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Biotechnology and its implications in brinjal improvement: A review

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Abstract
Eggplant, also known as brinjal or aubergine (*Solanum melongena* L.), is a major vegetable in many nations. Conventional breeding alone cannot bring the desired qualities and quantities from its wild species where the plants have the capacity to resist or tolerate the biotic and abiotic stress. For resistance to the most serious diseases, including bacterial and fungal wilts, nematodes, and shot and fruit borer pests, eggplant genetic resources have been evaluated. Due to sexual incompatibility, efforts to cross eggplants with their wild relatives lead to minimal outcomes. Nevertheless, the ability of eggplants to function well in tissue culture, particularly in plant regeneration, has made it possible to introduce biotechnology, in particular the manipulation of somaclonal variation, somatic hybridization, haplodisation and gene transfer. To achieve lines with enhanced resistance to little leaf disease and salt, Somaclonal variation was used. The needs can be fulfilled in both quantity and quality by molecular breeding and transgenics. Molecular markers like SSR, ISSR, RAPD, AFLP etc. and Genetic research in brinjal has gained traction over the past few years due to the usage of PCR-based markers.

Keywords: Eggplant, somaclonal variation, genome engineering, molecular markers, haploidisation

Introduction
Brinjal (*Solanum melongena* L.) An economically important vegetable production in several Asian and African countries. Eggplant is similar to tomato (*S. lycopersicum)* and pepper (*Capsicum annuum*) 12 chromosomal autogamous diploid (2n = 24). It is a good source of vitamins and minerals, especially iron, which makes it comparable to the overall nutritional value of tomatoes. (Kalloo, 1993) [36]. The dark purple ones are stated to be high in vitamin C in comparison with white brinjal (Tabing & Tiwari, 2018) [69]. Though the nutritional value of aubergines is lower than that of tomatoes, it is comparable with other popular vegetables (Grubben, 1977) [28]. In addition to being nutritionally rich, brinjal has many therapeutic applications, such as, by its decholestrolating activity due to the presence of polyunsaturated fatty acids, it helps to treat diabetes, asthma and liver complaints (Bhat, 2011; Santhosha *et al.*, 2017) [62]. The farmers were compelled by bacterial wilt to avoid growing not only brinjal in these fields, but also other solanaceous crops such as tomato, bell pepper and chilli. The rates of tolerance to biotic and abiotic tension are always inadequate. Besides hurdles in gene transmission from wild streams, the crop is infested with a plethora of biotic and abiotic stresses. Aubergines, particularly bacterial, fusarium and verticillium wilts, nematodes and insects, are vulnerable to numerous diseases and parasites (Sihachakr *et al.*, 1994) [66]. It exhibits partial, but often inadequate resistance to most of these pathogens (Messiaen, 1989; Daunay *et al.*, 1991). Economic deprivation is among the main causes of malnutrition in India. Their food is sometimes deficient in both quality and quantities owing to the poor socioeconomic standing of certain population groups. National obesity rates in India in 2010 were 14% for women and 18% for men, with some metropolitan areas at rates of up to 40%. Eggplant genetic advantages have been tested for tolerance to the most significant pathogens and pests (bacterial and fungal wilts, nematodes and borers of shooting and fruit).

Conventional breeding in eggplant
However, it is possible to cross wild eggplant ancestors with cultivated eggplants (Plazas *et al.*, 2016) [58]. Interspecific crosses, However, S.melongena and the other related Solanum species with desirable agronomic properties have often been limited by sexual barriers. (Behera and Singh, 2002). As an eggplant, it is vulnerable to a wide range of biotic and abiotic stress, and thus one of the main problems of traditional breeding and biotechnology is the insertion of resistance genes for increased yield and fruit quality. More than 25 species of genes solanum have been used for crossbreeding with cultivated brinjal till date and yielded with an unsatisfied success rate. Rotino *et al.*, 2014.

Interspecific hybrids of eggplants serve as rootstocks to protect plants from fungal and bacterial infestations. (Rotino *et al.*, 1997) [57]. It was also seen that S. Incanum and cultivated eggplant can be easily crossed and the interspecific hybrids resulting from it are fertile. (Ranil *et al.*, 2017) [58]. Importantly, the use of wild species also results in the transfer of genes with undesirable traits or genomic regions.

Table 1: List of eggplant species with resistance traits

Biotic stress	Source of resistance gene	reference
1. Bacterial wilt	*S.melongena* var *incanum, S.integrifolium* and *S. torvum S. hispidium, S. integrifolium, S. melongena* var *insanum, S. nigrum, S. sisymbrifolium, S. xanthocarpum, S. texanum, S. torvum S. integrifolium, S. torvum*	(Sugha, *et al*. 2002, Swarup, 2006, Yamakawa, 1982, Sheela, *et al*., 1984.) [67, 68]
2. Verticillium wilt, Fusarium wilt	*S.indicum, S. integrifolium, S. incanum S. caripense, S. periscum, S. scabrum, S. sisymbrifolium, S. torvum, S. integrifolium, S. melongena var insanum, S. nigrum, S. sisymbrifolium, S. torvum.*	Yamakawa & Mochizuki, 1979, Swarup, 2006, Sakata, *et al*. 1989, Fassuliotis & Dukes, 1972, Petrov *et al* 1989, Swarup, 2006 [3, 68, 64]
3. Little leaf:	*Solanum viarum S. incanum and S. sisymbrifolium S. viarum, S. incanum, S. sisymbrifolium, S. gilo, S. integrifolium*	Anjaneyulu & Ramkrishnan, 1968; Chakrabarti & Choudhury, 1974 Mayee & Munsi, 1973, Swarup, 2006 [68]
4. Cercospora solani:	*S. macrocarpon*	Madalageri *et al*., 1988
5. Shoot and fruit borer:	*Solanum sisymbrifolium, S. integrifolium, S. xanthoranpum, S. nigrum and S. khasianum S. integrifolium, S. sisymbrifolium, S. xanthocarpum, S. gilo S. xanthocarpum, S. khasianum, S. integrifolium, S. sisymbrifolium.*	Lal & Ahmad, 1965; Behera *et al*., 1999; Chadha, 1993, Swarup, 2006, Chelliah & Srinivasan, 1983, Sharma *et al*., 1980, Khan *et al* 1978 [68, 8]
6. Root Knot Nematode	*Solanum sisymbrifolium, Solanum torvum S. aethiopicum, S. torvum, S. sisymbrifoilim S. sisymbriifolium, S. torvum, S. aethiopicum, S. warscewiczii*	Naidu *et al*., 2006, Swarup, 2006 Fassuliotis & Dukes, 1972, Ahuja *et al*., 1987, Di Vito *et al* 1992, Daunay & Dalmasso, 1985, Hebert, 1985 [68]

These undesirable traits can be revoked for several generations of backcrossing, and features like fruit size can be easily restored for several generations of backcrossing. (Kouassi *et al*., 2016). While different approaches can produce good viable hybrids, wild relatives need to be used with caution because they can transfer undesirable characteristics on to hybrids. (such as strong susceptibility to Colletotrichum gloeosporioides and bitter taste, Due to the elevated amount of steroid saponin present in S. Turvum and S. Lineanum, respectively) in addition to characteristics that could be beneficial for hybrids (Kashyap *et al.*, 2003). Overall, the use of traditional breeding for the improvement of eggplant is limited by a range of barriers, such as drag linkage. (pre- and post-fertilization), etc. (Zulkarnain *et al.*, 2015). Transgenic technology can be seen as a genetic enhancement of eggplants to address the above-mentioned limitations of conventional breeding (Sharma *et al.*, 2002).

Somaclonal Variation

Eggplant reaction to *in vitro* cultivation and, in particular, its capacity to regenerate plants have been studied as a prerequisite for the implementation of any further biotechnological techniques. Several studies found that plants can be quickly regenerated in Aubergines by in somatic embryogenesis and vitro organogenesis (Kamat and Rao, 1978; Fassuliotis *et al.*, 1981; Alicchio *et al.*, 1982; Zelcer *et al.*, 1983; Sharma and Rajam, 1995a; Fari *et al.*, 1995a; Magioli *et al.*, 1998) (Matsuoka and Hinata, 1979; Gleddie *et al.*, 1983; Fobert and Webb, 1988; Ali *et al.*, 1991; Kalloo, 1993; Sharma and Rajam, 1995a, b; Yadav and Rajam, 1997, 1998) [36]. Cultivated explants (stem, hypocotyl, leaf, cotyledon, root) and cell suspension, but also anthers (Isouard *et al.*, 1979), protoplasts (Sihachakr *et al.*, 1993) [66] and isolated microspores (Miyoshi, 1996). Shoot regeneration has also been reported in various wild parents of the Aubergine: *S. khasianum*, *S. indicum*, *S. aviculare*, *S. aethiopicum* group gilo, *S.torvum*, *S. aviculare*. (Fassuliotis, 1975; Kashyap *et al.*, 1999, Bhatt *et al.*, 1979; ; Gleddie *et al.*, 1985; Rao *et al.*, 1988; Kowalczyk, 1983).

Haploidisation

In Brinjal, The haploid plants were collected from cultured anthers or isolated microspores through *in vitro* androgenesis. The first haploids of the brinjal were regenerated from a anther culture by the Chinese research group of haploid breeding (Raina and Iyer, 1973, Isouard *et al.*, 1979, Dumas de Vaulx and Chambonnet, 1982). For the further growth of cultivated anthers, a pretreatment time of 8 days at 35 C, in a medium containing 2, 4-D and kinetin, was required (Chambonnet, and Dumas de Vaulx 1982). The anther and genotype effect levels, as well as the conditions of culture, such as temperature and growth regulators in the culture medium, have been optimised for Regenerating plants (Rotino *et al.*, 1996) [57]. The use of *in vitro* androgenesis through anther culture culminated in the development of potent double haploid (DH) lines after colchicine therapy. Some of them managed to produce fruit at low temperatures. According to the genotype of the anther donor, fruit yield and quality can be decreased due to high consanguinity (Dore and Dumas de Vaulx, 1990) or increased in some lines. (Rotino, 1996) [57]. Haploid plants were also successfully regenerated from cultivated eggplant microspores (Gu, 1978; Miyoshi, 1996). The highest results were reached after 3 days of pretreatment, at 35 C, with a volume deficient in sucrose (Miyoshi, 1996). Sucrose appetite has been reported to suppress gametophytic growth and synthesis of cultivated microspore's DNA. During the initial culture a brief period of elevated temperature was observed to suppress the natural growth of the microspores, and thus induce androgenesis. Eggplant breeders are increasingly using *in vitro* androgenesis to quickly get fixed lines from heterozygous material, and to produce commercial F1 hybrids. The crosses are done manually, most of the time (Daunay *et al.*, 1997). However, eggplant has been described as functional male sterility. It is regulated by a single recessive gene which tends to be related to the coloured purple fruit gene (Phatak *et al.*, 1991).

Somatic Hybridization

In order to bypass possible sexual barriers or boost the fertility of hybrids normally obtained via traditional breeding methods, somatic hybridization experiments have been performed through protoplast fusion to promote the integration of agronomically significant traits from wild relatives into the cultivated eggplant. They were primarily resistant to diseases and pathogens and, in particular, to bacterial and fungal wilts, nematodes, mites and fruit borers. (Sihachakr *et al.*, 1994). Without comprehensive genetic or molecular information of the genes encoding for such characters, somatic hybridisation provides a way of increasing nuclear and cytoplasmic variability and transferring beneficial agronomic traits (Jones, 1988). The management of reliable protocols for protoplast isolation and culture, and plant regeneration in particular, is needed for protoplast fusion. In most cases, eggplant mesophyll tissues have been the predominant option since it is easy to achieve a high yield of protoplasts (Bhatt and Fassuliotis, 1981; Jia and Potrykus, 1981). It has also been shown that stems and petioles are proficient sources of protoplasts (Sihachakr and Ducreux, 1987; Sihachakr *et al.*, 1993). Fourvier *et al.* (1995) studied the first cytological events triggered as protoplasts divide to form plantlets, demonstrating that elevated cytoplasmic and nuclear activity followed cell wall formation and cell proliferation in eggplants. Both chemical and electrical fusion processes have been effectively used to create somatic eggplant hybrids with their wild relatives. The first somatic fusion of eggplant with S. Sisymbrifolium has regenerated 26 aneuploid somatic hybrids. They just had a S. Chloroplast from sisymbrifolium Genome and root knot nematodes were strongly resistant and potential resistant to spider mites (Gleddie *et al.*, 1986). However, their utility in a breeding programme is severely limited due to high hybrid sterility. A total of 10 and 19 Somatic Tetraploid eggplant Hybrids After chemical and electrical fusion respectively, *S.torvum* was retrieved (Guri and Sink, 1988a; Sihachakr *et al.*, 1989a)

Molecular Markers

In eggplants, phylogenetic maps and DNA markers were established by chloroplast DNA analysis. RAPDs, AFLP analyses, Isoenzymes and seed storage protein were also used to classify eggplants and their wild relatives. (Sakata *et al.*, 1991; Sakata and Lester, 1994, 1997; Isshiki *et al.*, 1994b, 1998) [61, 64] (Karihaloo *et al.*, 1995), (Mace *et al.*, 1999), (Isshiki *et al.*, 1994a; Karihaloo and Gottlieb, 1995), (Mennella *et al.*, 1999). Using RAPD markers, a partial genetic linkage map, consisting of 94 loci and 13 linkage groups spanning 716.7 cm in physical length, was established. This map was made of 168 F2 segregating progenies resulting from a Crossing between a line bearing fruit of a very high quality and a line resistant to bacterial wilt. Interestingly, two of the linkage groups could be resistant to bacteria, but additional codominant markers should be mapped to allow further QTL analyses to be carried out (Nunome *et al.*, 1998). A more recent genetic linkage map of eggplant RFLP for qualitative and Quantitative characteristics were built from a population of F2 segregation resulting from an interspecific cross With *S.linneanum* (Frary *et al.*, 2000).

Prospects of molecular farming in eggplant

Instead of using conventional bio-production processes involving yeast, bacteria and engineered insects and animal cells, plant molecular farming has emerged as a novel, innovative branch of plant biotechnology in which plants are equipped to generate vast quantities of recombinant pharmaceutical and industrial proteins. (Daniell *et al.*, 2001; Horn *et al.*, 2004; Obembe *et al.*, 2011). This unique system provides many advantages over traditional bio-production systems in terms of protection, size, cost-effectiveness and ease of distribution and storage. In the recent past, a variety of plant systems such as Arabidopsis, corn, carrot, tomato, etc. have been used as recombinant bio-factories for the expression of different proteins, including potential vaccines and pharmaceuticals, using different recombinant process modifications and using the most suitable methods and techniques (Nykiforuk *et al.*, 2006 Chung *et al.*, 2014 Rojas-Anaya *et al.*, 2009 Hiwasa-Tanase *et al.*, 2012).Furthermore, the emphasis was on the intermittent dissemination of virus-like particles (look-like viruses, but without viral genomic material), which could be a source of new vaccines (Marsian and Lomonossoff, 2016).

Genome engineering

Genome engineering involves a variety of techniques that make it possible to modify, delete, replace or implant unique genomic sequences of interest in the genome of a living organism into a targeted site (Gaj *et al.*, 2016). Unlike methods of genetic transformation that randomly inject a gene into a host genome, genome editing guides the insertions to site-specific locations. The availability of sequenced genomes and the emergence of highly efficient, complex editing technologies that guide endonucleases have permitted scientists to alter plant genomes directly at the location (Komor *et al.*, 2017). These technologies are based on the induction of double-stranded DNA cuts in the genomes of different species, including plants, which are then repaired either with homology-directed (HDR) repair or with non-homologous end-joining (NHEJ) (Rinaldo and Ayliffe, 2015). Four nuclease systems were primarily used, which are 1.ZFN = Zinc Finger Nucleases; 2. TALEN= Transcription Activator Like Effector Nucleases; 3.Meganucleases; 4. CRISPR Cas9= Clustered regularly inter-spaced Short Palandromic Repeats. ZFN, TALEN and CRISPR are more accurate and precise to edit the target sequence of DNA that Resistance to *Verticillium dahliae* and Ralstonia solanacearum from S. torvum was Meganucleases. Two outstanding reviews on the value of genome editing in vegetables, especially solanaceous food crops, have recently been published (Cardi *et al*., 2017; Van Eck, 2018).

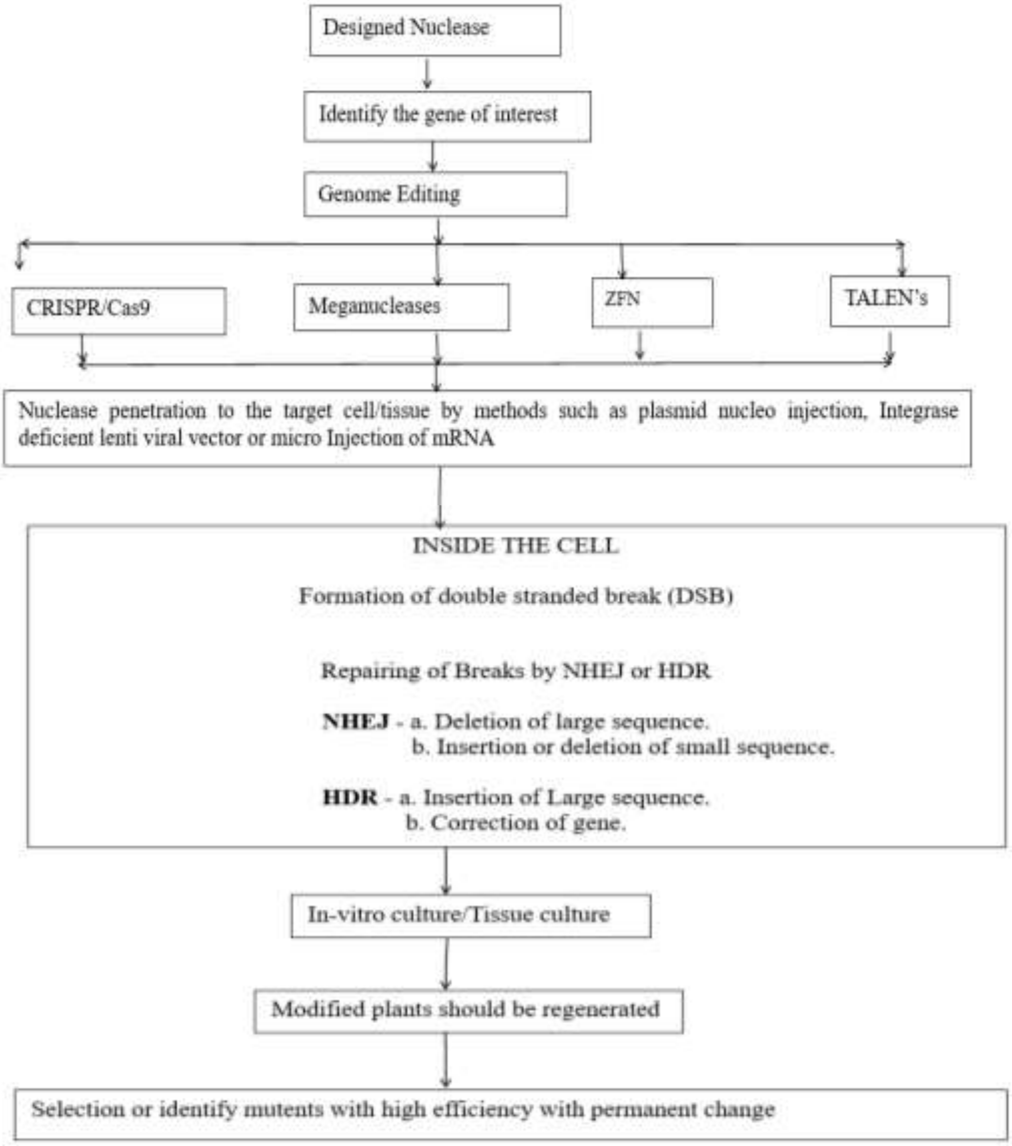

Fig 1: Flow chart of Steps relevant to the distribution of the engineered nuclease for genome editing.

Mutants have been developed that exhibit resistance to various pathogens, including Pseudomonas syringae pv. *Phytophthora capsici* and *Xanthomonas* spp. in tomatoes. Using the CRISPR / Cas-9 method to produced minor deletions in the SlDMR6-1 gene (Thomazella *et al.* (2016).

inserted Via electrofusion of protoplast in S. melongena. Genomic in situ hybridisation (GISH) has been used for the genome study of somatic hybrids (Collonnier *et al*., 2003). Introduced resistance of bacterial wilt from S. melongena by somatic hybridization into Solanum tuberosum (Yu *et al.* (2013). Bt brinjal is a transgenic brinjal introgressed with cry1Ac gene under the guidance of a high-level expression enhanced CaMV 35S promoter for successful built-in fruit and shoot borer control. The cry1Ac gene encodes the selectable marker genes for the insecticidal protein derived from the soil bacterium Bacillus thuringiensis, powered by enhanced CaMV 35S (promoter): the npt II (neomycin phosphotransferase) gene derived from the prokaryotic transposon Tn5 and the aad isolated from the transposon Tn7 gene.

Conclusion

In the improvement of vegetable crops, plant biotechnology has played a key role. Plant biotechnology is an ever-changing area, and a new technology is introduced to the plant biotechnology system every day. The assessment of eggplant genetic resources, so far largely focused on phenotypes, has revealed various useful characteristics in its wild relatives, but very few molecular marker data are available for characterisation. Efforts are being made to improve them by RFLP, RAPD and AFLP techniques. These studies should allow, firstly, the enrichment of the current genetic linkage map and the creation of new ones, and, secondly, the acceleration of the identification and isolation of pest and pathogen resistance markers and genes that are useful for MAS and other gene transfers. Vegetables such as tomatoes are a model for biotechnological science. Genetic advancement of eggplants is mainly based on conventional plant breeding Methods. As there are comprehensive studies on regeneration through organogenesis and somatic embryogenesis, the development of haploids and plant regeneration studies have greatly contributed to the eggplant. However, somatic hybridization has been widely studied in eggplants to address cross-incompatibility barriers, with the primary goal of adding useful agronomic traits from wild to cultivated species. In eggplant somatic hybrids, useful traits such as herbicide resistance, bacterial and fungal wilts, mites and nematodes have been successfully expressed (Sihachakr *et al*., 1994). There has been a significant increase in eggplant breeding in the last decade. In tissue culture and protoplasts, the ability of eggplants to react well has facilitated the use of numerous and effective biotechnology techniques for genetic resource management and eggplant improvement (Gunasekar & Kannan, 2018). Moreover, with the development of somatic hybrids, through increasing nuclear and cytoplasmic variability, new perspectives have opened up for the enrichment of current genetic pools. The majority of eggplant transformation experiments was mediated by transformations of agrobacterium, covering several biotic and abiotic difficulties. There is an immediate need, along with a broader adaptability to climate change, for eggplant varieties resistant to virus, fungal and bacterial pathogens. For the introduction of new genes encoding resistance to biotic and abiotic stresses, genetic engineering experiments in eggplants need to be further exploited, as only Bt endotoxin and parthenocarpy genes have been successfully applied so far in eggplants. Production of Bt brinjal will minimise production costs, along with minimal risk of adverse effects on health and the environment. Without further delay, release of Bt brinjal must be urged, as many countries Without any complications, have enabled the production and use of genetically modified crops. Inadequate research for the safety evaluation of Bt brinjal should be addressed, including clinical trials, according to regulatory authorities. Therefore, in effort to shift laboratory results to the fields, close cooperation between biotechnologists, plant breeders, plant pathologists and agronomists would be necessary.

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