Submergence tolerance of some modern rice cultivars at seedling and vegetative stages

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ABSTRACT

Twenty five Sri Lankan modern rice cultivars were screened for submergence tolerance at seedling and vegetative stages. An experiment was carried out at the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka during May to September, 2011 according to the randomized complete block design (RCBD) with 4 replicates and each replicate contained 20 plants to evaluate submergence tolerance of the rice cultivars were evaluated in two growth stages; seedling stage and vegetative stage. At seedling stage, germinated seedlings were grown for 2 weeks at normal growth conditions and 5-day, 9day and 14-day complete submergence stresses were given in separate experiments. Plants were allowed two-week-recovery period after each submergence stress. Plant height was recorded just before and after submergence stress, just after submergence stress and just after recovery period. Plant survival percentage was recorded after the recovery period. The experiment was repeated using 6 week old plants for vegetative stage. Among tested rice cultivars 100%, 32% and 24% of rice cultivars survived at 5-day, 9-day and 14-day submergence periods respectively at the seedling stage while 64%, 28% and 16% of rice cultivars survived at 5-day, 9-day and 14-day submergence periods respectively at vegetative stage. According to the Duncan's multiple range test at 14-day complete submergence, Bg 379/2 was the best tolerant cultivar among the tested cultivars at both seedling stage (20% survival rate) and vegetative stage (17.5% survival rate). All the survived rice cultivars were elongated (100%) under 14-day submergence stress at both seedling and vegetative stages compared to that of control plants. Some survived rice cultivars reduced the plant height at 5-day and 9-day complete submergence stresses at both seedling and vegetative stages compared to that of control plants but none of the cultivars reduced the plant height at 14-day complete submergence stress compared to that of control plants was able to survive. There was a correlation between height gain during submergence stress and survival percentage of rice cultivars which depends on both growth stage and submerged period. The correlation between elongation ability of rice cultivars under control condition and under stress condition was also depends on the growth stage and the submergence period.

Keywords: Complete submergence, seedling stage, survival rate, traditional rice, vegetative stage

Mostly rice is cultivated in upland, lowland, irrigated or deep-water conditions with little or no control of water levels. According to the Asian condition irrigated paddy occupies the vast area of paddy lands. Rain-fed lowland and deep-water rice together account for approximately 33% of global rice farmlands (Serres et al., 2010). In Sri Lanka rainfall patterns have changed over the last few decades due to climate change. Increasing rainfall variability has worsened yield divergence in rain-fed areas where consistent rainfall during the growing season is critical. This rain fed lowlands are highly vulnerable to monsoon flash floods and complete submergence (Serres et al., 2010). Submergence adversely affects rice growth and yield. It creates the damages to plants as consequences of; slow rates of gas exchange, severe shading by turbid water, mechanical damages due to strong flow rates and solute carrying capacity of flooded water (Michael and Phool, 2001). Mostly the rice is cultivated in wetland or lowland rice culture; the fields are flooded, leveled, and bunded before planting or transplanting. Water is supplied by natural rainfall,

floodwater, runoff from higher ground, or irrigation, and the fields typically remain flooded throughout much of the growing season, depending on rainfall or water availability. In Asia, most of the rice lands are grown under wetland conditions (Barker et al., 1985). Salinity (Kundu et al., 2010) droughts, floods, cyclones, tornadoes are the most prominent abiotic stresses that can cause severe damage to the rice crop. Floods occur principally in the major river deltas where one-third of Asia's rice crop is produced. High winds damage the rice crop by causing lodging. Sterility can occur in the flowering stage, rice shatters near harvest, and general flooding destroys infrastructure and causes delays in land preparation, planting, and harvesting. Although damage is more local than in the case of droughts, crop losses can be severe (Barker et al., 1985). The frequency of the cyclones developing in the Bay of Bengal is significant during the November-December period. The eastern parts of Sri Lanka are the most vulnerable to cyclones that pass over or close the island because they are the most affected sections particularly by storm surges. All low-lying areas of the country are

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affected directly by heavy rains, strong winds and lightning and indirectly by floods.

Sri Lanka has a bimodal climatology with the heaviest rains from October to December and subsidiary rains from April to June. Sri Lanka receives modest rainfall even in the remaining drier months (Zubair et al., 2008). There are two major paddy cultivation seasons in Sri Lanka. The principal cultivation season is known as 'Maha' and is from October to March. During this season, there is enough water to sustain the cultivation of all rice fields. The subsidiary cultivation season, known as 'Yala' is from April to September. Usually there is only enough water for cultivation of half of the land area compared to that for Maha (Zubair, 2002). Floods are caused by heavy down pour of rainwater particularly in all seasons, monsoons and inter monsoons. Thunderstorms (October-November 2006), Easterly waves (January 2007), westerly troughs and low level disturbances will cause flash or limited area floods in Sri Lanka. About 75 percent of the rice lands are located within inland valley systems and another 25 is located in coastal plains and associated flood plains (Panabokke, 1996). The low lying coastal belt of half bog and bog soils may experience inundation, floods and soil problems (Dhanapala, 2005). Sri Lanka reduces 10 percent of the total annual rice yield due to unexpected and uncontrolled floods during monsoon rains (Panabokke, 1996). Most of the modern rice varieties are not adapted to these flood conditions. Farmers suffer from regular yield loss when they grow modern rice varieties (Baltazar, 2008). When partially or completely submerged, most rice varieties display a moderate capacity to elongate leaves and the portion of stems that are trapped underwater. This elongation growth leads to a spindly plant that easily lodges when floodwaters recede. If the flood is deep, underwater elongation growth can exhaust energy reserves, causing death within a matter of days (Serres et al., 2010). Survival of rice seedlings decreased significantly under flooded conditions, but to a much lower extent in the tolerant genotypes. Under flooding, growth of both shoot and root started earlier in tolerant genotypes and proceeded faster than growth in intolerant ones. (Ismail et al., 2009). It is most likely that submergence-tolerant lowland rice cannot survive complete submergence for months, as deep-water rice does by maintaining photosynthetic organs above the water surface. Deep-water rice adapts to prolonged submergence as long as the rising flood never outdistances plant stature. On the other

hand, submergence-tolerant lowland rice acclimatizes to deep flash floods in which the water level rises well in advance of plant growth, but then falls within 2 weeks of the inundation. The two modes for submergence survival in rice are likely to have been acquired via a mechanism involving gene duplication at the *Sub1* locus in environments with distinct seasonal submergence regimes (Fukao *et al.*, 2007). The present study was carried out to identify the level of submergence tolerance of some modern rice cultivars at seedling and vegetative stages, to understand the submergence tolerance mechanism of selected rice cultivars and to understand the severity of submergence stress on growth stage of rice.

MATERIALS AND METHODS

The experiment was carried out at Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya during 2011May to September, 2011. Twenty five Sri Lankan modern rice cultivars were used for the experiment. The experiment was carried out at the Faculty of Agriculture, University of Ruhuna, Mapalana, Sri Lanka according to the randomized complete block design (RCBD) with 4 replicates and 20 plants were included for each replicate. Two week old seedlings (seedling stage) and 6 week old seedlings (vegetative stage) were subjected to 5 day, 9 day, and 14 day complete submergence conditions separately and control experiments were carried out all along the experiment. After complete submergence period plants were given two week recovery period under de-submerged conditions. In seedling stage submergence tolerance screening, germinated seeds were planted in trays (60 cm \times 90 cm) filled with homogenized soils according to the randomized complete block design with 4 replicates and 20 plants were included in each replicate. Plants were grown in normal growth conditions for two weeks. In vegetative stage submergence tolerance screening, germinated seeds were planted in plastic boxes $(15 \times 7.5 \times 15 \text{ cm})$ filled with homogenized soils according to the randomized complete block design with 4 replicates and each replicate contained 20 plants. Plants were grown for 6 weeks under normal growth conditions. Plants were completely submerged (so that the longest leaf of the plant is covered by water) in an outdoor concrete tank, for 5 days, 9 days and 14 days separately. After the complete submergence stress plants were given two week recovery period under normal growth conditions. Control plants were maintained without submergence stress.

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Average green plant height was measured both in stress plants and control plant just before submergence stress, just after submergence stress and after 2 week recovery period. Finally, plant dry matter weight was also measured. Data was analyzed using SAS statistical software. Submergence tolerance was identified as the survival percentage

Survival $\% = \frac{\lambda_2}{\lambda_1} \times 100$

 $\lambda_1 =$ Number of plants before submergence

 $\lambda_2 =$ Number of survived plants after recover

Effect of submergence was measured as the height reduction or height gain percentage of stressed plants compare to control plants during the relevant period.

 $\Delta = \mu_2 - \mu_1$

where,

- Δ = Height reduction or height gain during submergence
- μ_2 = Average height of plants just after the submergence stress
- μ_1 = Average height of the plants just before the submergence stress

Data Analysis

Analysis of information were taken up with the help of Analysis of variance and mean separation was done in 'Duncan's multiple range test' according to the randomized complete block design at the 0.05 significant level using SAS statistical software package v6.12 (**SAS** Institute Inc., 2000).

RESULTS AND DISCUSSION

There was a significant difference between control plants and submergence stressed plants in average plant height after 5 day, 9 day and 14 day submergence stresses. None of the rice cultivars were died after 5 day submergence stress. During submerged period 88% of rice cultivars elongated while only 12 % (At 402, At 307, At 306) of rice cultivars reduced plant height compared to that of control plants. All the three rice cultivars those reduced plant height during submergence scored less than 40% in survival rate while all the other rice cultivars elongated during submergence stress scored 40% -80% in survival rate. Among survived rice cultivars at 5 day completely submergence stress At 402, At 307, and At 306 have reduced the plant height compared to control plants while, At 307 was died at 9 day complete submergence conditions but At 402 and At 306 were able to survive while reducing the plant height compared to that of control plants even at 9 day completely submergence period. None of the rice cultivars reduced plant height during submergence stress was survived at 9 day submergence stress. All the submergence tolerant rice cultivars elongated compared to that of control plants at 5 day and 9 day submergence stress as well.

Rice cultivars namely At 308, At 402, At 401, At 307, At 306, At 303, At 353, At 362, Bg 305, Bg 352, Bg 300, Bg 359, Bw 452, Bw 453, Bw 272-6b, Bg 250, Bg 38 were died after 2 week recovery period following 9 day complete submergence stress. The highest plant height was recorded by rice cultivar Bw 452 under 9 day completely submergence stress but was not survived. Among survived cultivars 88.3% elongated during 9 day complete submerged period while 11.3% reduced their height.

None of the cultivars, those reduced their height during 5 day and 9 day complete submergence stress could survive at 14 day complete submergence stress. Among elongated rice cultivars compared to that of control plants at 14 day submergence stress, rice cultivar Bg 357 failed to survive. At 405 and Bw 364 remained the same as the control plants in plant height at 14 day submergence but could score 10%-20% survival rates. All 25 rice cultivars (100%) recovered after 5 day complete submergence stress while 32% cultivars survived after 9 day complete submergence stress and only 24% cultivars remained after 14 day complete submergence stress.

Bw 364 Bw 351 Bg 379/2 and those scored 79.17% survival rates under 5 day complete submergence stress were able to score 91.6% at 9 day complete submergence stress which showed strong acclimatization for prolong submergence stress (Table 1). Interestingly those cultivars were able to survive even at 14 day complete submergence stress.

According to the Duncan's multiple range test rice cultivar Bg 379/2 recorded the highest significant survival rate during each complete submergence stresses (91.67%-5 day, 92.83%-9 day, 20.00%-14 day) at seedling stage (Table 1).

Screening modern rice cultivars for submergence tolerance at vegetative stage

At vegetative stage, 64 % of tested rice cultivars were able to survive at 5 day complete submergence stress. This value was significantly less than that of seedling stage (100%). Contrarily to that the survival percentages at 9 day submergence (28%) and at 14 day

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			Seedling s	ling stage					Vegetative stage	e stage		
Variety	5 day submergence	lergence	9 day submergence	ergence	14 day submergence	ergence	5 day submergence		9 day submergence 14 day submergence	ergence	14 day subn	nergence
	Survival rate	HGt- HG¢	Survival rate	HGt- HGc	Survival rate	HGt- HGc	Survival rate	HGt- HGc	Survival rate	HGt- HGc	Survival rate	HGt- HG¢
At 308	16.67 ⁱ	0.18						0.17				
At 402	41.67^{fgh}	-0.95		-6.56				-1.07				
At 401	54.17^{def}	0.66					50.00^{bc}	3.81		2.22		
At 307	29.17^{ghi}	-1.34					17.00^{f}	-5.21		-3.66		
At 306	41.70^{fgh}	-0.52		-10.00				-1.80				
At 303	41.67^{fgh}	1.34					58.50^{ab}	2.45	25°	0.98		0.34
At 353	29.17^{ghi}	0.18					58.50^{ab}	1.51	25°	0.54		0.34
At 362	20.83^{hi}	-0.55					37.25^{cde}	-0.63		0.28		
Bg 305	25.00^{hi}	2.53						4.00				
Bg 352	29.17^{ghi}	0.12						2.02				
Bg 300	58.33 ^{cdef}	4.47		-0.04				0.02				
Bg 359	$50.00^{ m efg}$	3.62		1.03			21.00^{f}	0.08		-0.70		
Bg 360	66.67^{bcde}	2.93	45.825 ^b	1.32		-1.83		0.37				
Bg 304	$50.00^{ m efg}$	5.14	58.350 ^b	3.51	10.00^{bc}	4.46	58.25 ^{ab}	1.57	50.0°	0.13	10°	0.38
Bg 357	87.50^{ab}	9.34	50.000 ^b	7.08		-0.04	62.75^{ab}	2.62	60.0^{a}	1.44		0.53
Bw 364	79.17^{abc}	5.84	91.675ª	4.42	15.00^{ab}	-1.42	62.75^{ab}	4.30	60.0^{a}	2.96	$15^{\rm ab}$	1.70
Bw 351	79.17^{abc}	4.46	91.675ª	4.62	12.50^{bc}	2.60	58.50^{ab}	1.63	60.0^{a}	2.13	$12.5^{\rm cb}$	0.50
Bw 452	54.17^{def}	8.06		8.18			21.00^{f}	5.36		4.67		
Bw 453	37.50^{fghi}	7.74		4.48			41.50^{cd}	4.59		5.28		
Bw 272-6b	58.33 ^{cdef}	9.72		5.35			29.25^{def}	9.54		6.14		
At 354	91.67^{a}	5.40	54.175 ^b	3.64	07.50°	-1.83	29.25^{def}	3.79		2.70		
At 405	$75.00^{\rm abcd}$	4.00	58.350 ^b	3.75	10.00^{bc}	-1.27	$25.00^{\rm ef}$	5.46		1.82		
Bg 379/2	91.67^{a}	3.25	95.825ª	1.04	20.00^{a}	0.81	66.75^{a}	3.71	62.5^{a}	3.67	17.5^{a}	8.77
Bg 250	83.33^{ab}	5.90		4.55				5.31				
Bg 38	$75.00^{\rm abcd}$	7.61		4.35				6.02				

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submergence (16%) in vegetative stage plants were significantly higher than that of 9 day (32%) and 14 day (24%) submergence at seedling stage.

In survived cultivars height reduction compared to that of control plants could be seen at 5 day (At 402, At 307, At 306) and 9 day (At 307) submerged plants at vegetative stage. All the survived cultivars under 14 day complete submergence stress at vegetative stage gained plant height during the stress period compared to that of control plants.

Rice cultivar Bg 379/2 recorded the highest significant survival rate under each submergence stress (66.75%-5 day, 62.5%-9 day, 17.5%-14 day) at vegetative stage.

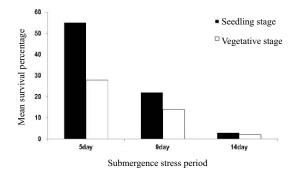


Fig. 1: Mean survival percentage of survived plants under 5 day 9 day and 14 day complete submergence stresses at seedling and vegetative stages.

Mean survival percentages were gradually decreased from 5 day to 14 day complete submergence stress periods (Fig.1). Mean survival percentage at seedling stage under each submergence stress period was significantly greater than that of at vegetative stage (Fig.1). It can be concluded that submergence damage is much severe at vegetative stage than that of at seedling stage under the same intensity of the stress (Fig.1).

There was a positive significant correlation between height gain of stressed plants *versus* survival percentage at 5 day and 14 day submergence stress at seedling stage and 9 day and 14 day submergence stress at vegetative stage (Table 2). This correlation was not significant at 9 day submergence stress at seedling stage and 5 day submergence stress at vegetative stage (Table 2).

In the present study 100%, 32% and 24% of rice cultivars were survived at 5 day, 9 day and 14 day submerged periods respectively at the seedling stage (Table 1). Cultivars survived at 14 day complete submergence stress were Bg 379/2, Bw 364, Bw 351, At 405, Bg 304, and At 354. Among those cultivars Bg 379/2 recorded the greatest tolerance (20%) under each three submerged periods (Table 1).

Among tested rice cultivars 64%, 28% and 16% of rice cultivars were survived at 5 day, 9 day and at 14 day submergence periods respectively at the vegetative stage (Table 1). Bg 379/2, Bw 364, Bw 351 and Bg 304 were the tolerant cultivars at 14 day complete submergence stress at vegetative stage while Bg 379/2 recorded the best survival percentage (17.5%) at each three submergence periods. Green plant height and seedling survival rate were considered as parameters for evaluation of submergence tolerance in rice at seedling and vegetative stages in the present study. Seedling vigor is a trait that expresses itself as an ability of seedlings to rapidly elongate after germination and emerge for escaping and surviving submergence stress. Cui et al. (2002) reported that good germination and fast early seedling growth were two major seedling-vigorrelated traits in rice.

Most of the cultivars (Bg 379/2, Bw 364, Bw 351, At 405, Bg 304, At 354) increased their height during submergence compared to that of control plants but some cultivars decreased their plant height for

		Seedling stage			Vegetative stage		
		5days	9days	14days	5days	9days	14days
Height gain versus survival percentage (In stressed plants)	Correlation coefficient (r)	0.620	0.394	0.582	0.292	0.420	0.643
	Probability (p)	0.001	0.051	0.004	0.156	0.036	0.001
Height gain of stressed plants versus control plants	Correlation coefficient (r)	0.398	0.771	0.530	0.167	0.347	0.795
	Probability (p)	0.049	0.001	0.006	0.420	0.089	.000

Table 2: Correlations between height gain versus survival percentages and height gain of stressed plants versus control plants

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survival under submergence stress compared to that of control plants. Fast shoot elongation apparently requires energy and therefore causes a risk of carbohydrate depletion under prolonged submergence due to the reduced photosynthesis. A study using fast shoot elongation growth as a basic parameter for screening rice cultivars under low dissolved oxygen levels suggested that fast shoot elongation is an adaptive response of water-seeded rice for acquiring oxygen (Won and Yoshida, 2000).

To examine if the seedlings elongated under submergence stress can recover with greening after release from the stress, we transferred the stressed seedlings to the normal growth conditions and survival percentages was calculated. All the cultivars survived at 14 day submergence stress (Bg 379/2, Bw 364, Bw 351, At 405, Bg 304, At 354) were elongated at both seedling and vegetative stages compared to that of control plants (Table 1) since submergence stress significantly promotes shoot elongation in young rice seedlings (Redona and Mackill, 1996; Ismail et al., 2009). Fast shoot elongation under submergence is beneficial during the post-germination stage when young seedlings experience hypoxia or anoxia (Won This trait is particularly and Yoshida, 2000). important during direct sowing of rice seeds in paddy field (Yamauchi et al., 2000; Magneschi and Perata, 2009). Rice plants adopting the "elongation strategy" can grow rapidly to reach above the water surface and start aerobic metabolism and photosynthesis (Kende et al., 1998; Voesenek et al., 2006).

Some rice cultivars survived while reducing the plant height (At 402, At 307, and At 306) compared to that of control plants only at 5 day and 9 day complete submergence periods at both seedling and vegetative stages but none of them were survived at 14 day complete submergence stress (Table 1). Reduced elongation during flash flood is advantageous for rice seedlings because elongated seedlings trend to lodge as soon as the water level recedes (Suge, 1985; Setter and Laureles, 1996). Submergence-induced elongation is an escape mechanism that helps submerged rice plants to regain contact with the aerobic environment (Arber, 1920). However the "quiescence strategy" that can help rice plants to maintain high levels of stored carbohydrates coupled with minimum shoot elongation has been considered a strategy for tolerance against submergence stress (Ito et al., 1999; Ram et al., 2002; Jackson and Ram, 2003; Das et al., 2005, Fukao et al., 2006).

The Sub1 locus is known to play a key role in submergence tolerance in rice according to the

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'quiescent strategy' that can help rice plants to maintain high levels of stored carbohydrates coupled with minimum shoot elongation (Fukao and Bailey-Serres, 2004, 2008; Fukao *et al.*, 2006; Xu *et al.*, 2006). However among tested rice cultivars none of the cultivars followed quiescent strategy (At 402, At 307, and At 306) under submergence stress was able to survive at the seedling or at the vegetative stage.

The mean survival percentages of rice cultivars under three different submergence stresses at seedling stage were significantly higher than that of vegetative stage (Fig.1). The submergence damage at vegetative stage was critical than that of at the seedling stage. The strength of correlation between height gain and survival percentage of rice cultivars at submergence stress was depended on the age of rice cultivar and duration of submergence stress (Table 2). There was a correlation between elongation ability of rice cultivars under control condition and under submergence stress and the strength of the correlation was also depended on the growth stage and the submerged period (Table 2). Submergence tolerance in rice has been referred to the trait that confers on rice plants at the vegetative stage an ability to survive stresses caused by flooding (Jackson and Ram, 2003). Rice plants die if they are fully submerged for a period longer than 2 weeks, because respiration is restricted when the oxygen and carbon dioxide supplies become limited (Boamfa et al., 2003). Among tested modern rice cultivars 24% cultivars were submergence tolerant at the seedling stage and 16% cultivars were tolerant at the vegetative stage under 14 day complete submergence stress. According to the Duncan's multiple range test, Bg 379/2 was the best tolerant cultivar at the seedling stage (20% survival rate) and vegetative stage (17.5% survival rate) at 14 day complete submergence stress. Rice cultivars survived at 14 day complete submergence stress (Bg 379/2, Bw 364, Bw 351, At 405, Bg 304, and At 354) can be considered as better cultivars to be grown in flood prone areas in Sri Lanka.

Submergence stress is much severe at vegetative stage than that of at seedling stage at the same submergence stress period for the same rice cultivar. Among tested rice cultivars submergence tolerant rice cultivars those reduced plant height during submergence stress could not survive at severe submergence stress conditions. There are correlations between height gain *versus* survival percentage and elongation ability at control conditions *versus* submergence stress conditions but the strength of the correlation depends on the growth stage of the rice cultivar and the submergence period. Submergence tolerance of rice cultivars

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