

Toxigenicity of *Fusarium* species causing wilt of chickpea*

by Subramaniam GOPALAKRISHNAN**

The toxicity of a devastating fungal pathogen, *Fusarium oxysporum* f. sp. *ciceri* (FOC) of chickpea (*Cicer arietinum*) the third most important legume crop in the world was studied. Five isolates were grown in liquid culture and the toxins were purified from the culture filtrates by solvent partitioning, solid phase extraction (SPE), thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Stages in purification were monitored by a live/dead assay employing chickpea cells. Time course experiments showed that for the isolate that produced the highest toxin titres (designated FOC 5) an incubation period of 12 days at 20°C was optimal. Some cultures of this isolate turned red during incubation and these were at least ten times more toxic than colourless cultures.

Shaking of culture filtrates of FOC 5 with ethyl acetate resulted in about half the toxic activity (50–55%) partitioning into the organic phase and 25–30% remaining in the aqueous phase. The activity of the aqueous phase was lost on freeze-drying suggesting a volatile compound. When the ethyl acetate phase was dissolved in aqueous acetonitrile and applied to C18 SPE cartridges, about 9% was not adsorbed and 35% could be eluted with methanol. Greater affinity was shown for Cyano SPE cartridges with 6% not adsorbed and 45% recoverable by elution in acetonitrile. Attempts at purification of the toxin(s) of adsorbed and non-adsorbed fractions from these reversed phase cartridges by HPLC did not yield pure products.

Recovery of activity of the ethyl acetate phase from flash chromatography on silica gel was >80%. However, HPLC demonstrated that several compounds were present.

Separation of components of the ethyl acetate phase or concentrated culture filtrates by TLC on silica-gel rather than using SPE, flash or reversed phase HPLC was more successful. Red spots corresponding to the active compound were scraped from TLC plates and eluted in acetonitrile. HPLC of the eluents on a cyano column with 10% acetonitrile as mobile phase demonstrated a single homogeneous peak with absorption maxima of 224 and 281 nm. The purified fraction was identified as 8-O-Methyl-fusarubin by NMR and mass spectral studies.

The four other isolates, identified by the International Crops Research Institute for the Semi-Arid Tropics as *F. oxysporum* f. sp. *ciceri* did not produce the red, toxic compound, throwing doubt on the correct identification of the isolates. When the sequences of ribosomal DNA of all five isolates was determined, the isolate that produced the red, toxic compound was identified by a BLAST search as *Fusarium acutatum*, three of the other isolates as *F. oxysporum* f. sp. *vasinfectum* and one as *F. oxysporum* f. sp. *vanillae*. ■

*PhD thesis 2004, University College London, UK.

**Email: krishnang@hotmail.com