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Influence of Bacterial Streak Disease Development on Changes in Phenolics, Soluble Amino Acids and Carbohydrates of Rice Leaves

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Abstract. The healthy leaves of rice cultivar 'BJ 1' resistant to bacterial leaf streak pathogen (*Xanthomonas translucens* f. sp. *oryzicola*) contained higher quantities of total phenolic compounds, reducing and nonreducing sugars than the susceptible cultivar 'IR 8', while the leaves of cultivar 'IR 8' possessed larger concentration of total soluble amino acids than the resistant cultivar 'BJ 1'.

In the leaves of cultivar 'BJ 1', the disease development caused an initial decrease in the concentration of phenols followed by an increase at later stages. As a result of inoculation, soluble carbohydrates and amino acids generally decreased in the leaves of resistant cultivar 'BJ 1', in contrast to an increase in their concentration in the leaves of cultivar 'IR 8'.

Bacterial leaf streak disease, caused by *Xanthomonas translucens* f. sp. *oryzicola* (FANG *et al.*) BRADBURY, produces orange to brown discolouration of the infected rice (*Oryza sativa* L.) leaves. Browning of the tissues during pathogenesis has been attributed to the oxidation of phenolic compounds (CRUICKSHANK and PERRIN 1964, THOMSON 1964), which are synthesized from simple sugars (NEISH 1964). Hence, any stress on carbohydrate metabolism will influence the phenolic concentration. The phenolics have often been correlated with the symptom production and defense mechanisms of the host tissues (FARKAS and KIRÁLY 1962). The toxicity of these phenolics to the pathogen has been shown to be reduced in presence of high soluble amino nitrogen (KIRKHAM 1954) thereby increasing the tissue susceptibility. The present investigation seeks to determine how the bacterial streak infection influences the changes in total quantities of phenolic compounds, soluble amino acids and sugars in both resistant and susceptible rice cultivars.

Materials and Methods

Rice cultivars, 'BJ 1' resistant and 'IR 8' susceptible to bacterial leaf streak disease were raised in circular earthen pots filled with 3.5 kg of rice field soil mixed with 2 g of ammonium sulphate. Forty day old plants inoculated by swabbing the leaves with a muslin cloth dipped in

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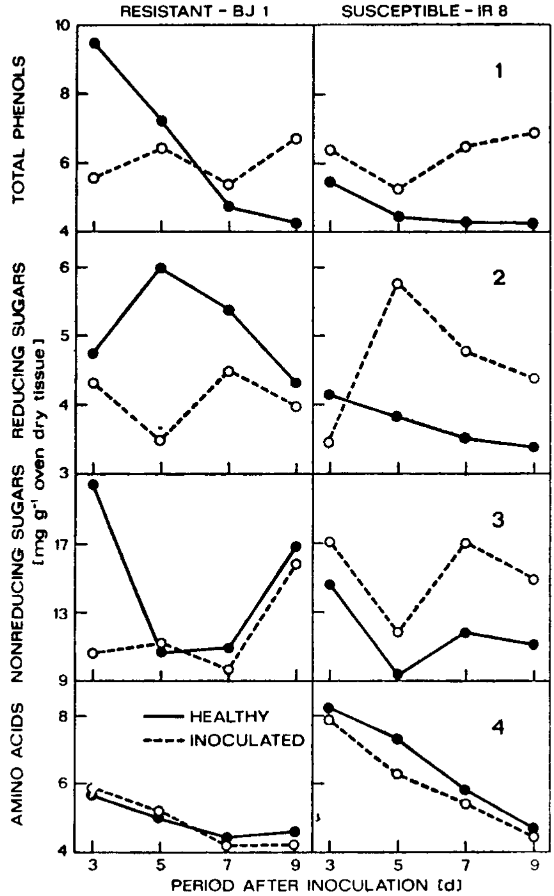

Fig. 1—4. Changes in total phenolic compounds (Fig. 1), reducing sugars (Fig. 2), nonreducing sugars (Fig. 3), and total soluble amino acids (Fig. 4) in leaves of both healthy and bacterial streak infected resistant ‚BJ 1' and susceptible ‚IR 8' rice cultivars.

a cell suspension (*ca.* 10^8 cells/ml) of *X. translucens* f. sp. *oryzicola* obtained from 48 h old cultures maintained on potato sucrose agar medium (Tagami and Mizukami 1962), were kept in a net house (25—30 °C). Leaf samples consisting of 10 g were collected 3, 5, 7, and 9 days after inoculation in the morning (8. *a.m.*) from both healthy and inoculated plants. As the symptom development was uniform throughout the leaf area, the whole leaves were used for the analyses. All the analyses were made in triplicates and the data represent the average of the values.

Leaves cut into small bits were extracted in boiling ethanol (Sridhar 1972) and the extract was used to estimate total phenols by employing Folin-Ciocalteau reagent (Bray and Thorpe 1954). Standards prepared from ferulic acid were used to calculate the unknowns.

Soluble amino acid in the extract was estimated using ninhydrin method of Moore and Stein (1948). Standards prepared from glutamic acid were used to calculate the samples.

Reducing sugar content of the extract was estimated by Nelson's method (Nelson 1944). For total soluble sugars the alcohol extract was hydrolyzed with 1 *N* H_2SO_4 on a hot water bath for 30 min at 49 °C (Inman 1962) and after neutralising it with 1 *N* NaOH, using methyl red indicator, total soluble sugar contents of the samples were estimated by the Nelson's method. Amounts of nonreducing sugars were calculated by subtracting the amount of reducing sugars from total soluble sugars. Results are expressed as glucose equivalents.

Results

Disease Development

Symptoms started to appear three days after inoculation in the form of water soaked translucent streaks. On the leaves of resistant cultivar 'BJ 1', the diseased tissue was discoloured to brown in the form of streaks and the lesion size was limited while, on the leaves of susceptible cultivar 'IR 8', the brown discolouration was delayed considerably allowing the formation of typical long brown streaks with profuse bacterial exudation, which appear between 7 and 10 days after inoculation.

Total Phenols

The concentration of total phenolic compounds was higher in the resistant cultivar 'BJ 1' than in the susceptible cultivar 'IR 8' (Fig. 1). The development of bacterial streak on the leaves of cultivar 'BJ 1' caused an initial reduction in the quantity of phenolic compounds corresponding to a decrease in soluble sugars. However, reduction in the quantity of phenolic compounds was followed by an increase in the later stages of disease development. Inoculated leaves of 'IR 8' possessed larger amounts of phenolic compounds than the healthy leaves with a concomitant increase in the synthesis of soluble sugars.

Soluble Carbohydrates

Leaves of cultivar 'BJ 1' contained increased levels of reducing (Fig. 2) and nonreducing (Fig. 3) sugars than those of cultivar 'IR 8'. Soluble carbohydrates, which provide the carbon skeleton for the increased synthesis of phenolics, amino acids and protein generally decreased in the diseased leaves of cultivar 'BJ 1', whereas disease development enhanced their concentration in the leaves of cultivar 'IR 8'.

Soluble Amino Acids

The susceptible cultivar 'IR 8' possessed higher concentration of amino acids than the resistant cultivar 'BJ 1' (Fig. 4). Inoculation of leaves with the streak pathogen decreased the amino acid content in cultivar 'IR 8' in all stages of disease development in spite of the fact that the photosynthetic products, soluble sugars are generally high in these tissues. Although the level of amino acids increased initially due to infection in the leaves of cultivar 'BJ 1', it decreased at the later stages of disease development.

Discussion

Augmented synthesis of phenolic compounds is characteristic of diseased plants and phenolics upon oxidation become highly reactive and are toxic to pathogens, thus inhibiting the development of pathogen in the tissues (Farkas and Kiraly 1962, Mahadevan 1966). The long narrow streaks caused by the bacterial infection finally turn brown in colour presumably due to phenolic oxidation. This process takes place at a faster rate in the resistant cultivars, while it is delayed in the susceptible cultivars allowing the lesions to develop well. Reduction of phenolic compounds in the leaves of resistant cultivar 'BJ 1' during the initial stages of disease development might be due to the rapid oxidation and incorporation into the higher class of molecules giving brown colour to the tissues. Hence, the rate and time at which the phenolic oxidation takes place in the diseased tissue determine resistance or susceptibility of the cultivars (Sridhar and Ou 1974).

Leaves of resistant cultivar contained higher amounts of total soluble sugars than the susceptible cultivar providing more precursors for the synthesis of phenolic compounds (Neish 1964). Since phenolic compounds increased in the inoculated leaves of resistant cultivar during the later stages of disease development with a concomitant reduction in sugars, it is suggested that the major part of the sugars is shunted to polyphenol synthesis. Besides this, it is likely that some of the carbohydrates might have been utilized for respiration, which is enhanced considerably in the diseased tissues (Uritani and Akazawa 1959, Uritani 1963), causing a decline in the soluble carbohydrates.

Inoculated leaves of susceptible cultivar synthesized more phenolics than the inoculated leaves of resistant cultivar (Byrde *et al.* 1960, Mahadevan 1964) and a high phenol level does not always mean resistance (Rubin and

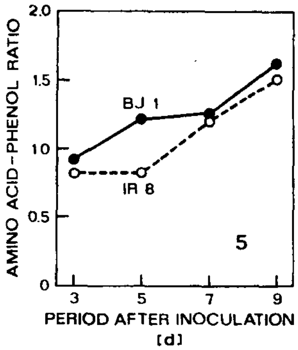

Fig. 5. Amino acid-phenol ratio in the bacterial streak infected resistant ‚BJ 1' and susceptible ‚IR 8' rice cultivars.

ARTSIKHOVSKAYA 1963). The susceptibility of leaves of 'IR 8' cultivar could be explained by the presence of a higher concentration of amino acids than in the leaves of resistant cultivar, which will help in the rapid breakdown of phenols (KIRKHAM 1954), thus reducing their toxicity. Hence, the ratio between total soluble amino acids and total phenolic compounds becomes important (Fig. 5). The amino acids — phenol ratio increases with the progress of the disease development. The inoculated leaves of cultivar 'BJ 1' are characterized by a higher ratio than that of diseased leaves of cultivar 'IR 8' and this phenomenon enhances the toxicity of phenolics in the disease syndrome which in turn restricts the disease development. The typical discolouration of the susceptible leaves was strongly delayed, suggesting the inhibition of phenolic oxidation itself.

The reduction of amino acids in the inoculated leaves might be attributed to the utilization by the pathogen, enhanced protein synthesis and reduced amino acid synthesis. In defending the parasite, the host requires additional energy and is provided by enhanced metabolism. The metabolic systems associated with the symptom expression by the plant are closely intertwined and any change in one of them will necessarily be reflected in resistance of the tissue.

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P. RANGA REDDY, R. SRIDHAR (Cuttack): **Vliv vývoje bakteriální pruhovitosti na změny fenolů, rozpustných aminokyselin a glycidů v listech rýže.** — Biol. Plant. 17 : 363—367, 1975.

Zdravé listy rýže cv. ‚BJ 1' odolné proti bakteriálnímu patogenu pruhovitosti (*Xanthomonas translucens* f. sp. *orizicola*) obsahovaly vyšší množství celkových fenolických sloučenin redukujících a neredukujících cukry než neodolný cv. ‚IR 8', zatímco listy kultivaru ‚IR 8' měly vyšší koncentraci celkových rozpustných aminokyselin než odolný cv. ‚BJ 1'.

V listech cv. ‚BJ 1' působila infekce počáteční snížení koncentrace fenolů, po kterém v pozdějších stádiích následoval vzestup. Očkováním se v listech odolného ‚BJ 1' obsah rozpustných glycidů a aminokyselin snížil a naopak v listech cv. ‚IR 8' jejich koncentrace stoupla.