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Biotechnological advances in leguminous vegetables: A review

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Abstract
Vegetable legumes (Garden Pea, French bean, Cowpea, Cluster bean, Lima bean, Winged bean etc.) are an integral part of a balanced human diet being main source of proteins. In addition, they also contain an appreciable amount of carbohydrates, vitamins, and minerals, along with various other health-promoting bioactive chemicals. The demand for both fresh and processed vegetable legumes is steadily increasing as consumers become more aware of the importance of a well-balanced diet. Therefore, sustaining optimum yields of vegetable legumes is extremely important but the main difficulties affecting their production are the relatively low increase in yields and the negative effects of biotic and abiotic stresses. Traditional crop improvement approaches are generally more laborious, time consuming and devoid of significant genetic gains. Therefore, these are inadequate for plant genome enhancement to develop new plant varieties. Recently, the invention of biotechnological tools has opened new avenues for research and development in vegetable legumes studies. Biotechnological tools provide three major aspects of genetic improvement of leguminous vegetables through tissue culture (*in vitro* regeneration, double haploid production *in vitro* mutagenesis, *in vitro* gene transfer, somatic hybridization, somaclonal variations), molecular breeding or marker assisted selection (MAS) (Marker assisted backcrossing, gene pyramiding, recurrent selection, genome-wide association mapping studies) and genetic engineering. However, recent advances in genome editing technology using clustered regularly interspaced short palindromic repeats (CRISPR), and CRISPR-associated (Cas 9) proteins have opened the door to a new plant breeding era. Genome editing technologies have many advantages over traditional agricultural methods, having simplicity and high specificity. Conventional breeding in conjunction with molecular breeding, genetic tools and resources enable vegetable breeders to scale up their research in the field of legume vegetable improvement. In the current paper, a comprehensive review on significant achievements in biotechnological advancement in vegetable legume breeding in India and abroad has been done.

Keywords: leguminous vegetables, MAS, tissue culture, genetic engineering, CRISPR

Introduction
Vegetables are one of the principal components of a balanced human diet. Their consumption is progressively increasing around the world as people become more aware of their importance for a well-balanced diet and their high content of health-promoting compounds. (Kader *et al*., 2004; Hounsome *et al*., 2008) [78, 68]. The recommended nutrient intakes for Indian males and females is 2730 & 2230 kcal energy, 60 & 55 g protein and 30 & 25 g fat respectively (ICMR, 2010) [71]. India has attained self-sufficiency in food crops a long time back during 1960's with the advent of green revolution. However, with the passage of time, improved living standards and awareness regarding dietary habits of the people, the focus has now been shifted towards nutritional security. Indian diet is rich in carbohydrates and fats but deficient in proteins. Legumes are considered important sources of plant protein, carbohydrates, essential minerals, vitamins, and a variety of other antioxidants and health-promoting compounds from a nutritional standpoint (Souci *et al*., 2000; Bouchenak and Lamri-Senhadji, 2013) [142, 27]. These days, consideration of vegetable legumes is growing new protein sources to meet the ever-increasing demand for vegetable proteins. Their consumption is mainly intended to provide a more balanced nutrition full of healthy compounds in addition to a primary protein source. Pea, cowpea and beans (Indian bean and French bean) are the important leguminous vegetables. Cluster bean, broad bean, lima bean, winged bean, and other beans are of lesser economic importance among them. (Dhaliwal, 2017) [43]. The green pods and seeds of legume vegetables are rich in proteins and carbohydrates.

The protein and carbohydrate content of 100 gm of edible fresh mass is 1.8 & 7.0g in common bean, 3.3 & 9.5g in cowpea, 5.4 & 14.5g in pea and 7.9 &17.6g in faba bean respectively (USDA, 2017) [114]. They are also an important source of essential micronutrients for humans, such as vitamins and minerals, which play a role in maintaining proper metabolic functions in cells and tissues as cofactors of metabolic reactions, coenzymes, gene regulators, and radical scavenging molecules (Bouchenak and Lamri-Senhadji, 2013; Septembre-Malaterre *et al*., 2017) [27, 132]. Therefore, increasing the use of legume vegetables and introducing new legume-based products that are affordable to low-income groups is necessary to alleviate poverty and malnutrition.

The adaptability and productivity of legume vegetables are limited by major abiotic stresses including (drought, heat, frost, chilling, waterlogging, salinity and mineral toxicities) leads the crop vulnerable to weeds, insects and diseases, which increase considerably the losses (Reddy *et al*., 2004; Mwang'ombe *et al*., 2007; Sekhon *et al*., 2019; Ojiewo *et al*., 2019**)** [114, 20, 131]. The conventional methods used for the genetic improvement of these crops are: pedigree, bulk, single seed descent (SSD), backcross method and mutation breeding. Due to the long term field trials, distant hybridization barriers, lengthy screening procedures and reliance on environmental factors,conventional breeding techniques are inadequate for plant genome enhancement to develop new plant varieties (Ahmar *et al*., 2020; Sekhon *et al*.,2019) [6, 131]. From 1980 onwards, the focus has been shifted from conventional to modern methods. With the advancement in non-conventional biotechnological approaches *viz*., tissue culture, molecular breeding or marker assisted selection (MAS) and genetic engineering. (Jacob *et al*., 2016; Pratap *et al*., 2018; Aditika *et al*., 2017; Dhaliwal *et al*., 2020) [72, 112, 4, 42] new avenues have been opened in legume research. In addition, recent advances in genome editing technology using clustered regularly interspaced short palindromic repeats(CRISPR), and CRISPR-associated (Cas9) proteins have opened the door to a new plant breeding era (Ahmar *et al*., 2020) [6]. Furthermore, a growing number of quantitative trait loci, candidate genes, and genes linked to abiotic and biotic resistance as well as agronomic traits have been reported, potentially allowing for faster progress in vegetable legume genetic improvement (Nagendra and Krishna rai., 2015; Jacob *et al*., 2016) [72]. The current status of biotechnological approaches in relation to biotic and abiotic stresses in legume vegetables is described in this review, as well as how these useful tools could be used to improve resistance to important constraints affecting legume vegetable crops.

Need of Biotechnological Approaches for Improvement in leguminous vegetables

Legume vegetables are highly prone to biotic stresses and are generally affected by a wide range of pathogens including fungi, bacteria, and viruses (Sekhon *et al*., 2019) [131]. Traditional breeding techniques may aid in the improvement of vegetable legume traits such as quality, nutrition, and yield, but not at the rate required. Moreover in conventional plant breeding, there are chances to skip the trait of interest and delay the time to develop new cultivars with desirable traits. Biotechnology involves the use of molecular markers, genetic engineering and tissue culture techniques to modify crop plants (Anonymous, 2015). The biotechnological approaches offer several advantages over conventional breeding methods (Afzal *et al*., 2020) [5]. Limitations of conventional breeding, such as linkage drag, sexual barrier in wide crosses, anti-nutritional factor, and so on, can be overcome efficiently and effectively using biotechnological approaches. Recent developments in molecular biology such as *in-vitro* mutagenesis, genetic engineering, DNA sequencing, molecular marker etc. foster new meaning, new dimension, and new potential (Singh *et al*., 2019) [136]. Scientists are using more cost-effective and improved molecular breeding techniques to improve the genomes of legume crops. To improve legume vegetables, various biotechnological approaches have been used. Based on their knowledge of DNA, scientists have been successful in isolating a target gene of interest, transferring it, and integrating it into the host species. Such approaches have been concisely discussed below:

Plant Tissue Culture

In general, Fabaceae species are difficult to regenerate *in vitro*, tend to be recalcitrant, and have high genotypic specificity. (Pratap *et al*., 2010) [109]. Tissue culture in legumes has been described as difficult on several instances (Anand *et al*., 2001; Chandra & Pental, 2003) [9]. Due to advances in molecular genetics, such as gene over-expression, gene suppression, promoter analysis, and T-DNA tagging, necessitate efficient transformation systems, reluctance to *in vitro* regeneration is a major constraint in transgenic plant production for many legumes. (Somers *et al*., 2003) [141]. Implementation of robust protocols for regeneration is therefore a necessary condition for both genetic transformation and other tissue-culture derived techniques to generate genetic diversity such as somaclonal variation, *in vitro* mutagenesis, doubled haploids culture, and somatic hybridization.

In vitro Regeneration

It is based on the ability of plant cells to differentiate into whole plants under specific culture conditions (Skrzypek, *et al*., 2012) [138]. Crop plants can multiply quickly under aseptic conditions due to organogenesis and somatic embryogenesis. It is a process whereby a cell or group of cells from somatic tissues such as roots, cotyledons, stems, leaves or reproductive organs form an embryo (Iantcheva *et al*., 2005) [70]. There are a number of studies in different legume vegetable crops which have reported successful protocols for *in vitro* regeneration (Table: 1). Plant regeneration in *Phaseolus* sp. was reviewed by Veltcheva *et al*., (2005) [151], and successful regeneration is reported mainly for *P. vulgaris* (de *Carvalho et al*., 2000; Santalla *et al*., 1998) [37, 126]. It was possible to regenerate from other *Phaseolus* species and was achieved in *P. coccineus* L. (Santalla *et al*., 1998) [126], *P. acutifolius* (Zambre *et al*., 1998) [159] and *P. polyanthus* (Zambre *et al*., 2001) [160].

Table 1: Type of explants used for *in vitro* regeneration of different legume vegetables

Species	Explant	References
Phaseolus vulgaris	(cv. Goldstar) Seeds cv. Carioca) Embryonic axes excised from mature seeds Apical meristems derived from seeds incubated overnight in MS-based medium (cv. Dark Red Kidney) Leaf discs and hypocotyls segments from 3- to 4- and 7-day-old seedlings Stab inoculation of nodal regions of germinating intact seedlings) Multiple buds from cotyledonary nodes, epicotyl Cotyledonary nodes excised from 7-day *in vitro* seedlings Embryogenic axes	Kim and Minamikawa (1997) [81] Aragao *et al*., (1992) [12] Russell *et al*., (1993) [124] Franklin *et al*., (1993) [53] Lewis and Bliss (1994) [89] Barros *et al*., (1997) [22] Thảo, *et al*., (2013) [147] Gatica *et al*., (2010) [58]
Vicia faba	Different sites on stem, stabbed to 2–3 mm depth	Siefkes-*et al*., (1995) [135]
Pisum sativum	(cv. Puget) Shoot apex, epicotyl and cotyledons Thin cell layers from nodes (Cvs. Greenfeast, Rondo) Immature embryonic axes lacking roots (cv. Puget) Cotyledonary nodes (Cvs. Bolero, Huka and Trounce) Immature cotyledons (cv. Puget) Cotyledonary meristems Immature embryonic axes and cotyledonary node Mature Seeds	Hussey *et al*., (1989) [69] Schroeder *et al*., (1993) [129] Davies *et al*., (1993) [39] Grant *et al*., (1995) [61] Bean *et al*., (1997) [24] Das *et al*., (2014) [38] Zhihui *et al*., (2009)

Double Haploids

In crop development programmes, haploids developed by *in vitro* cultivation of gametophytic cells, particularly male gametophytes, are extremely important. Breeders can create entirely homozygous genotypes from heterozygous parents in a single generation using doubled haploid (DH) breeding and the recombinant gametes can be fixed directly as fertile homozygous lines (Forster *et al*., 2007; Pratap, *et al*., 2006) [52, 111]. DH lines can be utilized for quick mapping population development, molecular marker-based linkage mapping, *in vitro* mutation breeding, and gene transfer. Above all, during the culture phase, *in vitro* screening for complicated features like drought, cold, and salinity tolerance can be done. (Pratap & Gupta, 2007) [110]. Among these, anther or microspore culture has been most frequently used owing to greater success and ease of getting instant doubled haploids. (Maluszynski, *et al*., 2003) [93]. Anther and microspore culture systems for various legume vegetables i.e. *Phaseolous* and fieldpea were developed by various workers (Munoz-Florez & Baudoin, 1994a, 1994b; Croser *et al*., 2005) [98, 99, 35]. Gosal and Bajaj (1988) [59] successfully induced callus from pea cultivar 'Bonneville' anthers as well as two breeding lines (T163 and P88).

***In vitro* mutagenesis**

In vitro mutagenesis offers opportunity for variation induction for the development of a number of improved varieties of vegetable legumes, advantages of high mutation frequency, handling of large populations and rapid cloning of selected variants. Mutagenesis during the culture phase, which results in the growth of plants that are not true to type following micropropagation and regeneration, is thus one of the useful sources of variety that breeders can exploit. Somaclonal variation and gametoclonal variation are the different types of variation which may occur naturally or be induced during the culture phase of an explant. *In vitro* selection of pea somaclones by pathogen-derived agents resulted in the discovery of somaclones with increased resistance to *F. solani* (Horacek *et al*., 2013) [61]. Tsyganov *et al*., (2007) [149] used EMS-induced mutagenesis to create a pea mutant with higher cadmium tolerance and accumulation. Genotype, nutrient content, and hormone supplements are the main factors that influence somaclonal variation (Khatun *et al*., 2003) [80]. Arias *et al*. (2010) [17] used Embryogenic axes from the Costa Rican common bean cultivars Bribr, Brunca, Guaym, Huetar, and Telire to establish a method for regeneration of the commercially important common bean (*Phaseolus vulgaris*) through mutagenesis under *in vitro* conditions.

***In vitro* gene transfer**

Advancements in genetic engineering of crop plants have ensured recovery of improved plants with genes introgressed in them from across the species barrier. As a result, transgenic plants in several vegetable legumes, have been developed. Through the development of insect-resistant cultivars and very strong built-in insecticidal characteristics equivalent to those of conventional pesticides, transgenics have the potential to dramatically improve the genetic component of integrated pest management (IPM). (Pratap *et al*., 2009) [113]. The development of transgenic plants across a wide range of legume species was reviewed by Atif *et al*., (2013) [18]. Direct gene transfer and agrobacterium-mediated gene transfer are two successful gene delivery techniques. While the latter technique has been shown to be the most effective genetic transformation system in the majority of species, some legumes are not agrobacterium hosts and therefore this system is not efficient for them (Abiri *et al*., 2014) [2]. Therefore, development of highly reproducible regeneration protocol is a prerequisite for widespread application of *in vitro* tissue culture techniques in vegetable legume improvement programmes. Ali *et al*., (2015) [7] employed transgenic pea plants to impart salt stress tolerance by overexpressing the Na+/H+ gene from *Arabidopsis thaliana.* Negawo used agrobacterium-mediated transformation to improve resistance to pests in pea (2015). In cowpea, conditions affecting genetic transformation were optimized by Popelka *et al*., (2006) [108] using different plant tissues as explants which was followed by several reports of successful genetic transformation in this crop for traits such as resistance to cowpea weevil (Solleti *et al*., 2008) [140] and pod borer (Higgins *et al*., 2012) [65], weed control (Citadin *et al*., 2013) [34] and salinity tolerance (Mishra *et al*., 2014) [96].

Somatic Hybridization

Through the generation of inter-specific and inter-generic hybrids, it is a significant tool for plant breeding and crop improvement programmes. The method entails fusing protoplasts from two different genomes, followed by the selection of appropriate somatic hybrid cells and hybrid plant regeneration (Evans and Bravo, 1988) [49]. Protoplast fusion is

an efficient method of transferring genes with desired traits from one species to another, and it is having an increasingly positive impact on crop improvement. (Brown and Thorpe, 1995) [29]. Campbell, 1997 [31] obtained intergeneric hybrids by protoplast fusion of the grass pea (*Lathyrus sativus* L.) possessing several interesting agronomic traits that were useful for *P. sativum*, especially in terms of disease resistance. Obando *et al*., 1990 [105]; Baudoin, 1992 [23] obtained interspecific hybrids by protoplast fusion of. *Phaseolus coccineus* L. (PC) and *Phaseolus polyanthus* Greenm. (PP) resistant to *Ascochyta* leaf blight, Bean Golden Mosaic Virus (BGMV), and Bean Fly in Common bean. Durieu and Ochatt (2000) [47] obtained somatic hybrids in Pea possessing stress tolerance and rust resistance.

Somaclonal variations

Somaclonal variations (SV) are genetic or epigenetic changes induced in plant cell which are important for crop improvement. In order to add desirable genetic diversity into the gene pool, induction of somaclonal variation is an alternative to traditional breeding and transgenic techniques. (Larkin and Scowcroft, 1981) [86]. Somaclonal variants can be somatically or genetically stable. (Qin-Mei and Li, 2012) [115]. On the other hand, epigenetic changes are temporary and reversible and not heritable (Meins, 1983) [94]. In Pea, resistance to *Ascochyta blight*, powdery mildew and *F. solani* was achieved through Somaclonal variation (Sharma and Kaushal, 2004; Horacek *et al*., 2013) [133, 61].

Marker Assisted Selection

Marker-assisted selection (MAS) can be used to improve the crop through manipulation of genomic regions that are involved in the desirable trait of interest through DNA markers (Gupta *et al*., 2010) [62]. The MAS has an advantage over visual phenotypic selection since the trait of interest is connected to a molecular marker, which improves the targeted trait's selection effectiveness (Jiang, 2013) [74]. MAS has shown its utility in crop plants for improvement of various traits by reducing the environmental effect and by increasing selection efficiency for a trait of interest (Tester and Langridge 2010) [146]. The availability of a wide range of molecular markers and high-density genetic linkage maps has expanded the field of traditional breeding for the identification of desirable lines with complex features using MAS (Ramesh *et al*., 2020) [117]. Markers i.e. Random Amplified Polymorphism (RAPD), Restriction Fragment Length Polymorphism RFLP), Amplified Fragment Length Polymorphism (AFLP), Sequence Characterized Amplified Region (SCAR) Simple Sequence Repeat (SSR), Sequence Tagged Sites(STS) and derivatives linked to biotic stresses have been identified and well characterized by several workers (Ouedraogo *et al*., 2002; Roman *et al*., 2002; Frew *et al*., 2002; Bouker *et al*., 2004) [107, 121, 54] including India (Taran *et al*., 2001; Gao *et al*., 2004) [145, 55]. Various QTLs, candidate genes, and genes have been reported for abiotic stress (Wu *et al*., 2014; Lee *et al*. 2014b) [154, 87], agronomic (Yuste-Lisbona *et al*., 2014a; Cruz-Izquierdo *et al*., 2012) [157, 36] and food quality related traits (Cichy *et al*., 2013; Krajewski *et al*., 2012) [33, [84]. As a result, genetic maps for many species were established in which potential resistance and/or tolerance loci or QTLs have been located (Tables 2, 3 and 4).

Various approaches of MAS: Under the umbrella of MAS, various molecular approaches are used, such as:

1. Marker-assisted backcrossing (MABC),
2. Marker-assisted gene pyramiding (MAGP),
3. Marker-assisted recurrent selection (MARS) and
4. Genomic –wide association mapping studies

These methods have been used in plant breeding to characterize genetic material and select individuals in the early segregating generation, allowing the breeding cycle to be completed faster and with more accuracy (Nadeem *et al*., 2018) [101].

Marker-assisted backcrossing (MABC)

After 6–7 generations of backcrossing, conventional backcrossing is a very useful approach for transferring oligogenic characteristics from donor parents to recipient parents by recovering the whole genome of recipient parents except the trait of interest. The MABC is a backcrossing approach that relies on molecular markers for assistance to aid in the selection of recipient parents and the recovery of their genomes (Holland, 2004). By introducing gene of interest or quantitative trait loci (QTLs) from donor parent to high yielding popular varieties, the MABC technique has been widely used to remove undesirable traits such as insect and disease susceptibility, anti-nutritional factors, and so on from high yielding popular varieties. (Ribaut *et al*., 2004). The close connection of markers with genes or QTLs is the foundation of MABC. Gao *et al*., (2004) [55] and Smykal *et al*., (2010) [139] have developed primers to assist in selection for PSbMV resistance to improve efficiency during cultivar development. The gene conferring resistance (*sbm-1)* was introgressed from cv. Lifter into PSbMV susceptible line using marker assisted backcross selection. Two varieties, IT93K-452-1 and IT89KD-288 were improved in Nigeria for Striga resistance at IITA by using MABC (Chamarthi *et al*., 2019) [32].

Marker-assisted gene pyramiding (MAGP)

Current breeding programs mainly focus on the development of lines governing resistance to biotic and abiotic stress Modern MAS methods involve pyramiding of different genes to accomplish such goals referred to as MAGP. In MAGP, two or more genes are chosen for pyramiding at the same time. Pyramiding several genes/QTLs from donor parent to recipient parent has been accomplished using a variety of methods, including recurrent selection, backcrossing, multiple-parent crossing, and complicated crossing (Gupta *et al*., 2010) [62]. The most relevant research has been done on common bean rust and anthracnose resistance. (Faleiro *et al*., 2004) [50]. Eleven rust resistant genes (*Ur-1* to *Ur-11*) were pyramided through MAGP approach into common bean cultivars, which also showed combined resistance to other diseases, such as BCMV, BGMV, common bacterial blight and anthracnose (Singh, 2001; Stavely, 2000) [137, 143]. Similarly, molecular markers linked to the majority of genes conferring anthracnose resistance (*Co-1* to *Co-10*) have been reported thereby providing the opportunity to pyramid them in a resistant cultivar through MAS (Kelly and Vallejo, 2004) [79].

Marker-assisted recurrent selection (MARS)

Recurrent selection is an effective plant breeding approach for improving quantitative traits through continuous crossings and selection procedure. Environmental changes, on the other hand, have a negative impact on its selection efficiency, resulting in a delayed breeding cycle. For the targeted traits in MARS, molecular markers are used at each generation level.

At every crossing and selection cycle, selective crossing is performed on selected individual plants. The decision is based on phenotypic information and marker scores. As a result, it improves recurrent selection efficiency and speeds up the breeding or selection cycle. The MARS has been actively utilized to enhance numerous genes or QTLs for polygenic characteristics such as crop yield, biotic and abiotic stress tolerance, and as a forward breeding method for biotic and abiotic stress tolerance. (Eathington *et al*., 2007). Cow pea varieties possessing drought tolerance (IITA Nigeria), grain quality and heat tolerance traits (Mondlane University (EMU), Mozambique), drought tolerance and resistance to Striga, nematodes and *Macrophomina* (ISRA) were developed through MARS by various workers and it was reviewed by Chamarthi *et al*., 2019 [32].

Genome- Wide Association Mapping Studies

The genome wide association mapping (GWAM) approach provides opportunity to explore the tremendous allelic diversity existing in natural germplasm (Deshmukh *et al*., 2014) [40]. A GWAM or linkage disequilibrium mapping (LDM) is used to evaluate associations between markers and trait (s) of interest scored across a large number of individuals. The advancement in genomic technologies has led to better understanding of the genetic basis of traits using GWAM. This technique provides high-resolution genetic variability mapping from germplasm sets that have gone through multiple rounds of recombination (Yu and Buckler, 2006) [156]. GWAM studies have been proved effective by identifying marker trait associations in Cowpea (Lucas *et al*., 2013; Burridge *et al*., 2017; Qin *et al*., 2017) [92, 30, 114] and Common bean (Villegas *et al*., 2017) [152]. The results from this study revealed QTL co-localizations between root traits and seed weight per plant, pod number and Striga tolerance (Burridge *et al*., 2017) [30].

Table 2: Molecular markers linked to desirable genes/QTL for biotic stress resistance in legume vegetables

Crop	Trait(s)	QTL/genes	Type of markers	References
Common bean	Resistance to common bacterial Blight Resistance to bean Common mosaic virus Resistance to anthracnose Resistance to white Mould Resistance to *Fusarium* wilt	QTL QTL, *I* *Are* QTL *PvPR1*, *PvPR2*	RAPD, SCAR, STS, SSR, RFLP, RAPD SCAR SCAR RAPD, AFLP RAPD	Taran *et al*., (2001) [145] Jung *et al*., (1997) [77] Adam-Blondon *et al*., (1994) [3] Kolkman and Kelly (2003) [83] Schneider *et al*., (2001) [128]
Cowpea	Resistance to *Striga gesneriodes* Resistance to *Thrips tabaci* and *Frankliniella schultzei*	*Rsg1* QTL	SCAR AFLP	Bouker *et al*., (2004) Muchero *et al*., (2010b) [97]
Pea	Resistance to powdery mildew Resistance to pea seed borne mosaic virus Resistance to *Fusarium* wilt Resistance to pea common mosaic virus Resistance to rust	*er* *Sbm-1* *Fw* *mo* *Sbm-1*	RFLP STS RFLP RFLP cDNA AFLP	Dirlewanger *et al*., (1994) [44] Frew *et al*., (2002) [54] Dirlewanger *et al*., (1994) [44] Dirlewanger *et al*., (1994) [44] Gao *et al*., (2004) [55]
Faba bean	Resistance tobroomrape Resistance to rust	*Oc1, Oc2,Oc3* *Uvf-1*	RAPD RAPD	Roman *et al*., (2002) [121] Avila *et al*., (2003) [19]

Table 3: QTLs, candidate genes, and genes for abiotic stress resistance in legume vegetables

Crop	Trait	QTL/Gene	References
Common bean	Drought stress response Lodging resistance	*DEGs* QTL	Wu *et al*., (2014) [154] Lee *et al*., (2014b) [87]
Faba bean	Frost resistance (leaf oleic acid content) Drought stress responses	QTL Gene	Arbaoui *et al*., (2008) [16] Abid *et al*., (2014) [1]
Pea	Salinity tolerance Frost tolerance	QTL QTL	Leonforte *et al*., (2014) [88] Klein *et al*., (2014) [82]

Table 4: QTLs, candidate genes, and genes for agronomic, and food quality related traits in legume vegetables

Crop	Trait	QTL/Gene	References
Common bean	Seed dimension, weight, color, and brightness, and number of seed per pod Width, thickness, length, size index, beak length and color of pod Canning quality and color retention	QTL QTL QTL	Yuste-Lisbona *et al*., (2014a) [157] Yuste-Lisbon *et al*., (2014b) [158] Cichy *et al*., (2013) [33]
Faba bean	Days to flowering, flowering length, pod length, number of seeds per pod and number of ovules per pod Vicine–convicine seed concentration	QTL QTL	Cruz-Izquierdo *et al*., (2012) [36] Khazaei *et al*., (2015)
Pea	Protein content Phytic acid concentration and iron bioavailability	QTL QTL	Krajewski *et al*., (2012) [84] Shunmugam *et al*., (2014) [134]

Genetic engineering

Crop improvement through genetic engineering has become a reality (Dunwell, 2000) [46]. Various transformation and regeneration protocols are now available in legume vegetables although in some cases the rate of recovery of transgenic lines is still low. *Agrobacterium tumefaciens* mediated

transformation of pea (Bean *et al*., 1997; Svabova *et al*., 2005) [24, 144], French bean (Nifantova *et al*., 2011) [103], Common bean (Amugune *et al*., 2011) [8], Cowpea (Garcia *et al*., 1987) [57] was an important breakthrough. Both micro-particle bombardment and *A. tumefaciens* (Li *et al*., 2004) have been used for DNA delivery into either embryogenic or organogenic cultures. Some vegetable legume cultivars have been transformed in order to enhance the resistance to biotic and abiotic stresses. Resistance to insects using *Bacillus thuringiensis* genes (Walker *et al*., 2000) [153] and viruses using pathogen-derived resistance (Aragao *et al*., 2002) [13], along with the introduction of constitutively expressed genes encoding pathogenesis-related (PR) proteins or phytoalexins (Samac *et al*., 2004) [125] have been reported in legume vegetables (Table 5). Bottinger *et al*., (2001) [26] were the first to use de novo regeneration with thidiazuron (TDZ) to create transgenic faba bean plants from modified tissues. Hanafy *et al*., (2005) [64] developed a second successful approach based on direct shoot organogenesis from meristematic cells of mature or immature embryo axes. Furthermore, Hanafy *et al*., (2013) [63] over-expressed a potato gene PR10a into faba bean cultivar Tattoo by *Agrobacterium tumefaciens* based upon direct shoot regeneration after transformation of meristematic cells derived from embryo axes, which enhanced tolerance to drought and salinity. Murdock (1992) [100] suggested the focus of studies on genetic transfer in cowpea for the development of improved bioassay systems to use in finding and testing specific insect resistance genes in order to identify specific genes that confer resistance to specific post flowering pests; attempting to make interspecific crosses between wild, insect-resistant *Vigna* species and cultivated *Vigna unguiculata*; and the genetic transformation of cowpea, using particle-mediated and *Agrobacterium*-mediated gene transfer. One of the early attempts in genetic transformation study was of Garcia *et al*., (1986) [56] using leaf discs inoculated with an *Agrobacterium tumefaciens* strain harbouring a Ti-plasmid-derived vector in which two copies of a chimaeric kanamycin resistance gene were found. By means of protoplast fusion and regeneration or by embryo-rescue assisted interspecific crossing e.g. resistance to black aphid in the related species *Vicia johannis* (Birch 1985) [25], could probably be introduced to *Vicia faba*. A number of investigators worked extensively on faba bean transformation and regeneration of transgenic plants (Schiemann and Eisenreich 1989; Ramsay and Kumar 1990; Bottinger *et al*., 2001; Hanafy *et al*., 2005) [127, 1118, 26, 64]. The first attempts to transfer foreign genes into faba bean were attempted using *Agrobacterium rhizogenes* containing the binary vector pGSGluc1 carrying nptII and uidA genes under the control of the bidirectional TR1/2 promoter (Schiemann and Eisenreich 1989) [127].

Table 5: List of some legume vegetables genetically engineered for biotic stress

Legume target	Biotic stress	Gene(s)	References
Phaseolus vulgaris	Bean golden mosaic virus (BGMV)	Rep-TrAP-REn, BC1 (viral genes)	Aragao *et al*., (1998) [14]
	Bean golden mosaic virus (BGMV) Drought	BGMV rep gene HVA1 gene	Faria *et al*., (2014) [51] Kwapata *et al*., (2012) [85]
Cowpea	Salinity	Vacoular Na+/H+ antiporter geneVrNHX1	Mishra *et al*., (2014) [96]
Pisum sativum	*Bruchus pisorum* Pea seed-borne mosaic virus (PSbMV) Alfalfa mosaic virus (AMV)	Alpha-amylase inhibitor (alpha-AI-1) Replicase (NIb) from PSbMV Coat protein from AMV	Schroeder *et al*.,(1995) [130] de Sousa-Majer *et al*., (2004) [41] Jones *et al*., (1998) [76] Timmerman-Vaughan *et al*., (2001) [148]

CRISPR/Cas9 and CRISPR/Cpf1 as Genetic Dissection Tools

The most easy, versatile, and precise approach of genetic manipulation in plants is CRISPR/Cas9-based gene editing. A Cas9 endonuclease and a guide RNA are the two crucial molecules (gRNA). CRISPR RNA (crRNA, a 20-nucleotide sequence complementary to the target DNA) and transactivating crRNA (tracrRNA), which acts as a binding scaffold for the Cas9 endonuclease, are the two short RNA molecules that make up the gRNA. Target site recognition by Cas9 requires the presence of a specific protospacer-adjacent motif (PAM) immediately flanking the target site. The canonical PAM associated with the most widely used Cas9 from *Streptococcus pyogenes* (*Sp*Cas9) is 5′-NGG-3′ (Jinek *et al*., 2012) [75]. This approach allows for a wide range of editing applications, including as insertions, deletions, and point mutations, without the use of donor DNA templates or double-stranded DNA breaks (Anzalone *et al*., 2019; Lin *et al*., 2020) [11, 91]. For transformation, including CRISPR/Cas9 gene editing, it is necessary to have the ability to deliver the DNA/RNA components, with the regeneration of an entire plant. Legumes are well-known for their resistance to the uptake and integration of foreign DNA, as well as their reluctance to regenerate. (Yadav *et al*., 2017; Ochatt *et al*., 2018) [155, 106]. This is compounded by the fact that although some legume tissues are transformable and some will regenerate, the two realities are not always in the same tissue. This is why, rather than simply developing a regeneration protocol, it is critical to build a transformation protocol that incorporates the transformation vector from the onset. Ji *et al*., (2019) [73] successfully applied the CRISPR-Cas9 system to disrupt the symbiosis receptor-like kinase (SYMRK) gene in Cowpea which is indispensable for both nodule and arbuscular mycorrhizal symbiosis. The introduction of gene-editing capabilities via CRISPR technology may address concerns and inspire greater study into vegetable legumes.

Conclusion

In modern agriculture, cross breeding, mutation breeding, and resistance breeding are the most common strategies for improving vegetables. Such time-consuming and tedious methods are insufficient to meet the growing global food demand. To deal with this challenge, marker-assisted breeding, tissue culture and transgenic approaches have been adopted. Crop breeding has been revolutionized by the development of biotechnological techniques. Genome editing technologies outperform traditional agriculture methods in terms of simplicity and specificity. Conventional breeding in conjunction with molecular breeding, genetic tools and resources enable vegetable breeders to scale up their research in the field of legume vegetable improvement.

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