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ORIGINAL PAPER

# Characterization of resistance to the bean weevil *Acanthoscelides obtectus* Say, 1831 (Coleoptera: Bruchidae) in common bean genotypes

**Edson L. L. Baldin[1] · Fernando M. Lara[2] · Roberto S. Camargo[1] · Luiz E. R. Pannuti[1]**

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**Abstract** The bean weevil *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Bruchidae) is one of the most serious pests of stored beans worldwide because of the damage it causes to grains within warehouses. The use of resistant genotypes may offer a control strategy for this pest. In the current study, we screened common bean genotypes of Andean American and Mesoamerican origin in laboratory and greenhouse bioassays to select the most promising beans for resistance to the bean weevil. In the laboratory, we evaluated number of eggs, period of development (egg-adult), number of emerged adults, dry weight of adults, and weight of consumed grains. In the greenhouse, number of pods per plant and number of grains per pod were evaluated. We also assessed the percentages of damaged pods per plant and damaged grains per pod. Combining the results obtained in the laboratory and greenhouse assays, the common bean genotypes Arc.1, Arc.2, Arc.1S, Arc.5S, and Arc.3S were identified as resistance expressing antibiosis against *A. obtectus*. The lowest percentages of damaged pods were found in the Arc.1 and Arc.1S genotypes, and their resistance to damage was apparently morphological (antixenotic) because they possessed structures that prevented contact between larvae and grains. The use of resistant genotypes in combination with other techniques may improve management of the weevil. Additionally, the resistant genotypes identified here can be used in breeding programs to develop common bean lines with resistance to *A. obtectus*.

**Keywords** Plant resistance · *Phaseolus vulgaris* · Stored bean · Greenhouse · Weevil

## Introduction

The consumption of the green pods and seeds of common bean (*Phaseolus vulgaris* L.) is increasing worldwide because beans constitute an important protein source for hundreds of million people (Broughton et al. 2003; Coelho et al. 2011; Zaugg et al. 2013). Although various cultivation practices are increasing the crop yields of beans, significant losses occur during storage (Schoonhoven and Cardona 1986; Wong-Corral et al. 2013). One cause of damage within the warehouse is bruchid attack, such as from the bean weevil *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Bruchidae), which is one of the most serious pests of stored beans worldwide (Ishimoto and Chrispeels 1996). The reproductive capacity of the weevil is high, and large populations can form over short periods. When attacking stored grains, the larvae destroy embryos and directly affect subsequent germination, in addition to causing commercial losses via reductions in bean weight and nutritional value (Schoonhoven and Cardona 1982; Gatehouse et al. 1987; Shade et al. 1987; Dobie et al. 1990; Ishimoto and Chrispeels 1996).

The most common practice of managing bean weevils is with chemical control using protective synthetic insecticides (organophosphates and pyrethroids) and fumigants (phosphine) (Lorini 1998; Weber 2001; Gusmão et al.

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✉ Luiz E. R. Pannuti
luizpannutixl@yahoo.com.br

[1] Department of Crop Protection, College of Agronomic Sciences, São Paulo State University, Botucatu, São Paulo State 18610-307, Brazil

[2] Department of Plant Protection, College of Agronomic and Veterinary Sciences, São Paulo State University, Jaboticabal, São Paulo State 14884-900, Brazil

2013). However, in addition to increasing the cost of production, chemical control becomes problematic when applications of insecticides occur close to product consumption because of the possible accumulation of toxic residues in grains (Baker et al. 2002; Kemabonta and Odebiyi 2005; Gusmão et al. 2013).

Because of the harmful chemical effects, the use of resistant genotypes provides desirable characteristics, including specificity for one or more pests, cumulative effects, action on successive generations of insects, persistence for several years, harmony with the environment, ease of incorporation into farm activities, absence of extra costs, and compatibility with other control tactics used in IPM (Painter 1951; Kogan 1975; Smith 2005).

Plant resistance is divided into three categories: antixenosis, antibiosis, and tolerance (Painter 1951; Panda and Khush 1995). Antixenosis is characterized by morphological or chemical factors that negatively affect the behavior of insects attempting to colonize a plant. The expression of antibiosis affects the biology of insects attempting to use a plant as a host, and tolerance is the ability of plants to resist or recover from an injury caused by an insect without affecting the biology or the behavior of the insect (Smith and Clement 2012).

Some wild accessions and improved varieties of common bean contain compounds, such as phytohemagglutinins, protease inhibitors, alpha-amylase inhibitors, and arcelin protein variants, that express antibiosis-type resistance to bean weevils (Sathe et al. 1984; Murdock et al. 1988; Kuroda et al. 1996; Chrispeels and Raikhel 1991; Kasahara et al. 1996; Grossi de Sá and Chrispeels 1997; Franco et al. 1999; Carlini and Grossi de Sá 2002; Velten et al. 2007). The effects of these chemicals on immature insects may include increased mortality caused by reduced lengths of developmental periods (Osborn et al. 1986; Cardona et al. 1990; Walter 1992). One of the most important of these chemicals is the protein arcelin, which is associated with bean resistance to bruchids. Arcelin is found in resistant wild varieties but is absent in many susceptible commercial and wild varieties (Romero Andreas et al. 1986; Cardona et al. 1990; Blair et al. 2010; Zaugg et al. 2013). Based on previous studies, arcelin, found in several wild genotypes, is associated with resistance in common beans (Osborn et al. 1986, 1988; Romero Andreas et al. 1986; Cardona et al. 1989, 1990; Minney et al. 1990; Velten et al. 2007), and types 1, 2, and 5 are the most resistant against bruchids (Barbosa et al. 1999; Baldin and Lara 2008; Sakthivelkumar et al. 2013). Arcelin prolongs immature development of *A. obtectus* by 15% (Schmale et al. 2002) and acts as a growth inhibitor on first instar larvae. It also affects bruchid female fitness by reducing female body mass (Velten et al. 2007).

Considering the damage caused by the bean weevil and the necessity to develop alternative control techniques, the current study utilized experiments conducted in both the laboratory and the greenhouse in order to characterize resistant bean genotypes to *A. obtectus*.

## Materials and methods

### Rearing *Acanthoscelides obtectus*

A rearing stock of *A. obtectus* was maintained in a chamber (25 ± 2 °C, RH = 70 ± 10%, and photoperiod of 12:12 h L:D) to supply insects in sufficient numbers to conduct the proposed tests. Clear glass flasks (750 mL) were used for rearing, which were closed at the top with a screw-on lid with a circular opening covered with a fine-mesh nylon screen for internal aeration. Each flask received approximately 0.3 kg of bean grains (cv. Bolinha) and approximately 300 unsexed adults. The grains in the flasks were periodically sifted, and the emerging adults were used to infest new flasks.

### Screening test

In a preliminary test, we evaluated 18 genotypes of common bean from different origins and with great genetic variability, some of them not previously assessed for this bruchid. The grains of genotypes were supplied by the Brazilian Agricultural Research Corporation (EMBRAPA) and are part of the company's germplasm bank. Their respective genealogies, origin, and justification for choice are described in Table 1. The Carioca Pitoco genotype was used as a susceptible standard, while Arc.2 was used as a resistant standard (Baldin and Lara 2004).

Materials from the plants of these genotypes were harvested and maintained in a greenhouse at 40 °C for approximately 3–5 days for subsequent drying and threshing of pods. After this period, the collected grains were placed in cold storage (2–3 °C) for 15 days to control potential previous pest infestations. Before the tests, the grains were maintained for 3–4 days under a 12 h photoperiod at 25 ± 2 °C and 70 ± 10% RH in a chamber to reach a hygroscopic balance.

The plastic containers used in the no-choice tests were transparent with fitted lids (5.0 cm height × 3.0 cm diameter). Ten grams of bean grains from each genotype was placed into the containers. The grain weight was assessed with an analytical balance to an accuracy of 0.001 g.

To begin the tests, six adults (randomly collected and up to 48 h old) were released into each container (Baldin and Lara 2008). The containers were capped and placed in the

**Table 1** Genotype, genealogy, and origin of 18 lines/cultivars of common bean

| Genotypes | Genealogy | Origin | History of resistance |
|---|---|---|---|
| Mesoamerican | | | |
| Porrillo 70 | Selection in crioula cultivar | CIAT | Commercial genotype/not evaluated |
| Xamego | (LM 20771/BAT 256)F1//(LM 20332/BAT 67)F1 | EMBRAPA | Commercial genotype/not evaluated |
| Diamante Negro | XAN 87/A 367 | EMBRAPA | Commercial genotype/not evaluated |
| Engopa 201 Ouro | Cultivar from BAG-CIAT | CIAT | Commercial genotype/not evaluated |
| Carioca | Selection of local cultivar from São Paulo | EMBRAPA | Commercial genotype/not evaluated |
| Rudá | Rudá Carioca × Rio Tibagi | EMBRAPA | Commercial genotype/not evaluated |
| Corrente | No information | EMBRAPA | Commercial genotype/not evaluated |
| Carioca Pitoco | Selection in material crioulo | CATI | Susceptible to *A. obtectus* (Baldin and Lara 2008) |
| Iapar 57 | Porrillo Sintético/Aeté 1-38//Cena 83-1/3/Iapar-BAC 32 | EMBRAPA | Commercial genotype/not evaluated |
| Onix | Porrillo Sintético/Turrialba 1//ICA Pijao/Negro Jamapa | EMBRAPA | Commercial genotype/not evaluated |
| Ipa6 | Rico-23 × Gordo | EMBRAPA | Commercial genotype/not evaluated |
| Genetic breeding | | | |
| Arc.1 | Line bred containing arcelin 1 | CIAT | Resistant to *Z. subfasciatus* (Lara 1997) |
| Arc.2 | Line bred containing arcelin 2 | CIAT | Resistant to *Z. subfasciatus* (Lara 1997) |
| Arc.3 | Line bred containing arcelin 3 | CIAT | Resistant to *Z. subfasciatus* (Lara 1997) |
| Arc.4 | Line bred containing arcelin 4 | CIAT | Resistant to *Z. subfasciatus* (Lara 1997) |
| Andean American | | | |
| Goiano Precoce | Crioula cultivar | EMBRAPA | Commercial genotype/not evaluated |
| Jalo Precoce | Massal selection made from Goiano Precoce cultivar | EMBRAPA | Commercial genotype/not evaluated |
| PR95105389 | BAG-IAC | IAC | Commercial genotype/not evaluated |
| Arc.1S[a] | Wild-bred containing arcelin 1 | CIAT | Resistant to *Z. subfasciatus* (Lara 1997) |
| Arc.3S[a] | Wild-bred containing arcelin 3 | CIAT | Resistant to *Z. subfasciatus* (Lara 1997) |
| Arc.5S[a] | Wild-bred containing arcelin 5 | CIAT | Resistant to *Z. subfasciatus* (Lara 1997) |

[a] Genotypes not assessed in screening but included in bioassays

chamber. The infestation proceeded for 7 days, after which the insects (dead or alive) were removed.

Fifteen days after the start of the infestation, the containers were evaluated daily to observe the developmental periods of the insects. The grains in each vial were sifted with an appropriate sieve, and the number of emerged insects per day per genotype was recorded. After counting, the emerged adults were placed in small glass vials (5.0 cm height × 2.2 cm diameter) and were immediately frozen (without loss of weight). At the end of the emergence period, the small glass vials containing the emerged weevils were opened, and the insects were oven-dried (50 °C) for 2 days; then, the dry weight was obtained with an analytical scale (0.001 g). The experimental design was completely randomized with eight replicates per genotype. Each container with 10 g of grains and six insects was a replicate.

## Test with selected genotypes

Based on the screening results and the availability of the grains, eight bean genotypes with variable levels of resistance were selected: Carioca Pitoco, Ipa 6, Porrilo 70, Onix, Arc.1, Arc.2, Arc.3, and Arc.4 (the latter four corresponded to lines bred that contained arcelin 1, 2, 3, and 4). The genotypes Arc.1S and Arc.3S were also included (wild genotypes containing arcelin 1 and 3, respectively) as resistant controls (Baldin and Lara 2008).

For grain infestation, six *A. obtectus* unsexed adults that were 48 h old were randomly collected and placed in plastic containers (5.0 cm height × 3.0 cm diameter) with 10 g of bean grains. The containers were capped and placed in the chamber (under the previously described conditions). The insects were removed after 7 days, as described for the screening tests.

To determine ovipositional preference, the total number of eggs was counted on day 20 after infestation with a stereoscopic microscope. Twenty-five days after the initial infestation, the numbers of emerged insects and the periods of development (egg-adult) were also determined. The dry weights of the insects and the consumption of the grains were assessed when the emergence was complete.

After counting, the emerged adults were placed in glass vials and weighed as described previously for the screening. To obtain the weight of consumed grain, the containers (infested and controls) were oven-dried (under the conditions previously described). After drying, the initial and final weights of the grains were adjusted according to the weights of the controls, and the difference in dry weight (consumption) was calculated. Eight replicates were used per genotype in a completely randomized design.

### Greenhouse test

Common bean seeds (selected genotypes) were sown in pots (20 L), and the plants (three/pot) were evaluated throughout their life cycles. The wild genotype Arc.5S (containing arcelin 5) was included in this test as resistant control (Baldin and Lara 2008).

After the maturation and drying of pods, a general cleaning was performed on each pot, consisting of the removal of dried leaves and branches. After counting the number of pods/plant/pot, adults of *A. obtectus* (48 h old) were released in a proportion of two individuals per pod. The plants were individually caged within tubular metallic structures (35 cm diameter × 60 cm height) and were covered with organdy fabric to prevent insect escape.

The infestation was maintained for 25 days, after which the pods were removed from the plants, placed in paper bags and placed in the chamber (under conditions described for the previous tests). After 60 days had elapsed from the initial infestation, the bags were opened, and the pods and grains of the different bean genotypes were evaluated.

The variables analyzed were the number of pods per plant, number of damaged pods per plant, number of grains per pod, and percentage of damaged grains per pod. Eight replicates were used per genotype (88 pots) in a completely randomized design.

### Statistical analyses

The data were subjected to ANOVA using the $F$ test. The normality and homogeneity of the data were assessed with the Shapiro–Wilk and Levene's tests, respectively. When the treatment effect was significant ($P \leq 0.05$), the means were separated using Fisher's LSD tests (PROC MIXED; SAS Institute 2002).

## Results

### Screening test

For the length of the *A. obtectus* developmental period (from egg to adult), the genotypes Arc.2 and Arc.1 were less suitable for *A. obtectus* larvae; they prolonged the phases of development in a manner that was different from most of the tested genotypes ($F = 6.31$; $df = 17, 162$; $P < 0.0001$). The genotype Arc.3 also caused a prolonged developmental period and differed from Corrente, Carioca Pitoco, Xamego, Engopa 201 Ouro, Goiano Precoce, Jalo Precoce, Porrillo 70, and Carioca (Table 2).

The mean number of emerged adults varied from 72.25 to 20.75 for Carioca Pitoco and Arc.2, respectively, and significant differences were detected among the genotypes ($F = 1.20$; $df = 17, 162$; $P = 0.0297$). However, the weights of the insects did not differ significantly among the genotypes ($F = 1.69$; $df = 17, 162$; $P = 0.0736$) (Table 2).

Based on the extended developmental periods (from egg to adult), the low number of emerged insects and the low weights of the insects (Table 2), the genotypes Arc.1S, Arc.3S, Arc.5S, Carioca Pitoco, Ipa 6, Porrillo 70, Onix, Arc1, Arc.2, Arc.3, and Arc.4 were selected for subsequent tests.

### Laboratory test

For *A. obtectus* oviposition (Table 3), no significant differences were detected regarding the number of eggs laid on each bean genotype ($F = 1.10$; $df = 9, 70$; $P = 0.3762$). For the developmental period (egg-adult), a significant difference was detected among the genotypes ($F = 27.28$; $df = 9, 70$; $P < 0.0001$) (Table 3), and the weevils subjected to the Arc.1S, Arc.2, and Arc.1 genotypes had significantly prolonged developmental periods.

The mean number of emerged adults varied from 69.88 to 103 for Arc.1 and Carioca Pitoco, respectively, but no significant differences were detected among the genotypes ($F = 0.41$; $df = 9, 70$; $P = 0.9283$). The dry weights of the adults ranged from 1.90 to 2.30 g, with significant differences among the genotypes ($F = 4.39$; $df = 9, 70$; $P < 0.0001$) (Table 3). The lowest mean adult weights were found on Arc.1S and Arc.3S; these values were different from Carioca Pitoco, Ipa 6, Arc.3, Porrillo 70, and

**Table 2** Period of development (egg-adult), number, and dry weight (mean ± SD) of adult *A. obtectus* on genotypes of common bean during no-choice tests (photoperiod = 12 h, $T = 25 \pm 2$ °C and RH = 70 ± 10%)

| Genotypes | Preliminary test | | |
|---|---|---|---|
| | Egg-adult[1] | No. of emerged adults[1] | Weight (mg)[1] |
| Carioca | 32.54 ± 0.50 d | 57.50 ± 5.42 abcd | 2.27 ± 0.08 a |
| Porrillo 70 | 32.66 ± 0.30 d | 35.25 ± 5.79 abcd | 2.11 ± 0.03 a |
| Jalo Precoce | 33.05 ± 0.32 cd | 47.25 ± 16.29 abcd | 2.23 ± 0.12 a |
| Goiano Precoce | 33.16 ± 0.57 cd | 56.50 ± 11.39 abcd | 2.40 ± 0.09 a |
| Engopa 201 Ouro | 33.23 ± 1.00 cd | 64.00 ± 23.37 abcd | 2.36 ± 0.06 a |
| Xamego | 33.32 ± 0.52 cd | 66.25 ± 10.69 abc | 2.19 ± 0.07 a |
| Carioca Pitoco | 33.44 ± 0.51 cd | 72.25 ± 21.03 a | 2.07 ± 0.08 a |
| Corrente | 33.52 ± 0.62 cd | 64.75 ± 20.00 abcd | 2.29 ± 0.07 a |
| PR95105389 | 33.61 ± 0.80 bcd | 36.25 ± 26.67 abcd | 2.22 ± 0.16 a |
| Rudá | 33.70 ± 0.43 bcd | 61.00 ± 17.51 abcd | 2.33 ± 0.07 a |
| Diamante Negro | 33.80 ± 0.85 bcd | 41.75 ± 12.38 abcd | 2.36 ± 0.11 a |
| Ônix | 33.99 ± 0.38 bcd | 26.75 ± 10.49 bcd | 2.33 ± 0.09 a |
| Iapar 57 | 34.12 ± 0.47 bcd | 38.50 ± 13.42 abcd | 2.21 ± 0.04 a |
| Ipa 6 | 35.35 ± 2.09 bc | 32.25 ± 15.48 abcd | 2.41 ± 0.12 a |
| Arc.4 | 35.45 ± 1.00 bc | 63.50 ± 19.20 abcd | 2.07 ± 0.06 a |
| Arc.3 | 35.96 ± 0.94 b | 24.75 ± 6.85 cd | 2.15 ± 0.17 a |
| Arc.1 | 39.12 ± 0.46 a | 70.05 ± 16.66 ab | 2.00 ± 0.10 a |
| Arc.2 | 40.34 ± 1.38 a | 20.75 ± 1.03 d | 2.15 ± 0.07 a |
| *P* | <0.0001 | 0.0297 | 0.0736 |

[1] The means within the columns followed by the same letters are not significantly different ($P > 0.05$, LSD test)

**Table 3** Number of eggs (mean ± SD), period of development (egg-adult) (DP), number of emerged adults (EA), dry weight of adults (DWA), and consumed weight of grains (CWG) of *A. obtectus* on genotypes of common beans in no-choice tests (photoperiod = 12 h; $T = 25 \pm 2$ °C and RH = 70 ± 10%)

| Genotype | No. of eggs[1] | DP (days) | EA | DWA (mg) | CWG (g) |
|---|---|---|---|---|---|
| Arc.4 | 113.13 ± 11.92 a | 38.21 ± 0.28 c | 92.63 ± 11.26 a | 2.06 ± 0.05 abc | 1.30 ± 0.16 a |
| Carioca Pitoco | 114.00 ± 13.37 a | 37.32 ± 0.39 cde | 103.00 ± 12.86 a | 2.23 ± 0.05 ab | 1.60 ± 0.35 a |
| Ipa 6 | 86.88 ± 16.90 a | 36.70 ± 0.22 def | 78.13 ± 16.55 a | 2.24 ± 0.05 a | 1.38 ± 0.42 a |
| Arc.3 | 89.75 ± 17.72 a | 37.43 ± 0.46 cde | 76.38 ± 15.48 a | 2.18 ± 0.10 ab | 1.50 ± 0.31 a |
| Porrillo 70 | 65.38 ± 15.09 a | 35.96 ± 0.43 f | 85.50 ± 17.10 a | 2.25 ± 0.10 a | 1.42 ± 0.25 a |
| Arc.1 | 79.75 ± 18.08 a | 39.67 ± 0.58 b | 69.88 ± 17.10 a | 2.06 ± 0.07 abc | 1.00 ± 0.23 a |
| Onix | 96.25 ± 17.77 a | 36.34 ± 0.54 ef | 87.75 ± 17.29 a | 2.30 ± 0.05 a | 1.65 ± 0.35 a |
| Arc.3S | 112.25 ± 20.12 a | 37.76 ± 0.33 cd | 93.75 ± 19.64 a | 1.90 ± 0.03 cd | 0.96 ± 0.19 a |
| Arc.2 | 86.38 ± 15.33 a | 40.63 ± 0.57 b | 75.00 ± 14.24 a | 1.99 ± 0.16 bc | 1.22 ± 0.22 a |
| Arc.1S | 116.13 ± 17.42 a | 43.65 ± 0.50 a | 85.00 ± 14.88 a | 1.73 ± 0.07 d | 0.90 ± 0.16 a |
| *P* | 0.3762 | <0.0001 | 0.9283 | <0.0001 | 0.5011 |

[1] The means within the columns followed by the same letters are not significantly different ($P > 0.05$, LSD test)

Onix genotypes. Grain consumption was not significantly different between the different genotypes ($F = 0.93$; $df = 9, 70$; $P = 0.5011$) (Table 3).

## Greenhouse test

The mean number of pods per plant varied from 4.17 to 16.33 for Arc.3S and Arc.2, respectively, but the differences were not significant among the genotypes ($F = 1.97$; $df = 10, 77$; $P = 0.0642$) (Table 4).

For the percentage of damaged pods per plant, the genotypes Arc.1 (8.97%) and Arc.1S (10%) were less damaged, producing different degrees of damage compared to Arc.4 (53.39%), Arc.3 (35.05%), and Carioca Pitoco (31.10%) (Table 4). The Arc.4 genotype had the highest percentage of damage, which was significantly different

**Table 4** Evaluation of pods and grains of bean genotypes infested with adult *A. obtectus* in no-choice tests in the greenhouse

| Genotypes | Pods | | Grains | |
|---|---|---|---|---|
| | No. pods/plant | Damaged pods/plant (%) | No. grains/pod | Damaged grains/pod (%) |
| Arc.1 | 9.11 ± 3.37 a | 8.97 ± 2.45 d | 3.70 ± 0.36 ab | 6.45 ± 3.13 c |
| Arc.1S | 6.00 ± 2.08 a | 10.00 ± 2.00 d | 1.58 ± 0.21 d | 11.19 ± 7.63 c |
| Arc.5S | 6.55 ± 2.15 a | 16.15 ± 5.62 cd | 2.41 ± 0.02 bcd | 8.27 ± 1.66 c |
| Ipa 6 | 4.33 ± 0.73 a | 17.13 ± 2.84 cd | 5.01 ± 0.24 a | 14.25 ± 4.58 bc |
| Arc.2 | 16.33 ± 3.03 a | 18.80 ± 3.68 bcd | 3.48 ± 033 abc | 11.86 ± 4.51 c |
| Porrillo 70 | 10.44 ± 1.37 a | 21.56 ± 13.19 bcd | 4.03 ± 0.24 a | 5.81 ± 3.62 c |
| Onix | 11.17 ± 5.26 a | 24.31 ± 4.39 bcd | 3.81 ± 0.49 ab | 16.85 ± 5.48 bc |
| Arc.3S | 4.17 ± 1.09 a | 26.06 ± 15.38 bcd | 2.28 ± 0.38 cd | 14.50 ± 6.64 bc |
| Carioca Pitoco | 5.89 ± 0.59 a | 31.10 ± 4.10 bc | 4.00 ± 0.24 a | 25.20 ± 4.75 ab |
| Arc.3 | 13.11 ± 4.97 a | 35.05 ± 7.12 b | 4.53 ± 0.06 a | 16.95 ± 5.14 bc |
| Arc.4 | 11.33 ± 2.34 a | 53.39 ± 1.56 a | 3.79 ± 0.14 ab | 34.71 ± 3.90 a |
| *P* | 0.0642 | 0.0005 | 0.0265 | 0.0004 |

[1] The means within the columns followed by the same letters are not significantly different ($P > 0.05$, LSD test)

from Arc.3 and Carioca Pitoco ($F = 4.53$; $df = 10, 77$; $P = 0.0005$). Regarding the intermediate mean values, the differences among the other genotypes were not significant (Table 4).

For the production of grains per pod, a significant difference was observed among the genotypes ($F = 13.73$; $df = 10, 77$; $P = 0.0265$). The genotypes Ipa 6, Arc.3, Porillo 70, and Carioca Pitoco were the most productive, whereas Arc.5S, Arc.1S, and Arc. 3S produced the lowest number of grains per pod (Table 4).

The percentage of damaged grains per pod ranged from 5.81 to 34.71%, with significant differences among the genotypes ($F = 4.56$; $df = 10, 77$; $P = 0.0004$). The genotypes Porillo 70, Arc.1, Arc.5S, Arc.1S, and Arc.2 had the lowest mean damage, whereas Arc.4 and Carioca Pitoco had the highest percentages of damaged grains per pod (Table 4).

## Discussion

Based on the screening test, the performance of *A. obtectus* varied in the different genotypes. The genotypes Arc.1, Arc.2, Arc.3, Arc.4, and IPA 6 resulted in prolonged developmental periods for the bean weevil (egg-adult), which suggests the occurrence of antibiosis (Smith and Clement 2012). The other genotypes showed intermediate behaviors or were susceptible.

To decrease insect consumption of host cultivars via antibiosis, plants may use toxic compounds that confer resistance by delaying the development of immature stages of pests (Panda and Khush 1995). Toxic components of plant defense include antibiotics, alkaloids, terpenes, cyanogenic glycosides, and proteins (Minney et al. 1990; Gepts 1999; Sakthivelkumar et al. 2013). The type of grain proteins that are typically associated with defense mechanisms are lectins, alpha-amylase inhibitors, proteinase inhibitors, inactivating-protein ribosomes, protein reserves (vicilin) of modified proteins, proteins involved in lipid transport, and glucanases (Chrispeels and Raikhel 1991; Kasahara et al. 1996; Grossi de Sá and Chrispeels 1997; Franco et al. 1999; Carlini and Grossi de Sá 2002). Other plant proteins that are involved in the complex mechanisms of plant defense include arcelins (Osborn et al. 1988; Lara 1998; Barbosa et al. 2000), chitinases (Herget et al. 1990), and some modified forms of storage proteins (Macedo et al. 1993; Sales et al. 2000). In our screening, a strong antibiosis to *A. obtectus* was verified, as a delay in the development of immature stages and a reduction in the number of emerged adults were observed.

Based on the results of using selected genotypes, Arc.1S, Arc.2, and Arc.1 delayed the developmental period of the weevil (egg-adult) by 43.65, 40.65, and 39.67 days, respectively. Conversely, with the genotypes Onix, Ipa 6, and Porillo 70, the developmental period was more rapid, at 36.70, 36.34, and 35.96 days, respectively, which also indicated susceptibility. Arcelin protein is typically associated with the resistance of common beans to bruchid beetles (Romero Andreas et al. 1986), which was found in the genotypes Arc.1 (arcelin, type 1), Arc.2 (arcelin, type 2), and Arc.1S (wild, arcelin, type 1) (Lara 1997). The resistance showed by these genotypes, in addition to the effects of arcelins 1 and 2, may also be affected by compounds such as lectins and alpha-amylase and proteinase inhibitors (Franco et al. 1999). Mazzoneto and Vendramim (2002) reported similar results in which common beans possessing arcelin showed antibiosis against *Zabrotes subfasciatus* (Boh., 1833) (Coleoptera: Bruchidae) by prolonging the developmental period (egg to adult), reducing male and female weights, reducing longevity, and decreasing fecundity.

In general, the great variability in the number of emerged insects observed in this study may be caused by larval mortality from contact with proteins with potential insecticidal properties, such as arcelin and vicilin, which are found in some leguminous plants. These proteins are bound in the midgut to chitinous structures (e.g., peritrophic membrane) that interfere with the assimilation of nutrients, which may cause the death of an insect (Amorim et al. 2008). In the current study, the mean number of emerged adults ranged from 69.88 to 103.00, with a tendency of lower emergence in the genotypes Arc.1, Arc.2, and Arc.3. When confined to seeds that contained these variants of the arcelin protein, significant increases in the biological cycle of the weevils *Z. subfasciatus* and *A. obtectus* have also been observed by other authors (Lara 1997, 1998; Wanderley et al. 1997; Barbosa et al. 2000; Baldin and Lara 2008).

The dry weight of adults (DWA) ranged from 1.73 to 2.30 mg. The lowest mean weight of adults was found with the genotype Arc.1S, which was in sharp contrast with the other genotypes, such as Carioca Pitoco (Fig. 1). Baldin and Lara (2008) also recorded lower adult weights from freshly harvested seeds of the genotypes Arc.5S, Arc.1S, and Arc.3S when compared with the susceptible Carioca. Similarly, the mean body weights of immature stages and newly emerged adult *A. obtectus* that develop in arcelin-containing beans are reduced when compared with arcelin-free controls (Velten et al. 2007).

The weight of grain consumption by the larvae ranged from 0.90 (Arc.1S) to 1.60 g (Carioca Pitoco) and was not different among the genotypes. These results are similar to those found by Mazzoneto and Boiça Júnior (1999), who evaluated common bean genotypes resistant to *Z. subfasciatus*.

According to Smith (2005), because *A. obtectus* larval consumption rates of different common bean genotypes were similar, the possibility of antixenosis is reduced, and the expression of antibiosis is likely, primarily in the genotypes Arc.1S and Arc.2. For the measure of damaged pods per plant in the greenhouse test, the genotypes Arc.4, Arc.3, and Carioca Pitoco had more damage and were significantly different from the genotypes Arc.1 and Arc.1S, which had the highest numbers of preserved pods.

During field infestations, female bruchids lay eggs on mature pods, and the larvae bore into seeds upon hatching (Howe and Currie 1964; Wightman and Southgate 1982).

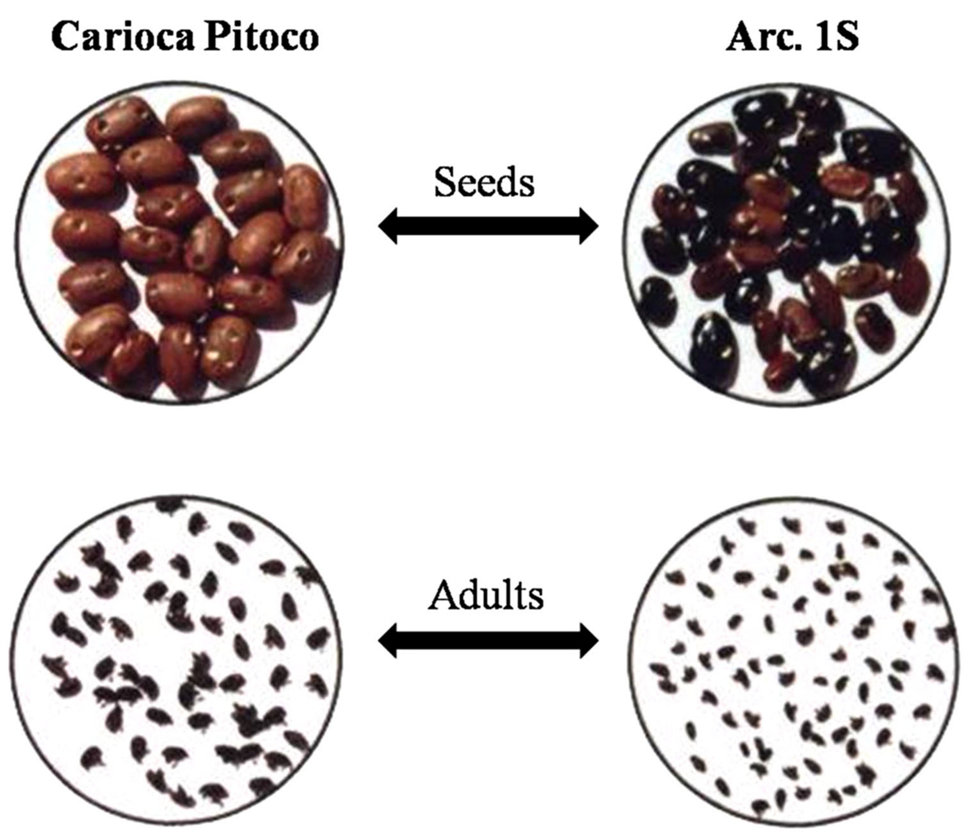


**Fig. 1** Adults of *A. obtectus* from susceptible (*left*) and antibiosis-type resistant (*right*) genotypes

This egg-laying behavior of the female results in a post-harvest infestation with serious implications for the storage of beans. Insects that multiply rapidly can cause high levels of damage and consequently, massive economic losses. Additionally, cross-infestation occurs when larvae move to non-infested grains in poor storage or sanitary conditions (Taylor 1981; Wong-Corral et al. 2013). Thus, pods could confer resistance to pest attack because of physical barriers, such as trichomes, surface waxes, and hardened tissues from sclerotinization, which are genetically regulated by biochemical processes (Apostolova et al. 2013). These authors argue that the natural resistance of bean varieties to *A. obtectus* is a complex process that is governed by several physical and chemical factors.

For the number of damaged grains per pod, the genotypes Porillo 70, Arc.1, Arc.5S, Arc.1S, and Arc.2 had the least damage, which suggested greater resistance to bean weevils. This resistance was based primarily on chemical factors, as discussed previously. However, for the Arc.1 and Arc.1S genotypes, the low percentage of damaged pods also suggested the presence of morphological resistance factors (antixenotic), such as pod surface (chemicals, trichomes, texture) and wall properties (lignified, thickened) which impose significant barriers to larval penetration (Fery and Cuthbert 1979; Fatunla and Badaru 1983; Messina 1984; Birch et al. 1989; Jackai et al. 2001, Clement et al. 2009).

Based on the results of the laboratory and greenhouse assays, the bean genotypes Arc.1, Arc.2, Arc.1S, Arc.5S, and Arc.3S are more resistant to *A. obtectus*. Because of the importance of common bean cultivation worldwide (Broughton et al. 2003) and the extended period of grain storage from harvest to consumption (Wong-Corral et al. 2013), the search for resistant genotypes to the bean weevil is of great importance. Although plant resistance to insects is an IPM strategy, few studies have characterized the lines of common bean that are resistant to this bruchid.

Thus, the determination of resistant genotypes described in this work could not only help reduce possible damages during cultivation and storage but also may minimize the use of synthetic insecticides, thereby decreasing their associated harmful effects. Additionally, the use of resistant genotypes might facilitate the creation of crop breeding programs to develop common bean genotypes that are resistant to *A. obtectus.*

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References


Amorim TML, Macedo LLP, Uchoa AF, Oliveira AS, Pitanga JCM, Macedo FP, Santos EA, Sales MP (2008) Proteolytic digestive enzymes and peritrophic membranes during the development of *Plodia interpunctella* (Lepidoptera: Piralidae): targets for the action of soybean trypsin inhibitor (SBTI) and chitin-binding vicilin (EvV). J Agric Food Chem 56:7738–7745

Apostolova E, Palagacheva N, Svetleva D, Mateeva A (2013) Investigations on the resistance of the some Bulgarian common bean genotypes towards bean weevil (*Acanthoscelides obtectus* Say). J Cent Eur Agric 14:1530–1540

Baker BP, Benbrook CM, Groth E, Lutz BK (2002) Pesticide residues in conventional, integrated pest management (IPM)-grown and organic foods: insights from three US data sets. Food Addit Contam 19:427–446

Baldin ELL, Lara FM (2004) Effect of storage temperature and bean genotypes on the resistance to *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Neotrop Entomol 33:365–369

Baldin ELL, Lara FM (2008) Resistance of stored bean varieties to *Acanthoscelides obtectus* (Coleoptera: Bruchidae). Insect Sci 15:317–326

Barbosa FR, Yokoyama M, Pereira PAA, Zimmermann FJP (1999) Effect of arcelin protein on the biology of *Zabrotes subfasciatus* (Boheman 1833) in dry beans. Pesqui Agropecu Bras 34:1805–1810

Barbosa FR, Yokoyama M, Pereira PAA, Zimmermann FJP (2000) Damage of *Zabrotes subfasciatus* (Boh.) (Coleoptera: Bruchidae) on common beans (*Phaseolus vulgaris* L.) lines containing arcelin. An Soc Entomol Bras 29:113–121

Birch ANE, Simmonds MSJ, Blaney WM (1989) Chemical interactions between bruchids and legumes. Mongr Syst Bot Missouri Bot Gard 29:781–809

Blair MW, Muñoz C, Buendía HF, Flower J, Bueno JM, Cardona C (2010) Genetic mapping of micro satellites markers around the arcelin bruchid resistance locus in common bean. Theor Appl Genet 121:393–402

Broughton WJ, Hernández G, Blair M, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (*Phaseolus* spp.)—model food legumes. Plant Soil 252:55–128

Cardona C, Posso CE, Kornegay J, Valor J, Serrano M (1989) Antibiosis effects of wild dry bean accessions on the mexican bean weevil and the bean weevil (Coleoptera: Bruchidae). J Econ Entomol 82:310–315

Cardona C, Kornegay J, Posso CE, Morales F, Ramirez H (1990) Comparative value of four arcelin variants in the development of dry bean lines resistant to the Mexican bean weevil. Entomol Exp Appl 56:197–206

Carlini CR, Grossi de Sá MF (2002) Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon 40:1515–1539

Chrispeels MJ, Raikhel NV (1991) Lectins, lectins genes and their role in plant defense. Plant Cell 3:1–19

Clement SL, Mcphee KE, Elberson LR, Evans MA (2009) Pea weevil, *Bruchus pisorum* L. (Coleoptera: Bruchidae), resistance in *Pisum sativum* × *Pisum fulvum* interspecific crosses. Plant Breed 128:478–485

Coelho SRM, Prudencio SH, Christ D, Sampaio SC (2011) Storage-induced changes in protein interactions and protein solubility in common beans. J Food Agric Environ 9:44–48

Dobie P, Dendy J, Sherman C, Padgham J, Wood A, Gatehouse AMR (1990) New sources of resistance to *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* Boheman (Coleoptera: Bruchidae) in nature seeds of five species of *Phaseolus*. J Stored Prod Res 26:177–186

Fatunla T, Badaru K (1983) Resistance of cowpea pods to *Callosobruchus maculatus* Fabr. J Agric Sci 100:205–209

Fery RL, Cuthbert FP Jr (1979) Measurement of pod-wall resistance to the cowpea curculio in the southernpea (*Vigna unguiculata* (L.) Walp.). Hort Sci 14:29–30

Franco OC, Melo FR, Silva MCM, Grossi de Sá MF (1999) Resistência de plantas a insetos: inibidores de enzimas digestivas e a obtenção de plantas resistentes. Biotecnologia Cienc Desenvolv 2:36–40

Gatehouse AMR, Dobie P, Hodges RJ, Meik J, Pusztai A, Boulter D (1987) Role of carbohydrates in insect resistance in *Phaseolus vulgaris*. J Insect Physiol 33:843–850

Gepts P (1999) Development of an integrated genetic linkage map in common bean (*Phaseolus vulgaris* L.) and its use. In: Singh S (ed) Bean breeding for the 21st century. Kluwer, Dordrecht, pp 53–91

Grossi de Sá MF, Chrispeels MJ (1997) Molecular cloning of bruchid (*Zabrotes subfasciatus*) α-amylase cDNA and interactions of the expressed enzyme with bean amylase inhibitors. Insect Biochem Mol Biol 27:271–281

Gusmão NMS, Oliveira JV, Navarro DM, Dutra KA, Silva WA, Wanderley MJA (2013) Contact and fumigant toxicity and repellency of *Eucalyptus citriodora* Hook., *Eucalyptus staigeriana* F., *Cymbopogon winterianus* Jowitt and *Foeniculum vulgare* Mill. essential oils in the management of *Callosobruchus maculatus* (Fabr.) (Coleoptera: Chrysomelidae, Bruchinae). J Stored Prod Res 54:41–47

Herget T, Schell J, Schreier PH (1990) Elicitor-specific induction of one member of the chitinase gene family in *Arachis hypogaea*. Mol Gen Genet 224:469–476

Howe RW, Currie JE (1964) Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. Bull Entomol Res 55:437–477

Ishimoto M, Chrispeels MJ (1996) Protective mechanism of the Mexican bean weevil against high levels of α-amylase inhibitor in the common bean. Plant Physiol 111:393–401

Jackai LEN, Nokoe S, Tayo BO, Koona P (2001) Influences on pod wall and seed defenses against the brown cowpea coreid bug, *Clavigralla tomentosicollis* Stal. (Hem., Coreidae), in wild and cultivated *Vigna* species. J Appl Entomol 125:277–286

Kasahara K, Hayashi K, Arakawa T, Philo JS, Wen J, Hara S, Yamagushi H (1996) Complete sequence, subunit structure, and complexes with pancreatic α-amylase of an α-amylase inhibitor from *Phaseolus vulgaris* white kidney beans. J Biochem 120:177–183

Kemabonta K, Odebiyi A (2005) Susceptibility of the life stages of *Callosobruchus maculatus* (Coleoptera: Bruchidae) to diflubenzuron in cowpea seeds. J Plant Dis Prot 112:193–199

Kogan M (1975) Plant resistance in pest management. In: Metcalf RL, Luckmann WH (eds) Introduction to insect pest management. Wiley, New York, pp 103–146

Kuroda M, Ishimoto M, Suzuki K, Kondo H, Abe K, Kitamura K, Arai S (1996) Oryzacystatins exhibit growth inhibitory and lethal effects on different species of bean insect pests *Callosobruchus chinensis* (Coleoptera) and *Riptortus clavatus* (Hemiptera). Biosci Biotechnol Biochem 60:209–212

Lara FM (1997) Resistance of wild and near isogenic bean lines with arcelin variants to *Zabrotes subfasciatus* (Boheman). I. Winter crop. An Soc Entomol Bras 26:551–560

Lara FM (1998) Resistance to *Zabrotes subfasciatus* (Boheman) in bean genotypes containing arcelin. III. Dry season. Cult Agron 7:25–40

Lorini I (1998) Controle integrado de pragas de grãos armazenados. Embrapa, Passo Fundo

Macedo MLR, Andrade LBS, Moraes RA, Xavier- Filho J (1993) Vicilin variants and the resistance of cowpea (*Vigna unguiculata*) seeds to the cowpea weevil (*Callosobruchus maculatus*). Comp Biochem Physiol 105C:89–94

Mazzoneto F, Boiça Júnior AL (1999) Determination of the types of resistance of bean genotypes to the attack of *Zabrotes subfasciatus* (Boh.) (Coleoptera: Bruchidae). An Soc Entomol Bras 28:307–311

Mazzoneto F, Vendramim JD (2002) Biological aspects of *Zabrotes subfasciatus* (Boh.) (Coleoptera: Bruchidae) on bean genotypes with and without arcelin. Neotrop Entomol 31:435–439

Messina FJ (1984) Influence of cowpea pod maturity on the oviposition choices and larval survival of a bruchid beetle *Callosobruchus maculatus*. Entomol Exp Appl 35:241–248

Minney BHP, Gatehouse AMR, Dobie P, Dendy CC, Gatehouse JA (1990) Biochemical bases of seed resistance to *Zabrotes subfasciatus* (bean weevil) in *Phaseolus vulgaris* (common bean); a mechanism for arcelin toxicity. J Insect Physiol 36:757–767

Murdock LL, Shade RE, Pomeroy MA (1988) Effects of E-64, a cysteine proteinase-inhibitor, on cowpea weevil growth, development, and fecundity. Environ Entomol 17:467–469

Osborn TC, Blake T, Gepts P, Bliss FA (1986) Bean arcelin 2. Genetic variation, inheritance and linkage relationships of a novel seed protein of *Phaseolus vulgaris* L. Theor Appl Genet 71:847–855

Osborn TC, Alexander DC, Sun SSM, Cardona C, Bliss FA (1988) Insecticidal activity and lectin homology of arcelin seed protein. Science 240:207–210

Painter RH (1951) Insect resistance in crop plants. McMillan, New York

Panda N, Khush GS (1995) Host plant resistance to insects. CAB International, Oxon

Romero Andreas J, Yandell BS, Bliss FA (1986) Bean arcelin. 1. Inheritance of a novel seed protein of *Phaseolus vulgaris* L. and its effect on seed composition. Theor Appl Genet 72:123–128

Sakthivelkumar S, Jesse MI, Veeramani V, Ramaraj P, Kathiravan K, Arumugama M, Janarthanana S (2013) Diversity and analysis of sequences encoded by arcelin genes from Indian wild pulses resistant to bruchids. Process Biochem 48:1697–1705

Sales MP, Gerhardt IR, Grossi de Sá MF, Xavier-Filho J (2000) Do legume storage proteins play a role in defending seeds against bruchids? Plant Physiol 124:515–522

SAS Institute (2002) SAS/STAT user's guide, version 8.1. SAS Institute, Cary

Sathe SK, Deshpande SS, Salunkhe DK (1984) Dry beans of *Phaseolus*. A Review. Part 1. Chemical composition: proteins. Crit Rev Food Sci Nutr 20:1–46

Schmale I, Wackers FL, Cardona C, Dorn S (2002) Field infestation of *Phaseolus vulgaris* by *Acanthoscelides obtectus* (Coleoptera: Bruchidae), parasitoid abundance, and consequences for storage pest control. Environ Entomol 31:859–863

Schoonhoven AV, Cardona C (1982) Low levels of resistance to the Mexican bean weevil in dry beans. J Econ Entomol 75:567–569

Schoonhoven AV, Cardona C (1986) Main insect pests of stored beans and their control. CIAT, Cali

Shade RE, Pratt RC, Pomeroy MA (1987) Development and mortality of the bean weevil, *Acanthoscelides obtectus* (Coleoptera: Bruchidae), on mature seeds of tepary beans, *Phaseolus acutifolius*, and common beans, *Phaseolus vulgaris*. Environ Entomol 16:1067–1070

Smith CM (2005) Plant resistance to arthropods. Springer Science & Business, Dordrecht

Smith CM, Clement SL (2012) Molecular bases of plant resistance to arthropods. Annu Rev Entomol 57:309–328

Taylor TA (1981) Distribution, ecology and importance of bruchids attacking grain legumes in Africa. In: Labeyrie V (ed) The ecology of bruchids attacking legumes (pulses). Springer Science & Business Media, Dordrecht, pp 199–203

Velten G, Rott AS, Cardona C, Dorn S (2007) The inhibitory effect of the natural seed storage protein arcelin on the development of *Acanthoscelides obtectus*. J Stored Prod Res 43:550–557

Walter A (1992) Un frijol silvestre protege los cultivos sin praguicidas. CIAT Int 11:3–4

Wanderley VS, Oliveira JV, Andrade MLA Jr (1997) Resistance of cultivars and lines of bean, *Phaseolus vulgaris* L., to *Zabrotes subfasciatus* (Boh.) (Coleoptera: Bruchidae). An Soc Entomol Bras 26:315–320

Weber E (2001) Armazenagem agrícola. Livraria e Editora Agropecuária, Guaíba

Wightman JA, Southgate BJ (1982) Egg morphology, host, and probable regions of origin of the bruchids (Coleoptera: Bruchidae) that infest stored pulses—an identification aid. N Z J Exp Agric 10:95–99

Wong-Corral FJ, Castañé C, Riudavets J (2013) Lethal effects of $CO_2$-modified atmospheres for the control of three Bruchidae species. J Stored Prod Res 55:62–67

Zaugg I, Magni C, Panzeri D, Daminati M, Bollini R, Benrey B, Bacher S, Sparvoli F (2013) QUES, a new *Phaseolus vulgaris* genotype resistant to common bean weevils, contains the arcelin-8 allele coding for new lectin-related variants. Theor Appl Genet 126:647–661