

# Application of Genetic Engineering in Breeding for Resistance to Storage Insect Pests

Assefa Amare Hagos (PhD)

EIAR, Mehoni Agricultural Research Center

Email: [assefaamare69@yahoo.com](mailto:assefaamare69@yahoo.com)

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**Abstract:** Reducing post-harvest storage losses through genetic improvement is both feasible and urgently needed to meet food demands in coming decades. A small but noticeable renaissance in the use of resistant varieties to minimize storage losses is taking place, especially in those ecologies where infrastructure for storage does not exist. To capitalize on genetic diversity for storage pest resistance, researchers have made significant progress in understanding the biochemical, biophysical, and genetic bases of host pest resistance, which is essential to ensure that the traits being selected meet with consumer demands. Traits that meet these criteria are now being mapped to confirm their role in resistance and to identify candidate genes using sequence homologies and proteomics. The introgression of resistance alleles from the same crop species, using marker-assisted selection or GE, will probably meet with greater public acceptance and possibly require less rigorous testing to document food safety than will the introduction of genetic material from other species. The real potential of this technology will be felt most in LDCs because the technology is packaged in the seed and should be designed to ensure that farmers have the option to recycle seed, a common practice for subsistence farmers. Modern gene technology can contribute to solve bruchid problem in *Vigna* species as seen in azuki bean, but its application is limited to the crops that basic technology related to genetic engineering is well established. Yet, commercial uses of the transgenic bruchid-resistant cultivars/lines require clarification of safety for human consumption as well as consumer acceptance.

**Keywords:** Genetic engineering, Resistance, Storage insect pests.

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## 1. INTRODUCTION

Genetic engineering can be defined as the transfer of genetic material from a different species (plant, bacterial or animal) or from a chemically synthesized gene into a target plant. The process of introducing a gene into an organism via recombinant DNA technology is known as transformation and recovered plant species are called as transgenic plants or genetically modified (GM) plants. Genetically engineered crops offers user-friendly, environment-friendly and consumer-friendly method of crop development to meet the demands of sustainable agriculture in the 21<sup>st</sup> century. Transgenic crops offers the prospect of many advantages; not just widening the potential pool of useful genes but also permitting the introduction of a number of different desirable genes at a single event and of reducing the time needed to introgress introduced characters into an elite genetic background( Hilder and Boulter 1999).

All plants possess a certain degree of resistance to insects, and so only, limited ranges of herbivores are able to feed on each individual species. This inherent resistance is based on various defence mechanisms, including a wide range of noxious secondary metabolites produced by the plant. Individual plants within one genus, or even one species, vary in their level of insect resistance, a fact long used by plant breeders to increase the insect resistance of crop cultivars. Bruchids or seed beetles or seed weevils (order Coleoptera, family Chysomelidae, and subfamily Bruchinae–formerly family Bruchidae) are major insect pests of stored legume seeds. These insects have been infesting seeds of starchy food legumes grown by human since the early time of agriculture (Southgate, 1979). The primary infestation occurs in the field, where bruchid adults lays eggs on pods after which larvae hatch, penetrate into the seed and feed on cotyledonary

and/or embryonic tissues. Damage in the field is only minor, but when such infected seeds are harvested and stored, the developing larvae/pupae continue to feed and eventually emerge from the seeds as adults, and cause secondary infestation

The secondary infestation more very damaging and usually results in total destruction of a seed lot if there is no protection. Seed damaged by bruchids are lost in seed weight, seed quality/nutrition and seed viability. As a consequence, seed lots become warm resulting in quality loss and mould growth (Rees, 2004). The damaged seeds are unsuitable for human consumption and for agricultural and commercial uses and may bring about negative publicity and lost in consumer trust in a product brand. Usually, chemicals is used to control the bruchids, but economic, health and environmental considerations favor using resistant varieties to manage these pests. Thus, improvement of bruchid resistance is given a priority in *Vigna* crops breeding programs around the world. Although many bruchid species attack legume seeds, azuki bean weevil (*Callosobruchus chinensis* L.), cowpea weevil (*C. maculatus* F.), common bean weevils (*Acanthoscelides obtectus* Say) and Mexican bean weevil (*Zabrotes subfasciatus* Boh.) rank among the most important insects of stored legumes, in term of damage.

Hence, the objective of this term paper was:

- Provided an overview of candidate storage insect resistance genes
- Explored different research information on transgenic storage insect pest resistance breeding

## 2. GENETIC ENGINEERING OF CROP PLANTS

Recombinant DNA technology offers the possibility of developing entirely new biological insecticides that retain the advantages of classical biological control agents, but have fewer of their drawbacks. However, commercial considerations have placed this technology beyond the reach of poorer sections of society, and has generated considerable public debate about its usefulness, effects on the non target organisms and the environment, thus preventing the use of an additional tool for increasing the production and productivity of crops. In addition to widening the pool of useful genes, genetic engineering also allows the use of several desirable genes in a single event and reduces the time to introgress novel genes into elite background. Biotechnology has provided several unique opportunities that include:

- Access to novel molecules,
- Ability to change the level of gene expression,
- Capability to change the expression pattern of genes, and
- Develop transgenic with different insecticidal genes.

However, transgenics are not a panacea for solving all the pest problems. There are some genuine or perceived concerns. The major limitations of transgenic plants are:

- secondary pests are not controlled in the absence of sprays for the major pests,
- need to control the secondary pests through chemical sprays will kill the natural enemies and thus offset one of the advantages of transgenics
- cost of producing and deployment of transgenics may be very high
- proximity to sprayed fields will reduce the benefits of transgenics
- insect migration may reduce the effectiveness of transgenics, and
- development of resistance in insect populations may limit the usefulness of transgenics.

Therefore, efficient deployment and management of transgenic plants in an effective manner will be an important prerequisite for sustainable use of biotechnology for crop improvement. As a result of advances in genetic transformation and gene expression during the last decade ( Horsch *et al.*, 1984), there has been a rapid progress in using genetic engineering for crop improvement, of which protection of crops against the insects is a major goal.

### 3. STORAGE INSECT-RESISTANCE GENES

The insect-resistance genes transferred into plants to date mainly target the insect digestive system. Most have been derived from a single species of bacterium or a range of higher plants, although some insect resistance genes from animals and other microorganisms have also recently been introduced into crop plants. However, the search for new genes is ongoing and aims to expand the range of insects affected, to combat the development of resistance in the target insects by identifying genes with different modes of action and to improve potency.

#### 3.1.Bt Insecticidal Proteins

Genetic variation for host plant resistance encodes a suite of survival traits that have evolved in plants over millions of years. Farmers, especially small-scale farmers, have selected directly or indirectly for traits of interest, including host plant resistance for storage pests (Lobell *et al.*, 2008). Primitive maize, for example, may have resulted from a cross between a perennial teosinte (*Zea diploperennis*) and eastern gamagrass (*Tripsacum dactyloides*), giving rise to a hybrid whose hard fruitcase inhibited oviposition by the maize weevil (Jepsen *et al.*, 2008). The domestication of other crops similarly involved selection for traits that enhanced production, processing and storage, while some variation in traits that are associated with storage pest resistance was maintained (Cannon, 1998).

Molecular maps for resistance to maize weevil have recently been developed (S Garcí'a-Lara, DJ Bergvinson, Abstract 154, 56th Maize Genetics Meeting, 11–14 March 2004, DF Mexico). QTL for maize weevil resistance, like those for resistance to fungi, explain only a small proportion of phenotypic variation, totaling just 25% over seven QTL. Given the close association between QTL for weevil resistance and cell wall components in maize, it seems possible that fortification of the pericarp cell wall through marker-assisted selection could deliver enhanced resistance to storage pests. Biochemical and biophysical bases for resistance Researchers addressing HPR against storage pests and diseases in food crops face the imposing challenge of enhancing resistance while maintaining the desired nutritional and processing qualities of the grain. For example, resistance biochemicals, such as soluble phenolics in sorghum (Ussuf *et al.*, 2001), may result in an unpleasant grain flavor. Potentially toxic or allergenic biochemicals will require extensive testing before their contents in food or feed can be increased (Warren *et al.*, 1996).

#### 3.2. $\alpha$ -Amylase Inhibitors

$\alpha$ -Amylases ( $\alpha$ -1, 4-glucan-4-glucanohydrolases) are widespread hydrolytic enzymes found in microorganisms, animals and plants. They catalyze the initial hydrolyses of  $\alpha$ -1,4-linked sugar polymers, such as starch and glycogen into shorter oligosaccharides, an important step towards transforming sugar polymers into single units that can be assimilated by the organism. Higher plants and animals produce a large number of different protein inhibitors of  $\alpha$ -amylases in order to regulate the activity of these enzymes (Morton *et al.*, 2000). However, these  $\alpha$ -AIs are used to generate transgenic plants that are resistant against insect pest (Dias *et al.*, 2010). The expression of the  $\alpha$ -AI gene encoding protein in plant system, such as pea (*Pisum sativum* L.) and azuki bean (*Vigna angularis* L.) showed promising effect against bruchid beetle pests (Coleoptera: Bruchidae) (Ishimoto and Kitamura, 1989).

#### 3.3. Arcelins

Post-harvest loss due to insect pests largely affects the overall food grain production and consumption and it is estimated to be 13%. Arcelins are antinutritional insecticidal seed storage proteins, found in the wild bean *Phaseolus vulgaris*, which have been shown to prevent infestation by post harvest insect pests such as bruchid beetles (Mourey *et al.*, 1998). Amino acid sequence comparison shows that arcelins belong to the bean lectin-like family, which includes the two types of phytohemagglutinin subunits (PHA-L and PHA-E) and  $\alpha$ -amylase inhibitors (Cardona *et al.*, 1990). Although the members of this protein family display similar tertiary structures, they differ in their biochemical properties, glycosylation patterns, quaternary structure and sugar binding specificities (Sharma *et al.*, 2004). Insecticidal properties of arcelins variants toward bruchid pests *Z. subfasciatus* has been reported (Olsnes AND Pihl, 1973), which is known to be one of the most important pests of stored beans.

### 3.4. Avidin as an Insecticidal Protein

Avidin has a strong insecticidal effect on many insects, although susceptibility varies widely between different insect species (apparently based on biotin requirements). Expression of avidin in transgenic maize initially aimed to produce the protein as a high-value product, but maize seed containing more than 0.1% avidin (of total protein) was fully resistant to larvae of three different coleopteran storage pests (Kramer *et al.*, 2000). The protein has also been expressed in other transgenic plants to confer pest resistance. Targeting of the foreign protein away from the cell cytoplasm (e.g. using targeting sequences from potato proteinase inhibitors; (Murray *et al.*, 2002) is important to avoid developmental abnormalities in the plants. No further development of this promising method has been reported.

## 4. BREEDING OF RESISTANCE TO BRUCHIDS (*CALLOSOBRUCHUS* SPP.) IN *VIGNA* CROPS

Bruchid beetles, *Callosobruchus chinensis* (L.) and *C. maculatus* (F.) are the most serious insect pests of *Vigna* crops during storage. Use of resistant cultivars is the best way to manage the bruchids. Bruchid resistant cowpea and mungbean have been developed and commercially used each with single resistance source. However, considering that enough time and evolutionary pressure may lead bruchids to overcome the resistance, new resistance sources are necessary. Genetics and mechanism of the resistance should be clarified and understood to develop multiple resistance cultivars. Gene technology may be a choice to develop bruchid resistance in *Vigna*

### 4.1. Genes for Bruchid Resistance

Coleopteran insects in the family *Bruchidae* cause serious cowpea grain losses in storage. *Callosobruchus maculatus* is key among these pests. Through conventional breeding efforts at IITA and elsewhere, modest levels of resistance to *C. maculatus* have been attained (Singh and Jackai, 1985). To enhance these modest resistance levels, efforts have also been underway to identify plant genes that affect *C. maculatus* development. The majority of artificial seed bioassays have involved the use of plant lectins (Murdock *et al.*, 1990; Omitogun *et al.*, 1999). Vicilins (7S seed storage proteins) and protease and  $\alpha$ -amylase inhibitors and  $\alpha$ -amylase inhibitor-like proteins (AIL), are also insecticidal to bruchids (Hilder *et al.*, 1987; Ishimoto *et al.*, 1999; Yunes *et al.*, 1998). Transgenic pea and azuki seeds containing the bean  $\alpha$ -amylase inhibitor are resistant to bruchid beetles (Shade *et al.*, 1994; Ishimoto *et al.*, 1996). Plans are underway to introduce this gene into modestly bruchid resistant IITA cowpea lines once the transformation system becomes routine. Various compounds are toxic to cowpea beetles. However, these toxins are more applicable in biocontrol than transgenic

### 4.2. Utilization Genetic Information in Breeding for Bruchid Resistance

#### 4.2.1. Azuki Bean

There are a few reports on genetics and breeding for bruchid resistance in azuki bean. Most of which are done by Japanese researchers. Breeding for bruchid resistance in azuki bean relies on other resistance *Vigna* species. Cultivated rice bean (*V. umbellata*) is considered the most useful source for the resistance in that it exhibits complete resistance against *C. analis*, *C. chinensis* and *C. maculatus* and yet their seeds are safe for human consumption, although cross compatibility between them is very low. The resistance in rice bean is due to biochemicals in seeds (Kashiwaba *et al.*, 2003). Three novel flavonoids with basic structure of naringenin isolated from rice bean seeds has inhibitory effects against growth and development of *C. chinensis* and *C. maculatus* (US patent 6,770,630B2). One naringenin derivative causes resistance to both bruchids and the second derivative causes resistance to only *C. chinensis* while the third one causes resistance to only *C. maculatus*. A mapping study in a population derived from rice bean x *V. nakashimae* revealed that bruchid resistance in rice bean is controlled by 4 QTLs (Somta *et al.*, 2006a). Two QTLs are co-localized and responsible for resistance to different bruchid species, while the other two express differential effects on *Callosobruchus* species.

Direct transfer of the resistance from rice bean to azuki bean is not successful due to genome incompatibility between them. A solution to this problem is to use bridging species. Bruchid-resistant azuki bean lines with rice bean as resistance donor have been developed using *V. nakashimae*, *V. riukiensis* and *V. tenuicauris* as bridging species (N. Tomooka, per com.), but not being commercially released. *V. nepalensis* (Tateishi & Maxted) is another useful resistance source of azuki bean resistance. It causes low damage and delay in emergence of bruchids. *V. nepalensis* is genetically and phenotypically similar to azuki bean. It is a species included in azuki bean complex, together with cultivated, wild and

weedy azuki bean (Vaughan *et al.*, 2005). Members in this species complex can be crossed readily with one another. Seed antibiosis in *V. nepalensis* causes resistance to *C. chinensis* and *C. maculatus* (Somta, 2005). QTL mapping revealed that the resistance in *V. nepalensis* is complex. Several QTLs conferring the resistance are linked to seed size QTLs. Increasing the resistance is accompanied by decreasing seed size. Yet some alleles from *V. nepalensis* contributed negative effects by promoting susceptibility (Somta *et al.*, 2007c). Maintaining bruchid resistance in large-seeded azuki bean progenies proved to be difficult, in this case.

#### 4.2.2. Cowpea

There are reports on genetics of cowpea resistance to *C. maculatus*. The first investigation used Tvu2027 as donor and it was found that maternal genotype determined the resistance through a major recessive gene and modifiers. Although paternal and embryo genotypic effects on the resistance were present in certain backcross combinations (Redden *et al.*, 1983). Genetic mapping for genes controlling *C. maculatus* resistance has been investigated. A major QTL accounted for up to 76% of the variation in the trait. Allele from the susceptible parent at a minor QTL also contributed the resistance. Several bruchid-resistance cowpea lines were developed using resistance genes from Tvu2027 and the resultant varieties were released to farmers in many countries (Singh, 2005).

#### 4.2.3. Blackgram

Studies on genetics and breeding for bruchid resistance in blackgram are very scarce. This may be because the crop is economically important only in the developing regions. As no resistance source of *C. maculatus* is identified in cultivated blackgram, the genetics of the resistance cannot be determined. However, inheritance of the resistance in wild blackgram revealed that the resistance is governed by two duplicated loci with resistance is dominance (Dongre *et al.*, 1996). Localization of the resistance gene(s) on genome map is in progress (N. Tomooka, per comm.). There has been no report on development of bruchid resistance in blackgram so far. Although blackgram is closely related to mungbean, transferring the resistance from blackgram into mungbean may be achieved only by genetic engineering due to a strong genetic barrier between the two species.

#### 4.2.4. Mungbean

TC1966 has been intensively used as the material for genetic study and breeding for bruchid resistance in mungbean. A single dominant gene, designated as *Br*. (Kitamura *et al.*, 1988), controls the resistance. DNA marker based studies enable researchers to localize the resistance (*Br*) gene. By using a small mapping population of 58 F<sub>2</sub> individuals, the gene is mapped onto linkage group (LG) 8 and flanked by RFLP (Restriction Fragment Length Polymorphism) marker pA882 and pM151. The marker pA882 is the nearest marker, 3.6 cM away from the gene (Young *et al.*, 1992). Quantitative trait loci (QTL) analysis revealed that this genome region contribute 87.5% of the total phenotypic variation (Young *et al.*, 1992). The resistance gene is narrowed down to 0.2 cM from RFLP marker Bng143 (Kaga and Ishimoto, 1998). Results from the same study also demonstrated that gene controlling vignatic acid A is not the same as that controlling the resistance, but rather co-segregating at the distance of 0.2cM apart. A BAC contig covering *Br* genomic region has been constructed (Kaga and Ishimoto, 1998). By using ACC41

as the resistance source, a major locus was found to confer resistance to *C. chinensis*, and RFLP marker mgM213 mapped on LG8 was identified as closely associated (1.3cM) with this locus (Miyagi *et al.*, 2004). STS (Sequence Tagged Site) markers (STSbr1 and STSbr2) co-segregating with this locus were also reported by the same authors. The resistance genes in TC1966 and ACC41 are likely to locate on the same locus or very closely linked because no segregation was observed in the progenies from a cross between them (Lambrides and Godwin, 2007).

Recently, resistance in cultivated mungbean has been reported. The resistance to *C. chinensis* and *C. maculatus* in V2709 and V2802 is monogenics (Somta *et al.*, 2007a). Although resistance gene in TC1966 has been used to develop mungbean resistant lines (Tomooka, *et al.*, 1992; Wattanasit and Pichitporn, 1996), no commercial resistance variety is being released to farmers. This is mainly due to uncertainty on safety of the resistance seeds for human consumption, as the biochemicals responsible for resistance has not yet been identified. Feeding test in mice using resistant mungbean derived from TC1966 demonstrated changes in blood biochemicals values, compared to the control mice (Miura *et al.*, 1996). Resistance in the cultivated form is safer in that human has consumed it for a period without report of detrimental effect. Yet, it is a higher yielder with less problematic in term of linkage drag of unwanted traits such as pod shattering



and indeterminate growth, as compared to the wild form. By employing V2709 as the resistance donor, a resistance mungbean cultivar, “Jangannogdu” was developed and officially released to farmers in Korea (Lee *et al.*, 2000). This is the only bruchid-resistant mungbean variety reported so far.

#### 4.3. Genetic Engineering to Improve Bruchid Resistance in *Vigna* Crop

Advance in transformation system and plant regeneration by tissue culture technique in legumes have made possible the development of bruchid-resistant cultivars. Proteinaceous  $\alpha$ -amylase inhibitor ( $\alpha$ AI) is a secondary metabolite that is widely present in seeds of most cereals and certain grain legumes. It confers resistance to *Callosobruchus* spp. in common bean (*P. vulgaris* L.). Transferring  $\alpha$ AI-1 gene from common bean was achieved and resulted in resistant transgenic plants in azuki bean (Ishimoto, *et al.*, 1996), pea (*Pisum sativum* L.) (Shade, *et al.*,

1994) and chickpea (*Cicer arietinum* L.) (Sarmah, *et al.*, 2004). The transgenic azuki bean is free from damage by *C. chinensis*, *C. maculatus* and *C. analis* (Ishimoto, *et al.*, 1996). Indeed, very recently,  $\alpha$ AI-1 transgenic mungbean was successfully produced, but there has been no report so far on test for bruchid resistance (Sonia, *et al.*, 2007).

Although genetic engineering is an effective and useful way to develop bruchid-resistance legumes, disadvantages of the technique exist. Firstly, it is not applicable in most *Vigna* crops such as mungbean, blackgram and cowpea because some protocols necessary for gene transferring are not yet well developed (Popelka *et al.*, 2004). Secondly, transgenic crops are not yet publicly accepted in terms of consumption and environmental safety. It was found that rats fed with transgenic peas containing  $\alpha$ AI-1 gene showed a lower dry matter digestibility but higher fecal and urinary output as compared to control rats, although growth and some nutritional performance variables were the same (Pusztai *et al.*, 1999).

### 5. WILL RESISTANCE TO STORAGE PESTS COMPROMISE GRAIN OR NUTRITIONAL QUALITY

This is a relevant question when consumer preferences, and the different resistance mechanisms and their potential impact on processing quality, are considered. Ideally, grain quality and resistance to storage pests should be developed in parallel so that consumers realize the full nutritional benefit of a crop. Protein quality can be improved through GE by increasing the content of lysine, an essential amino acid, through modification of the anticodon for tRNA<sup>Lys</sup> (Shahid *et al.*, 2009) or through the use of RNA-interference to enable the dominant expression of recessive mutants (e.g. o2) to increase lysine content (Segal *et al.*, 2003). Provided these events do not alter the grain texture, they could be combined with conventional sources of resistance (Dhliwayo and Pixley, 2003) or with GE-derived resistance [Alfonso *et al.*, 2003] to provide nutritious varieties that can be stored by farmers, especially those who depend on cereals for subsistence. Conclusions reducing post-harvest storage losses through genetic improvement is both feasible and urgently needed to meet food demands in coming decades. A small but noticeable renaissance in the use of resistant varieties to minimize storage losses is taking place, especially in those ecologies where infrastructure for storage does not exist. To capitalize on genetic diversity for storage pest resistance, researchers have made significant progress in understanding the biochemical, biophysical, and genetic bases of HPR, which is essential to ensure that the traits being selected meet with consumer demands. Traits that meet these criteria are now being mapped to confirm their role in resistance and to identify candidate genes using sequence homologies and proteomics. The introgression of resistance alleles from the same crop species, using marker-assisted selection or GE, will probably meet with greater public acceptance and possibly require less rigorous testing to document food safety than will the introduction of genetic material from other species. The real potential of this technology will be felt most in LDCs because the technology is packaged in the seed and should be designed to ensure that farmers have the option to recycle seed, a common practice for subsistence farmers.

### 6. SUMMERY

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