LR-5160
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REVIEW ARTICLE Legume Research- An International Journal

Effects of Biotic Stresses and Their Mitigation Strategies in Legumes: A Review

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10.18805/LR-5160

ABSTRACT

Legumes are crucial group of crops that are widely cultivated around the world for their protein rich seed and forage. The major impediments for boosting the output of legume crops are biotic and abiotic stresses. There is an urgent need to lessen the detrimental effects of these stresses on legume crops in order to boost the yield and production of legumes to address the nation's nutritional security issue. An attempt was made to gather published information on effects of biotic stress and mitigation strategies for developing resistant genotypes to maximize yield. This work was done at Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University Jalandhar in collaboration with other institutes. Systematic cum integrative review of research work done in different parts of World, particularly in India was comprehended. The literature search was done during August, 2022-February, 2023. About 150 review and research papers were screened from various data bases like ARCC journals, google scholar, research gate and scopus and 76 papers were used to write this paper. This review article presents a comprehensive documentation of the significant impacts of various biotic stresses on legume crops, along with the corresponding mitigation techniques. It highlights the potential effectiveness of integrating the pre-breeding approach with genomic breeding methods as a strategy for developing high-yielding cultivars that exhibit resistance to biotic stresses. By implementing this approach, legume productivity can be enhanced in regions with favourable growth conditions for these crops.

Key words: Biotic stress, GWAS, Legumes, MAS, QTL, Transgenic.

Lack of agricultural area, an increasing population and biotic/abiotic stresses impede agriculture and food production. Providing food for an expanding population is currently the greatest challenge. By 2050, the global population is deemed to surpass 10 billion, requiring a 60-100% increase in food supply (FAOSTAT, 2021). India is the leading producer, user and importer for legumes worldwide. To meet anticipated demand for pulses, production must increase from 755 kg/ha to 1.3-1.4 tonnes/ha, or 3-4 million hectares must be planted with pulses crops. Reducing post-harvest losses needs tremendous effort. To satisfy the demand of 30 million tonnes of pulses in 2030, India must boost its annual pulse output by 4.2%. This number warrants an examination of production restrictions and feasible remedies.

In addition to cereal, legumes are an essential fundamental food. Humans and animals can obtain proteins, minerals, dietary fibres and carbohydrates from common beans, peas and soya beans (Zargar *et al.,* 2017). Additionally, legumes can enhance soil quality by fixing atmospheric nitrogen and feed cattle (Meena and Lal, 2018). Chickpea, pigeon pea, mung bean, soya bean, common beans and peanut are the most important legume crops for meeting the dietary demands of people having nutritional deficiencies. However, due to climate change, increased disease and insect pressure reduces yield and quality of current legume cultivars (Moss *et al.,* 2020). Almost all the legumes are significantly impacted by biotic stress, which negatively impacts their development, production and nutritional value. Gaining a deeper knowledge of trait distribution across legume germplasm will aid in the exploitation of legume crop diversity and the determination of conservation priorities. Through traditional breeding, it

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How to cite this article: Sandhu, R., Bangarwa, S.K., Attri, M., Tiwari, S., Kohli, S., Fayaz, S. and Chaudhary, N. (2023). Effects of Biotic Stresses and Their Mitigation Strategies in Legumes: A Review. Legume Research. DOI: 10.18805/LR-5160.

Submitted: 23-04-2023 **Accepted:** 20-09-2023 **Online:** 09-10-2023

takes a considerable amount of time and endeavour to produce a plant with all the necessary characteristics. Due of these limiting variables, it may be practically hard to find a plant with all desirable accumulative features. In such cases, however, sophisticated approaches such as genome editing (GE), genome engineering, genome-wide association studies (GWAS) and omics technologies give options for rapidly introducing desirable characteristics into legume crops.

Aspects of biotic stress

Plants are exposed to biotic stressors induced by fungus, bacteria, insects, viruses, nematodes and weeds. All agricultural production systems must contain phyto pathogens and insect pests in an efficient and enduring manner. Resistance to available resistance genes and insecticides is continuously emerging in pathogens. Changes in host plant resistance, natural enemies and insect interactions may impact insect physiology and behaviour due to climate change (Dornez *et al.,* 2010). Increased CO_2 levels may exacerbate lepidopteron pod-borer and coleopteran defoliator infestations (Sharma *et al.,* 2006). Moreover, secondary pest infestation grew fast, resulting in a substantial loss of production (Sharma *et al.,* 2006).

Biotic stresses and their effects on major legume crops

Legume crops face an array of biotic stresses that severely impact their productivity and global food security. Insect herbivory and viral infections limit leaf area and hinder photosynthesis, while stress-inducing bacteria pose a threat to plant survival by disrupting nutrition delivery (Akram *et al.,* 2021). Temperature fluctuations influence pest activity and reproduction, contributing to a significant 20% annual yield loss from pests and illnesses in legume crops (Dhaliwal *et al.,* 2020). Particularly in Asia and Africa, leguminous crops can experience devastating losses of up to 100% under favorable biotic stress conditions (Singh *et al.,* 2022). This part of article explores the effects of biotic stresses on significant legume crops, shedding light on the severe damage inflicted by biotic stressors and severe yield losses caused by them.

The pod borer wreaks havoc on India's pigeon pea and chickpea harvests, resulting in substantial economic losses exceeding $30 million. Other factors like diseases, including collar rot, dry root rot, Pseudomonas syringae blight, stem/ white rot, Mycospaerella, wilt and yellow vein mosaic virus, further impact bean yield. Wilt can cause varying degrees of crop loss in legumes, ranging from zero to 100%, depending on the timing of infection (Pandey *et al.,* 2016). Additionally, the pod fly destroys 40% of pigeon pea pods (Singh and Singh, 2014). The sterility mosaic virus poses a significant threat to young pigeonpea plants, causing a staggering 95 to 100% loss in output, while older plants experience losses ranging from 26 to 97% (Gupta *et al.,* 2012). Pigeon pea production is further challenged by Fusarium wilt and sterility mosaic disease (Varshney *et al.,* 2013).

The situation is no different for soybean crops globally, with over 20 diseases affecting major soybean-producing nations. Root-knot nematodes alone account for a substantial 44.7% reduction in soybean productivity (Bassel *et al.,* 2012). Moreover, a myriad of insect pests, such as whitefly, jassids, aphids, the soybean leaf folder, green semi looper and leaf webber, alongside the pod borer, contribute to a 30-40% reduction in soybean productivity (Hesler *et al.,* 2018). Chickpea yields remain below their realizable potential, despite India's production of 1.0-1.2 tonnes per hectare, falling short of the predicted potential of over 2 t/ha.

The demand for plant-based meals, especially protein, cannot be fully met due to low chickpea yields, influenced by various biotic stresses like Fusarium wilt (FW), pod borer and ascochyta blight (AB). These stresses are the primary hindrances to improved chickpea yields and producer acceptance, with the potential to cause a complete loss in grain yield and quality (Iruela *et al.,* 2007; Li *et al.,* 2017). Timely action and robust protective measures are essential to safeguard chickpea crops and ensure sustained output. Chickpea-growing areas also face challenges from reniform, root-knot, root lesion and cyst-forming plant-parasitic nematodes.

The mung bean, despite its high yield potential of 2.5-3.0 t/ha, faces restricted productivity at only 0.5 t/ha, attributed to abiotic and biotic constraints, inadequate crop protection and limited access to enhanced seeds (Akram *et al.,* 2021). MYMD, a major mung bean virus disease, has caused significant economic losses, lowering India's yield by 85% (Prabhukarthikeyan *et al.,* 2017). Other fungal diseases like rhizoctonia root rot, anthracnose, powdery mildew and Cercospora leaf spot contribute to yield losses in mung bean crops (Singh *et al.,* 2013; Iqbal *et al.,* 2003; Bhat *et al.,* 2014; Mahesh Wari and Krishna, 2013). Halo blight is another emerging threat causing severe output losses and crop failure in Chinese mung bean farms (Sun *et al.,* 2017). India and Iran have also experienced significant yield reductions due to bacterial leaf spot caused by Xanthomonas phaseoli (Osdaghi, 2014; Kumar and Doshi, 2016).

Groundnut production faces challenges posed by insect pests such as pod borers, aphids and mites, as well as disease agents like leaf spots, rusts and toxin-producing Aspergillus fungus (Kumar and Kirti, 2015). Fungal diseases, including groundnut rust, early and late leaf spot, Sclerotium rolfsii and Aspergillus niger, further limit plant development and yield, causing up to 50% reduction in yield (Joshi *et al.,* 2020). Bud necrosis and Peanut mottle virus (PMV) present additional threats to groundnut crops and have gained popularity in India. Viruses alone can result in yield cuts of up to 60%, while minimal nematode-caused groundnut illnesses are reported in India (Kumar and Kirti, 2015). Root knot nematodes, however, have caused damage in various states. The plight of legume crops due to biotic stresses highlights the urgency to devise effective strategies to protect these essential crops, ensuring their resilience and contribution to global food production.

Mitigation strategies

Genetic resistance is the most appropriate strategy for mitigating biotic stress in legumes. But due to variable nature of resistance, pathogen strains and wide range of host plant, single approach is not sufficient. The narrow genetic base and appropriate screening approach of genetic resources are also a constraint for obtaining durable disease and insect resistance source in legumes. To utilize the full potential of legumes gene pool for resistance and to enrich legumes with genomic resources, integration of the pre-breeding approach with genomic breeding methods could be helpful for developing high yielding cultivars with biotic stress resistance for enhancement in legumes productivity in potential areas (Varshney *et al.,* 2018). Advanced biotechnological interventions (Fig 1) like whole genome sequencing, transcriptomics, proteomics, advanced mutagenesis methods, genome editing approaches and genome-wise association studies will be helpful in understanding the genetic interactions, proteins, metabolites involved in imparting resistance against biotic stresses.

Traditional breeding approaches

Conventional breeding is the most traditional method for agricultural plant development. The crop enhancement efforts rely on the inherent genetic diversity found in agricultural plants. A huge variety of agricultural plant types have emerged through traditional breeding. It is a time-consuming and tedious process because of the substantial selection abilities required at each phase. The primary goal of all crop enhancement projects is to obtain a huge and constant productivity. The abiotic and biotic stressors are significantly influencing crop output. For the evolution of a new variety or the improvement of existing varieties, conventional breeding techniques have been utilised in legumes. These techniques include the selection of resistant genetic resources through natural conditions, the crossing of resistant donor genotypes with susceptible genotypes for resistance transfer and the treatment of desired genotypes with physical (gamma radiation) or chemical (EMS, MMS, nitrogen mustards, imines) mutagens. The most precious assets for agricultural enhancement are genetic resources. Evaluation of characteristics-specific germplasm is the initial step in determining the genetic diversity necessary for certain traits. The conventional improvement technique necessitates the presence of naturally occurring diversity in crop genetic resources (CGR) and the examination of CGR to identify breeding resources. Until a new pathotype strain arises in nature, the utilisation of diverse genotypes is useful for developing resistant materials.

Genetic resistance

Identification of resistant cultivars and resistance mechanisms

Utilizing genetically resistant genotypes is the cheapest, environment friendly and sustainable method for combating soil-borne diseases. Regarding soil-borne diseases, there are a lesser number of resistant crop cultivars available. According to published research, several food crops are susceptible to root rot diseases caused by *Fusarium solani* (Tembhurne *et al.,* 2017), *F. oxysporum* (Chandra *et al.,* 2019), *Rhizoctonia solani* (Patro *et al*., 2018) and *Pythium* species (Syed *et al.,* 2020). However, *M. phaseolina*-resistant commercial cultivars have not yet been created, despite a few scientific attempts to identify the origins of resistance (Dhaliwal *et al.,* 2020). Researchers discovered few resistance cultivars against *M. phaseolina*, the pathogen

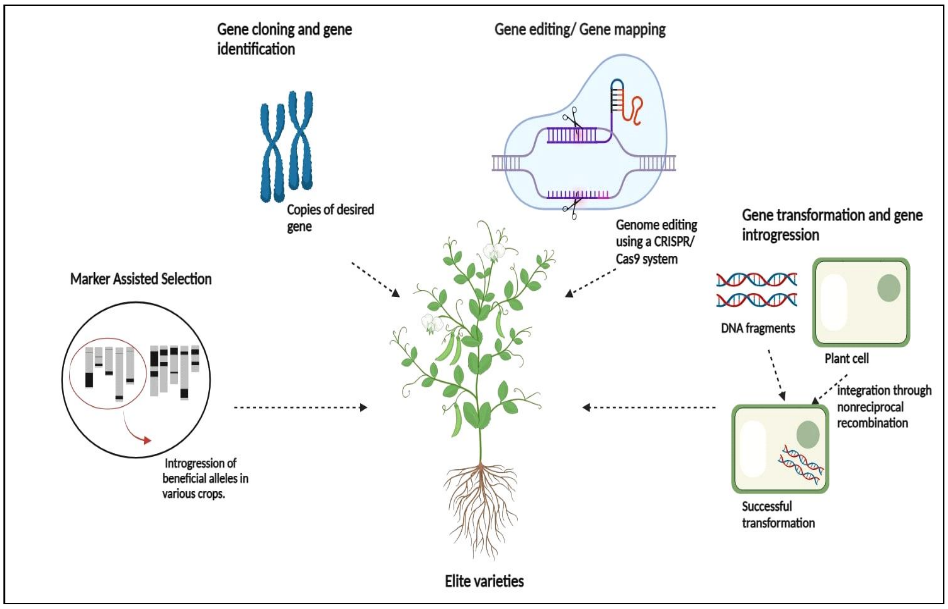

Fig 1: Advanced methods to enrich genomic resources in legumes for high-yielding cultivars with enhanced biotic stress resistance.

that causes dry root rot/charcoal rot in mung bean (Pandey *et al*., 2021), soybean (Pawlowski *et al.,* 2015) and chickpea (Gupta *et al.,* 2012). However, many variations are region-specific, necessitating testing across multiple locales and seasons. There are several sources of disease resistance originating from both transgressive segregation and other cultivars within the same species. The manner in which a host reacts to pathogen infection is a major determinant of its susceptibility or resistance. Several signalling molecules are activated when the host plant comes in contact with pathogen and, as a result, the plant's defensive response is initiated. The defensive systems consist of both structural and metabolic components. Every resistance breeding effort must comprehend which qualities are inherited and how they do so. For instance, resistance to charcoal rot is polygenic and defined by a continuous range of disease symptoms, *i.e*., extremely sensitive, moderately resistant and highly resistant (Talukdar *et al.,* 2009). The pattern of disease response as reflected by the length of necrosis was the subject of a study (da Silva *et al.,* 2019). Several findings reveal that numerous genes regulate charcoal rot resistance in soybeans. Ear rot (*Fusarium verticillioides*) shows a significant connection with genotype, polygenes play a significant impact for gaining resistance and no cultivar with complete immunity has been identified (Zila *et al.,* 2013). Infection with *Rhizoctonia solani* (Pierre and Bateman, 1967) and *Fusarium solani f. sp. phaseoli* (Kendra 1984) leads to formation of phytoalexins such as phaseolin, according to research. It has been demonstrated that resistant cultivars generate phytoalexins more rapidly and at a higher rate. There may be maternal effects on Fusarium root rot resistance in purple hypocotyls; hence, they may imply increase phytoalexin levels (Kendra 1984). Recent comprehensive genome expression profile investigations have provided us with insight into the mechanism of cultivar-level resistance. Differential expression of pectin degrading enzymes in different genotypes of rice following infection with R. solani demonstrated that the resistant genotypes were likely to have a lower degree of pathogen expression (Rao *et al.,* 2020). A list of biotic stress-resistant legume crop varieties is given in Table 1.

Recent advances in biotic stress mitigation

Gene modification in legume-pathogen interactions

Inbreeding frequently results in the transmission of unwanted traits that might compromise the desired outcome. Combining plant breeding with precise gene editing may increase the effectiveness of disease resistance deployment in plants. The capability and likelihood of introducing resistance traits to legumes through genome editing are rapidly growing. CRISPR/Cas9 has been identified in bacterial species that are capable of genome editing function as a form of protection against bacteriophages. CRISPR-Cas9 genome editing requires Cas9 and a tailored sg RNA.

Table 1: A list of biotic stress resistant legume crop varieties of recent past years.

Crop plant	Biotic stress	Pathogen	Resistant cultivar
Chickpea	Fusarium wilt	*Fusarium oxysporum* f. sp. *ciceris*	Pusa 5023, Pusa 1088
	Ascochyta blight	*Aschochyta rabiei*	Pusa 256, Uday Pusa 372, Pusa G 186
	Collar rot	*Sclerotium rolfsii* Sacc.	BDG 72, Co 3, Pusa 1105
Urd bean	Yellow Mosaic	Yellow Mosaic Virus	Pant U 30', 'NDU 99-3', 'Shekar 2', 'WBU 109',
	Powdery mildew	*Erysiphe polyponi* DC	KU 96-3', 'VBN 5', 'Ujala' 'VBN 5', 'Ujala' ('OBG 17') Powdery mildew resistant
Pigeonpea	Sterility mosaic disease and wilt	Sterility mosaic virus	'Azad', 'Narendra Arhar 1, 'Pusa 991', 'Pusa 992', 'TJT 501'
	Phytophthora blight	*Phytophthora drechsleri* f. sp. cajani	Pusa 2001',Pusa 2002
Cowpea	Root-knot nematode	*Meloidogyne* spp.	Pusa Sampada', 'UPC 628'
	Golden yellow mosaic	Yellow mosaic virus	Pusa Sukomal'
Horsegram	'Yellow mosaic	Yellow Mosaic Virus	VL Gahat 10'
	Macrophomina blight	*Macrophomina phaseolina*	'Birsa Kulthi 1'
	Powdery mildew and anthracnose		'PHG 9'
Mungbean	Yellow Mosaic	Yellow Mosaic Virus	'Pusa Vishal', 'PM 5', 'HUM 16', 'SML 668', 'RMG 492' Pusa 9531', 'Co 6', 'GG 2'

CRISPR/Cas9 was utilised to eliminate 2 ABC transporters, PxABCC2 and PxABCC3, in the lepidopteran pest *Plutella xylostella*, resulting in a higher degree of resistance to cry1Ac protoxin compared to strains that were sensitive (Guo *et al*., 2019). CRISPR/Cas9-mediated editing of the CYP6AE gene cluster in *Helicoverpa armigera* demonstrated that insect survival was reduced when exposed to insecticides and phytochemicals (Wang *et al.,* 2018). Investigations are being conducted into the development of CRISPR/Cas9 or TALEN entry vectors for gateway cloning in soybeans and *Medicago sativa truncatula* is also available (Curtin *et al.,* 2018). E-CRISP (Heigwer *et al.,* 2014) and CHOPCHOP (Montague *et al.,* 2014) are two new web-based tools for finding CRISPR-Cas9 target sites and off-target sites. In legumes, a tool for CRISPR/Cas9 design and a technique for gene editing in *Mycobacterium smegmatis* were created (Michno *et al.,* 2015). *Sclerotinia sclerotiorum* mutants with substantial loss of function were produced using CRISPR/cas9. Using the previously discovered Ssoah1 gene as the target gene, less damaging insertional gene mutants of soybean, *Brassica* spp. and tomato were produced (Li *et al.,* 2017) Similarly, the Avr4/6 genes of the pathogen were altered using gene editing to generate *Pytophthora sojae* mutants (Fang and Tyler, 2016). These investigations were crucial for elucidating the role of fungal and oomycete genes in pathogen pathogenesis. Recent study has showed that CRISPR/Cas9 genome editing of promoters may yield many cis-regulatory alleles and that quantitative variation is a beneficial breeding tool. Rodriguez-Leal *et al.* (2017) created a genetic approach that takes use of the transgenerational heritability of Cas9 activity in heterozygous loss-of-function mutant conditions. If we know the activity of cis-regulatory alleles, we may utilise this technique to screen QTLs for disease resistance and employ it as a breeding tool (Rodriguez-Leal *et al.,* 2017). This notion is made conceivable by epi-mutagenesis, a mechanism that rapidly creates DNA methylation variation by random demethylation. This capability to modify plant methylomes to create epigenetically different individuals may prove to be a beneficial breeding tool (Ji *et al.,* 2018). Although only a limited number of legume plants have currently undergone gene editing to enhance their resistance to pathogens, we anticipate that this technology will be increasingly utilized in the future to create disease-resistant legume plants that will enhance crop production.

Discovery of QTLs for disease resistance

QTL mapping is a method for linking plant disease resistance locus with resistant trait. The method facilitates the analysis of the roles of individual resistance loci, the specificity of resistance genes and the interactions between resistance genes, development stage and the environment. Few research has been conducted on the identification of QTLs for resistance to soil-borne diseases, particularly for common bean and cowpea (Muchero *et al.,* 2009). There are close to eight QTLs associated with cowpea resistance and one important QTL, Mac-2, accounts for over 40% of the resistance diversity (Muchero *et al.,* 2009). A marker associated with the pectin esterase inhibitor (PEI)-encoding 1 0853 gene has been identified. Pectins have a vital role in pathogen defence (Lionetti *et al.,* 2007), hence identified PEI as a phaseolina-resistant gene for M. cowpea. Identification of QTLs by GWAS (Genome Wide Association Mapping) has recently acquired importance for identifying resistance; nevertheless, little is known about soil-borne diseases. Some of the QTLs identified against biotic stress in legumes are summarised in Table 2.

Genetic engineering for crop insect/pests

Cry proteins can be expressed using genetic engineering techniques alone or in conjunction with other genes, vegetative insecticidal proteins (VIP), proteinase inhibitors and lectins for conferring insect pest resistance and the most recent biotechnological approaches, including RNAi and CRISPR/Cas9, are all viable options for long-term insect pest control. Genetic engineering approaches for developing insect resistance in legumes is illustrated using Fig 2.

Cry peptides

Crystal, also known as Cry toxin, insecticidal crystal toxins/proteins, crystalline inclusion and parasporal body, is obtained from a soil-dwelling gram-positive bacteria that creates crystals during sporulation. Insects belonging to the families Coleoptera, Diptera, Lepidoptera, Hymenoptera and Nematoda are harmed by cry toxins. They are safe for people, animals and natural insect predators.

Transgenic legumes

The cowpea aphid, *Aphis cracciovra*, has been observed to inflict considerable damage to the production of an important legume crop, chickpea. The overexpression of the agglutinin gene in chickpea can have a substantial impact on the survival and fertility of cowpea insects (Chakraborti *et al.,* 2009). In addition, transgenic pigeon pea expressing Glycine max trypsin inhibitor and Cry1Ab has been developed (Sharma *et al*., 2006), but it does not efficiently inhibit H. armigera (Sharma *et al.,* 2017). Transgenic chickpeas expressing an inhibitor of cowpea trypsin (Thu *et al.,* 2003) study with an a-amylase inhibitor (Sarmah *et al.,* 2004) demonstrated bruchid resistance. Pea weevil resistance was generated by producing an inhibitor of amylase in transgenic pea (Morton *et al.,* 2000). The introduction of Cry1Ab and Cry1Ac genes into pea cultivars (De Sousa-majer *et al*., 2007a), adzuki bean (Ishimoto *et al*., 1996) and chickpea (Ignacimuthu and Prakash, 2006) provided bruchid insect resistance. The insertion of Cry1Ab and Cry1Ac genes into pea cultivars (De Sousa-majer *et al*., 2007a), adzuki bean (Ishimoto *et al*., 1996) and chickpea (Ignacimuthu and Prakash, 2006) gave resistance against cowpea pests that were vulnerable to various different kinds of Bt crystal toxins. Luthia *et al.* (2013) introduced the AI-1 gene into the breeding line IT86D-1010 and the cultivar ‘Sasaque,’ both of which displayed 100 percent mortality of C. maculatus and C. chinensis larvae in the seeds of transgenic lines. List of transgenic legumes developed for insect resistance is given in Table 3.

Genetic engineering for transfer of disease resistance in legumes

Using transgenic technology, GE has developed the most effective alternative strategy for rapid development of resistance. Induction of resistance to yellow mosaic disease has been achieved by RNA interference/anti-sense RNA technology and pathogen-derived resistance in related species or genera (PDR). Haq *et al*. (2010) generated resistance for MYMIV utilising a gene construct of a soybean MYMIV isolate with an antisense Rep gene that has several roles, including viral replication, transcription and other protein synthesis processes connected to host DNA synthesis (Rouhibakhsh *et al*., 2011). The gene construct including the anti-Rep gene together with the soybean isolates DNA-A and DNA-B were co-inoculated in three legume hosts, *i.e*. cowpea (variety Pusa Komal), mungbean (variety PS16) and blackgram (variety T9) and the MYMV severity observed was 17.8%, 20% and 18.0%, respectively. Using RNA interference technique, transgenic cowpea with several RNAi constructs, including AC2, AC4 and AC2+AC4, was produced. Transgenic cowpea lines with AC2-hp and AC2+AC4- hp RNA displayed total resistance to (Kumar *et al*., 2017). Using agrobacterium-mediated gene transfer of wheat germin gene (gf-2.8) producing oxalate oxidase (OxO) that oxidises oxalic acid into CO_2 and H_2O_2, transgenic soybean with SWM (Sclerotinia sclerotiorum) resistance has been created. Increased OxO activity decreased disease

Table 2: List of QTL/marker analysis in legume–pathogen interaction studies.

Crop	Causal organism	QTL	Techniques	References
Pisum sativum L.	*Fusarium solani fsp. pisi (Fsp)*	*Fsp-Ps 2.1*	QTL mapping	(Coyne *et al.,* 2019)
	Uromyces pisi	Diversity arrays technology	*Up DSIV and Up DSII*	(Barilli *et al.,* 2018)
	Aphanomyces euteiches	*Ae-Ps7.6* and *Ae-Ps4.5*	QTL mapping	(Lavaud *et al.,* 2016)
	Erysiphe pisi	*er 1*	Deletion mapping	(Ganopoulos *et al.,* 2018)
Glycine max L.	*Xanthomonas axonopodis pv. glycines diaporthe phaseolorum, Cadophora gregata,*	GWAS utilizing SoySNP50k	SNPs	(Chang *et al.,* 2016)
	Phytophthora sojae	*RpsQ*	SSR	(Li *et al.,* 2017)
	Heterodera glycines	Novel QTL	QTL mapping	(Wen *et al.,* 2019)
	Aulacorthum solani	Novel Raso 2	SNP	(Lee *et al.,* 2015)
Vigna unguiculata L.	*Uromyces vignae*	*Auv-LS*	BSA	(Wu *et al.,* 2018)
	Meloidogyne javanica	*QRk-vu9.1*	QTL mapping	(Santos *et al.,* 2018)
Cicer arietinum L.	*Ascochyta rabiei*	*AB4.1*	WGS	(Li *et al.,* 2017)
Vicia faba L.	*Ascochyta fabae*	*Af3 and Af2*	QTL mapping	(Atienza *et al.,* 2016)
M. truncatula L.	*Aphanomyces euteiches*	2 QTLs	GWAS utilizing SNPs	(Bonhomme *et al.,* 2014)
	Phytophthora palmivora	*RAD1*	A. mapping	(Rey *et al.,* 2017)
Medicago sativa L.	*Verticillium alfalfa*	*17 SNPs*	BSA	(Zhang *et al.,* 2014)
Phaseolous vulgaris L.	*Colletotrichum lindemuthianum,*	17 and 21 effective SNPs	GWAS	(Perseguini *et al.,* 2016)

Table 3: Examples of transgenic legumes carrying transgenes for insect resistance.

Crop	Transgene	Target insect	Referances
Soyabean	*Cry 8 like*	Coleopteran-Holfrichia panallele	(Qin *et al.,* 2019)
	SpbPO-dsRNA (RNA i)	Lepidopteran	(Meng *et al.,* 2017)
Pigeon pea	Cry2Aa	Pod borer-H. armigera	(Singh *et al.,* 2018)
Chickpea	CryIIAa	Pod borer	(Sawardekar *et al.,* 2017)
	AI-1	Coleoptera-Chrysomelidae	(Luthia *et al.,* 2013)
Cowpea	Vip3A+Cry1Ab	Heliothis zea and Heliothis virescens	(Bommireddy *et al.,* 2011)
	Arcl on APA locus	Bruchids	(Grazziotin *et al.,* 2020)
Pea	Cry 1 Ab and Cry 1 Ac	Bruchids	(De Sousa-majer *et al.,* 2007)

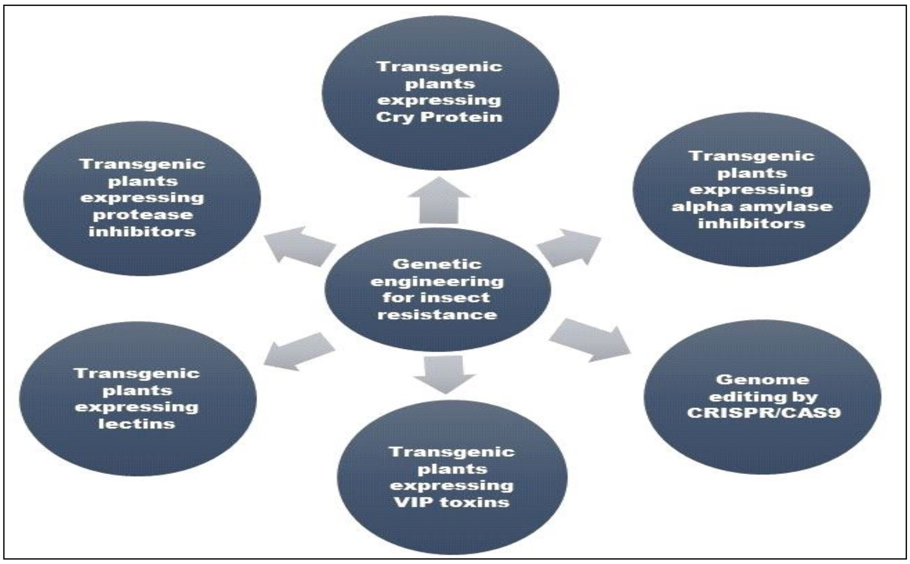

Fig 2: Genetic engineering approaches for developing insect resistance in legumes.

development and imparted SWM resistance to soybean plants (Akram *et al.,* 2021). Cober *et al*. (2003) analysed the transgenic line 80(30)-1 bearing the transgenic gene wheat germin gf-2.8 together with the normal soybean line 80(30)-9 and resistant and susceptible cultivars. Three years of field testing demonstrated that the transgenic lines have a lower disease severity index (DSI) than the regular line, which has the highest DSI.

CONCLUSION

Precise phenotyping is essential for finding a suitable source of resistance and developing segregating populations like F_2, backcross, RILs, NILs and other advanced populations for studying genetics and identifying stable genetic loci for disease resistance breeding programmes. Genomics assisted breeding tools like MAB and other variants like MABC, MARS, MAGP; genome-wide selection approaches like GS and GWAS; next generation mutagenesis strategies like TILLING, MutMap, MutMap+; genetic engineering approaches transgenesis, cisgenesis, next generation sequencing (NGS) platforms and the newly emerging genome-wide editing (CRISPRC as system) are potential methods for developing useful genomic resources for their extensive utilization in legumes breeding programs. Integrating genetic and genomic resources will help to produce the resistant cultivar needed for food and nutritional security.

Conflict of interest: None.

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