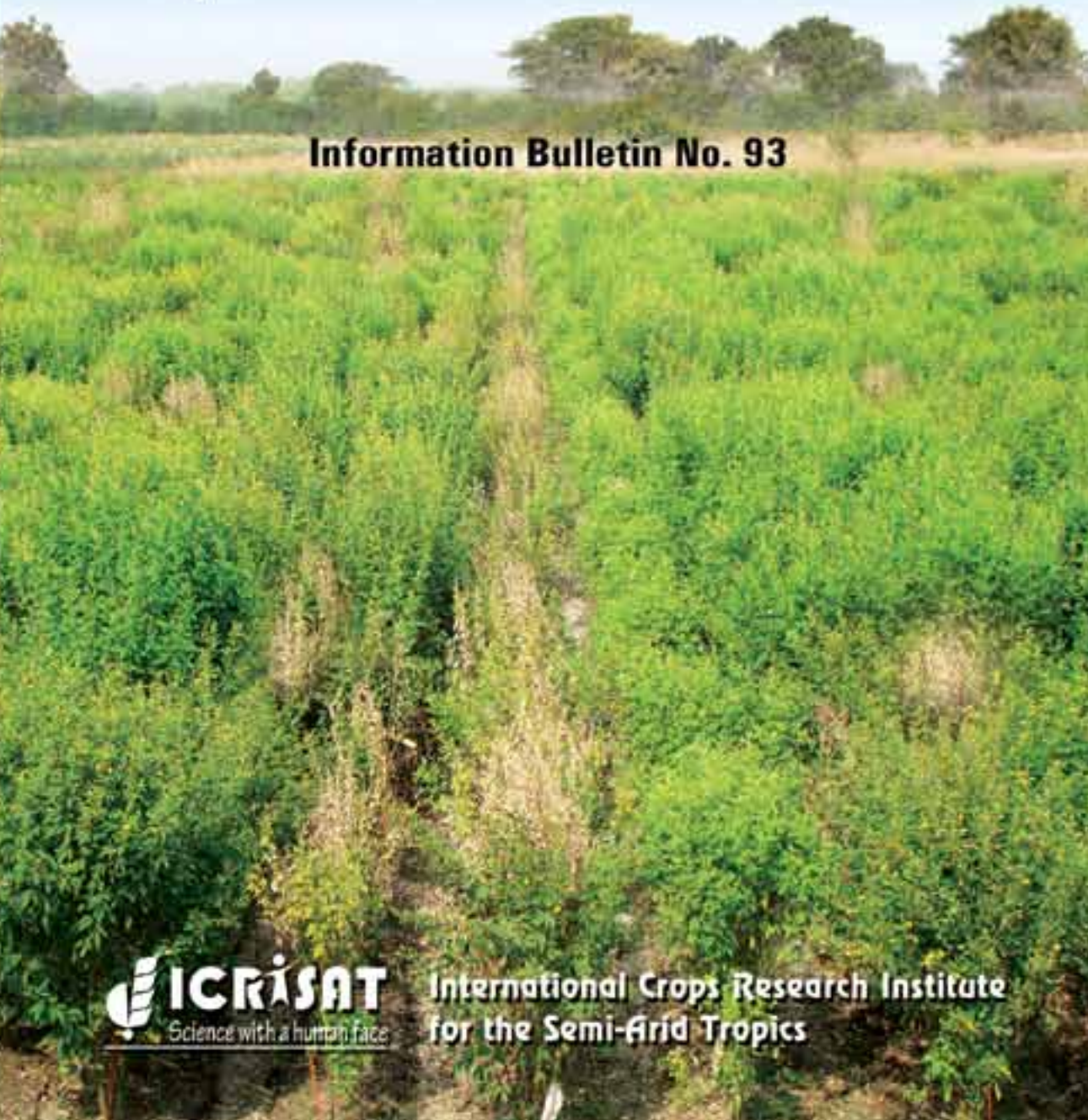
High Throughput Phenotyping of Pigeonpea Diseases

Stepwise Identification of Host Plant Resistance



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Abstract

Pigeonpea diseases are the major constraints in realizing the high yield potential of improved pigeonpea genotypes. Among diseases, fusarium wilt and sterility mosaic disease are the major constraints to pigeonpea production worldwide and phytophthora blight and alternaria blight are recently emerging as potential threats to pigeonpea production. A large volume of literature is available on studies related to various aspects of fusarium wilt, sterility mosaic disease, phytophthora blight and alternaria blight diseases including biology, epidemiology and management methods. In this bulletin, attempts have been made to briefly describe the distribution, economic importance, symptoms and causal organism. Detailed descriptions of stepwise evaluation of the screening techniques developed at ICRISAT for identification of resistant sources are provided. This bulletin will be useful to all researchers involved in the management of these diseases through host plant resistance.

High Throughput Phenotyping of Pigeonpea Diseases

Stepwise Identification of Host Plant Resistance

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Contents

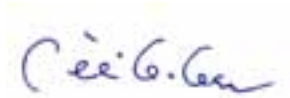
Foreword

Pigeonpea is attacked by many diseases irrespective of cropping systems and seasons. However, there are only few diseases that are more important than others in different pigeonpea growing regions of the world. ICRISAT's research focus has mainly been on managing the diseases of pigeonpea through host plant resistance (HPR), which involves developing effective and repeatable resistance screening techniques, identifying the sources of resistance, and breeding for resistance through collaborative efforts of pathologists.

As climate change further increases climate variability, there is an urgent need to develop HPR lines to enhance the livelihood of the poor. Hence the key to a sustainable future lies in improving crop productivity through ecologically friendly farming systems that are more effective in harnessing nature, and that will go a long way in enhancing the livelihoods. Therefore, developing appropriate strategies based on HPR for disease management under these situations in the future are critical.

In this bulletin, the authors have addressed globally important diseases and compiled the information on screening techniques so critical for identification of resistance and utilization in breeding for developing disease resistant cultivars. These techniques have been used to screen a large number of germplasm accessions and breeding lines to identify resistance and enhance resistance breeding. Screening techniques have also been refined and made simpler. It is well established that the most effective and economical method of controlling pigeonpea diseases is by growing resistant cultivars. It is in this context that a simple and reliable screening technique is required to easily discern resistant and susceptible lines.

I am sure that this bulletin will serve as a practical guide to pigeonpea researchers, scholars and especially those who have an interest in managing pigeonpea diseases through HPR.



William D Dar
Director General

Introduction

Pigeonpea (*Cajanus cajan* (L) Millsp.) is attacked by many soil borne and foliar diseases. As per the assessment of Nene et al. (1996), a total of 48 pathogens, including 34 fungi, 1 bacterium, 3 viruses and mycoplasma and 10 nematodes were reported from 28 countries until 1978. By 1995, the number increased to 210, which included 83 fungi, 4 bacteria, 19 viruses and mycoplasma and 104 nematodes, the maximum being from India. The number is still increasing but few are economically important and widespread causing heavy losses. The important diseases causing heavy losses are fusarium wilt, sterility mosaic disease (SMD), and new emerging disease phytopthora blight and alternaria blight. Among them fusarium wilt and SMD can cause losses up to 100%. They can cause disease in all the crop growing seasons.

Using fungicides and cultural practices for the management of the diseases under environmental conditions favorable to disease development is uneconomical and difficult to carry out. Of the available management approaches, host plant resistance (HPR) is the most reliable, economical and effective method for managing the diseases. Considerable efforts have been made by ICRISAT towards understanding the components of HPR such as biology and epidemiology, developing screening techniques, identifying resistance sources and utilizing these in breeding disease resistant lines. This bulletin describes the advances in host plant resistance to important diseases of pigeonpea and provides stepwise details of refined and repeatable screening techniques for identification of resistance sources and screening breeding materials. New sources of stable host plant resistance to diseases based on controlled environment and field screening techniques are also reported.

1. Fusarium wilt

1.1 Distribution and economic importance

Pigeonpea wilt is widely prevalent throughout the world but more important in India (Kannaiyan and Nene 1981) and in eastern Africa (Okiror 2002). The disease is seed borne and soil borne. The disease is reported to cause 30-100% loss in grain yield (Reddy et al. 1990) and may cause 100% yield losses in susceptible genotypes. The annual losses due to wilt have been estimated at $71 million in India and $5 million in eastern Africa.

1.2 Disease symptoms

Wilt symptoms usually appear when the crop is in flowering or podding stage (Fig. 1), but sometimes may be seen at seedling stage also. The most characteristic symptoms are browning or blackening of the xylem vessels (Fig. 2) and a purple band extending upwards from the base of the main stem. This band is more easily seen in pigeonpea with green stems than in those with colored stems. Partial wilting of the plant is also an indication of fusarium wilt. When young plants (1-2 months) die from wilt, they may not show the purple band symptom, but have obvious internal browning and blackening.

Fig. 1. Wilt infected adult plant.

Fig. 2. Browning or blackening of xylem vessels.

1.3 Causal organism

The causal organism of wilt disease of pigeonpea is *Fusarium udum* Butler. (Perfect stage: *Gibberella udum*). Mycelium may be parasitic or saprophytic and hyphae are hyaline, slender, much branched. Fungus produces macroconidia, microconidia and chlamydospores (Fig. 3). Macroconidia are 1-5 septate (predominantly 3 septate), curved to almost hooked and abundant in sporodochia. Microconidia are fusiform to reniform or oval and 0-1 septate. Chlamydospores are round or oval, thick walled, hyaline, sometimes in short chains, 5 to 10 µ in diameter. Perfect stage of pathogen (*G. udum*) needs further investigations. So far, 5 variants (strains) of *F.udum* have been identified and documented (Reddy et al. 1996, Mishra 2004).

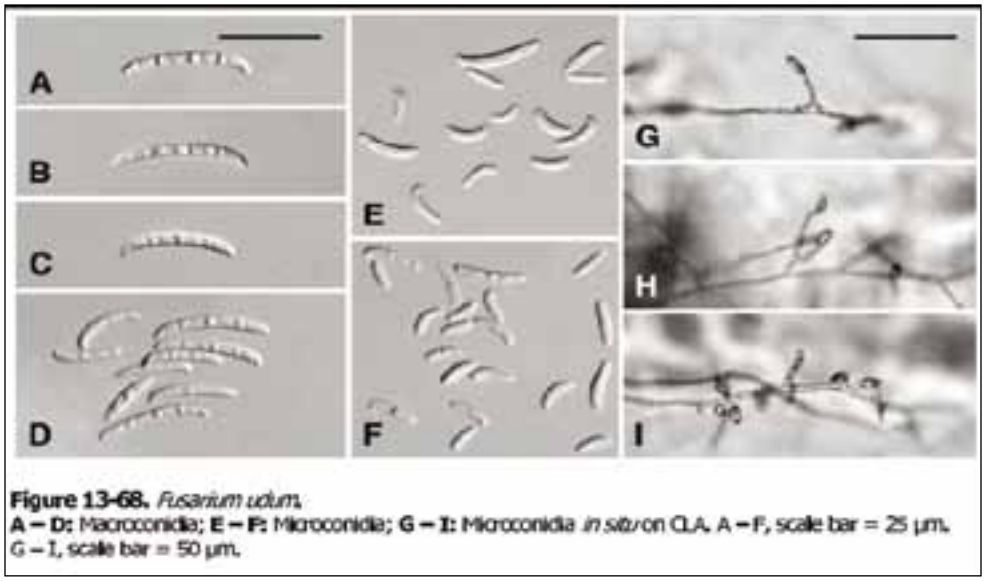


Fig. 3. Macro-and micro-conidia of Fusarium udum.
(Source: The Fusarium Laboratory Manual, John F. Leslie and Brett A. Summerell 2006).

1.4 Screening techniques

A number of screening techniques have been developed and modified over time for resistance screening of pigeonpea genotypes against *F. udum* in field, greenhouse and controlled environment. Nene et al. (1981) gave a detailed account on developing screening techniques for identifying resistant sources of fusarium wilt. Since then, screening techniques have been further refined and modified. Details of the refined screening techniques are as follows:

1.4.1 Field screening

Field screening for fusarium wilt resistance is done in the wilt sick plot. The detailed procedure for developing a wilt sick plot is as follows:

1. Select a well-drained plot and be sure that it is away from other pigeonpea grown plots in order to avoid the spread of inoculum.
2. Selected field should have grown the pigeonpea crop earlier and have traces of wilt incidences.
3. Incorporate the chopped stubbles of wilted plants collected from other fields into the selected field.
4. Plant highly susceptible genotype (eg, ICP 2376) in this plot.
5. There should be 10% wilted plants by the end of the season. Chop the tops of the living plants to allow fresh growth. These ratooned plants will show wilt after the new flush.
6. Wait for 30 days after ratooning and chop the whole crop and incorporate stubbles into the soil.
7. In the next season plant a susceptible genotype again and repeat steps 3 and 4. Now the wilt incidence should reach upto 80%. If the wilt incidence is less than this, repeat steps 3 and 4 for one more season.
8. In the next season screening of the test material can be done.
9. Planting of any other crop in this plot is not recommended.
10. Always use the selfed seed of the susceptible genotypes in the sick plot.
11. For screening, plant wilt susceptible variety of pigeonpea (eg, ICP 2376) after every fourth test row in the whole plot as a susceptible check. This will serve as infector and indicator row (Fig. 4).

Fig. 4. Wilt sick plot.

12. Also plant the wilt resistant check (eg, ICP 8863) along with susceptible check for comparison (Fig. 4).
13. Provide irrigation at flowering, if needed.
14. Periodical observations are recorded for wilt incidence at seedling, flowering and pod formation stages.

1.4.2 Greenhouse screening

Wilt resistant lines identified during the large scale resistance screening in wilt sick plot are confirmed for their resistance to wilt in the greenhouse. The most commonly used greenhouse screening techniques for fusarium wilt of pigeonpea are as follows:

1.4.2.1 Root dip screening

Raising of seedlings

- Pigeonpea seedlings of the test genotypes as well as susceptible genotype (eg, ICP 2376) are grown in polythene covers (Fig. 5a) filled with sterilized river sand in a greenhouse maintained at 25±2°C.
- Plastic bags are filled up to 2/3 of its volume with sterilized river sand.
- Before sowing, seeds are surface sterilized using 2% sodium hypochlorite for 2 minutes, rinsed in sterile water in order to wash off sodium hypochlorite, sow (5-6 seeds) in plastic bags and alow to grow for 8 days.

Inoculum preparation

- Single conidial isolate of *F. udum* isolated from naturally wilt infected pigeonpea plants isolated on potato dextrose agar (PDA) medium is used (Fig. 5b).

Fig. 5a. Raising of seedlings.

Fig. 5b. Single conidial isolate of F. udum.

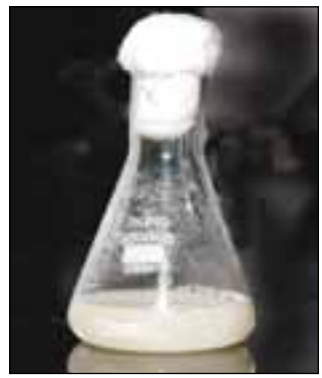


Fig. 5c. Mass multiplication on PD broth.

- The pathogen is mass multiplied on potato dextrose broth (PDB) in flasks kept on the shaker incubator at 25±1°C for 8 days with a 12 h photoperiod (Fig. 5c).
- Conidial suspension of *F. udum* is diluted with distilled water to maintain the threshold level of inoculum (6 x 10^5 spores/ml) using a haemocytometer.

Inoculation and transplanting

- The 8-day-old seedlings are carefully uprooted from polythene covers and the roots are washed under running water to remove excess sand (Fig. 5d).
- Root tips around 0.5 cm long are cut off to facilitate the entry of the pathogen into the host and are dipped in the churned inoculum suspension (6 x 10^5 spores/ ml) for 1-2 minutes (Fig. 5e).
- Inoculated seedlings are transplanted (Fig. 5f) into 12 cm pre-irrigated pots containing sterilized vertisol and sand (3:1).

Fig. 5d. Cleaning of seedlings.

Fig. 5e. Inoculation of pigeonpea seedlings.

Fig. 5f. Transplanting the seedlings after inoculation.

- Five inoculated seedlings are transplanted per pot and at least three replications are maintained for each test genotype as well as susceptible genotype.
- Uninoculated control is kept where root tips are dipped in sterile distilled water and transplanted into the pots.
- The plants are kept in the greenhouse at a temperature of 25± 2°C with 12 h natural light per day (Fig. 5g).
- Disease incidence is recorded periodically upto 60 days (Fig. 5h).

Fig. 5g. Wilt screening in greenhouse.

Fig. 5h. Disease expression.

1.4.2.2 Sick pot screening

Inoculum preparation

- Single conidial isolate of *F. udum* isolated from naturally wilt infected pigeonpea plants isolated on PDA medium is used (Fig. 5b).
- Prepare sand-pigeonpea flour medium (details provided in *Annexure 1*) in 250 ml conical flasks 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 (Fig. 6a).
- Mix 200 g of the fungus infested sand-pigeonpea flour medium with 2 kg autoclaved field soil (Fig. 6b) and place this mixture in a 15 cm plastic pot. Water the pot and keep for 2 days.

Fig. 6a. Sand-pigeonpea flour medium.

Fig. 6b. Mixing fungus infested sand-pigeonpea flour medium with autoclaved sand.

Sowing of seeds

- Sow 5-7 seeds of highly wilt-susceptible genotype in each sick pot (15 cm). After 60 days remove healthy plants and chop the wilted plants and incorporate them in the pot soil.

- Repeat the above steps, till we get >90% wilt incidence in susceptible genotypes (Fig. 6c). Pots are ready to be used for wilt screening when wilt incidence is >90%.

Screening and data recording

- Test genotypes can be screened in these sick pots along with susceptible and resistant checks, kept for comparison.
- The plants are monitored regularly for wilt symptoms.

Fig. 6c. Wilt expression in sick pot.

1.5 Disease incidence

Disease incidence is calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased seedlings}}{\text{Total no. of seedlings}} \times 100$$

Based on the disease incidence, genotypes are categorized for their reaction to fusarium wilt as follows:

Table 1. Disease reaction of wilt based on disease incidence.

Disease incidence (%)	Disease reaction
0-10	Resistant
10.1-20.0	Moderately resistant
20.1-40.0	Moderately susceptible
40.1-100	Susceptible

1.6 Resistant sources

Several wilt resistant genotypes have been identified and reported (Nene and Kannaiyan 1982, Zote et al. 1983, Zaveri et al. 1986, Konda et al. 1986). These sources of resistance can be used as resistant donors in pigeonpea wilt resistance breeding program. However, there is a need for better understanding of the inheritance of resistance, particularly in view of that fact that genotypes show different levels of resistance under field conditions. Some of the new sources of resistance reported against fusarium wilt are given in Table 2.

Table 2. New sources of resistance to fusarium wilt of pigeonpea.

Genotypes	**Remarks**	**References**
ICP 6739, ICP 8860, ICP 11015, ICP 13304, ICP 14638, ICP 14819	Resistant both in greenhouse and field	Sharma et al. 2012
ICP 7903, ICP 12031, ICP 12059, ICP 12841, ICP 13257, ICP 13258, ICP 12771, ICP 12775, ICP 7991, ICP 13618, ICP 14291, ICP 15137	Resistant both in greenhouse and field	Sharma and Pande 2011
IPA 16F, IPA 8F, IPA 9F and IPA 12F	Resistant to all the five variants of *F. udum* prevalent in India	Singh et al. 2011
IPA 204	Long duration wilt resistant variety	Chaudhary 2010
ICEAP 00040	Resistant (Kenya, Malawi and Tanzania)	Gwata et al. 2006
ICP 8863, ICP 9145, ICP 9174, ICP 12745, ICPL 333, BDN 1, ICPL 8363, ICPL 88047, BWR 370, DPPA 85-2, ICP 9168, ICP 11299, DPPA 85-3, DPPA 85-8, ICP 4769, DPPA 85-13, DPPA 85-14, ICP 10958, Bandapalera, C 11 (ICP 7118)	Moderately resistant	Reddy et al. 1993

2. Sterility mosaic disease

2.1 Distribution and economic importance

Sterility mosaic disease (SMD) is the second most important disease of pigeonpea and is most damaging in the Indian subcontinent, Bangladesh, Nepal, Sri Lanka and Myanmar. The annual yield losses due to SMD were estimated worth $282 million in Nepal and India (Kannaiyan et al. 1984).

2.2 Diagnostic symptoms

SMD is often referred to as “green plague” as the affected plants are green with excessive vegetative growth but with no flowers or pods. The disease spreads rapidly, leading to severe epidemics. SMD affected plants show characteristic mosaic symptoms that initially appear as vein clearing on leaves (Fig. 7). The leaves are small and show a light and dark green mosaic pattern (Fig. 8). In the field, the disease can be easily identified from a distance as patches of bushy, pale green plants without flowers or pods (Fig. 9).

Fig. 7. SMD infected plant.

Fig. 8. Light and dark green mosaic pattern.

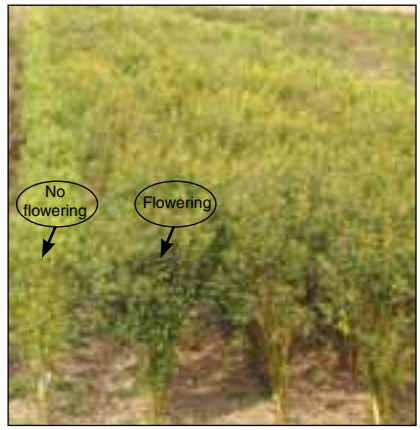


Fig. 9. SMD infected plants in field without flowers and pods.

2.3 Causal organism

SMD is caused by Pigeonpea sterility mosaic virus (PSMV). It is transmitted by an Eriophyid mite (*Aceria cajani*) (Kumar et al. 2000). A single Eriophyid mite vector is sufficient to transmit the disease (Fig. 10). Both pathogen and the mite vector are specific to *Cajanus cajan* and its wild relative *C. scarabaeoides* var. *scarabaeoides.*

Fig. 10. Eriophyid mite (magnified).

2.4 Screening techniques

2.4.1 Maintenance of pigeonpea SMD nursery

- Select a plot of adequate size and ensure that it is isolated from other pigeonpea fields to avoid spread of the viral inoculum from this plot to others.
- Ensure adequate shadow in the selected plot for the survival of Eriophyid mite (Fig. 11).
- Plant a susceptible genotype (eg, ICP 8863) in the selected plot four months earlier than the normal planting schedule of pigeonpea.
- Maintain a distance of at least 10-20 cm between the two rows.
- At the two leaf stage (10-15 days old seedlings) inoculate the pigeonpea plants using leaf stapling technique (Fig. 13b).

Fig. 11. Pigeonpea SMD maintenance nursery.

- Fold the SMD infected leaf on the primary leaf (from the above step) in such a way that its lower surface comes in contact with a primary leaf of susceptible seedlings; staple it in place.
- Irrigate the plot frequently to ensure the optimum temperature required for survival of mite for transmission of disease.
- Disease symptoms can be seen 2-3 weeks after inoculation.
- The plants will show 100% SMD infection 60 days after sowing.
- This nursery will serve as a source of inoculum for the screening of test entries in the field as well as in the greenhouse.
- Avoid spraying insecticides on the infector rows; test rows can be sprayed at flowering/ podding stage to control pests.

2.4.2 Field screening

1. Select an isolated plot or use a pigeonpea wilt sick plot.
2. Plant a susceptible genotype (eg, ICP 8863) on one side of the field to comprise an infector hedge. Rows should be planted across the wind direction and upwind of the field.
3. Plant the test genotype as well as susceptible check (eg, ICP 8863) in the field with row to row spacing of 75 cm and plant to plant spacing of 10 cm.
4. Plant the susceptible genotypes at frequent intervals after every 10 test rows. These will act as indicator/ infector rows to monitor the disease spread.
5. Inoculate 10-15 days old seedlings by leaf stapling technique and irrigate them whenever necessary.
6. The mites are carried from the infector hedge by the wind and thereby help in transmission of disease.
7. If the screening is successful, the indicator rows will develop nearly 100 % infection within a month (Fig. 12).
8. Prune the infector rows to stimulate fresh growth and to maintain the mite population for the next crop season.
9. Avoid spraying insecticides on the infector rows; test rows can be sprayed at flowering/ podding stage to control pests.

Fig. 12. Field screening.

10. Early observations are essential as some of the plants with mild mosaic or ring spot symptoms may recover later.
11. Record the observations of disease incidence twice, first in the seedling stage (30-40 days after planting) and again at maturity stage.

2.4.3 Greenhouse screening

2.4.3.1 Leaf stapling

Raising of seedlings

- Pigeonpea seedlings of the test genotypes as well as susceptible genotype (eg, ICP 8863) are grown in pots (12 cm) filled with sterilized alfisol and sand (9:1) in a greenhouse (Fig. 13a).
- Before sowing, seeds of test as well as susceptible genotypes are surface sterilized using 2% sodium hypochlorite for 2 minutes, then rinsed in sterile water in order to wash off sodium hypochlorite and are sown (5-6 seeds) in pots for 10-15 days.

Inoculation

- Young seedlings of 10-15 days are best suited for inoculation. Inoculation is carried out on the primary leaves at two leaf stage using leaf stapling technique.
- Single infected leaf is taken from the pigeonpea SMD nursery maintained on susceptible genotype and is stapled against the primary leaf of the test seedling in such a manner that the lower surface of the infected leaf is in contact with the test leaf (Fig. 13b). This will help in transmitting the mite from diseased to healthy leaf.

Fig. 13a. Raising of seedlings.

Fig. 13b. Inoculating with SMD.

- If the diseased leaves are too small, then use two diseased leaves for each primary leaf of the test plant. 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. Staple together the primary leaf and the two diseased leaflets. Stapling is done with the help of a small paper stapler.
- Record the data on time taken for symptom expression, number of plants infected and type of symptoms. Take the final observation after 60 days of sowing (Fig. 13d).

Fig. 13d. SMD expression (right).

2.5 Disease incidence

Disease incidence is recorded periodically and final disease incidence is calculated as described in section 1.5.

2.6 Resistant sources

Sources of resistance to SMD have been reported in germplasm and breeding lines of pigeonpea by various workers (Khare et al. 1994, Reddy et al. 1998, Gwata et al. 2006). Some of the recent SMD resistant genotypes are presented in Table 3.

Table 3. New sources of resistance to SMD of pigeonpea.

Genotypes	Reference
ICPs 3576, 7869, 9045, 11015, 11059, 11230, 11281, 11910, 14819, 14976, 15049	Sharma et al. 2012
ICPs 7893, 7897, 7899, 7991, 8535, 8692, 9157, 12009, 12019, 12090, 12139, 12747, 12806, 12808, 12813, 12819, 13107, 13229, 13257, 13277, 13625, 13774, 13965, 15176	Sharma and Pande 2011
ICP 7035	Rangaswamy et al. 2005

2.7 Combined resistance to wilt and SMD

Field screening can be done for both the diseases wilt and SMD in wilt sick plot. For identifying combined resistance to the two diseases (wilt and SMD) in the same genotypes, we follow a combination of techniques. First, develop a wilt sick plot as described in 1.4.1 for wilt screening; SMD screening can be done in the same sick plot as described in 2.4.2. Plant the test material along with susceptible checks for each disease. We recommend ICP 2376 (susceptible check to wilt) and ICP 8863 (resistant check to wilt) and vice versa for SMD, ie, ICP 8863 as a SMD susceptible check and ICP 2376 as a resistant check. These checks are planted after every eight-ten test rows. The rest of the procedure is as described earlier.

3. Phytophthora blight

3.1 Distribution and economic importance

Phytophthora blight (PB) is an economically important disease of pigeonpea. The disease has been reported from most pigeonpea growing areas in Asia, Africa, the Americas, Australia, Dominican Republic, Kenya, Panama and Puerto Rico. The recurrence of PB in the recent past was a major threat to pigeonpea production and productivity in the Deccan Plateau of India and was reported irrespective of cropping system, soil types and genotypes (Sharma et al. 2006, Pande et al. 2006). Information on worldwide losses caused by PB is not available, but the disease is of growing importance and has the potential to cause 100% yield losses in a susceptible genotype.

3.2 Diagnostic symptoms

The symptoms of the PB on pigeonpea have been described as stem rot, stem blight, stem canker and root rot (Pande et al. 2011). Early infection kills the whole plant (Fig. 14). Characteristic foliage blight symptoms are circular or irregular water soaked lesions on leaves, which become necrotic within a week when humidity is $\geq$ 80% and temperature is 20-30°C (Fig. 15). Stem symptoms appear as brown to dark brown lesions distinctly different from

Fig. 14. Early infection of PB.

Fig. 15. Water soaked lesions on leaves.

healthy green portions on main stem, branches and petioles (Fig. 16). The lesions on stems and branches increases rapidly, girdles, cracks and dries the stem (Fig. 17). Infected stem and branches break easily in the wind. Phloem vessels show smoky gray colored discoloration, however xylem vessels remain healthy (Fig. 18). The disease spreads rapidly during long spells of rain.

Fig. 16. Dark brown lesions on the stem.

Fig. 17. Stem showing breaking and cracks.

Fig. 18. Smoky gray colored discoloration of phloem vessels.

3.3 Causal organism

The PB is caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* (Pal et al., Kannaiyan et al.) The mycelium is intracellular, branched, hyaline, filamentous to slender aerial mycelium white and fluffy when grown on PDA, V8 juice agar or Oat meal agar medium, coenocytic when young (Fig. 19a) but later septate with thick plugs and abundant hyphal swellings (Fig. 19b) of 13-15 µ in diameter with tube like projections. Sporangia of *P. drechsleri* Tucker f. sp *cajani* (Pdc) are of proliferating type with sizes ranging from 42-83 × 28-48 µm (average 61.8 × 37.3 µm) (Fig. 20). The sporangial stalks are either narrowly tapered or widened somewhat at the base of the sporangium. Oogonium and oospore size show little variation (19-29 to 34-44 µm). Terminal and outer calary hyphal swellings with finger like projections are only observed at low temperatures (9-18°C). Chlamydospores were not formed on any media at any temperature (Kannaiyan et al. 1980).

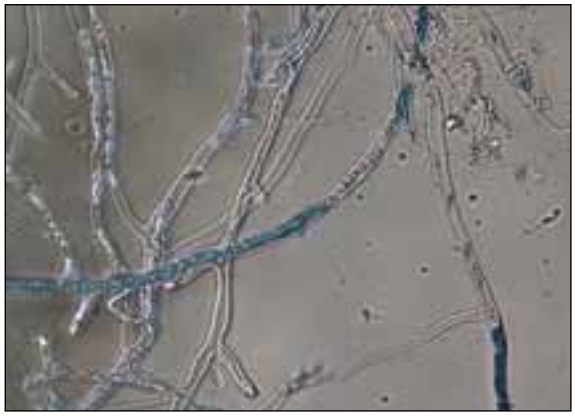


Fig. 19a. Coenocytic mycelium of Pdc.

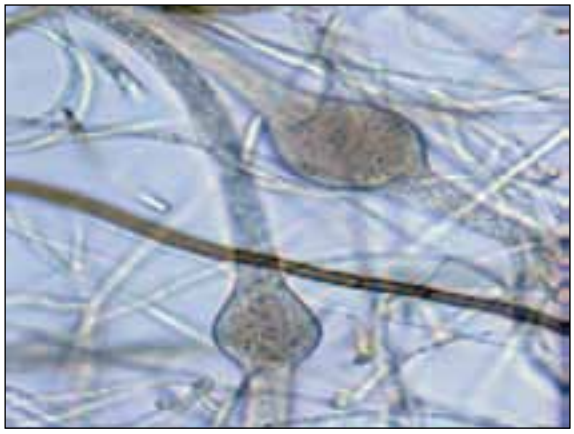


Fig. 19b. Hyphal swellings.

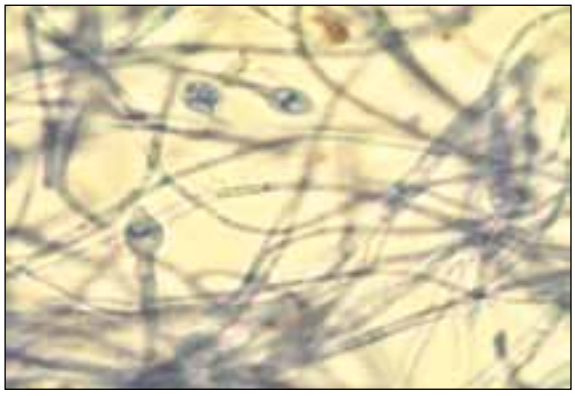


Fig. 20. Sporangia.

3.4 Screening techniques

3.4.1 Field screening

1. Select a well-levelled isolated plot where flood irrigation is possible.
2. Plant susceptible genotype (eg, ICP 7119) in the whole plot (Fig. 21).

Fig. 21. Sowing of susceptible genotype.

3. Inoculate all the plants 15 days after planting as given in the following steps.
4. Isolate *P. drechsleri* f. sp. *cajani* from infected pigeonpeas in your area on a specific medium V8 PARP (Pimaricin, Ampicillin, Rifampicin, Pentachloronitro benzene) medium (Fig. 22).
5. Prepare sand-pigeonpea flour medium (100 g) in 250 ml conical flasks and autoclave at 15 lb for 20 minutes. Inoculate each flask with the fungus and incubate at 25°C for 15 days with a 12 h photoperiod (Fig. 23).

Fig. 22. Pdc culture.

Fig. 23. Mass multiplication on sand-pigeonpea flour medium.

6. Rub a small amount of medium (from step 5) on the base of the stem of the 1-month old individual plants (Fig. 24). In another method, take the small amount of inoculum from step 5 and mix with soil near the collar region on 15 days old seedlings (Fig. 25). Follow steps 7-9 as given above.

Fig. 24. Rubbing medium on stem .

Fig. 25. Soil mixing.

7. Flood irrigate the field immediately after inoculation and again provide the frequent irrigations (twice a week), if dry weather is prevailing (Fig. 26).
8. Typical blight symptoms should appear in about 10 days.
9. One month later reinoculate the plants showing no symptoms (repeat 4-6).

Fig. 26. Temporary flooding after inoculation.

3.4.2 Greenhouse screening

3.4.2.1 Pot screening

3.4.2.1.1 Soil drenching

Raising of seedlings

- Pigeonpea seedlings of the test as well as susceptible genotypes are grown in the plastic pots (12 cm) filled with a mixture of sterilized alfisol and sand (9:1), kept in a greenhouse maintained at 28-30°C for 7-10 days (Fig. 27).
- Before sowing, seeds are surface sterilized using 2% sodium hypochlorite for 2 minutes, and rinsed in sterile water in order to wash off sodium hypochlorite.

Fig. 27. Raising of seedlings.

Inoculum preparation

- Obtain pure culture of Pdc isolated from naturally PB infected pigeonpea plants on specific medium (V8 PARP) (Fig. 22).
- Autoclave (15 lb; 20 min) V8 juice agar and pour the medium into petri dishes (20 ml per petri dish).
- Inoculate the medium in petri dishes with the fungus and incubate at 28-30°C for 1 week.
- Transfer 5 mm discs of the fungus growth to 100 ml autoclaved V8 juice broth in 250 ml flasks. Incubate at 28-30 °C for 2 weeks.
- The pathogen is mass multiplied on V8 broth or PDB (100 ml) in flasks kept on the shaker incubator at 25°C for 15 days with a 24 h photoperiod (Fig. 28).

Fig. 28. V8 broth.

Inoculation and transplanting

- Macerate the fungus inoculum (mycelial mat+broth) in a blender for 1-2 min by operating the blender intermittently.
- Dilute the suspension to get a final volume of 200 ml from each flask.
- Inoculate 7-10 days old seedlings by pouring 100 ml inoculum around the base of the seedlings in a pot (Fig. 29).
- Keep susceptible check, both inoculated and non-inoculated, with each batch of test genotypes.
- Water the pots 3-4 times at 2-3 h intervals daily.
- PB symptoms usually start appearing in 48 h. Take final observations 10 days after inoculation (Fig. 30).

Fig. 29. Soil drenching.

Fig. 30. Expression of symptoms.

3.4.2.1.2 Spray inoculation

Raising of seedlings

- Pigeonpea seedlings of the test as well as susceptible genotypes are grown in plastic pots (12 cm) filled with a mixture of sterilized alfisol and sand (9:1), kept in a greenhouse maintained at 28-30°C for 7-10 days (Fig. 27).
- Before sowing, seeds are surface sterilized using 2% sodium hypochlorite for 2 minutes, and rinsed in sterile water in order to wash off sodium hypochlorite.

Inoculum preparation

- Obtain pure culture of Pdc isolated from naturally PB infected pigeonpea plants on specific medium (V8 PARP).
- Autoclave (15 lb; 20 min) V8 juice agar and pour the medium into petri dishes (20 ml per petri dish).
- Inoculate the medium in petri dishes with the fungus and incubate at 28-30°C for 1 week.
- Transfer 5 mm discs of the fungus growth to 100 ml autoclaved V8 juice broth in 250 ml flasks. Incubate at 28-30 °C for 2 weeks.
- The pathogen is mass multiplied on V8 broth or PDB broth (100 ml) in flasks kept on the shaker incubator at 25°C for 15 days with a 24 h photoperiod.

Inoculation and transplanting

- Remove mycelial mat after 15 days from the flasks and wash twice with distilled water. Then macerate these mats with a small amount of water in a 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 100 ml water.
- Spray 7-10 days old seedlings in a pot (with hand sprayer) with 50 ml inoculum (Fig. 31a).
- Cover inoculated plants with polythene bags (Fig. 31b) to ensure high humidity for 48 h, then remove the bags and spray the plants with tap water every 2-3 h during the day until 96 h after inoculation. Afterwards, spray them 3 times a day until the final recording.

Fig. 31a. Spray inoculation.

Fig. 31b. Covering of inoculated plant with polythene bag.

- Initial symptoms can be seen on the 4th day after inoculation.
- Record the final data 10 days after inoculation.

3.5 Disease incidence

Disease incidence is recorded periodically and final disease incidence is calculated as described in section 1.5.

3.6 Resistant sources

Sources of durable genetic resistance to PB in pigeonpea are not available. The lack of a pigeonpea genotype resistant to PB is due to the difficulty in working with this host-specific Phytophthora in breeding programs because of frequent evolution of new pathotypes and co-existence of more than one pathotype at one location. Several sources of resistance to PB were identified in earlier studies conducted in different pigeonpea growing areas (Sharma et al. 1995, Reddy et al. 1990). Most of these lines were later found susceptible to PB under natural epiphytotic conditions in the Deccan Plateau (Sharma et al. 2006). However, a few lines found resistant to PB are listed in Table 4.

Table 4. Sources of resistance to Phytophthora blight of pigeonpea.

Genotypes	Remarks	Source
ICPs 7916, 12055, 12114, 12161, 13126, 15511, 7265, 6523, 15530, 7719, 7889, 8914, 9189, 10002, 12029, 7925, 12034, 12068, 12142, 12799, 12842, 13103, 13162, 13229, 13241, 13271, 13438, 13599, 13613, 13828, 13852, 14104, 15142, 15452	Resistant in the field and greenhouse	Sharma and Pande 2011
HPL 24-47, ICP 11376-5, ICP 11975, ICP 12730, ICP 12751, ICP 12755, ICPL 20093, ICPL 20096, ICPL 20099, ICPL 20100, ICPL 20101, ICPL 20104, ICPL 20105, ICPL 20109, ICPL 20114, ICPL 20115, ICPL 20122, ICPL 20124, ICPL 20125, ICPL 20126, ICPL 20127, ICPL 20128, ICPL 20135, ICPL 20136, ICPL 93179, ICPL 99044, KPBR 80-2-1, KPL 96053, KPBR 80-2-2-1	Resistant under natural epiphytotic conditions	Pande et al. 2006

4. Alternaria blight

4.1 Distribution and economic importance

The disease has been occurring in alarming proportions in recent years in major pigeonpea growing areas in India. It is affected by erratic rainfall pattern and is a serious problem in late sown (September), post rainy (pre-Rabi) pigeonpea crop, in India, Kenya and Puerto Rico. Precise information on the losses caused is not available. Disease incidence ranged from 20-80% irrespective of genotypes sown and the disease was to the extent of >80% in the improved pigeonpea genotypes and hybrids. Yield losses may reach 95% if the disease strikes in the early stages of the crop.

4.2 Diagnostic symptoms

The symptoms appear on all aerial parts of the plant (Leaves, stems, buds and pods). Symptoms on leaves are small, circular, necrotic spots that develop quickly, forming typical concentric rings (Fig. 32a). Later, these spots coalesce and cause blighting of leaves. The spots are initially light brown and later turn dark brown. On stems, spots are sunken, with concentric rings. In severe infection, defoliation and drying of infected leaves, branches and flower buds (Fig. 32b) is observed.

Fig. 32a. Concentric rings on the infected leaves.

Fig. 32b. Drying of flower and flower buds.

4.3 Causal organism

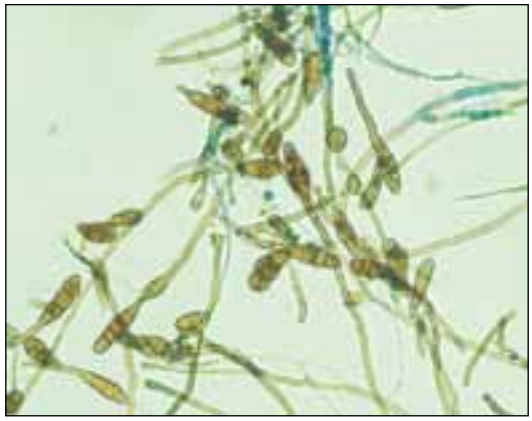


Fig. 33. Conidia.

Two species of *Alternaria* viz., *A. alternata* (Fr.) Keissler and *A. tenuissima* (Kauze ex Persoon) wilts are reported to cause leaf blight disease in India. The *A. tenuissima* colony is olive green with a black center. Conidiophores are short, arising singly. Conidia varied from 15.78 to 28.70 µm long and 8.03 to 13.47 µm wide. Very small beak (1.6 to 3.2 µm) or no beak was observed. Horizontal and vertical septation of conidia varied from four to six and two to four, respectively. The *A. alternata* produced brownish mycelia. Conidiophores were simple, olive brown, septate, variable in length with terminal conidia, which were solitary or in short chains. Mature conidia measure from 10-30 × 5-12 µm, short conical beak or beakless, narrowly ellipsoid to ovoid and elongated on branching chains (Fig. 33).

4.4 Screening techniques

4.4.1 Greenhouse screening

Spray inoculation

1. Fill 12 cm diameter plastic pots with sterilized soil. Sow 5-6 seeds per pot.
2. Obtain a pure culture of the fungus from infected pigeonpea in your area.
3. Autoclave (15lb; 20 min) PDA and pour the medium in petri dishes.
4. Inoculate the medium in petri dishes with the fungus and incubate at 28-30°C for one week.
5. Transfer 5 mm discs of the fungus growth to 100 ml autoclaved PDB in 250 ml flasks. Incubate at 28-30°C for two 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 blender for 1-2 min by operating the blender intermittently. Dilute this

suspension with tap water to get a final dilution with a conidial suspension of 5×10^5 conidia/ml (one mycelial mat in 200 ml water).

7. Spray 8-10 days old seedlings in a pot with 50 ml inoculum and incubate at 28-30°C in a greenhouse.
8. Cover inoculated plants with polythene bags to ensure high humidity for 48 h, and then remove the bags.
9. Ten days after inoculation, symptoms are observed on leaves.

4.5 Disease incidence

Disease incidence is recorded as given in section 1.5.

4.6 Resistant sources

A few resistant sources are reported by Nene et al. (1991) for Alternaria blight (DA 2, MA 128-1, MA 128-2, 20-105). No new sources of resistance have been reported in recent years.

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Appendix

1. Potato dextrose agar (PDA)

Peeled, sliced potatoes	-	200 g
Agar	-	20 g
Dextrose	-	20 g
Distilled water	-	1000 ml

Boil sliced potatoes in distilled water at 110°C for 10 min, collect the juice and add 20 g of dextrose and 20 g of agar to one litre of distilled water. Pour the PDA into flasks plugged with cotton plug and sterilize by autoclaving at 121°C at 15 lb pressure for 20 minutes. Before pouring PDA into petri plate, add streptomycin (antibiotic) with the concentration of 0.75 g per litre in order to avoid contamination of media.

2. Potato dextrose broth (PDB)

Peeled potatoes	-	200 g
Dextrose	-	20 g
Distilled water	-	1000 ml

Cut 200 g of peeled potatoes into small equal parts and add distilled water to it and boil for 10 minutes in an oven. Add 20 g of dextrose to 1 litre of the potato extract. Pour the PDB into flasks plugged with cotton plug and sterilize by autoclaving at 121°C at 15 lb pressure for 20 minutes.

3. Sand-pigeonpea flour medium

Sand	-	90 g
Pigeonpea	-	10 g
Distilled water	-	20 ml

Take a 250 ml conical flask and add 10 g pigeonpea flour with 90 g riverbed sand and add 20 ml distilled water to the flask.

4. V- 8 juice agar medium

V-8 juice agar medium is a product of the Campbell soup co. and it contains juices of tomato, carrot, celery, beet parsley, lettuce, spinach, watercress, vitamin C, and salts.

V- 8 juice	-	100 ml
$CaCO_3$	-	2 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	900 ml

Add this composition in distilled water, dissolve the chemicals and sterilize at 121°C, 15 lb pressure for 20 min. V8 agar consists of 20% V8 juice, 0.2% calcium carbonate, and 2% Difco agar, adjusted to pH 6.0-6.5 with sodium hydroxide. Cleared V8 broth contains V8 juice (100 ml), calcium carbonate (4 g), and distilled water (100 ml). Centrifuged this at 5000 g for 20 min, and filter the supernatant through Whattman no.1 filter paper. Dilute this up to 10 fold with glass distilled water, adjust pH to 6.0-6.5 and sterilize by autoclave at 15 psi for 20 min.

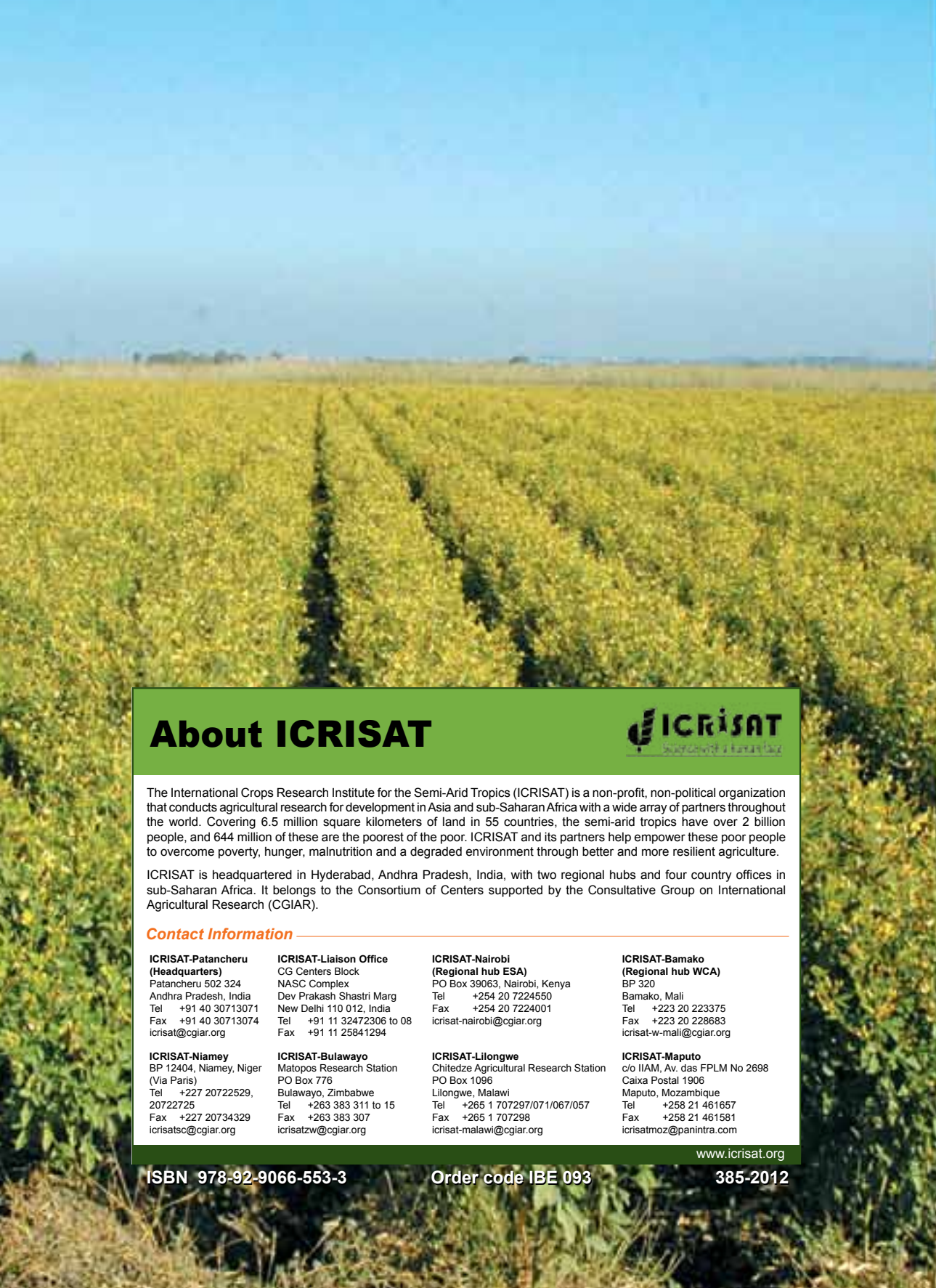
5. V8 PARP (Pimaricin Ampicillin Rifampicin Pentachloronitro benzene)

Pimaricin	-	400 µl/l
Ampicillin	-	250 mg/l
Rifampicin	-	1 ml/l
Pentachloronitro benzene (PCNB)	-	5 ml/l
V- 8 juice	-	100 ml
$CaCO_3$	-	2 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	900 ml

Preparation of PARP

Pimaricin	-	Prepare 2.5% aqueous solution of pimaricin.
Rifampicin	-	10 mg Rifampicin diluted in 1 ml DMSO
PCNB	-	1 gm PCNB diluted in 200 ml ethanol. Provide gentle heating in waterbath at 70°C.

Suspend 44.3 g of V8 agar in 100 ml distilled water and sterilize it. Add PARP in media at the time of pouring.



About ICRISAT

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a non-profit, non-political organization that conducts agricultural research for development in Asia and sub-Saharan Africa with a wide array of partners throughout the world. Covering 6.5 million square kilometers of land in 55 countries, the semi-arid tropics have over 2 billion people, and 644 million of these are the poorest of the poor. ICRISAT and its partners help empower these poor people to overcome poverty, hunger, malnutrition and a degraded environment through better and more resilient agriculture.

ICRISAT is headquartered in Hyderabad, Andhra Pradesh, India, with two regional hubs and four country offices in sub-Saharan Africa. It belongs to the Consortium of Centers supported by the Consultative Group on International Agricultural Research (CGIAR).

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