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Adaptive traits associated with tolerance to flash flooding during emergence and early seedling growth stages in rice

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Abstract

Identification of adaptive traits associated with flash flooding tolerance during the initial growth stages is one approach to enhance seedling establishment in direct-seeded rice. To identify these traits, dry seeds of 53 contrasting genotypes were sown in soil and watered normally (control) or submerged with 10 cm of water for 17 days. Subsequently, the plants kept under normal rice cultivation conditions for a further 7 days. Among these genotypes, nine lines had been developed for anaerobic germination and submergence tolerance (AG + Sub1) by IRRI. The length of coleoptile and mesocotyl were measured at different times of submergence period. Length and dry weight of shoot and root were measured during submergence and recovery periods. Emergence date and percentage of plants reaching the floodwater surface were monitored during submergence period. The results showed that tested genotypes were classified into four clusters based on coleoptile and mesocotyl lengths at different times of submergence period using Ward's method. The coleoptile and mesocotyl of IR06F561 genotype that placed individually in cluster 4 and AG + Sub1 lines in cluster 2 elongated more rapidly than other genotypes under control and submerged treatments. Rapid elongation of coleoptile under submergence conditions was significantly correlated with the rapid seed emergence and the percentage of plant reached the floodwater surface. Shoot dry weight during recovery period was significantly correlated with the traits of fast seed emergence, rapid coleoptile elongation and vigorous shoot growth under submergence conditions. In conclusion, the AG + *Sub1* lines (IR06F561) were identified having adaptive traits that contribute to flash flooding tolerance during emergence and early seedling growth stages.

Keywords: Avoidance mechanisms; rapid coleoptile elongation; seedling establishment, seedling vigor; shoot elongation.
Abbreviations: AG_anaerobic germination; DLTRS- dates of leaf tips reached the floodwater surface; EDS- emergence date of seed from soil surface; IRRI_International Rice Research Institute; PSRS- percentage of seedlings reached the floodwater's surface; Sub_submergence.

Introduction

Rice is among crop plants that exhibit a superior ability to survive in flood-prone ecosystems. Therefore, rice farmers in rainfed and irrigated areas are shifting to direct seeding from transplanted as it provides opportunities to reduce labour required and can result in earlier harvest. However, poor germination and uneven seedling establishment, which always associated with direct seeding method in non-levelled land and in rainfed areas, limit the large-scale adoption of this method (Gangwar et al., 2008; Ismail et al. 2009; Ella et al., 2011). Seed priming has long been established as an effective technique for ensuring rapid and uniform seed germination under less favourable conditions such as flooded soils. However, problems associated with costs and technique skills are frequently encountered. Breeding cultivars with tolerance to flooding during germination and early seedling establishment could be a better alternative and will help avoid these problems. However, the progress in enhancing tolerance of flooding during the initial growth stages was very limited and plagued by the lack of suitable donors with a high level of tolerance and knowledge about the physiological basis of tolerance. Only 0.23% of accessions and lines with greater tolerance of flooding during germination and early seedling growth were identified following large-scale screening of 8,000 gene bank accessions and breeding lines at IRRI. Furthermore, subsequent screening cycles further reduced the number to approximately 0.06% (Ismail et al., 2009; Angaji et al., 2010). This implies that the availability of suitable genetic donors will be a key to introduce tolerance to flooding conditions during the initial growth stages. Therefore, it is important to conduct more studies into the genotypic variation and to identify the important traits associated with tolerance of flooding during germination and seedling growth stages.

The coleoptile and mesocotyl elongations of a rice seedling contribute to the emergence ability of dry seeded rice. Under flooding conditions, rapid elongation of coleoptiles and mesocotyle could facilitate contact with air and subsequent

aeration of the growing radicle and leaves (Magneschi et al., 2009; Alibu et al., 2011). Variation in the elongation abilities of the coleoptile and mesocotyl under flooding conditions was observed among diverse rice genotypes, in which the tolerant genotypes produce longer coleoptiles and their mesocotyle and coleoptile elongate faster than those of sensitive genotypes (Setter et al., 1994; Biswas and Yamauchi, 1997; Biswas et al., 2002; Huang et al., 2003; Chung, 2010). Ling et al. (2004) evaluated a set of 81 recombinant inbred lines developed from a cross between slow coleoptile elongation (0.3 cm) genotype and the other with faster coleoptile elongation (3.1 cm), under a water depth of 20 cm using a coleoptiles elongation as the criterion for selecting tolerant accessions. They detected five quantitative trait loci (QTL), one each on chromosomes 1, 2 and 7, and two on chromosome 5 with great effect on coleoptile elongation rate under flooding conditions. Furthermore, the coleoptile of tolerant genotypes emerged above the soil surface, 3–4 days earlier than sensitive genotypes. Additionally, the tip of their first leaf reached the floodwater surface 2–3 days earlier (Ismail et al., 2009). Therefore, the elongations of the coleoptile and mesocotyl as well as the ability of the first leaf to reach floodwater surface as early were considered as important traits related to crop establishment and tolerance to flooding stress during germination stage (Magneschi et al., 2009). This implies that greater rates of coleoptile and mesocotyl elongation and rapid seedling emergence are adaptive traits that facilitate tolerant genotypes to escape or avoid complete submergence during seed emergence stage.

During seedling growth stage, most of rice genotypes can bear the complete submergence stress by two opposite strategies: escape and quiescence (Perata and Voesenek, 2007). Genotypes using the quiescent strategy, which controlled by a single polygenic locus submergence-1 (Sub1A) on chromosome 9, conserve energy and carbohydrates by restraining shoot elongation. With this strategy, substantial amounts of reserved carbohydrates are required for the regrowth of young shoots once the floodwater recedes. Without adequate stored carbohydrates, the regrowth may not be complete leading to the death of rice plants (Das et al., 2005, Fukao et al., 2006; Perata and Voesenek, 2007; Sarkar and Bhattacharjee, 2011; El-Hendawy et al., 2012). The elongation escape strategy, which is associated with fast shoot elongation and seedling vigor under submergence, would be beneficial as the leaves above water surface would provide diffusive pathways supplying gasses to under water tissues (Sarkar and Bhattacharjee, 2011; Vu et al., 2012). Vu et al. (2010) reported that seedling vigor evaluated based on the shoot elongation ability and the recovery rate after submergence could serve as a submergence escape or avoidance mechanism and help rice seedlings survive under submergence during the post-germination stage. Most importantly, the expression of *Sub1A* gene in elongating seedling genotypes suggested that it does not always 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; El-Hendawy et al., 2012).

The capacity of genotypes for rapid growth recovery after de-submergence is a desirable trait because it can assure early recovery and production of sufficient biomass for optimum productivity. Plant's ability to recover after de-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 (Kawano et al., 2009; Luo et al., 2011). 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. The present investigation attempted to identify the adaptive traits associated with tolerance of flooding during emergence and early seedling growth stage using different rice genotypes including AG+Sub1lines. Additionally, we further examined the relationship between these traits and recovery capacity during de-submergence.

Results

Genotype clustering based on Coleoptile and mesocotyl lengths

Coleoptile and mesocotyl lengths are the two reliable traits that could be used for evaluating rice genotypes under submergence stress during germination stage. Based on the variation of both traits at 4, 7, 10, 13, and 17 days of submergence period under control and submerged treatments, 53 genotypes were grouped into four clusters using Ward's method. Cluster 2, 3 and 4 included 6, 22 and 24 genotypes, respectively, whereas one genotype from AG + Sub1 lines (IR06F561) was grouped individually in cluster 1. Cluster 2 included 5 genotypes from AG + Sub1 lines (IR06F434, IR06F148, IR06F168, IR06F459 and IR07F323) and Kasalath. However, the last three genotypes from AG + Sub1 lines (IR07 F297, IR06F393 and IR06F463) were placed in cluster 4. The name of genotypes for each cluster is presented in Table 2.

Evaluation of genotypes based on coleoptile and mesocotyl elongation at different time of submergence period

The length of coleoptile and mesocotyl of IR06F561 genotype that individually placed in cluster 1 is a unique among tested genotypes. Coleoptile and mesocotyl of this genotype elongated more quickly, reaching 30.5 and 4.2 mm under control treatment, and 35.5 and 6.8 mm under submerged treatment at 7 days of submergence period, respectively (Fig. 1). The coleoptile and mesocotyl of genotypes that formed cluster 2 reached their maximum length at 7 days of submergence period similar to cluster 1, but a mean length of coleoptile and mesocotyl of genotypes in this cluster was significantly lower 1.0- to 2-fold than that of the genotype in cluster 1 under both treatments (Fig. 1). The coleoptile length of the genotype in cluster 1 at 7 days of submergence period was significantly higher 6.0- and 3.5-fold than that of the genotypes in cluster 4 at 10 days, and 4.9- and 3.8-fold than that of the genotypes in cluster 3 at 17 days of submergence period under control and submerged treatments, respectively. The mesocotyl length of the genotype in cluster 1 at 7 days of submergence period was significantly higher 1.8- and 2.8-fold than that of the of the genotypes in cluster 4 at 10 days and cluster 3 at 17 days of submergence period under control and submerged treatments, respectively (Fig. 1).

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

Gen. No.	Genotype name	Seed DW (g)	Country of origin	Gen. No.	Genotype name	Country of origin	Seed DW (g)
1	IR 06F148 (AG + Sub1)	44.1	Philippines	28	IR 24	Philippines	47.9
2	IR 06F168 (AG + Sub1)	21.9	Philippines	29	IR29	Philippines	44.6
3	IR 06F393 (AG + Sub1)	40.0	Philippines	30	IR 42	Philippines	37.3
4	IR 06F434 (AG + Sub1)	49.0	Philippines	31	IR 48	Philippines	44.0
5	IR 06F459 (AG + Sub1)	40.0	Philippines	32	IR 56	Philippines	46.5
6	IR 06F463 (AG + Sub1)	45.6	Philippines	33	IR 60	Philippines	40.3
7	IR 06F561 (AG + Sub1)	23.9	Philippines	34	IR 74	Philippines	44.9
8	IR 07F297 (AG + Sub1)	44.9	Philippines	35	ITA 212	Nigeria	49.7
9	IR 07F323 (AG + Sub1)	34.3	Philippines	36	Jhona 26	Pakistan	45.8
10	ARC 10177	41.6	India	37	Kasalath	India	34.1
11	BaranBoro	44.0	Bangladesh	38	Kataktara Da2	Bangladesh	47.1
12	BicoBranco	62.5	Brazil	39	LAC 23	Liberia	64.6
13	Black Gora (NCS12)	54.8	India	40	Mehr	Iran	44.0
14	C 22	44.5	Philippines	41	Milyang 55	Korea	41.6
15	Canela de Ferro	57.2	Brazil	42	Murungakayan 302	India	44.5
16	CG 17	45.4	Senegal	43	N 22	India	39.1
17	Chianung Si-Pi 661020	49.6	Taiwan	44	PachehaiPerumal	India	54.2
18	DA 28	48.3	Bangladesh	45	PadiLebat	Indonesia	37.3
19	DholiBoro	40.5	Bangladesh	46	PTB 30	India	59.1
20	Egyptian Jasmine	49.8	Egypt	47	Rathal	Sri Lanka	31.3
21	Fircoz	46.1	Iran	48	Sakha 103	Egypt	49.0
22	FR13A	57.3	Philippines	49	Surjamkuhi	India	37.9
23	Gharib	54.5	Iran	50	Tadukan	Philippines	36.6
24	Giza 177	53.7	Egypt	51	Tchampa	Iran	59.3
25	Giza 181	48.4	Egypt	52	Trembese	India	47.9
26	GotakGatik	51.8	Indonesia	53	WAB99-84 (FRF1)	Ivory Coast	57.0
27	IR 22	56.1	Philippines				

Table 2. Emergence date of seed from soil surface (EDS) under control (Con.) and submerged (Sub.) treatments, percentage of seedlings reached the floodwater's surface (PSRS) and dates of leaf tips reached the floodwater surface (DLTRS) under submerged treatment for different rice genotypes and individual cluster group.

Genotypes	Cluster	EDS		PSRS	DLTRS	Genotypes	Cluster	EDS		PSRS	DLTRS
		Con.	Sub.					Non-sub.	Sub.		
IR06 F561 (AG+Su1)	1	4.0	4.0	100.0	7.5	IR07 F297 (AG +Sub1)	4	4.0	6.0	20.0	17.0
IR06 F148 (AG +Sub1)	2	4.0	5.0	86.7	9.7	IR 06F393 (AG +Sub1)	4	4.7	5.7	50.0	11.7
IR06 F168 (AG +Sub1)	2	4.2	5.3	96.7	10.2	IR 06F463 (AG +Sub1)	4	4.7	5.0	53.3	11.5
IR06 F434 (AG +Sub1)	2	5.2	5.3	70.0	10.0	C22	4	6.0	8.0	50.0	12.0
IR06 F459 (AG +Sub1)	2	5.2	5.0	76.7	11.0	BaranBoro	4	5.8	5.8	43.3	11.0
IR07 F323 (AG +Sub1)	2	4.2	4.5	70.0	11.2	Black Gora(NCS12)	4	4.5	5.0	63.3	11.0
Kasalath	2	4.8	4.7	90.0	9.7	Canela de Ferro	4	6.0	7.3	43.3	14.2
ARC10177	3	7.0	8.7	46.7	14.7	CG17	4	4.7	4.7	36.7	12.5
BicoBranco	3	6.7	8.3	20.0	14.5	DA28	4	5.7	5.2	80.3	10.8
ChianungSi-Pi 661020	3	7.3	8.7	20.0	17.0	DholiBoro	4	5.8	6.2	36.7	12.0
Fircoz	3	7.8	9.5	10.0	16.7	Egyptian Jasmine	4	4.0	4.2	90.0	9.8
IR 42	3	7.2	8.0	23.3	17.0	FR13A	4	5.3	5.5	43.3	12.3
IR 74	3	7.7	9.0	20.0	17.0	Gharib	4	6.5	8.0	33.3	14.2
IR22	3	5.5	5.8	43.3	13.3	Giza 177	4	5.2	5.2	56.7	11.7
IR29	3	4.8	6.3	40.0	13.0	Giza 181	4	4.0	6.0	46.7	13.2
IR56	3	5.7	6.5	46.7	13.0	GotakGatik	4	5.5	5.7	43.3	13.5
ITA212	3	7.7	8.3	20.0	16.8	IR24	4	5.7	5.7	40.0	13.8
Jhona 26	3	7.8	9.8	0.0	0.0	IR48	4	5.7	5.5	80.3	12.3
LAC23	3	6.7	8.2	20.0	17.0	IR60	4	5.5	5.7	66.7	11.8
Mehr	3	8.0	10.0	0.0	0.0	Kataktara Da2	4	6.0	6.2	83.3	11.2
Milyang 55	3	7.0	7.0	16.7	16.7	PachehaiPerumal	4	5.0	7.8	70.0	13.0
MURUNGAKAYAN302	3	7.3	8.7	0.0	0.0	PTB30	4	4.3	4.8	70.0	10.2
N22	3	6.0	7.5	23.3	17.0	Surjamkuhi	4	5.2	5.7	76.7	11.2
PadiLebat	3	8.7	9.3	20.0	17.0	Tadukan	4	6.0	6.0	76.7	12.3
Rathal	3	6.7	8.0	10.0	16.3	Means for each individual cluster					
Sakha 103	3	6.7	8.5	23.3	15.7	Cluster 1		4.0 c	4.0 c	100.0a	7.5 d
Tchampa	3	7.7	8.7	20.0	17.0	Cluster 2		4.6 c	5.0 bc	81.7 b	10.3 c
Trembese	3	8.0	9.7	0.0	0.0	Cluster 3		7.1 a	8.3 a	20.2 d	13.0 a
WAB99-84(FRF1)	3	7.2	8.0	20.0	17.0	Cluster 4		5.2 b	5.9 b	56.4 c	12.3 b

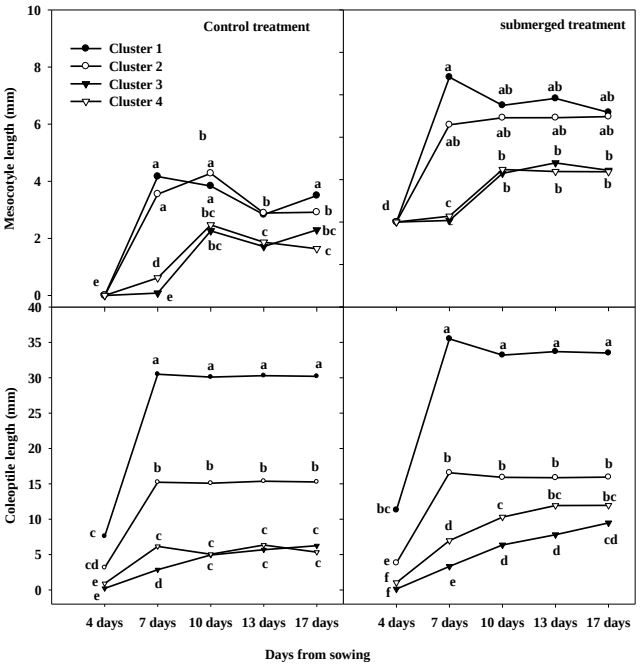

Fig 1. Coleoptile and mesocotyl lengths of four clusters group of genotypes at different days from sowing under control and submerged treatments. The value of each point was significantly different at $P < 0.05$).

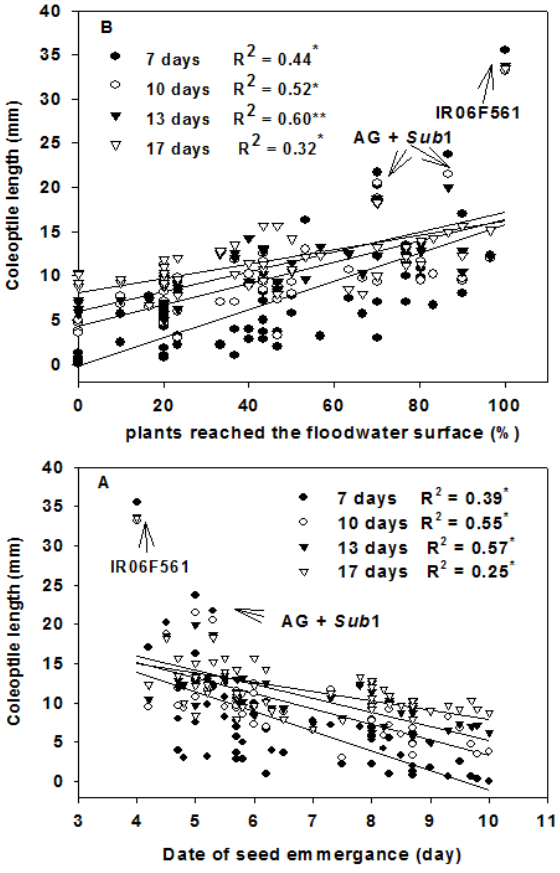

Fig 2. Relationships between coleoptile lengths measured at different time of submergence period and date of seed emergence from the soil surface (A) and percentage of plant reached the floodwater surface (B). Asterisks indicate significant differences ($P < 0.05$) between each two traits.

Evaluation of genotypes based on date of seed emergence from the soil surface and percentage of plants reached the floodwater's surface

According to the cluster analysis described above for coleoptile and mesocotyl length, the seeds of genotype in cluster 1 (IR06F561) started to appear from the soil surface earlier than the other genotypes under control and submerged treatments (4 days after sowing). The seeds of genotypes that formed clusters 2, 4 and 3 required 0.5, 1.2 and 3.1 additional days under control treatments, and 1.0, 1.9 and 4.3 additional days under submerged treatments to emergence from the soil surface when compared with the IR06F561 genotype (Table 2). Furthermore, the leaf tips of all plants that monitored during the entire period of submergence (100%) of the IR06F561 genotype came out above the floodwater surface within 7.5 days after sowing. Whereas, 81.7, 56.4 and 20.2% from the plants that monitored during the entire period of submergence of the genotypes in cluster 2, 4 and 3 reached the floodwater surface, and their leaf tips came out above the floodwater surface within 10.3, 12.3 and 13.0 days after sowing, respectively (Table 2).

Results of regression analysis showed that a close relationship existed between coleoptile lengths measured at different time of submergence period and the date of seed emergence from the soil surface (Fig. 2a) or percentage of plant reached the floodwater surface (Fig. 2b). However, these relationships were stronger for the coleoptile length measured at 7 and 10 of submergence period. Furthermore, the IR06F561 genotype was plotted in the area of long coleoptile length and earlier emergence date or high percentage of plant reached the floodwater surface. The genotypes from AG + Sub1 lines that placed in cluster 2 were plotted in area near from IR06F561 genotype (Fig. 2a, b).

Evaluation of genotypes based on shoot and root lengths

Significant variations for shoot and root lengths were observed among genotypes at 10, 13 and 17 days of submergence period and 7 days after recovery period (Tables 3 and 4). The IR06F561genotype, which had the longest coleoptile and mesocotyl lengths, produced also the longest shoot and root lengths at 10 and 13 days of submergence period under control and submerged treatments. The shoot and root lengths of AG + *Sub1* lines that placed in cluster 2, according to their coleoptile and mesocotyl lengths, were also much higher than the other genotypes, but it were still lower than that of IR06F561genotype. All genotypes that placed in cluster 3 produced the shortest shoot and root lengths at 10 and 13 days of submergence period under both conditions. For instance, under submerged treatment, the maximum shoot length of the genotypes in this cluster was less than 3.0 and 7.0 cm as compared with 16.5 and 30.1 cm for R06F561genotype at 10 and 13 days of submergence period, respectively. The maximum root length was less than 3 and 5 cm as compared with 7.1 and 8.5 cm for R06F561genotype at 10 and 13 days of submergence period, respectively (Tables 3 and 4). However, the shoot and root lengths of some genotypes in clusters 4 such as Baran Boro, Black Gora (NCS12), Egyptian Jasmine, Giza 177, Gotak Gatik, Pachehai Perumal, and PTB30 were competitive with IR06F561 genotype under both conditions. The shoot and root lengths of the most genotypes that formed cluster 3 were occasionally comparable to those of IR06F561 genotype only

Table 3. Shoot length of 53 contrasting rice genotypes at several times during submergence period and at 7 days after recovery period under control and submerged treatments.

Genotypes	Cluster	Shoot length under control treatments (cm)				Shoot length under submerged treatments (cm)			
		Submerged period			Recovery period	Submerged period			Recovery period
		10 days	13 days	17 days	7 days	10 days	13 days	17 days	7 days
IR06 F561 (AG+Su1)	1	19.0	32.0	49.6	56.8	16.5	30.1	43.2	50.4
IR06 F148 (AG +Sub1)	2	10.6	19.7	34.3	47.3	9.1	19.4	27.8	40.5
IR06 F168 (AG +Sub1)	2	11.1	20.3	35.0	46.6	6.5	20.2	28.4	41.6
IR06 F434 (AG +Sub1)	2	9.6	17.5	34.5	44.1	9.2	12.7	30.4	41.1
IR06 F459 (AG +Sub1)	2	9.3	18.5	29.6	45.6	7.2	13.8	29.3	39.3
IR07 F323 (AG +Sub1)	2	9.0	14.0	27.9	37.6	6.9	11.3	17.8	25.0
Kasalath	2	3.1	19.4	29.4	43.1	2.5	22.0	32.0	37.8
ARC10177	3	0.2	4.1	17.2	41.0	0.1	0.2	16.1	36.0
BicoBranco	3	1.4	7.0	36.0	45.5	2.5	4.4	22.2	37.1
ChianungSi-Pi 661020	3	0.2	1.3	15.9	37.7	0.5	0.6	11.0	33.0
Fircoz	3	0.2	1.5	15.0	38.7	0.1	2.9	18.5	31.8
IR 42	3	3.9	6.0	20.6	34.2	1.6	4.2	20.8	27.6
IR 74	3	0.3	1.5	18.6	37.5	0.1	0.8	17.3	26.2
IR22	3	2.0	8.9	20.8	34.5	1.4	6.5	18.1	33.4
IR29	3	2.0	7.4	17.3	35.9	0.6	3.3	18.1	23.7
IR56	3	1.2	7.9	25.5	37.7	0.7	6.0	26.7	32.1
ITA212	3	0.5	1.4	15.3	32.7	2.3	5.3	16.5	26.5
Jhona 26	3	0.6	1.1	13.2	37.7	0.1	0.8	3.4	30.0
LAC23	3	0.4	4.1	13.7	43.1	0.9	4.3	14.2	32.3
Mehr	3	1.1	1.1	18.8	42.5	0.1	0.8	5.8	33.8
Milyang 55	3	1.5	6.1	21.0	37.8	1.0	5.7	24.9	31.5
MURUNGAKAYAN302	3	1.1	1.4	15.7	49.0	0.6	1.4	8.3	35.1
N22	3	1.4	7.1	17.7	39.3	0.1	2.7	6.4	34.5
PadiLebat	3	0.4	1.6	17.5	35.5	0.1	1.1	19.9	32.3
Rathal	3	3.0	3.9	23.1	40.8	1.2	5.6	24.6	31.1
Sakha 103	3	1.5	3.4	18.3	52.1	2.2	2.8	18.5	43.4
Tchampa	3	0.3	3.0	19.4	38.1	0.1	0.2	9.9	38.3
Trembese	3	0.1	2.1	20.4	42.1	0.1	0.7	12.0	34.7
WAB99-84(FRF1)	3	0.5	3.9	19.7	45.1	0.5	3.7	18.8	32.8

Table 3. Continued

Genotypes	Cluster	Shoot length under control treatments (cm)				Shoot length under submerged treatments (cm)			
		Submerged period			Recovery period	Submerged period			Recovery period
		10 days	13 days	17 days	7 days	10 days	13 days	17 days	7 days
IR07 F297 (AG +Sub1)	4	4.2	4.2	23.6	37.0	5.3	5.3	9.6	24.1
IR 06F393 (AG +Sub1)	4	7.8	16.2	28.4	39.7	3.4	16.0	24.3	33.2
IR 06F463 (AG +Sub1)	4	7.6	16.6	27.5	41.1	7.4	12.4	24.8	36.6
C22	4	1.5	8.5	25.5	41.2	1.1	7.0	22.8	33.2
BaranBoro	4	1.6	14.2	33.7	51.2	0.5	10.4	27.5	38.7
Black Gora(NCS12)	4	2.2	11.3	26.5	43.4	1.8	16.4	31.3	37.8
Canela de Ferro	4	1.5	8.5	21.8	42.4	0.1	6.9	27.7	37.8
CG17	4	1.1	7.8	21.0	48.7	0.4	11.5	26.7	36.5
DA28	4	2.6	15.6	28.9	37.6	1.7	20.3	26.2	38.7
DholiBoro	4	1.4	11.4	27.5	45.0	0.8	6.7	16.7	38.6
Egyptian Jasmine	4	8.4	18.1	30.0	40.9	7.0	17.7	32.2	41.2
FR13A	4	6.2	13.3	37.4	47.3	5.5	11.1	29.2	39.3
Gharib	4	1.5	7.4	22.4	43.3	0.1	3.4	19.0	33.9
Giza 177	4	2.7	12.8	31.9	47.0	3.0	15.8	36.6	45.6
Giza 181	4	3.6	7.0	28.3	36.9	1.6	13.6	25.3	30.7
GotakGatik	4	1.5	9.4	23.2	48.5	0.6	7.6	23.5	43.2
IR24	4	1.5	10.5	25.3	38.0	0.8	7.3	24.4	33.2
IR48	4	1.9	10.2	21.9	38.7	2.7	8.6	26.6	34.3
IR60	4	1.4	7.1	22.5	37.4	1.3	8.8	24.8	32.0
Kataktara Da2	4	1.8	12.7	20.7	36.1	2.7	15.6	26.0	36.5
PachehaiPerumal	4	2.0	20.9	38.4	59.1	1.9	7.8	26.1	51.6
PTB30	4	1.7	16.8	28.0	49.8	2.1	18.0	31.3	46.6
Surjamkuhi	4	1.9	12.6	20.0	36.9	3.2	12.9	25.4	36.3
Tadukan	4	2.4	14.6	24.2	47.6	2.7	11.7	26.1	35.5
LSD (0.05)									
Sub. treatments (T)		ns	ns	1.8	3.6				
Genotypes (G)		3.1	6.0	5.6	7.2				
T × G		4.8	7.1	8.9	5.3				

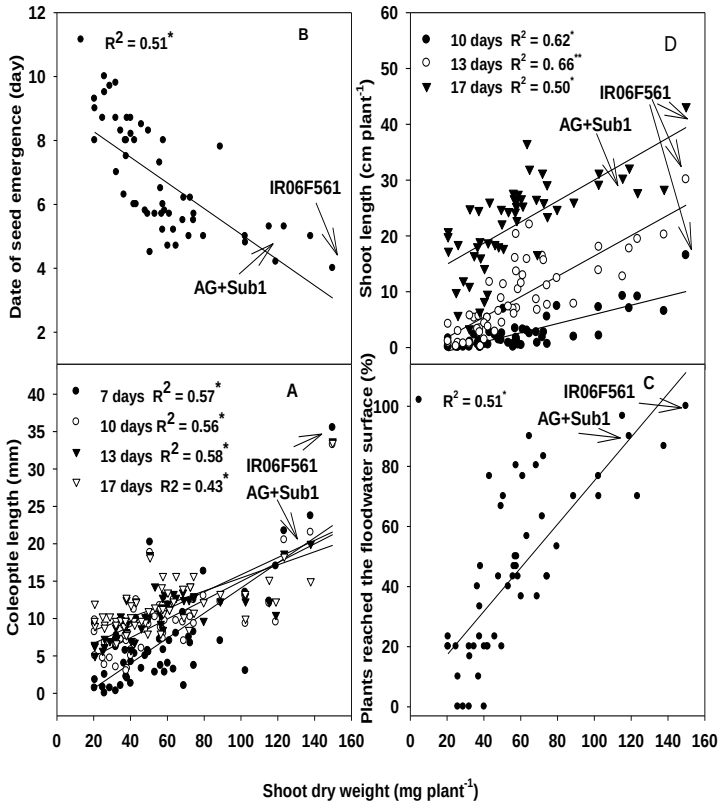

Fig 3. Relationships between shoot dry weight measured at 7 days after recovery period under submerged treatment and coleoptile lengths (A), date of seed emergence from the soil surface (B), percentage of plant reached the floodwater surface (C) and shoot length under submerged treatment. Asterisks indicate significant differences ($P < 0.05$) between each two traits.

under control treatment at 7 days after recovery period. However, significant differences between the shoot and root lengths of genotypes in cluster 3 and IR06F561 genotype were still observed at 17 days of submergence period and at 7 days after recovery period under submerged treatment (Tables 3 and 4).

Evaluation of genotypes based on shoot and root dry weight

Significant genotypic differences were observed in shoot and root dry weight at different times of submergence and recovery periods under control and submerged treatments (Tables 5 and 6). The IR06F561 showed the best performance in shoot and root dry weight among the genotypes especially at 10 and 13 days of submergence period under both conditions. For instance, the shoot dry weight of IR06F561 genotype at 10 days of submergence period was higher 2.2-, 16.3-and 7.4- fold under control treatment, and 2.1-, 8.7- and 4.6-fold under submerged treatment than that of the mean values for the genotypes in cluster 2, 3 and 4, respectively. At 13 days of submergence period, the shoot dry weight of IR06F561 genotype was higher 2.6-, 15.7- and 7.1-fold under control treatment, and 2.8-, 7.0- and 3.1-fold under submerged treatment than that of the mean values for the genotypes in cluster 2, 3 and 4, respectively. The gap differences in shoot and root dry weight between IR06F561 genotype and some other genotypes such as Egyptian Jasmine in cluster 4 and IR06F148, IR06F168, IR06F434 and IR06F459 in cluster 2 were narrowed at 13 days of submergence period and 7 days after recovery period especially under submerged treatment. However, the gap differences in shoot and root dry weight between IR06F561 genotype and all genotypes in cluster 3 were still wide enough at 13 days of submergence period and 7 days after recovery period under both conditions (Tables 5 and 6).

The shoot dry weight measured at 7 days after recovery period under submerged treatment was positively correlated with the coleoptile length measured at 7, 10, 13 and 17 days of submergence period (Fig. 3a), the date of seed emergence (Fig. 3b), the percentage of plant reached the floodwater surface (Fig. 3c), and the shoot length measured at 10, 13 and 17 days of submergence period (Fig. 3d) under submerged treatment. Furthermore, the IR06F561 genotype was plotted in the area of high shoot dry weight with long coleoptile length, earlier emergence date of seed, high percentage of plant reached the floodwater surface or long shoot length under submerged treatments. The genotypes from AG + Sub1 lines that placed in cluster 2 were plotted in area near from IR06F561 genotype.

Discussion

The first step toward genetic improvement and enhancing seedling establishment under flooding conditions is to identify the adaptive traits associated with flooding tolerance during the initial growth stages (germination, emergence, and seedling). In an earlier screening study, we indicated traits that are likely to be associated with anaerobic tolerance during germination and vegetative stages using the same contrasting rice genotypes that used in this study. In those studies, we found that rapid germination under anaerobic conditions is related to the traits of rapid water uptake and regulation of water uptake during rice seed imbibition (El-Hendawy et al., 2011). Traits associated with a quiescence strategy, such as minimum shoot elongation coupled with maintaining high levels of stored carbohydrates during submergence, play a key role in submergence tolerance in the vegetative stage (El-Hendawy et al., 2012). In the present study, we further identified the traits associated with flooding tolerance at emergence and early seedling stages.

Several studies have reported that rapid elongation of coleoptile and mesocotyl are important factors in seedling rice establishment under flooding conditions and the percentage of seedlings that reach the expanded second leaf stage is highly correlated with the time required for coleoptile and mesocotyl emergence after germination. In addition, considerable variation in coleoptile and mesocotyl elongation rates has been existed among rice genotypes (Setter et al., 1994; Huang et al., 2003; Fukuda et al., 2008). The tolerant genotypes elongate their coleoptile and mesocotyl more rapidly than intolerant genotypes in an attempt to enable the seedlings to make contact with the atmosphere and; thus, allow oxygen transport to roots through aerenchyma (Setter et al., 1994; Biswas and Yamauchi, 1997; Ling et al., 2004). Therefore, rapid elongation of the coleoptile and mesocotyl under complete submergence are necessary traits conferring flooding tolerance at the initial growth stage. The results of this study demonstrated that the coleoptile and mesocotyl of IR06F561 genotype that placed individually in cluster 1 elongated more rapidly and the coleoptile length of this genotype was much higher than other genotypes under control and submerged treatments (Fig. 1). The coleoptile and mesocotyl of genotypes in cluster 2, which include 5

genotypes from AG + Sub1 lines, were also elongated rapidly similar to IR06F561 genotype, whereas the coleoptile length of these genotypes was lower 1.0- to 2-fold than that of IR06F561genotype under both treatments with occasional comparable in mesocotyl length between both clusters. However, the coleoptile and mesocotyl of genotypes in clusters 3 and 4 elongated more slowly, and the lengths of both parts were also much lower as compared with IR06F561genotype (Fig. 1).

It is interesting to note that the initial dry seed weight of IR06F561genotype, which produced the longest coleoptile and mesocotyl lengths, was much lower than the other genotypes (Table 1). This result suggests that the rapid elongation of coleoptile and mesocotyl did not depend just on the total amount of food reserve in seed. This observation is in agreement with Fukuda et al. (2008), who reported that the differences in coleoptile length among genotypes were not depended just on the total amount of protein but also depended on the sucrose synthase activity per mg protein as well. The long-coleoptile genotypes have a better system to enhance sucrose synthase activity per mg protein than short-coleoptile genotypes. This system helps long-coleoptile genotypes to use energy more efficiently and enhance coleoptile growth under submerged conditions.

The results of this study also demonstrated that coleoptile lengths measured at different time of submergence period were negatively correlated with the date of seed emergence from the soil surface (Fig. 2a) and positively correlated with the percentage of plant reached the floodwater surface (Fig. 2b). These results indicate that rapid elongation of coleoptile under submergence conditions is correlated with the rapid seed emergence from the soil surface and in the same time is one reason for increasing the percentage of plant reached the floodwater surface. Therefore the IR06F561 genotype, which produced the longest coleoptile length, was plotted in the area of earlier seed emergence date and high percentage of plant reached the floodwater surface (Fig 2a, b). Conversely, the seeds of genotypes that produced the shortest coleoptile length, such as genotypes in cluster 3 and 4, emerged slowly from the soil surface under both conditions, and only 20.2 and 56.4 % of their plants reached the floodwater surface during submergence period. In addition, the leaf tips of the genotypes in clusters 3 and 4 did not came out above the floodwater surface until about 13.0 and 12.3 days after sowing, respectively, compared with 7.5 days for IR06F561 genotype and 10.3 days for genotypes in cluster 2 (Table 2).

Rapid emergence of leaf tips above floodwater surface, which is related with rapid elongation of coleoptile, can have a number of potential benefits such as the rapid restoration of contact with atmosphere which will promote gas exchange between submerged plant organs and atmosphere, aerate potentially submerged plant tissues, enhance potential for photosynthesis as a result of access to atmospheric CO_2 and ventilate of accumulated gaseous components such as ethylene, which at high concentration, interferes with normal root growth (Colmer, 2003; Pierik et al., 2009). Therefore, it is reasonable to assume that rapid elongation of coleoptile under submergence conditions can be considered as a target trait helping tolerant genotypes to escape or avoid complete submergence during the initial growth stage.

Vu et al. (2012) reported that the rapid and vigorous shoot elongation during submergence, which is constitutes an important trait in the elongation escape or avoidance strategy, has also been considered as a target trait to help tolerant genotypes to escape or avoid complete submergence during the early seedling growth stage. Rapid and vigorous shoot elongation under submergence conditions reflect the ability of intercalary meristems that respond quickly to flood water to escape from unfavorable stresses and suggested the high priority of photosynthesis during submergence period and initial recovery period by keeping normal metabolic pathways (Won and Yoshida, 2000; Cisse and Ejeta, 2003). In addition, this mechanism is advantageous for rice seedlings subjected to submergence stress just after germination (Vu et al., 2010). Cui et al. (2002) also reported that germination rate and early seedling growth were two major traits related to rapid shoot elongation. Furthermore, shoot elongation during submergence depends on genetic architecture and is affected by the state of seedling growth before submergence. The results of the present study confirm these findings and show that the IR06F561 genotype represents a typical model genotype, which uses fast and vigorous shoot elongation traits as key mechanisms to avoid or escape submergence stress during the early seedling stages. The shoot length and dry weight of this genotype was much higher than other genotypes especially at 10 and 13 days of submergence period under both conditions (Tables 3 and 5). Shoot length of some genotypes in cluster 4 such as Egyptian Jasmine and PTB 30 and the AG + sub1 genotypes in cluster 2 were competitive with those for IR06F561 genotype under both conditions. Therefore, these genotypes showed good recovery in terms of shoot dry weight after de-submergence. Conversely, the genotypes in clusters 3, which produced the shortest coleoptile, mesocotyl and shoot lengths during submergence period, showed much lower recovery in terms of shoot dry weight after de-submergence when compared with the tolerant and vigorous model genotype (IR06F561) (Tables 5 and 6).

The gene *Sub1* has the corresponding alleles of *Sub1A*, *Sub1B* and *Sub1C*. The *Sub1A* causes quiescence of growth through diminishing ethylene production and gibberellin responsiveness and vice versa. The *Sub1C* causes greater elongation of the shoot under submergence conditions (Fukao and Bailey-Serres, 2008; Bailey-Serres et al. 2010; Bailey-Serres and Voesenek, 2010). Earlier reports had suggested that *Sub1A* dominated over *Sub1C* triggered down regulation of *Sub1C*. Then it was likely that presence of *Sub1A* restricted shoot elongation under submergence ((Fukao et al., 2006; Xu et al., 2006). The results of this study and the studies of Vu et al. (2010) and Sarkar and Bhattacharjee (2011) confirmed that the presence of *Sub1A* does not always hinder the shoot elongation growth under submergence conditions at the early seedling growth stage. In this study, the rapid shoot elongation during submergence and rapid recovery during de-submergence in terms of shoot dry weight were occurred in most AG + sub1 lines.

Materials and Methods

Plant materials

This study was conducted in 2012 using 53 rice genotypes (*Oryza sativa* L.). The name of genotypes, their sources of origin and initial dry seed weight are listed in Table 1. These genotypes were chosen based on a wide diversity of origins and their representation of a wide range of variability. Among them, nine lines were developed by the IRRI for anaerobic germination (AG) and submergence tolerance (*Sub1*).

Experimental details

The experiment was carried out in a randomized complete block split plot design with three replicates. The treatments of

Table 4. Root length of 53 contrasting rice genotypes at several times during submergence period and at 7 days after recovery period under control and submerged treatments.

Genotypes	Cluster	Root length under control treatments (cm)				Root length under submerged treatments (cm)			
		Submerged period			Recovery period	Submerged period			Recovery period
		10 days	13 days	17 days	7 days	10 days	13 days	17 days	7 days
IR06 F561 (AG+Su1)	1	7.0	11.1	11.2	16.5	7.1	8.5	8.6	19.7
IR06 F148 (AG +Sub1)	2	8.7	9.2	10.9	20.0	6.3	7.3	8.8	17.3
IR06 F168 (AG +Sub1)	2	6.5	6.8	10.3	16.9	4.7	6.9	7.7	12.3
IR06 F434 (AG +Sub1)	2	4.2	7.4	8.2	11.8	3.5	4.4	6.6	13.8
IR06 F459 (AG +Sub1)	2	6.2	7.6	8.7	16.1	4.9	7.0	8.3	12.9
IR07 F323 (AG +Sub1)	2	6.8	8.1	10.0	11.0	6.4	7.5	7.6	10.2
Kasalath	2	4.6	9.1	9.9	13.7	3.5	9.1	9.6	11.1
ARC10177	3	0.6	3.0	5.6	10.4	0.0	0.0	5.2	8.1
BicoBranco	3	2.6	4.6	9.1	12.6	1.2	2.6	7.1	8.6
ChianungSi-Pi 661020	3	0.9	2.9	5.4	8.6	0.7	0.7	4.8	8.0
Fircoz	3	0.0	2.7	6.3	10.2	0.0	1.5	4.0	7.2
IR 42	3	4.4	4.7	9.5	11.4	1.3	4.3	6.1	7.2
IR 74	3	0.5	1.8	6.1	9.2	0.5	0.6	5.6	7.5
IR22	3	3.4	6.1	7.4	10.7	2.0	4.5	5.9	8.9
IR29	3	1.8	6.2	7.9	14.0	0.5	2.7	6.9	8.2
IR56	3	1.8	7.2	8.8	14.7	1.0	4.4	6.2	10.3
ITA212	3	2.1	2.9	7.3	9.4	2.3	4.0	6.1	7.2
Jhona 26	3	1.6	1.9	5.9	8.8	0.3	0.8	2.9	8.2
LAC23	3	1.3	3.7	7.6	10.3	0.5	1.4	4.6	8.3
Mehr	3	1.6	1.7	6.6	9.6	0.1	0.7	4.2	7.3
Milyang 55	3	2.0	4.9	7.3	10.0	1.3	4.1	6.7	7.9
MURUNGAKAYAN302	3	2.3	2.8	5.3	9.7	0.6	2.6	4.7	7.3
N22	3	1.7	6.5	7.8	13.4	0.1	2.3	4.5	10.9
PadiLebat	3	0.3	3.0	6.0	8.6	0.2	0.5	5.1	7.5
Rathal	3	3.4	4.3	9.7	11.4	1.4	2.2	6.4	6.9
Sakha 103	3	3.7	4.2	7.9	11.1	1.7	1.8	6.3	7.5
Tchampa	3	0.2	3.4	8.6	11.2	0.0	0.0	5.3	10.8
Trembese	3	0.8	2.6	7.6	11.8	0.1	1.5	5.4	10.0
WAB99-84(FRF1)	3	1.7	3.8	7.3	10.2	0.4	2.4	6.0	7.8

Table 4. Continued

Genotypes	Cluster	Root length under control treatments (cm)				Root length under submerged treatments (cm)			
		Submerged period			Recovery period	Submerged period			Recovery period
		10 days	13 days	17 days	7 days	10 days	13 days	17 days	7 days
IR07 F297 (AG +Sub1)	4	4.2	4.3	9.7	13.6	4.1	4.1	6.5	9.4
IR 06F393 (AG +Sub1)	4	5.6	6.6	9.3	20.4	3.1	5.8	6.1	7.5
IR 06F463 (AG +Sub1)	4	4.2	7.5	8.0	13.0	4.4	6.4	6.7	9.6
C22	4	2.4	5.6	7.4	12.0	0.7	3.0	6.9	10.0
BaranBoro	4	4.2	9.4	12.7	15.8	0.5	7.4	7.9	11.3
Black Gora(NCS12)	4	3.4	7.8	11.7	19.5	2.0	7.7	10.5	16.6
Canela de Ferro	4	2.4	6.1	8.6	14.7	0.4	3.5	6.9	10.3

CG17	4	1.8	5.9	8.7	13.3	1.6	5.8	6.0	12.6
DA28	4	3.4	8.0	12.2	19.3	1.3	6.1	7.7	14.4
DholiBoro	4	3.0	10.0	11.0	15.6	0.8	4.7	8.5	12.3
Egyptian Jasmine	4	6.3	6.8	8.3	18.4	2.9	5.9	7.9	12.8
FR13A	4	5.4	8.3	10.4	11.5	3.8	5.8	7.5	10.1
Gharib	4	0.8	4.2	6.6	10.6	0.1	2.0	4.4	7.1
Giza 177	4	4.3	7.3	9.6	14.2	1.2	5.3	6.9	9.2
Giza 181	4	4.9	5.3	9.4	12.3	1.3	5.7	7.2	8.4
GotakGatik	4	1.8	5.8	8.6	13.6	0.8	3.6	6.6	9.8
IR24	4	1.7	5.1	8.1	12.7	0.6	4.0	6.3	10.4
IR48	4	2.3	7.0	10.4	14.2	3.7	4.8	6.9	11.2
IR60	4	2.8	6.6	7.1	11.2	2.0	5.0	6.6	7.8
Kataktara Da2	4	2.6	6.6	7.7	16.1	2.6	6.0	6.9	14.9
PachehaiPerumal	4	2.9	9.3	9.5	16.6	1.6	4.8	6.6	12.4
PTB30	4	2.3	9.1	11.3	22.4	1.5	7.3	8.7	18.4
Surjamkuhi	4	2.1	8.9	10.2	16.6	3.5	5.4	7.1	14.1
Tadukan	4	2.9	6.2	7.8	10.6	2.5	4.8	6.2	10.3
LSD (0.05)									
Sub. treatments (T)		ns	ns	ns	1.8				
Genotypes (G)		0.8	1.1	3.5	4.8				
T × G		1.8	1.5	2.4	5.2				

Table 5. Shoot dry weight of 53 contrasting rice genotypes at several times during submergence period and at 7 days after recovery period under control and submerged treatments.

Genotypes	Cluster	Shoot dry weight under control treatments (mg)				Shoot dry weight under submerged treatments (mg)			
		Submerged period			Recovery period	Submerged period			Recovery period
		10 days	13 days	17 days	7 days	10 days	13 days	17 days	7 days
IR06 F561 (AG+Su1)	1	12.4	27.2	62.1	228.2	6.7	17.3	42.7	149.8
IR06 F148 (AG +Sub1)	2	6.7	13.2	57.9	210.5	3.8	7.9	25.6	137.9
IR06 F168 (AG +Sub1)	2	7.0	12.1	44.7	178.3	2.2	11.3	21.4	115.4
IR06 F434 (AG +Sub1)	2	5.2	10.7	51.1	136.3	2.8	5.7	18.6	123.5
IR06 F459 (AG +Sub1)	2	5.1	11.1	23.8	131.1	2.6	4.8	20.0	102.3
IR07 F323 (AG +Sub1)	2	7.0	11.3	34.1	126.2	3.0	6.7	13.2	50.6
Kasalath	2	2.4	11.1	24.9	86.3	1.3	8.0	23.1	64.8
ARC10177	3	0.3	2.2	10.8	59.4	0.1	0.3	7.3	38.2
BicoBranco	3	1.1	4.4	27.3	93.0	1.1	2.1	8.1	49.8
ChianungSi-Pi 661020	3	0.4	1.0	8.5	66.9	0.4	0.6	5.5	32.1
Fircoz	3	0.3	1.2	6.4	39.1	0.2	1.0	4.6	26.0
IR 42	3	1.6	2.0	11.3	57.5	0.4	1.7	6.3	20.6
IR 74	3	0.5	1.0	9.5	69.2	0.3	0.6	4.9	20.7
IR22	3	1.2	5.6	17.0	65.4	0.7	2.6	10.5	48.0
IR29	3	1.1	4.7	17.4	79.5	0.3	1.6	10.2	36.5
IR56	3	0.6	4.9	23.1	92.3	0.4	3.0	15.5	56.4
ITA212	3	0.7	1.2	9.8	62.3	0.8	2.0	9.4	34.7

Jhona 26	3	0.5	1.0	6.0	44.0	0.2	0.8	2.1	32.1
LAC23	3	0.6	2.5	9.3	71.6	0.5	1.4	5.2	40.3
Mehr	3	0.6	0.7	6.9	60.4	0.3	0.5	1.9	25.9
Milyang 55	3	1.0	3.8	15.3	72.3	0.6	2.1	13.7	32.4
MURUNGAKAYAN302	3	1.1	1.5	6.7	54.1	0.4	0.8	3.0	40.2
N22	3	0.6	3.0	10.2	52.6	0.2	1.0	2.7	37.7
PadiLebat	3	0.4	0.8	5.2	33.7	0.2	0.6	5.3	20.5
Rathal	3	1.3	2.0	22.4	80.9	0.6	1.8	7.9	37.2
Sakha 103	3	1.6	2.4	9.5	76.0	0.8	0.9	6.7	46.0
Tchampa	3	0.2	1.8	10.8	46.7	0.2	0.3	7.7	24.9
Trembese	3	0.4	1.2	12.2	64.5	0.2	0.6	3.9	28.8
WAB99-84(FRF1)	3	0.6	2.3	13.1	93.8	0.5	1.4	8.9	42.3

Table 5. Continued

Genotypes	Cluster	Shoot dry weight under control treatments (mg)				Shoot dry weight under submerged treatments (mg)			
		Submerged period			Recovery period	Submerged period			Recovery period
		10 days	13 days	17 days	7 days	10 days	13 days	17 days	7 days
IR07 F297 (AG +Sub1)	4	2.2	2.4	28.8	144.5	2.6	2.7	6.1	41.9
IR 06F393 (AG +Sub1)	4	3.7	6.7	29.5	129.2	0.9	4.0	10.9	57.0
IR 06F463 (AG +Sub1)	4	3.7	9.1	26.4	134.7	2.2	4.6	16.2	79.7
C22	4	0.5	4.1	18.2	83.6	0.5	2.9	14.1	57.9
BaranBoro	4	1.1	7.8	24.8	98.6	0.5	4.9	17.5	58.6
Black Gora(NCS12)	4	1.5	8.1	25.2	114.4	0.8	6.7	25.4	71.8
Canela de Ferro	4	0.9	4.7	16.6	79.8	0.3	2.4	10.9	55.9
CG17	4	0.5	4.9	16.7	86.0	0.4	3.6	13.4	60.3
DA28	4	1.3	8.5	29.4	84.5	0.7	8.7	19.7	57.5
DholiBoro	4	0.7	4.8	17.0	83.3	0.4	2.9	10.4	69.0
Egyptian Jasmine	4	6.7	13.9	34.0	198.0	2.0	6.4	24.1	119.0
FR13A	4	3.5	6.7	51.7	121.5	1.9	6.1	23.1	74.3
Gharib	4	0.5	3.7	12.7	65.4	0.2	1.5	10.2	37.8
Giza 177	4	1.4	7.1	19.8	96.0	1.3	4.6	15.4	63.4
Giza 181	4	3.5	3.9	30.9	128.1	0.9	4.8	17.9	57.6
GotakGatik	4	0.7	5.5	16.2	91.5	0.5	2.8	11.8	74.5
IR24	4	1.0	5.5	20.6	88.9	0.6	3.0	13.8	53.3
IR48	4	1.1	6.8	22.6	91.1	1.1	3.9	17.2	68.5
IR60	4	1.0	3.8	17.4	80.8	0.6	3.6	12.8	49.4
Kataktara Da2	4	0.9	8.0	17.3	80.5	0.9	5.8	16.3	72.5
PachehaiPerumal	4	1.1	11.5	37.5	143.8	0.7	4.3	15.4	88.8
PTB30	4	0.7	10.3	26.2	144.9	0.7	7.2	22.6	102.5
Surjamkuhi	4	1.1	7.7	17.0	82.5	1.1	4.6	17.8	61.2
Tadukan	4	1.0	5.4	16.5	76.9	0.9	3.8	12.3	43.1
LSD (0.05)									
Sub. treatments (T)		ns	1.1	3.4	8.6				
Genotypes (G)		2.3	2.6	8.8	10.3				
T × G		3.8	5.7	12.1	17.6				

Table 6. Root dry weight of 53 contrasting rice genotypes at several times during submergence period and at 7 days after recovery period under control and submerged treatments.

Genotypes	Cluster	Root dry weight under control treatments (mg)				Root dry weight under submerged treatments (mg)			
		Submerged period			Recovery period	Submerged period			Recovery period
		10 days	13 days	17 days	7 days	10 days	13 days	17 days	7 days
IR06 F561 (AG+Su1)	1	3.9	8.6	16.6	83.0	3.5	6.5	12.0	54.6
IR06 F148 (AG +Sub1)	2	2.4	3.4	17.4	68.3	2.6	3.2	7.6	57.0
IR06 F168 (AG +Sub1)	2	1.7	2.7	13.0	53.9	1.4	3.5	5.1	40.0
IR06 F434 (AG +Sub1)	2	1.6	2.7	16.9	42.7	2.1	3.1	6.5	45.7
IR06 F459 (AG +Sub1)	2	1.6	2.3	6.9	47.4	1.9	3.4	6.4	40.8
IR07 F323 (AG +Sub1)	2	2.7	3.5	8.8	31.3	1.9	3.7	4.2	14.1
Kasalath	2	1.4	4.0	9.5	24.2	0.7	2.9	6.5	23.2
ARC10177	3	0.1	1.1	3.4	19.6	0.1	0.2	2.8	12.0
BicoBranco	3	0.5	1.2	6.3	22.6	0.5	0.7	3.5	13.1
ChianungSi-Pi 661020	3	0.3	0.7	2.3	14.8	0.2	0.3	1.9	10.5
Fircoz	3	0.2	0.5	1.5	12.4	0.1	0.4	1.4	7.2
IR 42	3	0.7	1.4	4.5	16.3	0.3	1.3	2.7	6.6
IR 74	3	0.3	0.8	3.1	14.6	0.2	0.3	2.6	6.5
IR22	3	1.0	3.0	5.6	20.1	0.6	1.5	4.4	19.4
IR29	3	0.5	2.4	5.8	24.8	0.1	0.9	2.8	13.8
IR56	3	0.4	3.1	10.1	33.1	0.1	1.5	4.2	24.1
ITA212	3	0.4	1.0	2.7	16.2	0.7	1.4	3.1	10.4
Jhona 26	3	0.6	0.7	2.5	7.9	0.1	0.6	0.9	7.9
LAC23	3	0.3	1.3	3.6	18.4	0.2	0.4	2.5	10.5
Mehr	3	0.5	0.6	2.3	15.4	0.1	0.2	1.0	6.7
Milyang 55	3	0.5	1.9	4.4	19.8	0.4	1.5	5.7	8.8
MURUNGAKAYAN302	3	0.7	0.8	3.8	16.5	0.2	0.8	1.5	10.2
N22	3	0.4	1.7	4.3	18.6	0.1	0.7	1.7	16.2
PadiLebat	3	0.3	0.6	1.7	8.7	0.0	0.1	2.1	5.7
Rathal	3	0.6	1.0	6.8	34.7	0.3	0.8	3.1	12.0
Sakha 103	3	0.9	1.0	3.1	17.2	0.5	0.6	2.9	12.8
Tchampa	3	0.1	0.7	4.7	13.4	0.1	0.1	1.0	5.9
Trembese	3	0.2	0.7	3.8	21.8	0.0	0.2	1.7	9.9
WAB99-84(FRF1)	3	0.4	0.8	4.8	27.4	0.1	0.4	3.4	10.5

Table 6. Continued

Genotypes	Cluster	Root dry weight under control treatments (mg)				Root dry weight under submerged treatments (mg)			
		Submerged period			Recovery period	Submerged period			Recovery period
		10 days	13 days	17 days	7 days	10 days	13 days	17 days	7 days
IR07 F297 (AG +Sub1)	4	0.7	1.5	6.5	41.9	1.3	1.4	2.3	13.4
IR 06F393 (AG +Sub1)	4	1.4	2.0	10.0	43.0	0.7	2.4	3.0	14.3
IR 06F463 (AG +Sub1)	4	0.9	2.2	6.1	41.5	1.6	2.3	3.7	31.3
C22	4	0.4	2.2	6.8	33.8	0.2	1.0	3.7	21.0

BaranBoro	4	0.9	3.0	8.0	33.6	0.1	2.4	3.5	14.4
Black Gora(NCS12)	4	0.9	3.2	8.9	49.3	0.6	2.9	7.0	32.5
Canela de Ferro	4	0.4	1.8	5.3	32.4	0.1	0.8	2.9	19.9
CG17	4	0.3	2.1	6.5	30.8	0.2	1.6	3.8	22.3
DA28	4	0.9	3.2	11.4	37.0	0.3	3.1	4.8	32.8
DholiBoro	4	0.6	2.5	6.5	27.2	0.2	1.5	2.6	22.2
Egyptian Jasmine	4	2.6	4.2	8.6	70.4	1.6	4.3	7.3	48.0
FR13A	4	1.5	2.4	17.7	23.1	1.1	3.1	7.0	18.4
Gharib	4	0.2	1.1	3.0	21.5	0.1	0.4	1.4	11.8
Giza 177	4	1.2	3.3	5.5	30.6	0.6	1.7	4.1	20.0
Giza 181	4	1.7	1.9	8.6	40.9	1.0	3.1	6.6	19.1
GotakGatik	4	0.5	2.1	5.4	39.7	0.2	1.5	3.2	19.4
IR24	4	0.6	2.7	7.7	29.7	0.2	1.6	4.1	22.4
IR48	4	0.5	3.0	7.5	39.3	0.6	1.7	4.4	26.1
IR60	4	0.9	2.6	6.1	30.2	0.4	1.8	3.6	19.1
Kataktara Da2	4	0.7	3.1	5.4	26.5	0.6	1.8	3.5	27.2
PachehaiPerumal	4	0.8	4.9	13.2	71.1	0.5	2.4	4.5	30.5
PTB30	4	0.5	5.1	11.2	73.6	0.5	2.4	7.1	54.4
Surjamkuhi	4	0.7	3.7	6.5	37.6	0.8	1.8	4.8	25.2
Tadukan	4	0.7	2.0	5.1	20.5	0.4	1.5	2.6	11.1
LSD (0.05)									
Sub. treatments (T)		ns	ns	1.8	2.9				
Genotypes (G)		1.1	3.6	6.8	9.6				
T × G		1.9	4.3	8.8	12.1				

submergence and genotypes were randomly assigned to the main plot and subplots, respectively. Dry seeds of each genotype were sown directly at approximately 0.5-cm depth in plastic trays filled with nursery soil (pH, 5.0; N, 0.15%; P_2O_5, 0.27%; K_2O, 0.22%), with two seeds per nursery cell (2.4 × 2.4 × 4.5 cm). Nine nursery cells were used for each genotype and replicated three times. Immediately after sowing, the plastic trays were submerged in 10 cm tap water in a plastic container (85 × 56 × 20 cm) for 17 days. Another set of trays was maintained under control (non-flooded) conditions in an adjacent area. Subsequently, the seedlings of both flooding and control treatments were maintained under normal rice cultivation conditions for an additional 7 days as recovery period. The experiment was conducted in a laboratory growth chamber 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 daytime. The mean irradiation level at 50 cm above the water surface was 905 µmol $m^{-2}s^{-1}$ PAR. Humidity was maintained at 80%.

Measurements of growth

At the beginning of the experiments, four nursery cells from each genotype were monitored during submergence period within each replicate to record emergence date and the number of seedlings emerging from the water surface. At 4, 7, 10, 13, 17, and 24 days after sowing, two germinating seeds or seedlings form each replicate were selected from each genotype under both control and flooded conditions. Samples obtained from 4 to 17 days after sowing represent the submergence period, while samples obtained at 24 days after sowing represent the recovery period. The length of coleoptile and mesocotyl were measured at 4, 7, 10, 13 and 17 days after sowing, while the length and dry weigh of shoot and root were measured at 10, 13, 17, and 24 days after sowing. The length of the coleoptile was measured from the first node to the tip. The mesocotyl was measured from the point of emergence from the seed to the first node. Shoot length was measured from the base of the coleoptile to the tip of the leaf. Shoot and root dry weights were determined after drying at 80°C for 48 h.

Statistical analysis

The analysis of variance (ANOVA) was done appropriately for a randomized complete block split plot design with submergence treatments as the main plot, genotypes as the subplots and replicates as blocks. Mean square of the product between the submergence treatments and genotypes was used as the error term to test the interaction between both factors. Mean separation of treatment effects used Fisher's protected least significant differences (LSD) test. Statistical analysis was performed using CoStat Version 6.311 (CoHort software, Berkeley, CA 94701). Ward's minimum variance clustering method was used to classify genotypes into discrete clusters based on the length of coleoptile and mesocotyl at different time of submergence. The optimum number of clusters was determined by the sum of squares index (E) (Romersburg, 1988). Associations among different traits were examined by simple linear regression analysis using Sigma plot 8.0.

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