Effect of Nitrogenous Fertilizer on Biochemical Processes that Could Affect Lesion Size of Rice Blast

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ABSTRACT

It has been shown previously that nitrogenous fertilizers supplied to rice plants increase the size of rice blast lesions produced by the invasion of leaves by *Pyricularia oryzae*. Because the size of these lesions, particularly the width, may be limited by phenols and lignins synthesized by the invaded leaf, the relative activities of the key enzymes of lignin synthesis were examined. In the plants that were enriched with nitrogenous fertilizer, the activity of phenylalanine ammonia-lyase decreased 32%, and that of tyrosine ammonia-lyase by 22%. Lignin content decreased 53% in leaves that developed after the plants received nitrogen fertilizer.

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Susceptibility to rice blast is determined by the interaction between host and pathogen genes (12). However, the final size of the lesions depends on other factors. One such factor is the age of the tissue. *Pyricularia oryzae* Cav. forms large lesions on young tissue; whereas, the lesions on old tissue enlarge very slowly and remain small, and the enlargement in the direction of veins (width of lesion) is restricted (5). Because the cell walls of the veins of older leaves are usually heavily lignified (1), it is possible that the phenolic substances such as lignin and flavonoids could affect mycelial invasion. Mechanical resistance caused by thickening of vein-cell walls and the deposition of polyphenolic derivatives such as lignin could also restrict lesion size.

It is now well established that nitrogenous fertilizer increases the size, particularly the width, of the lesions produced on rice plants by *P. oryzae* (10). It is possible that rice plants amply supplied with nitrogenous fertilizer have a diminished ability to synthesize phenol substances and polyphenolic derivatives such as lignin.

This article reports the effect of nitrogenous fertilization of rice plants on the key enzyme activities of phenolic synthesis such as phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL), and peroxidase (7), and on the alteration of flavonoids and lignin contents.

MATERIALS AND METHODS.—Pregerminated seeds of rice (*Oryza sativa* L. 'Aichiasahi') were planted in an equal mixture of peat moss and sand in 5-cm pots and watered with one-fourth strength Kasugai's nutrient solution (8) until the fourth leaf stage. The seedlings were then transferred to 10-cm pots and watered with one-half strength nutrient solution. When the sixth leaf had completely developed, a solution containing 376 mg of ammonium sulfate was added to each pot.

Rice plants were raised in a greenhouse with supplemental fluorescent ("Gro-Lux") light at ca. 25 C. After the seventh leaf had completely developed, the desired leaves were harvested and analyzed.

Flavonoid content was measured by Sukizuka's method (8). Samples of equal fresh weight were boiled in 70% methanol for 2 min, homogenized, filtered, and washed with 70% methanol. The filtrates were volumetrically diluted to equal volumes, 70% methanol saturated with sodium carbonate was added, and the samples were assayed colorimetrically at 400 nm. The flavonoid content was expressed as rutin equivalents.

PAL and TAL were assayed by the following method. Sample leaves were homogenized in cold acetone with a Virtis 45 homogenizer for 2 min, filtered, and repeatedly washed with cold acetone. The samples were dried in vacuo for 2 hr and 100 mg of the resulting acetone powder from each sample was incubated for 3 hr at 30 C with 10 ml of 0.1 M Tris [tris(hydroxymethyl)amino methane] buffer (pH 8.8) containing 0.1% phenylalanine or tyrosine as a substrate. The *t*-cinnamic acid or *p*-coumaric acid produced was extracted with acidic ether. The ether was removed in vacuo and the residue was dissolved in 20 ml of 0.05 N NaOH (3, 6). The enzyme activity was expressed as optical density at 268 nm (*t*-cinnamic acid) and 333 nm (*p*-coumaric acid) per 3 hr per 5 mg of acetone powder.

Peroxidase activity was assayed as follows. One-gram samples of leaf tissue were each homogenized with 50 ml of cold 0.067 M phosphate buffer (pH 7.4), centrifuged at 12,800 *g* for 10 min, and the enzymes purified by repeated precipitation with equal volumes of cold acetone added to the supernatant in the same centrifuge tubes (4). After the acetone was removed in vacuo, the precipitates were each dissolved in 100 ml of distilled water. One-ml samples of these solutions were incubated for 1 hr at 25 C with 3 ml of 0.1 M Na-acetate buffer (pH 4.7), plus 1 ml of 1% *p*-phenylenediamine and 0.5 ml of 0.3% H_2O_2 solution. Enzyme activity was measured colorimetrically at 485 nm (11) and was expressed as optical density per 10 mg of fresh wt of leaf.

Lignin content was measured by the colloidal titration method (1). Dried samples were pretreated with alcohol-benzene (1:1) at room temperature for 1 hr, then treated with 1 N HCl at 100 C for 2 hr, followed by 0.2% Na_2CO_3 at 60 C for 2 hr, and then with 1 N H_2SO_4 at 100 C for 20 min. After the lignin was chlorinated, it was extracted with 3% Na_2SO_3 in 0.2 N NaOH and the extract volumetrically diluted. This solution was reacted with 0.005 N methyl glycol chitosan (MGC) for 1-2 min, and the remaining MGC was titrated with 0.0025 N potassium polyvinyl sulfate using toluidine-blue as an indicator. One ml of 0.0025 N potassium polyvinyl sulfate is equivalent to 0.475 mg of lignin in the graminaceous plant (1).

TABLE 1. Activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) in the seventh leaf of rice plants, some of which received additional nitrogenous fertilizer at the sixth leaf stage

Treatment	Exp. 1	Exp. 2	Exp. 3
		PAL activity[a]	
Additional N	0.185	0.150	0.165
No additional N	0.235	0.234	0.267
		TAL activity[b]	
Additional N	0.141	0.134	0.094
No additional N	0.176	0.157	0.141

[a] Optical density of *t*-cinnamic acid produced as measured at 268 nm.

[b] Optical density of *p*-coumaric acid produced as measured at 333 nm in 0.05 N NaOH.

Every experiment was repeated three to five times.

RESULTS AND DISCUSSION.—The leaves produced by the rice plants after they had been fertilized with additional nitrogen showed a 32% reduction in the activity of PAL and a 22% reduction in the activity of TAL (Table 1). It has been reported (2) that certain phenolic compounds, such as cinnamic and hydroxycinnamic acids are gradually released from a bound matrix when the acetone powders of plants are incubated with buffer solution. If the amount of these compounds were higher in the check plants than in the N-fertilized plants, their release in buffer could give misleading results. However, when the acetone powders of the rice leaves were incubated with buffer but without substrates, very little cinnamic or hydroxycinnamic acid was released from the samples taken from either set of plants. This observation agrees with an earlier report by Rahe et al. (9).

Nitrogenous fertilization decreased the flavonoid content of the seventh leaf by about 9%. This decrease could have resulted from the decreased activity of both PAL and TAL. Peroxidase activity was increased by nitrogen treatment.

It was expected that the reduction of phenolics would reflect a reduced lignin content. The reduction is very striking and the lignin content was reduced by about half in treated plants.

These results would indicate that the promotion of lesion enlargement by nitrogen fertilizer is due, in part, to the reduction of key enzyme activities and the resulting decrease in phenolics and lignin. Peroxidase activity in the treated plant was higher than in the check plant. This would suggest that the limiting factor for lignification is the content of phenolics and that peroxidase activity is not important after reaching a certain level. This point needs further study.

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