INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

BIOTECHNOLOGICAL METHODS IN SOYBEAN BREEDING

by

Venancio Arahana

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Agronomy

Under the Supervision of Professor George Graef

Lincoln, Nebraska

August, 2001

UMI Number: 3022617

UMI Microform 3022617

Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

DISSERTATION TITLE

Biotechnological Methods in Soybean Breeding

BY

Venancio Arahana

SUPERVISORY COMMITTEE:

	APPROVED	DATE
Signature	George L Graef	31 August 2001
Typed Name	Dr. George Graef	
Signature	James E. Specht	31 August 2001
Typed Name	Dr. James Specht	
Signature	Paul Staswick	31 August 2001
Typed Name	Dr. Paul Staswick	
Signature	James R. Steadman	31 Aug '01
Typed Name	Dr. James Steadman	
Signature		31 Aug '01
Typed Name	Dr. Tom Clemente	
Signature		
Typed Name		

GRADUATE COLLEGE
UNIVERSITY OF NEBRASKA

BIOTECHNOLOGICAL METHODS IN SOYBEAN BREEDING

Venancio Arahana, Ph.D.

University of Nebraska, 2001

Advisor: George Graef

Simple sequence repeat (SSR) markers were used to identify possible ancestors of current soybean cultivars or select individuals carrying a particular marker genotype in a backcross program. The use of transgenes to enhance resistance to *Sclerotinia sclerotiorum* in soybean was also investigated. Finally, the effects of genetic transformation in the seed storage protein profile of transgenic soybeans were evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Nineteen SSR markers putatively linked to quantitative trait loci (QTL) for resistance to *S. sclerotiorum* had been identified in soybean cultivars 'Corsoy 79', 'Dassel', 'Vinton 81', 'DSR173' and 'NKS19-90'. We used those linked markers to trace the origin of the marker alleles and QTL alleles in a pedigree study. In three cases the most likely source(s) of marker allele and QTL was determined. The favorable Satt424 marker allele on linkage group A2 in Corsoy 79 probably originated from Mandarin, Satt114 on linkage group F in NKS19-90 and Corsoy 79 most likely came from PI257.435, and Sat_109-Satt243 flanking a QTL on linkage group O in NKS19-90 and Vinton 81 probably originated from Mandarin or Mandarin Ottawa.

Markers Satt114, Sat_109 and Satt243 were employed for tracking the QTL linked to them in a marker assisted introgression program. The donor parents NKS19-90 and Vinton 81 were backcrossed to 'Williams 82'. Successful introgression of the QTL

from Vinton 81 was achieved since backcross progenies carrying marker alleles Sat_109 and Satt243 from Vinton 81 were more resistant than Williams 82. Introgression of QTL from NKS19-90 was prevented by recombination between marker allele and QTL and the reduced number of individuals recovered in each backcross generation.

Transferring the oxalyl-CoA decarboxylase gene from *Oxalobacter formigenes* into NKS19-90 and Vinton 81 did not improve resistance to the pathogen in these cultivars. It is hypothesized that a second gene of the oxalate degradation pathway is required.

Quantitative differences in the amounts of α subunits of β-conglycinin were detected between transgenic high-oleic soybeans, transformed with the FAD2-1 and Fat B genes under control of β-conglycinin promoter, and their wild type counterparts.

ACKNOWLEDGEMENTS

My indebted appreciation goes to my advisor Dr. George Graef for his constant guidance, encouragement and support throughout my graduate program. He not only provided me with academic advice but also with the financial support in the form of an assistantship, without which the completion of my studies would not have been possible.

My sincere gratitude goes to my committee: Drs. James Specht, James Steadman Paul Staswick and Thomas Clemente. They always had a helpful hand either by giving me advice, or allowing me to use the resources of their labs in specific stages of my research. Their comments and suggestions to improve the manuscript of this dissertation are also greatly appreciated.

My recognition also goes to Drs. Gautan Sarath and Nancy Caceres for their contribution to the fourth chapter of this dissertation. Also a big thanks to Ping who taught me the key points of protein manipulation in the lab.

An enormous thanks is for Becky Higgins and Kris Powers who helped with all the zillions of detached leaf assays I had to run. Beyond that they also gave me friendship.

My friends, mostly fellow graduate students, played an important role in maintaining my emotional balance. A special mention deserve Patricia Hain and Chestnut Pearl who permanently strove for making me feel at home. They gave me time, attention and support, and made me understand the real meaning of friendship.

The final thanks are for my family, my government, and other persons whose names are involuntarily omitted and yet I owe profound gratitude.

Venancio

TABLE OF CONTENTS

Page

GENERAL INTRODUCTION

Genetic improvement of soybean has depended upon genes available within the species *Glycine max* (L.) Merr (Palmer et al., 1996). Sexual hybridization has been used to recombine these genes, and elite genotypes have been identified. By using this approach an average genetic improvement of 12.5 kg/ha per year has been achieved in soybean as measured over 137 cultivars of hybrid origin released between 1939 and 1977 (Specht and Williams, 1984). Application of biotechnological methods to soybean breeding offers the prospect of accessing new sources of genetic variation and selection could be practiced based on the genotype as well as on the phenotype (Specht and Graef, 1996).

Biotechnology can help plant breeders in the creation of genetic variation by overcoming the barriers imposed by sexual incompatibilities. Tissue culture-based techniques such as embryo rescue, somatic hybridization and somaclonal variation have proven useful for this application in several plant species (Rodrigues et al., 2000; Grosser et al., 2000; Graybosch et al., 1987), however, the most promising approach seems to be plant transformation.

Plant transformation is a powerful tool for creating genetic variation by allowing the introduction of genes derived from unrelated plant species and even from other kingdoms into breeding programs (De Block, 1993). The potential of plant transformation in the creation of genetic variation is limited only by the availability of genes cloned and characterized and the existence of a suitable regeneration method for the species of interest (Specht and Graef, 1996).

The most commonly used method of plant transformation is via *Agrobacterium tumefasciens*. A regenerable explant is inoculated with the bacterium and cultured in an appropriate medium. The bacterium transfers the genetic material into a cell and integration of the gene takes place mediated by the Ti plasmid encoded information. By using this system Hinchee et al (1988) recovered soybean plantlets glyphosate tolerant and GUS positive starting with cotyledonary nodes from cultivar Pekin as explants. Di et al (1996) transformed soybean cv. Fayette with bean pod mottle virus (BPMV) coat protein precursor gene regenerating individuals completely resistant to the virus. Alteration of the soybean seed-oil composition is being pursued by antisense and cosupression technologies via *Agrobacterium* (Kinney et al., 2001; Buhr et al., 2001).

A second method of plant transformation consists of the delivery of alien DNA into host cells via bombardment of callus, shoot meristematic tissue or cell suspensions with small particles of gold or tungsten coated with the DNA construct (Finer et al., 1996). Several genes have been integrated into the soybean genome by this approach. Stewart et al. (1996) transformed soybean with a synthetic *Bacillus thuringiensis* cryIAc gene for resistance to insects. Maughan et al. (1999) expressed the bovine casein gene in soybean via biolistic DNA insertion. The most celebrated success in soybean transformation via bombardment was the generation of soybeans resistant to Glyphosate (Padgette et al., 1995).

Biotechnology also can help plant breeding in the process of selection. Use of molecular markers can accelerate introgression of qualitative as well as quantitative traits. Identification of molecular markers closely linked to or preferentially flanking the gene of interest would allow selection to be carried out based on the molecular marker

genotype (Paterson et al., 1991; Tanksley et al., 1989). The testing of progeny could be done at an early stage without the need to grow all plants to maturity and infection tests would be needed less frequently, leading to a more efficient selection process and speeding up the production of new cultivars (Simpson, 1999).

If a molecular map is available not only the tracking of the trait of interest during introgression is possible but also the monitoring of the whole genome of individuals, allowing for a more precise selection of the best individuals for continued breeding (Young and Tanksley, 1989a; Simpson, 1999). The result should be reducing the time needed to generate new cultivars and the efficient elimination of most of the donor parent genome around the gene of interest (Young and Tanksley, 1989b).

High-density molecular maps may enable complex genetic traits to be dissected in their Mendelian components (Lander and Botstein, 1989). Since in most of quantitative traits studied with molecular markers only relatively few genomic regions seem to account individually for a large proportion of the phenotype, the possibility of its use in marker assisted selection (MAS) programs is promising. High-density maps can also help in cloning the gene of interest.

Another application of molecular markers is in germplasm characterization by DNA fingerprinting (Simpson, 1999). Fingerprints are useful in discriminating between cultivars and closely related lines and also in determining the relationships between new accessions and those already present in germplasm banks. This can lead to a more efficient management of collections by eliminating duplicates or overlapping accessions. It has also legal implications in protection of the breeder's rights. DNA fingerprinting of parents can be helpful for predicting the performance of progeny as well (Jung, 2000).

This dissertation deals with the application of various biotechnological methods in soybean breeding. It has been divided in four chapters:

1. Tracing the origin of QTLs for resistance to *Sclerotinia sclerotiorum* in soybean ancestral lines.
2. Marker assisted introgression of QTL for resistance to *Sclerotinia sclerotiorum* in soybean.
3. Pyramiding QTL and transgenes to enhance resistance to *Sclerotinia sclerotiorum*.
4. Assessing changes in the seed protein profile of soybeans transformed with gene constructs containing the FAD2-1 or FAD2-1 + Fat B genes for fatty acid modification. The events are under the control of either β-phaseolin or β-congllycinin promoter.

REFERENCES

Buhr, T., S. Sato, F. Ebrahim, A. Xing, Y. Zhou, M. Mathiesen, B. Schweiger, A. Kinney, P. Staswick and T. Clemente. 2001. Nuclear-localized RNA transcripts efficiently down-regulate seed fatty acid genes in transgenic soybean. The Plant Cell. In press.

De Block, M. 1993. The cell Biology of plant transformation: current state, problems, prospects and the implications for the plant breeding. Euphytica 71:1-14.

Di, R., V. Purcell, G. B. Collins and S.A. Ghabriel. 1996. Production of transgenic soybean lines expressing the bean pod mottle virus coat protein precursor gene. Plant Cell Reports 15:746-750.

Finner, J.J., T.S. Cheng and D.P.S. Verma. 1996. Soybean transformation: technologies and progress. In: Soybean: genetics, molecular biology and biotechnology. D.P.S. Verma and R.C. Shoemaker (Eds.). CAB International. Pp. 249-262.

Graybosch, R.A., M.E.Edge and X. Delannay. 1987. Somaclonal variation in soybean plants regenerated from the cotyledonary node tissue culture system. Crop Sci. 27:803-806.

Grosser, J.W., P. Ollitrault and F.O. Olivares. 2000. Somatic hybridization in citrus: An effective tool to facilitate variety improvement. In Vitro Cellular and Developmental Biology Plant, 36:434-449.

Hinchee, M.A.W., D.V. Connor-Ward, C.A. Newell, R.E. McDonell, S.J. Sato, C.S. Gasser, D.A. Fishchhoff, D.B. Re, R.T. Fraley and R.B. Horsch. 1988. Production of transgenic soybean plants using Agrobacterium mediated DNA transfer. Bio/Technology 6:915-922.

Jung C. 2000. Molecular tools for plant breeding. In: Agricultural biotechnology in developing countries: towards optimizing the benefits for the poor. M. Qaim, A. F. Krattiger and J. von Braun (eds.). Kluwer Academic Publishers. Boston, pp. 25-37.

Kinney, A.J., R. Jung, and E.M. Herman. 2001. Cosuppression of the α subunits of β-conglycinin in transgenic soybean seeds induces the formation of endoplamic reticulum-derived protein bodies. The Plant Cell, 13: 1165-1178.

Lander, E.S. and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Maughan, P.J., R. Philip, M.J. Cho, J.M. Widholm, and L.O. Vodkin. 1999. Biolistic transformation, expression and inheritance of bovine beta-casein in soybean (Glycine max). In vitro Cellular and Developmental Biology Plant. 35:344-349.

Padgette, S.R., K.H. Kolacz, X. Delannay, D.B. Re, B.J. LaVallee, C.N. Tinius, W.K. Rhodes, Y.I. Otero, G.F. Barry, D.A. Eichholtz, V.M. Peschke, D.L. Nida, N.B. Taylor, and G.M.Kishore. 1995. Devlopment, Identification, and Chracterization of a Glyphosate-Tolerant Soybean Line. Crop Science. 135:1451-1461.

Palmer, R.G., T. Hymowitz and R.L. Nelson. 1996. Germplasm diversity within soybean. In: Soybean: Genetics, molecular biology and biotechnology. D.P.S. Verma and R.C. Shoemaker (eds.). CAB International. Pp. 1-35.

Paterson, A.H., S.D. Tanksley and E. Sorrells. 1991. DNA markers in plant improvement. Adv. in Agron. 46:40-90.

Rodrigues, O.B.M., M.I. de O. Penteado, C.B. do Valle. 2000. Embryo rescue of interspecific hybrids of Brachiaria spp. Plant Cell, Tissue and Organ Culture. 61:175-182.

Simpson, J. 1999. Molecular markers for crop improvement. In: Molecular biotechnology for plant food production. O. Paredes-Lopez (ed.). Technomic. Lancaster. Pp. 275-301.

Specht, J.E. and J.H.Williams. 1984. Contribution of genetic technology to soybean productivity: retrospect and prospect. In: Fehr, W.R. (ed.) Genetic contributions to yield gains of five major crop plants. CSSA Secial publication No. 7, Crop Science Society of America, Madison, Wisconsin, USA, pp. 49-74.

Specht, J.E., and G.L.Graef. 1996. Limitations and potentials of genetic manipulation of soybean. In:Soybean: Genetics, molecular biology and biotechnology. D.P.S. Verma and R.C. Shoemaker (eds.). CAB International. Pp. 91-106.

Stewart C.N. Jr., M.J. Adang, J.N. All, H.R. Boerma, G. Cardineau, D. Tucker, and W.A. Parrot. 1996. Genetic transformation, recovery and characterization of fertile soybean transgenic for a synthetic Bacillus thuringiensis CryIAc gene. Plant Physiology 112:121-129.

Tanksley, S.D., N.D: Young, A.H. Paterson and M.W. Bonierbale. 1989. RFLP mapping in plant breeding: new tools for an old science. Biotechnology 7:257-263.

Young N.D. and S.D. Tanksley, 1989a. Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor. Appl.Genet. 77:95-101.

Young, N.D. and S.D. Tanksley. 1989b. RFLP analysis of size of chromosomal segments retained around the tm-2 locus of tomato during backcross breeding. Theor. Appl. Genet. 77:353-359.

Tracing the Origin of QTLs for Resistance to *Sclerotinia sclerotiorum* In Soybean Ancestral Lines.

Venancio S. Arahana, George L. Graef*, James E. Specht, and James R. Steadman

V. S. Arahana, G. L. Graef, J. E. Specht, Dept. of Agronomy, Univ. of Nebraska, Lincoln, NE 68583-0915; and J. R. Steadman, Dept. of Plant Pathology. Published as Paper No. _____, Journal Series, Nebraska Agric. Res. Div. Project No. 12-255. Research supported by state and federal funds appropriated to the Agricultural Research Division and the University of Nebraska, and by grants received from the Nebraska Soybean Board. Received _____

*Corresponding author (E-mail ggraef1@unl.edu)

ABSTRACT

Nineteen Simple Sequence Repeat (SSR) markers, putatively linked to quantitative trait loci (QTLs) for sclerotinia stem rot resistance, were used to screen 45 soybean lines in a pedigree analysis. The objectives were (1) to trace the origin of the SSR alleles linked to the QTL alleles previously mapped to seven different linkage groups, in five soybean cultivars (Corsoy 79, NKS19-90, Vinton 81, Dassel and DSR 173) and (2) to determine if the phenotypes of the pedigree members were consistent with the traced QTL alleles. The 45 soybean lines were also screened for sclerotinia stem rot resistance using an excised leaf assay. A marker- phenotype association was the expected outcome for those lines carrying a particular QTL. For three of the five cultivars, presence of the favorable marker allele was consistently associated with smaller lesion size along the pedigree, thus allowing inference of the origin of the QTL for resistance to *Sclerotinia sclerotiorum* linked to those markers. The traced origin of the three confirmed QTLs was as follows: 1) The QTL linked to marker Satt424 (LG A2) in Corsoy 79 originated from Mandarin, 2) The QTL linked to markers Sat_109 and Satt243 (LG O) inVinton 81, originated from Mandarin Ottawa, and 3) The QTL linked to marker Satt114 (LG F) in NKS19-90, originated from PI 257.435. For the Dassel and DSR173 pedigrees, the marker allele associated with smaller lesion size in the cultivar was not consistently associated with smaller lesion size through the pedigree. This could be the result of a type I error in identifying the putative QTL, or there could have been recombination between the QTL and the marker locus. These results verify the previously identified QTLs, and show that QTLs with even relatively small effects on phenotype can

be traced through ancestral pedigrees or future crosses to facilitate development of resistant cultivars.

INTRODUCTION

Identifying resistance to *Sclerotinia sclerotiorum* in soybean has proven to be a difficult endeavor. Despite multiple screening studies, only partial resistance to *S. sclerotiorum* in soybean has been described (Kim et al., 1999, Boland and Hall, 1987). The inheritance of this partial resistance appears to be multilocus and complex (Kim and Diers, 2000). Using molecular markers, putative quantitative trait loci (QTLs) for resistance to this pathogen have been reported (Delaney et al., 1997, Kim and Diers, 2000, Arahana et al., 2001). Most of these QTL have yet to be confirmed.

Tracing the origin of a putative QTL allele back to its ancestral line source with molecular markers represents a form of QTL verification. Ancestors pass on blocks of their genome to derived cultivars, and these blocks can be tracked via the molecular marker loci that reside in those blocks. A marker-QTL association implies linkage. Since a marker allele-QTL allele phase, found in ancestral lines, tends to persist over meiotic events, the linkage phase may be retained in the derived cultivar, depending on degree of linkage. If a cultivar does not receive both the marker allele and its associated QTL phenotype it is assumed to be a recombinant cultivar (Lorenzen et al., 1995). In a previous study, several markers were found to be closely linked to putative QTLs (Arahana et al., 2001). The objective of this study was to track marker allele-QTL allele linkage phase present in a given cultivar back to the probable source ancestor. This was accomplished by evaluating the marker allele status and QTL phenotypes of all available cultivars in each pedigree.

MATERIALS AND METHODS:

Plant Material:

A total of 45 soybean cutivars, known to be members of the pedigrees of 'Corsoy 79', 'Vinton 81', 'Dassel', 'NKS19-90', and 'DSR173' (Bernard et al., 1988; Allen and Bhardwaj, 1987; Novartis Seeds, Inc., Mineapolis, MN; Dairyland Seed Co., Inc, West Bend, WI), were included in this study (Table 1 and Fig.1-3). All 45 cultivars were seeded on two different dates, into pots placed on benches in a greenhouse.

Screening for resistance to *S. sclerotiorum*:

Each 12-inch pot was thinned to four plants of a given cultivar. The experimental design for the detached leaf assay in the laboratory was an alpha lattice with each cultivar replicated four times per planting date, for a total of eight replications. In each replication, the 45 soybean cultivars were randomized to 12 pans, four leaves per pan. Pans were considered incomplete blocks. The experimental unit was one leaf excised from a single plant of each cultivar.

Plants at the V4 developmental stage (Fehr and Caviness, 1977) were used. The uppermost fully expanded trifoliolate leaf was removed from each plant and inoculated using an agar plug with isolate 143 of *Sclerotinia sclerotiorum,* following the protocol for a detached leaf assay as previously described (Arahana et al., 2001).

An initial screening of soybean lines was conducted using *S. sclerotiorum* isolate number 143 from soybean (J. Steadman, unpublished). After selection of parents for the QTL identification study (Arahana et al., 2001) based on their consistent partial resistance (smaller lesion size) rankings, a screen of the parental cultivars was conducted comparing reaction using two isolates from soybean. These isolates were selected from a

group of about 20 soybean isolates based on their virulence on Corsoy 79, and represent isolates with moderate and high virulence (Steadman et al., 1998). In addition, a screen of 100 recombinant imbred lines (RILs) from the Corsoy 79 x Williams 82 population was conducted with both isolates. No crossover interaction in reaction to the two isolates were observed, and isolate 143 was chosen because it generated a greater difference in lesion size in comparisons of the resistant parents versus the susceptible Williams 82 unpublished data).

SSR Marker Screening:

The 19 SSR markers identified by Arahana et al. (2001) putatively linked to QTLs for resistance to *Sclerotinia sclerotiorum* in soybean were used to trace the origin of the two marker alleles defining each SSR locus, back to their ancestral cultivars. DNA was extracted from leaves of each cultivar following the mini-extraction CTAB procedure (Saghai-Maroof et al., 1984). DNA amplification was performed using a PTC-100 thermocycler. The thermocycler profile and PCR reaction cocktail were as described previously (Arahana et al., 2001).

Data Analysis:

LSMEANS for lesion area were derived for each cultivar using the PROC MIXED procedure of SAS (SAS Institute, Inc., 1989), with PAN(REP) treated as a random effect. For each SSR marker, the association between lesion size phenotype and given SSR locus was determined by calculating single degree-of-freedom (i.e., allelic) contrasts between cultivars homozygous for an allele at a given SSR and those homozygous for the other allele. The origin of a QTL was ascribed to a particular

ancestor by determining if the marker allele in linkage phase with smaller lesion size was discernible in each parental generation at the pedigree.

RESULTS AND DISCUSSION:

Tracing the ancestral origin of a QTL using molecular markers can be a feasible task if (1) the marker is tightly linked to the QTL, (2) the pedigree of the cultivar bearing the favorable marker allele is complete and error-free, and (3) the magnitude of phenotypic variation explained by an individual QTL is large enough to allow the favorable allele to be traced in each step of the pedigree. To declare a particular cultivar as the ancestral source of a QTL, the association of the marker allele with the QTL allele must be consistently detected in the trace from the recipient to its presumed donor cultivar in a pedigree. Tracing the ancestral source of a QTL can serve as a form of QTL verification after a preliminary QTL discovery. We were able to show consistency in linkage phase between marker alleles and putative QTL alleles previously identified in analysis of recombinant inbred line populations and deduce their probable origin by pedigree analysis.

The results of ten SSR loci marking seven putative QTL on four linkage groups are reported in this study (Table 2). The QTLs for resistance to *S. sclerotiorum* detected on linkage groups A2 (Satt424), F (Satt114), and O (Satt243-Sat_109, Satt477 and Satt478) that were identified in a previous study (Arahana et al., 2001) also showed a significant association between marker allele and resistant phenotype in this pedigree analysis (Table 2). Other marker-QTL associations on linkae group D1a (Satt147-Satt129) and F (Satt510-Satt335) were not significant in this pedigree analysis. These putative QTLs may have been type I errors in the previous study.

MLG A2 (Satt424): This QTL exhibited an association between marker genotype and lesion size phenotype in the Corsoy 79 pedigree that could be traced back to Corsoy, Harosoy, then to Mandarin (Fig.1a). Thus, Mandarin is the probable source of the QTL allele for resistance to sclerotinia stem rot in Corsoy 79.

MLG F (Satt114): This QTL had previously been mapped to Linkage Group F with resistance alleles coming from cultivars NKS19-90 and Corsoy 79 (Arahana et al., 2001). The marker-allele trace in the NKS19-90 pedigree is not as clear as it is in the Corsoy 79 pedigree (Figs.1b and 2a). The relationship between lesion size and Satt114 genotype in the members of this pedigree was not consistent. This could be attributed to this QTL with small effects plus the non-genetic variation associated with the phenotypic screening for resistance. Recombination events between marker and QTL can also contribute to obscure the resistance response. In addition, some breeding lines present in the pedigree were no longer available for analysis in this study. The data indicate that PI 257435 or Mukden may be a source of the marker and QTL allele in NKS19-90. However, NKS1346 and B152 in this pedigree showed larger lesion sizes, but carried the (+) marker allele. Another possibility is that the QTL resistance allele in NKS19-90 comes from Adams (lesion size of 2.59 cm^2), via the L57-0034 and L66L-137 breeding lines and Pella. Recombination between the marker allele and QTL allele in the B152 x Pella cross may have resulted in the genotype and phenotype that is seen in NKS19-90.

For the Corsoy 79 pedigree, the association between marker and phenotype is more consistent (Figure. 1b). The origin of this QTL on LG F could be Mandarin, but Capital, and A.K. Harrow are also possible sources.