

A multiplex PCR for early detection of trichothecene and fumonisin producing fusarium species collected from India

M V Ramana, H S Murali and H V Batra

Defence Food Research Laboratory, India

Fusarium, which is common soil phytopathogenic fungi, to produce a number of different mycotoxins of the class of trichothecenes and fumonisins in stored food grains. Trichothecenes are a group of more than 40 compounds found commonly in grains. Trichothecenes are classified as macrocyclic or nonmacrocyclic, depending on the presence or absence of a macrocyclic ester. The most toxic nonmacrocyclic compounds are T-2toxin, Deoxynivalenol and fumonisins. Most of these are biological weapon toxins. These can be absorbed through intact skin causing systemic toxicity. Clinical symptoms may be present within seconds of exposure. Trichothecenes inhibits production of proteins and nucleic acids and also stimulates lipid peroxidation. Once the toxin crosses the plasma membrane barrier, interacts with a number of targets, including ribosomes and mitochondria and then inhibits electron transport activity contributing to cellular cytotoxicity.

In the present study, an attempt was made to develop a multiplex PCR for the rapid detection of the genes involved in toxin metabolic pathway viz., *tri5*, *tri6* and

fum1, *fum13* for trichothecene and fumonisin producing organisms respectively and *rDNA* gene for the specific detection of *Fusarium* genus as a internal control. The primers were designed for the individual mentioned genes to obtain adequate spatial product amplification for a clear resolution on agarose gel electrophoresis. The mPCR assay was standardized with the DNA extracted from pure cultures of toxigenic *Fusarium* strains obtained from culture collection centers, India. A total of 100 food samples comprising Paddy, corn and finger millet collected from southern parts of India, were analyzed by seed blot fungal isolation method, from which 85 *Fusarium* strains could be isolated. From among the isolated strains, 15 were showed positive signal for fumonisin metabolic pathway genes, 35 were trichothecene positive producing strains and rest stayed as negative for multiplex PCR assay. The PCR positive strains were cross checked by the HPTLC chemical analysis (CAMAG. Anchrome) for the presence of the toxin and this provided identical results.