INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0859/2019/22–1–35–42
DOI: 10.17957/IJAB/15.1030
http://www.fspublishers.org

Full Length Article

Morpho-Physiological Responses of Rice Towards Submergence Tolerance

Asmuni Mohd Ikmal, Ismail Amira and Abd Aziz Shamsudin Noraziyah*
Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia
*For correspondence: nora_aziz@ukm.edu.my

Abstract

Submergence has been a great abiotic stress in rice cultivation. In this study, fifteen rice genotypes and IR64-*Sub1* as control were evaluated under submergence at vegetative stage, which imposed for 14 days after 20 days of germination. Morpho-physiological traits such as change of chlorophyll content (CCC), elongation percentage (EP), coleoptile length (CL), survival rate (SR), photosynthesis rate (PR) and percentage of non-structural carbohydrate (NSC) were recorded before and after submergence imposition and analysed. The IR64-*Sub1* showed the highest SR (88.89%) followed by UKMRC2 (67.22%) and UKM-2 (65.93%) while MR264 had the lowest SR (8.89%). In most cycles, SR was positively correlated with CL and PR but negatively correlated with EP and CCC. Only NSCC showed significant correlation with SR ($p < 0.05$), however the correlation was inconsistent across cycles. EP plays a major role in affecting SR under submergence. Genotypes with high EP consumed high amount of NSC, causing them unable to recover after submergence. The best three genotypes (IR64-*Sub1*, UKMRC2 and UKM-2) adopted the quiescence strategy to survive under submergence by limiting their growth as shown by the low EP. Genotyping using *Sub1* closely linked markers found that no single genotype possess the *Sub1* locus. This suggests the existence of gene/s or QTL/s other than *Sub1* need to be discovered and survive under submergence of high yielding genotypes, UKMRC2 and UKM-2. UKMRC2 and UKM-2 can be recommended for submergence prone rice cultivating areas in Malaysia.

Keywords: *Sub1*; Survival rate; Photosynthesis; Elongation; Submergence

Introduction

Rice (*Oryza sativa* L.) is the main staple food consumed by almost 90% of the Asian countries' population. According to Malaysia's Rice Statistics 2014 (Department of Agriculture, 2015), 74% of total rice yield was from 59% of the planted areas, which are the eight granary areas across Peninsular Malaysia. However, the growth of rice was disrupted due to massive flood which covered almost whole granary areas and ruined the crop. Replanting is possible but most farmers cannot afford due to high cost involved for land preparation and seeds.

Drastic submergence will reduce the oxygen absorption and carbon dioxide, therefore will affect the whole process of photosynthesis despite of its semi-aquatic traits (Xu *et al*., 2006; Bailey-Serres *et al*., 2010; Gao *et al.*, 2018). In order to solve this matter, prioritizing the production of high yielding and submergence-resistant genotypes is important to enhance the stability of the paddy industry in the country. Xu and Mackill (1996) reported that rice is able to tolerate submergence with the presence of locus *Submergence 1* (*Sub1*) located on chromosome 9. *Sub1* is a polygenic locus that encode three ethylene responsive factor (ERF) genes, which are *Sub1*A, *Sub1*B and *Sub1*C. *Sub1*A gene provides the highest tolerance to submergence (Xu *et al*., 2006; Septiningsih *et al.*, 2009).

Several submergence tolerant rice varieties, such as Swarna-*Sub1*, Thadokkham1-*Sub1* and IR64-*Sub1* have been developed, by cross-breeding method with the assistance of molecular markers (Septiningsih *et al*., 2009). IR64-*Sub1*'s percentange of survival rate (SR) is 80–87% compared to the parent IR64 with survival percentage of 20–30%. Plant is unable to elongate and enlarge due to *Sub1*-mediated suppression of elongation which blocks the elongation of the shoots to conserve energy. The study at International Rice Research Institute (IRRI) also showed that the variety with high elongation rate such as IRRI 119-*Sub1* is capable of surviving and tolerate better than other relatively short *Sub1* varieties in floods with a water depth of 20–60 cm for consecutively 50 days (Septiningsih *et al*., 2009). This indicate that there is a positive interaction between *Sub1* locus and other morpho-physiological traits. Morpho-physiological traits are also linked with the ability to increase rice productivity in flooding areas besides the presence of the *Sub1* locus. According to Singh *et al.* (2011), improvement to submergence tolerance require a combination of *Sub1* locus and other morpho-physiological traits to produce more submergence tolerant variety in order to maintain the rice yield.

The current situation of massive flood in Malaysia is expected to be in a further extreme stake, causes the breeders to prioritize the submergence tolerant rice despite

of the preliminary level in studies. Although many high yielding rice genotypes have been successfully developed in the world and especially in Malaysia, but most of these genotypes are susceptible to abiotic stresses such as flood and drought (Shamsudin *et al*., 2016a; Swamy *et al*., 2017; Mohd Ikmal *et al*., 2018). Therefore, identification of promising parents and evaluation of morpho-physiological traits related to submergence is substantial prior to breeding. The objective of this study is to understand the interaction between morpho-physiological traits and rice genotypes survival mechanisms under submergence at vegetative stage. This study will provide more information on beneficial rice genotypes for cultivation in submergence prone areas and to discover potential genotypes as donor for breeding programmes, genomics and phenomics study.

Materials and Methods

Plant Materials and Experimental Design

Total of 16 rice genotypes comprising of traditional varieties (Langsat and Campa), modern cultivars (MR142, MR219, MR253, MR263, MR264, MR284, MRQ74) generated by Malaysia Agricultural Research and Development Instutute (MARDI), transgressive backcross lines (UKMRC2, UKMRC8, UKMRC9 and UKMRC11) derived from *Oryza rufipogon* (IRGC105491) × *Oryza sativa* cv. MR219 (Sabu *et al.*, 2006), drought tolerant pyramided lines (UKM-2 and UKM-5) developed through marker assisted QTL pyramiding technique as described by Shamsudin *et al.* (2016b) and a control cultivar, IR64-*Sub1* that highly tolerant to submergence (Septiningsih *et al*., 2009) were used in this study. Genotypes were arranged in randomized complete block design with three replications. Rice seeds were germinated on 15 cm height moistened soil in a pot measuring 20 cm height and 10 cm diameter. After 20 days of germination, seedlings in each pot were thinned to ten individuals. Trials were repeated for three times, where each repetition refered as "Cycle" and data were averaged.

Submergence at Vegetative Stage

On the 21st day after germination, pots were placed in a 63 cm height water tank and submergence was imposed. Protocols used to impose submergence were based on the previous study methods by Singh *et al*. (2014) and Iftekharuddaula *et al*. (2015) with slight modification. Submergence was maintained at full tank capacity for two weeks before desubmerged on the 15th day. Dissolved oxygen concentration and temperature of water were taken at 0800 on day 3, 7 and 13.

Data Collection and Analysis

Coleoptile length (CL) was recorded after five days from the first day of submerging seeds in test tubes. The lengths were measured in milimiter (mm) using vernier calipers. Survival rate (SR) was taken after ten days of de-submergence using formula used before by Ranawake *et al*. (2014). The elongation percentage (EP) was calculated as difference in seedling height (cm) before (PHB) and after (PHA) submergence (Yoshida *et al*., 1976). Chlorophyll content (CC) and photosynthesis rate (PR) was measured using SPAD-500 meter and LI-COR 6400XT respectively after two days of desubmergence. Change of chlorophyll content (CCC) calculated using formula below:

$$\text{CCC (\%)} = \frac{\text{CC before submergence - CC after submergence}}{\text{CC before submergence}} \times 100$$

To obtain non-structural carbohydrate (NSC) data, leaf samples were taken before submergence and a day after submergence. Protocols for obtaining NSC and its calculation followed method by Yoshida *et al*. (1976). Percentage of change in NSC (NSCC) was calculted by accounting the percentage of NSC before submergence (NSCB) and NSC after submergence (NSCA) using formula below:

$$\text{NSCC (\%)} = \frac{\text{NSC before submergence - NSC after submergence}}{\text{NSC before submergence}} \times 100$$

All data were analyzed using PBTools version 1.4 and Statistical Tools for Agricultural Research (STAR) software provided by IRRI freely on http://bbi.irri.org/. Correlation values were obtained using Minitab 17 to identify the relationship that exist between those traits. RStudio was used to compute correlation and produce the graphical correlation matrix by utilising the Corrplot package (https://cran.r-project.org/web/packages/corrplot/index.html).

Genotyping

Fresh leaf samples for each genotype were collected and extracted using cetyltrimethyl ammonium bromide (CTAB) protocols (Murray and Thompson, 1980) with some modifications. Agarose gel 1% was used to determine the DNA concentration that had been extracted by comparing DNA band with the standard marker λHindIII. Nanodrop spectrophotometer was also used to get more accurate estimation of DNA concentrations and purities. The stock solution of the DNA samples were diluted to concentration of 25 ng/μL by adding distilled water (dH_2O).

Polymerase chain reactions using primers were done using Mastercycler gradient (Eppendorf, Germany) (Table 1). Each PCR mixture in each well contained 1.5 μL of 10× Free $MgCl_2$ buffer, 1.0 μL of $MgCl_2$, 1.5 μL of 10 m*M* dNTPs, 1.0 μL each of 10 m*M* forward and reverse primers, 1.0 μL of 25 ng DNA template, 7.0 μL of dH_2O and 1.0 μL of 1U/μL *Taq* polymerase. Standard PCR profile was used except for the annealing temperature that varied among primers.

PCR products obtained were subjected to electrophoresis using 8% polyacrylamide gel at 110 V for one to two hours dependent on expected product sizes. Polyacrylamide gel then stained using RedSafe Nucleic

Acid Stain and exposed under ultraviolet (UV) light. Amplicon sizes were estimated in comparison with the 100 bp ladder loaded into wells at both ends of the gel.

Results

Phenotypic Analysis

All genotypes showed wide range of genetic variation in respect to these traits. Coefficients of variation (CV) for all traits in this study were between 18.21% to 96.6%. Plant height before (PHB) submergence had lowest CV (18.21%) while survival rate (SR) showed the highest CV (96.66%) than other traits. Three traits with medium CV were PHB (18.21%), plant height after (PHA) (19.26%) and chlorophyll content before (CCB) (18.93%). The remaining nine traits showed very high CV (>30%) (Table 2).

Abiotic Parameters

Concentration of dissolved oxygen in the water decreased from the first day to the last day of submergence (Fig. 1). The mean water temperature recorded was 31°C while pH was 7.80. Temperature of water ranged from 27–31°C during early morning, while 29–32°C in the afternoon.

Performance of Genotypes under Submergence

Initial measurement of plant height showed that PHB of all genotypes were around 24.00 cm to 32.00 cm. As observed, UKM-2 had the highest PHB of 32.86 cm while MR264 had the lowest (24.08 cm). For PHA, UKM-2 and MR264 were also the highest and lowest respectively. MR264 had the lowest elongation percentage (EP), which was 16.69 cm followed by the tolerant control, IR64-*Sub1* (21.25 cm) and MR219 (22.69 cm). The traditional variety, Langsat showed the highest EP (57.59%) among all genotypes all the value was significantly different from IR64-*Sub1* ($p < 0.05$). Another traditional variety, Campa also exhibited higher EP than IR64-*Sub1* but statistically insignificant (Table 3). UKMRC8, which derived from a cross between a modern cultivar, MR219 and a wild species of rice, *Oryza rufipogon* (IRGC105491) showed the second highest EP (43.58%) after Langsat. The modern cultivars namely MR142, MR263, MR284, MRQ74, UKMRC2 and UKMRC9 recorded EP between 27.00 to 33.00%. CCB of all genotypes were from 15.00 to 23.85 while for chlorophyll content after (CCA), the values varied from the lowest, 8.00 to the highest, 20.77. All genotypes had reduced chlorophyll content as shown by positive values of CCC except IR64-*Sub1*, which was the only genotype with increased chlorophyll content after submergence. UKMRC recorded the highest reduction of chlorophyll content (55.38%) and significantly different from IR64-*Sub1* (-4.74%). Campa and MR142 also expressed high values of CCC and were similar to UKMRC2. Apart of that, UKM-5 had apparently low CCC (12.22%) compared to other genotypes, which showed CCC values between 20.00 to 55.00%.

After submergence, all genotypes had reduced percentage of NSC compared to prior to submergence. The lowest reduction in NSC was seen for UKMRC9 with 46.60%, followed by UKMRC2 (49.60%) and IR64-*Sub1* (48.80%). Meanwhile, MRQ74 had the highest NSCC of 94.16% tailed by Langsat (89.77%) and MR263 (88.37%). Post-submergence photosynthesis rate (PR) showed that MR263 had the lowest PR (0.96 μmol m^{-1} s^{-1}) while UKMRC2 had the highest PR (8.36 μmol m^{-1} s^{-1}), which was significantly different from IR64-*Sub1*. As expected, IR64-*Sub1* had the highest percentage of SR (88.89%) compared to other genotypes.

Table 1: Markers used for genotyping of *Sub1* locus

Marker	Distance (cM)	Size (bp)	Annealing temperature (°C)
RM23662	0.4	149	55
RM5688	1.7	150	55
ART5	2.6	124	60
SC3	6.8	217	55
RM23877	6.3	327	55

cM =centimorgan, bp =base pair

Table 2: Descriptive statistics for all traits studied

Variable	Min	Max	Mean ± SE	Range	CV (%)
PHB	12.90	38.50	27.28 ± 0.41	25.60	18.21
PHA	18.34	56.80	35.74 ± 0.57	38.46	19.26
EP	1.80	116.85	32.31 ± 1.70	115.05	63.12
CCB	11.80	29.65	20.34 ± 0.32	17.85	18.93
CCA	3.87	25.00	12.60 ± 0.41	21.13	39.46
CCC	-86.72	82.07	36.32 ± 2.32	168.8	76.67
SR	0.00	100.00	38.14 ± 3.07	100.00	96.66
CL	4.06	41.55	24.33 ± 0.85	37.49	41.93
NSCB	1.10	19.60	11.00 ± 0.42	18.50	37.21
NSCA	0.08	9.56	2.90 ± 0.27	9.48	92.35
NSCC	-20.82	98.86	70.28 ± 2.74	119.68	38.15
PR	0.36	9.61	4.56 ± 0.38	9.25	57.15

Min =minimum, max =maximum, SE =standard error, CV =coefficient of variation, PHB =plant height before, PHA =plant height after, EP =elongation percentage, CCB =chlorophyll content before, CCA =chlorophyll content after, CCC =chlorophyll content change, SR =survival rate, CL =coleoptile length, NSCB =non-structural carbohydrate before, NSCA =non-structural carbohydrate after, NSCC =non-structural carbohydrate change, PR =photosynthesis rate

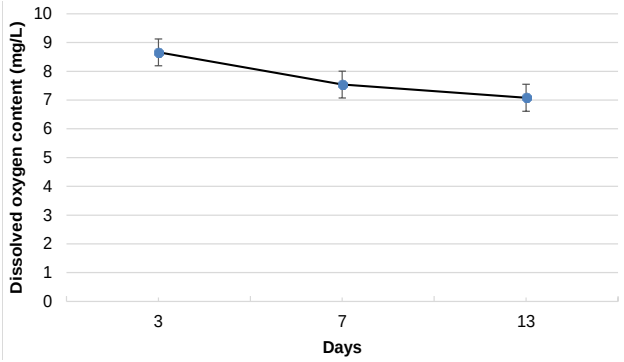

Fig. 1: Dissolved oxygen content (mg/L) from days 3 to 13

Table 3: Means of all traits for each genotype averaged over three cycles

Genotypes	PHB	PHA	EP	CCB	CCA	CCC	SR	CL	NSCB	NSCA	NSCC	PR
Campa	26.94^{ab}	35.65^{bcde}	32.78^{ab}	21.13^{a}	9.87^{b}	54.92^{a}	14.63^{d}	30.28^{ab}	7.60^{de}	3.27^{ab}	55.59^{a}	6.61^{abc}
IR64-*Sub1*	24.89^{b}	29.44^{de}	21.25^{b}	20.43^{ab}	20.77^{a}	-4.79^{c}	88.89^{a}	26.24^{abcd}	11.08^{bcde}	5.75^{a}	48.80^{a}	6.83^{ab}
Langsat	30.33^{ab}	47.69^{a}	57.59^{a}	21.55^{a}	13.54^{b}	37.16^{ab}	13.33^{d}	33.94^{a}	15.89^{ab}	1.62^{ab}	89.77^{a}	3.55^{abc}
MR142	28.81^{ab}	37.54^{bcd}	31.21^{ab}	21.07^{a}	9.34^{b}	54.98^{a}	26.88^{bcd}	29.63^{abc}	8.19^{de}	1.28^{ab}	83.36^{a}	2.69^{abc}
MR219	26.27^{ab}	31.70^{cde}	22.69^{b}	20.89^{ab}	14.14^{ab}	32.56^{abc}	11.38^{d}	17.44^{bcde}	8.59^{de}	2.35^{ab}	71.93^{a}	2.79^{abc}
MR253	25.78^{ab}	36.33^{bcde}	43.36^{ab}	23.85^{a}	12.70^{b}	47.39^{ab}	21.11^{bcd}	13.83^{de}	14.78^{abc}	2.14^{ab}	86.23^{a}	3.33^{abc}
MR263	25.13^{ab}	33.33^{cde}	33.70^{ab}	20.53^{ab}	9.00^{b}	53.99^{a}	17.04^{cd}	27.50^{abc}	17.02^{a}	2.15^{ab}	88.37^{a}	0.96^{c}
MR264	24.08^{b}	27.90^{e}	16.69^{b}	20.89^{ab}	10.46^{b}	48.76^{ab}	8.89^{d}	19.14^{bcde}	5.34^{e}	1.12^{ab}	52.20^{a}	5.84^{abc}
MR284	27.82^{ab}	35.15^{bcde}	28.21^{ab}	18.24^{ab}	12.72^{b}	28.05^{abc}	35.37^{bcd}	28.16^{abc}	11.39^{abcd}	4.79^{ab}	62.50^{a}	5.02^{abc}
MRQ74	24.76^{b}	31.17^{cde}	28.18^{ab}	19.27^{ab}	14.35^{ab}	22.95^{abc}	41.94^{abcd}	17.21^{cde}	12.80^{abcd}	0.65^{b}	94.16^{a}	5.72^{abc}
UKM-2	32.86^{a}	43.08^{ab}	32.22^{ab}	23.11^{a}	15.85^{ab}	31.75^{abc}	65.93^{abc}	29.59^{abc}	13.00^{abcd}	5.24^{ab}	60.46^{a}	2.29^{bc}
UKM-5	28.07^{ab}	35.59^{bcde}	29.17^{ab}	15.23^{b}	12.56^{b}	12.22^{bc}	45.00^{abcd}	28.08^{abc}	11.81^{abcd}	2.25^{ab}	76.32^{a}	6.52^{abc}
UKMRC11	27.97^{ab}	38.00^{bcd}	37.76^{ab}	20.58^{ab}	12.33^{b}	40.82^{ab}	54.72^{abcd}	28.13^{abc}	9.70^{cde}	1.77^{ab}	81.47^{a}	5.94^{abc}
UKMRC2	27.03^{ab}	34.93^{bcde}	31.10^{ab}	20.49^{ab}	8.97^{b}	55.38^{a}	67.22^{ab}	7.27^{e}	11.16^{abcde}	5.10^{ab}	49.60^{a}	8.36^{a}
UKMRC8	27.27^{ab}	38.55^{bc}	43.58^{ab}	19.15^{ab}	13.35^{b}	27.64^{abc}	51.24^{abcd}	26.00^{abcd}	9.77^{cde}	2.44^{ab}	77.09^{a}	1.53^{bc}
UKMRC9	28.47^{ab}	35.80^{bcde}	27.50^{ab}	19.00^{ab}	11.56^{b}	37.34^{ab}	46.67^{abcd}	26.83^{abc}	7.81^{de}	4.55^{ab}	46.60^{a}	5.01^{abc}

Means with the same letter at every column did not significantly different by Tukey's test (α=0.05)

UKMRC2 and the drought tolerant line, UKM-2 recorded the second and third highest SR, which were 67.22 and 65.93% respectively. Lowest SR was recorded by MR264 (8.89%), followed by MR219 (11.38%) and the two traditional varieties; Langsat (13.33%) and Campa (14.63%). For the submerged seeds, both the traditional varieties, Langsat and Campa recorded the longest and second longest CL, which were 33.94 and 30.28 mm respectively. The tolerant check, IR64-*Sub1* recorded CL of 26.24 mm while the susceptible check, MR219 had CL of 17.44 mm. The shortest CL was recorded by UKMRC2 (7.27 mm).

Correlation

In Cycle 1, SR was positively correlated with EP, CL, NSCC and PR but negatively correlated with CCC. However, only NSCC showed significant correlation (r=0.33, $p < 0.05$) in this cycle. Same result was observed in Cycle 2 and Cycle 3 where SR was also positively correlated with CL but negatively correlated with CCC. The results were inconsistent for EP and NSCC as SR showed negative correlation with these two traits in Cycle 2 and Cycle 3. CCC recorded significant negative correlation with NSCC in Cycle 1 but recorded insignificant low positive correlation in Cycle 3 (Fig. 2).

Genotyping

All the genotypes were tested for the presence of *Sub1* by using SSR markers, which linked to the QTL. The results showed that no tested genotypes possess the *Sub1* (Fig. 3).

Discussion

The relative variability of a population is measured by the coefficient of variation (CV) and desired CV for biological experiment is <10% (Acquaah, 2012). CV is a good tool to compare variation regardless of different units. High CV in SR and NSCA were because all these genotypes were from different background and have different level of submergence tolerance. The initial measurement of heights for all genotypes did not differed greatly suggesting identical growth rate after germination in tested genotypes.

The elongation of shoot will be suppressed via mediation of *Sub1* leading to the increment of SR due to limitation of carbohydrate usages as shown in IR64-*Sub1* (Sarkar *et al*., 2009; Singh *et al*., 2009). The rate of shoot elongation was one of the responses of plant to adapt during submergence using two different types of survival strategies depending on type of flood occur termed as quiescence and elongation (Luo *et al*., 2011). Elongation of shoot use enormous energy and carbohydrate (Voesenek *et al*., 2004; Pierik *et al*., 2009; Sarkar and Bhattacharjee, 2011) which affect recovery process after submergence and causing the plant to die (Das *et al*., 2005). The non-*Sub1* genotypes in this study, which elongated their shoot underwater such as Langsat and MR253 were among the less-surviving genotypes. Das *et al*. (2005) found that the susceptible genotypes had greater elongation than the tolerant genotype, ranging from 111 to 144%. Meanwhile, Sarkar and Bhattacharjee (2011) reported that Swarna is susceptible to submergence had greater shoot elongation than the tolerant Swarna-*Sub1*. However, MR264 which found to have the lowest EP also had the lowest SR. This might be due to the low percentage of NSCB which inhibit elongation process during submergence and low NSCA causing MR264 to have less energy for growth and recovery process during and after submergence. The desirable traits such as medium elongation and high production of NSC were found to be correlated with submergence tolerance (Das *et al*., 2005).

Elongation was identified as the main mechanism of survival for Campa, Langsat, MR253 and UKMRC8 under submergence. According to Metraux and Kende (1982), the internode length of plant will gradually increase up to three until five times along with the concentration of ethylene in

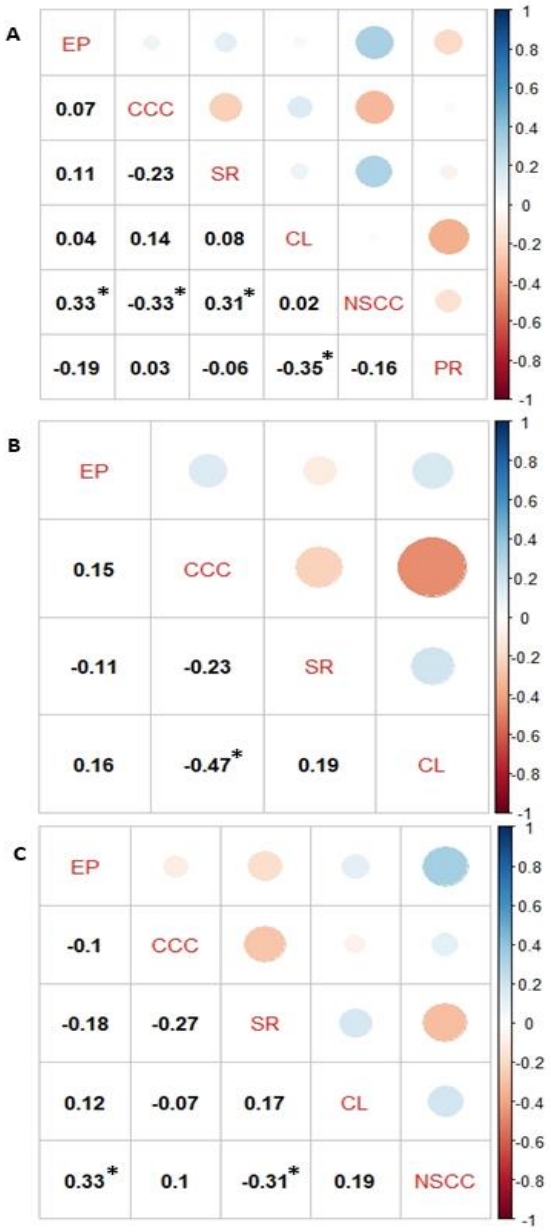

Fig. 2: Graphical correlation matrices for traits studied. (**A**) Cycle 1, (**B**) Cycle 2, (**C**) Cycle 3. EP =elongation percentage, CCC =chlorophyll content change, SR =survival rate, CL =coleoptile length, NSCC =non-structural carbohydrate change, PR =photosynthesis rate. *Significant correlation ($p < 0.05$)

the internodal up to 0.02 until 1.00 μL/L during submergence state for at least seven days both processes encourage plant height. Langsat might have experienced the accumulation of ethylene in its tissues leading to elongation at rate of 1.24 cm/day, showing its adaptability to deepwater flood as the other rice landraces. Rice landraces avoid complete submergence for survival by elongating its shoot to reach the water surface (Setter and Laureles, 1996; Sarkar and Bera, 1997). Due to accumulation of ethylene, expression of genes named as *SNORKEL 1* (*SK1*) and *SNORKEL 2* (*SK2*) was induced, which lead to GA biosynthesis or promotion of GA signal transduction that causes elongation of leaves and internodes. However, Langsat failed to reach the water surface to gain enough gaseous exchange for physiological processes during submergence which further lead to death after submergence.

Genotypes which maintain high NSC content after submergence (low NSCC) were able to produce new leaves rapidly and produce high amount of biomass during the recovery process after submergence (Panda *et al*., 2008; Sarkar and Bhattacharjee, 2011). Intolerant genotypes in this study, such as MR142, MR263 and MR219 have higher NSCC than the tolerant genotype, IR64-*Sub1*. Fukao *et al.* (2006) mentioned that under submergence, rice sucrose synthase genes namely *Sus1*, *Sus2* and *Sus3* and also α-amylase genes namely *Ramy3C*, *Ramy3D* and *Ramy3E* were highly induced. Induction of these genes lead to increase of energy consumption during submergence. Meanwhile, under the same condition, submergence tolerant genotype had lower expression of the genes which suggest the role of *Sub1A* for negative regulation of transcription of the genes.

Besides elongation, the other survival mechanism during submergence is quiescence applicable in short-term flood or flash flood. In order to enable photosynthesis in water, plant needs to store energy and preserve chlorophyll content, by that elongation will be inactivated (Ella *et al*., 2003; Das *et al*., 2005; Nagai *et al*., 2010; Winkel *et al*., 2013). During submergence, plant growth is the result from the accumulation of ethylene in tissues (Musgrave *et al*., 1972; Metraux and Kende, 1982). In order to stop the internodal elongation, the production of ethylene will be restrained by aminoacetic acid and aminoethoxivinylglycin (Metraux and Kende, 1982). Genotypes with moderate EP values with no significant difference from IR64-*Sub1* such as UKM-2, UKMRC9 and UKMRC2 are suitable for cultivation in areas with flood that last for less than two weeks. These genotypes will reduce EP to prevent energy loss and increase the SR after water level decreased (Sarkar *et al*., 1996; Das *et al*., 2005; Sarkar *et al*., 2009; Singh *et al*., 2009). IR64-*Sub1* have the *Sub1A* gene, elongation of leaves and internodes was inhibited by the action of *Slender rice-1* (*SLR1*) and *SLR1 Like-1* (*SLRL1*) genes which negatively regulating GA synthesis. Limited elongation reserves energy to be used after water recedes and to recover for survival. Another genotype with limited EP, UKMRC2 also had good SR but it was found to have no *Sub1* QTL.

Rapid coleoptile and mesocotyle elongation is needed during flooding at germination stage for the rice seed to survive (Magneschi and Perata, 2009; Takahashi *et al*., 2011). Anaerobic metabolic pathways such as glycolysis and fermentation are essential for coleoptile elongation (Setter and Ella, 1994; Kato-Noguchi and Kugimiya, 2003;

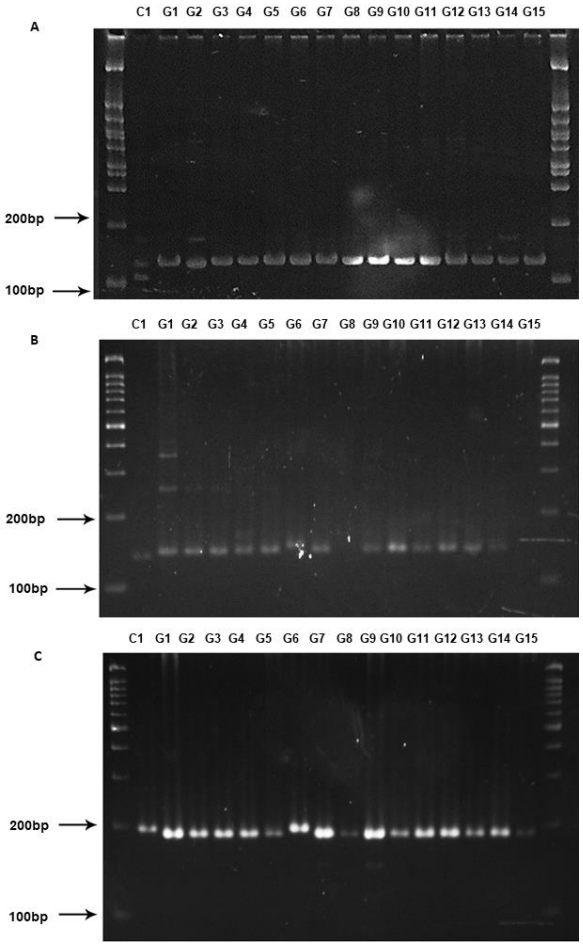

Fig. 3: PCR product after amplification using *Sub1* specific primers viewed using 3% agarose gel. (**A**) ART5, (**B**) RM8300, (**C**) RM23662, C1 =IR64-*Sub1*, G1 =MR219, G2 =Campa, G3 =MR284, G4 =Langsat, G5 =UKMRC11, G6 =MRQ74, G7 =MR264, G8 =MR253, G9 =UKMRC9, G10 =UKMRC8, G11 =UKM-5, G12 =MR263, G13 =UKMRC2, G14 =MR142, G15 =UKM-2

Takahashi *et al*., 2011). Alcoholic fermentation process takes place through two steps *i.e*., pyruvic decarbonization to acetaldehyde by pyruvic decarboxylase (PDC) and subsequent reduction by acetaldehyde to ethanol by alcohol dehydrogenase (ADH) (Hiroaki *et al*., 2006). Genotypes with short CL such as UKMRC2 might experience reduction of ADH activity in coleoptile that will reduce the rate of elongation during the submergence (Matsumura *et al*., 1995; Hiroaki *et al*., 2006). Adachi *et al*. (2015) also found that genotypes which adapted to hypoxic condition had longer CL than the control. However, the survival of seedling does not directly affected by CL because the content of NSC and CC at vegetative stage are more positively associated with SR (Ella and Ismail, 2006) added with medium elongation (Das *et al*., 2005).

Higher CCC values indicates a greater significant reduction in chlorophyll content before and after submergence. The high values of CCC for elongating genotypes may be due to high amount of ethylene production which stimulate the gene expression and activity of chlorophyllase enzyme involved in the process of chlorophyll breakdown (Jackson, 1987). This will also reduce the capacity of carbon dioxide fixation during and after the submergence thus distrupt the photosynthesis process (Sarkar *et al*., 2001; Ella *et al*., 2003; Singh *et al*., 2014). In this study, genotypes with lower CCC and EP such as UKM-2 and UKMRC8 had better SR than genotypes with higher CCC and EP. Sone *et al.* (2011) also found that a non-elongating cultivar able to survive after submergence by maintaining high chlorophyll content (CC). However, it was known that ethylene is important in plant growth process required for escape strategy to survive during submergence (Xu *et al.*, 2006; Hattori *et al*., 2009; Sarkar and Panda, 2009; Bailey-Serres and Voesenek, 2010; Bailey-Serres *et al*., 2010). Therefore, genotypes such as Campa, Langsat and MR253 elongated their shoot rapidly using this survival mechanism caused higher CCC.

The wild rice, *Oryza rufipogon* might possessed beneficial genes or QTLs for survival under submergence as the UKMRC lines showed higher SR compared to MR219. As generally known, wild species possess lots of vital genes for improvement of numerous traits. UKM-2 and UKM-5 with drought yield QTLs (*qDTYs*) also showed higher SR compared to MR219 indicate that *qDTYs* might be useful in enhancing tolerance level under submergence. Fukao *et al.* (2011) proved that *Sub1A* enables rice to recover from prolonged dehydration and involved in upregulation of genes associated with drought tolerance.

Survival of seedlings after submergence is likely to be associated with reduction of the CC. However, the association between these two traits was not strong for all three repetitions (r_1= -0.23, r_2= -0.23, r_3= -0.27; p>0.05). Genotypes which able to maintain high CC after submergence or had low CCC will be able to survive by producing more photosynthates after given recovery time. In the first repetition where NSCC was positively correlated with SR showed genotypes that consumed more energy and elongated more than the other genotypes able to survive. However, contrasting result were obtained in the third cycle where NSCC negatively correlated with SR while maintaining positive correlation with EP. This suggest that consumption of energy during submergence for elongation process is bad for survival after submergence. Weak association between SR and PR also suggest that survival after submergence is not likely to be influenced by the plant's ability to do photosynthesis. These results showed that there are complex interactions between all traits underlying survival under submergence.

Conclusion

Each genotype had different way to survive submergence by having distinct morphological and physiological traits.

Excessive usage of NSC for growth and elongation process brings drawbacks to seedlings' survival after submergence. Meanwhile, genotypes with limited EP and less NSCC are able to survive better which shows the importance of these two related traits for survival under submergence. Genotypes with no *Sub1*, but high survival rate such as UKM-2 and UKMRC2 might have other vital genes or QTL influencing its survival and crucial for researchers to exploit these materials for further studies such as QTL mapping.

Acknowledgements

Authors would like to thanks Universiti Kebangsaan Malaysia for research grants GUP-2017-033 and DCP-2017-004/2. The first author gratefully thanks Yayasan Canselor and Universiti Kebangsaan Malaysia for the Zamalah Yayasan Canselor scheme. Thank you to Prof. Dr. Wickneswari Ratnam and International Rice Research Institute (IRRI) for providing rice seeds. Last but not least, to all staff of laboratories and the glass houses of Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

References

Acquaah, G., 2012. Common statistical methods in plant breeding. *In*: *Principles of Plant Genetics and Breeding*, pp: 146–162. Acquaah, G. (Ed.). Blackwell Publishing, Malden, Oxford, UK

Adachi, Y., M. Sugiyama, J.I. Sakagami, A. Fukuda, M. Ohe and H. Watanabe, 2015. Seed germination and coleoptile growth of new rice lines adapted to hypoxic conditions. *Plant Prod. Sci.*, 18: 471–475

Bailey-Serres, J. and L.A.C.J. Voesenek, 2010. Life in the balance: a signaling network controlling survival of flooding. *Curr. Opin. Plant Biol*., 13: 489–494

Bailey-Serres, J., T. Fukao, P. Ronald, A. Ismail, S. Heuer and D. Mackill, 2010. Submergence tolerant rice: *Sub1*'s journey from landrace to modern cultivar. *Rice*, 3: 138–147

Das, K.K., R.K. Sarkar and A.M. Ismail, 2005. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. *Plant Sci*., 168: 131–136

Department of Agriculture, 2015. *Perangkaan Padi Malaysia 2014*, Department of Agriculture, Putrajaya, Malaysia

Ella, E.S. and A.M. Ismail, 2006. Seedling nutrient status before submergence affects survival after submergence in rice. *Crop Sci*., 46: 1673–1681

Ella, E.S., N. Kawano, Y. Yamauchi, K. Tanaka and A.M. Ismail, 2003. Blocking ethylene perception during submergence reduced chlorophyll degradation and improved seedling survival in rice. *Funct. Plant Biol*., 30: 813–819

Fukao, T., E. Yeung and J. Bailey-Serres, 2011. The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. *Plant Cell*, 23: 412–427

Fukao, T., K. Xu, P.C. Ronald and J. Bailey-Serres, 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

Gao, S.K., S.E. Yu, M. Wang, J.J. Meng, S.H. Tang, J.H. Ding, S. Li and Z.M. Miao, 2018. Effect of different controlled irrigation and drainage regimes on crop growth and water use in paddy rice. *Intl. J. Agric., Biol*., 20: 486–492

Hattori, Y., K. Nagai, S. Furukawa, X.J. Song, R. Kawano, H. Sakakibara, J. Wu, T. Matsumoto, A. Yoshimura, H. Kitano, M. Matsuoka, H. Mori and M. Ashikari, 2009. The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water. *Nature*, 460: 1026–1030

Hiroaki, S., M. Hideo, T. Tetsua, T. Nobuhiro and N. Mikio, 2006. A point mutation of *Adh1* gene is involved in the repression of coleoptile elongation under submergence in rice. *Breed. Sci*., 56: 69–74

Iftekharuddaula, K.M., H.U. Ahmed, S. Ghosal, Z.R. Moni, A. Amin and M.S. Ali, 2015. Development of new submergence tolerant rice variety for bangladesh using marker-assisted backcrossing. *Rice Sci*., 22: 16–26

Jackson, M.B., 1987. A structured evaluation of the involvement of ethylene and abscisic acid in plant responses to aeration stress. *In: Hormone Action in Plant Development – a Critical Appraisal*, pp: 189–199. Jackson, M.B., G.V. Hoad, J.R. Lenton and R.K. Atkin (Eds.). Butterworths, London

Kato-Noguchi, H. and T. Kugimiya, 2003. Preferential induction of alcohol dehydrogenase in coleoptiles of rice seedlings germinated in submergence condition. *Biol. Plant.*, 46: 153–155

Luo, F.L., K.A. Nagel, H. Scharr, B. Zeng, U. Schurr and S. Matsubara, 2011. Recovery dynamics of growth, photosynthesis and carbohydrate accumulation after de-submergence: A comparison between two wetland plants showing escape and quiescence strategies. *Ann. Bot*., 107: 49–63

Magneschi, L. and P. Perata, 2009. Rice germination and seedling growth in the absence of oxygen. *Ann. Bot*., 103: 181–196

Matsumura, H., T. Takano, K.T. Yoshida and G. Takeda, 1995. A rice mutant lacking alcohol dehydorogenase. *Breed. Sci*., 45: 365–367

Metraux, J.P. and H. Kende, 1982. The role of ethylene in the growth response of submerged deep water rice. *Plant Physiol*., 72: 441–446

Mohd Ikmal, A., Z. Nurasyikin, A. Kumar and A.A.S. Noraziyah, 2018. Evaluation of morpho-physiological traits of MRQ74 pyramided lines with drought yield QTLs. *Euphytica*, 214: 98

Murray, M.G. and W.F. Thompson, 1980. Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res*., 8: 4321–4325

Musgrave, A., M.B. Jackson and E. Ling, 1972. Callitriche stem elongation is controlled by ethylene and gibberellin. *Nat. New Biol.*, 238: 93–96

Nagai, K., Y. Hattori and M. Ashikari, 2010. Stunt or elongate? two opposite strategies by which rice adapts to floods. *J. Plant Res.*, 123: 303–309

Panda, D., S.G. Sharma and R.K. Sarkar, 2008. Chlorophyll fluorescence parameters, CO_2 photosynthetic rate and regeneration capacity as a result of complete submergence and subsequent re-emergence in rice (*Oryza sativa* L.). *Aquat. Bot*., 88: 127–133

Pierik, R., J.M.V. Aken and L.A.C.J. Voesenek, 2009. Is elongation-induced leaf emergence beneficial for submerged Rumex species? *Ann. Bot.*, 103: 353–357

Ranawake, A.L., U.G. Samarasinghe and S.G.J.N. Senanayake, 2014. Submergence tolerance of some modern rice cultivars at seedling and vegetative stages. *J. Crop Weed*, 10: 240–247

Sabu, K.K., M.Z. Abdullah, L.S. Lim and R. Wickneswari, 2006. Development and evaluation of advanced backcross families of rice for agronomically important traits. *Commun. Biomet. Crop Sci*., 1: 111–123

Sarkar, R.K. and B. Bhattacharjee, 2011. Rice genotypes with *Sub1* QTL differ in submergence tolerance, elongation ability during submergence and re-generation growth at re-emergence. *Rice*, 5: 1–8

Sarkar, R.K. and D. Panda, 2009. Distinction and characterisation of submergence tolerant and sensitive rice cultivars, probed by the fluorescence OJIP rise kinetics. *Funct. Plant Biol*., 36: 222–233

Sarkar, R.K. and S.K. Bera, 1997. A comparison of the submergence response of elongating and non-elongating flood tolerant deep water rice. *Indian Agric*., 41: 299–303

Sarkar, R.K., D. Panda, J.N. Reddy, S.S.C. Patnaik, D.J. Mackil and A.M. Ismail, 2009. Performance of submergence tolerant rice (*Oryza sativa*) genotypes carrying the Sub1 quantitative trait locus under stressed and non-stressed natural field conditions. *Indian J. Agric. Sci*., 79: 876–883

Sarkar, R.K., S. Das and I. Ravi, 2001. Changes in certain antioxidative enzymes and growth parameters as a result of complete submergence and subsequent re-aeration of rice cultivars differing in submergence tolerance. *J. Agron. Crop Sci*., 187: 69–74

Sarkar, R.K., R.N. De, J.N. Reddy and G. Ramakrishnayya, 1996. Studies on the submergence tolerance mechanism in relation to carbohydrate, chlorophyll and specific leaf weight in rice (*Oryza sativa* L.). *J. Plant Physiol*., 149: 623–625

Septiningsih, E.M., A.M. Pamplona, D.L. Sanchez, C.N. Neeraja, G.V. Vergara, S. Heuer, A.M. Ismail and D.J. Mackill, 2009. Development of submergence tolerant rice cultivars: the *Sub1* locus and beyond. *Ann. Bot*., 103: 151–160

Setter, T.L. and E.S. Ella, 1994. Relationship between coleoptile elongation and alcoholic fermentation in rice exposed to anoxia. I. Importance of treatment conditions and different tissues. *Ann. Bot*., 74: 265–271

Setter, T.L. and E.V. Laureles, 1996. The beneficial effect of reduced elongation growth on submergence tolerance of rice. *J. Exp. Bot*., 47: 1551–1559

Shamsudin, N.A.A., B.P.M. Swamy, W. Ratnam, M.T.S. Cruz, N. Sandhu, A.K. Raman and A. Kumar, 2016a. Pyramiding of drought yield QTLs into a high quality Malaysian rice cultivar MRQ74 improves yield under reproductive stage drought. *Rice*, 9: 21

Shamsudin, N.A.A., B.P.M. Swamy, W. Ratnam, M.T.S. Cruz, A. Raman and A. Kumar, 2016b. Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219. *BMC Genet*., 17: 1–14

Singh, S., D.J. Mackill and A.M. Ismail, 2014. Physiological basis of tolerance to complete submergence in rice involves genetic factors in addition to the *Sub1* gene. *AoB Plants*, 6: 1-20

Singh, S., D.J. Mackill and A.M. Ismail, 2011. Tolerance of longer-term partial stagnant flooding is independent of *SUB1* locus in rice. *Field Crops Res*., 121: 311–323

Singh, S., D.J. Mackill and A.M. Ismail, 2009. Responses of SUB1 rice introgression lines to submergence in the field: Yield and grain quality. *Field Crops Res*., 113: 12–23

Sone, C., O. Ito and J.I. Sakagami, 2011. Characterizing submergence survival strategy in rice *via* chlorophyll fluorescence. *J. Agron. Crop Sci.*, 198: 152–160

Swamy, B.P.M, N.A.A. Shamsudin, S. Rahman, R. Mauleon, W. Ratnam, M.T.S. Cruz and A. Kumar, 2017. Association Mapping of Yield and Yield-related Traits Under Reproductive Stage Drought Stress in Rice (*Oryza sativa* L.). *Rice*, 10: 21

Takahashi, H., H. Saika, H. Matsumura, Y. Nagamura, N. Tsutsumi1, N.K. Nishizawa and M. Nakazono, 2011. Cell division and cell elongation in the coleoptile of rice alcohol dehydrogenase 1-deficient mutant are reduced under complete submergence. *Ann. Bot*., 108: 253–261

Voesenek, L.A.C.J., J.H.G.M. Rijnders, A.J.M. Peeters, H.M.V.D. Steeg and H.D. Kroon, 2004. Plant hormones regulate fast shoot elongation under water: from genes to communities. *Ecology*, 85: 16–27

Winkel, A., T.D. Colmer, A.M. Ismail and O. Pedersen, 2013. Internal aeration of paddy field rice (*Oryza sativa*) during complete submergence importance of light and floodwater O_2. *New Phytol*., 197: 1193–1203

Xu, K. and D.J. Mackill, 1996. A major locus for submergence tolerance mapped on rice chromosome 9. *Mol. Breed.*, 2: 219–224

Xu, K., X. Xia, T. Fukao, P. Canlas, R. Maghirang-Rodriguez, S. Heuer, A.M. Ismail, J. Bailey-Serres, P.C. Ronald and D.J. Mackill, 2006. *Sub1A* is an ethylene response factor-like gene that confers submergence tolerance to rice. *Nature*, 442: 705–708

Yoshida, S., D. Forno, J. Cock and K.A. Gomez, 1976. *Laboratory Manual for Physiological Studies of Rice*. International Rice Research Institute, Philippines

[Received 20 Jun 2018; Accepted 25 Jan 2019; Published (online) 26 Apr 2019]