The genetic background of resistance to common bacterial blight in newly identified common bean lines on the basis of inheritance studies

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Summary: Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (Xcp). is a major disease problem of common bean (*Phaseolus vulgaris* L.). The inheritance of resistance in Xr1 and Xr2 lines to two isolates of Xcp was studied in the F2 and F3 populations from the crosses between these lines and the Masay variety (susceptible to Xcp). Segregation patterns indicated that different single recessive genes presumably in coupling phase linkage determined the resistance to the HUN and EK-11 strains of Xcp in both lines. The presence of some minor, modifying genes beside the monogenic genetic background of resistance was also observed. Xr1 and Xr2 lines represent valuable new monogenic genetic sources in resistance breeding to CBB.

Introduction

One of the most important diseases of common bean (*Phaseolus vulgaris* L.) is common bacterial blight (CBB), incited by *Xanthomonas campestris* pv. *phaseoli* (*Smith*) Dye (*Xcp*). CBB causes economic losses in bean producing regions worldwide, due to a reduction in seed yield and seed quality (*Yoshii*, 1980; *Saettler*, 1989). Investigations in the 1970-s and 1980-s generally proved that the pathogen *Xanthomonas campestris* pv. *phaseoli* is one of the most common stressor on bean in Hungary as well (*Velich* et al., 1994). Effective and ecological control for this disease seems most likely to be achieved through breeding resistant or tolerant cultivars (*Sanders & Schwarz*, 1980).

Resistance to CBB in beans may be determined by both major and minor, modifying genes. The reaction to the pathogen was found to be quantitatively inherited (*Coyne* and *Schuster*, 1974; *McElroy* et al., 1985; *Velich* et al., 1991). This horizontal resistance is poligenically inherited, and exhibits a nonrace-specific reaction to various races or strains (*Opio* et al., 1996), however host genotype x bacterial strain interactions have been reported by several authors (*Aggour* et al., 1989; *Arnaud-Santana* et al., 1993). Generally low to moderate heritabilities of the reactions to *Xcp* were found in different plant organs (*Ariyarathne*, 1994; *Arnaud-Santana* et al., 1994). The number of genes affecting resistance to *Xcp* found in several crosses were different, depending on the source of resistance (*Coyne* and *Schuster*, 1974; *Eskridge & Coyne*, 1996; *Musaana* et al., 1993; *Silva* et al., 1989). Transgressive segregation for higher levels of resistance to the *Xcp* pathogen was observed also in bean (*Valladares-Sanchez* et al., 1979). Differential leaf and pod resistance to *Xcp* (*Aggour* et al., 1989; *Mubaga* et al., 1991) have been reported, unlike to others who observed intermediate association for bean leaf and pod reaction (*Rava* et al., 1987; *Arnaud-Santana* et al., 1994). Germplasm with CBB resistance has been identified in P. vulgaris, and bean varieties and lines resistant or partially resistant to *Xcp* have been developed (*Coyne & Schuster*, 1969, 1974, 1983; *Yoshii* et al, 1978; *Saettler*, 1989). However, despite of extensive research on the development of CBB resistant varieties, high levels of resistance have not been achieved. The lack of high levels of CBB resistance in P. vulgaris germplasm encourages the process to screen Phaseolus species for resistance and to do research on interspecific hybridization.

The highest levels of CBB resistance have been reported in tepary bean, *Phaseolus acutifolius* (*Honma*, 1956; *Schuster* et al., 1983; *Drijfhout & Blok*, 1987). The genom of *P. acutifolius* is a valuable source for improving the level of resistance found in *P. vulgaris* germplasm. Several workers transferred genes from *P. acutifolius* into susceptible *P. vulgaris*, creating a range of bean genotypes with resistance to CBB, such as GN Nebraska #1 Sel. 27 (*Coyne & Schuster*, 1983), XAN lines (*Mc Elroy*, 1985), and navy bean lines (*Scott* and *Michaels*, 1992). *Xcp* resistance gene effects are predominantly additive (*Valladeres* et al., 1979; *Silva* et al., 1989), with dominance or partial dominance sometimes occurring (*Scott & Michaels*, 1988). Although a dominant major gene were reported to primarily determine the reactions to *Xcp* in tepary beans (*Drijfhout & Blok*, 1987) and major genes have been tagged in interspecific crosses

(*Park* et al., 1998), *McElroy* (1985) and later *Freytag* (1989) reported a quantitative pattern of inheritance of resistance in an interspecific cross with three genes involved in controlling resistance to *Xcp*. Accordingly, despite the original qualitative, race-specific resistance of *P. acutifolius* to different isolates of the CBB pathogen (*Zapate & Vidaver*, 1987; *Zaiter* et al., 1989; *Dursun* et al., 1994), the resistance of hybrids remained in most cases race-nonspecific in common bean backgrounds (*Opio* et al., 1996).

An intensive genetic program for bean improvement has been started in the eighties in Hungary. Resistance breeding and genetic research aimed at developing resistance to the most important diseases of hungarian bean production, halo blight (incited by *Pseudomonas syringae* pv. *phaseolicola*) and common bacterial blight. To expand the range of resistance sources 1500 varieties and lines were tested, however a great part of foreign varieties carrying resistance to *Xcp* were susceptible to the aggressive isolates in Hungary. Previous experiments based on a 9 parent diallel trial proved also the polygenic regulation of foliage resistance to *Xcp* (*Velich* et al., 1991). Recently some presumable new, monogenic resistance sources for CBB has been identified in the germplasm of Hungary (*Szarka*, 1993), namely the Xr1 and Xr2 lines. Experiments with Xr1, Xr2 along with the american Walley line suggested the presence of dominant allele(s), and similar genetic background of resistance to *Xcp*. In diallel populations the number of blocks taking part in resistance to *Xcp* were estimated to be 1.8 (F1) and 1.3 (F2), affirming the hypothesis of a single major gene system for CBB resistance affected by modifying genes (*Velich* et al., 1994). The origin of these lines is unknown, only one isozyme study, including european varieties, representatives from the andean and mesoamerican gene pool, a P. acutifolius line and two interspecific hybrids contributed to the identification of the genetic background of Xr1, Xr2 lines. Most isozyme patterns showed relatedness with european varieties, however relationship with P. acutifolius could be observed in two cases as well (*Békefi*, 1997). The objective of this study was to further investigate the genetic basis of resistance to *Xcp* of Xr1 and Xr2 lines, by making a new cross with a european susceptible variety (Masay), and testing an american *Xanthomonas* isolate (EK-11) along with the hungarian. Long-term goals are to tag and map disease resistance gene(s) of both lines controlling resistance to *Xcp*.

Materials and methods

Plant material

Inheritance studies were performed on F2 and F3 progeny of crosses between Xr1, Xr2 lines resistant to *Xcp* and the Masay french snapbean variety susceptible to *Xcp*, resistant to BCMV, anthracnosis and halo blight. Crosses were made by Jim Reiser in 1996, in UNL, Lincoln, NE. Clay pots of parental lines and 72, 85 F2 plants from the crosses Xr1xMasay and Xr2xMasay, respectively, were grown in 1997, and 40-40 randomly selected F3 families (15 plants per F3 family) were grown in 1998 in the greenhouse of UNL, Lincoln, NE. Five plants were planted in each pot in the F3 experiment. Composition of the potting medium was 15% soil, 25% peat, 30% vermiculite, and 30% sand. Plants were fertilized once a week with 200 mg·L-1 20-10-20 (NPK) fertilizer. Greenhouse temperatures ranged between 27±2 °C to 20±2 °C day/night, and the average natural day/night length were about 14/10 hours.

Inoculation. Selection for resistance to *Xcp* was done using two bacterial strains already tested in bean breeding programs of Nebraska or Hungary. The *Xcp* strain EK-11 isolated from common bean in Nebraska was provided by Dr. A.K. Vidaver, Dept. of Plant Pathology, UNL, Lincoln, NE, and strain HUN was provided by *János Szarka,* Hungary. The strains were cultured on MXP medium for 2-3 days, and then transferred to potassium phosphate buffer (pH 7.1), with the dilution to read 0.1 on a spectrophotometer at 640 nm, having 108 CFU/ml final concentration of each strain. The multiple-needle inoculation method (*Andrus*, 1948; *Valladares-Sanchez* et al., 1979) was used to inoculate the second fully expanded trifoliate leaf of each plant. A potassium phosphate buffered solution was used as a control. The disease reaction on the leaves were recorded 14, 17 and 21 days after inoculation. A 1 to 10 leaf reaction rating scale was based on necrosis, water soaking, and yellowed area on the inoculated leaf: 1 is 0-10%, 2 is 11% to 20%, 3 is 21% to 30%, 4 is 31% to 40%, 5 is 41% to 50%, 6 is 51% to 60%, 7 is 61% to 70%, 8 is 71% to 80%, 9 is 81% to 90%, and 10 is 91% to 100% of the inoculated area with symptoms. Plants scored 1 to 2 were considered resistant, and plants scored 3 to 10 were considered susceptible according to the ratings displayed by the resistant and susceptible parents.

Results and discussion

High and similar levels of resistance to both *Xcp* strains were expressed by Xr1 and Xr2 lines (below 10% inoculated leaf area with symptoms) (*Table 1.*). The lines were considered to be homozygous. Masay parent showed high susceptibility to the hungarian isolate (HUN) with almost 100% leaf area with symptoms. The Nebraskan EK-11 strain caused not so severe symptoms on Masay, although still classifying this variety to be susceptible to the isolate EK-11. There were some overlapping of plants in the parental distributions in case of EK-11, however disease ratings for the strain HUN separated them well. A high percentage of the F2 plants displayed parental genotypes. Frequency distributions suggested rather bimodal then continuous distributions, however clear bimodal distributions for disease reactions were not observed in the crosses (*Fig. 1.*). The involvement of one major gene, or few genes regulating resistance to *Xcp* in Xr1 and Xr2 lines could be concluded. Different hypothetical segregation ratios were analyzed

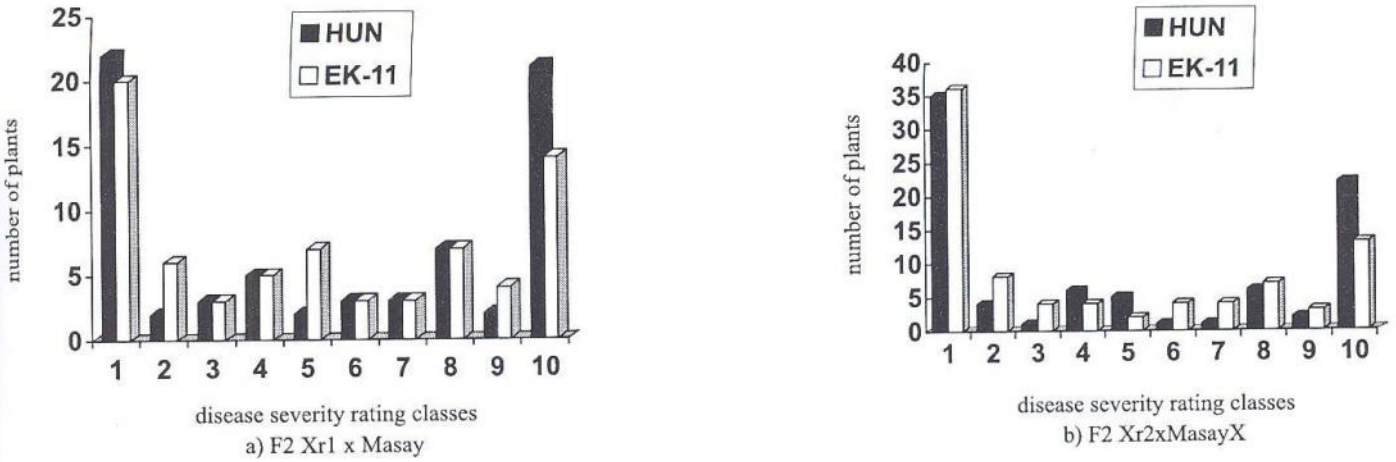

Figure 1 Disease reactions of the F2 populations from the crosses Xr1 x Masay (a) and Xr2 x Masay (b) to the Xcp isolates HUN, and EK-11.

assuming the interaction of few resistance genes (*Table 2.*). A good fit to a 7:9 ratio of resistant to susceptible plants was observed in both F2 populations, for both *Xcp* isolates, indicating that two complementary recessive genes might be involved in determining resistance to the HUN and EK-11 strains. However, in case of the disease reactions of the F2 progeny from the cross Xr1 x Masay, the resistant to susceptible 1:3 ratio could not be rejected as well. It was hypothesized that resistance to *Xcp* could be determined by only one recessive gene. The hypothesis of one recessive gene for resistance to both *Xcp* strains was confirmed in the F3 families of crosses derived from Xr1 x Masay and Xr2 x

Table 1 Frequency distributions of number of plants, mean values (percentage inoculated leaf area with common blight symptoms) and segregation of plants in F2 progenies for reactions to inoculation with two Xcp strains in Xr1 x Masay and Xr2 x Masay crosses.

Parent/ crosses	Strain	Number of plants in disease severity rating classes										Mean (%)	# of plants	
		1	2	3	4	5	6	7	8	9	10		R	S
Xr1 resistant parent	HUN	9		1								4.8	9	1
	EK11	9	1									6.0	10	0
Masay susceptible parent	HUN				5	1		1			4	94.5	0	11
	EK11								1	3	7	64.5	0	11
F2 Xr1xMasay	HUN	22	2	3	5	2	3	3	7	2	21	50.4	24	46
	EK11	20	6	3	5	7	3	3	7	4	14	45.9	26	46
Xr2 resistant parent	HUN	7	1									3.1	8	0
	EK11	8										4.4	8	0
Masay susceptible parent	HUN									1	9	99.0	0	10
	EK11	2	1	1	2	1	1	1		1	1	45.0	3	8
F2 Xr2xMasay	HUN	35	4	1	6	5	1	1	6	2	22	42.5	39	44
	EK11	36	8	4	4	2	4	4	7	3	13	39.1	44	41

Table 2 Chi-square tests for different segregation ratios of resistant and susceptible plants in the F2 population of crosses between Xr1/Xr2 lines (resistant to Xcp) and Masay (susceptible to Xcp) for reactions to two isolates of Xcp.

	Number of plants					
Population	Xcp strain	Observed R:S	Expected R:S	Expected ratio R:S	χ^2	P
F2 Xr1 x Masay	HUN	24:46	17.5:52.5	1:3	3.22	0.1-0.05
			30.6:39.4	7:9	2.53	0.25-0.1
			39.4:30.6	9:7	13.83	<0.001
	EK-11	26:46	18:54	1:3	4.74	0.05-0.025
			31.5:40.5	7:9	1.71	0.25-0.1
			40.5:31.5	9:7	11.86	<0.001
F2 Xr2 x Masay	HUN	39:44	20.7:62.3	1:3	21.8	<0.001
			36:47	7:9	0.44	0.75-0.5
			47:37	9:7	3.14	0.1-0.05
	EK-11	44:41	21.2:63.8	1:3	13.64	<0.001
			37.2:47.8	7:9	2.22	0.25-0.1
			47.8:37.2	9:7	0.69	0.5-0.25

Masay, based on the goodness of fit to a 1:2:1 ratio of families nonsegregating for resistance, segregating for resistance and susceptibility, and nonsegregating for susceptibility (*Table 3.*). Segregating families showed resistant phenotype in F2 generation. The segregation pattern of F3 families indicated that one gene determined the resistance to HUN and EK-11 strains in the cross Xr2 x common bean, and the limited sources of resistance, derived mostly from P. acutifolius. It would be useful to "tag" these genes for resistance to different *Xcp* strains with molecular markers, and to use these markers to increase the level of resistance to *Xcp* by pyramiding the genes present in these lines with other genes for resistance already identified in other *P. vulgaris* germplasm.

Table 3 Chi-square tests for different segregation ratios of F3 families nonsegregating for resistance (all R), segregating for resistance and susceptibility (Seg.), and nonsegregating for susceptibility (all S), derived from the crosses Xr1 x Masay and Xr2 x Masay.

		Number of F3 families					
Population	strain	all R	Seg.	all S	expected ratios	$\chi 2$	P
F3 Xr1 x Masay	HUN	7	24	9	1:2:1	1.35	0.7-0.5
					7:8:1	24.00	<0.001
	EK-11	12	23	5	1:2:1	3.35	0.3-0.1
					7:8:1	4.68	0.1-0.05
F3 Xr2 x Masay	HUN	14	17	10	1:2:1	1.97	0.5-0.3
					7:8:1	23.08	<0.001
	EK-11	11	24	5	1:2:1	3.40	0.3-0.1
					7:8:1	5.71	0.1-0.05

Masay as well, although not in accordance to the results based on its F2 population. This contradiction suggests the presence of some minor, modifying genes beside the monogenic genetic background of resistance, explaining also the lack of clear bimodal distribution of disease reactions in F2 populations. *Velich* et al. (1994) observed also modifying effects in F1 and F2 progenies of different crosses of Xr1 and Xr2 lines, however a dominant monogenic resistance of both lines to the HUN *Xcp* strain was proposed in that study. The value of resistance gene(s) for CBB in the lines Xr1 and Xr2 would be recommended to be investigated in different genetic backgrounds. Beside the dominant resistance genes to *Xcp* found in beans, there are several reports on different systems. A recessive gene for moderately resistance to some strains of *Xcp* was reported by *Adams* et al., (1988). A single recessive gene determined resistance and two complementary dominant genes determined susceptibility to three *Xcp* isolates has been found in different F2 population of crosses between resistant UNECA common bean mutant and susceptible common bean lines (*Dursun*, 1994).

Recombinants resistant to only one *Xcp* strain and susceptible to another strain were observed, indicating that different genes controlled the reaction to each *Xcp* strain. The observed segregations of different combinations of reactions to the two *Xcp* strains in both crosses showed coupling linkage between the genes controlling reactions to *Xcp* (data not shown). *Freytag* (1989), *Dursun* (1994), and *Park* (1998) reported also that three different genes in coupling phase linkage determined resistance to different *Xcp* isolates in tepary bean crosses.

Xr1 and Xr2 lines represent valuable new monogenic genetic sources in resistance breeding to CBB, also because of the complex genetic background of resistance to *Xcp* in

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