PIGEONPEA DISEASES

RESISTANCE–SCREENING TECHNIQUES

Information Bulletin No. 9

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS

Abstract

Nene, Y.L., Kannaiyan, J., and Reddy, M.V. 1981. **Pigeonpea diseases: resistance-screening techniques.** Information Bulletin No. 9. Patancheru, A.P., India: International Crops Research Institute for the Semi-Arid Tropics.

Over 50 diseases have been reported to affect pigeonpea (*Cajanus cajan* [L.] Millsp.), a widely grown tropical legume crop. Economically important diseases are wilt, Phytophthora blight, sterility mosaic, witches' broom, and rust. To assist plant breeders develop disease-resistant material, easy and effective techniques to screen germplasm and breeding material have been developed and standardized for some diseases. Details of these techniques are given, supported by 23 color illustrations and a nine-point rating scale for the ready identification of resistant breeding material.

Résumé

Nene, Y.L., Kannaiyan, J., Reddy, M.V. 1981. Pigeonpea diseases: resistance-screening techniques. **(Maladies du pois d'Angole: techniques de criblage pour la résistance.)** Information Bulletin No. 9. Patancheru, A.P., India: International Crops Research Institute for the Semi-Arid Tropics.

D'après les rapports scientifiques, le pois d'Angole (*Cajanus cajan* [L.] Millsp.), une légumineuse tropicale cultivée sur de vastes superficies, est affecté par plus de 50 maladies. Les plus importantes du point de vue économique sont le flétrissement, la brûlure causée par *Phytophthora,* la mosaïque stérilisante, le balai de sorcière et la rouille. En vue d'aider les sélectionneurs à mettre au point du matériel résistant aux maladies, des techniques faciles et efficaces destinées à cribler les ressources génétiques et le matériel de sélection ont été développées et uniformisées pour quelques maladies. Les détails de ces techniques, ainsi que 23 illustrations en couleurs et une échelle d'évaluation sur neuf points facilitant l'identification des lignées résistantes se retrouvent dans cette communication.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit scientific educational institute receiving support from a variety of donors through the Consultative Group on International Agricultural Research. Major financial support for ICRISAT's work in 1981 was provided by governments and agencies of Australia, Belgium, Canada, Federal Republic of Germany, France, Japan, Mexico, the Netherlands, Nigeria, Norway, Sweden, Switzerland, United Kingdom, United States, and from the following international and private organizations: European Economic Community, International Development Research Center, International Fertilizer Development Center, the United Nations Development Programme, the Leverhulme Trust, and the International Fund for Agricultural Development. All responsibility for the information in this publication rests with ICRISAT; where trade names are used this does not constitute endorsement of or discrimination against any product by the Institute.

Correct citation: NENE, Y.L., KANNAIYAN, J., and REDDY, M. V. 1981. Pigeonpea diseases: resistance-screening techniques. Information Bulletin No. 9. Patancheru, A.P., India: International Crops Research Institute for the Semi-Arid Tropics.

PIGEONPEA DISEASES

Resistance-Screening Techniques

Y. L. Nene, J. Kannaiyan, and M. V. Reddy

ICRISAT
Information Bulletin No. 9
International Crops Research Institute for the Semi-Arid Tropics
ICRISAT Patancheru P.O., Andhra Pradesh 502 324, India

October 1981

Contents

Introduction

Pigeonpea (*Cajanus cajan* [L.] Millsp.) is grown in the Indian subcontinent, Africa, Central America, Southeast Asia, and Australia. More than 50 diseases have been reported to affect pigeonpeas, but only a few are of economic importance. These include wilt (*Fusarium udum* Butler), Phytophthora blight (*Phytophthora drechsleri* Tucker f. sp. *cajani* Kannaiyan *et al.*), sterility mosaic (virus?), witches' broom (virus and mycoplasma?) and rust (*Uredo cajani* Syd.). Wilt is serious in the Indian subcontinent and eastern Africa; sterility mosaic and Phytophthora blight in the Indian subcontinent; and witches' broom and rust in Central America. In the late 1970s, ICRISAT pathologists focused their attention on wilt, Phytophthora blight, and sterility mosaic. To assist the breeders in developing disease-resistant material, easy and effective techniques to screen germplasm and breeding material have been developed and standardized. These techniques have already been reported briefly (Nene and Reddy 1976a, 1976b; Kannaiyan and Nene 1979; Kannaiyan et al. 1981; Nene et al. 1980). However, since procedural details were not described, it was considered desirable to bring together their detailed descriptions in this bulletin. We believe that these descriptions will be useful to pigeonpea researchers and others working on similar disease problems in other crops all over the world.

We use field techniques for large-scale screenings and glasshouse/net house/laboratory techniques for confirming resistances and for carrying out inheritance and race studies.

Though we use these techniques successfully at ICRISAT, innovations might be required to meet local needs.

Wilt (*Fusarium udum* Butler)

Wilt has been reported from Bangladesh, Grenada, Kenya, India, Indonesia, Malawi, Tanzania, Thailand, and Uganda. It causes severe losses in several regions of India and eastern Africa. Typical symptoms are yellowing, drooping, and drying of the leaves and presence of black streaks under the bark. Vascular tissue is discolored. Sometimes dark brown bands may be seen on the main stem (Figs. 1 and 2). The causal fungus *F. udum* is soilborne and survives in the left-over stubble for up to 3 years. It may also spread as an external surface contaminant on the seed.

Sick Plot (Fig. 3)

1. Select a well-drained plot of the size you require. Be sure that this plot is isolated

Figure 1.

Figure 2.

from other pigeonpea fields to avoid spread of the fungus inoculum from this plot to others. The plot should have had a pigeonpea crop before in which traces of wilt incidence must have been observed.

2. Incorporate into the soil chopped stubble of wilted plants from other fields.
3. Using selfed seed, plant a sole crop of a highly susceptible cultivar (e.g., ICP-2376, ICP-6997, No. 1258) in this plot. Ensure a good plant population. Carry out normal agronomic operations.
4. By the end of the season, there should be a minimum of 10% wilted plants. Chop off the tops of the living plants to allow fresh growth (ratoon). Many ratooned plants will show wilt after the new flush. Wait for 30 days after ratooning.
5. The whole crop should then be chopped and all the stubble incorporated into the soil. This will help increase the level of the fungus inoculum and thus make the soil "sick."
6. In the next season plant a susceptible cultivar again and repeat steps 2 and 3. At this time more than 80% wilt incidence may occur. However, if the wilt incidence is less than this repeat steps 2 and 3 for one more season.
7. In the next season (3rd or 4th year) screening of test material can be initiated. Plant every third row in the whole field with the susceptible line/cultivar. These rows will serve as checks and help in monitoring as well as maintaining wilt sickness of the plot (Fig. 4). The susceptible check rows should show between 90 and 100% wilt.

Figure 3.

Figure 4.

8. From the 4th/5th year onwards, every fifth row may be planted as a susceptible check. This will provide more rows for test material and at the same time maintain the level of wilt sickness.
9. Planting of any other crop in this plot is not recommended.
10. Ensure that the selfed seed of the susceptible lines/cultivars is always available for use in the sick plots.

Pot Screening (Sick Soil)

Direct sowing

1. Fill large (35-cm diameter) earthen or other suitable pots with soil from a pigeonpea field where wilt normally occurs. Bury the pots to soil level.
2. Obtain a pure culture of a pathogenic isolate of *F. udum* from infected pigeonpea in your area.
3. Prepare sand-pigeonpea flour medium in 250-ml conical flasks (mix 10 g pigeonpea flour with 90 g riverbed sand; add 20 ml distilled water in each flask) and autoclave at 15 lb for 20 min. Inoculate each flask with the fungus culture and incubate at room temperature (approx. 30°C) for 15 days.
4. Chop green pigeonpea aerial parts into small pieces (about 5 cm) and autoclave (15 lb; 20 min) 200 g material in a 1000-ml flask.
5. Mix 200 g of the fungus-infested sand-pigeonpea flour medium with 200 g of the autoclaved pigeonpea stem bits and incorporate this mixture in the top 15 cm soil in each pot (prepared under step 1). Water the pots and wait for 2 days.
6. Sow 50 seeds of a highly wilt-susceptible cultivar in each pot. After 60 days remove healthy plants. Then chop the wilted plants and incorporate them in the pot soil.

7. Repeat steps 5 and 6. If over 90% wilt incidence occurs, then the pots are ready for use in screening (Fig. 5).
8. During each screening include a susceptible check for comparison. One pot of a susceptible check for every 10 pots of test lines is adequate. (Note: Screening in pots requires only 2 months compared with 6-8 months in a sick plot.)

Transplanting

1. Obtain a pure culture of a pathogenic isolate of *F. udum* from infected pigeonpea in your area.
2. Prepare sand-pigeonpea flour medium in 250-ml conical flasks (mix 10 g pigeonpea flour with 90 g riverbed sand; add 20 ml distilled water in each flask) and autoclave at 15 lb for 20 min. Inoculate each flask with the fungus culture and incubate at room temperature (approx. 30°C) for 15 days.
3. Mix 200 g of the fungus-infested sand-pigeonpea flour medium with 2 kg autoclaved field soil (procure soil from a pigeonpea field where wilt has been seen) and place this mixture in a 15-cm plastic pot. Water the pot and wait for 2 days. Prepare as many pots as required.
4. Concurrently with step 2, fill polythene bags (25 × 16 cm) with autoclaved riverbed sand and sow 50 pigeonpea seeds of test entries in each bag.
5. When seedlings are 7–10 days old, remove them from the bags, injure their roots by trimming off the lower 2–3 cm portions, and transplant up to five seedlings into a plastic pot (see step 3; Figs. 6 and 7).

Figure 5.

Figure 6.

Figure 7.

6. Seedlings of both a susceptible and a resistant line should be included in the test each time.

Phytophthora Blight (*Phytophthora drechsleri* Tucker f. sp. *cajani* Kannaiyan et al.)

This disease has been reported from several states of India. Another species, *P. parasitica,* has been reported from Puerto Rico and causes a similar disease. It is possible that the disease occurs in other countries but has not been reported. The blight symptoms are seen in the form of lesions on stems and leaves (Figs. 8 and 9). Early infection kills the whole plant. How the fungus survives from one season to another is not clear, but the disease spreads rapidly during long spells of rain.

Figure 8.

Figure 9.

Field Screening

Stem inoculation

1. Select a well-leveled plot where flood irrigation is possible.
2. Plant all the test lines in the field following normal sowing procedures. Plant every tenth row with a susceptible cultivar. This will help in monitoring the success of the inoculation procedure. Inoculate all plants for the first time about 1 month after planting.
3. Obtain a pure culture of a pathogenic *Phytophthora drechsleri* f. sp. *cajani* from infected pigeonpeas in your area.
4. Autoclave (15 lb; 20 min) V-8 juice agar (V-8 juice 100 ml; $CaCO_3$ 2 g; agar 20 g; distilled water 900 ml) or pigeonpea infusion agar (infusion from 40 g pigeonpea seed; agar 20 g; distilled water to make up 1000 ml) and pour the medium into petri dishes (20 ml per petri dish).
5. Inoculate the medium in petri dishes (1 week before field inoculations) with the fungus.
6. Incubate the inoculated petri dishes at 28–30°C for 1 week (Fig. 10).
7. Remove the mycelial mats along with the medium from petri dishes and macerate them by gloved hand after adding 0.2% by weight carborundum (600-mesh). Addition of carborundum helps to produce microwounds at the time of inoculation.
8. Rub a small amount of the mycelial mash with fingers on the base of the stem of the 1-month-old individual plants (Fig. 11).
9. Flood-irrigate the field immediately after inoculation and again 1 week later (Fig. 12). The second irrigation is required only if dry weather prevails. Typical blight symptoms should appear in about 10 days.
10. One month later, reinoculate plants showing no symptoms after the first inoculation (repeat steps 3–8).

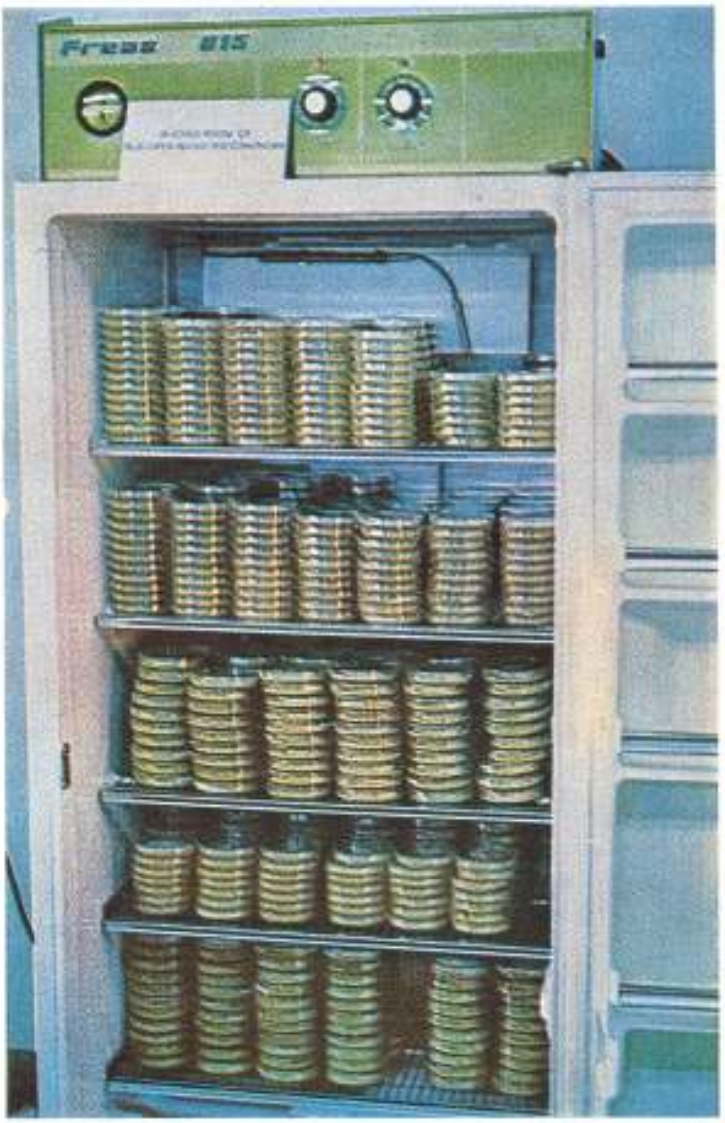

Figure 10.

Another stem inoculation procedure has been developed by Dr. J.S. Grewal and his colleagues at the Indian Agricultural Research Institute, New Delhi. Stems of adult plants are given a vertical cut with a sharp knife, and a little mycelial mash of the fungus is pushed under the bark. The wound is then covered with cellotape. Lesions develop from the point of inoculation in about 10 days.

Figure 11.

Figure 12.

Pot Screening

Soil drench

1. Obtain a pure culture of the fungus from infected pigeonpeas in your area.

2. Autoclave (15 lb; 20 min) V-8 juice agar or pigeonpea infusion agar (compositions given earlier) and pour the medium into petri dishes (20 ml per petri dish).

3. Inoculate the medium in petri dishes with the fungus and incubate at 28–30°C for 1 week.

4. Transfer 5-mm discs of the fungus growth to 100 ml autoclaved V-8 juice broth or pigeonpea infusion broth (see step 4 under stem inoculation procedure; composition same minus agar) in 250-ml flasks. Incubate at 28–30°C for 2 weeks.

5. Fill 20-cm diameter plastic pots with field soil (we use red Alfisol). Sow 25–30 seeds per pot.

6. Remove mycelial mats from the flasks (step 4) and wash them twice with water. Then macerate these mats with a small amount of water in a Waring blender for 1–2 min by operating the blender intermittently. Dilute this suspension with tap water to get a final dilution of one mycelial mat in 200 ml water.
7. Inoculate 5–10-day-old seedlings by pouring 100 ml inoculum (step 6) around the base of seedlings in a pot.
8. Keep susceptible (e.g., HY-3C) and resistant (e.g., BDN-1) checks, both inoculated and noninoculated, with each batch of germplasm or breeding material.
9. Water the pots liberally 3 times a day.
10. Phytophthora blight symptoms usually start appearing in 48 hours (Fig. 13). Take final observations 10 days after inoculation.

Figure 13.

Spray inoculation

1. Fill 20-cm-diameter plastic pots with field soil (we use red Alfisol). Sow 25–30 seeds per pot.
2. Obtain a pure culture of the fungus from infected pigeonpeas in your area.
3. Autoclave (15 lb; 20 min) V-8 juice agar or pigeonpea flour agar (compositions given earlier) and pour the medium in petri dishes (20 ml per petri dish).
4. Inoculate the medium in petri dishes with the fungus and incubate at 28–30°C for 1 week.
5. Transfer 5-mm discs of the fungus growth to 100 ml autoclaved V-8 juice broth or pigeonpea infusion broth in 250-ml flasks. Incubate at 28–30°C for 2 weeks.
6. Remove mycelial mats from the flasks and wash twice with distilled water. Then macerate these mats with a small amount of water in a Waring blender for 1–2 min by operating the blender intermittently. Dilute this suspension with tap water to get a final dilution of one mycelial mat in 200 ml water.
7. Spray 15–30-day-old seedlings (step 1) in a pot (hand sprayer) with 50 ml inoculum.
8. Cover inoculated plants with polythene bags to ensure high humidity for 48 hr. Then remove the bags and spray the plants with tap water every 2–3 hr during the day until 96 hr after inoculation. Afterward, these are sprayed 3 times a day until the final recording.
9. Initial symptoms can be seen on the 4th day after inoculation (Fig. 14). Record the final data 10 days after inoculation.

Sterility Mosaic

The viral nature of the sterility mosaic is yet to be fully established. The causal agent is transmitted by an eriophyid mite, *Aceria cajani* Channabasavanna (Fig. 15). The disease spreads through infective mites disseminated by wind. The disease has so far been reported only from Burma, India, and Thailand. Typical symptoms are mild mosaic

Figure 14.

Figure 15.

and either no or little flowering (Figs. 16 and 17). Pigeonpea and some of its wild relatives such as *Atylosia platycarpa* (L.) Benth. are the only recorded hosts of both the causal agent and the mite vector. The screening techniques described below are based on the transfer of infective mites from diseased to healthy leaves.

Figure 16. (Boxed area shows infected branch; note pale green color and absence of flowers.)

Figure 17.

Leaf Stapling

1. The availability of diseased pigeonpea leaves colonized with the mite vector, *Aceria cajani,* is essential for successful infections. Check a sample of diseased leaflet under a stereobinocular microscope for the presence of mites. Maintain pots with infected plants in a screen house or in partial shade in order to have a good source of inoculum. Do not depend on infected plants in the field as source of inoculum, especially at the beginning of the season, as the number of mites on such freshly infected plants is generally low and all the leaves do not carry mites.
2. Young seedlings (10–15 days old) are best suited for inoculation. Inoculation is carried out on the primary leaves.
3. In the "folded leaf" method of inoculation (Fig. 18), one diseased leaflet per

Figure 18.

primary leaf is used. Fold the diseased leaflet on the primary leaf in such a way that its lower surface comes in contact with a primary leaf of the test seedling and then staple with a small paper stapler (Max-10, Max Co. Ltd., Japan). (This is necessary because the mites are mostly on the lower surface and come into close contact with the test seedlings.)

4. Alternatively, if the diseased leaflets are too small, use two such leaflets for each primary leaf of the test seedling. Place the two diseased leaflets in such a way that the lower surface of one comes in contact with the lower surface of the primary leaf and the lower surface of the other with the upper surface of the primary leaf. Staple together the primary leaf and the two diseased leaflets (Figs. 19 and 20).

Figure 19.

5. Record the data on time taken for symptom expression (incubation period), number of plants infected, and type of symptom severity—ring spot (Fig. 21), mild mosaic, severe mosaic, partial or complete sterility. Take final observations at the flowering and podding stage.

Figure 20.

Figure 21.

6. In case of plants where the symptoms are doubtful, prune the plant to 50% of its height. The fresh growth will show symptoms more clearly in the case of infected plants.

Note: The leaflets usually dry out in 1 or 2 hr after stapling. When the diseased leaflets are drying the mites migrate onto the primary leaves of the test seedlings and feed on them and thus effect transmission. The injury made by stapling is minimal and does not produce any apparent effect on the inoculated seedling. The initial symptoms are usually seen in the freshly emerged trifoliate leaves after 5 days. The symptoms are not seen on the inoculated primary leaves.

The technique can be used for screening plants both in pots (Fig. 22) and fields.

Figure 22.

Spreader Rows

1. Plant a susceptible cultivar (e.g., BDN-1, C-11) in the field in widely spaced rows at least 4 months in advance of the regular planting time. The distance between two rows can be 10–20 m. Alternatively, a few rows can be grown on one side of the field to comprise an infector hedge (Fig. 23). Such planting also ensures the efficient spread of the disease. The rows should be planted across the wind direction and upwind of the field.
2. Inoculate the 10–15-day-old spreader row seedlings by the leaf-stapling technique. These plants will then be infected and the mites will multiply on them. These rows will then serve as a source of infection. Irrigate these rows as and when necessary.
3. Plant the test materials between the spreader rows at the normal planting time (Fig. 24). The mites are carried from the spreader rows to test rows by the wind and the causal agent is then transmitted.
4. Along with the test materials, plant rows with a susceptible cultivar at frequent intervals (e.g., one after every ten test rows) as indicator rows to monitor the extent of disease spread. If the screening is successful the indicator rows should develop nearly 100% infection within a month.
5. Prune the infector rows to stimulate fresh growth and to maintain the mite population.
6. When the infector rows are to be retained for more than one season, it is essential to use a pigeonpea cultivar that is resistant to wilt (e.g., NP (WR)-15) and is a perennial type.
7. Avoid spraying any insecticide on the infector rows. The test rows can be sprayed at flowering/podding stage to control pests.

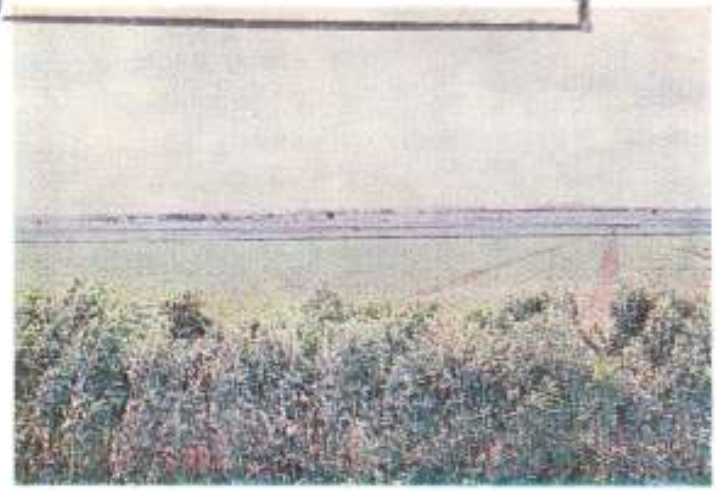

Figure 23.

Figure 24.

8. Record observations on disease incidence twice; first in the seedling stage (30–45 days after planting and again at maturity stage). Early observations are essential as some of the plants with mild mosaic or ring spot symptoms may recover later.

Multiple Disease Resistance

For identifying combined resistance to the three diseases (wilt, Phytophthora blight, and sterility mosaic) in the same genotypes, we follow a combination of techniques. First, develop a wilt-sick plot. In that plot use the spreader-row technique for sterility mosaic screening and the stem-inoculation technique for Phytophthora blight screening. Plant the

test material along with susceptible checks for each disease. We recommend HY-3C (resistant to wilt and mosaic; susceptible to blight), BDN-1 (resistant to wilt and blight; susceptible to mosaic), and No. 1258 (resistant to mosaic and blight; susceptible to wilt) as checks to be planted after every eight test rows.

Rating Scale

A 9-point scale divided into five categories is used at ICRISAT for easy scoring. Interpretation of the scale is as follows: 1, resistant; 3, moderately resistant; 5, tolerant; 7, moderately susceptible; 9, susceptible (Table 1).

Test lines with ratings 1–3 are considered acceptable for our breeding program; the 5 rating is acceptable only if lines with ratings 1 or 3 are not available; ratings 7–9 are not acceptable. Materials with ratings 3–5 can be improved in 2 years through screening and selfing resistant plant progenies.

Table 1. Rating scale for scoring.

Rating	Wilt	Blight	Sterility mosaic
1	No symptoms on any plant	No symptoms on any plant	No symptoms on any plant
3	10% or less mortality	Symptoms on 10% or fewer plants	Symptoms on 10% or fewer plants
5	11–20% mortality	Symptoms on 11–20% plants	Ring spot symptoms on most plants but disappearing with age; no sterility
7	20–50% mortality	Symptoms on 20–50% plants	Mild mosaic symptoms on most plants causing partial sterility
9	51% or more mortality	Symptoms on 51% or more plants	Severe mosaic on most plants; almost complete sterility

The rating scale in Table 1 is used only if the total number of plants of a test line varies between 20 and 50. The rating scale will be different if the plant number is more than 50.

Literature Cited

KANNAIYAN, J., and NENE, Y.L. 1979. Association of different *Fusarium* species with wilt disease of pigeonpea. Tropical Grain Legume Bulletin 15: 26-27.

KANNAIYAN, J., NENE, Y.L., RAJU, T.N., and SHEILA, V.K. 1981. Screening for resistance to Phytophthora blight of pigeonpea. Plant Disease 65: 61-62.

NENE, Y.L., KANNAIYAN, J., HAWARE, M.P., and REDDY, M.V. 1980. Review of the work done at ICRISAT on soilborne diseases of pigeonpea and chickpea. Pages 3–39 *in* Proceedings of the Consultants' Group Discussion on the Resistance to Soilborne Diseases of Legumes, 8–11 January 1979, Hyderabad, India.

NENE, Y.L., and REDDY, M.V. 1976a. A new technique to screen pigeonpea for resistance to sterility mosaic. Tropical Grain Legume Bulletin 5: 23.

NENE, Y.L., and REDDY, M.V. 1976b. Screening for resistance to sterility mosaic of pigeonpea. Plant Disease Reporter 60: 1034-1036.