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Common Bean (*Phaseolus vulgaris* L.) and the Bean Bruchid (*Zabrotes subfasciatus*): A Review

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ABSTRACT

Bean bruchid (Zabrotes subfasciatus) is one of the major constraints limiting common bean production in the low altitudes of tropical and subtropical areas. Understanding the biology, distribution and management of bean bruchid and the genetics and the physiological mechanisms underlying resistance in common bean is vital for the breeding to alleviate the adverse effects of the bean bruchid. In this literature review, research findings from the 1960s up to the most recent ones were included. The paper outlines the distribution, biology and management of bean bruchids. Then further discussion was given on breeding and genetics of resistance to bean bruchids and marker-assisted common bean breeding. This information can be utilized by common bean breeders who are working with bean bruchid.

Keywords: *Common bean, genetics and inheritance, marker assisted breeding, Zabrotes subfasciatus*

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes worldwide. According to data published in the Food and Agriculture organization (FAO) in 2014, the world dry bean production was estimated at 26.5 million tons that are produced from a cultivated area of 30.6 million hectares (FAOSTAT 2014). In 2010, out of the total beans produced, Latin America and the Caribbean constituted 24.4%, followed by Africa (17%) (FAOSTAT 2014). The total annual worldwide common bean production is about 12 million. Of which, 8 million tons are from Latin America and Africa. The contribution of Africa is about 2.5 million tons annually that makes the continent second major bean producer http://grainlegumes.cgiar.org/crops/common-bean/. The crop has a major role in household food and nutritional security. In Africa, the common bean can transform traditional subsistence farming to a market-oriented modern sector and makes a substantial contribution to the continent's economy. In eastern and southern Africa, the common bean is the most commonly-grown and consumed-grain legume and a good source of calories and dietary protein (Hillocks *et al*. 2006; Buruchara *et al*. 2011).

It is cultivated in a broad range of agro-ecologies and cropping systems, ranging from high potential to marginal and drought prone areas. The diverse bean growing conditions couples with specific preferences for particular bean types, seed colour and shape resulted in wide genetic diversity. In Ethiopia, it is the most widely-grown pulse crop, after the faba bean and an important source of income for many Ethiopian farmers (Asfaw*et al.* 2009). However, the productivity of the crop is below its potential, due to a number of abiotic and biotic stress factors. Most importantly, the bean bruchid (*Zabrotes subfasciatus*) is the most destructive insect pest, inflicting significant post-harvest losses of stored grain in Ethiopia. Different control options have been used to manage the losses caused by the insect. However, the development of cost-effective, environmentally-safe, sustainable and feasible control measures is the best option to manage bean bruchids in the common bean, particularly for the smallholder farmers. This paper presents a review of literature from a number of related studies and elaborates on the theoretical and practical aspects of the research. The first section provides information about the origin and domestication of the crop. The second section covers the biology, importance and control strategies of the bean bruchid. The third section comprises information about past research endeavours on breeding for bruchid resistance, the inheritance of the resistance and marker-assisted breeding.

Common Bean Origin and Domestication

A knowledge of the origin, domestication and diversification of cultivated and wild common bean species is useful for the characterization, conservation and deployment of available genetic resources. The genus *Phaseolus* belongs to the family Leguminosae and the subfamily Papilionoidea. The common bean is among the five domesticated *Phaseolus* species that are native to South America (Gepts & Debouck 1991). *Phaseolus vulgaris* and the majority of the cultivated and wild *Phaseolus* species have a diploid genome (2n=2x=22). Among all the species in the Leguminosae family, the common bean has a relatively small genome size (521.1 Mb) (Schmutz *et al*. 2014). Although the common bean is a self-pollinated species, the hybridization and the introgression of genes, from wild to cultivated beans species is easily to produce fertile and viable progenies (Singh 2001; Zizumbo-villarreal *et al*. 2005). However, incompatibility has been reported in some inter-gene pool crosses (Singh & Gutiérrez 1984; Burle *et al*. 2011).

The Common bean originated in the neo-tropics, and two independent centres of origin and domestication are reported (Gepts 1998). The multiple centres of domestication of the crop have resulted in two distinct major domestication gene pools, namely, the Mesoamerican and Andean gene pools (Singh *et al*. 1991b; c). The two gene pools are distinguished by their seed size and biochemical characteristics, including polymorphism in the phaseolins (seed-storage globulin proteins) (Gepts *et al*. 1988; Singh *et al*. 1991c, 1998; Haley *et al*. 1994; Velasquez &Gepts, 1994). The Mesoamerican gene pool can be further divided into three different races, namely, Mesoamerican (all small-seeded), Durango (medium-seeded semi-climber), and Jalisco (medium-seeded climber). Similarly, the Andean gene pool (all large-seeded) can be further divided into three races, namely, Nueva Granada, Peru and Chile (Singh *et al*. 1991a; Beebe *et al*. 2000; Blair *et al*. 2009). From its centres of origin, the crop has been disseminated to other parts of the world, such as Africa, Asia, Europe, and Oceania (Gepts & Bliss 1988). Common beans are believed to have been introduced to Africa in the 16th and 17th centuries, together with maize (Greenway 1945; Gentry 1969). The adaptation of the crop to different geographic regions, other than its centre of origin and domestication has led the crop to evolve different morphological, physiological and biochemical characteristics that have endowed the crop with abundant genetic variation (Gepts & Debouck 1991; Gepts 1998).

Since the introduction of common beans in Africa, farmers have developed and preserved important genotypes that are adapted to their local environments and their specific needs and this has led to the evolution of diverse morphological variants, called landraces (Wortman *et al*. 1998; Sperling 2001). In addition, the national bean research programs in many Africa countries, have been introducing a large number of new germplasms from different parts of the world (CIAT 2005). Consequently, the East African highlands have become the second centre of biodiversity for the common bean, due to its wide range of landrace diversity (Allen & Edje 1990; Wortman *et al*. 1998; Sperling 2001; Asfaw *et al*. 2009). Several researchers have reported on the co-existence of Andean and Mesoamerican gene pools in Africa (Martin & Adams 1987; Asfaw *et al*. 2009; Blair *et al*. 2010a; Okii *et al*. 2014; Tigist *et al*. 2019). Ethiopia and Kenya are among the major bean-producing countries in the sub-Saharan Africa, with highly diverse bean production systems (Hillocks et al., 2006; Asfaw et al., 2009).

Distribution and Biology the Bean Bruchids

The bean bruchids belongs to the order Coleoptera and the family bruchidae. There are two types of bruchids that commonly cause severe damage on stored beans, namely, *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say) (Cardona 2004). Both bruchid species are distributed worldwide in all bean-growing areas, but their prevalence is highly affected by the ambient temperature. *Zabrotes subfasciatus* originated in the tropical and sub-tropical regions of South and Central America, and they are prevalent in many other tropical and sub-tropical regions, especially East and Central Africa (Singh 1979; Abate & Ampofo 1996; Wortman *et al*. 1998; Alvarez *et al*. 2005). *Zabrotes subfasciatus* is more common in the low altitude areas, whereas *A. obtectus* is more frequent in the higher altitude areas (Cardona *et al*. 1989; Myers *et al*. 2001). The widespread occurrence of the pest in Ethiopia has also been reported by Negasi

(1994) and Wortman *et al*. (1998). Climate change will influence the patterns of the insect, with respect to their incidence and intensity. In the warmer areas of the country, Z. *subfasciatus* is the most important storage pest that causes serious grain losses.

The species *Z. subfasciatus* starts infestation in the stored seeds and adult longevity is relatively short (about 11 days). The females lay their eggs onto the dry seed and the eggs hatch on the seed coat. The first-instar larvae penetrate the seed coat and complete the life cycle inside the seed. The larvae of the species moult four times before pupating. During the last larval instar, the feeding and pupation cell becomes visible as a circular window in the seed, where the larvae feed on the lower testa surface. The male and female adult *Z. subfasciatus* can easily be differentiated by their colour and size. The female has cream-coloured spots on her elytra and are longer in size, while the male is short, with a pure grey colour. The insect completes its life cycle within 25-47 days i.e. 5-6 days for the egg, 14 days for the larva and 6-7 days for pupal stages. The females lay 36 eggs on average, and the adult life span is 10-13 days (Schoonhoven & Cardona 1986; Cardona *et al*. 1989).

Yield Loss Caused by the Bean Bruchid

Storage insect pests cause both quantitative and qualitative losses. Quantitative losses include the number of seeds eaten by the insect and the seed weight loss, whereas the grains that are contaminated by excrement or insect bodies cause qualitative losses (Schoonhoven & Cardona 1986; Jones 1999). The grain moisture content is directly correlated with bruchid infestation, where a seed moisture content of greater than 17% favours the rapid development of storage insects and fungi (*Aspergillus spp*., *Penicillium spp*. and *Phomopsis spp*) (Schoonhoven & Cardona 1986). The extent of the seed weight losses caused by bean bruchids depends on the storage period and storage conditions. On average, a 10-40% dry weight loss was reported, as a result of bean bruchid damage (Khamala 1978; Kiula and Karel 1985; Singh & Schwartz 2011) and the dry weight loss can reach up to 50-70% in most of the on-farm storage facilities, due to the lack of postharvest management practices (Khamala 1978; Lima 1987). Several researchers have reported on the extent of dry seed weight loss by bruchid in various countries in Africa. A mean of 30% stored bean damage, due to bean bruchids, has been reported in Rwanda, Burundi and Tanzania (Karel & Autrique 1989; Nahimana 1992). Similarly, a mean of 23% and 38% stored bean damage have been recorded in Uganda and Malawi, respectively (Karel & Autrique 1989; Kananji 2007). However, the highest bean damage of 73%, due to bean bruchids has been reported in Kenya (Karel & Autrique 1989). In Ethiopia, bean bruchids have caused an average of about 38% bean damage and 3.2% seed weight loss under farmer storage condition (Negasi 1994). Getu *et al*. (2003) and Araya & Getu(2009), on the other hand, reported that bean bruchids caused a grain weigh loss of up to 60% for beans stored from 3-6 months. The marketability, nutritional value, germination and seedling vigour of grains damaged by bean bruchid are significantly reduced (Singh & Schwartz 2011).

Management of the Bean Bruchids

Different types of control options have been used by farmers to keep the pest population below economic damage level. These include the sun-drying of the grains before storage, to reduce the grain moisture content, the cleaning and repairing of storage facilities, storing the grains with botanical pesticides or mixing them with small cereals, such as tef or ash, treating them with chemical insecticides and smoking the beans over a fire (Abate & Ampofo 1996; Tadesse *et al*. 2008). Nowadays, however, the cultural control practices are not used as often by farmers, because they now use chemical pesticides. Although insecticides are effective for bruchid control, smallholder farmers do not have separate storage structures for the fumigation of food grains and seeds. The development of environmentally-safe, sustainable, feasible and integrated pest control measures i.e. cultural, biological, host resistance and chemical, is vital for the control of bean bruchids.

Cultural and Physical Control Method

Farmers use different cultural methods to reduce the initial insect population. Proper drying before storage, the removal of all residues, the repair of storage structures and hygienic measures are the most common cultural practices for the control of bruchids(Tadesse & Eitecha 2000). Unlike *A. obtectus*, other pre-harvest cultural practices are not useful for controlling *Z. Subfaciatus* because the infestation begins while it is in storage. According to Quentin *et al*. (1991), shaking or tumbling the bean seeds several times per day controls bruchids by disrupting the larvae inside the seed and reducing the number of adults that emerge. Storing unthreshed beans is also practised, in order to reduce the damage caused by bean bruchids, as *Z. subfaciatus* prefers to lay their eggs on the seed coats (Abate & Ampofo 1996). The mixing of bean seeds with ash or small cereals, like tef and sorghum is also reported as one of control options for bean bruchids, as this affects insect's mobility and oviposition. However, these practices are effective if they are applied before infestation has taken place. The sun-drying of the grains followed by, sieving is a good technique to use against the storage pests of beans (Giga & Chinawda 1996). According to Songa & Rono(1998), this method has proved to be quite effective in reducing bruchid infestation, with no or minimal, effect on seed quality or germination. The use of cultural control measures is easy to implement, with minimal cost and limited labour. However, to be effective, long-term planning and careful timing is vital (Kananji 2007).

Biological Control Methods

Biological control is a useful and safe control option for storage pests, but very little research has been done on it thus far. Dinarmus basalis (Rondani) has been proved to be a promising parasitoid for *A. obtectus* and *Callosobruchus chinensis* (L.), which significantly reduces the population of bruchids(Islam & Kabir, 1995; Schmale *et al*. 2001). Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were found to be effective for the control of the maize weevil and cowpea bruchids in Ethiopia, under experimental conditions (Adane *et al*. 1997a; b, 1998).

Although promising results were recorded with parasitoids, its implementation under small-scale farmer storage conditions is difficult. Moreover, the incompatibility of the parasitoids with chemical control practices (Tadesse *et al*. 2008) has made the biological control of bean bruchid less appealing. Besides, the lack of knowledge and resources for rearing the parasitoids and the difficulty to parasitize the larva, as the larval are found inside the seed, limit the application of biological control for bruchid management (Kananji 2007).

Chemical Control

Chemical insecticides are usually used, either in the form of fumigation or dust formulations, against storage pests (Rai *et al*. 1987). Organophosphates and pyrethroid insecticides are the most commonly used chemicals for the control of storage insects. Smallholder farmers use dusts, while for large-scale storage facilities fumigation is more effective (Tadesse *et al*. 2008). In Ethiopia, there are many recommended insecticides against storage pests, but the most commonly, used insecticides are pirimiphos-methyl and malathion dust (Tadesse *et al*. 2008). It is important to have a knowledge of the application of insecticides and an awareness of the potential dangers caused by the chemicals (Jones 1999). However, most of the farmers in developing countries are exposed to insecticide toxicity, due to the improper handling and application of insecticides and the lack of awareness of the potential dangers in storage facilities. The other disadvantages of chemicals are environmental pollution and their effect on beneficial insects. In addition, the insects can develop a resistance to the chemicals

Breeding for Bean Bruchid Resistance

Host plant resistance is the basic component of integrated pest management, and it is a cheap, effective, sustainable and environmentally-safe method. Progress has been made on bruchid resistance breeding by scientists at CIAT. Schoonhoven & Cardona(1982) reported that almost all cultivated common bean cultivars and landraces lack resistance to *Z. subfasciatus.* However, several resistance genes were found in a few wild common bean accessions (Schoonhoven *et al*. 1983). One mechanism of resistance is believed to be antibiosis, which is conferred by the seed storage proteins produced by the APA (arcelin, phytohemagglutinin and α-amylase inhibitor) gene family (Schoonhoven *et al*. 1983; Acosta-Gallegos *et al*. 1998). Resistance, by using antibiosis, extends the time of adult emergence, insect growth and lifecycle. Reducing adult emergence, especially in the first and second instars larvae, in turn, results in reducing the weight of surviving adults (Osborn *et al*. 1988; Minney *et al*. 1990; Dorn *et al*. 2007). Although the APA proteins differ in their biochemical and physiological properties, their expressions show similar patterns (Moreno *et al*. 1990). Based on its protein size and electrophoresis patterns, arcelin is different from all the other APA proteins (Romero-Andreas *et al*. 1986).

Different variants of arcelin genes were identified and each variant was found to have a different effect on *Z. subfasciatus*. Currently, eight variants of arcelin (Arc-1 to Arc-8) have been identified (Osborn *et al*. 1986; Lioi &

Bollini 1989; Santino *et al*. 1991; Acosta-Gallegos *et al*. 1998; Zaugg *et al*. 2012). These arcelin variants are clustered in three groups, with the first group being composed of Arc-3 and Arc-4. These variants were found to be the progenitors of the rest of the variant genes. The second group consists of Arc-5 and Arc-7, while the third group includes Arc-1, Arc-2 and Arc-6 (Lioi *et al*. 2003). Arc-5 and Arc-1 confer the highest level of resistance to *Z. subfasciatus*in common bean accessions, followed by Arc-4, Arc-2 and Arc-3 in their order of importance (Cardona *et al*. 1990). The mode of action of arcelin is not well understood; however, some studies suggest that it might be due to a disruption of epithelial cells in the gut of the insect. Others have hypothesized that arcelin might provide the insects with a source of poorly digestible protein (Minney *et al*. 1990; Paes *et al*. 2000; Carlini & Grossi-de-Sa 2002). All the RAZ lines have been developed by CIAT, using Arc-1 variant lines through backcross breeding (Cardona *et al*. 1990).

Different national research programs have verified the resistance of RAZ lines. In Ethiopia, the screening of the RAZ lines and commercial bean cultivars has been done and it has been confirmed that most the CIAT accessions reveal high levels of resistance, compared to the commercial varieties. The resistant lines, such as RAZ-1, RAZ-7, RAZ-8 and RAZ-11, showed a stable resistance (Negasi & Abate 1992; Negasi 1994). Assefa(2010) studied the yield performance and the level resistance of advanced breeding resistant lines s of the common bean, which was developed by CIAT under field conditions in Ethiopia. It was found that all the advanced lines exhibited a good resistance to bean bruchids, but the yield performance of the lines was very poor, compared to the commercial varieties. According to Cardona(2005), the introgression of arcelin into commercial cultivars was effective against Zabrotes, but most of the RAZ lines had lower yields than their respective recurrent parents. Different RAZ lines were evaluated across different environments and they showed good potential for yield (Negash*et al*. 2014). The candidate genotypes, RAZ-42 was provisionally released in 2019 and after incorporating canning quality data the genotype will be released. The transfer of genes resistant to *A. obtectus*, from a genotype of tepary bean (*P. acutifolius*) into an African bean cultivar has also been reported (Mbogo *et al*. 2009; Kusolwa & Myers 2011). Even though some successful breeding efforts have been made to develop arcelin-derived resistant cultivars (Cardona *et al*. 1990; Cardona & Kornegay, 1999; Myers *et al*. 2001; Cardona 2004; Beneke 2010), RAZ-42 are the genotype provisionally released so far. Hence, it is essential to search for additional sources of bruchid resistance from landraces that are easier to use in a breeding program. Kananji(2007) collected a large number of landraces from farmers in Malawi and screened them for bruchid resistance. Malawian landrace K35 showed a good resistance to both bruchid species, while K25 exhibited a good resistance only to *Z. subfasciatus*. These two landraces showed resistance levels that were even better than the lines with arcelin (SMARC lines). A total of 300 common bean entries (landraces, resistant genotypes, improved varieties and breeding lines) were evaluated against *Z. subfasciatus* and the resistant genotypes, RAZ-11, RAZ-36, RAZ-2, RAZ-44, RAZ-120, RAZ-40 and MAZ-203, showed consistently complete resistance. Two other promising entries were also identified from the breeding lines (SCR-11) and landrace collections (NC-16) of Ethiopia (Tigist *et al*. 2018)

Inheritance of Resistance to Bean Bruchids

Understanding the inheritance of resistance to bean bruchids is crucial for developing a successful breeding program. Osborn *et al*.(1986) and Suzuki *et al*. (1995) studied the inheritance of resistance conferred by arcelin, using single F_2 seeds from crosses between lines that harbor the arcelin gene and cultivated lines that lack arcelin. The results confirmed that the resistance is genetically inherited in a simple Mendelian manner (Osborn *et al*. 1988). Kornegay *et al*. (1993) reported that arcelin is inherited as a monogenic dominant trait, which provides a higher level of resistance to bruchids when it is in the homozygous (Arc^+/Arc^+) state than in its heterozygous (Arc^+/Arc^-) state. This indicates that the transfer of the *Z. subfasciatus* resistant gene to commercial cultivars, through backcrossing would be easy. Resistance controlled by a single gene is liable to break down at some stage of the breeding cycle. Therefore, several resistance genes and/or QTLs from various sources could be used in resistance gene stacking to form a more stable and long-lasting resistance. The inheritance of the resistance gene to *A. obtectus*, which is obtained in the Malawi landraces was controlled by many genes (Kananji,2007).

Marker-Assisted Common Bean Breeding

Marker-assisted selection (MAS) is a procedure that has been developed to avoid the effects associated with the environment and in which selection is done by using phenotypic traits. The efficiency of phenotypic selection can be enhanced by the selection of genes through MAS (Francia *et al*. 2005). The selection process in MAS is assisted by molecular markers, which are not influenced by the environment and it can be detected at any stage of the plant's development. The application of these tools in the breeding programs increases the rate of genetic gain two folds compared to, the rate of gain by phenotypic selection (Ragot &Lee, 2007; Xu & Crouch, 2008).

Traditionally, selection for resistant lines can be done by using laboratory screening, which is tedious, time-consuming and requires large laboratory space and a large amount of seed, to undertake replicated trials. Selection can also be achieved by analysing the presence of the active arcelin gene, using either an immune essay or electrophoresis. Biochemical markers have been used to detect the presence of arcelin in small quantities of ground seed tissue. Protein based screening requires protein electrophoresis and arcelin-specific antibodies (Blair *et al*. 2002). However, these methods are time-consuming and expensive, due to the demanding protein extraction protocols. The MAS, on the other hand, is a simpler and more efficient tool in the development of bruchid resistant cultivars, thus it is essential to find more cost-effective and technically simpler resistance screening methods (Miklas *et al*. 2006). Marker-assisted breeding has been shown to be a valuable tool in the development of resistant cultivars.

DNA-based markers have been applied, to monitor the expression of the arcelin protein in breeding programs (Miklas *et al*. 2006). Several attempts have been made in different national and international research institutes to identify molecular markers that are tightly linked to the arcelin gene (Miklas *et al*. 2006; Blair *et al*. 2010b). The arcelin genes were mapped on Chromosome 4 of the common bean genome (Nodari *et al*. 1993). A total of sixty-eight genotypes, consisting of seven wild accessions, each representing the seven arcelin variants, were identified (Blair *et al*. 2002). Based on populations developed by crossing the resistance to Zabrotes (RAZ) lines and susceptible varieties, several Simple Sequence Repeat (SSR) markers associated with the arcelin gene were identified (Blair *et al*. 2002, 2010b). The region for the arcelin locus on chromosome 4 also reported by using SNP markers (Raatz*et al*. 2019). Genome-wide marker–trait associations of bruchid resistance were reported by Tigist *et al*. (2019) and the SNPs located on Pv4

and Pv7 were significantly associated with the two traits (percentages of adult emergence and seed weight loss). These newly-developed markers will be useful for marker- assisted selection and the introgression of arcelin, to develop bruchid resistant lines.

Conclusion

Although the common bean is an important crop both for food and export in the world, its production and productivity is low because of various yield-limiting factors, including bean bruchid infestation especially in Africa. Various control options, such as cultural, biological and chemical, have been used to control the insect. However, the above options have been found to be less appealing, due to issues related cost, health and environmental pollution, to technical aspects under smallholding farming systems. Therefore, the use of resistant varieties integrated, with other control methods has proved to be the best option, as it is an environmentally safe, sustainable and feasible control option. In addition to exotic resistance germplasm, the use of landraces which possess enormous genetic potential will be useful in the common bean breeding program to broaden the genetic basis of the crop. Hence, the evaluation of the landraces as sources of valuable genes for many insect and disease resistance, agronomic and physiological traits, would be important. The identification of new resistance genes and/or QTLs from different sources and mapping of these QTLs is therefore very vital.

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