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Post-germination seedling vigor under submergence and submergence-induced *SUB1A* gene expression in indica and japonica rice (*Oryza sativa* L.)

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Abstract

We evaluated post-germination seedling vigor in *indica* and *japonica* rice under submergence based on shoot elongation and recovery after submergence. The *japonica* cultivars showed more vigorous shoot elongation than *indica* cultivars under submergence in the dark, whereas an opposite response was observed under the light-dark submergence. Both Nipponbare and FR13A that are respectively known as submergence intolerant and tolerant cultivars showed vigorous shoot growth under submergence and high recovery after the stress, irrespective of the light/dark conditions. The results show that vigorous shoot growth enables rice seedlings to escape and survive submergence stress. RT-PCR analysis showed that expression of *SUB1A*, which is known as a key gene controlling submergence tolerance, was induced in elongating seedlings of FR13A and other *indica* cultivars including floating rice under submergence with light illumination. Our results suggest that light-induced *SUB1A* gene expression does not hinder the shoot elongation response under submergence in rice at the post-germination seedling stage.

Keywords: rice (*Oryza sativa* L.); post-germination seedling vigor; shoot elongation; *SUB1A* gene expression; submergence stress

Introduction

Wet-direct seeding has become popular in most of the rice growing countries, as it is more economical than the seedling transplanting method. In this cropping system, fast and vigorous seedling growth under submergence is a desirable trait not only to facilitate good emergence and optimum seedling establishment but also to increase competitive ability against weeds (Cisse and Ejeta, 2003; Zhou et al., 2007). Seedling vigor, in general, is a trait that expresses itself as an ability of seedlings to rapidly elongate after germination and emerge for escaping and surviving submergence stress. Cui et al. (2002) reported that good germination and fast early seedling growth were two major seedling-vigor-related traits in rice. Submergence stress in fact significantly promotes shoot elongation in young rice seedlings (Redona and Mackill, 1996; Ismail et al., 2009). A study using fast shoot elongation growth as a basic parameter for screening rice cultivars under low dissolved oxygen levels suggested that fast shoot elongation is an adaptive response of water-seeded rice for acquiring oxygen (Won and Yoshida, 2000). We further examined the significance of shoot elongation as a mechanism conferring seedling vigor under submergence during the post-germination stage in *indica* and *japonica* rice. Seedling growth and survival under submergence is influenced by various environmental factors including light levels (Ram et al., 2002). We therefore studied post-germination seedling vigor under both dark and light-dark submergence conditions in both *indica* and *japonica* cultivars. We adopted the test tube bioassay method in that seedling vigor is evaluated based on the ability of shoot elongation for escaping and surviving submergence stress (Manangkil et al., 2008). In this bioassay method it was suggested that seedling vigor of *japonica* cultivars was greater than that of *indica* cultivars. We therefore further examined this under light-dark submergence. The *Sub1* locus is known to play a key role in submergence tolerance in rice according to the ‘quiescent strategy’ that can help rice plants to maintain high levels of stored carbohydrates coupled with minimum shoot elongation and retention of chlorophyll (Fukao and Bailey-Serres, 2004, 2008; Fukao et al., 2006; Xu et al., 2006). Hence we studied expression of *SUB1A* gene in elongating rice seedlings under submergence.

Materials and methods

Plant materials and bioassay conditions for evaluating seedling vigor

Randomly selected 17 *indica* cultivars including two floating rice and 11 *japonica* cultivars were used for evaluating seedling vigor under submergence. Nipponbare was included as a high seedling vigor *japonica* check (Manangkil et al., 2008) and FR13A and IR42 as submergence tolerant and intolerant *indica* checks, respectively (Ella et al., 2003; Jackson and Ram, 2003). Kasalath was also included as a low seedling vigor *indica* check (Manangkil et al., 2008). Seedling vigor under submergence was evaluated based on shoot (coleoptile or first leaf) lengths of germinating seedlings after 5 days of submergen-

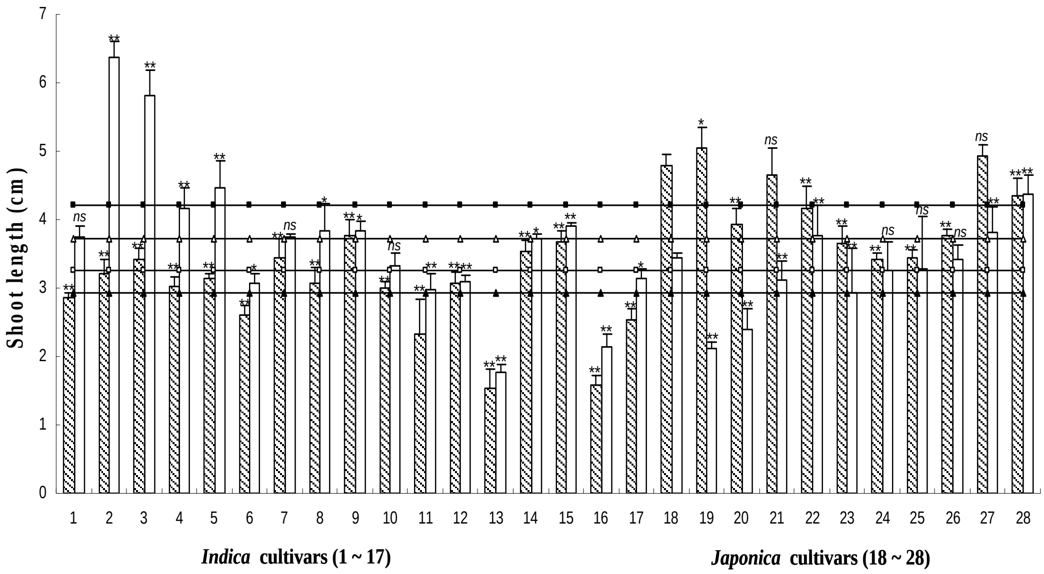

Fig 1. Shoot lengths of *indica* and *japonica* cultivars grown under submerged conditions in both dark and light-dark regimes. Cultivars with number 1 to 17 and 18 to 28 are *indica* and *japonica*, respectively. 1: Kasalath, 2: O luen chung, 3: PTB10, 4: 115, 5: PTB7, 6: Pachchai perumal, 7: 1034, 8: 415, 9: 437, 10: Pokkali, 11: KS282, 12: FR13A, 13: IR42, 14: Cula, 15: Habiganj-Aman II, 16: Chiem chanh, 17: Taichung Native 1, 18: Nipponbare, 19: Hitogokochi, 20: Kyounohana 1, 21: Yamadanishiki, 22: Jyousyu, 23: Ouu 2, 24: Hokushin 1, 25: Seniti, 26: Sakenohana 1, 27: Kokuryomiyako, 28: Koshihikari. Open bars: under the light-dark submergence, closed bars: under the dark submergence. Mean shoot lengths of *indica* and *japonica* are indicated by triangles (closed in the dark condition) and squares (open in the light-dark condition), respectively. Mean ± standard error from three independent experiments. **/* significantly different from Nipponbare at the 1% and 5% levels, respectively. *ns* - not significantly different from Nipponbare.

ce. Briefly, de-hulled seeds were subjected to overnight imbibition under running water to allow synchronous germination. Imbibed seeds were surface-sterilized with 1 % (w/v) solution of sodium hypochlorite (NaClO) for 10 min and rinsed in distilled water as described by (Boamfa et al., 2003). Sterilized seeds were allowed to germination in wet glass petri dishes (70-mm-diameter) in a dark incubator adjusted at 28 ºC for 3 days. The seeds were washed everyday with distilled water before germination. Ten germinating seeds per cultivar at the pigeon breast stage were transferred to a glass test tube filled with 10 cm deep distilled water. Uncovered test tubes were placed in an incubator at 28 ºC either in the darkness or in the 16 hd^{-1} photoperiod without changing water for 5 days. Shoot lengths were measured from the base to the tip of the shoots at the end of the 5-days stress period. Recovery rate (%) after submergence was measured by transferring the submerged seedlings to pots with soil placed under normal conditions (28ºC with 16hd^{-1} photoperiod) for additional 5 days. Submergence stress was given to the two groups of seedlings for 5 days, i.e. one group incubated under the dark submergence condition and the other group under the light-dark submergence condition. Plant height was also recorded after additional 5 days of growth under the normal conditions in pots with soil. Plant height was measured from the base to the top of the highest leaf at 3-4 leaf stage. For evaluating the diversity in seedling vigor among *japonica* cultivars, a total of 150 *japonica* cultivars including Nipponbare were subjected to the same test tube bioassay (Supplementary Table). The test tube bioassay was performed in the darkness.

Statistical analysis

All experiments were laid out following a complete randomized block design with three replications. Analysis of variance (ANOVA) was performed using Microsoft Exel Program. Least significant differences (LSD) were calculated using a software program Analyze-it + General 1.68 version. Computed F value was used to test the significance of the treatment effect and coeffcients of determination (R^2) were calculated among the three parameters, i.e. shoot length, recovery rate and plant height according to Gomez and Gomez (1984).

Profiling of SUB1A *gene expression by RT-PCR*

The same set of 17 *indica* and 11 *japonica* cultivars were used in the study of expression profiles of *SUB1A* gene. Sterilized seeds were allowed for germination in the dark at 28 ^{o}C for 3 days. Germinating seedlings were then transferred in test tubes with 10 cm deep distilled water (dark-submerged stress) and also under aerated normal conditions. After 5 days, seedling tissues were collected and frozen with liquid nitrogen. They were then subjected to RNA extraction using guianidine thiocyanate. The amount of transcripts was determined by reverse transcriptase polymerase chain reaction (RT-PCR) analysis using a first strand cDNA synthesis kit (TOYOBO, Osaka, Japan). The total template RNA samples for the cDNA synthesis were treated with DNaseI to remove contaminated DNA. RT-PCR was performed with specific primers for rice *SUB1A* gene, which were designed and synthesized by Invitro Lifetech Oriental (Nacalai). For each sample 4µl c-DNA template was added to 16µl reaction mixture conta-

Table 1. Correlation coefficients (R^2) among the three parameters used for evaluating seedling vigor under submergence. R^2 was calculated using the data shown in Fig 1 (shoot length in the test tube bioassay), Fig 2a (recovery rate) and Fig 2b (plant height). Dark and Light represent the data obtained in the dark and dark-light submerged conditions, respectively.

		Shoot length		Recovery rate		Plant height	
		Dark	Light	Dark	Light	Dark	Light
Shoot length	Dark	1	0.04	0.72	0.76	-0.21	0.10
	Light	0.04	1	-0.29	-0.27	0.63	0.75
Recovery rate	Dark	0.72	-0.29	1	0.98	-0.49	-0.36
	Light	0.76	-0.27	0.98	1	-0.50	-0.34
Plant height	Dark	-0.21	0.63	-0.49	-0.50	1	0.78
	Light	0.10	0.75	-0.36	-0.34	0.78	1

ining 1µl each of forward and reverse primer, 2µl 10 x buffer, 0.8µl $MgCl_2$, 1µl dNTPs, 10µl Q water and 0.2µl rTaq Polymerase to make a 20µl PCR mixture. RT-PCR was carried out by amplification with 30 cycles at 50°C (1 min) for annealing using a thermal cycler, Gene Amp PCR System 9700 (Applied Biosystem). Primer sequences were 5'-AGGTGAAAATGATGCAGG-3' (forward) and 5'-CT-TCCCCTGCATATGATATG-3' (reverse). Rice ubiquitin gene was used as an internal control.

Results

Evaluation of seedling vigor in indica and japonica cultivars based on their shoot elongation ability under submergence

We measured the shoot length as a parameter for evaluating seedling vigor in 17 *indica* cultivars including two floating rice and 11 *japonica* cultivars. Shoot length was measured after 5 days of submergence. In the dark, our observation suggested that the first leaf remained inside coleoptiles and shoot length represented coleoptile length. A mean shoot length of *japonica* cultivars (4.20 ± 0.22 cm) was significantly longer than *indica* cultivars (2.93 ± 0.19 cm) under the dark submergence condition (Fig. 1). Under the light-dark submergence condition, green leaf emerged out of coleoptiles, with the degree of emergence being varied depending on cultivars. Under this condition, we measured length of longer seedlings, either coleoptile or first leaf of the submerged seedlings. A mean shoot length of *japonica* cultivars (3.27 ± 0.15 cm) thus measured was shorter than that of *indica* cultivars (3.71 ± 0.19 cm). Nipponbare (#18 in Fig. 1) showed a much longer shoot length than FR13A (#12) under the dark submergence. Shoot length of FR13A was comparable to the mean of *indica* rice under the dark submergence, whereas shorter under the light-dark submergence. On the other hand, under the light-dark submergence condition, an opposite relation was observed. Particularly, shoot length of a *japonica* cultivar Hitogokochi (#19) was the longest under the dark and the second shortest after IR42 (#13) under the light-dark. Our results suggested that the observed differences in the shoot length of the *japonica* and *indica* cultivars under submergence could be ascribed to subspecies specific differences. We measured lengths of the first leaves remained inside the coleoptiles using check cultivars, Nipponbare and Kasalath. A mean length of the first leaf of Nipponbare (2.82 ± 0.15 cm) was greater than that of Kasalath (1.45 ± 0.16 cm) under the dark submergence. The result indicated that both the coleoptile length and the first leaf length inside the coleoptiles gave a good estimate of the ability of shoot elongation under the dark submergence. By contrast, the mean first leaf length of Nipponbare (1.8 cm ± 0.11) was shorter than that of Kasalath (2.3cm ± 0.12) in the light-dark submergence.

Recovery from the submergence stress

Fast shoot elongation apparently requires energy and therefore causes a risk of carbohydrate depletion under prolonged submergence due to the reduced photosynthesis. To examine if the seedlings elongated under submergence stress can recover with greening after release from the stress, we transferred the stressed seedlings to the normal conditions in pots with soil. Seedling recovery rate (%) was measured after 5 days of recovery period (Fig. 2a). The recovery rate was higher in rice seedlings subjected to the light-dark submergence than in those subjected to the dark submergence in both *indica* and *japonica*. A majority of *japonica* seedlings showed greater recovery rate than *indica* seedlings irrespective of the degree of stress. The submergence tolerant *indica* cultivar FR13A, however, showed the highest recovery rate of 100 % under both dark and light-dark submergence followed by Nipponbare at 83.3 % ± 1.5 under the dark and 93.3% ± 2.8 under the light-dark submergence. A positive correlation was observed between the shoot length measured under the dark submergence and the recovery rate of dark-submerged seedlings, while no significant correlation was observed between the shoot length measured under the light-dark submergence and the recovery rate of light-dark submerged seedlings (Table 1). Plant height of the seedlings recovered from both the dark and light-dark submergence was also measured after transferring them to the normal conditions for additional 5 days (Fig. 2b). The seedlings subjected to the submergence stress under the light-dark condition showed greater plant height after the 5-days recovery than the dark-submerged seedlings in both *indica* and *japonica* cultivars. A positive correlation was observed between the shoot length measured under the light-dark submergence and the plant height of light-dark submerged seedlings (Table 1). Plant height after the recovery period of *indica* cultivars was greater than that of *japonica* cultivars for both dark and light-dark submerged seedlings. No correlations were observed between the recovery rate and the plant height after the recovery period for both of the dark and

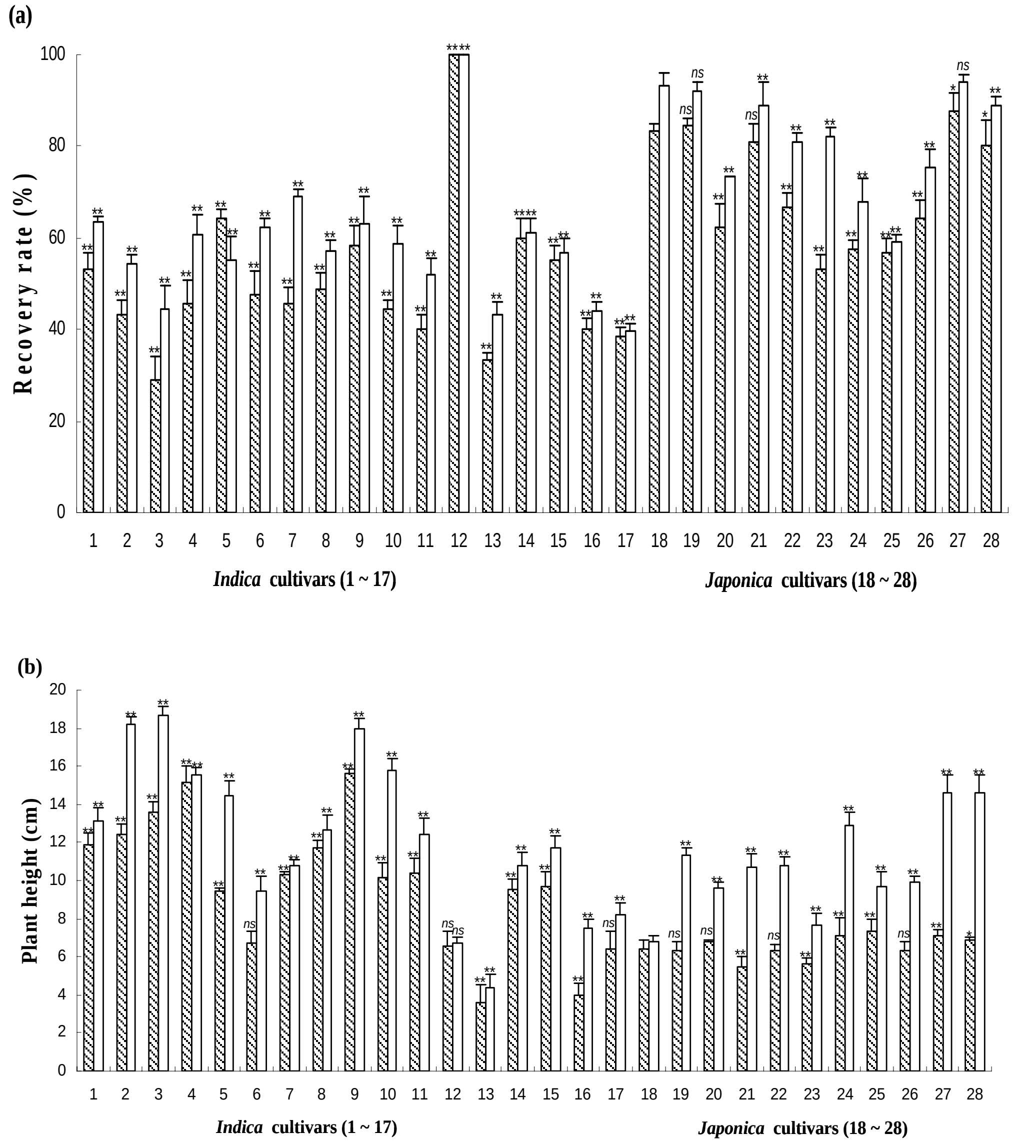

Fig 2. Recovery rate and plant height after the 5-days-recovery period of rice seedlings subjected to the 5-days dark and light-dark submergence stress. **(a)** Recovery rate (%) of 17 *indica* and 11 *japonica* cultivars. **(b)** Plant height measured after the 5-days recovery period. Shaded and open bars indicate seedlings submerged in the dark and the light-dark condition, respectively. Mean ± standard error from three independent experiments. **/* significantly different from Nipponbare at the 1% and 5% levels, respectively. *ns* - not significantly different from Nipponbare.

light-dark submerged *indica* and *japonica* seedlings (Table 1).

A large diversity in the seedling vigor under submergence in japonica cultivars

We further studied the diversity in the seedling vigor of a set of 150 *japonica* cultivars plus Kasalath as an *indica* check by adopting the test tube method. A large phenotypic variability was observed in the shoot length in the *japonica* cultivars under the dark submergence condition (Fig. 3 and supplementary Table). These *japonica* cultivars included both local and modern cultivars of eating rice and rice for brewing Japanese sake (rice wine). The modern cultivars showed a significantly (at the 1% level by Student's t test) greater mean shoot length than the domestic cultivars under the dark submergence condition in both eating (4.42 ± 0.10 cm vs 3.95 ± 0.06 cm) and sake-brewing rice (4.84 ± 0.13 cm vs 4.25 ± 0.07 cm).

Expression of SUB1A gene under submergence

The *Sub1* locus encoding ethylene-response-factor-like transcription factors plays an important role in submergence tolerance (Fukao et al., 2006; Xu et al., 2006). We studied transcript level of *SUB1A* gene in the set of 17 *indica* and 11 *japonica* cultivars grown under the same test tube bioassay conditions to examine if this gene was expressed in elongating seedlings under submergence. RT-PCR analysis using specific primers revealed clear induction of *SUB1A* gene expression in FR13A and five other *indica* cultivars including one floating rice and one non-floating rice that were subjected to submergence in the light-dark condition (Fig. 4a and 4b). The list of cultivars and origins

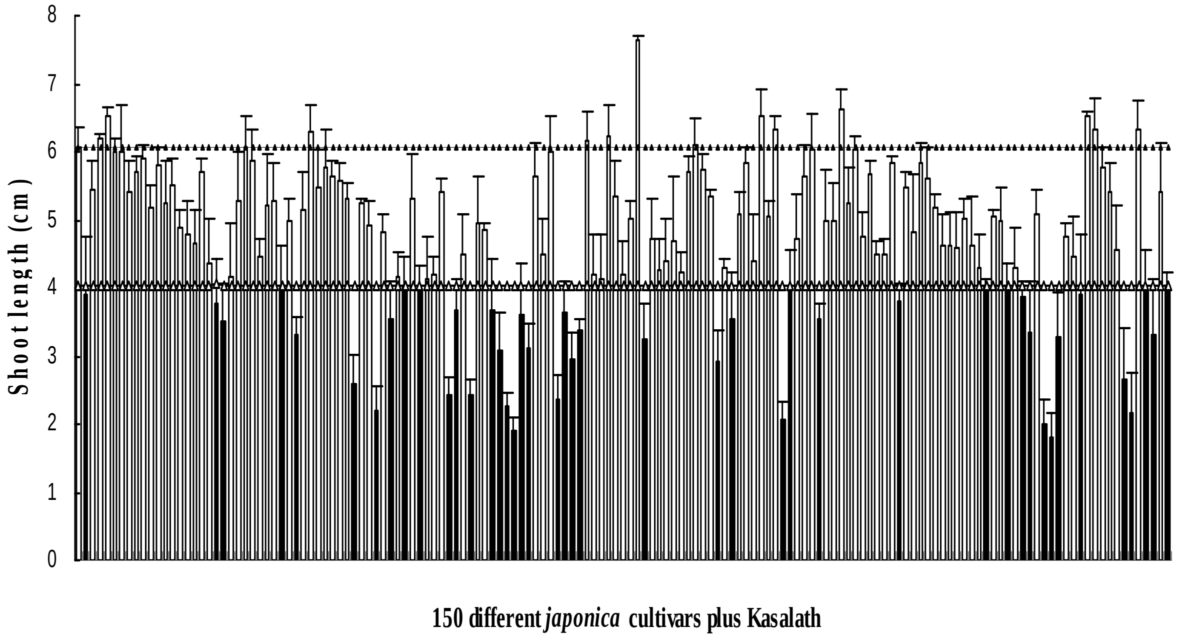

Fig 3. Shoot lengths of 150 *japonica* cultivars and one *indica* cultivar, Kasalath, subjected to the test tube bioassay in the dark. Closed and open triangles indicate means of Nipponbare (#1) and Kasalath (#151), respectively. Shaded and open bars indicate seedlings with shorter and longer shoot lengths than Kasalath, respectively. Mean ± standard error from three independent experiments. See supplementary Table.

expressing the *SUB1A* under submergence in the light-dark conditions are: 115 (origin from Taiwan); PTB7 (from India); Pachchai perumal (Srilanka); floating rice Cula and non-floating rice Chiem chanh (from Vietnam). *SUB1A* gene expression however was completely suppressed under the dark submergence. The level of expression in the floating rice appeared to be greater than that in FR13A (Fig. 4b). No gene expression was detected in all *japonica* cultivars examined.

Discussion

Rice plants can change metabolic pathways to adapt to the submerged environments (Setter et al., 1997). Fast shoot elongation under submergence is beneficial during the post-germination stage when young seedlings experience hypoxia or anoxia (Won and Yoshida, 2000). This trait is particularly important during direct sowing of rice seeds in paddy field (Yamauchi et al., 2000; Magneschi and Perata, 2009). For evaluating the post-germination seedling vigor under submergence, we adopted the test tube bioassay method that was devised to measure the ability of fast shoot elongation under submergence (Manangkil et al., 2008). A large variability exists in the shoot elongation ability under submergence among both *indica* and *japonica* cultivars (Figs. 1 and 3 and supplementary Table). *Japonica* cultivars showed greater shoot elongation ability than *indica* cultivars under dark submergence. Nipponbare known to be submergence intolerant due to the lack of functional *Sub1* locus showed fast shoot elongation particularly under dark submergence (Fig. 1). FR13A, a widely known submergence tolerant model cultivar (Ella et al., 2003; Jackson and Ram, 2003) showed a comparable level of shoot elongation to the mean of *indica* cultivars both in the dark and light-dark submergence conditions. Both FR13A and Nipponbare showed good recovery rates after release from the submergence stress (Fig. 2a). A positive correlation was found between the shoot length and the recovery rate of dark submerged seedlings and between the shoot length and the plant height of light-dark submerged seedlings in *indica* and *japonica* types (Table 1). The level of shoot elongation growth under dark submergence thus was correlated with the recovery rate after the stress, and that under light-dark submergence was correlated with the plant growth after the stress. The greater plant growth after release of the stress in the light-dark submerged seedlings might be due to photosynthesis during the light-dark submergence. These results support that fast shoot elongation is a key component acting both in *indica* and *japonica* rice grown under submergence and contributes to escape and survive submergence stress at the post-germination early seedling stage. Submergence tolerance in rice has been referred to the trait that confers on rice plants at the vegetative stage an ability to survive stresses caused by abrupt and temporal flooding, i.e. flash flood (Jackson and Ram, 2003). Rice plant die if they are fully submerged for a period longer than 2 weeks because respiration and photosynthesis are restricted when the oxygen and carbon dioxide supplies become limited (Boamfa et al., 2003). Therefore, the "quiescence strategy" that can help rice plants to maintain high levels of stored carbohydrates coupled with minimum shoot elongation and retention of chlorophyll has been considered a key mechanism for tolerance against submergence stress (Ito et al., 1999; Ram et al., 2002; Jackson and Ram, 2003; Das et al., 2005, Fukao et al., 2006). Reduced elongation during flash flood is also advantageous for rice seedlings because elongated seedlings trend to lodge as soon as the water level recedes (Suge, 1985; Setter and Laureles, 1996). The ethylene-response-factor-like genes located at the *Sub1* locus were shown to play a key role in the operation of this strategy (Fukao and Bailey-Serres, 2004, 2008; Fukao et al., 2006; Xu et al., 2006). We therefore examined the *SUB1A*

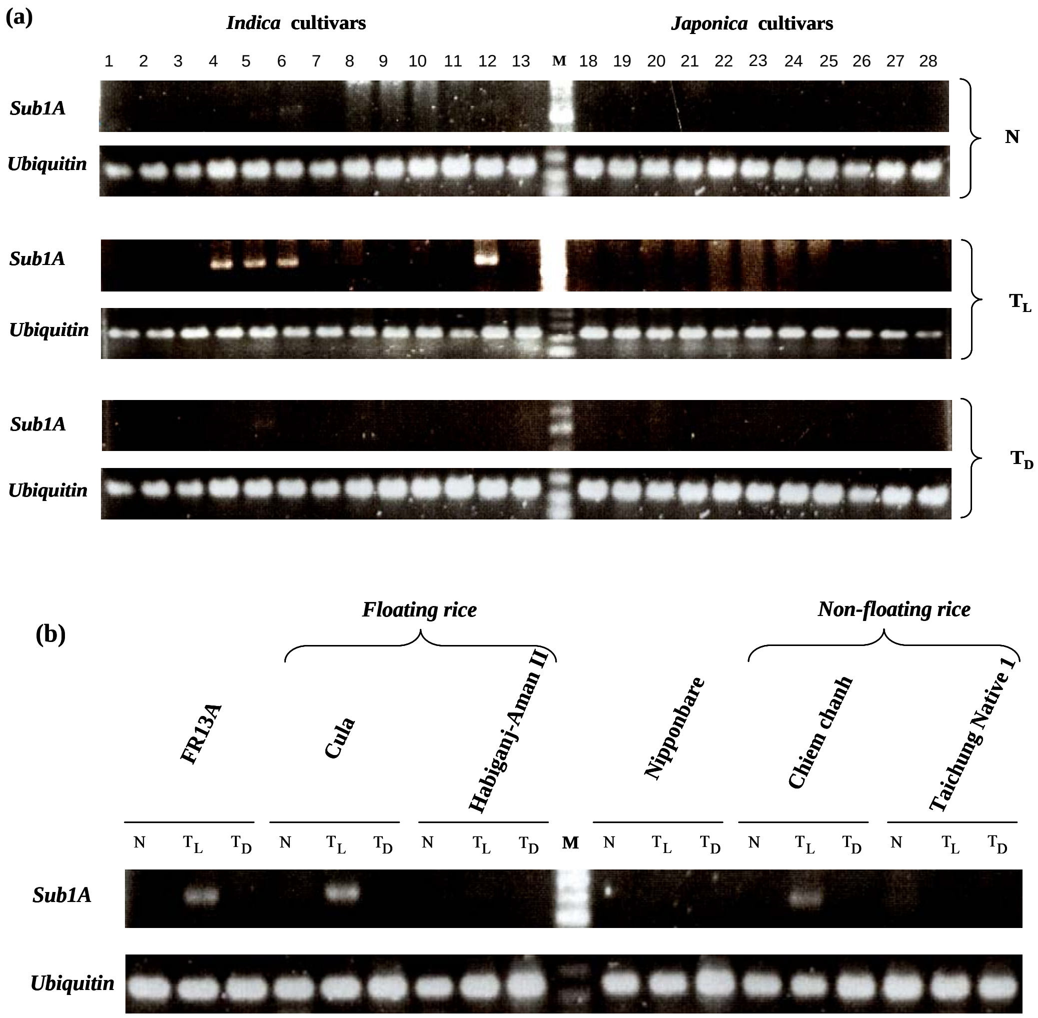

Fig 4. RT-PCR profiles of *SUB1A* transcripts in rice seedlings incubated under the dark and the light-dark submergence conditions. Amount of *SUB1A* transcripts was measured in (**a**) 13 *indica* and 11 *japonica* cultivars including Nipponbare (high seedling vigor cultivar), FR13A (submergence tolerant cultivar), Kasalath (low seedling vigor cultivar) and IR42 (submergence intolerant cultivar) and in (**b**) floating and non-floating rice. Ubiquitin was used as an internal control. N, TL and TD respectively represent non-submerged and light-dark and dark submerged seedlings. M indicates DNA size markers.

gene expression in a set of 17 *indica* and 11 *japonica* cultivars, all of which showed different levels of shoot elongation under submergence. RT-PCR analysis revealed strong induction of the *SUB1A* gene under submergence in the elongating seedlings of six *indica* cultivars including FR13A, one floating rice Cula and one non-floating rice cultivar Chiem chanh, while no transcripts were detected in the other *indica* and all *japonica* cultivars examined (Fig. 4). The observation that the expression of *SUB1A* gene occurs in the elongating seedlings under submergence suggests that *SUB1A* gene expression does not hinder the shoot elongation growth under submergence at the early seedling stage. Because no correlation was apparent between the level of *SUB1A* gene expression and the level of seedling vigor under submergence, it can also be suggested that *SUB1A* is not directly related to seedling vigor under submergence. Nipponbare, FR13A and other *indica* and *japonica* cultivars might possess yet unknown gene(s) that promote vigorous growth under submergence at the post-germination early seedling stage. Quantitative trait locus analysis is required to detect such genes. It was noted that expression of *SUB1A* gene was induced only under the light illumination. It is intriguing to examine the mechanism of light induction of the *SUB1A* gene expression under submergence. Submergence-induced elongation is an escape mechanism that helps submerged rice plants regain contact with the aerial environment (Arber, 1920). Rice plants adopting the so-called "elongation strategy" can grow rapidly to reach above the water surface and resume aerobic metabolism and photosynthesis (Kende et al., 1998; Voesenek et al., 2006). Deep-water or floating rice cultivars take this strategy to avoid submergence stress (Sauter, 2000). Perata and Voesenek (2007) discussed that differences in the level of submergence tolerance between and within species can only be partly explained by the *Sub1* locus. They further stated that the "quiescence strategy" does not necessarily lead to tolerance to low oxygen levels when plants are at the early seedling stage. According to Ismail et al. (2009), tolerance to flooding during germination is clearly not associated with tolerance of complete submergence during the vegetative stage. Our results support the contention that different mechanisms might be operative at different growth stages in response to submergence stress in rice.

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Supplementary Table: List of the 150 *japonica* cultivars and their shoot length.

No.	Designation	Name	Shoot length (cm)	SD
1	B083	Nipponbare	6.07	0.29
2	B117	Shinriki	3.92	0.83
3	B105	Sasasigure	5.46	0.41
4	A176	Yamadaho 132	6.19	0.07
5	B076	Matuyama Mitsui	6.53	0.12
6	B138	Yukigeshou	6.01	0.17
7	B049	Hyoukeika 56	6.00	0.68
8	B101	Reihou	5.40	0.45
9	A182	Yume Sansui	5.72	0.22
10	B027	Haya Ouzeki1	5.92	0.19

11	B109	Shinjyu	5.20	0.32
12	B131	Touno 1	5.80	0.28
13	B135	Yamabiko	5.25	0.62
14	B085	Norin 1	5.52	0.39
15	B087	Norin 21	4.87	0.28
16	B089	Norin 41	4.79	0.50
17	B075	Mangoku	4.64	0.49
18	B010	Asahi	5.71	0.18
19	B066	Koshikihikari	4.35	0.68
20	B019	Hujisaka 5	3.77	0.65
21	B139	Zenseki Wase	3.51	0.56
22	B100	Ouu 237	4.18	0.77
23	B043	Hokushin 1	5.28	0.71
24	B086	Norin 8	6.08	0.45
25	B020	Fukuhibiki	5.87	0.47
26	B003	Aichi Mikawanishiki 3	4.45	0.25
27	B004	Aikoku	5.21	0.74
28	B134	Wase Shinriki	5.29	0.56
29	B128	Tasensyou	3.99	0.65
30	A075	Kannomai	4.97	0.35
31	A028	Hattan 35	3.32	0.24
32	A030	Hattan Nishiki 1	5.15	0.56
33	A032	Hattangusa	6.31	0.38
34	A153	Shiratama	5.47	0.57
35	A094	Miyako	5.79	0.53
36	A116	Rokkou Nishiki	5.64	0.21
37	A099	Nadahikari	5.57	0.27
38	A006	Benkei	5.30	0.25
39	A078	Kikusui	2.60	0.42
40	A066	Ishikawa Sake 20	5.25	0.06
41	A115	Oyama Nishiki	4.93	0.36
42	A119	Saga Sake 12	2.20	0.36
43	A018	Ginnoyume	4.82	0.27
44	A133	Senbonnishiki	3.54	0.56
45	A031	Hattan Nishiki 2	4.17	0.34
46	A101	Nada Nishiki	4.02	0.44
47	B005	Ajimaru	5.30	0.67
48	A020	Gohykumangoku	3.96	0.38
49	A065	Isenishiki	4.14	0.61
50	A050	Hyoukei Sake 18	4.19	0.28
51	A048	Hyougo Yume Nishiki	5.40	0.21
52	A152	Shirakaba Nishiki	2.41	0.27
53	A154	Shirotae Nishiki	3.67	0.45
54	A043	Hitogokochi	4.89	0.61
55	A034	Hidahomare	2.42	0.23
56	A077	Kikusakae	4.94	0.71
57	A166	Tuyuhakaze	4.86	0.09
58	A160	Tamasakae	3.69	0.74
59	A089	Kuzuryuu	3.07	0.58
60	A167	Wakamizu	2.27	0.21
61	A118	Sachidama	1.90	0.20
62	A001	Aiyama	3.61	0.74
63	A011	Dewasansan	3.11	0.38
64	A073	Kairyou Shikou	5.65	0.49
65	A023	Hanafubuki	4.50	0.50
66	A112	Okuhomare	6.02	0.52
67	A046	Houhai	2.35	0.36
68	A082	Kojyou Nishiki	3.66	0.44
69	A110	Okayama Yaku Sake 48	2.97	0.37
70	A131	Gin Fubuki	3.38	0.16
71	A058	Hikei Sake 61	6.17	0.43
72	A063	Ipponzime	4.21	0.60
73	A132	Sakenohana 1	4.12	0.67
74	A095	Miyama Nishiki	6.22	0.46
75	A158	Takane Nishiki	5.36	0.51
76	A042	Hitachi sake 17	4.19	0.50
77	A015	Ginginga	5.03	0.26
78	A163	Hyoukei Sake 66	7.64	0.45
79	A162	Hyoukei Sake 65	3.26	0.52
80	B121	Shirayukihime	4.72	0.58
81	B064	Kiryuyoshi	4.27	0.47
82	A177	Yamadaho 1713	4.38	0.62
83	A168	Wataribune 2	4.69	0.97
84	A173	Wataribune	4.21	0.33

85	A017	Ginnosei	5.72	0.21
86	A027	Hattan 10	6.09	0.40
87	A071	Kairyou Hattan Nagare	5.74	0.22
88	A159	Takadawase	5.35	0.08
89	A092	Miki Nishiki	2.93	0.46
90	A144	Shin Yamadaho 2	4.28	0.16
91	A072	Kairyou Omachi	3.53	0.68
92	A079	Kinai Omachi	5.07	0.34
93	A013	Funaki Omachi	5.82	0.25
94	A048	Hyougo Omchi	4.39	0.69
95	A021	Gouriki	6.51	0.40
96	A045	Hokuriku 12	5.06	0.20
97	B005	Ajimaru	6.32	0.20
98	A142	Shin yamadaho 1	2.07	0.25
99	A120	Saikai 134	3.99	0.55
100	A081	Koi omachi	4.72	0.65
101	A134	Siga Wataribune 2	5.62	0.46
102	A080	Kinmon Nishiki	6.02	0.55
103	A087	Kuranohana	3.54	0.23
104	A093	Misatoshiki	4.98	0.75
105	A114	Omachi	4.99	0.55
106	A151	Shiragiku	6.63	0.30
107	A106	Nojyouho	5.24	0.53
108	A069	Iwai	6.03	0.20
109	A156	Tajima Gouriki	4.77	0.36
110	A035	Hidaminori	5.68	0.19
111	A012	Fukunohana	4.49	0.21
112	A047	Hyougo Kita Nishiki	4.49	0.22
113	B061	Kincyaku	5.85	0.08
114	B071	Kyoto Asahi	3.82	0.24
115	B006	Akebono	5.48	0.24
116	A181	Yamayuu 67	4.81	0.85
117	B094	Ooseto	5.83	0.29
118	B055	Kamenoo	5.61	0.46
119	B062	Kinmaze	5.17	0.20
120	B050	Hyoukei Ka 62	4.63	0.44
121	B067	Kougyoku	4.63	0.49
122	B122	Shirosenbon	4.59	0.53
123	B033	Hinomaru	5.03	0.27
124	B126	Takenari	4.61	0.75
125	B093	Ooita Mii 120	4.28	0.51
126	B091	Ooba	4.03	0.09
127	B133	Wase Asahi	5.04	0.12
128	B140	Yukigeshou	5.00	0.49
129	B107	Sen-iti	4.05	0.32
130	B051	Jyousyu	4.09	0.61
131	B052	Jukkoku	3.86	0.25
132	B132	Wakaba	3.33	0.75
133	B078	Mikawanishiki	5.09	0.36
134	B023	Ginbouzu	1.99	0.39
135	B053	Kamenoo 4	1.80	0.37
136	B111	Shinriki 245	3.28	0.67
137	B080	Moritawase 2	4.75	0.21
138	B079	Moritawase	4.47	0.59
139	B011	Banzai	3.92	0.85
140	B035	Hokuriku 20	6.51	0.07
141	B036	Hokuriku 52	6.33	0.44
142	C027	Hokushi Tami	5.76	0.30
143	B088	Norin 22	5.42	0.43
144	B022	Fusayoshi	4.57	0.63
145	B031	Hinohikari	2.67	0.73
146	B120	Shiranui	2.17	0.58
147	B102	Reimei	6.32	0.45
148	B065	Kizasa	3.97	0.59
149	B008	Akitsuho	3.32	0.80
150	B016	Chuusei Shinsenbon	5.40	0.73
151		Kasalath	4.02	0.22