Doctoral Dissertation

Involvement of Phenolics in Allelopathy and Submergence Tolerance of Rice (*Oryza sativa* L.) (Summary)

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Rice (*Oryza sativa* L.) is an essential food crop for billions of people, and it plays a crucial role in the relation of diet and health. Considered as one of the inevitably devastating consequences caused by climate change, damaged rice crops have increased tremendously around the world. Flood is considered as one of the major challenges for rice production, especially in the South and Southeast Asia that imposes major limitations on productivity and viability of agricultural crops worldwide. Among important crops, rice (*Oryza sativa* L.) is mostly affected by submergence stress because many rice landraces are primarily cultivated in lowland and flood-prone areas. Additionally, weed is also one of the most serious problems facing rice production around the world. Although herbicides are economical and effective, they cause devastating effects on the environment, human health and the high risk of herbicide resistance of weeds that may threaten sustainable crop production. To tackle the mentioned challenges in weed management, biologically novel treatments of weeds should be prioritized, one of which is using allelopathy as an effective and efficient manner due to its cost effective and eco-friendly aspects.

Rice has a process for overcoming the stress of flooding; a pathway that produces antioxidants as secondary metabolites, phenolics included. Moreover, some rice varieties release phenolics which might affect major weeds around rice plants. Therefore, it is important to recognize that finding phytochemicals – either those that can enhance the submergence tolerance of rice or those that suppress noxious weeds in the field - is necessary to maintain environmentally-friendly agriculture. To achieve the research objectives, four main experiments were conducted.

The identifications of the allelochemicals of dehulled rice, rice, and hulls of rice were carried out in laboratory. Phenolic acids in growth media of rice seeds and tested plants

(barnyardgrass, lettuce, radish) were identified and quantified using HPLC. The results showed that the inhibitory effects of dehulled rice were stronger than those of hulls and rice. The results also demonstrated that both rice and dehulled rice resulted in the decrease in lettuce shoot elongation, with 50.6 and 30.4%, respectively. The results revealed inhibitory effects of dehulled rice on root length, shoot height and survival rate of barnyardgrass, lettuce, and radish. Regarding phenolic identification, there were ten phenolic acids including gallic, protocatechuic, chlorogenic, vanillic, syringic, ferulic, benzoic, ellagic, cinnamic acids and vanillin detected in rice and dehulled rice root exudates, while syringic and ferulic acids were only detected in dehulled rice. Vanillin and vanillic acid were detected as allelochemicals in root exudates of rice and tested plants, indicating that vanillin and vanillic acid played the main role in alleopathic interaction of rice and the tested plants.

To clarify the allelopathic potential of vanillin and vanillic acid, the putative chemicals were purchased for treatments. The chemicals were applied with three concentrations of 50 ppm, 100 ppm and 200 ppm which were directly added to agar solution for seed germination. The recorded data revealed that most of the vanillin and vanillic acid treatments showed high inhibitory effects on germination rates and seedling growth of lettuce and radish, but strongly stimulated root elongation of rice. The phenolic concentration of 200 ppm was found to have the most negative effect on germination rate of lettuce. Survival rates of lettuce and radish were significantly reduced when exposed to various concentrations of phenolic treatments. Particularly, no lettuce was observed to survive after treated with vanillic acid (100 ppm), vanillin (100 ppm), and mixture (200 ppm). Moreover, vanillic acid also showed an inhibitory effect on the survival rate of barnyardgrass at 100 ppm treatment. The findings indicated the allelopathic potential of dehulled rice which can be used for identification of more phytotoxins to produce bioherbicides.

In addition, phenolic acid profiles of strong and weak submergence-tolerant rice varieties were identified and compared based on HPLC chromatograms. The results showed a high variation of germination rate (from 10 to 100%), shoot height (0.35 to 78.17 mm) and seedling vigor (0.05 to 72.83). Total phenolic and flavonoid contents of the strong tolerant cultivar significantly increased in the submergenc. There was a big difference in terms of number of phenolic acids found in the tolerant and susceptible 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 tolerant cultivar. Moreover, protocatechuic acid, chlorogenic acid, vanillic acid, benzoic acid and cinnamic acid significantly increased in the anaerobic flooding condition. The findings suggest that the phenolics increased in the strong tolerant varieties have a certain function in response and adaptation to anaerobic flooding condition.

Subsequently, the impacts of protocatechuic acid and vanillic acid on total phenolic, chlorophyll, malondialdehyde (MDA) contents and antioxidant enzyme activities of rice seedlings in submerged condition were evaluated. Amount of chlorophyll b after pre-soaked with 0.1 mM protocatechuic acid markedly increased by 40% compared to the untreated plants ($p < 0.05$) while that of chlorophyll a was negligibly different. Vanillic acid significantly reduced MDA quantity from 43.0±3.4 to 15.5±3.0 nmol/g fresh weight. By treating plant with protocatechuic acid and vanillic acid, the total endogenous phenolic and flavonoid contents, the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) were strongly promoted. Similarly, the expression of genes encoding for antioxidant enzymes was elevated. In addition, vanillic acid increased the expression level of APX gene in much higher levels than that of protocatechuic acid and their mixture, whilst no significant difference was observed in the other genes including SOD, CAT (catalase), GR (gluthione reductase), and POD (peroxidase).

The findings of this study highlighted the positive roles of protocatechuic acid and vanillic acid in photosynthetic and antioxidative processes in rice seedlings during submergence, potentially helping promote the submergence tolerance in rice.

Briefly, among detected phenolics, vanillic acid was the phytochemical possessing both allelopathic potential and enhancement of submergence tolerance of rice. With the high concentration (200 mM), such phenolic acid could suppress the germination and early emergence of weeds while the low concentration (0.1 mM) improved the physiological processes of rice under submergence by increasing chlorophyll content, enhancing antioxidant enzyme activities, and reducing lipid peroxidation. The findings in this study not only provide new information as to the physiological role of phenolics in rice, focusing on allelopathy and submergence tolerance, but also can be treated as the foundation to build protocols for agricultural practices - the involved phenolics can be applied in controlling weeds and boosting the submergence tolerance of rice.