# BREEDING COMMON BEANS FOR RESISTANCE TO BRUCHIDS

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## 1. Overview

The common bean weevil (*Acanthoscelides obtectus* Say) and the Mexican bean weevil [*Zabrotes subfasciatus* (Boheman)] are important storage pests of common beans (*Phaseolus vulgaris* L.) in tropical climates (Myers et al, 2021; Tigist et al., 2021). Both species belong to the chrysomelidae family, Bruchinae subfamily; the source of the generic term 'bruchid'. This subfamily includes approximately 1,650 species and is found worldwide (Yus-Ramos, 2014). The Mexican bean weevil is a serious bean pest in the Americas and East/Central Africa whereas the common bean weevil can infest beans in most tropical and subtropical regions. Back and Duckett (1918) noted that commercial bean production in the U.S. was concentrated in the northern States, in large part, to the avoid the damage caused by infestation of the common bean weevil in warmer regions of the country.

Cardona (1989) described important differences between the common bean weevil and the Mexican bean weevil. The Mexican bean weevil is better adapted to warmer temperatures and is more common in lower altitudes of the tropics. The common bean weevil is more frequently found at higher altitudes and latitudes, although it is also a pest in some warmer climates such as Puerto Rico. Araújo Soares et al. (2015) reported optimal temperature for pre-adult development of the common bean weevil was 30° C and optimal temperature for reproduction was 24° C. Another important difference noted by Cardona (1989) is oviposition behavior. Infestation and damage by the Mexican bean weevil only occur in storage. The Mexican bean weevil attaches eggs to the surface of the seed and does not oviposit in the field, whereas the common bean weevil scatters unattached eggs among stored seed or infests seed in the field by laying eggs on growing pods. Both weevils are short-lived (35-45 days) and mate and oviposit soon after emergence.

Seed infestation by the Mexican bean weevil can be managed in storage with appropriate sanitation techniques such as fumigation and/or storage in bruchid-proof containers (Myers et al., 2021). The chemicals used for seed fumigation are highly toxic and too expensive for use by smallholder farmers. The common bean weevil represents a greater challenge to manage than the Mexican bean weevil because infestation can begin in the field. Harvesting as soon as the bean crop reaches maturity can help to reduce infestation of the common bean weevil in the field (Schmale et al., 2002).

The development of bruchid resistant common bean cultivars will help to reduce post-harvest losses and contribute to a more stable supply of bean seed in developing countries. Mishra

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et al. (2018) noted that the use of resistant cultivars should form the backbone of integrated bruchid pest management practices. The following is a description and review of research techniques that have been used to breed beans for resistance to both the Mexican bean weevil and the common bean weevil.

## 2. Screening methods for resistance to bruchids in common beans

a. Mexican bean weevil - *Z. subfasciatus* (Boheman)

*Z. subfasciatus* (Boheman)

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Eggs of the Mexican bean weevil on the surface of bean seed.

Source: Juan Carlos Rosas

Schoonhoven and Cardona (1982) conducted trials to evaluate expressions of resistance under different infestation levels of the Mexican bean weevil and found that infesting 50 seeds with 7 pairs of newly emerged adults provided a sufficient level of infestation to detect differences in resistance between bean lines. Single replicates of each common bean germplasm accession were infested. Each group of 100 accessions included five replicates of the susceptible check cultivar 'Diacol-Calima'. Once an estimated 50% of the adults had emerged from the susceptible check cultivar, the samples were frozen, and the number of emerged adults were counted. The most resistant lines were retested using three replicates of 50 seeds infested with seven pairs of adults. Oviposition per female, adult emergence (counted every other day), percent emergence, duration of developmental stages, and dry weight of progeny were recorded. The data were analyzed using the log (x + 0.5) transformation.

Kornegay et al. (1993) conducted a study of the inheritance of resistance to the Mexican bean weevil by infesting small containers of seed with three adult pairs of weevils for 48 h. Number

of eggs laid per seed was limited to four with excess eggs removed. About six days after infestation, individual seeds were examined to count the number of larvae that had hatched and penetrated the seed coat. The number of hatched eggs, number of emerged adults, and days to adult emergence from oviposition were recorded for each seed. The data were used to determine percentage adult emergence and to calculate a reproductive index (RI) based on number of emerged adults, number of hatched eggs and days to adult emergence.

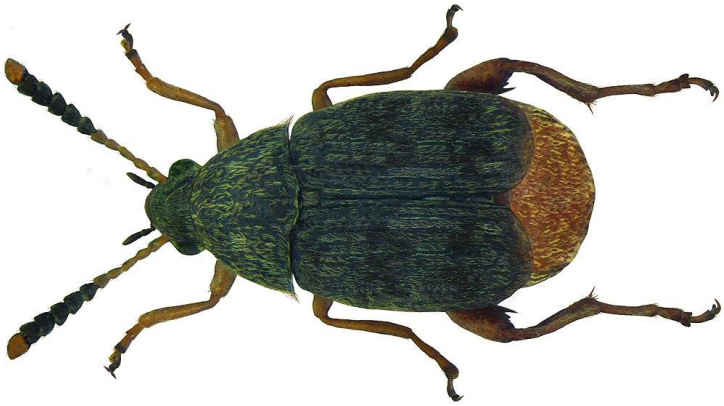
Blair et al. (2010) screened common bean lines for Mexican bean weevil resistance by infesting 15 seeds of each line with nine pairs (female and male) of adults from an insect colony maintained at CIAT. The seed was evaluated in mesh-covered, clear plastic vials (9-cm high × 1.7 cm in diameter). The walls of the vials were covered with sandpaper (No. 150) facing inward to prevent adults from laying eggs on the plastic surface of the container rather than the bean seed coat. Three replications of each line were evaluated in a rearing chamber at 27°C and 70% RH. Adults were removed 5 days after infestation when the counting of the number of eggs laid was initiated. The number of emerged adults was then evaluated over a 70-day period. The percentage of adult emergence was calculated based on the total number of adults emerged relative to the number of eggs laid.

Tigist et al. (2021) screened bean lines in Ethiopia for resistance to the Mexican bean weevil. Adult weevils were collected from infested seed to initiate the infestation and an unidentified susceptible bean cultivar was used to develop and maintain the colony. The mass rearing of the bruchid was conducted under an average room temperature of 27°C and a relative humidity of 70%. The bean seed to be evaluated was frozen at -20°C for four weeks to eliminate any prior infestation of weevils. Fifteen seeds of each bean line were placed in 6 cm x 7 cm transparent plastic jars. The lids of the plastic jars were perforated to provide ventilation and covered with mesh to prevent the escape of weevils. Each jar was infested with three pairs (female and male) of newly emerged adult weevils. A randomized complete block design (RCBD), with three replications was used. Ten days after infestation, the adult weevils were removed and the number of eggs on the surface of the seed was counted. The jars were monitored daily for the emergence of the next generation of adult weevils. After the first appearance of bruchids, the jars were monitored every 2 days, for recording purposes, and for the removal of newly emerged bruchids. The number of eggs per adult female (NE), the number of adult bruchids emerged (NAE) and seed damaged (% of seed with holes) were recorded. The percentage adult weevil emergence (PAE) was calculated, based on the total number of adults emerged compared with the number of eggs laid. The level of resistance of the bean lines was based on the percentage of adult emergence as described by Blair et al. (2010). The genotypes with adult emergence from 0-15% were classified as highly resistant (HR), those from 15-30% as resistant (R), those from 30-50% as intermediate resistance (IR) and those from 50-100% as susceptible (S). To ensure the homogeneity of variance, the data, based on count and percentage values, were transformed using natural log and arcsine transformation, respectively.

b. Common bean weevil - (*Acanthoscelides obtectus* Say)

Damage to bean seed caused by the common bean weevil.

Source: James Beaver



*Acanthoscelides obtectus* Say
Source: Udo Schmidt, CC BY-SA 2.0 <https://creativecommons.org/licenses/by-sa/2.0>, via Wikimedia Commons

Kornegay and Cardona (1991) conducted an inheritance study by screening individual seeds for reaction to the common bean weevil. Individual seeds were infested in small glass vials with three eggs. Glass balls (0.5 mm diameter) were mixed with the seed to improve larval penetration and prevent escapes. The number of adults that emerged from each seed was recorded and were collected as they emerged, oven-dried (24 hours at 50°C) and weighed. The CIAT breeding program evaluated at least 200 individual $F_2$ seed to be able to identify a few highly resistant segregants and suggested the development of a serological test for routine screening after more was learned about the biochemical basis of this resistance. It should be noted that the availability of effective molecular markers associated with common bean weevil resistance would also allow early generation selection for resistance.

Kusolwa and Myers (2011) multiplied and maintained a colony of the common bean weevil in Oregon using seed of the susceptible cultivar 'Rojo' to produce adult weevils for infestation. In screening for resistance, 15 adults were placed into glass containers containing 20-30 seeds. The caps of the containers were not completely closed to allow aeration but prevented the escape of adult weevils. The containers containing seeds and adult insects were placed in incubation trays and remained undisturbed for 12 days at 25 ± 3°C and ambient relative humidity, until the first adult emerged. After 12 days, the number of eggs laid was estimated using a magnifying lens, and adults were removed. In cases where no eggs were visible, samples were re-inoculated with new adults. The presence of a powdery/floury appearance on the surface of the beans was noted to confirm larvae penetration into the seeds and to identify escapes from infestation. The emergence of adults in each glass container was inspected daily and counted until 72 days after infestation.

Data were collected included the total number of adult weevils emerged after infestation, number of days for first adult emergence (DAE), number of days for 50% of total adults emerged, number of perforated seeds, severity of damage expressed by the number of seeds with 5 or more holes and percent seed weight loss. Frequency of adult emergence was determined by the total number adults emerged per day for the period of 72 days.

In Puerto Rico, Beaver et al. (2023) maintained a population of the common bean weevil by infesting a mixture of seed of the susceptible common bean cultivars 'Verano' and 'Badillo'. One quart (946 cc) Mason jars were partially filled with seed. The metal seals of the caps of the Mason jars were replaced with metal screens to allow aeration. At least 100 adults were placed in each Mason jar to maintain the colony and avoid inbreeding in the population. A layer of petroleum jelly was applied at the base of the plastic box containing the Mason jars to prevent predation of the weevils by ants and other insects. The days to the first emergence of adults varied depending on the ambient temperature. Screening began when large numbers of adults emerged, usually about 45 days after infestation. Grain dockage sieves with holes larger than the weevils but smaller than the bean seed were used to easily separate the adult weevils from the bean seed. Tapping the metal base of the sieve made the adults weevils temporarily quiescent and facilitated the handling of the insects. Placing the adults in a refrigerator for a short period of time also temporarily reduced their mobility. At the time of emergence of adults, new Mason jars of clean seed were infested with weevils. This helped to avoid the build-up of populations of pests such as mites that can be harmful to weevils.


Mason jars and small plastic containers are used in Puerto Rico to screen bean lines for resistance to the common bean weevil. Source: James Beaver

The technique used by Kusolwa et al. (2016) to screen beans for bruchid resistance involved infesting beans with at least one adult weevil per seed. Adult weevils were placed in 118-$cm^3$ plastic containers containing 10-25 seeds of each line. It is highly unlikely that females would be absent when infesting a container with at least 10 adults. The caps of the containers had 2 cm diameter holes covered by screens to allow aeration. The white bean cultivars 'Verano' and

'Morales' were included as susceptible checks. Two replications per bean line were used for the screening. Thirty days after infestation, the dead adults from the initial infestation were removed from each container. Seed of susceptible lines can show translucent panels of perforations at 30 days after infestation. In this case, the number of seeds with and without perforations were counted in each container. Additional evaluations of damaged seed were conducted between 45 and 60 days after infestation. In some studies, initial seed weight and seed weight at 60 DAI were measured to calculate percentage seed weight loss.

Schmale et al. (2002) sampled bean seed from small-scale farms over a 3-year period in the Cauca Valley of Colombia. Infestation of the seed with the common bean weevil was frequent (~ 90%) although the average initial level of infestation was only 16 weevils per 1000 seed. Emergence of adult weevils in the samples occurred over a three-week period with a normal distribution which suggested that initial infestation occurred during the last few weeks before harvest.

When screening beans for resistance, infesting seed at a rate of one adult common bean weevil per seed is a much higher level than expected under natural conditions. For a 100 seed weight of 20 g, this represents an initial density of 5,000 adults per kg of seed. At these high rates of infestation, genotypes that show no damage at 30 days after infestation are considered in Puerto Rico to have useful levels of resistance.

Screening seed in the laboratory for resistance does not allow the identification of mechanisms of resistance to field infestation of the common bean weevil. It also does not provide a measure of the effectiveness of resistance to what would be expected to be much lower levels of infestation shortly after the harvest of beans. Thus, a study was conducted in Puerto Rico over three seasons to measure the level of seed damage due to natural infestation after 90 days of storage. Samples of up to 100 g of seed were taken from each experimental unit shortly after harvest of field trials and placed in 946 cc plastic food storage containers from which the weevils could not enter or escape. After 90 days of storage, the percentage of damaged seed was measured. Resistant lines had a significantly lower frequency and level of seed damage than susceptible cultivars (Beaver et al., 2024).

Baldin et al. (2017) maintained a population of the common bean weevils in a chamber (25 ± 2° C, RH = 70 ± 10%, and photoperiod of 12:12 h L:D) to supply adults for bean screening trials in Brazil. Transparent glass flasks (750 mL) were used for rearing the weevil colony. The flasks were closed at the top with a screw-on lid with a circular opening covered with a fine-mesh nylon screen for aeration. Each flask contained approximately 0.3 kg of the susceptible bean cultivar 'Bolinha' and approximately 300 unsexed adults. Emerging adults from the seed in the flasks were periodically sifted and used to re-infest seed to maintain the colony.

In trials to screen bean lines for resistance, Baldin et al. (2017) used transparent plastic containers (5.0 cm height x 3.0 cm diameter) with fitted lids. 10 g of seed of each bean line was placed in a container. Each plastic container was infested with six adult weevils that were no more than 48 h old. The containers were capped and placed in a chamber. At 7 days after initial infestation, both dead and alive adult weevils were removed from the plastic containers. Beginning

15 days after initial infestation, the seed in the plastic containers were evaluated daily to observe the developing weevils. The seed in each container was sifted with a sieve, and the number of emerged insects was recorded. The emerged adults were placed in small glass vials (5.0 cm height x 2.2 cm diameter) and were immediately frozen to avoid the loss of weight. At the end of the emergence period, the weevils in the small glass vials were oven-dried at 50° C for 2 days and weighed. The experimental design was completely randomized with eight replications per bean line. Each plastic container was an experimental unit. The Carioca cultivar 'Pitoco' was used as a susceptible check and a line having Arcelin 2 was used as a resistant check (Baldin and Lara, 2004). The total number of eggs was counted with a stereoscopic microscope at 20 days after initial infestation to determine ovipositional preference. 25 days after the initial infestation, the numbers of emerged adults and the periods of development (egg-adult) were also determined. The dry weights of the weevils and the consumption of seed were measured when the emergence of the adults was complete. The seed in the vials at the end of emergence were oven-dried at 50° C for 2 days and weighed. The initial and final weights of the seed were adjusted according to the weights of the controls, and the difference in dry weight (consumption) was calculated.

Baldin et al. (2017) also conducted a greenhouse trial to evaluate natural infestation of the common bean weevil. Eight replicates were used per genotype in a completely randomized design. The experimental unit was an individual plant in a pot. After the plants in the greenhouse had reached harvest maturity, a general cleaning was performed on each pot by removing dried leaves, petioles, and branches. The number of pods/plants was counted, and the plants were individually caged within tubular metallic structures (35 cm diameter x 60 cm height). Two Adult (48 h old) common bean weevils per pod were released in each cage. The tubular structures were covered with organdy fabric to prevent the escape of weevils. 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). Sixty days after the initial infestation, the bags were opened, and the pods and seed of the different bean lines were evaluated. The variables analyzed were the number of pods per plant, number of damaged pods per plant, number of seeds per pod, and percentage of damaged seeds per pod.

Li et al. (2021) used an indoor weevil infestation method to screen common bean lines for resistance to the common bean weevil. The common bean weevils used in the study were collected from several locations in the Yunnan Province of China where severe bruchid damage had been observed. Two petri dishes of each line containing 20 seeds were screened for resistance. The seed of each bean line, and susceptible and resistant checks, were placed into 9-cm-diameter petri dishes and randomly placed on shelves. All the shelves were covered with a fine net. Thousands of the collected common bean weevil adults (male and female) were placed inside the net to mate freely to ensure that all the seeds were infested under the same conditions. The room temperature was maintained at 18 ~ 25 °C. The humidity was maintained at approximately 70%. After 100 days of exposure to the common bean weevil, the level of damage of each bean line was determined. The percentage of damaged seeds (PDS) and the total number of perforations per experimental unit were calculated. It should be noted that 100 days of exposure is sufficient for two generations of the weevil to be completed.

## 3. Sources of resistance to bruchids

### a. Common beans (*Phaseolus vulgaris*)

Schoonhoven and Cardona (1982) screened at CIAT more than 4,000 accessions of common beans for resistance to the Mexican bean weevil. Although significant differences among genotypes were observed using different resistance criteria, the levels of resistance were considered to be too low to have economic value.

The complex APA locus in some small-seeded wild common bean germplasm accessions includes genes for the seed proteins Arcelin (Arc), the lectin Phytohemagglutinin (PHA) and α-Amylase Inhibitor (α-AI) that provide insecticidal properties (antibiosis) to bean seed by reducing larval development and insect fertility and growth (Blair et al., 2010). Cardona (1989) reported that these wild common bean germplasm accessions possess different variants of the arcelin gene. Sources of the eight different variants include G 12882, G 24390 (*Arc-1*); G 12866 (*Arc-2*); G 12891, G 12895 & G 12942 (*Arc-3*); G 24371, G 24370, G 24369, G 24368, G 23676, G 23675, G 24391, G 12949, G 12952 & G 12953 (*Arc-4*), G 02771 (*Arc*-5), G 11051 (*Arc*-6), G 24582, G 24584 (*Arc-7*). A wild bean collected in Mexico identified as QUES was reported to the have *Arc-8* and resistance to both the Mexican and common bean weevil (Zaugg et al., 2012). Unfortunately, this report has limited value to bean breeders because no additional information was provided on how to obtain this potentially valuable genotype. Zaugg et al., (2012) did note that QUES had APA components like G12949 (*Arc-7*).

The different variants of arcelin have different effects on the Mexican bean weevil and confer distinct levels of resistance (Kornegay et al., 1993; Acosta et al., 1998; Tigist et al., 2021). Hartweck et al. (1997b) noted that the ranking of resistance of arcelin types to the Mexican bean weevil varied between studies. It was not determined if these differences were due to variability in virulence between biotypes of the Mexican bean weevil or to differences in screening techniques. The presence of the arcelin protein is inherited as a single dominant trait, where the homozygous state provides a higher level of resistance to bruchids (Blair et al., 2010).

Kornegay and Cardona (1991) reported that the wild bean accession G12492 (*Arc 4*) had high levels of resistance to the common bean weevil. Antibiosis resulting in delayed and reduced adult emergence, high mortality of late first instar larvae and reduced female fecundity were identified as the mechanisms of resistance. Results from an inheritance study suggested that resistance was conferred by two recessive complementary genes. Baldin et al. (2017) identified *Arc-1S* to have a strong antibiosis to the common bean weevil that resulted in a delay in the development of immature stages and a reduction in the number of emerged adults.

Bean breeders at CIAT developed numerous RAZ (Resistant to *Zabrotes subfasciatus*) bean breeding lines by backcrossing *Arc-1* into different market classes of beans (Cardona et al, 1990). Marker-assisted selection was used to develop MAZ lines. Tigist et al. (2018) reported that RAZ-11, RAZ-36, RAZ-2, RAZ-44, RAZ-120, RAZ-40 and MAZ-203 had high levels of resistance to the Mexican bean weevil in Ethiopia. Tigist et al. (2021) developed the Mexican bean weevil

resistant common beans using the Mesoamerican line RAZ 168 as the source of resistance. Broad sense heritability of traits related to Mexican bean weevil resistance ranged from 68.5%–93.9% and several breeding lines having high levels of resistance were identified.

Osborn et al. (2003) utilized the recurrent parents 'Sanilac' and 'Porillo 70' and backcrossing to develop white (SARC) and black (PARC) bean lines having different arcelin variants (*Arc-1*, *Arc-2*, *Arc-3*, *Arc-4*). The lines with *Arc-1* from the wild bean germplasm G12882, SARC1 and PARC1, expressed high levels of resistance to the Mexican bean weevil but low levels of resistance to the common bean weevil (Harmsen, 1989). In an attempt to increase arcelin content in the seed, SARC1 was crossed with the common bean breeding line MB11-29 that lacked phaseolin in the seed due to the introgression of a recessive allele from *P. coccineus* (Hartweck et al., 1997b). The breeding line SMARC-PN1 (PI 628627) from this cross expressed moderate levels of resistance to the common bean weevil and high levels of resistance to the Mexican bean weevil. The seed storage protein (phaseolin) deficiency found in SMARC-PN1 was reported by Taylor et al. (2008) to have resulted in improved sulfur amino acid content. The bruchid resistant germplasm line AO1012-29-3-3A which combines arcelin resistance from the wild tepary bean accession G40199 and the seed storage protein deficiency of SMARC-PN1 also showed higher levels of sulfur amino acids in the seed (Kusolwa et al., 2016).

Baldin et al. (2017) reported that in the field, female common bean weevils lay eggs on mature pods producing larvae that bore into seeds. They suggested that physical barriers, such as trichomes, surface waxes, and hardened or thicker tissues in pod walls may provide partial resistance.

In the highlands of Central America, another pest, the bean pod weevil (*Trichapion godmani* Wagner), lays eggs in the mesocarp of pods that produce larvae that burrow into immature bean seed (Cardona, 1989). It has not been determined if resistance mechanisms found in bean genotypes selected for resistance to the bean pod weevil (Blair et al. 2016; Garza et al., 2001) might provide some protection against field infestation of the common bean weevil and vice versa.

**b. Tepary beans (*Phaseolus acutifolius*)**

Mbogo et al. (2009) reported that the wild tepary bean line G40199 was resistant to both the common and Mexican bean weevil. This source of resistance was successfully used to develop common bean breeding lines with enhanced levels of resistance to this pest (Kusolwa et al., 2016).

Porch and Beaver (2022) screened thirty-four cultivated and 122 wild tepary bean genotypes from the tepary diversity panel (TDP) for resistance to the common bean weevil. All the cultivated common bean and tepary beans were susceptible at 60 days after infestation (DAI) whereas seven wild genotypes in the TDP had ≤ 10% damaged or soft seed at 90 DAI. Loss in initial seed weight ranged from 0.0-3.0% among the resistant wild tepary genotypes. The authors noted that valuable genetic diversity for bruchid resistance may have been lost during domestication of tepary bean. Introgression of this high level of resistance into common bean may be possible using bridging-parents that facilitate interspecific crosses (Barrera et al., 2020). The

wild tepary bean germplasm lines G40199, G40087 and G40253A were reported by Bornowski et al. (2023) to exhibit no damage by the common bean weevil at 60 days after infestation. Results from a GWAS conducted by Boronoski et al. (2023) found bruchid resistance to be associated with two cupin-1 domains on Pa07. Introgression of this novel source of resistance from tepary beans into common bean may be useful to complement the bruchid resistance conferred by the APA locus.

## 4. Conventional plant breeding techniques

Breeding for bruchid resistance in common bean using conventional techniques requires a long-term commitment. Simple backcrossing of arcelin variants in the APA locus has not proven effective in introgressing high levels of resistance to bruchids into common beans. Results from breeding efforts and QTL analyses suggest that additional genetic factors may contribute to high levels of resistance to bruchids (Mateo, 2016; Kamfwa et al., 2018). Although wild common bean lines containing *Arc-4* were reported to be highly resistant to the common bean weevil (Cardona et al. 1989), backcrossed common bean lines having *Arc-4* had low levels of resistance to this weevil (Harmsen, 1989). Kamfwa et al. (2018) reported that only 7% of the RILs derived from crosses with AO-1012-29-3-3A had high levels of resistance to the common bean weevil. This result is consistent with a low recovery rate of resistance to the common bean weevil in a cross between resistant wild common bean and cultivated common bean (Kornegay and Cardona, 1991). Similar low recovery rates of resistance have been obtained when crossing AO-1012-29-3-3A with ~13 African Andean bean landraces, with only 5% of the progenies being resistant (Mazala, 2023). Therefore, successful breeding for resistance to the common bean weevil may require the screening of larger populations of common beans and evaluations for resistance in later generations.

After selecting beans for highly heritable traits such as seed type, relative maturity and growth habit, breeding lines could be initially screened for bruchid resistance in the $F_4$ or later generations without replications but using multiple susceptible checks to confirm that the weevils uniformly infested the seed samples. The smaller number of lines expressing resistance can be confirmed in the following generations in replicated trials. Several cycles of selection may be necessary to overcome linkage drag in breeding for bruchid resistance that can result in progeny having lower yield potential or poor agronomic characteristics (Blair et al., 2010).

**Table 1**. Summary of sources of resistance to bruchids.

| Identity | Species | Source(s) of resistance | Bruchid | Reference |
|---|---|---|---|---|
| G12882, G24390 | *P. vulgaris* | *Arc-1* | *Z. subfasciatus* | Cardona (1989) |
| RAZ-168 | " | *Arc-1* | " | Tigist et al. (2018) |
| G12866 | " | *Arc-2* | " | Cardona (1989) |
| G12891, G12895, G12942 | " | *Arc-3* | " | " |
| G24371, G24370, G24369, G24368, G23676, G23675, G24391, G12949, G12952, G12953 | " | *Arc-4* | " | " |
| G02771 | " | *Arc-5* | " | " |
| G11051 | " | *Arc-6* | " | " |
| G24582, G24584 | " | *Arc-7* | " | " |
| SMARC-PN1 (PI 628627) | " | *Arc-1,* lack of phaseolin from an interspecific cross with *P. coccineus* | *Z. subfasciatus*, *A. obtectus* | Osborn et al. (2003) |
| G 40199 | *P. acutifolius* | APA complex locus on Pv04 | *Z. subfasciatus*, *A. obtectus* | Mbogo et al. (2009) |
| AO1012-29-3-3A (red kidney) | Interspecific (Pv x Pa) | G 40199, SMARC-PN1 | *Z. subfasciatus*, *A. obtectus* | Kusolwa et al. (2016) |
| PR1303-129 (black), PR1743-44 (small red) | *P. vulgaris* | AO1012-29-3-3A | *Z. subfasciatus*, *A. obtectus* | Beaver et al. (2024) |
| SUA-Red (dark red kidney), SUA-Karanga (light red kidney), SUA-Rosa (red speckled kidney) | *P. vulgaris* | AO1012-29-3-3A | *Z. subfasciatus*, *A. obtectus* | Myers et al. (2021) |
| Yellow and red mottled Andean bean breeding lines | *P. vulgaris* | AO1012-29-3-3A | *Z. subfasciatus*, *A. obtectus* | Mazala (2023) |
| G40199, G40087, G40253A | Wild *P.acutifolius* | Two cupin-1 domains on Pa07 | *A. obtectus* | Bornowski et al. (2023) |

Phenotypic screening of beans for resistance requires the maintenance of a colony of weevils. To avoid the buildup of pests and waste products in the colony, recently emerged adults should be transferred into containers with clean and uninfested seed. Sufficient numbers of adults should be transferred to avoid negative effects of genetic drift. Ideally, adults collected from the field can be occasionally added to the weevil colony to avoid inbreeding depression in the population. There is scarce information available regarding the population structure and genetic diversity of these bruchid species (e.g., biotypes). Ideally, promising breeding lines should be tested across different locations to ensure the identification of broad levels of resistance.

## 5. Mechanisms of resistance and molecular markers

Ishimoto et al. (1995) noted that the APA locus in cultivated bean genotypes have genes coding only for phytohaemagglutinin (PHA) and alpha-amylase inhibitor (α-AI) and are not resistant to the common and Mexican bean weevils. Blair et al. (2010) observed that the lack of success in breeding for bruchid resistance utilizing arcelin from the APA locus in wild beans may be due to a lack of understanding about the mechanisms of resistance and the organization of the APA locus. The complex APA locus in bean genotypes possessing arcelin can have gene duplications and deletions (Lioi et al. 2003) and copy number could be a factor in genetic resistance.

Blair et al. (2010) used phenotypic data from an Andean population segregating for *Arc-1* that showed a highly significant association with markers in the region of the APA locus where bruchid resistance was measured by the percentage of Mexican bean weevil adult emergence (PAE). The presence of the arcelin protein is inherited as a single dominant trait whereas the homozygous genotypes possess higher levels of resistance to bruchids (Kornegay et al. 1993). Blair et al. (2010) identified co-dominant and other microsatellite markers from the APA region that are potentially useful for marker-assisted selection for *Arc-1.* He noted that these markers may not be effective for other arcelin genes.

Using a gene-based approach, Mazaheri (2018) developed an α-AI-1 INS45 indel marker based on the annotated sequence of the α-amylase inhibitor gene linked to the complex APA locus, which amplified a DNA fragment that showed a 45 base pair insertion in the middle of a lectin Leg b domain. Mazala et al. (2023) reported that the α-AI indel marker was 100% accurate in identifying Andean bean lines resistant to the common bean weevil using the AO-1012- 29-3-3A source of resistance.

Kamfwa et al. (2018) identified three QTL for resistance to the common bean weevil in the interspecific line AO-1012- 29-3-3A on chromosomes Pv04 and on Pv06. One of the QTLs on Pv04 was previously reported as the arcelin, phytohemagglutinin and a-amylase, (APA) locus. Li et al. (2022) also reported a major QTL for resistance to the common bean weevil on Pv06 in a black bean germplasm accession from China encoding a bifunctional α-amylase/protease-inhibited protein. Kamfwa et al. (2018) noted that these results support previous studies (Kusolwa and Myers, 2011) that other resistance factors, in addition to arcelins encoded by the APA locus, from

wild common and tepary bean, may confer resistance to the common bean weevil. Pandurangan et al. (2016) reported that a single nucleotide polymorphism was likely introduced from *P. coccineus* into SMARC1-PN1 associated with the genotypic differences in β-phaseolin accumulation. Viscarra-Torrico et a. (2021) identified SNP markers on chromosomes Pv04 and Pv07 associated with the introgression of phaseolin and lectin deficiency into common beans.

Tigist et al. (2021) noted that laboratory screening for bruchid resistance is tedious and time-consuming, thus marker-assisted selection would be more efficient and cost effective and may allow selection for resistance in earlier generations than conventional breeding methods. Researchers at CIAT recently developed MAZ lines by crossing RAZ lines with different market classes of beans. The KASP marker BRU_00261 (Intertek ID: snpPV0007), located in the arcelin locus on the end of Pv04, was successful in identifying about 95% of the lines with resistance to the Mexican bean weevil in an $F_4$ Mesoamerican bean population derived from the cross 'SR 15 and MAZ 200' (Tigist et al., 2021).

## 6. Challenges

Bruchid resistant bean cultivars have not been widely deployed, therefore questions remain concerning the effectiveness of resistance when exposed to different biotypes of common bean and Mexican bean weevils. The bruchid resistant Andean bean germplasm AO1012-29-3-3A selected in Puerto Rico for resistance to the common bean weevil has proven to be an effective source of resistance to common bean weevil populations in Tanzania (Myers et al., 2021) and Zambia (Mazala, 2023). Beaver et al. (2024) reported that the bruchid resistant black bean breeding line PR1303-129 expressed resistance to the common bean weevil in Puerto Rico and Guatemala and the Mexican bean weevil in Honduras and the Dominican Republic.

It has also not been determined if or how quickly the weevils may develop tolerance and reproduce in the seed of resistant cultivars. Mayunga et al. (2023) found little genetic intraspecific variability among samples of the common bean and Mexican bean weevils collected in Tanzania. The authors noted that the lack of variability may be due in part to commercial trade of beans and the movement of infested seed within the country.

Molecular markers need to be effective in selection for resistance in different gene pools and market classes of beans. A better understanding of the mechanisms and genetics of sources of resistance is needed to develop more effective selection strategies. Agronomic practices such as timely harvesting and threshing of the crop and morphological traits such as cultivars having determinate (type I), or erect indeterminate (type IIA) growth habits may help to avoid exposure of the bean crop to the common bean weevil in the field and may complement genetic resistance by reducing initial pest population pressure during storage.

The potential effects of the introgression of bruchid resistance on the expression of other traits merits further study. Kusolwa et al. (2016) reported that the seed of the bruchid resistant Andean line AO-1012-29-3-3A had greater levels of threonine, proline, alanine, valine, lysine, methionine, and crude protein compared with the check cultivar 'Badillo'. Mazala et al. (2023) reported that bruchid resistance did not affect cooking time of Andean bean lines. However,

additional sensory studies (taste, texture, etc.) may be needed to ensure that these resistant varieties will not have consumer acceptability issues.

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