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Advances in Molecular Breeding for Bruchid (*Callosobruchus* spp.) Resistance in Mungbean [*Vigna radiata* (L.) Wilczek]: A Review

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ABSTRACT

Mungbean is one of the most important grain legumes with high-quality dietary protein in India as well in many other Asian countries. But the crop is severely affected by bruchids (*Callosobruchus* spp.) from field to storage condition. These storage pests not only affect the yield but also reduce the market value and quality of the crops. The chemical method of bruchid management is not economical and environmentally unsafe. So, the host-plant resistance to bruchids would be the best alternative and most sustainable way to control the bruchid. A very limited number of wild accessions and cultivated genotypes are available for the transfer of bruchid resistance gene through conventional breeding. Thus, insights into the molecular mechanism of resistance will help to find out the resistance genes/QTLs easily with the support of available genome sequence and that can be introgressed to the cultivated varieties through marker-assisted backcross breeding (MABB) approach. Therefore, in this review, we focused on QTL mapping, identification of novel QTLs, marker assisted-selection, genomics and transcriptomics study by using advanced molecular tools which will be very helpful for genomic-assisted breeding in mungbean for bruchid resistance.

Key words: Bruchid, Genomics, Marker, Mungbean, QTLs, Transcriptomics.

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the most important legume crops in many Asian countries including India and occupies the third position after chickpea and pigeon pea with respect to area and production. It is a cheap source of dietary protein (24-25%) and carbohydrate (56%) for human consumption (Anonymous, 2016). It plays an important role in sustainable agriculture by increasing the soil fertility through biological N-fixation. The production of mungbean is severely affected by the infestation of pulse beetles (Bruchid; Coleoptera, Bruchidae). Among the bruchids, *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.) cause heavy loss both in the field as well as in the storage (Bharati *et al*., 2017; Majhi and Mogali, 2020). The initial infestation originates in the field, where the adult beetles lay eggs on green pods and the larva bore through the pod and feed on the developing seed accounting for only 1-2% of damage. When the seeds are stored the insects continue to feed, emerge into adults and cause secondary infestation, which results in the total destruction of seeds within 3-4 months (Bharati *et al*., 2017; Sanhita *et al*., 2019).

Several methods are used for bruchid control such as storage under low temperature, solar irradiation of the grains, hermetic storage, use of biocontrol agents, use of botanical extracts and chemical treatment with methyl bromide, carbon disulfide, aluminum phosphide or other substances. Chemical control is effective but increases storage costs, harmful to humans and other animals and hazardous to the environment (Gbaye *et al*., 2011). Therefore, host-plant resistance to bruchids would be the most sustainable way to control the pest. Bruchid resistance in legumes depends on morphological barriers and secondary metabolites (amino acids, proteins or enzymes) and other toxic compounds interfering with growth, development or reproduction

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(Edwards and Singh, 2006). Although some sources of resistance have been identified, they are modified at the gene pool level for commercial cultivar release to develop bruchid-resistant mungbean. However, along with the desirable gene, undesired characters may be pronounced in the insect-resistant cultivar (War *et al*., 2017). To incorporate host-plant resistance in the breeding programs, it needs proper understanding about the lifecycle of bruchid, physical, biochemical and molecular basis of the resistance mechanism. Earlier it was noticed that only hybridization was conducted among the resistant and susceptible lines to transfer the resistant genes. But now due to the advancement of biotechnology, several innovative tools have been developed to study the molecular basis of resistance at the genomics and proteomics level to identify the candidate genes. Studies on bruchid-resistance in relation to the development of molecular markers have gained high thrust by minimizing the dependence on phenotypic data (Chen *et al*., 2007). The molecular markers for bruchid-resistance have increased the selection efficiency and concurrently reduce the number of selection tests as well as the cost required for screening (Schafleitner *et al*., 2016). A reliable genome size for achieving the correct coverage and estimating the percentage of repeated sequences of a genome has become an important parameter for planning next-generation sequencing (NGS) experiments (Mao *et al*., 2016). Therefore, in this review, we are providing insights into the different basis of bruchid resistance in mungbean *viz*., physical, biochemical and molecular with special attention to the molecular advancement in the direction of breeding programme. Modern plant breeding is dependent on the molecular tools for rapid identification and prediction of genes/QTLs and introgression of these QTLs. Hence, we focused on i) QTL mapping, ii) identification and annotation of novel genes/QTLs and iii) genomics and transcriptomics study.

Sources of bruchid resistances in mungbean

Among the cultivated *Vigna* species, only rice bean (*Vigna umbellata*) is resistant to bruchid. There are only a few bruchid-resistant mungbean varieties available today (Hong *et al*., 2015) and resistant lines adapted to the tropics are lacking. Recently, several resistant germplasms have been identiûed and used in the breeding programme. Two types of sources that we can get to incorporate the resistance gene into the cultivated species are discussed below:

Wild sources: Resistant but barrier in crossing

The wild accession of mungbean (*V. radiata var. sublobata*) TC1966 from Madagascar is resistant to many pulse beetles, including *C. chinensis*, *C. maculatus*, *C. phaseoli* and *Zabrotes subfasciatus* (Tripathy, 2016). A few Australian wild mungbean accessions ACC23 and ACC41 are resistant to *C. chinensis*, *C. maculatus* and *C. phaseoli* (Somta *et al*., 2007). TC1966 is crossed with *V. radiata* and the accession of this was introduced into the cultivated gene pool for bruchid resistance (Chen *et al*., 2013). The resistance factor of TC1966 depends on a single dominant gene (Ishimato *et al*., 1993; Chen *et al*., 2013). Sometimes introgression of resistant gene from the crop wild relatives (CWRs) becomes very useful for developing a resistant genotype when the wild accession is cross-compatible to the cultivated ones. Pandiyan *et al*. (2020) has screened out some introgression lines from RIL population developed from the cross of *V. radiata* and *V. umbellate* and also he made intra- and interspecific cross which can be further used for developing bruchid resistant cultivars with improved yield. Even though, few reports are available on the resistance genes, but the practical utility of this information in further breeding is very less. Therefore, proper understanding on the gene at molecular level is most urgent.

Cultivated sources: Available but very less

Bruchid resistance landraces V2709 and V2802 (moderately resistance) subjected to Pure line selection which led to the development of cultivar V2709BG and V2802BG and are highly resistant to both *C. chinensis* and *C. maculatus* (Somta *et al*., 2007). Mungbean accessions (LM 131, V 1123, LM 371 and STY 2633) have been reported to be moderately resistant to bruchid based on the percentage of survival (Somta *et al*., 2007). It was demonstrated that cultivated mungbean accessions, V2709 and V2802 are highly resistant to *C. chinensis* and *C. maculastus* (Somta *et al*., 2008; Majhi and Mogali, 2020). Majhi and Mogali (2020) conducted an experiment with *C. maculatus* to screen eight sets of breeding lines of mungbean. They used V-02-802 and V-02-709 as resistant donor for *C. maculatus***.** The result of the cross derivatives of V-02-802 × DGGV-7 and V-02-802 × DGGV-2 were shown resistant responses with a very low susceptibility index of 0.039 and 0.043, respectively as compared to the other breeding lines. The infestation level of C. *maculatus* is compared among the four genotypes after 45 days of force choice test is given in Fig 1.

Understanding the mechanism of bruchid resistance

Physical basis of bruchid resistance: Determined by shape, size, color and texture of seed and leaf

The first encounter between insect pests and host plants is the antixenosis mechanism of resistance through oviposition. It determines the resistance or susceptibility of the host plants basing on the host genes. Ovipositional probing is necessary for checking host suitability. Any detrimental effect on insect oviposition will have adverse effects on the subsequent generation of pests. Thus the suitability of the host plant determines the nutritional value and the absence of toxic compounds in the host plant will show how good it is for the progeny survival. Many non-preference traits are involved to avoid insect oviposition in both field and storage seeds (Petzold-Maxwell *et al*., 2011). Mainly biophysical characteristics *viz.,* spines, trichomes, pubescence *etc.* determine insect oviposition (War *et al*., 2013). To avoid further infestation by bruchid, the appropriate way to kill the insect eggs are required (Doss *et al*., 2000). Traits like seed color, texture, hardness, size and chemical constituents

mainly contributing to bruchid resistance (Somta *et al*., 2007; Sarkar and Bhattacharyya, 2015) (Table 1). The oviposition behavior of *C. maculatus* and *C. chinensis are* greatly affected by seed texture (Sarikarin *et al*., 1999). The rough surface of seeds prevents the female bruchid to lay on the seed surface rather than a smooth surface (Watt *et al*., 1977).

Biochemical basis of Bruchid resistance: Determined by amino acids, proteins, alkaloids and enzymes

Apart from various morphological characteristics, secondary metabolites are important in defending traits involved in plant defense against insect pests (Wisessing *et al*., 2008; War *et al*., 2013). They affect directly on pest biology and show antibiosis mechanism of resistance (War *et al*., 2012). An array of compounds found in seeds acts either additively or synergistically against insect pests including bruchids. These include vicilins, cysteine rich protein (*VrD1* or *VrCRP*), vignatic acids and para-amino-phenylalanine (Chen *et al*., 2002; Somta *et al*., 2007). Mungbean seeds contain lignins, quinines, alkaloids, saponins, non-protein amino acids and polysaccharides, and anti-nutritional seed proteins such as lectins, phytohemagglutinins (PHA) and proteinase inhibitors involved in resistance against bruchids. The α-amylase inhibitors interfere with bruchid digestive enzymes and can act as an important biocontrol agent against them (Wisessing *et al*., 2008) (Table 1). Trypsin inhibitors have been recorded in higher levels in bruchid resistant varieties in mungbean than the susceptible ones (Landerito *et al*., 1993). Plant lectins are carbohydrate-binding (glyco) proteins that reversibly bind to well-defined simple sugars or complex carbohydrates (Vandenborre *et al*., 2011). In legumes, lectins are accumulated in seeds and provide a potential defense against bruchids. Canavalin in the seed coat has detrimental effects on the development of bruchids (Oliveira *et al*., 1999). Two major D-galactose specific lectins (MBL-I and MBL-II) have been characterized by mungbean seeds (Suseelan *et al*., 1997), but their role in bruchid resistance has not been studied. Lectins from various plants have been reported to alter the growth and development of bruchids (Leite *et al*., 2005). Lectins such as Canatoxin from *Canavalia ensiformis* (L.), Zeatoxin from *Zea mays* seeds, seed lectin from *Talisia esculenta* Radlk., a galactose-specific lectin from African yam beans, *Sphenostylis stenocarpa* and a lectin from the marine red alga, *Gracilaria ornata Areschoug*, has been found highly toxic to *C. maculatus* (Macedo *et al*., 2007). They bind to the midgut proteins and reduce the α-amylase activity of *C. maculatus* larvae (Macedo *et al*., 2007). Accumulation of cyanogenic glycosides and phytic acid in mungbean seeds during seed maturation plays an important role in defense against bruchids (Lattanzio *et al*., 2005).

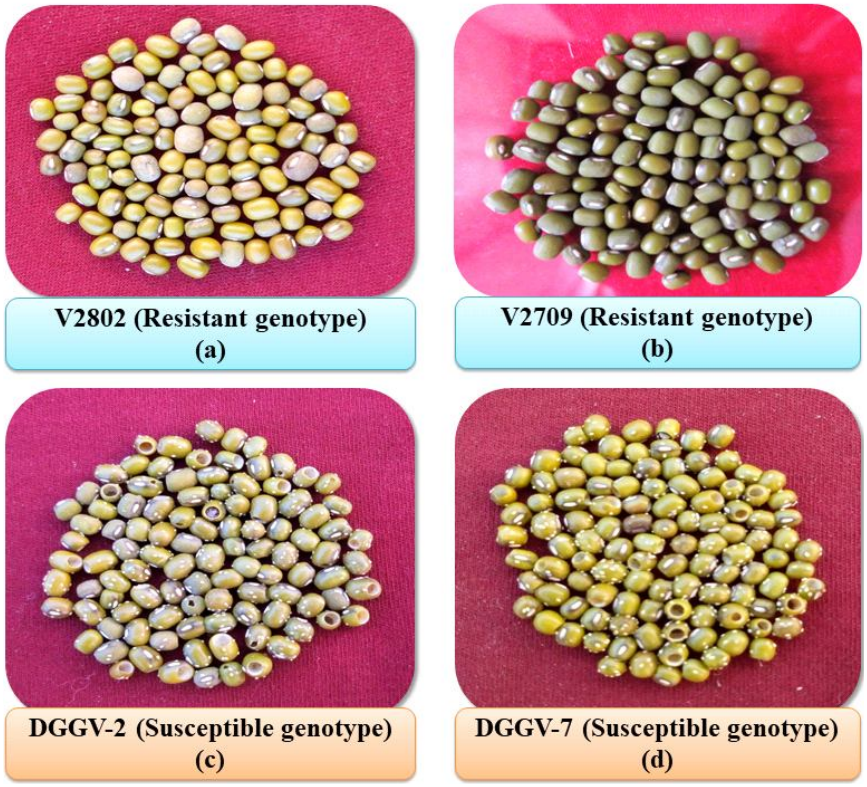

Fig 1: Genotypes (a) and (b) are the resistant sources for bruchid species *Callosobhuchus maculatus* and *C. chinensis* and commonly used as donor for development of resistant genotype. Genotypes (c) and (d) are susceptible to bruchid. The infestation level is compared among the four genotypes after 45 days of force choice test (Source: Modified from Majhi and Mogali, 2020).

Table 1: Different mechanisms of Bruchid resistance reported in several Mungbean genotypes, mapping populations and accessions.

Mechanisms of bruchid resistance	Mungbean genotypes, mapping populations and accessions	Characteristic features	Reference
Physical basis	TC1966	Seeds are covered with a network of parallel and transverse ridges, this makes the female bruchid relatively hesitant to lay eggs on seeds.	Fujii *et al*. (1989)
	VM2011 and VM3529	Dense hairs on the pods makes difficult for the adult to move over the pods and decrease the number of eggs laid and adult emergence from the pods.	AVRDC (1979, 1981)
	VM2164	The presence of a hard seed coat makes it unsuitable to feed.	AVRDC (1981)
	ACC23 and ACC41 (*V. radiata* subsp. *sublobata*) wild mung bean accessions	Presence of thick texture layer on the seeds might have acted as oviposition preventive barrier to bruchids.	Lambrides and Imrie (2000)
Biochemical basis	V2709, V2802, and VM2164	Possess high antibiosis mechanisms of resistance due to the presence of toxic secondary metabolites.	AVRDC (1992)
	VC6089	*VrD1* protein (*V. radiata*) defensin 1 is a cysteine-rich protein isolated from seeds that impart resistance against *C. maculatus*.	Chen *et al*. (2002)
	VC6089A, TC1966	Possesses some resistant-specific protein, polyprotein and aspartic proteinase.	Lin *et al*. (2016)
Molecular (genetic) basis	TC1966	14 linkage groups containing 153 RFLP markers were mapped having 9.3 cM as an average distance between the markers.	Young *et al*. (1992)
		RAPD markers utilized in conjunction with a RIL and near-isogenic line (NIL) mapping population using TC 1966.	Villareal *et al*. (1998)
		SSR marker is associated with a distance of <0.1 cM between DMB-SSR 158 and the major QTL in TC1966 was identified.	Chen *et al*. (2013)
	Cross derived population of 'Berken' and a wild mung bean genotype 'ACC41'	A linkage map was constructed using RFLP markers.	Humphry *et al*. (2002)
	RIL population between ACC41 (resistant) and Berken (susceptible)	Bacterial artificial chromosome (BAC) libraries with two polymerase chain reaction-based markers *STSbr1* and *STSbr2*, were analyzed and were found to be closely linked with a major locus conditioning bruchid resistance.	Miyagi *et al*. (2004)
	V. sublobata accession (sub2)	Polymerase chain reaction-based markers *STSbr1* and *STSbr2* were used for bruchid resistance, where *STSbr1* amplified a 225 bp fragment in all the resistant plants.	Sarkar *et al*. (2011)
	RIL population from the cross between 'Berken' and 'ACC41'	Mapping of *Br1* locus using the RIL population showed *Br1* on *LG9* between BM202 (0.7 cM) and Vr2-627 (1.7 cM).	Wang *et al*., (2016)
	RIL population involving 'TC1966' and mungbean yellow mosaic virus resistant variety 'NM92'	Using 200 RILs they identified 10 RAPD markers associated with bruchid resistance through bulked segregant analysis, of which four (OPW02, UBC223, OPU11 and OPV02) were closely linked. Seven codominant cleaved amplified polymorphisms developed from the identified RAPD markers showed tighter linkage with the Br gene than the original RAPD.	Chen *et al*. (2007)
	V2802	The mungbean SSR marker DMB-SSR 158 is perfectly associated with bruchid resistance in V2802.	Chotechung *et al*. (2011)

Table 1: Continue.....

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		By using the SSR marker, they found that DMB-SSR 158 marker on chromosome 5 is associated with polygalacturonase inhibitor genes (*Vradi05g03940-VrPGIP1* and *Vradi05g03950-VrPGIP2*) that account for resistance to bruchids. They concluded that the gene *VrPGIP1* could be the candidate gene for bruchid resistance in mungbean.	Chotechung *et al.* (2016)
	VC6089A	Bruchid resistance in a NIL VC6089A occurs due to the BURP (BNM2, USP, RD22, and PG1β) protein family. They further observed the higher expression of g39185 (resistant-specific protein), g34458 (gag/pol polyprotein), and g5551 (aspartic proteinase) in bruchid-resistant lines (VC6089A, TC1966, and RIL59).	Lin *et al.* (2016)
	V2709.	Identified two QTLs located between MB87 and SOPU11 for bruchid resistance in 'V2709'.	Hong *et al.* (2015)
	ACC41	In ACC41, a QTL accounting for about 98.5% of bruchid resistance was identified.	Mei *et al.* (2009)
	TC1966 and V2802	Populations derived from TC1966 and V2802 carry a strong QTL locus on chromosome 5 for bruchid resistance.	Schafleitner *et al.* (2016)

Molecular basis of bruchid resistance: Determined by alleles, genes and QTLs

Various resistance sources of bruchid are reported in wild species in TC1966 and plant breeders utilizing in crop improvement program for the release of resistance variety (Somta *et al*., 2007). In order to fasten the conventional breeding approach, molecular basis of evidence is necessary against the pest. Source of bruchid resistance in mungbean has been mapped using restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and single nucleotide polymorphism (SNP) markers (Chen *et al*., 2007; Chotechung *et al*., 2011). TC1966 accession was crossed with susceptible check VC3890 and the F_2 population analyzed through restriction fragment length polymorphism. Results revealed that the *Br* gene has a single locus using linkage group VIII, approximately 3.6 cM from the nearest RFLP marker (Young *et al*., 1992). Quantitative trait loci (QTL) analysis in TC1966 revealed that one major and two minor QTLs were responsible for bruchid resistance (Chen *et al*., 2013) (Table 1). A cysteine rich protein *VrCPR* was identified in TC1966 which shows lethal effect on *C. chinensis* grub (Chen *et al*., 2002). The proteomic study suggested that chitinase, beta-1,3-glucanase, peroxidase, provicilin and canavalin precursors play a role in bruchid resistance of mungbean (Khan *et al*., 2003).

Genomic-assisted resistance breeding

Conventional to molecular breeding: A shift from gene to QTLs

The art of distinguishing desirable traits and incorporating them into future generations is very imperative in Plant breeding. Since the practice of agriculture, farmers have been altering the genetic makeup of the crops unknowingly as they grow. Early farmers selected the best looking plants and seeds and saved them to plant for the next season. Then, once the science of genetics became better understood, plant breeders used what they knew about the genes of a plant to select for specific desirable traits (faster growth, higher yields, pest and disease resistance, larger seeds) to develop improved varieties. Wide hybridization is a reasonable strategy to ameliorate the eroded genetic base of a domesticated crop where the genetic variation is not available within the cultivable germ pool. As we have already discussed that rice bean (*V. umbellata*) is more resistant to the bruchid hence it can serve as a donor parent for introgressing this bruchid resistance gene in an inter-specific hybridization approach. Mungbean is cross-compatible with Rice bean, hence Mathivathana *et al*., (2019) developed a QTL mapping approach in a RIL population of *V. radiata* × *V. umbellata* to unfold the genomic region associated with the bruchid resistance. 108 $F_{3:9}$-derived lines, developed from the cross of susceptible accession of mungbean VRM (Gg)1 and the resistant rice bean accession TNAU RED, were established as RIL population for this QTL mapping.

Quantitative trait loci (QTL) Mapping: Connecting the genes by molecular markers

QTL mapping includes QTL confirmation, QTL validation and fine (or high resolution) mapping of the genes. Efforts have been made to construct linkage mapping by using RFLP and RAPD markers (Kaga and Ishimoto, 1998) and for studying genetic diversity in mungbean by RAPD along with inter simple sequence repeat profiles (Chattopadhyay *et al*., 2005). Meng *et al.* 2015 used QTL mapping and constructed a genetic linkage map. Inclusive composite interval mapping method was used to map the QTL for bruchid resistance (Li *et al*., 2007). Length of linkage groups and the tightly linked markers can be effectively used in marker-assisted selection, fine mapping and gene cloning. However, further in-depth investigations are needed in this area for developing the stable resistant variety. Kaewwongwal *et al.* (2017), investigated 77 DNA markers that were located near the *Br* locus on chromosome 5 of Mungbean and screened for polymorphisms between 'V2709' and 'KPS1' and 19 polymorphic markers were selected to construct a linkage map with a length of 84.12 cM. A single major QTL for *C. chinensis* (*qBrc5.1*) and *C. maculatus* (*qBrm5.1*) were detected by ICIM (Inclusive composite interval mapping). Results showed that, *qBrc5.1* and *qBrm5.1* were both located at 34.0 cM, between markers *VrID1* and *VrSSR017*. Results showed that additive and dominant effects of *qBrc5.1* were 49.07 and 45.00%, respectively, while those of *qBrm5.1* were 48.72 and 44.17%, respectively (Kaewwongwal *et al*., 2017). Among both QTL alleles, V2709 shows decrease in seed damage caused by bruchids, because both *qBrc5.1* and *qBrm5.1* were found in the same position and shows similar genetic effects for the resistance.

Kaga and Ishimoto (1998), mapped the '*Br*' gene by using the RFLP marker in TC1966 and they found that a region of 0.7cM between *Bng110* and *Bng143*. The Br gene is only 0.2 cM away from the *Bng143*. Chotechung *et al*., (2011) found that co-segregation of marker DMBSSR158 and *Br* genes in V2802 as analyzed in expressed sequence tag-simple sequence repeat (EST-SSR) marker. Later, Chotechung *et al*., (2016) reported a new gene *VrPGIP2* showing resistance to bruchid after a complete study of the genomic region of the Br gene that has a fragment of 38 kb segment on chromosome 5, and the *VrPGIP2* gene encodes for polygalacturonase-inhibiting protein. Furthermore, some genotypes *viz*., 'V2802', 'V1128', 'V2817', 'V2709' and 'TC1966' had the same *VrPGIP2* gene activity (Somta *et al*., 2007). However, further analysis revealed that there is no polymorphism in the EST-SSR marker DMB-SSR158, which is located on the *VrPGIP2* sequence, in genotype 'V2709' and bruchid-susceptible mungbean 'Kamphaeng Saen 1' (Chotechung *et al*., 2011). This information suggested that the gene or allele for bruchid resistance in 'V2709' is different from that of 'V2802'.

Development of markers accompanying with bruchid resistance genes

Traditionally available markers were not proved quantifiable information for resistance, so more information is needed on *Br* gene linked reliable markers for the breeding of bruchid-resistant mungbean varieties. *Br* gene usually associated with molecular markers associated with mapping the resistance genes that help to identify the factors underlying resistance against bruchids. Studies shown that gene-based or regulatory sequence-based markers could be efficient for this breeding program. Locus-linked RFLP and RAPD markers were also found more reliable for the selection. Resistance factors may occur because of changes in biochemical activity in susceptible and resistant lines, due to sequence variation or from expression differences of resistance genes. Polymorphic differences in resistant genes would provide reliable markers for resistance (Mao-Sen *et al*., 2016).The whole-genome sequence of a bruchid susceptible mungbean (*V. radiata* var. *radiata* VC1973A) was investigated in RILs population and annotates 14,500 genes (Kang *et al*., 2014). They also identified differentially expressed genes (DEGs) and nucleotide variations (NVs) in the promotor regions of DEGs and in the exons of sequence-changed protein genes (SCPs). The putative effects of DEGs and SCPs on bruchid resistance of mungbean were discussed and studies confirm that molecular markers derived from NVs could be used further for the selection of resistant lines (Kang *et al*., 2014). Mao-Sen and co-workers (2016) had identified linked markers (g779p, *g34480p* and *g34458p*) for resistant reaction from a RIL population between resistant and susceptible lines. Studies revealed that newly developed markers like *g779p* and *g34480p* exhibited 93.4% accuracy, which is far better than *g34458p*. Further studies confirmed with two bruchid-resistance-associated markers, the CAP marker *OPW02a4* and SSR marker *DMB-SSR158* (Chen *et al*., 2007; Chen *et al*., 2013) in 61 RILs revealed that, marker *DMB-SSR158* shows the highest accuracy of 98.3% in bruchid resistance mungbean.

Genome-transcriptome analysis: Searching novel alleles and gene annotation

The genome-transcriptome comparison study was carried out by Mao-Sen and co-workers (2016) for bruchid-resistance-associated genes by comparing the seed transcriptome of bruchid resistant (R) and susceptible (S) mungbean lines of two parental populations of NM92 (S) and TC1966 (R) and derived RILs. Two methods to RNA-seq were used for identifying DEGs. In the first approach, the number of transcripts per million (TPM) was calculated, results revealed that 22 up- and 6 downregulated genes were found in seeds of bruchid-resistant mungbean. Three upregulated genes namely *g4706*, *g34480* and *g42613* were detected in R-mungbean and two downregulated genes *viz*., *g40048* and *g41876* were detected in S-mungbean. In the second approach, DESeq analysis and total nine

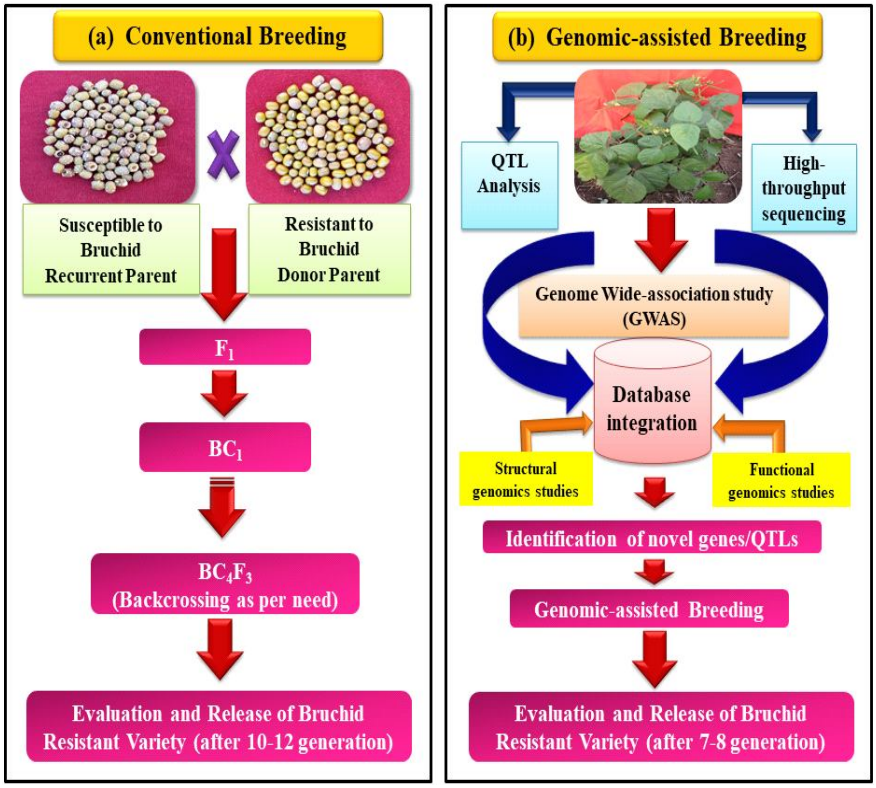

Fig 2: An illustration between (a) conventional plant breeding and; (b) genomic-assisted plant breeding approach for the development of mungbean genotypes resistant to bruchid. These two methods confirm the varietal development requires less duration in case of genomic assisted breeding approaches as compared to conventional breeding.

transcriptomes have identified 81 transcripts of 80 DEGs. Results show that a total of 31 were up- and 49 downregulated in bruchid-resistant mungbean. Furthermore, downregulated gene *g16371* was present in two splice forms, *g16371.t1* and *g16371.t2* (Chen *et al*., 2002). Further studies confirmed that 10 genes (*g24427*, *g34321*, *g4706*, *g34480*, *g28730*, *g17228*, *g9844*, *g39181*, *g39425*, *g42613*) were expressed only in bruchid resistant lines and three (*g40048*, *g35775*, *g2158*) were found in bruchid-susceptible lines (Mao-Sen *et al*., 2016).

Studies conducted by Chotechung and co-workers (2016) shown that, *VrPGIP2* is an intronless gene. It has 1,011 nucleotides and it translates into a 336-amino acid protein, which is similar to gene *LOC106760237* annotated by NCBI. Sequence alignment of *VrPGIP2* genes from 'V2802' and KPS1 shows that seven SNPs (at nucleotide positions 573, 958, 969, 972, 995, 1,003 and 1,008) present between the two mungbean accessions (Kaewwongwal *et al*., 2017). *VrPGIP1*gene was annotated by Kang *et al*., (2014) and it had three exons with an open reading frame of 1,302 nucleotides that encodes a 433-amino acid protein (Chotechung *et al*., 2016). However, the current mungbean genome annotation by NCBI showed that *VrPGIP1* (LOC106760236) is intronless, has 1,011 nucleotides and encodes a 336-amino acid protein, which is the same as *VrPGIP2*. Finally two new alleles named *VrPGIP1-1* and *VrPGIP2-2* were identified by sequencing *VrPGIP1* and *VrPGIP2* genes respectively from mungbean accession 'V2709' (Kang *et al*., 2014; Kaewwongwal *et al*., 2017). Again a recent finding by Kaewwongwal *et al*., (2020) revealed a second allele for *VrPGIP1* from ACC41, the resistant wild accession of mungbean (*V. radiata* var. *sublobata*).

FUTURE PROSPECTS AND CONCLUSION

Phenotypic selection needs special environmental conditions, if the environment is not suitable then there may be a chance that the susceptibility may not come for screening and selection, so the breeder may lose a season for it. Phenotypic based selection is also time-consuming, tedious, laborious and may be expensive. As the phenotypic data is environmentally sensitive, so it is necessary to use the molecular markers along with continuous backcrossing to increase the efficiency of selection and to reduce the number of selection tests as well as the cost required for screening. But, the selection through molecular markers shows more reliability in selection as it is not affected by the environment. Marker-assisted selection is genome-specific in nature, shows clear distinct allelic features, no detrimental effects on phenotypes and; easy assay and automation. A comparison between conventional and genomic-assisted plant breeding approach for the development of mungbean

genotypes resistant to bruchid is given in Fig 2. The development of successful bruchid resistance genotype in mungbean has attracted the attention of Plant Breeders, Entomologists and Biotechnologists due to its severe loss during storage. It is necessary to understand the developmental biology of bruchid before trying to access the resistance in the host genotype. The identified *Br* locus related molecular markers should validate properly to use in further breeding programme. Regulatory sequence-based or gene-based molecular markers would be the best for selecting bruchid resistant lines. Even though some resistant genotypes are identified for cultivation but still more research is necessary to give a sufficient number of resistant varieties to the farming society. Although some sources of resistance to bruchids are identified and used for crossing with high yielding varieties the difficulty is the transfer of undesirable characters with the resistance gene. Studies on bruchid resistance in relation to the development of molecular markers have gained high momentum. Further, bruchid resistance from black gram can be transferred into mungbean eficiently using interspeciûc or intraspeciûc crosses supported by bruchid resistance gene markers. RFLP and SSR markers have been widely used for mapping the bruchid-resistant gene in mungbean, owing to its complicated protocol to identify the resistance genes governing the trait. Due to the evolutionary force, bruchids may acclimatize to the newly formed resistant lines with single-gene resistance; thus, there is a prerequisite to identify and combine multiple resistant genes into the same cultivar through gene pyramiding strategies.

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