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Amino Acid and Phenolic Contents in Infected Leaves of Rice in Relation to Adult-Plant Resistance to Leaf Blast

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잎稻熱病에 對해 成體植物抵抗性을 지닌 벼의 感染葉에서 아미노산과 페놀化合物의 含量

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ABSTRACT

Ethanol-soluble amino acids and phenolics in healthy and blast-infected leaves of the susceptible rice cultivar Nakdong and adult-plant-resistant cultivar Dobong were quantitatively analyzed. At the 3 days after inoculation, the levels of soluble amino acids and phenolics in the infected fifth and eighth leaves of the two cultivars were similar to those of comparable healthy controls. As blast lesions appeared on the leaves at the 5 days after inoculation, the soluble amino acids and phenolics began to increase. At the 7 days after inoculation, the levels of amino acids and phenolics were about 1.5-3 times more than those in healthy controls at the five-and eight leaf stages. The adult-plant-resistant cultivar Dobong showed higher amounts of soluble amino acids and phenolics in both healthy and infected fifth and eighth leaves than did the susceptible cultivar Nakdong, although Dobong was less infected by *Pyricularia oryzae* than Nakdong. The pronounced increases in amino acids and phenolics in rice leaves of the cultivar Dobong during the blast infection may play an important role in the expression of adult-plant resistance to blast.

Key words: rice blast, adult-plant resistance, amino acids, phenolic compounds.

要 約

稻熱病에 對해 感受性을 보이는 낙동과 成體植物抵抗性을 보이는 도봉의 健全葉과 稻熱病 感染葉內의 에탄올可溶性 아미노산과 페놀化合物을 量的으로 分析하였다. 接種 3日後에는 可溶性 아미노산과 페놀化合物의 水準은 健全葉과 비슷하였다. 稻熱病 病斑이 接種 5日 後에 感染葉에 나타났을 때 可溶性 아미노산과 페놀化合物은 5葉期와 8葉期에서 增加하기 始作하였고, 接種 7日 後에서는 이들 化合物의 水準은 5葉期와 8葉期에서 健全葉보다 約 1.5~3倍 더 많았다. 稻熱病 感染葉와 비주 도봉이 稻熱病에 對해 낙동

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보다 덜 感染되었지만 5葉期와 8葉期의 健全葉과 感染葉에서 成體植物抵抗性 品種 도봉의 可溶性 아미
산 및 페놀化合物 含量은 感受性 品種 낙농의 含量보다 높았다. 버品種 도봉이 稻熱病에 感染동안 아미노
과 페놀化合物 含量이 현저하게 增加하여 成體植物抵抗性이 發現하는 데 重要한 역할을 할지 모른다.

INTRODUCTION

During fungal infection on plants, pathogens trigger the alteration in certain metabolic or nutritional processes leading to favorable or unfavorable conditions for fungal development within host tissues. Numerous reports have also been published on the appearance and accumulation of phenolic compounds in plant tissue in response to infection (8). Changes in levels of amino acids and phenolic compounds have also been studied in relation to resistance of host plants to pathogen (3,9).

In previous investigations, qualitative and quantitative differences in resistance to leaf blast among rice cultivars during plant development could be easily detected under controlled environmental conditions in terms of race specificity and effects of leaf and plant age (5). The different levels of quantitative resistance in the cultivars Nakdong and Dobong may depend on and or affect metabolic processes in blast-infected plants. In earlier study, we also suggest that carbohydrate metabolism is more impaired in blast-infected plants of susceptible rice cultivars than in adult-plant-resistant cultivars (4).

The objective of this research was to examine the changes in amino acid and phenolic contents in blast-infected rice plants of susceptible and adult-plant-resistant cultivars at different growth stages.

MATERIALS AND METHODS

Plant and inoculation. The rice (*Oryza sativa* L.) cultivars used were Nakdong, which is susceptible at all plant growth stages, and Dobong, which is susceptible at early growth stage, but resistant at late growth stage (5,6,7). The rice plants in greenhouse were grown in plastic pots (5x15x10cm) containing sterilized paddy soil mixed with the fertilizer. The basal fertilizer was applied at t 0.27-0.27-0.21g of actual N-P-K per pot. The ad tional 0.1g nitrogen fertilizer per pot was appl later to increase susceptibility to the blast infecti before inoculation. *Pyricularia oryzae* Cav. (r K1413) was cultured on oatmeal agar at 28±1°C 8 days. After removing the aerial mycelia on t oatmeal agar with a sterilized spatura, the cult was sporulated under fluorescent light at 28±1°C 48 hr. Plants at the five and eight leaf stages w inoculation by uniformly spraying a conidial susp sion (approximately 10^5 conidia ml^{-1}) of fungus by use of a laboratory glass sprayer. T inoculated plants were placed in a moist chamber 100% relative humidity and 28±1°C for 24 hr, a then transferred to a growth chamber with 80±1 relative humidity, illumination of 10,000 lux a 28±1°C. As a result, the leaf area infected w about 12% and 3% in Nakdong and about 9% a 1% in Dobong at the five and eight leaf stag respectively.

Analysis of total amino acids and phenoli One gram (fresh weight) of leaf tissue from heal and infected plants at five and eight leaf stages v harvested at 11 a.m., cut into 1-cm segments a boiled in 20 ml of 80% (v/v) ethanol for 10 n (three changes). The pigments in 50 ml of etha extracts were removed twice by shaking with 30 of petroleum benzine. Total amino acids w determined by the ninhydrin method of Yemm a Cocking (14) using glycine as a standard. Aliqu of the ethanol-soluble extracts before the treatm with petroleum benzine were used in determin the amount of total phenolic compounds by t method of Swain and Hillis (13).

RESULTS

Amino acids in healthy and infected plan Fig. 1 shows the levels of total ethanol-solu

nino acids in healthy and infected fifth and ghth leaves of the susceptible cultivar Nakdong id the adult-plant-resistant cultivar Dobong at arious intervals after inoculation with *P. oryzae*. At ve and eight leaf stages, the levels of total soluble nino acids in infected tissues of two cultivars ere similar to those of comparable healthy leaves until the 3 days after inoculation. As blast lesions appeared on the infected leaves at the 5 days after inoculation, the total soluble amino acids began to increase at five and eight leaf stages. At the 7 days after inoculation when rice leaves produced highly susceptible lesions, the levels of amino acids were about 1.5-3 times more than those in healthy

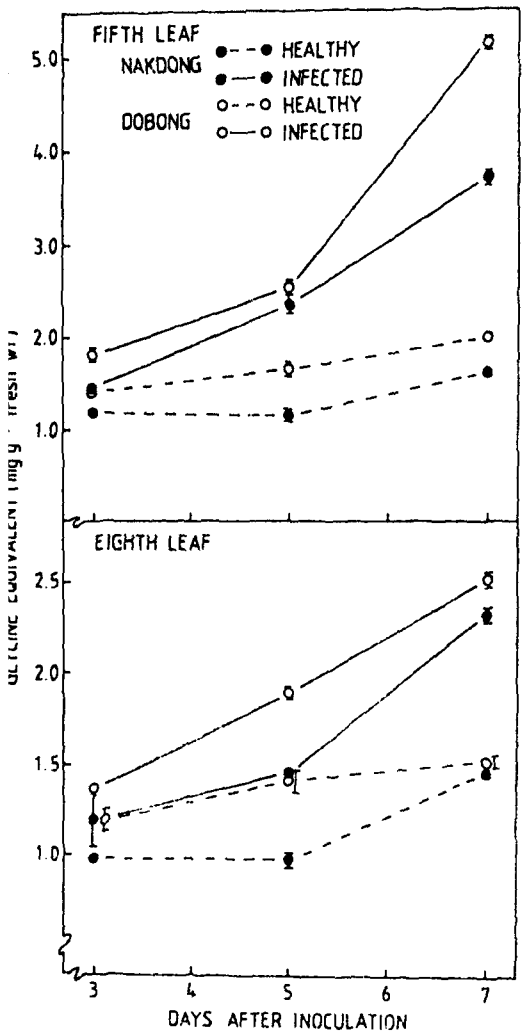

Fig. 1. Levels of total soluble amino acids in healthy and blast-infected fifth and eighth leaves of rice cultivars Nakdong (susceptible) and Dobong (adult-plant-resistant) at various intervals after inoculation at the five and eight leaf stages. The bar represents one standard deviation from the mean of three measurements. The leaf areas infected were about 12% and 3% in Nakdong and about 9% and 1% in Dobong at the five and eight leaf stages, respectively.

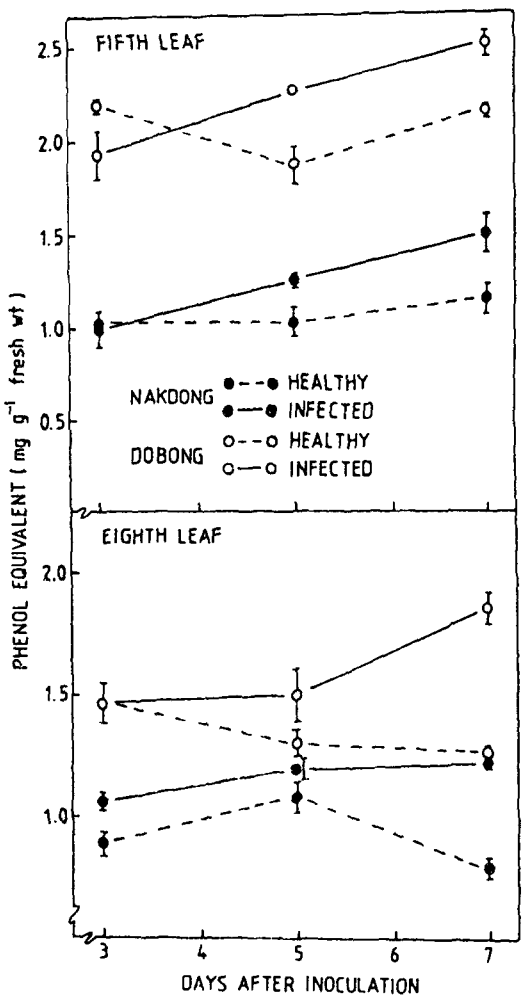

Fig. 2. Levels of total phenolics in healthy and blast-infected fifth and eighth leaves of rice cultivars Nakdong (susceptible) and and Dobong (adult-plant-resistant) at various intervals after inoculation at the five and eight leaf stagges. The bar represents one standard deviation from the mean of three measurements. The leaf areas infected were about 12% and 3% in Nakdong and about 9% and 1% in Dobong at the five and eight leaf stages, respectively.

controls at five and eight leaf stages. In infected fifth and eighth leaves, increases in the levels of total soluble amino acids were more pronounced in the adult-plant-resistant cultivar Dobong than those in the susceptible cultivar Nakdong throughout the experiment. No differences in levels of total amino acids were found between healthy fifth and eighth leaves of the two cultivars. The increases in total soluble amino acids in the infected fifth leaves were about twofold more than those in the infected eighth leaves.

Total phenolics in healthy and infected plants. The levels of total phenolics in healthy and infected leaves of the susceptible cultivar Nakdong and the adult-plant-resistant cultivar Dobong at various intervals after inoculation with *P. oryzae* are presented in Fig. 2. The adult-plant-resistant cultivar Dobong showed higher amounts of total phenolic compounds in both healthy and infected fifth and eighth leaves than did the susceptible cultivar Nakdong throughout the experiment. Differences in the levels of total phenolics between the two cultivars at the five leaf stage were higher than those at the eight leaf stage. No significant differences in the levels of total phenolics were observed between healthy and infected leaves of two cultivars until the 3, days after inoculation at five and eight leaf stages. However, slight increases in the levels of total phenolic compounds in the infected fifth and eighth leaves of two cultivars, especially the adult-plant-cultivar Dobong, were found at the 5 and 7 days after inoculation, as compared with healthy controls.

DISCUSSION

Our previous studies demonstrated that adult-plant resistance of rice to leaf blast could be detected and estimated by artificial inoculation with *P. oryzae* on rice plants at different leaf stages (5). The analytical experiments for total soluble amino acids and phenolic compounds in healthy and infected plants were done using rice plants at five and eight leaf stages, because seedling plants could be uniformly infected by artificial inoculati rather than mature plants.

The data obtained in this study demonstra that healthy leaves of the adult-plant-resist cultivar Dobong at five and eight leaf stages c tained higher amounts of total soluble phen compounds than did the susceptible cultivar bong. This finding suggests that a higher cont in phenolic compounds, presumed to be preforr inhibitory substances (12) present in plants, r play a significant role in the expression of ad plant resistance to blast. In rice-*Helminthospori oryzae* combination, Chattopadhyay and Bera reported that healthy leaves of resistant rice culti possessed more phenols than those of suscepti cultivar. When compared to the healthy lea increases in the levels of phenolic compounds w found in infected fifth and eighth leaves during 7 days after inoculation, particularly being m pronounced in the adult-plant-resistant Dob than in the susceptible Nakdong. The marked ac mulation of phenolic compounds in rice leaf tissue the cultivar Dobong at the eight leaf stage in sponse to blast infection could also account for possible production of antifungal phytoalexins, w some relation to the appearance of resistance later development stages of plants. Many anti-bl substances from the blast-infected leaves have b reported in recent years (1,10,11). Chattopadhy and Bera (2) demonstrated that an increase in t phenol content of rice leaves infected with *Helm thosporum oryzae* was more marked in the resist cultivar than in the susceptible cultivar.

The adult-plant-resistant cultivar Dobong show higher amount of soluble amino acids than did t susceptible cultivar Nakdong during the bl infection at five and eight leaf stages, althou Dobong was less infected by *P. oryzae* than Na dong. Increases in amounts of soluble amino aci in blast-infected leaves of the two cultivars m result from either the rapid decomposition of pr teins by host or fungus enzymes, or the stimulati of protein synthesis and accumulation of ami acids at the infection sites. The pronounced ri

f amino acids in infected fifth and eighth leaves of)obong could partly contribute to the synthesis of henolic compounds which arise from the aromatic mino acids such as phenylalanine and tyrosine. hese suggestions are well supported by our obser- ations that the increased rates of both amino acids nd phenolic compounds during blast infections at he five and eight leaf stages were more pronounced n the adult-plant-resistant cultivar Dobong than in he susceptible cultivar Nakdong. In addition, an nteresting fact is that carbohydrate metabolism vhich may be promoted in the direction favorable or blast development is more impaired in blast- nfected plants of susceptible Nakdong than in dult-plant-resistant Dobong (4).

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