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# Bruchid a Serious Pest on Pulse Crops: Its Control Measures and Breeding Advancements: A Review

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## ABSTRACT

Bruchid (*Callosobruchus spp*.) are the most destructive, notorious storage pest of pulses in the tropical and sub-tropical region. The yield losses are higher than half of the expected yield with in the short period of time. Bruchid initial infestation started in the field and shortly builds up during storage time and cause severe seed damage up to 100 per cent. Bruchid infestation ranges from 60 to 100% within two to three months of storage period. Among the different bruchid species, cowpea weevils (*C. maculatus* F.) and azuki bean weevils (*Callosobruchus chinensis* L.) are the most destructive storage pest of pulses. Though several options are now available to identify the elite genotypes against bruchid infestation, still the development of genotypes with sufficient level of host plant resistance is not achieved. In this pursuit, the present article has given a detailed review of the major species of bruchid, insect life style, management practices, screening technique, sources of resistance, novel breeding strategies and recent advancements including use of molecular markers in marker aided selection and QTL studies for bruchid resistance.

**Key words:** Bruchid, Control measures, Life cycle, Molecular markers, Phenotyping.

The genus *Vigna* belong to the family of *Fabaceae* and are the major sources of dietary proteins and essential amino acids (Asif *et al*., 2013). Major pulse crops include blackgram (*V. mungo*), greengram (*V. radiata*), adzuki bean (*V. angularis*), cowpea (*V. unguiculata*), rice bean (*Vigna umbellata*) and pigeon pea (*Cajanus cajan*). Globally, these crops serve as second most important group of crop plants next to cereals. These crops contain essential amino acids *viz.,* methionine, cysteine, threonine, tryptophan and lysine (Saxena *et al*., 2010). Thus, pulses supply a perfect combination of essential amino acids and minerals when taken along with cereals (Reddy, 2010).

Among the pulse-growing countries, India is one of the major producer, consumer and importer of pulse crops. India has contributed about 3.56 million tonnes per annum from the area of 5.44 million hectares with productivity of 655 kilograms per hectare (Project Coordinator's Report, 2018-19). However, the consumption of pulses has been increasing over the decades than domestic pulse production which leads to import of pulses. In addition to its production shortage, the net quantum of productivity and its economic value is constantly being affected by the storage pests, especially the bruchids (*Callosobruchus sp*.).

Bruchid, a notorious storage grain pest can render total damage to seeds, if not sufficient care (drying, fumigation and pesticide application *etc.,*) is undertaken during storage period. Presently none of the available varieties are resistant to bruchid. Bruchid damage to stored produces affect the quality, taints the taste of the grains and also if these bruchid are eaten along with the food may affect the human health thus the control measures to this storage pest are highly essential. Genetic improvement in pulse crops against storage grain pests is a challenging work for the scientists. Conventional breeding also does not provide any significant improvement due to scarcity of the resistant sources (donor genotypes) in the available germplasm. Molecular and biochemical techniques have been proved useful to generate markers to explore valuable genes in a set of germplasm. At this situation, breeders often try to trap the genetic variability that is available in the allied wild species through wide hybridization or through mutagenesis and genetic transformation techniques.

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## Major bruchid species of *Vigna* species

Bruchid beetle or bean weevil, *Callosobruchus spp*. (Coleoptera: Bruchidae), causes serious damage to several leguminous crops including cultivated *Vigna* species, such as greengram, blackgram, azuki bean and cowpea during storage (Fig 2). A number of Coleopteran *Callosobruchus* species (Bruchidae) infest food legumes and among those *C. maculatus, C. chinensis, C. analis* and *C. phaseolous* are most common. However, major bruchid beetles particularly cowpea weevils (*C. maculatus* F.) and azuki bean weevils (*Callosobruchus chinensis* L.) are the most destructive storage pests of pulses during storage (Talekar, 1988).

## Life cycle of bruchid

A female adult has been able to lay over hundreds of eggs on the seed surface and most of them will hatch. The larva starts emerging after 4 to 8 days of infestation, larva bores into the grains and starts feeding on the endosperm (Raina, 1970). During development, the larva feeds on the endosperm of the pulse grain, leaving a very thin layer of seed coat and exit when it matures. The symptom of damage depicted in Fig 1. The larval period ranges from 3 to 7 weeks, depending on the climatic conditions. The gestation period is typically longer in cool climate around 4 to 12 weeks to emerge. Bruchid requires 24 to 36 hours to mature completely once it emerges out. The lifespan of beetles is up to 10 to 14 days. The beetle tolerates a range of humidity and temperature, making it adaptable in climates worldwide.

## Various approaches to control bruchid

### Conventional approaches

Cultural control involves the manipulation of suitable host environment to eradicate the prevalence, growth and multiplication of storage pests. The cultural practices include removal of egg shells and dead larvae, removal of infested grains before storage of new grains, fumigation and disinfestation (Casida and Quistad, 1998). Traditionally, 10% dichloro diphenyl trichloro ethane (DDT) and/or 5% benzene hexachloride (BHC) dust at 6-8 sprays per 100 cubic feet of storage space were used for disinfestations (Upadhyay and Ahmad, 2011). These practices are considered to be the cheapest and most reliable techniques. However, these methods are constrained by a number of shortcomings such as, requirement for long-term planning and careful timing (Kananji, 2007). These methods are species-specific (Watson *et al.*, 1976). Further DDT and BHC are the banned pesticides.

Physical control are the treatment of the seeds and insects using physical agents, such as temperature, heat, moisture content and pressure (Sahadia and Aziz, 2011). Optimal environment required by each insect species *viz.,* temperature, humidity and photoperiod for its growth and perpetuation. The rate of the development of bruchid can be influenced by either lowering or raising the temperature and changing the relative humidity from its optimal range (Benz, 1987). In addition, raising and lowering the grain temperature to 60-65°C for a few minutes and to below 12°C can effectively kill all life stages *viz.,* eggs, larva and pupa of stored grain pests. The lowering of moisture content to below 9% can adversely affect the reproduction and development of stored insects in cowpea and moth bean (Upadhyay and Ahmad, 2011). Similarly, creating a low oxygen-controlled atmosphere kills stored product insects. A combination of low pressure and high temperature is more effective in killing the eggs, larvae of cowpea weevil and related bruchids in cowpea (Mbata *et al.*, 2005). The use of solar heating techniques has also been recommended for controlling bruchid beetles in mung bean and cowpea seeds (Moumouni *et al.*, 2014). In addition, inert dusting and exposure to ionising radiation could also be used. Insects coated with these make them to dehydrate and later it dies due to desiccation. Traditionally, six types of inert dusts viz., (sand and soil components, diatomaceous earth, silica aerogel, non-silica dusts, wood ash and particle films) are used (Wolfson *et al.*, 1991) with little variation in their performance. Alternatively, two kinds of ionising radiations, β- and γ-radiations, are also used to control insect pests in pulse grains. β-radiation is comparatively safer and easier to cope with because it can be turned on and off as per the will of the farmers, while an isotope-based γ-radiation radiates continuously and is hazardous for human health (Fields and Muir, 1996). Besides their beneficial aspects, these techniques have some limitations, such as their high-capital cost and loss of seed viability due to irradiation. However, there is no report available on the application of these techniques in bruchid pest management to date.

Biological control involves the utilization of living things to eradicate the bruchid population, known as biological control agents, to maintain the pest populations below damaging level. The biological agents *viz.,* predators, parasitoids and pathogens (Mahr *et al.*, 2008). *Hymenoptera* (*Dinarmus* spp.) parasitoids are commonly used to suppress *C. maculatus* population in blackgram (Soundararajan *et al.*, 2012). The *Apanteles flavipes* parasitoid is used to control bean weevil, *Callosobruchus chinensis* (Eliopoulos, 2006), while *Eupelmus vuilleti* is mostly used to manage the cowpea weevil, *C. maculatus* in cowpea (Cortesero *et al.*, 1997). Although biological agent-mediated control holds potential for controlling bruchids, their use by small-scale farmers could present some limitations (Kananji, 2007), including the supplementation of nutrition (such as honey, sugarcane or host larvae) to parasitoids, predators and pathogens to maintain their culture and effective application of these parasitoids, which depends upon the timing of the release and stages of larval development.

Phytochemical control which involves the utilization of plants and plant-derived products with the insecticidal and inhibitory activities against bruchid. It has been reported that 10 ml per kg of groundnut or coconut oil treatment has a toxic effect on eggs of *C. maculatus* (Raja and Inacimuthu, 2000). Neem (*Azadirachta indica*) products, is in the form of seed kernel extract (5%) with soap solution (0.1%), have also been found to be effective in controlling such pests. *C. chinensis* can be controlled effectively in chickpeas by treating with neem seed oil at 1 ml per 100 g of seeds. Tobacco powder is reported to be most effective in reducing *C. maculatus* egg hatching on stored cowpeas (Ofuya and Akhidue, 2005). Adenekan *et al.* (2013) reported that leaf, stem, root and flower powders of the drumstick tree (*Moringa oleifera*) could also be used as herbal insecticide to control the bruchid beetles in cowpea seeds under an optimal temperature of 30°C and relative humidity of 72%, because these herbal products reduced number of eggs laid, number of eggs hatched and mean adult emergence and prolonged the developmental periods.

Chemical pesticides, includes four chemical groups *viz.,* organo-chlorines, organo-phosphates, carbamates and pyrethroids, have been used in fields and storage for the management of storage pests, including bruchids (Megerssa, 2010). The use of spinosad, a natural bio-pesticide which is effective for the management of these storage pests both in laboratory and in field conditions (Sanon *et al.*, 2010). It reduces the emergence of *C. maculatus* from the seeds till 6 months in storage of cowpea. Scientists have found the effectiveness of chemical pesticides including dusts, fumigants and sprays for the prevention of bruchid pests (Harberd, 2004). However, the bruchid confers resistance to many traditional pesticides *viz.,* permethrin, lindane, pirimiphos-methyl, phostoxin, methylbromide and iodofenphos (Talukder, 2009) and their application at higher doses leads to the accumulation of toxic residues in the treated products. Furthermore, problems associated with chemical pesticides especially pesticide resistance, health hazards and environmental effects, have created a worldwide interest in the development of alternative approaches, such as exploitation of available host plant resistance through the tools of biotechnology and breeding for bruchid pest management in pulse crops.

**Fig 1:** Seed damage caused by bruchid infestation.

**Plant breeding approach**

Breeding progress depends upon various factors *viz.,* the magnitude of genetic variability available in germplasms, heritability of the desired traits and the selection pressure exerted (Falconer, 1989). Various genotypes of different pulses have been evaluated to obtain sources of host resistance to storage insects including bruchids. It was found that popular cultivars with improved yield (cultivated type) are more prone to these pests than the landraces or wild species. In various legumes, genes for complete resistance to bruchids have frequently been reported in wild relatives, such as *Vigna radiata* var. *sublobata* and *V. mungo* var. *sylvestris* (Chen *et al.*, 2007; Somta *et al.*, 2008; Souframanien *et al.*, 2010).

**Sources of bruchid resistance**

The wild relatives of crop species are one of the important sources of unexplored genes for crop improvement and greater availability of genetic diversity measured at both the biochemical and DNA level in wild species than their closely related species (Xu *et al*., 2000). Intensive modern breeding has contributed to a narrowing down the crop genepools as a few improved cultivars dominate large areas (Ladizinsky, 1985). Due to recent developments in gene transfer technology, genes from cross incompatible wild species can be involved in breeding activities. Therefore, evaluation of a broad range of wild species is an appropriate approach to explore genes which are unavailable in cultigens. The subgenus *Ceratotropis* of the genus *Vigna* is an important taxonomic group because it includes seven cultivated species, greengram [*Vigna radiata* (L.) Wilczek], blackgram [*V. mungo* (L.) Hepper], moth bean [*V. aconitifolia* (Jacquin) Maréchal], azuki bean [*V. angularis* (Willdenow) Ohwi and Ohashi], rice bean [*V. umbellata* (Thunberg) Ohwi and Ohashi], jungli bean [*V. trilobata* (L.) Verdcourt] and *V. reflexo-pilosa* (Lawn, 1995). The wild relatives of *Vigna radiata* var. *sublobata* (V1128, V2709, V2802 and TC1966)


**Fig 2:** Dimorphic picturization of *Callasobruchus maculatus.*

are the sources of resistance. It shows the resistant mechanism like reduced oviposition, less seed damage, low adult emergence and prolonged development period. Similarly, in blackgram resistance was noticed in the wild accessions of *Vigna mungo* var. *silvestris* (VM2164, VM2011, VM3529) (Souframanien *et al.,* 2010). The two Trombay varieties of blackgram namely TU 68 and TU 80 were found less susceptible to pulse beetle compared to other genotypes tested and can hold promise as genetic source in breeding programme of blackgram for bruchid pest resistance (Gopalaswamy *et al.,* 2016). In case of cowpea (*V. unguiculata*) some notable genotypes *viz.,* IT81D-994, Adom (CR-06-07) confers resistance against bruchid (Adam and Baidoo, 2008).

## Assessment methods for bruchid resistance

### Test insects

Adult beetles of bruchid are obtained from the stock culture maintained in plastic jars of containing blackgram or greengram grains. The mouth of the jar should be well sealed with muslin cloth and fastened tightly with the help of a rubber band or with lid with holes facilitating the better aeration for the beetles. Freshly emerged beetles from the stock culture are used for the screening experiments. Usually two types of approach are followed:

### No choice test

The pulse grains of (50 seeds) each variety is taken in a plastic container into which two to five pairs of freshly emerged bruchids are released. After introducing the bruchids into each jar, the mouth of the jar is secured with perforated lids for better aeration. The experiment need to be replicated under normal room temperature. After five days, the adults are removed and recorded data on oviposition. Later, they are allowed for the progeny development. Data on number of adults emerged is taken at 30-90 days after release of adults on the daily basis. Finally, per cent seed damage and per cent weight loss are recorded.

### Multi choice test

Pulse grains of all the test varieties were taken at equal quantity (30 grains) in single petri plates and randomly arranged in a circle in a plastic container. Five to ten pairs of freshly emerged bruchid were released in the middle of the circle giving a choice to adult beetles to settle and oviposition on their preferred grains. After allowing for five days, data on oviposition was recorded. At 30 - 90 days after release of insects, data on progeny emergence is recorded for each variety. Finally, per cent grain damage and weight loss are recorded.

### Biotechnological approaches

Biotechnological approaches involve transgenic genome modification and DNA marker-assisted breeding, can provide essential means of reducing yield loss and maintain the net production (Collard and Mackill, 2008). Improvements and some of the achievements in this field of bruchid resistance in pulse crops have been discussed below. Transgenic approaches with the optimization of *in vitro* propagation tools and advancements in genetic engineering techniques, the first transgenic crop was developed three decades ago (Eapen, 2008). Since then, the use of genetic transformation has been reported in most crops, including pulses, such as *Vigna unguiculata* (Popelka *et al.*, 2006), *Vigna mungo* (Saini *et al.*, 2003), *Cicer arietinum* (Fontana *et al.*, 1993), *Cajanus cajan* (Geetha *et al.*, 1999), *Vicia faba* (Ramsay and Kumar, 1990) and *Pisum sativum* (Schroeder *et al.*, 1993). Biochemical studies on pulses confirmed that lectins, the sugar-binding proteins normally found in the seeds are involved in plant defense mechanisms against bruchid (Peumans and Van Damme, 1995). Genes encoding for these proteins are members of the lectin multigene family, the most representative components being arcelins, phyto-hemagglutinins and α-amylase inhibitors (Lioi *et al.*, 2003). In addition, lectins, α-amylase inhibitors and protease inhibitors can retard insect growth and development when ingested, due to suppression of α-amylase activity and serine protease activity in the larval midgut of the weevils (Ussuf *et al.*, 2001). Among these, the α-amylase inhibitors are easily inactivated by cooking. Hence, the introduction of this gene into host plants could be regarded as a safe strategy for the development of transgenic bruchid-resistant crops. The transgenesis of the α-amylase inhibitor (*αAI-1*) gene, obtained from the common bean (*P. vulgaris*), was successfully achieved during the development of bruchid-resistant transgenic in the azuki bean (Ishimoto *et al.*, 1996), peas (Shade *et al.*, 1994), chickpea (Sarmah *et al.*, 2004) and mung bean (Sonia *et al.*, 2007). Interestingly, the transgenic azuki bean has not been damaged by *C. maculatus, C. chinensis* and *C. analis* (Ishimoto *et al.*, 1996). Even though it is an effective approach for the development of bruchid resistance in pulse crops, it has its own drawbacks. The expression of insecticidal proteins at target site may also be harmful for non-target organisms. In a biosafety study, it was seen that rats fed with transgenic peas containing *αAI-1* gene showed a higher faecal and urinary output with lower dry matter digestibility, as compared to control rats (Pusztai *et al.*, 1999). It has also been observed that broiler chickens fed with the same transgenic pea had lower growth, metabolic energy and starch digestibility (Li *et al.*, 2006). Therefore, it is still arguable as to whether the αAI-1 transgenic pulse is suitable for consumption or not.

## Molecular markers linked to bruchid resistance

Several studies were performed for identification of the DNA markers linked to bruchid resistance in different pulse crops. Hereditary analysis was performed in mung bean using F2, BC1F1 and F3 lines derived from V2709 (resistant variety, India) × Zhonglu 1 [susceptible variety, Asian Vegetable Research and Development Centre (AVRDC)] which showed that bruchid resistance of V2709- mung bean variety was

controlled by a single dominant locus (Sun *et al.*, 2008). Bulked segregants analysis (BSA) using 63 RAPD (random amplification of polymorphic DNA) primers and 113 sets of SSR (simple sequence repeat)/STS (sequence-tagged site) primers led to the identification of two markers, *OPC-06* and *STSbr2*, co-localised with the *Br2* locus conferring bruchid resistance in mung bean. Similarly, 10 RAPD markers associated with bruchid resistance genes were also identified through BSA in the F12 generation, derived from the cross, *V. radiata* cv Nm92 × *V. sublabata* cv TC1966 (Chen *et al.*, 2007). Among them, four tightly linked RAPD fragments were cloned and transformed into SCAR (Sequence Characterized Amplified Region) markers. One of the SCAR markers (OPW02a4; 1470 bp) was found to be linked with bruchid susceptibility. Later, Sarkar *et al.* (2011) employed two sets of STS-based markers (*STSbr1* and *STSbr2*) which were reported to be associated with the bruchid resistance in the Australian *V. radiata* var. *sublobata* (*ACC41*). Among them, only *STSbr1* confirmed to be associated with bruchid resistance in Indian *V. radiata* var. *sublobata* accession (*Sub2*) and also in some of the mung bean cultivars with contrasting host response to bruchid infestation. It behaves as a dominant marker among the Indian genotypes and is efficiently used for screening bruchid-resistant genotypes (Sarkar *et al.*, 2011). Two markers (*BMd8* and *BMd26*) were associated with bruchid resistance and low adult insect emergence, which could be very effective for MAS (marker assisted selection) in common bean reported by Blair *et al.* (2010). Chen *et al.* (2002) also identified a novel defensin encoded by mung bean c-DNA (complementary-DNA) having insecticide activity against bruchids, which could also be used as candidate gene for MAS.

## Mapping of QTLs linked to bruchid resistance

QTL analysis for resistance to *C. maculatus* and *C. chinensis* was performed in F2 and BC1F1 population of *Vigna nepalensis* (bruchid-resistant) × *V. angularis* (bruchid susceptible) (Somta *et al.*, 2008). The component traits such as percentage of seed damage and time required for adult emergence were measured as degree of resistance. Seven QTLs were detected from both the population, including five for resistance to *C. chinensis* and two for resistance to *C. maculatus*. A total of eight QTLs were detected. Two QTLs were for percentage of adult emergence, one on each LG2 and LG4, respectively. Similarly, six QTLs were for developmental period, including two QTLs on LG1, three QTLs on LG2 and one QTL on LG10. Very recently, two QTLs (*MB87-COPU11 and RP-COPU06*) for bruchid resistance (*C. chinensis*) have also been detected in mung bean (Hong *et al.*, 2015). Furthermore, in rice bean 11 numbers of QTLs associated with the percentage seed damage, developmental period and adult emergence have been identified (Venkataramana *et al.*, 2016) and found to be distributed all over 10 linkage groups of the molecular map. Schafleitner *et al.* (2016) also identified another major QTL for bruchid resistance in Chromosome 5 of Greengram genetic map based on recombinant inbred population. Two QTLs, Cmrae 1.1 and Cmrae1.2, were identified for percentage adult emergence, on linkage group (LG) 3 and 4 respectively. For developmental period, six QTLs were identified, with two QTLs (Cmrdp1.1 and Cmrdp1.2) on LG 1, three QTLs (Cmrdp1.3, Cmrdp1.4 and Cmrdp1.5) on LG 2 and one QTL (Cmrdp1.6) on LG 10 for *C. maculatus* on *Vigna mungo* var. silvestris (Souframanien *et al.,* 2010).

Mungbean (*Vigna radiata*) accession KPS1 contains VrPGIP2, which encodes a poly galacturonase inhibiting protein (PGIP), is the *Br* locus responsible for resistance to *C. chinensis* and *C. maculatus* mapped on chromosome 5. A single major QTL for C. chinensis (qBrc5.1) and a single major QTL for C. maculatus (qBrm5.1) were detected by ICIM (Kaewwongwal *et al.,* 2017).

### Utilization of GBS Platform for SNP discovery

*Callosobruchus* sp. infect *Vigna* at low levels in the field, multiply during grain storage and can destroy seed stocks in a few months. Resistance against bruchid beetles has been found in wild species *V. radiata* var. sublobata TC1966 and in cultivated greengram line V2802. Bruchid resistance data were obtained from recombinant inbred line populations TC1966 (*V. radiata* var. sublobata) × NM92 (F12) and V2802 (*V. radiata*) × NM94 (F7). More than 6,000 single nucleotide polymorphic markers were generated through genotyping by sequencing (GBS) for each of these populations and were used to map bruchid resistance genes. One highly significant quantitative trait locus (QTL) associated with bruchid resistance was mapped to chromosome 5 on genetic maps of both populations, suggesting that TC1966 and V2802 contain the same resistance locus. Co-segregation of all markers associated with resistance indicated the presence of only one major resistance QTL on chromosome 5, while QTL analysis based on physical map positions of the markers suggested the presence of multiple QTLs on different chromosomes. The diagnostic capacity of the identified molecular markers located in the QTL to correctly predict resistance was up to 100 %. (Schafleitner *et al*, 2016). Sequencing VrPGIP1 and VrPGIP2 in “V2709” revealed new alleles for both VrPGIP1 and VrPGIP2, named VrPGIP1-1 and VrPGIP2-2, respectively. VrPGIP2-2 has one single nucleotide polymorphism (SNP) at position 554 of wild type VrPGIP2. This SNP is a guanine to cystine substitution and causes a proline to arginine change at residue 185 in the VrPGIP2 of “V2709”. VrPGIP1-1 has 43 SNPs compared with wild type and “V2802” and 20 cause amino acid changes in VrPGIP1. One change is threonine to proline at residue 185 in VrPGIP1, which is the same as in VrPGIP2. Sequence alignments of VrPGIP2 and VrPGIP1 from “V2709” with common bean (*Phaseolus vulgaris*) PGIP2 revealed that residue 185 in VrPGIP2 and VrPGIP1 contributes to the secondary structures of proteins that affect interactions between PGIP and polygalacturonase and that some amino acid changes in VrPGIP1 also affect interactions between PGIP and polygalacturonase. Thus, tightly linked VrPGIP1 and VrPGIP2 are the likely genes at the Br locus that confer

bruchid resistance in mungbean "V2709" (Kaewwongwal *et al.,* 2017).

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