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 GA1 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, tocochromanols, and γ-oryzanol [8]. However, the detail of phenolic profile of rice during flooding germination has been poorly studied. Identification of phenolics involving 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 14th 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 mill. 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⁻¹ and stored at 4 °C for further analysis.

Estimation of total phenolic content

The total phenolic content was estimated based on the Folin-Ciocalteu method as following the procedure detailed in Ti et al. [9]. A mixture of 0.1 ml extract, 0.5 ml of Folin-Ciocalteu 10% and 0.4 ml Na₂CO₃ 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 AlCl₃ 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⁻¹) were filtered through 0.45 µm membrane filters and injected to a column RPC18 (250 mm x 4.6 mm x 5 µm) at a flow rate of 1 ml min⁻¹. 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 (LDS) 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      | Control (%) | 5 cm flood (%) | 10 cm flood (%) |
|-----|----------------|-------------|----------------|-----------------|
| 1   | Khang Dan      | 73.3def     | 83.3ae         | 100.0a          |
| 2   | OM6328         | 70.0def     | 93.3abc        | 96.7ab          |
| 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         | 26.7jik     | 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 | Control (mm) | 5 cm flood (mm) | 10 cm flood (mm) |
|-----|-----------|--------------|-----------------|------------------|
| 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       | 15.23def        | 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          | 12.17ch          |
| 29  | Bao Thai  | 43.83b       | 7.90hij         | 14.87def         |
| 30  | L bong    | 78.17a       | 15.66def        | 7.73il           |

**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          | 5.43j       |
| 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        | 10.33fg     |
| 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 conditions on 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 (µg 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        | 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; 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 to 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 "Koshihubo" 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", "Koshihubo" 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 5cm  | 0.41*      | 1       |         |            |         |         |
| GR 10cm | 0.26 ns    | 0.71**  | 1       |            |         |         |
| SV Control | 0.72**  | 0.54**  | 0.48*  | 1          |         |         |
| SV 5cm  | 0.33 ns    | 0.52**  | 0.45*  | 0.49*      | 1       |         |
| SV 10cm | 0.25 ns    | 0.32 ns | 0.47*  | 0.42*      | 0.89**  | 1       |

*: significant at $P < 0.05$; **: 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 \( \rho \)-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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