sugar content of germinating seeds under submerged as well as in saturated conditions during the first 5 days in rice. However, increasing rate of sugar content of submergence in susceptible and moderately tolerant varieties was much higher in primed seeds than that of in non-primed seeds under submerged condition.

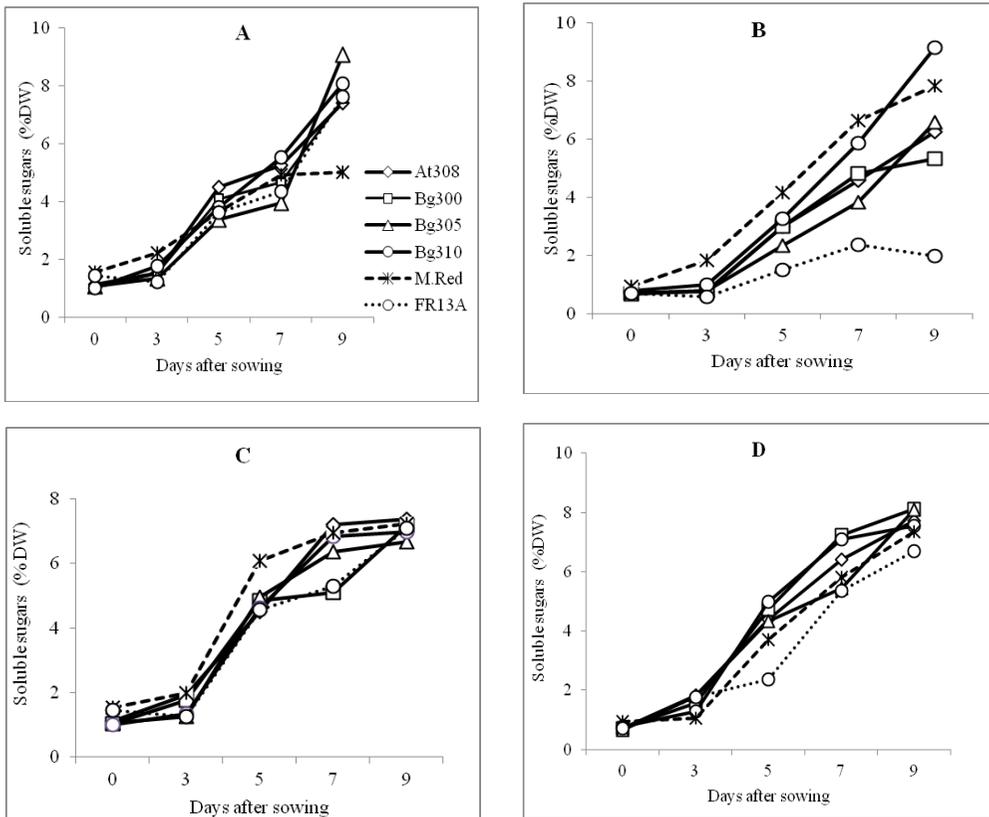


Figure 1. Effect of hydro priming on soluble sugar concentrations of 6 varieties with time after sowing under submerged and saturated conditions.[A. Submerged – primed, B. Submerged - non-primed, C. Saturated – primed, D. Saturated – non-primed. Data are means of 3 replicates, each of 25 seeds]

Effect of Hydro-priming on Starch Content

The starch in seeds significantly varied ($p < 0.05$) between submergence and saturated conditions, priming and non-priming treatment and among varieties from 3 to 5 days after sowing. Interaction effect of variety \times days after sowing varied between priming treatment (primed and non-primed) under both saturated and submerged conditions. Starch showed a reverse trend starting from day 0 to 9 where the concentration decreased with time in all varieties with a higher rate in saturated condition compared to submergence (Figure 2). There was no significant difference in starch degradation among varieties under submerged condition when seeds were primed (Figure 2 A). However, under submerged condition, when seeds were non-primed Mazhan Red showed a significant reduction in the starch concentration, with a rapid rate from day 0 to 5, compared to other varieties (Figure 2 B). In contrast, rate of starch degradation in FR 13A was significantly lower than that of in Mazhan Red over the specified period. All other varieties showed intermediate rate of starch degradation. Under saturated condition, both primed and non-primed seeds showed a similar pattern (Figures 2 C & D). These results indicated the effect of hydro-priming in accelerating starch degradation in AG susceptible and moderately-tolerant rice varieties during the early stage of seed germination under submergence.

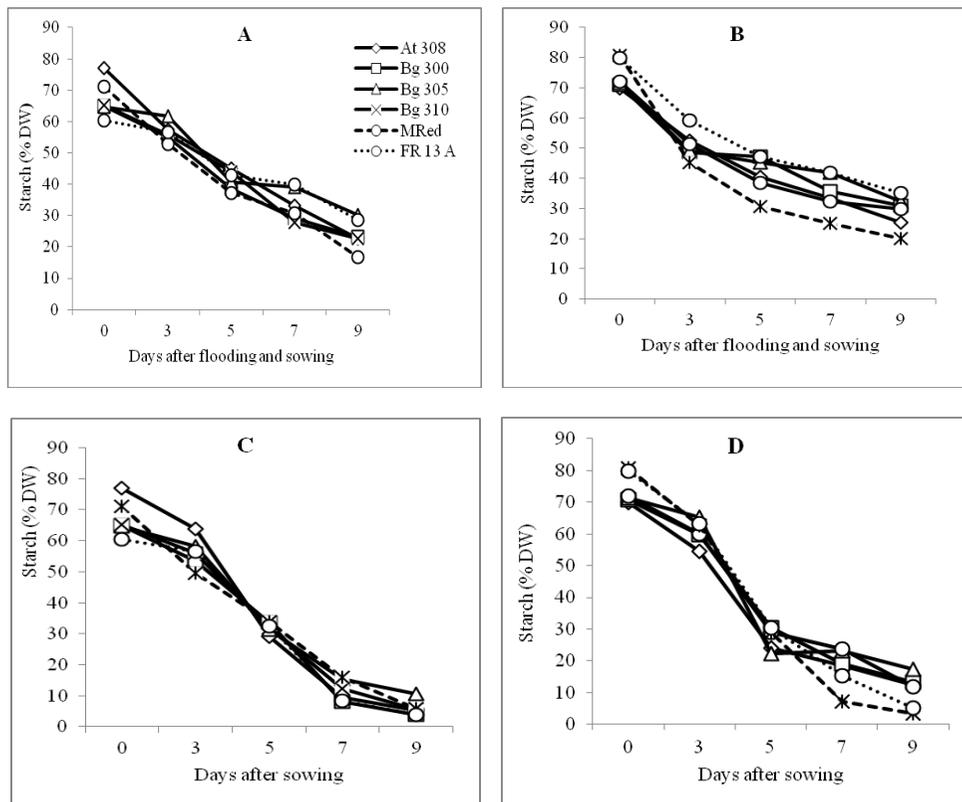


Figure 2. Effect of hydro-priming on starch content of 6 varieties with time after sowing under submerged and saturated conditions.

[A. Submerged – primed, B. Submerged - non-primed, C. Saturated – primed, D. Saturated – non-primed. Data are means of 3 replicates, each of 25 seeds]

Effect of Hydro-priming on α -amylase Content

Low levels of α -amylase activity were observed in the dry seeds of all varieties however, it increased progressively with time after sowing. In day 2 and 4 after sowing, the α -amylase activity was 2-3 times higher both with priming and non-priming under saturated condition compared to that of submerged condition (Figure 3). However, priming showed a significantly high α -amylase activity compared to non-priming, under submerged condition except with Mazhan Red and Bg 310 in day 2 and the increase in α -amylase activity in Bg 300 and FR 13 A with priming under submergence was much higher than that of Bg 310 and Mazhan Red (Figure. 3 A). Herath *et al.*, (2015) reported a significantly higher α -amylase activity in priming than non-priming treatment using IR 64, a susceptible variety to AG. Conversely, the α -amylase activity of non-primed seeds of Mazhan Red was comparatively higher at 4 days after sowing proving its ability to breakdown starch and to germinate under submerged condition (Figure 3 B). Ismail *et al.*, (2012) also reported high α -amylase activity of AG-tolerant cultivars ‘Khaiyan’ and Khao Hlan On’ compared to sensitive cultivars ‘FR 13A’ and ‘IR 42’ under submergence. There was no difference in α -amylase activity in primed and non-primed treatments among varieties under saturated condition in day 2 or 4 (Figures 3 C & D).

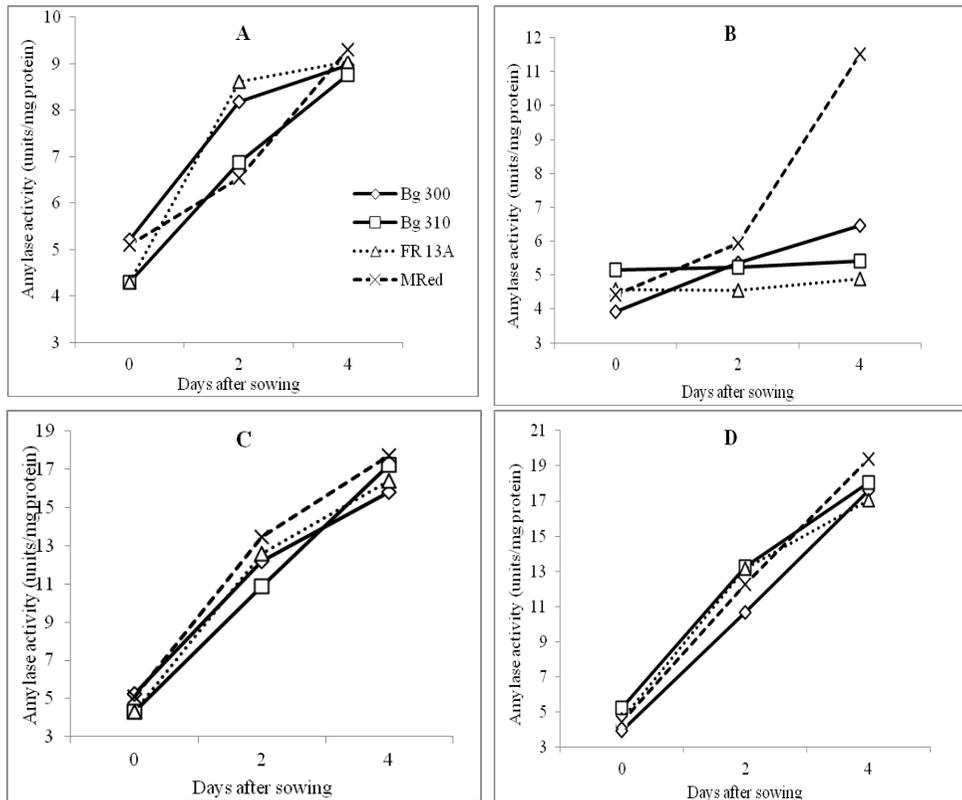


Figure 3. Effect of hydro-priming on α -amylase activity of 6 varieties with time after sowing under submerged and saturated conditions.

[A. Submerged – primed, B. Submerged - non-primed, C. Saturated – primed, D. Saturated – non-primed. Data are means of 3 replicates, each of 25 seeds]

Correlations Among Traits

Survival % was significantly and positively correlated with soluble sugar content at day 9 after sowing while α -amylase activity was negatively correlated with starch content at day 9 after sowing under submerged condition when seeds were primed (Table 5). Soluble sugars had a strong positive correlation with α -amylase activity while starch had negative correlations with both soluble sugars and α -amylase activity. These results showed the importance of high breakdown ability of starch, rapid synthesis of soluble sugars and high activity of α -amylase in rice seeds that germinate and survive under flooded condition with low O₂ stress. There were no significant correlations among those traits under saturated condition, except between soluble sugars and starch (Table 6). This also highlighted the importance of seed priming to enhance survival % by increasing the rate of soluble sugar synthesis, α -amylase activity and starch degradation of rice seeds germinated under submerged condition.

Table 5. Correlation coefficients (r) among different traits under submerged condition when seeds were primed

<i>Trait</i>	<i>Survival % (21 DAF)</i>	<i>Soluble sugar content (day 9)</i>	<i>Starch content (day 9)</i>	<i>Alpha amylase activity (day 4)</i>
Survival % (21 DAF)	1	0.74**	-0.51*	0.79**
Soluble sugar content (day 9)		1	-0.71**	0.80**
Starch content (day 9)			1	-0.57**
Alpha amylase activity (day 4)				1

* significant at $p=0.05$, **significant at $p=0.01$

Table 6. Correlation coefficients (r) among different traits under saturated condition when seeds were primed

<i>Trait</i>	<i>Survival % (21 DAF)</i>	<i>Soluble sugar content (day 9)</i>	<i>Starch content (day 9)</i>	<i>Alpha amylase activity (day 4)</i>
Survival % (21 DAF)	1	0.26	-0.11	-0.08
Soluble sugar content (day 9)		1	-0.77**	0.32
Starch content (day 9)			1	-0.24
Alpha amylase activity (day 4)				1

**significant at $p=0.01$

CONCLUSIONS

Three Sri Lankan rice varieties (Bg 300, Bg 310 and At 308) were moderately-tolerant to AG under submergence, while all other varieties are susceptible (low AG-tolerant) compared to Mazhan Red, the tolerant check under flooded condition. Hydro-priming increased the

survival (%) under submergence of susceptible and moderately-tolerant entries by 40%. Soluble sugar synthesis, starch degradation and α -amylase activity are accelerated under submerged conditions when seeds were hydro-primed. In all these varieties, survival rates correlated positively with soluble sugar ($r = 0.74$) and α -amylase activity ($r = 0.79$) and negatively with starch content ($r = -0.51$) under submerged condition when seeds were primed. These results emphasized the importance of seed priming to improve the stand establishment of rice seedlings under submerged condition. However, validation of this technique under farmer field conditions in large scale is required.

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