TABLE I

t°C	S_1 [a]	S_2	Log S_1/S_2	K_{exp}
0	9.8	1.0	0.9912	11.35
20	15.5	6.5	0.3774	9.08
30	19.0	9.0	0.3245	8.78

[a] S_1 for cobalt ammonium sulphate.

From figure 3, one can deduce

$$\text{Log } K = \log A + n \log S_1/S_2$$

The two constants A and n were calculated and were found to be 8.61 and 0.17 respectively.

The system is found to be of Roozeboom class I and behaves ideally over the entire concentration range of both the components.

The magnitude of the equilibrium distribution coefficient D_{eqm} of cobalt salt in nickel salt D_{eqm}(Co, Ni) is calculated and is equal on the average to 0.31; the magnitude of the D_{eqm}(Ni, Co) is equal on the average to 3.15

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MODIFICATION OF SUSCEPTIBILITY IN RICE TO BACTERIAL LEAF BLIGHT BY PHENOLS AND RELATED SUBSTANCES

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ABSTRACT

DL-Phenylalanine enhanced resistance of rice plants to *Xanthomonas campestris* pv. *oryzae* more effectively than shikimic acid, L-isomers of both phenylalanine and tyrosine, *p*-hydroxybenozoic, cinnamic, *p*-coumaric, caffeic, ferulic, vanillic and chlorogenic acids which are known to be present in rice plants when administered through the roots of seedlings.

INTRODUCTION

RICE cultivars reacting incompatibly to *Xanthomonas campestris* pv. *oryzae* (XCO) display brown discolouration of the tissue[1]. Although this bacterium is a typical vascular colonizer, microscopic examination of the diseased tissue revealed that the discolouration occurred not only in the vascular system, but also in the adjoining parenchymatous tissue[1]. This led to the postulation[2] that browning reaction is involved in the localization of the inoculum at the site of penetration.

Levels of phenols in XCO-infected rice leaves increased compared to healthy leaves[3,4]. This response is active especially in the resistant host-parasite combinations caused by genetic make-up of the host and/or due to certain environmental factors like potassium nutrition of the host[3] and light intensity[4]. Paradoxically, in our earlier experiments, we found that the healthy leaves of bacterial blight susceptible rice cultivar possessed higher amounts of phenols than the less-susceptible and resistant cultivars. Since phenolic metabolism is associated with tissue discolouration leading to necrosis and disease resistance[5,6], one major question is whether or not phenols are involved in bacterial blight resistance of rice. We therefore, investigated the effect of some of the native phenols of rice and their precursors on the development of bacterial leaf blight in rice plants.

Materials and Methods

Surface sterilized rice (*Oryza sativa*, cv. Taichung Native 1) seeds were germinated in Petri dishes kept in moist chamber. Soon after seed germination the lids were removed, irrigated with quarter strength of modified Hoagland's solution[7] and kept in insect-proof cages.

Ten-day-old seedlings were treated individually in test tubes (2 × 15 cm) containing 5 ml of different test chemicals for 24 hr at room temperature (28 ± 2° C). Control plants were treated similarly but with distilled water. Each seedling was treated as a replicate. At the end of the treatment, the top most leaves were inoculated with XCO as described earlier[3]. The seedlings were removed, their roots thoroughly washed in running tap water and transplanted in 2 l plastic pots containing half strength of modified Hoagland's solution at 5 seedlings per pot. The development of symptoms were recorded by measuring lesion length 15 days after inoculation. The experiment was repeated twice, and the data reported here represent the mean of 10 replicates in a typical experiment. Although there were some variations in the disease development between experiments, the trends in results were comparable.

Results and Discussion

If the accumulation of phenolics cause brown discolouration leading to disease resistance, we surmise that treatment of phenolics, native to rice leaves should resist the development of the parasite. Phenol-induced resistance to plants against fungal pathogen is not new in literature. For, pretreatment of susceptible cotton plants either with catechol or catechin[8] and tomato plants with catechol[9] conferred protection against *Fusarium* Wilt. In fact, most of the substances when fed to the rice seedlings markedly reduced their susceptibility to XCO infection (table 1). However, the degree of protection varied with the substance. One would naturally expect that the healthy leaves of highly susceptible cultivar Taichung Native 1, which possess higher amounts of phenolics to resist the disease more effectively than do the less-susceptible cultivars, if the resistance is related to phenol metabolism. It is possible that adequate concentration of phenols may not be present at the appropriate sites in the leaf tissue of highly susceptible cultivar to resist XCO.

DL-phenylalanine markedly inhibited the disease development but the naturally occurring L-isomer of both phenylalanine and tyrosine was not. Even though shikimic acid and *P*-hydroxybenzoic acid were toxic to the plants when fed at 10^{-3} M, these at 10^{-5} M markedly inhibited the disease development (table 1). Increase in phenylalanine ammonia-lyase (PAL) activity due to infection by XCO in susceptible rice leaves has been reported earlier[10,11]. The post-infectional increase in the enzyme activity was greater in resistant than in susceptible cultivar[10]. Therefore, we believe that phenolics synthesized by rice leaves from phenylalanine (figure 1) might enhance resistance. For, phenylalanine is not directly toxic to XCO. Nor does this bacterium possess PAL[12] to convert the amino acid to cinnamic acid derivatives.

Although L-phenylalanine was utilized more effectively by most of the plant species, Neish and his

Table 1

Inhibition of disease development in susceptible rice cultivar Taichung Native 1 by aromatic compounds

Treatment	Per cent inhibition over control (Concentration (M)) 10^{-3}	10^{-4}	10^{-5}
Shikimic acid	–	43	33
DL-Phenylalanine	77	72	68
L-Phenylalanine	54	42	33
L-Tyrosine	48	42	26
p-Hydroxybenzoic acid	–	46	31
Cinnamic acid	47	37	23
p-Coumaric acid	42	21	16
Caffeic acid	46	40	11
Ferulic acid	56	42	22
Vanillic acid	53	36	27
Chlorogenic acid	52	43	33

– Phytotoxic. The lesion was measured 15 days after inoclulation and the per cent inhibition over control was calculated. Data represent the mean of 10 replicates; the individual values were always within ± 5% of the mean.

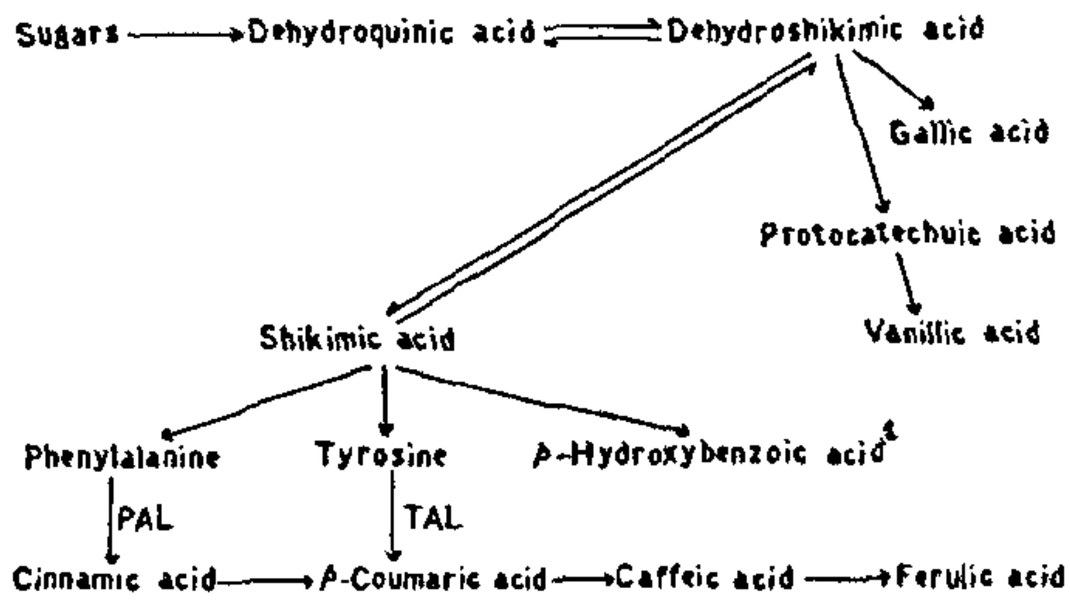

Figure 1. Pathway showing the synthesis of phenolic compounds. (*Known to occur in bacterial cultures.)

associates[13,14] proposed that both D- and L-isomers were converted to phenolics in a number of plants. But Kuc *et al*[15] showed that L-phenylalanine when infused into growing shoots of apple varieties did not influence the host reaction to *Venturia inaequalis*. However, both D- and DL-isomers induced the host resistance presumably by synthesizing phenolics from the DL-isomers[16].

No difference existed in the phenylalanine content of healthy leaves of resistant and susceptible rice cultivars[10]; its level actually increased in the susceptible cultivar infected by XCO[10,17], in contrast to a reduction in the resistant cultivar[10]. Presumably, the precursor is not a limiting factor for phenol synthesis in the susceptible leaves. Nevertheless, our results (table 1) clearly indicate that the ability of the susceptible rice cultivar to resist the disease development is enhanced more effectively when DL-phenylalanine was administered and to a lesser effect with L-isomer. Since the D-isomer has to be converted to L-isomer before the plant can utilize it, we believe that this conversion process eventually results in a regulated availability of the precursor for phenol synthesis without being rapidly incorporated into protein.

A variety of stimuli including light intensity, plant hormones, wounding and disease influence the induction of PAL[18]. Growing rice seedlings either in dark[19] or in low light intensity[4] which predispose them to fungal and bacterial infections, depresses the phenol content of the tissues, perhaps due to inhibition of PAL activity[20]. Analysis of the literature in the light of our results supports the hypothesis that primarily, the availability of phenylalanine and secondarily, the induction of PAL due to pathogenic stimulus and/or environmental factors are important 'velocity modulating factors' in the expression of resistance of rice plants to XCO.

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