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The use of RAPD amplified DNA as markers for virulence characteristics in soybean cyst nematode

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Summary – The feasibility was investigated for developing RAPD-based diagnostic dot blot tests to separate field isolates of soybean cyst nematode with different virulence to resistant cultivars of soybean. A RAPD primer was found that separated two northern Indiana isolates of the nematode with similar virulence toward resistant soybean lines, from two southern Indiana isolates with different virulence characteristics; and a molecular marker probe was developed for use with dot blots. The study demonstrates the feasibility for developing a RAPD-based test for use in diagnosing larger groups of field isolates of nematodes that share common virulence characteristics toward resistant cultivars.

Résumé – ***Utilisation d'ADN amplifié par RAPD comme marqueur génétique de la virulence du nématode à kystes du soja*** – En vue de reconnaître la virulence de certaines souches du nématode à kystes du soja envers des cultivars résistants de soja, il a été procédé à une évaluation des potentialités de discrimination de tests « dot-blot », après amplification de l'ADN par RAPD. Une amorce de RAPD sépare deux souches du nématode du nord de l'Indiana ayant des niveaux comparables de virulence envers des lignées résistantes de soja, de deux souches du sud de l'Indiana présentant des caractères de virulence différents. Une sonde moléculaire pour les tests « dot-blot » a été également mise au point. Cette étude montre la possibilité de mettre au point, à partir de RAPD, des tests permettant de reconnaître les grands groupes de souches du nématode ayant des caractères communs de virulence envers les cultivars résistants de soja.

Key-words : *Heterodera glycines* RAPD markers, Southern analysis, soybean cyst nematode.

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a serious pest of soybeans in many parts of the world. It was first reported in North America in 1954, in North Carolina, USA, and now is known to be widespread in soybean growing areas. Ferris *et al.* (1986) reported finding protein differences between southern and northern Indiana isolates in 2-D PAGE patterns. Faghihi *et al.* (1986) reported that among those isolates tested, SCN populations from the northern part of Indiana tended to be more virulent to resistant breeding lines of soybean than were isolates from farther south.

It has been demonstrated that DNA sequence variation can be detected by random amplified polymorphic DNA (RAPD) amplification (Williams *et al.*, 1990, 1993; Rafalski *et al.*, 1993), and RAPD amplification has been used to study differences in populations of *Heterodera schachtii* Schmidt (Caswell-Chen *et al.*, 1992). Although the RAPD technique has been utilized for genetic mapping, taxonomic identification, analyzing mixed genome samples, and other purposes (Hadrys *et al.*, 1992), our goal was to investigate the potential for generating molecular marker probes for use in a RAPD-based diagnostic test to separate field isolates of SCN with different virulence toward resistant soybean cultivars. For a feasibility test of this approach, it was necessary to select four field isolates of SCN, two of which shared a similar high degree of virulence toward a group of resistant soybean lines; and two of which were less virulent toward the same lines (for simplicity we will refer to these as virulence groups). The isolates would have to be sufficiently variable to generate different RAPD patterns, but some pattern commonalities would have to be found that would distinguish the more virulent pair of isolates from the less virulent.

Materials and methods

Assignment of four nematode field isolates to the two virulence groups was based on data from ongoing yearly laboratory tests of many field isolates with soybean breeding lines. Isolates NJ2 and NW6 are from Jasper County and White County respectively in the northern half of Indiana. Isolates SVI1 and SVI3 are isolates from widely separated areas in Vigo County in the southern half of Indiana. The original field locations of all four isolates are within a radius of 100 miles of each other. The number of replications for each isolate on each soybean line varied with the particular year of the test, but was always either four or five replications. The tests were carried out in glass tubes 2.5 cm diam. × 7.5 cm length, each of which held 10 ml water plus 1 ml of a

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water suspension of 3 000-4 000 eggs and juveniles of the test population (Faghihi *et al.*, 1986). For each test, one 4 to 6-day-old seedling was placed in the tube. A soil/sand (1 : 3) mixture was added to cover the roots, the tube labelled and placed in a basket with other tubes at 24 °C in a water bath. At the end of 28-30 days the females and cysts were separated from the roots placed on a sieve, by forcefully spraying with water. The females and cysts were recovered on ruled filter paper with a Büchner funnel and vacuum pump (Faghihi *et al.*, 1986). Race tests (Riggs & Schmidt, 1988) were carried out in a similar manner, using the standard differentials, with " Williams 82 " as the reference susceptible.

For each of the four field isolates of nematodes, 300 mg of clean cysts were ground to a powder in liquid nitrogen with a mortar and pestle that had been chilled on dry ice. The ground nematode powder was placed in a chilled sterile test tube and 2 ml of lysis buffer (0.5 M Tris-HCl [pH 8.0], 100 mM EDTA, 1 % Triton-X-100, 1 % β-mercaptoethanol, 100 µg/ml proteinase K) was added. The tube was placed at 56 °C for two hours, then cooled on ice and 1 ml placed in each of two 2-ml Eppendorf tubes. The DNA was extracted once with phenol and once with phenol/chloroform, and precipitated after addition of 0.1 vol of 3 M sodium acetate plus two volumes of ethanol, by 10 min centrifugation at 14 000 RPM (= 16 000 *g*). The precipitate was washed once with 70 % ethanol, dried by centrifugation under vacuum, and resuspended in 30 µl TE (10 mM Tris-HCl, 1 mM EDTA), pH 8. The DNA concentration was determined with the TKO 100 Mini Fluorometer (Hoefer Scientific Instruments, San Francisco, CA) and each preparation brought to a concentration of 0.5 µg/µl. RAPD amplification tests in which DNA template was used from 0.4 ng to 12 ng/25 µl total reaction volume indicated good RAPD amplification with 4-8 ng DNA, and DNA samples were diluted to 4 ng/µl with TE for subsequent use in all screening of RAPD primers. Prior to conclusion of the study, DNA was again extracted from newly collected cysts and used to confirm the usefulness of the B 19 primer.

Cyst preparations were made by a method similar to that of Caswell-Chen *et al.* (1992), except that InstaGene (BioRad) was used instead of Chelex 100 resin (Walsh *et al.*, 1991). A clean cyst or young female was ground in 20 µl cold TE buffer with a glass homogenizer (25 µl size), transferred to a 0.5 ml Eppendorf tube, and centrifuged at high speed for 3 min. After 15 µl of the supernatant was discarded, 25 µl InstaGene was added to the pellet, mixed, and the tube placed at 56 °C for 30 min. The sample was then vortexed for 10 s, placed in boiling water for 8 min, vortexed again for 10 s, centrifuged for 3 min at 14 000 RPM (= 16 000 *g*), and 1-2 µl of the supernatant used as the template in a 25 µl amplification reaction. When multiple cysts were used, the quantity of InstaGene was increased.

RAPD amplification was carried out with 60 10-mer oligonucleotide primers (Primer kits A, B, D, Operon Technologies Inc., Alameda, CA), using as template the extracted DNA from the four field isolates of SCN. Two thermocyclers were used at different times during the experiments, a Perkin Elmer DNA Thermal Cycler (Norwalk, CT) and a Coy Model 60 Tempcycler (Ann Arbor, MI). It is known that different thermal cyclers may produce markedly different results in RAPD amplification (Rafalski *et al.*, 1993) and we used both instruments for repeated testing of primers that produced patterns with potential use as molecular markers. Both thermal cyclers were programmed initially for 45 cycles of 20 s at 94 °C (denaturation), 40 s at 37 °C (annealing), and 1 min at 72 °C (extension). Various modifications of these settings were used during the course of the experiments. Reaction volumes were 25 µl and the reactions initially consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1 % Triton-X-100, 1.5 mM $MgCl_2$, 25 µM each dTTP, dCTP, dGTP, and dATP, 0.2 µM primer; 5-6 ng genomic DNA, and 1 unit of *Taq* DNA polymerase. For most tests 10X reaction buffer (containing KCl and Triton-X-100 and 100 mM $MgCl_2$ solution) were purchased from Promega (Madison, WI). For some experiments the buffer reagents were prepared in the laboratory. Solutions of 100 mM dNTPs were purchased from Boehringer Mannheim (Indianapolis, IN). The *Taq* DNA polymerase was from Promega. During the course of the experiments many alterations in the reaction mixture were tried in attempts to optimize reactions. Primer concentrations up to 1.0 µM were used. Control reactions which contained all reaction components except template DNA were included in every case, and all reactions were overlaid with mineral oil. The patterns of amplified products were analyzed by electrophoresis in 1.4 % TBE agarose gels run in 1 X TBE buffer at 70-80 V constant DC. Size markers were λ DNA/Hind III (BRL-Life Tecchnologies Inc., Gaithersburg, MD) and/or the BRL 123 bp DNA ladder. DNA bands were visualized by ethidium bromide staining, and gels were photographed.

For each primer that appeared to produce bands that might serve as potential markers to distinguish either virulence group, blots were prepared from gels containing amplified DNA obtained with the particular primer. Southern transfer of DNA fragments to GeneScreen Plus (Du Pont NEN) positively charged nylon membrane was done in the TransVac® (Hoefer) in 10 X SSC (3M NaCl, 0.3 M Sodium citrate).

To increase DNA from a specific RAPD pattern fragment for use as a hybridization probe, re-amplification of the DNA was carried out as previously described (White & Blake, 1991; Ferris *et al.*, 1993). The amplified DNA fragment of interest was excised from SeaPlaque agarose (FMC) and melted in 0.5-1 ml H_2O at

65 °C. This diluted DNA became the template for additional amplification by PCR. For this amplification, the thermocycler was programmed initially for 3 min at 94 °C, followed by 30 cyles of 1 min at 94 °C, 1 min at 43 °C, and 2 min at 72 °C. At times these parameters were changed in attempts to optimize the reaction. The band of interest was excised from 1.4 % TAE (40 mM Tris-acetate, 2 mM EDTA) agarose and concentrated by the GeneClean method (Bio 101, San Diego). DNA concentration of the probe was estimated by comparison of the UV fluorescence of a band on an ethidium bromide stained gel with the fluorescence of a known λ Hind III standard (Sambrook *et al.,* 1989). The diluted DNA from the excised DNA fragment (obtained as described above) was also used as a positive control in subsequent RAPD amplifications to produce blots for hybridization experiments to test the efficacy of that particular fragment as a marker probe.

Hybridization in formamide was essentially as described by Ausubel *et al.* (1994) except that the hybridization solution, which was 50 % formamide, was from Wahl and Berger (1987). The cleaned and concentrated DNA to be used as a radioactive probe was labeled with ^{32}P using the Prime-a-Gene labeling system (Promega). Unincorporated label was removed by column chromatography (Ausubel *et al.,* 1994). Incorporation efficiency was determined by a liquid scintillation counter. The amount of labelled probe (1 X 10^6 cpm/100 cm^2 of filter) added to the hybridization solution was calculated on the basis of the radioactivity of the probe at the time of use. After hybridization, washing was by standard methods (Ausubel *et al.,* 1994) and the nylon membrane containing the hybridized DNA was exposed to Kodak X-ray film for 1-4 hours.

Biotinylated probe for dot blots was prepared with the BioPrime DNA labeling system (BRL) according to the manufacturer's protocols. Unincorporated nucleotides were separated from the biotinylated probe using column chromotography (Ausubel *et al.,* 1994). As recommended, twelve five-drop fractions were collected and 1 µl from each fraction was spotted on nylon membrane. The DNA was stabilized by UV cross-linking and the peak of biotinylated DNA detected with the BRL PhotoGene nonradioactive detection system according to manufacturer's protocols. The biotinylated probe (1 µl/cm^2 of membrane) was used with the PhotoGene system for nonradioactive detection of hybridization in the dot blots (discussed below). PhotoGene® nylon membranes were used with this system, as well as all recommended buffers and solutions. Hybridization was carried out at 50 °C overnight. Washing of the membranes and binding the streptavidin-alkaline phosphatase conjugate were according to the manufacturer's protocol, the blot was incubated at room temperature (22 °C-24 °C) for 3 h and was subsequently exposed to Kodak diagnostic film for 5-15 min.

Dot blots were prepared with the Bio-Dot microfiltration apparatus (Bio-Rad) according to manufacturer's directions. The membrane was GeneScreen Plus when radio-labeled probe was to be used, and PhotoGene nylon for biotinylated probe. Buffers recommended for the particular membrane were used. For each dot blot sample to be used with a radio-labeled probe, 10 µl of amplified DNA was denatured by addition of 0.4 M NaOH plus 10 mM EDTA in a total volume of 200 µl. The sample was heated to 100 °C for 10 min prior to loading in a well. For each dot blot sample to be used with a biotinylated probe, 10 µl of amplified DNA was denatured for 15 min at room temperature with 200 µl of 1.5 M NaCl, 0.5 M NaOH, followed by neutralization with an equal volume (i.e., 210 µl) of 1.5 M NaCl, 1.0 M Tris-Cl (pH 7.5). The entire 420 µl sample was loaded in a well. Samples were pulled through the apparatus by applying a gentle vacuum.

Results

Determination of SCN virulence groups

An index of mature females was calculated by dividing the number of females on the test plant by the number that developed during the same test on a susceptible cultivar. By convention, the reaction was considered susceptible (+) if the number on the test isolate was equal to or greater than 10 % of the number on the susceptible cultivar, and was considered resistant (–) if the number was less than 10 % of the number on the susceptible plants (Riggs *et al.,* 1981; Faghihi *et al.,* 1986). The data for the four field isolates selected are given in Table 1. Data from the race tests (Riggs & Schmitt, 1988) are in Table 2. NJ2 is classified as race 8 and NW6 as race 10. Both SVI1 and SVI3 are classified as race 3.

Potential DNA markers

With most of the primers, diverse gel patterns were obtained for the four isolates, with each isolate often showing a unique pattern (e.g., Fig. 1A). Patterns from replicate samples from the same isolate were usually similar but not always. Often a band was present in one amplified product that seemed to be faint or missing in the replicate lane (e.g., see Fig. 1A, arrow). When diluted samples of the amplified products (1 : 25 v/v dilution in H_2O) were again amplified using the same primer, a faint band often became more prominent, showing that amplified DNA was present in the original product but not well visualized on the original gel by the ethidium bromide stain. We, therefore, routinely re-amplified the products in order not to be misled by fragments that were present but not visible on the gel. Amplification with eleven of the 60 primers tested resulted in one or more DNA fragments that appeared to be potential markers for separating the two northern populations from the two southern populations. Those primers

Table 1. *Data used for assignment of field isolates to virulence groups* *.

Soybean line	Cultivar Bedford	PI ** 209332	PI 87631	Cultivar D75-10710	PI 90763	Cultivar Pickett
Field isolate NJ2	(+) 78.6	(+) 61.6	(+) 90.2	(+) 33.1	(+) 86.4	(–) 3.3
NW6	(+) 66.2	(+) 19.7	(+) 49.7	(+) 19.9	(+) 22.9	(+) 13.6
SVI1	(–) 4.6	(–) 1.3	(–) 2.0	(–) 0	(–) 0.1	(–) 1.1
SVI3	(–) 8.3	(–) 2.7	(–) 9.3	(–) 1.9	(–) 2.7	(–) 1.8

* (+) = number of cysts equal to or more than 10 % of the number on the susceptible cultivar in the same test; (–) = number of cysts less than 10 % of the number on the susceptible cultivar. Numbers indicated are based on means for development of cysts on the differentials compared with the development on the susceptible cultivar in four or five replications (see text). Isolates NJ2 and NW6 are from northern Indiana, and isolates SVI1 and SVI3 are from southern Indiana.
** PI = Plant Introduction identification number assigned by the Plant Introduction Office, Germplasm Introduction and Evaluation Laboratory, USDA-ARS, Beltsville, MD, USA.

Table 2. *Data from race test**.

Soybean line	Cultivar Pickett	Cultivar Peking	PI ** 88788	PI 90763	Race
Field isolate NJ2	(–) 3.3	(–) 1.0	(–) 1.4	(+) 86.4	8
NW6	(+) 13.6	(–) 1.1	(–) 4.1	(+) 22.9	10
SVI1	(–) 1.1	(–) 0.0	(–) 0.0	(–) 0.1	3
SVI3	(–) 1.8	(–) 0.2	(–) 0.0	(–) 2.7	3

* (+) = number of cysts equal to or more than 10 % of the number on Williams 82 (susceptible cultivar) in the same test; (–) = number of cysts less than 10 % of the number on Williams 82. Numbers indicated are based on means for development of cysts on the differentials compared with the development on Williams 82 in five replications. Isolates NJ2 and NW6 are from northern Indiana, and isolates SVI1 and SVI3 are from southern Indiana.
** PI = Plant Introduction identification number assigned by the Plant Introduction Office, Germplasm Introduction and Evaluation Laboratory, USDA-ARS, Beltsville, MD, USA.

were : A04 (Fig. 1C), A11, A14 (Fig. 1B), B09, B13, B19 (Figs 1D, 2C), D01, D06, D08, D13 (Fig. 2A), and D18. Each of the DNA bands that appeared to be a potential marker was used as a hybridization probe for blots prepared from additional gels of amplification products obtained with the primer of interest. With most of the candidate markers, the probe either failed to hybridize with the similar sized, visible band from the second isolate of the virulence group, or it hybridized also with a band that was not visible on the ethidium bromide stained gel, and that was present (unseen until probed) in an isolate of the other virulence group. The hybridization test was repeated several times (two to six or more) for those probes that appeared to be diagnostic. In one case (the 0.62 kb probe fragment obtained with primer D13, Fig. 2A, B), the probe hybridized with bands in the northern populations only, in three out of four tests. In the fourth test, however, faint hybridization occurred with invisible bands (with respect to the gel) in an isolate of the other virulence group (data not shown). The 1 kb probe obtained with the B19 primer (sequence = ACCCCCGAAG) was the only one to hybridize consistently (many tests) only with the target bands and to show a satisfactory pattern without reamplification (Figs 1D, 2C, D).

For a rapid screening test, the ability to use as template simple mashed preparations of one or a few cysts would be desirable. We therefore used primer B19 in RAPD amplifications for which InstaGene preparations made from single cysts served as the template. Often, however, the expected amplification did not occur in the sample preparations from the two northern populations, and when it did, the bands were less distinct than when extracted DNA was the template. With aliquots of preparations made from four cysts and ten cysts, results were similarly erratic. Occasionally, in contrast to our results with extracted and quantified DNA, faint bands were present in preparations made from the two southern populations.

Dot blot tests using product from B19 RAPD amplification of quantified extracted DNA templates, followed by hybridization with ^{32}P labelled B19 marker probe were consistently successful (Fig. 3). Identical results were obtained with biotin-labelled B19 marker probe and the signal detected on X-ray film by chemiluminescence. Both kinds of labeled probes were further test-

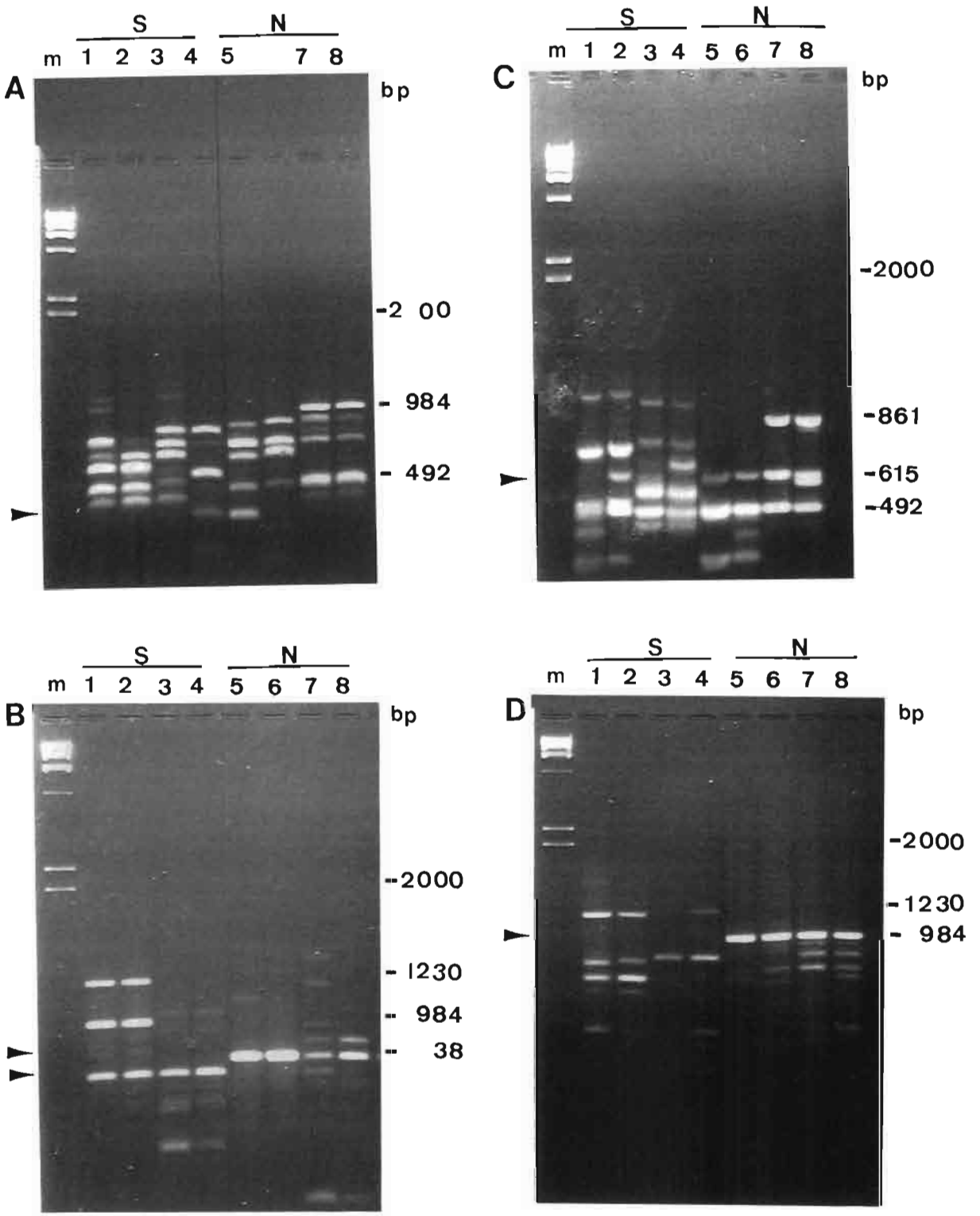

Fig. 1. *DNA gel patterns for all isolates following RAPD amplification with four different primers : (A) B15, (B) A14, (C) A04, and (D) B19. Lanes 1, 2 = SVI1, lanes 3, 4 = SVI3, lanes 5, 6 = NJ2, lanes 7, 8 = NW6, lane m = λ DNA/Hind III, S = south, N = north. In A, arrow shows example of a band present in one replicate sample of an isolate (NJ2), but missing in the second replicate. In B, C, D, arrows show possible diagnostic fragments for S or N isolates.*

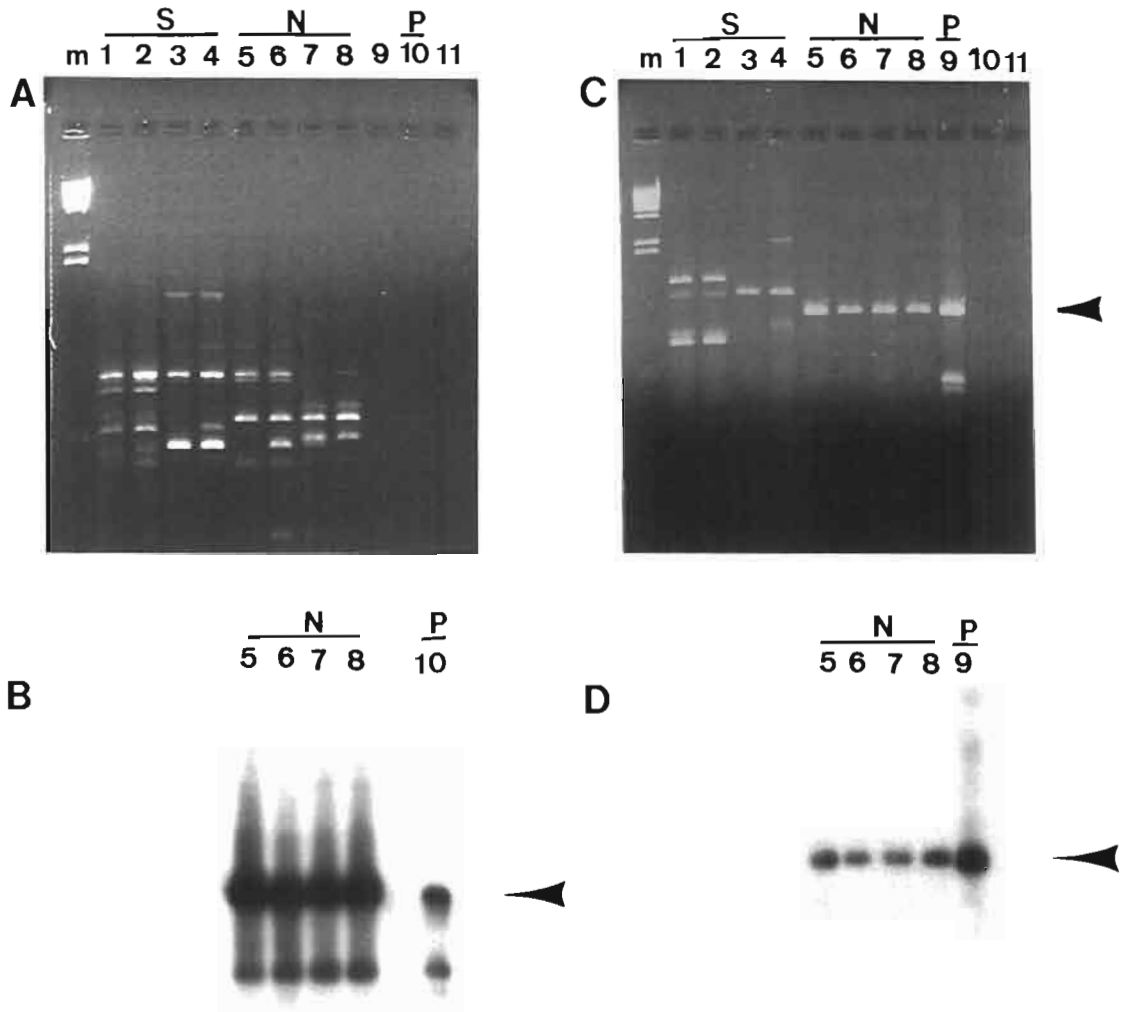

Fig. 2. *A : DNA gel pattern for all isolates following RAPD amplification with primer D13, lane 10 = positive control (pattern not visible on gel), lane 11 = negative control; B : Southern blot of gel shown in 2A probed with 0.62 kb D13 fragment obtained as described in text. C, DNA gel pattern for all isolates following RAPD amplification with primer B19, lane 9 = positive control, lane 10 = negative control; D, Southern blot of gel shown in 2C probed with 1 kb B19 diagnostic fragment. Lanes 1, 2 = SVI1, lanes 3, 4 = SVI3, lanes 5, 6 = NJ2, lanes 7, 8 = NW6, lane m = λ DNA/Hind III, S = south, N = north, P = positive control, arrows (0.62 kb, 2B and 1 kb, 2D) indicate hybridized probe fragments for the northern isolates (NJ2 and NW6).*

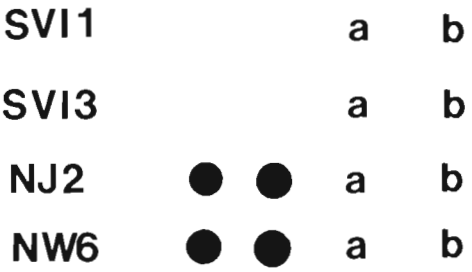

Fig. 3. *Dot blots of DNA amplified with B19 primer, hybridized with radiolabeled B19 diagnostic probe. Two blots (a & b) for each isolate.* (Above : isolates SVI1 and SVI3; below : isolates NJ2 and NW6.)

ed in 20-30 experiments with dot blots of B19 RAPD amplification products obtained with cyst preparation as template, in place of extracted DNA. Whenever we achieved amplification (as indicated on gels), dot blots hybridized with either kind of labeled probe to produce a visible signal on X-ray film in 15 min to 3 h.

Discussion

Nematologists have long sought molecular markers to diagnose races (Riggs & Schmidt, 1988) of soybean cyst nematode, but success has proved elusive. Molecular differences reported among races tend to be restricted to comparisons of a single population of each race (Podzol

& Noel, 1984; Huettel, 1986; Kalinski & Huettel, 1988). In a recent preliminary study of the use of RAPDs to differentiate three races in populations from Minnesota and Missouri, it was found that similarities in banding patterns were more dependent on geographical origin than on race (Nevin Young, pers. comm.; *SON Molecular Biology Newsletter,* Vol. 5). It is our view that soybean growers' interest in SCN race stems from their desire to know which resistant varieties to plant in their soybean fields. Inasmuch as we know that field populations in Indiana differ in their pathogenicity to resistant soybean lines, our goal was to select two geographically separated populations with very similar virulence to soybean lines we have tested, and to compare them with two populations that are less virulent to these lines. If molecular markers could be found to separate these two sets of populations, then it might be feasible to extend the study to find, for most populations in our region, useful markers that would provide guidance to growers in their selection of resistant varieties.

The variability we found in RAPD patterns among the different isolates (e.g., Fig. 1), plus the fact that we were able to develop a molecular marker probe to separate our two sets of populations, make it likely that testing more 10-mer primers would lead to a larger number of useful markers. These could be used as probes in a RAPD-based test to diagnose particularly virulent populations of SCN in a given geographic area. It is feasible to contemplate a panel of probes to be used as markers for virulence of field populations to different groups of resistant cultivars. To be useful to growers, a series of ongoing tests would be necessary to determine virulence of a sample of field populations toward an evolving group of resistant cultivars likely to be grown in a given area. Such an activity could best be undertaken by a commercial testing laboratory, which would also have the facilities to automate the search for suitable DNA marker probes. Because infraspecific differences in SCN genomic DNA are presumably small, it is not likely that long stretches of DNA will differ among isolates, and therefore the tests with DNA marker probes will probably have to include RAPD amplification or PCR.

The difficulty we found in RAPD amplification with cyst preparations as template, instead of extracted DNA, has been reported by others who have attempted RAPD amplification from single nematodes (Cenis, 1993; Kennedy *et al.,* 1995). Recent publications (Ellsworth *et al.,* 1993; Rafalski *et al.,* 1993; Park *et al.,* 1994) have emphasized the drastic influence of small differences in amplification conditions on the RAPD amplification product. The factors that have been found to be most important include an invariant primer/template concentration ratio across experiments, total amount of template DNA, variations in $MgCl_2$, and cycling parameters. Munthali *et al.* (1992) considered the amount of template critical for reproducibility, and Rafalski *et al.* (1993) presented evidence that for their templates, reproducible results were possible only with genomic DNA concentrations between 5-25 ng DNA per 25 µl reaction. Their theoretical explanation was that, under favorable conditions, the 10-mer primer will not bind to a site with a single base difference in the binding site, whereas too low template concentration forces the process into stochastic behavior. According to Ellsworth *et al.* (1993), a low concentration of template facilitates artifactual large fragments, probably reflecting the availability of binding sites and/or their relative affinities. It is likely that our inconsistencies with cyst preparations as template for the RAPD amplifications arose from low template concentration and/or variability in template concentration among preparations, although we tried to eliminate variability by the use of aliquots of multiple cyst preparations. Ellsworth *et al.* (1993) stressed the importance of spectrophotometric analysis to standardize DNA template. We concur with the view that DNA template concentration should always be reported in publications when extracted DNA is used as a RAPD template. Elsworth *et al.* (1993) also suggested that each oligonucleotide primer/template combination may require optimization with respect to amplification conditions, in order to produce consistency. A need for such optimization may account for the differences between replicate lanes 3 and 4 in our Fig. 1A, and for similar inconsistencies we have observed in RAPD papers in the literature. Furthermore, optimization might make it possible to use the D13 0.62 kb fragment (Fig. 2B) as a marker probe, inasmuch as we found it to be satisfactory in three out of four experiments.

Non-radioactive labelling of the probe would be desirable for a test to be widely used in diagnostic clinics, and radiolabeled and biotinylated probes were equally effective for hybridization of dot blots in our laboratory. In such a setting, dot blots would be preferable to the use of agarose gels. We discarded several candidate marker probes which appeared (based on gels only) to be diagnostic, prior to hybridization tests. Based on our experience, we recommend caution in interpretation of RAPD pattern differences based solely on visualization of patterns in agarose gels. Recent publications (Park & Kohel, 1994; Elsworth *et al.,* 1993; Rafalski, pers. comm.) stress the need for hybridization to confirm the equivalency of bands and to visualize RAPD bands too weak to be visible on gels. This would be true, as well, for investigators who use RAPD data to infer genetic relationships among populations and species. Our routine procedure of reamplification often enabled us to discover the presence of bands unseen on the first gel, prior to doing a hybridization test for a candidate probe.

The approach we have suggested and tested for using RAPD markers to diagnose virulence characteristics of field populations of SCN, could be used also for other nematode species whose field populations exhibit different virulence patterns to resistant cultivars of their crop

hosts. To be most useful for routine use for diagnosing field populations, it would be desirable to be able to use in the RAPD amplification simple template preparations made from one or a few nematodes; and this aspect needs more research. Nevertheless, even if this problem cannot be resolved, it would still be feasible to extract DNA from nematodes taken from a field of interest, for use with a panel of marker probes chosen to diagnose virulence toward resistant cultivars. Such a test could provide growers with guidance more quickly than a traditional screening test, of the kind that many soybean growers now request for determination of race in soybean cyst nematode. In Indiana, SCN race information is not predictive of behaviour of a particular field population toward new resistant lines and cultivars, as is indicated by the fact that our two northern populations belong to different races, but to the same virulence group when tested with our soybean lines and cultivars.

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