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Differential growth response of rice genotypes based on quiescence mechanism under flash flooding stress

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Abstract

The main strategies enabling rice plants to cope with flash flooding stress require growth regulation during submergence and subsequent rapid growth recovery after de-submergence. The objective of this study was to characterize the response of 56 diverse contrasting rice genotypes to submergence and their recovery following de-submergence. Among these genotypes, nine lines had been developed for anaerobic germination and submergence tolerance (AG + *Sub1*) by IRRI. Fourteen-day-old plants were submerged completely in water for 7 days. Subsequently, the plants were kept under normal rice-cultivation conditions as the control for a further period of 5 days. The tested genotypes were generally classified into three clusters based on shoot elongation rate of submerged to non-submerged treatments (ratio) during submergence period and chlorophyll contents during recovery period using Ward's method. The genotypes in clusters I include most of AG + *Sub1* lines and tolerant genotype FR13A adapted to submergence stress, which get the benefits of quiescence mechanism during submergence coupled with maintenance of higher chlorophyll content during recovery period. In contrast, the cluster III spanned most of intolerant genotypes such as IR42 by enhancing shoot elongation through escape mechanism in response to submergence. This mechanism negatively affected the plant growth recovery due to a great reduction of chlorophyll contents during the recovery period. The genotypes placed in cluster II followed the similar trend as cluster I during the submergence and recovery periods in addition of increases in shoot fresh weight during submergence period. This finding suggests that other mechanisms along with quiescence might be associated with submergence stress in the genotypes placed in cluster II. In conclusion, the contrasting rice genotypes expressed differential growth responses in genotypes with lower shoot elongation ratio using different quiescence strategies during submergence period.

Keywords: AG+*Sub1*, cluster analysis, escape strategy, growth recovery, seedling vigor.
Abbreviations: AG_anaerobic germination; IRRI_International Rice Research Institute; Sub_submergence.

Introduction

Submergence stress is a common environmental challenge for agriculture sustainability in many regions throughout the world. Partial-to-complete submergence of aerial organs considerably reduces the growth and survival of most crop plants. The negative impact of submergence on economic plants is mainly related to a poor gas exchange under water through impeding biochemical activities such as aerobic respiration and photosynthesis (Das et al., 2005; Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009). Acclimation responses to these conditions are species-specific and genotype-specific. Modification of morphology and anatomy of shoots and switching the energy conversion modes from aerobic to anaerobic respiration can ameliorate the negative effects of submergence (Fukao et al., 2006; Mommer et al., 2007). Studies on *Oryza sativa*, which is an adapt species to submergence conditions, has revealed that most of genotypes can bear the submergence stress by two contrasting strategies: escape and quiescence (Jackson and Ram, 2003; Perata and Voesenek, 2007). Genotypes using the escape strategy respond to submergence by enhancing shoot elongation to expose their leaf tips above the water surface. This strategy is disadvantageous under flash flood condition because faster shoot elongation competes with maintenance processes that are necessary for survival during submergence as well as for growth resumption during de-submergence (Ram et al., 2002; Panda et al., 2008). In tolerant genotypes, this strategy of using cell elongation and heightened carbohydrate metabolism is repressed. Furthermore, rapid elongation by Low Oxygen Escape Syndrome (LOES) can restore contact between leaves and air but can also result in death if carbohydrate reserves deplete before emergence in leaves above the water surface. This mechanism is expected to be effective only when flood waters become shallow. This mechanism is expressed by *Sub1C*. However, genotypes using the quiescence strategy conserve energy and carbohydrates by restraining shoot elongation. Therefore, the flash flood tolerance of rice genotypes can be enhanced by selecting lines that exhibit quiescent strategy. A single polygenic locus submergence-1 (*Sub1*) on chromosome 9 has been known to play a key role for flash flood tolerance in rice according to the quiescent strategy. The gene *Sub1* has the corresponding alleles of *Sub1A*, *Sub1B* and *Sub1C*. The submergence-induced *Sub1A* gene helps genotypes to maintain high levels of stored carbohydrates coupled with minimum shoot elongation during submergence and recommences the initiation of leaf development and retention of chlorophyll upon de-submergence, which was shown in a submergence-tolerant genotype model FR13A (Xu et al., 2006; Fukao and Bailey-Serres, 2008). However, genotypes lacking *Sub1A* such as submergence-intolerant genotype model IR42 rapidly consume leaf starch and soluble sugars due to the rapid elongation of shoots during submergence (Fukao et al., 2006).

Most of genetic variation studies on submergence tolerance have revealed that slow shoot elongation during submergence is always related to the high flash flood tolerance and the expression of *Sub1A* gene. In contrast, few reports have described that the slow shoot elongation during submergence, is not always linked with high flash flood tolerance (Jackson and Ram, 2003; Perata and Voesenek, 2007). Enhancement in the genotypic tolerance to anaerobic conditions during germination is much more inexpensive for poor farmers in the developing countries and is more feasible for adoption on a larger scale than other management practices. Unfortunately, very limited success has been achieved from previous efforts to improve the tolerance of genotypes for anaerobic conditions during germination (Jiang et al., 2004). For instance, Angaji et al. (2009) reported that tolerance to flooding during germination seems relatively rare in rice. After screening over 8000 gene bank accessions, elite breeding lines, and genotypes, they identified few genotypes with greater ability to germinate under flooding condition. Only 0.23% of all accessions were identified with a reasonably high level of tolerance in the initial screening. In an earlier study, we demonstrated that most AG (Anaerobic Germination) + *Sub1* lines germinated more rapidly under anaerobic conditions in fifty-eight contrasting rice genotypes (El-Hendawy et al., 2011).

Furthermore, the expression of *Sub1A* gene occurring in the elongating seedling genotypes suggested that *Sub1A* gene expression does not hinder shoot elongation growth under submergence in the early seedling stage and the rapid elongation was not linked with low tolerance (Vu et al., 2010). AG+*Sub1* lines show superior germination facility under anaerobic condition; however, their submergence tolerances are not known.

In this study, we further examined the significance of quiescence and escape mechanisms in flash flood tolerance using different rice genotypes including AG+*Sub1* lines. The damage caused by submergence is generally not apparent immediately at early seedling stage. It manifests itself only after water recedes during recovery from complete submergence. When rice plants are submerged by flash floods, they experience two different environmental changes: anaerobic conditions during submergence and aerobic conditions after de-submergence. Therefore, flash flood tolerant plants should be quickly adaptable to these two different environments. The capacity of genotypes for rapid growth recovery after submergence is a desirable trait because it can assure early recovery and production of sufficient biomass for optimum productivity. A plant's ability to recover after submergence is mainly related to its shoot elongation responses to submergence (escape or quiescence strategy), carbohydrate content before and after submergence, and photosynthetic capacity during the initial recovery period (Das et al., 2005; Panda et al., 2008; Kawano et al., 2009; Luo et al., 2011). Sone et al. (2011) described higher chlorophyll contents of rice leaves in quiescence than in escape under submergence.

The studies in rice have shown that shoot elongation of the escape strategy resulted in promoting consumption of carbohydrate and subsequently reduced recovery of submergence and vice versa with the quiescence strategy. However, Luo et al. (2011) found that although stem elongation of the escape strategy depleted the carbohydrate storage in *Alternanthera philoxeroides* during submergence, this species quickly resumed growth after de-submergence because of the high priority on photosynthesis and carbohydrate accumulation during the initial recovery period. This finding implies that certain protective mechanisms might operate during recovery from complete submergence and these mechanisms might differ from those operating during submergence. Therefore, we further examined the recovery capacity of different genotypes after submergence by monitoring chlorophyll and biomass accumulation during 5 days of de-submergence.

Results

Cluster analysis

The shoot elongation rate during submergence and chlorophyll content during recovery period are the two reliable traits could be used for evaluating rice genotypes under submergence stress. The 56 genotypes were grouped into three clusters using Ward's method, based on chlorophyll contents and the ratios of submerged to non-submerged treatments of shoot elongation rate during submergence period (7 days submergence) and during recovery period (5 days de-submergence) (Fig. 1). Clusters I, II and III included 29, 8 and 19 genotypes, respectively. Seven genotypes from AG + *Sub1* lines and the model tolerant genotype FR13A were grouped in cluster I. Two genotypes from AG + *Sub1* lines were placed in cluster II. However, the model susceptible genotype IR42 was placed in cluster III (Fig. 1).

To identify the characterization of each cluster group during submergence and recovery periods, cluster means of shoot elongation rate and increase in shoot fresh weight during submergence period, and chlorophyll content in leaves during recovery period are summarized in Table 2.

Cluster I was characterized by genotypes with lower shoot elongation rates (1.05 ± 0.35 cm d^{-1}) and moderate increases in shoot fresh weight (9.78 ± 1.83 mg d^{-1}) during submergence period coupled with maintenance a higher chlorophyll contents (3.38 ± 0.69 µg mg^{-1} fresh weight) during recovery period. In contrast, cluster III was characterized by genotypes with higher shoot elongation rates (2.10 ± 0.54 cm d^{-1}) and less increases in shoot fresh weight (8.09 ± 1.09 mg d^{-1}) during submergence period coupled with low chlorophyll contents (2.21 ± 0.45 µg mg^{-1} fresh weight) during recovery period. However, cluster II was characterized by genotypes with lower shoot elongation rates (1.05 ± 0.47 cm d^{-1}) as same as Cluster I and high increases in shoot fresh weight (12.09 ± 3.12 mg d^{-1}) during submergence period coupled with maintenance a higher chlorophyll contents (3.13 ± 0.22 µg mg^{-1} fresh weight) during recovery period (Table 2).

Evaluation of genotypes based on shoot elongation rate during submergence and recovery periods

Significant variation for measurements of shoots was observed among genotypes during submergence and recovery periods (Table 3). For instance, the shoot elongation rates during submergence period varied from 0.11 to 2.79 and from 0.10 to 3.80 cm d^{-1} among genotypes under non-submerged and submerged treatments, respectively. During recovery period, the shoot elongation rates varied from 0.08 to 3.21 cm d^{-1} under non-submerged treatments and from 0.02 to 1.78 cm d^{-1} under submerged treatments (Table 3). During submergence period, non-submerged treatments increased the shoot elongation rate of susceptible genotype IR42 more than tolerant genotype FR13A and vice versa in submerged

Table 1. List of rice genotypes used in this study.

Gen. No.	Genotype name	Country of origin	Gen. No.	Genotype name	Country of origin
1	IR 06F148 (AG + Sub1)	Philippines	29	IR 42	Philippines
2	IR 06F168 (AG + Sub1)	Philippines	30	IR 48	Philippines
3	IR 06F393 (AG + Sub1)	Philippines	31	IR 56	Philippines
4	IR 06F434 (AG + Sub1)	Philippines	32	IR 60	Philippines
5	IR 06F459 (AG + Sub1)	Philippines	33	IR 74	Philippines
6	IR 06F463 (AG + Sub1)	Philippines	34	ITA 212	Nigeria
7	IR 06F561 (AG + Sub1)	Philippines	35	Jhona 26	Pakistan
8	IR 07F297 (AG + Sub1)	Philippines	36	Kasalath	India
9	IR 07F323 (AG + Sub1)	Philippines	37	Kataktara Da2	Bangladesh
10	ARC 10177	India	38	Khao Kap Xang	Thailand
11	Baran Boro	Bangladesh	39	LAC 23	Liberia
12	Bico Branco	Brazil	40	Mehr	Iran
13	Black Gora (NCS12)	India	41	Milyang 55	Korea
14	C 22	Philippines	42	Murungakayan 302	India
15	Canela de Ferro	Brazil	43	N 22	India
16	CG 17	Senegal	44	NP 125	India
17	Chianung Si-Pi 661020	Taiwan	45	Pachehai Perumal	India
18	DA 28	Bangladesh	46	Padi Lebat	Indonesia
19	Dholi Boro	Bangladesh	47	PTB 30	India
20	Egyptian Jasmine	Egypt	48	Rathal	Sri Lanka
21	Fircoz	Iran	49	Rikutou Nourin21	Japan
22	FR13A	Philippines	50	Sakha 103	Egypt
23	Gharib	Iran	51	Shai-kuh	China
24	Giza 177	Egypt	52	Surjamkuhi	India
25	Giza 181	Egypt	53	Tadukan	Philippines
26	Gotak Gatik	Indonesia	54	Tchampa	Iran
27	IR 22	Philippines	55	Trembese	India
28	IR 24	Philippines	56	WAB99-84 (FRF1)	Ivory Coast

Table 2. Characteristics of each individual clusters during submergence and recovery periods, and 1 day before submergence for submerged treatments. Data are mean ± of SE for individual clusters.

Parameter	Clusters		
	I	II	III
Shoot elongation rate during 7 days of submergence (cm d^{-1})	1.05 ± 0.35	1.05 ± 0.40	2.10 ± 0.54
Increase in shoot fresh weight during 7 days of submergence (mg d^{-1})	9.78 ± 1.83	12.09 ± 3.12	8.09 ± 1.09
Chlorophyll contents at 5 days of desubmergence (µg mg^{-1} FW)	3.38 ± 0.69	3.13 ± 0.22	2.21 ± 0.45

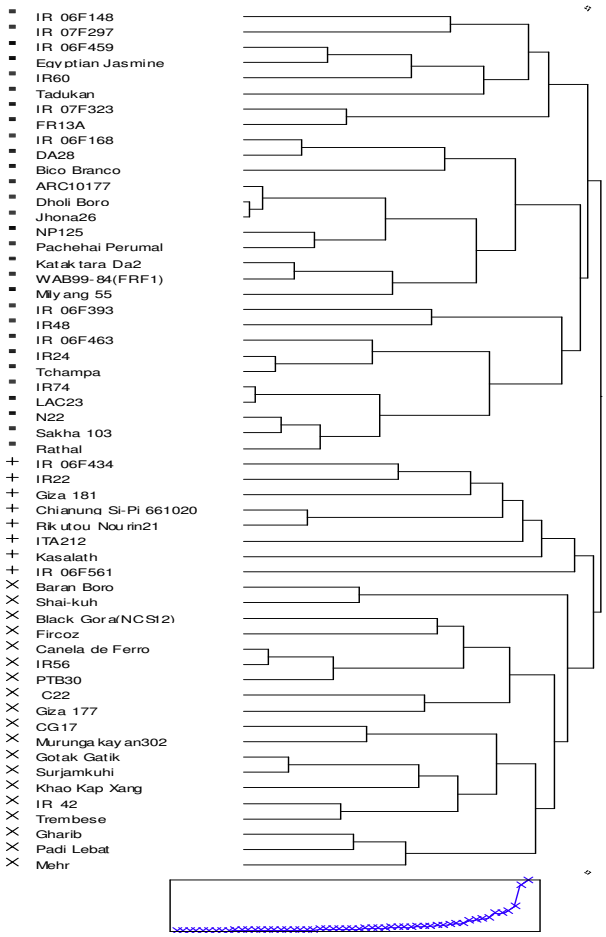

Fig 1. Hierarchical cluster analysis of the 56 rice genotypes using ratios of submerged to non-submerged treatments for shoot elongation rate during submergence period and chlorophyll content during recovery period for submerged treatments.

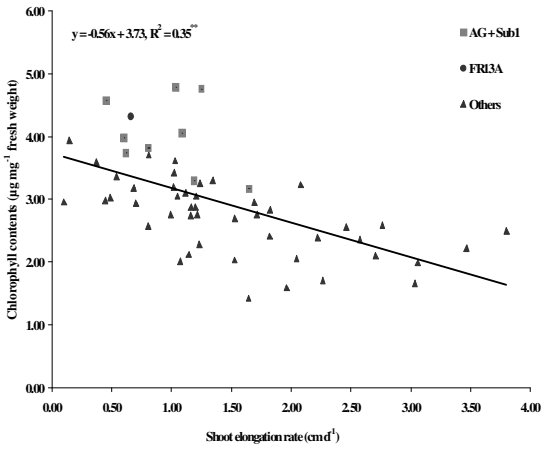

Fig 2. Relationships between shoot elongation rate during 7 days of submergence and chlorophyll contents at 5 days after de-submergence. Asterisks indicate significant differences ($P \leq 0.01$) between two variables.

treatments. The similar behavior of shoot elongation rate, like the one observed in model genotype FR13A, was found in 12, 3 and 1 genotypes in clusters I, II and III, respectively, in which the shoot elongation rate in these genotypes followed the quiescence strategy during submergence period (Table 3). Shoot elongation rate of all AG + *Sub1* lines in cluster I followed the same pattern as FR13A, with the exception of IR06F148 which showed an intermediate elongation rate (1.25 cm d^{-1}).

However, shoot elongation rate of IR06F434 in cluster II followed the same pattern as IR42, in which the shoot elongation rate followed the escape strategy. The shoot elongation rate under submerged treatments for IR06F434 and IR42 were 1.65 and 1.69 cm d^{-1}, respectively (Table 3). It is interesting to note that, during submergence period, the shoot of all AG + *Sub1* lines was elongated lower than that of FR13A under non-submerged treatments. However, during recovery period, the shoot of all AG + *Sub1* lines were elongated faster than that of FR13A under submerged treatments, with the exception of IR07F323 and IR06F434 (Table 3).

Evaluation of genotypes based on increase in shoot fresh weight during submergence and recovery periods

Large variations in measurements of shoot fresh weight were observed between genotypes during submergence and recovery periods. For instance, under submerged treatments, the increases in shoot fresh weight ranged from 0.3 to 23.5 and from 0.4 to 28.1 mg d^{-1} during submergence period and recovery period, respectively (Table 4).

The ratio of increases in shoot fresh weight of submerged treatment to non-submerged treatment ranged from 0.01 to 0.68 during submergence period and from 0.01 to 1.35 during recovery period (Table 4). At one day before submergence, the tolerant genotype FR13A had a higher shoot fresh weight (286 mg $plant^{-1}$) than intolerant genotype IR42 (122 mg $plant^{-1}$). During submergence period, susceptible genotype IR42 showed a higher increases in shoot fresh weight (6.4 mg d^{-1}) than a tolerant genotype FR13A (4.3 mg d^{-1}) under submerged treatment and vice versa under non-submerged treatment. All AG + *Sub1* lines and most genotypes in the different cluster groups showed a higher increase in shoot fresh weight than FR13A under submerged treatment and vice versa under non-submerged treatment.

During recovery period, increases in shoot fresh weight in most AG + *Sub1* lines of cluster I were occasionally comparable to those for FR13A under submerged treatments. However, the response was different for two of AG + *Sub1* lines which placed under cluster II. During submergence period, the ratios of increase in shoot fresh weight of submerged to non-submerged treatment for most genotypes in three cluster groups were higher than those obtained for FR13A, with the highest values being found in AG + *Sub1* lines. During recovery period, this ratio for most AG + *Sub1* lines was still higher than those obtained for FR13A (Table 4).

Evaluation of genotypes based on chlorophyll content during submergence and recovery periods

Under non-submerged treatments and 1 day before submergence, the AG + *Sub1* lines and susceptible genotype IR42 invariably displayed higher chlorophyll contents than tolerant genotype FR13A (Table 5). During submergence period, chlorophyll contents for 6 genotypes of cluster I,

including 4 genotypes from AG + *Sub1* lines, under submerged treatments were occasionally comparable to those obtained for FR13A. However, submerged treatment showed substantially reduced chlorophyll contents in other genotypes as found in IR42 (Table 5). During recovery period, chlorophyll contents for most genotypes in cluster I and II under submerged treatments were occasionally comparable to those contents in FR13A. However, submerged treatment showed substantially reduced chlorophyll contents in all genotypes in cluster III. Chlorophyll contents during recovery period were negatively correlated with the shoot elongation rate during submergence period (r = -0.35) ($P < 0.01$) (Fig. 2). Most AG + *Sub1* lines and FR13A were plotted in the area of slow shoot elongation rate and high chlorophyll contents among the tested genotypes.

Discussion

Based on the variations in growth characteristics during submergence period, escape and quiescence strategies are two contrasting mechanisms involved in the adaptation of contrasting rice genotypes to submergence stress (Perata and Voesenek, 2007; Bailey-Serres and Voesenek, 2008). Plants with quiescence mechanism are characterized by minimum shoot elongation, which enable them to use the energy economically and to recover quickly once the water receded. The ethylene-response-factor-like genes located at the *Sub1* locus were shown to play a key role in the operation of this mechanism (Fukao and Bailey-Serres, 2008). In contrast, plants with an escape mechanism respond to submergence by enhanced shoot elongation. Although the escape mechanism is an advantage under partial or prolonged submergence, in which the shoot elongation allows plants to resume aerobic metabolism and photosynthetic fixation of CO_2 by raising their shoots above water, a major disadvantage of this mechanism is that elongated seedlings uses energy and consumes carbohydrates in developed leaves before the submergence stress. The plants trend to lodge as soon as the water level recedes affects the recovery growth in young seedlings (Das et al., 2005; Fukao et al., 2006; Fukao and Bailey-Serres, 2008; Panda et al., 2008; Sakagami et al., 2009; Vu et al., 2010). In this study, these mechanisms have been tested in different contrasting rice genotypes including 9 new lines adapted to both anaerobic and submergence stress (AG + *Sub1*) by measuring shoot elongation rate, increase in shoot fresh weight and chlorophyll contents during submergence and recovery periods. The tested genotypes were generally classified into three clusters based on the ratios of submerged treatment to non-submerged treatment of shoot elongation rate during submergence period and chlorophyll contents during recovery period using Ward's method (Fig. 1). Cluster I was characterized by genotypes with slow shoot elongation rate during submergence period with maintenance of chlorophyll contents during recovery period and vice versa, observed in cluster III. The genotypes in cluster II followed the similar trend as cluster I, in addition of increases in shoot fresh weight during submergence period (Table 2). Generally speaking, the main mechanism inherent in most genotypes in clusters I and II, which enables them to cope the submergence stress, is the quiescence strategy. However, the genotypes placed in cluster III typically adapted to submergence stress using an escape strategy which is negatively adaptation to flash flood stress. Importantly, although the two types of mechanisms of submergence tolerance have been separated between the three clusters, some genotypes within each cluster showed different mechanism from those observed generally in each cluster. Although the genotypes placed in clusters I and II used quiescence strategy and the genotypes placed in cluster III used escape strategy based on their shoot elongation rate for submerged treatment during submergence period, some genotypes in cluster I (e.g. IR48, Rathal and WAB99-84) and cluster II (e.g. IR 06F434 and Kasalath) showed a higher shoot elongation rate as seen for the susceptible model IR42. In contrast, some genotypes in cluster III (e.g. Khao Kap Xang and PTB30) showed a lower shoot elongation rate as seen for the model tolerant FR13A (Table 3). In general, the shoot elongation rate of genotypes mentioned above showed similar trends under both submerged and non-submerged treatments during submergence period. However, the model tolerant FR13A showed a higher shoot elongation rate under non-submerged treatment, while it shifted toward a lower degree under submerged treatment. However, a reverse response was found in the susceptible model IR42 (Table 3). These inconsistencies in response to submergence stress may be due to the shoot elongation rate under submerged treatments, which may be associated with their elongation under non-submerged treatments. Results of the regression analysis showed a close relationship between shoot elongation rate of submerged and non-submerged treatments for each cluster during submergence period. However, these relationships were stronger for cluster II ($r = 0.77$, $P < 0.01$) and cluster III ($r = 0.52$, $P < 0.01$) than that in cluster I ($r = 0.30$, $P \leq 0.065$) (Fig 3). Compared to cluster I and III, the cluster II had relatively higher increases in shoot fresh weight for submerged treatment during submergence period (Table 2). Furthermore, increases in shoot fresh weight of submerged treatments significantly correlated with shoot elongation rate of submerged treatment during submergence period ($r = 0.57$ $P \leq 0.05$) and shoot fresh weight at 1 day before submergence ($r = 0.49$, $P \leq 0.05$) in cluster II. No correlation was found in cluster I and III (Figs 4 and 5). This finding implies that other mechanisms such as improving photosynthesis and anaerobic metabolism under water along with quiescence mechanisms might be associated with submergence stress in the genotypes in cluster II. Two AG + *Sub1* lines (IR06F434 and IR06F561) in cluster II expressed different responses during submergence compare to other AG+ *Sub1* lines placed in cluster I in shoot biomass increase, which needs to be more characterized in further studies. In addition, increases in shoot fresh weight in the genotypes placed in cluster II may be related to seedling vigor before submergence due to significant correlation between increase in shoot fresh weight and shoot fresh weight at 1 day before submergence (Fig. 5). These results are largely in agreement with Vu et al. (2010), who reported that the vigorous shoot growth before submergence treatments which enables rice seedlings to escape and survive the submergence stress. Other studies also found that pre-submergence stored carbohydrate are reported to be associated with enhanced survival under flooding conditions, possibly by supplying the required energy for maintenance of metabolism through anaerobic respiration (Sarkar et al., 2006). In addition, the characteristic of two genotypes in cluster II (IR 06F434 and Kasalath) show that the faster shoot elongation of submerged treatments during submergence period of both genotypes concomitant with increase in shoot fresh weight (Table 3). This result also indicates that, in some cases, plants may balance between different mechanisms during submergence period to survive, which could be beneficial for submergence tolerance. Manzur et al. (2009) also reported that a forage legume shows two different flexible mechanisms under submergence stress.

Table 3. Shoot height, shoot elongation rate, and the ratio of shoot elongation rate of submerged treatments to non-submerged treatments during 7 days of submergence and 5 days of desubmergence for different rice genotypes under non-submerged and submerged treatments.

Genotypes	Cluster groups	Shoot height at 1 day before submergence	Shoot elongation rate (cm d^{-1})				Sub./ Non-sub. ratio	
			During 7 days of sub.		During 5 days of de-sub.		During 7 days of sub.	During 5 days of de-sub.
			Non-submergence	Submergence	Non-submergence	Submergence		
IR 06F148	1	32.9	0.72	1.25	0.71	0.31	1.74	0.44
IR 06F168	1	35.3	0.66	0.62	0.38	0.28	0.94	0.74
IR 06F393	1	36.5	0.24	0.81	0.93	0.93	3.32	1.00
IR 06F459	1	36.8	0.61	0.61	1.85	0.19	1.00	0.10
IR 06F463	1	36.2	0.41	1.09	2.05	0.28	2.68	0.13
IR 07F297	1	29.2	0.49	1.04	0.80	0.50	2.13	0.62
IR 07F323	1	28.5	0.80	0.46	0.10	0.03	0.57	0.32
ARC10177	1	24.3	0.64	0.81	1.06	0.80	1.27	0.75
Bico Branco	1	45.2	0.30	0.10	0.28	0.83	0.33	2.95
DA28	1	39.7	1.15	0.99	2.35	0.27	0.86	0.12
Dholi Boro	1	44.7	0.58	0.81	3.21	0.82	1.39	0.26
Egyptian Jasmine	1	37.0	0.31	0.37	0.28	0.10	1.19	0.35
FR13A	1	40.0	1.37	0.66	1.41	0.12	0.48	0.09
IR24	1	36.8	0.27	0.69	0.08	0.12	2.53	1.60
IR48	1	33.0	0.54	2.08	0.95	0.20	3.84	0.21
IR60	1	43.7	0.89	1.21	0.61	0.03	1.35	0.06
IR74	1	29.9	0.49	1.03	0.91	0.56	2.08	0.61
Jhona26	1	43.3	0.76	1.02	0.24	0.48	1.34	1.98
Kataktara Da2	1	36.9	1.06	1.53	0.81	0.46	1.45	0.57
LAC23	1	36.6	0.57	1.16	1.10	0.36	2.03	0.32
Milyang 55	1	30.3	0.71	1.05	0.81	0.67	1.48	0.82
N22	1	40.5	0.55	1.24	2.26	0.95	2.25	0.42
NP125	1	49.5	1.07	1.21	0.14	0.03	1.14	0.26
Pachehai Perumal	1	52.3	1.19	1.20	1.68	0.04	1.01	0.02
Rathal	1	31.5	1.11	2.22	1.29	0.13	2.00	0.10
Sakha 103	1	49.2	0.61	1.34	0.44	0.56	2.22	1.29
Tadukan	1	36.8	0.41	0.15	0.74	1.08	0.36	1.46
Tchampa	1	42.6	0.44	1.16	0.19	0.03	2.67	0.13
WAB99-84(FRF1)	1	34.9	1.71	2.58	1.40	0.28	1.51	0.20
IR 06F434	2	37.0	0.42	1.65	1.40	0.04	3.93	0.03
IR 06F561	2	44.2	0.16	1.19	1.62	0.42	7.24	0.26
Chianung Si-Pi 661020	2	37.6	0.28	1.02	0.26	0.11	3.67	0.42
Giza 181	2	33.8	0.11	0.49	0.30	0.14	4.57	0.47
IR22	2	34.3	0.12	0.54	0.91	0.03	4.39	0.03
ITA212	2	32.6	0.22	1.12	0.65	0.10	5.18	0.15
Kasalath	2	39.5	0.35	1.71	1.15	0.02	4.90	0.02
Rikutou Nourin21	2	34.3	0.20	0.70	0.33	0.24	3.58	0.75
Baran Boro	3	41.0	1.82	1.64	0.33	0.60	0.90	1.79
Black Gora(NCS12)	3	37.0	1.49	3.06	0.63	0.19	2.05	0.30
C22	3	33.3	0.87	2.46	0.36	0.21	2.82	0.57
Canela de Ferro	3	36.8	1.25	3.04	1.68	0.03	2.42	0.02
CG17	3	37.5	1.62	2.71	1.47	0.43	1.67	0.29
Fircoz	3	42.1	0.83	2.27	0.76	0.41	2.73	0.53
Gharib	3	33.5	0.89	1.83	1.15	0.05	2.04	0.04
Giza 177	3	49.7	0.38	1.23	0.17	0.15	3.24	0.91
Gotak Gatik	3	41.3	1.58	2.05	0.82	0.04	1.30	0.05
IR 42	3	28.6	0.89	1.69	0.20	0.39	1.89	1.93
IR56	3	40.8	1.52	3.47	2.38	0.17	2.28	0.07
Khao Kap Xang	3	40.7	0.34	0.45	1.01	0.73	1.32	0.72
Mehr	3	41.4	1.09	2.76	1.72	0.40	2.54	0.23
Murungakayan302	3	41.6	2.79	3.80	0.21	0.18	1.36	0.86
Padi Lebat	3	44.6	0.84	1.82	1.10	0.71	2.17	0.65
PTB30	3	36.5	0.46	1.07	0.69	0.03	2.32	0.04
Shai-kuh	3	40.1	1.63	1.96	2.29	1.78	1.21	0.78
Surjamkuhi	3	34.3	1.23	1.53	0.90	0.22	1.24	0.24
Trembese	3	42.9	0.68	1.14	0.70	0.87	1.67	1.24

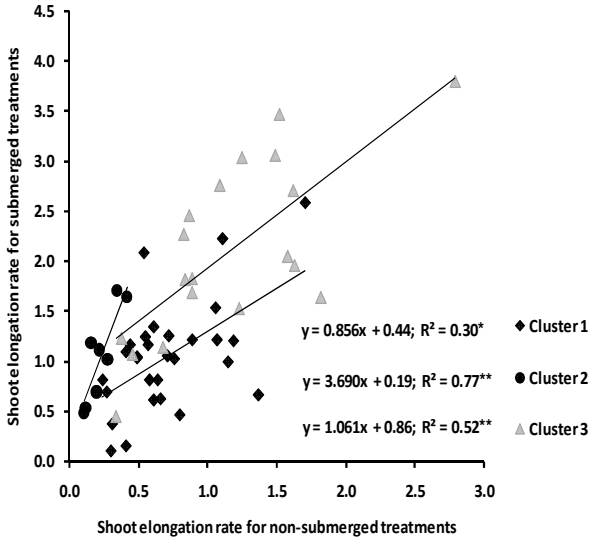

Fig 3. Relationships between shoot elongation rate for non-submerged treatments and for submerged treatments during submergence period. Asterisks indicate significant differences ($P < 0.01$) between two variables.

The plants are adapted to partial submergence by escape strategy, while they shift towards a non-elongating quiescent strategy by utilizing stored reserves when completely submerged. The capability of a rapid regrowth following de-submergence is another mechanism which plays an important role in the submergence tolerance of plants. Submergence tolerance by escape and quiescence strategies does not only require the corresponding regulation of growth and carbohydrate consumption during submergence period but also entails coordinated recovery of photosynthesis and growth following de-submergence (Sarkar et al., 2006; Panda et al., 2008; Luo et al., 2011).

In this study, the genotypes followed the quiescence strategy during submergence period, recovered more quickly than those followed the escape strategy after de-submergence. The growth recovery during de-submergence in this study is consistent with a lesser decrease in chlorophyll content during the first 5 days of de-submergence. The cluster I and II had higher chlorophyll contents during recovery period than cluster III (Table 2). In addition, a negative relationship was found between shoot elongation rate during submergence period and chlorophyll content during recovery period (r = 0.35, $P \leq 0.05$) (Fig 2).

These results are largely in agreement with Sone et al. (2011), who found that the non-shoot-elongating cultivar of rice coped with submergence by maintaining high chlorophyll content in the leaves, while the shoot-elongating cultivar were characterized by significant reduction in chlorophyll contents during recovery period. The lower chlorophyll contents of the de-submerged plants during the recovery period, presumably caused by structural and/or functional damage to chloroplasts (Panda et al., 2006), which may delay the recovery of carbohydrate accumulation and growth in these plants. Luo et al. (2011) reported that the growth recovery of the de-submerged plants must be strongly dependent on newly synthesized photo-assimilates, when carbohydrate storage consumed by shoot elongation during submergence. Ella et al. (2003) also reported that the high negative correlation between malondialdehyde contents at day 1 of recovery and chlorophyll contents at day 3 of recovery is another observation suggesting the importance of photosynthetic capacity for seedlings to survive during de-submergence.

Materials and methods

Plant materials

This study was conducted in 2011 using 56 rice (*Oryza sativa* L.) genotypes (Table 1). They were chosen based on their wide diversity of origins and their representation of widely various characters. Among them, nine lines were developed by the IRRI for anaerobic germination (AG) and submergence tolerance (*Sub*1). In addition, FR13A with a designated *Sub1* gene and IR42 without the *Sub1* gene were used as submergence-tolerant and submergence-intolerant genotypes, respectively.

Experimental details

Pre-germinated seeds were sown at the depth of 1 cm from the soil surface in a plastic tray (27.5 × 27.5 × 2.5 cm) filled with dried clay soil from a paddy field. For submerged treatments, 14-day-old seedlings were submerged completely in 80-cm-deep water in an acrylic glass container (3 m × 3 m × 1.3 m) for 7 days. Subsequently, the seedlings were re-exposed to air for 5 days. Non-submerged treatments were maintained under normal rice-cultivation conditions, with 5 cm of stagnant water above soil. The seedlings were grown before and after submergence and maintained after withdrawal of submergence in a laboratory growth chamber. The temperature was kept at 28°C from 6:00 to 18:00 h and at 25°C from 18:00 to 6:00 h. Artificial light was provided for 12 h during the day time. The mean irradiation level at 50 cm above water surface was 905 µmol m^{-2} s^{-1} PAR. The growth chamber humidity was maintained at 80% during the experiment period.

Growth and chlorophyll analysis

The experimental design was a randomized complete block design with four replications. Two plants per replication were selected from each genotype to measure the height and fresh weight of shoot at 1 day before and after submergence, and 1 day after de-submergence. Chlorophyll contents in active leaf blades were also determined at three set times using the extraction method. Each sample (about 10 mg FW^{-1}) was extracted in 80% ethanol and then enclosed in test tubes containing 10 ml of 80% (v/v) ethanol. To prevent chlorophyll degradation, the test tubes were placed in a dark cabinet for 48 h in a room at 4 °C. The chlorophyll contents were determined using spectrophotometry at 645 and 663 nm. The chlorophyll contents were expressed (µg mg^{-1} fresh weight) according to the following equation (Arnon, 1949).
Total chlorophyll content = [20.2 (A645) + 8.02(A663)]

Statistical analysis

All measurements in this study were analyzed using the ANOVA appropriate for a randomized complete block design with four replications. The data of ratios of submerged treatment to non-submerged treatments for shoot elongation rate during submergence period and chlorophyll content during recovery period were used for cluster analysis. Ward's minimum variance clustering method was used to classify

Table 4. Shoot fresh weight, increase in shoot fresh weight, and the ratio of increase in shoot fresh weight of submerged treatments to non-submerged treatments during 7 days of submergence and 5 days of desubmergence for different rice genotypes under non-submerged and submerged.

Genotypes	Cluster groups	Shoot fresh weight at 1 day before submergence	Increase in shoot fresh weight (mg d^{-1})				Sub./ Non-sub. ratio	
			During 7 days of sub.		During 5 days of de-sub.		During 7 days of sub.	During 5 days of de-sub.
			Non-submergence	Submergence	Non-submergence	Submergence		
IR 06F148	1	159	27.6	15.7	38.1	15.4	0.57	0.40
IR 06F168	1	196	33.5	12.6	25.9	10.9	0.38	0.42
IR 06F393	1	215	26.7	10.4	52.9	18.8	0.39	0.35
IR 06F459	1	198	28.3	9.7	63.2	6.5	0.34	0.10
IR 06F463	1	225	33.0	13.9	41.3	7.3	0.42	0.18
IR 07F297	1	178	21.1	14.3	52.6	16.9	0.68	0.32
IR 07F323	1	170	24.4	4.6	16.0	16.1	0.19	1.01
ARC10177	1	107	13.9	5.5	34.1	22.9	0.39	0.67
Bico Branco	1	228	25.2	3.0	35.9	23.2	0.12	0.65
DA28	1	217	36.1	3.4	45.9	22.7	0.09	0.49
Dholi Boro	1	206	36.6	7.8	55.9	2.7	0.21	0.05
Egyptian Jasmine	1	192	32.5	14.5	25.1	12.6	0.45	0.50
FR13A	1	286	45.9	4.3	79.6	19.0	0.09	0.24
IR24	1	177	25.0	15.5	12.2	7.8	0.62	0.64
IR48	1	171	31.0	20.2	53.8	2.5	0.65	0.05
IR60	1	217	30.5	8.6	23.5	19.9	0.28	0.85
IR74	1	143	23.7	9.6	26.0	7.5	0.41	0.29
Jhona26	1	228	44.0	15.4	46.6	4.4	0.35	0.09
Kataktara Da2	1	221	27.5	9.8	35.5	8.3	0.36	0.23
LAC23	1	198	32.0	7.4	28.7	16.4	0.23	0.57
Milyang 55	1	149	25.2	7.4	44.6	21.1	0.29	0.47
N22	1	168	23.2	9.4	54.0	8.1	0.40	0.15
NP125	1	263	62.8	8.8	50.0	1.7	0.14	0.03
Pachehai Perumal	1	292	51.5	18.3	98.9	6.5	0.36	0.07
Rathal	1	126	23.7	1.8	54.9	8.1	0.07	0.15
Sakha 103	1	221	36.8	7.5	53.8	10.0	0.20	0.19
Tadukan	1	247	23.7	3.6	44.8	8.6	0.15	0.19
champa	1	195	36.5	12.3	20.1	7.6	0.34	0.38
WAB99-84(FRF1)	1	194	35.4	8.2	57.4	8.5	0.23	0.15
IR 06F434	2	234	36.7	17.9	63.8	2.8	0.49	0.04
IR 06F561	2	239	37.1	19.3	56.9	0.4	0.52	0.01
Chianung Si-Pi 661020	2	167	25.8	11.1	23.9	14.2	0.43	0.59
Giza 181	2	183	20.8	3.0	15.0	20.3	0.14	1.35
IR22	2	170	23.7	8.8	28.1	7.6	0.37	0.27
ITA212	2	186	29.3	12.4	36.8	2.0	0.42	0.06
Kasalath	2	178	28.1	13.3	33.7	6.4	0.47	0.19
Rikutou Nourin21	2	151	24.2	10.9	27.3	10.4	0.45	0.38
Baran Boro	3	241	37.2	3.1	34.8	1.7	0.08	0.05
Black Gora(NCS12)	3	147	24.6	11.8	26.6	7.6	0.48	0.28
C22	3	109	19.9	8.9	35.7	7.1	0.45	0.20
Canela de Ferro	3	202	44.6	19.3	70.1	4.4	0.43	0.06
CG17	3	192	44.4	3.8	11.9	8.0	0.09	0.67
Fircoz	3	179	23.5	4.7	34.0	8.7	0.20	0.25
Gharib	3	176	29.5	11.9	48.1	13.6	0.40	0.28
Giza 177	3	216	33.7	23.5	64.2	5.0	0.70	0.08
Gotak Gatik	3	211	45.0	0.3	20.6	7.7	0.01	0.37
IR 42	3	122	23.7	6.4	13.6	7.0	0.27	0.51
IR56	3	196	41.2	5.6	59.3	6.0	0.13	0.10
Khao Kap Xang	3	141	19.4	6.1	24.9	18.8	0.31	0.76
Mehr	3	185	29.3	5.3	51.2	5.6	0.18	0.11
Murungakayan302	3	209	38.6	10.6	59.0	5.9	0.28	0.10
Padi Lebat	3	157	27.8	1.0	43.9	6.0	0.04	0.14
PTB30	3	136	19.0	11.2	34.8	14.2	0.59	0.41
Shai-kuh	3	220	51.8	13.2	91.5	21.4	0.25	0.23
Surjamkuhi	3	171	26.7	5.3	29.8	4.5	0.20	0.15
Trembese	3	193	39.7	1.8	59.4	28.1	0.04	0.47

Table 5. Chlorophyll content and the ratio of chlorophyll content of submerged treatments to non-submerged treatments during 7 days of submergence and 5 days of desubmergence for different rice genotypes under non-submerged and submerged.

Genotypes	Cluster groups	Chlorophyll content at 1 day before submergence	Chlorophyll content (µg mg^{-1}FW)				Sub./ Non-sub. ratio	
			During 7 days of sub.		During 5 days of de-sub.		During 7 days of sub.	During 5 days of de-sub.
			Non-submergence	Submergence	Non-submergence	Submergence		
IR 06F148	1	6.06	6.91	1.82	5.62	4.76	0.26	0.85
IR 06F168	1	5.28	6.76	1.85	5.82	3.73	0.27	0.64
IR 06F393	1	5.32	6.43	1.61	4.95	3.82	0.25	0.77
IR 06F459	1	5.69	4.80	2.18	5.24	3.98	0.45	0.76
IR 06F463	1	6.03	6.09	2.39	5.93	4.06	0.39	0.68
IR 07F297	1	5.95	5.76	2.62	6.06	4.78	0.45	0.79
IR 07F323	1	5.43	6.63	2.97	4.92	4.57	0.45	0.93
ARC10177	1	5.24	4.60	1.87	5.53	3.70	0.41	0.67
Bico Branco	1	4.18	5.18	1.53	4.52	2.96	0.29	0.65
DA28	1	5.14	4.70	0.87	4.41	2.75	0.18	0.62
Dholi Boro	1	4.67	4.49	1.20	3.87	2.57	0.27	0.67
Egyptian Jasmine	1	5.03	5.46	2.11	4.58	3.59	0.39	0.78
FR13A	1	4.50	4.90	2.18	4.80	4.32	0.44	0.90
IR24	1	5.22	5.90	1.57	4.97	3.18	0.27	0.64
IR48	1	4.77	6.05	1.72	4.38	3.23	0.28	0.74
IR60	1	4.81	5.43	1.19	4.18	3.05	0.22	0.73
IR74	1	5.06	6.63	1.51	5.95	3.61	0.23	0.61
Jhona26	1	4.77	5.44	1.50	4.84	3.19	0.28	0.66
Kataktara Da2	1	5.63	5.35	1.30	4.32	2.69	0.24	0.62
LAC23	1	4.65	4.60	1.81	4.40	2.73	0.39	0.62
Milyang 55	1	4.30	5.88	1.66	5.17	3.05	0.28	0.59
N22	1	4.50	5.07	0.81	5.17	3.26	0.16	0.63
NP125	1	5.41	4.37	1.81	4.22	2.75	0.41	0.65
Pachehai Perumal	1	4.80	5.94	1.72	4.32	2.88	0.29	0.67
Rathal	1	4.99	4.58	1.90	3.71	2.38	0.41	0.64
Sakha 103	1	5.23	6.32	1.55	5.06	3.31	0.24	0.65
	1	6.53	6.59	1.90	5.31	3.94	0.29	0.74
Tchampa	1	4.59	4.70	2.10	4.45	2.88	0.45	0.65
WAB99-84(FRF1)	1	4.45	4.14	1.18	3.71	2.36	0.29	0.64
IR 06F434	2	5.37	5.40	1.68	4.94	3.16	0.31	0.64
IR 06F561	2	5.31	5.57	1.98	4.52	3.29	0.36	0.73
Chianung Si-Pi 661020	2	5.61	5.23	1.56	5.79	3.42	0.30	0.59
Giza 181	2	5.19	4.98	1.98	5.30	3.03	0.40	0.57
IR22	2	5.29	6.85	1.48	5.15	3.36	0.22	0.65
ITA212	2	4.98	5.80	2.04	4.66	3.11	0.35	0.67
Kasalath	2	4.83	5.87	1.71	6.26	2.76	0.29	0.44
Rikutou Nourin21	2	5.32	6.00	1.33	5.19	2.94	0.22	0.57
Baran Boro	3	5.23	5.12	1.12	4.98	1.42	0.22	0.29
Black Gora(NCS12)	3	5.61	5.16	1.79	4.91	1.98	0.35	0.40
C22	3	4.91	4.72	1.29	5.18	2.55	0.27	0.49
Canela de Ferro	3	4.61	4.07	1.62	3.50	1.66	0.40	0.47
CG17	3	4.05	5.39	2.10	4.69	2.09	0.39	0.45
Fircoz	3	4.70	5.91	1.91	4.41	1.70	0.32	0.39
Gharib	3	4.69	5.44	1.38	5.04	2.82	0.25	0.56
Giza 177	3	4.90	5.81	1.88	5.12	2.28	0.32	0.45
Gotak Gatik	3	5.37	4.83	1.26	4.25	2.06	0.26	0.48
IR 42	3	5.71	6.43	1.41	5.75	2.95	0.22	0.51
IR56	3	5.44	5.63	2.07	4.88	2.23	0.37	0.46
Khao Kap Xang	3	5.68	6.52	1.48	5.49	2.98	0.23	0.54
Mehr	3	5.42	4.84	0.82	4.72	2.59	0.17	0.55
Murungakayan302	3	4.59	5.42	1.92	5.83	2.50	0.35	0.43
Padi Lebat	3	4.65	5.19	1.08	4.55	2.41	0.21	0.53
PTB30	3	4.62	4.17	1.60	4.51	2.00	0.38	0.44
Shai-kuh	3	5.55	4.63	1.75	5.64	1.59	0.38	0.28
Surjamkuhi	3	4.09	5.28	0.59	4.09	2.03	0.11	0.50
Trembese	3	4.54	4.69	2.09	4.00	2.12	0.45	0.53

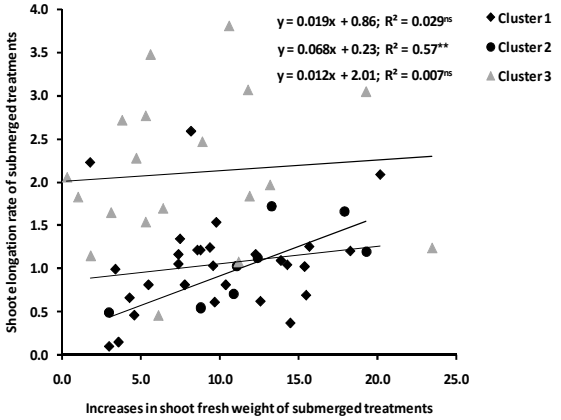

Fig 4. Relationships between increases in shoot fresh weight of submerged treatments and shoot elongation rate of submerged treatments during submergence period. Asterisks indicate significant differences (P < 0.01) between two variables.

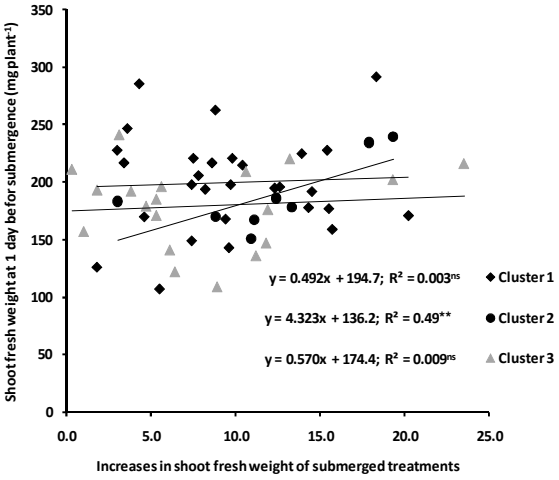

Fig 5. Relationships between increases in shoot fresh weight of submerged treatments during submergence period and shoot fresh weight at 1 day before submergence. Asterisks indicate significant differences (P < 0.01) between two variables.

genotypes into discrete clusters (Romersburg, 1988). The optimum number of clusters was determined by the sum of squares index (E) (Romersburg, 1988). Linear regression analyses were performed to investigate the relationship between different measurements during submergence and recovery period for three cluster groups. Regression analyses were performed using Microsoft Excel 2007.

Conclusion

In conclusion, quiescence and escape strategies are two mechanisms identified with tested rice genotypes for adaptation to flash flood tolerance. The genotypes followed quiescence strategy during submergence period had higher chlorophyll contents during recovery period than genotypes followed escape strategy. Other different mechanisms along with quiescence strategy might be associated with increasing of biomass production under submergence in genotypes placed in cluster II. Finally, flash flood tolerance does not only associate with growth behavior during submergence period but also entails coordinated recovery of photosynthesis and growth during de-submergence period.

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