

structure with plants arranged like a thatched house allowed the rainwater to run-off quickly, which otherwise would have accumulated on the pods, and such situation prevailed during the drying in the second set in 1998. Further, the drying method does not call for any special skill in making the drying structures, and the cost involved in raising the structures is also low (Rs 200 or US\$ 4.2 approx. for drying of 100 kg pods), and the same poles can be used year after year. The technology may be a boon to the small farmers, particularly those cultivating summer groundnut. Due to improper drying methodology and poor storage conditions the farmers are deprived of a chance to store their own quality produce for sowing in the next summer season. The NRCG method showed its superiority in terms of retention of seed viability and seed quality, over the other three drying methods, more specifically when pods experienced rain while drying in the field.

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## References

**Abdul-Baki, A.A., and Anderson, J.D. 1973.** Relationship between decarboxylation of glutamic acid and vigour in soybean seed. *Crop Science* 13:222-226.

**DOR (Directorate of Oilseeds Research). 1983.** Simple and efficient post-harvest technique for increasing seed viability of the rabi/summer groundnut. *Directorate of Oilseeds Research Newsletter* 2:1-5.

**ISTA (International Seed Testing Association). 1993.** International rules for seed testing. *Seed Science and Technology* 13:322-441.

**Nautiyal, P.C., Ravindra, V., and Joshi, Y.C. 1990.** Varietal and seasonal variation in Spanish groundnut. *Indian Journal of Agricultural Sciences* 60:143-145.

**Nautiyal, P.C., and Zala, P.V. 1991.** Effect of drying methods on seed viability and seedling vigour in Spanish groundnut. *Seed Science and Technology* 19:451-459.

## Biotechnology

### Molecular Diversity in *Trichoderma* Isolates with Potential for Biocontrol of *Aspergillus flavus* Infection in Groundnut

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The species of the genus *Trichoderma* are known to be potential biocontrol agents for several soilborne plant pathogens (Papavizas 1985). During the past two years we have identified several *Trichoderma* isolates that have shown strong antagonism to *Aspergillus flavus* infecting groundnut (*Arachis hypogaea*) and some of these isolates have been used as potential biocontrol agents in greenhouse and field experiments (Desai et al. 2000, Anjaiah et al., in press). One of the mechanisms of biocontrol of plant pathogens with *Trichoderma* is known as mycoparasitism where *Trichoderma* recognizes and attaches to the pathogenic fungus and begins to excrete extracellular hydrolytic enzymes, such as chitinases,  $\beta$ -1,3-glucanases, proteases, and lipases. These enzymes act on the cell walls of the fungi and thus cause lysis. *Trichoderma* spp are difficult to distinguish morphologically (Bissett 1991), and it is not yet well known whether the ability for biocontrol is a general property of the genus *Trichoderma* or a specific attribute of some species only. The molecular diversity among the species will help in characterizing the isolates for different modes of biocontrol ability and their deployment for effective control of plant pathogens. Using random amplified polymorphic DNA (RAPD) fingerprinting we studied genetic diversity in 17 *Trichoderma* isolates belonging to different species used in biocontrol of *A. flavus* infection in groundnut. The in vitro antagonistic characteristics of these isolates were reported earlier (Desai et al. 2000).

Genomic DNA was isolated from 17 selected isolates of *Trichoderma* species belonging to five species aggregates, *viride*, *hamatum*, *harzianum*, *auroviride*, and *longibrachiatum* (Desai et al. 2000) using the method described by Arisan-Atac et al. (1995). RAPD fingerprinting analysis was performed using three primers (GAGGTGGNGGNTCT, [GACA]<sub>4</sub>, and [GAG]<sub>5</sub>). All three primers led to the amplification of 6-10 fragments. A dendrogram (Fig. 1) based on average linkage cluster analysis, using Jaccard test was prepared using the

combined data from all three primers for the 17 isolates. The results indicated that the 17 *Trichoderma* isolates could be broadly classified into two groups. All *T. harzianum* isolates were classified into group I, and others in group II. In group I, there were minor variations, which could be related to the source of origin from different climatic zones. There were 3 subgroups in group II. Subgroup I: *T.auroviride* (T 18 Udaipur), which was different from *T.hamatum* and *T.viride*. Subgroup II: *T. hamatum* (T 5 and T 6 NRCG), *Trichoderma* spp (T 10 NRCG), *T.viride* (T 25 ICRISAT, T 17 Udaipur, and T 27 NARDI), and *T. longihrachiatum* (T 16 Udaipur). Subgroup III: *T. viride* (T 20 and T 22 Akola). It was interesting to note that all *T.hamatum* isolates were similar to *T.viride* isolates; however, *T.auroviride* (T 18) was different from others.

Molecular analysis of genomic DNA from these *Trichoderma* isolates also revealed the presence of chitinase gene in polymerase chain reaction (PCR) using primers designed in the conserved regions of the gene that often contributed to the biocontrol ability (data not

shown). Some of these *Trichoderma* isolates were shown to be effective not only for reduction of seed and peg infection by *A. flavus* but also reduced *A. flavus* population in the rhizosphere of groundnut (data not shown). Selected *Trichoderma* isolates from this study are being used in field experiments to evaluate their biocontrol potential against aflatoxin contamination in groundnut. Strain typing by RAPD fingerprinting offers a quick and convenient method for screening a large number of isolates for their biocontrol potential against *A. flavus*.

## References

Anjaiah, V., Thakur, R.P., Rao, V.P., Sharma, K.K., Cornells, P., and Koedam, N. (In press.) A biological control approach making use of rhizobacteria and soil fungi for soil-borne post harvest infection of *Aspergillus flavus* in groundnut. In Proceedings of biological control of fungal and bacterial plant pathogens - Biocontrol 2000. Spain: IOBC/WPRS-EFPP Working Group, University of Sevilla.

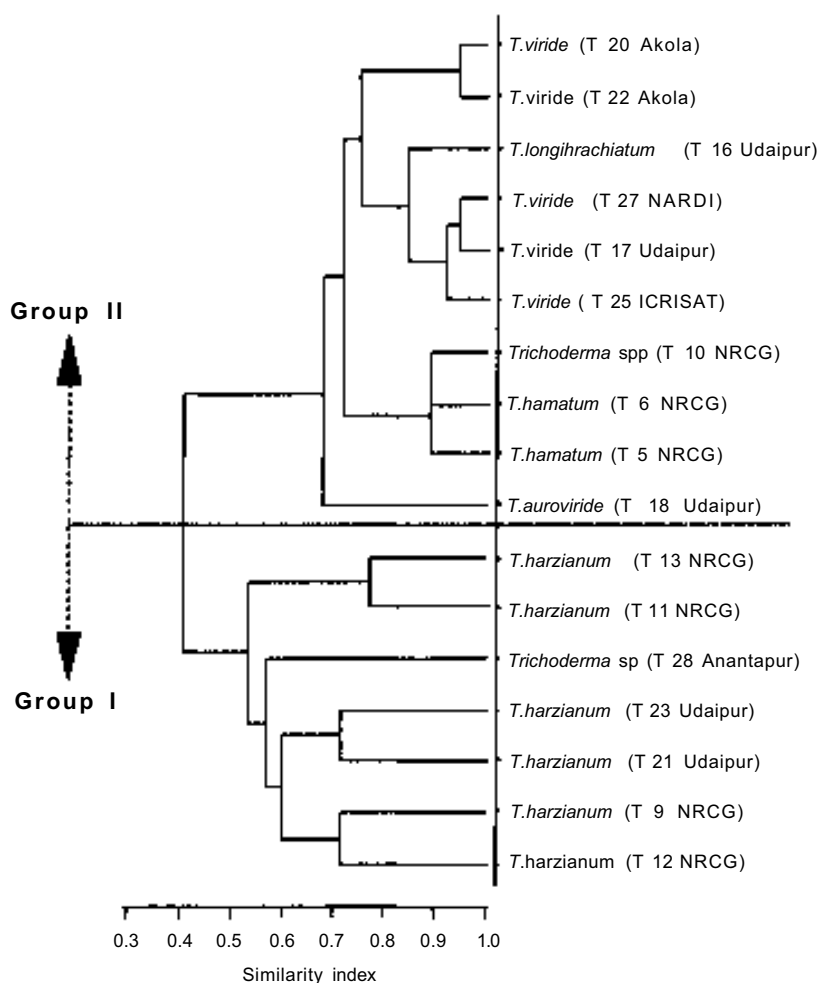


Figure 1. Dendrogram showing relative genetic similarities between strains and species of *Trichoderma*, calculated from a RAPD fingerprinting-[0/I]-data matrix including all three primers by average linkage cluster analysis using GENSTAT software.

**Arisan-Atac, I., Heidenreich, E., and Kubicek, C.P. 1995.** Randomly amplified polymorphic DNA fingerprinting identifies subgroups of *Trichoderma viride* and other *Trichoderma* sp. capable of chestnut blight control. FEMS Microbiology Letters 126:249-256.

**Bissett, J. 1991.** A revision of the genus *Trichoderma*. II. Intrageneric classification. Canadian Journal of Botany 69:2357-2372.

**Desai, S., Thakur, R.P., Rao, V.P., and Anjaiah, V. 2000.** Characterization of isolates of *Trichoderma* for biocontrol potential against *Aspergillus flavus* infection in groundnut. International *Arachis* Newsletter 20:57-59.

**Papavizas, C.G. 1985.** *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-54.

## Pathology

### Control of Foliar Diseases of Groundnut Using Inorganic and Metal Salts

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Late leaf spot (LLS) caused by *Phaeoisariopsis personata* and rust caused by *Puccinia arachidis* are the two major foliar diseases of groundnut (*Arachis hypogaea*) which usually occur together and significantly reduce the crop yield (Subrahmanyam et al. 1995). Desirable levels of host plant resistance against these two diseases are not available in cultivars commonly grown by farmers. Fungicidal control of LLS and rust is expensive and requires alternative strategies for management of these destructive diseases. Several inorganic and metal salts possess anti-fungal activity and control pre-harvest and postharvest diseases caused by pathogenic fungi (Reuveni et al. 1995, Olivier et al. 1998). These chemicals were also proved to elicit the plant defense mechanisms against invasion of pathogenic fungi (Gamil 1995). We studied the effects of 33 inorganic and metal salts on the germination of conidia of *P.personata* and urediniospores of *P. arachidis* under in vitro conditions and the efficacy of

selected salts to reduce the incidence of LLS and rust in detached leaf bioassay.

### In vitro antimicrobial assay

Inorganic and metal salts at a final concentration of  $10^{-2}$  M and  $10^{-3}$  M were evaluated for their inhibitory effects on germination of *P.personata* conidia and *P.arachidis* urediniospores. Conidia and urediniospores were taken separately onto cavity slides and mixed with respective salt solutions. The final concentration of conidia and urediniospores was  $30,000 \text{ ml}^{-1}$ . Conidia and urediniospores suspended in sterile double distilled water were treated as controls. Three replications were maintained for each treatment and the experiment was repeated once. The slides were incubated in a humid chamber at  $23 \pm 1^\circ\text{C}$  in dark. Conidia and urediniospores were observed for germination at 24 h and 8 h after incubation, respectively. In each replication, one hundred spores were observed randomly for germination and the percentage inhibition with respect to control was calculated separately for each treatment. The differences between the percentage inhibition values in two sets of experiments were not significant and hence were pooled and subjected to analysis of variance.

Of the 33 salts tested, chromium trioxide, cupric sulfate, ferric chloride, nickel chloride, and zinc chloride at  $10^{-3}$  M concentration had significant inhibitory activity ( $P=0.01$ ) against both *P.personata* and *P.arachidis*. At the same concentration sodium carbonate and sodium molybdate were effective against *P.personata* alone, and ammonium dihydrogen orthophosphate and cobalt chloride were effective against *P.arachidis* alone. Ammonium fluoride, ammonium sulfate, ammonium tartrate, borax, calcium chloride, calcium sulfate, magnesium chloride, magnesium nitrate, sodium carbonate, sodium citrate, sodium fluoride, and sodium phosphate were inhibitory to both the test fungi, only at  $10^{-2}$  M concentration (Table 1).

### Detached leaf bioassay

Chromium trioxide, cupric sulfate, ferric chloride, nickel chloride, and zinc chloride at  $10^{-3}$  M concentration were tested to control the development of LLS and rust on detached groundnut leaves of the susceptible genotype TMV 2 (Subrahmanyam et al. 1983). Cultures of *P.personata* and *P.arachidis* were maintained on detached leaves of genotype TMV 2.

Conidia and urediniospores suspended in sterile double distilled water at a concentration of  $20,000 \text{ ml}^{-1}$