THE EXPRESSION AND INHERITANCE OF RESISTANCE TO *ACANTHOSCELIDES OBTECTUS* (BRUCHIDAE) IN SOUTH AFRICAN DRY BEAN CULTIVARS

by

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CHAPTER 1

INTRODUCTION

Common bean (*Phaseolus vulgaris* L) is a warm season, annual legume which consists of several types and classes. While dry beans are produced for consumption as a grain, green beans are bred and produced for the consumption of the green pods. Common bean is produced mainly in developing countries where it represents a major source of dietary protein, especially in the absence of animal or fish protein sources. In sub-Saharan Africa, beans are produced mainly by resource poor subsistence farmers. Under these low input conditions, beans are more vulnerable to attack by insects and diseases, whilst they are also influenced by environmental stress conditions such as drought and low soil fertility (Miklas *et al.,* 2006). Stored bean seed is vulnerable to attack by bean bruchids, forcing these farmers to sell the crops early in the season when prices are low.

Stored bean seed is commonly attacked by bean bruchids, leading to considerable losses in quality and quantity of the product. *Acanthoscelides obtectus*, commonly known as the common bean weevil and *Zabrotes subfasciatus*, commonly known as the Mexican bean weevil are the most important insect pests of stored bean seed (Schoonhoven & Cardona, 1986; Kornegay & Cardona, 1991a; Parsons & Credland, 2003). *A. obtectus* is commonly found at higher altitude, whereas *Z. subfasciatus* remains a serious threat in warmer climates.

On a commercial level bean bruchids are effectively controlled by means of chemical disinfestation and/or protection. On a small scale level there are a variety of measures employed in the on-farm disinfestation and protection of stored bean seed. On-farm chemical control measures do often not have the desired effect due to a lack of information and low levels of literacy among subsistence farmers. Traditional on-farm control measures such as the addition of ash, vegetable oil and dust or physical measures such as bean tumbling or heat treatment, can be effective, although labour intensive and only effective for small quantities of seed.

Host plant resistance would be a better form of control, if it does not have a negative effect on human nutrition. High levels of resistance were found against both bruchid species when Schoonhoven *et al.* (1983) tested 210 wild bean accessions from the CIAT germplasm bank. A novel, previously unreported protein in common bean was found in the accession PI 325690. When crude proteins from these accessions were subjected to SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis), unique protein bands were detected in several of the accessions, as reported by Osborn *et al.* (1986). These protein variants were referred to as arcelin. Arcelin derived resistance against *Z. subfasciatus* was transferred from the wild beans through a backcross breeding program, to develop the RAZ-breeding lines (Cardona *et al.,* 1990). Hartweck and Osborn (1997) developed the SMARC breeding lines with altered protein content (no phaseolin) and with a higher reported resistance against *A. obtectus*.

Up to date little or no research has been done in the development of bruchid resistance in the stored seed of dry bean cultivars in South Africa. Therefore the objectives of this study were:

- To transfer resistance against *A. obtectus* into susceptible commercial South African dry bean cultivars in a backcross breeding programme.
- Selection of breeding lines with increased resistance against *A. obtectus* through bioassays with adult insects of the species.
- Development of a recombinant inbred line population, which could be used in future research for near infrared spectroscopy or any other technique requiring the separation of resistant and susceptible seed in a wide genetic background.
- Testing the yield ability of the developed breeding lines (from backcrossing) and comparing it to that of the susceptible commercial parents and arcelin donor parent in the breeding scheme.
- Evaluating the resistance of the backcross progeny against *A. obtectus* compared to that of the susceptible commercial parents and the arcelin donor parent.

- Evaluation of the backcross progeny for seed size and colour (suitable for use as red speckled sugar beans) and identifying backcross progeny equal or better in all characteristics than the commercial parents.

CHAPTER 2

LITERATURE REVIEW

2.1 COMMON BEANS (*Phaseolus vulgaris* L)

Beans refer to the food legumes of the genus *Phaseolus*, family *Leguminosae*, subfamily *Papilionoideae*, tribe *Phaseoleae*, subtribe *Phaseolineae.* Distributed mainly in the Americas and sub-Saharan Africa, the genus *Phaseolus* contains some 50 wild-growing species and five domesticated species, such as common bean (*Phaseolus vulgaris* L), lima bean (*P.lunatus* L), runner bean (*P. coccineus* L), tepary bean (*P. acutifolius* A. Gray) and the year bean (*P. polyanthus* Greenman) amongst others.

Cultivars of common bean stem from two different centres of domestication, *i.e.* the southern Andes and Mesoamerica (Gepts, 2001). All species of the genus are diploid and most have 22 chromosomes ($2n = 2x = 22$). An aneuploid reduction to 20 chromosomes is found in a few species. Consisting of 625 Mbp per haploid genome, the genome of the common bean is one of the smallest in the legume family (Gepts, 2001). Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia and USDA, Pullman, Washington, USA, hold large germplasm collections of domesticated and wild forms of beans, and the National Botanical Garden, Meise, Belgium holds the reference collection (Gepts, 2001).

Common bean is a warm-season, annual legume, consisting of several types and classes. Emergence occurs with a segment of the hypocotyl arching up through the soil, between the developing root (anchored radicle) and the large cotyledons at the end of the hypocotyl. The hypocotyls arch straightens (once the cotyledons emerge) and the first two unifoliate leaves expand from the cotyledon node along the stem and terminal bud. All subsequent leaves are trifoliate and develop from terminal or axillary buds. Flower colours vary from white, pink or purple.

With less than one percent natural crossing, beans are normally self-pollinating. There are two growth types in common bean: determinate (concentrated flowering, stem terminates in a cluster of flowers) and indeterminate (flowering over a protracted period, growth continues during and after flowering) (Smoliak *et al.,* 1990).

Consumed worldwide, common bean is the most important food legume and in developing countries it provides an important source of protein (22%), vitamins (folate), and minerals (Ca, Cu, Fe, Mg, Mn, and Zn) for human diets. First world countries recognise beans for their nutritional contribution in targeting problems such as cancer, diabetes, and heart diseases (Broughton *et al.,* 2003). Within common bean two major usage types exist. Dry beans are produced for consumption as a grain, whereas green beans (also known as French or snap beans) are bred and produced for their pod qualities and consumed while the pods are still green.

Common bean is produced worldwide, with a global bean harvest of 24 million tons annually (Popelka *et al.,* 2004). Accounting for nearly half of the global output, Latin America is the most important bean-producing region with 8 million hectares used for bean production. In sub-Saharan Africa, beans provide the main source of dietary protein for more than 70 million people. Distribution of beans in sub-Saharan Africa is illustrated in Figure 2.1. Raised mostly by women for subsistence and the market, more than 3.5 million hectares (a quarter of the global output), are cultivated annually (CIAT, 2005). In South Africa a total of 42 200 hectares produced an estimated 63 560 tons in 2008 (DPO, 2008) with the contributions from the different provinces shown in Table 2.1

Table 2.1 Contributions from the different provinces to the dry bean production of South Africa during the 2008 planting season (DPO, 2008)

Province	Ha %	Ton %	Usage type
Limpopo	10	16	Previously seed production area, currently mainly commercial production
Mpumalanga/Gauteng	31.5	30	Commercial production area (Lowveld area - seed production)
North-West	8	7	Commercial production area
Free State	43	39	Commercial production area
KwaZulu-Natal	7	7	Commercial production area
Western Cape	0.5	1	Seed production area (breeder and basic seed plantings)
TOTAL	42200ha	63 510t*	* Scenario figure

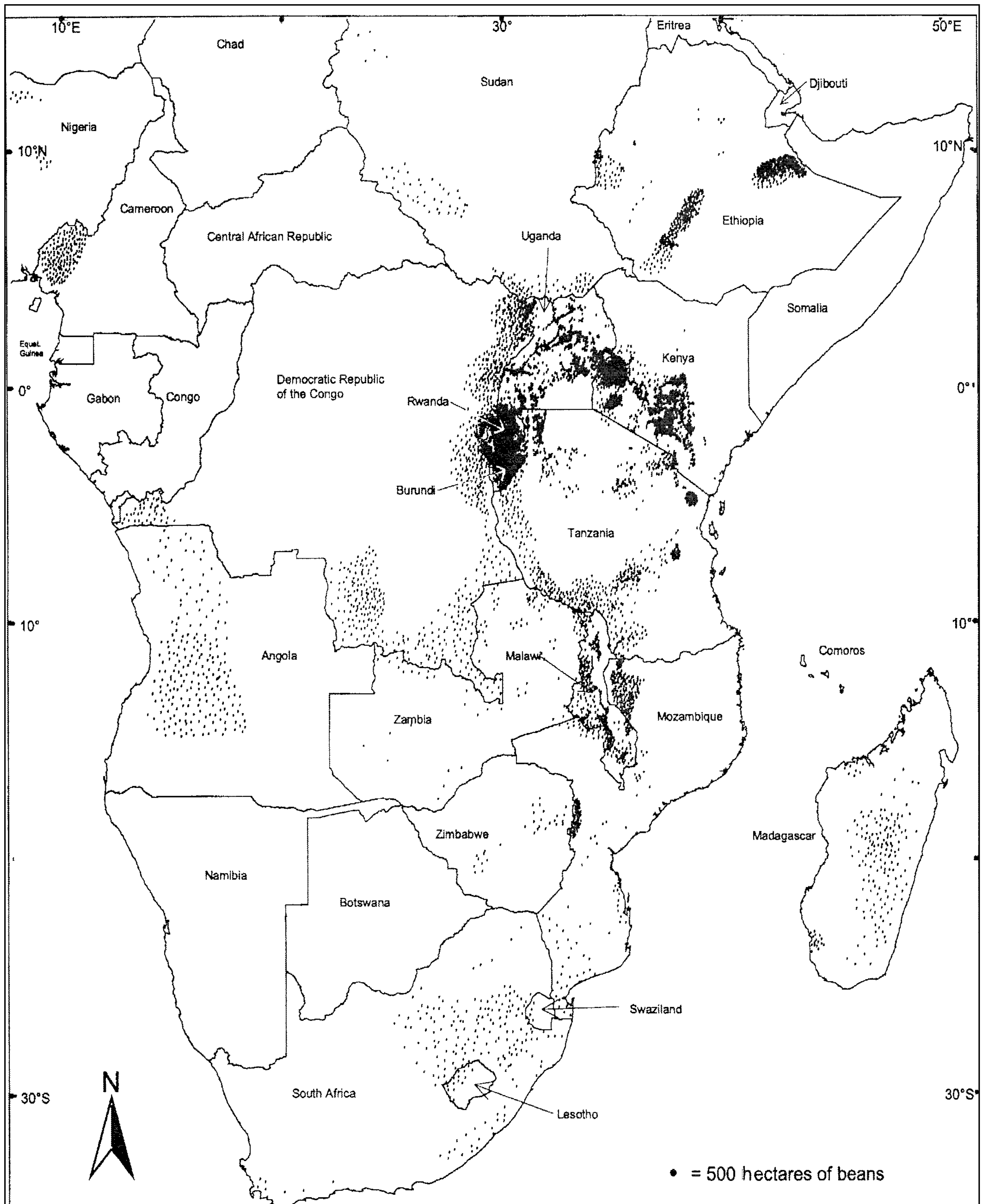

Figure 2.1 Map showing distribution of bean production (stippled areas) in sub-Saharan Africa (Wortman *et al.,* 2004)

2.2 INSECT PESTS OF STORED DRY BEAN SEED

Zabrotes subfasciatus (Boheman), commonly known as the Mexican bean weevil (Figure 2.3) and *Acanthoscelides obtectus* (Say), commonly known as the Common bean weevil (Figure 2.4), are the most important insect pests of stored beans around the world. Today both pests are distributed worldwide. These species (generally referred to as bruchids), both belong to the order Coleoptera and the family Bruchidae and both species cause severe damage to stored beans (Schoonhoven & Cardona, 1986). *A. obtectus* females can lay their eggs both in the field (in cracks of growing pods) and amongst stored beans, scattering their eggs between bean seed (Schoonhoven & Cardona, 1986; Kornegay & Cardona, 1991a; Parsons & Credland, 2003). In most bruchid species (including *Z. subfasciatus)* the female adheres individual eggs to the dry seeds and in sodoing the fitness of the offspring is predetermined, because the hatched larvae must complete its life cycle in the seed to which it was adhered. With *A. obtectus* (Parsons and Credland, 2003), a small number of eggs are adhered to the seeds, whilst the majority of the eggs are released freely among the seeds. The first instar larvae select the host (seed) for the remaining stages of development, because it can move freely among the seeds (Parsons & Credland, 2003). Adults of *A. obtectus* are larger than those of *Z. subfasciatus*. The male and the female of *Z. subfasciatus* are easily differentiated (the female is larger than the male with cream coloured spots on her elytra and the male is uniform grey in colour).

According to Schoonhoven & Cardona (1986) sexual differentiation is more difficult in *A. obtectus*, since size and colouring are the same, but possible, as illustrated by Nahdy (1994). Where the male has even grey or white pubescence of the pygidium, the female has a denser lateral pubescence on the pygidial midline, leaving four brown patches of the cuticle more exposed than the rest. This characteristic is used with a fair degree of accuracy to sex *A. obtectus* adults. In both *A. obtectus* and *Z. subfasciatus* the female insects lays eggs that hatch and the first- instar larvae penetrate and develop inside the seed in a growth chamber. The larvae of both the species moult four times before pupating.

A characteristic round “window” which originates from the growth and feeding chamber, becomes visible in the testa of the seed during the last larval instar. The adults eclose by pushing out this “window” and shortly thereafter mating occurs, followed by oviposition (Schoonhoven & Cardona, 1986). Damage to the bean seed is manifested by these typical escape “windows” made on the surface of the seed by the emerging adults (Koona *et al*., 2006).

As stated by Schoonhoven & Cardona (1986), both species originated in South America and according to studies by Alvarez *et al.* (2005), *A. obtectus* migrated from Andean America to Mexico relatively recently. Because of movements in stocks of bean seed, most bruchid species are now distributed worldwide (Kornegay & Cardona, 1991a; Alvarez *et al.,* 2005). Where *Z. subfasciatus* is found mostly in warmer areas, *A. obtectus* is generally found at higher altitudes in the tropical regions and throughout temperate climates in general (Schoonhoven & Cardona, 1986; Kornegay & Cardona, 1991a). For this reason, *A. obtectus* is the main storage pest of common beans in South Africa (Figure 2.2). *Z. subfasciatus* has almost completely replaced *A. obtectus* as the main pest in regions with low elevation and high temperatures, where at high elevation and cooler temperatures the replacement of *A. obtectus* has been much lower. This suggests that *A. obtectus* is a much stronger competitor at lower temperatures, than *Z. subfasciatus* (Schoonhoven & Cardona, 1986). Estay *et al*. (2009) predicted changes in the equilibrium status of *Tribolium confusum* (Coleoptera: Tenebrionidae) and *Collosobruchus chinensis* (Coleoptera: Bruchidae) due to climate change in a study that examined the link between invasive insect species and climate change. It was illustrated that new suitable habitats may become available for specific insect species, due to climatic changes. This could imply a possible shift in geographical occurrence and relative importance of the storage pests of common beans. With global climate change and earth-warming on the increase, *Z. subfasciatus* could in future become the dominant of the two species over ever-increasing areas.

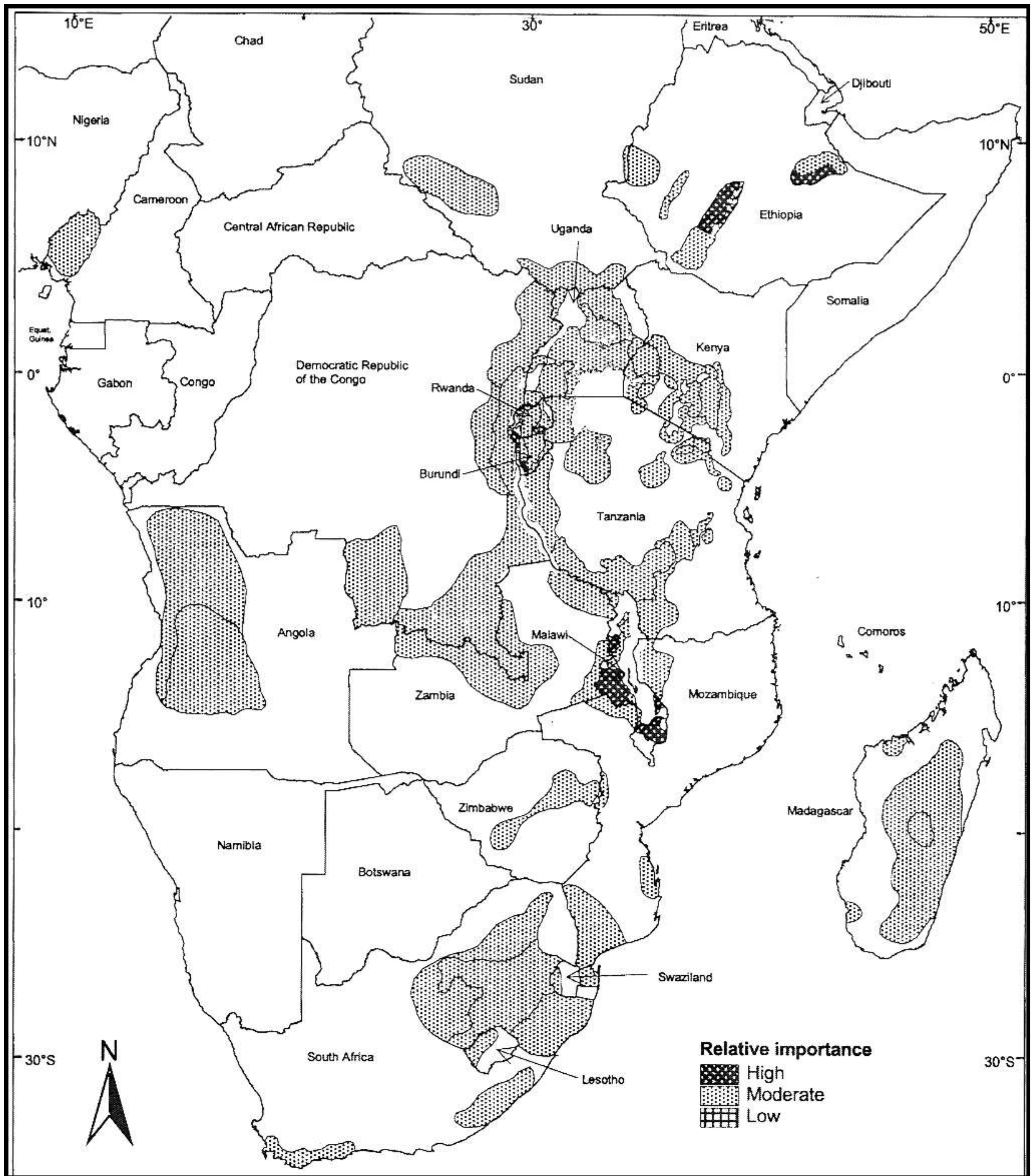

Figure 2.2 Map showing relative importance of *Acanthoscelides obtectus* in Sub Saharan Africa (Wortman *et al*., 2004)

Figure 2.3 *Zabrotes subfasciatus* (Mexican bean weevil)
Photo source: flyaqis.mov.vic.gov.au/padil/beetles.html

Figure 2.4 *Acanthoscelides obtectus* (Common bean weevil)
Photo source: www.padil.gov.au/viewPestDiagnosticImages.asp.

2.3 DAMAGE DUE TO BRUCHID INFESTATION

Because losses due to bruchid infestation could be directly correlated to the period of storage, there are no reliable studies that indicate the losses accurately. According to Schoonhoven and Cardona (1986) a grain loss of 7.4% was measured after a storage period of 45 days based on work done by CIAT workers in 30 warehouses in Colombia. In a study by Schmale *et al.* (2002), an average of 14% damage was reported during a 16-week storage period and bean losses of up to 40% has been reported in Tanzania due to bruchid infestation whilst 38% loss was recorded in Malawi (Kananji, 2007). According to Schoonhoven and Cardona (1986), insects in stored bean seed cause two types of losses. These are quantitative losses due to the number of seed, or pieces of seed eaten by the insects (Figure 5) and qualitative losses are due to excrement or insects cadavers in the grain. When stored at relatively high humidity (>17%), the grain is also an excellent medium for rapid development of both insect larvae and fungi such as *Aspergillus* spp., *Penicillium* spp. and *Phomopsis* spp. Attack is prevented when seed is stored at lower relative humidity (<14%). Because of bruchid attack, the quality of bean seed deteriorates and is therefore not marketable, which causes economic losses to producers and quality losses to consumers (Schoonhoven & Cardona, 1986).

Many farmers plant their own harvested bean seed (Chipungahelo *et al*., 2001). This practice causes losses in crop productivity because of lower germination of damaged seeds, therefore requiring a higher seeding rate to compensate for low population numbers.

Plants originating from bruchid-damaged seed are also more susceptible to powdery mildew, reducing plant development and yield. In East Africa one of the most important reasons why farmers do not wish to grow large quantities of beans is the fear of bruchid attack in their small farm storage facilities. To avoid large scale storage losses due to bruchid infestation these farmers will sell most of their beans shortly after harvest (Nchimbi-Msolla & Misangu, 2001). As demonstrated by Schoonhoven and Cardona (1986), this is also the situation in Mexico and Latin America. Here farmers are forced to

sell their crops when prices are low during the harvest months (prices dropped 49.3% just before harvest in July 1984). If the farmers can store their bean seed until the lean months, they can get higher prices and this will stabilize bean prices in general by providing a more stable supply. An economic benefit to both bean growers and consumers can therefore be gained by techniques that allow beans to be kept free from insect pests.

Figure 2.5 Damage to common bean seed caused by bean weevil infestation
Photo source: commons.wikimedia.org/wiki/Image:Bonenkever_Acanthoscelides obtectus.jpg

2.4 METHODS OF BRUCHID CONTROL

Although not practiced in South Africa, South African bean cultivars are sold in many parts of Africa and therefore an account of small-farmer bruchid control measures are provided. Maintaining strict cleanliness in storage sites is the first step in controlling insect pests, but other control measures are available to producers. Control of bruchid attack is achieved on two levels *i.e.* domestic and small farmer level and large commercial level.

2.4.1 DOMESTIC AND SMALL FARMER LEVEL OF CONTROL

2.4.1.1 Bean /ash mixtures

A mechanical method of control is to store beans mixed with ash. Scoonhoven and Cardona (1986) and Proctor (1994) state that bruchids have difficulty in infesting because the spaces between the seeds are filled with ash. Bruchid populations can decrease with the application of ash, with 20% of the weight of the seed regarded as being the optimal mixture. Once beans have been infested, this method will not be effective, but good results can be achieved in preventing initial bruchid infestation. With this method of control, there is no reduction in germination ability of seed. However, since large quantities of ash are required and being very labour intensive, this method is only suitable for small lots of seed.

2.4.1.2 Bean/inert dust or bean/sand mixtures

According to Proctor (1994) laterit, clay dust, quicklime etc. mixed in a 0.1-50% ratio with bean seed will have the same effect as wood ash, but beans will have to be cleaned before consumption. Sand can be added in proportions of 40-100% of the volume of seed or used as a top layer 2-7 cm thick with the same effect. These methods can only be used on small lots, because of the big quantities of dust/sand required, making this method very labour intensive.

2.4.1.3 Control by vegetable oils

An effective method of protecting stored beans against bruchid attack is by coating seed with edible vegetable oil. The following oils at specified rates can be added to stored bean seed: peanut oil (5 ml/kg); coconut oil (5 - 10 ml/kg); palm oil (5 - 10 ml/kg); sesame oil (5 ml/kg) and neem kernel oil (2 - 3 ml/kg). All of these oils are cheap and easily obtainable. According to Schoonhoven and Cardona (1986) and Proctor (1994), the oil seems to be toxic to the embryos of the bruchids inside the eggs. The oviposition of both bruchid species is therefore disturbed.

Effective control can be obtained when applying 10 ml of oil/ kg of beans. The results of one study shows that untreated beans and beans treated with up to 10 ml of oil/ kg of beans have the same germination even after a storage period of six months (Schoonhoven & Cardona, 1986). The results of another study shows that cheaper crude oils are more effective in protecting bean seed against bruchid attack than the more expensive refined oils. Beans treated with vegetable oil can be consumed or planted as seed. If seed are well mixed with the oil, seed can be protected against bruchid attack for up to six months (Schoonhoven & Cardona, 1986; Proctor, 1994).

2.4.1.4 Control by harvesting techniques

When the oviposition behaviour of bruchids is taken into account, field infestation can be reduced. Storing beans in the pods can minimize *Z. subfasciatus* attacks because this bruchid prefers laying eggs on shelled seed. By harvesting earlier and reducing exposure time, *A. obtectus* can be controlled, since it can infest beans in the field by laying eggs in and on the pods (Schoonhoven & Cardona, 1986). Schoonhoven & Cardona (1986), found that attacks by *A. obtectus* increased by 472% when delaying harvesting and threshing by 10 days. This shows the importance of threshing directly after harvest maturity to eliminate ovipositioning on the pods by insects coming from the field.

2.4.1.5 Low temperature control

By reducing storage temperatures to <10°C, growth and reproduction of bruchids are significantly affected because they are adapted to higher temperatures of 20-32°C. Bruchids in any stage of its life-cycle are completely eliminated by storing seed in a freezer or freezer compartment (Schoonhoven & Cardona, 1986; Proctor, 1994). This storage practice would only be effective and practical for small quantities of seed.

2.4.1.6 Use of heat and smoke

To slow down the development of bruchids and prevent re-infestation, beans in their pods are stored in bundles over kitchen fires. Bean seed can also be spread out in the sun to kill larvae in the seed. Although it changes the taste of beans, smoking with dried hot pepper

(*Capsicum* sp.) has a good effect. Storage containers (*e.g.* mud silos) can be smoked out before storage (Proctor, 1994).

2.4.1.7 Impregnated bags

In a study by Koona *et al*. (2006), a four to six-fold decrease in seed damage by bruchids was reported when beans were stored in jute bags impregnated with aqueous extracts from two insecticidal plants, *Chenopodium ambrosioides* and *Lantana camara.*

2.4.1.8 Bean tumbling

Quentin *et al.* (1991) determined that periodic tumbling of beans in a suitable container such as half-filled jars, buckets or gunnysacks reduced *A. obtectus* populations by 97% relative to the stationary controls. As it takes 24 hours for the larvae of *A. obtectus* to bore into a dry red kidney bean, these authors found that periodic tumbling of the beans repeatedly forced larvae to start new entry holes and caused the larvae to die of exhaustion or get crushed by tumbling beans.

2.4.1.9 Biological control

The use of microorganisms, food traps and planting of varieties more tolerant to storage pests will, according to Proctor (1994), grow in importance in farm level storage in the future. In a study by Schmale *et al*. (2002), results indicate that by combining arcelin resistance with biological control by *Dinarmus basalis* (Rondani) of the order Hymenoptera and family Pteromalidae, bruchid damage was kept below 1%, compared to the 4.7% when only the arcelin free standard is used.

2.4.1.10 Chemical methods

A number of problems arose when chemical insecticides for stored products were introduced on farm level. Studies have shown that mistakes are commonly made with the use of chemical insecticides. These include the choice of inadequate products, application of degraded or inadequately formulated insecticides and inadequate application rates. Studies in West Africa have shown that dealers selling these products are not informed well enough to advise farmers regarding correct application, labelling is sometimes not

sufficient enough to prevent misuse and a high percentage of farmers are illiterate which causes problems in the sharing of technical information (Proctor, 1994).

2.4.1.11 Dustable powders

According to Proctor (1994), dustable powders are mostly used at small farmer level in Africa. The following formulations (active ingredient shown) are commonly used to treat individual bags, stacks of bags or as layers between the stored products:
Organophosphorous compounds**:** Fenitrotion; Pirimiphos-methyl; Chlorpyrifos-methyl; Methacrifos: Malathion.
Pyrethroids**:** Deltemethrin; Permethrin; Fenvalerate; Cyfluthrin.

As stated by Schmale *et al.* (2002), insecticide use needs to be safe for both the user and the consumer. The proper handling of the contact chemical and low or no residue level at the time of consumption is essential, but very difficult to achieve at small farmer level.

2.4.1.12 Fumigation

As many beans are not stored in airtight containers in Africa, Asia and South America, fumigation seems to be an effective measure for pest control as it is cost effective and no residue is left on the stored produce. Because of poor sealing, the desired effect can rarely be achieved and people and animals are exposed to severe health risks in large parts of Africa. This is mostly due to the incorrect application of fumigants by untrained farmers (Proctor, 1994) and must therefore be entirely discouraged.

2.4.2 COMMERCIAL LEVEL OF CONTROL

Commercial level of pest control is possible in large scale in warehouses by the following two methods *i.e.* disinfestation and/or protection.

2.4.2.1 Disinfestation

With this method the infestation present at the time of treatment is eliminated. No residues remain and beans can be consumed, but seed can also be reinfested after

treatment. Phosphine (Aluminium Phosphide) and Methyl Bromide are most commonly used because of their high toxicity and penetrating ability. Bean stacks are covered with plastic sheets and sealed against the ground to prevent the gas escaping. Stacks are treated with four to five tablets per cubic meter of beans in well-sealed warehouses or six to 12 tablets in silos that are more ventilated in the case of Phosphine. Methyl bromide is applied at 0.5 kg / 28 m^3 of bean seed. This method is not recommended for seed that is destined for planting, as germination can be affected by high temperatures during disinfestation (Schoonhoven & Cardona, 1986).

In South Africa mainly commercial farmers produce beans, and after harvest the beans are marketed through grain traders. Farmers generally do not store their crop for long periods and storage, pre-packing and distribution are done by the traders. These storage facilities are generally well maintained and qualified personnel carry out the disinfestations. Cleanliness of the storage facility is the most important method of reducing losses due to storage pests in these facilities (C. Porter, Afgri Handling and Storage, Mpumalanga, personal communication). Losses are kept to a minimum by keeping to a strict fumigation programme. Beans are fumigated with Phosphine, with good results (S. Visser, Umgeni Products, KwaZulu Natal and M. de la Rey, Starke Ayres Kaalfontein, personal communications).

2.4.2.2 Protection of stored grain against bruchid attack.

For protection, bean seed is treated with a chemical product that has a residual effect. Beans treated with a protectant can be inedible and only suitable for planting, but will not be reinfested during the time of the residual action of the chemical. There are three main protectants that can be used with good results at the following rates: Malathion (8-12 ppm as a powder or 1-2 ml l^{-1} water); Lindane (2-4 ppm as a powder) and Pyrethrins (1 kg of 60% commercial product per 600 kg of stored beans). Lindane is very toxic to humans and no beans that are to be eaten should be treated with it.

2.5 RESISTANCE TO BRUCHIDS IN COMMON BEAN

Although control of bruchids is possible by addition of chemicals, dust, oils etc. it is often not ideal to add foreign substances to the product near to the time of consumption. Resistance, if not negatively influencing human nutrition, would be a better method of control. Simmonds *et al*. (1989) described six stages in the bruchid-legume interaction where resistance may occur, with resistance shown as: no oviposition; disrupted embryo development; larvae failing to penetrate the testa; larvae dying within the cotyledon; failure of pupation or adult emergence and the reduced fitness of adult insects. Schoonhoven and Cardona (1982) reported low levels of resistance in dry bean when more than 4000 accessions were screened for resistance against *Z. subfasciatus.* Although significant differences were found between varieties, the levels of resistance were too low to be of economic value. Subsequently, when a further 210 wild accessions sourced from the germplasm bank of CIAT were tested for resistance to *Z. subfasciatus* and *A. obtectus,* Schoonhoven *et al.* (1983) found high levels of resistance to each species of seed weevil. Antibiosis resistance was expressed as reduced oviposition, prolonged larval development period and reduced insect progeny weight. This resistance was found in the Mexican wild non-cultivated form of beans and weedy types. The weedy types are a regressive form or hybrid between wild and cultivated forms (Schoonhoven *et al.,* 1983). Although small seed size was related to resistance, Schoonhoven *et al.* (1983) argued that other factors were likely to be more important.

2.6 ARCELIN

According to Osborn *et al.* (1986), it was shown that wild bean forms indigenous to Central America and South America could potentially be sourced for protein variants to be used in the genetic improvement of bean cultivars. A novel, previously unreported protein in common bean, was recorded in accession PI 325690. Crude proteins from seeds of these wild bean accessions from Mexico were analyzed by one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoretic patterns showing unique protein bands were detected in several of the

accessions, as found by Osborn *et al.* (1986). They detected four protein variants with electrophoretic mobilities similar to each other, but also different from phaseolin and lectin, the other major seed proteins. These previously undescribed variants were named arcelin proteins (named after Arcelia, the town in Mexico where the accessions were collected), and designated arcelin 1, 2, 3 and 4. Today seven variants of arcelin are described. Lioi and Bollini (1989) described a fifth variant, arcelin-5, based on electrophoretic variation. As reported by Blair *et al*. (2002) arcelin- 6 was described by Santino *et al.* (1991) and arcelin- 7 by Acosta-Gallegos *et al.* (1998).

2.6.1 Introduction to arcelin

Lectins (sugar-binding proteins) are expressed at low levels in many vegetative tissues, but in roots, tubers and bark it accumulates in amounts that are more substantial. Involved in plant defence against bruchids, lectins accumulate in seed tissues in legumes. In the *Phaseolus* genus, the lectin locus has evolved extensively and contains a multigene family that codes for up to three major components. Normally occurring in wild and cultivated accessions, phytohemagglutinin (PHA) and α- amylase inhibitor (αAI) are the most representative members of the lectin family in the common bean (Sales *et al.*, 2000; Lioi *et al.,* 2003). Found only in wild accessions from Mexico, arcelin (Arc) is the third protein. All of these proteins are synthesized only in the embryonic axis and cotyledons during seed formation (Osborn *et al.,* 1986). Being the only member of the storage protein family that binds carbohydrates, PHA is the only true lectin. Where such activity is totally absent in αAI and where Arc may retain only a weak carbohydrate binding activity, they are referred to as lectin related proteins (Lioi *et al.,* 2003). Encoded by a family of different genes, each of the arcelin variants is composed of several polypeptides. As shown by Goossens *et al.* (1999) arcelin-5 protein, is encoded by four different genes. According to Osborn *et al*. (1988), there is a tight linkage between genes controlling arcelin expression (<0.3% recombination) to those controlling PHA expression. Arc and PHA have a few characteristics in common. Both are glycoproteins with similar amino acid composition. The two proteins are also related antigenically and have the same developmental timing of accumulation (Osborn *et al.,* 1988). These researchers also found that Arc had some hemaglutinating activity, which is a

characteristic that is associated with lectins. A few features distinguish Arc from PHA. According to Osborn *et al.* (1988) Arc has a more basic iso-electric point than PHA, a greater number of basic amino acid residues, additional cysteine residues and one methionine residue, which PHA lacks. Where a small component of native Arc protein was tetrameric, most of the arcelin preparation was dimeric. Native PHA is a tetramer of subunits. A further characteristic distinguishing Arc from PHA is the hemaglutinating activity of Arc, which is specific only for some pronase-treated erythrocytes. It does not agglutinate native erythrocytes, nor does it bind to thyroglobulin as do PHA (Osborn *et al.,* 1988).

According to Hartweck *et al*. (1991) arcelin-1 is the most thoroughly characterized of the protein variants. Janzen *et al.* (1976) found that their effect on seed-feeding insects is another difference between arcelin-1 and PHA. PHA is involved in the specificity of host-insect interactions between *Phaseolus* species and seed feeding insects. PHA does not provide protection against *P. vulgaris* herbivore, but does against seed feeding pests from other *Phaseolus* species. Arcelin-1 protects seed against *P. vulgaris* pests and the different variants are associated with different levels and types of resistance against *Z. subfasciatus* or *A. obtectus* (Osborn *et al.,* 1988).

2.6.2 Toxicity of arcelin

Carlini and Grossi-de-Sá (2002) pointed out that despite extensive investigations the mechanism of action of arcelins is still controversial. Osborn *et al*. (1988) postulated that arcelin could be toxic, whereas Hugo *et al.* (1990) stated that it could be indigestible, thus causing larval starvation. Recent studies were conducted on larval tissues to understand why arcelin is an insecticidal factor for *Z. subfasciatus*, but not for *A. obtectus.* Although the mechanism of action of arcelin is not known, Paes *et al.* (2000) postulated that the toxicity of arcelins may be based on specific binding to glycoconjugates or proteins somewhere in the gut of the insect (in analogy with phytohemagglutinin, a protein toxic to mammals by virtue of its binding to the intestinal epithelium). In their study, Paes *et al.* (2000) studied the effects of dietary arcelin on the structure of the larval gut and determined the distribution of arcelin in larval tissues with specific antibodies. Traversing

the cells that line the gut, arcelin-1 had a severe deleterious effect (destruction of the epithelial cells) on the gut of *Z. subfasciatus,* but not on the gut of *A. obtectus* and arcelin was present in the haemolymph of *Z. subfasciatus.*

The insecticidal activity of the arcelin-5 variant was investigated by Goossens *et al.* (2000) who observed that no correlation could be established between the presence of arcelin-5 and the insecticidal effects observed in the highly resistant G02771 accession. Artificial seed, into which purified arcelin-5 protein was incorporated, was used in insect feeding assays. It was found that even elevated levels of arcelin-5 were not sufficient to obtain adequate levels of resistance against *Z. subfasciatus.* It was stated by Goossens *et al.* (2000) that as resistance is clearly closely linked to the presence of the arcelin-1 or arcelin-5 loci, arcelins would remain useful markers in breeding programmes aiming to incorporate high levels of resistance against *Z. subfasciatus* in *P.vulgaris* cultivars.

In studying the potential effects of arcelin on domesticated animals and humans, Pusztai *et al.* (1993) evaluated the then recently released RAZ 2 line (Arc-1). The proteins of the RAZ-2 line were less digestible and less well utilised than other high quality animal proteins, and the resultant growth of laboratory rats was somewhat retarded. However, the anti-nutritional effects of this line were found to be less than with most bean varieties and could easily be abolished by heating the fully hydrated beans at 100°C for 10 min (Pusztai *et al.,* 1993; Singh, 2001).

2.6.3 Inheritance of arcelin and resistance against *A. obtectus*

It was established that differences in arcelin polypeptide expression is inherited as a monogenic trait. This was seen after analysis of single F2 seeds from crosses among arcelin containing lines and between cultivated lines (without arcelin) and arcelin containing lines (Osborn *et al.,* 1986; Suzuki *et al*., 1995) and arcelin expression is controlled genetically in a simple Mendelian fashion (Osborn *et al.,* 1988). These researchers also found that when present with other arcelin alleles, these alleles are co-dominant and presence is dominant over absence of arcelin. Resistance against *Z. subfasciatus* can be transferred to commercial bean types by employing a backcross

breeding procedure (Kornegay & Cardona, 1991b), but Hugo *et al*. (1990) argued that the bases of resistance to the two pest species were different.

Arcelin seemed to be ineffective against *A. obtectus,* and it has been difficult to obtain resistant progeny from crosses between cultivated varieties and the resistant wild accessions. Resistance levels also seemed to decrease as generations progressed (Kornegay & Cardona, 1991b). Inheritance of resistance against *A. obtectus* in wild beans was studied to understand the genetics of resistance. One accession with resistance against *Z. subfasciatus* (G 12952) was crossed to two susceptible varieties with different seed size. Reciprocal F1 and individual F2 seed were evaluated to determine the number of days to adult emergence (DAE), and emerged adult weight. Resistance levels in the F1 were very similar to that of the susceptible cultivars. In the F2, very few of the individuals showed the resistance levels of G 12952 against *Z. subfasciatus* and showed a continuous, but skewed distribution pattern from a low to high DAE. Resistance was found to be inherited as two complimentary recessive genes, when the frequency distributions were divided into discrete categories, based on adult response. Resistance levels was also lower in the F3 generation (compared to F2 evaluations), but still did not fall into the susceptible category, indicating that resistant genotypes were almost stable (as can be expected with recessively inherited traits). Seed size was also found to be negatively correlated with adult weight, but not with DAE (Kornegay & Cardona, 1991b).

2.7 BREEDING FOR RESISTANT GENOTYPES IN COMMON BEAN

In breeding for host plant resistance the initial step in a breeding programme would be to assemble a wide assortment of germplasm of desirable species. Sources of germplasm could be commercial cultivars, advanced breeding lines or landraces, containing useful genes. The purpose of selection is to identify and propagate individuals or groups of genotypes and to distinguish genetic variation from environmentally based variability.

Hybridization is a breeding method of cross-pollination between genetically different parents, achieving gene recombination (Sleper & Poelman, 2006). Following cross-pollination, segregating populations are grown, from which pure lines are selected after homozygosity is reached. From the segregating population, lines with a combination of desirable genes from both parents are selected. Selected lines are evaluated for the presence of desirable genes by means of a progeny test and then increased as a new cultivar.

2.7.1 Insect feeding tests

As described by Kananji (2007) two tests are mainly used to determine resistance of germplasm to storage pests. With the no-choice method the insects are restricted in their choice of seed sample. This method is widely used in laboratory screening of genotypes for resistance against storage pests according to the same author. Seed samples are placed in small bottles and are then infested with adult insects of known age and sex. With this testing method, antibiosis is measured and data on emerging adult insects as well as development period are taken. With the free-choice test, the insects can infest the seed samples of their choice. The ability of a specific variety to repel insects (antixenosis) is usually measured with this method. A large number of adults can be introduced into a container where many varieties have to be tested.

2.7.2 History of breeding for bruchid resistance

Breeding for bruchid resistance in common beans began at CIAT in 1982. These researchers followed a selection strategy consisting of infesting bulked F2 populations with large numbers of bruchid adults. Seeds with the least damage were selected and grown out in the field, harvested in bulk and re-infested. Some breeding lines with acceptable levels of resistance were obtained, by continuing this procedure for several generations. All of these lines were undesirable because of their small seed size and growth habit. Many small seeded materials escape infestation, because bruchids seemed to prefer large seeded material. A different strategy was called for. The F2 populations of crosses between cultivated beans and resistant wild types were first planted in the field, and individual plant selections were then made on basis of acceptable agronomical traits.

Half the F3 seed from the individual F2 plants was infested with adult bruchids and the other half planted in the field. The selections showing the highest levels of resistance were harvested on an individual plant basis, with the susceptible plants being eliminated (Cardona *et al.,* 1990). The procedure was then repeated in the hope of obtaining bush and climbing bean lines with larger grain types and acceptable levels of resistance (Kornegay & Cardona, 1991a). According to Cardona *et al.* (1990), within the series the level of resistance in the variants is progressively lower, ranging from Arcelin-5 > Arcelin-4 > Arcelin-1 > Arcelin-2 > Arcelin-6 > Arcelin-3, if in the background of the wild progenitor. In the cultivated background the alleles that provide the most resistance are Arcelin-1 > Arcelin-2 > Arcelin-5 > Arcelin-3 > Arcelin-4. Arcelin (a partially dominant gene), provides its highest level of resistance to bruchids in the homozygous form. Arc+/Arc+ in individual seeds are more resistant than Arc+/Arc-.

Researchers at CIAT have widely used the Arc-1 variant in their breeding programmes to create resistant breeding lines, like the RAZ breeding lines, through backcrossing and gene transfer (Cardona *et al.,* 1990). Using serological techniques to detect the presence of arcelin and replicated insect feeding tests, to measure resistance levels, these researchers used a backcross-breeding program to transfer resistance against the Mexican bean weevil from wild beans to bean cultivars. Schoonhoven & Cardona (1982) described the techniques to maintain insect cultures and testing of breeding lines and accessions. Using a randomised complete block design, they tested four to five replicates by infesting 50 seeds per replicate with seven pairs of adult bruchids (Cardona *et al.,* 1990). The data they recorded was days to adult emergence, percentage emergence, weight of adult progeny, and percentage seed damaged after 55 days of infestation. Wild germplasm with reported resistance were subjected to SDS-PAGE analysis for the presence of arcelin. Seed that contained arcelin was sown in a greenhouse, and a bulk of each accession selected and tested for resistance to Mexican bean weevil.

Some 118 breeding materials were tested for resistance for two consecutive generations. Germplasm containing arcelin was used as donor parents and the cultivated parents were Mexican bean weevil susceptible commercial varieties with different growth habits, seed

sizes and colours (Cardona *et al.,* 1990). Table 2.2 shows the breeding scheme followed in the backcross breeding programme. Insect feeding tests in the early generations were substituted by serological tests to detect the presence of arcelin and advance lines were tested in replicated feeding trials.

Table 2.2 Breeding scheme used to improve beans for resistance against Mexican bean weevil (Cardona *et al.,* 1990)

	Resistant (arcelin donor parent) X **Susceptible** (recurrent parent)
F1:	Backcrossed to susceptible parent
BC1F1:	Serological tests in 10-20 seeds per cross. Arc+ is backcrossed to susceptible parent.
BC2F1:	Serological tests in 10-20 seeds per cross. Arc+ seeds are planted and individual plants are selected in the field.
BC2F2:	Serological tests in 10-20 seeds per plant selected in BC2F1. Homozygous Arc+ are planted in progeny rows in the field and selected for agronomical characteristics.
BC2F3:	Seeds are submitted to replicated feeding tests with the insect. Resistant progeny are planted in the field and selected for agronomical characteristics.
BC2F4:	Best lines are coded "RAZ".

Arcelin has been transferred and incorporated from wild accessions into cultivars and breeding lines with acceptable agronomical and commercial qualities, so now it is no longer necessary to use the wild accessions as parents. Because the wild ancestral traits has been removed through the backcross breeding program, transferring the resistance from one bean grain class to another can now be accomplished by simple crosses (Cardona *et al.,* 1990).

Because Hartweck and Osborn (1997) found that the arcelin concentration in some backcross lines were not effective against *A. obtectus*, these researchers from the University of Wisconsin, Madison, USA aimed to increase the concentration of arcelin proteins by genetically manipulating the quantity of other seed specific proteins. Phaseolin and PHA are the major storage proteins of common bean cultivars and make up 40-60% and 6-12% respectively of the total seed protein. Arcelin can contribute approximately 50% of the total protein in Sanilac backcross lines, as stated by Hartweck and Osborn (1997). Although these proteins account for the majority of the total protein, the absence of any or all of these proteins is compensated for by the remaining protein fractions, thus the total protein concentration in the seed remains the same or increases, as stated by Hartweck and Osborn (1997). Delaney and Bliss, (1991), also found that the amount of phaseolin is reduced by 50% when arcelin is artificially introduced. These researchers developed backcross lines in a 'Sanilac' background which contained phaseolin (PP lines), or the null allele for phaseolin (PN lines) in combination with alleles for either the Arc-1, -2 or -4 variants (SMARC lines) or PHA variants from 'Bunsi', 'Protop P-1' or 'Viva' (SMPHA lines) (Breeding scheme shown in Figure .2.6). Because the phytohemagglutinin gene is tightly linked to the arcelin gene, selection for arcelin variants could be performed by selection of phytohemagglutinin using a blood cell agglutination assay (Hartweck & Osborn, 1997; Osborn *et al.,* 2003).

The concentrations of arcelin, phaseolin and different PHA variant proteins in these lines were determined and paired PN and PP lines were compared (Hartweck & Osborn, 1997). SMARC and SMPHA lines were derived in pairs of phaseolin containing and phaseolin null lines. Parental SMARC and SMPHA lines were grown in a replicated greenhouse trial and measured for total protein, PHA, arcelin dimer, phaseolin, as well as agronomic characteristics.

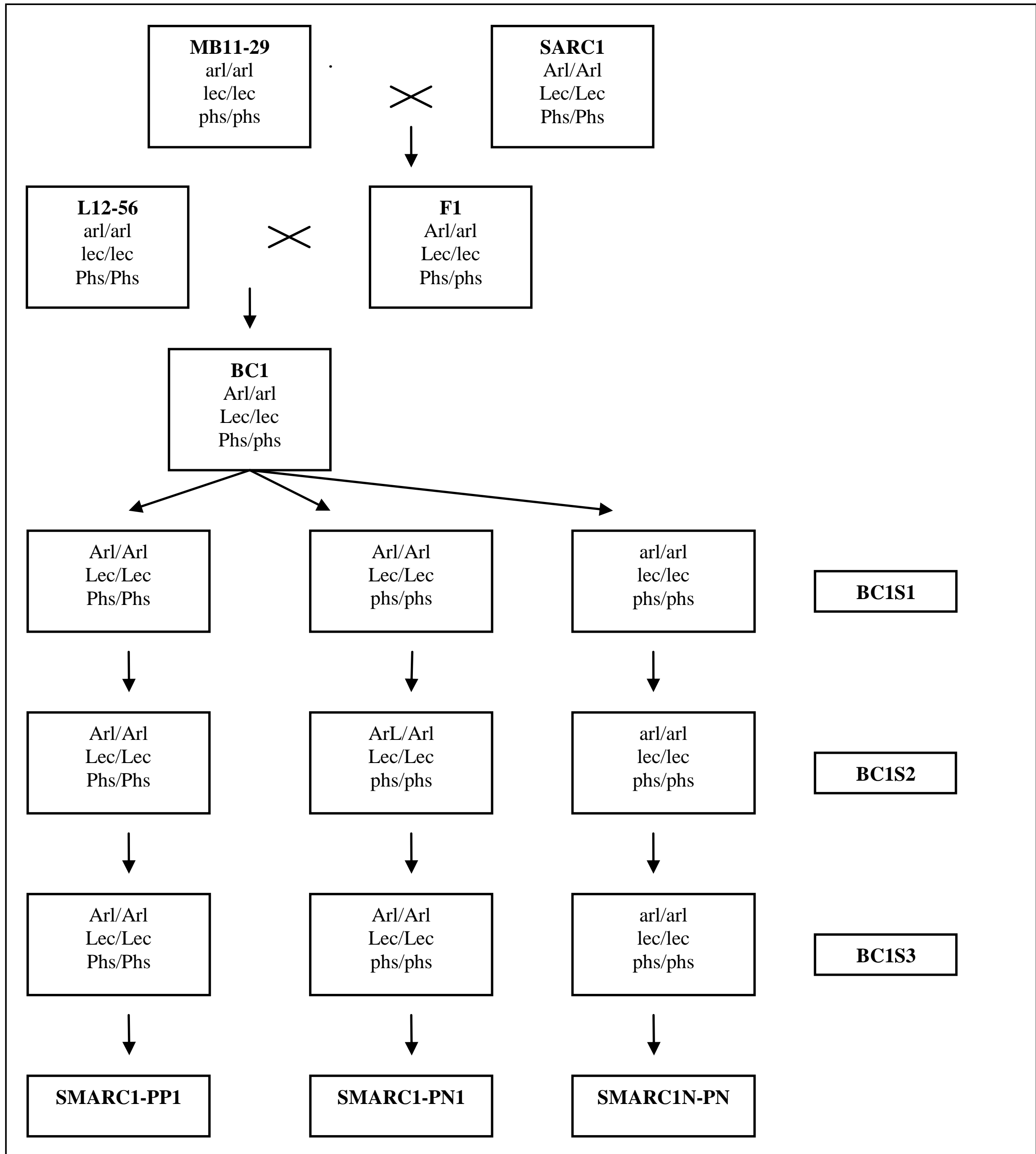

Figure 2.6 Breeding scheme used to develop one set of SMARC1 lines. Genotypic symbols for Arcelin, PHA and Phaseolin are Arl, Lec, and Phs respectively (Hartweck *et al*., 1997)

Hartweck & Osborn, (1997) found that there was a significant increase of arcelin in two of four pairs of SMARC lines and PHA concentration was significantly higher in four of five pairs of SMPHA lines. These researchers postulated that the changes in seed protein composition might improve resistance to bruchids.

In a separate study, these lines were tested for resistance to both *A. obtectus* and *Z. subfasciatus* by measuring total adult emergence and days to adult emergence and an index of susceptibility ratings (Hartweck *et al.,* 1997). All insect trials were conducted at CIAT, Cali, Colombia, where the lines were tested in a randomised complete block design with three replications. Two to three pairs of *Z. subfasciatus* were used to infest 10-15 seeds, with *A. obtectus*, each replication had 10 seeds, and each seed was infested with three eggs. The most important factor for resistance level in the SMARC lines were arcelin type, with SMARC-1 (arcelin 1) lines being the most resistant, SMARC-2 (arcelin 2) lines intermediate and SMARC-4 (arcelin 4) lines the least resistant (Hartweck *et al.* 1997). In resistance to *A. obtectus,* the absence of phaseolin was an important factor in the development of the SMARC lines. The lines with phaseolin had a 50% higher adult emergence than the SMARC-1 lines, which also had the highest resistance against both bruchids (Hartweck *et al*., 1997).

SARC-1 to-4 in a Sanilac background (white navy type seed), PARC-1 to-4 in a Porrillo 70 background (black seeded lines), SMARC-1 PN-1 to SMARC-4 PN-4 in a Sanilac background, SMARC1N-PN1 in a Sanilac background, were registered in 2003. These lines can be used in studying the effects of different seed protein compositions, or as parents for the development of bruchid resistant cultivars (Osborn *et al.*, 2003). Despite the release of this breeding lines, no suitable resistant varieties, meeting farmers specific requirements, has been developed thus far (Kananji, 2007).

2.8 USE OF DNA MARKERS FOR SELECTION OF ARCELIN-DERIVED BRUCHID RESISTANCE

Seed protein assay is a method of selecting for arcelin-based resistance by detecting arcelin in small quantities of ground seed tissue. Protein electrophoresis and arcelin-specific antibodies are required for this process (Blair *et al.,* 2002). As stated by Miklas *et al.* (2006), the use of arcelin as biochemical marker represents one of the first true uses of marker-based selection in common bean. Limitations of the protein based selection is that it is time consuming and not compatible with DNA-based marker systems and Miklas *et al.* (2006) proposed the need of new molecular markers for the arcelin resistance gene.

A study was conducted to replace the protein-based selection with a genetic assay, whereby closely linked microsatellite or SCAR markers for arcelin or related proteins are used (Blair *et al.,* 2002). To establish a high throughput DNA marker system to screen for arcelin-based bruchid resistance, two DNA extraction techniques (rapid, high-throughput alkaline lysis “microprep” or standard organic separation “miniprep”) were used. Increasing of the efficiency of breeding for multiple constrained resistance and pyramiding of bruchid resistance, was the long-term objective of this study (Blair *et al.,* 2002).

These researchers used 68 genotypes in total with seven wild accessions representing the seven-arcelin variants; 28 advanced breeding lines from the resistance program and 28 bruchid susceptible parental lines (Blair *et al.,* 2002). Leaf tissue discs for the “microprep” and newly emerged trifoliates for the “miniprep” were harvested from the greenhouse. Another set of 791 F4 and F5 progeny lines from RAZ x Susceptible parents were cultivated in the field and DNA extracted from leaf tissue, and used for microsatellite amplification. Samples extracted with the second technique had a low storage period and degraded easily (low DNA quality). Five microsatellites have been amplified up to date (Blair *et al.,* 2002). Microsatellite allele diversity varied for the different markers, with Clon41 detecting the most alleles (11), while Bd15 and BMd26 detected three each. Diversity within the subgroups for the different microsatellites also

varied. The existence of linkage disequilibrium between microsatellites and arcelin locus was indicated by the pattern of diversity. These markers can be used to select for greater recombination and to break linkage drag associated with this locus (poor plant vigour of arcelin-derived lines) (Blair *et al.,* 2002).

2.9 USE OF NEAR INFRARED SPECTROSCOPY FOR SELECTION OF ARCELIN-DERIVED BRUCHID RESISTANCE

Replicated insect feeding tests are widely used as a method to determine levels of resistance against both *A. obtectus* and *Z. subfasciatus.* SDS-PAGE was used by many researchers to determine the presence/absence of arcelin in seeds from common bean in inheritance studies and breeding programs aimed at improving host plant resistance against bean bruchids (Cardona *et al.,* 1990, Hartweck & Osborn, 1997; Meyers *et al*., 2000). Because of limitations in the protein based selection method, Blair *et al.* (2002) reported on development work done with microsatellite markers. In this study, little evidence could be found on the use of Near Infrared Spectroscopy (NIR) for the selection of arcelin based bruchid resistance. NIR spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range 780-2500 nm. The concentrations of constituents such as water, protein, fat and carbohydrate can be determined in principal by classical absorption spectroscopy, but as the chemical information of most food groups are obscured by changes in the spectra by physical properties, NIR becomes a secondary method requiring calibration against a reference method for the constituent of interest. As Osborne (1988) pointed out, the major advantages of NIR lie in the fact that usually no sample preparation is required and therefore analysis is very simple and fast. Several constituents can also be measured concurrently.

NIR is extensively used for quality selection in wheat breeding programmes. NIR has application in determining protein content of wheat, allowing the rapid screening of large numbers of lines for this characteristic. The ultimate application in a wheat-breeding programme would be the direct prediction of functional quality including flour yield, damaged starch, water absorption, dough development time and extensibility and loaf

volume (Osborne, 1988). In common bean NIR application is established in the determining of moisture, starch, protein and fat (Hermida *et al.* 2006). In soybeans NIR have been applied in the determination of protein, moisture, oil, crude fibre, tripsin inhibitor, P, Na, Ca, Mg, K, 7S/11S globulins, protein denaturation, 11S globulin, N-solubility index and stachyose as tabled by Ozaki *et al*. (2007).

2.10 BRUCHID RESISTANCE IN TEPARY BEAN (*Phaseolus acutifolius* A. Gray)

While *P. acutifolius* and *P. parfifolius* Freytag can be crossed without embryo rescue to produce fully fertile progeny, embryo rescue is required for at least two generations when these species are crossed with common bean (Singh, 2001; Meyers *et al.,* 2001). To restore the fertility of the hybrids, one or more backcrosses to the recurrent common bean parent are often required. According to Mejía-Jiménez *et al.* (1994), with *P. acutifolius* as the female parent of the initial F1 cross, and/or the first backcrossing of *P. vulgaris* x *P. acutifolius* hybrid onto *P. acutifolius*, will often be more difficult than using *P. vulgaris* as the female parent in the initial cross and backcrossing the interspecies hybrid onto *P. vulgaris*. In addition to recovering of fertility and higher number of hybrid progeny, the choice of parents and use of congruity backcross (backcrossing to each of the species alternately), facilitate interspecific crosses of common beans and tepary beans (Singh, 2001).

The Biotechnology Unit at CIAT developed and used a double congruity backcross technique to develop fertile interspecific *P. vulgaris – P. acutifolius* (common x tepary) bean hybrids using the tepary genotype N1576, which is shown to be a genotype competent to Agrobacterium- mediated genetic transformation (CIAT, 2005). G 40199, a tepary accession which is an excellent source of resistance against the bean weevil *A. obtectus,* but not against *Z. subfasciatus* (Zambre *et al.*, 2005)*,* was involved in some of these crosses. Several progeny lines containing both *P. vulgaris* and *P. acutifolius* cytoplasm with very high levels of antibiosis resistance to *A. obtectus* were identified in 2002 and 2003. In 2004, they placed emphasis upon the reconfirmation of resistance in previously selected progeny lines. High levels of resistance to the insect (< 20 percentage

adult emergence) were found in one hybrid containing *P. vulgaris* cytoplasm and seven containing *P. acutifolius* cytoplasm (CIAT, 2005). Resistant seeds were multiplied in a greenhouse and some showed high levels of resistance against *A. obtectus* (<20% in replicated tests). Interspecific hybrids with *P. vulgaris* cytoplasm (susceptible) and also some with *P. acutifolius* cytoplasm were tested and after multiplication of selected seeds replicated reconfirmation tests showed intermediate resistance (20-50% adult emergence) in some of the hybrids (CIAT, 2005). Testing of individual seeds to detect segregation in interspecific hybrids continued (a tedious but important and necessary process). Resistant seeds were multiplied, but did not germinate. Two double congruent hybrids with *P. acutifolius* cytoplasm were selected for further testing (CIAT, 2005).

CHAPTER 3

DEVELOPMENT OF EXPERIMENTAL LINES WITH INCREASED RESISTANCE AGAINST *Acanthoscelides obtectus*

3.1 INTRODUCTION

Consumed worldwide, common bean is the most important food legume and in developing countries it provides an important source of protein, vitamins and minerals in human diets. Dry bean storage is compromised by attack from two major insect species in the Bruchidae seed weevils namely common bean weevil (*Acanthoscelides obtectus*) and the Mexican bean weevil (*Zabrotes subfasciatus*) (Kananji, 2007). Without control measures losses can be severe to both commercial and subsistence farmers. Being difficult to quantify, because of the direct correlation between storage period and damage level, losses as much as 40% were reported in Tanzania by Schmale *et al.* (2002).

While many traditional methods of control do exist, these methods are all labour intensive and only practical for small quantities of beans. Commercial methods of control are effective, but there is still the possibility of re-infestation. In South Africa, *A. obtectus* is the major pest of stored beans, and although well-controlled in most storage facilities, host plant resistance would lead to a saving on chemicals needed to control these storage pests. While resistance against *Z. subfasciatus,* as conferred by arcelin, can easily be transferred through backcrossing, the transfer of resistance against *A. obtectus* is more complex, with plant resistance levels decreasing as generations progress (Kornegay & Cardona, 1991b).

Hybridisation is used to transfer genes that control insect resistance from a wild form to a cultivar. The inheritance of the resistance traits and the level of resistance conferred by the genes, will determine the selection strategy used. Pedigree, single seed descent or backcross breeding methods can be used to transfer simply inherited traits.

Cardona *et al.* (1990) employed a backcross breeding programme to transfer resistance against the Mexican bean weevil from wild beans into bean varieties, developing the RAZ breeding lines. Hartweck and Osborn (1997), in developing the SMARC breeding lines with genetically altered arcelin and phaseolin concentration, also used the backcross breeding method of gene transfer.

The aims of this study were:

1) To transfer resistance against *A. obtectus* into susceptible commercial South African dry bean cultivars in a backcross breeding programme.
2) Selection of breeding lines with increased resistance against *A. obtectus* through bioassays with adult insects of the species.
3) Development of a recombinant inbred line population, which could be used in future research for near infrared spectroscopy or any other technique requiring the separation of resistant and susceptible seed in a wide genetic background.

3.2 MATERIALS AND METHODS

3.2.1 Plant materials

The following cultivars and semi-commercial varieties were used to develop bruchid resistant breeding lines:

SMARC4-PN1: Researchers at the University of Wisconsin, Madison (Hartweck & Osborn, 1997), developed this breeding line with reported resistance against *A. obtectus*. The female parent in the initial cross was experimental line MB11-29, which contained null alleles for arcelin, phytohemagglutinin and phaseolin and consists of 72% “Sanilac” germplasm. The male parent in the initial cross was a common bean line SARC4, which contained the arcelin variant protein Arc-4 (Hartweck *et al.*, 1991). This breeding line was used as arcelin donor parent.

PAN 118: A local red speckled dry bean variety that is well suited to low input or subsistence farming conditions. This variety has excellent resistance to diseases such as rust (*Uromyces appendiculatus)*, angular leaf spot (*Phaeoisariopsis*

griseola) and BCMV (bean common mosaic virus), and is cultivated in disease prone areas of Southern Africa. This variety was included in the study because of its disease resistance.

PAN 128: A local red speckled dry bean variety cultivated in all dry bean production areas, but very competitive in the eastern Free State and western production areas of South-Africa. This variety was included in the study because of its high yield and adaptability.

PAN 9249: A large seeded variety adapted to most of the dry bean production areas of South Africa. This variety has a high yield and disease resistance and was included in the study because of its high yield potential.

PAN 107: A variety that was never released commercially but was bred to replace the Natal speckled sugar landrace cultivars Umvoti and Wartburg, which were quick maturing, but low yielding varieties. These varieties were also small seeded with low resistance to diseases such as rust, angular leaf spot and BCMV. This variety was an improvement on the landraces in terms of seed size, yield and quality, but without improvement in disease resistance. This variety was selected for inclusion in this study, because it is a source of quick maturity, without the negative association of small seed (A. Jarvie, PANNAR, personal communication, 2009)

AV 275: Although never registered, this Natal red speckled sugar breeding line was used successfully in the breeding programme as a source of tolerance to low soil fertility. This breeding line was not commercialised, as seed size and shape was not acceptable for subsistence farming (A. Jarvie, PANNAR, personal communication, 2009).

AV 1337: Although successfully used in a breeding programme, this breeding line has never been commercialised. It is a rust resistant backcross recovery from PAN 148, a moderately large seeded sugar bean cultivar which has been marketed and produced in most of Southern Africa's major dry bean production areas. Large quantities of seed from this cultivar are exported to African countries, including Zimbabwe and Zambia. This cultivar, with its good agronomic characteristics, has been marketed for more than ten years in southern Africa. This cultivar was

included in the trial because of its popularity among southern African bean producers.

3.2.2 Artificial hybridisation procedure

Backcross breeding programmes have successfully been implemented in previous studies to incorporate resistance from wild bean germplasm against Z. *subfasciatus* and to a lesser degree against *A. obtectus* (Cardona *et al.,* 1990; Hartweck *et al.,* 1997). Because *P. vulgaris* is highly self-pollinating, the main method of developing genetic variability is by artificial hybridisation. The different techniques for artificial hybridisation has been described by Genchev (2007), where ease of use, speed and efficiency remained the deciding factor in determining the best method to use. Efficiency largely depends on factors such as the fact that the stigma and pollen should be close in contact for as long as possible to ensure fertilisation, and environmental factors such as temperature and humidity that are responsible for the rate of desiccation of the pollen and stigma. Genchev (2007) described preparation of the female flower bud with and without emasculation. Pollination methods described with emasculation include the rubbing, hooking and insertion method. With the clamping method the left-hand wing is pressed down, resulting in the protruding of the unpollinated stigma from the keel. The stigma from the male parent is pulled out and hooked behind the stigma to be pollinated. The hooking method without emasculation was used in this study, because it has the advantage of the stigma from the female parent being prevented from pulling back in the keel (by hooking), while the loose stigma from the male parent can be hooked onto the stigma of the bud about to be pollinated.

3.2.3 Insect rearing

The technique to maintain insect cultures and to test breeding lines and cultivated varieties, as described by Schoonhoven and Cardona (1982), was modified for the purpose of this study. All experiments were conducted indoors, and although climate was

not controlled, temperatures were monitored. The original *A. obtectus* adults were collected at the PANNAR Research Station at Greytown. This original population was multiplied and used for breeding done at this locality. For breeding done at the PANNAR Research Station at Delmas, an insect population was collected and reared at Delmas. Insects were reared on a commercial large seeded common bean variety, susceptible to attack by *A. obtectus.* Insect populations were reared in new 1 l glass jars, holding approximately 1 kg of dry bean seed each and with nylon mesh fitted to the metal tops, to ensure aeration. Adult bruchids were allowed to infest the seed for 20 days and were then removed. When new adult insects eclosed after 35-38 days, they were sieved out with a 4 mm sieve. These 1-2 day old adults were then used to re-infest fresh seed and thus the rearing of new adult insects was continued. These one day old insects were also used in the evaluation of F2 progeny lines for resistance. This process was repeated to ensure a permanent source of mature bruchids to conduct the insect feeding trials. If a sufficient number of new adults eclosed, they were sieved out one day after eclosion and used in the experiments.

3.2.4 Bioassay with adult insects

To determine resistance, progeny lines from the initial cross and the back-crosses were evaluated in a bioassay with adult *A. obtectus* insects. Bioassays were laid out in a completely randomized design without replication. As only a small amount of seed were available for testing, replication of the experiment was not possible. Although climate was not controlled, all bioassays were conducted indoors, with temperature monitored. Twenty F2 seeds were placed in small plastic containers and fitted with nylon mesh to ensure aeration. *A. obtectus* can infest pods in the field, but because all breeding work was conducted in a glasshouse, seeds were found to be clean of bruchid infestation. The 20 F2 seeds were artificially infested with ten newly emerged adult bruchids. As fitness of the adult insects used were important, only adult insects of the same age were used in the evaluation process. Adult bruchids were allowed to infest the seed for 20 days and were then removed. When the new adult bruchids emerged after 35-38 days, they were

removed from the seed and total emergence numbers recorded. Total adult emergence was used to quantify resistance.

3.2.5 Development of a recombinant inbred line reference population

To explore the possible use of NIR Spectroscopy as a selection method for resistance against *A. obtectus,* a Recombinant Inbred Line (RIL) population was developed during 2007. Two susceptible commercial varieties (PAN128 and PAN9249), one susceptible semi-commercial variety (PAN107) and two susceptible advanced breeding lines (AV275 and AV1337) were artificially crossed with the resistant breeding line (SMARC4-PN1). Seed from the original crosses were harvested and self pollinated. The F2 seeds were advanced through single seed descent without selection to the F5 generation. From the F2 generation onwards two seeds per plant were harvested. One seed was replanted to form the next generation, while the second seed was stored as backup of the particular generation. This procedure was repeated to the F5. Two to four single plants from each F5 family were harvested to form F6 RI lines. A total of 95 RI lines were developed. Twenty seeds from each RIL were randomly selected and evaluated for resistance against *A. obtectus* in a bioassay with adult insects (Appendix 2). The most resistant and susceptible lines were separated firstly by ranking F5 family means and then choosing F6 individuals within those families based on their individual emergence data. Means were calculated for each F5 family group (within the pedigrees). This was done because at an F5 level of homozygosity, family means should be a reliable indication of performance. These means were ranked and the six most resistant and most susceptible families selected. F6 individuals within selected families were selected based on individual emergence data. Using this methodology, the 10 most resistant and most susceptible individual RI lines were selected.

3.2.6 Development of resistant Near Isogenic Lines

Seven parents were planted in the glasshouse at the PANNAR Research Station at Greytown during 2005. These were PAN118, PAN128, PAN9249 (susceptible commercial varieties), PAN107 (a susceptible semi-commercial variety) and AV275 and AV1337 (susceptible advanced breeding lines). The resistant donor parent was SMARC4-PN1. A backcross breeding scheme was followed as outlined in Figure 3.1. Crosses were made between the resistant donor parent and susceptible varieties. The resulting F1 seed was harvested and replanted with the susceptible recurrent parents.

In Backcross 1 all the F1 plants were crossed with the recurrent parents. BC1F1 seed was harvested, replanted and self-pollinated. Individual BC1F2 plants were harvested and evaluated in Bioassay 1 (Appendix 3) to determine resistant backcross progeny lines from Backcross 1. The two best plants per pedigree were selected based on data from Bioassay 1. The selection criterion was the total adult insects of *A.obtectus* emerging from the 20 seeds of the BC1F2 plants after artificial infestation with 10 adult insects of the species.

In Backcross 2 the two best individual selected BC1F2 plants were crossed with the recurrent parents. The BC2F1 seed was harvested, replanted and self-pollinated. The F3 generation was harvested on an individual plant basis and evaluated in Bioassay 2 (Appendix 3). From Bioassay 2 resistant backcross progeny lines from Backcross 2 were selected. The two best plants per pedigree were selected based on data from Bioassay 2. Selection criteria were total adult emergence of *A. obtectus* from the 20 F3 seeds following artificial infestation with 10 adult insects of the species.

In Backcross 3 the two best individual selected BC2F2 plants (Bioassay 2) were crossed with the recurrent parents. The resultant BC3F1 seed was harvested and stored at the PANNAR Research Station pending a progeny test on the BC2 donor parents. The BC2 donor parents were self-pollinated and all the seed harvested of each plant (now F4) were evaluated in Progeny Test 1.

Progeny Test 1 was a replicated yield trial, where yield data, seed size and usage type was recorded. Seed harvested from the trial plots were subjected to Bioassay 3 (Appendix 4) to determine the resistant entries.

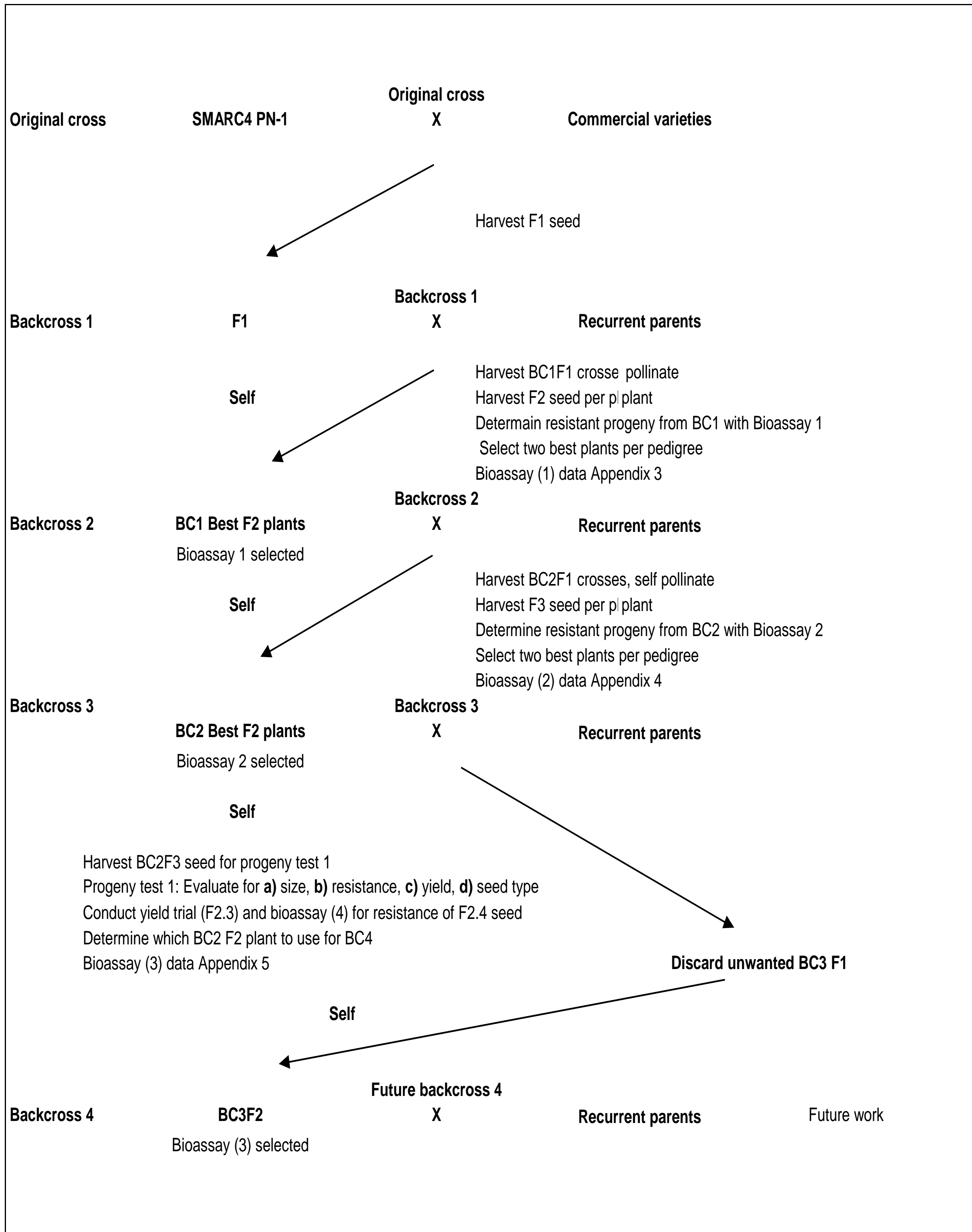

Figure 3.1 Breeding scheme used for development of Near Isogenic Lines

3.3 RESULTS AND DISCUSSION

3.3.1 Development of Recombinant Inbred Lines (RIL)

A population of 95 F6 RI lines was developed. The population was subjected to bruchid feeding in a bioassay (Appendix 2) to differentiate between the resistant and susceptible individual lines in the population.

The 10 most resistant and 10 most susceptible lines were selected (Table 3.1). These 20 individual lines represented a total of 20% of the population. By selecting the most resistant and most susceptible lines from the population, heterozygous lines are eliminated.

Table 3.1 Selected RILs from the population representing the most resistant and susceptible lines

Most resistant lines			Most susceptible lines		
Pedigree	RI lines	Adult emergence per 20 seeds	Pedigree	RI lines	Adult emergence per 20 seeds
AV275*1/SMARC4PN1	08GD018-2	**9**	PAN118*1/SMARC4PN1	08GD005-2	**43**
PAN118*1/SMARC4PN1	08GD006-3	**10**	PAN118*1/SMARC4PN1	08GD004-1	**45**
PAN118*1/SMARC4PN1	08GD007-2	**10**	PAN118*1/SMARC4PN1	08GD005-3	**55**
PAN107*1/SMARC4PN1	08GD021-2	**10**	PAN9249*1/SMARC4PN1	08GD033-1	**59**
AV275*1/SMARC4PN1	08GD018-1	**11**	PAN118*1/SMARC4PN1	08GD005-1	**65**
PAN118*1/SMARC4PN1	08GD007-1	**13**	PAN107*1/SMARC4PN1	08GD019-2	**67**
PAN107*1/SMARC4PN1	08GD022-2	**14**	PAN118*1/SMARC4PN1	08GD004-3	**71**
PAN107*1/SMARC4PN1	08GD022-1	**16**	PAN107*1/SMARC4PN1	08GD019-4	**76**
PAN118*1/SMARC4PN1	08GD006-2	**18**	PAN107*1/SMARC4PN1	08GD002-1	**87**
PAN107*1/SMARC4PN1	08GD026-1	**18**	PAN107*1/SMARC4PN1	08GD020-1	**89**

3.3.2 Development of resistant Near Isogenic Lines (NIL)

Resistance against *A. obtectus* in the SMARC4-PN1 line is conferred through the Arc-4 variant of the arcelin gene (Hartweck & Osborn, 1997). The resistance gene was

transferred to the susceptible varieties and breeding lines through artificial hybridisation in a backcross breeding programme. Six pedigrees were developed in this study. Kornegay and Cardona (1991b) found that resistance levels seem to decrease as generations progressed. Adult emergence data from this study confirmed this finding, with higher adult emergence recorded in later generations. Average adult emergence from the selected backcross progeny lines recorded in Bioassay 1 (Appendix 3) was two adult insects per 20 seeds tested. In Bioassay 2 (Appendix 4) the average adult emergence from selected backcross progeny lines was 25 adult insects per 20 seeds tested. In Bioassay 3 (Appendix 5) the average adult emergence from the selected backcross progeny lines was 35 adult insects per 20 seeds tested.

Selection criteria after each round of backcrossing was for resistance against *A. obtectus* as quantified through a bioassay with adult insects of the species and adult emergence recorded. Results of Bioassay 1 are shown in Appendix 3. Total adult emergence counts were generally low in this bioassay. The highest adult emergence count for a plant selected was eight adults per 20 seeds tested in pedigree PAN107*1/SMARC4PN1. The lowest adult emergence count was zero adults per 20 seeds tested in the three pedigrees AV1337*1/SMARC4PN, PAN118*1/SMARC4PN1 and AV275*1/SMARC4PN1. A zero adult emergence count was not always considered absolute resistance. An absence of any emerging adult insects could also be due to an escape and therefore zero counts were not always selected. The selected plants were used as donor parents in Backcross 2.

Bioassay 2 showed higher total adult emergence counts than the previous bioassay (Appendix 4). This could be due to the difference in geographical location and climatic conditions between the testing locations. In studies where live adults of *A. obtectus* and *Z. subfasciatus* were used in the testing of resistance in plant material, insect cultures were maintained at 27°C and 70% RH as described by Schoonhoven and Cardona (1982). This would suggest that these insect species have very specific temperature requirements for optimal development. Differences in climatic conditions and geographic position between Greytown and Delmas are shown in Appendix 6. The highest adult emergence count for plants selected was 57 adults per 20 seeds evaluated for pedigree

PAN9249*1/SMARC4PN1. The lowest adult emergence counts selected was zero adults per 20 seeds evaluated for pedigrees PAN118*1/SMARC4PN1 and PAN107*1/SMARC4PN1. Adult emergence recorded in the selected plants was lower in all the pedigrees compared to that of the adult emergence recorded for the recurrent parents. This finding would indicate an improved resistance in the selected lines over that of the susceptible recurrent parents.

For Bioassay 3 the highest adult emergence count for the plants selected was lower than those for Bioassay 2. The highest adult emergence count for a plant selected was 44 adults per 20 seeds evaluated for pedigree PAN9249*1/SMARC4PN1. This pedigree also had the highest adult emergence count for plants selected in Bioassay 2 and is a poor parental combination for selection of bruchid resistant breeding lines. The lowest adult emergence count was recorded for pedigrees AV1337*1/SMARC4PN1 and AV275*1/SMARC4PN1 with nine adults per 20 seeds evaluated. These two pedigrees also had the lowest adult emergence counts in the first bioassay, making them good parental combinations for the selection of bruchid resistant breeding lines. Adult emergence data from this bioassay showed a higher resistance against *A. obtectus* feeding in the selected breeding lines than in the recurrent parents. This finding would indicate the presence of the arcelin gene in selected lines conferring resistance against feeding by this insect species, as was set out in the objectives of this study.

3.4 CONCLUSIONS

As confirmed by these results, it was possible to select plants with improved resistance against *A. obtectus* compared to the susceptible recurrent parents. Some parental combinations showed a higher degree of resistance than other combinations. The adult emergence data over generations confirms the findings of Kornegay and Cardona (1991b). These researchers found that resistance levels seemed to decrease as generations progressed. No evidence could be found in the literature of NIR application in bruchid resistance breeding in common bean, even though it is used routinely for the determination of soya and dry bean protein. For this novel application a RIL population was developed that can serve as reference lines for any technique (including NIR, but not

limited to) that requires the separation of resistant from susceptible in a wide genetic background. These RILs were at an F6 stage and have hopefully fixed their reaction to bruchid infestation. Dry bean varieties with improved resistance against bean bruchids do not always meet with farmer's demands for yield and seed characteristics (Kananji, 2007). The result of this breeding effort is the development of an F3 generation which must be evaluated against the commercial varieties for seed size, yield, seed usage type and resistance against *A. obtectus.*

Appendix 1. RIL populations developed showing insect feeding data and most resistant and most susceptible lines selected

F6 RI line	Pedigree	Adult emergence per 20 seeds	F5 Family mean	Selections
08GD001-1	AV1337*1/SMARC4PN1	20		
08GD001-2	AV1337*1/SMARC4PN1	56	35	
08GD001-3	AV1337*1/SMARC4PN1	29		
08GD002-1	AV1337*1/SMARC4PN1	87		**S**
08GD002-2	AV1337*1/SMARC4PN1	30	46	
08GD002-3	AV1337*1/SMARC4PN1	20		
08GD003-1	AV1337*1/SMARC4PN1	23	23	
08GD004-1	PAN118*1/SMARC4PN1	45		**S**
08GD004-2	PAN118*1/SMARC4PN1	41	52	
08GD004-3	PAN118*1/SMARC4PN1	71		**S**
08GD005-1	PAN118*1/SMARC4PN1	65		**S**
08GD005-2	PAN118*1/SMARC4PN1	43	54	**S**
08GD005-3	PAN118*1/SMARC4PN1	55		**S**
08GD006-1	PAN118*1/SMARC4PN1	32		
08GD006-2	PAN118*1/SMARC4PN1	18	20	**R**
08GD006-3	PAN118*1/SMARC4PN1	10		**R**
08GD007-1	PAN118*1/SMARC4PN1	13	12	**R**
08GD007-2	PAN118*1/SMARC4PN1	10		**R**
08GD008-1	PAN118*1/SMARC4PN1	18		
08GD008-2	PAN118*1/SMARC4PN1	34	37	
08GD008-3	PAN118*1/SMARC4PN1	60		
08GD009-1	PAN118*1/SMARC4PN1	0	0	
08GD010-1	PAN128*1/SMARC4PN1	34	34	
08GD010-2	PAN128*1/SMARC4PN1	33		
08GD011-1	PAN128*1/SMARC4PN1	11		
08GD011-2	PAN128*1/SMARC4PN1	59	38	
08GD011-3	PAN128*1/SMARC4PN1	44		
08GD012-1	PAN128*1/SMARC4PN1	34	31	
08GD012-2	PAN128*1/SMARC4PN1	28		
08GD013-1	PAN128*1/SMARC4PN1	10	27	
08GD013-2	PAN128*1/SMARC4PN1	44		
08GD014-1	AV275*1/SMARC4PN1	28		
08GD014-2	AV275*1/SMARC4PN1	26	34	
08GD014-3	AV275*1/SMARC4PN1	48		
08GD014-4	AV275*1/SMARC4PN1	33		
08GD015-1	AV275*1/SMARC4PN1	34	34	
08GD016-1	AV275*1/SMARC4PN1	83	38	

08GD016-2	AV275*1/SMARC4PN1	10		
08GD016-3	AV275*1/SMARC4PN1	22		
08GD016-4	AV275*1/SMARC4PN1	36		
08GD017-1	AV275*1/SMARC4PN1	42	28	
08GD017-2	AV275*1/SMARC4PN1	10		
08GD017-3	AV275*1/SMARC4PN1	31		
08GD018-1	AV275*1/SMARC4PN1	11	10	**R**
08GD018-2	AV275*1/SMARC4PN1	9		**R**
08GD019-1	PAN107*1/SMARC4PN1	25	47	
08GD019-2	PAN107*1/SMARC4PN1	67		**S**
08GD019-3	PAN107*1/SMARC4PN1	21		
08GD019-4	PAN107*1/SMARC4PN1	76		**S**
08GD020-1	PAN107*1/SMARC4PN1	89	50	**S**
08GD020-2	PAN107*1/SMARC4PN1	10		
08GD021-1	PAN107*1/SMARC4PN1	19	15	
08GD021-2	PAN107*1/SMARC4PN1	10		**R**
08GD022-1	PAN107*1/SMARC4PN1	16	15	**R**
08GD022-2	PAN107*1/SMARC4PN1	14		**R**
08GD023-1	PAN107*1/SMARC4PN1	28	45	
08GD023-2	PAN107*1/SMARC4PN1	61		
08GD024-1	PAN107*1/SMARC4PN1	10	25	
08GD024-2	PAN107*1/SMARC4PN1	15		
08GD024-3	PAN107*1/SMARC4PN1	60		
08GD024-4	PAN107*1/SMARC4PN1	16		
08GD025-1	PAN107*1/SMARC4PN1	33	32	
08GD025-2	PAN107*1/SMARC4PN1	29		
08GD025-3	PAN107*1/SMARC4PN1	53		
08GD025-4	PAN107*1/SMARC4PN1	12		
08GD026-1	PAN107*1/SMARC4PN1	18	18	**R**
08GD027-1	PAN107*1/SMARC4PN1	12	22	
08GD027-2	PAN107*1/SMARC4PN1	16		
08GD027-3	PAN107*1/SMARC4PN1	39		
08GD028-1	PAN107*1/SMARC4PN1	13	41	
08GD028-2	PAN107*1/SMARC4PN1	69		
08GD029-1	PAN107*1/SMARC4PN1	10	21	
08GD029-2	PAN107*1/SMARC4PN1	14		
08GD029-3	PAN107*1/SMARC4PN1	47		
08GD029-4	PAN107*1/SMARC4PN1	13		
08GD030-1	PAN107*1/SMARC4PN1	55	44	
08GD030-2	PAN107*1/SMARC4PN1	32		
08GD031-1	PAN9249*1/SMARC4PN1	10	29	
08GD031-2	PAN9249*1/SMARC4PN1	47		
08GD032-1	PAN9249*1/SMARC4PN1	17	33	
08GD032-2	PAN9249*1/SMARC4PN1	49		
08GD032-3	PAN9249*1/SMARC4PN1	32		
08GD033-1	PAN9249*1/SMARC4PN1	59	46	**S**
08GD033-2	PAN9249*1/SMARC4PN1	32		

08GD034-1	PAN9249*1/SMARC4PN1	34	
			23
08GD034-2	PAN9249*1/SMARC4PN1	20	
08GD034-3	PAN9249*1/SMARC4PN1	16	
08GD035-1	PAN9249*1/SMARC4PN1	10	
08GD035-2	PAN9249*1/SMARC4PN1	44	
			34
08GD035-3	PAN9249*1/SMARC4PN1	66	
08GD035-4	PAN9249*1/SMARC4PN1	15	
08GD036-1	PAN9249*1/SMARC4PN1	15	
08GD036-2	PAN9249*1/SMARC4PN1	60	
			32
08GD036-3	PAN9249*1/SMARC4PN1	17	
08GD036-4	PAN9249*1/SMARC4PN1	36	

Appendix 2. Bioassay 1: Number of adults of *Acanthoscelides obtectus* emerging from 20 seeds after infestation

Pedigree	Code	Lab Plot Bioassay 1	Adult emergence per 20 seeds	Selection
AV1337/SMARC4-PN1	BRUX941	07P L001	0	
AV1337/SMARC4-PN1	BRUX941	07P L002	0	
AV1337/SMARC4-PN1	BRUX941	07P L003	0	
AV1337/SMARC4-PN1	BRUX941	07P L004	0	
AV1337/SMARC4-PN1	BRUX941	07P L005	15	
AV1337/SMARC4-PN1	BRUX941	07P L006	1	
AV1337/SMARC4-PN1	BRUX941	07P L007	1	
AV1337/SMARC4-PN1	BRUX941	07P L008	0	
AV1337/SMARC4-PN1	BRUX941	07P L009	0	
PAN 148	PAN 148	07P L010	0	
AV1337/SMARC4-PN1	BRUX941	07P L011	4	
AV1337/SMARC4-PN1	BRUX941	07P L012	8	
AV1337/SMARC4-PN1	BRUX941	07P L013	0	
AV1337/SMARC4-PN1	BRUX941	07P L014	0	
AV1337/SMARC4-PN1	BRUX941	07P L015	11	
AV1337/SMARC4-PN1	BRUX941	07P L016	0	
AV1337/SMARC4-PN1	BRUX941	07P L017	61	
AV1337/SMARC4-PN1	BRUX941	07P L018	10	
AV1337/SMARC4-PN1	BRUX941	07P L019	0	
AV1337/SMARC4-PN1	BRUX941	07P L020	0	
AV1337/SMARC4-PN1	BRUX941	07P L021	4	
AV1337/SMARC4-PN1	BRUX941	07P L022	29	
RAZ 42	RAZ 42	07P L023	0	
PAN 9275	PAN 9275	07P L024	0	
AV1337*1/SMARC4PN1	BRUX1021	07P L025	19	
AV1337*1/SMARC4PN1	BRUX1021	07P L026	48	
AV1337*1/SMARC4PN1	BRUX1021	07P L027	2	**BC1**
AV1337*1/SMARC4PN1	BRUX1021	07P L028	0	**BC1**
AV1337*1/SMARC4PN1	BRUX1021	07P L029	9	
PAN118*1/SMARC4PN1	BRUX1022	07P L030	2	**BC1**
PAN118*1/SMARC4PN1	BRUX1022	07P L031	3	
PAN118*1/SMARC4PN1	BRUX1022	07P L032	0	**BC1**
PAN118*1/SMARC4PN1	BRUX1022	07P L033	22	

Pedigree	Code	Lab Plot Bioassay 1	Adult emergence	Selection
PAN118*1/SMARC4PN1	BRUX1022	07P L034	11	
PAN 148	PAN 148	07P L035	0	
PAN 148	PAN 148	07P L036	**0**	
PAN128*1/SMARC4PN1	BRUX1099	07P L037	6	
PAN128*1/SMARC4PN1	BRUX1099	07P L038	16	
PAN128*1/SMARC4PN1	BRUX1099	07P L039	7	**BC1**
PAN128*1/SMARC4PN1	BRUX1099	07P L040	0	
PAN128*1/SMARC4PN1	BRUX1099	07P L041	4	
PAN 148	PAN 148	07P L042	0	
PAN 148	PAN 148	07P L043	0	
PAN128*1/SMARC4PN1	BRUX1099	07P L044	1	**BC1**
PAN128*1/SMARC4PN1	BRUX1099	07P L045	3	
PAN128*1/SMARC4PN1	BRUX1099	07P L046	15	
PAN128*1/SMARC4PN1	BRUX1099	07P L047	33	
PAN128*1/SMARC4PN1	BRUX1099	07P L048	0	
PAN 148	PAN 148	07P L049	0	
PAN128*1/SMARC4PN1	BRUX1099	07P L050	11	
PAN128*1/SMARC4PN1	BRUX1099	07P L051	13	
PAN128*1/SMARC4PN1	BRUX1099	07P L052	15	
PAN 148	PAN 148	07P L053	0	
PAN128*1/SMARC4PN1	BRUX1099	07P L054	10	
PAN 148	PAN 148	07P L055	0	
PAN128*1/SMARC4PN1	BRUX1099	07P L056	17	
AV275*1/SMARC4PN1	BRUX1100	07P L057	2	**BC1**
PAN 148	PAN 148	07P L058	0	
AV275*1/SMARC4PN1	BRUX1100	07P L059	13	
AV275*1/SMARC4PN1	BRUX1100	07P L060	43	
AV275*1/SMARC4PN1	BRUX1100	07P L061	0	
AV275*1/SMARC4PN1	BRUX1100	07P L062	21	
AV275*1/SMARC4PN1	BRUX1100	07P L063	0	
AV275*1/SMARC4PN1	BRUX1100	07P L064	0	
AV275*1/SMARC4PN1	BRUX1100	07P L065	0	
AV275*1/SMARC4PN1	BRUX1100	07P L066	32	
AV275*1/SMARC4PN1	BRUX1100	07P L067	32	
AV275*1/SMARC4PN1	BRUX1100	07P L068	0	
AV275*1/SMARC4PN1	BRUX1100	07P L069	45	
AV275*1/SMARC4PN1	BRUX1100	07P L070	8	
AV275*1/SMARC4PN1	BRUX1100	07P L071	69	

Pedigree	Code	Lab Plot Bioassay 1	Adult emergence	Selection
PAN 148	PAN 148	07P L072	**0**	
PAN 148	PAN 148	07P L073	0	
PAN 148	PAN 148	07P L074	**0**	
AV275*1/SMARC4PN1	BRUX1100	07P L075	0	**BC1**
AV275*1/SMARC4PN1	BRUX1100	07P L076	7	
AV275*1/SMARC4PN1	BRUX1100	07P L077	11	
PAN 148	PAN 148	07P L078	0	
PAN107*1/SMARC4PN1	BRUX1101	07P L079	2	
PAN107*1/SMARC4PN1	BRUX1101	07P L080	10	
PAN107*1/SMARC4PN1	BRUX1101	07P L081	6	
PAN107*1/SMARC4PN1	BRUX1101	07P L082	1	**BC1**
PAN107*1/SMARC4PN1	BRUX1101	07P L083	34	
PAN107*1/SMARC4PN1	BRUX1101	07P L084	13	
PAN107*1/SMARC4PN1	BRUX1101	07P L085	14	
PAN107*1/SMARC4PN1	BRUX1101	07P L086	28	
PAN107*1/SMARC4PN1	BRUX1101	07P L087	26	
PAN107*1/SMARC4PN1	BRUX1101	07P L088	17	
PAN107*1/SMARC4PN1	BRUX1101	07P L089	93	
PAN107*1/SMARC4PN1	BRUX1101	07P L090	4	
PAN107*1/SMARC4PN1	BRUX1101	07P L091	9	
PAN107*1/SMARC4PN1	BRUX1101	07P L092	8	**BC1**
PAN107*1/SMARC4PN1	BRUX1101	07P L093	49	
PAN107*1/SMARC4PN1	BRUX1101	07P L094	6	
PAN107*1/SMARC4PN1	BRUX1101	07P L095	3	
PAN107*1/SMARC4PN1	BRUX1101	07P L096	9	
PAN107*1/SMARC4PN1	BRUX1101	07P L097	71	
PAN107*1/SMARC4PN1	BRUX1101	07P L098	21	
PAN107*1/SMARC4PN1	BRUX1101	07P L099	18	
PAN107*1/SMARC4PN1	BRUX1101	07P L100	4	
PAN107*1/SMARC4PN1	BRUX1101	07P L101	51	
PAN107*1/SMARC4PN1	BRUX1101	07P L102	0	
PAN107*1/SMARC4PN1	BRUX1101	07P L103	3	
PAN107*1/SMARC4PN1	BRUX1101	07P L104	11	
PAN 148	PAN 148	07P L105	0	
PAN107*1/SMARC4PN1	BRUX1101	07P L106	4	
PAN107*1/SMARC4PN1	BRUX1101	07P L107	78	
PAN107*1/SMARC4PN1	BRUX1101	07P L108	15	
PAN107*1/SMARC4PN1	BRUX1101	07P L109	36	

Pedigree	**Code**	**Lab Plot Bioassay 1**	**Adult emergence**	**Selection**
PAN107*1/SMARC4PN1	BRUX1101	07P L110	28	
PAN 148	PAN 148	07P L111	0	
PAN 148	PAN 148	07P L112	**0**	
PAN9249*1/SMARC4PN1	BRUX1102	07P L113	52	
PAN9249*1/SMARC4PN1	BRUX1102	07P L114	13	
PAN9249*1/SMARC4PN1	BRUX1102	07P L115	11	
PAN9249*1/SMARC4PN1	BRUX1102	07P L116	68	
PAN9249*1/SMARC4PN1	BRUX1102	07P L117	31	
PAN9249*1/SMARC4PN1	BRUX1102	07P L118	29	
PAN9249*1/SMARC4PN1	BRUX1102	07P L119	14	
PAN9249*1/SMARC4PN1	BRUX1102	07P L120	14	
PAN9249*1/SMARC4PN1	BRUX1102	07P L121	21	
PAN9249*1/SMARC4PN1	BRUX1102	07P L122	84	
PAN 148	PAN 148	07P L123	0	
PAN 148	PAN 148	07P L124	0	
PAN9249*1/SMARC4PN1	BRUX1102	07P L125	24	
PAN9249*1/SMARC4PN1	BRUX1102	07P L126	56	
PAN9249*1/SMARC4PN1	BRUX1102	07P L127	17	
PAN9249*1/SMARC4PN1	BRUX1102	07P L128	44	
PAN9249*1/SMARC4PN1	BRUX1102	07P L129	11	
PAN9249*1/SMARC4PN1	BRUX1102	07P L130	4	
PAN9249*1/SMARC4PN1	BRUX1102	07P L131	99	
PAN 148	PAN 148	07P L132	0	
PAN9249*1/SMARC4PN1	BRUX1102	07P L133	1	**BC1**
PAN9249*1/SMARC4PN1	BRUX1102	07P L134	1	**BC1**
PAN9249*1/SMARC4PN1	BRUX1102	07P L135	38	
PAN9249*1/SMARC4PN1	BRUX1102	07P L136	23	
PAN9249*1/SMARC4PN1	BRUX1102	07P L137	51	

Appendix 3. Bioassay 2: Number of adults of *Acanthoscelides obtectus* emerging from 20 seeds after infestation

Pedigree	Code	Lab plot Bioassay 1	Lab plot Bioassay 2	Adult emergence	Selection
AV1337*1/SMARC4PN1	BRUX1021	07P L027	07PD099	41	**BC2**
AV1337*1/SMARC4PN1	BRUX1021	07P L027	07PD100	48	
AV1337*1/SMARC4PN1	BRUX1021	07P L027	07PD101	60	
AV1337*1/SMARC4PN1	BRUX1021	07P L027	07PD102	50	
AV1337*1/SMARC4PN1	BRUX1021	07P L027	07PD103	67	
AV1337*1/SMARC4PN1	BRUX1021	07P L028	07PD104	76	
AV1337*1/SMARC4PN1	BRUX1021	07P L028	07PD105	75	
AV1337*1/SMARC4PN1	BRUX1021	07P L028	07PD106	82	
AV1337*1/SMARC4PN1	BRUX1021	07P L028	07PD107	88	
AV1337*1/SMARC4PN1	BRUX1021	07P L028	07PD108	5	**BC2**
	AV1337		07PD109	105	
	AV1337		07PD110	75	
	AV1337		07PD111	100	
	AV1337		07PD112	103	
	AV1337		07PD113	100	
PAN118*1/SMARC4PN1	BRUX1022	07P L030	07PD114	102	
PAN118*1/SMARC4PN1	BRUX1022	07P L030	07PD115	149	
PAN118*1/SMARC4PN1	BRUX1022	07P L030	07PD116	82	
PAN118*1/SMARC4PN1	BRUX1022	07P L030	07PD117	0	**BC2**
PAN118*1/SMARC4PN1	BRUX1022	07P L030	07PD118	48	
PAN118*1/SMARC4PN1	BRUX1022	07P L032	07PD119	118	
PAN118*1/SMARC4PN1	BRUX1022	07P L032	07PD120	106	
PAN118*1/SMARC4PN1	BRUX1022	07P L032	07PD121	76	
PAN118*1/SMARC4PN1	BRUX1022	07P L032	07PD122	61	
PAN118*1/SMARC4PN1	BRUX1022	07P L032	07PD123	23	**BC2**
	PAN 118		07PD124	106	
	PAN 118		07PD125	100	
	PAN 118		07PD126	128	
	PAN 118		07PD127	87	
	PAN 118		07PD128	153	
PAN128*1/SMARC4PN1	BRUX1099	07P L039	07PD129	94	
PAN128*1/SMARC4PN1	BRUX1099	07P L039	07PD130	74	
PAN128*1/SMARC4PN1	BRUX1099	07P L039	07PD131	4	**BC2**

Pedigree	Code	Lab plot Bioassay 1	Lab plot Bioassay 2	Adult emergence	Selection
PAN128*1/SMARC4PN1	BRUX1099	07P L039	07PD132	18	**BC2**
PAN128*1/SMARC4PN1	BRUX1099	07P L039	07PD133	68	
PAN128*1/SMARC4PN1	BRUX1099	07P L044	07PD134	70	
PAN128*1/SMARC4PN1	BRUX1099	07P L044	07PD135	80	
PAN128*1/SMARC4PN1	BRUX1099	07P L044	07PD136	42	
PAN128*1/SMARC4PN1	BRUX1099	07P L044	07PD137	59	
PAN128*1/SMARC4PN1	BRUX1099	07P L044	07PD138	53	
	PAN 128		07PD139	72	
	PAN 128		07PD140	72	
	PAN 128		07PD141	78	
	PAN 128		07PD142	62	
	PAN 128		07PD143	114	
AV275*1/SMARC4PN1	BRUX1100	07P L057	07PD144	101	
AV275*1/SMARC4PN1	BRUX1100	07P L057	07PD145	42	**BC2**
AV275*1/SMARC4PN1	BRUX1100	07P L057	07PD146	54	
AV275*1/SMARC4PN1	BRUX1100	07P L057	07PD147	78	
AV275*1/SMARC4PN1	BRUX1100	07P L057	07PD148	73	
AV275*1/SMARC4PN1	BRUX1100	07P L075	07PD149	97	
AV275*1/SMARC4PN1	BRUX1100	07P L075	07PD150	44	**BC2**
AV275*1/SMARC4PN1	BRUX1100	07P L075	07PD151	77	
AV275*1/SMARC4PN1	BRUX1100	07P L075	07PD152	89	
AV275*1/SMARC4PN1	BRUX1100	07P L075	07PD153	53	
	AV 275		07PD154	91	
	AV 275		07PD155	85	
	AV 275		07PD156	88	
	AV 275		07PD157	75	
	AV 275		07PD158	89	
PAN107*1/SMARC4PN1	BRUX1101	07P L082	07PD159	70	
PAN107*1/SMARC4PN1	BRUX1101	07P L082	07PD160	102	
PAN107*1/SMARC4PN1	BRUX1101	07P L082	07PD161	109	
PAN107*1/SMARC4PN1	BRUX1101	07P L082	07PD162	84	
PAN107*1/SMARC4PN1	BRUX1101	07P L082	07PD163	145	
PAN107*1/SMARC4PN1	BRUX1101	07P L092	07PD164	90	
PAN107*1/SMARC4PN1	BRUX1101	07P L092	07PD165	113	
PAN107*1/SMARC4PN1	BRUX1101	07P L092	07PD166	104	
PAN107*1/SMARC4PN1	BRUX1101	07P L092	07PD167	95	
PAN107*1/SMARC4PN1	BRUX1101	07P L092	07PD168	0	**BC2**
	PAN 107		07PD169	105	

Pedigree	Code	Lab plot Bioassay 1	Lab plot Bioassay 2	Adult emergence	Selection
	PAN 107		07PD170	76	
	PAN 107		07PD171	86	
	PAN 107		07PD172	90	
	PAN 107		07PD173	89	
PAN9249*1/SMARC4PN1	BRUX1102	07P L133	07PD174	101	
PAN9249*1/SMARC4PN1	BRUX1102	07P L133	07PD175	64	
PAN9249*1/SMARC4PN1	BRUX1102	07P L133	07PD176	83	
PAN9249*1/SMARC4PN1	BRUX1102	07P L133	07PD177	76	
PAN9249*1/SMARC4PN1	BRUX1102	07P L133	07PD178	57	**BC2**
PAN9249*1/SMARC4PN1	BRUX1102	07P L134	07PD179	74	
PAN9249*1/SMARC4PN1	BRUX1102	07P L134	07PD180	42	**BC2**
PAN9249*1/SMARC4PN1	BRUX1102	07P L134	07PD181	87	
PAN9249*1/SMARC4PN1	BRUX1102	07P L134	07PD182	63	
PAN9249*1/SMARC4PN1	BRUX1102	07P L134	07PD183	165	
	PAN 9249		07PD184	149	
	PAN 9249		07PD185	125	
	PAN 9249		07PD186	133	
	PAN 9249		07PD187	98	
	PAN 9249		07PD188	124	
	SMARC4PN1		07PD189	42	
	SMARC4PN1		07PD190	33	
	SMARC4PN1		07PD191	41	
	SMARC4PN1		07PD192	40	
	SMARC4PN1		07PD193	35	

Appendix 4. Bioassay 3: Number of adults of *Acanthoscelides obtectus* emerging from 20 seeds after infestation

Pedigree	Code	Lab plot Bioassay 1	Lab plot Bioassay 2	Lab plot Bioassay 3	Adult emergence per 20 seeds	Selection
AV1337*1/SMARC4PN1	BRUX1021	07P L027	07PD099	09PD001	32	
AV1337*1/SMARC4PN1	BRUX1021	07P L027	07PD108	09PD002	9	**BC3**
	AV1337			09PD003	95	
PAN118*1/SMARC4PN1	BRUX1022	07P L030	07PD117	09PD004	34	**BC3**
PAN118*1/SMARC4PN1	BRUX1022	07P L032	07PD123	09PD005	44	
	PAN 118			09PD006	102	
PAN128*1/SMARC4PN1	BRUX1099	07P L039	07PD131	09PD007	26	**BC3**
PAN128*1/SMARC4PN1	BRUX1099	07P L039	07PD132	09PD008	64	
	PAN 128			09PD009	113	
AV275*1/SMARC4PN1	BRUX1100	07P L057	07PD145	09PD010	39	
AV275*1/SMARC4PN1	BRUX1100	07P L075	07PD150	09PD011	9	**BC3**
	AV 275			09PD012	177	
PAN107*1/SMARC4PN1	BRUX1101	07P L092	07PD168	09PD013	44	**BC3**
	PAN 107			09PD014	57	
PAN9249*1/SMARC4PN1	BRUX1102	07P L133	07PD178	09PD015	34	**BC3**
PAN9249*1/SMARC4PN1	BRUX1102	07P L134	07PD180	09PD016	45	
	PAN 9249			09PD017	120	
	SMARC4PN1			09PD018	21	

Appendix 5. Geographical and climatic data for Greytown and Delmas (South African Weather Service, 2009)

	Latitude	Longitude	Elevation masl	Average annual rainfall	Average maximum temperature	Average minimum temperature
Greytown	29° 4' 01S	30° 34' 60E	1015 masl	844 mm	26°C	11°C
Delmas	26° 8' 60S	28° 40' 60E	1560 masl	713 mm	22°C	10°C

CHAPTER 4

EVALUATION OF YIELD AND AGRONOMIC CHARACTERISTICS OF NEAR ISOGENIC LINES AND PARENTAL GENOTYPES

4.1 INTRODUCTION

The two bruchid species, *Acanthoscelides obtectus* and *Zabrotes subfasciatus* remain a very serious threat wherever common bean is cultivated. Arcelin containing breeding lines (SMARC and RAZ coded breeding lines) are unlikely to be accepted by small-scale farmers. The reason being the fact that these varieties are all small seeded and not adapted to local growing conditions (Kananji, 2007). Resistance against *A. obtectus* was transferred to six adapted, high yielding South African dry bean cultivars.

In this study 11 BC2F3 near isogenic lines were selected containing increased levels of resistance against *A. obtectus.* These lines were developed through backcrossing followed by progeny testing where the two best plants per pedigree were selected based on bioassays with adult insects of *A. obtectus* (see Chapter 3 for details).

For the developed breeding lines to be commercially acceptable or of use in a breeding programme certain criteria should be met. These are i) the breeding lines should have better resistance against *A. obtectus* than the susceptible commercial parents, as evaluated through bioassays with adult insects of the species; ii) Seed characteristics such as size and colour should be acceptable as red speckled sugar bean type; iii) Seed yield of the breeding lines should be the same or higher than the susceptible commercial parents as determined through a replicated yield trial.

The aim of this study was:

1) To test the yield ability of the backcross progeny lines and comparing it to that of the susceptible commercial parents and arcelin donor parent in the breeding scheme.
2) Evaluating the resistance of the backcross progeny lines against *A. obtectus* compared to that of the susceptible commercial parents and the arcelin donor parent.
3) Evaluation of the backcross progeny lines for seed size and colour (suitable for use as red speckled sugar beans).
4) Identifying backcross progeny lines equal or better in all characteristics than the commercial parents.

4.2 MATERIALS AND METHODS

4.2.1 Plant materials

The characteristics of the susceptible commercial parents (AV1337; PAN118; PAN128; AV275; PAN107 and PAN9249), as well as the arcelin donor parent (SMARC4-PN1) used in the development of the progeny lines were discussed in the materials and methods section of Chapter 3. The following breeding lines and commercial varieties were evaluated:

BRUX1021-1 and BRUX1021-2: The two best backcross progeny lines selected from pedigree AV1337*2/SMARC4PN1. Selections were made from bruchid adult emergence data from Bioassay 2 (Appendix 4).

BRUX1022-1 and BRUX1022-2: The two best backcross progeny lines selected from pedigree PAN118*2/SMARC4PN1. Selections were made from bruchid adult emergence data from Bioassay 2 (Appendix 4).

BRUX1099-1 and BRUX1099-2: The two best backcross progeny lines selected from pedigree PAN128*2/SMARC4PN1. Selections were made from bruchid adult emergence data from Bioassay 2 (Appendix 4).

BRUX1100-1 and BRUX1100-2: The two best backcross progeny lines selected from pedigree AV275*2/SMARC4PN1. Selections were made from bruchid adult emergence data from Bioassay 2 (Appendix 4).

BRUX1101: The best backcross progeny line selected from pedigree PAN107*2/SMARC4PN1. Selection was made from bruchid adult emergence data from Bioassay 2 (Appendix 4).

BRUX1102-1 and BRUX1102-2: The two best backcross progeny lines selected from pedigree PAN9249*2/SMARC4PN1. Selections were made from bruchid adult emergence data from Bioassay 2 (Appendix 4).

PAN 123: A commercial small white dry bean variety included in the evaluation because of its high yield and lodging resistance as well as seed size comparable to SMARC4-PN1.

PAN 185: A commercial small white dry bean variety included in the evaluation because of its high yield and disease resistance as well as seed size comparable to SMARC4-PN1.

4.2.2 Yield trial

A yield trial, consisting of 20 entries with three replicates was conducted at the PANNAR Research Station at Delmas. The purpose of the trial was to determine the yield ability of the near isogenic lines. Seed yield of the breeding lines was measured against the yield of the parents used in the development of the lines. The trial was conducted under protected growing conditions in a greenhouse. The trial was planted on 15 December 2008. The greenhouse was fitted with a pad and fan cooling system, and an electric heating system with no artificial light sources installed. The maximum temperature was regulated at 28°C and the minimum temperature at 15°C. The plants were grown in plastic containers with a volume of 20l. The plastic containers were filled with a general use potting soil mixture.

Three plants were grown per container with each container treated as an experimental unit. Irrigation was electronically managed with a Hunter XC 401 dripper system. Plants were irrigated at a rate of one liter of water/day/container for the first 40 days (onset of flowering). Flower colour (correlated to seed colour) and plant growth habit was recorded for all the entries in the trial for later analysis. The irrigation rate was increased to 2l of water/day/container from day 40 up to physiological pod maturity. When the pods were at physiological maturity, irrigation was withdrawn and plants were allowed to dry out. The dry pods were harvested in bulk per container, air dried to 13% moisture content and the mass determined. The harvesting date for the yield trial was 9 March 2009.

4.2.3 Evaluation of seed characteristics

The seed harvested in the yield trial was used in the determination of seed size and colour. In the determination of seed size, 100 randomly selected seeds from each entry in all three replications were weighed to determine 100-seed mass at 13% moisture. Seed colour acceptable for use as red speckled sugar bean type was determined by using the same seed sample. Seed from the backcross progeny lines was visually compared to the commercial parents and rated “yes” if the colour was comparable to the commercial parent or “no” if the colour was different from the commercial parent.

4.2.4 Bioassay for resistance against *A. obtectus*

Methodology for the rearing of insect cultures and artificial infestation was described by Schoonhoven and Cardona (1982). All the entries in the yield trial were evaluated for resistance against *A. obtectus* in a replicated insect feeding test during April 2009. This test was conducted indoors with temperature control. From the 100- seed mass samples a sub-sample of 20 undamaged seeds were selected. As the seed were harvested in a greenhouse it was assumed not to have been infested by the insect species earlier. The 20 seeds from each sample were placed in small plastic containers and fitted with nylon mesh on top to ensure aeration. The seeds were artificially infested with 1day-old, newly emerged adult bruchids. Since fitness of the adult insects used was important, only adult

insects of the same age were used in the evaluation process. Adult bruchids were allowed to infest the seed for 20 days and then removed. When the new adult bruchids emerged after 35-38 days, they were removed from the seed and total emergence recorded for all the entries in all three replications. Total adult emergence was used to quantify resistance.

4.2.5 Experimental design and data analysis

For the yield trial and replicated insect feeding test the 20 entries were tested in a randomized complete block design with three replications. As small quantities of seed were available for the yield trial, the trial was not repeated at different localities. Entries were randomized using a randomization table for a four row x five column trial layout. Data from the yield trial was analyzed using AGROBASE (2000) software (Appendices 7-9). Entries were ranked for yield ability and the means separated by least significant difference for comparison. Entries were compared within pedigrees for yield, bruchid resistance, seed size and seed colour.

4.3 RESULTS AND DISCUSSION

4.3.1 Evaluation of arcelin donor and commercial parents for six agronomic characteristics

There were eight commercial and semi-commercial varieties included in the yield trial and the bioassay to determine resistance against bruchid feeding (Table 4.1). The arcelin donor parent SMARC4-PN1 was also included as resistant check. The entries were evaluated and ranked (Table 4.2) for yield, adult bruchid emergence count, seed size, seed colour, flower colour and growth habit. The coefficient of variance (CV) for the yield trial and seed size evaluation was 11.23% and 1.67% respectively, making the trial results very reliable for interpretation. The CV for the adult emergence trial was higher at 20.66%, indicating that it is a more variable attribute to evaluate.

PAN 9249 was the highest yielding variety and had the biggest seed size. PAN 123 had the lowest yield with the smallest seed size. The resistant check SMARC4-PN1 had the lowest adult emergence and the second smallest seed size of the commercial and semi-commercial varieties. There were significant (P≤0.05) differences in yield between the resistant check SMARC4-PN1 and PAN 9249, PAN 118, AV 275 and PAN 128. The resistant check SMARC4-PN1 had a lower adult bruchid emergence count than all the commercial and semi-commercial varieties, with significant (P≤0.05) differences between the resistant check and AV 275; PAN 9249; PAN 128; PAN 118 and AV 1337. All the commercial and semi-commercial varieties had significantly (P≤0.05) bigger seed size than the resistant check SMARC4-PN1, except for PAN 123 with a smaller seed size.

Table 4.1 Evaluation of semi-commercial; commercial and arcelin donor parents for six agronomic characteristics

Entries	Yield (g/pot)	Adult emergence	Seed size (g/100 seeds)	Seed colour	Flower colour	Growth habit
SMARC4-PN1	82.97	27.00	20.33	w	W	I
AV 1337	79.20	88.33	31.97	sp	W	I
PAN 118	116.67	98.00	41.23	sp	W	I
PAN 128	108.93	103.33	40.93	sp	P	I
AV 275	115.37	116.67	50.57	sp	P	D
PAN 107	74.70	50.00	63.07	sp	P	I
PAN 9249	129.37	115.00	67.40	sp	P	I
PAN 123	66.90	39.00	19.37	w	W	D
PAN 185	102.27	43.33	24.63	w	W	D
Grand mean	97.14	54.62	37.98			
CV	11.23	20.66	1.67			
LSD (0.05)	24.14	24.98	1.40			

w = white; sp = red speckled seed; p = purple; D = determinate; I = indeterminate

Table 4.2 Ranking of the 20 trial entries for three measured characteristics

Trial entries	Mean yield (g/pot)	Yield ranking	Mean adult emergence (no of emerged adults)	Adult emergence ranking	Mean seed size (g/100 seeds)	Seed size ranking
SMARC4-PN1	82.97	15	27.00	18	20.33	19
AV1337	115.37	16	88.33	5	31.97	15
BRUX1021-1	86.10	14	35.00	16	29.37	17
BRUX1021-2	91.23	13	16.67	19	30.87	16
PAN118	116.67	3	98.00	4	41.23	6
BRUX1022-1	100.07	10	40.00	13	37.57	9
BRUX1022-2	97.90	11	45.33	9	32.80	12
PAN 128	108.93	6	103.33	3	40.93	7
BRUX1099-1	106.07	7	30.00	17	32.57	13
BRUX1099-2	91.53	12	57.00	6	36.63	10
AV 275	115.37	4	116.67	1	50.57	3
BRUX1100-1	67.93	18	37.33	15	50.43	4
BRUX1100-2	149.77	1	11.67	20	33.23	11
PAN 107	74.70	17	50.00	8	63.07	2

Trial entries	Mean yield (g/pot)	Yield ranking	Mean adult emergence (no of emerged adults)	Adult emergence ranking	Mean seed size (g/100 seeds)	Seed size ranking
SMARC4-PN1	82.97	15	27.00	18	20.33	19
BRUX1101	65.03	20	44.00	11	43.77	5
PAN 9249	129.37	2	115.00	2	67.40	1
BRUX1102-1	110.37	5	44.67	10	32.50	14
BRUX1102-2	100.50	9	50.00	7	40.47	8
PAN 123	66.90	19	39.00	14	19.37	20
PAN 185	102.27	8	43.33	12	24.63	18
Grand mean	97.14		54.62		37.98	
CV	11.23		20.66		1.67	
$LSD_{(0.05)}$	24.14		24.98		1.40	

Ranking: 1 = highest yield, highest emergence count, largest seed; 20 = lowest yield, lowest emergence count, smallest seed

4.3.2 Comparison of parents and progeny for pedigree AV1337*2/SMARC4PN1

For ease of comparison, results of the yield trial are discussed per pedigree. The semi-commercial parent AV1337 had a significantly (P≤0.05) higher yield than the two backcross progeny lines in the yield trial (Table 4.3). There was no significant difference in the yield between the backcross progeny lines. The means for adult emergence of the backcross progeny lines was significantly (P≤0.05) lower than that of the commercial parent. Although seed size was significantly smaller (P≤0.05) for the backcross progeny lines than the commercial parent, seed colour of seed from both the backcross progeny lines could be compared to that of the commercial parent. There was no significant difference in seed size between the two backcross progeny lines.

Table 4.3 Comparison of parents and progeny for pedigree AV1337*2/SMARC4PN1

Entries	Yield (g/pot)	Adult emergence	Seed size (g/100 seeds)	Seed colour
AV1337	115.37 (a)	116.67 (a)	50.57 (a)	Y
SMARC4-PN1	82.97	27.00	20.33	N
BRUX1021-1	86.10 (b)	35.00 (b)	29.37 (b)	Y
BRUX1021-2	91.23 (b)	16.67 (b)	30.87 (b)	Y
Grand mean	97.143	54.617	37.985	
CV	11.23	20.66	1.67	
LSD(0.05)	24.14	24.98	1.40	

Means followed by the letters (b) differ significantly (P≤0.05) from the means of the parents denoted by the letter (a); Y = red speckled sugar bean seed type; N = not red speckled sugar seed type

4.3.3 Comparison of parents and progeny for pedigree PAN118*2/SMARC4PN1

There was no significant difference in yield between the commercial parent PAN118 and the backcross progeny lines (Table 4.4). Both progeny entries showed a significantly ($P \leq 0.05$) lower mean adult emergence compared to the commercial parent in the bioassay. The lowest mean adult emergence of the two progeny entries was for BRUX1022-1 with 40 adults emerging per 20 seeds. There was no significant difference in mean adult emergence between the donor parent SMARC4-PN1 and the backcross progeny lines. Seed size of the commercial parent was significantly ($P \leq 0.05$) larger than the two progeny entries, indicating that the parent phenotype was not recovered fully for this characteristic. The seed size of BRUX1022-1 was significantly ($P \leq 0.05$) larger than that of BRUX1022-2. Seed colour of both progeny entries compared well to that of the commercial parent.

Table 4.4 Comparison of parents and progeny for pedigree PAN118*2/SMARC4PN1

Entries	Yield (g/pot)	Adult emergence	Seed size (g/100 seeds)	Seed colour
PAN118	116.67	98.00 (a)	41.23 (a)	Y
SMARC4-PN1	82.97	27.00	20.33	N
BRUX1022-1	100.07	40.00 (b)	37.57 (b)	Y
BRUX1022-2	97.90	45.33 (b)	32.80 (c)	Y
Grand mean	97.14	54.61	37.99	
CV	11.23	20.66	1.67	
$LSD_{(0.05)}$	24.14	24.98	1.40	

Means followed by the letters (b) and (c) differ significantly ($P \leq 0.05$) from the means of the parents denoted by the letter (a); Y = red speckled sugar bean seed type; N = not red speckled sugar seed type

4.3.4 Comparison of parents and progeny for pedigree PAN128*2/SMARC4PN1

There were no significant differences in the mean yield between the commercial parent PAN128 and the two progeny entries (Table 4.5) in the yield trial. The mean adult emergence of the progeny entries was significantly (P≤0.05) lower than that of the commercial parent in the bioassay. Mean adult emergence for BRUX1099-2 was significantly higher (P≤0.05) than that for SMARC4-PN1 in the bioassay. Mean seed size of the progeny entries was significantly (P≤0.05) smaller than that of the commercial parent. Seed colour for both of the backcross progeny lines did not compare to the commercial parent.

Table 4.5 Comparison of parents and progeny for pedigree PAN128*2/SMARC4PN1

Entries	Yield (g/pot)	Adult emergence	Seed size (g/100 seeds)	Seed colour
PAN 128	108.93	103.33 (a)	40.93 (a)	Y
SMARC4-PN1	82.97	27.00 (a)	20.33	N
BRUX1099-1	106.07	30.00 (b)	32.57 (b)	N
BRUX1099-2	91.53	57.00 (c) (b)	36.63 (c)	N
Grand mean	97.143	54.617	37.985	
CV	11.23	20.66	1.67	
$LSD_{(0.05)}$	24.14	24.98	1.40	

Means followed by the letters (b) and (c) differ significantly (P≤0.05) from the means of the parents denoted by the letter (a); Y = red speckled sugar bean seed type; N = not red speckled sugar seed type

4.3.5 Comparison of parents and progeny for pedigree AV275*2/SMARC4PN1

Mean yield for BRUX1100-2 was significantly (P≤0.05) higher than that of the semi-commercial parent AV275 and progeny entry BRUX1100-1 in the yield trial (Table 4.6). Both progeny lines showed significantly lower (P≤0.05) adult emergence than that of the semi-commercial parent in the bioassay. There were also significant differences (P≤0.05) in adult emergence between the two progeny lines. Seed size of the commercial parent was significantly larger (P≤0.05) than backcross progeny line BRUX1100-2. There was no significant difference in the size of the seed between the commercial parent and BRUX1100-1 and the progeny seed size of 50.43g/100 seeds could be commercially acceptable. Although BRUX1100-2 had the highest mean yield and lowest mean adult emergence, seed colour did not compare to that of the commercial parent. Seed colour of BRUX1100-2 compared well to that of the commercial parent.

Table 4.6 Comparison of parents and progeny for pedigree AV275*2/SMARC4PN1

Entries	Yield (g/pot)		Adult emergence		Seed size (g/100 seeds)		Seed colour
AV 275	115.37	(a)	116.67	(a)	50.57	(a)	Y
SMARC4-PN1	82.97		27.00		20.33		N
BRUX1100-1	67.93	(b)	37.33	(b)	50.43	(a)	Y
BRUX1100-2	149.77	(c)	11.67	(c)	33.23	(b)	N
Grand mean	97.143		54.617		37.985		
CV	11.23		20.66		1.67		
LSD(0.05)	24.14		24.98		1.40		

Means followed by the letters (b) and (c) differ significantly (P≤0.05) from the means of the parents denoted by the letter (a); Y = red speckled sugar bean seed type; N = not red speckled sugar seed type

4.3.6 Comparison of parents and progeny for pedigree PAN107*2/SMARC4PN1

There were no significant differences ($P \leq 0.05$) between the mean yield of the commercial parent PAN107 and the progeny entry (Table 4.7). For adult emergence as determined with the bioassay (Table 4.2) there were no significant differences ($P \leq 0.05$) between the mean adult emergence of the commercial parent PAN107 and the backcross progeny line or the donor parent SMARC4-PN1. Although seed size of the backcross progeny line was significantly smaller ($P \leq 0.05$) than that of the commercial parent, the seed colour of the progeny compared well to that of the commercial parent.

Table 4.7 Comparison of parents and progeny for pedigree PAN107*2/SMARC4PN1

Entries	Yield (g/pot)	Adult emergence	Seed size (g/100 seeds)	Seed colour
PAN 107	74.70	50.00	63.07 (a)	Y
SMARC4-PN1	82.97	27.00	20.33	N
BRUX1101	65.03	44.00	43.77 (b)	Y
Grand mean	97.14	54.62	37.99	
CV	11.23	20.66	1.67	
$LSD_{(0.05)}$	24.14	24.98	1.40	

Means followed by the letters (b) differ significantly ($P \leq 0.05$) from the means of the parents denoted by the letter (a); Y = red speckled sugar bean seed type; N = not red speckled sugar seed type

4.3.7 Comparison of parents and progeny for pedigree PAN9249*2/SMARC4PN1

The large seeded commercial parent PAN9249 ranked second for yield and first for seed size. This commercial variety also had the second highest adult emergence in the bioassay (Table 4.2). There were significant differences ($P \leq 0.05$) between the mean yield of the commercial parent and the backcross progeny line BRUX1102-2 (Table 4.8). The mean adult emergence for the progeny entries in the bioassay was significantly lower ($P \leq 0.05$) than that of the commercial parent. The donor parent SMARC4-PN1 had a significantly lower adult emergence than progeny line BRUX1102-2. Seed size of the commercial parent was significantly larger ($P \leq 0.05$) than that of both the backcross progeny lines, with significant differences between the seed size of the two backcross progeny lines. While the seed colour of BRUX1102-1 compared well to that of the commercial parent, the seed colour of BRUX1102-2 did not compare to that of the commercial parent PAN 9249.

Table 4.8 Comparison of parents and progeny for pedigree PAN9249*2/SMARC4PN1

Entries	Yield (g/pot)	Adult emergence	Seed size (g/100 seeds)	Seed colour
PAN 9249	129.37 (a)	115.00 (a)	67.40 (a)	Y
SMARC4-PN1	82.97	27.00 (a)	20.33	N
BRUX1102-1	110.37	44.67 (b)	32.50 (b)	Y
BRUX1102-2	100.50 (b)	50.00 (b) (b)	40.47 (c)	N
Grand mean	97.143	54.617	37.985	
CV	11.23	20.66	1.67	
$LSD_{(0.05)}$	24.14	24.98	1.40	

Progeny means followed by the letters (b) and (c) differ significantly ($P \leq 0.05$) from the means of the parents denoted by the letter (a); Y = red speckled sugar bean seed type; N = not red speckled sugar seed type

4.3.8 Correlation between three dry bean characteristics

The results revealed a moderate ($P \leq 0.05$) positive correlation between 100-seed mass and adult emergence (Table 4.9). The 100-seed mass was determined as a measure of seed size in this study. This does not support the finding of Kananji (2007) which showed no significant correlation between seed size and adult bruchid emergence for both *A. obtectus* and *Z. subfasciatus*.

Table 4.9 Correlation matrix between three host-plant characteristics

	Yield	100-seed mass
100-seed mass	0.145	
Adult emergence	0.209	0.523*

* $P \leq 0.05$

4.4 CONCLUSIONS

In this study the yield ability, resistance against *Acanthoscelides obtectus,* seed size and seed colour of the selected backcross progeny lines (Chapter 3) were evaluated and compared to that of the commercial and semi-commercial parents and the arcelin donor parent. The high CV for the adult emergence trial would indicate the need for a fourth replication to reduce variability and to generate more reliable data for interpretation. Alternatively a larger sample size for both bruchids and seed could reduce variability, if the origin of the variability was related to male: female ratios in the infestation. All bioassays were done on single-plant basis resulting in small seed samples available for evaluation. An additional replication would therefore be the best method to improve the CV. The data showed that seven NIL's had yields similar to that of the commercial parents. One of the backcross progeny lines had a significantly better yield than the commercial parent as indicated from the yield trial data. The same backcross progeny line

also had the lowest adult bruchid emergence for all entries in the bioassay. For adult bruchid emergence ten backcross progeny lines had significantly better resistance against *A. obtectus* than that of the commercial parents. The seed size evaluation showed that ten backcross progeny lines had significantly smaller seed size than the commercial parents and seven backcross progeny lines had seed colour that could be compared to that of the commercial parents. These findings showed that with the backcrossing procedure, progress was made in the recovery of many of the parental traits. However, the highest yielding backcross progeny line from these trials still lacked the seed size and colour of the commercial parent. The combination of all the important parental traits along with good bruchid resistance was not attained in a single backcross progeny lines after two back crosses. Another one or possibly two cycles of backcrossing would be necessary for the further recovery of these characteristics, and for the recovery of the more complex traits such as seed yield and seed size, some inter-crossing may be required.

APPENDIX 6

A N A L Y S I S O F V A R I A N C E

10/25/2009

Dependent variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	59	31540.187			
BLOC	2	97.210	48.605	0.41	0.6674
ENTRY	19	26924.594	1417.084	11.92	0.0000
Residual	38	4518.383	118.905		

Grand mean = 97.143 R-squared = 0.8567 C.V. = 11.23%

LSD for ENTRY = 24.14 S.E.D. = 8.9034 Heritability = 0.916

t (1-sided a=0.005, 38 df) = 2.7116 MSE = 118.90482

ENTRY

Level	Averages --- Y ---	Cv	Rank	
11	149.77	11.4	1	brux1100-2
17	129.37	7.1	2	pan9249
6	116.67	8.3	3	pan118
12	115.37	8.9	4	av275
15	110.37	10.7	5	brux1102-1
9	108.93	14.1	6	pan128
7	106.07	10.8	7	brux1099-1
19	102.27	11.9	8	pan185
16	100.50	9.4	9	brux1102-2
4	100.07	6.4	10	brux1022-1
5	97.90	5.8	11	brux1022-2
8	91.53	3.0	12	brux1099-2
2	91.23	10.0	13	brux1021-2
1	86.10	1.8	14	brux1021-1
18	82.97	19.7	15	smarc4-pn1
3	79.20	15.2	16	av1337
14	74.70	20.1	17	pan107
10	67.93	15.8	18	brux1100-1
20	66.90	8.3	19	pan123
13	65.03	10.5	20	brux1101

APPENDIX 7

A N A L Y S I S   O F   V A R I A N C E

10/25/2009

Dependent variable: HUNDRED

Source	df	SS	MS	F-value	Pr> F
Total	59	8946.157			
BLOC	2	5.953	2.977	7.42	0.0019
ENTRY	19	8924.963	469.735	1171.23	0.0000
Residual	38	15.240	0.401		

Grand mean = 37.985   R-squared = 0.9983   C.V. = 1.67%

LSD for ENTRY = 1.4021   S.E.D. = 0.5171   Heritability = 0.999
t (1-sided a=0.005, 38 df) = 2.7116   MSE = 0.40106

ENTRY

Level	Averages --- Y ---	Cv	Rank	
17	67.40	0.7	1	pan9249
14	63.07	1.3	2	pan107
12	50.57	0.5	3	av275
10	50.43	4.4	4	brux1100-1
13	43.77	0.7	5	brux1101
6	41.23	0.1	6	pan118
9	40.93	0.6	7	pan128
16	40.47	1.6	8	brux1102-2
4	37.57	0.8	9	brux1022-1
8	36.63	2.3	10	brux1099-2
11	33.23	0.6	11	brux1100-2
5	32.80	3.2	12	brux1022-2
7	32.57	1.2	13	brux1099-1
15	32.50	1.5	14	brux1102-1
3	31.97	1.0	15	av1337
2	30.87	3.2	16	brux1021-2
1	29.37	1.6	17	brux1021-1
19	24.63	0.8	18	pan185
18	20.33	3.2	19	smarc4-pn1
20	19.37	2.1	20	pan123

APPENDIX 8

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                   A N A L Y S I S   O F   V A R I A N C E
10/25/2009

 Dependent variable: ADULTEMERG

              Source   df         SS            MS      F-value   Pr> F
ÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄ
               Total   59     62438.183
                BLOC    2       127.633         63.817     0.50  0.6099
               ENTRY   19     57470.183       3024.746    23.75  0.0000
            Residual   38      4840.367        127.378
ÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄ
Grand mean = 54.617        R-squared = 0.9225        C.V. = 20.66%
ÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄÄ

LSD for ENTRY = 24.98 74       S.E.D. = 9.2151     Heritability = 0.958
  t (1-sided a=0.005, 38 df) = 2.7116   MSE = 127.37807


 ENTRY
        Averages
Level  --- Y ---  Cv     Rank

  12    116.67   25.5       1 av275
  17    115.00   20.3       2 pan9249
   9    103.33   14.5       3 pan128
   6     98.00   12.8       4 pan118
   3     88.33   13.1       5 av1337
   8     57.00   14.4       6 brux1099-2
  16     50.00   15.6       7 brux1102-2
  14     50.00   10.0       8 pan107
   5     45.33   12.1       9 brux1022-2
  15     44.67   13.1      10 brux1102-1
  13     44.00   10.4      11 brux1101
  19     43.33   10.4      12 pan185
   4     40.00   23.8      13 brux1022-1
  20     39.00   16.0      14 pan123
  10     37.33   13.7      15 brux1100-1
   1     35.00   18.7      16 brux1021-1
   7     30.00   13.3      17 brux1099-1
  18     27.00   24.3      18 smarc4-pn1
   2     16.67   18.3      19 brux1021-2
  11     11.67   30.1      20 brux1100-2
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CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Common bean (*Phaseolus vulgaris*) is a food legume produced worldwide and an important source of dietary protein to people in many developing countries. The stored grain of common bean is susceptible to attack by the bruchid species *Zabrotes subfasciatus* and *Acanthoscelides obtectus.* Host plant resistance together with chemical or biological control would be effective in protecting stored bean seed against bruchid attack.

Although some parental combinations showed a higher degree of resistance against bruchid attack, it was possible to select progeny lines with better resistance than the susceptible commercial parents. This would suggest the successful transfer of resistance conferred by the arcelin protein. This selection was done through a bioassay with adult insects of *A. obtectus*. The adult emergence data over generations confirms the findings of Kornegay and Cardona (1991b) that resistance levels seemed to decrease as generations progressed.

A population of 95 F6 recombinant inbred lines was developed from which the 10 most resistant and 10 most susceptible (as determined through a bioassay with adult insects of the species *A. obtectus*) were selected. This RIL population was developed to serve as reference lines for NIR or any other technique that requires the separation of resistant from susceptible in a wide genetic background in a future study.

A replicated yield trial was conducted to test the yield ability of the developed NIL's against the yield of the commercial parents in the breeding programme. Small amounts of seed limited the trial to one locality. Data from the yield trial indicated that seven NIL's had yields comparable to that of the commercial parents, while backcross progeny line BRUX1100-2 had a significantly ($P \leq 0.05$) higher yield than the semi-commercial parent AV 275.

For the adult emergence test the high CV would indicate the need for larger samples for both adult bruchids and seed to reduce variability. This is especially true if the origin of the variability is male: female ratio's in the bioassay (influencing number of eggs deposited and number of subsequent emerged adult insects). All bioassays were done on a single plant basis, with limited seed available for experimentation. The addition of a fourth replication in the adult emergence test would therefore be recommended to reduce variability. For adult bruchid emergence ten backcross progeny lines had significantly ($P \leq 0.05$) higher resistance levels against *A. obtectus* than that of the arcelin donor parent. Backcross progeny line BRUX1100-2 had the lowest adult bruchid emergence for all entries in the bioassay.

With the backcrossing procedure, progress was made in the recovery of many of the parental traits. The seed size evaluation showed that ten backcross progeny lines had significantly ($P \leq 0.05$) smaller seed size than the commercial parents and seven backcross progeny lines had seed colour that was comparable to that of the commercial parents.

The combination of all the important parental traits along with good bruchid resistance was not attained in any backcross progeny lines after only two backcrosses. Another one or possibly two cycles of backcrossing would be necessary for the further recovery of all these characteristics, and for the recovery of the more complex traits such as seed yield and seed size, some inter-crossing between backcross progeny lines may be required.

CHAPTER 6

SUMMARY

Key terms: Common bean; *Phaseolus vulgaris*; *Acanthoscelides obtectus*; *Zabrotes subfasciatus;* bruchids; arcelin; bean breeding; adult emergence; yield trial; seed size.

- This study was undertaken to transfer resistance against *Acanthoscelides obtectus* into South African dry bean cultivars; to select breeding lines with increased resistance against *A. obtectus* through bioassay with adult insects of the species; to develop a recombinant inbred line population, which could be used in future research for near infrared spectroscopy or any other technique; to test the yield ability, resistance against *A. obtectus,* seed size and colour of the developed breeding lines (progeny) and comparing it to that of the susceptible commercial parents and arcelin donor parent in the breeding scheme and to identify backcross progeny lines equal or better in all characteristics than the commercial parents.

- Crosses were made between six commercial and semi-commercial South African dry bean cultivars (recurrent parents) and the SMARC4-PN1 breeding line (arcelin donor parent). Parental characteristics were recovered in a backcross breeding programme. Bioassays were conducted with adult *A. obtectus* insects to identify resistant backcross progeny lines. The most resistant backcross progeny lines were selected for evaluation in a yield trial, and bioassay, against the commercial parents. A population of 95 F6 RI lines was developed from which the 10 most resistant and 10 most susceptible (as determined through a bioassay with adult insects of the species *A. obtectus*) were selected.

- A replicated yield trial was conducted and one RI line (BRUX1100-2) was shown to have significantly higher yield ability than the commercial parents. A replicated bruchid emergence test was conducted where one RI line (BRUX1100-2) was shown to have significantly higher bruchid resistance than the arcelin donor

parent. The seed size evaluation showed that ten backcross progeny lines had significantly smaller seed size than the commercial parents and seven backcross progeny lines had seed colour that could be compared to that of the commercial parents.

- The combination of all the important parental traits along with good bruchid resistance was not attained in any single backcross progeny line with only two backcrosses. Another one or possibly two cycles of backcrossing would be necessary for the further recovery all of these characteristics.

OPSOMMING

Sleutel woorde: Droëbone; *Phaseolus vulgaris*; *Acanthoscelides obtectus*; *Zabrotes subfasciatus;* bruchids; arcelin; droëboon teling; volwasse insek verskyning; opbrengs proef; saad grootte.

Die doelstellings van hierdie studie was om weerstand teen *A.obtectus* oor te dra na Suid-Afrikaanse droëboon kultivars; om teellyne te selekteer met verhoogde weerstand teen *A.obtectus* deur middel van bioassesering met volwasse insekte van die spesie; die ontwikkeling van 'n gerekombineerde ingeteelde lyn populasie wat in toekomstige navorsing gebruik kan word in naby infrarooi spektroskopie of ander toepassings; die toets van die opbrengs vermoë en weerstand teen *A. obtectus*, asook die evaluasie van saadgrootte en kleur van die ontwikkelde lyne (nageslag) teenoor die kommersiële ouers en arcelin skenker ouer in die teelprogram en die identifikasie van nageslag wat gelyk aan of beter is as die ouers vir alle gemete eienskappe.

Kruisings is gemaak tussen ses kommersiële en semi-kommersiële Suid-Afrikaanse droëboon kultivars (herhalende ouers) en SMARC4-PN1 teellyn (arcelin skenker ouer). Die eienskappe is herwin in 'n terugkruisingsprogram. Bioasseserings is gedoen met volwasse *A. obtectus* insekte om nageslag met weerstand teen insek voeding te identifiseer. Uit die program is nageslag geselekteer met die hoogste weerstandsvlakke, om te evalueer in 'n opbrengsproef en bioassesering teenoor die kommersiële ouers.'n Populasie bestaande uit 95 F6 RI lyne is ontwikkel waarvan die 10 mees vatbare en 10 mees bestande lyne (soos bepaal deur middel van 'n bioassesering met volwasse *A. obtectus* insekte) geselekteer is.

'n Gerepliseerde opbrengs proef is onderneem waaruit een RI lyn (BRUX1100-2) geïdentifiseer is met 'n betekenisvolle hoër opbrengsvermoë as die kommersiële ouers. 'n Herhaalde bioassesering met volwasse *A. obtectus* insekte is onderneem waaruit een RI lyn (BRUX1100-2) geïdentifiseer is met betekenisvolle beter bruchid weerstand as die arcelin skenker ouer. Die saad grootte en kleur evaluasie het getoon dat tien van die

nageslag betekenisvolle kleiner saadgrootte het, terwyl sewe van die nageslag saadkleur het wat goed met die van die ouerlyne kan vergelyk.

Dit was nie moontlik om al die belangrike ouereienskappe, tesame met goeie bruchid weerstand na slegs twee terugkruisings, te herwin nie. ‘n Verdere een of selfs twee terugkruisings sal nodig wees om al die belangrikke ouereienskappe ten volle te herwin.

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