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Changes in Amino Acids and Phenols in Limabean (Phaseolus lunatus) Varieties Resistant and Susceptible to Stem Rot Disease (Macrophomina Phaseolina)

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ABSTRACT

Limabean plants resistant (PLJ-1) and susceptible (PLJ-5) to stem rot (Macrophomina Phaseolina) were grown in sick soil (inoculated with organisms) and leaves collected at pre-infectional, and late infectional stages were analysed for amino acids, phenols and their profile by paper chromatography and TLC respectively. Resistant variety (PLJ-1) exhibited lower levels of total amino acids and phenols except at pre-infectional stage. A continuous increase in free amino acids and phenols was observed in infected plants of susceptible (PLJ-1) variety from pre-infectional (I) to late-infectional stage (III) while reverse was true for amino acids in uninfected plants of resistent variety. At early infectional stage (disease initiation) the resistant plants had higher levels of cystine and arginine plus histidine. All the phenols were higher in resistant plants at pre-infectional stage (i). Catechol and chlorogenic acid were found to be higher in resistant plants at all stages.

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INTRODUCTION

In limabean an important pulse crop of Gujarat, Ashy stem blight or stem rot is one of major soil borne diseases causing extensive loss in yield. A severe outbreak of this disease was observed during 1987 at the Pulse Research Farm, GAU campus, Junagadh and in many farmer's fields in Junagadh district.

Phenolic compounds and amino acids are reported to be involved in resistance to fungal diseases (Satyavir and Grewal, 1975; Reddy and Rao, 1977) based on the differences in their concentrations in plant tissues between resistant and susceptible varieties (Murthy and Bagyaraj, 1978; Sindhan and Jaglan, 1987)and because of the changes in their levels around the onset and progress of disease (Srivastava and Dhavan, 1985; Singh and Srivastava, 1988). The present experiments describe preliminary studies on the phenolics and amino acids in leaf tissues of limabean plants in response to stem rot caused by Macrophomina phaseolina at different disease stages.

MATERIALS AND METHODS

The seeds of limabean varieties resistant (PLJ-1) and susceptible (PLJ-5) to stem rot were sown in pots where the soil has been inoculated with Macrophomina phaseolina. It was reported that fungal growth appears within 10-12 days of sowing and that within another 6-8 day period the infection spreads leading to wilting and death of the plant (Jadeja, 1989). The leaf samples were therefore collected at pre-infectional (8 days after sowing; DAS) early infectional (10 DAS) and late infectional (14 DAS) stages. The leaf samples were extracted in 80% alchohol and partially purified by extraction with chloroform to remove the pigments. The free amino acids in this extract were estimated by ninhydrin method (Lea and Takahashi, 1966) and total phenols by folin's method(Mahadevan and Shridhar, 1986) respectively. Amino acid

profile was screened by paper chromatography (Block and Zweig, 1958) and phenol profile was screened by thin layer chromatography (Harborne, 1973).

RESULTS AND DISCUSSION

Resistant variety (PLJ-1) of limabean had higher levels of free amino acids at the pre-infectional stage compared to susceptible (PLJ-5) variety (Table1). In the infected plants of susceptible variety the amino acid concentration increased while in uninfected resistant plants there was a fall in the same. Higher levels of free amino acids in diseased plant tissues have been reported by other workers (Vidhyasekaran, 1977) and same may be for utilisation by the fungus.

The concentration of total phenolic compounds were more or less similar at the pre-infectional stage in both the varieties (Table 1). The same decreased during infection in susceptible plants while in resistant plants there was a fall during early infectional stage alone. There are many reports (Sharma, 1983; Saxena *et al.*, 1987) of increased levels of phenolic compounds in resistant plants and hardly any reports of a decrease in phenols in resistant plants. It might be that more phenols are produced by the susceptible plants or released by fungal glycosidases while the disease is in progress.

A screening of the selected phenolics in the leaf extract indicated (Table 2) that all the phenols were higher in resistant plants at pre-infectional stage. They also had higher levels of chlorogenic acid at early infectional and late infectional stages, while susceptible plants registered an increase in caffeic acid and resorcinol plus orcinol during late infectional stage. These results indicate that quantitative studies on the individual phenolics may throw light on the involvement of specific phenols in disease resistance.

The data on levels of individual amino acids in leaf tissues (Table 3) showed that generally all the amino acids were present in higher concentrations in the resistant plants at the pre-infectional stage except cystine. At both the early and late-infectional stages the reverse was true i.e. higher levels of all the amino acids were seen in susceptible plants except cystine and arginine plus histidine at early infectional stage.

TABLE 1

Leaf free amino acids and phenols in stem rot resistant and susceptible varieties of limabean.

Stages of disease	*Resistant variety PLJ-1*	*Susceptible variety PLJ-5*
	Free amino acids (mg/g fresh leaf)	
I	4.69	2.67
II	3.14	3.60
III	2.28	3.94
	Phenols (mg/g fresh leaf)	
I	0.673	0.611
II	0.560	0.654
III	0.580	0.756

Values are means of two daplicate analysis.

I Pre-infectional stage. II Early-infectional stage.

III Late-infectional stage.

TABLE 2

Relative amounts (abundance) of phenols in stem rot resistant and susceptible varieties of limabean.

	Stages of disease	*Resistant variety PLJ-1*	*Susceptible variety PLJ-5*
Caffeic	I	++	---
acid	II	+	+
	III	+	+++
Resorcinol	I	++	-
+	II	+	++
orcinol	III	+	+++
Catechol	I	++	---
	II	++	+
	III	++	++
Chlorogenic	I	+++	---
acid	II	+++	++
	III	++	+

+++ = much; ++ = less; + = least; -- = absent

I Pre - infectional stage II Early-infectional stage

III Late-infectional stage.

The levels of amino acids increased from pre-infectional to early infectional stage in resistant plants except cystine. From early to late-infectional stage all the amino acids increased in susceptible plants. The reverse was noticed in resistant plants except for tryptophan and phenylalanine. Thus during the late stage of disease the pattern of changes in the individual amino acids differed in both the varieties. At late infectional stage most of the amino acids were present in higher concentration in susceptible plants.

Earlier studies on wilt in chickpea (Mandavia and Parameswaran, 1991) showed that cystine was higher in leaves of resistant plants than that in susceptible plants especially at the early infectional period. In the present studies also levels were higher in resistant plants compared to susceptible plants at early infectional stage. In susceptible plants cystine levels were higher at pre-infectional stage. This fell down to very low levels during early infectional stage followed by an increase in the later period. These changes may be related to the infection process as such changes were not observed in resistant plants. Other workers have suggested roles of cystine in disease resistance to gram blight (Satyavir and Grewal, 1975b), and cystine to fusarium wilt in a Cajanus cajan (Murthy and Bagyaraj, 1978b), and to Helminthosporium nodylosum in finger millet (Vidhyasekaran, 1977).

The amino acids cystine and arginine plas histidine were higher in resistant plants at early infectional stage and may be contributing in disease resistance.

TABLE 3

Individual amino acids in leaves of stem rot resistant and susceptible varieties of limabean (Micromoles/g. fresh leaf).

	Stages of disease	*Resistant variety PLJ-1*	*Susceptible variety PLJ-5*
(a) Aromatic amino acids :			
Tyrosine	I	3.46	0.35
	II	1.30	2.44
	III	0.80	4.76
Tryptophan	I	1.60	0.29
	II	0.23	0.92
	III	1.44	2.60
Phenylalanine	I	0.92	0.12
	II	0.19	0.39
	III	0.65	1.66
(b) Sulphar containing amino acids :			
Cystine	I	2.57	8.03
	II	2.81	1.88
	III	1.96	4.39
Methionine	I	1.13	0.25
	II	0.27	0.50
	III	0.30	1.05
(c) Other amino acids :			
Arginine	I	1.4	0.30
+	II	1.3	0.86
histidine	III	0.58	2.37
Aspartic acid	I	3.40	0.99
	II	1.41	2.42
	III	0.77	5.08
Glutamic acid	I	1.60	0.41
	II	0.74	1.15
	III	0.39	2.45
Leucine	I	0.42	0.11
	II	0.076	0.24
	III	0.11	0.30

Each value is means of two observations.

I Pre - infectional stage II Early-infectional stage

III Late-infectional stage.

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