

Marker-assisted Selection for Combining Resistance to Bacterial Spot and Bacterial Speck in Tomato

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ABSTRACT. The lack of resistance to bacterial diseases increases both the financial cost and environmental impact of tomato (*Lycopersicon esculentum* Mill.) production while reducing yield and quality. Because several bacterial diseases can be present in the same field, developing varieties with resistance to multiple diseases is a desirable goal. Bacterial spot (caused by four *Xanthomonas* Dowson species) and bacterial speck (caused by *Pseudomonas syringae* pv. *tomato* Young, Dye and Wilkie) are two economically important diseases of tomato with a worldwide distribution. The resistance gene *Pto* confers a hypersensitive response (HR) to race 0 strains of the bacterial speck pathogen. The locus *Rx3* explains up to 41% of the variation for resistance to bacterial spot race T1 in field trials, and is associated with HR following infiltration. Both *Pto* and *Rx3* are linked in repulsion phase on chromosome 5. We made a cross between two elite breeding lines, Ohio 981205 carrying *Pto* and Ohio 9834 carrying *Rx3*, to develop an F₂ population and subsequent inbred generations. Marker-assisted selection (MAS) was applied to the F₂ progeny and to F_{2,3} families in order to select for coupling-phase resistance. Thirteen homozygous progeny from 419 F₂ plants and 20 homozygous families from 3716 F₃ plants were obtained. Resistance was confirmed in all selected families based on HR in greenhouse screens using bacterial speck race 0 and bacterial spot race T1 isolates. Resistance to bacterial spot race T1 was confirmed in the field for 33 of the selected families. All selected families were also resistant to bacterial speck in the field. MAS was an efficient tool to select for desirable recombination events and pyramid resistance.

Bacterial spot of tomato is a disease complex with five races, T1 to T5, described (Jones et al., 2000, 2004a). It is caused by as many as four species of bacteria [*Xanthomonas euvesicatoria* ex Doidge, *X. vesicatoria* ex Doidge, *X. perforans* Jones et al.), and *X. gardneri* Šutic (Doidge, 1921; Jones et al., 2004b; Šutic, 1957)] with taxonomic divisions modified from Vauterin et al. (1995) as described by Jones et al. (2004b). Bacterial spot of tomato occurs throughout the world wherever tomatoes are grown and environmental conditions are favorable for disease development (Stall, 1995). Bacterial spot affects leaves, stems and fruits, and causes both yield and fruit grade losses through defoliation and fruit lesions (Scott, 1997). The resistance to bacterial spot is incompletely or quantitatively inherited.

Bacterial speck of tomato, caused by *Pseudomonas syringae* pv. *tomato*, mainly occurs on foliage and fruit, and causes yield loss in field and greenhouse grown tomatoes. Two races, 0 and 1, have been reported to date (Scott, 1997). Genetic studies indicated that single dominant genes control resistance to bacterial speck. Four genes, *Pto-1* to *Pto-4*, have been reported (Pilowsky and Zutra, 1986; Pitblado and MacNeill, 1983; Stockinger and Walling, 1994; Tanksley et al., 1996). *Pto-1* is also generally referred to as *Pto* and has been cloned (Martin et al., 1993). The resistance gene *Pto* is widely deployed in both fresh market and processing tomato varieties.

The lack of resistance to bacterial spot in tomato varieties leads to frequent application of copper sprays and the failure of growers to adopt disease forecasting tools that minimize control sprays for fungal pathogens. Disease forecasting models for tomato do not predict bacterial infection and growers default to a calendar application of copper and fungicide tank mixes. The lack of resistance to bacterial diseases therefore adds to the cost of production beyond loss of yield and quality and results in the use of more pesticides than are necessary to control fungal diseases. Although the development of varieties with resistance to multiple bacterial diseases is a desirable goal, the process has been difficult due to the necessity of selecting for multiple diseases and the emergence of new species and races.

Marker-assisted selection (MAS) offers an opportunity to circumvent some of the problems associated with phenotypic selection for resistance to multiple bacterial pathogens and races. MAS has been successfully used to select for single qualitative or quantitative traits in many crops. These include the extensive use of an acid phosphatase isozyme polymorphism to select for root-knot nematode (*Meloidogyne* spp.) resistance in tomato (Carboni et al., 1995; Medina-Filho and Stevens, 1980; Rick and Fobes, 1974). Other applications of MAS to disease and pest resistance breeding include Potato Virus Y in *Solanum tuberosum* L. (Hamalainen et al., 1997); southwestern corn borer (*Diatraea grandiosella* Dyar) in *Zea mays* L. (Willcox et al., 2002); and downy mildew [*Plasmopara halstedii* (Farl.) Berl. et de Toni] on *Helianthus annuus* L. (Brahm et al., 2000). Markers have been used to combine multiple genes for rust [*Uromyces appendiculatus* (Pers.) Unger] resistance in *Phaseolus vulgaris* L. (Kelly et al., 1993) and bacterial blight [*Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings] resistance genes in *Oryza sativa* L. (Huang et al., 1997; Yoshimura et al., 1995). Quality traits that have been manipulated through MAS include high-molecular-weight glutenin in *Triticum aestivum* L. (Ahmad, 2000); the waxy genes

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in *O. sativa* (Ramalingam et al., 2002); seed size in *Glycine max* (L.) Merr. (Hoeck et al., 2003); and fiber strength in *Gossypium hirsutum* L. (Zhang et al., 2003). The potential for combining resistance and quality traits makes MAS an appealing strategy for increasing the efficiency of plant breeding.

A previous study indicated that three quantitative trait loci (QTL) in Hawaii 7998 confer a hypersensitive response (HR) to race T1 of bacterial spot (Yu et al., 1995). *Rx1* and *Rx2* are located on the top and bottom of chromosome 1, respectively, and *Rx3* is located on chromosome 5. Only the *Rx3* locus has been demonstrated to provide resistance in the field against T1 strains, and it explains as much as 41% of the variation for resistance (Yang et al., 2005). Both *Pto* and *Rx3* are located on chromosome 5, but are derived from different sources and the linkage of desirable alleles is therefore in repulsion phase. The use of phenotypic selection to combine resistance is complicated by the need to identify recombinant plants, to distinguish recombinants from plants that are heterozygous for alleles from both parents, and to select for homozygous resistance in coupling phase. Selection based on markers allows for efficient classification while reducing the resources necessary for phenotypic evaluation. The objective of this study was to use MAS to select coupling phase recombinants in order to develop lines with resistance to both diseases.

Materials and Methods

PLANT MATERIAL. Two inbred backcross lines (IBLs) were used to pyramid the resistance gene, *Pto*, and the locus, *Rx3*. Ohio 9834 is an IBL derived from Hawaii 7998 and Ohio 88119 carrying the *Rx3* locus for partial resistance to race T1 of bacterial spot (Francis and Miller, 2004; Yang et al., 2005) but no resistance to bacterial speck. Ohio 981205 is an IBL in the Ohio 88119 genetic background carrying *Pto* for resistance to race 0 of bacterial speck but no resistance to bacterial spot. The Ohio 88119 genetic background was chosen due to its concentrated early set and use as an elite line in commercial tomato hybrids (Berry et al., 1995; Francis et al., 2002). A cross was made between Ohio 9834 and Ohio 981205 to develop an F_2 population and subsequent inbred generations.

Individual F_2 plants were grown in 288 Square Plug Tray Deep (Landmark Plastic Corp., Akron, Ohio) with PRO-MIX (Premier Brands, Yonkers, N.Y.) in the greenhouse for DNA isolation and selection. Selected F_2 plants were transplanted into 7.6-L pots, allowed to set fruit, and seeds were collected. Seed from selected $F_{2,3}$ families was again sown in 288 cell trays, and homozygous individuals were selected from heterozygous families prior to transplant into the field.

DNA ISOLATION AND MARKER ANALYSIS. Genomic DNA was isolated from fresh young leaves 96 samples at a time. To prevent drying of samples, 7.5 μ L ddH₂O was added to each well of a flat-bottom 96-well plate (96-APF-1CO; Rainin Instrument Co., Woburn, Mass.) and the plate was kept on ice. A leaf disk from each individual plant was collected using a hole punch. After all tissue samples were collected, 100 μ L of 0.25 N NaOH was added to each well and the leaf disks were ground 3–5 min using a 96-prong seed crusher (PerkinElmer, Norton, Ohio). Following grinding, a 7.5- μ L aliquot of each extract was transferred to a 96-well plate containing 75 μ L of ice-cold neutralization solution (0.05 M Tris-HCl pH 7.0, 0.1 mM EDTA) in each well. The neutralized solution is ready for use as DNA template in PCR applications and can be stored at -20°C for at least 1 month.

Two PCR-based DNA markers were used to genotype individuals. Marker *Pto* (forward primer: 5'-ATCTACCCACAATGAGCATGAGCTG-3', reverse primer: 5'-GTGCATACTC-CAGTTTCCAC-3') was designed according to the sequence of the cloned gene *Pto* (Coaker and Francis, 2004; Martin et al., 1993). Of three markers (TOM49, *Rx3*-L1, and CosOH73) linked to the *Rx3* locus, marker *Rx3*-L1 (forward primer: 5'-CTCCGAGCGAAGAGTCTAGAGTC-3', reverse primer: 5'-GAAGGCAAAGGAAAAGGAGAAGGATGG-3') explains the highest phenotypic variation (Yang et al., 2005) and therefore was used for selection. PCR reactions were conducted in a 20- μ L volume consisting of 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM MgCl₂, 50 μ M of each dNTP, 0.3 μ M primers, 2 μ L genomic DNA template and 1 unit of *Taq* DNA polymerase. Reactions were heated at 94 $^\circ\text{C}$ for 2 min followed by 36 cycles of 1 min at 94 $^\circ\text{C}$, 1 min at 60 $^\circ\text{C}$, and a 2-min extension at 72 $^\circ\text{C}$. Final reactions were extended at 72 $^\circ\text{C}$ for 5 min. Amplification was performed in a programmable thermal controller (PTC-100; MJ Research, Watertown, Mass.). Polymorphisms were detected as cut amplified polymorphisms (CAP). The PCR products were digested with *Rsa* I for *Pto* and *Bsr*BI for *Rx3*-L1 according to the enzyme manufacturers' protocols. Fragments were separated using 2% agarose gels (Biotechnology Grade 3:1 agarose; Amresco, Solon, Ohio), stained with ethidium bromide, and photographed using the Syngene BioImaging System (Cambridge, U.K.).

Recombination between markers was estimated using maximum-likelihood (Allard, 1956).

GREENHOUSE AND FIELD EXPERIMENTS. A replicated randomized complete-block design was used for greenhouse disease evaluation. Ohio 88119 was used as a susceptible control for both diseases, Ohio 9834 was used as a resistant control for bacterial spot race T1, and Ohio 981205 was used as a resistant control for bacterial speck race 0. In the greenhouse, five $F_{3,4}$ plants of each selected family and controls were grown in each of two blocks using PRO-MIX with fertilizer and water supplied as needed. Two strains, *Xcv* 89 and *Xcv* 110c, of bacterial spot race T1 and one strain, DC3000, of bacterial speck race 0 were used for infiltration. The inoculum concentration was adjusted to $\approx 2 \times 10^8$ cfu/mL for strains *Xcv* 89 and *Xcv* 110c, and 4×10^7 cfu/mL for strain DC3000. The plants were misted with water 1 h before inoculation. The inoculum was infiltrated through the back of a fully expanded leaflet on the third true leaf using a 3-mL syringe without needle when plants were at the fourth true-leaf stage. The inoculated plants were kept at 20–25 $^\circ\text{C}$ in a humid environment. HR was recorded 24–72 h after inoculation.

In the field, 20 plants of each selected $F_{2,3}$, $F_{3,4}$, and controls were grown in each of two blocks at The Ohio State Univ.'s Vegetable Crops Branch in Fremont, Ohio, using conventional practices (Precheur, 2000) in 2002 and 2003. In 2002, the plants were inoculated with a T1 strain of bacterial spot and the disease was scored using 1–12 scale as described previously (Horsfall and Barratt, 1945; Scott et al., 1995). Early blight [*Alternaria solani* (Ell. & Mart.) Jones & Grout] naturally occurred and spread in the field in 2002, this disease was also scored using the same scale. Early blight and bacterial spot were distinguished based on characteristics of the lesions as follows: concentric rings are present in early blight lesions in contrast to black lesions found in bacterial spot infected plants; early blight lesions are surrounded by a yellow hallow in contrast to the absence of a halo or a light green halo found surrounding bacterial spot lesions. In 2003, the field was naturally infected by bacterial speck. The reactions of plants to this disease were recorded as resistant or susceptible.

STATISTICAL ANALYSIS. Analysis of variance (ANOVA) and mean separations were performed using the General Linear Model procedure of SAS (version 8.1; SAS Institute, Cary, N.C.). Mean separations were based on the least significant difference (LSD) after a significant F test in the ANOVA. Genotype was considered as a fixed-effect variable, while block was considered as a random-effect variable.

Results

SELECTION OF COUPLING PHASE RECOMBINANTS USING DNA MARKERS. A total of 419 F_2 plants were subject to DNA marker analysis for two CAP markers, Pto and Rx3-L1. Primers for marker Pto amplified a 552-base pair (bp) fragment from both resistant and susceptible parents. The restriction enzyme *Rsa*I did not cut the PCR product from the resistant parent Ohio 981205, but cut the PCR product from the susceptible parent Ohio 9834 into two fragments, 113 bp and 439 bp (Fig. 1A). Primers for marker Rx3-L1 amplified a 323-bp fragment from both resistant and susceptible parents. The restriction enzyme *Bsr*BI did not cut the PCR product from the bacterial spot susceptible parent Ohio 981205, but digested the PCR product from the resistant parent Ohio 9834 into two fragments, 275 and 48 bp (Fig. 1B). Both markers are co-dominant. The segregation ratio for both markers in the population was consistent with the expected 1:2:1 ratio at $P = 0.05$ ($\chi^2 = 1.57$ for Pto, $\chi^2 = 5.77$ for Rx3-L1).

Based on marker genotypes, 13 F_2 plants were homozygous for desirable recombination events, and thus represent the fusion of two recombinant gametes. These 13 F_2 individuals were transplanted and grown in the greenhouse as a source of $F_{2,3}$ seed for field evaluation. A further 94 families were heterozygous for desirable recombination events. The estimate of recombination between markers was 36.8 ± 2.2 cM for this cross. To obtain a

larger population of plants homozygous for desirable coupling phase recombination events, 20 F_2 individuals heterozygous for favorable recombination events were randomly selected and grown in the greenhouse to develop $F_{2,3}$ families. A total of 3176 plants in the 20 $F_{2,3}$ families were genotyped and homozygous recombinant plants were transplanted to the field. At least 40 homozygotes were selected for each family and randomly divided between two plots in the field.

HYPERSENSITIVE RESPONSE IN THE PARENTAL GENOTYPES AND SELECTED FAMILIES. Seed was saved from individual F_3 plants in the field and $F_{3,4}$ plants from each selection were used for greenhouse evaluation of resistance. Five plants from each family were infiltrated with strain DC3000 for bacterial speck race 0 and strains *Xcv* 89 and *Xcv* 110c for bacterial spot race T1. For bacterial speck, all selected plants and Ohio 981205 showed HR within 24 h. For bacterial spot, however, HR was unambiguous 48–72 h after infiltration in the selected families. The susceptible control Ohio 88119 and line Ohio 981205 showed water-soaked disease lesions 5 d after infiltration. Hawaii 7998 showed HR within 24 h and Ohio 9834 (*Rx3*) showed HR 48 h after infiltration, with a reaction that was quantitatively distinct from the reaction of Hawaii 7998. It is not clear what caused the delay in HR in Ohio 9834 and selected lines, though it is clear that resistance in Hawaii 7998 is conferred by genes in addition to *Rx3* (Yang et al., 2005; Yu et al., 1995).

PERFORMANCE OF SELECTED FAMILIES FOR DISEASE RESISTANCE IN THE FIELD. Although we confirmed HR to race T1 strains in the selected families, the relationship between HR and resistance in the field is not well established. Somodi et al. (1996) found the correlation between HR and field susceptibility was $r = 0.31$. For this reason we chose to verify resistance under field conditions. The 33 selected $F_{2,3}$ families and three controls, Ohio 88119, Ohio 9834, and Ohio 981205, were grown in a replicated trial inoculated with race T1 strains of bacterial spot. The susceptible

controls, Ohio 88119 and Ohio 981205, had high disease ratings, whereas the resistant line Ohio 9834 had a relatively low rating. All selected families had significantly lower disease ratings than the susceptible controls (Table 1). Only one family (SG01-3002) had a disease rating that was significantly higher than Ohio 9834. All other families had equal or lower disease ratings than Ohio 9834. Seventeen of the families had significantly lower disease ratings than Ohio 9834 (Table 1).

Due to the natural occurrence and spread of early blight in 2002, the severity of this disease was also scored. All families showed partial resistance in the field relative to susceptible controls (Table 1). The correlation between resistance to bacterial spot and early blight was significant, but low ($r = 0.59$, $P < 0.0001$). The close agreement between greenhouse results and field performance suggest that the presence of early blight did not confound our assessment of bacterial spot resistance (Table 1).

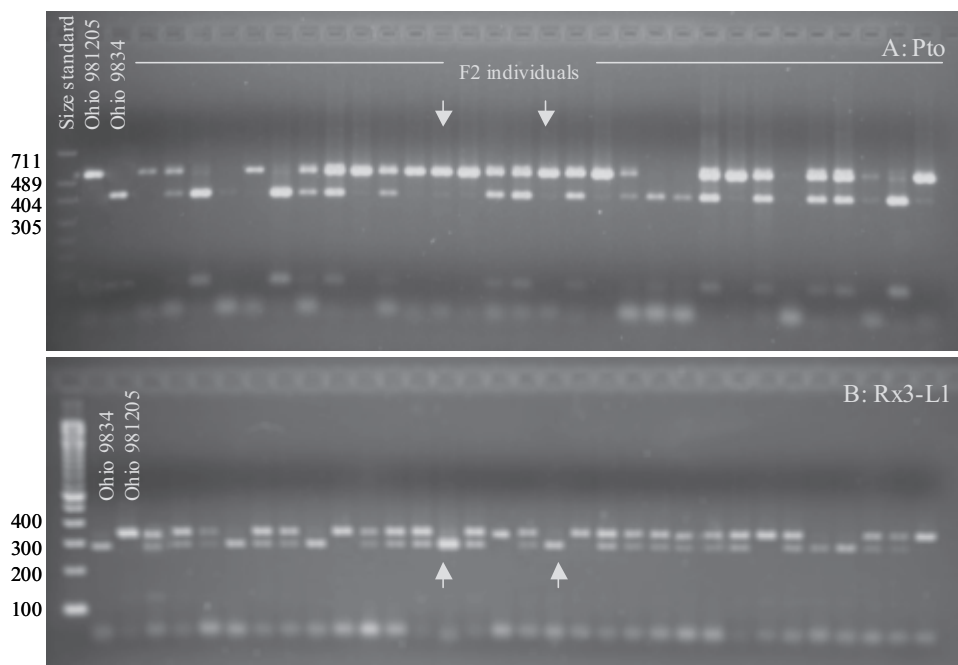


Fig. 1. Molecular marker banding patterns for tomato parental lines, Ohio 981205 and Ohio 9834, and F_2 progeny. For the marker Pto (A), fragment sizes are 552 bp for the Ohio 981205 allele (resistant) and 113 bp and 439 bp for the Ohio 9834 allele (susceptible). Fragment sizes for marker Rx3-L1 (B) are 323 bp for the bacterial spot susceptible parent, Ohio 981205, and 275 bp and 48 bp for the resistant parent, Ohio 9834. Recombinant individuals with resistance in coupling phase are indicated by arrows.

Table 1. Response of tomato lines to bacterial speck and bacterial spot based on hypersensitive reaction in the greenhouse and resistance in the field.

Genotype	Greenhouse rating ^z		Field rating		
	Bacterial speck	Bacterial spot	Bacterial speck ^y	Bacterial spot ^x	Early blight ^w
SG01-3000	HR	HR	R	4.00 a	4.00 ab
SG01-3034	HR	HR	R	4.00 a	3.50 a
SG01-3011	HR	HR	R	4.25 ab	4.50 a-c
SG01-3004	HR	HR	R	4.50 a-c	4.50 a-c
SG01-3008	HR	HR	R	4.50 a-c	5.00 a-d
SG01-3023	HR	HR	R	4.75 a-d	6.00 c-e
SG01-3028	HR	HR	R	4.75 a-d	4.50 a-c
SG01-3003	HR	HR	R	5.00 a-e	4.50 a-c
SG01-3010	HR	HR	R	5.00 a-e	6.00 c-e
SG01-3015	HR	HR	R	5.00 a-e	7.00 e
SG01-3020	HR	HR	R	5.00 a-e	4.50 a-c
SG01-3022	HR	HR	R	5.00 a-e	4.50 a-c
SG01-3029	HR	HR	R	5.00 a-e	5.50 b-e
SG01-3006	HR	HR	-	5.25 b-f	5.00 a-d
SG01-3021	HR	HR	-	5.25 b-f	4.50 a-c
SG01-3030	HR	HR	-	5.25 b-f	5.50 b-e
SG01-3032	HR	HR	-	5.25 b-f	5.00 a-d
SG01-3013	HR	HR	-	5.50 c-f	4.50 a-c
SG01-3001	HR	HR	-	5.75 d-g	5.50 b-e
SG01-3005	HR	HR	-	5.75 d-g	5.00 a-d
SG01-3027	HR	HR	-	5.75 d-g	6.00 c-e
SG01-3014	HR	HR	-	6.00 e-g	6.50 de
SG01-3016	HR	HR	-	6.00 e-g	6.00 c-e
SG01-3017	HR	HR	-	6.00 e-g	6.50 de
SG01-3018	HR	HR	-	6.00 e-g	6.00 c-e
SG01-3019	HR	HR	-	6.00 e-g	6.50 de
SG01-3024	HR	HR	-	6.00 e-g	6.50 de
SG01-3031	HR	HR	-	6.00 e-g	6.00 c-e
SG01-3025	HR	HR	-	6.25 fg	6.50 de
SG01-3035	HR	HR	-	6.25 fg	6.00 c-e
SG01-3012	HR	HR	-	6.50 g	5.50 b-e
SG01-3026	HR	HR	-	6.50 g	5.50 b-e
SG01-3002	HR	HR	-	7.75 h	7.00 e
Ohio 9834	S	HR	S	6.50 g	6.00 c-e
Ohio 88119	S	S	S	9.00 i	7.00 e
Ohio 981205	HR	S	R	9.00 i	6.50 de
Mean					5.67
5.51					
LSD ^v 0.05				1.06	1.80
LSD 0.3				0.59	1.01

^zHR = hypersensitive response, S = susceptible.

^yBacterial speck resistance was scored qualitatively as R = resistant or S = susceptible in the field in 2003. Families that were not evaluated in 2003 are indicated "-".

^xBacterial spot ratings were taken in the field in 2002 using a modified Horsfall-Barratt (1945) system in which 1 = 0%, 2 = 0% to 3%, 3 = 3% to 6%, 4 = 6% to 12%, 5 = 12% to 25%, 6 = 25% to 50%, 7 = 50% to 75%, 8 = 75% to 87%, 9 = 87% to 94%, 10 = 94% to 97%, 11 = 97% to 100%, and 12 = 100% diseased tissue.

^wEarly blight ratings followed the scale used for bacterial spot.

^vMean separation in columns are based on Duncan's multiple range test at $P \leq 0.05$, least significant difference (LSD) at $P = 0.05$ and $P = 0.3$.

The selected 13 $F_{3,4}$ lines and three controls, Ohio 88119, Ohio 9834, and Ohio 981205, were grown in the field in Fremont, Ohio, in Summer 2003. Facilitated by the cool and humid weather in the early summer, bacterial speck naturally spread in the field. All selected lines and the control Ohio 981205 showed resistance to this infection while Ohio 88119 and Ohio 9834 were susceptible (Table 1).

Discussion

Breeding for resistance to bacterial spot is difficult due to several factors, including the presence of multiple species and races of the pathogen, the lack of an easy method to screen plants for resistance, and the lack of available disease resistance in commercially acceptable genetic backgrounds. A strategy that alternates MAS with field selection offers an opportunity to select for resistance and horticultural performance. The relative efficiency of MAS in producing gain under selection is one of the major concerns for using DNA markers in a breeding program. MAS may produce high efficiencies compared to phenotypic selection when using a marker that is tightly linked to a qualitative trait, when the selection unit is a single plant rather than a replicated line, and when the proportion of phenotypic variance explained by the marker is high (Francis et al., 2003). In the case of *Pto*, the target gene is the marker and heritability is expected to approach 1. However, the success of MAS for a QTL is dependent on several factors including linkage distance and the strength of the QTL (Dudley, 1993; Kearsey and Farquhar, 1998). There is a possibility of losing the QTL-marker association when flanking markers are not used (Dudley, 1993; Willcox et al., 2002; Zhou et al., 2003). In this study we applied MAS to the *Rx3* locus, a major QTL from Hawaii 7998 that confers field resistance to race T1 of bacterial spot. Three markers with the order of TOM49, *Rx3-L1*, and *CosOH73* show significant association with resistance. Marker *Rx3-L1* explains a higher phenotypic variation than the two flanking markers (Yang et al., 2005), and in this study, successfully selected individuals showing resistance in selected families.

Markers *Pto* and *Rx3-L1* were separated by 36.8 cM, and the selected families we tested reacted with an HR when challenged with T1 strains of the bacterial spot pathogen. This observation suggests that the *Rx3* locus is distal to *Rx3-L1* or very closely linked in a proximal position, relative to *Pto*. The distance between loci in centimorgans will be dependent on genetic backgrounds. For example, the estimate of recombination in the Ohio 9834 x

Ohio 981205 cross was larger than the 28.8 cM estimated for the reference *L. esculentum* x *L. pennellii* (Corr.) D'Arcy population (Tanksley et al., 1992), which may be attributed to effects of the wide cross. We have observed recombination distances between *Pto* and *Rx3-L1* as low as 14 cM in some crosses (unpublished data). Given this range of recombination, phenotypic selection

for *Pto* and *Rx3* in coupling phase would require the evaluation of ≈ 11 individuals from between 62 (assuming $r = 0.37$) and 168 (assuming $r = 0.14$) F_3 families in order to have a 95% probability of detecting at least one family segregating for individuals that were homozygous resistant for both *Pto* and *Rx3*. Thus phenotypic selection based on screening a minimum of 680 F_3 individuals would be required assuming 37 cM, assuming only half of the recombination events are favorable (resistance in coupling phase), and assuming a 25% frequency of recovering the favorable homozygous event in $F_{2,3}$ individuals (Sedcole, 1977). The ability to identify favorable recombinant events in the heterozygous condition gives MAS an advantage for pyramiding these genes. However, the loose linkage between *Pto* and *Rx3* will require verification of resistance following subsequent crosses.

In the Great Lakes region, multiple bacterial diseases, including bacterial spot, bacterial speck, and bacterial canker [*Clavibacter michiganensis* ssp. *michiganensis* (Smith) Davis], occur simultaneously due to high humidity and frequent rains during the tomato-growing season. These diseases cause yield and quality loss and they are difficult to control. Pyramiding resistance from difference sources into a breeding line or hybrid offers a potential strategy to reduce the impact of bacterial disease. We demonstrate the feasibility of combining *Pto* and *Rx3* through MAS as a first step in developing varieties with resistance to multiple bacterial diseases. The *Rx3* locus is from a poorly adapted *L. esculentum* line Hawaii 7998 while *Pto* can be traced back to its origin of PI 370093 (Pitblado and Kerr, 1980). To combine these two unlinked loci required introgression of the resistance loci and selection of favorable coupling phase recombination events. Both processes were facilitated by combining MAS and field selection. Marker-assisted selection was of particular use in selecting coupling phase recombination to pyramid *Pto* with a QTL for resistance to race T1 of bacterial spot. The families developed in this study will be of use in efforts to breed for multiple resistances to bacterial disease.

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