An Isozyme Marker for Resistance to Root-Knot Nematode in Sugarbeet

M. H. Yu,* L. M. Pakish, and H. Zhou

ABSTRACT

Root-knot nematode (*Meloidogyne* spp.) is a destructive pest of sugarbeet (*Beta vulgaris* L.) that reduces production in infested areas and is difficult to manage. Identification of nematode-resistant plants is a time-consuming process that is subject to genotype–environment interaction. Development of resistant cultivars/hybrids is the most effective control. This study was conducted to establish a rapid and effective screening technique to detect a large number of sugarbeet genotypes with resistance to *Meloidogyne* spp. A nematode-resistant sugarbeet germplasm line, Mi-1 *Beta*, was previously developed using J2 inoculation and screening procedures. Leaf and cotyledon extractions were used in diagnosis. Phosphoglucomutase (PGM) was found to be a potentially useful isozyme marker of resistance in Mi-1 *Beta* and derived lines in starch gel electrophoresis. Seven banding patterns (four resistant and three susceptible) were produced. All susceptible plants shared the banding pattern of the resistant strains, except for a single PGM band. If demonstrated to be tightly linked to nematode resistance, this novel PGM isozyme marker will accelerate breeding sugarbeet with resistance to root-knot nematode.

The root-knot nematode, *Meloidogyne* spp., is an economically important sugarbeet (*Beta vulgaris* L.) pest that is difficult to control in production fields. Previously, soil fumigation was the most reliable technique. Root-knot nematode is now yield-limiting due to continuing loss and restriction of soil fumigants, thereby preventing farmers from growing sugarbeet crops profitably. Host-plant resistance to root-knot nematode is, therefore, the most environmentally safe means to alleviate sugarbeet production problems and nematicide hazards.

The wild sea beet [*B. vulgaris* ssp. *maritima* (L.) Arcang] source of root-knot nematode resistance is effective against multiple species of *Meloidogyne* spp. (Yu et al., 1999). Introgression of root-knot nematode resistance from wild beet to cultivated sugarbeet has been achieved using second-stage juveniles (J2) inoculation, screening, and pollination procedures in the greenhouse. Improved sugarbeet-breeding materials with resistance to root-knot nematode were further selected for tap-root conformation and root-yield performance.

The process of transferring resistant genes from noncultivated taxa or species to the cultivated genomes, and selecting productive genotypes to become hybrid parents, always has been time-consuming and labor-intensive. A simpler and more accurate screening procedure has been sought for accelerating sugarbeet root-knot nematode-resistance breeding. An isozyme pattern of phosphoglucomutase (PGM) has been shown to be associated with root-knot nematode resistance found in the Mi-1 *Beta* germplasm lines. The PGM isozyme marker can be employed to easily identify individual Mi-1 *Beta* genotypes with resistance to *Meloidogyne* spp.

The objective of this study was to establish a rapid and effective screening procedure to detect a large number of sugarbeet genotypes with resistance to root-knot nematode.

MATERIALS AND METHODS

The *Beta* materials with resistance to *Meloidogyne* spp. used in this research were the Mi-1 germplasm (PI 593237; Yu, 1997), progeny of its hybrid crosses to sugarbeet, and susceptible controls (sugarbeet 1783 and 4500) available in the USDA-ARS program. Stecklings (seedlings) of the susceptible sugarbeet lines C37, C78, C80, and R876-89-5NB were obtained from R.T. Lewellen, USDA-ARS, Salinas. Crosses to transfer root-knot nematode resistance from wild beet to sugarbeet were conducted in the greenhouse and laboratory. Test plants were examined for root gall formations 35 to 40 d after inoculation of early-stage beet seedlings with 1200 *M. incognita* J2 per plant (Yu et al., 1999). Individual plants producing none or fewer than 10 small (<1 mm) root galls were classified as resistant. All other plants were classified as susceptible. Seeds were germinated in steam-sterilized sand.

Tissue of cotyledons and expanding young leaves was collected from greenhouse or field (Spence farm in Salinas, CA, sandy loam soil classification) plants, from seedling stage to full maturation for isozyme analysis. A minimum of 55 plants per germplasm line were examined. Samples, about 5 mm^2 in size, were ground in pre-chilled mortars with 0.1-mL extraction buffer. The extraction buffer consisted of 0.1 *M* Tris (*tris*-[hydroxymethyl]aminomethane)–HCl, pH 7.0, 2.45 *M* of glycerol, 1.25 m*M* of PVP-40T (polyvinylpyrrolidone), 0.5% (v/v) Triton X-100, and 125 m*M* 2-mercaptoethanol, the latter added just before use (modified from Aicher and Saunders, 1990). Paper wicks (11 by 2 mm; Northfork Products, Syracuse, IN) were soaked in each sample extract and stored at 4°C.

Buffers and the phosphoglucomutase stain were adapted from systems described by Van Geyt and Smed (1984) with a slight modification. The gel buffer consisted of 5 m*M* of histidinemonohydrochloride, pH 7.0, and the electrode buffer was 0.2 *M* of trisodium citrate-2 H_2O, pH 7.0. The staining solution for the isozyme PGM consisted of 25 mL of 0.1 *M* Tris–HCl buffer, pH 8.0; 5 m*M* of α-D-glucose-1-phosphate (glucose-1-P); 7 units glucose-6-phosphate dehydrogenase (G-6-PDH); 5 m*M* of β-nicotinamide adenine dinucleotide phosphate (NADP); 2 mL of 0.025 *M* $MgCl_2$; 1 m*M* of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT); and 0.13 m*M* of phenazine methosulfate (PMS).

The gel was prepared using a 1:2:1 mixture of hydrolyzed starch (Pasteur Merieux Connaught, Charlotte, NC), hydrolyzed potato starch (Starch-Art, Smithville, TX), and po-

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Abbreviations: PGM, phosphoglucomutase; PMS, phenazine methosulfate.

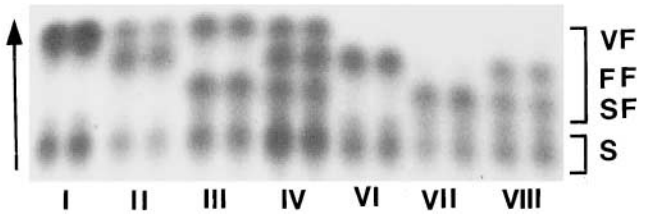

Fig. 1. A phosphoglucomutase-stained starch gel showing seven banding types induced from root-knot nematode-resistant Mi-1 sugarbeet plants (left to right, two samples each: Types I to IV are resistant, Types VI to VIII are susceptible; VF = very fast, FF = fast fast, SF = slow fast, and S = slower band).

tato starch (Sigma-Aldrich, St. Louis, MO) dissolved in gel buffer. The gel solution was heated in a microwave oven until translucent, degassed using a vacuum pump, and poured into trays. After cooling in the refrigerator, the gel was loaded with the sample wicks. The gel tray with loaded samples (including two tracking dye wicks) was placed in a refrigerated, horizontal gel electrophoresis apparatus (Model H4; Gibco-BRL Products, Life Technologies, Grand Island, NY). A constant current of 55 to 65 mA (65 to 85 volts) was applied to the gel. Wicks were removed from the gel 10 to 15 min after the start of the run. Electrophoresis was carried out for 5 1/2 to 6 h, until the tracking dye had moved 5 to 7 cm from the cathodal end. The gel was sliced in 1.5-mm-thick slabs with monofilament thread, and developed in PGM stain with an agar overlay (made up of 2% agar solution in 25 m*M* of $MgCl_2$). They were incubated in the dark at 37°C for about 1 h. Isozyme banding patterns were recorded for analysis. Photographs were taken using the Video Copy Processor (P67UA; Ultra-Violet Products, Upland, CA).

RESULTS AND DISCUSSION

In addition to phosphoglucomutase, some 20 other enzyme activity stains were investigated with the purpose of discriminating *Meloidogyne* spp. resistant genotypes in Mi-1 *Beta*, progeny of its hybrid crosses, and other sugarbeet populations. The tested enzymes were aconitase (ACO), acid phosphatase (ACP), alcohol dehydrogenase (ADH), adenylate kinase (AK), arginine aminopeptidase (AMP), diaphorase (DIA), endopetidase (EP), esterase (EST), fructose-1,6-diphosphatase (FDP), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), hexokinase (HK), isocitrate dehydrogenase (ICD), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), peptidase with leucyl-alanine (PEP-LA), 6-phosphogluconate dehydrogenase (PGD), phosphoglucoisomerase (GPI), and shikimate dehydrogenase (SKDH) (Siciliano and Shaw, 1976; Vallejos, 1983; Van Geyt and Smed, 1984; Acquaah, 1992). Among these isozymes, AK and MDH induced isozyme bands in some populations of sugarbeet progeny. However, their banding patterns were inconclusive to detect resistance or susceptibility in the donor plants.

It was determined from the starch gel electrophoresis study that an isozyme of PGM is diagnostic for resistance to *Meloidogyne* spp. in Mi-1 *Beta* genotype. Activity of PGM could be shown in two zones on the gel. The first, or faster, zone was polymorphic and consisted of up to three bands, very fast (VF), fast fast (FF), and slow fast (SF) (Fig. 1). The zymogram of *Meloidogyne* spp.-resistant Mi-1 *Beta* and its derived progeny comprised four resistant and three susceptible banding types: Type I had only the VF band, Type II had VF and FF bands, Type III had VF and SF bands, and Type IV had all three bands. Among the four banding types, Type II had the highest frequency (Table 1) in the current nematode-resistant sugarbeet population.

The zymogram of susceptible individuals in the same Mi-1 breeding population exhibited three banding patterns, but they all lacked the VF band: Type VI had FF band, Type VII had SF band, and Type VIII had both bands (Fig. 1). This demonstrated that the high mobility (not low molecular weight) VF band on the starch gel zymogram of PGM may be a useful marker for root-knot nematode resistance in this population. Among susceptible plants, the Type VI banding appeared most frequently (Table 1). The susceptible parental lines (C78 and R876-89-5, etc.) and the control plants (sugarbeet 1783 and 4500) used in this breeding program had the three banding patterns of the nonresistant Mi-1 derivatives (Table 1). This indicated that susceptible individuals from both the Mi-1 germplasm lines and sugarbeet cultivars produced the same range of zymograms.

The second (or slower) banding zone consisted of one or two slower (S) bands (Fig. 1) that showed no polymorphism in any of the entries studied. Generally,

Table 1. Number (percent) of plants in *Beta* cultigens that exhibited different phosphoglucomutase-stained band patterns in starch gel electrophoresis.

		Banding types (%)							
***Beta* germplasm**	**Sample size**	**I**	**II**	**III**	**IV**	**V†**	**VI**	**VII**	**VIII**
Mi-1 beet germplasm lines:									
Initial Mi-1 synthetic‡	**105**	**35.2**	**38.1**	**10.5**			**15.2**		**1.0**
Resistant derivatives F_2	**120**	**13.3**	**47.5**	**2.5**	**0.8**		**30.0**	**0.8**	**5.0**
Nonresistant derivatives	**87**						**67.8**	**3.5**	**28.7**
Susceptible sugarbeet cultivars and pollinators:									
C37	**55**						**100**		
C78	**80**						**68.7**	**1.3**	**30.0**
C80	**80**						**51.3**	**5.0**	**43.8**
R876-89-5NB	**80**						**86.2**		**13.8**
1783§	**83**						**12.0**	**39.8**	**48.2**
4500§	**71**						**100**		

† A putative Type V banding pattern (if present, would show zero bands in the first zone) has not been observed in the samples investigated.
‡ Increased from inter-pollination of the selected Mi-1 beet plants that carried a high level of resistance to root-knot nematode in isolation chambers.
§ Sugarbeet plants used as susceptible control.

one S band was present in the slower zone from leaf samples, whereas cotyledons exhibited one or two bands depending on the environment and plant age. All cotyledons from field-grown plants exhibited two bands, while those from the greenhouse showed either one or both bands. Older (yellowed) cotyledons usually exhibited only one band. The S bands did not have apparent diagnostic value to sugarbeet root-knot nematode resistance.

The PGM bands (from leaf extractions) detected in the starch gel were stable at all stages of growth tested. The cotyledon was an excellent tissue for isozyme diagnosis. Samples collected from 6 to 29 d after sowing (in greenhouse) showed readable and consistent isozyme bandings, but the bands were clearest when cotyledons were collected between 14 and 21 d after planting. In contrast, if expanding young leaves (at any growing stage of the plant) were used for sample extractions, banding remained clear and readable, that is, the VF and FF bands remained distinct, throughout the whole growth period of the beet plant.

It is interesting to find that the Mi-1 host resistance to multiple species (six species, nine races) of *Meloidogyne* nematode (Yu et al., 1999) was indexed by the presence of a single VF band (Fig. 1; Table 1). The detection of this PGM isozyme marker, which is an expression of a new putative gene, is a potentially useful and practical means to identify *Meloidogyne* spp.-resistant Mi-1 genotypes in the sugarbeet root-knot nematode-resistance breeding program. The laboratory test results agreed with those obtained from simultaneous *M. incognita* J2 inoculation study of the same donor plants (an estimated 2000 entries) in the greenhouse. All test plants that exhibited a VF band in PGM staining were resistant to the root-knot nematode demonstrating that the PGM marker is a reliable indicator of *Meloidogyne* spp. resistance in Mi-1. This isozyme marker may avoid any uncertainties (if occurring) in the classification of nematode-resistant plants in greenhouse inoculation studies, which is based on gall counts (e.g., zero or <10 galls/seedling) as the criterion for resistance.

The investigation of PGM activities in the genus *Beta* has generated useful information in plant science. In sugarbeet, *Pgm1* was found to segregate independently from loci *B* (annual growth habit) and *R* (hypocotyl color) (Aicher and Saunders, 1990). Convincing evidence was obtained on the location of linked pairs of loci *Pgm1-Mdh2* and *Mdh1-Gdh2* in different *B. vulgaris* chromosomes (Denisova and Levites, 1999). Genetic deviation in Californian wild beets was found to have two different origins: (i) *Beta vulgaris*, evolved from subspecies *vulgaris* and *maritima* hybridized populations, and (ii) *Beta macrocarpa*, genetically almost identical to the European accessions (Bartsch and Ellstrand, 1999).

Isozyme markers still are useful as simple, inexpensive means for detection of gene introgression and recombination, for comparative mapping, and for determination of genetic diversity and phylogenetic relationships among plant species (Hart and Langston, 1977; Hoffman, 1999; Horandl et al., 2000). The starch gel electrophoresis employed in the present study demonstrated the utility of PGM as a simple and rapid assay for a putative marker for the selection of Mi-1 *Beta* individuals resistant to *Meloidogyne* spp. This marker will facilitate and greatly benefit sugarbeet root-knot nematode-resistance breeding programs in the future if tightly linked to resistance to *Meloidogyne* spp.

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