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Review Article

Improvement of Soybean Crop for Yield, Stress Tolerance, and Value-Added Products Using a Transgenic Approach

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Soybean (*Glycine max*) is an economically important crop, ranking first among the edible oilseed crops in the world due to its oil content and nutritional value. Besides, it is used as a dietary supplement and a source of pharmaceuticals. The recent rapid climate changes and increasing global population have led to increasing demand for vegetable oil. In the recent past, advances in the field of plant biotechnology have revolutionized agricultural practices at a global level to enhance the yield of crops. This technology not only makes an impact on the agricultural market but also opens up new corridors for agriculture-related industrial applications of this important crop. Therefore, in the last two decades, soybean has gained attention for genetic improvement with remarkable developments in the manipulations of genes for the induction of desired characteristics. In this review, we introduced the transgenic approach as a promising tool for the improvement of soybean oilseed quality and productivity. Then, the enhancement of nutritional and pharmaceutical value together with biotic and abiotic stress-resistant ability was summarized and compared. The methods and strategies for achieving soybean crops with improved abiotic stress tolerance, productivity, and pharmaceutics are categorized to help with future research.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is an important annual edible oilseed crop belonging to the *Fabaceae* family. It is the fourth largest crop in the world, mainly cultivated in tropical and subtropical regions [1]. At a global level, the soybean crop is one of the major and cheapest sources of vegetable protein for millions of people and animals. Being high in protein content and having very less or no starch, it is good food for diabetics [2]. In some parts of the world, it is consumed in the form of soymilk (whitish liquid extract from the seed), tofu (curd prepared from seed extract), soy sauce, and some other fermented products. Besides, it is an attractive crop for biodiesel production [3]. The crop also plays a key role in biological nitrogen fixation and the improvement of soil fertility [4]. At a global level, there is a tremendous increase in demand for soybean oil and food products. On the other hand, climate change and biotic and abiotic stress exposure cause pressure on agriculture and result in the decline of yield. Therefore, there is a need for improved soybean cultivars which can manage growth and yield under stressful environment.

Several soybean cultivars are available but with constricted germplasm [5]. The gene pool of soybean requires the incorporation of multiple genes for its improvement. There are rich genetic resources in wild relatives of soybean, but incompatibility issues make it difficult to transfer useful traits via interspecific crosses [6]. Therefore, genetic engineering is essential for the introduction of elite genes and to

achieve the improvement of soybean cultivars with desirable traits.

Over the past decade, numerous studies on the application of biotechnological approaches have been reported [7]. The transgenic methods take a relatively short duration for gene manipulation and field-testing period for new variety establishment. In contrast, conventional breeding programs require extensive backcrossing and time duration [8]. Genes responsible for biotic and abiotic stress tolerance and other yield-related traits have already been identified, and gene transformation methods were applied for crop improvement [9]. The whole-genome sequence, availability of genomic information, and improved technologies have widened the knowledge of the relationship among genes, their functionality, and their vital applications in the improvement of soybean germplasm [10].

In this review, we specifically introduced the applications of transgenic approaches for the improvement of soybean crops which can be employed in the fulfillment of edible oil and seed protein demand of growing population and livestock. Moreover, the optimization strategy of metabolic pathways for improvement of dietary supplements, lipid and protein quality and content, pharmaceuticals, biotic and abiotic stress tolerance, and herbicide resistance was discussed. In addition, the current cultivation status of genetically modified soybean cultivars was also summarized.

2. Economic Perspective of Soybean

Soybean is the leading economic edible oilseed crop worldwide and is a source of protein for human and animal consumption and a significant crop for biodiesel production [11, 12]. Besides oil and protein, soybean seeds consist of about 22-23% carbohydrates, 3–6% fiber, and 3–6% minerals [13]. It is cultivated in tropical and subtropical regions of the world. More than 80 countries in the world are producers of soybean; among them, Brazil, United States, India, Argentina, and China are major soybean producers. Its production in the world accounted for 360.993 million metric tons in 2018-2019 [14]. Generally, out of total soybean production, 85% contributes to oil and meal production. The meal of soybean contains 50% of protein which also remains intact after oil extraction. Among these, farm animals consume 19% of the meal [12]. Besides this, it is also used as dog food [15]. Therefore, soybean is widely consumed as feed for farm animals [16, 17]. In addition to regular routine foods, humans also use soybean and their products in day-to-day life. These products include soybean oil, soy sauce, soymilk, tofu, soy meal, soy flour, protein, tempeh, and soy lecithin [18]. In Japan, the pods of soybean are boiled and served with salt as "edamame" which is minimally processed food [19]. In India, soybean seeds and meal are used as a vegetable [20]. To compensate for low lysine deficiency in cereal proteins, soybean protein products (10–20%) are added to flour [21]. In addition, soybean is also used in the production of biodiesel fuel, crayons, cleanup solvents, biodegradable paints/varnishes, fiberglass, etc. [11, 22].

Different cosmetics, oils, soap, plastics, crayons, resins, and inks can beprepared from soybean. It is also used to prepare different solvents and clothes. Saponin is one of the important chemical components to regulate different human disorders, and soybean is the major source of it [23]. The different biomolecules such as phytic acid, daidzein, genistein, and isoflavones obtained from soybean are useful for cancer, cardiovascular diseases [24, 25], and various other disorders. Soybean food is important to lower cholesterol and reduce heart and diabetes diseases with other health benefits [26]. The phytic acid obtained from soybean showed antidiabetic, anti-inflammatory, and anticancer properties and acted as a good chelator for mineral absorption [27–30]. Due to diverse applications in different fields, it boosts world trade and the economy. Soybean is an important crop in the sense of farmers' socioeconomic status; therefore, it attains first position among oilseed crops. The versatile applications of soybean made it a hot topic of genetic engineering for further improvement. The various purposes to apply the transgenic approach in soybeans are given in Figure 1, and the transformation details and outcomes of the developed transgenic soybean are given in Table 1.

3. Enhancement of Dietary Supplements Using a Transgenic Approach

3.1. Oil and Fatty Acid Content. Soybean oil has several health benefits such as relatively high smoke point, richness in heart-healthy fats, support in bone health, high content of omega-3 fatty acids, and promotion of skin health. These features have increased global demand for soybean oil and stretched the attention of researchers. The research was targeted towards the development of transgenic approach for increasing the oil content of soybean seeds by manipulating substrates and enzymes involved in oil-producing pathways. Conversely, this approach showed less success as increased oil content reduced protein content and vice versa. The overexpression of fungal diacylglycerol acetyltransferase (DGAT2) protein in soybean resulted in a 1.5% increase in seed oil content without any reduction in protein content [49]. This DGAT2 protein converts diacylglycerols (DAGs) to triglycerols (TAGs). The transgenic lines' overexpressed DGAT2 proteins were tested in about 63 locations within the United States and Argentina for five growing seasons. In another example, overexpression of yeast sphingolipid compensation (SLC1) protein in seeds resulted in a 3.2% increase in the oil content of somatic embryos and transgenic plants with a 1.5% increase in seed oil content [44]. This protein possesses lysophosphatidic acid transferase activity which converts lysophosphatidic acid into phosphatidic acid. As per the pricing of soybean oil, a 1.5% increase in soy oil adds ~1.2 billion USD to the US economy. For further enhancement in oil content, it is essential to target other related metabolic pathways and metabolic engineering strategies.

The application of transgenic techniques offers new tools to improve soybean genotypes in terms of superior soy oil used for food and other industrial applications. Such nutritional advancements can be achieved by directed manipulation of fatty acid synthesis which will modify the content of fatty acids naturally present in soy oil. High amounts of oleic acid and low

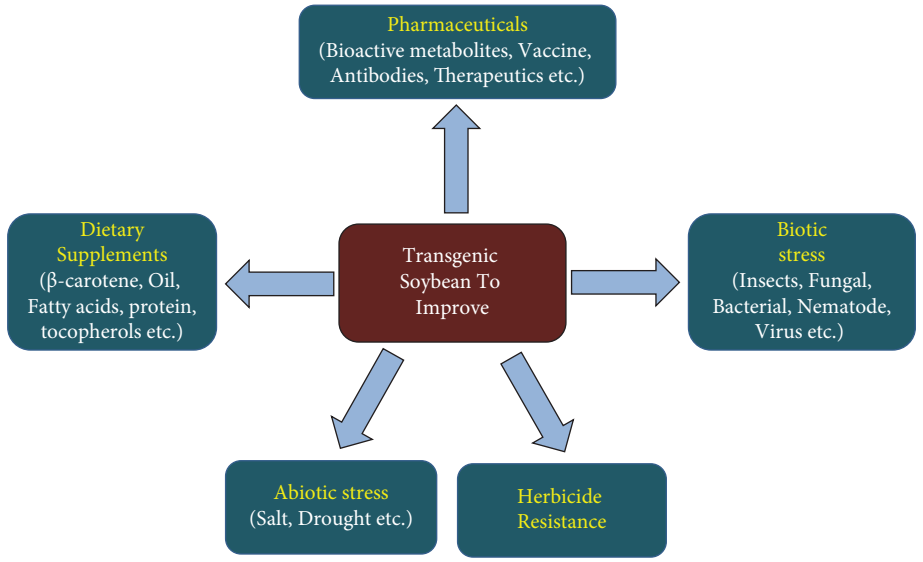

Figure 1: Transgenic approach used for the enhancement of potential traits of soybean crop.

amounts of linolenic acid (LA) containing soy oil must be targeted for genetic engineering. The LA accounts for 10–13% of the total fatty acid content of soy oil. It reduces the oxidative stability of oil and causes rancidity and decreased shelf life. The GmFAD3 gene family plays an important role in LA biosynthesis. Flores et al. [103] used gene silencing techniques to suppress GmFAD3 with the help of a single RNAi construct. This resulted in soybeans with low linolenic acid (below 2%). Soy oil contains a high amount of oleic acid (~18%) having good nutritious value. However, there was a decrease in yield due to alteration in fatty acid profiles in vegetative tissues of soybeans. To overcome this Delta-12 fatty acid desaturase (FAD2-1A and -1B), gene was downregulated [34]. The resulting seeds showed an increase in ~80% OA content without altering the fatty acid content of vegetative tissues. For confectionery applications, a combination of elevated OA and stearic acid content is essential. In another study, downregulation of Delta-12 oleate desaturase (GmFAD2-1) gene using *Agrobacterium tumefaciens* mediated gene transfer and antisense RNA method in Williams 82 variety of soybean. The resulting transgenic plants showed a tremendous increase in oleic acid content (51.71%) and a significant reduction in palmitic acid content (to <3%) in total seed oil content. More importantly, there was no structural difference in fatty acids of transgenic and wild plant oil extracts. Similarly, Monsanto [104] targeted the FAD2 gene along with the Acyl-ACP thioesterase (FATB) gene encoding enzyme in the biosynthetic pathway of soybean fatty acid synthesis. The suppression of these two genes resulted in a high level of oleic acid (monounsaturated) and lower levels of palmitic acid (saturated) and linoleic acid (polyunsaturated). The field trials of transgenic soybean (MON 87705) were taken from 17 sites across 10 states of U.S. soybean-producing regions. Interestingly, this transgenic plant confers tolerance to glyphosate [104].

To mitigate the high ω-3 fatty acid demand, private companies released several oleic, linoleic, and STA-improved cultivars. Monsanto [104] developed the transgenic soybean cultivar "Vistive Gold™ (MON87705)" having increased oleic acid content and released it in Columbia, Australia, Canada, Indonesia, European Union, USA, New Zealand, Japan, Singapore, Mexico, Philippines, Taiwan, South Korea, Vietnam, and MON87705-$\times$MON87708$\times$MON89788 and MON 87705$\times$MON 87708$\times$MON 89788 were released in Canada. Cultivar with increased oleic acid and linoleic acid was released in Mexico, Taiwan, South Korea, and European Union. The other cultivars of Monsanto company MON 87769$\times$MON 89788 were released in South Korea, Mexico, and Taiwan, and MON87769 was released in European Union, Indonesia, Australia, Canada, Columbia, New Zealand, Japan, South Korea, USA, Vietnam, Mexico, Philippines, and Taiwan which are rich in STA. The Dupont-released transgenic soybean cultivars G94-1, G94-19, and G168 have increased oleic acid content in Japan, Australia, USA, New Zealand, and Canada, and Treus™ and Plenish™ (DP305423) were released in Japan, Australia, China, Canada, European Union, South Korea, New Zealand, USA, Mexico, Singapore, Taiwan, Philippines, and South Africa, while Treus™ (DP 305423$\times$GTS 40-3-2) was released in Japan, Argentina, Philippines, Canada, South Korea, China, Taiwan, Mexico, and South Africa [35].

Table 1: Summary of improved soybean cultivars, target genes, delivery methods, and outcomes of soybean transgenics.

Traits *Dietary supplements*	Cultivar	Gene	Gene source	Delivery method	Outcome	Reference
Oil and fatty acid content	*Glycine max*	*DGAT2*	*Umbelopsis* (formerly *Mortierella*) *ramanniana*	*Agrobacterium*-mediated	Increased oil that shows no major impact on protein content or yield; increase in oil of 1.5% (by weight) in the mature seed	Lardizabel et al. [24]
	Glycine max cv. Jack	*GmFAD3*	*Glycine max* GmFAD3 RNAi silencing	*Agrobacterium*-mediated	LA contents below 2%	Flores et al. [26]
	Glycine max	*FAD2-1A and -1B*	*Glycine max* FAD2-1A and -1B ribozyme-terminated antisense	*Agrobacterium*-mediated	Ribozyme downregulates endogenous gene expression; oleic acid levels, greater than 85%, and saturated fatty acids levels, less than 6%	Buhr et al. [27]
	Glycine max cv. Thorne	Δ^6 *desaturase*	*Borago officinalis*	*Agrobacterium*-mediated	Converts LA and α-linolenic; GLA to ~27% and SDA to ~3% in seed oil (ALA) to GLA and SDA	Sato et al. [31]; Clement et al. [32]
	Glycine max cv. Williams 82	Delta-12 oleate desaturase GmFad2-1b gene	*Glycine max cv* Williams 82,	*Agrobacterium*-mediated	Increase in oleic acid (up to 51.71%) and a reduction in palmitic acid (to <3%) in their seed oil content	Zhang et al. [33]
Protein and amino acid content	*Glycine max* cv. Jack	β-Casein	Bovine milk protein, β-casein	Particle bombardment	Produces bovine β-casein; seed accumulated 0.1–0.4%	Maughan et al. [34]
	Glycine max cv. Maverick	O-acetylserine sulfhydrylase (OASS)	Overexpressed	*Agrobacterium*-mediated	Increase in the level of protein-bound cysteine (58–74%) and free cysteine (22–32%)	Kim et al. [35]
	Glycine max cv. Jack	Zein	*Zea mays*	*Agrobacterium*-mediated	20% increase in methionine and a 15–35% increase in cysteine	Dinkins et al. [36]
	Glycine max	AK and DHDPS	*E. coli and Corynebacterium*	*Agrobacterium*-mediated	Increase of free lysine >100-fold and total seed lysine 5-fold	Falco et al. [30]
	Glycine max cv. A3525	Aspartate kinase (AK)	*Xenorhabdus bovienii*	*Agrobacterium*-mediated	Increase in threonine and free amino acid levels by 100-fold and 3.5-fold, respectively	Qi et al. [37]
	Glycine max cv. Zigongdongdou (ZD)	Cystathionine γ-synthase (AtD-CGS)	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Met incorporated into proteins, increase in mature seeds by 1.8- and 2.3-fold, respectively	Song et al. [38]

Table 1: Continued.

Traits	Cultivar	Gene	Gene source	Delivery method	Outcome	Reference
Dietary supplements						
Tocopherols	*Glycine max* cv. A3244	Homogentisate phytyltransferase (HPT)	*Synechocystis* sp.	*Agrobacterium*-mediated	Increase of tocopherol by 15-fold	Karunanandaa et al. [39]
	Glycine max cv. Iksannamulkong	Homogentisate geranylgeranyl transferase (HGGT)	*Oryza sativa*	*Agrobacterium*-mediated	Enhanced vitamin E and all forms of tocopherol levels	Kim et al. [40]
	Glycine max	*VTE3* and *VTE4*	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Seeds accumulated >95% α-tocopherol	Van Eenennaam et al. [41]
	Glycine max cv. Jack	γ-Tocopherol methyltransferase (γ-TMT)	*Perilla frutescens*	Particle bombardment	10.4-fold increase in α-tocopherol content and a 14.9-fold increase in β-tocopherol content in seeds	Tavva et al. [42]
β-Carotene	*Glycine max* cv. Kwangan	Phytoene synthase-2A-carotene desaturase	Capsicum phytoene synthase (Psy) and Pantoea carotene desaturase (CrtI)	*Agrobacterium*-mediated	Transgenic seeds accumulated 146 mg/g of total carotenoids, of which 112 mg/g (77%) was β-carotene	Kim et al. [43]
	Glycine max L. cv. Jack	Phytoene synthase gene (crtB)	*Pantoea ananatis*	Particle bombardment	Plastids accumulated 845 μg β-carotene g^{-1} dry seed weight	Schmidt et al. [44]
	Glycine max cv. Jack	Phytoene synthase gene (crtB), ketolase genes (crtW from *Brevundimonas* sp. strain SD212 and bkt1 from *Haematococcus pluvialis*)	*Pantoea ananatis*, *Brevundimonas* sp. strain SD212, *Haematococcus pluvialis*	Particle bombardment	Transgenic seeds accumulated higher astaxanthin, lutein, β carotene, phytoene, α-carotene, lycopene, and β-cryptoxanthin	Pierce et al. [45]

Table 1: Continued.

Traits	Cultivar	Gene	Gene source	Delivery method	Outcome	Reference
Dietary supplements						
Pharmaceuticals						
Vaccines	*Glycine max* cv Williams 82, *W82 cell suspension cultures*	Hepatitis B surface antigen (HBsAg)	Hepatitis B virus	*Agrobacterium*-mediated	Plant-expressed HBsAg was retained intracellularly; HBsAg titers were obtained with soybean suspension cultures 20–22 mg/L	Smith et al. [46]
	Glycine max cv. Thorne	FanC	*E. coli*	*Agrobacterium*-mediated	Accumulation of FanC antigen; ~0.4% TSP in both leaves and seeds	Piller et al. [47]
	Glycine max cv. Jack	Enterotoxigenic heat-labile toxin B subunit (LTB)	*E. coli*	Particle bombardment	Immunogenic and partial protection against LT challenge in mice; transgenic produces 2.4% total seed protein at maturity	Moravec et al. [48]
	Glycine max cv. Jack and Kunitz	Nucleocapsid protein (PRRSV-ORF7)	PRRS virus	*Agrobacterium*-mediated	Accumulated nucleocapsid protein (PRRSV-ORF7); transgenic plants accumulated to 0. 64% of TSP	Vimolmangkang et al. [49]
	Glycine max	Enterotoxin B (mSEB)	*S. aureus*	*Agrobacterium*-mediated	Seed extracts containing mSEB showed an immune response; ~76 theoretical doses of human vaccine per single soybean seed	Laura et al. [50]
Antibodies	*Glycine max*	Monoclonal antibody	Humanized monoclonal anti-herpes simplex virus 2 (HSV-2) antibody	Particle acceleration method	Antibody in leaf tissue	Zeitlin et al. [51]

Table 1: Continued.

Traits	Cultivar	Gene	Gene source	Delivery method	Outcome	Reference
Dietary supplements						
	Glycine max	Growth hormone (hGH)	Human	*Agrobacterium*-mediated	Expressed the mature form of hGH; 0.0008% TSP	Russell et al. [52]
	Glycine max cv. Sichuan	Human basic fibroblast growth factor (bFGF)	Human fetal brain cDNAs	*Agrobacterium*-mediated	Accumulated human basic fibroblast growth factor (bFGF); 2.3% total seed protein at maturity	Ding et al. [53]
	Glycine max cv. Jack	Novokinin peptide gene	Ovalbumin	Whisker-mediated transformation	Transgenic soybean accumulates Novokinin (0.5% of TSP)	Yamada et al. [54]
	Glycine max cv. Jack	Suppression of the α/α' subunit of β-conglycinin storage protein synthesis	*Glycine max*	Particle bombardment	Suppression of the α/α' subunit of β-conglycinin (>7% (w/w) of the total protein in seeds)	Schmidt and Herman [55]
Therapeutics	*Glycine max* cv. Williams 82	Human thyroglobulin gene (hTG)	Human	*Agrobacterium*-mediated	Produces thyroglobulin ~1.5% of total soluble seed protein	Powell et al. [56]
	Glycine max	Human growth hormone (hGH)	Human	Particle bombardment	Expressed the mature form of hGH in their seeds; bioactive hGH up to 2.9% of the total soluble seed protein content	Cunha et al. [57]
	Glycine max cv. Conquista	Human proinsulin gene	Human	Particle bombardment	Plants expressed the proinsulin gene and accumulated in mature seed	Cunha et al. [58]
	Glycine max cv. BR16	Human coagulation factor IX (*hFIX*)	Human	Particle bombardment	Accumulated hFIX protein to seed; 0.23% of TSP	Cunha et al. [59]
	Glycine max cv. Jack	Epidermal growth factor (EGF)	Human	Particle bombardment	EGF protein produced in soybean seed; accumulated a range of 6.7 ± 3.1 to 129.0 ± 36.7 μg EGF/g of dry soybean seed	He et al. [60]
Bioactive metabolites	*Glycine max* cv. Jack	C1 and R transcription factors	*Zea mays*	Particle bombardment	Increased levels of isoflavones	Yu et al. [61]

Table 1: Continued.

Traits	Cultivar	Gene	Gene source	Delivery method	Outcome	Reference
Dietary supplements						
Abiotic stress tolerance						
Salt tolerance	*Glycine max* cv. DongNong-50	WRKY transcription factors	*Medicago sativa*	*Agrobacterium*-mediated	Overexpression improves salt tolerance in soybean	Wang et al. [62]
	Glycine max cv. DT26	H^+-pyrophosphatase gene (*AtAVP1*) and Na^+/H^+ antiporter gene (*AtNHX1*)	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Enhances salt tolerance	Nguyen et al. [63]
	Glycine max cv. Bert	*AtMYB44*	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Enhanced drought/salt stress tolerance	Seo et al. [64]
	Glycine max cv. Kwangankong	*AtABF3*	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Overexpression conferred drought and salt tolerance	Kim et al. [65]
	Glycine max	*GmFDL19*	*Glycine max*	*Agrobacterium*-mediated	Enhanced drought and salt tolerance	Li et al. [66]
Drought tolerance	*Glycine max* cv. Conquista	*soyBiPD* gene	*Glycine max*	Particle bombardment	Increased drought resistance	Valente et al. [67]
	Glycine max	*P5CR*	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Increased drought resistance	De Ronde et al. [68,69]
	Glycine max cv. BR16	*AtDREB1A*	*Arabidopsis thaliana*	Particle-bombardment	Increased drought resistance	de Paiva Rolla et al. [70]
	Glycine max cv. BR16	*AtDREB1A, AtDREB2CA, AtAREB1* transcription factors	*Arabidopsis thaliana*	Particle-bombardment	Increased drought resistance	Fuganti-Pagliarini et al. [71]
	Glycine max cv. Zhonghuang 20	*LOS5/ABA3*	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Increased drought resistance; seed yield of transgenic plants is at least 21% at field	Li et al. [72]
Biotic stress tolerance						
Viral	*Glycine max* cv. Fayette	Coat protein precursor (CP-P) gene	Bean pod mottle virus (BPMV)	*Agrobacterium*-mediated	Increased resistance	Di et al. [73]
	Glycine max cv. Jack	Capsid polyprotein (pCP) gene	Bean pod mottle virus (BPMV)	Particle bombardment	Increased resistance	Reddy et al. [74]
	Glycine max cv. 9341	Coat protein gene	Soybean mosaic virus (SMV)	*Agrobacterium*-mediated	Highly resistant to SMV	Wang et al. [75]
	Glycine max cv. Jack	Coat protein gene	Soybean dwarf virus (SbDV)	Particle bombardment	Increased resistance to SbDV	Tougou et al. [76,77]
	Glycine max cv. Williams 82	*GmAKT2*	*Glycine max*	*Agrobacterium*-mediated	Enhances SMV resistance	Zhou et al. [78]
	Glycine max cv. Tianlong, Huachun, Huachun, Williams 82, Jack and Kwangan	HC-Pro gene	Soybean mosaic virus	*Agrobacterium*-mediated	Enhances SMV resistance	Gao et al. [79]; Kim et al. [80]

Table 1: Continued.

Traits	Cultivar	Gene	Gene source	Delivery method	Outcome	Reference
Dietary supplements						
Fungal	*Glycine max* cv. BR-16	Oxalate decarboxylase (*OXDC*)	*Flammulina* sp.	Particle bombardment	Enhances white mould resistance	Cunha et al. [81]
	Glycine max cv. Heinong-35, Hefeng-35, Dongnong-42 and Jilin-30	Chitinase (CHI) and the barley ribosome-inactivating protein (RIP)	Bean and barley	*Agrobacterium*-mediated	Enhances fungal disease resistance	Li et al. [82]
	Glycine max cv. Williams 82	Antibody gene encoding scFv	Hybridoma cell line	*Agrobacterium*-mediated	Enhances foliar sudden death syndrome resistance	Brar and Bhattacharyya [83]
	Glycine max cv. Williams 82	Non-host resistance (NHR) gene	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Enhances resistance to rust disease	Langenbach et al. [84]
	Glycine max	*CcRpp1*	*Cajanus cajan*	Particle bombardment	Showed full resistance to Asian soybean rust	Kawashima et al. [85]
	Glycine max cv. Shennong 9	hrf2	*Xanthomonas oryzae* pv. oryzicola	*Agrobacterium*-mediated	Enhances resistance to *P. sojae*	Niu et al. [86]
Nematode	*Glycine max* cv. Jack and Chapman	Major sperm protein (MSP) gene	*Heterodera glycines*	Particle bombardment	Control of the soybean cyst nematode	Steeves et al. [87]
	Glycine max cv. KS4607	Cpn-1, Y25, and Prp-17	*Heterodera glycines*	*Agrobacterium*-mediated	Control of the soybean cyst nematode	Li et al. [88]
	Glycine max cv. Williams 82	Tyrosine phosphatase (TP) and mitochondrial stress-70 protein precursor (MSP)	*Meloidogyne incognita*	*Agrobacterium*-mediated	Decreased root-knot nematodes; decreased by >90%	Ibrahim et al. [89]
	Glycine max cv. Williams 82	*SAMT1*	*Glycine max*	*Agrobacterium*-mediated	Resistance to soybean cyst nematode	Lin et al. [90]
	Glycine max cv. Williams 82	*PAD4*	*Arabidopsis thaliana*	*Agrobacterium*-mediated	Resistance to soybean cyst nematode and root-knot nematode	Youssef et al. [91]
	Glycine max cv. Williams 82	CLE-receptor	*Glycine max*	*Agrobacterium*-mediated	Enhances resistance to soybean cyst nematode	Guo et al. [92]
	Glycine max cv. JackX	*HgY25, HgPrp17*	*Heterodera glycines*	Particle Inflow gun	Enhances resistance to soybean cyst nematode	Tian et al. [93]

Table 1: Continued.

Traits	Cultivar	Gene	Gene source	Delivery method	Outcome	Reference
Dietary supplements						
Insect	*Glycine max* cv. Williams 82, MB80-281	*Cry1A(b)*	*Bacillus thuringiensis* (Bt)	Microprojectile bombardment	Increased insect resistance	Parrott et al. [94]
	Glycine max cv. Jack	*Cry1Ac*	*Bacillus thuringiensis* (Bt)	Particle bombardment	Increased insect resistance to lepidopteran populations	Stewart et al. [95]
	Glycine max cv. Jack	*Cry1Ac*	*Bacillus thuringiensis* (Bt)	Particle bombardment	Increased insect resistance to lepidopteron populations	Walker et al. [96]
	Glycine max	*Cry1A*	*Bacillus thuringiensis* (Bt)	*Agrobacterium*-mediated	Increased insect resistance to lepidopteron populations	Miklos et al. [97]
	Glycine max cv. IAS5	*Cry1Ac*	*Bacillus thuringiensis* (Bt)	Particle bombardment	Increased insect resistance to *A. gemmatalis*	Homrich et al. [98]
	Glycine max	*Cry1A*	*Bacillus thuringiensis* (Bt)	*Agrobacterium*-mediated	Increased insect resistance to lepidopteron populations	Mcpherson and MacRae [99]
	Soybean Roundup Ready®	*EPSPS*	*Agrobacterium* spp. strain CP4	Particle acceleration method	Tolerant to herbicide glyphosate	Padgette et al. [100]
	Glycine max cv. CV127	*csr1-2*	*Arabidopsis thaliana*	Particle bombardment	Tolerant to herbicides of the imidazolinone chemical class	Homrich et al. [101]
	Glycine max cv. Thorne	Dicamba monooxygenase	*Pseudomonas maltophilia* (strain DI-6)	*Agrobacterium*-mediated	Resistance to treatment with dicamba	Behrens et al. [102]

3.2. Protein and Amino Acid Content. An increase in demand for dietary protein with diverse amino acid content stretches researchers' attention towards plant systems [36]. Plant system is an alternative cost-effective platform to synthesize protein and amino acids than the conventional one. It can express complex proteins on a large scale with proper molecular folding and modifications. Soybean is one of the important crops reported as a rich source of protein in their seed and highly utilized as a human and animal food [37, 38]. Therefore, it was a soft target of recombinant technology to make a biofactory not only for dietary supplements but also for pharmaceuticals [24]. The expression of recombinant protein in soybean provides advantages like no contamination and infection risk, high innate protein content, specialized compartments for proteins storage, low production cost, long-term storage of proteins, reduced cost of cold storage, and simple purification process over the prokaryotic expression system [36, 37].

To develop a transgenic soybean and utilize the seed as a biofactory, Cho et al. [105] constructed an expression cassette with seed-specific lectin promoter and, using β-glucuronidase, confirmed its expression. Later on, Maughan et al. [39] produced bovine β-casein (0.1–0.4% of TSP) with the same expression cassette, while Philip et al. [40] demonstrated β-casein localization in the vacuole, posttranslational modification, and purification from transgenic soybean seed commercial value for target-specific expression of proteins.

Soybean lacks the sulfur-containing amino acid cysteine and methionine. Therefore, Kim et al. [41] overexpressed the O-acetylserine sulfhydrylase (OASS) gene in the soybean "Maverick" cultivar through *Agrobacterium*-mediated gene transfer. They reported 58–74% of protein-bound cysteine and 22–32% of free cysteine in the seed. Dinkins et al. [42] overexpressed the maize zein gene via *Agrobacterium*-mediated gene transfer in soybean "Jack" cultivar seeds. They reported normal protein composition with an increase in 15–35% cysteine and 12–20% methionine. To enhance the dietary essential amino acid lysine in Soybean, Falco et al. [36] transferred enzymes aspartokinase and dihydrodipicolinate acid synthase obtained from *E. coli* and *Corynebacterium* via *Agrobacterium*-mediated gene transfer. The engineered lysine biosynthetic pathway reported an increase in free lysine >100-fold and total seed lysine 5-fold. To increase threonine level, Qi et al. [106] transferred aspartate kinases obtained from *Xenorhabdus bovienii* in "A3525" soybean cultivar with seed-specific promoter via *Agrobacterium*-mediated gene transfer. They reported no change in seed germination and morphological trait with an increase in 3.5-fold total free amino acid and 100-fold threonine levels.

Recently, Song et al. [107] expressed *Arabidopsis* cystathionine γ-synthase (AtD-CGS) as a Met-insensitive form with seed-specific promoter legumin B4 in the "Zigongdongdou" soybean cultivar. They found an increase in methionine content 3.8–7-fold in developing transgenic green seeds and 1.8- and 2.3-fold in mature dry seeds with an increase in other amino acids and total protein content. However, positive regulation of Met content regulates other amino acid synthesis and gives higher protein content in seed. From the above examples, soybean proves as an efficient alternative platform for protein and amino acid synthesis with increased nutritional value without altering its agronomic traits when grown in field conditions.

3.3. Tocopherols. Based on the methyl group present in tocopherol molecule, soybean seed tocopherols were classified as α, β, γ, and δ; among them, α-tocopherol has the highest nutritional value. These are the most essential molecules collectively called vitamin E and are considered one of the fat-soluble antioxidants. There is an increase in deficiencies associated with vitamin E, and its demand in the market increased tremendously. The soybean seed was a rich source of δ and γ-tocopherols and was mainly utilized for commercial oil stabilization. Therefore, the recombinant technology approach is mainly utilized to enhance the tocopherol content in soybean seeds. However, a slight increase in tocopherol content was reported in transgenic seeds in which homogentisate phytyltransferase (HPT) as the first tocopherol synthesis pathway enzyme encoding gene was overexpressed [43]. A modest 1.5-fold increase in total tocopherol content was reported by transferring the same gene with seed-specific promoters obtained from *Synechocystis* and *Arabidopsis* [45]. Later on, expression of the chorismate mutase-prephenate dehydrogenase (TYRA) gene obtained from bacteria with seed-specific promoters resulted in >10-fold enhancement in total vitamin E content.

To address the low tocopherol content issue in transgenic plants, Kim et al. [108] transformed rice tocopherol biosynthetic gene homogentisate geranylgeranyl transferase (HGGT) via *Agrobacterium*-mediated gene transfer with the constitutive and seed-specific promoter. The transgenic lines overexpressing the HGGT gene reported increased tocopherol content in addition to newly reported tocotrienols as an exceptional β-form in soybean.

To increase the α-tocopherol, Van Eenennaam et al. [109] transformed VTE3 and VTE4 genes using the *Agrobacterium*-mediated gene transfer method obtained from *Arabidopsis* which was responsible for tocopherol head group methylation. The expression of these genes in transgenic soybean seed reported more than a 95% increase of α-tocopherol content without disturbing the total tocopherol content of the seed. However, it demonstrates tocopherol shift from other forms to α-form where α-form amplifies the 5-fold activity of vitamin E. This research demonstrates increased vitamin E content in soybean oil and makes soybean an important dietary supplement for consumers that enhances its commercial value.

The α-tocopherol is one of the important bioactive molecules synthesized from γ-tocopherol, but it is necessary to have an adequate amount of final step catalyzing enzyme γ-tocopherol methyltransferase (γ-TMT) in the seed. Soybean oil is the major edible vegetable oil consumed, so manipulating the tocopherol biosynthetic pathway in soybean seed to convert tocopherols into a more active α-tocopherol form could have significant health benefits. Therefore, Tavva et al. [58] transformed γ-TMT gene

obtained from *Perilla frutescens* in soybean "Jack cultivar" with the seed-specific promoter. They found an increase in α-tocopherol content 10.4-fold and β-tocopherol up to 14.9-fold in transgenic seeds which showed 4.8-fold higher vitamin E activity. In addition, they reported that α-tocopherol restored seed storage and seed germination activity by minimizing oxidative damage caused due to lipid peroxidation. The above mentioned attempts demonstrate that the enhancement of total tocopherol and α-tocopherol content in transgenic soybean can eradicate the deficiencies caused due to low levels of vitamin E. However, to date, it is in the primary phase of commercialization; if commercialized, it could be used as a potential dietary supplement.

3.4. β-Carotene. β-Carotene is one of the important dietary supplements and works as a precursor molecule for vitamin A biosynthesis. Its limitations cause vitamin A-associated diseases like xerophthalmia, night blindness, breakdown of the human immune system [110], and fatal instances in pregnant women. These were major problems concerning β-carotene in many developing countries where single crops were utilized for dietary supplements [111]. Therefore, transgenic plants can be an alternative platform to enhance the level of β-carotene (provitamin A) in crop plants to mitigate this problem. Kim et al. [47] utilized a transgenic approach to enhance β-carotene levels in the soybean "Kwangan" cultivar. They manipulated the carotenoid biosynthetic pathway by overexpressing the phytoene synthase-2A-carotene desaturase (PAC) gene prepared by fusing Capsicum phytoene synthase (Psy) and Pantoea carotene desaturase (CrtI) gene. This expression cassette was transferred by *Agrobacterium*-mediated gene transformation with linked CaMV-35S (35S) or β-conglycinin (β) seed-specific promoter. They obtained 19 for β-PAC and 18 for 35S-PAC transgenic lines. Among them, the β-PAC line found the highest amount of carotenoid (146 mg/g of total carotenoids), ~62-fold higher than non-transgenic seeds; from them (112 mg/g), 77% was β-carotene. Therefore, they have demonstrated β-carotene production in soybean through genetic manipulation of the carotenoid pathway.

In another example, the *phytoene synthase gene (crtB)* was obtained from *Pantoea ananatis* bacteria combined with seed-specific chloroplast targeting peptide cassette and transferred in soybean "Jack" cultivar via particle bombardment method. It was found that the transgenic line overexpressing crtB gene accumulated 845 μg β-carotene g^{-1} dry seed weight. They reported β-carotene and α-carotene in a 12 : 1 ratio. In addition, a shift in oil content as an increase in oleic acid and a decrease in linoleic acid was reported while a 4% (w/w) increase in seed protein content was reported [38]. However, increased β-carotene, protein, and oleic acid content improved the nutritional quality of soybean seed.

To increase the intake of pink or red ketocarotenoids, canthaxanthin, and astaxanthin in poultry and aquaculture produce, animal feed should be a rich source of this. The conventional feed and delivery system is unable to reach this demand. Therefore, the highly utilized animal feed component soybean could be the efficient source and cost-effective delivery platform after synthesizing this carotenoid in it. Therefore, Pierce et al. [46] transformed the soybean "Jack" cultivar with the first step regulating the carotenoid pathway phytoene synthase enzyme (crtB) gene obtained from *Pantoea ananatis* via particle bombardment method. To synthesize ketocarotenoids, they fused this gene with ketolase genes (crtW) obtained from *Brevundimonas* sp. strain SD212, bkt1 gene obtained from *Haematococcus pluvialis*, and seed-specific promoter. The result reported almost 52 μg canthaxanthin per gram seed dry weight along with astaxanthin, lutein, β-carotene, phytoene, α-carotene, lycopene, and β-cryptoxanthin as a product of carotenoid pathway in the transgenic seed. Counterpart non-transgenic seed reported only lutein. However, transgenic soybean showed their ability to synthesize ketocarotenoids as an alternative source of costly animal feed additives [46]. After all these successful attempts of nutritionally rich transgenic soybean development, it is still in the primary phase of commercialization, but in the future, it could be released as a biofortified food and have nutritional benefits for humans and farm animals.

4. Improvement in Pharmaceuticals Using a Transgenic Approach

Increase in market demand for pharmaceutically important proteins and enzymes led to the discovery of rDNA technology in the early 1970s which gained popularity among biopharmaceutical, medicinal, food, nutritional, and agricultural industries. In the early days, the prokaryotic expression system was highly utilized as a protein expression system. Since the 1970s, *E. coli* has been widely utilized for insulin production [112]. Later on, fungal and yeast cells were utilized as expression platforms to fulfill the complex posttranslational modification demands. However, the problems associated with them were chances of contamination, failure for complex posttranslational modification, and inappropriate glycosylation. These problems were solved by transgenic animals, but manufacturing costs,social and ethical issues associated with them make this platform unfeasible for most protein production. The discovery of plant cell culture techniques and bioreactors that overcome all these limitations attracts attention towards plants as an efficient platform for pharmaceutical production. Soybean is one of the widely utilized crops for food, food products, and fodder in humans and livestock; therefore, it attracts the attention of researchers to produce pharmaceutically important compounds.

4.1. Vaccines. An increase in human bacterial and viral diseases severely affects the human population, and therefore vaccines are the landmarks to overcome this. However, there is a problem to reach every person in highly populated countries. Plants are used as an efficient platform for protein production and also attract attention to the production of edible vaccines. Therefore, soybean seeds and seed-based

products like soymilk are highly utilized in diet and are considered a perfect source to produce vaccines and their delivery. Soybean seeds can store vaccine antigens without degradation for many years at room temperature [48, 50, 51] which reduces the need and cost of cold storage. Smith et al. [52] expressed hepatitis B surface antigen (HBsAg) in *Glycine max* L. Merr. cv. Williams 82 through *Agrobacterium*-mediated gene transformation method to resolve the problem of its immunogenicity which requires complex and substantial posttranslational modifications. They obtained 20–22 mg/L HBsAg from soybean cell suspension culture and batch culture, which was synthesized intracellularly. The plant-derived antigen consisted predominantly of disulfide cross-linked HBsAg protein (p24(s)) dimers, which were all membrane-associated. Piller et al. [57] constitutively overexpressed the *E. coli* FanC antigen by *Agrobacterium*-mediated gene transfer method in soybeans and reported stable accumulation up to ~0.4% TSP in both leaves and seeds. The Enterotoxigenic *E. coli* (ETEC) and (K99) strains are considered a major cause of enteric disease-diarrhea in humans and livestock. If untreated, it causes death; therefore, it is regularly vaccinated in pregnant livestock to induce innate immunity and protect new infants. Recently, Garg et al. [53] also showed chloroplasts targeting FanC, a vaccine subunit antigen in soybean, by using a gene transformation approach. Therefore, to control the several *E. coli* diseases, an edible vaccine with an *E. coli* fimbrial subunit protein is a potential approach to increase effectiveness to induce mucosal immunity against pathogen attacks on site.

To increase the efficacy of antigen administered orally and nasally, mucosal adjuvant is required because it generates systemic immunity when delivered at mucosal surfaces. Therefore, in search for a powerful mucosal adjuvant, *E. coli* heat-labile toxin A (LTA) and B (LTB) subunit has the potential as an antigen and powerful mucosal adjuvant [113]. Therefore, Moravec et al. [54] demonstrated the production of a powerful mucosal adjuvant by transferring *E. coli* Enterotoxigenic heat-labile toxin B subunit (LTB) to soybean cultivar "Jack" by the particle bombardment method. The administration of seed extract containing LTB orally to mice and found a response of mucosal IgA anti-LTB and systemic IgG antibody against it. They reported an increase in antibody response 500-fold against bacterial FigHt antigen administered with soybean-derived LTB, demonstrating the functional ability of soybean-derived LTB as an oral adjuvant.

To solve the problem of the porcine reproductive and respiratory syndrome (PRRS) among breeding swineherds because current vaccines against it did not show efficacy when applied on the field, Vimolmangkang et al. [37] overexpressed a nucleocapsid protein (PRRSV-ORF7) of PRRS virus in soybean cultivars Jack and Kunitz by the *Agrobacterium*-mediated gene transformation method. They found 0.64% TSP in transgenic seed and induced specific humoral and mucosal immune responses against PRRSV-ORF7 in mice administered with this seed extract without adjuvant. Similarly, Hudson et al. [114] expressed *S. aureus* enterotoxin B (mSEB) via the *Agrobacterium*-mediated transformation method in soybean and characterized its non-toxic form as a model vaccine candidate. The soy-mSEB vaccine was produced at a high vaccine-to-biomass ratio, which resulted in approximately 76 theoretical doses of human vaccine per single soybean seed. They also assessed the subcellular environmental impact on protein accumulation and stability by localizing it extracellularly and intracellularly and found no change in the accumulation and stability of mSEB protein. The produced model vaccine also compared bacterial expression system-produced protein and native protein and found that they are immunologically and biochemically similar. Administration of mSEB-containing seed extract to mice for immunization showed an immune response within 14 days after the first administration. However, the transgenic soybean proteins show their efficacy as a vaccine, but commercializing and using them as a potential renewable oral vaccine needs more research.

4.2. Antibodies. The development of antibody technology has made significant strides in biotechnology, with applications ranging from disease diagnosis to therapeutic production. To mitigate antibodies, monoclonal antibodies (mAbs) played a major role in the advancement of the pharmaceutical field to produce mAb-based therapeutics and diagnostic systems. The plant is an efficient platform and has great potential to produce therapeutic use antibodies. Zeitlin et al. [59] demonstrated the production of humanized monoclonal anti-herpes simplex virus 2 (HSV-2) antibodies in transgenic soybean. It was an attempt to develop an efficient method of mucosal immunoprotection to overcome the problem of sexually transmitted diseases. They expressed humanized HSV-2 mAbs in soybean by transferring its gene by the particle acceleration method and found that these are expressed in soybean leaf tissue. They applied both soybean-produced and mammalian cell-produced HSV-2 mAbs to human semen and cervical mucus for 24 hours in a murine model and found that both antibodies diffuse in human cervical mucus, and they are similar in their stability and showed efficacy to prevent vaginal HSV-2 infection.

4.3. Therapeutics. An increase in health issues in the human population boosts the protein therapeutic market. However, the high cost of protein therapeutics because of the lengthy manufacturing and purification process limits utilization. Its potential use and its growth in the future largely depend on resolving the therapeutic delivery and lengthy manufacturing process problem that could reflect its low cost. To overcome these problems, protein-rich plants attract attention as a cost-effective alternative platform for synthesizing complex therapeutic proteins. The transgenic soybean is one of the best platforms for therapeutic protein production because of the high protein content in seed, low production cost, safe to use, and simple purification method.

There is increasing demand for human growth hormone (hGH) as therapeutic, and only the *E. coli* system-produced hGH makes it unaffordable to common people for therapy. To lower its price, Russell et al. [56] transferred human growth hormone (hGH) synthesizing gene in soybean through *Agrobacterium*-mediated gene transfer with

soybean seed-specific promoter 7S β-conglycinin and constitutive 35S promoter. They found 0.0008% TSP hGH accumulation in the seed, but it was a negligible amount. Later on, Cunha et al. [55] expressed the same gene with a strong expression cassette of 7S α' subunit of β-conglycinin promoter and α-coixin signal peptide. They found 2.9% TSP fully active hGH accumulation in seed and localized in seed protein storage vacuole. This increasing bioactive hGH could lead these soybean expression systems as an economically feasible alternative for large-scale therapeutic molecule production.

Ding et al. [60] expressed another highly valued therapeutic human basic fibroblast growth factor (bFGF) used for tissue repair, wound healing, and treatment of cardiovascular and neurodegenerative diseases in soybean seeds. They transferred bFGF cDNAs obtained from the human fetal brain with soybean seed-specific G1 promoter and endogenous signal sequence by the *Agrobacterium*-mediated gene transfer method in soybean "Sichuan" cultivar. The expression of this therapeutic in seed accumulated ~2.3% of TSP, and administered seed extract to mice showed its mitogenic activity.

Goeddel et al. [112] first demonstrated the transgenic expression of insulin in *E. coli* since it has been exploited as an important therapeutic agent. The therapeutic production from different expression systems has remained with high costs and contamination risk. Therefore, to resolve these problems of insulin expression, Cunha et al. [93] used a soybean expression system. They transferred the human proinsulin gene with seed-specific promoter γ-kafirin derived from sorghum in soybean cultivar "Conquista" through the particle bombardment method. To target transgenic proinsulin expression in soybean seed, they used α-coixin PSV signal peptide in the expression cassette. The transgenic proinsulin was stably expressed in soybean seed and remained stable at ambient storage conditions for up to seven years.

Yamada et al. [101] utilized this soybean expression system to produce cardiovascular therapeutics. One of them is Novokinin which has a vasorelaxation property and controls blood pressure [61]. They expressed the Novokinin peptide gene in the soybean "Jack" cultivar via the whisker-mediated transformation method with the modified β-conglycinin promoter. They found that transgenic seed accumulated 0.5% of total seed protein. Further, they administered a single dose of transgenic seed raw defatted flour formulation, purified formulation, orally to the hypertensive rat group and reported decreased systolic blood pressure. Cunha et al. [115] overexpressed human coagulation factor IX (hFIX) in soybean seed to prove a cost-effective method for overcoming the problem of the higher cost of current intravenous infusion of plasma-derived or other systems producing hFIX protein to arrest and prevent hemorrhage and its low quantity production. Using the particle bombardment method, they transferred hFIX to the soybean "BR16" cultivar. For targeting this protein in the seed, they used soy 7S promoter and coixin signal peptide. The transgenic hFIX accumulated to 0.23% of TSP. The transgenic seed-produced purified protein showed their efficacy and activity of blood clotting up to 1.4% of normal plasma and is stable for up to six years at $22 \pm 2^{\circ}$C storage conditions. Powell et al. [116] demonstrated the ability of soybean to produce complex and large subunit proteins where currently utilized expression systems fail to produce them. They transferred the human thyroglobulin (hTG) gene in soybean "Williams 82" cultivar via the *Agrobacterium*-mediated gene transfer method. The hTG is a homodimeric protein of 660 kDa and is utilized as a source of standard protein and thyroid disease diagnosis. For the above use, this protein required proper folding and posttranslational modification. Until now, the expression system fulfills required hTG demands; therefore, hTG is derived from surgically removed thyroid tissue and cadaver utilized commercially but still has a problem of heterogeneity and lack of uniformity in producer-purified hTG because the used kit showed the variation in result. For the potential use of the soybean-derived expression system, they used 7S promoter and seed targeting endogenous hTG signal peptide. Transgenic seed accumulated full-length hTG dimeric protein of 1.5% of TSP over generations and reported functionality through ELISA kits. Therefore, they successfully demonstrated the soybean-derived expression system as one of the most efficient systems to produce the biggest hTG functional protein than other plants reported to date, and it proves their practicality and capability as a refractory to express any proteins than conventional systems.

The ability of soybean for protein production and storage stretches expectations to enhance its level, and it may be possible by applying protein distribution knowledge. Therefore, Schmidt and Herman [117] demonstrated a proteome rebalancing approach to enhance recombinant protein levels in seeds. For this, they expressed green fluorescent protein (GFP) in soybeans with suppressing β-conglycinin to observe green fluorescent yield and found that it was increased ~4-fold while the increase in protein level was >7% of TSP. Thus, the proteome rebalancing strategy is one of the important strategies that could help to develop transgenic lines that can increase levels of recombinant proteins in the future. Recently, He et al. [118] expressed human epidermal growth factor (EGF) in soybean "Jack" cultivar to address the life-threatening disease necrotizing enterocolitis (NEC) of premature infants caused due to gut microbiome. Necrotizing enterocolitis is treated by surgically removing diseased and dead tissues. In infants, necrotizing enterocolitis is reduced by EGF as an intestinotrophic growth factor found in the mother's breast milk, salvia, and amniotic fluid. The expression cassette of the human EGF gene has an ER signal tag at the N' terminal and a seed-specific codon bombarded on soybean embryos. They found 6.7 to 129 μg EGF/g protein in dry soybean seed. This level is biologically significant and showed activity like commercially available EGF. Proteomic and immunoblot assay confirmed its relatedness to human EGF protein. This work highlights the practicability of soybean seeds as a biofactory for therapeutic molecule production.

All these examples confirmed the plant as a promising alternative system for valuable recombinant therapeutic production. Recently, Israeli company Protalix Biotherapeutics developed the first plant-derived therapeutic

product as an enzymatic drug ELELYSO™ to treat Type 1 Gaucher's disease and it reached marketable status [119]. This confirms the intrinsic ability of plant systems for the demanding scale production of pharmaceuticals and therapeutics with competitive low cost and high quality.

4.4. Bioactive Metabolites. Plants are a rich source of bioactive metabolites that are utilized for the production of different medicines [46]. The advancement in biotechnology makes it possible to enhance these bioactive molecules from plant cells through cell suspension culture and well-designed bioreactors. The advancement of the transgenic approach makes it possible to manipulate the desired metabolite pathway from the cell for continuous synthesis of these metabolites in the cell [118, 120]. Yu et al. [121] engineered a phenylpropanoid biosynthetic pathway to increase soybean isoflavone levels. They transferred maize C1 and R transcription factors in the "Jack" cultivar by the particle bombardment method to activate the phenylpropanoid biosynthetic pathway, which in turn increased the daidzein level and decreased genistein level in the seeds. The daidzein, genistein, and isoflavones obtained from soybean are useful againstcancer and cardiovascular diseases [25] and various other disorders. The expression of C1 and R transcription factors in soybean seed increased isoflavone levels by simultaneous co-suppression of flavanone 3-hydroxylase that blocked the anthocyanin branch of the pathway [25]. It well demonstrates that the combinational phenomenon of the C1/R transcription factor induces gene activation and inhibition of competing pathways providing a useful tool to enhance isoflavone accumulation in soybean seed [62, 121].

5. Enhancement of Abiotic Stress Tolerance Using a Transgenic Approach

The change in climate leads to an increase in abiotic and biotic stresses in crop plants [63]. Among the abiotic stresses, salinity, drought, cold, heat, UV rays, and heavy metals are the major factors severely influencing crop plants. Increased abiotic stresses and decreased crop production raise the problem of food security in near future. If it continues, today's agriculture will fail to fulfill the growing population demands. It was estimated that agricultural production needs to increase by up to 70% in upcoming years to meet the demand of the increasing population and livestock [64, 66]. Thinking of the future increasing population, sustainable agriculture is the big challenge in front of us. The stresses not only directly influence crop plants but also indirectly influence crop plants by reducing the cultivable agricultural land area. This is because of climate change, availability of fresh water for irrigation, use of chemical fertilizers and pesticides, soil erosion, and intrusion of seawater [122]. It leads to detrimental effects on crop yield and productivity by reducing crop plant growth and development [72]. To face these challenges, transgenic soybean is an alternative strategy to make stress-tolerant plants or genotypes for better yield and production.

5.1. Salt Tolerance. Soybean is a leading cash crop. However, soil salinity and sodicity significantly affect its production. The salt in soil and water negatively affects the physiology and growth of soybean which subsequently reduces yield. Therefore, it is classified as a moderately salt-sensitive glycophyte [71]. Several salt stress-responsive genes were identified and characterized in soybean, but very few of them were successful in field trials. An overexpression of MSWRKY11 of alfalfa in the DongNong-50 cultivar of soybean by the *Agrobacterium*-mediated gene transfer method increased the salt tolerance of soybean seedlings [123]. Upon salt exposure, the transgenic plants showed longer hypocotyls, high content of chlorophyll, proline, catalase, superoxide dismutase, and soluble sugars, and reduced ROS (H_2O_2 and O_2^-) and MDA content than non-transgenic plants. In addition, the agronomic traits like plant height, pods per plant, seed weight, and seeds per plant were significantly higher in transgenic plants than in non-transgenic plants [123].

Two transporter genes from *Arabidopsis* (vacuolar H^+ pyrophosphatase 1 and vacuolar Na^+/H^+ antiporter 1) were co-expressed in soybean. These genes help in the compartmentalization of toxic sodium ions from the cytoplasm to vacuoles. The co-expressed transgenics are more tolerant to short-term salt stress than individually expressed genes and non-transgenic plants [67].

Another transcription factor AtMYB44 involved in abscisic acid-induced stomatal closure is a well-known salt-responsible gene. This gene was overexpressed in soybean by the cotyledonary-node method. The resulting homozygous transgenic lines showed dwarf phenotype throughout the growth period in greenhouse conditions and improved salt and drought stress tolerance as compared to non-transgenic lines. In field conditions, transgenic plants showed a reduction in growth; however, after harvesting, they showed higher yield, and their amino acid and fatty acid content and compositions were at par with non-transgenic plants. These transgenic lines also showed improved environmental stress tolerance [70].

The GmFDL19 is a member of the basic leucine zipper family (bZIP) which acts as a promoter of flowering in soybean. Upon overexpression, it induced early flowering in transgenic soybean. It also showed enhanced drought and salt stress. Its expression is induced by ABA, PEG, and high salinity [68]. Although many genes have been identified that can improve salt tolerance in plants and some salt-tolerant crops have been developed, progress has been rather limited regarding producing such crops for field conditions or commercialization.

5.2. Drought Tolerance. Drought is the major abiotic constraint, which severely affects crop growth and productivity [69]. Soybean possesses a set of adaptive mechanisms to ameliorate drought-induced damage [65]. Incorporation of these traits in soybean germplasm for enhanced tolerance and yield is essential. In model plants like *Arabidopsis* and tobacco, overexpression of a single gene showed potential against drought stress [85]. However, it has to be translated into multiple crop plants [65, 124]. The introduction of

binding protein (BiP), an endoplasmic reticulum localized molecular chaperone, into soybean showed improved drought stress tolerance [125]. The transgenic lines showed a decrease in wilting of leaves, water potential, and stomatal closure under drought stress. Overexpression of a transcription factor, DREB1A in soybean, showed improvement in the total number of pods, number of pods with seeds, and number of seeds at field level under drought stress [83]. Moreover, in the greenhouse, due to the lower transpiration rate, the survival rate of transgenic plants was increased. One more transcription factor, AREB (ABA-responsive element binding), was introduced in soybean, and transgenic lines were tested under irrigated and non-irrigated field conditions. The results showed that the transgenic line 1Ea2939 has high water use efficiency and leaf area index and the oil and protein content was not affected [85]. The LOS5/ABA3 overexpression using constitutive super promoter resulted in at least a 21% increase in seed yield of transgenic lines under drought stress conditions in the field [65]. Transgenic soybean expressing P5CR, encoding L-Δ1-pyrroline5-carboxylate reductase, which catalyzes the final step in proline biosynthesis, under the control of an inducible heat shock promoter was more tolerant to drought and high temperature than non-transgenic plants [84, 126].

The *Arabidopsis* ABF3 transcription factor (ABRE binding factor) is overexpressed in soybean using *Agrobacterium*-mediated gene transformation. The results showed that the transgenic lines conferred salt and drought stress under low watering conditions [86]. Although many genes have been identified that can improve drought tolerance in plants and some drought-tolerant crops have been developed, progress has been rather limited regarding producing such crops for field conditions or commercialization.

6. Enhancement of Biotic Stress Tolerance Using a Transgenic Approach

Biotic stresses including insect pests, fungi, bacteria, viruses, and nematodes are the prime challenge for sustainable agriculture and food production as they may decline the forthcoming yields in crop plants by 60–70% [73, 75]. The United States, Brazil, and Argentina are the three major soybean-growing countries in the world where more than 50% of all soybeans are harvested. Such geographic distributions impeded the spread of pathogens and diseases. Hence, soybean can be hampered by several different pathogens and diseases, including fungi, bacteria [76], viruses, pests, and nematodes. The pathogen's attack is usually tissue-specific and/or can cause damage to roots, stems, leaves, pods, and seeds [75, 77]. The insect-pest and disease control management are currently paying attention to agronomic practices such as the use of cultivating resistant genotypes, genetically improved varieties, planting under tillage, wide row planting, and rotation with non-host specific crop varieties [127]. The use of chemical spray remedies has poor success because of low penetration rate, dose-dependent tissue damage, and uneven application due to an already formed canopy cover; additionally, the chemical application cannot be cost-effective for farmers and can be harmful to the environment. There has been little achievement with conventional plant breeding approaches for insect pests and disease resistance in soybean [128]. Recently, biotechnological approaches have emerged to produce genetically modified soybeans (transgenic) that have disease resistance [129]. Therefore, insect pest and disease resistance in crop plants is an additional area of interest for both researchers and farmers.

6.1. Virus Tolerance. The viral diseases are most widespread in crop plants worldwide. The development of viral resistance with the use of a transgenic approach has been studied a little more extensively in soybean. Successful resistance to several viruses in several crop species has been achieved through pathogen-derived resistance with the use of viral coat proteins and interference with viral assembly. Di et al. [130] developed a bean pod mottle virus (BPMV) resistant soybean by introducing a BPMV coat protein and by inserting a BPMV capsid polyprotein into the soybean genome. The events generated in BPMV resistance in soybean were analyzing the infectivity assays and exhibiting systemic infection which resulted in no visible symptoms. The infection of soybean mosaic virus (SMV) can cause yield loss by 90%; to overcome yield loss, the development of SMV-resistant soybean is important. To produce genetically modified soybean lines resistant to SMV, the plants contain a coat protein gene and the 3′ UTR from SMV. The coat protein gene and the 3′ UTR sequences were screened in transgenic lines, and two of the soybean lines were highly resistant to SMV. These results suggested that this is the first report of stable SMV resistance in genetically modified soybean [131]. Tougou et al. [132] studied soybean dwarf virus (SbDV) and developed SbDV-resistant soybean plants through the sense coat protein gene with overexpression of SbDV-CP mRNA, or repression accumulation of SbDV-CP mRNA, and siRNA by RNA analysis. The results of these transgenic lines remained with no symptoms. Similarly, Tougou et al. [81] achieved RNA silencing to develop resistance for SbDV using inverted repeat-SbDV coat proteins spaced by a β-glucuronidase sequence. Upon infection with SbDV, the transgenic soybean plants were symptomless, which was suggesting that the SbDV-resistance was achieved through an RNA silencing-mediated approach.

6.2. Bacterial Tolerance. Bacterial diseases in plants may affect roots, stems, and leaves or can be impeded internally without external symptoms [76, 82]. Hence, in soybean, bacterial diseases are most prevalent that occur mainly in the early-season juvenile and young leaves [75]. Particularly, the bacterial blight of soybean is the most common soybean bacterial disease caused by the bacterium species *Pseudomonas syringae* pv. *glycinea*, and it is most prevalent in the early season at the juvenile stage. In addition, there are several bacterial diseases in soybean resulting in poor seed quality and loss in potential yield [133]. On the other hand, there has been a promising achievement in the development of bacterial disease resistance in rice, tomato, banana, and tobacco crop plants using biotechnological approaches

[134]. The literature survey suggested that there have been few efforts made on the development of bacterial disease resistance for soybean [87]. Therefore, the present research may facilitate new approaches for the development of bacterial disease resistance in soybean crops.

6.3. Fungal Tolerance. Fungal pathogens are the most frequent in soybean crops and therefore represent targets for the development of fungal disease-resistant varieties. *Sclerotinia* stem rot (SSR) fungal pathogen is caused by *Sclerotinia sclerotiorum*, and it is the most commonly found pathogen in soybeans [135] cultivated in the countries of the United States and Brazil. The pathogen has been associated with the presence of oxalic acid (OA), and the metabolism of OA is correlated with fungal resistance [136]. Cunha et al. [93] studied OA-dependent SSR resistance and developed transgenic soybean lines with the use of overexpressed oxalate decarboxylase enzymes (OXDC). A significant reduction of disease severity was achieved with the expression of OXDC, and the higher levels of OXDC exhibited complete SSR resistance suggesting the feasibility of this approach.

An alternative technology demonstrated by Li et al. [89] was to develop multigene resistance by overexpressing multiple anti-fungal genes such as bean chitinase (CHI) and the barley ribosome-inactivating protein (RIP). Efforts were made to produce successful transgenic fungal-resistant soybean by the overexpression of both genes, also controlling fungal diseases through the use of the single-chain variable fragment (scFv) antibody approach, and similarly, Brar and Bhattacharya [77] demonstrated control of *Fusarium virguliforme* which is responsible for sudden death syndrome (SDS) in soybean through the antibody approach. Use of the pathogenic toxin Tox1 as a target, soybeans were transformed with an antibody gene encoding scFv anti-FvTox1 with improved foliar SDS resistance. The developed biotechnological strategy could be beneficial for resistance to other plant pathogen-induced diseases.

Developing durable non-host resistance (NHR) for crop protection often necessitates the identification and transfer of NHR-linked genes to the target crop. Langenbach et al. [128] demonstrated the identification of genes associated with NHR and transfer of NHR-linked genes in *Arabidopsis thaliana*-exhibited *Phakopsora pachyrhizi* fungus causing Asian soybean rust (ASR) disease resistance. But Asian soybean rust disease is only treatable with fungicides, and the developed transgenic soybean lines are becoming less effective due to the emergence of fungicide resistance. To date, there are no commercial soybean cultivars with long-lasting resistance to *P. pachyrhizi*, and although soybean resistance loci have been mapped, no resistance genes have been explored. The introduction of CcRpp1 (*Cajanus cajan* Resistance against *Phakopsora pachyrhizi* 1) gene from pigeon pea to soybean resulted in better resistance against *P. pachyrhizi* [73]. The transgenic soybean lines were produced by introducing the desired DNA sequence into the embryogenic callus using particle gun bombardment. The most devastating and widespread oomycete pathogen *Phytophthora sojae* causes *Phytophthora* root and stem rot (PRR) disease throughout soybean-producing regions worldwide. Niu et al. [129] introduced the $harpin_{Xooc}$-encoding hrf2 gene isolated from rice bacterial pathogen *Xanthomonas oryzae* into soybean for resistance to *P. sojae* pathogen by eliciting multiple defense responses mediated by different signaling pathways. The pathogen resistance assessed through molecular analysis confirmed the integration and expression of hrf2 in the produced transgenic soybean. The potential functional role of the hrf2 gene in plant resistance against *P. sojae* makes it a promising tool for broadening disease resistance to other pathogens in soybean. Therefore, this is an important step in the direction of developing the first commercially available transgenic fungal-resistant soybean cultivar.

6.4. Nematode Tolerance. Nematode microorganisms are noticeably devastating parasites causing crop productivity loss of approximately $157 billion (USD) worldwide [90]. Recent techniques used to combat agricultural losses due to nematode parasites include the use of nematicides, cultivation techniques, and varieties with spontaneous resistance. The majority of yield loss can be attributed to infection of the root-knot nematodes (RKNs) and soybean cyst nematodes (SCNs) caused by *Meloidogyne* and *Heterodera*, respectively [137].

RNA interference (RNAi) has become a powerful tool for silencing genes in crop plants. An approach that has emerged in recent years is the use of RNA interference (RNAi) to target genes of feeding nematodes for resistance in soybean. Also, Steeves et al. [99] demonstrated the efficacy of an RNAi-based strategy to develop resistance against SCN. The ingestion of plant-derived dsRNAs inactivating targeted genes can be attributed to producing nematode resistance by silencing essential nematode genes. Gheysen and Vanholme [138] and Lilley et al. [139] discovered that there are a variety of candidate genes known to be important for nematode survival, and many of these were implemented candidates through planta RNAi strategies to control nematode diseases. The nematode-resistant transgenic soybeans were produced by introducing an RNAi expression vector containing inverted repeats of a cDNA clone of the SCN major sperm protein (MSP). RNA-associated gene silencing was produced in the cyst nematode by ingestion of dsRNA molecules and resulted in ~75% suppression of reproductive capabilities nematodes in the host. Recently, Tian et al. [140] reported that the expression of hairpin RNAi constructs derived from two SCN genes (HgY25 and HgPrp17) related to reproduction and fitness enhances resistance to SCN in developed transgenic soybean. Similarly, RNAi was used to interrupt genes associated with root-knot nematode (RKN) gall formation to provide resistance to soybean [94]. Moreover, genes encoding tyrosine phosphatase (TP) and mitochondrial stress-70 protein precursor (MSP) were stably expressed in soybean roots, and following infection with RKN, the number of galls was decreased by >90%, and nematode proliferation within roots was reduced 5-fold over in the developed transgenic soybean roots over control. Nematode infection activates plant defense responses

mediated by salicylic acid (SA). The overexpression of GmSAMT1 affects the development of soybean cyst nematode, which contributes to the plant's resistance to this pest [126]. Therefore, the developed transgenic strategies exhibited nematode resistance in soybean.

6.5. Insect Tolerance. Insect resistance in plants through applying chemical insecticides has brought about considerable protection to crop yield [96]. Unfortunately, extensive use of chemical insecticides has resulted in the eradication of beneficial insects, environmental degradation, and adverse effects on human health and other organisms. As farmers move forward to achieve greater crop productivity, it will be imperative to replace chemical inputs with safer alternatives to manage insect pests in agricultural ecosystems [141]. Within agricultural biotechnology, insect resistance is a prime research area that has the potential to improve agricultural productivity and provide many alternatives to insecticides while being effective against pests, innocuous to non-target organisms, and cost-effective. Nowadays, biotechnology has enabled the creation of genetically modified plants for insect resistance on a commercial scale, and one of the most extensive strategies for insect resistance in soybeans involves the *Bacillus thuringiensis* (Bt) gene [87, 97].

In the last decade, *Bacillus thuringiensis* (Bt) is a common bacterium found in the environment that has been used as a biological control agent against lepidopteran insects for more than 50 years. Bt targets a class of compounds responsible for an insecticidal activity known as crystalline proteins or cry proteins (Cry1) that are highly toxic after ingestion [98]. Particularly, the Cry1 toxins are involved in the disruption of the midgut cellular membranes of insects leading to subsequent cell death. One of the major advantages of using Bt genes for insect resistance in transgenic crops is the eradication of *Lepidoptera dipteran* and *Coleopteran* class of insects [142]. Therefore, inserting Bt toxins into plants, by genetic transformation, is an attractive strategy for developing insect-resistant transgenic crops.

Using Bt toxin, to date, several different plant species have been genetically modified to confer insect resistance. So far, the Bt trait has been commercialized in cotton and maize, while it is still progressing in soybean. Transformation of soybean with Bt to induce resistance to *Lepidoptera* has been performed, and by 1994, fertile transformed soybeans containing a synthetic Bt (Cry1A (b)) were generated [143, 144]. The synthetic Bt (Cry1A (b)) gene caused complete *A. gemmatalis* larval mortality and significantly reduced *Pseudoplusia includens* and *Helicoverpa zea* larval survival. The detached leaf bioassays exhibit that the transgenic soybean lines were resistant to multiple soybean insects with <3% leaf defoliation compared to 20% observed in conventionally produced lepidopteran resistance [144]. Walker et al. [145] used a relevant model by evaluating soybean lines modified with Cry1Ac for resistance to *Lepidoptera* under field conditions. Likewise, Macrae et al. [146] and Mcpherson and MacRae [141] have been evaluating Bt soybean lines for resistance against *Lepidoptera* in the field for over 2 years. In this case, soybean plants expressing Bt Cry1Ac showed <1.5% defoliation when compared to 53% defoliation in control plants. Soybean lines expressing a cry1A gene with a high degree of resistance against *Pseudoplusia includens*, *Helicoverpa zea*, and *Anticarsia gemmatalis* were reported by Miklos et al. [147]. Similarly, soybean expressing a synthetic Bt cry1Ac gene was highly toxic to *A. gemmatalis*; in addition, the Bt cry1Ac transgene does not affect the agronomic performance and yield in soybean by evaluating in vitro and field bioassays [148, 149]. Application of transgenic (Bt) soybean has become apparent through gene pyramiding strategies (Bt Cry1Ac) with native plant resistance genes to increase plant resistance against insect resistance. Gene pyramiding through several quantitative trait loci (QTLs) from soybean genotypes has been produced which results in antixenosis and antibiosis resistance towards *Lepidoptera* [100, 150], and Walker et al. [151] reported the development of transgenic soybean lines by combining QTLs with synthetic Cry1Ac. Therefore, Bt in consortium with other pesticides has the potential to drastically reduce the consumption of chemical pesticides; however, it will be important to continue research and have a development model for the future generation technique, to ensure that insects do not rapidly adapt to resistance.

7. Enhancement of Herbicide Resistance Using a Transgenic Approach

The amino acids required for plants from aromatic amino acids are the essential amino acids synthesized through the shikimate biosynthetic pathway. 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS) is one of the important enzymes of the aromatic amino acid biosynthesis pathway [102, 152]. The highly growing plants regulate this pathway continuously to synthesize higher amounts of aromatic amino acids to fulfill the needs of growing cells. Therefore, they showed their higher efficiency of nutrient uptake from soil. Among these plants, unwanted herbs include weeds also growing with agriculture crops that have a faster growth rate because of faster nutrient uptake. It makes soil nutrients unavailable for growing crops and hampers crop growth [153]. To control these unwanted herbs, in 1970, glyphosate was discovered [154], and it was utilized commercially in 1974. They achieved success by showing their strong ability to control these fast-growing unwanted herbs by blocking the aromatic amino acid synthesizing enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glyphosate used in the USA reached up to 4.5 million kg until 1995. However, simultaneously, to a certain extent, it also inhibits crop 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzymes which leads to a decrease in crop yield. Due to the fame and wide use of glyphosate as an herbicide by farmers, it became a target of recombinant DNA technology to develop herbicide-resistant soybean.

Padgette et al. [155] utilized recombinant DNA technology to transfer herbicide-resistant EPSPS gene isolated from *Agrobacterium* spp. strain CP4 via the particle acceleration method in soybean. The developed transgenic soybean plant meets the plant demands of aromatic amino

acids and other essential metabolites necessary for growth and development in presence of glyphosate. Later on, Monsanto commercialized transgenically developed Roundup Ready® herbicide tolerant soybean crop as one of the first commercially available transgenic crops. The expression of EPSPS in transgenic soybean provides resistance to glyphosate (Roundup™). The fame of these soybeans grew with farmers since Roundup™ could be applied to a field of Roundup Ready® soybeans to considerably decline weed populations without harming soybean crops. This inbuilt property of resistance of Roundup Ready® soybeans was easy to use and presented many advantages to farmers. In addition, they offer wider application than currently used herbicides in soybean in terms of growth stage-specific response and effective control of larger weeds. These factors make this technology popular for effective weed control.

After Monsanto's success with Roundup Ready® soybean, Liberty Link® soybean was another herbicide-tolerant soybean cultivar released by Bayer Crop Science. They attempted to develop glufosinate ammonium, another type of herbicide-tolerant cultivar, to overcome the problem of decreased photosynthesis and inhibition of glutamine amino acid synthesis in soybean crops. For this, they transformed the phosphinothricin-*N*-acetyltransferase (PAT) gene isolated from *Streptomyces viridochromogenes*. PAT is a glutamine synthetase inhibitor that binds to glutamate, making plants resistant. The phosphinothricin herbicides have an active part of glufosinate ammonium (Basta®, Ignite®, Rely®, Liberty®, Harvest®, and Finale®) which is used to control different weeds when crop grows or for total plant control on uncultivable land. The glufosinate ammonium-based herbicides have a different mode of action than glyphosate-based herbicides, and the emerging Liberty Link® provides an alternative strategy for farmers to control weeds.

Several herbicide-tolerant soybeans were developed and are in various stages of development. However, for effective control of the weed population, Mathesius et al. [156] developed glyphosate and acetolactate synthase (ALS)-inhibiting herbicide-tolerant soybean cultivar. These soybean cultivars express the glyphosate acetyltransferase (GAT4601) and modified versions of soybean acetolactate synthase (GMHRA) proteins. This was a different approach than previous genetic modification where multiple genes were utilized to confer two-herbicide tolerance. German company BASF developed an imidazolinone class of herbicide-tolerant soybean utilizing the same technology. Inhibition of the native enzymes by imidazolinone herbicides causes plant death. Therefore, they transferred the imidazolinone-tolerant AHAS-large subunit (csr1-2) gene obtained from *Arabidopsis thaliana* in soybean. This helps in the synthesis of valine, leucine, and isoleucine branched-chain essential amino acids [157]. The Embrapa (the Brazilian Agricultural Research Corporation) researcher group developed imidazolinone chemical class herbicide-tolerant soybean cultivar CV127 (Cultivance) having csr1-2 gene which was approved for commercial release by National Technical Commission on Biosecurity in Brazil [120].

Monsanto has developed a dicamba herbicide-resistant soybean cultivar [158]. Dicamba (3, 6-dichloro-2-methoxy benzoic acid) eco-friendly broadleaf weed control highly used low-priced herbicide. To develop a dicamba-resistant plant, dicamba monooxygenase (DMO) gene obtained from bacteria was transformed in soybean. Soybean with transformed gene neutralized dicamba impact on plants. It is now in the final stage of commercialization.

Hydroxyphenylpyruvate dioxygenase (HPPD) is responsible for the synthesis of plastoquinones through the catabolism of tyrosine and further regulating photosynthesis, tocopherols, and carotenoid biosynthesis. Some herbicide inhibits this enzyme and leads to decreased plant growth. However, Syngenta and Bayer Crop Science companies developed HPPD-inhibiting herbicides and glufosinate-resistant transgenic soybean cultivars cooperatively [159]. The transformation of gene acetohydroxyacid synthase (AHAS) isolated from *Arabidopsis* in soybean confers imazapyr herbicide resistance while the introduction of 4-hydroxyphenylpyruvate dioxygenase (HPPD) isolated from *Pseudomonas fluorescens* confer isoxaflutole herbicide resistance. The same phosphinothricin herbicide resistance was achieved by transferring the bialaphos-resistant soil bacteria phosphinothricin *N*-acetyltransferase (PAT) gene [160–162]. However, these new emerging multiple herbicide tolerance approaches are efficient tools for farmers to control weeds that developed resistance against single herbicides. In the future, it is expected to introduce another novel innovative approach for generating GM soybeans having the ability to control not only weed but also increase weed resistance and eliminate environmental causes and cost issues.

8. Impact on Root Nodulation

Root nodulation is a crucial process for biological nitrogen (N_2) fixation in legume plants [163]. Gwata et al. [164] suggested that the process of root nodulation could be used to rapidly identify genetic segregants resulting from selection in plant breeding programs and biotechnological strategies. Symbiotic atmospheric nitrogen fixation is a multifaceted physiological process subjected to the interaction of genetic elements in legumes and rhizobia and *Frankia* [165, 166]. Hayashi et al. [166] reported that the formation of symbiotic root nodules in soybean (*Glycine max*) is regulated by numerous host genes like Rj (rj) genes; however, molecular cloning of Rj genes is vulnerable due to large genome size and complicated genome structure. For instance, *Sinorhizobium fredii* is a fast-growing *Rhizobium* that can establish a nitrogen-fixing symbiosis in soybean legume crops. In a limited set of soybean genotypes, the root nodulation with *S. fredii* strains showed a higher level of specificity. The previous literature exhibited the dominant gene Rfg1 in soybeans that restrict root nodulation with *S. fredii* strains (USDA193, USDA257, and USDA205) [167]. A gene GmEXPB2 is preferentially expressed at the early stage of root nodule development. In contrast,

overexpression of GmEXPB2 noticeably modified soybean root architecture such as an increase in size and several cortical cells, root hair density, and size of root hair zone [156]. Likewise, the AP2, GRF5, and C3H family TF regulatory networks were associated with early root nodule development, and nodule maturation was associated with GRAS, LBD41, and ARR18 family TFs in soybean crops. This attempt supported the experimental confirmation in the future to determine key gene targets for genetic engineering approaches to optimize root nodule development and enhance nitrogen fixation for higher productivity [168]. Hayashi et al. [166] reported that the particular Rj genotypes can exclude nodulation with indigenous *Bradyrhizobium* strains that have vital importance in agriculture for improving the efficiency of *B. japonicum* strains that reveal efficient nitrogen fixation in soybean.

9. Current Cultivation Status of Genetically Modified Soybean

ISAAA [169] report revealed that genetically modified soybean cultivation occupied about 50% of the world which covers about 94.1 million hectare area; of the global 189.8 million hectare GM area, a 3% rise in the cultivation rate or 2.7 million hectare more area comes under the cultivation as compared with 2016. Out of 94.1 million hectares, 69.7 hectares comprise herbicide tolerance (HT) and about 24.4 million hectares cover insect resistance and/or herbicide tolerance (IR/HT) (Intacta™) soybean; there was a rise of 4% or 9,79,000 hectares in 2017. The slant attributes were deployed magnificently in Argentina, Paraguay, and Uruguay and utmost without any alteration in Brazil. Worldwide countries that planted genetically modified soybean were as follows: USA (34 million hectares), Brazil (33.7 million hectares), Argentina (18.1 million hectares), Paraguay (2.7 million hectares), Canada (2.5 million hectares), Bolivia (1.2 million hectares), Uruguay (1.1 million hectares), Chile (1,000 hectares), and South Africa (243,000 hectares). The transgenic soybean is grown in the USA, Brazil, South Africa, and Bolivia but is not cultivated in Mexico due to legal restrictions.

Genetically modified soybean was not planted in Costa Rica in 2017; instead, crops were planted for export depending on the seed demands. [170] In 2017, approximately 77% of the global soybean hectarage, equivalent to 121.5 million hectares, consisted of genetically modified soybean crops [161]. Notably, Brazil approved four trials for genetically modified soybean planting that year, which included a stacked trait providing resistance against both insects and herbicides (IR (lep)/HT glyphosate), as well as three herbicide-tolerant events that involved resistance to glyphosate, glufosinate, and methozone, respectively, stacked with IR (lep). There were 14 recombinant soybean traits approved for food and feed in 2017.

The impact of the genetically modified herbicide-tolerant soybean technology boosts the gross income of the farm. The rise in the farm grains is the result of a reduction in the production cost and or decrease in the expenditure needed for weed control, especially herbicides. An increase in yield gain occurred from the enhancement in the level of weed control, and the average farm income setback has been higher, in countries such as Romania, Mexico, and Bolivia. In 2009, a second generation of genetically modified herbicide-tolerant soybean became available for commercial soybean cultivators in the USA and Canada. This technology provides the equivalent tolerance as the first generation as well as is proven as cost-effective with high-yielding potential.

The potential reflects in the form of higher gross farm income benefits. Genetically modified herbicide-tolerant soybeans have also enabled the assumptions of no-tillage production systems, shortening the production cycle [153]. This advantage has facilitated a lot of farmers in South Africa to plant the crop of soybean straightaway after a wheat crop in the same growing season. The second crop, in addition to traditional "one crop" soybean production, has added significantly to farm income and the volumes of soybean production in countries like Argentina and Paraguay. As a whole, in 2016, genetically modified herbicide-tolerant technology in soybean (excluding second generation "Intacta" soybeans) has increased gross farm income by $ 4.37 billion, and since 1996, it has delivered $54.6 billion of additional farm income [171, 172]. Of the total collective income gains from using genetically modified herbicide-tolerant soybeans, $24.6 billion (45%) has been due to yield gains/second crop benefits, and the balance, 55%, has been due to cost saving [173].

The combination of genetically modified herbicide-tolerant and insect-resistant (Intacta) (to glyphosate) soybean was first grown commercially in 2013, in South Africa. During the first four years, the technology covered about 49.6 million hectares and attributed an additional $5.2 billion to the gross farm income of soybean farmers in Argentina, Brazil, Paraguay, and Uruguay, with higher yields and decreased cost of production. The genetically modified soybean helped the increase in income benefits for farmers during 1996–2016 which was about US $ 59.7 billion for 2016 itself [173].

10. Conclusion and Future Prospects

As estimated, by 2050, the world population and livestock demand for grain will rise by 40–70% for oil and protein or nutrient-rich diets. While considering the economic use of soybeans and increasing population demand, it is necessary to enhance the traits that have the potential to give higher grain yield with increased levels of protein, oil, and other nutrient content. The identification of various agricultural strategies to improve abiotic stress, herbicide, insect, and disease resistance will make it possible for farmers to obtain better yields with reduced ecological inputs. The increase in the nutritional value of soybean oil will significantly raise the value and worth of soy as a food crop to meet the needs of an increasing population and benefit human health. Utilizing the full perspective of the soybean as an efficient expression platform will depend on overcoming the problems of efficient transformation methods, stable transgene expression levels, organ-specific expression, developing efficient purification methods, obtaining interest from pharmaceutical partners, and overcoming issues related to commercialization. The production of vaccines, antibodies, and other

therapeutic proteins will indubitably help to control diseases not only in humans but also in livestock and is necessary to continue its development over the upcoming future. Therefore, overall, in the future, the demand for soybean oil and soybean protein will be fulfilled through efficient soybean grain production with a transgenic approach.

Data Availability

This MS is a review based on the published reports that are referred and listed in the reference lists of the MS. Data are thus available as a secondary document.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

DBS and TDN conceptualized the research. DBS, GCN, and MRC wrote the manuscript. DBS and MRC prepared the final version of the manuscript. TDN, DBS, GCN, and MRC revised the manuscript.

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References

[1] A. Saryoko, K. Homma, I. Lubis, and T. Shiraiwa, "Plant development and yield components under a tropical environment in soybean cultivars with temperate and tropical origins," *Plant Production Science*, vol. 20, no. 4, pp. 375–383, 2017.

[2] I. C. Baianu, T. You, D. M. Costescu, P. R. Lozano, V. Prisecaru, and R. L. Nelson, "High-resolution nuclear magnetic resonance and near-infrared determination of soybean oil, protein, and amino acid residues in soybean seeds," in *Oil Extraction and Analysis*, pp. 193–240, AOCS Publishing, Urbana, IL, USA, 2019.

[3] A. Churasia, J. Singh, and A. Kumar, "Production of biodiesel from soybean oil biomass as renewable energy source," *Journal of Environmental Biology*, vol. 37, no. 6, pp. 1303–1307, 2016.

[4] I. A. Ciampitti and F. Salvagiotti, "Soybeans and biological nitrogen fixation: a review," *Better Crops with Plant Food*, vol. 102, no. 3, pp. 5–7, 2018.

[5] P. P. Chee and C. Y. Hu, "Transgenic soybean (Glycine max) transgenic crops I," in *Biotechnology in Agriculture and Forestry*, Y. P. S. Bajaj, Ed., Springer, Berlin, Heidelberg, 2000.

[6] M. H. Bodanese-Zanettini, M. S. Lauxen, S. N. C. Richter et al., "Wide hybridization between Brazilian soybean cultivars and wild perennial relatives," *Theoretical and Applied Genetics*, vol. 93–93, no. 5-6, pp. 703–709, 1996.

[7] M. N. Ishaq and B. O. Ehirim, "Improving soybean productivity using biotechnology approach in Nigeria," *World Journal of Agricultural Sciences*, vol. 2, no. 2, pp. 13–18, 2014.

[8] A. Pratap, S. K. Gupta, J. Kumar, and R. K. Solanki, "Soybean," in *Technological Innovations in Major World Oil Crops*Springer, New York, NY, USA, 2012.

[9] N. Tripathi, M. K. Tripathi, S. Tiwari, and D. K. Payasi, "Molecular breeding to overcome biotic stresses in soybean: update," *Plants*, vol. 11, no. 15, 2022.

[10] J. Han, B. Guo, Y. Guo, B. Zhang, X. Wang, and L. J. Qiu, "Creation of early flowering germplasm of soybean by CRISPR/Cas9 technology," *Frontiers of Plant Science*, vol. 10, 2019.

[11] M. R. Amirjani, "Effect of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean," *American Journal of Plant Physiology*, vol. 5, no. 6, pp. 350–360, 2010.

[12] Fao, "Livestock's long shadow: environmental issues and options," 2016, https://www.fao.org/home/en/.

[13] L. Z. Wang, "Soybean: a world-wide crop," in *Proceedings of the World Soybean Research Conference V*, B. Napompeth, Ed., Kasetsart University Press, Chiang Mai, Thailand, 1997.

[14] Sopa, *The Soybean Processor Association of India*, Indore, Madhya Pradesh, 2018.

[15] E. W. Lusas and M. N. Riaz, "Soy protein products: processing and use," *Journal of Nutrition*, vol. 125, no. 3 Suppl, pp. 573S–580S, 1995.

[16] V. Heuzé, H. Thiollet, G. Tran, M. Lessire, and F. Lebas, "Soybean hulls," *Feedipedia, a Programme by INRA, CIRAD*, AFZ and FAO, Rome, Italy, 2017.

[17] V. Heuzé, G. Tran, P. Nozière, M. Lessire, and F. Lebas, "Soybean seeds," *Feedipedia, a Programme by INRA, CIRAD*, AFZ and FAO, Rome, Italy, 2017.

[18] K. I. Chen, M. H. Erh, N. W. Su, W. H. Liu, C. C. Chou, and K. C. Cheng, "Soyfoods and soybean products: from traditional use to modern applications," *Applied Microbiology and Biotechnology*, vol. 96, no. 1, pp. 9–22, 2012.

[19] Y. Takahashi and T. Ohyama, "Production and consumption of green vegetable soybeans Edamame," *Soybeans: Cultivation, Uses and Nutrition*, pp. 425–442, Nova Science Publishers, Inc, Hauppauge, NY, USA, 2011.

[20] G. Dukariya, S. Shah, G. Singh, and A. Kumar, "Soybean and its products: nutritional and health benefits," *Journal of Nutritional Science and Healthy Diet*, vol. 1, no. 2, pp. 22–29, 2020.

[21] D. Sharma, G. Raakhi, and I. Joshi, "Nutrient analysis of raw and processed soybean and development of value-added soybean noodle," *Inventi Rapid: Life Style*, vol. 1, pp. 1–5, 2014.

[22] S. Marking, "The checkoff-supportednew-uses payoff begins," *Soybean Digest*, vol. 58, no. 3, pp. 9–21, 1998.

[23] T. Moses, K. K. Papadopoulou, and A. Osbourn, "Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives," *Critical Reviews in Biochemistry and Molecular Biology*, vol. 49, no. 6, pp. 439–462, 2014.

[24] L. Ridges, R. Sunderland, K. Moerman, B. Meyer, L. Astheimer, and P. Howe, "Cholesterol lowering benefits of soy and linseed enriched foods," *Asia Pacific Journal of Clinical Nutrition*, vol. 10, no. 3, pp. 204–211, 2001.

[25] F. M. Sacks, A. Lichtenstein, L. Van Horn, W. Harris, and P. Kris-Etherton, "Winston and American heart association nutrition committee, "soy protein, isoflavones, and cardiovascular health: an American heart association science

advisory for professionals from the nutrition committee circulation," *American Heart Association Nutrition Committee*, vol. 113, no. 7, 2006.

[26] Fda, *Food Labeling Health Claims Soy Protein and Coronary Heart Disease*, US Food and Drug Administration Federal Register, Washington, DC, USA, 1999.

[27] J. H. Yoon, L. U. Thompson, and D. Jenkins, "The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response," *The American Journal of Clinical Nutrition*, vol. 38, no. 6, pp. 835–842, 1983.

[28] R. F. Hurrell, M. A. Juillerat, M. B. Reddy, S. R. Lynch, S. A. Dassenko, and J. D. Cook, "Soy protein, phytate, and iron absorption in humans," *The American Journal of Clinical Nutrition*, vol. 56, no. 3, pp. 573–588, 1992.

[29] I. Vucenik, A. M. Shamsuddin, and A. Kalam, "Cancer inhibition by inositol hexaphosphate (IP6) and inositol: from laboratory to clinic," *Journal of Nutrition*, vol. 133, no. 11, pp. S3778–S3784, 2003.

[30] M. Sudheer Kumar, B. Sridhar Reddy, S. Kiran Babu, P. M. Bhilegaonkar, A. Shirwaikar, and M. K. Unnikrishnan, "Antiinflammatory and antiulcer activities of phytic acid in rats," *Indian Journal of Experimental Biology*, vol. 42, no. 2, pp. 179–185, 2004.

[31] S. Sato, A. Xing, X. Ye et al., "Production of γ-linolenic acid and stearidonic acid in seeds of marker-free transgenic soybean," *Crop Science*, vol. 44, no. 2, pp. 646–652, 2004.

[32] T. E. Clemente and E. B. Cahoon, "Soybean oil: genetic approaches for modification of functionality and total content," *Plant Physiology*, vol. 151, no. 3, pp. 1030–1040, 2009.

[33] L. Zhang, X. D. Yang, Y. Y. Zhang et al., "Changes in oleic acid content of transgenic soybeans by antisense RNA mediated posttranscriptional gene silencing," *International Journal of Genomics*, vol. 2014, Article ID 921950, 8 pages, 2014.

[34] T. Buhr, S. Sato, F. Ebrahim et al., "Ribozyme termination of RNA transcripts down-regulate seed fatty acid genes in transgenic soybean," *The Plant Journal*, vol. 30, no. 2, pp. 155–163, 2002.

[35] M. Garg, N. Sharma, S. Sharma et al., "Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world," *Frontiers in Nutrition*, vol. 5, no. 12, 2018.

[36] S. C. Falco, T. Guida, M. Locke et al., "Transgenic canola and soybean seeds with increased lysine," *Nature Biotechnology*, vol. 13, no. 6, pp. 577–582, 1995.

[37] S. Vimolmangkang, K. Gasic, R. Soria-Guerra, S. Rosales-Mendoza, L. Moreno-Fierros, and S. S. Korban, "Expression of the nucleocapsid protein of Porcine Reproductive and Respiratory Syndrome Virus in soybean seed yields an immunogenic antigenic protein," *Planta*, vol. 235, no. 3, pp. 513–522, 2012.

[38] M. A. Schmidt, W. A. Parrott, D. F. Hildebrand et al., "Transgenic soya bean seeds accumulating β-carotene exhibit the collateral enhancements of oleate and protein content traits," *Plant Biotechnology Journal*, vol. 13, no. 4, pp. 590–600, 2015.

[39] P. J. Maughan, R. Philip, M. J. Cho, J. M. Widholm, and L. O. Vodkin, "Biolistic transformation, expression, and inheritance of bovine β-casein in soybean (Glycine max)," *In Vitro Cellular and Developmental Biology - Plant*, vol. 35, no. 4, pp. 344–349, 1999.

[40] R. Philip, D. W. Darnowski, P. J. Maughan, and L.. , O. Vodkin, "Processing and localization of bovine β-casein expressed in transgenic soybean seeds under control of a soybean lectin expression cassette," *Plant Science*, vol. 161, no. 2, pp. 323–335, 2001.

[41] W. S. Kim, D. Chronis, M. Juergens et al., "Transgenic soybean plants overexpressing O-acetylserinesulfhydrylase accumulate enhanced levels of cysteine and Bowman-Birk protease inhibitor in seeds," *Planta*, vol. 235, no. 1, pp. 13–23, 2012a.

[42] R. D. Dinkins, M. S. Srinivasa Reddy, C. A. Meurer et al., "Increased sulfur amino acids in soybean plants overexpressing the maize 15 kDazein protein," *In Vitro Cellular and Developmental Biology - Plant*, vol. 37, no. 6, pp. 742–747, 2001.

[43] B. Savidge, J. D. Weiss, Y. H. H. Wong et al., "Dusty postbeittenmiller, henry E valentin, "isolation and characterization of homogentisate phytyltransferase genes from Synechocystis sp. PCC 6803 and Arabidopsis," *Plant Physiology*, vol. 129, no. 1, pp. 321–332, 2002.

[44] S. S. Rao and D. Hildebrand, "Changes in oil content of transgenic soybeans expressing the yeast SLC1 gene," *Lipids*, vol. 44, no. 10, pp. 945–951, 2009.

[45] B. Karunanandaa, Q. Qi, M. Hao et al., "Metabolically engineered oilseed crops with enhanced seed tocopherol," *Metabolic Engineering*, vol. 7, no. 5-6, pp. 384–400, 2005.

[46] E. C. Pierce, P. R. LaFayette, M. A. Ortega, B. L. Joyce, D. A. Kopsell, and W. A. Parrott, "Ketocarotenoid production in soybean seeds through metabolic engineering," *PLoS One*, vol. 10, no. 9, Article ID e0138196, 2015.

[47] M. J. Kim, J. K. Kim, H. J. Kim et al., "Genetic modification of the soybean to enhance the β-carotene content through seed-specific expression," *PLoS One*, vol. 7, no. 10, Article ID e48287, 2012.

[48] J. L. Oakes, K. L. Bost, and K. J. Piller, "Stability of a soybean seed-derived vaccine antigen following long-term storage, processing and transport in the absence of a cold chain," *Journal of the Science of Food and Agriculture*, vol. 89, no. 13, pp. 2191–2199, 2009.

[49] K. Lardizabal, R. Effertz, C. Levering et al., "Expression of Umbelopsis ramanniana DGAT2A in seed increases oil in soybean," *Plant Physiology*, vol. 148, no. 1, pp. 89–96, 2008.

[50] N. B. Cunha, A. Arajo, A. Leite, A. M. Murad, G. R. Vianna, and E. L. Rech, "Short communication Correct targeting of proinsulin in protein storage vacuoles of transgenic soybean seeds," *Genetics and Molecular Research*, vol. 9, no. 2, pp. 1163–1170, 2010.

[51] Z. D. Cunha, J. M. Savoie, and A. Pardo-Giménez, "Soybean the main nitrogen source in cultivation substrates of edible and medicinal mushrooms," *Soybean and Nutrition*, vol. 22, pp. 433–452, 2011.

[52] M. L. Smith, H. S. Mason, and M. L. Shuler, "Hepatitis B surface antigen (HbsAg) expression in plant cell culture: kinetics of antigen accumulation in batch culture and its intracellular form," *Biotechnology and Bioengineering*, vol. 80, no. 7, pp. 812–822, 2002.

[53] R. Garg, M. Tolbert, J. L. Oakes, T. E. Clemente, K. L. Bost, and K. J. Piller, "Chloroplast targeting of FanC, the major antigenic subunit of Escherichia coli K99 fimbriae, in transgenic soybean," *Plant Cell Reports*, vol. 26, no. 7, pp. 1011–1023, 2007.

[54] T. Moravec, M. A. Schmidt, E. M. Herman, and T. Woodford-Thomas, "Production of Escherichia coli heat labile toxin (LT) B subunit in soybean seed and analysis of its immunogenicity as an oral vaccine," *Vaccine*, vol. 25, no. 9, pp. 1647–1657, 2007.

[55] N. B. Cunha, A. M. Murad, T. M. Cipriano et al., "Expression of functional recombinant human growth hormone in transgenic soybean seeds," *Transgenic Research*, vol. 20, no. 4, pp. 811–826, 2011a.

[56] D. A. Russell, L. A. Spatola, T. Dian et al., "Host limits to accurate human growth hormone production in multiple plant systems," *Biotechnology and Bioengineering*, vol. 91, no. 4, pp. 522–782, 2005.

[57] K. J. Piller, T. E. Clemente, S. M. Jun et al., "Expression and immunogenicity of an Escherichia coli K99 fimbriae subunit antigen in soybean," *Planta*, vol. 222, no. 1, pp. 6–18, 2005.

[58] V. S. Tavva, Y. H. Kim, I. A. Kagan, R. D. Dinkins, K.-H. Kim, and G. B. Collins, "Increased α-tocopherol content in soybean seed overexpressing the Perilla frutescens γ -tocopherol methyltransferase gene," *Plant Cell Reports*, vol. 26, no. 1, pp. 61–70, 2006.

[59] L. Zeitlin, S. S. Olmsted, T. R. Moench et al., "A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes," *Nature Biotechnology*, vol. 16, no. 13, pp. 1361–1364, 1998.

[60] S. H. Ding, L. Y. Huang, Y. D. Wang, H. C. Sun, and Z. H. Xiang, "High-level expression of basic fibroblast growth factor in transgenic soybean seeds and characterization of its biological activity," *Biotechnology Letters*, vol. 28, no. 12, pp. 869–875, 2006.

[61] N. Matoba, H. Usui, H. Fujita, and M. Yoshikawa, "A novel anti-hypertensive peptide derived from ovalbumin induces nitric oxide-mediated vasorelaxation in an isolated SHR mesenteric artery," *FEBS Letters*, vol. 452, no. 3, pp. 181–184, 1999.

[62] L. Křížová, K. Dadáková, J. Kašparovská, and T. Kašparovský, "Isoflavones," *Molecules*, vol. 24, no. 6, 2019.

[63] C. Kissoudis, R. Chowdhury, S. van Heusden et al., "Combined biotic and abiotic stress resistance in tomato," *Euphytica*, vol. 202, no. 2, pp. 317–332, 2015.

[64] D. Tilman, C. Balzer, J. Hill, and B. L. Befort, "Global food demand and the sustainable intensification of agriculture," *Proceedings of the National Academy of Sciences*, vol. 108, no. 50, pp. 20260–20264, 2011.

[65] Y. Li, J. Zhang, J. Zhang et al., "Expression of an Arabidopsis molybdenum cofactor sulphurase gene in soybean enhances drought tolerance and increases yield under field conditions," *Plant Biotechnology Journal*, vol. 11, no. 6, pp. 747–758, 2013.

[66] N. Alexandratos and J. Bruinsma, *World Agriculture towards 2030/2050: The 2012 Revision*, FAO, Rome, 2012.

[67] N. T. Nguyen, H. T. Vu, T. T. Nguyen et al., "Co-Expression of Arabidopsis AtAVP1 and AtNHX1 to improve salt tolerance in soybean," *Crop Science*, vol. 59, no. 3, pp. 1133–1143, 2019.

[68] Y. Li, Q. Chen, H. Nan et al., "Overexpression of GmFDL19 enhances tolerance to drought and salt stresses in soybean," *PLoS One*, vol. 12, no. 6, Article ID e0179554, 2017.

[69] K. E. Trenberth, A. Dai, G. van der Schrier et al., "Global warming and changes in drought," *Nature Climate Change*, vol. 4, no. 1, pp. 17–22, 2014.

[70] J. S. Seo, H. B. Sohn, K. Noh et al., "Expression of the Arabidopsis AtMYB44 gene confers drought/salt-stress tolerance in transgenic soybean," *Molecular Breeding*, vol. 29, no. 3, pp. 601–608, 2012.

[71] D. B. Shelke, G. C. Nikalje, M. R. Chambhare, B. N. Zaware, S. Penna, and T. D. Nikam, "Na^+ and Cl^- induce differential physiological, biochemical responses and metabolite modulations in vitro in contrasting salt-tolerant soybean genotypes," *3Biotech*, vol. 9, no. 3, 2019.

[72] D. B. Shelke, M. Pandey, G. C. Nikalje, B. N. Zaware, P. Suprasanna, and T. D. Nikam, "Salt responsive physiological, photosynthetic and biochemical attributes at early seedling stage for screening soybean genotypes," *Plant Physiology and Biochemistry*, vol. 118, pp. 519–528, 2017.

[73] C. G. Kawashima, G. A. Guimarães, S. R. Nogueira et al., "A pigeonpea gene confers resistance to Asian soybean rust in soybean," *Nature Biotechnology*, vol. 34, no. 6, pp. 661–665, 2016.

[74] M. S. S. Reddy, S. A. Ghabrial, C. T. Redmond, R. D. Dinkins, and G. B. Collins, "Resistance to Bean pod mottle virus in transgenic soybean lines expressing the capsid polyprotein," *Phytopathology*, vol. 91, no. 9, pp. 831–838, 2001.

[75] H. P. Esse, T. L. Reuber, D. Does, and D. van der Does, "Genetic modification to improve disease resistance in crops," *New Phytologist*, vol. 225, no. 1, pp. 70–86, 2020.

[76] O. Wally and Z. K. Punja, "Genetic engineering for increasing fungal and bacterial disease resistance in crop plants," *GM Crops*, vol. 1, no. 4, pp. 199–206, 2010.

[77] H. K. Brar and M. K. Bhattacharyya, "Expression of a single-chainvariable-fragment antibody against a Fusarium virguliforme toxin peptide enhances tolerance to sudden death syndrome in transgenic soybean plants," *Molecular Plant-Microbe Interactions*, vol. 25, no. 6, pp. 817–824, 2012.

[78] L. Zhou, H. He, R. Liu, Q. Han, H. Shou, and B. Liu, "Overexpression of GmAKT2 potassium channel enhances resistance to soybean mosaic virus," *BMC Plant Biology*, vol. 14, no. 1, http://www.biomedcentral.com/1471-2229/14/154, 2014.

[79] L. Gao, X. Ding, K. Li et al., "Characterization of Soybean mosaic virus resistance derived from inverted repeat-SMV-HC-Pro genes in multiple soybean cultivars," *Theoretical and Applied Genetics*, vol. 128, no. 8, pp. 1489–1505, 2015.

[80] H. J. Kim, M. J. Kim, J. H. Pak et al., "RNAi-mediated Soybean mosaic virus (SMV) resistance of a Korean Soybean cultivar," *Plant Biotechnology Reports*, vol. 10, no. 5, pp. 257–267, 2016.

[81] M. Tougou, N. Yamagishi, N. Furutani, Y. Shizukawa, Y. Takahata, and S. Hidaka, "Soybean dwarf virus-resistant transgenic soybeans with the sense coat protein gene," *Plant Cell Reports*, vol. 26, no. 11, pp. 1967–1975, 2007.

[82] M. F. Grossi-de-Sa, B. P. Patrícia, and R. F. Rodrigo, "Genetically modified soybean for insect-pest and disease control," *Soybean-molecular Aspects of Breeding*, vol. 4, pp. 429–452, 2011.

[83] A. A. de Paiva Rolla, J. de Fátima Corrêa Carvalho, R. Fuganti-Pagliarini et al., "Phenotyping soybean plants transformed with rd29A: AtDREB1A for drought tolerance in the greenhouse and field," *Transgenic Research*, vol. 23, no. 1, pp. 75–87, 2014.

[84] J. A. De Ronde, W. A. Cress, G. H. J. Krüger, R. J. Strasser, and J. Van Staden, "Photosynthetic response of transgenic soybean plants, containing an Arabidopsis P5CR gene, during heat and drought stress," *Journal of Plant Physiology*, vol. 161, no. 11, pp. 1211–1224, 2004b.

[85] R. Fuganti-Pagliarini, L. C. Ferreira, F. A. Rodrigues et al., "Characterization of soybean genetically modified for drought tolerance in field conditions," *Frontiers in. Plant Science*, vol. 8, 2017.

[86] H. J. Kim, H. S. Cho, J. H. Pak et al., "Confirmation of drought tolerance of ectopically expressed AtABF3 gene in

soybean," *Molecules and Cells*, vol. 41, no. 5, pp. 413–422, 2018.

[87] S. Martins-Salles, V. Machado, L. Massochin-Pinto, and L. M. Fiuza, "Genetically modified soybean expressing insecticidal protein (Cry1Ac): management risk and perspectives," *FACETS*, vol. 2, no. 1, pp. 496–512, 2017.

[88] J. Li, T. C. Todd, T. R. Oakley, J. Lee, and H. N. Trick, "Host-derived suppression of nematode reproductive and fitness genes decreases fecundity of Heterodera glycines Ichinohe," *Planta*, vol. 232, no. 3, pp. 775–785, 2010.

[89] H. Y. Li, Y. M. Zhu, Q. Chen et al., "Production of transgenic soybean plants with two anti-fungal protein genes via Agrobacterium and particle Bombardment," *Biologia Plantarum*, vol. 48, no. 3, pp. 367–374, 2004.

[90] C. H. Laura, C. L. Kevin, L. B. Kenneth, and J. P. Kenneth, "Advancements in transgenic soy: from field to bedside," *A Comprehensive Survey of International Soybean Research -Genetics, Physiology, Agronomy and Nitrogen Relationships*, 2013.

[91] R. M. Youssef, M. H. MacDonald, E. P. Brewer, G. R. Bauchan, K. H. Kim, and B. F. Matthews, "Ectopic expression of AtPAD4 broadens resistance of soybean to soybean cyst and root-knot nematodes," *BMC Plant Biology*, vol. 13, no. 1, 2013.

[92] X. Guo, D. Chronis, C. M. De La Torre, J. Smeda, X. Wang, and M. G. Mitchum, "Enhanced resistance to soybean cyst nematode Heterodera glycines in transgenic soybean by silencing putative CLE receptors," *Plant Biotechnology Journal*, vol. 13, no. 6, pp. 801–810, 2015.

[93] W. G. Cunha, M. L. P. Tinoco, H. L. Pancoti, R. E. Ribeiro, and F. J. L. Aragão, "High resistance to Sclerotinia sclerotiorum in transgenic soybean plants transformed to express an oxalate decarboxylase gene," *Plant Pathology*, vol. 59, no. 4, pp. 654–660, 2010b.

[94] H. M. Ibrahim, N. W. Alkharouf, S. L. Meyer et al., "Post-transcriptional gene silencing of root-knot nematode in transformed soybean roots," *Experimental Parasitology*, vol. 127, no. 1, pp. 90–99, 2011.

[95] J. Lin, M. Mazarei, N. Zhao et al., "Overexpression of a soybean salicylic acid methyltransferase gene confers resistance to soybean cyst nematode," *Plant Biotechnology Journal*, vol. 11, no. 9, pp. 1135–1145, 2013.

[96] J. Onofre, M. O. Gaytán, A. Pe ̃na-Carde ̃na et al., "Identification of aminopeptidase-N2 as a Cry2Ab binding protein in Manduca sexta," *Peptides*, vol. 98, no. 1, pp. 93–98, 2017.

[97] D. Qin, X. Y. Liu, C. Miceli, Q. Zhang, and P. W. Wang, "Soybean plants expressing the Bacillus thuringiensis cry8-like gene show resistance to Holotrichia parallela," *BMC Biotechnology*, vol. 19, no. 1, pp. 66–12, 2019.

[98] M. Peferöen, "Progress and prospects for field use of Bt genes in crops," *Trends in Biotechnology*, vol. 15, no. 5, pp. 173–177, 1997.

[99] R. M. Steeves, T. C. Todd, J. S. Essig, and H. N. Trick, "Transgenic soybeans expressing siRNAs specific to a major sperm protein gene suppress Heterodera glycines reproduction," *Functional Plant Biology*, vol. 33, no. 11, pp. 991–999, 2006.

[100] P. B. Cregan, T. Jarvik, A. L. Bush et al., "An integrated genetic linkage map of the soybean genome," *Crop Science*, vol. 39, no. 5, pp. 1464–1490, 1999.

[101] Y. Yamada, K. Nishizawa, M. Yokoo et al., "Anti-hypertensive activity of genetically modified soybean seeds accumulating novokinin," *Peptides*, vol. 29, no. 3, pp. 331–337, 2008.

[102] V. Tzin and G. Galili, "The biosynthetic pathways for shikimate and aromatic amino acids in Arabidopsis thaliana," *The Arabidopsis book/American Society of Plant Biologists*, vol. 8, 2010.

[103] T. Flores, O. Karpova, X. Su et al., "Silencing of GmFAD3 gene by siRNA leads to low alpha-linolenic acids (18:3) of fad3- mutant phenotype in soybean [Glycine max (Merr.)]," *Transgenic Research*, vol. 17, no. 5, pp. 839–850, 2008.

[104] USDA-APHIS, "Monsanto improved fatty acid profile MON 87705 soybean petition 09-201p: final environment assessment," 2010.

[105] M. J. Cho, J. M. Widholm, and L. O. Vodkin, "Cassettes for seed-specific expression tested in transformed embryogenie cultures of soybean," *Plant Molecular Biology Reporter*, vol. 13, pp. 225–269, 1995.

[106] Q. Qi, J. Huang, J. Crowley et al., "Metabolically engineered soybean seed with enhanced threonine levels: biochemical characterization and seed-specific expression of lysine-insensitive variants of aspartate kinases from the enteric bacterium Xenorhabdus bovienii," *Plant Biotechnology Journal*, vol. 9, no. 2, pp. 193–204, 2011.

[107] S. Song, W. Hou, I. Godo et al., "Soybean seeds expressing feedback-insensitive cystathionine γ-synthase exhibit a higher content of methionine," *Journal of Experimental Botany*, vol. 64, no. 7, pp. 1917–1926, 2013.

[108] Y. H. Kim, Y. Y. Lee, Y. H. Kim et al., "Antioxidant activity and inhibition of lipid peroxidation in germinating seeds of transgenic soybean expressing OsHGGT," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 2, pp. 584–591, 2011.

[109] A. L. Van Eenennaam, K. Lincoln, T. P. Durrett et al., "Engineering vitamin E content: from Arabidopsis mutant to soy oil," *The Plant Cell Online*, vol. 15, no. 12, pp. 3007–3019, 2003.

[110] R. Zimmermann and M. Qaim, "Potential health benefits of Golden Rice: a Philippine case study," *Food Policy*, vol. 29, no. 2, pp. 147–168, 2004.

[111] A. Sommer and F. R. Davidson, "Assessment and control of vitamin A deficiency: the annecy accords," *Journal of Nutrition*, vol. 132, no. 9, pp. 2845S–2850S, 2002.

[112] D. V. Goeddel, D. G. Kleid, F. Bolivar et al., "Expression in Escherichia coli of chemically synthesized genes for human insulin," *Proceedings of the National Academy of Sciences*, vol. 76, no. 1, pp. 106–110, 1979.

[113] E. T. Ryan, T. I. Crean, M. John, J. R. Butterton, J. D. Clements, and S. B. Calderwood, "In vivo expression and immunoadjuvancy of a mutant of heat-labile enterotoxin of Escherichia coli in vaccine and vector strains of Vibriocholerae," *Infection and Immunity*, vol. 67, no. 4, pp. 1694–1701, 1999.

[114] L. C. Hudson, R. Garg, K. L. Bost, and K. J. Piller, "Soybean seeds: a practical host for the production of functional subunit vaccines," *BioMed Research International*, vol. 2014, Article ID 340804, 13 pages, 2014.

[115] N. B. Cunha, A. M. Murad, G. L. Ramos et al., "Accumulation of functional recombinant human coagulation factor IX in transgenic soybean seeds," *Transgenic Research*, vol. 20, no. 4, pp. 841–855, 2011.

[116] R. Powell, L. C. Hudson, K. C. Lambirth et al., "Recombinant expression of homodimeric 660 kDa human thyroglobulin in soybean seeds: an alternative source of human thyroglobulin," *Plant Cell Reports*, vol. 30, no. 7, pp. 1327–1338, 2011.

[117] M. A. Schmidt and E. M. Herman, "Proteome rebalancing in soybean seeds can be exploited to enhance foreign protein

accumulation," *Plant Biotechnology Journal*, vol. 6, no. 8, pp. 832–842, 2008.

[118] Y. He, M. A. Schmidt, C. Erwin et al., "Transgenic soybean production of bioactive human epidermal growth factor (EGF)," *PLoS One*, vol. 11, no. 6, Article ID e0157034, 2016.

[119] M. J. Paul, A. Y. Teh, R. M. Twyman, and J. K. Ma, "Target product selection - where can Molecular Pharming make the difference?" *Current Pharmaceutical Design*, vol. 19, no. 31, pp. 5478–5485, 2013.

[120] M. S. Homrich, B. Wiebke-Strohm, R. L. M. Weber, and M. H. Bodanese-Zanettini, "Soybean genetic transformation: a valuable tool for the functional study of genes and the production of agronomically improved plants," *Genetics and Molecular Biology*, vol. 35, no. 4, pp. 998–1010, 2012.

[121] O. Yu, J. Shi, A. O. Hession, C. A. Maxwell, B. McGonigle, and J. T. Odell, "Metabolic engineering to increase isoflavone biosynthesis in soybean seed," *Phytochemistry*, vol. 63, no. 7, pp. 753–763, 2003.

[122] J. You and Z. Chan, "ROS Regulation during abiotic stress responses in crop plants," *Frontiers of Plant Science*, vol. 6, 2015.

[123] Y. Wang, L. Jiang, J. Chen et al., "Overexpression of the alfalfa WRKY11 gene enhances salt tolerance in soybean," *PLoS One*, vol. 13, no. 2, Article ID e0192382, 2018.

[124] C. Liang et al., "Genetically modified crops with drought tolerance: achievements, challenges, and perspectives," in *Drought Stress Tolerance in Plants*, Springer, Berlin, Germany, 2016.

[125] M. A. S. Valente, J. A. Q. A. Faria, J. R. L. Soares-Ramos et al., "The ER luminal binding protein (BiP) mediates an increase in drought tolerance in soybean and delays drought-induced leaf senescence in soybean and tobacco," *Journal of Experimental Botany*, vol. 60, no. 2, pp. 533–546, 2009.

[126] J. A. De Ronde, R. N. Laurie, T. Caetano, M. M. Greyling, and I. Kerepesi, "Comparative study between transgenic and non-transgenic soybean lines proved transgenic lines to be more drought tolerant," *Euphytica*, vol. 138, no. 2, pp. 123–132, 2004a.

[127] D. B. Shelke, M. R. Chambhare, and H. Sonawane, "Fungal endophytes: potential benefits of their future use in plant stress tolerance and agriculture," in *Beneficial Microorganisms in Agriculture*, pp. 177–209, Springer, Singapore, 2022.

[128] C. Langenbach, H. Schultheiss, M. Rosendahl, N. Tresch, U. Conrath, and K. Goellner, "Interspecies gene transfer provides soybean resistance to a fungal pathogen," *Plant Biotechnology Journal*, vol. 14, no. 2, pp. 699–708, 2016.

[129] L. Niu, J. Yang, J. Zhang et al., "Introduction of the harpinXooc-encoding gene hrf2 in soybean enhances resistance against the oomycete pathogen Phytophthora sojae," *Transgenic Research*, vol. 28, 2019.

[130] R. Di, V. Purcell, G. B. Collins, and S. A. Ghabrial, "Production of transgenic soybean lines expressing the bean pod mottle virus coat protein precursor gene," *Plant Cell Reports*, vol. 15, no. 10, pp. 746–750, 1996.

[131] X. Wang, A. L. Eggenberger, F. W. Nutter Jr, and J. H. Hill, "Pathogen-derived transgenic resistance to soybean mosaic virus in soybean," *Molecular Breeding*, vol. 8, no. 2, pp. 119–127, 2001.

[132] M. Tougou, N. Furutani, N. Yamagishi, Y. Shizukawa, Y. Takahata, and S. Hidaka, "Development of resistant transgenic soybeans with inverted repeat-coat protein genes of soybean dwarf virus," *Plant Cell Reports*, vol. 25, no. 11, pp. 1213–1218, 2006.

[133] I. P. Budde and M. S. Ullrich, "Interactions of *Pseudomonas syringae* pv. glycinea with host and nonhost plants in relation to temperature and phytotoxin synthesis," *Molecular Plant-microbe Interactions*, vol. 13, no. 9, pp. 951–961, 2000.

[134] M. Tohidfar and K. Solmaz, "Transgenic crops with an improved resistance to biotic stresses A review," *Biotechnology, Agronomy, Society and Environment*, vol. 19, 2015.

[135] A. Ranjan, N. M. Westrick, S. Jain et al., "Resistance against Sclerotinia sclerotiorum in soybean involves a reprogramming of the phenylpropanoid pathway and up-regulation of antifungal activity targeting ergosterol biosynthesis," *Plant Biotechnology Journal*, vol. 17, no. 8, pp. 1567–1581, 2019.

[136] V. Kumar, A. Chattopadhyay, S. Ghosh et al., "Improving nutritional quality and fungal tolerance in soya bean and grass pea by expressing an oxalate decarboxylase," *Plant Biotechnology Journal*, vol. 14, no. 6, pp. 1394–1405, 2016.

[137] P. K. Kandoth, N. Ithal, J. Recknor et al., "The soybean Rhg1 locus for resistance to the soybean cyst nematode Heterodera glycines regulates the expression of a large number of stress-anddefense-related genes in degenerating feeding cells," *Plant Physiology*, vol. 155, no. 4, pp. 1960–1975, 2011.

[138] G. Gheysen and B. Vanholme, "RNAi from plants to nematodes," *Trends in Biotechnology*, vol. 25, no. 3, pp. 89–92, 2007.

[139] C. J. Lilley, M. Bakhetia, W. L. Charlton, and P. E. Urwin, "Recent progress in the development of RNA interference for plant parasitic nematodes," *Molecular Plant Pathology*, vol. 8, no. 5, pp. 701–711, 2007.

[140] B. Tian, J. Li, L. O. Vodkin, T. C. Todd, J. J. Finer, and H. N. Trick, "Host-derived gene silencing of parasite fitness genes improves resistance to soybean cyst nematodes in stable transgenic soybean," *Theoretical and Applied Genetics*, vol. 132, no. 9, pp. 2651–2662, 2019.

[141] R. M. Mcpherson and T. C. MacRae, "Evaluation of transgenic soybean exhibiting high expression of a synthetic <I>Bacillus thuringiensis cry1A</I> transgene for suppressing Lepidopteran population densities and crop injury," *Journal of Economic Entomology*, vol. 102, no. 4, pp. 1640–1648, 2009.

[142] Z. Hongyu, Y. Ziniu, and D. Wangxi, "Composition and ecological distribution of Cry protein and their genotypes of Bacillus thuringiensis isolates from warehouses in China," *Journal of Invertebrate Pathology*, vol. 76, no. 3, pp. 191–197, 2000.

[143] W. A. Parrott, J. N. All, M. J. Adang, M. A. Bailey, H. R. Boerma, and C. N. Stewart, "Recovery and evaluationof soybean plants transgenic for a Bacillus thuringiensis var. kurstaki insecticide gene," *In Vitro Cellular and Developmental Biology - Plant*, vol. 30, no. 3, pp. 144–149, 1994.

[144] C. N. Stewart Jr, M. J. Adang, J. N. All et al., "Genetic transformation, recovery, and characterization of fertile soybean transgenic for a synthetic Bacillus thuringiensis cryIAc gene," *Plant Physiology*, vol. 112, no. 1, pp. 121–129, 1996.

[145] D. R. Walker, J. N. All, R. M. Mcpherson, H. R. Boerma, and W. A. Parrott, "Field evaluation of soybean engineered with a synthetic <I>cry1Ac</I> transgene for resistance to corn earworm, soybean looper, velvetbean caterpillar (Lepidoptera: noctuidae), and lesser cornstalk borer (Lepidoptera: Pyralidae)," *Journal of Economic Entomology*, vol. 93, no. 3, pp. 613–622, 2000.

[146] T. C. Macrae, M. E. Baur, D. J. Boethel et al., "Laboratory and field evaluations of transgenic soybean exhibiting high-dose

expression of a synthetic Bacillus thuringiensis cry1A gene for control of Lepidoptera," *Journal of Economic Entomology*, vol. 98, no. 2, pp. 577–587, 2005.

[147] J. A. Miklos, M. F. Alibhai, S. A. Bledig et al., "Characterization of soybean exhibiting high expression of a synthetic Bacillus thuringiensis cry1A transgene that confers a high degree of resistance to Lepidopteran pests," *Crop Science*, vol. 47, no. 1, pp. 148–157, 2007.

[148] M. S. Homrich, L. M. P. Passaglia, J. F. Pereira et al., "Resistance to Anticarsia gemmatalis Hübner (Lepidoptera, Noctuidae) in transgenic soybean (Glycine max (L) Merrill Fabales, Fabaceae) cultivar IAS5 expressing a modified Cry1Ac endotoxin," *Genetics and Molecular Biology*, vol. 31, no. 2, pp. 522–531, 2008a.

[149] M. S. Homrich, L. M. P. Passaglia, J. F. Pereira et al., "Agronomic performance, chromosomal stability and resistance to velvetbean caterpillar of transgenic soybean expressing cry1Ac gene," *Pesquisa Agropecuária Brasileira*, vol. 43, no. 7, pp. 801–807, 2008b.

[150] B. G. Rector, J. N. All, W. A. Parrott, and H. R. Boerma, "Quantitative trait loci for antibiosis resistance to corn earworm in soybean," *Crop Science*, vol. 40, no. 1, pp. 233–238, 2000.

[151] D. R. Walker, J. M. Narvel, H. R. Boerma, J. N. All, and W. A. Parrott, "A QTL that enhances and broadens Bt insect resistance in soybean," *Theoretical and Applied Genetics*, vol. 109, no. 5, pp. 1051–1057, 2004.

[152] H. Maeda and N. Dudareva, "The shikimate pathway and aromatic amino acid biosynthesis in plants," *Annual Review of Plant Biology*, vol. 63, no. 1, pp. 73–105, 2012.

[153] V. K. Nandula, "Herbicide resistance traits in maize and soybean: current status and future outlook," *Plants*, vol. 8, no. 9, 2019.

[154] J. E. Franz, M. K. Mao, and J. A. Sikorski, *Glyphosate: A Unique Global Pesticide*, American Chemical Society, Washington, DC, USA, 1996.

[155] S. R. Padgette, K. H. Kolacz, X. Delannay et al., "Development, identification, and characterization of a glyphosate-tolerant soybean line," *Crop Science*, vol. 35, no. 5, pp. 1451–1461, 1995.

[156] C. A. Mathesius, J. F. Barnett, R. F. Cressman et al., "Safety assessment of a modified acetolactate synthase protein (GM-HRA) used as a selectable marker in genetically modified soybeans," *Regulatory Toxicology and Pharmacology*, vol. 55, no. 3, pp. 309–320, 2009.

[157] Y. T. Lee and R. G. Duggleby, "Mutagenesis studies on the sensitivity of Escherichia coliacetohydroxyacid synthase II to herbicides and valine," *Biochemical Journal*, vol. 350, no. 1, pp. 69–73, 2000.

[158] M. R. Behrens, N. Mutlu, S. Chakraborty et al., "Dicamba Resistance: enlarging and preserving biotechnology-based weed management strategies," *Science*, vol. 316, no. 5828, pp. 1185–1188, 2007.

[159] A. Van Almsick, "New HPPD-inhibitors – a proven mode of action as a new hope to solve current weed problems," *Outlooks on Pest Management*, vol. 20, no. 1, pp. 27–30, 2009.

[160] F. J. L. Aragão, L. Sarokin, G. R. Vianna, and E. L. Rech, "Selection of transgenic meristematic cells utilizing a herbicidal molecule results in the recovery of fertile transgenic soybean [Glycine max (L.) Merril] plants at a high frequency," *Theoretical and Applied Genetics*, vol. 101, no. 1-2, pp. 1–6, 2000.

[161] N. Dufourmantel, M. Dubald, M. Matringe et al., "Generation and characterization of soybean and marker-free tobacco plastid transformants over-expressing a bacterial 4-hydroxyphenylpyruvate dioxygenase which provides strong herbicide tolerance," *Plant Biotechnology Journal*, vol. 5, no. 1, pp. 118–133, 2007.

[162] Y. Kita, M. S. Hanafy, M. Deguchi et al., "Generation and characterization of herbicide-resistant soybean plants expressing novel phosphinothricin N-acetyltransferase genes," *Breeding Science*, vol. 59, no. 3, pp. 245–251, 2009.

[163] X. Li, J. Zhao, Z. Tan, R. Zeng, and H. Liao, "GmEXPB2, a cell wall b-expansin, affects soybean nodulation through modifying root architecture and promoting nodule formation and development," *Plant Physiology*, vol. 169, pp. 2640–2653, 2015.

[164] E. T. Gwata, D. S. Wofford, K. J. Boote, and H. Mushoriwa, "Determination of effective nodulation in early juvenile soybean plants for genetic and biotechnology studies," *African Journal of Biotechnology*, vol. 2, no. 11, pp. 417–420, 2003.

[165] S. Adhikari, S. Damodaran, and S. Subramanian, "Lateral root and nodule transcriptomes of soybean," *Data*, vol. 4, no. 2, 2019.

[166] M. Hayashi, Y. Saeki, M. Haga, K. Harada, H. Kouchi, and Y. Umehara, "Rj (rj) genes involved in nitrogen-fixing root nodule formation in soybean," *Breeding Science*, vol. 61, no. 5, pp. 544–553, 2012.

[167] Y. Fan, J. Liu, S. Lyu, Q. Wang, S. Yang, and H. Zhu, "The soybean Rfg1 gene restricts nodulation by Sinorhizobium fredii USDA193," *Frontiers of Plant Science*, vol. 8, 2017.

[168] S. Smita, J. Kiehne, S. Adhikari, E. Zeng, Q. Ma, and S. Subramanian, "Gene regulatory networks associated with lateral root and nodule development in soybean," *Silico Plants*, vol. 2, no. 1, pp. 1–15, 2020.

[169] Isaaa, *The International Service for the Acquisition of Agri-Biotech Applications (ISAAA)*, Global Status of Commercialized Biotech/GM Crops, Philippines, 2017.

[170] X. Liu, L. Yu, W. Li et al., "Comparison of country-level cropland areas between ESA-CCI land cover maps and FAOSTAT data," *International Journal of Remote Sensing*, vol. 39, no. 20, pp. 6631–6645, 2018.

[171] R. Alcántara-de la Cruz, G. M. Oliveira, L. B. Carvalho, and M. F. Silva, "Herbicide resistance in Brazil: status, impacts, and future challenges," in *Pests, Weeds and Diseases in Agricultural Crop and Animal Husbandry Production*Intech Open, London, UK, 2020.

[172] N. H. Thaher, H. H. Odame, and V. Henson-Apollonio, "A case study of management of IPRS in soybean biotechnology: evidence from Brazil and a successful coexistence in Canada and USA," *Drake Journal of Agricultural Law*, vol. 26, 2021.

[173] G. Brookes and P. Barfoot, "Farm income and production impacts of using GM crop technology 1996–2016," *GM Crops & Food*, vol. 9, no. 2, pp. 59–89, 2018.