

## Involvement of Phenolic Compounds in Anaerobic Flooding Germination of Rice (*Oryza sativa* L.)

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**Abstract** By this study, thirty rice varieties were evaluated for anaerobic flooding tolerance using the direct sowing method. Phenolic profiles of strong and weak tolerant varieties were identified and compared based on HPLC chromatograms. The germination rates and shoot heights of rice were recorded for calculating the seedling vigor, which indicate the tolerant ability of rice in flooding condition. The results revealed a high variation of germination rate (10.01 to 100%), shoot height (0.35 to 78.17 mm) and seedling vigor (0.05 to 72.83). There was a high correlation between ( $r = 0.71$ ) germination rate in 5 cm and 10 cm flood. Phenolic and flavonoid contents of the strong tolerant cultivar significantly and proportionally increased in the flooding levels (5 cm and 10 cm). There was a total difference in terms of number of phenolic acids found in the strong and weak tolerant varieties. In particular, six phenolic acids (gallic acid, catechol, caffeic acid, syringic acid, vanillin, and ellagic acid) were only identified with high concentration in the strong tolerant cultivar. The findings suggest that the phenolics presented in the strong tolerant varieties probably have a certain function in response and adaptation to anaerobic flooding condition. Further researches on exogenous application of these phenolic acids to increase the flooding tolerant level of rice should be continued at both green house and field treatments.

### Introduction

Rice (*Oryza sativa* L.) is an essential food crop for billions of people, and it plays a crucial role in the relation between the diet and health. Recently, severe environmental stress conditions have increased and expanded to crop production areas globally, especially rice lands because of reverse climate change impacts. As a result, the sustainability of rice has been highly threatened. Flood is consider one of the major challenges for rice production, especially in the South and Southeast Asia. There is approximately 16% of rice production area covered by waterlogging annually [1]. Flooding causing yield loss is a primary stress constraint to rice production, especially in rainfed lowland areas of the tropics [2]. Particularly, this type of natural disaster during germination stage strongly can affect the growth and productivity of rice. Numerous of reports on the importance of the germination stage of crops have been published [3]. This phase is extremely vulnerable to change of environmental conditions. However, if a plant can successfully overcome the stress conditions at such a stage, it will be stronger during growth and development.

Besides gene functions in flooding tolerance of rice, the phytochemical pathway is also an interesting issue for rice researchers. Kende et al. [4] and Ismail et al. [5] reported the vital role of ethylene and gibberellic acid (GA) in elongation of internodes of rice under water; ethylene and GA<sub>1</sub> concentrations increased 50 times and 4 times, respectively, during submergence. In addition, rice plants have another pathways for overcoming the stress of flooding related to the production of antioxidants as secondary metabolites such as, phenolics [6]. Phenolic compounds are secondary metabolites of plants, with different activities including protection against pathogens and predators,

protection against ultraviolet radiation or other environmental stressors [7]. Several compounds with antioxidant activity have been identified in rice, including phenolic compounds, tocopherols, tocotrienols, and  $\gamma$ -oryzanol [8]. However, the detail of phenolic profile of rice during flooding germination has been poorly studied. Identification of phenolics involving in submergence tolerance in rice is really necessary to maintain sustainable production of rice, especially in flooding areas. Therefore, this study was conducted to examine the effect of flooding conditions on the phenolic profiles of strong and weak flooding tolerance rice varieties at the germination stage.

## **Materials and methods**

### **Plant materials**

Thirty rice varieties were obtained from the Cuu Long Delta Rice Research Institute, Vietnam.

### **Screening of germination under flooding conditions**

The germination test experiment was a randomized complete block design with three treatments of flooding including 0 cm (control), 5 cm and 10 cm flooded with water. Dry seeds were directly sown in soil at 2 cm depth in plastic trays. The numbers of germinated seeds were recorded after 14 days. The germination rates were calculated and expressed as percentages. The heights of emerged seedlings at 14<sup>th</sup> day were also measured. Seedling vigor index was calculated as (germination rate x seedling height)/100. The two rice varieties which had the highest and lowest seedling vigor index levels were selected for comparing their phenolic profiles.

### **Phenolic extraction**

Whole germinating seedlings were dried at 30 °C in a convection oven (MOV-212F, Sanyo, Japan) and then ground into powder using a coffee miller. Samples (500 mg) were extracted with a 40 ml mixture of methanol: HCl (80%: 0.1%) for 4 h. The extract was condensed using a rotary evaporator. The dried extract was diluted in methanol to make a concentration of 1 mg ml<sup>-1</sup> and stored at 4 °C for further analysis.

### **Estimation of total phenolic content**

The total phenolic content was estimated based on the Folin-Ceicalteu method as following the procedure detailed in Ti et al. [9]. A mixture of 0.1 ml extract, 0.5 ml of Folin-Ceicalteu 10% and 0.4 ml Na<sub>2</sub>CO<sub>3</sub> 7.5% was mixed in a microtube and incubated for 30 min at room temperature. The absorbance of the reaction was measured at 765 nm using a spectrophotometer (HACH DR/4000U - Japan). Total phenolic content was expressed as mg gallic acid equivalent (GAE) per gram dry weight (DW).

### **Determination of total flavonoid content**

The flavonoid content of extracts was estimated following the method of Djeridane et al. [10] with some minor alternations. An equal volume of extract and AlCl<sub>3</sub> 2% was mixed in a test tube and left at room temperature for 15 min. The absorption was measured at 430 nm using a spectrophotometer (HACH DR/4000U-Japan) against methanol as a blank reading. Total flavonoid content was calculated based on the linear equation of rutin standard and expressed as mg of rutin equivalents (RE) per gram DW.

### **Identification of phenolic acids of seedling extracts**

To identify the phenolic components, the Jasco HPLC system, consisting of a LC-Net II/ ADC, a UV-2075 Plus and a PU-2089 Plus, was employed. The extracts (1 mg ml<sup>-1</sup>) were filtered through 0.45  $\mu$ m membrane filters and injected to a column RPC18 (250 mm x 4.6 mm x 5  $\mu$ m) at a flow rate of 1 ml min<sup>-1</sup>. The mobile phases included absolute methanol (A) and 0.1% acid acetic (B). Gradient elution process was set up with the mobile phase A increased from 5 - 10% for 5 min, then increased from 10 to 90% for next 45 min, the last 10 minute was 100% A. The peaks of samples were compared, identified and calculated based on 15 phenolic standards.

## Statistical analysis

All data analyses were done using CROPSTAT 7.2 statistical software and ANOVA with the least significant difference (LSD) at the 0.05 level. Means were compared with Duncan's Multiple Range Test.

## Results

### Screening anaerobic germination of different varieties under flooding conditions

The submerged screening result of 30 rice varieties at germination stage is shown in Table 1. There were only ten varieties obtaining a germination rate higher than 90% in the control (saturated water) treatment. The lowest germination rate was the "BV5" variety in both the control and the 5 cm flooding treatments with only 10%. There were 4 varieties consisting of "T1", "Xn1", "Bao Thai" and "L bong" which showed the highest percentage of germination (100%) in 5 cm flooding treatment. For the 10 cm flooding treatment, "Khang Dan", "MNR2", "Koshihubo", "Q5", "Xn1" and "L bong" had the highest germination rate (100%).

**Table 1.** Germination rates of different varieties at 14 days after sowing

No.	Varieties	Germination rates (%)		
		Control	5cm flood	10 cm flood
1	Khang Dan	73.3def	83.3ae	100.0a
2	OM6328	70.0def	93.3abc	96.67ab
3	MNR2	63.33efg	90.0ad	100.0a
4	OM8108	13.3kl	80.0bf	90.0ad
5	OM4900	33.3ij	76.7cg	90.0ad
6	OM6677	40.0hi	83.3ae	83.3ad
7	OM8104	16.7kl	80.0bf	73.3def
8	OM5629	26.7ijk	60.0gh	93.3abc
9	OM8105	60.0fg	86.7ae	76.7cf
10	OM5900	20.0jkl	73.3dg	90.0ad
11	T5	90.0abc	83.3ae	86.7ad
12	T8	40.0hi	40.0ij	43.3g
13	BV5	10.0l	26.7j	60.0f
14	T4	90.0abc	60.0gh	63.3ef
15	K1	80.0bcd	63.3fgh	83.3ad
16	T3	40.0hi	80.0bf	73.3def
17	Koshihubo	96.7a	96.7ab	100.0a
18	T7	53.3gh	40.0ij	30.0g
19	T2	76.7cde	50.0hi	76.7cf
20	HTS1	60.0fg	73.3dg	76.7cf
21	OM6162	96.7a	83.3ae	80.0be
22	Jasmine	90.0abc	60.0gh	83.3ad
23	IR64Sub1	80.0bcd	70.0efg	76.7cf
24	T1	90.0abc	100.0a	63.3ef
25	OM7345	90.0abc	93.3abc	96.7ab
26	Q5	96.7a	86.7ae	100.0a
27	BT	36.7i	86.7ae	90.0ad
28	Xn1	93.3ab	100.0a	100.0a
29	Bao Thai	76.7cde	100.0a	96.7ab
30	L bong	93.3ab	100.0a	100.0a
LSD 5%		4.3	4.79	4.55
CV (%)		13.23	12.1	10.70

Means in column with the same letter are not significant difference at  $P < 0.05$

There was a considerable variation in shoot height of rice seedlings in both flooding treatments and varieties (Table 2). The highest variability was “L bong” in control (78.17 mm) and reduced to 15.66 mm and 7.73 mm under 5 cm and 10 cm flooding conditions, respectively. Besides that, the lowest variety on shoot height in the control was “OM8108” with 0.35 mm, and this value increased to 4.2 mm and 5.8 mm in 5 cm and 10 cm flooding conditions, respectively. In this experiment, the highest value was “Koshihubo”, which achieved 69.37 mm in 5 cm flood and 63.00 mm in 10 cm flood.

**Table 2.** Shoot heights of different varieties at 14 days after sowing

No.	Varieties	Shoot heights (mm)		
		Control	5 cm flood	10 cm flood
1	Khang Dan	28.74c	9.97gh	12.03ei
2	OM6328	9.56hi	24.13b	14.30def
3	MNR2	21.07d	23.93b	18.10cd
4	OM8108	0.35l	4.20kl	5.80jm
5	OM4900	5.73jk	15.23ef	12.30eh
6	OM6677	15.97f	8.00hij	11.17fi
7	OM8104	7.75ij	11.30g	10.97fi
8	OM5629	1.47l	8.73hi	17.93cd
9	OM8105	7.03j	21.57a	15.87cde
10	OM5900	11.67gh	7.30ij	12.90efg
11	T5	28.00c	17.27cde	22.50b
12	T8	11.60gh	8.30hij	1.73mn
13	BV5	3.93k	2.47lmn	8.87gj
14	T4	6.27j	2.70lmn	8.33hk
15	K1	12.83g	18.23c	19.50bc
16	T3	10.97gh	18.10c	13.17efg
17	Koshihubo	56.17a	69.37a	63.00a
18	T7	3.80k	1.63mn	1.53mn
19	T2	26.97c	3.27lm	15.30cf
20	HTS1	17.63ef	17.83cd	13.03efg
21	OM6162	43.57b	2.73lmn	0.87n
22	Jasmine	18.73e	0.67n	1.07n
23	IR64Sub1	7.00j	0.97mn	1.17n
24	T1	22.27d	9.90gh	4.50kn
25	OM7345	34.10a	13.73f	2.17mn
26	Q5	53.07a	25.87b	32.57a
27	BT	12.43g	6.10jk	3.83lmn
28	Xn1	58.77a	18.90c	12.17eh
29	Bao Thai	43.83b	7.90hij	14.87def
30	L bong	78.17a	15.66def	7.73il
LSD		0.69	0.7	1.21
CV (%)		6.0	10.20	18.50

*Means in column with the same letter are not significant difference at  $P < 0.05$*

Seedling vigor (SV) was also determined under anaerobic conditions combining both different flooding conditions and shoot height (Table 3). In the control treatment, the highest SV values, which were more than 30.00, were obtained from “Koshihubo”, “OM6162”, “OM7345”, “Q5”, “Xn1”, “Bao Thai”, and “L bong”. However, in flooding treatments, most of these varieties had a significant decrease of SV values, except “Koshihubo” variety which had an increase from 54.34 in control to 66.96 and 63.00 in 5 cm and 10 cm flooding treatments, respectively. Three varieties showed the lowest seedling vigor index ( $<1.00$ ) in flooding treatments were “T7”, “Jasmine”, and

“IR64sub1”. From this result, the varieties “Koshihubo” and “Jasmine” were selected as strong (S) and weak (W) tolerance, respectively, for comparing different phenolic profiles in submerged treatments.

**Table 3.** Seedling vigor index of different varieties at 14 days after sowing

No.	Varieties	Seedling vigor		
		Control	5 cm flood	10 cm flood
1	Khang Dan	21.03d	8.25hi	12.03dh
2	OM6328	6.73gh	22.48b	13.85cg
3	MNR2	13.31ef	21.52b	18.10bc
4	OM8108	0.05j	3.38kl	5.23ijk
5	OM4900	1.91hij	11.65fg	11.2fgh
6	OM6677	6.40gh	6.69ij	9.31ghi
7	OM8104	1.28ij	9.00hi	8.37hij
8	OM5629	0.42j	5.25jk	16.93bcd
9	OM8105	4.24hij	18.64c	12.20dh
10	OM5900	2.34hij	5.36jk	11.42eh
11	T5	25.17a	14.48de	19.58bc
12	T8	4.58hij	3.38kl	0.74k
13	BV5	0.393j	0.65m	5.43ij
14	T4	5.43hi	1.60lm	5.26ijk
15	K1	10.27fg	11.50fg	16.29be
16	T3	4.31hij	14.38de	9.72fi
17	Koshihubo	54.34b	66.96a	63.00a
18	T7	2.01hij	0.66m	0.48k
19	T2	20.72d	1.60lm	11.78eh
20	HTS1	10.55fg	12.89ef	10.33fgh
21	OM6162	42.17a	2.27lm	0.70k
22	Jasmine	16.88de	0.41m	0.90k
23	IR64Sub1	5.67hi	0.66m	0.91k
24	T1	19.99d	9.90gh	2.83k
25	OM7345	30.69c	12.84ef	2.08k
26	Q5	51.34b	22.29b	32.57a
27	BT	4.56hij	5.36jk	3.50jk
28	Xn1	54.85b	18.9c	12.17dh
29	Bao Thai	33.53c	7.90hi	14.46cf
30	L bong	72.83a	15.66d	7.73hij
LSD		1.30	0.70	1.367
CV (%)		14.3	12.1	23.40

*Means with the same letter are not significant difference at  $P < 0.05$*

#### **The effect of flooding conditionson the total contents of phenolic compounds in anaerobic germination of rice varieties**

Table 4 compares the production of phenolics and flavonoids during germination between two strong and weak tolerant varieties under submerged stress conditions including in water in depths of 5 cm and 10 cm. Obviously, the strong variety produced far more phenolics than the weak variety. For the strong tolerant variety, the total phenolic content tremendously rose by four times and even six times after rice seeds were directly sown in 5 cm and 10 cm deep water, respectively, compared to the control. In contrast, although the control treatment of the weak tolerant variety had higher total phenolic content than that of the strong tolerant variety, this number significantly reduced under 5 cm flooding treatment and did not change in 10 cm treatment.

**Table 4.** Changes of total phenolic and flavonoid contents of the strong and weak flooding-tolerance varieties

Treatment	Phenolic content (mg GAE/g dry weight)	Flavonoid content mg RU/g dry weight
S-Control	0.71±0.03 d	0.264±0.024 c
S-5 cm flooded	2.89±0.12 b	0.282±0.017 c
S-10 cm flooded	4.42±0.18 a	0.734±0.033 a
W-Control	1.61±0.04 c	0.271±0.029 c
W-5 cm flooded	0.62±0.10 d	0.072±0.007 d
W-10 cm flooded	1.65±0.04 c	0.398±0.011 b

*Means±SE with the same letters in the same row are not significant difference at  $P < 0.05$ ; S: strong tolerant variety; W: weak tolerant variety.*

Regarding the total flavonoid content, there was no significant difference between the control and the 5 cm flooding treatment in the strong tolerant variety (Table 4). However, it was approximately three-fold increase in 10 cm flooding treatment. In addition, it could be clearly observed that the flavonoid content of the strong variety was two times higher than in the weak variety under 10 cm flooding treatment.

The dynamic change of phenolic components of rice seedlings during flooding is shown in Table 5. It is observed that there was dramatic difference in both types and concentrations of phenolics identified in strong and weak flooding-tolerant varieties. Ten phenolic acids, including gallic acid, protocatechuic acid, catechol, chlorogenic, vanillic acid, caffeic acid, vanillin, benzoic acid, ellagic acid, and cinnamic acid were found in the strong tolerant variety while those in weak variety had only seven types in the 10 cm flooding treatment. Particularly, gallic acid, catechol, caffeic acid, syringic acid, vanillin, and ellagic acid were produced when the strong variety was sown directly in 10 cm flood. These phenolics were not detected in the weak tolerant variety during water treatments, except vanillin. Moreover, protocatechuic acid, chlorogenic acid, vanillic acid, benzoic acid and cinnamic acid significantly increased in the anaerobic flooding condition.

**Table 5.** Phenolic acids and concentrations ( $\mu\text{g g}^{-1}$  DW) of strong and weak flooding tolerance varieties

Phenolics	S-Control	S-5cm	S-10cm	W-Control	W-5cm	W-10cm
GA	nd	7.22±0.11	22.30±8.68	nd	nd	nd
PA	10.24±1.64c	27.88±3.22b	66.61±3.52a	nd	6.74±0.16c	15.00±0.69c
CL	nd	nd	8.52±0.35	nd	nd	nd
CH	29.06±10.34bc	77.81±4.54a	52.32±5.73b	20.47±10.59c	12.43±0.26c	8.42±1.13c
VA	16.22±6.56bc	46.67±1.35a	30.62±4.63b	12.55±6.51c	6.60±1.01c	5.65±0.20c
CA	nd	nd	18.59±6.61	nd	nd	nd
SyA	nd	17.54±0.80	nd	nd	nd	nd
VN	nd	nd	27.23±19.86	8.67±2.15	5.25±0.62	9.59±0.10
p-CA	nd	nd	nd	nd	nd	6.05±1.75
BA	194.34±0.47bc	128.59±1.77c	343.63±0.73a	221.83±39.35b	234.51±32.99b	217.88±17.53b
EA	nd	8.48±0.43	10.54±0.51	nd	nd	nd
CiA	8.50±0.80b	8.03±0.33b	24.75±0.89a	7.99±0.88b	20.04±2.71a	11.05±3.43b

*Means±SE with the same letters in the same row are not significant difference at  $P < 0.05$ ; nd: not detected; S: strong tolerant variety; W: weak tolerant variety; DW: dry weight; GA: Gallic acid; PA: Protocatechuic acid; CL: Catechol; CH: Chlorogenic; VA: Vanillic acid; CA: Caffeic acid; SyA: Syringic acid; VN: Vanillin; p-CA: p-coumaric acid; BA: Benzoic acid; EA: Ellagic acid; CiA: Cinnamic acid.*

## Discussion

### Flooding and seedling vigor

Morphologically, the most important escape strategy in the submerged period of rice is coleoptile elongation, which assists the plant gain a high level of oxygen in the water surface [11]. Although most of rice varieties are able to germinate under anaerobic or flooding conditions, the elongation of coleoptile highly links to the tolerance capacity of individual varieties [5,12,13]. In 5 cm flooding treatment, only "Koshihuko" variety showed a shoot height higher than water level after 14 days flood. This value was not significantly different in the 10 cm flooding treatment. Actually, numerous studies have explained the mechanism of coleoptile elongation. The role of ethylene hormone was mostly focused on in this complex process. Ethylene promotes the growth of coleoptile under deep water where oxygen concentration is in shortage, and higher ethylene levels mean faster and longer coleoptile elongation [5,14]. However, the mechanism of ethylene production under anoxic condition is unclear because oxygen is the main factor for activating the enzyme 1-aminocyclopropane-1-carboxylic acid in the process of ethylene synthesis [15].

Another submerged-tolerant indicator in rice is seedling vigor. It is considered to be a measurable trait for evaluating the uniformity, speed and emergence of seed germination. Seedling vigor is defined as the ability of seeds to generate seedlings under abnormal environmental conditions [16]. In this study, the seedling vigor had a great variation, which was from 0.05 to 72.83, and was heavily dependent on rice variety. In addition, in many varieties ("OM6328", "MNR2", "OM8108", "OM4900", "OM6677", "OM8104", "OM5629", "OM8105", "OM5900", "BV5", "K1", "T3", "Koshihuko" and "HTS1") the seedling vigor was promoted by flooding treatments. Submergence stress dramatically promotes the elongation of coleoptile during germination. Coleoptile elongation is closely correlated with the increase of ADH activity in rice seedlings of both *Indica* and *Japonica* rice. Particularly, Vu et al. [17] reported an increase of *ADH1*, *ADH2* and *ALDH2a* gene expression in submergence stress. In fact, seedling vigor has a strong relationship with germination rate. The results of correlation analysis, in Table 6, show that there was a high correlation between germination rate in 5 cm deep water and 10 cm deep water ( $r = 0.71$ ,  $P < 0.01$ ). In addition, a positive correlation coefficient ( $r = 0.52$ ,  $P < 0.01$ ) between seedling vigor and germination rate under 5 cm flooding treatment was found.

**Table 6.** Correlation (r) of seedling vigor index and germination rates of different varieties at 14 days after sowing

	GR Control	GR 5cm	GR 10cm	SV Control	SV 5cm	SV 10cm
GR Control	1					
GR 5cm	0.41 <sup>*</sup>	1				
GR 10cm	0.26 <sup>ns</sup>	0.71 <sup>**</sup>	1			
SV Control	0.72 <sup>**</sup>	0.54 <sup>**</sup>	0.48 <sup>*</sup>	1		
SV 5cm	0.33 <sup>ns</sup>	0.52 <sup>**</sup>	0.45 <sup>*</sup>	0.49 <sup>*</sup>	1	
SV 10cm	0.25 <sup>ns</sup>	0.32 <sup>ns</sup>	0.47 <sup>*</sup>	0.42 <sup>*</sup>	0.89 <sup>**</sup>	1

<sup>\*</sup>: significant at  $P < 0.05$ , <sup>\*\*</sup>: significant at  $P < 0.01$ ; ns: not significant; SV: seedling vigor; GR: germination rates.

### Anaerobic flooding germination and phenolic compounds of rice

Under stress conditions, the phenolic content, one of the secondary metabolites of plants, increases as a natural response to adapt to or react with environmental changes [18]. In submergence, the lack of oxygen results in high accumulation of radical oxygen species (ROS), which cause damage to plant cells in many degenerative processes [6] including lipid peroxidation, DNA damage and metabolic disorders [19]. Moreover, phenolic compounds were proved to have the strongest radical scavenging capacity and the most effective neutralization of ROS [1,6].

To study a possible involvement of phenolic alteration under submergence, we compared the levels of total phenolic and flavonoid contents and identified phenolic components of the strong

("Koshihubo") and weak ("Jasmine") tolerant rice cultivars. The result revealed a considerable increment of total phenolic and flavonoid contents of the strong submerged-tolerant varieties compared to weak varieties under anaerobic submerged germination of rice. Banerjee et al. [1] had a similar result of total phenolic content in three rice varieties including "Swarna", "FR13A" and "Swarna Sub1A" which were submerged sensitive, submerged tolerant and containing *Sub1A* varieties, respectively. Furthermore, Ramakrishna and Ravishankar [18] also observed dramatic increase of flavonoids polyphenols and anthocyanins during salt stress of *Hordeum vulgare*, *Cakile maritime* and *Grevillea* sp., respectively. Joseph et al. [19] highlighted the total rise of phenolics in five rice cultivars in India, and the highest rate of phenolic change was found in the "Orkazhama" variety. In a study by Boscaiu et al. [6], the accumulation of phenolics and flavonoids was recorded during a stressful period in a Mediterranean climate. That study revealed the high correlation between phenolic and flavonoid contents and water stress. Although, the phenolic content of the weak tolerant variety was higher than the strong one in normal condition (control), the accumulated phenolics was significantly lower in both 5 cm and 10 cm flooding treatments.

Regarding the phenolic components of rice in flooding germination, there are few reports. Therefore, this study was more focused on identification of phenolic profiles of the strong and weak flooding tolerant varieties. The results reported here provide the valuable information for plant physiologists and rice breeders. Variable concentrations of gallic acid, catechol, caffeic acid, syringic acid, vanillin, and ellagic acid were only found in the strong tolerant variety after flooding treatment. This means that these phenolic acids probably relate to flooding tolerance of rice. In point of fact, these phenolic acids are commonly found in rice and other plants [20]. However, the concentrations were low and their presence was in bound form, which is linked to another sugar or ester [21]. Phenolics are not the main factor to assist rice to escape from flooding condition or shoot elongation, but the high accumulation of such secondary metabolite partly protects plant cells from physical injuries during exposure to stress conditions, especially submergence. For instance, Quan et al. [22] reported the presence of only *p*-hydroxybenzoic acid in the tolerant rice variety after drought stress treatment.

## Conclusions

The study pointed out some potential flooding tolerant rice varieties in 30 tested varieties based on the seeding vigor index. In addition, our results showed that the flooding condition caused a considerable increase in the total phenolic and flavonoid contents in strong submerged-tolerant rice varieties. Moreover, the phenolic acids consisting of gallic acid, catechol, caffeic acid, syringic acid, vanillin, and ellagic acid were only identified in the strong one. It is suggested that these phenolic acids seem to involve in flooding tolerance of rice at the germination stage, and they can be applied as exogenous sources to enhance flooding or submergence tolerance in rice.

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