

## CONTROL OF MYCOFLORA ASSOCIATED WITH PIGEONPEA SEEDS

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### ABSTRACT

Associated with the seeds of four field-grown pigeonpea cultivars, *Alternaria* sp., *Aspergillus flavus*, *A. niger*, *Fusarium* spp., and *Rhizoctonia bataticola* were predominant. Cultivars NP-69 (late) and Prabhat (extra early) harbored more fungi than did T-21 (early) or ICP-1 (mid). Genotypic differences rather than weather during pod maturity, or different storage periods seem to influence the intensity of seed-borne mycoflora. Greater reduction in seed germination was observed in cultivars NP-69 and Prabhat which had higher frequency of mycoflora, especially *Aspergillus* spp.

Seed treatment with Benlate T at 3 g/kg provided complete control of all seed-borne fungi with no adverse effect on germination. This treatment can be recommended for controlling seed-borne mycoflora to ensure safe international exchange of seed.

Pigeonpea (*Cajanus cajan* (L.) Millsp.), an important legume of India and few countries in central America and eastern Africa, is prone to damage by several fungal diseases through different stages of growth (Nene, 1978). The role of seed-borne mycoflora in adversely affecting the stand and health of the crop has been fairly well understood in many legumes including pigeonpea (Khare, 1974; Mishra and Kanaujia, 1972; and Suryanarayana, 1976). Wilt causing *Fusarium* sp. could be borne on pigeonpea seed (Shukla and Bhargava, 1976). Preliminary tests on some pigeonpea cultivars grown at ICRISAT revealed the presence of several seed-borne fungi. Weather during the seed set and development, and the duration of seed storage vary considerably as cultivars differ widely in maturity. Thus differences in flowering and podding time could influence the range and intensity of mycoflora carried by the seed. Therefore studies were undertaken to identify the mycoflora associated with the seed of four cultivars of different maturity groups and to find an efficient fungicide to control the mycoflora. This information is important in view of the large scale seed exchange between ICRISAT and its cooperators all over the world.

## MATERIALS AND METHODS

Four cultivars—viz., Prabhat, T-21, ICP-1 and NP-69—were chosen to represent extra early, early, mid and late maturity groups, respectively. Sown in June/July, 1976 they were ready for harvest between November 1976 and April 1977 depending on time to maturity (Table 1). Dry pods were harvested, hand-thrashed and the seeds obtained were stored in air-tight plastic jars (10 x 7 x 6 cm) in the laboratory. Just prior to the next sowing season (June 1977), samples of 400 seeds were drawn from each cultivar. These samples were subjected to the standard "blotter test" as recommended by International Seed Testing Association (1966) to assess the range and intensity of the mycoflora present. Nonsurface-sterilized seeds were incubated on moistened sterilized blotter paper discs (10 seeds/disc) placed inside sterilized plastic petri plates for 7 days at 25°C under 12-hr regime of alternate near UV and darkness. After a week of incubation, the seeds were examined for the presence of mycoflora. Germination of seeds was also recorded.

Data on weather prevailing during the month-long period preceding the harvest were obtained from the Agroclimatology Unit of ICRISAT Center.

Seed of cultivar ICP-1 was used in seed treatment studies. Based on an initial screening of nine fungicides, three chosen for further evaluation. They were :

- 1) methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate (benomyl 50%) (Benlate)
- 2) bis (dimethylthio-carbamoyl) disulfide (thiram 75%) (Thiride)
- 3) methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate (benomyl 30%) + bis (dimethylthio-carbamoyl) disulfide (thiram 30%) (benomyl-thiram) (Benlate T).

Seeds were dry dressed at two rates—2 and 3 g fungicide/kg of seed. For each treatment 400 seeds were used and an untreated lot consisting of 400 seeds was maintained as check. After treatment, seeds were plated on potato-dextrose-agar (10 seeds/petri plate) and incubated for 7 days at 30°C. The plates were then examined for mycoflora and seed germination was also recorded.

## RESULTS AND DISCUSSION

Maximum ambient temperature during the month preceding harvest of early and mid maturity cultivars (T-21 and ICP-1) was relatively low compared to extra early (Prabhat) and late (NP-69) cultivars (Table 1). Minimum ambient temperature appeared to be particularly low for ICP-1 while it exceeded 20°C for NP-69. Cultivars Prabhat and T-21 received 21 and 29.7 mm rainfall during the month preceding harvest, whereas NP-69 and ICP-1 received either scanty (1.8 mm) or no rainfall. Relative humidity was also distinctly higher for cvs. T-21 and Prabhat than for the other two. Relative humidity was least for NP-69.

Table 1. Maturity characteristics of pigeonpea cultivars and the weather data of one month preceding harvest

| Cultivar<br>(maturity<br>group) | Age in days at        |                      | Date of               |                    | Weather Data of one month preceding harvest <sup>a</sup> |                     |                    |                  |      |
|---------------------------------|-----------------------|----------------------|-----------------------|--------------------|--|---------------------|--------------------|------------------|------|
|                                 | 50%<br>flow-<br>ering | 75%<br>matu-<br>rity | Plant-<br>ing<br>1976 | Harvest<br>1976/77 | Temperature<br>(° C)                                     | R.H. b (%)          | Morning<br>Evening | Rainfall<br>(mm) |      |
| Prabhath<br>(Extra<br>early)    | 69                    | 120                  | July 5                | Nov. 15            | 30.4<br>(22.5-33.4)                                      | 19.1<br>(15.2-23.5) | 82<br>(51-97)      | 42<br>(23-95)    | 21.0 |
| T-21<br>(Early)                 | 85                    | 132                  | July 5                | Dec. 1             | 29.0<br>(22.5-31.5)                                      | 19.2<br>(14.7-22.0) | 92<br>(80-97)      | 52<br>(26-95)    | 29.7 |
| ICP-1<br>(mid)                  | 126                   | 196                  | June 28               | Jan. 20            | 29.3<br>(26.5-31.0)                                      | 13.5<br>(10.0-16.0) | 77<br>(58-91)      | 24<br>(13-39)    | 0.0  |
| NP-69<br>(Late)                 | 176                   | 275                  | June 29               | Apr. 10            | 36.8<br>(33.0-39.8)                                      | 21.7<br>(18.5-25.1) | 64<br>(35-85)      | 24<br>(15-50)    | 1.8  |

<sup>a</sup> = Values in parantheses indicate the range.

<sup>b</sup> = R.H. - Relative humidity

Table 2. Percent seed germination of mycoflora associated with seeds of four pigeonpea cultivars before these were sown in field <sup>a</sup>

| Cultivar | Percent germination | Percent seed with mycoflora <sup>c</sup> | Frequency of mycoflora (%) <sup>d</sup> |                       |                          |                           | Other fungi |                               |
|----------|---------------------|--|---|-----------------------|--------------------------|---------------------------|-------------|-------------------------------|
|          |                     |  | <i>Fusarium</i> spp.                    | <i>Alternaria</i> sp. | <i>Aspergillus niger</i> | <i>Aspergillus flavus</i> |             | <i>Rhizoctonia bataticola</i> |
| Prabhat  | 68.2                | 69.3                                     | 0.0                                     | 0.7                   | 34.7                     | 15.7                      | 16.7        | 1.2                           |
| T-21     | 88.5                | 37.8                                     | 7.5                                     | 5.5                   | 0.0                      | 0.0                       | 22.7        | 1.9                           |
| ICP-1    | 91.2                | 42.8                                     | 0.5                                     | 2.5                   | 3.7                      | 0.2                       | 37.7        | 0.0                           |
| NP-69    | 46.5                | 92.7                                     | 3.0                                     | 2.2                   | 58.2                     | 8.7                       | 18.7        | 1.7                           |

<sup>a</sup> Cultivars Prabhat, T-21, ICP-1 and NP-69 were stored in laboratory for 210, 194, 144 and 64 days, respectively before sowing in field.

<sup>b</sup> For each cultivar 400 seeds were used.

<sup>c</sup> Includes the wilt causing *F. udum*.

<sup>d</sup> Includes *Rhizopus* sp., *Penicillium* sp., and *Sclerotium rolfsii*.

From the data presented in Table 2, it is evident that 92.7% of the seeds of cv. NP-69, and 69.3% of the seeds of cv. Prabhat harboured fungi. In contrast, only about 40% seeds of the other two cultivars were associated with mycoflora. *Aspergillus niger* was the dominant fungus in seeds of cvs. Prabhat and NP-69 (34.7% and 58.2%), while *Rhizoctonia bataticola* was more common in seeds of cvs. T-21 and ICP-1 (22.7% and 37.7%, respectively). *Aspergillus flavus* was negligible in cvs. T-21 and ICP-1, but in cvs. Prabhat and NP-69, it was 15.7% and 8.7%, respectively. The other fungi that occurred at lower frequencies (less than 10%) included species of *Fusarium* (including the wilt fungus *F. udum*), *Alternaria*, *Rhizopus*, *Penicillium*, and *Sclerotium rolfsii*. The percent seed germination was higher in cvs. T-21 and ICP-1 (88.5 and 91.2%) than in Prabhat and NP-69 (68.2 and 46.5%).

Control of *Fusarium* spp. was accomplished at the lower rate (2 g/kg) with all the three fungicides (Table 3). Benlate seemed ineffective in controlling other fungi at either rate. Though Thiride (2 g and 3 g/kg) and Benlate T (2 g/kg) reduced seed-borne fungi to less than 1%, complete control was achieved only with Benlate T at the higher rate (3 g/kg). Germination of seed was not reduced at this higher rate.

Relatively higher frequency of seed-borne mycoflora was observed in the late cv. NP-69 and the extra early cv. Prabhat. The month preceding the harvest of NP-69 had relatively high temperatures (maximum and minimum) and low humidity. In the case of the extra early cv. Prabhat, such a difference could be observed only in the case of maximum temperature. Nevertheless, the role of these variations of weather factors in seed contamination appears negligible since higher temperature and lower relative humidity should generally tend to lower the intensity of seed-borne mycoflora. The duration of storage also showed no relationship with the frequency of mycoflora (Table 2, foot note a). It appears, therefore, that the genotypes differ in their susceptibilities to the mycoflora.

In the two cultivars (Prabhat and NP-69) which recorded distinctly higher frequency of mycoflora, a marked reduction in seed germination was observed. The relatively higher frequency of *Aspergillus niger* and *A. flavus* observed in these cultivars could be implicated in seed spoilage with consequent reduction in germination. *Rhizoctonia bataticola* which occurred to the extent of 37.7% in ICP-1 did not appear critical since more than 90% of the seeds germinated (Table 2).

Benlate T at 3 g/kg controlled all fungi, including the wilt fungus *F. udum*, without adverse effect on seed germination (Table 3). Effective control of seed-borne *Fusarium* in chickpea (*F. oxysporum* f. sp. *ciceri*) and in flax (*F. oxysporum* f. sp. *lini*) has been shown possible through seed dressing with Benlate T (Haware *et al.*, 1978 and Tu and Cheng, 1976). Seed treatment with Benlate T (3 g/kg) can, therefore, be recommended to control the seedborne mycoflora of pigeonpea to ensure safe international exchange of seed.

Table 3. Effect of fungicidal treatment of pigeonpea seed on seed-borne mycoflora and seed germination <sup>a</sup>

| Fungicide and rate<br>(g/kg seed) | Fusarium<br>(%) | Other fungi<br>(%) | Germination<br>(%) |
|-----------------------------------|-----------------|--------------------|--------------------|
| Benlate (2)                       | 0.0             | 18.3               | 83.8               |
| Benlate (3)                       | 0.0             | 14.0               | 84.0               |
| Thiride (2)                       | 0.0             | 0.3                | 80.5               |
| Thiride (3)                       | 0.0             | 0.8                | 78.3               |
| Benlate T (2)                     | 0.0             | 0.8                | 78.5               |
| Benlate T (3)                     | 0.0             | 0.0                | 78.3               |
| Non-treated                       | 0.8             | 61.8               | 79.3               |

<sup>a</sup> For each treatment, 400 seeds of cv. ICP-1 were used.

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