

Traits Associated with the Escape Strategy are Responsible for Flash Flooding Tolerance of Rice during the Emergence and Seedling Stages

S. EL-HENDAWY^{1,2*}, C. SONE³, O. ITO³ and J.-I. SAKAGAMI³

¹Plant Production Department, College of Food and Agriculture Sciences, King Saud University, 11451 Riyadh, Saudi Arabia

²Agronomy Department, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

³Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki 305-8686, Japan

(Received 8 June 2014; Accepted 1 October 2014;

Communicated by X.F. Zhang)

To identify the adaptive traits responsible for flooding tolerance during the initial growth stages of rice, 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 were kept under normal rice cultivation conditions for a further 7 days. Cluster analysis showed that 53 genotypes were divided into three groups based on emergence date, percentage of plants reaching the water's surface, maximum coleoptile length, shoot elongation rate during submergence and increases in shoot dry weight after de-submergence. Twelve genotypes were placed in cluster 1 and characterized by fast emergence, rapid coleoptile elongation, and vigorous shoot growth under control and submergence conditions. The genotypes in cluster 1 attained also a higher increase in shoot dry weight at different time of submergence and de-submergence than the genotypes in clusters 2 and 3. A significant correlation was observed between the increase in shoot dry weight and traits related with fast and vigorous shoot elongation and coleoptile. In conclusion, flooding tolerance during initial growth stages were mainly due to major submergence avoidance or escape mechanisms, and crop establishment of direct-seeded rice in flood-prone areas is accomplished by harnessing reserves for fast shoot elongation.

Keywords: coleoptile elongation, crop establishment, early flooding, quiescence strategy, seedling vigour

Introduction

Poor germination and seedling establishment are among the main constraints that restrict rice large-scale adaptation in flood-prone areas, especially when farmers are interested in achieving the advantages of the direct seeding method, which is more economical and operationally simpler than the seedling transplanting method (Ismail et al. 2009; Ella et al. 2011). Unfortunately, very limited success has been achieved from previous efforts to improve the tolerance of genotypes under flooding conditions during the initial growth

* Corresponding author; E-mails: shendawy@yahoo.com; mosalah@ksu.edu.sa; shendawy2003@gmail.com

stages, i.e. germination, emergence, and early seedling growth stages. For example, large-scale screening of 8,000 diverse rice accessions and breeding lines revealed only 0.23% had great ability to germinate and establish under flooding conditions. Most importantly, in subsequent screening cycles this number was reduced to approximately 0.06% (Angaji et al. 2010). Therefore, it is important to investigate the genotypic variation and understand the mechanisms of flooding tolerance during the initial growth stages in rice. Clarifying the adaptive traits that are associated with flooding tolerance during the initial growth stages is the first step toward genetic improvement and could enhance crop establishment of direct-seeded rice in flood-prone areas.

Coleoptile is a morphologically important structure played a key role in enhancing seedling establishment under flooding conditions (Alibu et al. 2011). Under flooding conditions, the coleoptile elongated quickly to enable the seedling to contact with the atmosphere and thus allow oxygen to be transported to underwater tissues (Huang et al. 2003). Furthermore, different rice genotypes showed considerable variation in coleoptile elongation abilities under flooding conditions (Huang et al. 2003; Chung 2010), a relationship between the degree of anaerobic coleoptile extension and tolerance to flooding stress was observed, and the tolerant genotypes had much longer coleoptiles than others (Biswas et al. 2002). Therefore, coleoptile lengths might be potential measurement criteria to select genotypes with improved seedling establishment under flooding conditions.

Based on growth stages and plant characteristics, the genotypes adapted to complete submergence by two contrasting strategies: escape or quiescence (Perata and Voesenek, 2007). During germination stage, the tolerant genotypes emerged from the soil surface 3–4 days before the sensitive one and reached the floodwater surface 2–3 days earlier (Ismail et al. 2009). Therefore, fast seed emergence and rapid coleoptile elongation were the two basic adaptive traits that help tolerant genotypes escape or avoid complete submergence during this stage (Magneschi et al. 2009). In contrast, traits associated with a quiescent strategy such as minimal shoot elongation coupled with maintaining high levels of stored carbohydrates under submergence has been considered as major traits controlling submergence tolerance during the early seedling growth stage (Das et al. 2005). A major feature of this mechanism during this stage is that the shoot does not elongate to conserve energy and reuses for generation of new tissues after de-submergence (El-Hendawy et al. 2012). A single polygenic locus submergence-1 (*Sub1*) on chromosome 9 has been known to play a key role for submergence tolerance in rice according to the quiescent strategy. The submergence-induced *Sub1A* gene helps genotypes to maintain high levels of stored carbohydrates coupled with minimum shoot elongation during submergence and coordinates the recovery of photosynthesis, growth, and carbohydrate-partitioning following de-submergence, which shown in a submergence-tolerant genotype model FR13A (Fukao et al. 2006). However, Sarkar and Bhattacharjee (2011) and Vu et al. (2010) reported that rapid shoot elongation under submergence is advantageous and constitutes one important trait in the elongation escape strategy during the early seedling growth stage. They also reported that the *Sub1* gene expression does not always hinder shoot elongation growth under submergence in the early seedling growth stage. Therefore, different submergence tolerance mechanism could be operative at different growth

stages. Hence, the objective of this study was to establish which of the traits related to either quiescence or escape strategy showed the most reliable associations with flash flooding tolerance in rice during emergence and early seedling growth stage using different rice genotypes including nine lines developed for anaerobic germination and submergence tolerance (AG + Sub1) by IRRI. The AG + Sub1 lines were evaluated for superior germination facility under anaerobic condition and for submergence tolerance during late seedling growth stages (El-Hendawy et al. 2011, 2012); however, their submergence tolerances during initial growth stages are not known.

Materials and Methods

Plant materials

This study was conducted in 2012 using 53 upland rice genotypes (*Oryza sativa* L.). The name of genotypes was listed in Table S1*. 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*). 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

Seeds of each genotype were dry-seeded at approximately 0.5-cm depth in plastic trays filled with nursery soil (pH, 5.0; N, 0.15%; P₂O₅, 0.27%; K₂O, 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, both the flooding and control treatments were maintained under normal rice cultivation conditions for an additional 7 days. 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

Three nursery cells (one from each replicate) from each genotype were selected from either control or flooded treatment at 4, 7, 10, 13, 17, and 24 days after dry seeding to measure the length of the coleoptile and shoot. Samples obtained from 4 to 17 days after dry seeding represented the submergence period, while samples obtained at 24 days after dry seeding represented the de-submergence period. The length of the coleoptile was

* Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

measured from the first node to the tip. Shoot length was measured from the base of the coleoptile to the tip of the leaf. Shoot dry weights were determined after drying at 80 °C for 48 h.

Statistical analysis

The data was statistically analyzed using a randomized complete block design with three replications. Analysis of variance (ANOVA) was used to analyze the data for the response of variables. The means between genotypes were compared using Duncan's multiple test. Probability levels lower than 0.05 were held to be significant. The data of emergence date, percentage of plants reaching the water's surface, maximum coleoptile length, shoot elongation rate during submergence and increases in shoot dry weight after de-submergence were used for cluster analysis. Ward's minimum variance clustering method was used to classify genotypes into discrete clusters. The optimum number of clusters was determined by the sum of squares index (E) (Romersburg 1988). Associations among different traits were examined by simple correlation using the General Linear Model (GLM) procedure implemented in SAS (SAS Institute 2004).

Results

Cluster analysis

The 53 genotypes were grouped into three clusters using Ward's method based on date of emergence, percentage of plants reaching the water's surface, maximum coleoptile length, shoot elongation rate during submergence and increases in shoot dry weight after de-submergence. Table 1 shows the characterization of each cluster group under non-submerged and submerged treatments.

The seeds of genotypes in cluster 1 emerged from the soil surface 1.5 and 2.1 days under non-submerged treatments, and 1.7 and 2.8 days under submerged treatments before the seeds of genotypes in clusters 2 and 3, respectively. The percentage of plants that reached the floodwater's surface for the genotypes in cluster 1 was 77.8%, compared with 42.0 for the genotypes in clusters 2 and 19.7% for the genotypes in clusters 3. The leaf tips of genotypes in clusters 2 and 3 required an additional 3.2 and 5.3 days to emerge above surface of water, respectively, compared with the genotypes in cluster 1 (Table 1).

The maximum coleoptile length of genotypes in clusters 2 and 3 was decreased by 39.4 and 37.9% under non-submerged treatments and 29.2 and 35.1% under submerged treatments, and they required an additional 2.5 and 3.1 days under non-submerged treatments and 3.7 and 4.6 days under submerged treatments to reach the maximum length, respectively, as compared with the genotypes in cluster 1 (Table 1).

Under both conditions, the genotypes in cluster 1 attained a higher rate of shoot elongation between 10 and 13 days of submergence than the genotypes in clusters 2 and 3 and vice versa between 17 and 24 days of de-submergence. Between 13 and 17 days of submergence, the similar behaviour of shoot elongation rate observed among the three clus-

Table 1. Characteristics of each individual cluster under non-submerged and submerged treatments

Treatments	Traits	Clusters group		
		Cluster 1	Cluster 2	Cluster 3
Non-submergence	Emergence date (days)	4.62 ± 0.66	6.10 ± 1.01	6.68 ± 1.43
	Maximum coleoptile length (mm)	12.40 ± 2.01	7.51 ± 1.11	7.70 ± 1.66
	Days to reach maximum coleoptile length (days)	9.00 ± 1.52	11.52 ± 2.16	12.08 ± 2.11
	Shoot elongation rate between 10 and 13 days of sub. (cm d ⁻¹)	3.55 ± 1.08	2.21 ± 1.06	0.82 ± 0.16
	Shoot elongation rate between 13 and 17 days of sub. (cm d ⁻¹)	3.28 ± 0.94	3.92 ± 0.99	3.59 ± 0.71
	Shoot elongation rate between 17 and 24 days of de-sub. (cm d ⁻¹)	1.86 ± 0.57	2.61 ± 0.79	2.96 ± 0.83
	Increase in shoot dry weight between 10 and 13 days of sub. (mg d ⁻¹)	2.30 ± 0.66	1.21 ± 0.36	0.49 ± 0.14
	Increase in shoot dry weight between 13 and 17 days of sub. (mg d ⁻¹)	5.59 ± 1.62	3.76 ± 1.01	2.65 ± 0.64
	Increase in shoot dry weight between 17 and 24 days of de-sub. (mg d ⁻¹)	15.08 ± 2.61	9.63 ± 2.10	8.32 ± 2.2
Submergence	Emergence date (days)	5.15 ± 1.05	6.88 ± 1.45	7.99 ± 1.73
	% of plants that reached the flood water's surface (%)	77.81 ± 10.5	41.97 ± 4.10	19.72 ± 2.01
	Days to reach surface water (day)	10.52 ± 1.32	13.71 ± 2.15	15.79 ± 3.19
	Maximum coleoptile length (mm)	17.00 ± 2.53	12.03 ± 2.11	11.03 ± 1.92
	Days to reach maximum coleoptile length (days)	10.00 ± 1.83	13.72 ± 2.05	14.58 ± 3.12
	Shoot elongation rate between 10 and 13 days of sub. (cm d ⁻¹)	4.56 ± 0.98	1.70 ± 0.17	0.60 ± 0.14
	Shoot elongation rate between 13 and 17 days of sub. (cm d ⁻¹)	2.73 ± 0.68	4.18 ± 0.58	1.96 ± 0.38
	Shoot elongation rate between 17 and 24 days of de-sub. (cm d ⁻¹)	1.40 ± 0.18	1.71 ± 0.33	2.94 ± 0.83
	Increase in shoot dry weight between 10 and 13 days of sub. (mg d ⁻¹)	1.96 ± 0.34	0.68 ± 0.14	0.29 ± 0.05
	Increase in shoot dry weight between 13 and 17 days of sub. (mg d ⁻¹)	3.64 ± 0.79	2.33 ± 0.95	1.07 ± 0.31
	Increase in shoot dry weight between 17 and 24 days of de-sub. (mg d ⁻¹)	9.52 ± 1.93	6.08 ± 1.19	4.36 ± 0.92

Data are mean ± of standard deviations for each individual cluster.

ters group under non-submerged treatments, whereas the genotypes in cluster 2 attained a higher rate of shoot elongation (4.18 cm d^{-1}) than the genotypes in cluster 1 (2.73 cm d^{-1}) and cluster 3 (1.96 cm d^{-1}) under submerged treatments (Table 1).

The genotypes in cluster 1 attained also a higher increase in shoot dry weight at different time of submergence and de-submergence than the genotypes in clusters 2 and 3. For instance, under submerged treatments, the increase in shoot dry weight of the genotypes in clusters 2 and 3 was decreased by 63.3 and 85.2% between 10 and 13 days of submergence, 36.0 and 70.6% between 13 and 17 days of submergence, and 36.1 and 54.2% between 17 and 24 days of de-submergence, respectively, when compared with the genotypes in cluster 1 (Table 1).

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

The seeds of most genotypes in cluster 1 emerged from the soil surface within 4 and 5 days following dry seeding under non-submerged and submerged treatments, respectively. The seeds of genotypes No. 7, 20, 37 and 46 in this cluster were emerged rapidly and emerged from the soil surface in the same day under both conditions. In addition, the percentage of plants that reached the floodwater's surface was more than 80.0% in about 58.0% of the total genotypes that formed this cluster, and the leaf tips of these genotypes emerged above surface water within 10 days following dry seeding. Although, the seeds of genotypes No. 3 and 25 in cluster 1 emerged rapidly under both conditions, the percentage of plants that reached the floodwater's surface of both genotypes was less than 50%. The similar behaviour was found in the genotypes No. 6, 11, 16, 19, 22, 24, 26, 28, 29 and 32 in cluster 2 and genotype No. 27 in cluster 3 (Table S1). Although the genotypes No. 4 and 5 were placed in cluster 2 and genotype No. 9 was placed in cluster 3, the seeds of these lines emerged rapidly and the percentage of plants that reached the floodwater's surface was occasionally comparable to those the most genotypes in cluster 1. The seeds of all genotypes in cluster 3, with the exception of the genotypes No. 9 and 27, were emerged slowly under both conditions, and the leaf tips of the most of genotypes in this cluster did not reach the floodwater's surface during submergence period (Table S1).

Evaluation of genotypes based on coleoptile elongation

The genotype No. 7 in cluster 1 produced the maximum coleoptile length within 7 days following dry seeding under both conditions. The maximum coleoptile length of three genotypes in cluster 1 (No. 1, 2 and 20), three genotypes in cluster 2 (No. 4, 5 and 6) and one genotype in cluster 3 (No. 9) was occasionally comparable to those that of genotype No. 7 and the coleoptile reached the maximum length within also 7 days following dry seeding under both conditions. However, the coleoptile of the other genotypes in cluster 2 and 3 reached the maximum length at 13 or 17 days following dry seeding (Table S1).

Evaluation of genotypes based on shoot elongation rate

Between 10 and 13 days of submergence, the shoot of about 83.0% of the total genotypes in cluster 1 elongated at a rate higher than 3.0 cm d^{-1} under non-submerged treatment and 3.5 cm d^{-1} under submerged treatment, whereas the shoot of about 83.0% of the total genotypes in cluster 3 elongated at a rate less than 1.5 cm d^{-1} under both conditions (Table S2). Between 13 and 17 days of submergence, the shoot of almost genotypes elongated at the same rate under non-submerged treatment, whereas the shoot of genotypes in cluster 3 still elongated at a lower rate under submerged treatment when compared with the genotypes in clusters 1 and 2. Between 17 and 24 days of de-submergence, the shoot of most genotypes in cluster 1 elongated at a rate lower than 2 cm d^{-1} , whereas the shoot of most genotypes in cluster 3 elongated at a rate higher than 3.5 cm d^{-1} under both conditions. The behaviour of shoot elongation rate of genotypes in cluster 2 was similar to those that of genotypes in cluster 1 under submerged treatment and cluster 3 under non-submerged treatment (Table S2).

Evaluation of genotypes based on increase in shoot dry weight

The genotypes which their seeds emerged rapidly from the soil surface and their coleoptile and shoot elongated faster during submergence period such as most genotypes in cluster 1 attained also a high increase in shoot dry weight under both conditions. However, the most genotypes in cluster 3, which their seeds emerged slowly from the soil surface and their coleoptile and shoot showed lesser elongation, attained a lower increase in shoot dry weight at different time of submergence and de-submergence especially under submerged treatment. Increase in shoot dry weight for genotypes No. 4, 5 and 6 in cluster 2 were occasionally comparable to those of most genotypes in cluster 1 during de-submergence (Table S3).

Correlation studies

The percentage of plants that reached the floodwater's surface was negatively and positively correlated with emergence date and maximum coleoptile length, respectively. Shoot elongation rate and increase in shoot dry weight under submerged treatments and between 10 and 13 days of submergence correlated negatively with emergence date and positively with the percentage of plants that reached the floodwater's surface and maximum coleoptile length. Increases in shoot dry weight between 13 and 17 days of submergence and between 17 and 24 days of de-submergence still correlated negatively with emergence date and positively with the percentage of plants that reached the floodwater's surface and maximum coleoptile length. Increases in shoot dry weight at different time correlated positively with shoot elongation rate between 10 and 13 days of submergence and negatively with shoot elongation rate between 17 and 24 days of de-submergence (Table 2).

Table 2. Correlation coefficients among different traits measured at different stage and submergence conditions

Traits		% plants that reach to surface	Shoot elongation rate under submergence			Increase in shoot dry weight under submergence		
			10–13 days	13–17 days	17–24 days	10–13 days	13–17 days	17–24 days
Submergence	Emergence date	-0.81***	-0.75**	-0.07 ^{ns}	0.59*	-0.74**	-0.76**	-0.64**
	% plants that reach to surface	–	0.76**	0.08 ^{ns}	-0.48*	0.81***	0.78**	0.71**
	Maximum coleoptile length	0.68**	0.48*	-0.09 ^{ns}	-0.32*	0.69**	0.68**	0.76**
	Shoot elongation rate between 10 and 13 days of sub.	–	–	-0.09 ^{ns}	-0.49*	0.88***	0.68**	0.48*
	Shoot elongation rate between 13 and 17 days of sub.	–	-0.01 ^{ns}	–	-0.45*	-0.05 ^{ns}	0.31*	0.01 ^{ns}
	Shoot elongation rate between 17 and 24 days of de-sub.	–	-0.49*	-0.45*	–	-0.42*	-0.54*	-0.31*

*, **, ***, ^{ns}: significant at $P \leq 0.05, 0.01, 0.001$ and non-significant, respectively

Discussion

Previous studies have reported that rapid emergence is a trait indispensable for early germination and good stand establishment under submergence conditions (Ismail et al. 2009). The results of this study also indicated clear differences among genotypes in seed emergence date and percentage of plants that reached the floodwater's surface under submerged conditions. The seeds of most genotypes in cluster 1 emerged rapidly in both non-submerged and submerged treatments, with more than 77.8% of their plants emerging above the water's surface during submergence. However, the most genotypes in cluster 3 exhibited a lower percentage of plants that reached the floodwater's surface (less than 20%) and their seeds emerged slowly under both conditions (Tables 1 and S1). In addition, the correlation between seed emergence date and percentage of plants reaching the floodwater's surface were strong and negative (Table 2). It is thus reasonable to assume that rapid emergence of seed from the soil surface under submergence conditions can be considered as a basic trait component helps tolerant genotypes to escape or avoid complete submergence during early germination. This is because rapid emergence could facilitate rapid restoration of contact with air, consequently improving internal aeration for aerobic respiration and allowing partial aerial photosynthesis. The rapid emergence of most genotypes in cluster 1 may be due to the ability of these genotypes to degrade the starch of seeds into soluble sugars faster under low-oxygen stress, which presumably provided the substrates necessary for generating the energy required for growth and maintenance processes (Ismail et al. 2009).

Rapid elongation of the coleoptile under complete submergence is also considered to be trait conferring establishment of tolerance at the initial growth stage. Several studies have used this trait as the criterion for selecting tolerant rice accessions, and the tolerant genotypes produce longer coleoptiles under submergence conditions for providing oxygen to roots through aerenchyma (Biswas et al. 2002; Alibu et al. 2011). The results of this study revealed that the genotypes in cluster 1 had the longest coleoptile (Table 1) and the coleoptile of most genotypes in this cluster reached the maximum length within 7 days following dry seeding in both conditions (Table 1). However, 58.3% of the total genotypes in cluster 3 attained a shortest coleoptile length and the coleoptile reached the maximum length at 17 days following dry seeding (Table S1). In addition, the percentage of plants that reached the floodwater's surface correlated positively with maximum coleoptile length (Table 2). These results indicate that rapid coleoptile elongation can be considered a more reliable trait for submergence escape or avoidance during the initial growth stage by enabling rice seedlings to rapidly reach the water's surface and allowing oxygen uptake into underwater tissues. These results are largely in agreement with Alibu et al. (2011), who reported that inherent differences in genotypic coleoptile elongation manifested more clearly under submergence conditions. The results of this study also showed that the ability of coleoptile to elongate rapidly under submerged treatment is not likely to be explained in terms of *Sub1* genes. Therefore, the AG + *Sub1* lines were separated in the three clusters. This finding is largely in agreement with Jackson (2008), who

reported that ethylene not *Sub1* genes promotes coleoptile elongation under flooding conditions and higher ethylene levels could mean faster extension growth.

Maintenance of high dry matter levels coupled with minimum shoot elongation are two reliable traits that play key roles in the quiescence strategy, which enables plants to economically use energy and to quickly recover once the water recedes. However, fast and vigorous shoot elongation are the two major traits strongly associated with submergence avoidance or escape strategy, which allow plants to re-establish air contact (El-Hendawy et al. 2012; Nishiuchi et al. 2012). The question that arises here is which traits related to either the quiescence or the escape strategy enable rice genotypes to cope with submergence stress during the initial growth stages? A number of studies have reported that traits related to submergence avoidance or escape strategy are key mechanisms for seedling establishment, particularly when floods occur after direct seeding. The results of the present study confirm these findings and show that the most genotypes in cluster 1 represent typical model genotypes, which use fast and vigorous shoot elongation traits as key mechanisms to avoid or escape submergence stress during the early seedling stages. The genotypes in this cluster attained a higher rate of shoot elongation between 10 and 13 days of submergence than the genotypes in clusters 2 and 3 and vice versa between 17 and 24 days of de-submergence (Tables 1 and S2). Furthermore, the genotypes in cluster 1 attained a higher increase in shoot dry weight at different time of submergence and de-submergence than the genotypes in clusters 2 and 3 (Tables 1 and S3). In addition, increases in shoot dry weight at different time correlated positively with shoot elongation rate between 10 and 13 days of submergence (Table 2). Together, these results indicate that fast and vigorous growth under submergence conditions are major traits controlling submergence tolerance at the early seedling stage.

In contrast to fast and vigorous shoot elongation, quiescence strategy traits have been considered indispensable for quick recovery following submergence because plants using this strategy can save energy and resources during submergence, which could positively affect the generation of new tissues after de-submergence. The results of the present study confirm this strategy and show that the most genotypes in cluster 3 represent a typical model for quiescence strategy, which showing both minimum shoot elongation under submergence and good recovery following submergence (Tables S1, S2, S3, S4 and S5). The ethylene-response-factor-like genes located at the *Sub1* locus were shown to play a key role in the operation of this strategy (Vu et al. 2010). The AG + *Sub1* lines (No. 8 and 9 in cluster 3) followed the quiescence strategy in this study. However, Perata and Voesenek (2007) stated that the quiescence strategy does not necessarily lead to submergence tolerance at the early seedling stages after germination. The present study confirms this hypothesis when comparing the increase in shoot dry weight of the genotypes in cluster 1 with the genotypes in clusters 2 and 3 especially between 10 and 13 days of submergence (Tables 1, S3 and S5). The most genotypes in cluster 1 attained a higher increase in shoot elongation and also higher increase in shoot dry weight and vice versa for most genotypes in cluster 3 (Tables S1, S2, S3, S4 and S5). These results indicate that rapid shoot elongation at the early seedling stage appears to be a favorable trait that strongly improves inward diffusion of oxygen and the rate of photosynthesis through re-

storing contact of leaves with the atmosphere above the water's surface. It is interesting to note that the rapid shoot elongation during submergence occurred also in AG + Sub1 lines (No. 1, 2, 3 and 7 in cluster 1). This finding indicates that presence of Sub1 locus does not always hinder the shoot elongation growth under submergence conditions at the early seedling growth stage. This is in agreement with the results of Vu et al. (2010) and Sarkar and Bhattacharjee (2011).

Acknowledgements

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding this Research Group No. (RG-1435-032).

References

- Alibu, S., Saito, Y., Shiwachi, H., Irie, K. 2011. Relationship between coleoptile and mesocotyl elongation of upland rice (*Oryza sativa* L.) seedlings under submergence and soil-sand culture. *African J. Agric. Res.* **31**:6463–6472.
- Angaji, S.A., Septiningsih, E.M., Mackill, D.J., Ismail, A.M. 2010. QTLs associated with tolerance of flooding during germination in rice (*Oryza sativa* L.). *Euphytica* **172**:159–168.
- Biswas, J.K., Islam, M.S., Yasmeen, R., Pervia, S., Shahjahan, M.S., Alam, S. 2002. Relative contribution of the coleoptile and the first leaf length to seedling establishment in rice (*Oryza sativa* L.) as affected by anaerobic seedling in two different soil. *Pakistan J. Biol. Sci.* **5**:413–415.
- Chung, N.J. 2010. Elongation habit of mesocotyls and coleoptiles in weedy rice with high emergence ability in direct-seeding on dry paddy fields. *Crop Pasture Sci.* **61**:911–917.
- Das, K.K., Sarkar, R.K., Ismail, A.M. 2005. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. *Plant Sci.* **168**:131–136.
- El-Hendawy, S.E., Sone, C., Ito, O., Sakagami, J.I. 2011. Evaluation of germination ability in rice seeds under anaerobic conditions by cluster analysis. *Res. J. Seed Sci.* **4**:82–93.
- El-Hendawy, S.E., Sone, C., Ito, O., Sakagami, J.I. 2012. Differential growth response of rice genotypes based on quiescence mechanism under flash flooding stress. *Aust. J. Crop Sci.* **6**:1587–1597.
- Ella, E.S., Dionisio-Sese, M.L., Ismail, A.M. 2011. Seed pre-treatment in rice reduces damage, enhances carbohydrate mobilization and improves emergence and seedling establishment under flooded conditions. *AoB Plants* plr007 doi:10.1093/aobla/plr007 <http://aobplants.oxfordjournals.org/>
- Fukao, T., Xu, K., Ronald, P.C., Bailey-Serres, J. 2006. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *Plant Cell* **18**:2021–2034.
- Huang, S.B., Greenway, H., Colmer, T.D. 2003. Anoxia tolerance in rice seedlings: exogenous glucose improves growth of an anoxia-'intolerant', but not of a 'tolerant' genotype. *J. Exp. Bot.* **54**:2363–2373.
- Ismail, A.M., Ella, E.S., Vergara, G.V., Mackill, D.J. 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). *Annals Bot.* **103**:197–209.
- Jackson, M.B. 2008. Ethylene-promoted elongation: an adaptation to submergence stress. *Annals of Bot.* **101**:229–248.
- Magneschi, L., Kudahettige, R.L., Alpi, A., Perata, P. 2009. Comparative analysis of anoxic coleoptile elongation in rice varieties: relationship between coleoptile length and carbohydrate levels, fermentative metabolism and anaerobic gene expression. *Plant Biol.* **11**:561–573.
- Nishiuchi, S., Yamauchi, T., Takahashi, H., Kotula, L., Nakazon, M. 2012. Mechanisms for coping with submergence and waterlogging in rice. *Rice* **5**:1–14.

- Perata, P., Voesenek, L.A.C.J. 2007. Submergence tolerance in rice requires Sub1A, an ethylene-response-factor-like gene. *Trend Plant Sci.* **12**:43–46.
- Romersburg, H.C. 1988. Cluster analysis for researchers. Lifetime Learning Publications. Belmont, CA, USA.
- Sarkar, R.K., Bhattacharjee, B. 2011. Rice genotypes with SUB1 QTL differ in submergence tolerance, elongation ability during submergence and re-generation growth at re-emergence. *Rice* **5**:1–11.
- SAS 2004. The SAS System for Windows, Version 9.1.3. SAS Inst., Cary, NC, USA
- Vu, H.T.T., Manangkil, O.E., Mori, N., Yoshida, S., Nakamura, C. 2010. Post-germination seedling vigour under submergence and submergence-induced *SUB1A* gene expression in indica and japonica rice (*Oryza sativa* L.). *Aust. J. Crop Sci.* **4**:264–272.

Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at <http://www.akademai.com/content/120427/>

Electronic Supplementary *Table S1*. Emergence date under non-submerged (Non.) and submerged (Sub.) treatments, percentage of plants that reached the flood water's surface and dates to reach flood water surface under submerged treatment, maximum coleoptile length and days required to reach this maximum under both conditions for different rice genotypes

Electronic Supplementary *Table S2*. Shoot elongation rate of the three clusters group of 53 contrasting rice genotypes at several times during submergence and at 7 days after de-submergence under non-submerged and submerged treatments

Electronic Supplementary *Table S3*. Increase in shoot dry weight of the three clusters group of 53 contrasting rice genotypes at several times during submergence and at 7 days after de-submergence under non-submerged and submerged treatments

Electronic Supplementary *Table S4*. Increase in shoot length of the three clusters group of 53 contrasting rice genotypes at several times during submergence and at 7 days after de-submergence under non-submerged and submerged treatments

Electronic Supplementary *Table S5*. Increase in shoot dry weight of the three clusters group of 53 contrasting rice genotypes at several times during submergence and at 7 days after de-submergence under non-submerged and submerged treatments