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Bruchid Resistance in Pulses: A Review

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ABSTRACT

The pulse crops are crucial for human nutrition not only because of their rich source of proteins but also for their dietary fibres, complex carbohydrates, resistant starch, vitamin complex and minerals. Pulse crops include mungbean, urdbean, redgram, bengalgram, adzuki bean and cowpea which are agriculturally and economically important crops in tropical and subtropical regions. Bruchid beetles, [*Callosobruchus chinensis* (L.)] and [*C. maculatus* (F.)] are the most serious insect pests of *Vigna* crops especially during storage. Infestation starts in the field and carried to storage godowns, where sometimes it causes total destruction of the seeds within six months. The damage is caused by the grubs, which feed on the entire grain contents leaving only the shell behind. The seeds thus loss its viability and they are rendered unfit for human consumption. Use of resistant cultivars is the best way to manage the bruchids. Hence, the resistant accessions can be effectively used as promising donors for developing bruchid resistant varieties which would ensure food security by reducing postharvest losses under storage conditions.

Key words: Bruchids, Genetics, Pulses, Resistance, Screening methods.

Pulses are rich source of protein (20 to 25%) and have ability to fix atmospheric nitrogen in the soil (30-150 kg ha^{-1}). Since more than 80% of the pulses area is under stressed rainfed environment, the quality seed of improved varieties has emerged as the most vital input for enhancing pulses production in India. Further, insect-pests, diseases and weeds are the major factors which are limiting the pulses productivity.

Despite the importance of food security, stored pulses suffer damage by insects with bruchid being the most important post-harvest insect pest as reported in the tropics (Ndong *et al.*, 2012). Bruchids or seed beetles or seed weevils (order: Coleoptera; family: Chysomelidae; subfamily: Bruchinae; formerly family: Bruchidae) are major insect pests of stored legume seeds. Out of about 1300 species of seed beetle in the family Bruchidae, 20 are recognized as pests usually in stored legume seeds (Talekar, 1988). Bruchids attack almost all edible legumes, including mungbean, urdbean, cowpea, pigeon pea and lentil and are cosmopolitan in distribution encompassing Australia and Oceania, Europe, Asia, Africa and America (Rees, 2004). Bruchids infestation results in weight loss, low germination and nutritional changes in seeds, thereby reducing the market value, rendering it unfit for human consumption, agricultural and commercial uses (Talekar, 1988; Rees, 2004; Oke and Akintunde, 2013; Duan *et al.*, 2014).

Although chemical control and integrated pest management (IPM) remain the most effective means of controlling bruchids in large scale storage, use of resistant sources is a reliable, eco-friendly and prerequisite for sustainable grain protection (Sarwar and Tofique, 2006). To reduce the seed loss due to bruchids in pulses, it is essential to determine the source of resistance so that resistant factors can be utilized in breeding programme.

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Lifecycle and ecology of bruchids

The life cycle and ecology of both *C. maculatus* and *C. chinensis* are similar. The initial infestation originates in the field. The eggs are strongly attached to pods and seeds. In the field, eggs are laid on pods and in storage directly on the seeds. Bruchids lay 1-3 eggs/seed and greater seed size accommodates still more number of eggs. Yellow coloured seeds are preferred than green or black coloured seeds for oviposition and bruchid development. Hatching of the eggs takes place after 6 days of oviposition (Devi and Devi, 2014). The larvae/grubs penetrate into the seed and develop inside the seed by feeding the grain contents. Larvae excavate an emergence tunnel to the seed surface, forming a translucent window in the seed coat for the adult emergence. The adult *C. maculatus* males and females both have an average life span of seven days under laboratory conditions and only a few can survive more than two weeks (Southgate, 1979 and Fatima *et al.*, 2016). The oviposition by *C. maculatus* reaches a peak within two days after the commencement of oviposition and declines over time. Bruchids do not feed during the brief adult stage and reproduction depends mainly on the resources. The insect

accesses during larval stages from a single seed, with severe competition from other larvae (Guedes *et al.*, 2007). To avoid fierce larval competition in future which may occur even inside the seeds, allowing only the emergence of larvae with good fitness females lay a large number of eggs, depending on the substrate quality that the female perceives. A good quality substrate will lead to good larval fitness and a successful population build-up. Female bruchids have the ability to check the host suitability, deposit proper egg load and discriminate the eggs laid by other females on a seed (Guedes and Yack, 2016). The females use various tactile, chemical and physical cues to choose suitable egg-laying substrate. These include multiple sensory modalities, egg-marking pheromone and larval feeding vibrations from the seed. However, the chemical cues last only for a few weeks and are influenced by environmental factors. The vibration cues and egg-marking pheromone play an important role in reducing egg density on a seed and in turn, minimizing larval competition.

Seed loss caused by bruchids

The insect females deposit their eggs on seed coat and embryogenesis is completed after 3 to 5 days (Beck and Blumer, 2011). After hatching, the larvae penetrate the cotyledons where they develop by consuming the energy reserves of cotyledons, reducing both the quantity and quality of seeds, making them unfit for planting, marketing and human consumption (Ali *et al.*, 2004). Adult emergence occurs after 25-30 days (Oliveria *et al.*, 2009). Due to the bruchids high fertility, ability to re-infest and short generation times, even low initial infestation rates can lead to tremendous damage (Yamane, 2013). A single beetle is able to cause 3.5% weight loss in cowpea seeds (Tembo *et al.*, 2016). The proportion of loss caused by this pest is 25-30% in the field and 80% in the store within 6-8 months (Hill, 1990). The loss in quality is due to contamination with insect exudates, eggs, dead insects and holes, conversion of seed contents. However, the losses due to bruchids vary from crop to crop (Kananji, 2007; Swella and Mushobozy, 2007).

Screening methods for bruchid resistance

Freshly emerged insects are used for screening the pulses genotypes for identification of resistance response against bruchids infestation. The culture of bruchid beetles is normally maintained in plastic jars of one liter capacity containing fresh mungbean seeds. The rim of the plastic jar is covered with muslin cloth and fastened tightly with a rubber band. The beetles of stock culture are released in the plastic jar for mass multiplication at 30±2°C and 70±5% relative humidity. The emergence of new insects is expected after 25 days from the date of beetle release depending on temperature, humidity and insect species. Sub-culturing of this beetle is done at regular intervals so as to maintain a continuous supply of insects for experiments throughout the period of study.

Bioassay for bruchid resistance

There are two screening methods:
1. Free choice test.
2. No choice test.

Free choice test

Free choice test is the preliminary screening method and is normally carried out unreplicated. Pulses genotypes are screened by this method for assessing comparative resistance to bruchids under laboratory condition (30±2°C and 70±5% RH). In free choice test, 50 seeds of each accession are kept in a separate petridishes (4.5 cm diameter and 1 cm depth) and are kept open in a common cage. After arranging the petridishes having seeds inside the cage, 100 pairs of freshly emerged adults are to be released at the centre of the cage and covered with muslin cloth. These insects are allowed to remain there for the purpose of oviposition for three days. Three days after the release of adult beetles, seeds with eggs will be recorded and each petridishes are covered with lid. Observations on per cent survival of adults, mean developmental period and the susceptibility index are recorded as described by Howe (1971).

No choice test

The pulses genotypes having resistant response identified in the preliminary screening under 'free choice test' are alone selected for further confirmation of resistance under 'no choice test'. In this test, twenty five seeds of each entry are kept separately in a plastic container (6 cm × 4 cm, height × diameter) and air circulation is provided through a 1 cm diameter hole in the lid, that is covered with nylon mesh. Male and female bruchids are sexed morphologically as described by Raina (1970). Three pairs of well characterized and freshly emerged male and female adults of bruchid are released in the plastic container and allowed for oviposition. The insects are allowed to remain there for the purpose of oviposition for three days and subsequently the insects are removed. Three days after the release of adult beetles, observations are recorded on the number of eggs laid. Infested seeds are kept under laboratory conditions at 30±2°C and 70±5% RH. The number of emerged adults and damaged seeds are counted daily. The observations are continued for next 40 days (Duraimurugan *et al.*, 2014).

Genetics of bruchid resistance

Knowledge on the genetics of bruchid resistance is necessary to understand the mechanisms of gene action controlling the resistance to bruchid infestation among the available pulses genotypes. Breeding to combine seed and pod resistance has been explored to reduce losses associated with bruchid infestation in pulses. Understanding the genetic basis of bruchid resistance in pulses is crucial for designing suitable breeding strategies for the generation of bruchid-resistant cultivars in pulses.

The genetic control of resistance to bruchids may be either monogenic / oligogenic or polygenic in nature and is further influenced by four kinds of gene actions namely additive, dominance, epistatic and cytoplasmic (Aslam *et al.*, 2006). In mungbean, resistance to the bruchids, *C. chinensis* and *C. maculatus*, is conferred by an oligogene (Somta *et al.*, 2007). In addition, Kaga and Ishimoto (1998) observed that bruchid resistance in *V. radiata* var. *sublobata* (TC1966) was also controlled by single dominant gene. In contrast to these findings, Somta *et al.* (2007) reported that bruchid resistance in the mungbean is controlled by a major gene, with varying degree of expressivity. A polygenic pattern of inheritance has also been reported in some of the pulse crops, including rice bean, mungbean, urdbean and adzuki bean. In many crop plants, polygenic host resistance showed greater durability and stability than those of monogenic control across the environments, because of their higher endurance to the effects of genetic changes in insects as well as climate change (Panigrahi *et al.*, 2013).

Genetics of bruchids resistance in urdbean

Urdbean is one of the most important food legume crop cultivated in India. It constitutes the major source of dietary protein for majority of the Indians. The seeds are stored by farmers, traders and millers for consumption, sowing and processing. [*Callosobruchus maculatus* (F.)] (Coleoptera: Bruchidae), a storage pest, causes severe infestation from the field itself. It can cause considerable damage in urdbean both in terms of quality and quantity. The traits such as reduced oviposition, less seed damage, low adult emergence and prolonged developmental period for the development of pulse bruchid are attributed to the tolerance in wild species of urdbean (Soundararajan *et al.*, 2013).

Studies on genetics and breeding for bruchid resistance in urdbean 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* was identified in cultivated urdbean, the genetics of the resistance could not be determined. However, inheritance of the resistance in wild urdbean revealed that the resistance was governed by duplicated gene with dominant gene action (Dongre *et al.*, 1996). Localization of the resistance gene (s) on genome map is in progress. There has been no report on development of bruchid resistance in urdbean so far. Although urdbean is closely related to mungbean, transferring the resistance from urdbean into mungbean may be achieved only by genetic engineering due to a strong genetic barrier between the two species.

Biochemical in urdbean seeds is responsible for resistance to *C. chinensis* (Talekar and Lin, 1992). A proteinaceous factor, novel 40-kDa peptide isolated from urdbean 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 urdbean has not yet been determined. Since wild urdbean is immune to several important bruchid species, the resistance factor (s) is worth to be identified.

Mechanism of bruchid resistance in cowpea

It has been reported a high anti-nutrient levels, mainly antitryptic and antiamylasic activity in bruchid resistant cowpea lines (Piergiovanni *et al.*, 1994). The results of the experiment showed that a high activity of a single inhibitor class (porcine amylase, bacillus amylase, bovine chymotrypsin and trypsin) was typical of the bruchid susceptible lines, leading to the conclusion that breeding for high protein inhibitor content could be an effective way of obtaining cowpea lines improved for the naturally resistance to storage pest attack. It has been reported that seeds of the common bean are resistant to Adzuki bean weevil largely because of the presence of α-amylase inhibitor (αAI-1) and seed protein that is toxic to the larvae. To control αAI-1 tolerant bruchid species such as *Acanthoscelides obtectus* and to avoid the development of resistance to αAI-1, varieties carrying this transgene should be protected with additional control measures in order to strengthen the crops resistant to bruchid species (Shade *et al.*, 1996). The compounds in the seed coat (tannins, flavonoids, total phenolic content and anti oxidant activity) and in the cotyledons (carbohydrates, proteins and α-amylase inhibitory activity) conferred resistance to bruchid infestation (Miesho *et al.*, 2017). Most of the improved varieties are obtained by crossing bruchid resistance sources with those susceptible but with desirable characteristics to spread bruchid-resistance within other varieties.

Several bruchid-resistance cowpea lines have been developed using resistance genes from TVu-2027 and the varieties have been released to farmers in many countries. TVu-2027 is the single source of bruchid resistance and hence there are reasons to believe that bruchid could rapidly evolve to break the resistance. According to Shade *et al.* (1996), after selection on resistant cowpea seeds for over 53 generations, *C. maculatus* was able to develop a new biotype to overcome resistance in Tvu-2027. Therefore, for durable insect-resistance, new sources of resistance are necessary for developing multiple resistance lines.

Sources of bruchid resistance

Breeding progress depends upon various factors, including the magnitude of genetic variability among the germplasms, heritability of the desired traits and the selection pressure exerted (Falconer, 1989). Thus, higher the accuracy in selecting the parents for a given trait, higher would be the genetic gain to be expected. Various genotypes of different pulses have been evaluated to obtain sources of host resistance to bruchids infestation. It was found that popular cultivars with improved yield (cultivated type) are more prone to these pests than the landraces and/or wild species (Lale and Kolo, 1998). 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* (Souframanien *et al.*, 2010). Resistance to the cowpea bruchid, *C. maculatus* has also been recorded in cultivated cowpea. Although genes conferring complete resistance to these pests have rarely been seen in cultivated

species (Acosta-Gallegos *et al*., 2007), few cultivars of some pulses including cowpea, urdbean, field pea, chickpea and pigeon pea have some trait(s) that provide complete resistance to bruchid pests. The genetic sources for resistance against bruchid infestation in pulse crops have been studied as follow.

Source of bruchid resistance in mungbean

Only a few sources of mungbean are resistant to bruchids have been found. Initially, TC 1966 a wild mungbean collected in Madagascar, showed complete resistance to *C. maculates* and *C. chinensis* (Fuji and Miyazaki, 1987; Fuji *et al*., 1989). The World Vegetable Center screened about 100 mungbean accessions against *C. chinensis* and all possessed resistance against the pest. In these accessions, only 10-20% seeds had bruchid eggs and the seeds are rough textured with hard testa (AVRDC, 1990a). F_2 dervied from crosses between bruchid resistance mungbean TC 1966 and high yielding but susceptible mungbean breeding lines showed moderately resistance to bruchids and genetically controlled (AVRDC, 1990b). The resistance in TC1966 was controlled by a single dominant gene (Kitamura *et al*., 1988; Fuji *et al*., 1989). Later, several resistant lines were developed successfully using TC1966 as resistant donor (Tomooka *et al*., 1992; Watanasit and Pichitporn, 1996). However, wild species have been reported to have harmful anti-nutrients for humans and could possess unwanted wild characters due to linkage drag. For an example, mice fed with artificial diets containing bruchid resistant lines having wild mungbean, TC1966 as the resistance source showed unwanted changes in blood biochemical value (Miura *et al*., 1996). Pod shattering has also been reported in a commercial variety developed from TC1966 (Watanasit and Pichitporn, 1996). Then the World Vegetable Center identified two mungbean lines, V2709 and V2803 with complete resistance to bruchids and these lines were used to transfer the resistance to other cultivars (Talekar and Lin, 1981, 1992; AVRDC, 1991). Out of 500 mungbean accessions screened to *C. chinensis* two entries V2802 B-G and V1128 B-BL were free from bruchid infestation. V2802B-G was a selection from the resistance accession, V2802 (AVRDC, 1990b). Line V2709 has been used as a bruchid resistant source in Korea to develop bruchid resistant variety, Jangan (Hong *et al*., 2015) and in China to develop bruchid resistant lines such as Zhonglv3, Zhonglv4 and Zhonglv6 (Yao *et al*., 2015). Two cultivated lines, V1128 and V2817 also showed to have resistance to *C. maculatus* (Somta *et al*., 2008). Sarkar *et al.* (2011) reported the existence of bruchid resistance in IC 333175, IC 325770, IC 329030, Dantan Sonamung, RS4, RMG11 and Khargonel and their use as the source to develop bruchid resistance in mungbean. Presently, mungbean breeders and entomologists have only few mungbean accessions, which include TC 1966, ACC 41, V2709, V 2802, V 1128, V 2817, ACC 23 and Indian *sublobata* (sub2) as sources of resistance against bruchids (Somta *et al*., 2007, 2008; Mei *et al*., 2009; Sarkar *et al*., 2011).

Sources of bruchid resistance in urdbean

Urdbean 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* was identified in cultivated urdbean, but wild urdbean (*V. mungo* var. *silvestris*) was shown to be completely resistant to *C. maculatus* and other bruchid species such as *C. chinensis, C. analis, C. phaseoli* and *Z. subfasciatus* (Kashiwaba *et al*., 2003). However, incomplete resistance to bruchid was also reported in wild urdbean (Dongre *et al*., 1996). Urdbean is considered to be among the most resistance species. Based on the index of susceptibility, three accessions *viz*., UH 82-5, IC 8219 and SPS 143 (susceptibility index of 0.055, 0.057 and 0.058, respectively) were found to be moderately resistant (Duraimurugan *et al.,* 2014).

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 bruchids and it prevails in Africa where the cowpea has been 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. maculates* and only 3 landraces *viz*., TVu11952, Tvu11953 and Tvu2027 were found to have resistance against bruchid infestation (Singh *et al*., 1982). However, these 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* (Singh and Ng, 1990). The result of the screening 50 cowpea resistant genotypes to *C. maculatus* (De Castro *et al*., 2013) showed that seven cowpea genotypes *viz*., IT85 F-2687, MN05-841 B-49, MNC99-508-1, MNC99-510-8, TVu 1593, Canapuzinho-1-2 and Sanzi Sambili exhibited non-preference-type of resistance for the oviposition and feeding of *C. maculatus*.

Biochemical and molecular aspects of bruchid resistance

Considering the harmful effect of insecticides, it is necessary to develop resistant genotypes in order to reduce damage caused by bruchid beetles. Dick and Credland (1984) observed that oviposition preference is influenced by host availability to a greater extent and has nothing to do with the actual resistance nature of an accession. The reduction in per cent adult emergence in urdbean is an indication of the presence of unfavourable chemical constituents inside the cotyledons. Further, such unfavourable chemicals present inside a seed directly affect the development of a

growing grub resulting in prolongation of developmental period (Dongre *et al.,* 1996).

Biochemical studies on pulses confirmed that lectins, the sugar-binding proteins normally found in the seeds are involved in plant defence mechanisms against bruchids (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). The transgenesis of the α-amylase inhibitor gene, obtained from the common bean (*P. vulgaris*), was successfully achieved during the development of bruchid-resistant transgenic in the adzuki bean (*V. angularis*), pea (*P. sativum*), chickpea (*C. arietinum*) and mungbean (*V. radiata*).

CONCLUSION

All economically important *Vigna* crops are susceptible to bruchids. Sources of resistance in *Vigna* crops are rare, while wild *Vigna* shows wider arrays of resistance. Genetics of the bruchid resistance can be either simple or complex. There appear constraints in using wild *Vigna* as resistance sources because gene exchange between wild and cultivated genotypes is difficult due to genetic barriers. More importantly, defence 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 adzuki bean, but its application is limited to the crops that basic technology related to genetic engineering is well established. In storage, the damage is essentially caused by *C. maculatus* which is the main postharvest pest of legumes. Control strategies such as biological, chemical, cultural and mechanical methods, may not adequately address the problem of bruchid damage.

Consequently, genetic improvement of pulse varieties for bruchid resistance is the most cost effective and long-term measure to limit the damage of this pest. However, the genetic development of varieties for bruchid resistance might not be achieved due to linkage drag, biotype variation, lack of inter specific compatibility and narrow genetic base in the gene pools. Moreover, the sources of resistance are very few among the large cultivated varieties, while wild species of some cultivars are known to have multiple resistance mechanisms against bruchid. Successful development of bruchid-resistant cultivars will result in reduced usage of chemical pesticides and increased activity of natural bio control agents and thus sustained yield by culminating losses in storage. The use of host-plant-resistant cultivars will form the backbone of integrated bruchid pest management in the future. However, many resistance-related issues, such as linkage drag and durability-resistant alleles in the context of insect adaptation have to be unravelled. Thus, combined use of inexpensive cultural practices and physical treatments and phyto-inhibitors along with host-plant resistance during storage should be the strategy of IPM to reduce the losses due to bruchids.

Conflict of interest

All authors declared that there is no conflict of interest.

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