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Genetics and Breeding of Resistance to Bruchids (*Callosobruchus* spp.) in *Vigna* Crops: A Review

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ABSTRACT

Vigna crops include mungbean (*V. radiata*), blackgram (*V. mungo*), azuki bean (*V. angularis*) and cowpea (*V. unguiculata*) are agriculturally and economically important crops in tropical and subtropical Asia and/or Africa. 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 comercially 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 neccessary. 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*. In this paper we review sources, mechanism, genetics and breeding of resistance to *C. chinensis* and *C. maculatus* in *Vigna* crops with the emphasis on mungbean, blackgram, azuki bean and cowpea.

Keywords: Bruchid resistance, *Callosobruchus* spp., Legumes, *Vigna* spp.

INTRODUCTION

The genus *Vigna* falls within the tribe *Phaseoleae* and family *Fabaceae*. Species in this taxon mainly distribute in pan-tropical Asia and Africa. Seven *Vigna* species are widely cultivated and known as food legume crops. Of these, five is of Asian origin (subgenus *Ceratotropis*) and two is of African origin (subgenus *Vigna*). The Asian *Vigna* comprises mungbean or green gram (*V. radiata* (L.) Wilczek), blackgram or urd bean (*V. mungo* (L.) Hepper), azuki bean or red bean (*V. angularis* (Willd.) Ohwi & Ohashi), rice bean or red bean (*V. umbellata* (Thunb.) Ohwi & Ohashi) and moth bean (*V. aconitifolia* (Jacq.) Maréchal). The African *Vigna* comprises cowpea (*V. unguiculata* (L.) Walp.) and bambara groundnut (*V. subterranea* (L.) Verdc.). These crops are adapted to agroclimatic condition and fit well into many cropping systems in the regions. Young pods, seeds and sprouts from the crops are important sources of proteins, vitamins and minerals for common people in the regions, while leaves are used as fodder in animal farming. Generally,

seed yield of *Vigna* crops is still low due to poor genetic background, insect and disease damages, and production management.

Bruchids or seed beetles or seed weevils (order Coleoptera, family Chysomelidae, 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 (Fig. 1). 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.

In this paper we reviewed the genetics and breeding for bruchid resistance in *Vigna* crops with emphasis on mungbean, blackgram, azuki bean and cowpea, the four *Vigna* crops that are most intensively used for research on bruchid resistance in the genus.

BRUCHIDS AS THE MOST DESTRUCTIVE STORAGE PEST OF *VIGNA* SPECIES

C. chinensis and *C. maculatus* are the most serious pests of stored seeds of the *Vigna* crops (Fig. 1). They cause huge economic damage under conditions of tropical subsistence agriculture (Southgate, 1979; Rees, 2004). Geographical distribution of both bruchids is now worldwide, but especially devastating in the tropics. They have a similar life cycle and ecology. Population development of both insect species is rapid with the life cycle of about 20 to 30 days. With suitable host and optimum weather condition of about 30 and 70% r.h., the life cycle is only 22.3 and 24 days, respectively for *C. chinensis* and *C. maculatus* (Raina, 1970). The two bruchid species are quite common as to which *Vigna* species they attack. For example both bruchids are able to feed on mungbean and cowpea but not on rice bean. However, differences in *Vigna* host specific exists, e.g. *C. chinensis* fails to feed on blackgram, while *C. maculatus* can.

Figure 1 Bruchid (*Callosobruchus* spp.) infestation to *Vigna* seeds during storage.

Legume researchers have been seeking for sources of bruchid resistance in both cultivated and wild *Vigna* species, although resistance in the cultivated ones is rare. All *Vigna* crops, except rice bean are susceptible to either one or both of insect species. Host-pest relationship between *Vigna* crops and the beetles is given in Table 1.

There are a few reports on resistance to *C. chinensis* and *C. maculatus* in wild *Vigna* (Fujii and Miyazaki, 1987; Fujii *et al*., 1989; Singh and Ng, 1990; Tomooka *et al*., 2000; Kashiwaba *et al*., 2003). The following information is extracted from these reports. By and large, resistance is present in wild progenitors and relatives of cultivated *Vigna*. There exists high variation in the reaction to bruchid species in wild *Vigna* species. A *Vigna* species may possess resistance to both or one of the two bruchid species. For example *V. subramaniana* showed resistance to both bruchids, while *V. trinervia* exhibited resistance to only C. *chinensis*. Intraspecific variation for bruchid resistance in a *Vigna* species also appears. For example, an accession of *V. hirtella* complex showed no damage by *C. maculatus* but partially damage by *C. chinensis*, while susceptible accessions to both bruchids also exist. Response to bruchids in wild *Vigna* species is summarized in Table 2. With exception to wild progenitors of the of the *Vigna* crops, not many of these wild species can be used as resistance source by breeding because of their genetic isolation. Cross compatibility among some of these wild species and cultivated species is yet to be studied.

Table 1 Relationship between *Vigna* crops and bruchids (*Callosobruchus* spp.) predators.

***Vigna* crop**	**Species**	**Bruchid species***	
		C. chinensis	***C. maculatus***
Mungbean	*V. radiata* var. *radiata* (L.) Wilczek	√	√
Blackgram	*V. mungo* var. *mungo* (L.) Hepper	√	×
Azuki bean	*V. angularis* var. *angularis* (Willd.) Ohwi & Ohashi	√	√
Cowpea	*V. unguiculata* var. *unguiculata* (L.) Walp.	√	√
Rice bean	*V. umbellata* (Thunb.) Ohwi & Ohashi	×	×
Moth bean	*V. aconitifolia* (Jacq.) Maréchal	√	√
Bambara groundnut	*V. subterranea* (L.) Verdc.	√	√

*√ = bruchid is able to feed on host seeds, × = bruchid is unable to feed on host seeds

Table 2 Wild relatives of *Vigna* crops with some accessions carrying resistance to bruchid (*Callosobruchus* spp.) predators*.

Species	**Bruchid species**	
	C. chinensis	***C. maculatus***
Asian *Vigna* (subgenus *Ceratotropis*)		
V. hirtella	×	×
V. mungo var. *silvestris* (wild ancestor of blackgram)	×	×
V. minima	×	×
V. riukiuensis	×	×
V. nepalensis	×	×
V. radiata var. *sublobata* (wild ancestor of mungbean)	×	×
V. reflexo-pilosa var. *reflexo-pilosa* (wild ancestor of var. *glabra*)	×	√
V. subramaniana	×	×
V. trinervea	×	√
V. umbellata var. *gracilis* (wild ancestor of rice bean)	×	×
African *Vigna* (subgenus *Vigna*)		
V. oblongifolia	?	×
V. luteola	?	×
V. reticulate	?	×
V. vexillata	?	×

*Compiled from the results of Kashiwaba *et al*. (2000) Tomooka *et al*. (2000) and Singh and Ng (1990).

× = some resistant accessions found, √ = no resistant accession found, ? = no information

Seed defense chemicals is among the factors causing bruchid resistance. Seven chemicals isolated from seeds of various *Vigna* species have lethal effect against bruchids (Table 3). Some of the chemicals are novel and unique. The resistance in *Vigna* species is either a result of a single component or a combination of chemicals. A resistance is usually conditioned by a single gene and thus can be easily moved into a cultivar (Kitamura *et al*., 1988; Tomooka *et al*., 1992; Somta *et al*., 2007b). Resistance due to a combination of chemicals encoded by different loci was reported by Somta *et al*. (2006a, 2007c) and expected to be difficult to incorporate into a cultivar.

In general, seed defense chemicals are badly taste and/or toxic to humans as well as to seed predators and thus are always selected against during plant domestication to neutralize or minimize their effects. The co-evolution between bruchids and their food sources together with the mutation of bruchid strains during the course of evolution led the bruchids to be able to detoxify the defense chemicals and eventually use the seed that was previously toxic to them as their exclusive food plant.

Table 3 Potential biochemical metabolites in seeds of *Vigna* species against growth and development of bruchids, *Callosobruchus* spp.

***Vigna* species**	**Metabolite**	**Bruchids**	**References**
V. radiata var. *sublobata*	Cyclopeptide alkaloids (vignatic acids A and B)	*C. chinensis*	Sugawara *et al*. (1996)
	GIF-5	*C. chinensis*	Kaga *et al*. (2000)
	Defensin (cysteine-rich protein (*VrD1* or *VrCRP*))	*C. maculatus*	Chen *et al*. (2002)
V. mungo var. *mungo*	Protein (a novel 40-kDa single polypeptide)	*C. chinensis*	Wang *et al*. (1999)
V. umbellata (cultivated; Menaga)	Flavonoids (naringenins)	*C. chinensis*	US patent 6,770,630B2
		C. maculatus	US patent 6,770,630B2
V. unguiculata (resistant lines related to Tvu2027)	Vicilins (7-S storage globulins)	*C. maculatus*	Macedo *et al*. (1993)
V. vexillata (TVnu72)	*para*-aminophenylalamine	*C. maculatus*	Birch *et al*., 1986

GENETICS AND BREEDING FOR BRUCHID RESISTANCE IN MUNGBEAN (*V. RADIATA*)

Mungbean is widely grown in South and Southeast Asia, and becomes familiar to farmers in Australia, America and Canada. It is the most economically important *Vigna* crop in Asia. Mungbean seed is rich in protein (25-30%), amino acid, vitamins and minerals. It is cooked into several kinds of food such as soup, cake, noodle, sweets, bread and biscuits. Green pod, green seed and sprout are consumed as vegetable. Mungbean sprout is now gaining popularity as an ingredient in the western cuisine. Plant parts are also used as fodder. Because of rapid growth and early maturity (can be harvested within 60 to 90 days after planting), it is a component of many cropping systems in drier and warmer climates in the tropics and subtropics.

The world production area of mungbean is about 5.5 million ha (Weinburger, 2003). India is the largest producer of about 2.9 million ha and most products are used domestically. China, Myanmar, Vietnam and Thailand are the main exporters of mungbean grain and products.

Sources of bruchid resistance in mungbean

In cultivated mungbean, 4 accessions were reported to be resistant to both *C. chinensis* and *C. maculatus* from screening of thousands of mungbean landraces (Somta et al., 2007a; Talekar and Lin, 1992). Earlier screening of 525 AVRDC mungbean germplasm failed to identify resistance accessions (Talekar and Lin, 1981), but later screening of 500 more accessions resulted in identification of three accessions, V1128, V2709 and V2802 showing moderate to high level of *C. chinensis* resistance (AVRDC, 1990a; AVRDC, 1990b; Talekar and Lin, 1992). The three accessions are also effective against *C. maculatus* (Somta *et al*., 2007a; Somta *et al*., 2007b). Additional screening of about 1,000 mungbean landraces against the weevils showed no resistance source (Tomooka, *et al*., 2000; Somta *et al*., 2005, unpublished data). However, a new effective resistance source, V2817 was found immune to both bruchids (Somta *et al*., 2007a).

Historically, a bruchid resistance mungbean, TC1966 was first found in a wild relative (*V. radiata* var. *sublobata*) after screening a few accessions. TC1966 showed complete resistant against various bruchids, such as *C. analis, C. chinensis, C. maculatus, C. phaseoli* and *Z. subfasciatus* (Fujii and Miyazaki, 1987; Fujii *et al*., 1989; Lambrides and Imries, 2000; Kashiwaba *et al*., 2003). Lambrides and Imries (2000) reported resistance in two additional accessions of wild mungbean, ACC41 and ACC23. However, TC1966 and ACC41 are susceptible to Australian strains of *C. maculatus* (Lambrides and Godwin, 2007)

Apart from wild mungbean, several wild *Vigna* species closely related to mungbean, e.g. wild blackgram (*V. mungo* var. *silvestris*) and *V. subramaniana* also possess resistance to bruchids (Tomooka *et al*., 2000) and may be useful in breeding for resistance mungbean.

Mechanisms of bruchid resistance in mungbean

Biochemicals in seeds confer resistance to bruchids in mungbean, but the basis of the resistance is complex and ambiguous. Resistance chemical factors have been isolated and identified from isogenic lines carrying resistance from TC1966. Two novel cyclopeptide alkaloids, named as vignatic acids A and B, were isolated (Sugawara *et al*., 1996). Although, vignatic acids A showed resistance to *C. chinensis* infestation, it is not the principal factor responsible for the resistance (Kaga and Ishimoto, 1998). A peptide compound "GIF-5" toxic to the bruchids was also identified from a similar material that was used for isolating vignatic acids (Kaga *et al*., 2000).

A cysteine-rich protein (*VrCRP* or *VrD1*) of the plant defensin family shown to be lethal to C. *chinensis* larvae, has been isolated from resistant mungbean carrying the resistance gene from TC1966 (Chen *et al*., 2002). *VrD1* insecticidal activity has its basis in the inhibition of a polysaccharide hydrolysis (Liu *et al*., 2006). Chen *et al*. (2002) tried to prove that *VrD1* is not the product of the bruchid resistance gene. Thus the basis for the resistance in TC1966 is still inconclusive. While the resistance in cultivars V1128, V2709, V2802 and V2817 is due to antibiosis (Talekar and Lin, 1992; Somta *et al*., 2007a), but the responsible chemical(s) has yet to be determined.

Utilization of genetic information in breeding for bruchid resistance in mungbean

TC1966 has been intensively used as the material for genetic study and breeding for bruchid resistance in mungbean. The resistance is controlled by a single dominant gene, designated as *Br*. (Kitamura *et al*., 1988). DNA marker based studies enable researchers to localize the resistance (*Br*) gene. By using a small mapping population of 58 F_2 individuals, the gene is mapped onto linkage group (LG) 8 and franked 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). The resistance gene from V2709 is being investigated molecularly using microsatellite (simple sequence repeat or SSR) and STS markers (Hong, *et al*., 2006)

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 it has been consumed by human for a period of time 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. However, single resistance cultivar is considered less durable, as the insects co-evolve with the host plants and can usually overcome the resistance sooner or later. In a recent study, *C. maculatus* reared on resistant mungbean seeds carrying the *Br* locus from TC1966 for 5 consecutive generations showed high fecundity and a positive growth throughout the time course (Lin *et al*., 2005). Development of multiple resistant cultivars is an effective way to slow down the evolution of the resistance.

GENETIC AND BREEDING FOR BRUCHID RESISTANCE IN BLACKGRAM (*V. MUNGO*)

Blackgram is grown largely in South and Southeast Asia but in a less extent, comparing to mungbean. India, Burma and Thailand are the main producers. Cultivation and uses of blackgram are similar to those of mungbean. Sprouts produced from blackgram gain more popularity due to longer shelf life.

Sources of bruchid resistance in blackgram

Blackgram is known to immune to *C. chinensis* but susceptible to *C. maculatus*. However, it prolongs developmental period of *C. maculatus*. The bruchids may require as long as 53 days to complete their life cycle which is more than twice as it did in mungbean (Tomooka *et al*., 2000). This mode of resistance may be useful in limiting the rate of multiplication and reducing the population growth resulting in considerable reduction in seed loss during storage.

No source of resistance to *C. maculatus* is identified in cultivated blackgram, but wild blackgram (*V. mungo* var. *silvestris*) is shown to be completely resistant to *C. maculatus* and other bruchid species such as *C. chinensis*, *C. analis*, *C. phaseoli*, and *Z. subfasciatus* (Fujii *et al*., 1989; Dongre *et al*., 1996; Tomooka *et al*., 2000; Kashiwaba *et al*., 2003), although an accession with incomplete resistance

is also reported (Dongre *et al.*, 1996). It is considered to be among the most resistance species.

Mechanism of bruchid resistance in blackgram

Biochemical in blackgram seeds is responsible for resistance to *C. chinensis* (Talekar and Lin, 1992). A proteinous factor, novel 40-kDa peptide isolated from blackgram caused lethality to the bruchids (Wang *et al.*, 1999). The peptide is neither α-amylase nor protease inhibitors. The mechanism of the resistance in wild blackgram has not yet been determined. Since wild blackgram is immune to several important bruchid species, the resistance factor(s) is worth to be identified

Utilization of genetic information in breeding for bruchid resistance in blackgram

Studies on genetics and breeding for bruchid resistance in blackgram are very scarce. This may be due to the fact that 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.

GENETICS AND BREEDING FOR BRUCHID RESISTANCE IN AZUKI BEAN (*V. ANGULARIS*)

Azuki bean is an economically important legume in East Asia. The bean is very popular in Japan, China, Korea and Taiwan, which Japan is the main consumers. It is the second most important legume crop after soybean in Japan and Korea. Azuki bean is a major ingredient in almost all sweets especially in ceremonial foods in Japan. In Nepal, young pods are consumed as vegetable, (Vaughan *et al*., 2005)

China is the largest producer with the cultivated areas of about 470,000 ha and annual production of about 700,000 tons (Vaughan *et al*., 2005). The bean is grown as a cash crop in Australia, Canada, New Zealand and the USA.

Sources of bruchid resistance in azuki bean

Azuki bean is a primary host of *C. chinensis*. There has been no report on resistance to *C. chinensis* and *C. maculatus* in both cultivated varieties and wild form (*V. angularis* var. *nipponensis*). Screening for the resistance using several hundred accessions of cultivated and wild azuki bean is futile (Vaughan *et al*., 2005). Incorporating azuki bean germplasm with wide geographical distribution may lead to identifying of effective sources. However, several wild *Vigna* closely related to azuki bean show resistance to bruchids (Tomooka *et al*., 2000). They are *V. hirtella, V. minima, V. nepalensis, V. riukiuensis, V. trinervia* and *V. umbellata*.

Utilization of genetic information in breeding for bruchid resistance in 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; Somta *et al*., 2006b). 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. riukiuensis* 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. napelensis* 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.

GENETICS AND BREEDING FOR BRUCHID RESISTANCE IN COWPEA (*V. UNGUICULATA*)

Cowpea is the most economically important *Vigna* crop grown in the world. It is widely cultivated in semi-arid tropics spanning Asia and Africa, especially the latter, and is also popular in North and South America. Owing to its drought tolerant and warm weather adaptive, cowpea performs in the dry regions better than the other food legumes. It is a useful component in traditional cropping systems. It can be intercropped with cereals, cane, cotton and plantation crops (Singh, 2005). Cowpea

seeds play important role as a source of protein, minerals and vitamins in daily diets for hundred millions of poor people in the Africa. Dry seeds, young leaves, green pods and green seeds are eaten. Plant parts are used as fodder, silage or hay to feed livestock.

The estimated growing area for cowpea in the world is more than 14 million ha with annual production of about 4.5 million tons (Singh, 2005). Nigeria is the largest producer and consumer of cowpea with about 5 million ha area and about 2.4 million tons produced annually.

Sources of bruchid resistance in cowpea

C. maculatus is the most serious pest of stored cowpea due to the fact that cowpea is the primary host of this bruchids, and it prevails in Africa where the cowpea is originated and largely grown. Resistance sources in cowpea are very rare. At the International Institute of Tropical Agriculture (IITA), Nigeria, more than 15,000 accessions of the world cowpea collection were screened against *C. maculatus*, only 3 landraces, TVu11952, Tvu11953 and Tvu2027 were found to be resistant (Singh *et al*., 1982). All the three accessions showed only a moderate level of resistance. Investigation in wild *Vigna* relatives of cowpea resulted in identifying several accessions of *V. vexillata*, *V. reticulata*, *V. oblongifolia* and *V. luteola* carrying resistance to *C. maculatus* (Birch *et al*., 1986; Singh and Ng, 1990).

Mechanism of bruchid resistance in cowpea

Resistance to *C. maculatus* in cowpea is due to seed biochemicals, but the basic chemicals responsible for the resistance has long been ambiguous since the resistance sources came from only Tvu2027. Recently, a seed storage protein, vicilins (7-S globulins) was found to involve at least in part in the resistance of Tvu2027 (Macedo *et al*., 1993). The vicilins from resistant cowpea seeds are resistant to midgut digestive enzymes of the bruchids. This lower rate of hydrolysis causes the resistance through reducing the availability of nutrients necessary for growth and development of larval bruchids (Fermino *et al*., 1996). In addition, vicilins isolated from cotyledons of the resistant cowpea seeds showed deleterious effects on development and survival of *C. maculatus*, whereas the same chemicals isolated from axial tissue had no effect against the bruchids (Domingues *et al*., 2006).

Since cowpea is mainly produced in Africa where *C. maculatus* is dominant, there is a lack of information on cowpea resistant to *C. chinensis*, the bruchids attacks cowpea in Asia.

Utilization of genetic information in breeding for bruchid resistance in 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). However, by using Tvu2027, TVu11952 and Tvu11953 as resistance sources, Singh *et al*. (1985) and Kitch (1987)

showed that the resistance inherited as two recessive genes. The genes were designated as rm_1 and rm_2 (Singh *et al*., 1985). All the 3 accessions possessed the same resistance genes (Kitch, 1987).

Genetic mapping for genes controlling *C. maculatus* resistance has been investigated. Four QTLs were found associating with the resistance (Fatokun, 2002). 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. In another report, SSR marker Vm50 was found to closely associate with the delay in emergence of *C. maculatus* with 20% variation explained (Fatokun, 2000).

Several bruchid-resistance cowpea lines were developed using resistance genes from Tvu2027 and the resultant varieties were released to farmers in many countries (Singh, *et al*., 1996; Singh, 2005). However, since the resistance comes from only a single source (Tvu2027), there are reasons to believe that bruchids can soon evolve to break the resistance. Shade *et al*. (1996) reported that *C. maculatus* was able to develop a biotype to overcome Tvu2027, after selection on resistant cowpea seeds for over 53 generations. Thus new sources of resistance are necessary for developing multiple resistance cultivars for durable resistance.

Genetic engineering as an alternative method to improve bruchid resistance in *Vigna* crops

Advance in transformation system and plant regeneration by tissue culture technique in legumes have made possible the development of bruchid-resistant cultivars. Proteinaceous α-amylase inhibitor (αAI) 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 αAI-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). In deed, very recently, αAI-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 αAI-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). Recent investigations showed that broiler chickens fed with transgenic pea expressing αAI-1 had lower growth, starch digestibility and metabolizable energy (Li *et al*., 2006), whereas pigs fed with the same transgenic pea had lower dry matter digestibility due to reduced starch digestion (Collins *et al*., 2006). Therefore, it is still arguable

whether the αAI-1 transgenic legume is safe for human and animal consumption, although anti-nutritional property of proteinaceous factors such as α-amylase inhibitor may be inactivated by heat.

GENERAL DISCUSSION

All economically important *Vigna* crops are susceptible to bruchids. Sources of resistance in *Vigna* crops are rare, while wild *Vigna* show wider arrays of resistance. Genetics of the resistance can be either simple or complex. There appear constraints in using wild *Vigna* as resistance sources as gene exchange between wild and cultivated genotypes is difficult due to genetic barriers. More importantly, defense chemicals in the wild *Vigna* is not confirmed as safe for human consumption. 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.

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