Isolation of isoflavonoid phytoalexins from seeds of *Phaseolus vulgaris* L.

S.S. Gnanamanickam[1]

Centre for Advanced Study in Botany, University of Madras, Madras 600005 (India), 2 June 1978

Summary. Bean seeds challenged with *Rhizoctonia solani* or exposed to natural microflora yielded the isoflavonoid phytoalexins: phaseollin, phaseollidin, phaseollinisoflavan, coumestrol and kievitone. The data suggest that seeds may have the major host-defence factors of a plant.

Most of these ground parts of bean, *Phaseolus vulgaris* L., produce the isoflavonoid compounds, phaseollin, phaseollidin, phaseollinisoflavan, coumestrol and kievitone in response to fungal, viral and bacterial invasions[2-5]. These compounds possess antifungal property, and our previous study[4] has shown that these isoflavonoids also have antibacterial properties. Keen[6] reported a seed technique by which he isolated phytoalexins from germinated seeds of plants contaminated by the naturally occurring microflora. With this technique, however, green bean yielded only phaseollin. I used a modified seed technique, where bean seeds challenged with *R. solani* or other microflora yielded large amounts of the isoflavonoid phytoalexins. Commercially purchased 'Red Kidney' bean seeds were soaked in water for 1-2 h and placed in stainless steel trays lined with moist filter paper. A thick mycelial suspension of *R. solani* (7-day-old culture) was added at the rate of 100 ml/100 g seed and mixed; otherwise the seeds were allowed to be colonized by the naturally present microflora. The seeds were completely infested and turned yellowish brown when incubated in dark for 7 days. Control seeds (heat killed and incubated likewise) did not show any change in colour. The seeds were immersed in ethanol (95%), ground in a blender at the maximum speed for 1 min, and filtered through Whatman No. 1 filter paper. The ethanol extract was concentrated to about 50 ml using a rotary vacuum evaporator and extracted in 5 vol. of ethyl acetate[4]. The crude extract was chromatographed on TLC plates. The fluorescing bands were removed and purified through further TLC in 2 more solvent systems (table 1) before their purity was assessed by spectral method[4].

The incubated living (but not dead) seeds, colonized either by *R. solani* or by the natural microbial populations, produced 5 fluorescing bands (table 1). These compounds were identified as phaseollin, phaseollidin, phaseollinisoflavan, coumestrol and kievitone by co-chromatography.

These phytoalexins are inhibitory to the growth of *Pseudomonas phaseolicola*[4,7] (causal agent of bean halo blight disease) and *R. solani*[2], and therefore appear to be the host-defence mechanisms in *P. vulgaris*. The data presented here indicate that infested bean seeds are capable of producing major phytoalexins of the plant and when sufficient quantities of seeds are used, yield fairly large amounts of these isoflavonoids (table 2).

This technique is a valuable tool, especially if space for plant growth is limited. The fact these isoflavonoids like coumestrol and phaseollin are toxic to animals[8,9], contamination of seeds by microorganisms involves risk to consumers.

Table 1. R_f-values and different properties used in the identification of the phytoalexins produced by bean seeds

Solvent	Phaseollin	Phaseollidin	Phaseollin-isoflavan	Coumestrol	Kievitone
Chloroform + methanol (25+1)	0.67	0.45	0.38	0.23	0.10
Chloroform + ethanol (100+3)	0.50	0.37	0.27	0.12	0.06
Benzene + ethyl acetate + methanol (25+8+4)	0.85	Not tested	0.75	Not tested	0.55
UV-absorption spectra (nm)					
λ_{max} EtOH	280 280-286 sh 312-315	281 287	280 284 sh 310 sh	243 307, 343	290 327 sh
λ_{max} NaOH	281, 290	290	290, 341	250, 280 310, 385	337

Table 2. Yield of isoflavonoids by the seed technique (µg/g seed material)

Treatment	Phaseollin	Phaseollidin	Phaseollin-isoflavan	Coumestrol	Kievitone
Seed × *R. solani*	78	14	110	305	146
Seed × natural microflora	51	Not detected	102	160	125

1 The author thanks Dr Suresh S. Patil of University of Hawaii for his guidance and provision of laboratory facilities. Gift of phytoalexin samples by Dr S. Pueppke and Dr N.T. Keen is gratefully acknowledged. East-West Center, Honolulu, Hawaii, USA, is thanked for the financial assistance.

2 D.A. Smith, H.D. Van Etten and D.F. Bateman, Physiol. Pl. Path. *5*, 51 (1975).
3 R.S. Burden, J.A. Bailey and G.W. Dawson, Tetrahedron Lett. *41*, 4175 (1972).
4 S.S. Gnanamanickam and S.S. Patil, Physiol. Pl. Path. *10*, 159 (1977).
5 F.M. Lyon and R.K.S. Wood, Physiol. Pl. Path. *6*, 117 (1975).
6 N.T. Keen, Phytopathology *62*, 795 (1972).
7 S.S. Patil and S.S. Gnanamanickam, Nature *259*, 486 (1976).
8 H.D. Van Etten, Phytopathology *62*, 795 (1972).
9 E.M. Bickoff, A.N. Booth, R.L. Lyman, A.L. Livingston, C.R. Thomspon and G.D. Kohler, J. agric. Fd Chem. *6*, 536 (1958).