CAJANOL ACCUMULATION IN STEM CALLUS TISSUE OF *CAJANUS CAJAN* INFILTRATED WITH SPORE SUSPENSION OF *BOTRYTIS CINEREA*

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At least 100 plant species representing 21 families have been shown to accumulate phytoalexins in response to microbial infection[1-3]. Etiolated stems of pigeonpea challenged with *Helminthosporium carbonum* produce four isoflavone phytoalexins, formononetin, genistein, and cajanin, and an isoflavanone, cajanol, which was also reported from the roots and sliced non-sterile seeds[4-6]. Accumulation of cajanol in stem callus tissue of *Cajanus cajan* infiltrated with spore suspension of *Botrytis cinerea*, a non-pathogen of pigeonpea, is reported.

For phytoalexin elicitation, callus tissues were induced from the stem (*C. cajan* cv Prabhat) by standard procedures on Linsmaier-Skoog[7] agar medium containing 3 mg indole acetic acid, 3 mg naphthalene acetic acid and 0.1 kg kinetin per litre. Callus tissues were subcultured every 4 weeks on 25 ml medium for 6 times before use.

B. cinerea was grown on potato sucrose agar medium for 8–10 days at 25°C. Inoculums were prepared by suspending spores in sterile distilled water (5×10^5 spores/ml). Well-grown callus tissues (10 g fresh wt/flask) were infiltrated with 1 ml/flask of the spore suspension. The controls were treated with sterile distilled water.

The infected callus exhibited a necrotic response within 2 days of infiltration. The callus tissues, incubated for 2 days after infiltration of *B. cinerea* were harvested and freeze-dried. The dried material (4 g dry wt) was extracted with ethyl acetate. The extract was evaporated to dryness to give 60.5 mg of yellow residue. The residue was dissolved in a mixture of acetonitrile and distilled water (1:1, v/v) and analysed by reversed phase high performance liquid chromatography[4]. Antifungal activity of HPLC fractions was assayed by standard *Cladosporium* bioassay[2]. Active fractions were pooled, dried *in vacuo* and subjected to UV, MS and NMR spectral analysis[4-6].

The antifungal compound was obtained as white needles from aqueous methanol with m.p. 149°C. On MS analysis it gave m/z, M^+316.0421 ($C_{17}H_{16}O_6$ requires 316.0524) and there were fragments at m/z 167 (M^+-149), 150 (M^+-166), 135 (M^+-181) and 107 (M^+-209). The UV spectrum in methanol had absorption maxima at λ 229 nm (log ε 4.52), 287 (4.48) and a shoulder at 335 nm (3.67). The ^{1}H-NMR spectrum (figure 1) in deuterated chloroform ($CDCl_3$) showed a singlet at δ 12.236 (IH) characteristic of the phenolic-OH. Proton singlets at δ 3.764 (3H) confirmed the presence of two methoxyl ($-OCH_3$) groups in the molecule. The ^{1}H-NMR also showed doublets at δ 6.933 (IH, J = 8.3 Hz), 6.433 (IH, J = 2.3 Hz), 6.081 (IH, J = 2.3 Hz), 6.008 (IH, J = 2.3 Hz) and double doublets at δ 6.370 (IH, J = 4.60, 10.75 Hz), 4.530 (IH, J = 10.75, 12.05 Hz), 4.425 (IH, J = 4.60, 10.75 Hz), 4.295 (IH, J = 4.60, 12.05 Hz). Irradiation of the doublet at δ 6.933 reduced the double doublets at δ 6.370 to a doublet with coupling constant J = 2.3 Hz. Nuclear Overhauser enhancement (NOE) experiments showed that irradiation of the methoxyl protons at δ 3.764 and 3.827 enhanced proton signals at δ 6.433, 6.008 and 6.081. All the foregoing data are well in accord with an already reported isoflavanone, cajanol from etiolated stem of pigeonpea challenged with *H. carbonum* and nonsterile sliced seeds[4,6].

It is obvious from the results that *B. cinerea* induces phytoalexin synthesis in stem tissues either by secreting some elicitor or by dislocating the endogeneous elicitor from the stem tissues since no cajanol was detected in the stem tissues treated with sterile distilled water. Phytoalexins accumulate in plants when challenged with certain avirulent pathogens or treatment with components isolated from both avirulent and virulent pathogens[3,8].

Accumulation of cajanol in the stem tissues is further strengthened by a similar report on accumu-

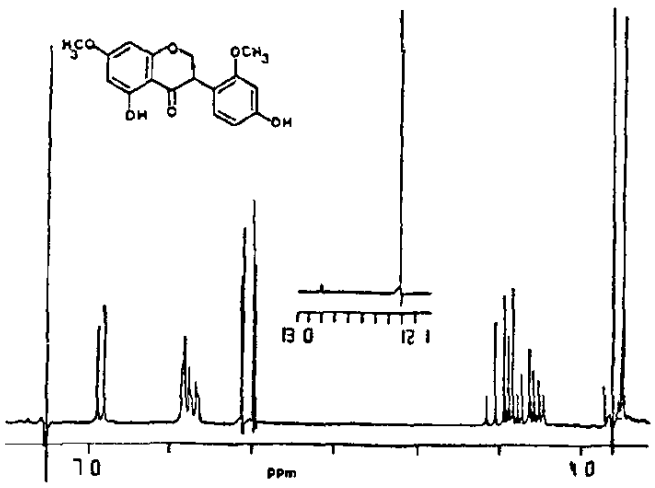

Figure 1. ^{1}H-NMR spectrum of cajanol in $CDCl_3$ (deuterated chloroform).

lation of cajanol, cajanin and other related phytoalexins in etiolated stem of pigeonpea challenged with *H. carbonum*[5,6].

Phytoalexin surveys in the Papillionoideae, a subfamily of the Leguminoseae suggest that cajanol is of exceptionally rare occurrence. Indeed, apart from *C. cajan*, this isoflavanone has only been obtained from the hypocotyls of *Stizolobium deeringianum* (tribe Erythrineae) challenged with *H. carbonum*. There it co-occurs with various other isoflavanoids including genistein, 2 hydroxygenistein, dalbergioidin and a third isoflavanone provisionally identified as isoferreirin, (5,7,4'-trihydroxy-2'-methoxy isoflavanone). In *S. deeringianum*, the latter compound might represent the immediate biosynthetic precursor of cajanol[6].

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CHLOROPHYLL CONTENT IN RICE AS INFLUENCED BY THE ROOT-KNOT NEMATODE, *MELOIDOGYNE GRAMINICOLA* INFECTION

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THE root-knot nematode, *Meloidogyne graminicola* is one of the limiting factors in rice production. The symptoms of infection and root damage are expressed as stunting and chlorosis in plants[1]. Mohanty *et al*[2] observed a reduction (20–39.5%) in chlorophyll *a* and *b* fractions due to infection by this nematode in a susceptible rice variety. Such reductions were also observed in the root, root-lesion and lance nematode infections in rice[3,4]. In the present studies, changes in chlorophyll *a* and *b* fractions occurring in the resistant and susceptible rice varieties, following infection by the root-knot nematode, *M. graminicola* have been estimated.

Five rice varieties [MW 10, IR 36, Udaya (resistant), Annapurna and Parijat (susceptible)] were selected and the seeds of each variety were sown in 30 polypots containing 200 g steam sterilized soil at the rate of one seed/pot. When the seedlings were five days old, 15 seedlings from each variety were inoculated with 100 infective juveniles/pot. Thirty days after inoculation, leaves from both inoculated and uninoculated plants of each variety were clipped separately and soaked in 80% acetone, ground and extracted. The extract was centrifuged at 12,000 *g* for 5 min to allow the debris to settle. The supernatant was decanted and the volume made up to 25 ml with the addition of 80% acetone. The absorbance of the extract was recorded on spectrophotometer (Spectronic 20) and the chlorophyll was expressed[5] as mg/g. (i) Chlorophyll $a = 0.0127 \times OD663 - 0.00269 \times OD645$, and (ii) Chlorophyll $b = 0.0229 \times OD645 - 0.00468 \times OD663$, where OD (optical density) was estimated by absorbance by chlorophyll.

The *a* fraction of chlorophyll in the uninoculated plants ranged from 1.86 mg/g in Udaya to 2.50 mg/g in Parijat and in the inoculated plants, from 1.55 mg/g in MW 10 to 2.67 mg/g in Udaya (table 1). There was an increase by 43.5% in *a* fraction of chlorophyll due to nematode infestation in Udaya while in the other varieties there was a decrease from 2.2 in IR 36 to 20.8% in Annapurna.

The *b* fraction of chlorophyll in uninoculated plants ranged from 0.74 mg/g in Udaya to 0.88 mg/g in IR 36 and Parijat (table 1). In the inoculated plants, the *b* fraction ranged from 0.59 mg/g in MW 10 to 0.92 mg/g in Udaya. As with the *a* fraction, the *b* fraction of chlorophyll increased in Udaya by 24.3% while in the other varieties there was a decrease from 4.5% in Parijat to 28.6% in Annapurna.

These results confirmed the earlier reports on the reduction in chlorophyll *a* and *b* fractions (by 20 to 39.5%) due to this nematode infection in susceptible rice varieties[2]. Such reductions were also reported