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Introgression of Novel Alleles for Partial Resistance to Big Vein Disease from *Lactuca virosa* into Cultivated Lettuce

Ryan J. Hayes[1] and Edward J. Ryder

United States Department of Agriculture, Agricultural Research Service, Crop Improvement and Protection Unit, 1636 E. Alisal Street, Salinas, CA 93905

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Abstract. **Big vein is an economically damaging disease of lettuce (*Lactuca sativa* L.) incited by Mirafiori lettuce big vein virus, which is vectored by the soil-borne fungus *Olpidium brassicae* (Woronin) P.A. Dang. Resistance to this disease is needed because no feasible cultural control methods have been identified. Partial resistance is available within cultivated lettuce and is expressed as delayed appearance of symptoms in combination with a reduced percentage of symptomatic plants. Complete resistance has been identified only in accessions of *L. virosa* L., an incongruent wild relative of lettuce. Resistance from *L. virosa* has not been introgressed into lettuce. The objective of this research was to determine whether big vein resistance from *L. virosa* can be introgressed into lettuce. Progenies of backcross (BC) hybrids between *L. virosa* and *L. sativa* cultivars were greenhouse tested for big vein resistance over four generations of self-pollination. Selected plants from resistant BC families were used as parents to create BC_2 progeny from crosses with high partial-resistant cultivars, intermediate partial-resistant cultivars, and susceptible cultivars to test for the presence of transgressive segregants. Experiments were conducted in the greenhouse by infecting seedlings with *O. brassicae* zoospores collected from big vein symptomatic plants. Plots were evaluated for area under the disease progress curve and the percentage of symptomatic plants; asymptomatic plants from resistant families were retained in every generation. Complete resistance to big vein was not recovered, and may be the result of insufficient sampling of BCF_2 progeny or linkage between resistance alleles and alleles causing incongruity. Variation for partial resistance was observed in all BC generations, and transgressive segregants were identified among BC_2 families from crosses using partially resistant and susceptible parents. This research suggests that *L. virosa* contains alleles that confer partial resistance to big vein when introgressed into an *L. sativa* background, and these alleles are distinct from those present in partially resistant lettuce cultivars. Alternative breeding strategies should be pursued to introgress complete resistance from *L. virosa* into cultivated lettuce.**

Big vein is an economically damaging disease of lettuce (*Lactuca sativa* L.). Symptoms include chlorosis surrounding the vascular bundles and leaf stiffening that can disrupt normal head development, resulting in a reduced frequency of marketable heads. Big vein is caused by a virus, vectored by the soil-borne fungus *Olpidium brassicae* (Woronin) P.A. Dang (Jagger and Chandler, 1934). The disease-causing virus is reported to be Mirafiori Lettuce Big Vein Virus (MLBVV) (Lot et al., 2002; Roggero et al., 2003a). Big vein is most prevalent in cool wet soils (Campbell and Grogan, 1963; Westerlund et al., 1978a, b), and increases with continuous lettuce production without rotation. Consequently, big vein consistently occurs at high levels during spring production in California's coastal growing districts, and during winter production in Arizona.

Controlling big vein is important for sustaining production of quality lettuce in California and Arizona, where 99% of U.S. lettuce production occurs (Anonymous, 2006). No economically viable methods of cultural control are currently available. Therefore, effective long-term control of big vein disease is best accomplished through genetic resistance. Within cultivated lettuce, partially resistant cultivars are available that have a reduced frequency of symptomatic plants or symptom expression that is delayed until plants reach market maturity (Ryder and Robinson, 1995). Cultivars with this type of resistance have improved marketable yields in fields infested with big vein (Ryder 1979). However, progress in increasing the level of partial resistance or the development of complete resistance has been slow. Wild *Lactuca* species represent an important source of useful alleles that could be used to achieve higher levels of resistance to big vein. Among the wild relatives of lettuce, complete resistance to big vein has only been identified in accessions of *L. virosa* L. (Bos and Huijberts, 1990; Hayes et al., 2006), but this resistance has not been introgressed into lettuce cultivars. Although *L. sativa* and *L. virosa* are both 2n = 2x = 18, introgression of *L. virosa* genes is complicated by extreme sterility in the hybrid progeny. *L. virosa* is evolutionarily divergent from *L. sativa* (Koopman et al., 1998, 2001), and introgression has required bridge crosses with *L. serriola*, colchicine doubling, or embryo rescue (Eenink et al., 1982; Maisonneuve et al., 1995; Thompson and Ryder, 1961). The objective of this research was to determine whether big vein resistance from *L. virosa* can be introgressed into cultivated lettuce.

Materials and Methods

Backcross (BC) *L. virosa* × *L. sativa* hybrids generated from embryo rescue were provided by B. Maisonneuve (INRA, Montfavet, France) for these experiments. The term backcross in this report refers only to crossing *L. virosa* × *L. sativa* hybrid plants of any generation of self-pollination back to any *L. sativa* genotype. The *L. sativa* parent used to create the original interspecific F_1 was not necessarily used in subsequent crosses. The *L. virosa* parent was from accession IVT280, which was reported to be completely resistant to big vein (Bos and Huijberts, 1990; Hayes et al., 2006). The *L. sativa* parents used in crosses to IVT280 and to create the BC generation were the butterhead cultivars Girelle, Melina, and Galore, and the red oak leaf cultivar Cocarde. Every effort was made to include these parents in all tests; however, some experiments did not include a complete set of parents because of limited seed quantity. Starting with 13 unselected BCF_2 families, selection for big vein resistance was conducted through four generations from self-pollination (Table 1). Selection was practiced by retaining seed from the asymptomatic plants of resistant families. The seed from each plant was maintained separately. An exception was made when saving seed from $BCF_{2:3}$ plants; seed from all plants within each selected family was massed to create a $BCF_{2:4}$ generation. Different numbers of replications and control cultivars were used in evaluations of each BC generation. The BCF_2 generation was tested in an unreplicated greenhouse trial with recurrent parents and the high partial-resistant (HPR) cultivar Pavane (Latin) and the susceptible cultivar Great Lakes 65 (iceberg). The $BCF_{2:3}$ families were evaluated with their parents and three replications of 'Pavane' and 'Great Lakes 65'. The $BCF_{2:4}$ generation was tested along with 'Pavane', the HPR cultivar Pacific (iceberg), and 'Great Lakes 65', all having two replications. The $BCF_{4:5}$ lines were tested with three replications along with their parents, 'Pavane', 'Great Lakes 65', and the HPR cultivar Margarita (butterhead). Based

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[1]To whom reprint requests should be addressed; e-mail rhayes@pw.ars.usda.gov

Table 1. Generation, breeding activity, the number tested, and the number retained during selection for big vein resistance in backcross (BC) and BC_2 *L. virosa* × *L. sativa* progeny.

Generation	Breeding activity	No. tested	No. retained
	Selection within BC progeny		
BCF_2	Inoculate and select for resistance	13 families	81 plants from 5 families
$BCF_{2:3}$	Inoculate and select for resistance	81 families	31 families
$BCF_{2:4}$	Inoculate and select for resistance	31 families	21 plants from 5 families
$BCF_{4:5}$	Inoculate and select for resistance	21 families	NA
	Development and selection within BC_2 progeny		
BC_2F_1	Cross selected BC parents to high partial-resistant, intermediate partial-resistant, and susceptible *L. sativa* cultivars	NA	NA
BC_2F_2	Randomly select plants for seed increase	NA	183 plants from 5 families
$BC_2F_{2:3}$	Inoculate and select putative transgressive segregants	183 families	212 plants from 65 families
$BC_2F_{3:4}$	Inoculate and confirm transgressive segregants	212 families	NA

NA, Not applicable.

on the results from evaluating BC progeny, BC_2 families from crosses with two BC *L. virosa* × *L. sativa* hybrid breeding lines were generated for further testing (Table 1). Asymptomatic plants from $BCF_{2:3}$ HPR family 00–366–3 were crossed to susceptible 'Salinas 88' and the HPR cultivars Pacific and Pavane. Asymptomatic plants from the intermediate partial-resistant (IPR) family 00–366–9 was crossed to IPR cultivar Clemente (romaine) and 'Salinas 88'. A random selection of BC_2F_2 plants were grown to seed, and the resulting $BC_2F_{2:3}$ families were tested in an unreplicated greenhouse trial along with *L. sativa* parents, 'Great Lakes 65', and IVT280 to identify putative transgressive segregants. Seed was saved from each asymptomatic plant from families identified as putative transgressive segregant families. The resulting $BC_2F_{3:4}$ families were divided into two equivalent sets by sampling equal numbers of lines derived from each $BC_2F_{2:3}$ family. Each set was then evaluated for big vein resistance in independent greenhouse experiments to confirm the existence of transgressive segregants. These experiments used three replications of each $BC_2F_{3:4}$ family and nine replications of 00–366–3, 00–366–9, 'Pavane', 'Pacific', 'Clemente', 'Salinas 88', and *L. virosa* accession IVT280.

All experiments were conducted using a greenhouse assay (Ryder and Robinson, 1995). Inoculum was produced in the greenhouse by growing big vein symptomatic plants in 15-cm pots containing *O. brassicae*-infested field soil collected from the U.S. Department of Agriculture, Agricultural Research Service research station in Salinas, Calif. The field soil used to grow symptomatic plants was collected from the same location for each experiment, and reverse transcription–polymerase chain reaction of MLBVV isolates obtained from plants grown in this soil were closely related to other MLBVV isolates in California, Arizona, Europe, and Japan (Hayes et al., 2006). At the time of inoculation, a suspension of more than 30,000 *O. brassicae* zoospores/mL was prepared from six symptomatic plants by macerating the roots in water. Seedlings were germinated in potting mix containing 2 field soil : 1 sand and grown for 3 weeks. Inoculations were conducted by watering these seedlings with the zoospore suspension on two separate occasions, each separated by 48 h. Twelve seedlings per plot were subsequently transplanted into 8-cm pots containing field soil, except in experiments with $BC_2F_{3:4}$ families in which eight seedlings were transplanted per plot. Plants were grown in a greenhouse maintained at 18 °C, and the proportion of plants per plot with leaf vein banding typical of big vein disease was recorded every 7 d for up to 8 weeks and was used to calculate the area under the disease progress curve (AUDPC).

Data were analyzed using the PROC Mixed procedure of SAS (2004, version 9.1) with entries (families, control cultivars, parents, and accessions) as a fixed effect and replications as random effects. In experiments in which hybrid families were not replicated, replication of control cultivars and parents was used to estimate the error needed for significance testing. The data from evaluations of $BC_2F_{3:4}$ families had skewed distributions and were analyzed using nonparametric methods. For comparison among parents and among pedigrees, the median AUDPC was calculated for each parent and for each pedigree. The range AUDPC (range = maximum AUDPC – minimum AUDPC) was also calculated for the families within each pedigree to estimate the amount of variation. To identify transgressive segregants, the data were analyzed using analysis of variance-type statistics of ranked data using the PROC Mixed procedure in SAS (2004, version 9.1) (Brunner et al., 2002; Shah and Madden, 2004). The relative marginal effects (RME), a value ranging from 0 to 1, were calculated as the mean rank divided by the number of plots (experimental subjects). The LD_CI macro was used in Proc Mixed to calculate the RME and 95% confidence intervals of RME for detection of statistical difference among entries. To control for increased type 1 error associated with multiple testing, the confidence interval used an alpha of 0.05 divided by the number of statistical comparisons within each pedigree. These comparisons were made only after significant differences were identified by Proc Mixed. The analysis was conducted separately for each experiment, where entries (families, parents, and accession) were fixed effects and replications were random effects. Transgressive segregants were identified as $BC_2F_{3:4}$ families that were significantly more resistant than both parents. The percentage of transgressive segregants was calculated for each pedigree.

Results

Greenhouse evaluation of unselected BCF_2 families identified variation among families for resistance to big vein, with percent symptomatic plants ranging from 9% to 80% (data not shown). No families with complete resistance, as seen in *L. virosa* accession IVT280, were observed. Twenty-five percent of the families had fewer symptomatic plants than the HPR control cultivar Pavane (17% symptomatic), and 83% percent of the families had fewer symptomatic plants then the susceptible control 'Great Lakes 65' (42% symptomatic plants). The three recurrent parents tested—'Girelle', 'Milena', and 'Cocarde'—were all more susceptible than 'Great Lakes 65'.

Variation for AUDPC and percent symptomatic plants was identified in 81 $BCF_{2:3}$ families previously selected for partial resistance (Fig. 1). The AUDPC of families ranged from 1.3 to 76.1, and 88% of the families were not significantly different from 'Pavane' (Fig. 1). *L. virosa* accession IVT280 had no symptomatic plants (Fig. 1). The recurrent parents in this experiment all had more than 83% symptomatic plants and AUDPC values more than 53.5 (Fig. 1). Considering just the families derived from 00–366, it is apparent that many $BCF_{2:3}$ families derived from this BCF_2 family have a high level of partial resistance to big vein. None of these families were significantly different from 'Pavane', and four of five families with lower AUDPCs than 'Pavane' were all derived from 00–366 (Fig. 1). Furthermore, the best-performing family (00–366–3) was derived from BCF_2 family 00–366. Family 00–366 was produced from the cross (IVT280 × 'Cocarde') × 'Galore', and in this experiment 00–366–3 was significantly more resistant than the parent cultivar Cocarde (Fig. 1). The susceptible parent 'Galore' was not included in this experiment.

The AUDPCs in the $BCF_{2:4}$ generation ranged from 2.3 to 39.5 (data not shown). Although the differences in the AUDPC were not significant for this experiment, the most resistant family (02–2367) was created from a mass seed lot of asymptomatic plants from 00–366–3. Despite the lack of significant differences in the $BCF_{2:4}$ generation, 21 $BCF_{2:4}$ plants were selected from the five best-performing families for further testing in

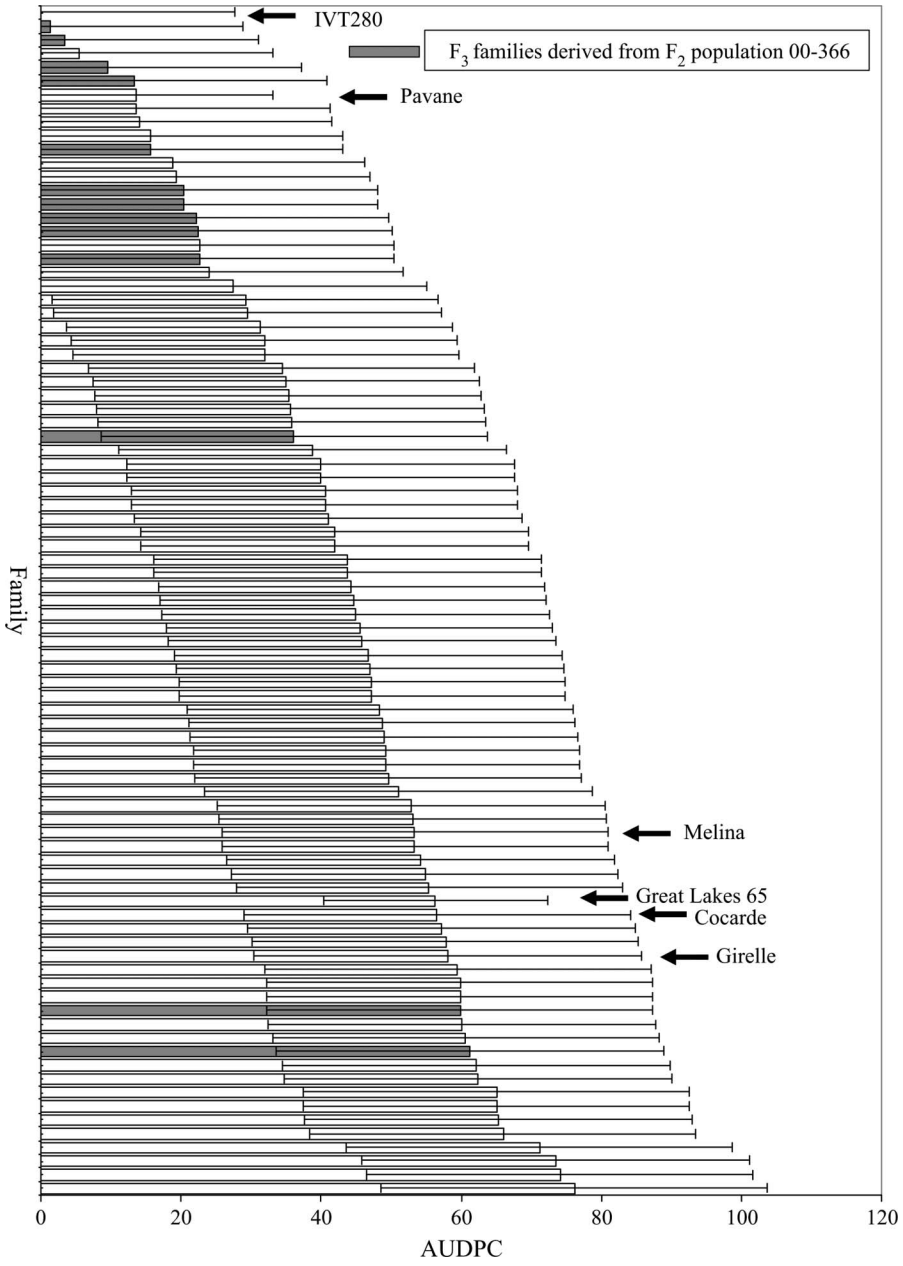

Fig. 1. Variation for big vein area under the disease progress curve (AUDPC) for 81 $BCF_{2:3}$ *L. virosa* × *L. sativa* hybrid families; *L. virosa* parent accession IVT280; *L. sativa* parent cultivars Melina, Cocarde, and Girelle; and the *L. sativa* control cultivars Pavane and Great Lakes 65 in a greenhouse experiment. Error bars indicate 95% confidence intervals.

the $BCF_{4:5}$ generation. Of the $BCF_{4:5}$ families tested, family 02–2367–1, a family descended from 00–366–3, had the lowest AUDPC in the experiment (Table 2). Other families derived from 00–366–3 also performed well in this experiment. This includes families 02–2367–2 and –4, which had the lowest and second lowest percentage of symptomatic plants (Table 2). The AUDPC of every line derived from 02–2367 was significantly lower than 'Galore', one of this family's *L. sativa* parents (Table 2). Other lines that can be traced back to BCF_2 family 00–366 also had low levels of disease. This includes the second best-performing family for AUDPC, family 02–2371–4, which is a derivative from 00–366–9 (Table 2).

Variation for AUDPC and the percentage of symptomatic plants was detected among $BC_2F_{2:3}$ families that have 00–366–3 and 00–366–9 as parents (data not shown). The range of AUDPCs of these families in all pedigrees extended to zero, and included many families that had less disease than the *L. sativa* and BC *L. virosa* × *L. sativa* hybrid parents (data not shown). These $BC_2F_{2:3}$ families were considered putative transgressive segregants. The existence of transgressive segregants was confirmed in the $BC_2F_{3:4}$ generation (Table 3). Families from 00–366–3 × 'Pavane' and 00–366–9 × 'Clemente' had transgressive segregants in both experiments (Table 3). Transgressive segregants were identified in 00–366–9 × 'Salinas 88' only in expt. 1 and in 00–366–3 × 'Pacific' only in expt. 2 (Table 3). Variation in the percentage of transgressive segregants was observed, and appears to result from interaction between the parent genotypes and experiment. There does not appear to be a relationship between the percentage of transgressive segregants and a pedigree's median AUDPC or family range. Rather, the percentage of transgressive segregants is more closely related to the amount of disease in the parents with which the $BC_2F_{3:4}$ families were compared. For example, identification of transgressive segregants in expt. 2 in 00–366–3 × 'Pacific' and not in expt. 1 is likely the result of higher amounts of big vein disease in 'Pacific' in expt. 2. No transgressive segregants were found in 00–366–9 × 'Salinas 88', and lower percentages were found in 00–366–9 × 'Clemente' in expt. 2. This can be explained by a far reduced amount of disease in 00–366–9 in expt. 2. Regardless, it seems likely that transgressive segregants are present in these populations, and suggests that the *L. sativa* parents and the *L. virosa* × *L. sativa* hybrid parents differ in alleles for partial resistance to big vein.

Discussion

Big vein is an economically damaging disease of lettuce with limited options for cultural control. Consequently, resistant cultivars are the best approach to reduce the damage resulting from this disease. Partial resistance is available within cultivated lettuce and has been described as a reduction in the number of symptomatic plants or a delay in symptom expression sufficiently past market maturity to reduce economic loss (Ryder and Robinson, 1995). Field and greenhouse testing methods have been developed to select for this form of resistance (Ryder and Robinson, 1995), and cultivars with HPR have been released (Ryder and Robinson, 1995). Although complete resistance from *L. virosa* was not recovered in hybrid progeny, high levels of partial resistance were identified in *L. virosa* × *L. sativa* hybrids. Line 00–366–3 had the least amount of disease in the $BCF_{2:3}$ generation, and gave rise to $BCF_{2:4}$ family 02–2367 and $BCF_{4:5}$ lines 02–2367–1,–2, and –4, which had the least amount of disease or were among the lines with the least amount of disease in their generation. Clearly, 00–366–3 has a high level of partial resistance to big vein. When 00–366–3 was used as a parent in crosses to HPR *L. sativa* cultivars, families significantly more resistant than both parents were identified. Because the resistant *L. virosa* × *L. sativa* hybrid parent used in this cross was developed by backcrossing into susceptible cultivars, the apparent existence of transgressive segregants in crosses to HPR *L. sativa* strongly suggests that *L. virosa* contributed unique alleles that confer partial resistance to big vein. The breeding line 00–366–9 does not appear to be as resistant as 00–366–3, but families that were significantly more resistant than 00–366–9, 'Clemente', and 'Salinas 88' were identified. This indicates that other families derived from 00–366 may be useful for

Table 2. Big vein area under the disease progress curve (AUDPC) and percent symptomatic plants in $BC_2F_{4:5}$ *L. virosa* × *L. sativa* hybrids and cultivars Pavane, Margarita, and Great Lakes 65.

			AUDPC		
				95% CI	
Line	No. of plants tested	Percent symptomatic plants	Mean	Lower	Upper
02–2367–1	34	50	3.9	0.0	9.9
Pavane	70	33	3.9	0.0	8.3
02–2371–4	32	50	4.5	0.0	10.6
02–2377–1	27	37	4.7	0.0	10.8
02–2367–2	34	27	4.8	0.0	10.9
02–2367–4	35	32	5.4	0.0	11.5
02–2371–5	36	64	6.8	0.7	12.9
02–2377–3	34	44	7.2	2.0	12.5
02–2385–5	12	33	7.3	0.0	17.9
02–2377–2	36	50	7.7	1.6	13.8
02–2367–3	35	49	7.8	1.7	13.9
02–2376–1	34	41	8.4	2.4	14.5
02–2376–4	35	57	8.6	2.5	14.7
02–2385–3	2	50	8.8	0.0	19.3
Margarita	58	47	9.0	4.7	13.3
02–2366–2	34	44	9.2	3.1	15.3
02–2366–5	34	44	9.7	3.6	15.8
02–2371–2	36	50	9.8	3.7	15.9
02–2366–1	34	44	11.2	5.1	17.3
02–2377–4	9	67	11.7	1.1	22.2
02–2366–4	33	58	12.1	6.0	18.2
02–2376–2	22	64	12.3	4.9	19.8
02–2366–3	36	61	14.5	8.4	20.6
02–2371–6	11	54	15.6	5.0	26.2
02–2376–3	12	75	19.0	8.4	29.5
Great Lakes 65	72	76	20.5	16.2	24.8
Ravel	11	91	21.6	11.1	32.2
02–2371–1	34	71	22.9	16.8	29.0
02–2371–3	12	75	29.8	19.2	40.3
Galore	12	83	33.8	23.3	44.4
02–2368–1	2	100	38.5	27.9	49.1

CI, confidence interval.

big vein resistance breeding. Furthermore, numerous BC families with distinct pedigrees were selected through four generations of self-pollination, but were not used to generate BC_2 progeny because their level of partial resistance was not as great as 00–366–3. Although the fact that they were more susceptible than 00–366–3 further substantiates the value of 00–366–3, it is likely that several BC lines in addition to 00–366–3 have useful big vein resistance alleles derived from *L. virosa*.

Low frequencies of $BC_2F_{3:4}$ families were identified as transgressive segregants, which were defined as families significantly more resistant than both parents. However, higher numbers of transgressive segregants may exist in the $BC_2F_{3:4}$ populations than were identified using this method. The greenhouse screen used in this research is intended to detect variation for delayed symptom expression or reduced frequency of symptom expression and, to accomplish this, does not maximize disease pressure. The lack of sufficiently high levels of big vein disease pressure may have inhibited our ability to maximize genetic variation, and reduced our ability to detect statistical differences in populations in which parents and families have low levels of disease resulting from high levels of resistance. The $BC_2F_{3:4}$ generation had highly skewed distributions, and it is likely that more genetic variation is available at the resistant end of the distribution. It is possible that more transgressive segregants would be identified if this variation could be exposed. New screens with higher levels of disease pressure are needed, and could be developed by increasing *O. brassicae* concentrations, increasing the duration of *O. brassicae* exposure, and using environments more conducive for virus infection and symptom expression.

The hybrid breeding lines developed in this research will likely be useful for breeding new cultivars with partial resistance to big vein. Current crisphead cultivars that are grown in California and have partial big vein resistance are closely related, and introgression of novel alleles for partial resistance to big vein can be used to increase the current level of resistance. Furthermore, the lettuce industry desires cultivars that combine big vein resistance, high yield, and freedom from physiological defects. In this regard, the BC_2 families from crosses with 'Salinas 88' are particularly useful. 'Salinas 88' is an important parent for breeding new cultivars with high yield and quality, and numerous families with big vein resistance significantly better than 'Salinas 88' were identified. These families are an important resource for developing new big vein-resistant cultivars with a genetically distinct background.

Table 3. Median and range of area under the disease progress curve (AUDPC) for $BC_2F_{3:4}$ *L. virosa* × *L. sativa* hybrids families and their parents and the percentage of transgressive segregants in two greenhouse experiments.

	Expt. 1				Expt. 2			
		AUDPC		Percent		AUDPC		Percent
Entry	No. of families tested	Median	Family range	transgressive segregants[z]	No. of families tested	Median	Family range	transgressive segregants
Families								
High × High								
03–366–3 × Pavane	29	6.6	31.0	4.5	22	2.5	13.1	4.5
03–366–3 × Pacific	16	5.4	29.3	0.0	17	5.7	38.1	5.8
High × Susceptible								
03–366–3 × Salinas 88	12	4.6	28.4	0.0	12	5.5	16.0	0.0
Intermediate × Intermediate								
03–366–9 × Clemente	35	5.7	37.0	25.7	34	5.2	42.5	5.3
Intermediate × Susceptible								
03–366–9 × Salinas 88	18	11.3	29.8	11.1	17	5.3	24.5	0.0
Parents								
IVT280		0.0				0.0		
00–366–3		7.0				7.9		
Pavane		6.0				11.8		
Pacific		4.8				10.1		
Clemente		9.3				15.3		
00–366–9		11.4				1.3		
Salinas 88		22.3				23.9		

[z]Transgressive segregants were detected as families significantly different from both parents using the 95% confidence interval of the relative marginal effect.

Developing immunity to big vein is an important goal for resistance breeding, because this type of resistance can eliminate inoculum increases while decreasing economic loss. Immunity from wild *Lactuca* species would be desirable for this reason (Hayes et al., 2006). Immunity or complete resistance was not recovered in the hybrids evaluated for this research (Fig. 1, Table 2), which may be the result of selection methods that do not identify and eliminate symptomless genotypes from the population. Recently, ELISA and DNA-based detection methods of MLBVV have become available (Hayes et al., 2006; Latham et al., 2004; Navarro et al., 2004; Roggero et al., 2003b). These methods may improve selection efficiency by identifying symptomless virus-containing plants. However, these methods were not available when these experiments were conducted. Additionally, greenhouse testing methods that reduce the number of escapes would be valuable for selecting immune genotypes.

Many useful traits exist in *L. virosa*, and introgression has been conducted through recurrent backcrossing that followed bridge crosses with *L. serriola*, colchicine doubling, or embryo rescue (Eenink et al., 1982: Maisonneuve et al., 1995; Thompson and Ryder, 1961). Successful introgression using recurrent backcross breeding with distant wild relatives is typically limited to introgression of single genes (Haghighi and Ascher, 1988; Mejia–Jimenez et al., 1994). In contrast, efforts to introgress multigene traits frequently fail. The inheritance of big vein resistance is not known in *L. virosa*, and partial resistance in *L. sativa* is presumed to be oligogenic (Ryder, 1980). Although the distribution and relationship among alleles for partial resistance and genes for complete resistance in *L. virosa* are not known at this time, it is possible that several genes of varying effects will need to be introgressed from *L. virosa* for the recovery of complete resistance in hybrid breeding lines. If the small number of original BC families did not represent all genes responsible for the complete resistance of *L. virosa* IVT280, then genetic drift may explain why complete resistance was not recovered in hybrid progeny. Additionally, selection before evaluating for big vein resistance may have eliminated resistance alleles. Introgression may also fail because of linkage between desirable genes and genes that prevent further sexual reproduction, such as those causing death, sterility, or sexual incompatibilities (Haghighi and Ascher, 1988). All these phenotypes were observed in the *L. virosa* × *L. sativa* hybrids used for this research and may also explain why complete resistance from *L. virosa* was not recovered. Alternative hybridization strategies such as congruity backcrossing (Haghighi and Ascher, 1988; Mejia-Jimenez et al., 1994) have been designed to break linkages between introgression barriers and desirable alleles. These approaches should be pursued to increase the number of big vein resistance genes introgressed from *L. virosa* into cultivated lettuce.

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