Techniques for Screening Sorghums for Resistance to *Striga*

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Abstract

Vasudeva Rao, M.J. 1985. Techniques for screening sorghums for resistance to *Striga.* Information Bulletin no. 20. Patancheru, A.P. 502324, India: International Crops Research Institute for the Semi-Arid Tropics.

Striga is a root parasite of cereals and legumes which causes serious losses to host crops. Breeding genotypes of host crops with resistance to this parasite is recognized as the most economic way to combat *Striga.* However, the breeding progress has been slowed by the absence of valid screening techniques. In this bulletin all laboratory and field methodologies currently used for screening *Striga* resistance are described in detail. New screening techniques such as the three-stage methodology and the checkerboard layout techniques are discussed at length. Several suggestions are offered to assist the development and management of Striga-sick fields.

Resume

Vasudeva Rao, M.J. 1985. *(Techniques de criblage des sorghos pour la resistance au* Striga.) Techniques for screening sorghums for resistance to *Striga.* Information Bulletin no. 20. Patancheru, A.P. 502324, India : International Crops Research Institute for the Semi-Arid Tropics.

Le *Striga* est un parasite des racines des cultures cerealieres et legumieres qui cause des pertes importantes de recolte. La selection de genotypes de plantes-hdtes resistantes au *Striga* est generalement reconnue comme le moyen le plus economique de lutter contre ce parasite. Cependant, les progres dans la selection ont *ete* lents en raison du manque de techniques de criblage valables. Ce bulletin expose en detail toutes les methodes de criblage utilises actuellement au laboratoire et au champ pour la resistance au *Striga.* De nouvelles techniques de criblage sont examinees, en particulier la methode des essais en trois etapes et les dispositifs en damier. Plusieurs suggestions sont faites pour la preparation et le maintien des parcelles infestees par le *Striga.*

Cover photographs: *Striga hermonthica* (left) and *Striga asiatica.*

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Techniques for Screening Sorghums for Resistance to *Striga*

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Introduction

Striga spp. are root parasites of cereals and legumes. They cause serious economic losses to a range of host plants, primarily sorghum and pearl millet. Although more than 60 species have been described in the genus *Striga,* only seven are considered economically important: S. *hermonthica,* S. *asiatica, S. densiflora, S. euphrasioides, S. aspera, S. forbesii* (all specific to cereals); and S. *gesnerioides* (specific to dicots). Breeding genotypes resistant to *Striga* is recognized as the most economic way to avert the losses caused by *Striga.*

Precise and reliable screening techniques are indispensable prerequisites in breeding for resistance to any yield reducer. In the case of the parasitic weed *Striga,* the establishment on a host root and its subsequent emergence are influenced by complex interactions between the *Striga,* the host, and edaphic and atmospheric environmental factors. The development and use of efficient screening systems in *Striga* resistance breeding has been slow, but with the increasing urgency to alleviate Striga-caused losses all over the semi-arid world, there has been increased emphasis on breeding Striga-resistant sorghum and millet varieties. Accompanying this breeding work is an increased need to develop efficient resistance screening systems. This publication describes the prevailing screening systems and discusses their usefulness.

Influence of the host root in determining the fate of the parasite's establishment occurs in three developmental stages of the parasite: seed germination, haustorial establishment, and subsequent growth and reproduction of the *Striga* plant. Low stimulant production, mechanical barriers, and antibiosis have been identified as mechanisms conferring *Striga* resistance in sorghum. The latter two were termed anti-haustorial factors (Saunders 1933, 1942). To determine host resistance, researchers have adopted either of two approaches: screening for an individual mechanism (primarily in the laboratory), or screening for low emergence frequency of *Striga* plants, which may involve one or more resistance mechanisms (mostly in the field).

Techniques to Identify Low Stimulant Production

Sorghum varieties or plants whose roots produce either none or very little of a stimulant which triggers the germination of preconditioned *Striga* seeds may be identified using these techniques. In general, they have three stages:

- root exudate production and extraction,
- *Striga* seed preconditioning, and
- assessing the germination of preconditioned *Striga* seeds by exposure to the root exudate.

Double Pot Technique

The double pot technique (Figure 1) was developed by Parker et al. (1977) and has been extensively used to identify low stimulant producing sorghum lines (Vasudeva Rao 1984; Vasudeva Rao et al. 1983b). However, it has been used to a lesser extent to screen pearl millet lines (Parker et al. 1977). There are three stages in this procedure:

Root Exudate Production

Sorghum seeds are surface sterilized using 1% sodium hypochlorite solution for 25 minutes, washed with distilled water until the chlorine odor disappears, and incubated for 24 hours at 25°C for germination. Fifteen germinated sorghum seeds are placed on 150 g washed and heat sterilized quartz sand in a 6 cm diameter container (Figure 1a) with a perforated base (small pot, carton, ice cream cup, or styrofoam cup). Another 100 g of sand is added. This is then placed in another identical container without perforations and 25 ml of distilled water added. The 'double pots' are then maintained by adding 15 ml distilled water daily. The excess water which drains into the outer pot is discarded.

The root exudate (solution in the root zone) will be ready for germination assay after the seventh day. The root exudate is removed from each pot with a suction pump. The seedling container is held tightly on a funnel which is placed inside a conical flask with a side tube connected to a suction pump. The funnel stem ends in a test tube inside the conical flask. The root exudate is collected in the test tube when the suction is started (Figure 1 b).

Preconditioning *Striga* Seed

This stage is to induce physiological changes (preconditioning) in the *Striga* seeds so that they respond to the germination stimulant.

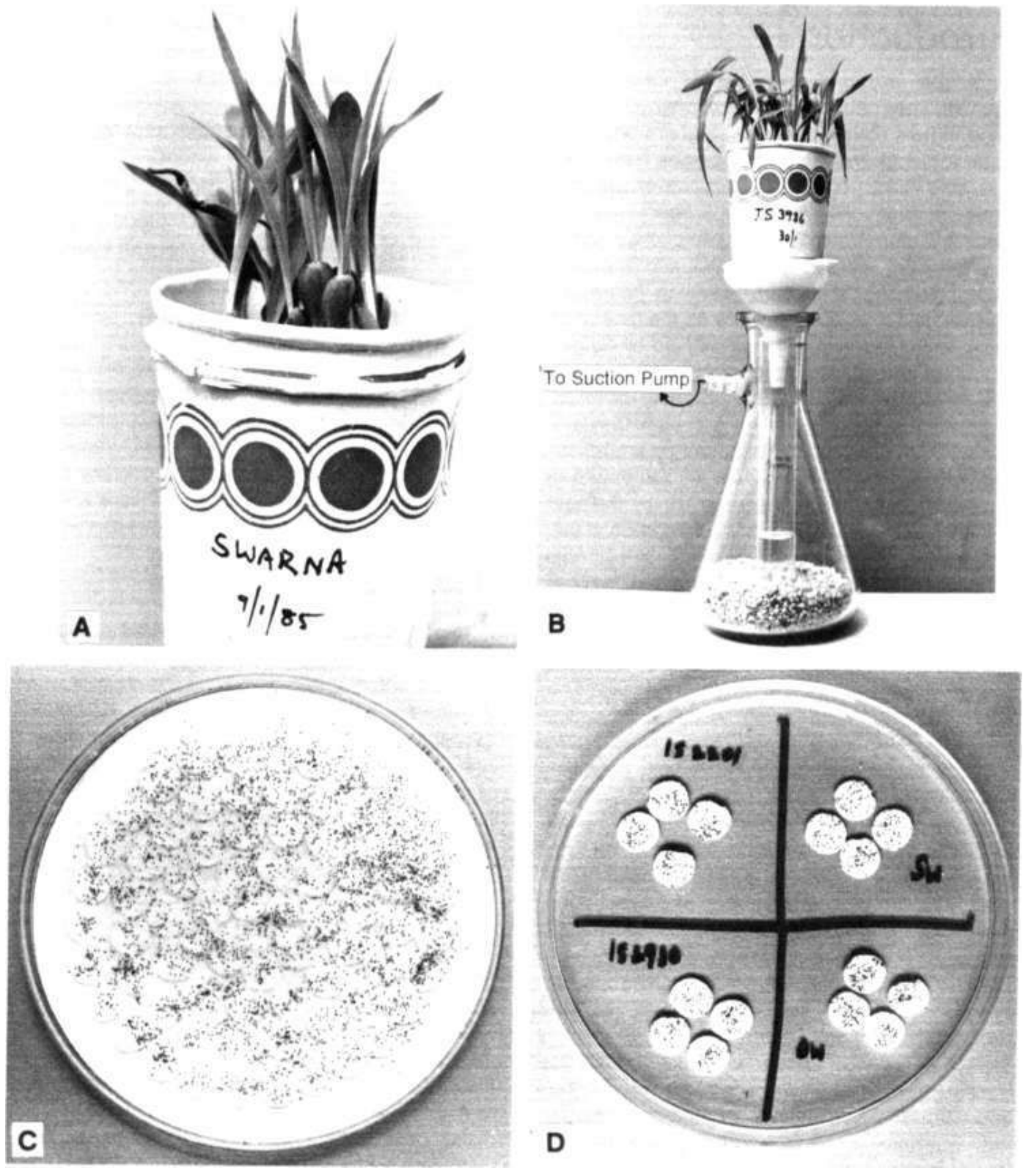

Figure 1. Double pot technique: a. The double pot. b. Root exudate, c. Preconditioned Striga seeds, d. Germination assay, e. Microdosimeter with disposable tip.

Striga seeds are surface sterilized by soaking in a 1 % sodium hypochlorite solution for 5 minutes, then washed with distilled water on filter paper in a Buchner funnel until the chlorine odor disappears. The seeds are then dried and stored in small corked vials. Two layers of full circles of glass fiber filter paper are placed in a petri dish lid and wet with distilled water. Small discs cut from a stack of glass fiber filter paper are arranged on the moist paper in the petri dish lid. The sterilized, dried *Striga* seeds are carefully sprinkled on the discs (approximately 25/disc) (Figure 1c).

The entire petri dish is then wet with more distilled water so that the *Striga* seeds are sufficiently moistened. The base of the petri dish is used to cover the bigger half (lid), and sets of the petri dishes are placed horizontally (so that no portion of the petri dish gets excess moisture) in an incubator in the dark. The *Striga* seeds are incubated between 23 ° and 33 °C for 10-14 days, after which they become responsive to the germination stimulant from the host root. At lower temperatures, more preconditioning days are required. Germination responses of different *Striga* species and samples to different preconditioning temperatures and durations vary and have specific response peaks.

Root Exudate Testing for Stimulant Presence

Discs containing the preconditioned *Striga* seed are removed from pretreatment dishes and excess moisture removed by dabbing on a dry filter paper. Four replicates of each *Striga* sample are used for every exudate sample to be tested. It is best to take one disc from each of four different pretreatment dishes. Up to five areas can be marked in each petri dish with a felt-tip marker so that multiple exudates can be tested in a single petri dish (Figure 1d). Normally, two replicate pots are used for each host line and exudates from the two replications are tested in seperate petri dishes.

An exact quantity (20µl) of the freshly drawn root exudate is applied to each disc using a standardized micrometer syringe, micropipette, or microdosimeter (Figure 1e). Disposable plastic tips are used only once and changed for each exudate. The petri dishes are covered, enclosed in polyethylene bags, and incubated in darkness at 33 °C for 24 hours. At the end of the 24-hour period, the number of germinated *Striga* seeds and the total number of seeds per disc are counted. Germination percentages are calculated.

In order to compare host genotypes across batches, a high stimulant producing variety is introduced as a check in every batch. The germination percentage obtained from the root exudate of test entries is expressed as a percent of that obtained from the check variety root exudate in that batch. At ICRISAT, where nearly 15000 sorghum germplasm lines have been screened for their stimulant production, Swarna (released name CSV-1) has been used as the high stimulant check. To check the spontaneous germination of *Striga* seeds, a water check is also included. This check uses water drawn through sand in the double pot system, but the sand is not planted with any host plant. For the screening to be effective, the high stimulant check must give high levels of germination and the water check must give no germination.

Pasteur Pipette Technique

This technique is used to assay root exudates of single plants for their ability to stimulate germination of preconditioned *Striga* seeds (Parker et al. 1977). The technique is useful in genetic studies involving segregating material. Sterilized sorghum seeds are germinated in petri dishes as in the double pot technique, and transferred to sand-filled petri dishes where the radicles are permitted to grow straight down for 1 -2 days. Seedlings are then transferred to Pasteur pipettes (Figure 2) with sealed tips which are filled with distilled water. Seedlings are held in the pipettes with filter paper or cotton. The pipettes with the seedlings are mounted in a rack, enclosed in a black polythene bag, kept in darkness at 25-33 °C for two days, and then transferred to normal light conditions. The root solution in the pipette could be assayed after 3-4 days for its ability to germinate *Striga* seeds as described in the double pot technique.

Sandwich TeChnique

Preconditioned *Striga* seeds placed between two glass fiber filter paper discs, referred to as a sandwich (Figure 3b), are also used to screen sorghum roots for *Striga* stimulant production (Eplee 1975). Sandwiches are placed either on host seedling roots grown in petri plates, on moist filter papers placed in petri plates in which host seedlings are

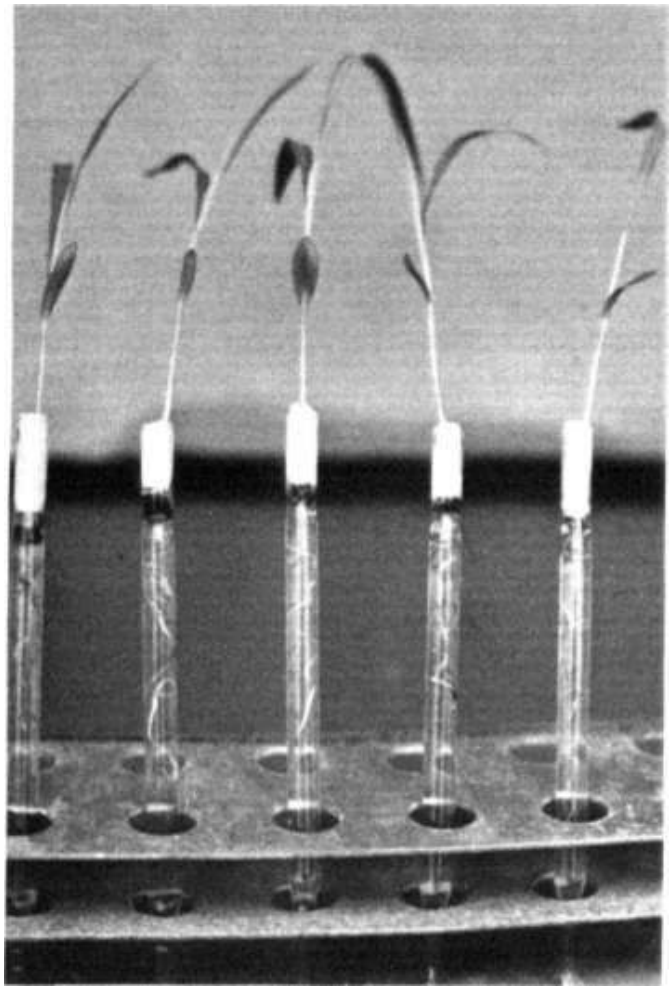

Figure 2. Pasteur pipette technique.

grown (but away from the host roots), or even buried in the soil in pots without actual contact with the host roots. To do this, the potted soil is separated from the pots by gently inverting and tapping the pot at proper soil moisture (Figure 3a).

Striga sandwiches are gently pressed on the exposed soil surface at various depths and the pot is remade by lowering the pot on the inverted potted soil. *Striga* germination counts in the sandwiches are made by removing the sandwiches from the pots after 24-48 hours. This is a useful technique to study the effects of host root stimulant on *Striga* seed germination after it has interacted with the soil. The Second International *Striga* Workshop concluded that this technique has not given consistent correlation with the double pot technique, but could perhaps correlate with field resistance if its sensitivity is reduced to get better differentials between test varieties (ICRISAT 1983b).

'Eplee Bag' Technique

This technique involves germinating *Striga* seeds in 'Eplee bags' buried at various soil depths in pots or in field plots. The Eplee bags (Figure 4b) are made by tieing approximately 100 *Striga* seeds in a 3 x 3 cm piece of 'nitex' nylon monofilament screen cloth with a mesh opening of 116 microns (Eplee

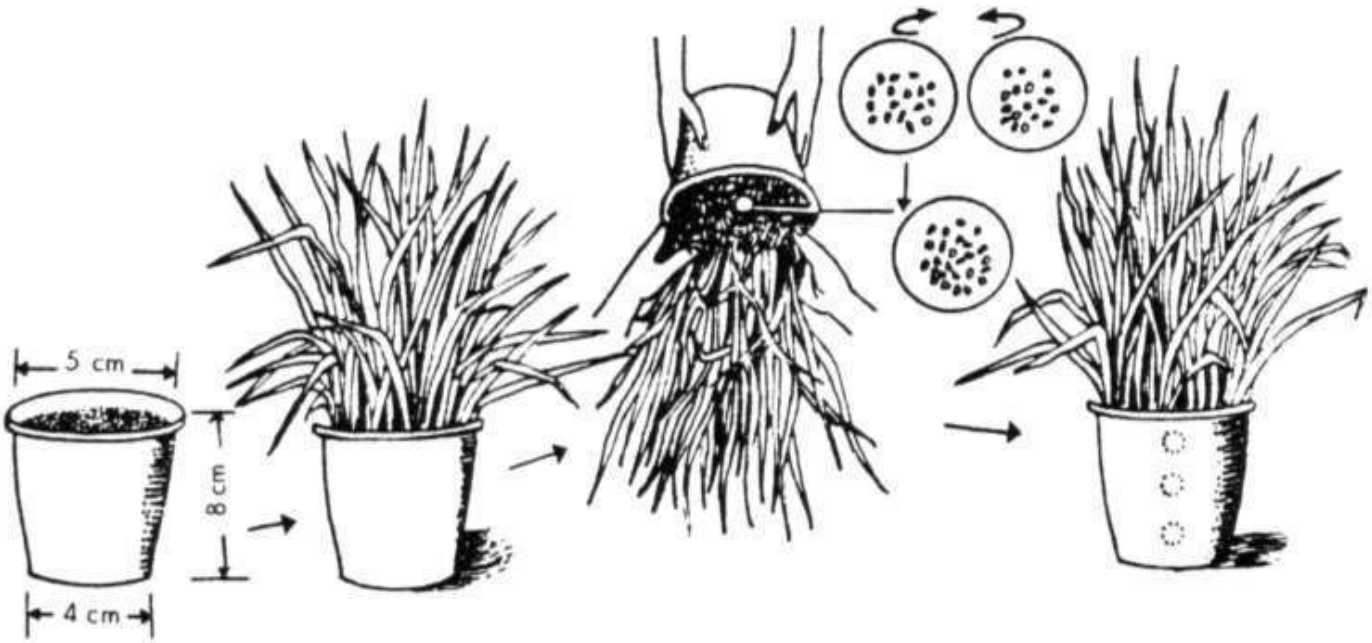

Figure 3a. Stages in germination assay using sandwiches (Source: M. M. Hosmani, University of Agricultural Sciences, Bangalore, India).

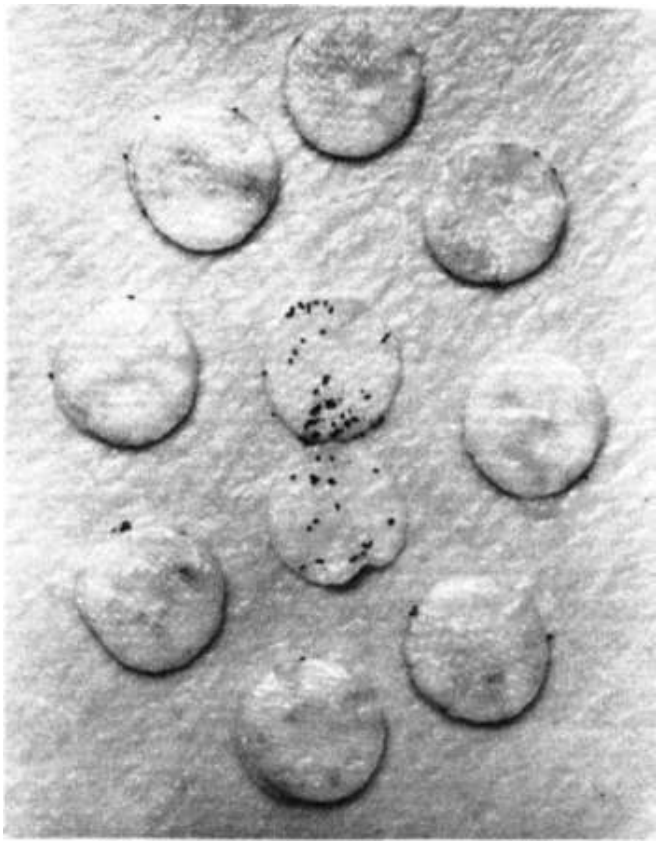

Figure 3b. *Striga* sandwiches using discs of filter paper. Center sandwich is open to show seeds.

1975). The pores in the cloth allow ample passage of the gases, moisture, and the stimulant into the bags to allow seed germination. The bags are placed in a hole in the soil at the desired depth and distance away from the plant, and the hole is refilled. A string attached to the bag before burial is used to recover it. To determine the *Striga* seed germination, the retrived bags are opened, the germinated and ungerminated seeds counted, and the germination percentage calculated as a percent of the total seeds in the bag.

The Eplee bag technique has been extensively used to study the effects of ethylene and other synthetic germination stimulants on *Striga* seed germination in situ. Currently no published information is available on the use of Eplee bags for host resistance screening, but it would be interesting to see if this technique could be used. The advantages of this technique are the visual assessment of *Striga* seed germination after the germination stimulant has interacted with the soil, and reduced disturbance to a host plant either in the pot or field. In addition, this technique can be used at any stage of crop growth.

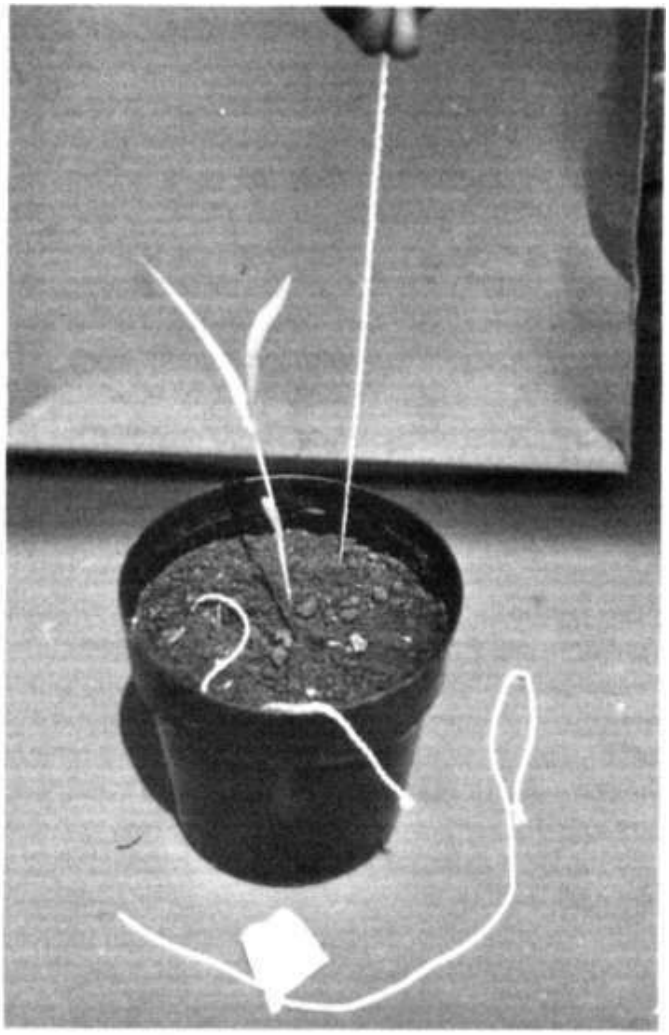

Figure 4a. The Eplee bag technique.

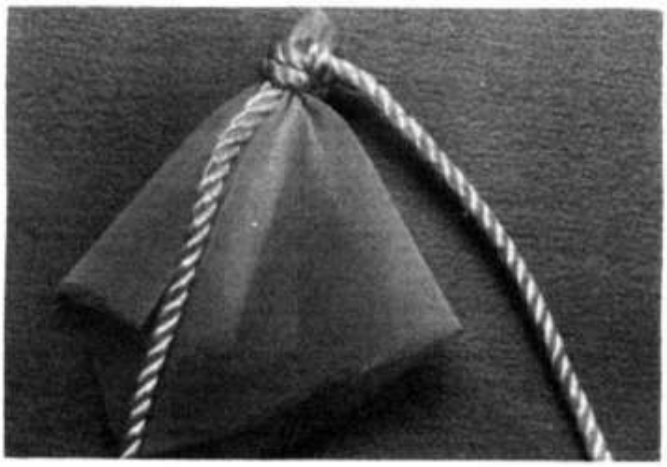

Figure 4b. Eplee bag.

Techniques to Identify Anti-Haustorial Factors

Two factors—mechanical and chemical—are known to confer resistance to *Striga* parasitization

in sorghum roots. The mechanical factors create an obstruction, while the chemical factors create a physiological hinderance during the process of *Striga* parasitization. The mechanical factor works through a timing mechanism to induce early thickening of the root endodermal cell walls and deposition of silica crystals in those walls. The same thickening occurs later in susceptible host roots (ICRISAT 1978). It is difficult to clearly differentiate the effects of these two factors because some chemical factors like lignins mechanically block parasitization, thus becoming mechanical hinderances. Together, these have been named as 'anti-haustorial' factors and are reported to be independent of the low stimulant mechanism.

Although these mechanisms were predicted based on limited experimentation (Saunders 1933, 1942), proper techniques to screen sorghum lines for anti-haustorial factors were reported only recently (ICRISAT 1978). Although this laboratory technique, named 'root slope technique', does not recognize and measure the effects of chemical resistance, it does permit the chemical factors to act and their effects to be measured along with the mechanical factors. Briefly the procedure is as follows:

- Sorghum seedlings are grown on moist filter paper and the root growth monitored for 15 days (i.e., 1- to 15-day-old roots are identified).
- Ready-to-germinate *Striga* seeds are inoculated on root regions of different ages.
- Susceptible host-root exudate or synthetic stimulant is applied to germinate the *Striga* seeds.
- After 7-10 days, the stage of establishment of the *Striga* haustoria on the host roots is determined by counting the number of *Striga* seedlings attached to the host roots and the number of successful penetrations. A penetration is counted as successful if the haustorium has penetrated the host xylem vessel. The *Striga*-penetration index is calculated as the ratio between the number of successful penetrations and the number of haustoria attached to the host xylem vessels.

Using this technique a resistant (N-13) and a susceptible hybrid (CSH-1) were compared (ICRISAT 1978) for the Striga-penetration index (Figure 5). Up to 6 days of age N-13 and CSH-1 were roughly equally susceptible, but after 6 days N-13

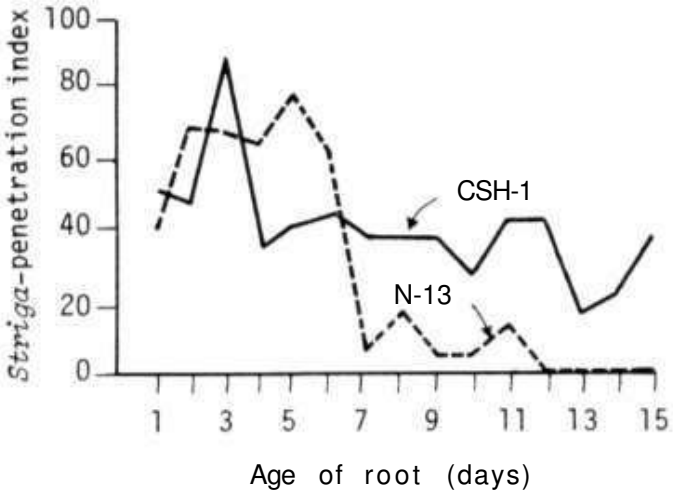

Figure 5. Striga-penetration index of sorghum cultivars N 13 and CSH 1, as determined by root slope technique. (Source: ICRISAT 1978).

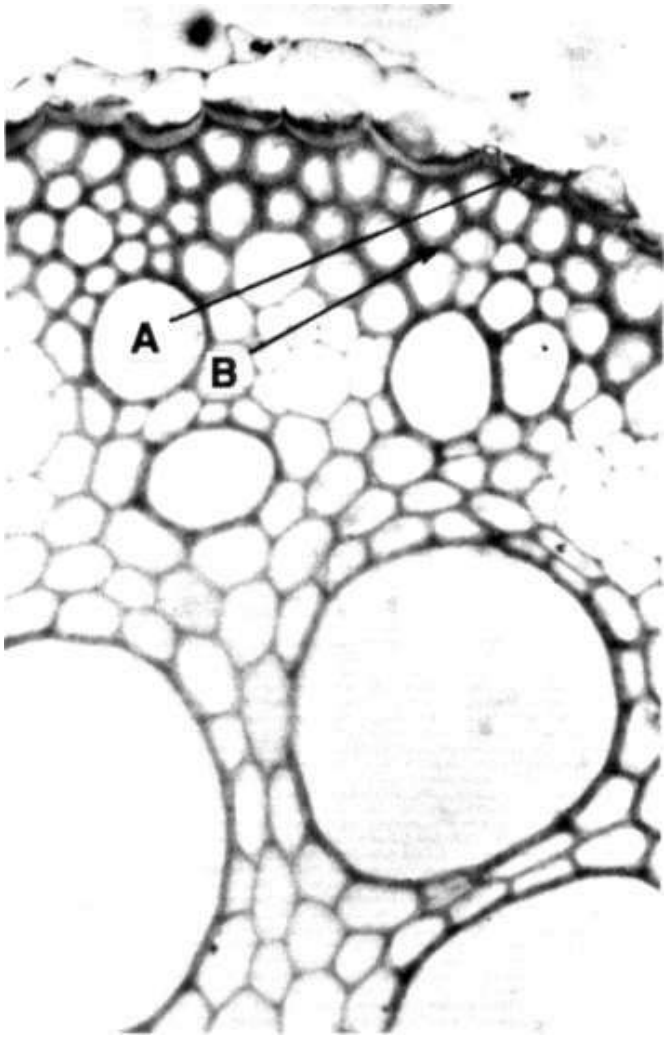

Figure 6. Root cross section of resistant sorghum variety N 13 (x320): a. Lignified endordermal layer, b. Thickened pericycle cell wall.

became highly resistant to *Striga* parasitization, whereas CSH-1 continued to show susceptibility even up to 15 days.

Maiti and his colleagues screened 10 sorghum lines for anti-haustorial factors (Maiti et al. 1984). Their procedure was to grow host seedlings in 200 ml paper cups in sterilized coarse sand mixed with *Striga* seed for 4 weeks. The seedlings were removed from the cups and the roots gently washed. The *Striga-host* attachment region was fixed in a FPA mixture and processed through an absolute alcohol and tertiary butanol series for microtome sectioning. Three to five attachments were studied per host variety for the degree of endodermal cell wall thickening, presence of silica crystals in the endodermal cells, and the degree of thickening of the pericycle walls on lateral roots of comparable age. Endodermal cell wall and pericycle wall thicknesses (Figure 6) were measured using stage and occular micrometers and scored as high, intermediate, or low on the basis of the thickness of one or both. To study the timing of the lignification process, anatomy of the host roots was examined in the absence of *Striga* in a time-course study. The endodermal cell wall thickening in the host root sections was examined at 7,14,21, and 28 days by staining the sections with safranin ortoluidine blue.

Techniques to Identify Resistance in Pots

Screening for resistance in pots, while not a replacement for field screening, is a useful adjunct because of superior control over the pot environment. Host plants are grown in pots filled with soil mixed with a known quantity of *Striga* seed and the resistance evaluated by counting the subterranean or emerged *Striga.* Pot techniques also have a distinct advantage over the currently available laboratory techniques: they can screen for the final effect of all mechanisms while laboratory techniques screen for one or more mechanisms. Pots of various shapes and sizes have been used by different researchers, and there is no agreement about the method and depth for sowing of *Striga* seeds. Even the quantity of *Striga* seeds to be used per kg of soil has varied between authors. The Second International *Striga* Workshop (ICRISAT 1983b) concluded that it is important to standardize the number of *Striga* seeds per pot.

Yaduraju and Hosmani (1980) used 30 cm diameter earthen pots filled with black clay loam soil to screen 28 sorghum cultivars. *Striga* seeds from two different seasons were mixed together and sown at the rate of 0.5 g/pot and placed 8-10 cm below the soil surface. The *Striga* resistance was determined based on the number of emerged *Striga* 80 and 100 days after sowing.

Parker and Dixon (1983) reported developing a 'poly bag' technique (Figure 7) which involved growing *Striga* on sorghum or millet roots in flattened transparent polyethylene bags to observe *Striga* growth repeatedly under a microscope without danger of desiccation or microbial contamination (Figure 7b). A 9 x 30 cm sterilized (autoclaved) glass fiber filter paper was spread inside a 15 x 30 cm standard 120 gauge polyethylene bag and moistened with 12 ml distilled water. About 2000 surface sterilized *Striga* seeds were sprinkled on the paper, the poly bag flattened, and the top folded and held over a piece of cane for support. Several such bags were suspended and incubated for 7-10 days in rectangular plastic buckets to precondition the *Striga* (Figure 7a).

A slit was made just below the cane and a single pregerminated host seed was introduced through the slit. The bags were held in the dark for 3-4 days after which the seedlings emerged through the slit. The buckets were shrouded with black polyethylene to exclude light from the roots and the row of shoots was allowed to emerge through a slit in the center line of the bucket. Seedlings received nutrient solution through the slit at the top, or the bottom corners were cut for irrigation by suspending a set of bags in the nutrient solution. Parker and Dixon (1983) reported that they could maintain sorghum in poly bags up to 90 days and record *Striga* numbers and monitor growth. They reported that progress of the attachment could be observed repeatedly by hand lens or under a microscope, and small samples for preservation or sectioning could be removed by cutting through the bag without damage to the whole system.

Vasudeva Rao et al. (1983b) reported the development of an improved pot screening technique named 'seed pan' technique. The host lines were grown in shallow seed pans (Figure 8) approximately 35 cm top diameter, 15 cm bottom diameter, and 15 cm high, accomodating about 2.5 kg of a 1:1 mixture of sand and clay soils. The shape and size of the pans concentrated the host roots and provided a shallower soil depth (Figure 8a), which favored a higher frequency of *Striga* establishment.

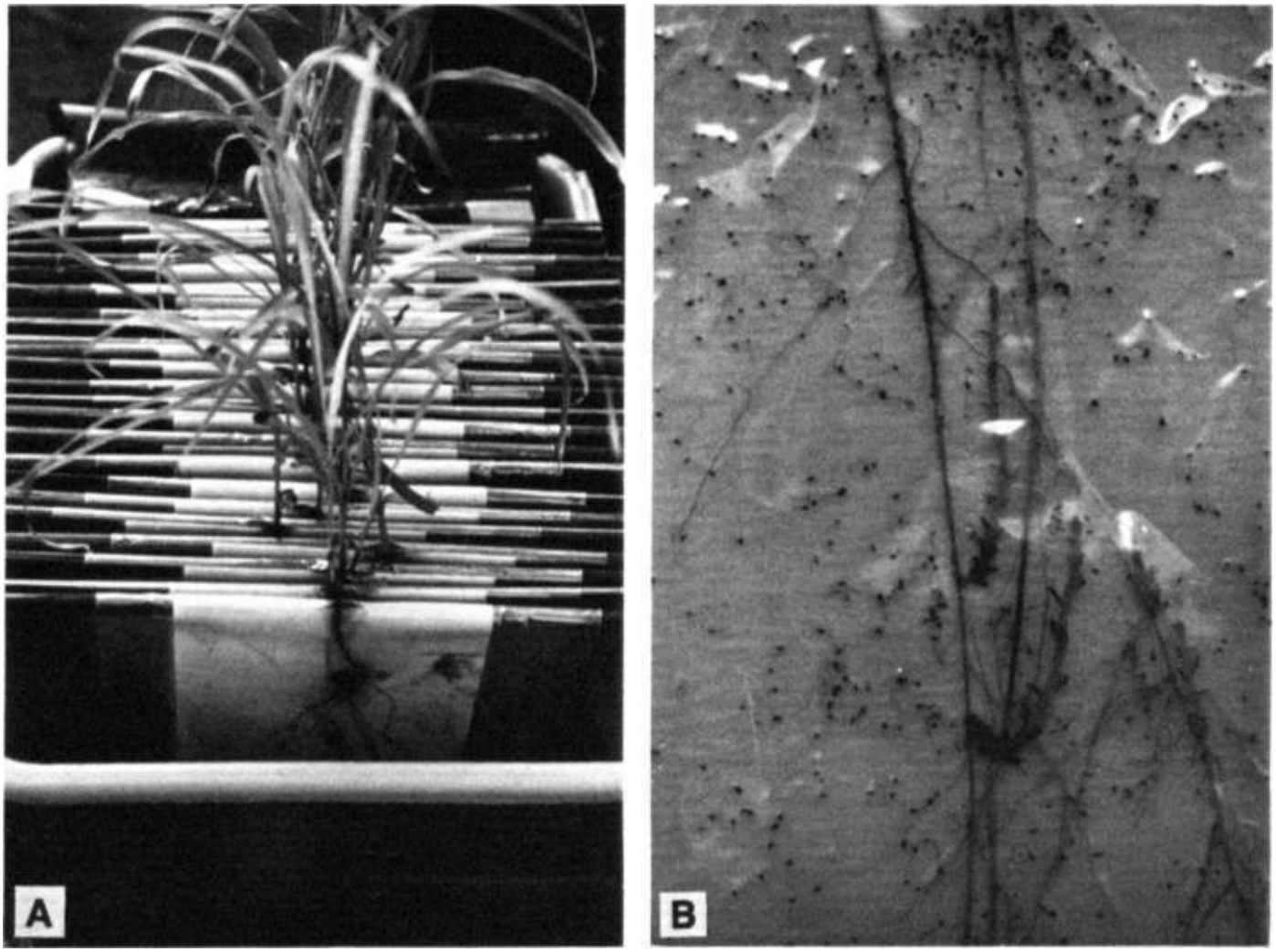

Figure 7. Poly bag technique, a. Poly bags suspended in a bucket, b. *Striga* growing on sorghum roots.

Striga seeds pretested in the laboratory for germination were sown 10-15 days before planting the test material and kept watered to condition them in situ before the arrival of host roots. About 0.1 g *Striga* seed was used per pan.

To obtain uniform infestation across the pans, the *Striga* was mixed with the amount of soil required for the entire experiment and then pans were filled equally by weight. Reaction of the test lines to *Striga* was measured by uprooting the host plant at about 50 days after sowing and counting the subterranean *Striga* initials (Figure 8c). Although counting subterranean *Striga* is a laborious process, it is advantageous because it gives the plant breeder the resistance reactions of the test entries long before they are ready for harvesting in the field.

Alternatively, the host can be allowed to grow longer and the emerged *Striga* counted 80-90 days after sowing. The soil in the seed pan is insufficient for growing plants more than 50 days, but a wooden flat 30 x 30 x 15 cm has recently been standardized for this purpose (Figure 8d). Several experiments have now been carried out at ICRISAT Center using seed pan and wooden flats, and consistent results have been obtained to differentiate resistant and susceptible genotypes (Figures 8b, 8d).

The seed pan technique has also been used at ICRISAT Center for screening sorghum lines in a stimulant positive situation, i.e., by providing artificial or natural stimulant to all the pans 5,10,15,20, and 25 days after sowing the host. For this, seedlings of a high stimulant variety, Swarna, were grown 10 days before sowing the host in plastic trays with holes in a quartz sand medium. Exudate was obtained by excess irrigation and draining the root solution into buckets. Such freshly drawn root exudate was uniformly applied to all seed pans. In this set up only the resistance due to low stimulant production is nullified by the external application of

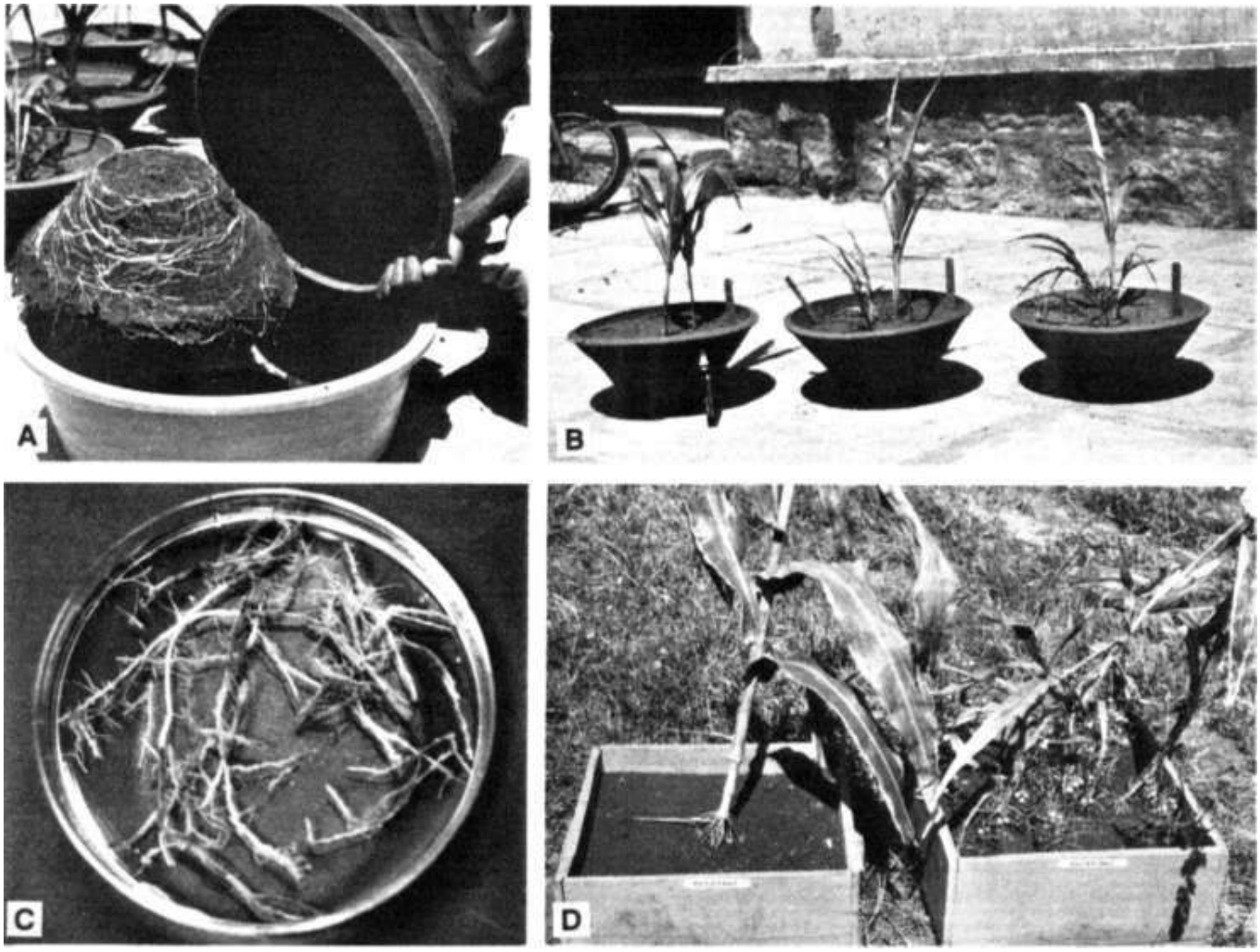

Figure 8. Seed pan technique, a. Concentration of the sorghum roots, b. Resistant plant (left) and susceptible plants, c. Subterranean *Striga* picked during counting, d. Resistant plant (left) and susceptible plant in wooden flats.

stimulant. Any resistance observed should then be due to mechanisms other than low stimulant production.

Sowing depth for *Striga* seeds in pots to get maximum infestation has been studied by many researchers. Agabawi and Younis (1965) reported that maximum *Striga* shoots were produced when seeds were sown on the surface, and as the depth of sowing increased, parasite emergence was significantly reduced, with no *Striga* emerging when sown at 20 and 25 cm below the soil surface. Kambal (1977) found that mixing 0.2 g *Striga* seed per pot gave best results. Parker, however, observed that mixing 0.2 g *Striga* seed with the top 3 cm soil in pots resulted in good *Striga* emergence (Parker et al. 1978). Ramaiah (1983) reported using about 1300 *Striga* seeds per kg of soil in the top 2.3 cm soil layer in large pots holding 5 kg soil. Planting host seeds about 3 cm below the surface gave good results.

Techniques to Identify Resistance in the Field

Despite improvements in laboratory and pot screening techniques, field screening to identify resistant types is indispensible because in the final analysis, resistance in the field is what matters to farmers. However, field screening is often problematic due to nonuniform *Striga* infestations and variable environmental influences. The primary problems in field screening are:

- variable infestations as measured by the emerged *Striga* counts from year to year in the

same field, and from spot to spot in any field in any one year,

- significant environmental influence on *Striga* infestation,
- the high coefficients of variability observed in screening trials, and
- the inability to get predetermined graded levels of *Striga* infestation.

Field screening for *Striga* resistance can be done under either natural or artificially induced infestations. Because of as yet unexplained microvariations in the soil environment within the experimental area, even in artificially infested fields, variation in *Striga* numbers between plots is common on the same host variety. As an example of this variability, *Striga* count data on CSH-1, a susceptible commercial hybrid, obtained in checkerboard layout trials conducted at five locations in India over 3 years are presented in Table 1. Plot to plot variability as seen in the range of *Striga* numbers per m^2 is enormous, and this infestation variability significantly influences the validity of the screening data. At Bijapur and Akola (India), where trials were conducted for three consecutive years, the mean infestation levels were different each year.

Table 1. Variability in *Striga asiatica* infestation in CSH-1 in 10 checkerboard trials in India.

Trial no.	Season and year[1]	Location	No. of plots	*Striga* counts/m^2 Min.	Max.	Mean
53	R 81	Akola	40	87.2	851.4	362.3
53	R 81	B. Sagar	40	0.0	119.8	39.6
53	PR 81	Bijapur	40	4.3	82.3	33.1
71	R 82	Akola	40	4.4	253.0	44.6
71	PR 82	Bijapur	40	189.4	817.2	500.6
71	R 82	Patancheru	40	54.4	255.7	153.2
71	R 82	Parbhani	40	9.5	75.7	27.3
71	R 82	Indore	40	5.6	1309.0	277.0
81	R 83	Akola	110	16.6	333.3	143.1
81	PR 83	Bijapur	60	0.7	210.0	71.2

1. R - Rainy season; PR = Postrainy season.

Three-Stage Field Screening Methodology

In order to circumvent the problems of variable infestation in *Striga-sick* fields, a 'three-stage' screening methodology (Figure 9) was developed at ICRISAT Center, Patancheru, and successfully used in resistance breeding projects (Vasudeva Rao et al. 1981,1982a, 1982b, 1983a, 1983b). The methodology measures and accounts for variable

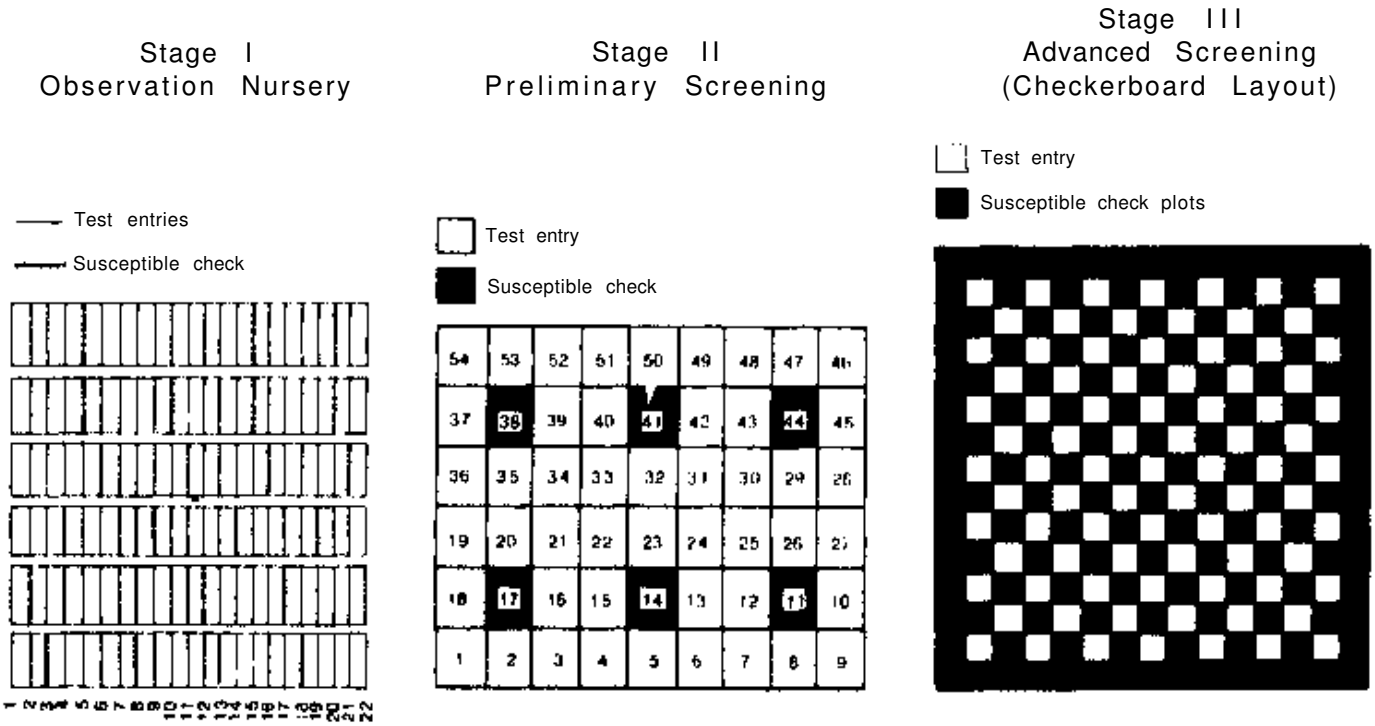

Figure 9. Three-stage screening methodology for *Striga* resistance breeding (Source: ICRISAT 1983).

Striga infestation in a field by providing in the layout frequently repeated plots of a susceptible cultivar, and increasing the frequency as tested material is advanced through three stages: an observation nursery, a preliminary screening stage, and an advanced screening stage. Specific field layouts and statistical procedures have been developed for the three stages.

Layouts

Observation Nursery

This stage is an unreplicated trial of a large number of test entries with a frequently repeated susceptible check grown in the sick field. Test entries may be grown in two- or three-row plots in one or multiple location nurseries.

Preliminary Screening

The second stage of testing includes those nursery entries that were agronomically good in stage I and displayed some resistance. The entries are tested in three-row plots and are replicated at least three times. Check plots, using a Strga-susceptible variety, are systematically arranged so that every test entry plot has one check plot adjacent to it. This arrangement results in each replication being divided into units of nine plots. Each unit will have eight plots of test lines surrounding one plot of a susceptible check line.

Advanced Screening

The third and final stage tests the entries selected from the preliminary screening in larger plots with four susceptible check plots surrounding each test entry plot. Each entry is tested in a five-row plot so that yield estimates and *Striga* reactions are obtained from a fairly reliable plot size, with border effects minimized. The entire trial is planted on all four sides with a strip of a susceptible check variety. The field, which looks checkered with alternating susceptible and test entry plots in both the directions, is therefore called a checkerboard layout. This is not an experimental design in the statistical sense, but rather a field layout within which some of the standard experimental designs can be used.

Data Collection, Analysis, and Interpretation

Observation Nursery

Data on morphological characters and *Striga* counts or *Striga* scores (see next section on indices of *Striga* resistance) are obtained on the host lines which appear uniform. *Striga* counts of the test line are standardized by expressing the reaction as a percent of the average of the nearest two susceptible check plots. Highly susceptible lines are rejected based on *high Striga* reactions relative to the appropriate check plots. If a line is segregating, selection can be made within the line and tested again in the observation nursery stage.

Preliminary Screening

Since each entry is grown in three-row plots, *Striga* counts and other characters are recorded on the central row. Trials are classified into three groups based on *Striga* counts in the susceptible check plots of all the units.

All-Zero Trials

If all the check plots register a zero *Striga* count it indicates that *Striga* has not appeared and the trial is of no use in evaluating *Striga* reactions of test entries. However, the trial could be used for yield evaluation.

Some-Zero Trials

In this group of stage II trials, *Striga* appeared in some check plots and not in others. Standard statistical analytical procedures of this data result in very high CVs, and the conclusions are unreliable. In order not to discard the results from these partially infested trials, a system of data interpretation called single unit comparison is used (Vasudeva Rao et al. 1983b). This system treats each unit of nine plots (with a susceptible check in the center) as an independent unit and conclusions are drawn by examining the data unit by unit. This system has proved very useful in partially infested trials.

The following criteria, based on counts of emerged *Striga* plants, are used to determine the resistance of a test entry.

- Check plots must show high *Striga* counts to make the comparison valid.

- Test entry *Striga* reaction should be less than 10% of the check plot in the unit.
- Test entry should qualify as resistant in all the replications at a location.
- Test entry should be selected across several locations.
- Averages within or among locations should not be used.

Test entries are then classified into one of six categories based on *Striga* reaction:

- Confirmed resistant (R). Has less than 10% of the *Striga* count of the unit control plot which shows a high *Striga* count. The entry must show a valid resistance reaction across all replications and locations.
- Confirmed susceptible (S). Shows more than 10% of the *Striga* count of the control. This group also includes those that are infested irrespective of the infestation in the unit check.
- Control low, therefore comparison not reliable (NR). Comparison not valid because the control had low *Striga* counts.
- Resistant/susceptible (R/S). Entries that show either resistance or susceptibility across replications or locations. Reactions in this category may be an indication of *Striga* 'strain' differences.
- Resistant/not reliable (R/NR). Shows different combinations of the first three reaction categories, and needs retesting.
- Susceptible/not reliable (S/NR). Shows different combinations of the first three reaction categories, and can be rejected because it is susceptible in some replications.

Classification into these six categories using the single unit comparison system, based on a set of empirical criteria, is highly useful for evaluating *Striga* resistance (Vasudeva Rao 1983b).

No-Zero Trials

In these trials, the *Striga* infestation is high in the suceptible check plots of all the units. Reactions of test entries are computed as a percentage of the *Striga* counts in the susceptible check of the same unit. These data can then be analysed according to the design used. The mean infestation level in the trial (the site mean) could be obtained by averaging the *Striga* counts of all check plots and expressing it as counts per m^2. Invariably the *Striga* counts data are skewed. Logarthmic transformation of the *Striga* counts in a 90 entry stage II trial from Akola, Maharashtra, India, conducted during the 1981 rainy season (Table 2), indicated that with log transformation and adjustment for variability the distribution of the counts became near normal (Figure 10) and therefore improved the CVs considerably (Vasudeva Rao et al. 1982a).

Table 2. Coefficients of variability in a 90 entry stage II trial as influenced by logarithm transformation and adjustment for variability.

Data treatment	CV%
Untransformed *Striga* counts (STC)	75.40
Log(STC)	25.33
STC % adjacent check	115.47
(Log STC) % adjacent check	31.28

Advanced Screening

Strga-caused grain yield losses have been difficult to quantify because of nonuniform *Striga* distribution and effects. The checkerboard layout provides a method to estimate grain yields from replicated test entry plots, and at the same time, a way to monitor, estimate, and utilize the information on *Striga* infestation in the susceptible check plots which are regularly interspersed in the experimental area. In addition to being used at ICRISAT Center, Patancheru; ICRISAT Burkina Faso (K.V. Ramaiah, ICRISAT, personal communication, 1984); and the All India Coordinated Sorghum Improvement Project for coordinated *Striga* resistance trials (ICAR 1983,1984); the checkerboard layout has also been tried as a screening technique for other yield reducers like drought (B.V. Subba Reddy, ICRISAT, personal communication, 1984) and charcoal rot (S. Pande, ICRISAT, personal communication, 1984) at ICRISAT Center. Because five-row plots are used at this stage of screening, *Striga* counts, grain yield, and other ancillary characters are recorded on the central three rows. Checkerboard layout data is analysed as follows:

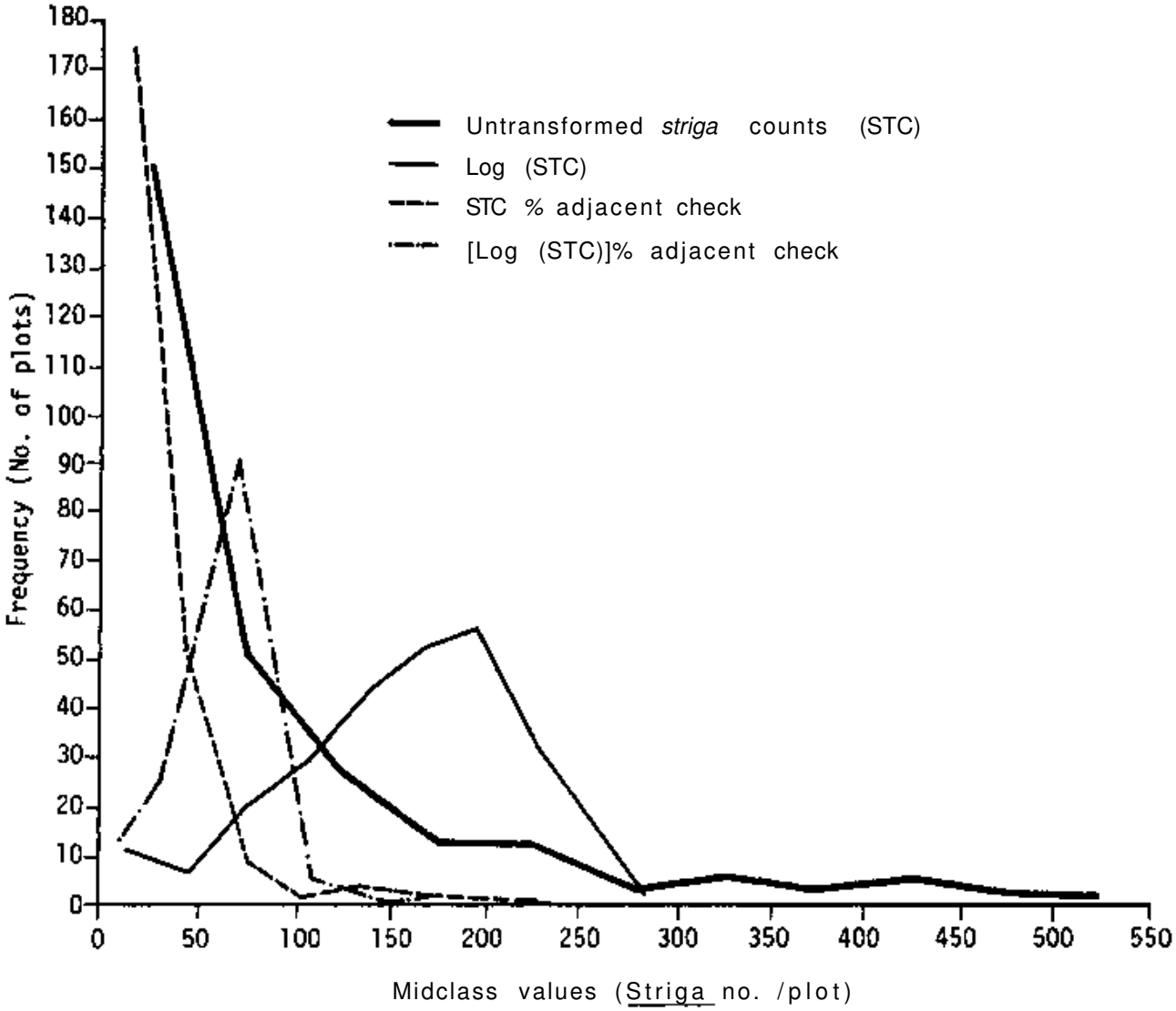

Figure 10. Frequency distribution of *Striga* counts from a stage II trial with and without transformation.

Measures of Infestation Levels and Yield Loss

It is necessary to first study the infestation level and its effect on the yield of the susceptible host before any valid conclusions are drawn about the *Striga* resistance of the test entries.

- Mean and Range. Mean infestation level in the trial is obtained by averaging the *Striga* counts in all the susceptible check plots in the experimental area excluding the border strip plots. The means are then compared across locations, and trials rejected if the values are low (ICAR 1984). The range of *Striga* numbers and yields in the susceptible check plots (minimum and maximum) indicate the variability of the infestation and control yields. For a trial to be valid, all susceptible check plots should have at least some *Striga* plants. Even if some check plots have no *Striga* plants, the positional check average (see next section) should not be zeros.

- Correlation Coefficient. A correlation coefficient is obtained between *Striga* numbers and plot yields of only the susceptible check plots. This indicates the relationship between *Striga* infestation level and yield of the susceptible control. Since *Striga* is a yield reducer, a negative correlation between these two characters should be expected, which is what happens if there is enough infestation.

- Coefficient of Determination (R^2) and Crop Loss Estimates. R^2 is the square of the correlation coefficient. This coefficient indicates the proportion of variation in the yield of the susceptible check variety which is explained by the variation in *Striga* counts alone. Usually this value is low because even in *Striga-sick* fields, there are factors other than *Striga* which cause yield variation. It may be useful to monitor the nutrient and moisture levels in the susceptible check plots which could then be added to the multiple regression equation. Crop loss in the susceptible check variety can be calculated at the minimum, mean, and maximum *Striga* loads observed in the experimental area based on the regression equations (M.J. Vasudeva Rao, V.L. Chidley, and L.R. House. Crop loss estimates in sorghum caused by *Striga asiatica.* In preparation).

Analysis of *Striga* Reactions of Test Entries

Striga reactions of test entries are calculated as a ratio of *Striga* numbers in the test entry plots and the positional check average expressed as a percentage. The positional check average is the average *Striga* counts of the four susceptible check plots surrounding each test entry plot. This value is a reliable estimate of the potential infestation level in the test entry plot if instead of the test entry a susceptible check were to have been grown. The presence of many susceptible check plots in the trial permits measuring the variability of *Striga* infestation.

The method of adjusting the test entry plot values by using the covariance on neighboring plots (NEPLOT analysis) originally suggested by Papadakis (1937) and reviewed by Bartlett (1978) was found to be useful (Gilliver et al. in press). Iterations are done using the end and side plots for each test entry plot in a stepwise manner before the design analysis. The test entry plots are iterated first using end plots alone, then side plots alone, then end and side plots seperately, and finally end and side plots together. The trials are analysed as completely randomized designs and the adjusted means, corresponding to the lowest coefficient of variation, are used for statistical interpretation to differentiate *Striga* reactions of test entries.

Coefficients of variation obtained by the nearest neighbor plot analysis of four advanced trials using the actual and logarithm transformed *Striga* counts (Table 3) indicated that log transformation considerably reduced the coefficients of variation in all four trials. In two of the four trials, the CVs were beyond acceptable limits of 20% when the iterations were not used. However, CVs were reduced to below 20% during the process of iterations, thus making the trial conclusions more useful.

In addition to NEPLOT analysis, empirical screening of the test entries by plot assessment has also been developed for checkerboard layout data (Gilliver et al. in press). In this system of data analysis, test entries are selected by plot assessment as resistant only when a sufficiently high intensity of *Striga* is present in the susceptible check plots around it and the *Striga* intensity in the test entry plots is low. When the *Striga* numbers in the check plots fall below a predetermined (high

Table 3. Coefficients of variability obtained in four checkerboard trials with and without transformation.

	Trial 1[1]		Trial 2		Trial 3		Trial 4	
Iteration	No trans-formation	Log trans-formation	No trans-formation	Log trans-formation	No trans-formation	Log trans-formation	No trans-formation	Log trans-formation
No local control	130.02	34.01	78.40	11.38	88.16	26.83	120.71	26.74
Ends alone	128.45(6)[2]	34.10(3)	78.66(3)	11.16(2)	57.40(3)	17.89(3)	119.26(4)	21.67(3)
Sides alone	125.59(6)	33.16(3)	73.53(4)	10.14(3)	72.13(4)	19.22(3)	108.20(4)	20.08(3)
Ends and sides separately	118.99(4)	32.17(3)	73.89(4)	10.12(2)	57.41(3)	17.23(4)	105.29(4)	19.28(4)
Ends and sides together	112.15(5)	31.19(3)	74.48(4)	10.12(3)	58.63(4)	16.98(4)	104.97(5)	18.91(5)

1. Trials 1,2, and 3 are from Bhavanisagar, Akola, and Bijapur rainy season 1981 respectively and Trial 4 is from Bhavanisagar summer season 1982.
2. Figures in parentheses are the number of cycles of iteration.

intensity) level, an assessment for judging highly resistant entries is not carried out, but test entries are rejected if the resistance is too low.

Analysis of Test Entry Grain Yield Data

Grain yields from the replicated test entry plots, which are also randomized within each replication, are used for statistical analysis. A randomized block design analysis of only the test entry plots can give the treatment means, standard errors, and critical difference estimates for yield potential estimation and test entry comparisons. However, while expressing the test entry yields, it would be useful to also present yields of the susceptible control.

As an example, results from the ICRISAT multilocation *Striga* resistance checkerboard layout trials conducted during the 1982 rainy season are presented in Table 4 (ICRISAT 1983a).

Other Layouts

Hanumantha Rao (1982) used a field arrangement in which each test entry plot (3 rows of 3 m) was located between plots (guard-row plots) of a susceptible check variety (1 row of 3 m). *Striga* were counted in the susceptible check variety on a 1 m length and in the middle row of the test entry plot. Ramaiah (1984) proposed a sandwich layout in which two rows of a susceptible variety are planted on either side of two test varieties. Resistance is based on the *Striga* numbers in the test entry expressed as a percentage of the *Striga* numbers

Table 4. *Striga* reactions (SR)[1] and grain yields (kg/ha) of 15 breeding lines and 5 source lines in multilocation testing (checkerboard layout, rainy season 1982).

		ICRISAT Center		Akola		Indore		Parbhani		Bijapur		Mean	
Origin	Pedigree	SR	Grain yield	SR	Gram yield	SR	Grain yield	SR	Grain yield	SR	Grain yield	SR	Gram yield
SAR 1	(555 x 168)1-1	01	3970	4.2	2280	0.2	1540	5.7	2250	1.4	2130	1.9	2430
SAR 2	(555 x 168)-16	05	3370	39	2590	1.3	1600	10.2	1960	1.8	2120	30	2330
SAR 5	(148 x555)-1.2	0.7	3960	3.6	2470	12.4	990	6.2	1960	2.6	1530	42	2180
SAR 6	(148 x555)-33 1-3	0.2	3680	3.2	2180	1.0	2130	13 3	2450	0.5	1910	30	2450
SAR 9	[SRN 4841 x (WABC x P-3)]-7.3	11	4840	1.5	1070	4.9	2350	4.0	2080	12.8	1420	41	2350
SAR 10	[555 x (PDxCS-3541) 29-3]-5-2-1	05	4110	1.1	2580	0.6	2620	6.8	2620	0.8	2170	1.7	2500
SAR 11	(555 x Awash-1050)-22	2.2	1560	5.7	1780	11 1	820	11.5	1670	3.9	1730	6.0	1510
SAR 12	(SRN-4841 xSPV-104)-17'	41	3500	5.3	1040	88	2160	93	2210	8.3	1130	7.1	2010
SAR 13	(555 x 168)-1	0.8	4600	1.7	2270	1.1	1730	5.6	1960	2.1	1600	2.0	2430
SAR 14	(Framida x 1481-21-2-2-4	0.3	4370	4.7	1070	13 0	710	3.8	2120	1.7	1300	3.9	1910
SAR 15	(555 x 168)-23-2-2-3-2	0.8	4210	5.0	2400	4.6	1760	28.6	1710	1.3	2140	6.9	2440
SAR 16	(555 x 168)-19 2-7	0.8	4340	5.2	2700	18.2	660	8.7	2450	2.3	1500	6.4	2330
SAR 17	(N-13 x 269)-5-2	3.2	2660	10.3	2210	10.1	860	2.6	2000	6.2	1710	5.7	1890
SAR 18	(N-13x 2KX6)-1-2-1-2	3.2	4210	20.9	1670	4.2	988	9.9	2080	11 7	1130	8.7	2020
T-233B	T-233B	128 1	2360	141.3	1660	156.9	710	31.1	1870	23.5	780	85.6	1470
N-13	N-13	0.1	1290	0.3	2190	1.1	770	2.9	1370	08	2850	09	1690
555	555	4.9	1170	10 3	2530	10 3	1030	4.0	1 710	1 2	2150	52	1720
SRN 4841	SRN 4841	114	2210	68	0	10.3	1280	110	460	22.7	1230	10.5	1040
IS-4202	IS-4202	0.2	1690	3.8	2220	1.4	1970	43	1920	2.9	860	2.1	1730
IS-7471	IS-7471	0.8	0	12.9	0	25.5	0	95	0	0.9	1638	8.4	330
CSH-1[2]	Mean	230	4040	67	1310	222	1470	36	2130	501	800		
(Suscep-	Min	82		7		4		13		189			
tible	Max	434		380		1047		101		817			
control)	r	-0.43**		-0.49"		-0.57"		-l0.39'		0 32*			

1. SR of test entries = emerged *Striga* counts as percentage ot control averaged over two replications.
2. SR of CSH-1 = emerged *Striga/m*^{2} averaged over 40 CSH-1 plots in tthe checkerboard Yield of CSH-1 also averaged over 40 plots
3. 'r' correletion between *Striga* count and yield/plot.

(Source: ICRISAT 1983).

in the adjacent susceptible variety. Among the various layouts proposed and tried, only the 3-stage methodology using the checkerboard layout at the advanced screening stage has been found useful in *Striga* resistance breeding.

Indices of *Striga* Resistance

Two main criteria have been used as indices of *Striga* resistance: *Striga* numbers and grain yield. *Striga* numbers are reported in three ways:

- *Striga* score. Scoring on a 0-5 or a 0-10(0 = no *Striga)* scale is useful when it is not possible to physically count the numerous *Striga* plants that emerge.
- *Striga* counts. Counting the number of *Striga* plants that emerge above ground with or without uprooting them, at one, two, or three stages of crop growth, is a valid way of representing the *Striga* reactions of varieties. *Striga* counts are expressed as the number of *Striga* plants per unit area or per host plant.
- *Striga* index. This is a weighted average of *Striga* counts and the heights of *Striga* plants (Hanumantha Rao 1982). The weights are *Striga* numbers in each height category. *Striga* resistance of test varieties in guard-row-plots is calculated as a ratio of the *Striga* index of the test variety to that of the average of the two guard, rows. *Striga* index does not appear to have any advantage over *Striga* numbers because heights change with time and measuring *Striga* heights is laborious. However, it could be useful when studying relative growths of *Striga* in different varieties or where treatment effects result in differential growth of *Striga.*

Among the three indices of *Striga* resistance, emerged *Striga* numbers appear to be the best, but the validity of emerged *Striga* numbers has been questioned because the numbers that emerge above ground is an unknown percentage of the actual subterranean *Striga* numbers on the host roots. However, in screening trials, it could be assumed that this unknown percentage is the same across all genotypes.

Although grain yield in *Striga-sick* fields has often been used to define resistance, *Striga* resistance should be defined based only on low or nil *Striga* counts compared to the susceptible check. Considering grain yield along with *Striga* counts could lead to confusion between 'resistance' and 'tolerance. Also, grain yield in *Striga-sick* fields is influenced by many other factors in addition to *Striga.*

Strigra-Sick Field Management

Research on the development and management of *Striga-sick* fields has received little attention in the past. However, the following guidelines could be useful for managing *Striga-sick* fields:

- Ideally, a *Striga-sick* field should have good surface and subsurface drainage, with soil of low clay content and few weeds.
- *Striga* plants should be collected every year both from the *Striga-sick* field and other fields, and distributed uniformly over the sick field. Pure *Striga* seed could be distributed by mixing with sand to make up the bulk. The dried *Striga* plants (trash) also contain large quantities of seed and should be incorporated into the soil. *Striga* distribution should be carried out after the initial plowing. After the *Striga* seed distribution only a shallow discing should be done to incorporate the *Striga* seed in the top 5-10 cm of soil. Pot tests have indicated that *Striga* from deeper levels have a lesser chance of emergence.
- *Striga-sick* fields should receive lower doses of fertilizers, particularly N.
- All mechanical operations, and weeding in particular, should be completed before the time *Striga* is expected to emerge, about 25-30 days after sowing.
- It is essential that all volunteer host plants are removed before the test material is sown. A volunteer susceptible host plant occuring in a plot of a resistant test entry could significantly skew screening results. One way to overcome this problem is to have two sick fields and fallow or rotate with a non-host crop in alternate years. A trap crop should not be planted if a rotation is used.

- Implements and other agents of *Striga* seed dispersal should be thorougly cleaned and all soil removed before they leave the *Striga-sick* field after every operation.

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