Evaluation of Soybean Resistance to Sclerotinia Stem Rot Using Reciprocal Grafting

T. D. Vuong, Research Associate, Department of Crop Sciences, University of Illinois; and G. L. Hartman, Research Plant Pathologist, USDA-ARS and Department of Crop Sciences, University of Illinois, 1101 W. Peabody Drive, Urbana, IL 61801

ABSTRACT

Sclerotinia stem rot of soybean is one of the major soybean diseases in the north central region of the United States. One disease management option is to plant cultivars that have resistance. Some sources of partial resistance have been identified, but information pertaining to the nature of resistance is limited. The objective of this study was to determine if the expression of resistance is dictated by shoots of resistant plants and if this can be altered by using resistant and susceptible soybean genotypes grafted in different shoot and rootstock combinations of self-, single-, or double-shoot grafts. After successful grafts were made, several experiments were conducted using different inoculation techniques and soybean genotypes. In one experiment, cotyledons were inoculated with a plug of fungal mycelium, plants were incubated in a mist chamber for 23 h, and plant survival was recorded over time. Based on seven grafting combinations of cross- and self-grafted plants using two soybean cultivars, grafts with NKS19-90 (partially resistant) as shoots had greater (P ≤ 0.05) plant survival at 3, 4, and 5 days after inoculation than the other graft combinations. In another experiment, a total of 17 graft combinations were generated using resistant plant introductions and two susceptible cultivars. Resistant self-grafts of the plant introductions had greater (P ≤ 0.05) plant survival (mean = 75%) than self-grafts of the susceptible cultivars (mean = 15%) at 5 days after inoculation. Inter-genotypic grafts with resistant shoots had greater (P ≤ 0.05) plant survival (mean = 65%) than those in reciprocal combinations (mean = 8%) 5 days after inoculation. A cut stem inoculation method was used to test graft combinations of one resistant and two susceptible cultivars. Grafts with susceptible shoots of cvs. Williams 82 and Asgrow 2242 had greater (P < 0.05) lesion lengths (mean = 13.2 cm) than shoots of NKS19-90 (mean = 9.2 cm) regardless of the rootstock 15 days after inoculation. In a double-graft experiment, shoots of both NKS19-90 and Williams 82 were grafted to either NKS19-90 or Williams 82 rootstocks. Regardless of the rootstock, the shoots of Williams 82 died while shoots of NKS19-90 survived. For all the experiments, resistance was greater when the grafted shoot came from a resistant source on a susceptible rootstock compared with the reciprocal combination regardless of the type of grafting technique or inoculation method.
and susceptible soybean genotypes showed that resistance to brown stem rot was conditioned by roots and not shoots (2). Although Sclerotinia stem rot is primarily known as a stem disease of soybean, there is no indication whether disease resistance associated with stems is the result of stem and/or root response to infection. It is possible that under certain conditions, root or basal stem infection could occur by germination of sclerotia residing in the soil into infectious mycelia. The objective of this study was to determine if the expression of resistance is dictated by shoots of resistant plants and if this can be altered by using resistant and susceptible soybean genotypes grafted in different shoot and rootstock combinations of self-, single-, or double-shoot grafts.

**MATERIALS AND METHODS**

**Plant growth conditions and inoculum preparation**. Seeds were sown in a steam-disinfected soil:sand (1:1) mix in a greenhouse set at 27 ± 2°C and 16-h photoperiod with a photosynthetically active radiation of 434 µmol·m⁻²·s⁻¹ (LI-170 Quantum/ Radiometer/Photometer, Lambda Instrument Corp., Lincoln, NE). At 5 to 6 days after sowing, seedlings were grafted and placed in a mist chamber at 100% relative humidity and 25 ± 2°C for 6 to 7 days, then returned to the same greenhouse bench where seeds were sown and kept for 2 days before being transferred to another greenhouse room for inoculation. To inoculate plants, mycelial plugs (0.5 cm in diameter) were cut from the edges of 24-h-old colonies of *S. sclerotiorum* isolate 15H7T (isolated from a soybean seed lot obtained from Story City, IA, in 1996 and stored as a stock culture on water agar at 4°C). This isolate has consistently produced significant levels of infection through 6 years of use (G. L. Hartman, unpublished).

**Cotyledon inoculation of single-shoot grafts of cultivars**. Twenty seeds of three soybean cultivars, cv. NK S19-90, a partially resistant genotype (15), and cvs. Asgrow 2242 and Williams 82 (15), susceptible genotypes, were germinated in a 1:1 sand:soil mix in three separate rows in 26 x 52 cm plastic trays. At 5 to 6 days after planting, half of the seedlings were reciprocally single-shoot grafted between resistant and susceptible genotypes; the remaining seedlings were self-grafted. For grafting, hypocotyls were cut 1 cm above the soil line and shoots were placed on the rootstock using a V-shaped graft (3). The grafts were held in place by 1-cm sections of 3-mm-diameter plastic drinking straws. In this experiment, there were seven grafting combinations. Three were self-grafted plants; two were cv. NK S19-90 as shoots with the two other cultivars as roots; and two were cv. NK S19-90 as rootstock with the two other cultivars as shoots. There were 7 to 10 plants per replication.

To inoculate plants, a single plug was placed mycelial side down on one cotyledon against the stem of each seedling. All seedlings and the insides of plastic domes were lightly atomized with water using a hand-held sprayer and covered. The dome-covered trays were placed under black mesh cloth (80% light reduction) at 20 ± 2°C for 21 to 23 h. The domes were removed and the trays were returned to the original greenhouse bench, where seeds were germinated for evaluation of disease response. The number of seedlings that survived was counted daily over 5 days, and the percentage of plants that survived was calculated. The experiment was a randomized complete block design (RCBD) with three replications, and it was performed twice.

**Cotyledon inoculation of single-shoot grafts of PIs and cultivars**. This experiment was as previously described for the single-shoot grafts of cultivars except that different genotypes were used. A total of 17 graft combinations were generated using resistant plant introductions PI549.076A, PI503.336, and PI194.639, and two susceptible cultivars, Alpha andMerit. There were 12 reciprocal grafts representing the grafts of the three PIs and the two cultivars and five self-grafts. The procedures of single-shoot grafting, cotyledon inoculation, and disease assessment were performed following the methods previously described. There were 7 to 10 plants per graft combination. The experiment was an RCBD with three replications. Values for the area under survival curve (AUSC) were calculated as follows:

\[ AUSC = \frac{\sum_{i=1}^{n} (X_{i+1} + X_i)/2}{n}, \]

where \( X_i \) is percent survival at the i-th observation, \( t_i \) is time (days after inoculation) at the i-th observation, and \( n \) is total number of observations.

**Cut stem inoculation of single-shoot grafts**. Eight seeds each of NK S19-90, Asgrow 2242, and Williams 82 were germinated in 15-cm clay pots. Five 6-day-old healthy seedlings were single-shoot grafted as previously described. There were seven graft combinations, which included three self-grafts, and NK S19-90 used either as rootstock or shoots for each of the cultivars. Each graft combination was performed in each pot. Grafted plants were incubated for 6 days in a mist chamber at 25 ± 2°C. The plants were transferred to an adjacent bench and allowed to grow for 4 weeks.

A cut stem inoculation method was used to evaluate resistance when the plants were 1 month old and the fourth trifoliolate leaf was fully expanded. Stems were severed with a razor blade 0.5 cm above either the third or fourth node. A mycelial plug as previously described was placed mycelial side down on the wound. Inoculated plants were incubated in a mist chamber at 20°C under high humidity for 48 h. Infected plants were transferred to an adjacent bench. Lesion length of each stem was measured daily until day 15 after inoculation, and these data were used for analysis. There were five plants per treatment. The experiment was an RCBD with three replications, and it was performed twice.

**Cut stem inoculation of double-shoot grafts of cultivars**. In addition to the single-shoot technique, a double-shoot technique also was employed. Two shoots, NK S19-90 (R) and Williams 82 (S), were grafted to one rootstock, either NK S19-90 or Williams 82. Two to three grafts were made in each of two pots (replications) to make a total of five to seven double-shoot grafts for each combination. Self-grafts and plants that were not grafted were included. Incubation and inoculation procedures were performed as previously described. Disease symptoms including wilting and death of plants were observed daily for each graft and the controls until 5 days after inoculation.

**Statistical analysis**. An analysis of variance of the percent survival, AUSC values, and lesion lengths was completed using the general linear model procedure (PROC **Table 1. Analyses of variance for two grafting experiments of soybean inoculated with Sclerotinia sclerotiorum**

<table>
<thead>
<tr>
<th>Source of variation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P &gt; F</th>
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<tr>
<td>Cotyledon inoculation</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Trial</td>
<td>1</td>
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<td>Cut stem inoculation</td>
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<td></td>
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<tr>
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<td>Replication (trial)</td>
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<tr>
<td>Combination</td>
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<td>12.58</td>
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<tr>
<td>Trial x combination</td>
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<td>0.27</td>
<td>0.11</td>
<td>0.994</td>
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</tbody>
</table>

<sup>a</sup> The cotyledon inoculation experiment is based on plant survival 5 days after inoculation (DAI) and the cut stem inoculation methods is based on lesion lengths measured 15 DAI.

<sup>b</sup> Cv. NK S19-90, a partially resistant genotype (15), and cvs. Asgrow 2242 and Williams 82, susceptible genotypes (15), were reciprocally single-shoot grafted between resistant and susceptible genotypes, self-grafted, or not grafted. There were seven grafting combinations. Three were self-grafted plants; two were with cv. NK S19-90 as shoots with the two other cultivars as roots, and two were cv. NK S19-90 as rootstock with the two other cultivars as shoots. There were 7 to 10 plants per replication. Data from noninoculated controls were not used in the analysis.
GLM) in SAS (SAS Institute, Cary, NC) with trials and replications treated as random effects and genotypes and grafting combinations as fixed effects. Means were compared with Fisher’s protected least significant difference (LSD) values.

RESULTS

Cotyledon inoculation of single-shoot grafts of cultivars. Disease symptoms appeared on inoculated cotyledons 23 h after incubation. Infected areas expanded to the node causing water-soaking of the stem and wilting of leaves within 2 days after inoculation (DAI) followed by death of the plants. The experiment × treatment interaction was not significant, and data from the two trials were combined (Table 1). Based on seven grafting combinations of cross- and self-grafted cultivars, there was no significant difference for plant survival for the first 2 days (Fig. 1). At 3 DAI, a sharp decline in plant survival occurred in all grafts with susceptible shoots; all grafts that used NKS19-90 as shoots had significantly ($P < 0.05$) more plants survive (90%), regardless of the genotype used as the rootstock. At 5 DAI, only 5% of the plants that had susceptible shoots survived, while all grafts that used NKS19-90 as shoots had more plants ($P < 0.05$) survive (40%).

Cotyledon inoculation of single-shoot grafts of PIs and cultivars. Mean AUSC values for grafts using resistant PI shoots and susceptible cultivar rootstocks (AUSC = 331) were greater ($P < 0.05$) than those in reciprocal combinations (AUSC = 183). In self-grafts, plant survival of Alpha and Merit declined more than resistant self-grafts of PI194.639, PI503.336, and PI194.639 (Table 2). The self-grafts of PI194.639 had greater ($P < 0.05$) AUSC values than PI549.076A, but not greater than PI503.336. At 5 DAI, the percent survivals of Alpha, Merit, PI549.076A, PI194.639, and PI194.639 were, respectively, 9, 20, 65, 77, and 84%.

Cut stem inoculation of single-shoot grafts. All infected plants had bleached stem symptoms extending down from the point of inoculation. The experiment × treatment interaction was not significant, and data from the two trials were combined (Table 1). Stem lesion lengths were less after inoculation for grafts with shoots of NKS19-90 than with shoots of Asgrow 2242 and Williams 82 (Table 3). Initially, stem lesion lengths were not different among grafts; however, differences were observed from 10 to 15 DAI. When comparing lesion lengths between grafted resistant and susceptible shoots, resistant shoot grafts were not different, but grafts with susceptible shoots of cv. Williams 82 and Asgrow 2242 had larger ($P < 0.05$) lesions (mean = 13.2 cm) than resistant shoots (mean = 9.2 cm) regardless of the rootstock.

Cut stem inoculation of double-shoot grafts of cultivars. Regardless of the root-
stock, all susceptible shoots of Williams 82 became infected and died 3 DAI; meanwhile, the resistant shoots of NKS19-90 showed no disease symptoms and survived (Fig. 2A and B). Symptoms on Williams 82 plants began as early as 1 DAI as leaves became flaccid and wilted 2 DAI.

**DISCUSSION**

Several studies have demonstrated the association of shoots with physiological traits in soybean when using grafting techniques. For example, hypernodulating mutants were reported to be regulated by factors in plant shoots rather than roots (6,7,10), as it was shown that autoregulation of nodulation in root systems may involve the presence of shoot-derived inhibitor(s) as well as activator(s) (6). Shoot regulated the decline in the rate of nitrogen fixation (17) due to physiological changes in its components. Factors in shoots of soybean (PI 416,937) primarily controlled salt tolerance (1). However, using similar grafting techniques to study resistance mechanisms of two soilborne soybean diseases, it was concluded that roots played a major role in resistance to sudden death syndrome (18) and brown stem rot (2).

Sclerotinia stem rot of soybeans is primarily an aboveground stem disease, although the fungus is known to infect roots of other crops such as sunflowers (13), and it has been observed to cause basal infection of soybeans as well (G. L. Hartman, data not published). In this study, we evaluated soybean response to *S. sclerotiorum* infection using different shoot and rootstock combinations in either self-, single-, or double-shoot grafts and soybean genotypes. We did not observe in any of these graft combinations, or in noninoculated controls, graft incompatibility related to genotype, and we concluded that the observed plant death was due to the disease. Based on more than 25 graft combinations and two inoculation methods, resistance was greater when the grafted shoot came from a resistant source on a susceptible rootstock compared with the reciprocal combination regardless of the type of grafting technique or inoculation method. In addition, when using double-shoot grafts of one susceptible and one resistant shoot, the susceptible shoot died while the resistant shoot survived regardless of the genotype of the rootstock. Although it seems that shoots are associated with the expression of resistance to *S. sclerotiorum*, there are additional questions related to the role of root resistance in cases where the plant is infected through the roots or the crown.

The different grafting techniques, single- and double-shoot, provided a useful tool to show that *S. sclerotiorum* resistance in soybean originates from shoots and not from roots. More research needs to be conducted to determine the nature of these resistance factors and if they also can be found in roots when the basal portion of the stem or roots is infected. Future studies that investigate the interaction of resistance factors in the plant to oxalic acid produced by the fungus, which has been shown to be important in symptom development in dry beans, soybean, and sunflower (20,22,23), may note that a localized defense response is more likely than a defense product dependent on roots based on our experiments here with different grafting combinations.

**LITERATURE CITED**


