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Screening Techniques for Ascochyta Blight of Chickpea

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A joint contribution from ICRISAT and ICARDA centers

Blight caused by *Ascochyta rabiei* (Pass.) Lab. is the major disease of chickpea in North Africa, West Asia and South and East Europe. The use of resistant cultivars is considered to be the best way of controlling the disease. Though considerable work has been done on the development of screening techniques, rating scales and other related aspects, simple and reliable techniques for large-scale field screening were not available. The earlier methods of inoculation for field screening consisted of spraying with a pycnidiospore suspension from the pure culture of the fungus and covering the plants with "Sarkanda" screens or cloth tents, or spreading the diseased chickpea debris, or dried culture of the fungus over the plants, or mixing the diseased plant debris with seed at the time of sowing and depending on natural rainfall for disease development (Luthra *et al.* 1938; Grewal and Vir 1974; Bedi 1949).

These methods were not adequate for large-scale field screening of germplasm lines and breeding materials. Similarly, greenhouse or pot culture screening techniques for large-scale screening were not available. Lack of reliable field-screening techniques for large-scale screening of germplasm lines and breeding materials, hampered the progress on resistance breeding. A need for further standardization of both field and laboratory screening techniques as well as rating scales was felt. The results of the field and greenhouse screening techniques and the rating scales standardized for evaluating the materials are presented in this paper.

Field-Screening Techniques

During the 1977-78 season, when research on ascochyta blight of chickpea started at ICARDA, a great need was felt for a simple, efficient and reliable

field-screening technique. Based on the experience gained during the 1977-78 season, when a spray with pycnidiospore suspension prepared from the naturally infected plants under natural rainfall conditions did not provide sufficient disease pressure (none of the susceptible checks were killed), debris method of inoculation (Luthra *et al.* 1938; Sattar and Hafiz 1951) coupled with supplementary sprinkler irrigation was considered. The method was tested during the 1978-79 season and the results were excellent. Singh *et al.* (1981) described the technique which involved a combination of inter-planting a susceptible spreader line, scattering between rows infected debris collected from the previous season, spraying pycnidiospores harvested from the infected plants of a susceptible line, and providing sprinkler irrigation. This technique has been further standardized (Reddy *et al.* 1980) and the results of the experiments and observations made are presented here.

Type of Debris

During the 1978-79 season, diseased debris was cut into small bits and was used for inoculation, whereas during the 1979-80 and 1980-81 seasons diseased stalks as such were used. Based on the disease severity obtained during these 3 years, it appears that the latter is superior to the former.

Spreader Rows vs. Whole Plot Inoculation

The disease severity was compared between plots with only the spreader rows (every third or fifth) inoculated and the entirely inoculated plots. The disease spread and severity was found to be greater in entirely inoculated plots than plots where only the spreader rows were inoculated. The disease spread was slightly faster in plots where a spreader row was sown after every two test rows than in those where spreader rows were sown after every four test rows.

Frequency of Indicator-cum-Spreader Rows

The disease severity in plots where the indicator-cum-spreader rows sown after every 2, 4 and 8 test rows were inoculated and in those where the entire plots were inoculated was found to be the same under sprinkler irrigation. This suggests that spreader rows in the debris method of inoculation do not play a significant role in disease spread and serve mainly as indicator rows.

Row Direction

Planting of rows in East-West or North-South direction was found to have no effect on disease severity in plots that were inoculated entirely. In plots where only the spreader rows were inoculated, the disease spread was slightly faster in plots where the rows were planted in a North-South direction as the wind direction was East-West.

Date of inoculation: Inoculations with diseased debris from December through

early February were found to give equally severe infections with sprinkler irrigation under ICARDA farm conditions. Inoculation after February without sprinkler irrigation was found unreliable.

Methods of inoculation: The efficiency of different methods of inoculation with and without sprinkler irrigation was compared. The inoculation methods compared were (i) diseased debris, (ii) pycnidiospore suspension from pure culture of the fungus, and (iii) freshly infected plants. Under sprinkler irrigation all the three methods gave equally severe infection. Without sprinkler irrigation, the debris method was found to be superior. The optimum time for immersing infected plants in water for the maximum release of spores was found to be one hour.

Flat vs. Ridge Planting

Observations on flat and ridge plantings indicated a comparatively fast and severe disease development in flat plantings.

Effect of Irrigation Water

To determine whether the water used for sprinkler irrigation has any effect on disease development, the germination of spores in irrigation water and sterile distilled water was compared, but no appreciable difference was found.

Viability of Pycnidiospores in Diseased Debris

The viability of pycnidiospores was studied in (i) 1-year-old diseased debris stored dry, (ii) diseased debris left over in the field from previous season, (iii) freshly infected plants, and (iv) pure culture of 10-day-old fungus. The percent germination of spores after 12 hr incubation in the above treatments was 9, 55, 56 and 59, respectively. But the germination of spores from infected debris increased with time indicating that they needed more time for germination.

Effect of Plant Spacing

To determine if the plant spacing has any effect on resistance, reaction of a set of lines that included six resistant, four tolerant, nine susceptible, and six highly susceptible lines was studied at two inter-row spacings of 20 and 30 cm. No difference was found (Reddy and Singh 1980).

The method of inoculation with a pycnidiospore suspension of the fungus followed by covering the inoculated plants with screens or cloth tents used by Luthra *et al.* (1938) and Grewal and Vir (1974) is not practicable for large-scale adoption. The debris method of inoculation (Luthra *et al.* 1938, 1941; Sattar and Hafiz 1951; Vedysheva 1966) and the use of dried cultures of the fungus (Bedi 1949) under Syrian conditions where the rainfall is low, were found inadequate for creating high disease pressure.

Uniform inoculation in the first fortnight of February with coarse diseased

debris of chickpea collected from the previous season coupled with sprinkler irrigation when necessary was found to be simple, efficient and reliable for a large-scale field screening.

Bedi (1949) claimed that the method of using dried cultures of the fungus was superior to the debris method because in the debris method, inoculations had to be delayed until sufficient fresh blighted material became available and the diseased material from the previous season could not be relied upon due to the low viability of the fungus. The present study indicated that the debris collected in the previous season can safely be used. Luthra *et al.* (1938) and Kaiser (1973) claimed that the fungus in diseased debris remained viable for more than 3 years.

Further, the debris method can be more useful in places where no laboratory facilities or pathologists are available. The only lacuna in this method is that there is no exact monitoring of either the quantity or quality of the inoculum being used every year. But this may be a blessing in disguise as it is more close to what happens in nature. The fact that none of the lines changed their reactions significantly during the 3 years of screening indicates that this does not pose a problem as far as the reliability of the results is concerned. In field inoculations, the pycnidiospore suspension spray either from a pure culture of the fungus or freshly infected plants can play a supplementary role when the disease severity is low due to insufficient diseased debris or any other reason.

Greenhouse or Pot Culture Screening Technique

The greenhouse or pot culture techniques used earlier for chickpea blight consisted of inoculating 10- to 40-day old plants grown in pots by spraying with a pycnidiospore suspension prepared from the pure culture of the fungus and by incubating them in humidity chambers for 2 to 6 days (Kaiser 1973; Chauhan and Sinha 1973; Vir and Grewal 1974). At ICRISAT Center, near Hyderabad, India where asochyta blight does not occur naturally, a need was felt for a greenhouse-screening technique to screen large numbers of germplasm. Initially, the usual methods of inoculating the potted plants and incubating them in humidity chambers were tried but were found unsatisfactory.

Isolation Plant Propagator Method

An isolation plant propagator manufactured by Burkard Manufacturing Co. Ltd., Rickmansorth, Herts, U.K., originally designed for the growing of healthy barley seedlings for epidemiological studies on powdery mildew was found to be very ideal for screening chickpea for ascochyta blight at ICRISAT Center. The unit mainly consists of a motor, four chambers in two tiers with filtered air being supplied to each of them through thick plastic pipes. Each chamber consists of a metallic tray covered with a wooden plank with holes for placing the pots. The

pot assembly consists of a stem that rests in the tray and passes through the bottom of the pot and with a plastic cover with two small holes in the top. The water is fed to the pot with cotton wicks. Each tray accommodates 30 pots and thus each unit 120 pots. When the motor is on, filtered air is sucked in and distributed to the trays filled with water to a certain level, becomes cooled, passes through the stems and builds up a pressure under the cover so that no external spores can enter the pots. High humidity maintained under the covers was found to help the blight development.

To facilitate better growth of chickpea seedlings, they were placed in a glasshouse where the temperature was maintained around 25°C through the help of fans and desert coolers. The unit was slightly modified by providing additional light to the lower chambers with four, 4-ft long 60 watt fluorescent tubes at the bottom of each of the two top trays. Using two such units, about 8000 lines were screened during a 3-year period.

For screening the germplasm, 10- to 15-day old seedlings of each accession (10 seedlings) in a single pot were inoculated by spraying with a spore suspension from a pure culture of the fungus. For inoculations, 10- to 15-day old culture multiplied on chickpea seed meal dextrose broth (80 g chickpea seed meal, 20 g dextrose, 1 liter water) and incubated at 20-25°C with 12 hr intermittent light was used. The concentration of spores in the suspension was 20,000 to 40,000/cc. Approximately 1.5 cc of spore suspension was sprayed on each seedling. Immediately after inoculation, the seedlings were covered with plastic covers. Symptoms usually developed in 4-6 days and the susceptible lines were completely killed in 10-15 days after inoculation. The technique can be very useful in studies on races.

Plastic House Screening

A plastic house provided with a perfo-irrigation system and temperature maintained at 20-25°C was found to be extremely suitable for pot culture screening at ICARDA Center. 10- to 15-day old plants grown in pots were inoculated by spraying with a pycnidiospore suspension of the pure culture of the fungus.

After inoculation, the perfo-irrigation was run for half an hour twice a day for 5 days. The symptoms appeared 7-10 days after inoculation and the susceptible lines were killed within 1 month after inoculation. Good correlation was found between disease ratings in field and plastic house screenings.

Disease Rating Scales

Six rating scales have been devised and used by various workers for scoring the blight severity (Aujla 1964; Aujla and Bedi 1967; Morrall and McKenzie 1974; Grewal and Vir 1974). To facilitate rapid evaluation of lines under pot culture conditions, Reddy and Nene (1978, 1979) evolved a 9-point scale. The scale has been described in the paper by Nene in these proceedings.

Singh *et al.* (1981) devised a modified 9-point scale for scoring the materials under field conditions where:

1 = No lesions visible on any plant (highly resistant)
3 = Lesions visible on less than 10% plants, no stem girdling (resistant)
5 = Lesions visible on up to 25% plants, stem girdling on less than 10% plants, but little damage (tolerant)
7 = Lesions present on most plants, stem girdling on less than 50% plants, resulting in the death of a few plants and causing considerable damage (susceptible)
9 = Lesions profuse on all plants, stem girdling present on more than 50% plants, and death of most plants (highly susceptible).

Though the 9-point rating scales were found to be very simple and practicable for a large-scale field evaluation of materials grown in a row or plot, some difficulty was experienced in rating the individual plants in segregating populations as the exact quantification of damage done to various parts of the plant was not specified under each reaction category. Even though chickpea is a highly self-pollinated crop, considerable variation in the reaction of individual plants of a line was observed. Leaf and stem lesion types and their combination considered by earlier workers (Aujla 1964; Grewal and Vir 1974) for categorizing the lines was found to be inadequate as considerable variation within and among the plants of a line was observed for such reaction. Further, the infection on pods was not considered in any of the previous scales. In order to arrive at a more accurate quantitative rating scale specifying the amount of damage done to various parts of the plant under each reaction category, the damage caused to various parts of the plant in lines representative of each reaction category was measured. The results are presented in Table 1. Based on these results a diagrammatic representation of 1-9 scale is proposed (Fig. 1).

Summary

Blight caused by *Ascochyta rabiei* (Pass.) Lab. is a major disease of chickpea in North Africa, West Asia, Pakistan and southern and eastern Europe. Importance of resistant cultivars in control of blight has been emphasized ever since early reports of the disease were made. Since simple and reliable screening techniques are essential for a successful resistance breeding program, a need was felt for further standardization of field and greenhouse screening techniques and rating scales.

A simple, efficient and reliable field-screening technique has been standardized at ICARDA and is being used for large-scale field screening of germplasm lines and breeding materials. The technique consists of sowing a susceptible cultivar in the field at frequent intervals to serve as indicator-cum-spreader line,

Table 1
A quantitative 9-point rating scale for Ascochyta blight of chickpea.

Disease Rating	Reaction category	Percent buds killed	Percent foliage infected	Percent stems with lesions	Percent stems broken	Stem lesion type	Leaf lesion type	Percent pods with lesions
1	Highly Resistant (HR)	Nil	Nil	Nil	Nil			Nil
2	Highly Resistant-Resistant (HR-R)	Nil	1.0	Nil	Nil		Necrotic with no or very few pycnidia	Nil
3	Resistant (R)	0–2.5	5.0	80.0	5.0	No lesions girdling	Necrotic with few pycnidia	5.0
4	Resistant-Tolerant (R-T)	0–5.0	20.0	80.0	15.0	2 mm long girdling	Necrotic with few pycnidia	15.0
5	Tolerant (T)	10.0	40.0	100.0	40.0	2 mm long girdling	Necrotic with large number of pycnidia	40.0
6	Tolerant-Susceptible (T-S)	25.0	50.0	100.0	50.0	2 mm long girdling	Necrotic with large number of pycnidia	50.0
7	Susceptible (S)	40.0	75.0	100.0	75.0	75% girdling	Necrotic with large number of pycnidia	75.0
8	Susceptible-Highly Susceptible (S-HS)	100.0	90.0	100.0	100.0	100% girdling	Necrotic with large number of pycnidia	100.0
9	Highly Susceptible (HS)	Plants completely killed						100.0

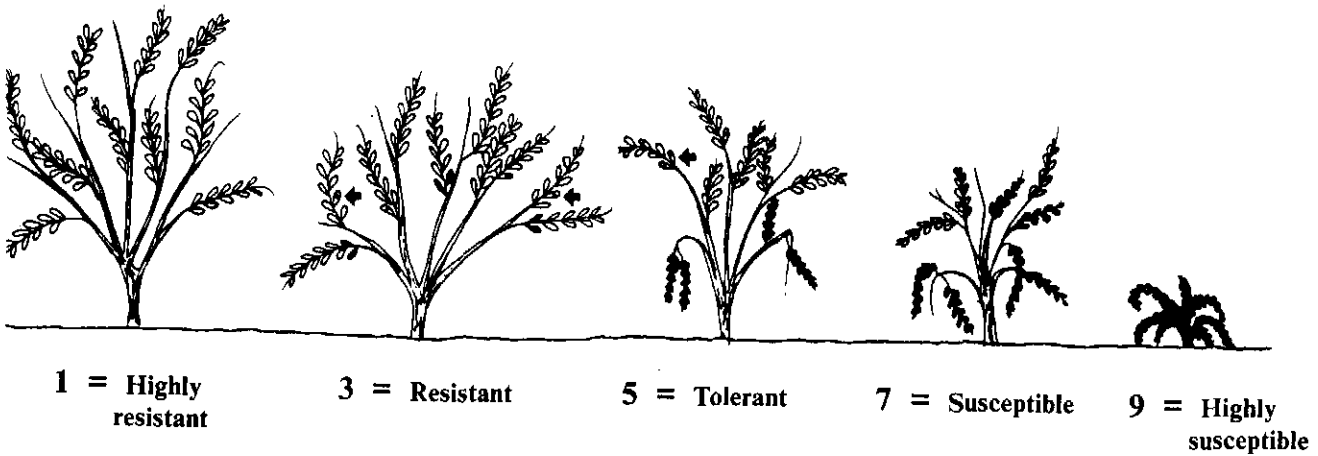

Figure 1. Diagrammatic representation of a 9-point rating scale for Ascochyta blight of chickpeas in the field.

inoculating the plants by uniformly scattering the diseased plant debris collected from the previous season throughout the field and providing sprinkler irrigation as and when necessary.

Satisfactory greenhouse and pot culture techniques were also standardized for the screening of germplasm. An isolation-plant propagator was found suitable for screening the germplasm round the year at ICRISAT center, where blight does not occur naturally. A plastic house provided with perfo-irrigation facility and temperature control was found to be very effective for pot culture studies at ICARDA. These techniques could also be useful at other locations.

A good correlation was found between the results of field screening by debris method of inoculation and the plastic house screening using the fungus pycnidiospore suspension.

A 9-point disease rating scale was devised and is being used for field and pot culture observations. A more comprehensive quantitative scale for scoring the segregating populations is proposed.

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