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Review

Genetic Engineering of Eggplant (*Solanum melongena* L.): Progress, Controversy and Potential

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Abstract: Eggplant (*Solanum melongena*) is the third most important vegetable in Asia and of considerable importance in the Mediterranean belt. Although global eggplant production has been increasing in recent years, productivity is limited due to insects, diseases, and abiotic stresses. Genetic engineering offers new traits to eggplant, such as seedless parthenocarpic fruits, varieties adapted to extreme climatic events (i.e., sub- or supra-optimal temperatures), transcription factor regulation, overexpressing osmolytes, antimicrobial peptides, *Bacillus thuringiensis* (*Bt*) endotoxins, etc. Such traits either do not occur naturally in eggplant or are difficult to incorporate by conventional breeding. With controversies, *Bt*-expressing eggplant varieties resistant to eggplant fruit and shoot borers have already been adopted for commercial cultivation in Bangladesh. However, to maximize the benefits of transgenic technology, future studies should emphasize testing transgenic plants under conditions that mimic field conditions and focus on the plant's reproductive stage. In addition, the availability of the whole genome sequence, along with an efficient in vitro regeneration system and suitable morphological features, would make the eggplant an alternative model plant in which to study different aspects of plant biology in the near future.

Keywords: eggplant; *Solanum melongena*; genetic engineering; *Agrobacterium*; transgenic plant

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1. Introduction

Eggplant (*Solanum melongena* L; family: Solanaceae) is an important vegetable crop that is cultivated for its fruits. It is also known as brinjal, aubergine, melongene, garden egg, or guinea squash in different countries. Recent statistics from the Food and Agriculture Organization (FAO) show that the eggplant ranks fifth, after tomatoes, onions, cucumbers and gherkins, and cabbages in terms of total global production, with 52.3 million tons being produced in 2017. In China and the Indian subcontinent, it is one of the most popular and principal vegetable crops and is grown throughout these regions, except at higher altitudes. It ranks as the third most consumed vegetable in China, India, and Bangladesh, where roughly 40% of the global population resides. Eggplant is also produced in the Mediterranean basin to a lesser—but still important—extent. Global eggplant production is concentrated in five leading countries, namely China, India, Egypt, Turkey, and Iran (~93% of the total global production; Table 1). However, the yield in these countries is much lower compared to European countries [1,2].

Eggplant was originally domesticated in what is now India and Bangladesh [3]. Nowadays, the crop has been bred to be cultivated year-round in various agro-climatic regions all over the world. Despite its perennial nature, it is grown as an annual crop. Eggplant fruits exhibit a wide range of shapes and colors, ranging from long club-like to egg shaped to ovoid shaped fruits, displaying various colors spanning from different shades of purple, yellow, and green, to white. Most of the commercially important varieties

of eggplant have been developed from the long-established types that originated from tropical India and China.

Table 1. Global status of brinjal production in 2017.

Country	Production (ton)	Contribution (%)	Acreage (ha)	Yield (kg/ha) [§]
China	32,883,567	62.9	784,966	41,892 (12)
India	12,510,000	23.9	733,000	17,067 (46)
Egypt	1,307,793	2.5	48,253	27,103 (29)
Turkey	883,917	1.7	25,592	34,538 (17)
Iran	654,149	1.3	21,225	30,776 (20)
Indonesia	535,436	1.0	43,905	12,195 (59)
Japan	307,800	0.6	9160	33,603 (18)
Italy	286,473	0.5	9449	30,318 (21)
Philippines	241,901	0.5	21,446	11,280 (64)
Bangladesh *	226,000	0.4	31,556	7924 (71)
Global	52,309,119	100	1,858,253	28,149

Ref: FAOSTAT 2019 [2]; * BBS 2014 [1]; [§] Number in parenthesis is the country rank based on yield (kg/ha).

According to the International Treaty on Plant Genetic Resources for Food and Agriculture, eggplant is one of the 35 crops considered to be most important for food security [4]. In Bangladesh and India, eggplant is mainly cultivated on small family farms and is an important source of nutrition and income for many resource-constrained farmers. Their overall economic security and well-being depend on the crop [5]. The crop still produces a yield (though reduced) when under stress conditions; therefore, it is a preferred crop of marginal and resource-poor farmers in almost all agro-ecological zones in this region.

Nutritionally, eggplants contain relatively low amounts of proteins, fats, soluble carbohydrates, important vitamins, and minerals. However, they are very rich in fiber. They are highly beneficial for the regulation of blood sugar levels, and for reducing plasma cholesterol levels, and aortic cholesterol content [6]. Additionally, the high levels of anthocyanins found in many cultivars exhibit numerous beneficial effects on human health.

The eggplant yield in major producing countries is much lower than in developed countries, as the adoption of various agricultural practices in developed countries has increased eggplant yields. It is important to develop improved varieties that are adapted to local climatic conditions for producing economically profitable yields. Thus, breeding objectives include: the exploitation of hybrid vigor for increasing productivity; resistance against insect pests, including fruit and shoot borers; resistance against wilt and other diseases, including those of viral origin; sustainability of yield under extreme climatic events; and the development of locally preferred cultivars. Eggplant improvement initiatives have been undertaken by various research institutes which benefit from classical breeding. These can also be complemented by a new generation breeding approach—genetic engineering—which breaks the barrier of gene transfer between unrelated organisms. Several excellent reviews have described the progress that has been made towards culturing eggplant in vitro [7]. However, the status on the progress in genetic engineering [8,9] requires updating. This review summarizes the application of genetic transformation techniques in eggplant from their time of implementation to the present. The aims of such work are discussed, including factors affecting transformation, transgenic technology for resistance against pests, disease, and abiotic stresses, along with controversies for commercial cultivation, and the future prospects of eggplant as a crop plant model organism, following the availability of its whole genome sequence.

2. Eggplant Production Constraints

The yield of eggplant is much lower in two major growing countries, India and Bangladesh, than the global average (Table 1). Nearly 80% of the eggplant production area is cultivated by small, marginal, and resource-poor farmers in India [10]. These cultivators are vulnerable to environmental production constraints such as irrigation shortages and in-

festations of insects and diseases. By contrast, the growing eggplant in developed countries is quite 'high-tech' with the control of temperature, light, irrigation, hormone applications, and by using improved varieties adapted to the specific cultivation environment.

2.1. Biotic Stresses

Eggplant is a versatile crop that is well-adapted to different agro-climatic environments. Thus, it is exposed to a number of insects and diseases that cause significant economic loss. In the tropics and subtropics, the eggplant fruit and shoot borer (EFSB), *Leucinodes orbonalis*, is the single most devastating insect causing up to 65% yield loss [11]. The EFSB larvae (especially third- and fourth- instars) bore into tender shoots, resulting in the plant ultimately wilting and becoming unable to bear fruits. In addition, the larvae bore into fruits, making them unmarketable. The occurrence of the pest inside the plant body saves them from insecticidal contact. Escaping from insecticides and a lack of natural resistance in cross-compatible species make EFSB the major eggplant pest. Furthermore, eggplant fruit borers (*Helicoverpa armigera*), stem borers (*Euzophera perticella*), hadda beetles (*Epilachna vigintiopunctata*), as well as some sucking aphids and parasitic nematodes (*Meloidogyne* spp), are important pests affecting eggplant cultivation.

Soil pathogens can also pose a significant obstacle to eggplant cultivation, particularly when the soil moisture content is high. Wilting caused by a number of fungal genera such as *Fusarium*, *Verticillium*, *Rhizoctonia*, *Sclerotium*, and *Phytophthora* cause considerable loss in crop yield. Wilts are characterized by the yellowing of foliage and drooping of the apical shoot to the ultimate death of plants. Infection of *Pythium spp*, *Phytophthora parasitica*, *R. solani*, and *Sclerotium rolfsii* causes sudden wilting of seedlings in nursery beds. Besides, bacterial wilts caused by *Pseudomonas solanacearum* are of economic significance [12].

Aphid-transmitted viral disease (caused by Potato Virus Y) exhibits mosaic mottling of the leaves and stunting of plants. Plants show stunted growth with deformed leaves. Among other viruses that affect eggplant are the *Cucumber mosaic virus*, *Potyvirus*, *Eggplant mottled crinkle virus*, and *Alfalfa mosaic virus*. The little leaf disease of eggplant is caused by mycoplasma and is transmitted through sap by insects. The plants show chlorosis in young leaves which are followed by axillary bud proliferation. The affected plants show a reduction in the above-ground plant parts, such as leaves and nodes with a bushy appearance. In cases of severe infection, the plants become sterile, and flower and fruit setting are negligible. The affected plants are severely stunted. The disease has been reported from all eggplant growing areas. Diseased plants virtually do not produce any marketable fruits and overall cause up to 90% yield losses in certain cases.

2.2. Abiotic Stresses

Climatic conditions are extremely important for optimal growth of eggplant and better-quality fruits. Drastic variation in climate conditions negatively affects eggplant in terms of production as well as quality (Figure 1). It has been reported that exposure to salinity stress at an early stage significantly reduces the fruit yield in eggplant [13]. The optimal temperature for the growth and development of eggplant ranges from 22 °C to 30 °C. Eggplant is very sensitive to cold temperatures. Low temperature during winter limits root growth, reduces plant vigor, and produces deformed fruits. The plant is very susceptible to severe frost. Temperatures above the optimal temperature limit plant growth, increase flower drop and poor fruit settings, reduce productivity, and damage quality. Drought also reduces photosynthesis and subsequent growth of plants. It has been reported that the effect of transient drought can be as devastating as continuous drought on yield in many crops [14]. Despite being transient, early water shortage can significantly stifle plant growth, and the impact of this was found to be negative on photosynthetic capacity, even when normal conditions are restored [15]. At the field level, such transient drought episodes may occur, even in many intensive eggplant cultivation areas due to irrigation water shortage (e.g., reduced water flow in the river, lowering groundwater level). The simultaneous exposure to drought and heat (common in field conditions) significantly

reduces the quality (bitter-tasting fruits) and marketability (poor color formation and sun scalding, the formation of brown areas on fruit surfaces that are exposed to the sun) of the fruit. Heat and drought stress promote the development of a fungal disease on eggplant, which spreads rapidly through the crop. Eggplant is a high-water plant, though it does not withstand waterlogging. Exposure to waterlogging causes defoliation and flower dropping and accelerates pathogen infestation associated with wilting and rotting disease.

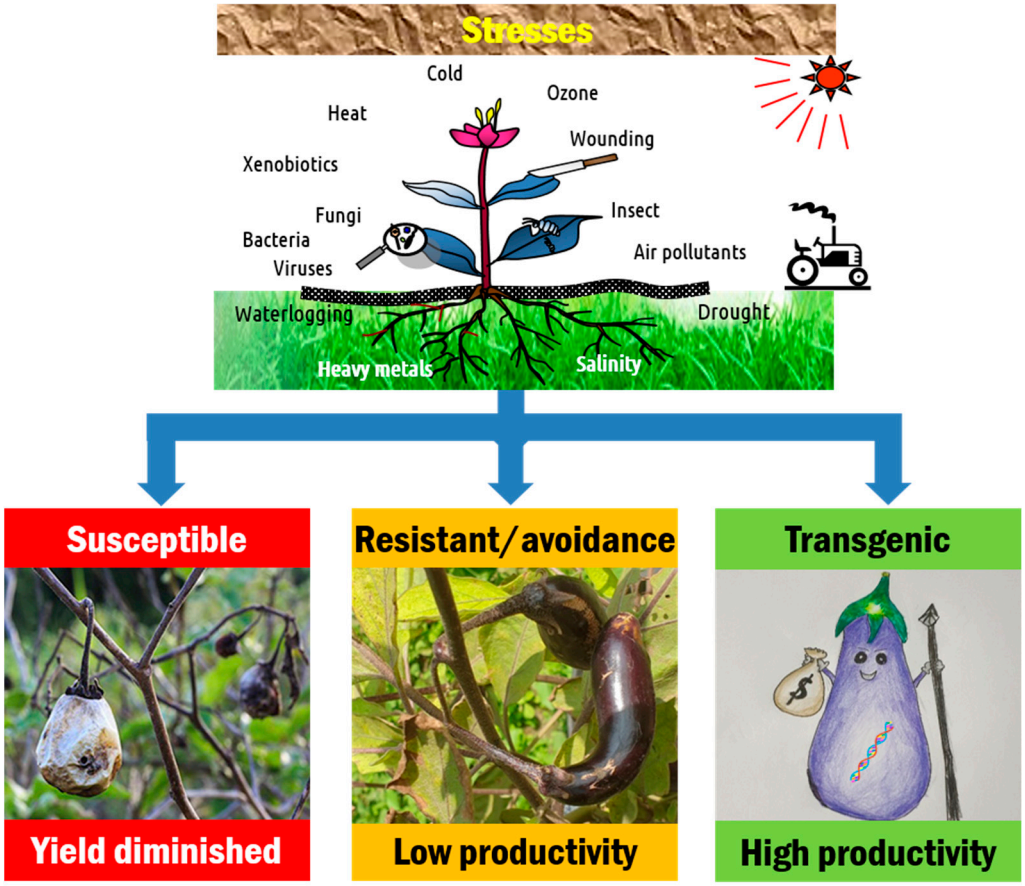

Figure 1. Stress, response, and production potential of crops. Most high-yielding cultivars are poorly adapted to environmental extremes, while landraces are able to tolerate or avoid stresses and are low-yielding. Due to poor recombination of these desirable traits through conventional hybridization, direct introduction of a single or several genes (transgenic) to modern cultivars conferring stress tolerance would be a feasible solution to sustain yield under changing agricultural conditions.

Due to global climate changes and various anthropogenic activities, the occurrence of environmental stresses that limit yield is frequent in major eggplant producing areas. These include a rise in temperature, reduced rainfall and river flow, increased salinity, and a shift in rainfall pattern [16]. The occurrence of abiotic stresses such as drought and temperature vary unpredictably across different years and within fields. As eggplant is moderately sensitive to some of these stresses, there is an urgent need of its genetic improvement to withstand stress throughout its life cycle in order to meet the necessary economic threshold. This is particularly important for developing countries like Bangladesh, China, and India, where subsistent farmers are the major eggplant producers.

3. Quality and Other Issues

Eggplant fruits contain the highest amount of chlorogenic acid (5-O-caffeoyl-quinic acid, or CGA) among major vegetables, fruits, and plant products, providing significant amounts of CGA to the diet [17]. CGA is beneficial to human health for its antioxidant, anticarcinogenic, antiinflammatory, cardioprotective, antiobesity, and antidiabetic properties.

Nevertheless, there is a wide variation in eggplant CGA content, ranging from 0.15–2.8% by dry weight. Some wild relatives of eggplant, such as *S. incanum*, contain much higher levels of CGA. These have raised interest in the development of new 'healthier' eggplant varieties with high CGA content. Several breeding strategies have been proposed for this, including exploitation of the natural variation, development of hybrids with good agronomic and commercial characteristics, as well as interspecific hybridization to introgress favorable alleles from the wild species into the genetic background of cultivated eggplant.

The absence of seeds in most fruits generally is appreciated by consumers both of fresh consumption and in processed form. Seedlessness is a major quality trait of the fruits when seeds are hard or exhibit a negative taste. The commercial ripeness of eggplant fruits precedes its physiological maturity. Thus, the presence of seeds considerably depreciates the value of fruits for both the fresh and the processed market. The presence of seeds causes a faster and more intense browning of the fruit flesh upon cutting. Additionally, increased synthesis of saponin and solasonin compounds is observed to cause a bitter taste and a harder flesh [18]. Thus, seedlessness can increase the palatability of eggplant to consumers. Seedless parthenocarpic eggplant has been produced through the inclusion of natural mutants [19] in the modern breeding program and genetic engineering. However, the non-genetic approach, such as spraying plant growth regulators, is the most commonly applied procedure.

4. Biotechnological Approaches for Genetic Improvement

Cultivated eggplant has been primarily subjected to intensive breeding programs, in China, India, Bangladesh, Western Europe, Turkey, and Japan, with intensive production of this crop. Many open-pollinated as well as F1 hybrids, with differentiated phenotypes, are the result of breeding works held in the last thirty plus years. The main breeding objectives are yield or tolerance to biotic and abiotic stresses (waterlogging, drought, low or high temperatures, salinity), and improvement of quality (uniformity, intense color, lack of prickles). However, cultivated eggplant genotypes often have insufficient levels of resistance to biotic and abiotic stresses. Genetic resources of this species have been assessed for resistance to its most serious diseases and pests [20]. The attempts at crossing eggplant with its wild relatives had limited success due to sexual incompatibilities.

Somaclonal variation is a possible way of exploiting genetic variation induced in vitro. In eggplant, somaclonal variants for resistance to atrazine [21] and culture filtrate of *Verticillium* have been reported [22,23]. In vitro induction of mutation has been successful by applying dimethilsulfonate in meristem culture [24]. The mutant progeny showed a number of variations in morphologic traits. For example, some lines exhibited superior fruit productivity in comparison to control. Heritable somaclonal variations in leaf and fruit shapes have been reported in plants derived from somatic embryos induced by 2,4 D or NAA [25]. However, the practical application of somaclonal variation in eggplant is still limited.

Conventional approaches to breeding crop plants with improved pest resistance and abiotic stress tolerances have so far met limited success due to their laborious and time-consuming nature. On the other hand, high-yielding varieties generally require high input and are not well adapted to environmental extremities. Until recently, improved tolerance of most high-yielding varieties to abiotic stress has been achieved by crossing with landraces or wild relatives. In general, the introduction of genes to high-yielding varieties for conferring tolerance to abiotic stresses through crossing with landraces typically shows poor recombination [26]. Abiotic stress tolerance genes may be linked with undesirable traits, such as low yield, and are eliminated during selection for higher yield. Some cultivars are adapted to grow in a particular (narrow) season and are able to avoid stresses. These may have a small share of the overall productivity of a geographic area. Direct introduction of a single or several genes (transgenic) to modern cultivars would be feasible to confer tolerance to stress factors and sustain yield under stress conditions (Figure 1). Transferring of gene(s) from heterologous species provides the means of selec-

tively introducing/modifying new traits into crop plants and expanding the gene pool beyond what has been available to conventional breeding approaches. Plant genetic transformation offers the opportunity for breeding various crops through complementing or accelerating conventional breeding methods [27]. Genetic engineering approaches have been applied to the eggplant. In the subsequent sections, we discuss the journey of eggplant transformation—from the beginning of transformation dealing with its technical aspects to the commercial adoption of the transgenic crop—as well as the application of the latest technology, the genome editing approach (Figure 2).

4.1. Technical Aspects of Genetic Transformation in Eggplant

To date, all genetic transformation experiments in eggplant have been carried out through *Agrobacterium*, except for a couple of reports that have used a biolistic system (discussed later). The first report on successful genetic transformation through *Agrobacterium tumefaciens* was in 1988 by Guri and Sinks [28]. They transformed in vitro grown eggplant leaves with a pMON200 vector containing the *nptII* gene. Many researchers have worked to establish the transformation protocol as well as to develop stable transgenic plants for inclusion in breeding programs. However, a comprehensive study to optimize the affecting common factors (genotype, explants, *Agrobacterium* strains, selection markers, culture conditions) in a single study is still missing. A summary on various parameters of eggplant transformation is given in Table 2.

4.1.1. Mode of Plant Regeneration for Transformation

Efficient transformation methods essentially require good control of the plant regeneration from the infected explants through either direct or indirect organogenesis. A recent review summarizes various modes of plant regeneration in eggplants [7]. Availability of efficient and genetic transformation-compatible protocol is crucial for the transformation studies undertaken. Eggplants are very amenable to direct shoot regeneration from various tissues, including cotyledons, hypocotyls, and leaves. This is much simpler and less time-consuming than the callus-mediated regeneration system applied in many plants. Thus, these tissues are the widely used explants in the *Agrobacterium*-mediated transformation of eggplant. However, the regeneration efficiency is affected by different factors, such as growth regulators, explant type, and genotype. Most of the organogenesis systems reported are based on supplementing culture media with auxins and cytokinins, either alone or in combination. The number of regenerated shoots per explant was generally low (approximately seven shoots per explant), although much higher than those from callus.

Callus induction and plant regeneration was reported from various explants, including leaf, cotyledon, hypocotyl, anther, and isolated microspores [29–32]. Regenerable calli are generally amenable to genetic transformation. However, a few reports are available on the use of callus to infect with *Agrobacterium* and to subsequent development of transgenic plants. An *Agrobacterium*-mediated transformation system has been tested to evaluate the effect of a binary vector system [33]. Pre-cultured in vitro leaf explants grown were co-cultivated and allowed to form white friable callus. In a selection medium, the shoots were regenerated from the calli. About 13% of the infected in vitro leaves formed resistant callus and 55% of the callus formed shoots leading to an overall transformation efficiency of 7.6%. The regenerated shoots were able to form roots in the presence of 100 mg/L kanamycin and grow normally in the greenhouse. Co-cultivation and subsequent callus-mediated plant regeneration from cotyledon and hypocotyl explant have also been reported in several Brazilian eggplant cultivars [34].

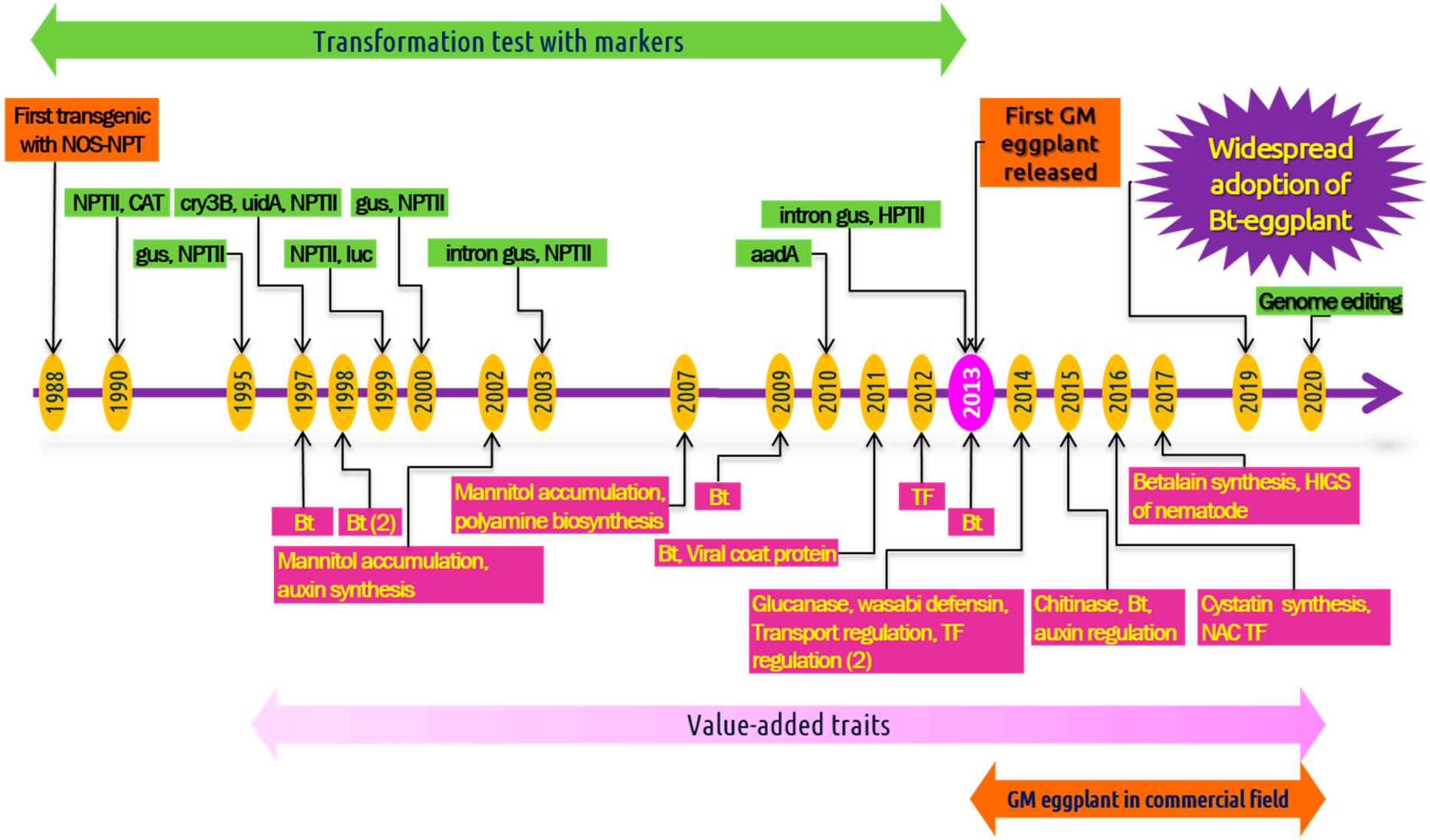

Figure 2. Timeline of the engineering of eggplant—from the first report to the commercialization. Multiple publications in the same year are mentioned in parenthesis.

Table 2. Common factors tested for the *Agrobacterium*-mediated and biolistic genetic transformation in eggplant.

Cultivar	Explant	*Agrobacterium* Strain	Vector	Gene	Selection Marker	Frequency	Ref
Black Beauty	In vitro leaves	715 and pCIB10	pMON200	NOS-NPT	Kanamycin	nc	[28]
Picenfiia	Leaves (callus)	GV3101	pBCATI	NPTII, CAT	Kanamycin, 100 mg/L	8%	[33]
Kecskeméti lila	Somatic embryo from cotyledon	C58C1 Rif^R	pGSGluc1	*gus*, NPTII	Kanamycin, 100 mg/L	nc	[35]
Hibush	Stem, leaves	Q10, Q20, Q30, Q40, Q201-204	pRU01, PRU02, PRU03	cryIIIB, uidA, NPTII	Kanamycin, 50 mg/L	21%	[36]
Pusa Purple Long	Cotyledonary leaves	EHA 105	pBT 1291, pBinAR	cry1Ab	Kanamycin, 100 mg/L	nc	[37]
Hibush	Leaves (callus)	LBA4404	pBI121	NPTII, luc	Kanamycin, 100 mg/L	3%	[38]
F-100, Emb	Cotyledon, leaves	C58C1	pTSM-3.1800	*gus*, NPTII	Kanamycin, 50 mg/L	5–23%	[34]
MEBH 11, MEBH 9, Kalpatru, Rohini	Root	LBA4404	pBAL2	*Gus*-intron, NPTII	Kanamycin, 100 mg/L	nc	[39]
Eggplant	Stem (chloroplast)	Biolistic	pPRV111A	aadA	Spectinomycin, 300 mg/L	9%	[40]
Jamuni Gola, Pusa Kranti, Azad Kranti, Arka Samhitha, Hisar Shyamal	Seed (*in planta*)	EHA 105	CAMBIA 1301-bar	*gus*A-intron, hptII	BASTA®, 100 mg/L	46%	[41]
Pusa purple long	Cotyledone, leaves	LBA4404	pCAMBARchi11	Endochitinase (I)	Hygromycin, 20 mg/L	10–20%	[42]

nc = not counted.

It is noteworthy that the time required (8–12 weeks) for the differentiation and development of transformed shoots may lead to a reduction of the organogenic potential and subsequent recovery of transgenic shoots. The efforts to transform callus tissues with *luc* genes has been reported [38]. However, the experiment was intended to observe the expression and the incorporation of *luc* gene and LUC activity in the transgenic eggplant throughout a year. No stable transgenic plants were produced from the callus in this study. Considering all reports published to date, it appears that the use of callus is not the primary method of choice in eggplant transformation.

Regeneration in eggplant has been also obtained by somatic embryogenesis directly from different explant tissues or indirectly from callus [43], but the development of somatic embryos into plants still remains difficult, being highly dependent on the genotype. The difference in morphogenic potential within single explants has also been reported [44]. These render the process less utilized for plant transformation.

4.1.2. Choice of Explants for Genetic Transformation

The majority of the researchers used in vitro grown seedlings as the starting materials for *Agrobacterium*-mediated transformation. Exploiting the capability of direct shoot organogenesis, cotyledon [36], young leaf [28], or stem segments [28,36] were used, which are less complex compared to callus-mediated organogenesis or somatic embryogenesis.

A single report is available to use leaves from ex vitro plants. The leaves from in vitro as well as four-week-old greenhouse-grown plants have also been tested to infect with *Agrobacterium* [33]. Damage of the explants during sterilization procedure makes it particularly susceptible to *A. tumifaciens* infection, causing excessive colonization of the leaf tissue. No explants survived after six weeks. Another project utilizes root explants to infect with *Agrobacterium* followed by the induction of transgenic callus [39]. Calli were subcultured for shoot-bud initiation and produced *gus*-positive stable transgenic plants selected in kanamycin.

In planta transformation is an alternative genetic transformation method, which does not essentially involve in vitro culture of plant cells or tissues. Advantages of this method include the absence of somaclonal variation, and less labor and time required. Generation of the microscopic path on target tissue allows DNA to transfer from *Agrobacterium* to targeted plant cells having regeneration potential. In the model plant *Arabidopsis*, it is the most widely used technique for gene transfer. The explants used in this method include hypocotyls and cotyledon [45], germinating seeds [46,47], floral buds [48], shoot apical nodes [49], mature embryos [50], and fruits [51]. Successful application of the direct seed transformation has been reported in seeds of *Brassica napus*, and *B. rapa* [52,53]. In eggplant, only a single report is available utilizing this approach, which was performed by Subramanyam et al. [41]. They vacuum-infiltrated *Agrobacterium* suspension into eggplant seeds followed by sonication. A transformation frequency of 40–46% was observed in different cultivars in terms of *gus* expression in seedlings.

4.1.3. Choice of Vectors for Eggplant Transformation

The first successful genetic transformation of eggplant via *Agrobacterium* was achieved using a co-integrated vector [28]. These authors also failed to obtain any transgenic plant using a binary vector system. Co-integrated vectors or hybrid Ti plasmids are among the first types of DNA construct devised for *Agrobacterium*-mediated transformation. The requirement of long homologies between the Ti plasmid and the *E. coli* plasmids (pBR322-based Intermediate vectors) makes them difficult to engineer. In addition, this system is relatively inefficient compared to the binary vector. Therefore, co-integrated vectors are not widely used nowadays in plant genetic transformation. In eggplant, that is the only report for the co-integrated system, while later works were done with a binary vector system (Table 2).

4.1.4. Targeting Transgene to an Organelle

Transfer of foreign genes to plant organelles such as the mitochondria and plastid appears to be a powerful tool to obtain plants with new traits and to study fundamental aspects of their function. This approach receives great attention nowadays because of its superior performance over the conventional and more commonly used nuclear transformation system. Organelles such as mitochondria and chloroplast mimic a prokaryotic system. The codon usage is also of prokaryotic nature. As a result, the expression of the prokaryotic gene(s) in these organelles without further modification is possible. As the chloroplasts are maternally inherited, the flow of transgene does not occur through pollen dispersal. In addition, the absence of gene silencing, strong predictable transgene expression, and its application in molecular farming, both in pharmaceutical and nutraceuticals, are some of the many advantages [54]. Only one report is available to introduce a gene to the plastid genome [40]. This was carried out by bombardment of green stem segments with pPRV111A plastid expression vector carrying the *aadA* gene encoding aminoglycoside 3′-adenylyltransferase. RT-PCR analysis confirmed the transplastomic expression of the *aadA* gene with an overall transformation frequency of 9%. Regardless of transformation frequency, the protocol shows the potential application of this important tool.

4.2. Application of Transgenic Technology in Eggplant

To date, researchers have introduced several genes in eggplant to confer agronomically important traits. A summary of these traits is provided in Figure 3 and Tables 2–6 and discussed in the following sections.

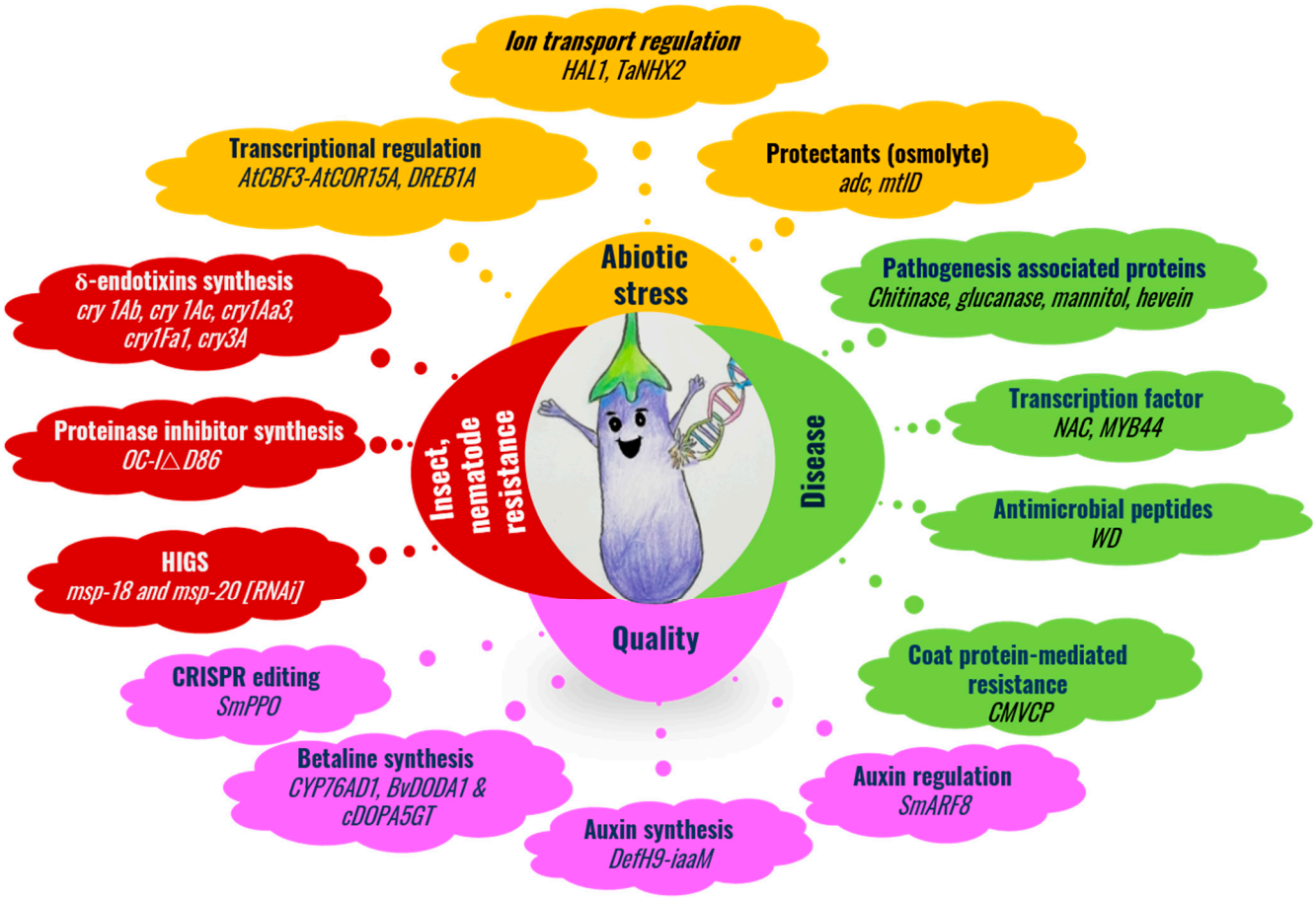

Figure 3. Approaches and genes used in the engineering of eggplant for various agronomic traits.

4.2.1. Transformation for Insect Resistance

Eggplant is infested by a number of pests, causing significant yield loss. These include fruit and shoot borers (FSB), epilachna beetles (hadda), stem borers, red spider mites, and jassids. Among them, the FSB is the most devastating and virtually unmanageable. Farmers require applications of high doses of insecticides. On the other hand, 'classical' genetic sources of resistance to insects are scarce within eggplant germplasm [55]. This makes it challenging to develop superior cultivars that are resistant to major insects and diseases by conventional breeding methods. As chemical control is the only method of limiting insects, consumers often have no alternative but to accept insect-damaged and infested fruit or fruits containing high pesticide residues.

Bacillus thuringiensis (*Bt*) is a Gram-positive, soil-dwelling bacterium. Many strains of *Bt* produce δ-endotoxins during sporulation, which are crystal proteins with insecticidal activity. *Bt* (obtained by fermentation) has been widely applied through sprays or ground applications as an insecticide by farmers worldwide over the last 45 years, particularly in organic farming. Their primary action is to lyse midgut epithelial cells by penetrating them into the target membrane and forming pores [56]. Molecular biologists take this opportunity to engineer crops against insect attacks. Based on the codon usages by plants, synthetic or semi-synthetic gene constructs have been made to facilitate transfer and expression in crop plants. As an insect-susceptible crop, eggplant has been genetically modified to express insecticidal crystal protein (*cry*) genes over the last decades [37,57,58]. This allows the generation of EFSB-resistant transgenic eggplant lines. A summary of insect-resistant traits introduced in eggplant is given in Table 3.

Table 3. Insect resistant transgenic eggplant.

Promoter::Gene	Target Insect	Cry Protein Level	Key Results	Ref
CaMV 35s::cry1Aa3	EFSB (*Leucinodes orbonalis*) Lepidoptera	30.9–44.3 ng/g (fresh leaves); 20.5–35.7 ng/g (fruits)	• Exhibited higher ESFB larval mortality.	[58]
CaMV 35s::cry1Fa1	EFSB (*Leucinodes orbonalis*) Lepidoptera	Not determined	• Protected against EFSB.	[59]
CaMV 35s::cry1Ac	EFSB (*Leucinodes orbonalis*) Lepidoptera	2.46–4.33 ng/mL in leaves	• Exhibited significant larval mortality and reduced growth of any surviving larva.	[57]
CaMV 35s::cry1Ab	EFSB (*Leucinodes orbonalis*) Lepidoptera	125–142 ng/mg in soluble protein	• Exhibited significant insecticidal activity and is correlated to the Cry1Ab protein expression.	[37]
CaMV 35s::cry3A	Colorado potato beetle (*Leptinotarsa decemlineata*)	Not determined	• Showed resistance (69% of the transgenic plants).	[60]
CaMV 35s::cry3A	Colorado potato beetle (*Leptinotarsa decemlineata*)	Not determined	• Produced toxin (61% transgenic plants). Most larvae dead within 72 h of feeding. Few survivors had stunted growth.	[61]

More than 200 Cry proteins have been discovered, belonging to at least 50 subgroups. The tertiary structures of six different three-domain Cry proteins, Cry1Aa, Cry2Aa, Cry3Aa, Cry3Bb, Cry4Aa, and Cry4Ba have been determined by X-ray crystallography. These proteins are of a narrow host range. Nevertheless, the relative toxicity of each Cry protein varies widely among insect species [62,63]. Generally, *Bt* toxins are expressed in plants using strong constitutive promoters such as the CaMV 35S or derivatives. In some cases, wound-inducible promoters such as the pathogenesis-related PR-1a gene [64] or the *A. tumefaciens* mannopine synthase [65] or tissue-specific (a maize PEPC promoter expressed in green tissues and a maize pollen-specific promoter [66]) were also used. However, in eggplant, only CaMV35s promoter is used to express the *Bt* genes (Table 3). Different versions of the *cry* gene have been transferred to various eggplant varieties such as the cry1Ab in *cv* Pusa Purple Long [37], cry1Ac in *cv* Kashi Taru [57], and cry1F in Manjarigota, Ruchira, Poona selection, and Krishna kathi [67,68]. These works resulted in substantial resistance of eggplant against laboratory-reared larvae of FSB (*Leucinodes orbonalis*). In addition, transgenic expression of the *cry3A* gene makes eggplant resistant to the Colorado potato beetle, *Leptinotarsa decemlineata* [60,61]. It is noteworthy that all these controlled experiments were performed using laboratory-reared insects and actual performance may differ under field conditions.

4.2.2. Transformation for Nematode Resistance

Eggplant is infected by a number of parasitic root-knot nematode species, including *Meloidogyne incognita* and M. javanica. Additionally, nematode infection makes the crop more susceptible to other diseases, such as bacterial wilt and fusarium wilt. Overall, these cause substantial yield losses [69,70]. The control measures include chemical, biological, and cultural practices. However, plants can be engineered to prevent nematode development inside the plant body. So far, only two reports are available. In the first one, transgenic eggplant expressing a modified rice cystatin (*OC-IΔD86*) gene under the control of the root-specific promoter inhibited nematode growth by 78% [71].

Host-induced gene silencing (HIGS) has also been tested to restrict nematode growth in eggplant. *msp-18* and *msp-20* genes, specific to dorsal and subventral esophageal glands of M. incognita, respectively, have been targeted to silence in transgenic eggplant [72]. It has been hypothesized that the cell sap containing bioactive dsRNA/siRNA species will be translocated from plant to nematode through amphidial aperture or stylet lumen during the nematode penetration. Transgenic expression of siRNAs specific to msp-18 and msp-20 caused transcriptional alteration of unrelated effectors such as CWMEs in feeding juveniles and females of *M. incognita*. The authors also showed that the nematode absorbs plant molecules during their exploratory probing phase on the plant surface. Eventually, the transgenic eggplants showed an improved nematode resistance.

4.2.3. Genetic Engineering for Disease Resistance

Plants protect themselves from pathogen infections through a number of preformed primary barriers, such as constitutively expressed waxes, cell wall reinforced with callose, lignin and suberin, antimicrobial peptides, proteins, and various secondary metabolites. These elements serve as the initial impediments to pathogens. Once the primary barriers are broken by the pathogen, plants exhibit a 'defense response' through a number of mechanisms, including the rigidification of cell walls, production of reactive oxygen species (ROS), synthesis of secondary metabolites, and ultimately, programmed cell death. These responses are generally governed by a group of so called 'pathogenesis-related' (PR) proteins. Irrespective of plant species, PR proteins are classified into 17 families based on their sequence homologies, serologic or immunologic relationships, and enzymatic properties [73]. Among these, β-1,3-glucanase enzymes, PR-2 family members, received considerable attention. This enzyme not only has antimicrobial potential but also plays a role in the growth and development of plants [74]. The application of corresponding

genes has been tested in genetic engineering for enhancing disease resistance in crop plants, including eggplant (Table 4).

Plant β-1,3-glucanases (EC 3.2.1.39) hydrolyze the cell walls of fungal pathogens by degrading β b-1,3/1,6-glucans. Generally, the degradation of the fungal cell is also accompanied by the activities of chitinase isozymes (EC 3.2.1.14), which breaks the bond between the C1 and C4 of two consecutive N-acetylglucosamines of chitins in the fungal cell wall. Degradation of those two molecules causes the lysis of invading fungal hypha and helps plants to stop infection [75].

Table 4. Transgenic eggplant resistant to diseases.

Promoter::Gene	Target Trait	Key Results	Ref
CaMV 35s:: SmMYB44	Bacterial wilt	• Overexpression of SmMYB44 induced spermidine synthase gene expression and spermidine content, increasing the resistance to bacterial (*Ralstonia solanacearum*) wilt. • RNAi-mediated down regulation of SmMYB44 resulted in susceptibility proved the mechanism.	[76]
not mentioned::hevein	Fungal wilts tolerance	• Transformed plants were better in tolerating *Fusarium oxysporum* challenge.	[77]
CaMV 35s::chi	Fungal wilts tolerance	• Showed higher chitinase activity. • Expressed higher level of fungal resistance to the wilts caused by *Verticillium dahlia* and *Fusarium oxysporum* revealed by root-dip assay and assay with spores mixed to soil.	[42]
CaMV 35s::glu	Fungal wilts tolerance	• Expressed higher glucanase activity. • Showed lower wilting in transgenic plants challenged by *V. dahlia* and *F. oxysporum* spores (10^8/mL) using root-dip and soil-mix method.	[78]
CaMV 35s:: WD	Early blight (*Alternaria solani*) tolerance	• Necrosis of stem and leaves, stem softening and dropping of leaves and the plant were 70% of wild-type plants, and 30–55% of transgenics.	[79]
CaMV35s::mtlD	Fungal wilt resistance	• Transgenic plants had 0.1–0.5 µmol/g fwt of mannitol, while it was not detected in untransformed plants. • Increased resistance against fungal wilts caused by F. oxysporum, V. dahliae and R. solani in vitro and ex vitro.	[80]
CaMV 35s::CMV-CP	Virus resistance (*Cucumber mosaic virus*)	• 65–70% of transgenic plants (of different lines) were resistant to Cucumber mosaic virus.	[81]

Individual transgenic eggplants were generated by introducing an alfalfa glucanase gene [78]. Kanamycin-selected T1 seeds (selfed primary T0 plants) showed typical 3:1 transgene segregation, suggesting single-copy transgene integration. In vitro grown seedlings from both transgenic and wild-type plants were challenged by the addition of *V. dahlia* and

F. oxysporum spores (10^8/mL). The transgenic plants exhibited delayed wilting compared to untransformed plants. Some of the transgenics survived after one month of inoculation and showed new leaf development. In contrast, no wildtype survived more than 10 days. A similar result was obtained when one-month-old seedlings were challenged by those spores in soil condition. The degree of fungal resistance was variable among the transgenic lines. In another work, a class I rice endochitinase was constitutively expressed in transgenic eggplant [42]. Transgene expression corresponded to higher chitinase activity and delayed appearance of disease by five–seven days when treated with *V. dahlia* and *F. oxysporum* spores (10^8/mL) in vitro or in vivo. Hevein is a small (4.7 kDa) cysteine-rich protein abundant in rubber latex, has a carbohydrate-binding property, and inhibits many chitin-containing fungi. Transgenic expression of hevein has also been reported to show tolerance against *Fusarium oxysporum* in vivo [77].

Plant responses to pathogen attacks are complex, and the functions of many PR proteins remain obscure. Mannitol, a six-carbon non-cyclic sugar alcohol, plays an important role in the storage of carbon and energy, regulation of coenzymes, osmoregulation, and free-radical scavenging. Mannitol dehydrogenase, the key enzyme of mannitol biosynthesis, shows a high degree of homology with the ELI3 class of PR proteins. This indicates that some metabolic enzymes and PR proteins have overlapping functions [82] in disease response. Transgenic eggplant overexpressing a bacterial mannitol phosphate dehydrogenase (*mtlD*) gene resulted in high mannitol accumulation. When the plants were challenged with wilt-causing fungi—*Fusarium oxysporum*, *Verticillium dahliae*, and *Rhizoctonia solani*—transgenic plants showed greater resistance compared to the non-transgenic controls under both in vitro and ex vitro growth conditions. Although the role of mannitol in enhancing fungal disease resistance is unclear, it is possible that mannitol may be involved in protecting the host cells from osmotic stress resulting from the restriction of water up-take by wilt fungus [83].

Besides cell wall degrading enzymes, plants synthesize small antimicrobial proteins or peptides (AMP), which have activity against phytopathogens. The protective effect of exogenously applied AMPs has been reported in several plant tissues [84–86]. However, in situ production of AMP in plants through transgene expression would be a promising tool to protect plants against diseases caused by microorganisms. Transgenic plants expressing natural AMP have been reported to exhibit resistance against broad-spectrum disease [87,88]. In some cases, natural AMPs exhibit several drawbacks. Therefore, initiatives were undertaken to design them rationally. These designed AMP showed antimicrobial activity in plants [89–91]. In eggplant, the only transgenic application reported is the overexpression of the *Wasabi defensin (wd)* gene, which was isolated from *Wasabia japonica*—a Japanese horseradish [79]. In vitro grown transgenic seedlings were challenged with *Alternaria solani* (which causes early blight in eggplant) to evaluate their fungal resistance. Wild-type plants were infected shortly, with plants wilting in two weeks. However, transgenic plants were less damaged (30–55%). Inoculation of *Alternaria solani* on detached leaves of wild-type plants caused damage to most parts of the leaf tissue, while the damage was mostly confined to the site of inoculation in transgenic leaves.

While NAC transcription factors are involved in coordinating responses to attacks by fungi, bacteria, and viruses, the role of this gene from eggplant (*SmNAC*) has been studied [92]. Overexpression of *SmNAC* decreases the accumulation of the plant-immune signaling molecule, salicylic acid (SA). Additionally, the expression of *ICS1*, a gene involved in SA biosynthesis, was also reduced. The transgenic plants exhibit reduced resistance to bacterial wilt caused by *Ralstonia solanacearum*. When the gene is silenced using RNAi (*SmNAC-RNAi*), the transgenic plants showed resistance against the wilt. On the other hand, non-transgenic plants exhibited wilt symptoms after seven days of inoculation. Exogenous application of SA on overexpressing lines showed no wilt symptoms whereas RNAi lines had symptoms. However, wilting of all plants after 20 days suggests that *SmNAC* negatively affects the resistance. Besides, spermidine has also been reported to be linked to resistance to *R. solanacearum* [76]. A transcription factor, *SmMYB44*, induces *SmSPDS* expression

through direct interaction with its promoter. Overexpression of *SmMYB44* resulted in resistance to the bacterial wilt.

Viral disease in crop plants bears significant economic significance. Based on viral genes and sequences, various transgenic approaches were tested for the resistance. These include coat protein (CP)-mediated resistance, replicase-mediated resistance, rep protein-mediated resistance, and RNA-mediated resistance against RNA viruses [93]. In addition, non-viral sources of genes have also been used to generate transgenic plant expressing antiviral antibodies [94].

CP-mediated viral resistance in plants is the oldest transgenic plant of its kind [95,96]. Although the mechanism of protection conferred by CP is unclear, the level of protection in transgenic plants varies from immunity to delay or attenuation of symptoms. In some cases, protection is broad and effective against several strains of the virus from which the CP gene is derived, or even against closely related virus species [96]. It has been hypothesized that interaction between the transgenic CP and the CP of the challenging virus is a consequence of disease prevention. To date, only CP-mediated resistance was applied in eggplant to test resistance against *Cucumber mosaic virus*. The CP gene of the *Cucumber mosaic virus* was transformed in eggplant for induction of virus resistance. In addition, 65–70% of transgenic plants of different lines were resistant to the *Cucumber mosaic virus*.

Disease-resistant eggplant has been developed through PR proteins, antimicrobial peptides, and immunization. Although these are among the most attractive and potentially rewarding ways to combat plant pathogens, the results presented here are of a preliminary nature. Clearer evidence is necessary for the sustainability of the traits under field conditions before introducing them to a breeding program. In addition, a detailed study to understand the mechanism of the participation of corresponding proteins/metabolites to disease resistance is important.

4.2.4. Transformation for Abiotic Stress Tolerance

Reduction in eggplant productivity as a consequence of severe climatic events prompts researchers to take the initiative to develop abiotic stress-tolerant transgenic eggplants (Table 5 and references therein). Like other crops, economically sustainable production of eggplants is vulnerable to drought, salinity, and low- and high-temperature regimes. Significant progress has been made in understanding the mechanism of abiotic stress tolerance and the key regulator genes that could potentially be involved in the processes of tolerance [27]. Based on this knowledge, attempts are being taken to develop tolerant eggplants through genetic engineering. To date, only a few reports have been published on the development of a transgenic eggplant showing abiotic stress tolerance, including cold, heat, salinity, and osmotic stresses. The molecular mechanisms exploited for these include modulations of ion transport, direct synthesis of osmolytes, polyamines, and transcriptional regulation.

The salt tolerance of crop plants depends mostly on their ability to restrict Na^+ and Cl^- uptake and sequester these ions into vacuoles when entering the tissues. This can be governed by the NHX antiporter. These secondary ion transporters transfer the Na^+ or K^+ across a membrane in exchange for protons (H^+). This exchange activity is driven by the H+ electrochemical gradient generated by the vacuolar membrane H^+-ATPase and H^+-pyrophosphatase [97]. Engineering of the transporter has been carried out in eggplant. The only available report tested the expression of a wheat Na^+/H^+ antiporter gene (*TaNHX2*) driven by a double cauliflower mosaic virus (CaMV) 35S promoter [98]. The transgenic plants had an improved ROS scavenging capacity and higher fresh and dry weight under salt stress. When the plants were irrigated with increasing concentrations of NaCl (0, 50, 100, and 150 mM) for seven days followed by 200 mM NaCl for a period of three weeks, they appeared close to normal, whereas non-transformed plants were chlorotic with retarded growth and ultimate death. However, the effects were not monitored further.

Table 5. List of abiotic stress-tolerant transgenic eggplant developed so far.

Promoter::Gene	Target Trait	Key Results	Ref
CaMV35s::HAL1	Salt tolerance	• Minor difference in growth reduction exposed to 50 mM NaCl.	[99]
AtRD29A::AtCBF3 and AtRD29A::AtCOR15A	Chilling tolerance	• Slower withering of the leaves of the homozygous transgenic plants after exposure to cold (2 ± 1 °C). Proline content and catalase and peroxidase activities were significantly increased.	[100]
rd29A::DREB1A	Moisture stress tolerance	• Fifteen-day of water stress, control plants were completely dried, but the transformed plants were in normal condition.	[101]
CaMV35s::adc	Multiple abiotic stresses tolerance	• Seed germination, shoot length and fresh weight were significantly higher in T1 plants exposed to salt (150 mM), heat (45 °C), cold (4–6 °C), osmotic (7.5% PEG) or cadmium (1 mM).	[102]
CaMV35s::mtlD	Multiple abiotic stresses tolerance	• Higher germination rate of transgenic seeds under 200 mM salt-amended MS medium. • The detached leaves of transgenic plants withstand desiccation. • Better growth under chilling (6–8 °C for 10 days), and salt (200 mM NaCl in hydroponics).	[103]

Maintaining adequate K^+ concentrations and the production of compatible organic solutes, such as glycine betaine and proline, are also required for salt tolerance [104]. Apart from the integral transporter proteins, their modulators that the change cation transport systems have been applied to transgenic plants using *HAL1* gene of *Saccharomyces cerevisiae*. *HAL1* is involved in the regulation of K^+ transport in yeast. Overexpression of *HAL1* in *Saccharomyces cerevisiae* increased salt tolerance [105]. *Agrobacterium*-mediated transformation of this gene in eggplant significantly enhanced salt tolerance at the cellular and whole plant levels [99]. Severe growth inhibition (in terms of fresh weight) was observed when the wild-type calli were grown in a medium containing 150 mM of NaCl. However, the growth reduction is little in transgenic callus. The greenhouse trial of a single transgenic line (T2) showed no effect in fresh weight under moderate salt concentrations (25 mM). However, at a higher salt concentration (50–100 mM NaCl), plant growth was significantly better in transgenic lines compared to wildtypes. Beneficial effects of transgenic overexpression of the *HAL1* gene have been reported in in vitro grown shoot apex of melon [106], callus culture, and a whole tomato plant [107]. In spite of the apparent growth benefits of transgenic eggplant in a moderate level of salt under greenhouse conditions, more studies are needed to use this candidate gene from an agronomic viewpoint.

Imposition of stresses, especially those disrupt cellular osmotic status, generally makes proteins and cellular organelles unstable. For instance, effective osmotic adjustment makes plants tolerant for temporary or prolonged periods of water shortage. Plants exposed to salinity, drought, and cold induce the synthesis of a group of small solute molecules—so cold 'compatible solutes' of 'osmolyte' help plants to survive osmotic stress. These include amino acids (proline, γ-aminobutyric acid, aspartic acid), proteins, carbohydrates (fructan, sucrose, glucose, starch mannitol, trehalose, raffinose, polyols), polyamines (glycine betaine, alanine betaine), and organic acids [108]. At high concentrations, these compatible solutes contribute to the lowering of the osmotic potential ($\Psi\pi$) and allow water to move into the cells. This maintains turgor pressure (Ψp) and enhances tolerance of the tissue to low soil water potentials [109]. These solutes also sequester water molecules, protect cell membranes and protein complexes, and allow the metabolic machinery to continue functioning [110].

To date, attempts have been made to develop transgenic eggplant only to increase the synthesis of mannitol [103] and polyamine [102]. In the first report, expression of an *E. coli* mannitol-1-phosphodehydrogenase (*mtlD*) gene in eggplant confers salinity, cold, and osmotic stress tolerance. Germination of seeds from T0 plants was tested in vitro with 200 mM NaCl or 7.5% PEG (MW 8000) to induce salt and drought, respectively. Wild-type seeds failed to germinate in either medium, while transgenic seeds from different lines

germinated on the salt/PEG-amended medium. The germination rate varied (18–64%) in different lines. On hydroponics, the transgenic lines were green and grew well with salt (200 mM NaCl) stress, and exhibited longer shoot, root, and leaf lamina compared to control seedlings. The root system of salt-stressed transgenic lines, in particular, was healthier than that of control seedlings which showed extensive necrosis of the leaves. The seedling growth of the transgenic plants (based on the height, root, and leaf lamina length) was also better than the wildtype in hydroponic culture amended with PEG (to induce drought/osmotic stress). In this work, the effect of soil-drying was not tested, and a detached leaf assay was conducted instead. Detached leaves placed on a dry filter in Petri plates from control started drying within 24 h and contained less water, while transgenic leaves were still fresh even after 24 h of drying. However, this experiment is not sufficient to show the actual drought tolerance. Moreover, exposure of the plants to 6–8 °C for ten days showed better growth. Although the authors claimed enhanced tolerance to these stresses, they did not estimate cellular mannitol content and there is a lack of quantitative data in several aspects of stress-assay.

Polyamines are other osmolyte compounds, which are small aliphatic molecules that bear a positive charge at cellular pH [108]. Polyamines are accumulated during stress conditions, although their exact mode of action at the molecular level is not clearly understood. Arginine decarboxylase (*adc*) gene has been overexpressed in transgenic eggplant to increase polyamine biosynthesis [80]. ADC pathway produces putrescine from arginine. Spermine and spermidine are two important polyamines produced from putrescine by the subsequent addition of aminopropyl groups derived from S-adenosylmethionine. Constitutive overexpression of the *adc* gene resulted in an increased arginine decarboxylase and ornithine decarboxylase enzyme activity in the transgenic plants. Consequently, there was a significant increase in putrescine (3–7 fold) and spermidine (3–5 fold) levels, particularly in the conjugated form. In vitro germination test showed that the wild-type seeds failed to germinate under salinity, drought, heat, and cadmium stress. Although a few seeds were germinated in cold, they failed to grow further. The authors showed that the transgenic seeds had a germination rate of 50–80% under the same condition. The early growth of the seedlings was challenged by those stresses. Transgenic plants had a significantly higher shoot height and fresh weight compared to the wild-type plants. The difference in dry weights was mostly non-significant.

Abiotic stresses have a strong impact on the expression of regulatory genes, most notably transcription factors (TF). TFs bind to cis-regulatory DNA sequences and influence the transcription of specific genes. A particular TF may bind to multiple genes, and each gene may be controlled by multiple TFs to perform coordinated gene function. The TFs form complex networks that may control from one to thousands of genes in response to conditions inside or outside of the cell. Altered expression of some stress-related transcription factors induces changes in stress-associated metabolite levels [27]. As master regulators of gene expression, TFs are expected to be excellent candidates for modifying complex traits in crop plants, and TF-based technologies are likely to be a prominent part of the next generation of successful biotechnology crops.

The DREB1A/CBF3 transcriptional activator gene regulates the expression of genes containing C-repeat/Dehydration responsive element (DRE). In eggplant, a DREB1A gene was transferred by *Agrobacterium*-mediated transformation [101]. Although the author showed that the transgenic plant can withstand drought stress for a prolonged period, analyses of morphological and biochemical parameters are not supportive. Cold acclimation through ABA-independent pathway involving C-repeat binding factors CBF1, CBF2, and CBF3 have been studied in model plants. Upon exposure to cold, expression of CBF3 is induced by the upstream transcriptional activators ICEs. This, in turn, starts the expression of CBF-targeted, cold-regulated (COR) genes to form a complex gene network system that imparts chilling and/or freezing tolerance in *Arabidopsis thaliana*. Although the pathway is conserved in many plant species, components, and functions of CBFs and their regulons in cold-sensitive plants are imperfect or deficient. Transgenic eggplant was

developed expressing *AtCBF3* and *AtCOR15A* genes [100]. Cold exposure (2 °C) resulted in slowed and lessened withering in two homozygous independent transgenic lines. The cold tolerance is also characterized by increased proline content and higher levels of catalase and peroxidase activities.

It is noteworthy that constitutive over-expression of most TF genes, including CBF3/DREB1A in transgenic model plants, resulted in phenotypic abnormality, including growth retardation. Therefore, both works used stress-inducible *rd29A* promoters to drive the DREB1A/CBF3 and *AtCOR15A* gene [101,111].

The regulations of the above-mentioned mechanisms are very complex in plants grown in natural environments exposed to a variety of stresses. Expression of a single gene producing certain protein/metabolite in transgenic plants could promote a dramatic enhancement in stress tolerance. However, the genetic manipulation of a crop species with individual transgenes could lead to a slight improvement in tolerance level. This may not lead directly to a new salt-tolerant cultivar but would be sufficient from a breeding point of view.

4.2.5. Engineering for Other Traits Associated with Quality and Productivity

Control of Fruit Development

Fruit setting in eggplants is one of the vulnerable points negatively affected by adverse environmental conditions such as sub and/or supra-optimal temperatures, humidity, and drought [112]. These conditions ultimately affect eggplant productivity. Apart from the regulation of the physiological processes by genetic engineering that might have a negative effect, an 'escaping' approach is also applied for eggplant breeding. Fruit setting without fertilization (parthenocarpy) may play a profound role in sustaining productivity under uncertain environmental conditions. Studies on natural and experimental parthenocarpy revealed that they are largely regulated by endogenously synthesized hormones, most notably auxins in ovules and placenta. Since then, parthenocarpy has been induced by exogenous plant growth regulators, or by gene introduction (Table 6).

Table 6. Transgenic eggplant resistant for productivity and quality.

Promoter::Gene	Target Trait	Key Results	Ref
EEF48::crtB	β-carotene accumulation	• Accumulation of β-carotene in fruits was 30-fold higher than that of the untransformed plant.	[113]
CaMV 35s:: Cas9-*SmPPO*	Fruit flesh browning	• Editing of three PPO genes (SmelPPO4, SmelPPO5, and SmelPPO6) using CRISPR/Cas9 results in reduces fruit flesh browning.	[114]
CaMV 35s:: SmARF8 [RNAi]	Parthenocarpic fruit	• Silencing SmARF8 gene produced seedless parthenocarpic fruit.	[19]
pDefH9:: DefH9-iaaM	Parthenocarpic fruit	• Increased fruit productivity by 33% and were always seedless.	[115]

Hormonal treatments for the production process are expensive due to the cost of both chemicals and labor. On the other hand, the many parthenocarpic varieties have low-frequency fruit settings and reduced fruit sizes [116]. Often the parthenocarpic trait is polygenic [117]. It is, therefore, difficult to introduce in superior commercial varieties. All these lead to a genetic engineering approach to develop parthenocarpic eggplant through transgenic technology. It has been observed that there is a positive correlation between fruit setting and growth, and an increased level of auxins in the developing ovules and embryos [118]. This finding is further verified by the successful induction of parthenocarpic fruits by external application of phytohormones to flower buds. Gene manipulations for direct synthesis or regulation in the upstream pathways (transcriptional regulation) are the two approaches utilized for developing parthenocarpy in eggplant. The *iaaM* gene

(codes for a tryptophan monoxygenase) is isolated from *Pseudomonas syringae pv. Savastanoi* produces indolacetamide that, in turn, is either chemically or enzymatically converted to the auxin indole-3-acetic acid. A placenta and ovule-specific promoter were isolated from the *DefH9* gene from *Anthirrinum majus*. The *iaaM* gene driven by the *DefH9* promoter has been introduced to transgenic eggplant to enhance auxin biosynthesis in the ovule and placenta. As a result, parthenocarpic seedless fruits were produced by emasculated flowers [119]. When the flowers were allowed to pollinate, a normal fruit setting was observed that contained seeds.

To test the effect of adverse environmental conditions on productivity, winter production was compared in an unheated greenhouse in Italy [120]. The trial also includes two non-transgenic control hybrids and a commercial parthenocarpic cultivar (cv Talina). Hybrids were either treated or untreated with a commercial formulation of phytohormones to induce fruit set and growth. The fruit productivity of the non-transgenic hybrids was significantly influenced by hormonal treatment. Cold weather negatively affected fruit setting through fertilization. By contrast, hormone spray did not influence the productivity of the transgenic DefH9-iaaM hybrids. The fruit productivity in transgenic hybrids was ca. 25% higher. This was also concomitant with a 10% reduction in cultivation cost, mainly due to the labor needed for the hormonal sprays.

Early spring production (March to the first half of May) in the Mediterranean region is affected by low temperatures, in which fruit-set and growth are limited. DefH9-iaaM transgenic hybrids produced six-fold higher fruits during early production [115]. The average fruit weight was significantly higher in the transgenic eggplants. The transgenic fruits were always seedless and of normal shape and size during the whole harvesting period. During the whole spring production cycle, the transgenic hybrids showed 46% and 37% higher yield compared to open-pollinated parthenocarpic cultivars and commercial hybrid check varieties (control), respectively.

A summer production trial was conducted in an open field during the optimal period of eggplant cultivation. Based on the data from ten harvests, 37% higher yield was observed in hybrids compared to control. Increased productivity was associated with both the higher number of fruits/plants and the increased weight of transgenic fruits. Fruit quality is also improved through the lack of seeds and placental cavity.

Apart from the expression of auxin-biosynthetic genes in ovaries and ovules to produce auxins directly, control of the activity of transcription factors (the auxin response factors; ARFs) is also another approach to induce parthenocarpy. ARFs influence the expression of auxin-responsive genes by interacting with auxin/indole acetic acid (Aux/IAA) proteins [121]. They have been shown to be involved in the regulation of various aspects of fruit development [111] and play diverse roles in flower and fruit development in tomato plants [121]. It has been shown that the ARF8 has an important role in regulating fruit initiation in tomatoes [122]. In eggplant, SmARF8 is highly expressed in the immature buds, and expression decreased approximately sixfold in 'bud flowers'. Interestingly, a spontaneous mutant eggplant that produces parthenocarpic fruit exhibits much lower SmARF8 expression (1/3-fold) in immature buds. Down-regulation of SmARF8 in eggplant by RNA interference exhibited parthenocarpy in unfertilized flowers [19]. The fruits were seedless but did not differ from wild-type fruits in other traits. Wild-type plants failed to set fruits upon emasculation. However, normal seeded fruits were developed when pollinated (both RNAi transgenic lines and wildtype). The parthenocarpic phenotypes in Arabidopsis arf8-1 and arf8-4 mutants were partially complemented through expressing SmARF8 full-length cDNA. This suggests that the SmARF8 is a negative regulator of parthenocarpy.

Metabolic Engineering

Increased accumulation of health-promoting secondary metabolites in crop plants is one of the targets of genetic engineering. Anthocyanin, betalain, beta-carotene, lutein, zeaxanthin, and polyphenols are among the many such compounds. Naturally, secondary metabolites perform many essential roles in defense, environmental interactions, and reproductive processes.

However, progress in heterologous metabolic engineering was hindered primarily due to the lack of knowledge of the link between metabolites and the activities of key enzymes that regulate the metabolic pathway. Once the gene(s) and their regulations have been identified, successful metabolic engineering could lead to an improved crop.

Betalains are important due to their antioxidant activity, health-promoting properties, and wide use as food colorants and dietary supplements. Heterologous betalain production was successful for the first time in eggplant, tomato, and potato. Simultaneous expression of three genes from the betalain pathway, namely, the cytochrome *P450 CYP76AD1*, *BvDODA1 dioxygenase*, and the *cDOPA5GT glycosyltransferase*, resulted in entirely red betalain accumulation in whole plants, including fruit flesh and skin [123]. The authors also observed that enhanced betalain accumulation in tobacco resulted in significantly enhanced resistance toward gray mold (*Botrytis cinerea*), a plant pathogen responsible for major crop losses. This change in color is different from the modification of pathways generating flavonoid/anthocyanin or carotenoid pigments and opens up an opportunity for the development of new ornamental varieties, innovative sources for commercial betalain production, as well as the utilization of these pigments in crop protection.

Chlorogenic acid (GCA)-rich eggplant could be good for health. However, oxidation of CGA by polyphenol oxidases causes fruits to brown upon cutting. A pathway for the biosynthesis of CGA has been characterized in tomatoes. Overexpression of hydroxycinnamoyl-CoA quinate: hydroxycinnamoyl transferase (HQT) resulted in a higher accumulation of CGA in tomato. This was not accompanied by the levels of other soluble phenolics and to show improved antioxidant capacity and resistance to infection by a bacterial pathogen [124]. This opens the door to use similar approaches in the close relative, eggplant. Experimental results indicate that fruit flesh browning is associated more with higher polyphenol oxidase (PPO) activity rather than the higher CGA content [125]. Therefore, simultaneous selection for low PPO activity and high CGA content could result in materials with greater functional quality and low browning. A recent report on the introduction of carotenoid biosynthesis pathway showed the transgenic fruits having 1.50 μg g^{-1} FW of β-carotene [113]. Although the level was 30-fold higher than the wild-type controls, this amount is not nutritionally significant. Thus, more research is needed for a biofortification target such as the overexpression of *Orange* gene or genome editing for it.

5. Promoters—The Drivers of Transgenes

Compiling all transgenic eggplant data, it was revealed that 80% of the transgenes are driven by the constitutively expressed CaMV35s promoter. On the other hand, Tub2, AtRD29A, Ub10, pDefH9 promoters have been used in some cases (Tables 2–6 and reference therein). CaMV35s is a strong constitutively expressed promoter. However, it has several disadvantages, as the promoter is not active in all tissues and cell types, and sometimes it does not express well under stress conditions. It has been reported that the 35S promoter may affect the expression of nearby genes along with the downstream transgene, possibly via its enhancer regions [126]. Moreover, public acceptance of viral DNA maybe not be preferred during a commercial release of transgenic crops. Thus, native promoters could be useful in developing future transgenic crops. Following the availability of the whole genome sequence of eggplant, all genes and their putative regulatory sequences have been revealed. Functional characterization of these would be useful in developing a more stable GM eggplant with more public acceptability.

6. Genome Editing in Eggplant

The CRISPR-Cas system is an adaptive immune mechanism discovered in bacteria and Archaea for precise defense against invading bacteriophages and exogenous plasmids. Later, this technology was successfully used to engineer human, animal, and plant genomes. The Cas9 protein generates a double-strand break in the DNA that triggers cellular DNA repair mechanisms. If a homologous repair template is available, the error-prone non-homologous end-joining process introduces random insertions/deletions/substitutions.

This generally causes disruption of gene function. If the donor DNA template homologous to the sequence surrounding the double-strand break site is available, the error-free homology-directed repair mechanism creates precise mutations. Therefore, precise gene modification, such as gene knock-in, deletion, or mutation, is possible [127]. So far, only a single report is available on the knock-out of three target PPO genes (*SmelPPO4*, *SmelPPO5*, and *SmelPPO6*). PPOs catalyze the oxidization of polyphenols, which are responsible for the browning of the eggplant fruit flesh after cutting. Reduced browning is a desirable trait for both industrial processing and fresh consumption. The induced mutations were very precise and stably inherited in the T1 and T2 progeny without causing any off-target effect. Reduced PPO activity and consequent browning were observed [114]. This demonstration would surely pave the way for more targets in eggplant.

7. Commercial Cultivation of Transgenic Eggplant: Adoption and Controversy

Insects are a major problem of economic importance in eggplant cultivation (see Section 2.1). Their management causes severe health and environmental issues. Considering these, Bangladesh adopted the first transgenic eggplant for commercial cultivation that expresses the *Cry1Ac* gene and provides resistance against EFSB. The Maharashtra Hybrid Seeds Company (Mahyco), an India-based company through a joint venture with Monsanto, generated *Bt* primary transformant by incorporating the *Cry1Ac* gene. The transgene is driven by transcriptional control of the enhanced CaMV35S promoter (P-E35S). The *Cry1Ac* gene then has been transferred to nine Bangladeshi open-pollinated varieties through conventional hybridization. Among these, four varieties, namely, BARI *Bt* begun-1, BARI *Bt* begun-2, BARI *Bt* begun-3, and BARI *Bt* begun-4, were released for experimental cultivation at the farmer level in 2013.

In India, it has been shown that cultivation of hybrid *Bt* eggplant would result in an increase in yield of 37% with a reduction in total insecticide use of about 42% over non-*Bt* hybrids [5]. Simulations show that the aggregate economic surplus gains of hybrid *Bt* varieties could be around US$108 million per year [128]. All three parties—the cultivators, the innovating company—will share these gains. In particular, the availability of open-pollinated *Bt* varieties makes the technology more accessible, especially for resource-poor farmers. Despite the extensive research on the applicability of *Bt* eggplant carried out in India, attempts at commercialization failed there due to huge controversy. Until there is a political, scientific, and societal consensus, commercial cultivation is halted. In the Philippines, commercial release was also stalled by court order, considering possible health hazards.

Given the advantages of *Bt* eggplant reported from studies in India and the Philippines, Bangladeshi scientists have started to analyze the safety of *Bt* brinjal. Nevertheless, the decision on the commercial release of *Bt* eggplant in Bangladesh faced many controversies at home and in neighboring countries. This opposition is not so much against genetic engineering technology. The primary concern is related to the possible dependence of local seed companies on imported technologies. In addition, the impacts on human health and gene flow are other concerns. Moreover, the lack of an appropriate labeling system in the Bangladeshi markets makes many consumers uncomfortable and not want to consume.

To satisfy cultivators and consumers, Bangladesh Agricultural Research Institute (BARI) conducted a number of field trials in seven regional agricultural research stations in Rangpur, Jessore, Mymensingh, Tangail, Bogra, Dinajpur, and Jamalpur districts. Trial results suggested a significant yield increase in *Bt* eggplant compared to their conventional counterparts. In addition to pest infestation and yield studies, a variety of safety studies were conducted for *Bt* eggplant in order to comply with local regulatory policies. It has been shown that the Cry1Ac protein causes no adverse effects on human cells [129], wild and domesticated animals, birds, fishes, soil microbes, and non-target insects, including beneficial insects [130,131]. The safety of Cry1Ac proteins is attributed to the mode of action, specificity, and digestibility. It was observed that *Bt* eggplant is substantially equivalent to food from non-*Bt*s. Followed by a series of discussions with scientific, agricultural, and regulatory experts, *Bt* eggplant was released for limited commercial cultivation in

2013. The countrywide performance of the *Bt* eggplant will be realized in recent years. Nevertheless, the incorporation of the useful transgene in open-pollinated genetically modified eggplant (OP GMOs) could provide an easily adaptable, scale-neutral option for the farmers. Since little or no changes are required in existing crop management practices, OP GMOs could provide significant economic benefits to the subsistent farmers when such seeds are available to them at a reasonable cost.

Updates on Commercial Cultivation of Bt-Eggplant in Bangladesh

Following government approval in 2013, 20 selected farmers were given the opportunity for field cultivation. The adoption has increased rapidly in each year. By 2018, the technology reached 27,012 farmers, which is about 17% of the total eggplant farmers of the country [132]. Such a rapid and widespread rate of adoption is associated with very favorable socioeconomic benefits. In a demonstration trial by BARI, insect infestation was less than 1% *Bt* eggplant compared to 48–57% in non-*Bt* lines [133]. A separate two-year experiment (2016–17) revealed that fruit infestation in *Bt* lines was 0–2.3%, whereas it was 37–46% in their counterpart. It is noteworthy that the yield was significantly higher in insecticide-applied *Bt*-eggplants compared to the insecticide-free fields. This indicates that *Bt*-plants are also affected by non-target insects such as whiteflies, thrips, and mites that affect plant health and subsequent fruit weight [134]. Some studies also showed that the cultivation of *Bt*-eggplant provides significant economic benefits to farmers [132]. The good start of the *Bt*-eggplant plays a vital role in the future of crop biotechnology.

8. Eggplant for Science: The Future Model Crop Plant for Gene Functions?

In order to conduct research works quickly and to spend human and financial resources efficiently, model plants offer several unique advantages. To be a model system, an organism needs to be amenable to genetic transformation, genetically tractable, morphologically suitable for laboratory cultivation, and known at the gene level. The availability of efficient and straightforward regeneration protocols, especially via direct organogenesis, makes eggplant a primary choice of plant for genetic engineering. As previously mentioned, the good frequency of transformation by *Agrobacterium* and several morphological characteristics make eggplant an attractive plant for conducting experiments. Eggplant has a short maturity time; leaves and flowers are large and easy to emasculate. It is self-pollinating and bears large size fruits that contain numerous seeds. Parthenocarpic fruits can be produced in eggplant by hormone treatment or by introducing mutant genes without any negative pleiotropic effect. Moreover, the recently decoded whole genome sequence of eggplant [135,136] reveals that the size (1155 Mb) is closely related to crop plants such as potato (844 Mb) and tomato (950 Mb), but much smaller than model plant-tobacco (4500 Mb). Obviously, smaller genome size and the genomic nature of eggplant might appear as an advantage in the near future.

Arabidopsis has been serving as a model plant for cellular and molecular biology since the availability of its whole genome in 2000. The global research community and an extensive collection of mutants established this plant in a very good position to understand gene functions. Tobacco (another member of the Solanaceae family) has also long been a model plant in studying plant physiology and molecular biology due to its genetic information, size, ease of genetic transformation and crossing, short life cycle, and ability to obtain thousands of seeds per cross. However, eggplant, with the advent of less complex and smaller genome size, offers a more practical opportunity to observe the development of edible fruit, which is lacking in tobacco (Table 7). The recent development of the CRISPR/Cas9 system allows the introduction of site-specific double-stranded DNA breaks. Such targeted mutagenesis will surely boost the development of mutants in eggplant. The development of a highly efficient genetic transformation system and gene expression studies would establish eggplant as a preferred model system in the near future in order to investigate genetics, physiology, development, and evolution. In addition, the high content

of free radical scavengers (anthocyanins and polyphenols) in eggplant fruits might be an interesting aspect of functional food research.

Table 7. Comparison of traits to be considered as a model plant for cellular and molecular biology of flowering plants.

Traits	*Arabidopsis thaliana*	*Nicotiana tabacum*	*Solanum melongena*
Genome size	125 Mb	4400–4600 Mb	~1155 Mb
Ploidy	Diploid	Allotetraploid	Diploid
Chromosome number	10 (n = 5)	48 (n = 12)	24 (n = 12)
Life cycle	6 weeks	12 weeks	16 weeks
Seed production	Numerous	Numerous	Numerous
Space requirement	Very low	Moderate	Moderate
Genetic transformation efficiency	High	High	Moderate to high
Whole genome sequence	Available	Available	Available
Availability of mutant lines	Very high	Limited	Few
True-to-type micropropagation	Not easy	Very handy	Very handy
Commercial significance	No	Yes	Yes
Edible fruits	No	No	Yes
Research community	All over the world	All over the world	Limited countries

9. Conclusions

This review summarizes various factors and objectives of the applications of genetic engineering to eggplant. A meta-analysis of the factors affecting transformation such as explant, Agrobacterium strains, promoters to drive target genes, traits that are attempted to improve, and geographical location of the research community, specifically those who developed transgenic eggplant, is given in Figure 4. This summary picture would be helpful in selecting an appropriate methodology for future research. In addition, trends in the acceleration of eggplant research in recent years would be helpful in improving this important vegetable as a food and a candidate for molecular farming.

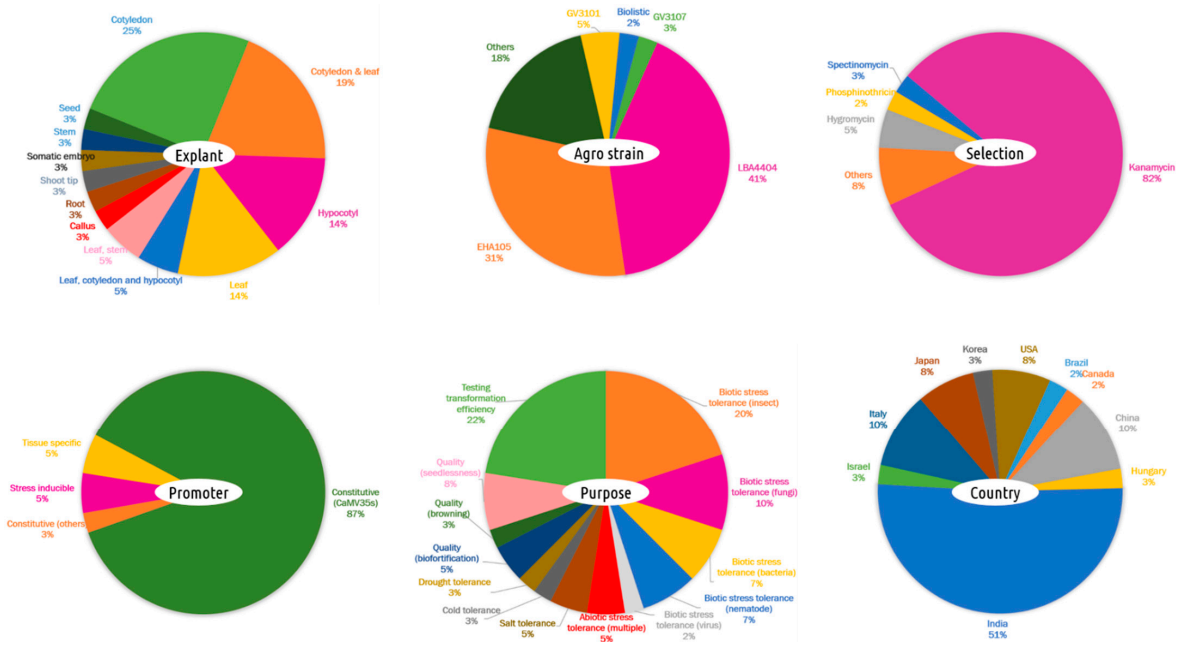

Figure 4. A meta-analysis of literature reports showing the relative contribution of various components of transgenic eggplant. From top left: explant, agrobacterium strains, and selection marker used for transformation. From bottom left: relative percentage of various promoters used, the purpose of transformation, and country-wise contribution of transgenic plant development. Data were retrieved by a manual search of the Scopus and PubMed database (up to early 2021). The journal articles only reported stable development of transgenic eggplant were considered in this analysis.

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References

1. BBS. *Statistical Database of the Bangladesh Bureau of Statistics*; B.B.o. Statistics, Ed.; Bangladesh Bureau of Statistics (BBS): Dhaka, Bangladesh, 2014.
2. FAOSTAT. *Statistical Database of the Food and Agriculture Organization of the United Nations*; F.a.A.O.o.t.U. Nations, Ed.; Online database; 2019. Available online: http://www.fao.org/faostat/en/#data (accessed on 10 April 2021).
3. Hui, Y.H.; Sherkat, F. *Handbook of Food Science, Technology, and Engineering-4 Volume Set*; CRC Press: Boca Raton, FL, USA, 2005.
4. FAO. *International Treaty on Plant Genetic Resources for Food and Agriculture*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2009.
5. Kumar, S.; Prasanna, P.; Wankhades, S. Potential benefits of bt brinjal in India-an economic assessment. *Agric. Econ. Res. Rev.* **2011**, *24*, 83–90.
6. Jorge, P.A.R.; Neyra, L.C.; Osaki, R.M.; de Almeida, E.; Bragagnolos, N. Effect of eggplant on plasma lipid levels, lipidic peroxidation and reversion of endothelial dysfunction in experimental hypercholesterolemia. *Arq. Bras. Cardiol.* **1998**, *70*, 87–91. [CrossRef]
7. Sidhu, M.; Dhatt, A.; Sidhus, G. Plant regeneration in eggplant (*Solanum melongena* L.): A review. *Afr. J. Biotechnol.* **2014**, *13*, 714–722.
8. Collonnier, C.; Fock, I.; Kashyap, V.; Rotino, G.; Daunay, M.; Lian, Y.; Mariska, I.; Rajam, M.; Servaes, A.; Ducreux, G.; et al. Applications of biotechnology in eggplant. *Plant Cell Tiss. Org. Cult.* **2001**, *65*, 91–107. [CrossRef]
9. Kashyap, V.; Kumar, S.V.; Collonnier, C.; Fusari, F.; Haicour, R.; Rotino, G.; Sihachakr, D.; Rajam, M. Biotechnology of eggplant. *Sci. Hortic.* **2003**, *97*, 1–25. [CrossRef]
10. Choudhary, B.; Gaur, K. *The Development and Regulation of Bt Brinjal in India (Eggplant/Aubergine), in ISAAA Briefs*; International Service for the Acquisition of Agri-Biotech Applications: New Delhi, India, 2009.
11. Mall, N.; Pandey, R.; Singh, S.; Singhs, S. Seasonal incidence of insect-pests and estimation of the losses caused by shoot and fruit borer on brinjal. *Indian J. Entomol.* **1992**, *54*, 241–247.
12. Singh, B.K.; Singh, S.; Yadavs, S.M. Some important plant pathogenic disease of brinjal (*Solenum melongena* L.) and their management. *Plant Patho. J.* **2014**, *13*, 208–213. [CrossRef]
13. Akinci, I.E.; Akinci, S.; Yilmaz, K.; Dikici, H. Response of eggplant varieties (*Solanum melongena*) to salinity in germination and seedling stages. *N. Z. J. Crop Hortic. Sci.* **2004**, *32*, 193–200. [CrossRef]
14. Monti, A.; Brugnoli, E.; Scartazza, A.; Amaducci, M.T. The effect of transient and continuous drought on yield, photosynthesis and carbon isotope discrimination in sugar beet (*Beta vulgaris* L.). *J. Exp. Bot.* **2006**, *57*, 1253–1262. [CrossRef]
15. Brown, K.F.; Messem, A.B.; Dunham, R.J.; Biscoe, P.V. Effect of drought on growth and water use of sugar beet. *J. Agric. Sci.* **1987**, *109*, 421–435. [CrossRef]
16. IPCC. *Climate Change 2007—The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC)*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2007.
17. Plazas, M.; Andújar, I.; Vilanova, S.; Hurtado, M.; Gramazio, P.; Herráiz, F.J.; Prohens, J. Breeding for chlorogenic acid content in eggplant: Interest and prospects. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2013**, *41*, 26–35. [CrossRef]
18. Sánchez-Díaz, M.; Aparicio-Tejo, P.; González-Murua, C.; Peñas, J.I. The effect of NaCl salinity and water stress with polyethylene glycol on nitrogen fixation, stomatal response and transpiration of *Medicago sativa*, *Trifolium repens* and *Trifolium brachycalycinum* (subclover). *Physiol. Plant.* **1982**, *54*, 361–366. [CrossRef]
19. Du, L.; Bao, C.; Hu, T.; Zhu, Q.; Hu, H.; He, Q.; Mao, W. SmARF8, a transcription factor involved in parthenocarpy in eggplant. *Mol. Genet. Genom.* **2015**, *291*, 93–105. [CrossRef] [PubMed]
20. Sękara, A.; Cebula, S.; Kunickis, E. Cultivated eggplants–origin, breeding objectives and genetic resources, a review. *Folia Hortic.* **2007**, *19*, 97–114.
21. Farooqui, M.A.; Rao, A.V.; Jayasree, T.; Sadanandam, A. Induction of atrazine resistance and somatic embryogenesis in *Solanum melongena*. *Theor. Appl. Genet.* **1997**, *95*, 702–705. [CrossRef]
22. Alicchio, R. Somaclonal variation in eggplant (*Solanum melongena* L.). In *Somaclonal Variation in Crop Improvement I*; Springer: Berlin/Heidelberg, Germany, 1990; pp. 416–434.
23. Rotino, G.; Falavigna, A.; Restainos, F. In vitro selection of eggplant cells resistant to culture filtrate of *Verticillium dahliae* Kleb. and regeneration of plants. *Capsicum Newsl.* **1987**, *6*, 94–95.
24. Dorica, B. The study of "in vitro" induced somaclonal variations in eggplants (*Solanum melongena*). *Buletinul USAMV ClujNapoca Seria Zootennie şi Biotehnologii* **2006**, *62*, 178–181.

25. Hitomi, A.; Amagai, H.; Ezura, H. The influence of auxin type on the array of somaclonal variants generated from somatic embryogenesis of eggplant, *Solanum melongena* L. *Plant Breed.* **1998**, *117*, 379–383. [CrossRef]
26. Vikram, P.; Swamy, B.P.M.; Dixit, S.; Singh, R.; Singh, B.P.; Miro, B.; Kohli, A.; Henry, A.; Singh, N.K.; Kumar, A. Drought susceptibility of modern rice varieties: An effect of linkage of drought tolerance with undesirable traits. *Sci. Rep.* **2015**, *5*, 14799. [CrossRef] [PubMed]
27. Alam, I.; Kim, K.-H.; Sharmin, S.A.; Kim, Y.-G.; Lee, B.-H. Advances in the molecular breeding of forage crops for abiotic stress tolerance. *J. Plant Biotechnol.* **2010**, *37*, 425–441. [CrossRef]
28. Guri, A.; Sinks, K.C. *Agrobacterium* transformation of eggplant. *J. Plant Physiol.* **1988**, *133*, 52–55. [CrossRef]
29. Başay, S.; Ellialtıoğlus, Ş. Effect of genotypical factors on the effectiveness of anther culture in eggplant (*Solanum melongena* L.). *Turk. J. Biol.* **2013**, *37*, 499–505. [CrossRef]
30. Corral-Martínez, P.; Seguí-Simarros, J. Efficient production of callus-derived doubled haploids through isolated microspore culture in eggplant (*Solanum melongena* L.). *Euphytica* **2012**, *187*, 47–61. [CrossRef]
31. Miyoshi, K. Callus induction and plantlet formation through culture of isolated microspores of eggplant (*Solanum melongena* L.). *Plant Cell Rep.* **1996**, *15*, 391–395. [CrossRef]
32. Zayova, E.; Vassilevska-Ivanova, R.; Kraptchev, B.; Stoeva, D. Indirect shoot organogenesis of eggplant (*Solanum melongena* L.). *J. Cent. Eur. Agric.* **2012**, *13*, 446–457.
33. Rotino, G.L.; Gleddies, S. Transformation of eggplant (*Solanum melongena* L.) using a binary *Agrobacterium tumefaciens* vector. *Plant Cell Rep.* **1990**, *9*, 26–29. [CrossRef] [PubMed]
34. Magioli, C.; Pinheiro, M.M.; Mansurs, E. Establishment of an efficient *Agrobacterium*-mediated transformation system for eggplant and study of a potential biotechnologically useful promoter. *J. Plant Biotechnol.* **2000**, *2*, 43–49.
35. Fári, M.; Nagy, I.; Csányi, M.; Mitykó, J.; Andrásfalvy, A. *Agrobacterium* mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (*Solanum melongena* L. cv. 'Kecskeméti lila'). *Plant Cell Rep.* **1995**, *15*, 82–86. [CrossRef]
36. Billings, S.; Jelenkovic, G.; Chin, C.-K.; Eberhardt, J. The effect of growth regulators and antibiotics on eggplant transformation. *J. Am. Soc. Hort. Sci.* **1997**, *122*, 158–162. [CrossRef]
37. Kumar, P.A.; Mandaokar, A.; Sreenivasu, K.; Chakrabarti, S.K.; Bisaria, S.; Sharma, S.R.; Kaur, S.; Sharma, R.P. Insect-resistant transgenic brinjal plants. *Mol. Breed.* **1998**, *4*, 33–37. [CrossRef]
38. Hanyu, H.; Murata, A.; Park, E.Y.; Okabe, M.; Billings, S.; Jelenkovic, G.; Pedersen, H.; Chin, C.-K. Stability of luciferase gene expression in a long term period in transgenic eggplant, *Solanum melongena*. *Plant Biotechnol.* **1999**, *16*, 403–407. [CrossRef]
39. Franklin, G.; Lakshmis, S.G. *Agrobacterium tumefaciens*-mediated transformation of eggplant (*Solanum melongena* L.) using root explants. *Plant Cell Rep.* **2003**, *21*, 549–554. [CrossRef]
40. Singh, A.K.; Verma, S.S.; Bansal, K.C. Plastid transformation in eggplant (*Solanum melongena* L.). *Transgenic Res.* **2010**, *19*, 113–119. [CrossRef] [PubMed]
41. Subramanyam, K.; Rajesh, M.; Jaganath, B.; Vasuki, A.; Theboral, J.; Elayaraja, D.; Karthik, S.; Manickavasagam, M.; Ganapathi, A. Assessment of factors influencing the *Agrobacterium*-mediated in planta seed transformation of brinjal (*Solanum melongena* L.). *Appl. Biochem. Biotechnol.* **2013**, *171*, 450–468. [CrossRef] [PubMed]
42. Singh, D.; Haicour, R.; Sihachakr, D.; Rajams, M.V. Expression of rice chitinase gene in transgenic eggplant confers resistance to fungal wilts. *Indian J. Biotechnol.* **2015**, *14*, 233–240.
43. Khan, H.; Faisal, M.; Aniss, M. Plant regeneration via somatic embryogensis in callus culuture of *Solanum melongena* L. *Phytomorphology* **2008**, *58*, 153–157.
44. Rajam, M.V.; Sharmas, P. Genotype, explant and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). *J. Exp. Bot.* **1995**, *46*, 135–141.
45. Beranová, M.; Rakouský, S.; Vávrová, Z.; Skalicky, T. Sonication assisted *Agrobacterium*-mediated transformation enhances the transformation efficiency in flax (*Linum usitatissimum* L.). *Plant Cell Tissue Organ Cult.* **2008**, *94*, 253–259. [CrossRef]
46. Keshamma, E.; Rohini, S.; Rao, K.S.; Madhusudhan, B.; Kumars, M.U. Tissue culture-independent in planta transformation strategy: An *Agrobacterium tumefaciens*-mediated gene transfer method to overcome recalcitrance in cotton (*Gossypium hirsutum* L.). *J. Cotton Sci.* **2008**, *12*, 264–272.
47. Park, B.-J.; Liu, Z.; Kanno, A.; Kameya, T. Transformation of radish (*Raphanus sativus* L.) via sonication and vacuum infiltration of germinated seeds with *Agrobacterium* harboring a group 3 LEA gene from *B. napus*. *Plant Cell Rep.* **2005**, *24*, 494–500. [CrossRef]
48. Tague, B.W.; Mantis, J. In planta Agrobacterium-mediated transformation by vacuum infiltration. In *Arabidopsis Protocols*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 215–223.
49. Weeks, J.T.; Ye, J.; Rommens, C.M. Development of an in planta method for transformation of alfalfa (*Medicago sativa*). *Transgenic Res.* **2008**, *17*, 587–597. [CrossRef]
50. Lin, J.; Zhou, B.; Yang, Y.; Mei, J.; Zhao, X.; Guo, X.; Huang, X.; Tang, D.; Liu, X. Piercing and vacuum infiltration of the mature embryo: A simplified method for *Agrobacterium*-mediated transformation of indica rice. *Plant Cell Rep.* **2009**, *28*, 1065–1074. [CrossRef]
51. Yasmeen, A.; Mirza, B.; Inayatullah, S.; Safdar, N.; Jamil, M.; Ali, S.; Choudhry, M.F. *In planta* transformation of tomato. *Plant Mol. Biol. Rep.* **2009**, *27*, 20–28. [CrossRef]

52. He, Y.; Bai, J.; Wu, F.; Mao, Y. In planta transformation of Brassica rapa and B. napus via vernalization-infiltration methods. *Protocol. Exch.* **2013**. [CrossRef]
53. Song, L.; Zhao, D.-G.; Wu, Y.-J.; Tian, X.-E. A simplified seed transformation method for obtaining transgenic *Brassica napus* plants. *Agric. Sci. Chin.* **2009**, *8*, 658–663. [CrossRef]
54. Bhattacharya, A.; Kumar, A.; Desai, N.; Parikh, S. Organelle transformation. *Methods Mol. Biol.* **2012**, *877*, 401–406.
55. Rotino, G.; Perri, E.; Acciarri, N.; Sunseri, F.; Arpaias, S. Development of eggplant varietal resistance to insects and diseases via plant breeding. *Adv. Hortic. Sci.* **1997**, *11*, 193–201.
56. Bravo, A.; Gill, S.S.; Soberón, M. Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. *Toxicon* **2007**, *49*, 423–435. [CrossRef] [PubMed]
57. Pal, J.K.; Singh, M.; Rai, M.; Satpathy, S.; Singh, D.V.; Kumars, S. Development and bioassay of *CryiAc*-transgenic eggplant (*Solarium melongena* L.) resistant to shoot and fruit borer. *J. Hortic. Sci. Biotechnol.* **2009**, *84*, 434–438. [CrossRef]
58. Rai, N.P.; Rai, G.K.; Kumar, S.; Kumari, N.; Singh, M. Shoot and fruit borer resistant transgenic eggplant (*Solanum melongena* L.) expressing *cry1Aa3* gene: Development and bioassay. *Crop Protect.* **2013**, *53*, 37–45. [CrossRef]
59. Shrivastava, D.; Dalal, M.; Nain, V.; Sharma, P.; Kumars, P.A. Targeted Integration of *Bacillus thuringiensis* delta-Endotoxin *cry1Fa1* in Brinjal (*Solanum melongena* L.). *Curr. Trends Biotechnol. Pharm.* **2011**, *5*, 1149–1156.
60. Jelenkovic, G.; Billings, S.; Chen, Q.; Lashomb, J.; Hamilton, G.; Ghidiu, G. Transformation of eggplant with synthetic *cryIIIA* gene produces a high level of resistance to the Colorado potato beetle. *J. Am. Soc. Hort. Sci.* **1998**, *123*, 19–25. [CrossRef]
61. Arpaia, S.; Mennella, G.; Onofaro, V.; Perri, E.; Sunseri, F.; Rotino, G.L. Production of transgenic eggplant (*Solanum melongena* L.) resistant to Colorado potato beetle (*Leptinotarsa decemlineata* Say). *Theor. Appl. Genet.* **1997**, *95*, 329–334. [CrossRef]
62. Li, H.; Bouwers, G. Toxicity of *Bacillus thuringiensis* Cry proteins to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in South Africa. *J. Invertebr. Pathol.* **2012**, *109*, 110–116. [CrossRef] [PubMed]
63. Perez-Guerrero, S.; Aldebis, H.K.; Vargas-Osunas, E. Toxicity of six *Bacillus thuringiensis* Cry proteins against the olive moth *Prays oleae*. *Bull. Insectol.* **2012**, *65*, 119–121.
64. Williams, S.; Friedrich, L.; Dincher, S.; Carozzi, N.; Kessmann, H.; Ward, E.; Rylas, J. Chemical regulation of Bacillus thuringiensis∂-endotoxin expression in transgenic plants. *Nat. Biotechnol.* **1992**, *10*, 540–543. [CrossRef]
65. Vaeck, M.; Reynaerts, A.; Hofte, H.; Jansens, S.; de Beuckeleer, M.; Dean, C.; Zabeau, M.; Montagu, M.V.; Leemanss, J. Transgenic plants protected from insect attack. *Nature* **1987**, *328*, 33–37. [CrossRef]
66. Koziel, M.G.; Beland, G.L.; Bowman, C.; Carozzi, N.B.; Crenshaw, R.; Crossland, L.; Dawson, J.; Desai, N.; Hill, M.; Kadwell, S.; et al. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Biotechnology* **1993**, *11*, 194–200. [CrossRef]
67. Jadhav, M.; Jadhav, A.; Pawar, B.; Kale, A.; Kutes, N. *Agrobacterium*-mediated genetic transformation of Brinjal with *cry1F* gene for resistance against shoot and fruit borer. *J. Crop Improv.* **2015**, *29*, 518–527. [CrossRef]
68. Shruti, B.K.; Roy, R.; Kumar, P.A.; Roy, S.P. Gene integration of *cry1f* in, brinjal for resistance against fruit and shoot borer. *J. Adv. Zool* **2015**, *36*, 83–89.
69. Firoz, A.; P, S.; P, P.S.; Anwar, F.; Sharmila, P.; Saradhi, P.P. No more recalcitrant: Chickpea regeneration and genetic transformation. *Afr. J. Biotechnol.* **2010**, *9*, 782–797. [CrossRef]
70. Koenning, S.R.; Overstreet, C.; Noling, J.W.; Donald, P.A.; Becker, J.O.; Fortnum, B.A. Fortnums, Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. *J. Nematol.* **1999**, *31*, 587–618.
71. Papolu, P.K.; Dutta, T.K.; Tyagi, N.; Urwin, P.E.; Lilley, C.J.; Rao, U. Expression of a cystatin transgene in eggplant provides resistance to root-knot nematode, *Meloidogyne incognita*. *Front. Plant Sci.* **2016**, *7*, 1122. [CrossRef] [PubMed]
72. Shivakumara, T.N.; Chaudhary, S.; Kamaraju, D.; Dutta, T.K.; Papolu, P.K.; Banakar, P.; Sreevathsa, R.; Singh, B.; Manjaiah, K.M.; Rao, U. Host-induced silencing of two pharyngeal gland genes conferred transcriptional alteration of cell wall-modifying enzymes of meloidogyne incognita vis-á-vis perturbed nematode infectivity in eggplant. *Front. Plant Sci.* **2017**, *8*, 8. [CrossRef] [PubMed]
73. Veluthakkal, R.; Dasguptas, M.G. Pathogenesis-related genes and proteins in forest tree species. *Trees Struct. Funct.* **2010**, *24*, 993–1006. [CrossRef]
74. Balasubramanian, V.; Vashisht, D.; Cletus, J.; Sakthivels, N. Plant β-1,3-glucanases: Their biological functions and transgenic expression against phytopathogenic fungi. *Biotechnol. Lett.* **2012**, *34*, 1983–1990. [CrossRef] [PubMed]
75. Mohammadi, M.; Karr, A.L. β-1,3-Glucanase and chitinase activities in soybean root nodules. *J. Plant Physiol.* **2002**, *159*, 245–256. [CrossRef]
76. Qiu, Z.; Yan, S.; Xia, B.; Jiang, J.; Yu, B.; Lei, J.; Chen, C.; Chen, L.; Yang, Y.; Wang, Y.; et al. The eggplant transcription factor MYB44 enhances resistance to bacterial wilt by activating the expression of spermidine synthase. *J. Exp. Bot.* **2019**, *70*, 5343–5354. [CrossRef]
77. Bhat, S.G.; Arulananthu, G.; Rajesh, G.; Rameshs, N. *Agrobacterium*-mediated transformation of brinjal (*Solanum melongena* L.) using fungal resistant gene. *Electron. J. Plant Breed.* **2020**, *11*, 160–168.
78. Singh, D.; Ambroise, A.; Haicour, R.; Sihachakr, D.; Rajam, M.V. Increased resistance to fungal wilts in transgenic eggplant expressing alfalfa glucanase gene. *Physiol. Mol. Biol. Plants* **2014**, *20*, 143–150. [CrossRef]
79. Darwish, N.A.; Khan, R.S.; Ntui, V.O.; Nakamura, I.; Miis, M. Generation of selectable marker-free transgenic eggplant resistant to *Alternaria solani* using the R/RS site-specific recombination system. *Plant Cell Rep.* **2014**, *33*, 411–421. [CrossRef] [PubMed]

80. Prabhavathi, V.; Rajam, M. Mannitol-accumulating transgenic eggplants exhibit enhanced resistance to fungal wilts. *Plant Sci.* **2007**, *173*, 50–54. [CrossRef]
81. Pratap, D.; Kumar, S.; Raj, S.K.; Sharmas, A.K. *Agrobacterium*-mediated transformation of eggplant (*Solanum melongena* L.) using cotyledon explants and coat protein gene of *Cucumber mosaic virus*. *Indian J. Biotechnol.* **2011**, *10*, 19–24.
82. Stoop, J.M.; Williamson, J.D.; Pharr, D.M. Mannitol metabolism in plants: A method for coping with stress. *Trends Plant Sci.* **1996**, *1*, 139–144. [CrossRef]
83. Shen, B.; Jensen, R.G.; Bohnert, H.J. Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. *Plant Physiol.* **1997**, *113*, 1177–1183. [CrossRef] [PubMed]
84. Ali, G.S.; Reddys, A.S.N. Inhibition of fungal and bacterial plant pathogens by synthetic peptides: In vitro growth inhibition, interaction between peptides and inhibition of disease progression. *Mol. Plant-Microbe Interact.* **2000**, *13*, 847–859. [CrossRef] [PubMed]
85. López-García, B.; González-Candelas, L.; Pérez-Payá, E.; Marcos, J.F. Identification and characterization of a hexapeptide with activity against phytopathogenic fungi that cause postharvest decay in fruits. *Mol. Plant-Microbe Interact.* **2000**, *13*, 837–846. [CrossRef] [PubMed]
86. López-García, B.; Pérez-Payá, E.; Marcos, J.F. Identification of novel hexapeptides bioactive against phytopathogenic fungi through screening of a synthetic peptide combinatorial library. *Appl. Environ. Microbiol.* **2002**, *68*, 2453–2460. [CrossRef]
87. Allefs, S.J.H.M.; De Jong, E.R.; Florack, D.E.A.; Hoogendoorn, C.; Stiekema, W.J. Erwinia soft rot resistance of potato cultivars expressing antimicrobial peptide tachyplesin I. *Mol. Breed.* **1996**, *2*, 97–105. [CrossRef]
88. Ponti, D.; Mangoni, M.L.; Mignogna, G.; Simmaco, M.; Barra, D. An amphibian antimicrobial peptide variant expressed in Nicotiana tabacum confers resistance to phytopathogens. *Biochem. J.* **2003**, *370*, 121–127. [CrossRef]
89. Alan, A.R.; Blowers, A.; Earle, E.D. Expression of a magainin-type antimicrobial peptide gene (MSI-99) in tomato enhances resistance to bacterial speck disease. *Plant Cell Rep.* **2004**, *22*, 388–396. [CrossRef] [PubMed]
90. Osusky, M.; Zhou, G.; Osuska, L.; Hancock, R.E.; Kay, W.W.; Misra, S. Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance to phytopathogens. *Nat. Biotech.* **2000**, *18*, 1162–1166. [CrossRef]
91. Yevtushenko, D.P.; Misras, S. Comparison of pathogen-induced expression and efficacy of two amphibian antimicrobial peptides, MsrA2 and temporin A, for engineering wide-spectrum disease resistance in tobacco. *Plant Biotechnol. J.* **2007**, *5*, 720–734. [CrossRef]
92. Na, C.; Shuanghua, W.; Jinglong, F.; Bihao, C.; Jianjun, L.; Changming, C.; Jin, J. Overexpression of the eggplant (*Solanum melongena*) NAC family transcription factor smnac suppresses resistance to bacterial wilt. *Sci. Rep.* **2016**, *6*, 31568. [CrossRef]
93. Prins, M.; Laimer, M.; Noris, E.; Schubert, J.; Wassenegger, M.; Tepfer, M. Strategies for antiviral resistance in transgenic plants. *Mol. Plant Pathol.* **2008**, *9*, 73–83. [CrossRef]
94. Ziegler, A.; Torrances, L. Applications of recombinant antibodies in plant pathology. *Mol. Plant Pathol.* **2002**, *3*, 401–407. [CrossRef] [PubMed]
95. Abel, P.P.; Nelson, R.S.; De, B.; Hoffmann, N.; Rogers, S.G.; Fraley, R.T.; Beachy, R.N. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* **1986**, *232*, 738–743. [CrossRef] [PubMed]
96. Lomonossoff, G.P. Pathogen-derived resistance to plant viruses. *Annu. Rev. Phytopathol.* **1995**, *33*, 323–343. [CrossRef] [PubMed]
97. Blumwald, E.; Aharon, G.S.; Apse, M.P. Sodium transport in plant cells. *Biochim. Biophys. Acta* **2000**, *1465*, 140–151. [CrossRef]
98. Yarra, R.; Kirti, P.B. Expressing class I wheat NHX (*TaNHX2*) gene in eggplant (*Solanum melongena* L.) improves plant performance under saline condition. *Funct. Integr. Genom.* **2019**, *19*, 541–554. [CrossRef]
99. Kumar, S.K.; Sivanesan, I.; Murugesan, K.; Jeong, B.R.; Hwang, S.J. Enhancing salt tolerance in eggplant by introduction of foreign halotolerance gene, *HAL1* isolated from yeast. *Hortic. Environ. Biotechnol.* **2014**, *55*, 222–229. [CrossRef]
100. Wan, F.; Pan, Y.; Li, J.; Chen, X.; Pan, Y.; Wang, Y.; Tian, S.; Zhang, X. Heterologous expression of *Arabidopsis C-repeat binding factor 3 (AtCBF3)* and *cold-regulated 15A (AtCOR15A)* enhanced chilling tolerance in transgenic eggplant (*Solanum melongena* L.). *Plant Cell Rep.* **2014**, *33*, 1951–1961. [CrossRef]
101. Sagare, D.B.; Mohantys, I. Development of moisture stress tolerant brinjal cv. Utkal Anushree (*Solanum melongena* L.) using *Agrobacterium* mediated gene transformation. *J. Agric. Sci.* **2012**, *4*, 141. [CrossRef]
102. Prabhavathi, V.R.; Rajams, M.V. Polyamine accumulation in transgenic eggplant enhances tolerance to multiple abiotic stresses and fungal resistance. *Plant Biotechnol.* **2007**, *24*, 273–282. [CrossRef]
103. Prabhavathi, V.; Yadav, J.; Kumar, P.; Rajam, M. Abiotic stress tolerance in transgenic eggplant (*Solanum melongena* L.) by introduction of bacterial mannitol phosphodehydrogenase gene. *Mol. Breed.* **2002**, *9*, 137–147. [CrossRef]
104. Alam, I.; Sharmin, S.A.; Kim, K.-H.; Kim, Y.-G.; Lee, J.J.; Bahk, J.D.; Lee, B.-H. Comparative proteomic approach to identify proteins involved in flooding combined with salinity stress in soybean. *Plant Soil* **2011**, *346*, 45–62. [CrossRef]
105. Serrano, R. Salt tolerance in plants and microorganisms: Toxicity targets and defense responses. *Int. Rev. Cytol.* **1996**, *165*, 1–52.
106. Bordas, M.; Montesinos, C.; Dabauza, M.; Salvador, A.; Roig, L.A.; Serrano, R.; Moreno, V. Transfer of the yeast salt tolerance gene *HAL1* to *Cucumis melo* L. cultivars and in vitro evaluation of salt tolerance. *Transgenic Res.* **1997**, *6*, 41–50. [CrossRef] [PubMed]
107. Gisbert, C.; Rus, A.M.; Bolarín, M.C.; López-Coronado, J.M.; Arrillaga, I.; Montesinos, C.; Caro, M.; Serrano, R.; Moreno, V. The yeast *HAL1* gene improves salt tolerance of transgenic tomato. *Plant Physiol.* **2000**, *123*, 393–402. [CrossRef]

108. Krasensky, J.; Jonaks, C. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.* **2012**, *63*, 1593–1608. [CrossRef]
109. Bray, E.A. Molecular responses to water deficit. *Plant Physiol.* **1993**, *103*, 1035–1040. [CrossRef]
110. Chaves, M.M.; Maroco, J.P.; Pereira, J.S. Understanding plant responses to drought- from genes to the whole plant. *Funct. Plant Biol.* **2003**, *30*, 239–264. [CrossRef] [PubMed]
111. Guilfoyle, T.J.; Hagens, G. Auxin response factors. *Curr. Opin. Plant Biol.* **2007**, *10*, 453–460. [CrossRef]
112. Karapanos, I.C.; Mahmood, S.; Thanopouloss, C. Fruit set in solanaceous vegetable crops as affected by floral and environmental factors. *Eur. J. Plant Sci. Biotechnol.* **2008**, *2*, 88–105.
113. Mishiba, K.-I.; Nishida, K.; Inoue, N.; Fujiwara, T.; Teranishi, S.; Iwata, Y.; Takeda, S.; Koizumi, N. Genetic engineering of eggplant accumulating β-carotene in fruit. *Plant Cell Rep.* **2020**, *39*, 1029–1039. [CrossRef] [PubMed]
114. Maioli, A.; Gianoglio, S.; Moglia, A.; Acquadro, A.; Valentino, D.; Milani, A.M.; Prohens, J.; Orzaez, D.; Granell, A.; Lanteri, S.; et al. Simultaneous CRISPR/Cas9 editing of three PPO genesreduces fruit flesh browning in *Solanum melongena* L. *Front. Plant Sci.* **2020**, *11*, 1883. [CrossRef] [PubMed]
115. Acciarri, N.; Restaino, F.; Vitelli, G.; Perrone, D.; Zottini, M.; Pandolfini, T.; Spena, A.; Rotino, G.L. Genetically modified parthenocarpic eggplants: Improved fruit productivity under both greenhouse and open field cultivation. *BMC Biotechnol.* **2002**, *2*, 4. [CrossRef] [PubMed]
116. Mapelli, S.; Frova, C.; Torti, G.; Soressi, G.P. Relationship between set, development and activities of growth regulators in tomato fruits. *Plant Cell Physiol.* **1978**, *19*, 1281–1288.
117. Tian, S.B.; Liu, F.Z.; Wang, Y.Q.; Luo, Z.Y.; Chen, Y.K.; Liu, J.S.; Lian, Y. Genetic analysis of parthenocarpy in eggplant. *Acta Hortic. Sin.* **2003**, *30*, 413–416.
118. Archbold, D.D.; Denniss, F.G. Strawberry receptacle growth and endogenous IAA content as affected by growth regulator application and achene removal. *J. Am. Soc. Hort. Sci.* **1985**, *110*, 816–820.
119. Rotino, G.L.; Perri, E.; Zottini, M.; Sommer, H.; Spena, A. Genetic engineering of parthenocarpic plants. *Nat. Biotech.* **1997**, *15*, 1398–1401. [CrossRef] [PubMed]
120. Donzella, G.; Spena, A.; Rotino, G.L. Transgenic parthenocarpic eggplants: Superior germplasm for increased winter production. *Mol. Breed.* **2000**, *6*, 79–86. [CrossRef]
121. Kumar, R.; Tyagi, A.K.; Sharma, A.K. Genome-wide analysis of auxin response factor (ARF) gene family from tomato and analysis of their role in flower and fruit development. *Mol. Genet. Genom.* **2011**, *285*, 245–260. [CrossRef] [PubMed]
122. Goetz, M.; Hooper, L.C.; Johnson, S.D.; Rodrigues, J.C.M.; Vivian-Smith, A.; Koltunow, A.M. Expression of Aberrant Forms of AUXIN RESPONSE FACTOR8 Stimulates Parthenocarpy in Arabidopsis and Tomato. *Plant Physiol.* **2007**, *145*, 351–366. [CrossRef]
123. Polturak, G.; Grossman, N.; Vela-Corcia, D.; Dong, Y.; Nudel, A.; Pliner, M.; Levy, M.; Rogachev, I.; Aharoni, A. Engineered gray mold resistance, antioxidant capacity, and pigmentation in betalain-producing crops and ornamentals. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 9062–9067. [CrossRef]
124. Niggeweg, R.; Michael, A.J.; Martin, C. Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nat. Biotech.* **2004**, *22*, 746–754. [CrossRef]
125. Prohens, J.; Rodríguez-Burruezo, A.; Raigón, M.D.; Nuezs, F. Total phenolic concentration and browning susceptibility in a collection of different varietal types and hybrids of eggplant: Implications for breeding for higher nutritional quality and reduced browning. *J. Am. Soc. Hort. Sci.* **2007**, *132*, 638–646. [CrossRef]
126. Yoo, S.Y.; Bomblies, K.; Yoo, S.K.; Yang, J.W.; Choi, M.S.; Lee, J.S.; Weigel, D.; Ahn, J.H. The 35S promoter used in a selectable marker gene of a plant transformation vector affects the expression of the transgene. *Planta* **2005**, *221*, 523–530. [CrossRef]
127. Yin, K.; Gao, C.; Qiu, J.-L. Progress and prospects in plant genome editing. *Nat. Plants* **2017**, *3*, 17107. [CrossRef]
128. Krishna, V.V.; Qaims, M. Potential impacts of Bt eggplant on economic surplus and farmers' health in India. *Agric. Econ.* **2008**, *38*, 167–180. [CrossRef]
129. Mesnage, R.; Clair, E.; Gress, S.; Then, C.; Szekacs, A.; Séralini, G.-E. Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. *J. Appl. Toxicol.* **2013**, *33*, 695–699. [CrossRef] [PubMed]
130. Guo, Y.; Feng, Y.; Ge, Y.; Tetreau, G.; Chen, X.; Dong, X.; Shi, W. The cultivation of Bt corn producing Cry1Ac toxins does not adversely affect non-target arthropods. *PLoS ONE* **2014**, *9*, e114228. [CrossRef] [PubMed]
131. Zhou, D.; Xu, L.; Gao, S.; Guo, J.; Luo, J.; You, Q.; Ques, Y. Cry1Ac transgenic sugarcane does not affect the diversity of microbial communities and has no significant effect on enzyme activities in rhizosphere soil within one crop season. *Front. Plant Sci.* **2016**, *7*, 265. [CrossRef] [PubMed]
132. Shelton, A.M.; Hossain, M.J.; Paranjape, V.; Azad, A.K.; Rahman, M.L.; Khan, A.S.M.M.R.; Prodhan, M.Z.H.; Rashid, M.A.; Majumder, R.; Hussain, S.S.; et al. Bt Eggplant project in Bangladesh: History, present status, and future direction. *Front. Bioeng. Biotechnol.* **2018**, *6*, 106. [CrossRef] [PubMed]
133. Mondal, R.I.; Quamruzzaman, A.K.M.; Hasan, K.; Khanam, D. The journey of Bt eggplant in Bangladesh. In Proceedings of the 4th Annual South. Asia Biosafety Conference, Hyderabad, India, 19–21 September 2016.
134. Prodhan, M.Z.H.; Hasan, M.T.; Chowdhury, M.M.I.; Alam, M.S.; Rahman, M.L.; Azad, A.K.; Hossain, M.J.; Naranjo, S.E.; Shelton, A.M. Bt eggplant (*Solanum melongena* L.) in Bangladesh: Fruit production and control of eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenee), effects on non-target arthropods and economic returns. *PLoS ONE* **2018**, *13*, e0205713. [CrossRef]

135. Hirakawa, H.; Shirasawa, K.; Miyatake, K.; Nunome, T.; Negoro, S.; Ohyama, A.; Yamaguchi, H.; Sato, S.; Isobe, S.; Tabata, S.; et al. Draft genome sequence of eggplant (*Solanum melongena* L.): The representative *Solanum* species indigenous to the Old World. *DNA Res.* **2014**, *21*, 649–660. [CrossRef] [PubMed]
136. Gramazio, P.; Yan, H.; Hasing, T.; Vilanova, S.; Prohens, J.; Bombarely, A. Whole-Genome Resequencing of Seven Eggplant (*Solanum melongena*) and One Wild Relative (*S. incanum*) Accessions Provides New Insights and Breeding Tools for Eggplant Enhancement. *Front. Plant. Sci.* **2019**, *10*, 1220. [CrossRef]