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Bruchid resistance in mung bean: An overview

S. MAHATO[1], M. JENA[2], P. MOHANTY[2], C. BEHERA[2], N. SENAPATI[2], G. B. DASH[2], P. K. NAYAK[2], D. LENKA[2] AND SWAPAN K. TRIPATHY[3]*

[1]Department of Entomology, College of Agriculture, OUAT, Bhubaneswar (India)
[2]Department of Agricultural Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar (India)
[3]Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar(India)

**swapankumartripathy@gmail.com*

ABSTRACT

Bruchid is a major threat to mung bean in storage. No cultivated variety of mung bean is free from bruchid attack. However, a wild form of mung bean (TC 1966) and few pure line selections (V2709BG and V2802BG) of Indian origin land races are completely immune to both *Callosobruchus chinensis* and *C. maculatus*. Bruchid resistance is controlled by a single dominant gene (Br) and it is mapped on chromosome 8. Conventional back cross breeding coupled with molecular marker aided selection can accelerate the breeding process for bruchid resistance. A number of toxic antimetabolic compounds (secondary metabolites, antinutritional seed proteins, protein inhibitors, enzyme inhibitors etc) have insecticidal activity in different food legumes. Among these, a gene from common bean encoding alpha amylase inhibitor-1(á AI-1) has been largely attempted for genetic transformation in mung bean. Mung bean is recalcitrant to *in vitro* culture process. Therefore, efficient reproducible plant regeneration protocol and novel technique for transfer of the resistance gene(s) driven by a suitable seed specific promoter is needed for successful genetic transformation. But, its potential for acceptance by farmers relies on clarification of safety for human consumption.

Key words: Bruchid, mung bean, source of resistance, inheritance, breeding strategy

INTRODUCTION

India accounts for about 45% of total world production of mung bean [*Vigna radiata* (L.) wilczek] 2n=22. It is the third largest pulse crop in the country after bengal gram and red gram. On dry weight basis, mung bean seeds possess high degree of digestibility and contain 17.2-29.9% protein (average 22.83%), 1-1.5% fat, 3.5-4.5% fibre, 4.5-5.5% ash and 60-65% carbohydrate. The seed protein is rich in lysine but deficient in methionine, cystine and cysteine. Besides, the seeds harbour higher amount of vit.-B, potassium, iron, phosphorus and calcium. It bears traces of antinutritional factors without any health hazards as compared to other grain legumes. A sizeable proportion of the produce is damaged due to bruchids in storage. The loss may be as high as 50% by *Callosobruchus chinensis* (Damte and Dawd, 2006) or even can reach up to 100% (Somta *et al*., 2006) in cultivated varieties. Fumigation or dusting with insecticides is the common practice to control bruchids. Besides, use of a variety of plant extracts, biocides (Koona and Dom, 2005), parasitoids (Ngamo *et al*. 2007) and plant resistance factors (Ignacimuthu *et al*. 2000) can be eco-

friendly and cost effective. However, exploring and use of host plant resistance to bruchids could be a viable option. None of the available cultivated varieties has resistance to bruchids. In course of evolution of pulse crops, most of the valuable genes have been eroded owing to their continuous minimal cultivation under rainfed situation in marginal and sub-marginal lands with poor fertility condition. This loss of genetic diversity in the crop has also been accompanied by further loss of genes found in wild relatives and progenitors of the crop species. Breeders often resort to trap the genetic variability from allied species through wide hybridization or create novel plant types through mutagenesis and genetic transformation. In this pursuit, we present the source and mechanism of host resistance, mode of inheritance and genetic improvement of mung bean for bruchid resistance.

SOURCE AND MECHANISM OF RESISTANCE

Almost all legumes except rice bean (*Vigna umbellata*) are prone to bruchid attack. However, an accession TC 1966 of wild mung bean (*V. radiata* var. *sublobata*) is reported to be immune to *Callosobruchus chinensis, C. maculatus*, *C. analis, C. phaseoli* and *Z. subfasciatus* (Fujii and Miyazaki 1987, Kashiwaba *et al.,* 2003). While, a few Australian wild mung bean accessions ACC23 and ACC41 are resistant to *C. chinensis, C. maculatus* and *C. phaseoli*. Further, pure line selection of moderately bruchid resistant mung bean landraces (V2709 and V2802) led to development of cv. V2709BG and V2802BG which are completely immune to both *C. chinensis* and *C. maculatus* (Somta *et al.,* 2007). Besides, a mung bean accession V2817 out of thousands of *Vigna* accessions, is also found immune to *C. chinensis* and *C. maculatus* (Somta *et al*., 2007). In addition, four mung bean accessions (LM 131, V 1123, LM 371 and STY 2633) have been reported to be moderately resistant to pulse beetle based on percentage of survival.,

Host plant resistance to bruchids involved either antibiosis, antixenosis (non- preference) and/ or tolerance. The resistance mechanism may involve morphological adaptations as direct defense and also physiological and/or biochemical mechanisms affecting the insects' cellular processes, growth and development (Edwards and Singh 2006). Legumes adapts a number of defense systems e.g., distinct pod and seed characteristics as morphological barriers and inherent production of secondary metabolites and anti-nutritional compounds to cause anti-metabolic activity in bruchids (Panda and Khush 1995) leading to death (Singh 2002).

Large and dull seeds are more preferred for oviposition than small and shiny seeds in mung bean. Besides, least insect infestation is associated with hard seed coat in few varieties against *Callosobruchus analis* in mung bean (Deeba *et al*., 2006) as compared to soft and thin seed coat varieties (Shaheen *et al.,* 2006). Some of the legume seeds are a rich source of toxic secondary metabolites (e.g, lignins, tannins, quinines, alkaloids, phytate, saponins, cysteine rich protein, non-protein amino acids and polysaccharides) and antinutritional seed proteins (lectins, arcelins, vicilin, phaseolin, phytohaemaglutinins (PHA), trypsin inhibitors, enzyme inhibitors), cyanogenic glycosides, and phytic acid that accumulate in the cotyledons of seeds during seed maturation (Singh 2002; Lattanzio *et al*. 2005). Saponin, phytic acid and trypsin inhibitor (TI) activity increased with increased storage period of seed (Modgil and Mehta 1997). Each such factor is conditioned by different single genes (Somta *et al*. 2006). Talekar and Lin (1992) reported resistance to *C. chinensis* in cultivated mung bean accessions V2709 and V2802 is offered by secondary metabolites causing antibiosis in the cotyledons.

A group of carbohydrate-binding storage proteins (lectins) are known to reduce fecundity of insects and have proteolytic activity in the insects mid gut leading to block nutrient assimilation. A variety of isoforms of lectin (isolectins) do exist which differs in distinct carbohydrate specificity, charge, mobility on polyacrylamide gel and biological property (Correia *et al*., 2008). Resistance mechanism against *Callosobruchus* spp. due to á-amylase inhibitor in common bean (*P. vulgaris* L.) and trypsin inhibitor (CpTI) in cowpea (*Vigna*

unguiculata) have been demonstrated (Edwards and Singh 2006). á-amylase and protease inhibitors seriously impair carbohydrate and protein metabolism due to inactivation of the respective digestive enzymes in the insect gut cells. Vignatic acid A (an alkaloid) isolated from isogenic lines carrying bruchid resistance gene of wild mung bean TC1966 is reported to be resistant to *C. chinensis*(Sugawara *et al*. 1996). Besides, a peptide compound "GIF-5" toxic to the bruchids has been identified from the similar source material (Kaga *et al*., 2000). A cysteine rich protein (VrCRP) has been isolated from seed coat of bruchid resistant wild mung bean genotype TC 1966 which has insecticidal (lethal to *C. chinensis*), fungicidal and bactericidal activity. Mung bean seeds containing 0.2% VrCRP completely inhibit the development of bruchid larvae. Isolation and transgenic transfer of such plant origin multifunctional VrCRP gene to popular high yielding food legume genotypes can confer bruchid resistance (Chen *et al*. 2002).

Seeds of *Cajanus albicans* and *Vigna bourneae* are reported to have bruchid resistance due to higher levels of trypsin/chymotrypsin inhibitors (Ignacimuthu *et al*. 2000). Highest trypsin inhibitor and chymotrypsin inhibitor activities have been reported in cowpea genotype GC82-7, whereas highest proteinase inhibitor and á-amylase inhibitor activities are inherent to cv. TV 7(Marconi *et al*. 1997). Besides, SDS-PAGE analysis of seed proteins of *Dolichos lablab* was shown to have high level of vicilins, lectins and á-amylase inhibitors that offered resistance to *C. maculatus*. In addition, protease inhibitors as well as phytic acids are abundant in tepary bean (*Phaseolus acutifolius*) seeds (Campos *et al*. 2004). Wild *Lablab purpureus* seeds contain arcelin- a natural insecticidal molecule. A 33kDa arcelin protein resisting to bruchids has been identified in wild *Phaseolus vulgaris* (Osborn *et al*. 1988). Gene specific primers synthesized from arcelin mRNA sequence (Yamada *et al*. 2005) can be used to identify genotypes with high arcelin content (Sakthivelkumar *et al*. 2014). Besides, flavonoids isolated from rice bean seeds have inhibitory effects against growth and development of *C. chinensis* and *C. maculatus* (Somta *et al*. 2006). Soybean seeds are resistant to *Callosobruchus* species due to presence of anti-nutritional factors (phenols and 4-5 times more trypsin inhibitors) than cowpea and chickpea. Thus, host plants can develop inherent nutritional, physiological and ecological hurdles on the insects (Acosta-Gallegos *et al*. 2008).

INHERITANCE AND BREEDING STRATEGY

Understanding the genetics of bruchid resistance and its mode of inheritance is necessary in order to develop an efficient and effective breeding programme. The genetic control of resistance may be monogenic and oligogenic for insects like *C. chinensis* and *C. maculatus* in mung bean (Chen *et al*. 2007, Somta *et al.* 2007). Bruchid resistance in the erstwhile mentioned wild mung bean TC 1966 is controlled by a single dominant gene, Br (Fujii *et al*. 1989). Besides, both the Indian origin resistant purelines V2709BG and V2802BG derived from local selection harbour the same dominant gene (Br) with modifier effects (Somta *et al.,* 2007). This was confirmed by the resistance pattern in F_1, F_2 and BC generations derived from crosses of above resistant accessions with a popular Thailand bruchid susceptible mung bean cultivar KPS 1. Recently, Hong *et al*. (2015) studied inheritance pattern of bruchid resistance using Jangan mung bean and a wild relative, TC 1966 as resistance source (R) and cv. Sunhwa as the susceptible parent (S). Resistance reaction in F_1 and F_2 individuals from two crosses (S × R and R × R combinations) was confirmed to be due to a single dominant gene.

Bruchid resistance gene (Br) screened for *C. chinensis* was mapped to linkage group 8 in mung bean wild accession TC 1966 using small F_2 mapping population (58 plants) of cross VC 3890 x TC 1966 and the Br gene was reported to be flanked by RFLP markers A882 and R26 at 3.6cM from the former and 6.5cM from the latter respectively (Young *et al*.,1992). Unfortunately, these markers are not found satisfactory when applied at the advanced mung bean generation. Further, a more tightly linked RFLP marker Bng143 was identified at a distance of just 0.2cm from Br

gene (Kaga and Ishimoto, 1998). Such a marker was also shown to be co-segregating with the gene for Vignaic acid A. Besides, screening of genomic DNA library made it possible to identify a BAC contig covering Br genomic region in chromosome 8 (Miyagi *et al.* 2004). Miyagi *et al*. (2004) mapped a RFLP marker mgM213 on LG8 which was just 1.3cm away from the major locus conferring resistance to *C. chinensis*. STS (Sequence Tagged Site) markers (STSbr1 and STSbr2) co-segregating with this locus have been also reported by the same authors. The resistance genes in TC 1966 and ACC 41 are most probably 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). Kaga and Ishimoto (1998) mapped the bruchid resistance gene to chromosome 8 using 414 NILs derived from BC20F2 involving TC 1966. Lambridge *et al*.,(2000) and Miyagi *et al*. (2004) also confirmed that the Br gene is located on chromosome 8 using RAPD and SSR markers respectively in 67 RILs derived from a cross Berken x Acc 41.

Mung bean breeders face formidable challenges in developing new cultivars with bruchid resistance. The wild mung bean accession TC 1966 was by and large used as resistance source for mung bean breeding against bruchid infestation. But unfortunately, the resulting resistant lines suffer a serious setback due to linkage drag of unfavourable traits like pod dehiscence and indeterminant growth as compared to TC 1966 (Watanasit and Pichitporn 1996). Besides, TC 1966 is reported to be nutritionally poor due to higher level of glutamic-oxalacetic transaminase activity making it not safe for human consumption (Miura *et al.* 1996). Although resistance gene in TC1966 has been used to develop mung bean resistant lines (Tomooka *et al*. 1992), no commercial resistance variety is being released to farmers. This is mainly due to uncertainty on safety of the resistance seeds for human consumption. Besides, inter-specific cross-incompatibility pose problem for transfer of resistance gene (available in wild species) to cultivated varieties. Therefore, many researchers used the erstwhile mentioned mung bean accessions i.e., V2709BG and V2802BG as alternative resistance sources for breeding programme. Conventional breeding coupled with molecular marker aided selection can accelerate the breeding process.

Bruchid resistance is a complex trait owing to diversity of *Callosobruchus* species and varied mechanisms of host plant resistance. The á AI-1 gene from common bean (*Phaseolus vulgaris*) has been considered to be a strong candidate gene to confer resistance to several *Callosobruchus* spp. (Chrispeel *et al*. 1998). This was successfully integrated into the mung bean genome using Agrobacterium mediated genetic transformation (Sonia *et al*. 2007). Besides, a number of blocks of genes (QTLs) are associated for resistance to bruchids. Young *et al.* (1992) mapped the QTL for bruchid resistance in Chromosome 8 with LOD value 15.3 which explains 87.5% of total phenotypic variation for the trait. Fatokun (2002) identified four QTLs associated with the resistance to bruchids in cowpea. Among these, a major QTL accounted for up to 76% of the variation in the trait. Conversion of well-adapted high yielding varieties to bruchid resistant genotype using marker aided back cross breeding may pave the way for success.

CONCLUSION

Mung bean is a highly preferred legume crop owing to its shorter maturity duration, high protein content with traces of antinutritional factors and high degree of digestibility as compared to other food legumes. It can enrich soil fertility by atmospheric nitrogen fixation. But, a significant proportion of seeds in storage is liable to bruchid damage. Genetic and biochemical mechanisms, mode of inheritance and genomic analysis underlying bruchid resistance have been established. But, no significant achievement for development of bruchid resistant varieties has been made so far owing to extremely narrow and shrinking genetic base in the available primary gene pool. Wild forms of mung bean and a few allied species of *Vigna* retain the resistance mechanism, but these are not aminable to harness bruchid resistance due to linkage drag and cross

incompatibility. Transfer of the bruchid resistance gene (á AI-1) to mung bean from allied spp. (*Phaseolus vulgaris*) has been attempted. But, it poses a serious setback in terms of clarification of safety standards for human consumption.

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