academicJournals

Vol. 13(12), pp. 1314-1322, 19 March, 2014
DOI: 10.5897/AJB2013.13021
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Identification and utility of sequence related amplified polymorphism (SRAP) markers linked to bacterial wilt resistance genes in potato

Zhi Yanping, Li Hui, Zhang Hairui, and Gang Gao*

School of Life Science, Shanxi Normal University, Linfen041004, Shanxi, China.

Received 11 July, 2013; Accepted 26 February, 2014

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most economically important diseases affecting potato (*Solanum tuberosum*). It is necessary to develop more molecular markers for potential use in potato genetic research. A highly resistant primitive cultivated species *Solanum phureja* was employed to generate a F1 mapping population to perform the bulked segregant analysis (BSA) for screening and identifying of sequence related amplified polymorphism (SRAP) markers linked to the potato resistance to bacterial wilt. A linkage map containing 23 DNA markers distributed on three linkage groups, and covering a genetic distance of 111 cm with an average distance of 5.8 cm between two markers was developed. Two SRAP markers, Me2em5 linked in repulsion phase and Me2em2 in coupling phase, flanked the resistance genes at genetic distances of 3.5 and 3.7 cm distance, respectively. These markers and two others were used for early seedling selection in a BC1 population. The results show that this marker system could be used in marker assisted selection (MAS) breeding program.

Key words: Sequence related amplified polymorphism (SRAP) marker, potato, bacterial wilt.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most widely spread and very destructive plant disease, causing enormous economic losses. The vascular pathogen enter and colonize the plant vascular (xylem) system, disrupting water transport, and causing the characteristic symptoms of wilting, and often vascular discoloration, and death of aerial tissues (He, 1983; Hayward, 1991). The bacterial parasite cause diseases on over 400 plant species, including many crops such as potato, tomato and eggplant. The severity of attacks by this soil-borne vascular parasite is known to vary considerably according to climate, farming practices, soil type and geographic locations (Guidot et al., 2007). Control of wilt diseases is also complicated by the scarcity of sources of disease-resistant host germplasm, and the soil and vascular habitats of the pathogen (Hayward, 1991; Esposito et al., 2008). Despite decades of interests in the pathology, epidemiology and control of bacterial wilt, very little is known about the sources and resistance of the disease and about the genetic or molecular mechanisms under-

*Corresponding author. E-mail: sxnuzyp@126.com.

lying host plant resistance (Qu, 1996). Some nuclear SSR alleles derived from the wild species *S. chacoense* appeared to be related to bacterial wilt resistance (Chen et al.,2013).

The mode of inheritance of resistance in some tetraploid primitively cultivated species is not yet clearly known. Therefore, it is difficult to select bacterial wilt resistant clones or varieties in potato breeding. Although, method of biological control could be used in some situations to combat the pathogens, development of resistant cultivars is usually the best agronomic solution. Disease resistance breeding has traditionally been done by phenotypic selection. Efficiency of phenotypic selec-tion is reduced by variability in the pathogen, infection and disease development. Molecular markers tightly lin-ked to the resistance genes can eliminate these sources of phenotypic variation to enable more efficient breeding strategies in potato improvement (Li et al., 2013; Virupaksh et al., 2012). Marker-assisted selection can be helpful to bacterial wilt resistance breeding. We have identified some molecular markers like AFLP and RAPD makers that linked to potato resistance, these markers were found not to be effective to screen a large population containing up to 200 true potato seeds in the actual application (Gao et al., 2002, 2005). To accelerate introgression of the resistance into cultivated lines, it would be desirable to have more and better molecular markers to perform selection of those possible resistance genes.

Sequence-related amplified polymorphism (SRAP) technology has been recognized as a new and useful molecular marker system for mapping and gene tagging in many crops plants (Valdez-Ojeda et al., 2008; Cao et al., 2011; Niu et al., 2011; Guo et al., 2012; Deng et al., 2013). They are also useful in positional cloning of genes and in elucidating the genetic mode of complex traits that do not display Mendelian segregation (Levi, 2002). Marker-facilitated selection would be particularly effective for pyramidding more resistance genes to provide effective and potentially stable resistance to disease (Martin et al., 1991).

The objectives of this study were to determine the feasibility of using SRAP makers to develop a molecular linkage map of the *S. tuberosum*, and to identify marker loci associated with bacterial wilt. Both of these objectives were met, and we identified the SRAP markers that proved useful in selecting for the gene conferring bacterial wilt resistance in a BC1 population. A longer-term goal of this project will be to use closely linked molecular markers to introduce these resistant loci into potato cultivars as a potential strategy to control potato bacterial wilt disease in the field. In this study, the set of markers provide an impactful marker combination for use in a marker assisted selection (MAS) breeding program to identify genotypes containing resistance from original potato cultivars. Taken together, this study provides and sheds light into potential directions for development of novel management strategies for molecular breeding to controlling wilt diseases.

MATERIALS AND METHODS

Plant materials

One genotype ED13, derived from the cross hybridization between 772102.37 (resistant) and USW7589.2 (susceptible) collected both from the Department of Plant Breeding, Wageningen University, the Netherlands was used as the resistant parent. The susceptible parent was derived from USW5337.3 × *Solanum phureja* (provided by Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, China). A segregating F1 population (Figure 4A) composed of 230 seedlings from hybrid true potato seed (TPS) was used for mapping the SRAP markers. Another segregating BC1 population were generated by first backcrossing a CE26 (susceptible, derived from a cross USW 5337.3 × 772102.37) with the parent 772102.37 for assessing the utility of candidate SRAP markers in identifying bacterial wilt-resistant BC1 plants (Figure 4B).

Wounded root plus soil inoculation

Inocula for potato plants at a concentration of 1×10^8 cfu/ml were made from cultures grown on BPG plates at 28°C for 48 h using SMM without agar and were incubated at 28°C for 4 h. Potato plants with six to eight fully expanded leaves were inoculated by pouring 50 ml of bacterial suspension into the soil around the base of the stem with wounding the roots with a knife. 15 potato seedlings were inoculated with the strain PO41 of the bacterium (provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China). The virulent, wild colony type of *R. solanacearum* cultures was selected on 2, 3, 5-tetrazolium chloride medium (TZC) and used for inoculation. Inoculated plants were cultivated at 28°C under a 16/8 h (light/dark) photoperiod in a growth chamber and were not watered five days before and after inoculation. Control plants were mock-inoculated with sterile water. Each treated plant was rated daily for disease for 21 days after inoculation. Symptoms were scored daily on a 0 to 5 disease index, where 0 indicates no disease, 1 indicates 1 to 10% of leaves wilted, 2 indicates 10 to 25% of leaves wilted, 3 indicates 25 to 50% of leaves wilted, 4 indicates 50 to 75% of leaves wilted, and 5 indi-cates 75 to 100% of leaves wilted. Each experiment contained 16 plants per treatment, and experiments were repeated at least three times. Leave tissues of the susceptible were sampled 4 to 7 dpi into liquid nitrogen for DNA extraction, while the resistant samples were collected 8 to 14 dpi due to slower disease development in this resistant host.

Sequence-related amplified polymorphism analysis

DNA was isolated according to Ducreux et al. (2008). Bulk segregant analysis (BSA) (Michelmore et al., 1991) was used to identify SRAP markers linked to the bacterial wilt resistance gene. For BSA, resistant and susceptible DNA bulks were composed of 10 most resistant (disease index 0) plants and most susceptible (disease index 5), respectively. SRAP analysis was conducted according to previously established protocols with minor modifications (Li et al., 2001). The PCR reaction was set up in a final volume of 20 µL containing 50 ng of DNA, 5.0 pmoL of primer, 200 mM dNTPs, 1.5 mM $MgCl_2$, and 0.5U of Taq polymerase (Sangon Biotech Co. Ltd., Shanghai, China) in 1× Taq buffer. The PCR program included an initial denaturing at 94°C for 3 min followed by 8 cycles of 94°C for 30 s, 37°C for 30 s, and at 72°C for 90 s and then 35 cycles of 94°C for 30 s, 48°C for 30 s, and 72°C for 90 s with a final extension of 72°C for 10 min. PCR products were separated using 8% denaturing polyacryamide gel electrophoresis and visualized by fast silver staining (Bassam et al., 1991).

Table 1. The primer sequences of SRAP used in this study.

Forward primer		Reverse primer	
me1	TGAGTCCAAACCGGATA	em1	GACTGCGTACGAATTAAT
me2	TGAGTCCAAACCGGAGC	em2	GACTGCGTACGAATTTGC
me3	TGAGTCCAAACCGGAAT	em3	GACTGCGTACGAATTGAC
me4	TGAGTCCAAACCGGACC	em4	GACTGCGTACGAATTTGA
me5	TGAGTCCAAACCGGAAG	em5	GACTGCGTACGAATTCGA
me6	TGAGTCCAAACCGGTAA	em6	GACTGCGTACGAATTAAC
me7	TGAGTCCAAACCGGACG	em7	GACTGCGTACGAATTGCA
		em8	GACTGCGTACGAATTCAA

The primer combinations (Table 1) that generated polymorphic bands between the bulks were tested on the bulked individuals to eliminate false-positive markers.

Linkage analysis of markers

Several markers obtained previously were employed before the construction of linkage map to increase reliability; those include three AFLP, one RAPD markers. Preliminary screening for candidate seedling was carried out on the two populations using these markers. As expected, more than 80% of the individual genotype was detected to contain all or one relevant marker DNA fragment. Based on the preliminary selection, Linkage analysis was performed with the JoinMap software (Van Ooijen and Voorips, 2001). Prior to linkage analysis segregation rates, SRAP fragments that ranged in size of 75 to 500 bp were scored for marker. Presence or absence of each polymorphic fragment was coded as “1” and “0”, where “1” indicated the presence of a specific allele, and “0” indicated its absence. For each segregating marker, a chi-square test was performed to fit for deviation from the 1:1 expected segregation ratio in a testcross or BC1 population under the ‘locus genotypic frequency’ command. Markers were sorted on the basis of the chi-squares test with a P -value of <0.05; and skewed markers were excluded from the analysis. All markers were analyzed for linkage, and recombination fractions were converted into map distances (centimorgans) while employing the Kosambi mapping function.

Logarithm of the odds ratio for linkage (LOD) scores of 2.0 to 6.0 were used for grouping of markers, followed by high threshold LOD scores of 7.0 to 10.0 for final mapping of markers in each linkage group. Loci showing weak or suspect linkages were removed from the analysis.

Assessing the utility of a marker set in identifying bacterial wilt-resistant BC1 plants

Forty-one (41) BC1 plants were generated by first backcrossing bacterial wilt-susceptible CE26 genotype with the recurrent parent 772102.37. The marker set identified here were used to select for those progeny that presumably contain the corresponding DNA allele conferring bacterial-wilt resistance, and then three times strict phenotypic identification (the disease index was scored for wilt symptoms as described earlier) were employed to exanimate their identical degree. Spearman correlation coefficient was used to analyze the correlation between the molecular markers and the disease parameters statistical software package S-Plus Version 6. Probabilities <0.01 were considered as significant. All p values were based on two-sided tests, and the differences were considered statistically significant when the p value was ≤ 0.05. All molecular detection and phenotypic identification were repeated three times.

RESULTS

Polymorphism and screening of SRAP markers

100 primer combinations were first tested for selective amplification of DNA fragments from the resistant and susceptible bulks to screening the polymorphism in the diploid mapping population. Prior to linkage analysis segregation rates, SRAP fragments that ranged in size of 75 to 500 bp were scored for marker. Fifty-six (56) of the polymorphic SRAP primer combinations amplified inconsistent band patterns per line. This inconsistency may have been the results of residual heterozygosity or the amplification of similar sequences in two separate genomic regions. It may also result from the use of polyacrylamide gels, which have a higher resolution power than most agarose gels. A total of 314 unambiguous bands were amplified by the 38 of 54 SRAP primer combinations, of which 187 bands were polymorphic (59.55%) and ranged in size from 50 to 1000 bp. The number of polymorphic fragments for each SRAP primer combination varied from 2 to 15, with an average of 6.0 fragments per primer combination. Polymorphisms were amplified with 38 primer pairs (67.8%) resulting in 146 polymorphic bands between the resistant and susceptible parental genotypes. Based on these results, primer pairs that generated polymorphic bands were tested on the resistant and susceptible bulks.

SRAP bands present in one pool and absent in the other were regarded as candidate markers linked to bacterial wilt resistance. SRAP fragments that ranged in size of 75 to 500 bp were scored for marker. A relatively small number of these primer combinations (4 of 54 pairs; 7.41%) were not suitable for the mapping experiment within the tested population because of the lack of polymorphism in size of 75 to 500 bp. Hence, out of the original 56 primer pairs, only 30 combinations were used. However, three (5.56%) of these combinations were skewed from the expected 1:1 segregation ratio and had to be excluded from the final linkage analysis. Twenty-seven (27) primer combinations produced 86 SRAP candidate markers that were used in the next linkage analysis.

Identification of SRAP markers linkage map

In order to obtain more closely linked markers and to avoid any possible mapping errors, the stringent linkage analysis criteria used with the JoinMap analysis (Van Ooijen et al., 2001) resulted in linkage groups. We compared for each primer combination, all primer pairs that generated polymorphic bands were tested on the resistant and susceptible bulks. SRAP bands present in one pool and absent in the other were regarded as candidate markers linked to bacterial wilt resistance. These SRAP markers were uniquely present in one of the donor parents and in the F1 individual genotypes. Although, a total of eighty-six SRAP markers were suitable for the mapping analysis using the first filial generation population, 23 (26.7%) of these markers were skewed from the expected 1:1 segregation ratio and had to be excluded from the final linkage analysis. Sixty-three (63) SRAP markers were used in the final linkage analysis. Five markers (5.8%) did not show strong linkage to our aim, and they were excluded from the analysis. A total of 58 SRAP markers were analyzed for linkage. Of these, 23 could be mapped with high confidence on the linkage map, four markers were clustered on linkage group 1, and seven markers were on linkage group 2 and twelve on linkage group 3 (Figures 1 and 2).

Markers linked in coupling or repulsion phase

Among the twelve polymorphic bands on linkage group 3, we found four markers tightly linked to the resistance locus, two of them linked in coupling phase and the others linked in repulsion phase. The primer combinations me2em5 and me5em2 (Table 1) were respectively detected, a band polymorphic between the resistant bulk and the susceptible bulk, since the marker band named as Me2em5 and Me5em2 was present in all of the susceptible plants in the bulks. They were screened out from the F1 population segregating for bacterial wilt resistance. When the phenotype identification was performed, this marker also co-segregated with disease reaction in the same fashion. And the linkage was further tested in the BC1 plants. The band was present in most of the susceptible genotypes and absent in the resistant ones. These results indicated a tight linkage between the marker me1em2 and the dominant susceptibility allele in the repulsion phase. The markers linked in repulsion phase exhibited similar electrophoretic images (Figures 2 and 3); While the primer combinations me2em2 and me5em1 detected approximately two close-migrating concomitant polymorphic bands between the resistant and susceptible individuals (Figure 2). These double-banded marker linked in coupling phase were designated Me2em2 and Me5em1, respectively.

The two polymorphic bands appeared in most of the resistant genotypes of the F1 population, and of which this proportion basically fits the expected 1:1 ratio, after all, as there are some medium-resistant or medium-susceptible genotypes in the population detected by phenoltype identification. As expected, the band types in the gel were present reproducibly in the BC1 population (Figure 3).

Practical utility of the markers

In previous studies, several markers including AFLP, RAPD and SSR markers were described to link with bacterial wilt resistance loci, but did not determine their usefulness well in MAS programs. Here, we tested 41 BC1 plants inoculated with the pathogen for the presence or absence of the new SRAP markers. Four markers were used to select for those progeny that presumably contain the corresponding DNA allele conferring bacterial-wilt resistance following three times strict phenotypic identification (Figure 3). As shown in Figure 3A, of the 17 plants whose disease index were rated as 0 (highly resistant), 11 were detected to contain all four markers as homozygous genotypes, six plants were heterozygous for the four markers, in which five plants were detected to contain three markers, while one plant contained only one SRAP marker. Of the 15 plants whose disease indexes were rated as 5 (highly susceptible), 14 were detected to contain the repulsion marker. These results suggest that there is at least one major locus in the four marker alleles, because there were one or two markers detected in other nine plants whose disease indexes showed mildly resistant or mildly susceptible. These results were consistent with the expectation.

DISCUSSION

Although, SRAP markers are feasible to generate polymorphic (Levi et al., 2006; Poczai et al., 2013), a relatively small number of these primer combinations were not suitable for the mapping experiment within the tested population because of the lack of polymorphism in size of 75 to 500 bp. Hence, out of the original 56 primer pairs, only 30 combinations were used. However, three (5.56%) of these combinations were skewed from the expected 1:1 segregation ratio and had to be excluded from the final linkage analysis. These SRAP markers proved to be enough efficient and reliable in the mapping analysis in this study. We initially used bulk segregant analysis (BSA) strategy (Michelmore et al., 1991) to identify SRAPs. BSA has been widely used in many crop species for detecting markers linked to genes conferring disease resistance (Hyten et al., 2009) and is a powerful method for identifying molecular markers that show association with a gene of interest or a specific region of the genome (Ren et al., 2012; Salinas et al., 2013).

Segregation distortion is widespread in plant populations and is a common feature of plant genetic linkage maps; it is frequent in progeny derived from interspecific crosses and distortion tends to increase with increasing

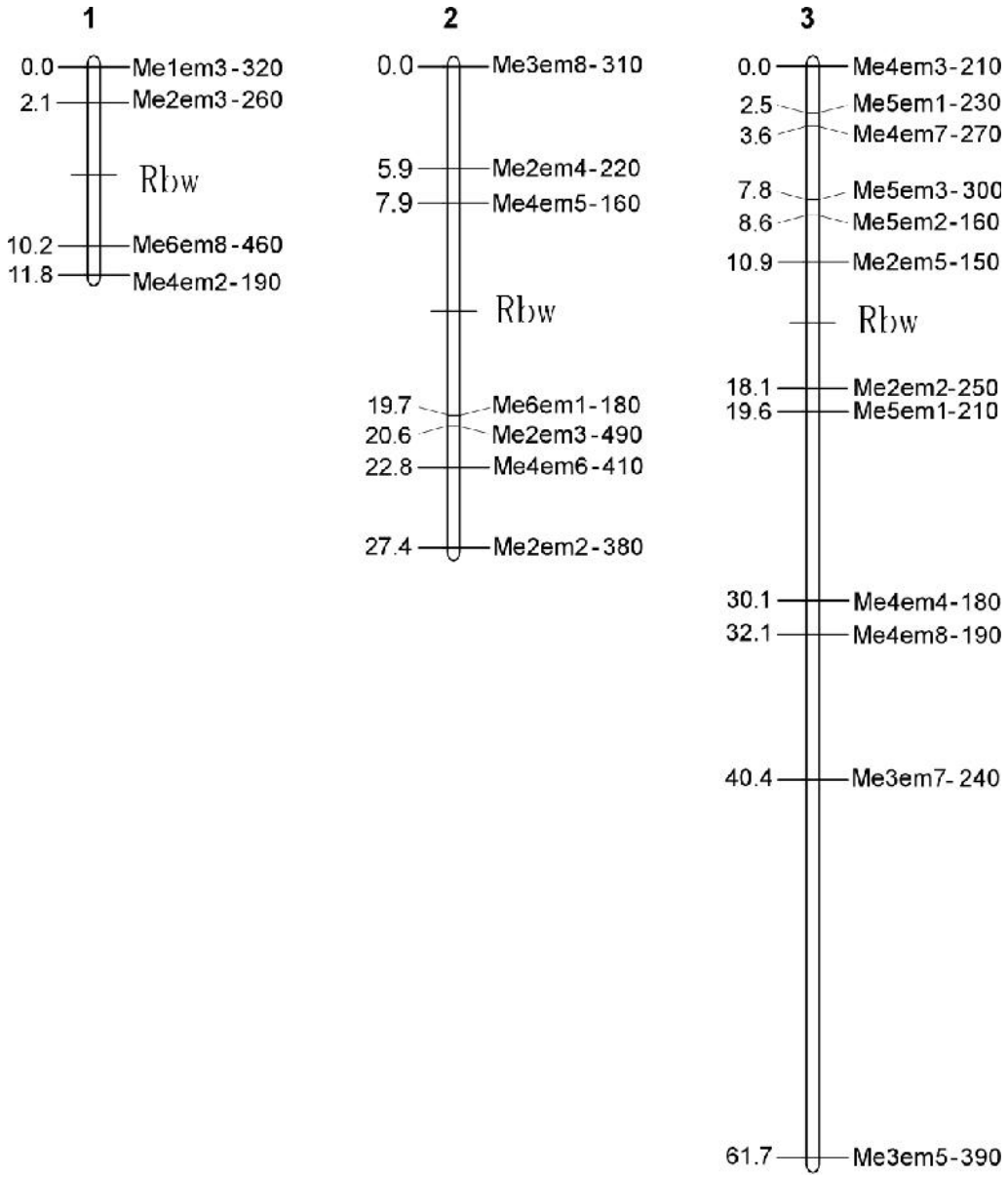

Figure 1. Genetic linkage groups of F1 population constructed with SRAP markers using the JoinMap software. Distances in centiMorgans are indicated to the left and marker names to the right followed by the fragment size in nucleotide bases. Me2em5 and Me2em2 flanked the resistance gene locus (Rbw) at genetic distances of 3.5 and 3.7 cm distance, respectively.

numbers of meioses (Zhang et al., 2012).

In this study, 16.8% of the total loci showed segregation distortion ($P < 0.05$), which is larger than other reports on cotton, wheat and *Aegilops tauschii* (Faris et al., 1998; Lin et al., 2005; Guo et al., 2007; Kumar et al., 2007; Yu et al., 2007). Skewed segregation frequently occurs in populations derived from interspecific crosses and may be influenced by many factors, such as the differential genes controlling the reproduction processes, meiotic drive controlling unique structural features and genetic properties rendering selective advantage or disadvantage to its respective gametes or zygotes (Lyttle, 1991; Buckler et al., 1999). Both biological factors and technical problems potentially contribute to segregation distortion. Integration of distorted segregation markers in linkage construction possibly lead to untrue distance between the adjacent markers in linkage groups (Weber et al., 2003; Lu et al., 2012). Therefore, in order to increase accuracy of the genetic map constructed, the distortion segregation markers were ignored in this study. It has been proven that highly skewed markers may contribute to overestimation of recombination frequency and to

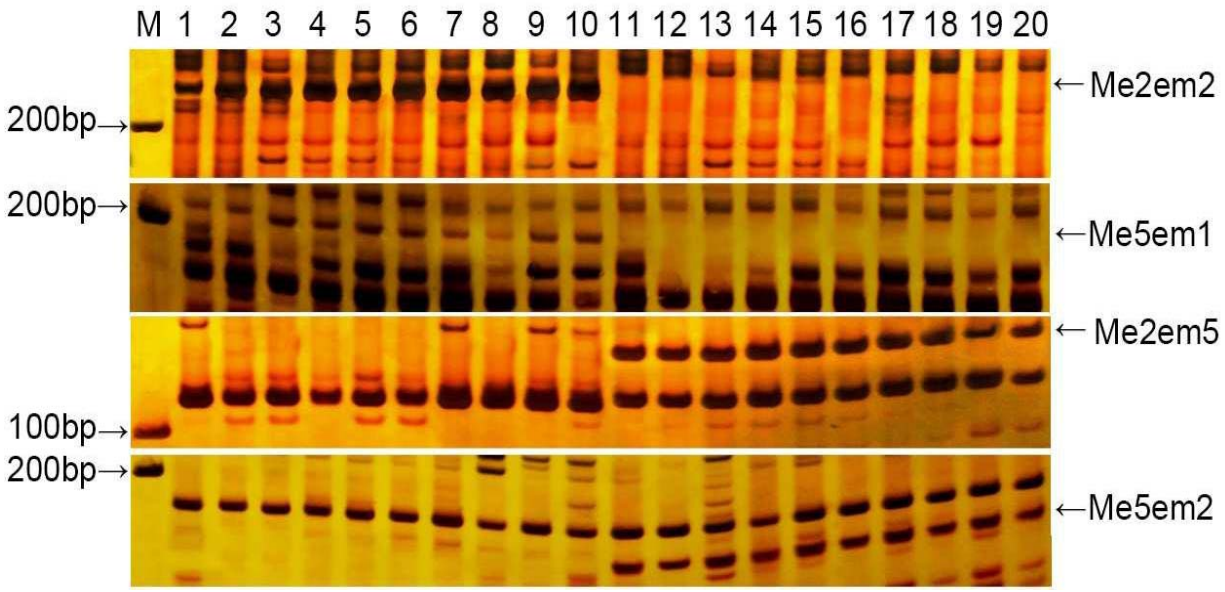

Figure 2. Samples of markers detected by primer combination Me2em2, Me5em1, Me2em5 and Me5em2 in the resistant and susceptible DNA bulks. Lanes: 1 to 10, most resistant (disease index 0) genotypes. 11 to 20, most susceptible (disease index 5) genotypes. Standard size markers are given on left side.

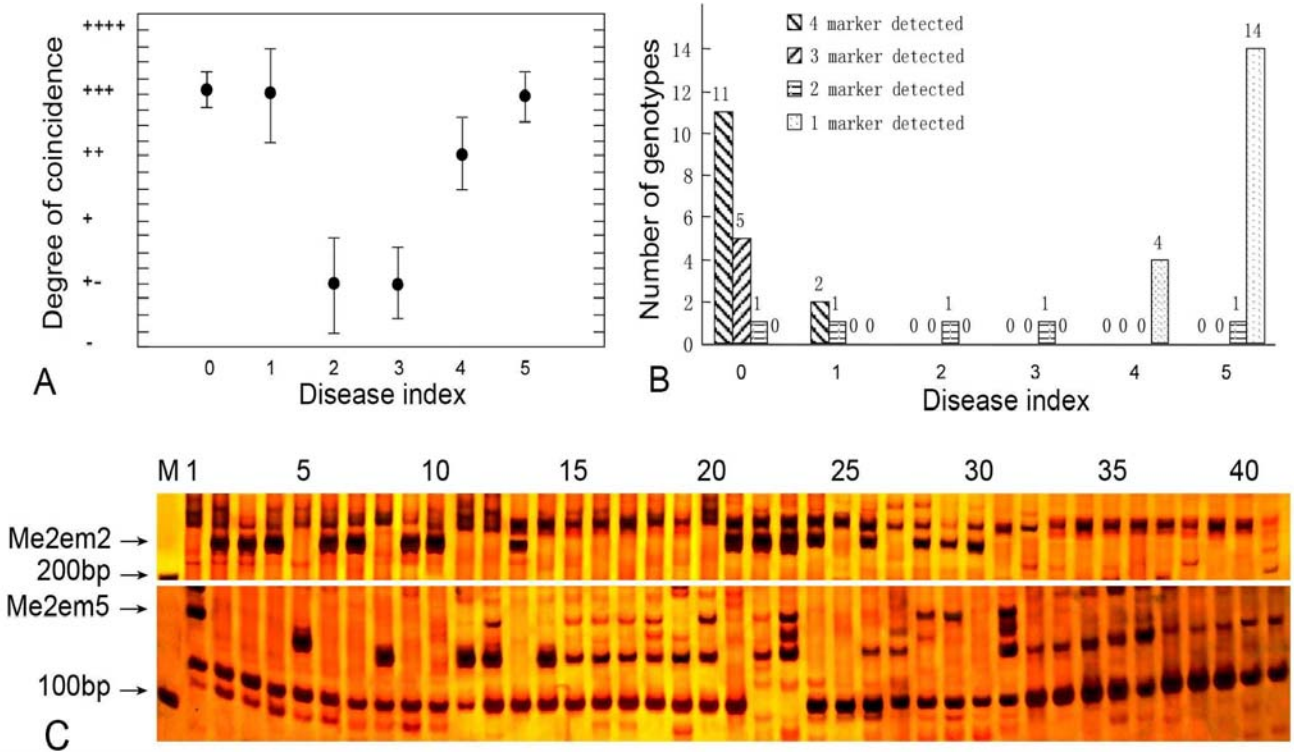

Figure 3. Assessment of the utility of 4 markers in a segregating BC1 population. A) Spearman correlation coefficient analysis for degree of coincidence between the molecular markers and the disease parameters. For disease index, 0 = no disease, 1 = 1 to 10% of leaves wilted, 2 = 10 to 25% of leaves wilted, 3 = 25 to 50% of leaves wilted, 4 = 50 to 75% of leaves wilted, and 5 = 75 to 100% of leaves wilted, or plant death 21 dpi; B) Distribution of markers genotype data from bacterial wilt resistant polymorphism and phenotype data from potato BC1 population derived from the bacterial wilt-susceptible CE26 genotype with the recurrent parent 772102.37. The results of one of three independent experiments were shown; C) Electrophoresis patterns of polymerase chain reaction-amplified with genomic DNA of 20 genotypes. M, molecular marker showed by arrows followed by base number; Lanes 1 to 10, F1 plants; lanes 11 to 20, susceptible F1 plants. Primer combinations are given on left side.

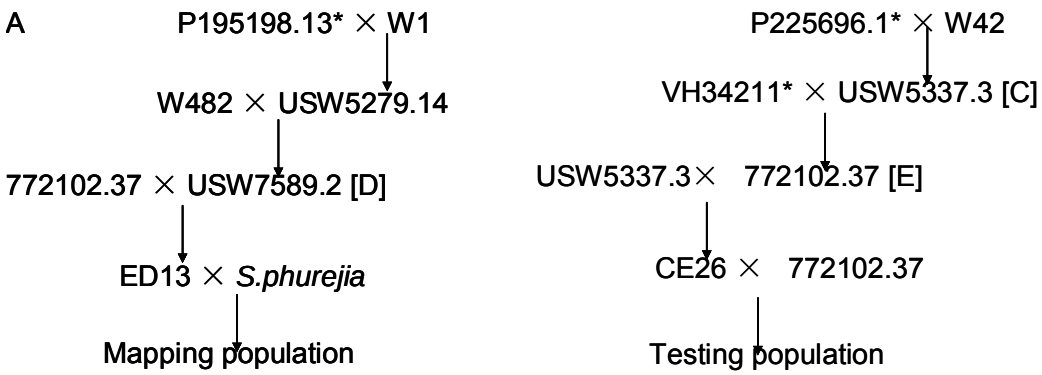

Figure 4. Genetic background of the plant materials used in this study. A) The mapping population composed of 230 genotypes; B) The testing population composed of 47 genotypes. *Pedigree from *S. phurejia* and *S. vernei* involved (Qu, 1996).

loose linkages between markers while they may cause the merging of two linkage groups (Saliba-Colombani et al., 2000). Thus, in this study, the skewed markers had to be excluded from the mapping analysis.

Five markers (5.8%) did not show strong linkage to our aim, and they were excluded from the analysis. Conversely, they may cause the merging of two linkage groups. Although, this phenomenon was manifested in others (Levi et al., 2006; Saliba-Colombani et al., 2000) and our results, we speculate that these molecular markers in group 1 or 2 may represent additional resistance genes; they are more likely to link within other different chromosomes. There may be of different origin derived from those remoter ancestors. This result suggested that the bacterial wilt resistance may be controlled by quantitative trait loci, or there may be more loci exist in potato genome. A recent report on resistance to *R. solanacearum* in eggplant (Lebeau et al., 2013) seems to be able to support this speculation. It is worthy that a more in-depth genetic analysis of bacterial wilt resistance in potato, especially in tetrapoid potato, needs to be considered. The markers Me2em2, Me5em1, Me2em5 and Me5em2 found associated with the locus can be used readily for marker assisted selection, helping to introgress the recessive resistance allele of this gene into cultivated lines. Although, these are not codominant markers, the linkage in coupling phase of Me2em2, Me5em1 and in repulsion phase of marker Me2em5 and Me5em2 to the resistance allele, makes it possible to identify almost all *R. solanacearum* isolates.

Use of these markers might circumvent in many cases progeny testing of resistant plants, thereby reducing in half the time required to develop bacterial wilt resistant lines. Since the BC1 plants generated did not contain the same level of resistance found in the mapping population, as the smaller population was more likely to exhibit a larger segregation distortion and different genetic recombination; it was possible that a certain deviation of the matching degree occurred between the symptomatic phenotypic identification and molecular marker detection. It was not surprising that two moderately susceptible genotypes were detected to contain a molecular marker. Similar results were confirmed in others of similar experiments in eggplant (Lebeau et al., 2013). Using spearman correlation for analysis we found that there was not very strong correlation existed between the SRAP markers (4 primer recombination) and bacterial wilt disease scores. On the interpretation of the results, we inferred that the symptom and disease scores were not significantly correlated with the numbers of the SRAP markers used here. In general, the correlation between symptom scores and gene functional status measures should be stronger than the correlation between disease scores and gene number measures. After all, the other markers in linkage groups 1 and 2 were not used for correlation analysis. It is worth paying more attentions to that those markers should become the object of special consideration in MAS breeding program because the contribution from the gene loci linked with the markers is not inconsiderable.

It has been demonstrated that SRAP markers have good coverage of the genome and was able to rapidly detect markers linked to the resistance gene (Guo et al., 2012; Lu et al., 2012; Zhao et al., 2012). The results in this study suggest that the resistance to bacterial wilt is not simply inherited, but possibly controlled by a series of genes. Here, we identified the SRAP markers that proved useful in selecting for the gene conferring bacterial wilt resistance in a BC1 potato population. The set of markers provide a robust marker combination for use in MAS breeding program to identify genotypes containing the relative allele conferring bacterial wilt resistance in potato cultivars.

Conclusion

In this study, based on an F1 segregating generation, we identified the SRAP markers that proved useful in selecting for the gene conferring bacterial wilt resistance

in a BC1 potato population. The set of markers provide a robust marker combination for use in MAS breeding program to identify genotypes containing the relative allele conferring bacterial wilt resistance in potato cultivars.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by a high-tech industrialization project of the Department of Education of Shanxi Province (2010011) and a program of the Science and Technology Agency of Shanxi Province (2012011032-2) and a project of National Natural Science Fund (31260344). The authors thank the membership of the potato breeding group of Institute of Vegetables and flowers, Chinese Academy of Agricultural Sciences, and Dr. Yuan Yuxiang from Institute of Horticulture, Henan Academy of Agricultural Sciences, for their technical assistance and helpful suggestions.

REFERENCES

Bassam BJ, Gaetano-Anollé G, Gresshoff PM (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels.Anal.Biochem.196:80-83.

Buckler ES, Phelps-Durr TL, Buckler CS, Dawe RK, Doebley JF, Holtsford TP (1999). Meiotic drive of chromosomal knobs reshaped the maize genome. Genetics, 153:415-426.

Cao K, Wang LR, Zhu GR, Fang WC, Chen CW, Zhao P (2011). Construction of a linkage map and identification of resistance gene analog markers for root-knot nematodes in wild peach,Prunus kansuensis. J. Am. Soc. Hortic. Sci. 136:190-197.

Chen L, Guo X, Xie C, He L, Cai X, Tian L, Song B, Liu J (2013). Nuclear and cytoplasmic genome components of Solanum tuberosum S. chacoense somatic hybrids and three SSR alleles related to bacterial wilt resistance. [Epub ahead of print].

Deng CL, Qin RY, Gao J, Jia YY, Ren YX, Gao WJ, Lu LD (2013). SRAP analysis of DNA base sequence changes in lotus mutants induced by Fe+ implantation. Genet. Mol. Res. 12:335-343.

Ducreux LJ, Morris WL, Prosser IM, Morris JA, Beale MH, Wright F, Shepherd T, Bryan GJ, Hedley PE, Taylor MA (2008). Expression profiling of potato germplasm differentiated in quality traits leads to the identification of candidate flavour and texture genes. J. Exp. Bot. 59:4219-4231.

Esposito N, Ovchinnikova OG, Barone A, Zoina A, Holst O, Evidente A (2008). Host and non-host plant response to bacterial wilt in potato: role of the lipopolysaccharide isolated from Ralstonia solanacearum and molecular analysis of plant-pathogen interaction. Chem. Biodivers. 5:2662-2675.

Faris JD, Laddomada B, Gill BS (1998). Molecular mapping segregation distortion loci in Aegilops tauschii. Genetics, 149:319-327.

Gao G, Jin L, Qu D, Lian Y, Yan G, Duan J (2005). Identification of common AFLP markers linked with resistance to bacterial wilt. Acta.Bot.Boreali-Occidentalia Sinica, 25:269-274.

Gao G, Qu DY, Lian Y, Jin LP, Yan GQ, Feng LX (2002). A RAPD marker linked to the potato resistance to bacterial wilt. Acta.Phytopathologica Sinica, 32:267-271.

Guidot A, Prior P, Schoenfeld J, Carrere S, Genin S, Boucher C (2007). Genomic structure and phylogeny of the plant pathogen Ralstonia solanacearum inferred from gene distribution analysis. J. Bacteriol. 189:377-387.

Guo HL, Xuan JP, Liu JX, Zhang YM, Zheng YQ (2012). Association of molecular markers with cold tolerance and green period in zoysiagrass (Zoysia Willd). Breed. Sci. 62:320-327.

Guo WZ, Cai CP, Wang CB, Han ZG, Song XL, Wang K, Niu XW, Wang C, Lu KY, Shi B, Zhang TZ (2007). A microsatellite-based, gene-rich linkage map reveals genome structure, function and evolution in Gossypium. Genetics.176:527-541.

Hayward AC (1991). Biology and epidemiology of bacterial wilt caused by Pseudomonas solanacearum. Annu. Rev. Phytopathol. 29:65-87.

He LY, Sequeira L, Kelmen A (1983). Characterization of strains of Pseudomonas solanacearum from China. Plant. Dis. 67:1357-1361.

Hyten DL, Smith JR, Frederick RD, Tucker ML, Song Q, Cregan PB (2009). Bulked segregant analysis using the Golden Gate assay to locate the Rpp3 locus that confers resistance to soybean rust in soybean. Crop. Sci. 49:265–271.

Kumar S, Gill BS, Faris JD (2007). Identification and characterization of segregation distortion loci along chromosome 5B in tetraploid wheat. Mol. Genet. Genomics. 278:187-196.

Lebeau A, Gouy M, Daunay MC, Wicker E, Chiroleu F, Prior P, Frary A, Dintinger J (2013). Genetic mapping of a major dominant gene for resistance to Ralstonia solanacearum in eggplant. Theor. Appl. Genet. 126(1):143-158.

Levi A, Thomas E, Joobeur T, Zhang X, Davis A (2002). A genetic linkage map for watermelon derived from a testcross population: (Citrullus lanatus var citroides x C. lanatus var lanatus) x Citrullus colocynthis. Theor. Appl. Genet. 105:555-563.

Levi A, Thomas CE, Trebitsh T, Salman A, King J, Karalius J, Newman M, Reddy OUK, Xu Y, Zhang X (2006). An extended linkage map for watermelon based on SRAP, AFLP, SSR, ISSR, and RAPD markers. J. Am. Soc. Hortic. Sci. 131:393-402.

Li G, Quiros CF (2001). Sequence-related amplified polymorphism (SRAP) a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica.Theor. Appl. Genet. 103:455-461.

Lin ZX, He DH, Zhang XL, Nie YC, Guo XP, Feng CD, Stewart JM (2005). Linkage map construction and mapping QTLs for cotton fiber quality using SRAP, SSR and RAPD. Plant Breeding, 124:180-187.

Lu JJ, Wang S, Zhao HY, Liu JJ, Wang HZ (2012). Genetic linkage map of EST-SSR and SRAP markers in the endangered Chinese endemic herb Dendrobium (Orchidaceae). Genet. Mol. Res. 11:4654-4667.

Li L, Tacke E, Hofferbert HR, Lübeck J, Strahwald J, Draffehn AM, Walkemeier B, Gebhardt C (2013). Validation of candidate gene markers for marker-assisted selection of potato cultivars with improved tuber quality. Front. Plant Sci. 126(4):1039-52.

Lyttle TW (1991). Segregation distorters. Annual Review of Genetics, 25:551-557.

Martin GB, Williams JG, Tanksley SD (1991). Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci U S A.88:2336-2340.

Michelmore RW, Paran I, Kesseli RV (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis:A rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. USA. 88:9828-9832.

Niu Z, Klindworth DL, Friesen TL, Chao S, Jin Y, Cai X, Xu SS (2011). Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics. 187:1011-21.

Poczai P, Varga I, Laos M, Cseh A, Bell N, Valkonen J P, Hyvönen J (2013). Advances in plant gene-targeted and functional markers: a review. Plant Methods.Published online 2013 February 13. doi: 10.1186/1746-4811-9-6.

Qu DY (1996). Use of unreduced gametes of diploid potato for true potato seed production through 4x-2x crosses. Wageningen:Plant Breeding. p.104.

Ren Y, Li Z, He Z, Wu L, Bai B, Lan C, Wang C, Zhou G, Zhu H, Xia X (2012). QTL mapping of adult-plant resistances to stripe rust and leaf rust in Chinese wheat cultivar Bainong 64.Theor. Appl. Genet. 125:1253-1262.

Saliba-Colombani V, Causse M, Gervais L, Philouze J (2000). Efficiency of RFLP, RAPD and AFLP markers for the construction of an intraspecific map of the tomato genome. Genome, 43:29-40.

Salinas M, Capel C, Alba JM, Mora B, Cuartero J, Fernández-Muñoz R,

Lozano R, Capel J (2013). Genetic mapping of two QTL from the wild tomato Solanum pimpinellifolium L controlling resistance against two-spotted spider mite (Tetranychus urticae Koch). Theor. Appl. Genet. 126:83-92.

Valdez-Ojeda R, JL Hernández-Stefanoni M, Aguilar-Espinosa R, Rivera-Madrid R, Quiros CF (2008). Assessing morphological and genetic variation in annatto (Bixa orellana L.) by sequence-related amplified polymorphism and cluster analysis. Hort. Sci, 43:2013-2017.

Van Ooijen JW,Voorrips RE (2001). JoinMap Version3.0: Software for the Calculation of Genetic Linkage Maps. Wageningen: Plant Res. Intl.

Virupaksh U, Patil J, Gopal B, Singh P (2012). Improvement for Bacterial Wilt Resistance in Potato By Conventional and Biotechnological Approaches. Agric. Res. 1(4):299–316.

Weber CA, Moore GA, Deng ZN, Gmitterr FG (2003). Mapping freeze tolerance quantitative trait loci in a Citrus grandis × Poncirus trifoliata F1 pseudo-testcross using molecular markers. J. Am. Soc. Hortic. Sci. 128:508-514.

Yu JW, Yu SX, Lu CR, Wang W, Fan SL, Song MZ, Lin ZX, Zhang XL, Zhang JF (2007). High-density linkage map of cultivated allotetraploid cotton based on SSR, TRAP, SRAP and AFLP markers. J. Integr. Plant. Biol. 49:716-724.

Zhang S, Gao M, Zaitlin D (2012). Molecular linkage mapping and marker-trait associations with nlrpt, a downy mildew resistance gene in Nicotiana langsdorffii. Frontiers in Plant. Sci. 3:1-16.

Zhao N, Zhai H, Yu XX, Liu ZS, He SZ, Li Q, Ma DF, Liu QC (2012). Development of SRAP markers linked to a gene for stem nematode resistance in sweet potato, *Ipomoea batatas* (L) Lam. J. Integr. Agri. 12:414-419.