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1) Screening for cyst nematode resistance in soybean breeding.

As the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, has become a serious pest of soybeans in the USA, development of resistant cultivars has received greater attention. This necessitates screening of large numbers of plant progenies to locate SCN-resistant isolates in segregating generations. Ross and Brim (1957) used a double-row method to detect SCN-resistant strains of soybeans. The conventional method of screening now involves growing one or two plants in a small pot containing soil adequately infested with SCN. After 30 days, the plant roots are exposed by gently shaking to remove the soil and then counting white females (cysts) on the roots. The plants are scored 0 through 4 based on the number of cysts on the roots of a plant (Epps and Hartwig, 1972); 0: no cyst, 1: 1-5 cysts, 2: 6-10 cysts, 3: 11-30 cysts and 4: 30+ cysts. In field screening, the plant rows are scored 1 through 5 for yellow appearance, plant height and poor yield performances. During the course of our screening for SCN, we have encountered several difficulties which lead us to try various methods seeking repeatable results. Here we report some of the common problems in screening for SCN and how we dealt with them.

Greenhouse screening

Level of inoculum: Inoculum containing an adequate number of cysts with viable eggs and larvae is most necessary for greenhouse screening. Ordinarily, infested soil is collected from a problem field in the fall and cyst counts are made for soil samples. An elutriator is used to extract the cysts. Cysts with viable eggs and larvae sink to the bottom. Cysts which float are devoid of or have few eggs. To determine the number of cysts needed for screening, a test was conducted with four inoculum levels of a mixture of races on two soybean varieties. The results are given in Table 1.

Table 1. Number of cyst nematodes on roots per plant (mean of 6 plants)

	Cyst infestation/100 g of soil				
Cultivar	5	10	20	30	40
Essex	26	52	61	77	86
Forrest	20	31	52	78	88

At least 10 good cysts per 100 g of soil are needed for proper screening. If inoculum potential is low, the number of cysts per unit of soil can be increased by growing a mixture of two or more susceptible varieties in greenhouse soil for about 35 days. Under good temperature and moisture conditions, the cyst number will increase from 10 to 100 per 100 g of soil in one

generation. A very high level of inoculum may distort the results. A high inoculum level can be satisfactorily diluted by mixing with sterile soil. Our results indicated that 20-30 cysts per 100 g of soil were best inoculum for pot screening.

Temperature: Temperature has a pronounced effect on SCN larval emergence and development (Slack and Hamblen, 1961; Ross, 1964). Our best results were obtained when plants were grown at 27.5°C. At a lower temperature, a longer time was required to complete the life cycle. At temperatures above 27.5°C, there were fewer cysts on the roots although the white cysts appeared earlier. Cyst development was reduced drastically when temperatures went above 35°C.

Number of days for SCN development: To determine the number of days required for SCN development and an optimum period required for scoring, the cultivar 'Essex' was grown in the greenhouse in SCN-infested soil at 27.5°C (± 1°C). Cysts were removed from the roots by a strong jet of water on an 80 mesh sieve. Cyst count was made by using a stereoscopic microscope. The data are presented in Table 2.

Table 2. Number of cyst nematodes on roots/plant (variety Essex: mean of 3 plants)

	Days after planting					
	20	24	26	28	30	35
Cyst number/plant	9	79	276	160	120	96

Cysts first appeared on roots 20 days after seeds were planted. The number of cysts increased for 6 consecutive days, but decreased thereafter. Since seedlings take 6-8 days to develop an adequate root system, 26 days are required to produce maximum root infestation for optimum observation at 27.5°C. The decline of cyst after that period is due to falling off of the cysts from the roots.

Black seed coat: Several of the resistant soybean PI lines which are used in breeding programs are black seeded and have poor germination if seeded directly. Similar problem is encountered in the black-seeded segregates following hybridization. To increase germination, the seed was scarified (a) with a knife and (b) by swirling vigorously for a minute in a beaker with 150-grade sandpaper all around. The data are presented in Table 3. Scarification by either method improved germination of black-coated seed. Scarification of individual seed with a knife did a better job than did scarification with sandpaper; however, it involved longer time.

Table 3. Effect of scarification on seed germination

Genotype	Percent germination (7 days after planting)		
	Control	Scarified with knife	Scarified with paper
PI 88788	62	86	78
PI 89772	52	80	72

Field Screening

Level of inoculum: The most difficult problem in field screening is the development and maintenance of a uniformly high inoculum potential in the soil, which is very necessary. The inoculum level can be increased up to 20 times during the season by growing a susceptible variety. It may increase little if a variety resistant to one or more races of SCN is grown (Anand, 1981). Thus, the inoculum level will vary with the level of resistance of the previously grown variety. Therefore, it is necessary to grow one or more susceptible varieties uniformly in the test field the preceding year. Planting soybeans in narrow rows aids rapid inoculum increase. A healthy crop stimulates SCN development. A poor crop due to drought or very low fertility may decrease the cyst count. It would also be desirable to establish cyst nursery in a field with uniform soil type. In light sandy soils, cysts develop well and are preferred over loam soils for nursery plots. Heavy soils are not suitable for SCN development.

Time of screening: SCN has no effect on the germination and stand of the crop. Early field symptoms appear about 4 weeks after planting. Susceptible lines are slightly shorter and have yellowish leaves, typical symptoms of nitrogen deficiency. Leaf yellowing is less pronounced later. The best time for scoring SCN resistance appears to be about 6 weeks after planting. Droughty conditions aggravate expression of susceptible symptoms whereas heavy rains have an opposite effect. Lines can be evaluated again at maturity, based on plant height and general performance. Field scores are recorded as 1 through 5. One is considered highly resistant and 5 highly susceptible with no seed production. Check cultivars grown at regular intervals (1 in 10 rows) provide a measure of SCN infestation in the field.

Comparison of greenhouse and field screening

Eighty-one advance lines were screened in the greenhouse and in the field cyst nursery. Scoring by the two procedures was highly correlated ($r = .63^{**}$) indicating that both the methods gave similar results.

References

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