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

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벼 잎의 炭水化物, 아미노산, 페놀化合物 含量과 잎 稻熱病에 對한 成體植物 抵抗性과의 關係

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ABSTRACT

Six rice cultivars showing various types of resistance or susceptibility to *Pyricularia oryzae* in the field were used to study the relationship between susceptibility and contents of carbohydrates, amino acids and phenolics at different plant maturities and leaf ages under controlled environmental conditions. No consistent differences between the susceptible cultivars and adult-plant-resistant cultivars were found in terms of the contents of individual carbohydrates, total amino acids and phenolics in rice leaves throughout the plant development. Only the adult-plant-resistant cultivar Dobong had lower contents of inositol throughout the plant development than the other cultivars. The amounts of sucrose, inositol, glucose, and fructose increased in all tested cultivars at eight leaf stage than those at five leaf stage, but slightly decreased at twelve leaf stage. In contrast, total amino acids and phenolics gradually decreased during plant development. With increasing age of rice leaves, the amounts of total soluble amino acids and phenolics gradually increased in healthy fifth leaf tissues, although there were no significant differences between the cultivars. In particular, a high level of phenolics existed in old fifth leaves of the cultivar Dobong. These results suggest that increased resistance to blast of matured rice plants and old leaves may be the two different phenomina derived from the physiological changes occurring during plant development and leaf senescence, probably functioning differently in *P. oryzae* development.

Key words: rice blast, adult-plant resistance, carbohydrates, amino acids, phenolics.

要 約

圃場에서 稻熱病에 對하여 抵抗性이 다른 6個의 벼 品種을 一定한 環境條件에서 재배하여 벼 잎에 含有되어 있는 炭水化物, 아미노산 및 페놀화합물 含量과 植物의 여러 發育時期 및 벼 잎의 나이가 어떠한 關係가 있는지 硏究하였다. 벼가 發育하는 동안 벼 잎에 含有되어 있는 炭水化物, 총 아미노산 및 페놀化合物의 含量에 있어서 感受性 品種과 成體植物抵抗性 品種간에 有意差는 없었다. 단지 成體植物抵抗性 品

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種인 도봉만이 植物이 發育하는 동안 다른 品種에 比해 inositol 含量이 가장 적었다. sucrose, inositol, glucose 및 fructose 含量은 모든 品種에서 5葉期보다 8葉期에서 增加하였으나 12葉期에서는 약간 減少하였다. 반면에 총 아미노산과 페놀化合物은 植物이 發育하는 동안 점차로 減少하였다. 健全한 5葉이 老衰함에 따라 可溶性 아미노산과 페놀化合物의 含量은 점차 增加하였으나 品種간의 有意差는 없었다. 特히 도봉品種의 노쇠한 5葉에서 페놀化合物이 가장 높았다. 벼가 成熟하여감에 따라 또한 老衰한 벼 잎에서 稻熱病에 對하여 抵抗性이 增加되는 것은 아마도 稻熱病의 發生에 相異하게 作用하는 벼의 發育과 벼 잎이 老衰하는 동안에 일어나는 生理的인 變化에서 起因하는 相異한 現象일지도 모른다.

INTRODUCTION

The biochemical constituent such as carbohydrates, amino acids, and phenolics in leaf tissues of plant genotypes may play an important role in the expression of resistance or susceptibility to plant diseases. Horsfall and Dimond (2) proposed that sugar content of plant tissue leads to a nutritional status favorable or unfavorable for fungal development. Amino acids which are not only substrates for pathogen but show a direct fungistatic effect may be involved in the metabolism of host resulting in an increased resistance (13). Another property of amino acids may have an influence on the germination and growth of conidia on the leaf surface prior to penetration (11). Furthermore, it has been assumed that phenolic compounds and their oxidation products are accumulated in plant in response to pathogenic infection and injury and exhibit antimicrobial properties to plant pathogen (10).

In previous investigations, qualitative and quantitative differences in resistance or susceptibility to leaf blast between rice cultivars during plant development could be detected and evaluated under controlled environmental conditions on the basis of race specificity and effects of plant and leaf age (3). The relative ranks of resistance were consistently in the order Jinju < Nakdong < Palkeum < Jinheung < Dobong < Nongbaek at five leaf stage.

This research was initiated to determine if the contents of soluble carbohydrates, amino acids and phenolics in healthy leaf tissue of rice plants are related to quantitative differences in resistance or susceptibility to rice blast.

MATERIALS AND METHODS

Cultivation of rice plants. The rice (*Oryza sativa* L.) cultivars used were: (i) Nakdong and Jinju, susceptible at all plant growth stages (ii) Palkeum, Jinheung and Dobong, susceptible at early growth stage, but resistant at late growth stage, and (iii) Nongbaek, resistant at all growth stages (3,4,5). The rice plants in greenhouse were grown in plastic pots (5x15x10cm) containing sterilized paddy soil mixed with the fertilizer. The fertilizer was applied at the rate of 0.27-0.27-0.21g of actual N-P-K per pot. Six seedlings at the five leaf stage were transplanted in the Wagner's pot (1/5000a) with fertilizer of 0.326-0.392-0.234g of actual N-P-K per pot. Additional 0.26g nitrogen and 0.1g kalium per pot were applied later for tillering during cultivation of rice plants.

Extraction and determination of total ethanol-soluble carbohydrates, amino acids, and phenolics. One gram (fresh weight) of leaf tissue from healthy rice plants at various leaf stages and fifth leaves of different ages was harvested at 11 a.m., cut into 1-cm segments and boiled in 20ml of 80% (v/v) ethanol for 10 minutes (three changes). The pigments in 50ml of ethanol extracts were removed twice by shaking with 30ml of petroleum benzine. Aliquots of the ethanol-soluble extracts before the treatment with petroleum benzine were used in determining the amount of total phenolic compounds by the method of Swain and Hillis (12). The ethanol-soluble fractions were stored at $-20^{\circ}C$ until analyzed for total, individual carbohydrates and total amino acids. Total carbohydrates were determined in aliquots of the ethanol-water fractions according to the phenol-sulphuric acid method of Dubois et al. (1) using glucose as a standard. The contents of total amino acid were determined by ninhydrin method of Yemm and Cocking (16) using glycine as a standard. The remaining ethanol-soluble fractions were analyzed for individual carbohydrates.

Analysis of individual carbohydrates. *Ion exchange chromatography.* The ethanol water fractions extracted from the leaf tissue were vacuum-evaporated to dryness at 36°C and taken up in a known volume of double distilled water and dichloromethane (2:1, v/v). The water fractions containing the carbohydrates, organic acids, and amino acids were applied to ion exchange columns filled with Dowex 50W (counter ion H^+, 200-400 mesh) and Amberlite IRA 68 (counter ion OH^-, 16-50 mesh). The eluates from both columns were vacuum-evaporated to dryness at 40°C and resuspended in a small volume of the double-distilled water and dichloromethane (1:1, v/v) to dissolve the residue completely. The supernatant water fractions were stored in -20°C until use for gas chromatographic analysis of carbohydrates.

Gas chromatography. Analysis of carbohydrates by gas chromatography was carried out using the water fraction to which phenyl-β-D-glucopyranoside had been added as an internal standard. The lyophilized samples were dissolved in 0.2ml of 2.5% hydroxylamine in pyridine and heated at 80 to 85°C for 20 min. The resulting oximes were converted to their trimethylsilyl ethers by the addition of 0.1ml of hexamethyldisilazane (HMDS) and 0.1ml of trimethylchlorosilane (TMCS) and heating at 80 to 85°C for 2 min. Samples were taken to dryness under a stream of nitrogen and then centrafuged three times with 1 ml of hexane. The clear supernatant samples was injected into the gas chromatograph (Packard, Model 419) equipped with a flame ionization detector using a 180cm, 2-mm O.D. glass column which was packed with 2.3% OV 17 on chromosorb W-AW (60-80 mesh ASTM). The carbohydrates were detected using a temperature program which was delay of 2 min and 5°C min^{-1} from 120°C to 280°C following postinjection interval of 5 min. The carrier gas was nitrogen at flow rate of 30ml min^{-1}. The injector and detector block temperature was 290°C. The carbohydrates were identified by comparison of their retention time with those of standard compounds, followed by co-chromatography. The different detector sensitivities were compensated using a correction factor for each carbohydrate. Each sample was chromatographed three times, but no sample replication was possible.

RESULTS

Carbohydrates in the leaves at various growth stages. Individual ethanol-soluble carbohydrates in healthy leaf tissue at various leaf stages were identified and quantitatively determined by gas chromatography (Fig. 1.). The levels of all soluble carbohydrates which existed in healthy leaf tissue of rice plants greatly increased in all cultivars at the eight leaf stage compared to those at early leaf stage, but declined to some degree at the twelve leaf stage. However, the polyol inositol contents in the adult-plant-resistant cultivars Palkeum and Dobong consistently increased during the development of plants. In particular, the cultivar Dobong with a high adult-plant resistance contained the lowest amount of inositol among the other tested cultivars. No consistent differences between the cultivars, related to their susceptibility or resistance to leaf blast, were found in the amounts of soluble carbohydrates from rice leaf tissue.

Amino acids at various growth stages and leaf ages. Figures 2 and 3 show the levels of total soluble amino acids as mg g^{-1} fresh weight of healthy leaves of rice plants in relation to plant maturity and age of fifth leaves. No significant differences in the amounts of total amino acids were observed between the cultivars during plant development and a slight decrease was found at the twelve leaf stage. With aging of fifth leaves, the amounts of total soluble amino acids increased regardless of rice cultivars.

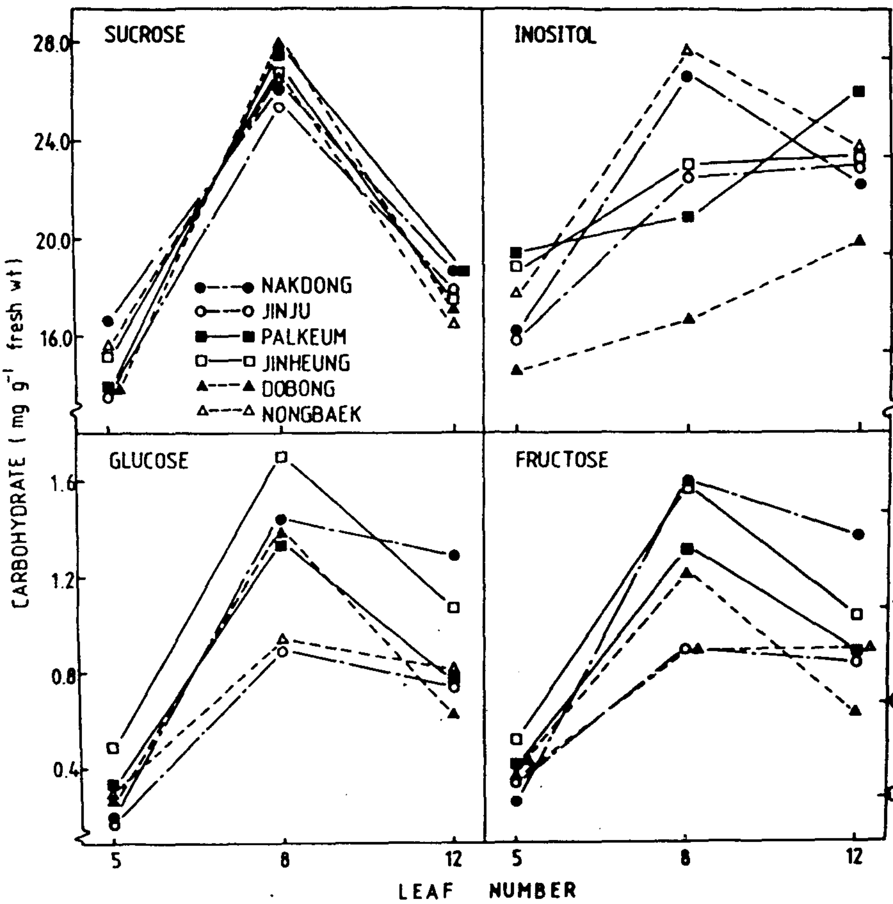

Fig. 1. Levels of sucrose, inositol, glucose, and fructose in healthy leaves of six rice cultivars at various developmental stages.

Total phenolics at various growth stages and leaf ages. The amounts of total phenolic compounds in healthy leaf tissue declined in all cultivars during plant development, but slightly increased at later leaf stage (Fig. 4). No consistent differences between the cultivars were found in the levels of total phenolics in healthy leaf tissue with increasing stages. Figure 5 shows changes in amount of total phenolics in healthy leaf tissue with increasing age of fifth leaves. The levels of total phenolics increased considerably at the 32-day old fifth leaves. In particular, the level of phenolics in the cultivar Dobong was higher than those of the other cultivars. No significant differences between the cultivars were found in the amounts of total phenolics with aging of fifth leaves.

DISCUSSION

No consistent differences between the susceptible cultivars and adult-plant-resistant cultivars were found in terms of the contents of individual carbohydrates, total amino acids, and phenolics in rice leaves throughout the plant development. These results imply that the quantitative differences in resistance or susceptibility to leaf blast may not be explained by the different levels of carbohydrates, amino aicds, and phenolics of leaf tissue. Only the adult-plant-resistant cultivar Dobong showed lower content of inoistol throughout the plant development than the other cultivars. The low level of the cyclitol inositol known to be a membrane constituent and reserve substance (6)

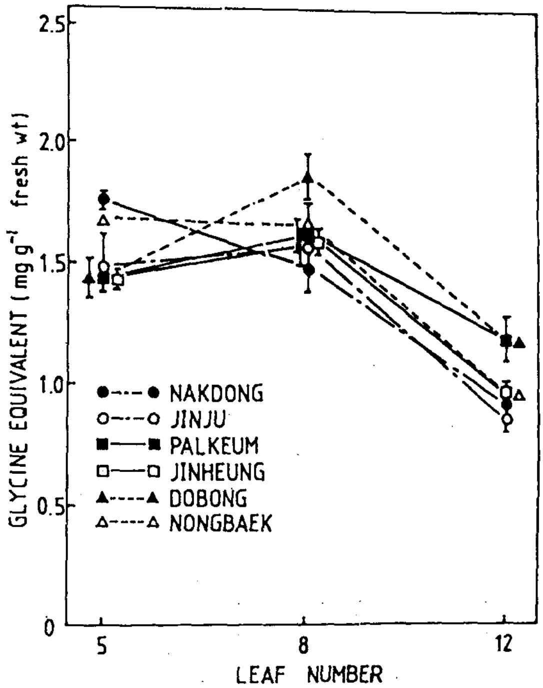

Fig. 2. Levels of total soluble amino acids in healthy leaves of six rice cultivars at various developmental stages. The bar represents one standard deviation from the mean of three measurements.

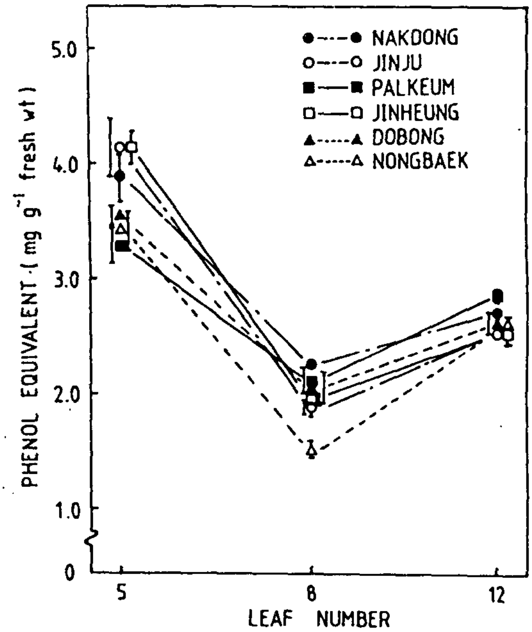

Fig. 4. Levels of total phenolics in healthy leaves of six rice cultivars at various developmental stages. The bar represents one standard deviation from the mean of three measurements.

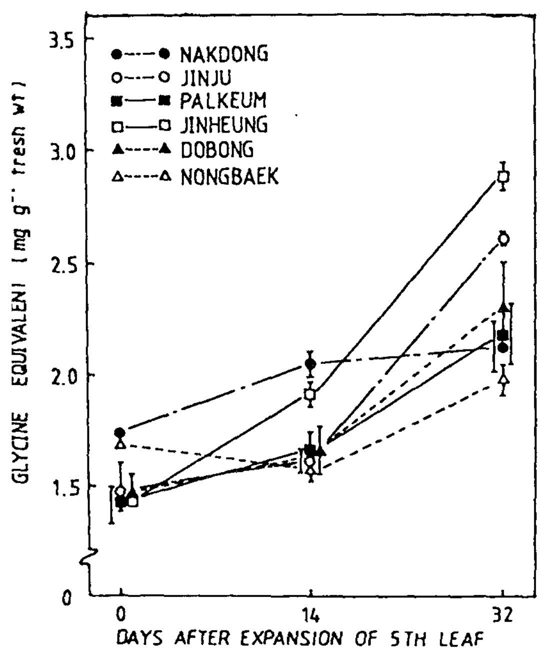

Fig. 3. Levels of total soluble amino acids in healthy fifth leaves from seedlings of six rice cultivars of different ages. The bar represents one standard deviation from the mean of three measurements.

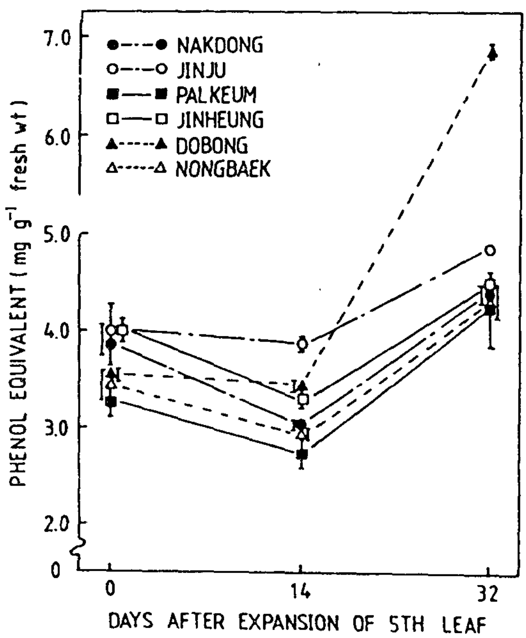

Fig. 5. Levels of total phenolics in healthy fifth leaves from seedlings of six rice cultivars of different ages. The bar represents one standard deviation from the mean of three measurements.

may play a role in the expression of resistance to blast in the cultivar Dobong throughout the plant development.

The amounts of sucróse, inositol, glucose, and fructose increased in all tested cultivars at eight leaf stage than those at five leaf stage, but slightly decreased at twelve leaf stage. In contrast, total amino acids and phenolics gradually decreased during plant development. Our previous study demonstrated that these rice cultivars became resistant to leaf blast as the plants became older, althought this phenomenon was more pronounced in those with adult-plant resistance (3). Increased resistance of rice plants with increasing age may be therefore related to a non-specific gradual alteration of various metabolisms during plant development. Furthermore, morphological changes and increase of silicon contents in leaves of adult plants may also contribute to adult-plant resistance of rice to blast (14). In particular, the fact that all cultivars contain low amounts of phenolics in leaf tissue of mature plants could not account for any relationship between the level of phenolics and adult-plant resistance, provided that phenolic compounds may play an important role in disease resistance (10).

An interesting fact is that, with increasing age of rice leaves, the amounts of total soluble amino acids and phenolics gradually increased in healthy fifth leaf tissues. Our previous results showed that the fifth leaves of all tested cultivars, even in compatible rice-blast combinations, became increasingly resistant to blast as these leaves became older (3). In view of these two facts, it seems likely that either the different levels of or a particular one of amino acids and phenolics may partly affect the production of preformed anti-blast substances in relation to blast infection during leaf senescence. Recently, Matsuyama and Wakimoto (7, 8) found an anti-blast substance which accumulated after inoculation mainly in lower rice leaves resistant to blast.

Our results suggest that increased resistance to blast of mature rice plants and old leaves may be the two different phenomena derived from the physiological changes occurring during plant development and leaf senescence (9, 15), probably functioning differently in *P. oryzae* development.

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