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A quantitative trait locus influencing tolerance to Phytophthora root rot in the soybean cultivar 'Conrad'

Changren Weng · Kangfu Yu · Terry R. Anderson · Vaino Poysa

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Abstract 'Conrad', a soybean cultivar tolerant to Phytophthora root rot (PRR), and 'OX760-6-1', a breeding line with low tolerance to PRR, were crossed. F_2 derived recombinant inbred lines were advanced to F_6 to generate a population through single-seed descent. This population was used to identify quantitative trait loci (QTLs) influencing PRR tolerance in 'Conrad'. A total of 99 simple sequence repeat (SSR), or microsatellite, markers that were polymorphic and clearly segregated in the F_6 mapping population were used for QTL detection. Based on the data of PRR in the field at two planting locations, Woodslee and Weaver, for the years 2000 and 2001, one putative QTL, designated as Qsatt414-596, was detected using MapMaker/QTL. Qsatt414-596 was flanked by two SSR markers from the linkage group MLG J, Satt414 and Satt596. Satt414 and Satt596 were also detected to be significantly ($P < 0.005$) associated with PRR using the SAS GLM procedure and were estimated to explain 13.7% and 21.5% of the total phenotypic variance, respectively.

C. Weng (✉)
Wayne State University, 110 E. Warren Ave. Rm#311, Detroit, MI 48201, USA
e-mail: wengc@karmanos.org

K. Yu · T. R. Anderson · V. Poysa
Agriculture and Agri-food Canada, Greenhouse and Processing Crop Research Centre, 2585 Highway 20 E, Harrow, ON, CanadaN0R 1G0

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Introduction

Phytophthora root rot (PRR) of soybean (*Glycine max* (L.) Merr.) caused by *Phytophthora sojae* Kauf. & Gerd. is most severe in low, poorly drained, clay soil, and has caused yield losses since it was found in North America more than 50 years ago (Bernard et al., 1957; Kaufmann and Gerdemann, 1958; Hildebrand, 1959). In the past, control of PRR has primarily relied on uses of soybean cultivars with race-specific resistance (Faris and Sabo, 1989) but in recent years, isolates with novel virulences have been identified in field surveys (Dorrance et al., 2003a; Keeling, 1982; Anderson and Buzzell, 1992; Forster et al., 1994; Schmitthenner et al., 1994; Abney et al., 1997). Fourteen dominant *Rps* genes conferring resistance to PRR have been identified at eight loci (Burnham et al., 2003), but none of them controls all races and virulence types (Dorrance et al., 2003a). Resistance has been suggested to follow a gene-for-gene mechanism, and intensive use of race-specific resistance for control has promoted selection for new races that are virulent to the current resistance genes (Drenth et al., 1996). Use of tolerant cultivars possessing general tolerance (also termed partial resistance (Dorrance et al., 2003b), field resistance

or general resistance (Burnham et al., 2003) may be an alternative to decrease the selection pressure for virulent races (Schafer, 1971; Thomison et al., 1988). Tolerance provides some level of protection against all races of *P. sojae* (Schmitthenner, 1985; Tooley and Grau, 1984a, b). Tolerance to PRR is inherited in a quantitative trait manner and this trait is influenced by several quantitative trait loci (QTLs) (Buzzell and Anderson, 1982; Walker and Schmitthenner, 1984; St. Martin et al., 1991; Glover and Scott, 1998; Kyle and Nickell, 1998). Mapping these QTLs and identifying markers tightly linked with them in tolerant lines may facilitate breeding soybean for PRR tolerance by means of marker-assisted selection (MAS). In soybean, QTLs for many agronomic traits have been mapped, including plant height (Mansur et al., 1996), aluminium tolerance (Bianchi-Hall et al., 2000), soybean cyst nematode tolerance (Meksem et al., 2001; Schuster et al., 2001; Yue et al., 2001a, b), and white mould tolerance (Arahana et al., 2001; Kim and Diers, 2000). Kassem et al. (2006) reported 61 QTLs for six traits (soybean sudden death syndrome, resistance to soybean cyst nematode, seed yield, foliar trigonelline content, seed isoflavones, and resistance to manganese toxicity) of soybean. Our study reports on identifying and mapping QTLs for tolerance to PRR.

Recently, simple sequence repeat (SSR), or microsatellite markers using polymerase chain reaction (PCR) procedures have been used extensively for soybean gene mapping and QTL mapping due to their superiority, availability, and reliability. SSR markers are particularly suitable for soybean due to their abundance in the soybean genome. So far, more than 1,000 different SSR loci distributed throughout the soybean genome have been identified, and sequences of primers for those SSR loci are available in the soybean database SoyBase at the internet site: http://129.186.26.94/SSR.html constructed by the USDA-ARS Plant Genome Program, Cornell University, and Iowa State University. Recent development of integrated linkage maps of soybean (Cregan et al., 1999; Song et al., 2004) involving a total of 1015 SSR markers greatly facilitates research on soybean gene and QTL mapping.

The soybean cultivar 'Conrad' (Fehr and Sabo, 1989), is susceptible to all *P. sojae* races that were maintained at the Greenhouse and Processing Crop Research Center (Anderson and Buzzell, 1992). In field trials on *P. sojae* infested soil, however, the percentage of plants killed by PRR was 2% for 'Conrad' and 40% for 'Amsoy 71' (Buzzell and Anderson, 1982). As 'Amsoy 71' was determined to be moderately tolerant to PRR in previous trials, 'Conrad' can be considered tolerant to PRR. Dorrance et al. (2003b) evaluated the partial resistance of 12 soybean cultivars including 'Conrad' at seven site-year combinations. They found that 'Conrad' possessed partial resistance or tolerance to PRR and the partial resistance may provide protection when plants were subjected to diverse *P. sojae* populations. In field studies on the tolerance of 'Conrad' to PRR, the number of QTLs controlling the tolerance was estimated to be 3–4 based on the results from backcrosses (unpublished data). Burnham et al. (2003) used three recombinant inbreed line (RIL) populations derived from crosses between 'Conrad' and susceptible soybean lines to detect partial resistance QTL to PRR at the seedling stage using solution culture in growth chambers. Two such QTLs were mapped on linkage groups MLG F and MLG D1b + W. A literature search indicated that there were no reports of QTL analysis of tolerance to PRR based on data from field trials. In this study, we report the identification of one putative QTL influencing PRR tolerance in 'Conrad' using an F_6 population consisting of 62 recombinant inbred lines, and SSR markers that are linked to this QTL based on the data of PRR tolerance in the field at two locations for the years 2000 and 2001.

Materials and methods

Plant materials and DNA samples

The mapping population, consisting of 62 F_2 derived F_6 RILs of soybean, *Glycine max* (L.) Merr, was developed by single-seed descent from a cross between 'Conrad' and 'OX760–6-1'. 'Conrad' is tolerant to PRR in the field, while 'OX760-6-1', a breeding line developed at the Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, Harrow, has very low tolerance to PRR under field conditions. There are no known single genes for resistance to PRR in either parent. The 62 F_6 RILs were grown in a greenhouse, leaf samples were taken from each line, and DNA was

isolated from the leaf samples according to the procedures described by Yu et al. (1999).

Disease tolerance evaluation

Tolerance to PRR was determined at two field sites (Woodslee and Weaver) in 2000 and one field site (Woodslee) in 2001. The Woodslee site used in both years has been planted with soybeans since 1975 and has been used to evaluate tolerance to PRR of recommended soybean cultivars in Ontario since that time. The Weaver site is a grower's field found to have a high incidence of PRR in past surveys (Anderson and Buzzell, 1992). Both sites consist of Brookston clay loam without tile drainage and receive minimum tillage. Tolerance is based on the number of seedlings and plants that develop symptoms of PRR between complete emergence (3–4 weeks after planting) to approximately the R3 growth stage (Fehr et al., 1971) and is referred to as plant loss. Plant loss under these conditions has been related to tolerance in previous studies (Buzzell and Anderson, 1982). Seedlings that were wilted or dead when emergence was determined, were considered emerged and included in emergence totals. Wilted plants with leaves attached and a characteristic brown lesion extending from the soil line into the lower internodes were considered to have PRR and not included in the final plant stand counts. Plants with brown lesions that developed from the lower nodes were considered to be infected with the stem canker organism *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* (Athow & Caldwell) and were not considered as lost plants.

Plots consisted of single rows 3 m in length with 50 seeds per row and an inter-row spacing of 50 cm. Plots were replicated twice. In 2000, plots were sown in the field on 9th June in a randomised block design at both locations and numbers of emerged plants determined after 21 days. On 8th August the number of plants not severely infected with PRR (plant stand) was counted. In 2001, plots were sown on 11th June in a completely random design; the number of emerged plants was determined after 21 days, and plant stand was counted on 9th August. Percentage plant loss per plot was calculated as follows: percentage plant loss = [(number of emerged plants − plant stand)/number of emerged plants] × 100%. Weeds were controlled in 2000 by Dual (Syngenta Crop Protection Canada Inc.) at 2.64 kg ai/ha plus Pursuit (BASF Canada Inc.) at 0.1 kg ai/ha and in 2001 by Dual-Magnum (Syngenta Crop Protection Canada Inc.) at 1.5 kg ai/ha plus Pursuit at 0.312 kg ai/ha.

PCR analysis

Oligonucleotide sequences for PCR primers were obtained from the SoyBase internet site: http://129.186.26.94/ssr.html. One oligonucleotide of each primer pair was tailed at its 5′ end with the florescence-labeled M13 universal forward primer sequence (19 bp) to facilitatesignal detection without the need for labelling every primer. PCR reactions were in 10 µl mixes containing 25 ng genomic DNA, 1.5 mM Mg^{2+}, 0.2 mM each dNTP, 1 × PCR buffer (10 mM Tris–HCl, pH8.3, 50 mM KCl, and 0.0001% (w/v) gelatine), 0.05 µM tailed SSR primer pair, 0.05 µM M13 forward primer, and 0.4 units of *Taq* DNA Polymerase (GIBCO BRL). The amplification temperature profiles were as follows: 2 min at 94°C, followed by 35 cycles of 45 s at 94°C, 45 s at 47°C, and 45 s at 72°C, and then 5 min at 72°C. The PCR products were separated on 6% sequencing gel and were detected using The LI-COR Global Edition IR^2 System.

Linkage and QTL analysis

Linkages among the SSR markers were analysed using computer software MAPMAKER/EXP 3.0 (Lander et al., 1987). The Kosambi mapping function was used to calculate map distances. An LOD threshold of 4.0 and a maximum distance of 30 centimorgan (cM) were used to group and order the SSR markers. The associations between markers and the PRR tolerance were detected using the SAS GLM procedure. The QTL search between marker intervals was carried out using computer software MapMaker/QTL. An LOD threshold of 2.0 was used.

Results

Disease tolerance analysis

The difference in climate between the two years may have affected the severity of the disease in the disease tolerance evaluation experiments. At Woodslee, the

rainfall (286.0 mm) in the soybean-growing season (June 9–August 8) for the year 2000 was much higher than that (72.6 mm) in the growing period (June 11–August 9) for the year 2001. The average percentage loss for the F_6 population for 2000 (15.6%) was higher than that for 2001 (8.1%), and the range of the percentage loss for 2000 (0.0–76.1%) was much wider than that for 2001 (0.0–31.2%) (Table 1). Likewise, the percentage losses were 11.2% for 'Conrad' and 61.0% for 'OX760-6-1' for 2000, and 4.7% for 'Conrad' and 10.0% for 'OX760-6-1' for 2001 (Table 1).

Linkage analysis

A hundred and ten out of 288 SSR primer pairs were polymorphic between the two parents, 'OX760-6-1' and 'Conrad', and 99 out of the 110 amplified clearly segregating banding patterns when genotyping the 62 F_6 lines. Sixty of these 99 segregating SSR markers were mapped to 22 linkage groups belonging to 19 of the 20 chromosomes/linkage groups designated by Cregan et al. (1999). These 22 linkage groups covered a total of 940 cM, which accounted for about 31% of the total genome length of 3,000 cM estimated by Shoemaker and Olson (1993). If the potential coverage of the unlinked SSR markers was included, the covered proportion could be significantly higher.

QTL analysis

One QTL, designated as Qsatt414-596, was detected in the Satt414-Satt596 interval of linkage group MLG J designated by Cregan et al. (1999) with interval mapping using MapMaker/QTL. Qsatt414-596 was mapped at a slightly different locus for different locations and years (Fig. 1). To confirm the interval mapping results, we used SAS GLM procedure to analyse the association between single SSR markers and PRR phenotypes. Marker Satt596 showed significant ($P < 0.005$) association with PRR for both locations and both years and explained 13.7, 14.8, and 21.5% of the total phenotypic variance for Woodslee in 2000, Woodslee in 2001, and Weaver in 2000, respectively. Marker Satt414 was only significantly ($P < 0.005$) associated with PRR for Woodslee in 2001 and explained 18.4% of the total phenotypic variance (Table 1).

Discussion

The QTL of our field study was mapped on linkage group MLG J while the two QTLs detected by Burnham et al. (2003) in the greenhouse study using vermiculite as the growth medium were on linkage groups MLG F and MLG D1b + W. Four factors could have accounted for these different results. First, plants may activate different partial resistance mechanisms, controlled by different QTLs at different growth stages and/or different growth environments. Second, while the same tolerant source was used in both studies, the susceptible parents differed. Williams, Harosoy and Sloan, which were used by Burnham et al. (2003), might possess the tolerance QTL identified in this study. Third, the *P. sojae* strain composition in the field of our study is likely to be more complicated than the single strain *P. sojae* isolate OH25 used by Burnham et al. (2003), which is

Table 1 Percentage plant loss for parents, range of plant loss for F_6 population, and SSR markers associated with PRR at two locations for the years 2000 and 2001

Year	Location	% Plant loss of parents		Range of loss for F_6	SSR marker	R^2 (%)[a]	P[b]	LOD[c]
		Conrad	OX760-6					
2000	Woodslee	11.23	60.99	0.00–76.13	Satt596	13.7	0.0040	1.76
	Weaver	3.33	17.86	0.00–29.44	Satt596	21.5	0.0002	2.80
2001	Woodslee	4.73	10.00	0.00–31.16	Satt596	14.8	0.0026	2.16
					Satt414	18.4	0.0009	2.23

[a] R^2 is the proportion of the phenotypic data explained by the marker locus.

[b] P is the type I error probability.

[c] LOD is the logarithm of odds for interval mapping.

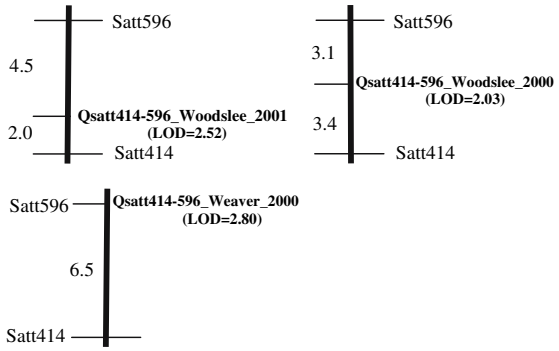

Fig. 1 The QTL of this study on linkage group MLG J as designated by Cregan et al. (1999) for the three location-year combinations.

likely to result in identification of different QTLs. Fourth, our experiment was a field trial which is considered to have a larger random error. In fact, our study did cover the interval on linkage group MLG D1b + W and one end of the interval on linkage group MLG F where Burnham et al. (2003) had located the two QTLs. Five SSRs were in the MLG D1b + W interval, they are Satt157, Satt428, Satt282, Satt041, and Sat_089 and they covered 44 cM of the linkage group MLG D1b + W. Another five were in the MLG F interval, they are Satt030, Satt040, Satt569, Satt252, and Satt510 and cover 108.3 cM of the linkage group MLG F. Satt252 was estimated to have some degree of association with a QTL at a type I error probability of 0.0476, which was not significant using our criteria.

The SSR markers linked to the putative QTLs could be useful for MAS for PRR tolerance. The putative QTL, Qsatt414–596 detected using data of 2001 at Woodslee, was flanked by Satt414 (2.0 cM) and Satt596 (4.5 cM). The accuracy of genotyping in MAS was theoretically estimated to be 97.8% or 94.3% (since a double cross-over is needed to disassociate both markers from the associated QTL) if Satt414 or Satt596 is used singly, and 99.8% if both Satt414 and Satt596 are used. However, location of Qsatt414-596 needs to be confirmed with more markers located both inside the Satt414-Satt596 interval and outside the Satt414-Satt596 interval at the Satt596 side.

Although the relatively small sample size could reduce the power of detection, QTLs with large effects, however, can still be detected. In our study, Qsatt414-596 explained 13.7% and 21.5% of the total phenotypic variance for 2000 at Woodslee and for 2000 at Weaver, respectively, and was detected at a type I error rate of 0.0009 and an LOD of 2.23 for 2001 at Woodslee and at a type I error rate of 0.0002 and an LOD score of 2.80 for 2000 at Weaver. Moreover, although only 62 F_6 RILs were used for QTLs detection, the results were based on the disease tolerance evaluation using two replications with 50 seeds for each replication for each location and each of the two years, totalling 18,600 plants. The result that Qsatt414-596 was consistently detected for both years and at both locations indicates that the QTL identification was unlikely to be a random event. However, before Satt414 and Satt596 can be used for map-based cloning, a finer map for the QTL and molecular markers needs to be constructed with a larger sample size.

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References

Abney TS, Melgar JC, Richards TL, Scott DH, Grogan J, Young J (1997) New races of *Phytophthora sojae* with Rps1d virulence. Plant Dis 81:653–655.

Anderson TR, Buzzell RI (1992) Diversity and frequency of races of *Phytophthora sojae* f. sp. *glycinea* in soybean field in Essex County, Ontario, 1980–1989. Plant Dis 76:587–589.

Arahana VS, Graef GL, Specht JE, Steadman JR, Eskridge KM (2001) Identification of QTLs for resistance to *Scleritinia sclerotiorum* in soybean. Crop Sci 41:180–188.

Bernard RL, Smith PE, Kaufmann MJ, Schmitthenner AF (1957) Inheritance of resistance to Phytophthora root rot and stem rot in the soybean. Agron J 49:391.

Bianchi-Hall CM, Carter TE, Bailey JMA, Main MAR, Rufty TW, Ashley DA, Boerma HR, Arellano C, Hussey RS, Parrott WA (2000) Aluminum tolerance associated with quantitative trait loci derived from soybean PI 416937 in hydroponics. Crop Sci 40:538–545.

Burnham KD, Dorrance AE, VanToai TT, St. Martin SK (2003) Quantitative trait loci for partial resistance to *Phytophthora sojae* in soybean. Crop Sci 43:1610–1617.

Buzzell RI, Anderson TR (1982) Plant loss response of soybean cultivars to *Phytophthora megasperma* f. sp. *glycinea* under field conditions. Plant Dis 66:1146–1148

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kathler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39:1464–1490

Dorrance AE, McClure SA, deSilva A (2003a) Pathogenic diversity of *Phytophthora sojae* in Ohio soybean fields. Plant Dis 87:139–146

Dorrance AE, McClure SA, St. Martin SK (2003b) Effect of partial resistance on *Phytophthora* stem rot incidence and yield of soybean in Ohio. Plant Dis 87:308–312

Drenth A, Whisson SC, Maclean DJ, Irwin JAG, Obst NR, Ryley MJ (1996) The evolution of races of *Phytophthora sojae* in Australia. Genetics 86:163–169

Faris MA, Sabo FE (1989) The systematics of *Phytophthora sojae* and *P. megasperma*. Can J Bot 67:1442

Fehr WR, Cavines CE, Burmood DT, Pennington JS (1971) Stages of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci 11:929–931

Fehr WR, Cianzio SR, Voss BK, Schultz SP (1989) Registration of 'Conrad' soybean. Crop Sci 29:830

Forster H, Tyler BM, Coffey MD (1994). *Phytophthora sojae* races have arisen by clonal evolution and by rare outcrosses. Mol Plant-Microbe Interact 7:780–791

Glover KD, Scott RA (1998) Heritability and phenotypic variation of tolerance to Phytophthora root rot of soybean. Crop Sci 38:1495–1500

Hildebrand AA (1959) A root and stalk rot of soybean caused by *Phytophthora megasperma* var. *sojae* var. nov. Can J Bot 37:927–957

Kassem MA, Shultz J, Meksem K, Cho Y, Wood AJ, Iqbal MJ, Lightfood DA (2006) An updated 'Essex' by 'Forrest' linkage map and first composite interval map of QTL underlying six soybean traits. Theor Appl Genet 113:1015–1026

Kaufmann MJ, Gerdemann JW (1958) Root and stem rot of soybean caused by *Phytophthora sojae* n. sp. Phytopathology 48:201–208

Keeling BL (1982) Four new physiologic races of *Phytophthora sojae* n. sp. *Glycinea*. Plant Dis 66:334–335

Kim HS, Diers BW (2000) Inheritance of partial resistance to *Sclerotinia* stem rot in soybean. Crop Sci 40:55–61

Kyle DE, Nickell CD (1998) Genetic analysis of tolerance to *Phytophthora sojae* in the soybean cultivar, Jack. Soybean Genetics Newsletter 25:124–125

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MapMaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Mansur LM, Orf JH, Chase K, Jarvik T, Cregan PB, Lark KG (1996) Genetic mapping of agronomic traits using recombinant inbred lines of soybean. Crop Sci 36:1327–1336

Meksem K, Pantazopoulos P, Njiti VN, Hyten LD, Arelli PR, Lightfoot DA (2001) 'Forrest' resistance to the soybean cyst nematode is bigenic: saturation mapping of the *Rhg1* and *Rhg4* loci. Theor Appl Genet 103:710–717

Schafer JF (1971) Tolerance to plant disease. Annu Rev Phytopathol 9:235–252

Schmitthenner AF (1985) Problems and progress in control of Phytophthora root rot of soybean. Plant Dis 69:362–368

Schmitthenner AF, Hobe M, Bhat RG (1994) *Phytophthora sojae* races in Ohio over a 10-year interval. Plant Dis 78:269–276

Schuster I, Abdelnoor RV, Marin SRR, Carvalho VP, Kiihl RAS, Silva JFV, Sediyama CS, Barros EG, Moreira MA (2001) Identification of a new major QTL associated with resistance to soybean cyst nematode (*Heterodera glycines*). Theor Appl Genet 102:91–96

Shoemaker RC, Olson TC (1993) Molecular linkage map of soybean (*Glycine max* L. Merr.). In: O'Brien SJ (ed), Genetic maps: locus maps of complex genomes. Cold Spring Harbor Laboratory Press, New York, p 6.131–6.138

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

St Martin SK, Scott DR, Schmittchenner AF, McBlain BA (1991) Relationship between tolerance to Phytophthora rot and soybean yield. Plant Breed 113:331–334

Thomison PR, Thomas CA, Kenworthy WJ, Mcintosh MS (1988) Evidence of pathogen specificity in tolerance of soybean cultivars to Phytophthora rot. Crop Sci 28:714–715

Tooley PW, Grau CR (1984a) Field characterisation of rate-reducing resistance to *Phytophthora megasperma* f. sp. *Glycinea* in soybean. Phytopathology 74:1201–1208

Tooley PW, Grau CR (1984b) The relationship between rate-reducing resistance to *Phytophthora megasperma* f. sp. *Glycinea* and yield of soybean. Phytopathology 74:1209–1216

Walker AK, Schmitthenner AF (1984) Heritability of tolerance to Phytophthora rot in soybean. Crop Sci 24:490–491

Yu K, Park SJ, Poysa V (1999) Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus* and *Vigna*). Genome 42:27–34

Yue P, Sleper DA, Arelli P (2001) Mapping resistance to multiple races of *Heterodera glycines* in soybean PI 89772. Crop Sci 41:1589–1595

Yue P, Arelli P, Sleper DA (2001) Molecular characterization of resistance to *Heterodera glycines* in soybean PI 438489B. Theor Appl Genet 102:921–928