

Evaluation of Soybean Varieties and Breeding Lines for Resistance to Soybean Cyst Nematode and Their Copy Number Variation at *Rhg1* Locus

TECHNICAL REPORT

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a. Background Information

Soybean cyst nematode (SCN; *Heterodera glycines*), a major pest affecting soybean, significantly impacts yield in infested fields. Effective management of SCN relies heavily on the development and deployment of resistant soybean varieties, which is considered as a sustainable and environmentally friendly strategy. In North Dakota, SCN populations HG type 0, HG type 7 and HG type 2.5.7 are prevalent, posing a challenge to soybean production. Major sources of SCN resistance that are widely used to develop resistant soybean varieties include Peking and PI 88788. However, the overuse of these limited resistance sources has led to the emergence of more virulent SCN populations capable of overcoming this resistance. So, it is crucial to explore new resistance sources and thoroughly evaluate soybean breeding lines and commercial varieties for resistance to SCN. The *Rhg1* locus, derived from PI 88788, is known to confer strong resistance to SCN. More than 90% of soybean varieties in the United States utilize PI 88788 as their resistance source against SCN. Previous research has shown that the copy number at the *Rhg1* locus determines the level of resistance to SCN. Therefore, evaluation of the resistance responses of soybean breeding lines and commercial varieties to SCN populations help to select SCN resistant soybeans, and assessing copy number at *Rhg1* locus facilitate rapid selection of soybean lines with increased SCN resistance.

b. Research Objectives

1. Evaluate 40 commercial soybean varieties for their resistance responses to two common SCN populations detected in North Dakota.
2. Evaluate 100 NDSU breeding lines for their resistance levels to two common SCN populations detected in North Dakota.
3. Assess their copy number variation for rapidly selecting the lines with the *rhg1* resistance gene against SCN.

c. Materials and Methods

A total of 152 soybean breeding lines from the NDSU breeding program (117) and commercial soybean varieties (35) from various companies and growers were acquired and used. Out of these, 12 varieties and all breeding lines were screened for resistance reactions to SCN population HG type 7 and 23 varieties were screened for HG type 0. All of these soybean breeding lines and varieties were screened for resistance reactions to SCN population HG type

2.5.7. The HG type 2.5.7 population, which has a higher ability to reproduce on the major SCN resistance source PI 88788 and its derivative lines, was collected from Richland County, ND, and HG type 0 and 7 was collected from Traill County, ND.

Five days old pre-germinated seedlings from each of the soybean breeding lines and varieties were planted in 100 cc of pasteurized river sand in cone-tainers, which were arranged in a completely randomized design with four replicates. Each plant was inoculated with 2,000 SCN eggs at the time of planting and grown in a controlled growth chamber maintained at 27°C with a 16-hour daylight period for 32 days. Then white females formed on each plant were extracted and counted, and the numbers of white females in the four replicates were averaged to determine the mean number of white females. This mean was then used to calculate the Female Index (FI) according to the formula: $FI = (\text{mean number of white females produced on a tested soybean line} / \text{mean number of white females on the susceptible check, Barnes}) \times 100\%$. Based on the FI values, soybean varieties and lines were categorized for their resistance responses into four groups, as described by Schmitt and Shannon (1992): resistant (R) (FI < 10%), moderately resistant (MR) (FI = 11% to < 30%), moderately susceptible (MS) (FI = 30% to < 60%), or susceptible (S) (FI ≥ 60%).

To determine copy number variations at the *Rhg1* locus, a real-time quantitative PCR (qPCR) assay was used. This assay was optimized for annealing temperature, primer concentration, and DNA template concentration, and validated using 12 soybean accessions with known copy numbers at the *Rhg1* locus. Genomic DNA was extracted from the leaf tissues of 10-day-old soybean plants. A total of 50 DNA samples were prepared from the soybean breeding lines from the NDSU breeding program.

The qPCR was conducted using an internal control gene, a heat-shock protein gene (*hsp*). The qPCR reaction was performed in a 10 µl volume (containing 5 µl of 2× Sso Advanced SYBR Mastermix, 0.2 µl each of forward and reverse primers (10 mM), 3.1 µl of nuclease-free H₂O, and 1.5 µl of template DNA). The amplification program included an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, and annealing at 60°C for 1 minute. Relative quantification was determined using the $2^{-\Delta\Delta CT}$ method to calculate the copy number based on the reference check, Williams 82 with a single copy of *Rhg1* repeat. The copy numbers of the 50 breeding lines obtained by qPCR assay were then compared with the resistance responses to two SCN populations, HG type 7 and HG type 2.5.7, to assess their degree of association. Correlation analysis was conducted between the female indexes of the respective HG types and the copy numbers from qPCR assay to evaluate if the copy number could serve as an indicator to determine the resistance level to SCN.

d. Research Results and Discussion

Among the 35 commercial soybean varieties tested for HG type 0 or 7, nine varieties were resistant (FI: 0.0 to 9.6%), eight varieties were moderately resistant (FI: 14.5 to 27.1%), 13 varieties were moderately susceptible (FI: 33.5 to 58.5%), and the remaining five varieties were susceptible (FI: 60.2 to 89.6%) (Figure 1). For another SCN population HG type 2.5.7, six varieties were resistant (FI: 2.4 to 8.7%), seven varieties were moderately resistant (FI: 15.2 to 29.9%), 10 varieties were moderately susceptible (FI: 32.3 to 59.8%), and the remaining 12 varieties were susceptible (FI: 60.5 to 120.2%) (Figure 1). Interestingly, 12 of the varieties were resistant or moderately resistant to both the SCN populations (HG type 2.5.7 and HG type 0/7).

Likewise, among the 117 NDSU breeding lines screened for HG type 7, six lines were resistant (FI: 7.8 to 9.9%), 34 lines were moderately resistant (FI: 12.9 to 29.9%), 22 lines were

moderately susceptible (FI: 34.8 to 59.6%), and the remaining 55 lines were susceptible (FI: 60.0 to 91.3%) (Figure 2). For HG type 2.5.7, none of the lines were resistant, 25 lines were moderately resistant (FI: 22.0 to 28.7%), 18 lines were moderately susceptible (FI: 30.5 to 59.8%), and the remaining 74 lines were susceptible (FI: 60.1 to 90.1%) (Figure 2). ND21-11516(GT) had the lowest female index value for both HG type 7 (7.8%) and HG type 2.5.7 (22.0%). A total of 25 breeding lines among the 117 breeding lines showed resistant or moderately resistant reaction to both the HG types (Figure 3).

The majority of the 152 soybean lines and varieties tested for the two SCN populations were susceptible or moderately susceptible. Comparatively, more resistant and moderately resistant soybeans with lower female index values were identified when screened for HG type 0/7 compared to HG type 2.5.7. This disparity is likely due to the different genetic diversities of the SCN populations, each exhibiting unique capabilities in parasitizing soybean lines. The higher susceptibility observed in lines screened for HG type 2.5.7 indicates a direct association between pathogen virulence and the SCN population's ability to overcome resistance, leading to an increased presence of SCN white females on the root system.

The *Rhg1* locus copy numbers among the 50 breeding lines ranged from 1 to 11. Six lines had 11 copies, seven had 10 copies, and the remaining 37 had a single copy (Figure 4). Lines with high copy numbers (10 or 11) were either resistant or moderately resistant to HG type 7 and moderately resistant or moderately susceptible to HG type 2.5.7 (Figure 5). Majority of the lines with a single copy exhibited moderately susceptible or susceptible response to HG type 7 and exhibited susceptible response to HG type 2.5.7. A strong negative correlation was observed between the female indexes and copy numbers for both HG type 7 ($r = -0.780$) and HG type 2.5.7 ($r = -0.922$), suggesting that an increased copy number at *Rhg1* is indicative of greater resistance to SCN. One line with a single copy at *Rhg1* locus was moderately resistant to HG type 2.5.7 and two lines with a single copy at *Rhg1* locus were moderately resistant to HG type 7. This suggests further experiments on SCN resistance phenotyping and investigation into the potential co-expression or genetic interaction of different SCN resistance genes in these lines.

e. Benefits to ND Soybean Farmers and Industry

This study identified commercial varieties and breeding lines with resistance to SCN. Twelve commercial varieties among the 35 commercial varieties showed resistant or moderately resistant response to both HG types, offering valuable options for farmers to select resistant varieties for infested fields, reducing yield losses and ensuring stable production. Additionally, 25 breeding lines among the 117 breeding lines were resistant or moderately resistant to both HG types, providing breeders with a pool of candidates for developing new SCN-resistant varieties. The determination of *Rhg1* copy number further aids in the rapid selection of breeding lines with increased resistance to SCN. The integration of both phenotypic and molecular methodologies in this research has facilitated the exploration of potential correlations between the resistance response and copy number variation at the *Rhg1* locus. The findings of these two approaches appear to be mutually corroborative. A manuscript on this topic has been prepared for submission to a journal for publication for unrestricted use to disseminate the research findings.

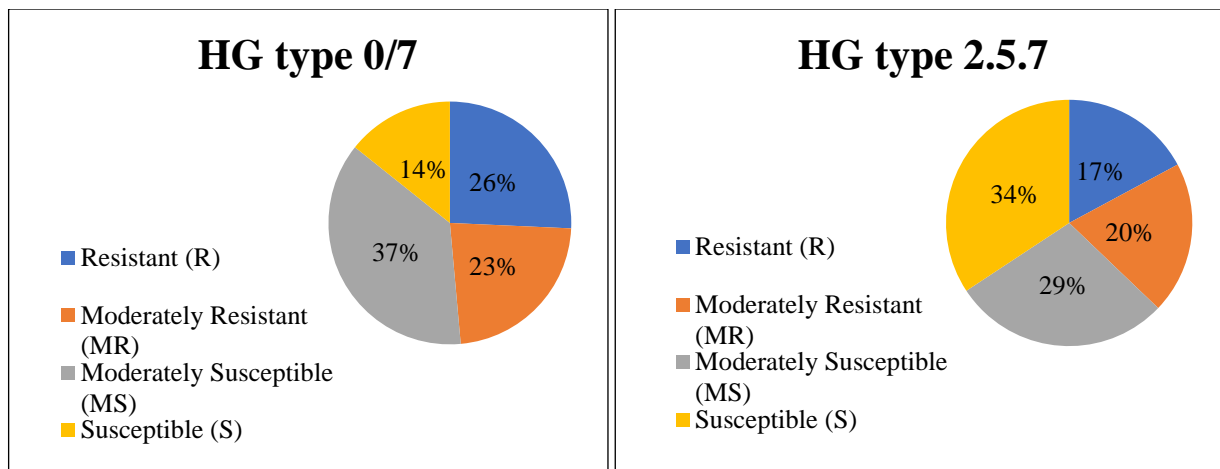


Figure 1. Resistance responses of 35 commercial soybean varieties from companies and growers to HG type 0/HG type 7 and HG type 2.5.7 isolated from soybean fields in ND.

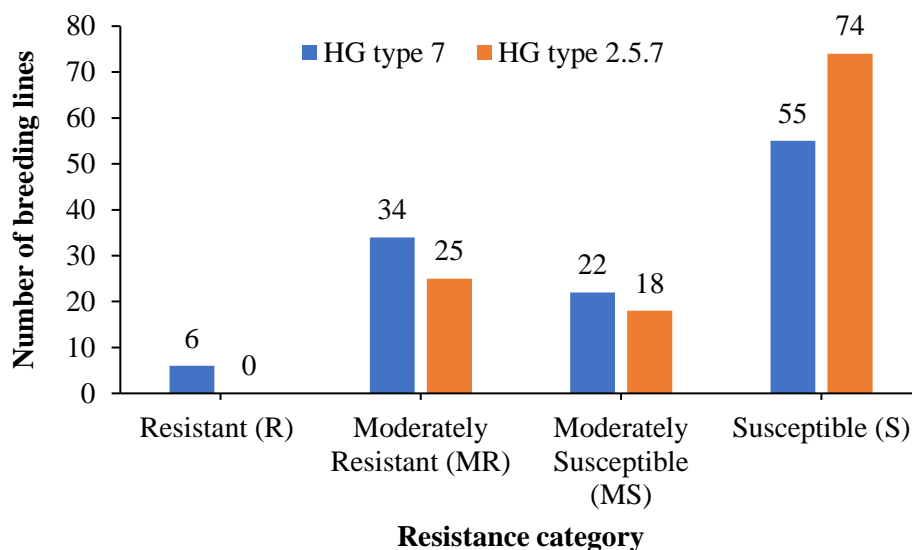


Figure 2. Distribution of 117 soybean breeding lines across four resistance categories: resistant (R) (FI < 10%), moderately resistant (MR) (FI = 10 to <30%), moderately susceptible (MS) (FI = 30 to <60%), and susceptible (S) (FI ≥ 60%), based on the criteria established by Schmitt and Shannon (1992), for HG types 2.5.7 and 7 isolated from soybean fields in ND.

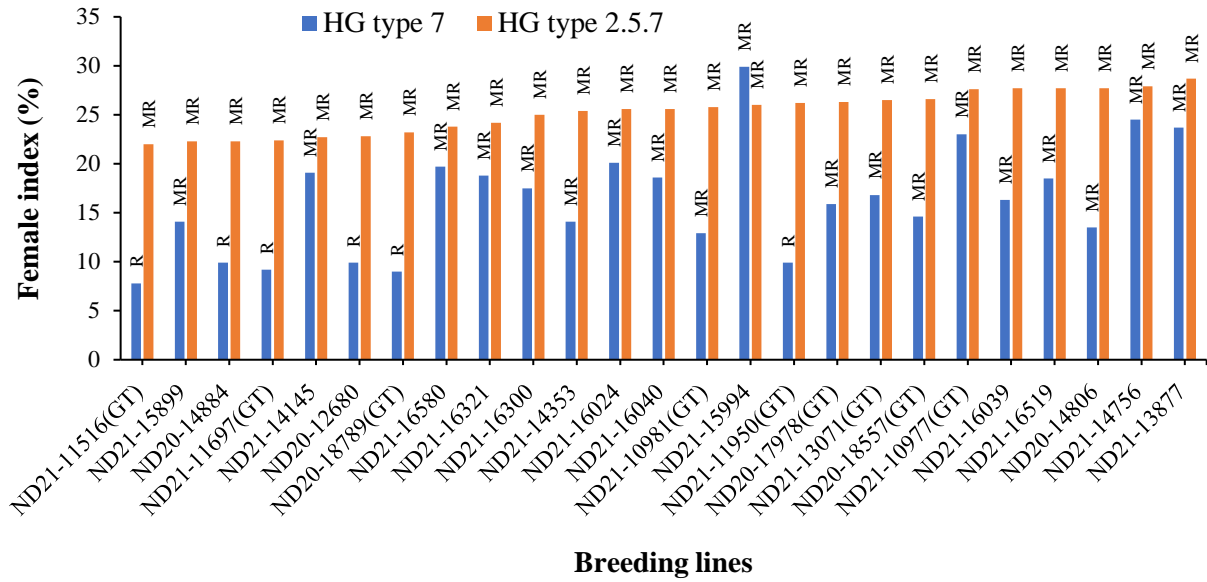


Figure 3. Resistance responses of 25 breeding lines that showed resistant or moderately resistant response to both HG type 7 and 2.5.7 collected from soybean fields in ND.

Copy number variation at *Rhg1*

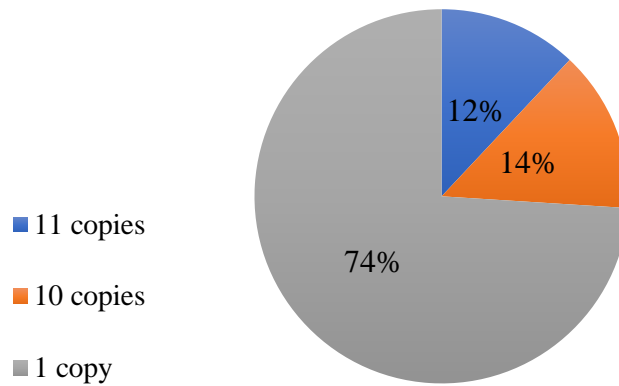


Figure 4. Copy number variation at the *Rhg1* locus among 50 randomly selected breeding lines from the NDSU soybean breeding program.

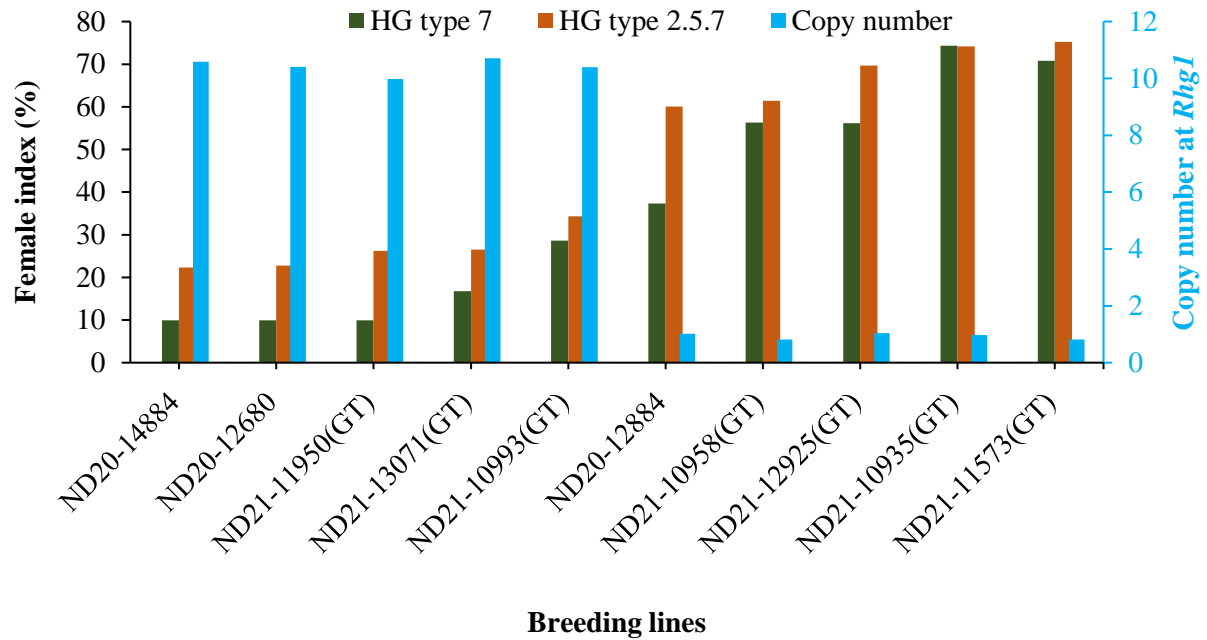


Figure 5. Relationship between female indexes (%) and copy numbers at *RhgI* locus of 10 randomly selected breeding lines. The primary y-axis represents the female index, while the secondary y-axis represents the copy number at the *RhgI* locus.