

Genetic sources of bruchid resistance in soybean: A review

Ulemu, M.M., Kyamanywa, S. & Tukamuhabwa, P.

Department of Agricultural Production, School of Agricultural Sciences, College of Agriculture and Environmental Sciences, Makerere University of Kampala, P. O. Box 7062, Kampala, Uganda

Corresponding author: ulemsiska@gmail.com

Abstract

Soybean (*Glycine max*) has two traits that make it hugely popular in the world; its high protein content (40%) and oil (20%) making it a vital industrial crop. Considerable systematic research focusing on breeding for grain yield improvement, foliage insect pest resistance and pod shattering leading to the release of a number of varieties has been done world wide. Landmark achievements have also been made on adoption of these improved varieties to the extent that almost all soybean grown in Uganda is improved. However, little research geared towards storage pests has been done not only in Uganda but also worldwide because the crop had no issues of storage pests until recently in Australia and now in Uganda. Unlike field pests and diseases, damage caused by pests on stored products is completely irreversible making it very significant. In most parts of Africa, soybeans are stored together with other legumes. Such storage systems with little or no use of control measures would allow cross infestations by storage pests from other legumes such as cowpeas. The major problem in storage for legumes are bruchids. This problem of bruchids if it is not dealt with in a sustainable manner it might pull down all the progress and achievements in place in the Uganda soybean breeding programme since 1930s. In this review we present research status on bruchids and further highlight knowledge gaps in soybean research as pertaining to breeding for bruchid resistance.

Key words: Bruchid, resistance sources, soybean, Uganda

Résumé

Le soja (*Glycine max*) présente deux traits qui le rendent extrêmement connu dans le monde; sa haute teneur en protéines (40%) et son huile (20%) faisant d'elle une culture industrielle vitale. Des recherches systématiques considérables ont été effectuées au niveau mondial et sont basées sur l'amélioration du rendement des grains, la résistance des feuilles aux insectes nuisibles et l'égrenage des gousses conduisant à la dissémination d'un certain nombre de variétés. Des réalisations historiques ont également été faites sur l'adoption des variétés améliorées jusqu'à ce que tout le soja cultivé en Ouganda soit amélioré. Toutefois, peu de recherches sur les ravageurs post récolte ont été faites non seulement en Ouganda mais aussi au niveau mondial parce que la culture n'avait pas de problèmes de ravageurs de

stockage jusqu'à récemment en Australie et maintenant en Ouganda. Contrairement aux ravageurs et pathologies des champs, les dommages causés par les parasites sur les produits stockés sont complètement irréversibles, ce qui les rend très importants. Dans la plupart des régions d'Afrique, le soja est stocké avec d'autres légumineuses. De tels systèmes de stockage avec peu ou pas de mesures de contrôle favoriseraient des infestations par des parasites de stockage provenant d'autres légumineuses telles que le niébé. Le problème majeur dans le stockage des légumineuses constitue les bruches. S'il n'est pas traité de manière durable, le problème des bruches pourrait réduire à néant tous les progrès et les réalisations du programme de sélection du soja en Ouganda depuis les années 1930. Dans cette revue de littérature, nous présentons l'état des recherches sur les bruches et soulignons les lacunes dans les recherches sur la sélection du soja pour la résistance aux bruches.

Mots clés: Bruches, souches de résistance, soja, Ouganda

Background

Soybean was introduced in Uganda from both the United States and South Africa between 1908 and 1913 (Tukamuhabwa *et al.*, 2010). Two traits make soybean (*Glycine max*) hugely popular in the world; its high protein (40%) and oil (20%) content, and these make it an important industrial crop in Africa. In fact soybean produces the highest amount of protein per unit area among crops (Tukamuhabwa *et al.*, 2010). Soybean in Uganda is grown throughout the country with the Northern region being the highest (66.6%) producer (UBOS, 2010). Considerable systematic research focusing on breeding for grain yield improvement, foliage insect pest resistance and pod shattering leading to the release of a number of varieties has been done world wide. Landmark achievements have also been made on adoption of these improved varieties such that almost all soybean grown in Uganda is improved type. However, little research geared towards storage pests has been done so far not only in Uganda but also world wide because the crop had no apparent issues of storage pests until recently in Australia and now in Uganda. Reports by Srinives *et al.* (2007) and other researchers indicate that soybean is rarely attacked by storage pests. As such the International Institute for Tropical Agriculture (IITA) which does a lot of research on soybean in Africa, does not have soybean storage pest on the priority research areas. Nevertheless, recent reports by Rees (2010) in Australia and Saruchi and Thakur (2014) in Palampur India indicate that soybean was attacked by soybean bruchid (*Bruchidius mackenziei* Kingsolver) and cowpea bruchid (*Callosobruchus maculatus*), respectively. Similarly in Uganda, Tukamuhabwa and Obua (personal communication 2015) have reported damage of soybean seed in storage in three varieties (Maksoy 2N, Maksoy 3N and Maksoy 4N) at MUARIK and Namulonge Research Institute (Kampala). This review article describes and discusses research status on bruchids and further highlights knowledge gaps in soybean research as pertaining to breeding for bruchid resistance.

Literature summary

Bruchids and their management. There are about 1300 species of seed beetles in the family Bruchidae. Of these 20 are recognized as pests in stored legume seeds especially in

developing countries (Southgate, 1981; Credland, 2000). According to Credland (2000), four species are of cosmopolitan importance and these are *Callosobruchus maculatus*, *C. chinensis*, *Acanthoscelides obtectus* and *Zabrotes subfasciatus*. However, Southgate (1979) reported that in addition to these four, other species of *Callosobruchus* including *C. analis*, *C. rhodesianus*, and *C. subinnotatus* constitute a secondary group of storage pests while *Bruchus pisorium*, *B. rufimans* and *Bruchidius atrolineatus* are important as pests in the field and early stages of storage. Out of these, three species have been reported on soybeans; *C. analis* in Indonesia (Naito, 1999), *C. maculatus* in Australia and India (Saruchi *et al.*, 2014) and *C. mackenziei* in Australia (Rees, 2010).

Reports on losses due to bruchid damage vary from country to country, due to the environment in which these losses occur and as such accurate data on the scale of damage are hard to find. For example, for common beans in Malawi losses up to 38% have been reported. Uganda reported up to 8% while Kenya and Tanzania reported as high as 78% within six months of storage (Kananji, 2007). These losses can be substantial quantitatively and qualitatively, thus reducing the degree of usefulness and making the seeds unfit either for planting or for human consumption (Singh, 2009). Bruchids are therefore a major obstacle to achieving food security in developing countries.

The essence of any pest control measure is to reduce its biological fitness, which finally contributes to control of the pest population. Management of bruchids which are predominant storage pests is not simple, not only for the developing countries but even in countries such as Australia, which have access to conventional fumigants and chemical insecticides. The success of treatments has not always been established and work on bruchid control is still in progress (Credland, 2000; Srinives *et al.*, 2007). Over the years, several methods of bruchid control have been employed by farmers and researchers have identified some efficient control methods for the storage pests. Some of these methods range from store hygiene, physical, cultural, biological, chemical control methods and use of inert materials. Chemical control appeared to be the most effective and efficient control method (Adebowale and Adedire, 2006) but it has adverse effect on both man and environment. Chemicals require a recurring expenditure and for their safe use, appropriate level of education is required (Singh, 2009). Extensive use of chemical pesticides increases the cost of the product; reduce the population of natural enemies (parasites and predators) and leads to development of pesticide resistance (Singh, 2009). To reduce these problems, bruchids have also been controlled using biological measures. These involve the application of pathogens, a range of invertebrate predators, parasites and parasitoids (Dent, 2000) which have been used with some degree of success in storage pests management particularly in bruchids. Amevoin *et al.* (2007) reported that a parasitoid *Dinarmus basalis* was used to control cowpea bruchids in West Africa farmers' stores. The parasitoid reduced damage from 30 to 10%. Soundararajan *et al.* (2012) demonstrated the use of the same bio control agent (*Dinarmus* spp.) for control of *C. maculatus* in blackgram. A few attempts were made in India for control of bruchids (Soundararajan *et al.*, 2012). However Dent (2000) argues that application of biocontrol presents a great deal of challenges in that continuous monitoring and continuous rearing of the bio agent is required. The time of introduction of bio control agent has to be well studied and understood taking into account the initial rate of seed infestation by the Bruchids. The

cost of reared natural enemies must be judged in terms of the value of the crop protected by using the agent and in comparison to the cost of competing pest control options such as chemicals. Dent (2000) further states that biological agents may also have some environmental impact because once introduced and established, it is often very difficult and impossible to eradicate.

Furthermore to avoid the negative effects of pesticides, physical control of bruchids has also been tried and reported. Ofuya *et al.* (1993) used physical means to manage *C. maculatus* and *A. obtectus* where 100% nitrogen (N₂) at 25-32°C respectively and at 70 ±5% relative humidity was used. The mortality of adults, eggs, larvae and pupae of the cowpea bruchid, *Callosobruchus maculatus* (F.) and the bean bruchid, *Acanthoscelides obtectus* was investigated. Irrespective of temperature, all adults of both bruchids were killed within 1 day of exposure to pure N₂ atmosphere. All eggs were killed in 5 days at 25°C and in 3 days at 32°C. Complete mortality of larvae and pupae was observed in 9 days at 25°C and in 5 days at 32°C, except with young larvae of *A. obtectus*, which were all killed in 5 days at 25°C and in 3 days at 32°C. Exposure to an atmosphere of 100% N₂ could therefore provide a rapid means of disinfestation of stored grain legumes from *C. maculatus* and *A. obtectus*. This type of method is definitely out of reach for the smallholder farmers and middle scale processors, but is applicable in large storage facilities.

Another method for managing bruchids is the use of host plant resistance (HPR). Use of host plant resistance to manage bruchids was reported by Amusa *et al.* (2013) in beans. Host plant resistance has the advantages of being sustainable, environmentally friendly and being cost effective. However little or no work has been done on soybean. Literature has shown that bruchids in stored seed are a major problem because of their ability to re-infest stored seed. However if host plant resistance is to be used proper identification of the pest is a prerequisite. Steps are under way to identify the new pest attacking soybean in Uganda.

Incidence and severity of the Bruchid spp on soybean. Bruchids are the most important pests of stored grain legumes. They are important because their infestation starts from the field and carries on along the value-chain, their damage is irreversible and direct on the economic part (grain) (Kananji, 2007). Even low initial infestation rates can lead to tremendous damage because of the beetles' high fertility and short generation times (Takashi, 2013). Each emerging female for example in *C. maculatus* quickly finds a mate and, if food is readily available, produces about 100 offspring. Takashi (2013) further states that in cowpea one generation takes about a month and after three or four generations, losses due to *C. maculatus* are very severe. Bruchids cause overall weight loss; loss of seed viability and altered nutritional quality due to the presence of insect frass, excrement and dead insects in and on the seed. Bruchids in stored seed are a major problem because of their ability to re-infest stored seed. A single beetle is able to cause 3.5% weight loss in cowpea seeds (Booker, 1967). In other grain legumes losses of up to 100% have been reported after 3-6 months of storage (Ofuya *et al.*, 1993; Credland, 2000).

In developing countries bruchids become a big issue because most subsistence farmers rely on traditional storage structures under the same roof, which are especially vulnerable to

bruchid attack and lead to cross infestations among stored products which are sharing a common pest (Naito, 1999). For example, Graham bean beetle (*Callosobruchus analis*) is reported to be a serious pest of soybean in Indonesia due to cross infestations (Naito, 1999). Naito (1999) further reports that due to damage by insect pests Indonesia imports a lot of soybean. To control this bruchid, research was carried out to develop low cost technologies from which rice husks ash emerged to be the best option. The rice husk ash prevented oviposition, suppressed population growth, had the least number of adult emergence and showed least seed damage. Mortality of the bruchids was observed to last up to 3 months after treatment. It was suspected that high content of silica in rice husk made it highly effective in controlling *C. analis* (Naito, 1999). However the mixture required to get the best results is 1- 10% of seed weight. This method is therefore a challenge in those areas where rice is not a major crop because a lot of rice husks are required.

Literature review has demonstrated that there is a knowledge gap in terms of ascribing damage by bruchids to soybean. Reports of soybean being attacked by *C maculatus*, *C. chinensis* and *Bruchidius kingslover* in Australia, Indonesia and Uganda lack substantial information and do not answer the question how much damage is caused by these bruchids on soybean in the farmers' field and what is the distribution of the pest in Uganda. Therefore the issue is lack of information on the abundance, spread and percent losses associated with the pest necessitates research into this area.

Sources of resistance to bruchids. Breeding progress depends on the magnitude of genetic variability within the germplasm, heritability of the trait under question and the level of selection intensity applied (Keneni *et al.*, 2011). The higher the levels of these components for a given trait, the higher will be the genetic gain expected from each cycle of selection. In exploration for new sources of host plant resistance (HPR), in South America especially Brazil, extensive research on HPR against stink bugs has been conducted. But in US especially, the southern states, lepidopteran foliage feeders have been the focus of HPR research (McPherson *et al.*, 2008). In Uganda the focus has been on agronomic traits (Tukamuhabwa *et al.*, 2010). Due to the preference for agronomic traits and disease research, soybean germplasm has not been extensively explored for resistance to storage pests. No cultivar of soybean showing resistance against storage pests has been released so far in the world (Bansal *et al.*, 2013). There is a need to identify sources of resistance against bruchids in soybeans though the selection for resistance against bruchids is relatively time consuming and labor intensive (Somta *et al.*, 2008).

Singh (2009) reports that so far no cultivated legume has been reported to be immune to bruchids. In general genes for complete resistance to insect pests and storage insects in particular are of rare occurrence in nature for cultivated species (Singh, 2009). However they have often been reported in species of wild relatives for a number of legume crops (Somta *et al.*, 2006). Nevertheless, a few cases of complete resistance were also reported in cultivated or germplasm collections of haricot bean (Ishimoto *et al.*, 1995), field pea, cowpea, black gram and chickpea (Keneni 2011). In most cases, insect resistance has been found in the unimproved traditional germplasm (Singh, 2009).

In studies done by Talekar (1987) where host plant resistance to insects attacking soybean and mungbean in the tropics was sought for no resistance was found for bruchids in soybean but in mungbean. However in a comparative study on the varietal preference and developmental behaviour of *C. maculatus* on 13 different soybean varieties done by Saruchi and Thakur (2014), results revealed that on the basis of developmental behaviour among all the genotypes, one genotype (bragg) was totally resistant; 5 were found relatively resistant while other 7 varieties were found susceptible to *C. maculatus*. These results were in agreement with Amusa *et al.* (2013) and Laphale *et al.* (2012) who found resistance to cowpea bruchid in a cultivated variety even though a number of reports indicate that varieties with improved yield are more susceptible than landraces (Keneni, 2011).

From the review it shows that resistance to bruchids in soybeans can be found in available germplasm either in Uganda or elsewhere and these findings further indicate that there is genetic variability in genotypes with respect to bruchid resistance which once identified can be utilized in both conventional breeding programmes and marker assisted breeding. So far no sources of resistance in cultivated soybean have been identified or characterized and there has been no study on the genetic control of the resistance to storage pests in soybean (Srinives *et al.*, 2007). The identification of sources of resistance to bruchids and their mechanisms would lead to reduced post harvest losses due to bruchids. This therefore calls for detailed studies starting from farm level on this new pest and host plant.

Mechanism of resistance to storage insect pests. Insect pest resistance for storage insect pests comprises two important mechanisms, antixenosis and antibiosis (Keneni, 2011). However, the processes of resistance involve morphological, physiological and/or biochemical mechanisms. Desroches *et al.* (1995) found that the seed coat in a faba bean (*Vicia faba*) acts like a physical barrier against penetration by *C. chinensis* and *C. maculatus*. They found that only 45–58% of the neonate larvae perforated through the seed coat to the cotyledons. A similar type of resistance against *C. maculatus* was also reported on cowpea (Edde *et al.*, 2003). On the other hand, Lale and Kolo (1998) observed that resistance to *C. maculatus* in three cultivars of cowpea was conferred mainly by a combination of reduced oviposition and egg-hatching which may be a reflection of chemical rather than physical characteristics of the seed coat. Host plants may also pose nutritional, physiological and ecological hurdles on the insects.

The impact of antixenosis on the population dynamics is no less complex, with some of the effects paralleling those of antibiosis (Thomas and Waage, 1996). For instance, reduced oviposition through non-preference is equivalent to reduced fecundity, and it can also increase larval movement thereby slowing development time or increasing juvenile mortality. Increased emigration from the crop due to antixenosis has the equivalent effect of increased adult mortality (Thomas and Waage, 1996). Overall, therefore, not only different mechanisms but also different strengths of resistance may be required to effect equivalent levels of population suppression of pests with different life histories.

Plant resistance to insects is rarely totally dependent on a single mechanism, there are often overlaps between the morphological and biochemical bases of resistance, e.g. trichomes

that exude substances that are toxic to insects as in some species of *Nicotiana* which exude alkaloids toxic to aphids (Thurston *et al.*, 1966). Amusa *et al.* (2013) and Laphale *et al.* (2012) reported that different cowpea genotypes possessed different characteristics and therefore response to bruchid infestation was different and went further to indicate that some characteristics which include seed size, testa thickness and hardness in the genotypes may influence cowpea seed response to bruchid attacks.

Some studies have attributed grain resistance to differences in grain size (mass) and asserted that the larger grains supply more food and space for insect growth and that the smaller grains or grains with less mass offer more resistance to pests attack than the larger grains (Singh, 1974). However, this is only true to some extent in some genotypes, because in a study by Amusa *et al.* (2013) two genotypes (IT99K-494-6 and IT81D-994) showed no significant difference in the grain size, yet had different levels of tolerance to the bruchid. This indicated that grain size did not affect the genotype's resistance to the bruchid attack. The rate of insect population is known to be affected by the resistance a particular genotype or variety offers, by causing a reduction in the rate of oviposition through physical or mechanical barrier (Semple, 1992). The barrier may either limit access into the grain or make it unsuitable for oviposition. The barrier may make it difficult for eggs to adhere to the seed or prevent the larva's penetration into the seed when they are hatched (Laphale *et al.*, 2012). The physical characteristics of seeds can determine the acceptability for oviposition but may not be related to the antibiotic nature of the seed (Messina and Renwick, 1985). Nwanze *et al.* (1975) showed that rough seeds were less acceptable to *C. maculatus* than smooth ones. On the other hand, Murdock *et al.* (1997) indicated that varieties with smooth and glossy seed coat constantly are more resistant than rough seeded varieties suggesting that other factor besides seed coat appearance affect cowpea's resistance to bruchid infestation. In a soybean study with 13 genotypes all the genotypes were highly preferred by *C. maculatus* for egg laying with the exception of Harasoya which had an intermediate surface texture (Saruchi and Thakur, 2014). Saruchi and Thakur (2014) further explained that the variation in egg laying was attributed to seed coat texture and physical characteristics of the genotypes. In general however, egg counts have not been shown to be predictive enough in resistance studies as other variables such as percent adult emergence, TDT, growth (susceptibility) index and percent loss in weight (Saruchi and Thakur, 2014).

From the review done, plant resistance to insects is rarely totally dependent on a single mechanism; there are often overlaps between the morphological and biochemical bases of resistance. Even though morphological factors may contribute towards insect resistance, these only cannot be used to predict plant resistance mechanism. Furthermore, crops confer different resistance mechanisms for different pests. Therefore there is a need to study soybean resistance mechanism to bruchids so that the information can be used in breeding programs.

Perspective. To establish a viable breeding programme for resistance to bruchids in soybeans, an effective and adaptable source of resistance needs to be identified. Currently characterization of existing soybean germplasm for resistance to bruchids in Uganda is under way at Makerere University in Kampala, Uganda. In addition the study will assess

the importance of bruchids on soybean seed at farm household level in major soybean growing areas of Uganda; determine the mode of gene action and inheritance of resistance to bruchids in soybean and finally identify Quantitative Trait Loci controlling resistance to bruchids in Soybean. This research will therefore provide starting material for the breeding program. Subsequently, resistant varieties, with traits preferred by farmers will be developed and deployed to farmers. This will contribute significantly to the national goal of improving food security and nutrition in Uganda and the region.

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