Theor Appl Genet (1997) 95 : 1018–1023

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Bruchid resistance of common bean lines having an altered seed protein composition

Received: 2 April 1997 / Accepted: 20 May 1997

Abstract Arcelin seed proteins of common bean (*Phaseolus vulgaris* L.) are toxic to one of the most damaging pests of bean seeds, *Zabrotes subfasciatus* (Boheman), but they appear to have little effect on another important bean pest, *Acanthoscelides obtectus* (Say), when introduced into standard cultivars by backcrossing. With the goal of increasing arcelin concentration to improve resistance, we modified seed-protein composition by introducing a null allele for the major seed protein, phaseolin, into lines (SMARC1, 2 and 4) or three phytohemagglutinin types (SMPHA lines). These lines were tested for resistance to both insects by measuring percentage insect emergence (%E) and days-to-adult emergence (DAE). For SMARC lines, arcelin type was the most important factor in resistance levels, with SMARC1 lines being most resistant, SMARC2 lines intermediate, and SMARC4 lines the least resistant to both bruchids. Additionally, the absence of phaseolin was a significant factor in the resistance of SMARC lines to *A. obtectus*. SMARC1 lines without phaseolin had half the percentage insect emergence of lines with phaseolin. SMARC1 lines with an altered seed composition had the highest levels of resistance to both bruchids of any large-seeded line reported to-date.

Key words *Phaseolus vulgaris* · Arcelin · Insect resistance · *Zabrotes subfasciatus* · *Acanthoscelides obtectus*

Communicated by G. Wenzel

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Introduction

The common bean (*Phaseolus vulgaris* L.) seed protein, arcelin, has been shown to be toxic to one of the major storage pests of common bean, *Zabrotes subfasciatus* (Boheman) (Osborn et al. 1988a). Six allelic variants of arcelin have been reported (Osborn et al. 1986; Kornegay et al. 1993; Suzuki et al. 1995), and five have been well characterized: arcelin-2 and arcelin-5 variants contain dimeric arcelin protein, arcelin-3 and -4 variants contain tetrameric proteins, and the arcelin-1 variant contains both dimeric and tetrameric proteins (Osborn et al. 1988b; Hartweck et al. 1991; Goossens et al. 1994). Wild bean accessions containing the arcelin variants were ranked by variant for resistance to *Z. subfasciatus* as: $4 = 5 > 1 > 2 > 3$ (Cardona et al. 1990; Fory et al. 1996). However, after backcrossing alleles for the arcelin proteins into cultivated lines, the variants were ranked $1 > 2 > 3 = 4$ (Harmsen 1989; Cardona et al. 1990). Wild lines containing arcelin-4 are also highly resistant to *Acanthoscelides obtectus* (Say) (Schoonhoven et al. 1983; Cardona et al. 1989), another major storage pest of common bean; and although backcross lines containing arcelin had little resistance to this insect (Osborn et al. 1988a; Harmsen, 1989) altering the protein composition of arcelin-containing seeds might improve resistance.

To test the effects of altered protein composition on resistance, we developed backcross lines in a 'Sanilac' background which contained phaseolin (PP lines), or null alleles for phaseolin (PN), in combination with alleles for either the arcelin-1, -2 or -4 variants (SMARC lines) or PHA variants from 'Bunsi,' 'Protop P-1,' or 'Viva' (SMPHA lines) (Hartweck and Osborn 1997). The SMPHA lines were developed because arcelin and PHA are related members of the phytohemagglutinin-arcelin-α-amylase inhibitor gene family which plays an important role in plant defense against pests (Osborn et al. 1988a; Chrispeels and

Raikhel 1991; Hartweck et al. 1991; Mirkov et al. 1994). Because of tight linkage between these genes, arcelin variants also contain linked phytohemagglutin and α-amylase inhibitor alleles inherited as a block from parents (Osborn et al. 1986; Suzuki et al. 1995). In SMARC and SMPHA lines, the concentrations of arcelin dimer, phaseolin, and the different PHA variant proteins were determined and seed-specific traits were measured for SMARC and SMPHA lines (Hartweck and Osborn 1997). The objective of the present study was to test SMARC and SMPHA lines for resistance to *Z. subfasciatus* and *A. obtectus*.

Materials and methods

Development of lines

Lines which contained either arcelin or PHA proteins were named based on the nature of their protein variant; either SMARC1, SMARC2 or SMARC4 for lines containing arcelin-1, -2 or -4 variants, respectively, or SMPHAB, SMPHAP or SMPHAV for lines containing PHA-B, PHA-P, and PHA-V variants, respectively. For each variant, two sets of paired lines were constructed which contained either a Sanilac phaseolin allele (PP lines) or a null allele for phaseolin (PN lines). For one set, a third line was similarly derived which contained null alleles for all three proteins (N-PN lines). The development and protein analysis of these lines is described elsewhere (Hartweck and Osborn 1997).

Insect bioassays

Seeds produced in a greenhouse in Wisconsin were bulked and sent to CIAT, Cali, Colombia, where the insect trials were conducted. Two susceptible lines, ICA-Pijao and UI 111, and a resistant wild accession containing arc-4, G12952 (Cardona et al. 1990), were included in the experiment. Insects were reared and maintained as previously described (Schoonhoven and Cardona 1982). All experiments were conducted at 27°C and 70% RH in a controlled environment chamber. Lines were tested in a randomized complete block with three replications using 2–3 pairs of *Z. subfasciatus* to infest 10–15 seeds. For several lines (SMARC2N-PN, SMARC4N-PN, and SMPHABN-PN), only 1–5 seeds were infested with one pair of *Z. subfasciatus* for each replication. Tests using *A. obtectus* were performed similarly except that each replication had ten seeds and each seed was infested with three eggs. For SMARC2N-PN1 and SMPHABN-PN lines, 1–4 seeds per replication were used.

Resistance parameters were measured as previously described (Schoonhoven et al. 1983; Cardona et al. 1990) and included: days-to-adult-emergence (DAE), percentage emergence (%E) and an index of susceptibility (IS) ratings. DAE values were log-transformed and percentage emergence scores were transformed using arcsin ($\sqrt{\text{proportion emerged}}$). Original values are reported with significance levels corresponding to those of the transformed data. The index of susceptibility scores was calculated as described previously (Cardona et al. 1990). For *Z. subfasciatus*, IS values were calculated as $\{[\log(\text{progeny per infesting female})]/\text{DAE}\} \times 100$, and for *A. obtectus*, IS was calculated as $\{[\log(\text{percent emergence})]/\text{DAE}\} \times 100$.

Statistical analyses

Analyses of variance were performed using the general linear model (GLM) procedure of SAS (SAS Institute Inc. 1982). Due to differences in the variation of measured traits, SMARC and SMPHA lines were analyzed separately. For tests of paired PN and PP lines, a nested design was used for the analysis of variance (Damon and Harvey 1987) with the protein variant type (arc-1, -2, and -4, PHA-B, -P, and -V) as the main factor and the phaseolin type (PP and PN) as the nested factor. Both protein and phaseolin effects were considered fixed. Only data from parental SARC, or Bunsi, Viva and Protop, and PP and PN lines were used in the nested analysis. Comparisons between individual PN and PP lines were performed with *t*-tests using the phaseolin-type error term as an estimator of variance. A randomized complete block design was used to calculate LSDs for comparisons between all SMARC or SMPHA lines and parents. The relationships between the quantity of arcelin or PHA (Hartweck and Osborn 1997) and insect resistance measurements were determined by regression analyses using SAS regression procedure with protein concentration as the independent variable and the insect resistance score as the dependent variable.

Results

Resistance to *Z. subfasciatus*

In most SMARC and parental SARC lines, *Z. subfasciatus* %*E* was decreased and adult emergence was delayed compared to Sanilac (Table 1). SMARC1 had the lowest percentage emergence (most resistant), SMARC2 lines were intermediate, while SMARC4 lines had the highest percentage emergence. Based on DAE, the variants were ranked SMARC1 (most resistant) > SMARC4 > SMARC2 (least resistant). For IS scores, which take both resistance parameters into account, the lines were ranked SMARC1 > SMARC2 > SMARC4.

In the analysis of variance of SMARC-PN and -PP lines, the presence or absence of phaseolin did not have a significant effect on %E and the only significant differences from paired *t*-tests were that SMARC2-PN2 and SMARC4-PN2 had a greater %E than their paired PP lines. The presence or absence of phaseolin was significant in the analysis of variance for DAE, with increased DAE associated with the absence of phaseolin, but these differences were not significant in individual paired *t*-tests.

None of the SMPHA lines had the high level of resistance found in SMARC lines (Table 2). Most %E and DAE scores were similar to, or indicated less resistance than, Sanilac. In two cases, PN lines were significantly different from their paired PP lines (SMPHAP-PN1 and a reduced %E and SMPHAV-PN1 had a reduced DAE) though the effect of phaseolin was not significant in the analyses of variance.

Resistance to *A. obtectus*

SMARC lines had less resistance to *A. obtectus* than to *Z. subfasciatus* (Table 1). The SMARC1 lines had the

Table 1 Means and least-significant differences for percentage emergence (%E), days-to-adult-emergence (DAE) and index of susceptibility (IS) of *Z. subfasciatus*- and *A. obtectus*-infested seeds of SMARC, parental, and check lines

Lines	*Z. subfasciatus*			*A. obtectus*		
	%E	DAE	IS	%E	DAE	IS
SMARC1-PN1	3.7	50.3	0.2	***29.7***[a]	39.0	8.6
SMARC1-PP1	3.7	49.3	0.3	***66.0***	39.7	10.5
SMARC1-PN2	10.3	49.3	2.0	***36.3***	36.7	9.7
SMARC1-PP2	5.7	48.0	1.6	***63.3***	38.3	10.8
SARC1	3.7	48.7	0.2	42.0	39.0	9.6
SMARC1N-PN	86.7	35.0	8.8	78.3	36.0	12.2
SMARC2-PN1	41.3	39.0	4.9	63.3	37.7	10.9
SMARC2-PP1	46.7	38.3	6.2	66.0	38.3	10.9
SMARC2-PN2	***68.0***	38.3	6.6	55.0	38.3	10.4
SMARC2-PP2	***40.0***	38.7	6.4	63.0	36.7	11.3
SARC2	42.0	43.0	5.1	67.3	39.7	10.6
SMARC2N-PN	97.0	34.3	11.2	89.0	37.0	11.9
SMARC4-PN1	85.3	45.7	6.9	75.3	***38.0***	11.4
SMARC4-PP1	80.3	41.7	6.3	71.7	***35.0***	12.5
SMARC4-PN2	***81.0***	45.3	7.0	80.3	36.0	12.1
SMARC4-PP2	***62.3***	45.3	6.3	67.0	35.7	11.8
SARC4	64.3	45.7	6.2	73.3	36.3	11.8
SMARC4N-PN	100.0	33.0	6.5	ND[b]	ND	ND
MB11-29	88.7	34.3	9.5	85.0	33.7	13.1
Sanilac	91.3	34.0	9.0	81.3	34.3	12.8
L12-56	91.7	34.3	8.8	65.3	36.7	11.4
UI 111	96.0	32.0	10.8	70.3	32.6	13.1
ICA-Pijao	95.0	33.3	9.5	81.3	32.3	13.6
G 12952	6.7	63.0	0.8	3.6	75.0	1.7
LSD[c]	14.8	4.0	0.7	16.4	1.6	0.4

[a] Comparisons between PN and PP lines were performed with *t*-tests with $P = 0.05$. Paired PN and PP lines which were significantly different are in bold italic
[b] ND = not determined
[c] Least-significant differences were calculated for all lines with $P = 0.05$

Table 2 Means and least-significant differences for percentage emergence (%E), days-to-adult-emergence (DAE) and index of susceptibility (IS) of *Z. subfasciatus*- and *A. obtectus*-infested seeds of SMPHA and Bunsi, Protop and Viva

Lines	*Z. subfasciatus*			*A. obtectus*		
	%E	DAE	IS	%E	DAE	IS
SMPHAB-PN1[a]	89.7	33.7	10.4	74.7	34.7	12.3
SMPHAB-PP1	96.0	34.0	9.9	78.0	34.7	12.5
Bunsi	91.3	33.7	9.8	67.3	34.3	13.0
SMPHABN-PN	83.3a	33.7	10.9	65.3	36.3	12.4
SMPHAP-PN1	***84.7***[b]	33.0	10.5	80.3	33.7	13.1
SMPHAP-PP1	***95.7***	33.7	10.9	65.3	34.3	12.1
SMPHAP-PN2	97.0	33.7	10.7	76.3	34.0	12.8
SMPHAP-PP2	96.3	34.3	9.2	84.0	34.0	11.9
Protop P-1	97.3	32.7	10.5	69.3	33.0	12.8
SMPHAPN-PN	90.3	33.7	9.6	83.3	35.7	12.4
SMPHAV-PN1	91.3	***32.0***	11.0	81.0	33.7	13.0
SMPHAV-PP1	91.7	***35.3***	8.4	77.0	34.0	12.7
SMPHAV-PN2	93.7	33.7	10.2	76.3	34.0	12.8
SMPHAV-PP2	98.0	35.7f	8.5	84.0	34.7	13.4
Viva	98.0	35.7	8.5	84.0	33.3	13.4
SMPHAVN-PN	85.7	34.7	9.1	60.0	34.0	12.3
LSD[c]	7.5	1.1	0.7	13.5	1.4	0.1

[a] There was only one set of SMPHAB lines
[b] See Table 1 for explanation
[c] Least-significant differences were calculated for all lines with $P = 0.05$. Parental checks and controls were used in the analyses of variance. See Table 1 for means of these lines

lowest %E scores, followed by SMARC2 lines and then by SMARC4 lines. Phaseolin-type was a significant factor in the analysis of variance for %E. This was probably due to a large significant reduction in %E for SMARC1-PN lines compared to their corresponding PP lines (Table 1). Paired differences between PN and PP lines in *A. obtectus* %E were not significant for SMARC2 and SMARC4 lines. In the analysis of variance, the presence of phaseolin did not have a significant effect on DAE, but one of the SMARC4-PN lines was significantly higher relative to its paired PP line. Based on IS scores, the SMARC1-PN lines, along with SARC1, were ranked as more resistant than the SMARC1-PP, SMARC2 and SMARC4 lines. The SMARC2 lines were slightly more resistant than the SMARC4 lines.

For the SMPHA lines, there were no significant differences in the analysis of variance or in *t*-tests between paired lines for *A. obtectus* %E, DAE and IS (Table 2). However, among the SMPHA lines, there were some examples of decreased %E (60–67%) compared to Sanilac (85%). SMPHAB-PN1, SMPHAVN-PN, SMPHAP-PP1, Bunsi and Protop had a lower %E and SMPHABN-PN and SMPLAPN-PN had a higher DAE than Sanilac.

Resistance to *Z. subfasciatus* and *A. obtectus*

The IS scores for resistance to both bruchids are presented graphically in Fig. 1. SMARC1 lines gave the highest levels of resistance to both bruchids, though the level of resistance to *Z. subfasciatus* was much greater than that for *A. obtectus*. The SMARC2 and SMARC4 lines had intermediate levels of resistance, while the SMPHA lines were not resistant.

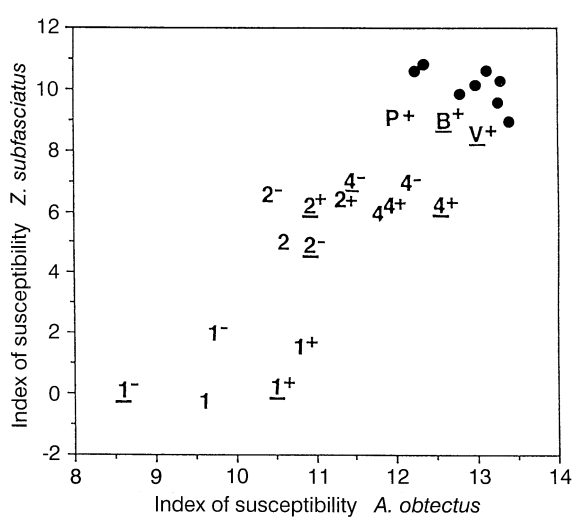

Fig. 1 Scatter diagram of the IS scores of SMARC and SMPHA lines to *Z. subfasciatus* are plotted against the *A. obtectus* IS scores. SMARC lines are numbered (*1, 2 and 4*) and SMPHA lines are represented as *dots*. Phaseolin-containing lines are designated + and null lines as −. The first set of paired lines are *underlined*; for example, SMARC1-PP1 and SMARC1-PN1 are represented as 1 + and 1 − and the second set of lines (SMARC1-PP2 and SMARC1-PN2) are represented as 1 + and 1 −. The parental SARC lines are represented by just a number (e.g. 1 = SARC1)

Protein quantity and resistance

Regression models were constructed for SMARC and SMPHA lines using protein quantity as the independent variable and the insect resistance score as the dependent variable. Coefficients of determination were calculated to show how much of the variation in a particular resistant measurement could be explained by variation in protein concentration. Differences in arcelin concentration did account for significant amounts of variation in both *Z. subfasciatus* and *A. obtectus* %E measurements (33% and 30%, respectively). There was no significant effect on variation for *Z. subfasciatus* DAE and a smaller, though significant, effect on the variation of *A. obtectus* DAE (17%). For PHA, 14–15% of *Z. subfasciatus* %E, DAE and IS score variation could be attributed to variation in PHA concentration; however, PHA concentration did not have a significant effect on *A. obtectus* resistance scores.

Discussion

SMARC lines containing the different arcelin variants had different levels of resistance to *Z. subfasciatus*. The SMARC1 lines were highly resistant, the SMARC2 lines were intermediate, and the SMARC4 lines had only low levels of resistance. Backcross lines containing the three arcelin variants have been similarly ranked in past studies for resistance to *Z. subfasciatus* (Harmsen 1989; Cardona et al. 1990). There were similar rankings for resistance to *A. obtectus*, although the affects were reduced.

The high levels of resistance to *Z. subfasciatus* in SMARC1 lines might be due to the unique composition of this arcelin variant compared to the others. The presence of both dimer and tetramer proteins in arcelin-1-containing lines may have enhanced the resistance response over that of SMARC2 and SMARC4 lines which contain only a single arcelin protein type (Hartweck et al. 1991). The dimer proteins of arcelin-1 and -2 are almost identical except that arcelin-2 has one less potential glycosylation site (John and Long 1990) and the two arcelin-1 tetramer proteins are 77 and 93% identical to arcelin-4 at the N-terminal end of the proteins (Hartweck et al. 1991). These small differences between the proteins, or other factors, might also contribute to the observed resistance responses.

The arcelin locus is tightly linked and closely related to PHA and α-amylase inhibitor (Osborn et al.

1988a; Chrispeels and Raikhel 1991; Hartweck et al. 1991; Mirkov et al. 1994). PHA was thought to confer resistance to the Cowpea weevil, *Callosobruchus maculatus* (Janzen et al. 1976), but more recent purification techniques have shown that PHA was not responsible for the observed antibiosis (Huesing et al. 1991). Although the common-bean α-amylase inhibitor in transgenic pea confers resistance to pea insects (Shade et al. 1994; Schroeder et al.1995), Fory et al. (1996) found that it had only a minor role in the resistance of common bean to *Z. subfasciatus* and *A. obtectus*. Arcelin has been shown to be an important factor in the resistance of the common bean to *Z. subfasciatus* based on a testing of artificial seeds and backcross lines (Osborn et al. 1988a; Harmsen 1989; Cardona et al. 1990; results reported here). However, it appears to confer only low levels of resistance to *A. obtectus* (Osborn et al. 1988a; Harmsen 1989).

Resistance to *A. obtectus* was significantly increased in some of our arcelin-containing lines which also carried null alleles for phaseolin. In these lines, the absence of phaseolin was compensated by arcelin and/or other proteins (Hartweck and Osborn 1997) and differences in arcelin dimer concentration explained approximately 30% of the variation in percentage emergence scores. Additionally, reduced phaseolin concentration, per se, might be an important component in resistance because it is an easily digested, nutritious protein for bruchids (Minney et al. 1990).

Although some wild beans containing arcelin are highly resistant to *A. obtectus* (Schoonhoven et al. 1983; Kornegay and Cardona 1991; Fory et al. 1996), most of this resistance was lost after backcrossing arcelin alleles into cultivated lines (Osborn et al. 1988a; Harmsen 1989). Our results show that the resistance of cultivated beans containing arcelin can be dramatically increased by genetically removing phaseolin. In lines combining the arcelin-1 and the phaseolin null alleles, the percentage of *A. obtectus* emergence decreased to half the level found in phaseolin-containing paired lines. These lines have the highest levels of resistance to both *Z. subfasciatus* and *A. obtectus* of any large-seeded bean line developed to-date. Further improvements in resistance may be possible by selection for resistance within these materials, or by using them as parents for population improvement.

Acknowledgements The authors gratefully acknowledge the assistance to Dr. Brian S. Yandell and thank Mr. Ken Kmeicik for many helpful suggestions. This work was funded in part by WARF grant awarded through the Graduate School, University of Wisconsin-Madison.

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