**Resistance of Soybean Cultivars to a New Root-lesion Nematode Species in North Dakota**

TECHNICAL REPORT

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 Root-lesion nematodes (*Pratylenchus* spp.) are one of the more destructive groups of plant-parasitic nematodes worldwide. These soil-borne endoparasitic pathogens have a wide host range, which includes soybean plants. In North Dakota, during 2015 and 2016, soil surveys of different soybean fields were conducted to determine the prevalence and distribution of plant-parasitic nematodes. During the surveys six soil samples collected from a soybean field in Richland County showed that root-lesion nematodes were present in all of these samples with population densities ranging from 125 to 2,000 per kg of soil. Morphological and molecular tests revealed that this nematode differs from other known species of root-lesion nematodes in both morphology and DNA sequences. In 2021, this new species of root-lesion nematode was named as *Pratylenchus dakotaensis*, paying homage to the state in which it was discovered.

 Previous greenhouse studies to ascertain resistance levels of 20 soybean cultivars to *P. dakotaensis,* revealed that the resistance levels of those cultivars varied from moderately resistant to susceptible. However, none of the cultivars tested were resistant against *P. dakotaensis*. Hence the objectives of this study were to evaluate ten additional soybean cultivars to determine the levels of resistance to this new root-lesion nematode species (*P. dakotaensis*) detected in North Dakota and also to develop a real-time PCR assay to detect and quantify this new species directly in DNA extracts from field soil.

 To achieve the objectives, soil samples were collected from the Richland County soybean field in which *P. dakotaensis* was discovered. Soil samples were then thoroughly mixed together into a composite sample to ensure even distribution of nematodes. Nematodes were then extracted from three subsamples to determine the initial population density. Ten cultivars used in the region were selected for resistance tests, including 0916R2X, 50-90, 50948N, H06X7, LS-1138NRR2X, ND Benson, ND Stutsman, NS 1492NR2, NS 61624NXR2, and SB-8807N (Table 1). Additionally, the local cultivar Barnes was used as a positive control and an unplanted control was used as a negative control. Moreover, the cultivar NS 1911NR2 was used as a susceptible check as it was found to be the most susceptible cultivar in all trials of our previous experiments.

 In this study during experiment setup, seeds from each of the cultivars were pre-germinated in a petri dish for 4 to 5 days. Two trials were conducted. For trial 1, the pre-germinated seeds were planted in large cone-type containers containing soil (≈ 500g of soil) naturally infested with *P. dakotaensis*. Each cultivar was planted in five replicates. The plants were then grown in a greenhouse room for 15 weeks at 22 °C and a photoperiod of 16 hours. To confirm the results, the experiment was also repeated. For trial 2, the pre-germinated seeds were planted in small cone-type containers containing soil (≈ 170g of soil) naturally infested with *P. dakotaensis*. Similar to trial 1 five replicates of each cultivar were also planted for trial 2 and the plants were incubated in a greenhouse room at the same greenhouse conditions. Trial 2 was harvested at nine weeks after planting as our previous greenhouse studies demonstrated that resistance rating did not vary significantly at nine weeks with small containers compared to 15 weeks with large containers.

 During harvest, the above-soil part of each plant was cut off, and soil and roots were collected for nematode extraction. The plant roots were cut into 1 to 2 cm pieces and mixed thoroughly with the soil; nematodes were then extracted from both roots and soil using the Whitehead tray nematode extraction method. The extracted nematodes were then accumulated in a 20-µm-aperture sieve and collected in a nematode suspension vial. Root-lesion nematodes were identified and quantified based on their morphological feature under a microscope. The postharvest densities of root-lesion nematodes were then averaged across the five replicates of each cultivar and tallied as the total number of individuals per kg of soil and roots. The average reproductive factor was determined by dividing the average postharvest population density by the initial population density (Table 1).

 Resistance ratings were scaled on the basis of average postharvest population density of *P. dakotaensis* in a test line relative to the average postharvest population density in the susceptible check (NS 1911NR2). Ratio of the postharvest population density in the test line relative to the susceptible check was calculated and expressed as a percentage (Table 2). The host ranking for each cultivar was then categorized into four classes based on the ratio. The four resistance rating classes were resistant = R (average postharvest population density ≤ 25% of the susceptible check), moderately resistant = MR (26-50%), moderately susceptible = MS (51-75%), and susceptible = S (≥ 76%). Results from both trials were also combined by averaging the ratios of postharvest population densities across the two trials (Figure 1).

 To develop a quantitative real-time PCR (qPCR) assay for detecting and quantifying this new species in DNA extracts from field soil, the primer pair (IC-ITS1F/IC-ITS1R) designed from our previous work was tested for its specificity using both nematode individuals (*P. scribneri, P. neglectus and P. penetrans*) and soil DNA extracts (seven field soil samples). A two-fold serial dilution using DNA extract from a single nematode was prepared to determine the detection sensitivity. Varying number of *P. dakotaensis* (1, 4, 16, 64, and 256) were picked and added to 0.5 g of autoclaved soil. DNA extraction was done in triplicate for each level of infestation using the Qiagen DNeasy PowerSoil Kit and qPCR reaction was performed in triplicate for each of the DNA extracts to generate the standard curve. Melting curve analysis was done to monitor the specificity of the assay. A total of 15 soil samples were collected from a soybean field in Richland County. Nematodes were manually extracted from 200 g of soil using sugar centrifugation method and counted twice under a microscope. DNA was extracted directly from each soil sample and assayed by the qPCR and standard curve. Correlation analysis was conducted to determine the relationship between the nematode numbers determined by the qPCR assay and by the traditional microscopic method.

 The resistance rating results from the first and second trials were similar. However, the average postharvest population densities and reproductive factors varied between the two trials. In trial 1, the average postharvest population density and reproductive factor values of *P. dakotaensis* ranged from 3,236 (cultivar name: 50-90) to 6,930 (NS 61624NXR2) nematodes per kg of soil and roots and from 2.02 to 4.33, respectively. In trial 2, these two parameters ranged from 1,482 (0916R2X) to 2,235 (NS 1911NR2) nematodes per kg of soil and roots and from 1.35 to 2.03, respectively. The variation in these two parameters could be attributed to the difference in initial population densities between the two trials. The initial population density for trial 1 was 1,600 nematodes per kg of soil, whereas for trial 2 it was 1,100 nematodes per kg of soil. Additionally, trial 2 was harvested earlier than trial 1. Thus, as expected all of the cultivars in trial 2 had lower postharvest population densities and reproductive factors compared to trial 1. Nonetheless, similar trend was evident between the two trials. For example, cultivars that had higher reproduction in trial 1 also had higher reproduction in trial 2.

 All of the cultivars tested had the same resistance rating in both trials except one cultivar (50948N) having a resistance rating of moderately susceptible in trial 1 but susceptible in trial 2 (Table 2). However, it is important to note that, the ratio of postharvest population density for 50948N was only 7% higher in trial 2 than that in trial 1. The resistance ratings of the cultivars tested varied from moderately susceptible to susceptible in both trials. Combined results of the two trials revealed that three cultivars including 0916R2X (ratio: 67%), 50-90 (68%), and 50948N (71%) were moderately susceptible and the remaining seven cultivars were all susceptible to *P. dakotaensis* (Figure 1). These susceptible cultivars include H06X7 (84%), LS-1138NRR2X (81%), ND Benson (94%), ND Stutsman (88%), NS 1492NR2 (105%), NS 61624NXR2 (126%), and SB-8807N (90%). The positive control Barnes (86%) was rated as a susceptible cultivar. None of the cultivars tested were resistant or moderately resistant.

 The qPCR assay detected DNA only specific to *P. dakotaensis* with Cq values ranging from 19.86 ± 0.10 to 29.48 ± 0.44. No fluorescent signals were detected from the non-target nematode species and soil DNA extracts. The qPCR assay could detect an equivalent of 1/32 of the DNA of a single nematode. Melting curve analysis showed a single melting peak at 81.5o C (Figure 2A). A standard curve (y = -3.5528x + 28.352) was generated, showing a high amplification efficiency (E= 91%) and a strong correlation between the numbers of nematodes added to autoclaved soil and the numbers determined by theqPCR(R² = 0.99) (Figure 2B). A good, positive correlation was observed between the numbers of nematodes obtained from the 15 field soil samples through manual counting and the corresponding qPCR counts (y = 1.6825x - 334.97, R² = 0.7847) (Figure 3).

 The resistance rating results provide us with an insight into the virulence of the new root-lesion nematode species, *P dakotaensis*, on commercial soybean cultivars. Further research is necessary to evaluate more cultivars to identify soybeans with resistance against *P. dakotaensis*. Such research would be useful to identify best performing resistant cultivars with which growers can manage the nematode to minimize yield loss. The qPCR assay provides a rapid and efficient method for *P. dakotaensis* detection and quantification in place of time-consuming steps of conventional nematode extraction, microscopic identification and counting. The developed assay does not require any taxonomical expertise, has immense practical significance and thus, can serve as a valuable diagnostic tool for providing informed decisions to growers.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | Average postharvest population density | Reproductive factor |
| Cultivar ID | Company | Maturity Group | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| 0916R2X | Channel | 0.9 | 3,335 | 1,482 | 2.08 | 1.35 |
| 50-90 | Proseed | N/A | 3,236 | 1,647 | 2.02 | 1.50 |
| 50948N | Integra Seed | 0.9 | 3,430 | 1,694 | 2.14 | 1.54 |
| Barnes | NDSU breeding | 0.3 | 4,112 | 2,118 | 2.57 | 1.93 |
| H06X7 | Hefty Seed | 0.6 | 4,243 | 1,835 | 2.65 | 1.67 |
| LS-1138NRR2X | Legacy Seed | 1.1 | 3,983 | 1,859 | 2.49 | 1.69 |
| ND Benson | NDSU breeding | 0.4 | 4,610 | 2,206 | 2.88 | 2.01 |
| ND Stutsman | NDSU breeding | 0.7 | 4,211 | 2,165 | 2.63 | 1.97 |
| NS 1492NR2 | NorthStar Genetics | 1.4 | 5,490 | 2,059 | 3.43 | 1.87 |
| NS 1911NR2 | NorthStar Genetics | 1.9 | 4,986 | 2,235 | 3.12 | 2.03 |
| NS 61624NXR2 | NorthStar Genetics | 1.6 | 6,930 | 2,165 | 4.33 | 1.97 |
| SB-8807N | Thunder seed | 0.7 | 4,380 | 2,118 | 2.74 | 1.93 |
| Non-planted control | - | - | 1,450 | 541 | 0.91 | 0.49 |

Table 1. Postharvest population density and reproductive factor of the new root-lesion nematode species (*Pratylenchus dakotaensis*) on each of the soybean cultivars and controls in two trials conducted.

\*\*Postharvest population density was obtained by averaging five replicates of each of the cultivars. Reproductive factor was determined by dividing the average postharvest population density by the initial population density.

Table 2. Host ranking of ten soybean cultivars, the positive control “Barnes” and the susceptible check NS 1911NR2 to the new root-lesion nematode species (*Pratylenchus dakotaensis*).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Cultivar ID | Company | Maturity Group | Trial 1 | Trial 2 |
| Ratio (%) | Resistance rating | Ratio (%) | Resistance rating |
| 0916R2X | Channel | 0.9 | 66.9 | MS | 66.3 | MS |
| 50-90 | Proseed | N/A | 64.9 | MS | 73.7 | MS |
| 50948N | Integra Seed | 0.9 | 68.8 | MS | 75.8 | S |
| Barnes | NDSU breeding | 0.3 | 82.5 | S | 94.7 | S |
| H06X7 | Hefty Seed | 0.6 | 85.1 | S | 82.1 | S |
| LS-1138NRR2X | Legacy Seed | 1.1 | 79.9 | S | 83.2 | S |
| ND Benson | NDSU breeding | 0.4 | 92.5 | S | 98.7 | S |
| ND Stutsman | NDSU breeding | 0.7 | 84.5 | S | 96.8 | S |
| NS 1492NR2 | NorthStar Genetics | 1.4 | 110.1 | S | 92.1 | S |
| NS 1911NR2 | NorthStar Genetics | 1.9 | 100.0 | S | 100.0 | S |
| NS 61624NXR2 | NorthStar Genetics | 1.6 | 139.0 | S | 96.8 | S |
| SB-8807N | Thunder seed | 0.7 | 87.8 | S | 94.7 | S |
| Non-planted control |  | - | 29.1 | - | 24.2 | - |

\*\* Ratio of postharvest population densities (%) = (postharvest population density in a test line/ postharvest population density of the susceptible check) x 100. The ratio for each cultivar and the non-planted control was then averaged across the five replicates. The rating was categorized based on the ratio: Resistant = R (postharvest population density ≤ 25 % of the susceptible check), Moderately Resistant = MR (26-50 %), Moderately Susceptible = MS (51-75 %), and Susceptible = S (≥ 76 %).

Figure 1. Classification of the resistance responses of ten soybean cultivars to the new root-lesion nematode species (*Pratylenchus dakotaensis*)based on the two trials.

**A.**



**B.**

Figure 2. **A:** *Pratylenchus dakotaensis* melting curve profile. No amplification was observed for negative controls. **B**: The standard curve, regression equation, and co-efficient of determination (R2) for the qPCR assay developed for detection and quantification of *P. dakotaensis* in soil. Quantification cycle (Cq) was plotted against the log of the number of *P. dakotaensis* (1, 4, 16, 64, and 256) added to 0.5 g of autoclaved soil. Each red dot represents an average of three biological replicates for each treatment run in triplicate. Amplification efficiency (E) = 10(1/−m) – 1, where m is the slope of the regression equation.

Figure 3. A correlation curve for nematode quantification from 15 naturally infested field soil samples between the numbers of *Pratylenchus dakotaensis* determined by qPCR and by sugar extraction and microscopic counting method. Each dot for y-axis represents the mean of three independent biological replicates run in triplicate and the corresponding dot for x-axis represents the average of nematode counts in each extraction manually counted twice.