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Soybean improvement through stress resistance and new plant breeding technologies

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Soybean belongs to the Leguminosae family having great nutritional value. It is considered to be a multipurpose crop used as food, feed, and fuel. Soybean as BNF (Biological Nitrogen Fixation) plant increases soil fertility through root nodule bacteria. Conventional breeding was used for improvement in crops in the past. But now scientists are working on soybean improvement through Genetic engineering (GE) to satisfy the global food demand. Genetic engineering methods i.e. gene silencing and transgenesis have reduced many risks and helped to increase soybean resilience. Recently, new plant breeding technologies (NBPTs) like transcription activator-like effector nucleases, zinc finger nucleases, and clustered regularly interspaced short palindromic repeats (CRISPR Cas9) appeared that are the basis for genetic improvement in soybean. These NBPTs proved beneficial in the improvement of soybean through precision genome engineering and gene functional characterization. These NBPTs have also covered the ethical and public acceptance problems about GE and transgenesis in soybean. In this review, we have provided a comprehensive note about stress resistance, nutritional enhancement of transgenic soybean, GE, and NBPTs, and their prospects.

Key words: *soybean, new plant breeding technologies, genetic engineering, stress resistance*

INTRODUCTION

Soybean (*Glycine max* L. Merr) is vast oil seed crop that is a legume. It belongs to the Fabaceae family. Soybean is vital crop in lots of areas across the world, including Argentina, Brazil, China, Japan, the United States, and Vietnam. It is rich source of protein and has an elevated level of nutritional value present in it. Soybeans are also important agriculturally as well as economically. Soybean has saturated and unsaturated fatty acid. Saturated fatty acids include Stearic acid and Palmitic acid. Unsaturated fatty acid includes linoleic acid, oleic acid, and linolenic acid. Humans consume soybean as soymilk, which is a high-level supplement to the body. Due to the better profile of protein, it is used in chick-rearing and animal feed. Considering its usefulness, we must improve our crops over time. These changes have been made through different techniques, different breeding methods, and genetic engineering. According to the U.S. Department of Agriculture, Agricultural Research Service (USDA) (fdc.nal.usda.gov), Soybean is source of 60% oil and protein consumption. It is the fourth largest field crop by volume. Soybean production is affected by abiotic stresses such as drought, floods, salinity, and heavy metals. It is important to know plant response against different stresses at molecular level for effective management of crops. The molecular mechanisms of stress tolerance are complex and require omics-level information for effective understanding. (Deshmukh et al., 2014) Soybean production is not only affected by environmental factors such as drought, floods, salinity, and heavy metals. It also faces the challenge of adapting to non-traditional regions. Over the years, several methods for editing plant genomes have been developed, i.e. using mega-nucleases, ZFNs and TALENs. But there are some limitations such as low B. editing efficiency, complex vector assembly, and off-target mutations. In this context, Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein (CRISPR Cas9) came into being and it is widely used due to flexibility and easily identifying the site to be modified. Despite significant progress in traditional plant breeding, the development of improved plant varieties has been slow given the increased demand for food due to a rapidly growing world population (Wei et al., 2015). Genes which are active during abiotic stresses were divided into two groups. The products of the first group are effector proteins that protect the cell membrane system, retain water, control ionic homeostasis, and scavenge reactive oxygen species. These proteins have enzymes which are required for Osmo protectants, LEA proteins, molecular chaperones, aquaporins, and detoxification enzymes. The second class of products is regulatory proteins that are essential for signal transduction, signal perception, and transcriptional regulation of gene expression. Several transcription factor families are induced by drought and salt stress, such as the B. DREB, ERF, WRKY, MYB, bZIP, and NAC families (Wei et al., 2009). Drought and salt tolerance of transgenic plants were improved after the transfer of DREB1A and AtMYB2 into Arabidopsis. Alfin1, a PHD finger protein, identified as a salt-inducible transcription factor, enhances stress tolerance by ectopic expression in transgenic plants (Wei et al., 2009). With advancement in genome sequencing technology, and good knowledge of agronomic traits which are linked with agronomic regions can make progress in molecular breeding in soybean. Due to the high demand for soybean, metabolomics has been applied to know plant responses to the completely different organic phenomena and abiotic stresses and to boost soybean yield. Currently, there's a requirement for the event of procedure tools and databases for processing and analysis of metabolic data. In our gift study, we tend to factorially manipulate the antioxidant synthesis pathway in soybean plants victimization associated with economical Agrobacterium-mediated transformation methodology and the commission recombinant gene (Kim et al., 2012). However, Soybean production is effected by many stresses such as insect pests, viral infection, weeds, and anti-nutritional factors (Rahman et al., 2022). For the sustainable production of soybeans, various strategies are used to control pests, viruses,

weeds, and dietary impacts. Since the 19th century, traditional breeding and transgenic technology has been combined for improvement in soybean. Therefore, this review focuses on soybean improvement through genetic engineering (GE) and NPBT. GE and NPBT enhanced features. In addition, ethical issues, potential goals and regulation of genome-edited soybeans are highlighted (Rahman et al., 2022).

STRESS RESISTANCE

Two types of stresses can affect soybean production. (i) Biotic stress which includes insects, viruses, and nematodes (ii) Abiotic stress which includes salinity, drought, flooding, and temperature. These stresses affect the plant physiology, morphology, and architecture thus reducing the agronomical importance of the plant. To handle these stresses, it is essential to make plants resistant by using techniques of genetic engineering. Some resistant genes against different stresses have been identified which are discussed below:

ABIOTIC STRESS

Salinity

Soil salinity has now become a global issue and it highly affects the growth of crops and plants and it is increasing day by day. It is considered, that in 2050 approximately 50% of cultivated land will suffer from this severe stress. Salinity stress is negatively correlated with the growth and metabolism of soybean. The parameters like photosynthesis, growth, and gas exchange showed a drastic reduction due to high salt concentration. Salinity mainly affects the photosynthesis phenomenon of soybean by accumulating high concentrations of Na+ and Cl- that inhibit photosynthesis. Salinity affects the growth of soybean by posing a reduction in root and shoot dry and fresh weights (Ullah et al., 2019). The agronomic traits of soybean including height, number of branches, biomass, number of internodes, leaf size, number of pods, and weight per plant negatively affected by salinity. Soybean belongs to the family Leguminosae, it forms root nodules which help in atmospheric nitrogen fixation, high salt concentrations reduce the aerobic respiration of nitrogen-fixing bacteria, which in return decreases the level of leghemoglobin in root nodules and thus decreases energy that helps in nitrogen fixation (Phang et al., 2008). Different breeding strategies are used to find out resistance genes against salinity. AtNHX1 gene introduced into soybean through genetic engineering has great potential against salt stress (Li et al., 2010). PgTIP1 gene is transformed into soybean hybrid strain 4076(F5), obtained by crossing two cultivars showing salt and drought tolerance at the physiological and molecular levels(An et al., 2018). It is also considered that minor genes control the salt tolerance in soybean(Qingyun et al., 2004).

Drought

Drought stress is a threatening issue to soybean production globally, roots and root nodules are important characteristics of soybean that help in detecting drought stress (Kunert et al., 2016). Drought poses anatomical and physiological changes in soybean and also changes its architecture, acute drought stress affects the root system of soybean by a reduction in its biomass and length up to 76% and diameter decreases up to 46% at even exposure to low stress as a result slender roots are formed (de Souza et al., 2021). Soybean is a BNF (Biological Nitrogen Fixation) plant, many countries worldwide depend on soybean to cope up the crop N requirement, still and all drought stress posing harm to plant metabolic process, specifically BNF by reducing their yield and efficiency. Many strategies are adopted to cope with drought stress including different breeding methods, selection of elite strains

against drought stress, and genetic engineering (de Freitas et al., 2022). Heat shock TF (Transcription Factor) and heat shock protein-encoding genes that help the plant against drought, many up-regulated and down-regulated GmNAC genes are found in the NAC TF family of soybean against drought stress (Le et al., 2012). A comparative study was taken out on drought-tolerant, susceptible and flood-tolerant and susceptible genotypes, drought stress increases the number and size of plastoglobules that help the plant to survive the drought, in plastoglobules, there are fibrillin proteins. fibrillin proteins, FBN1a, 1b, and 7a have a potential role in soybean response to excess water and drought stress (Mutava et al., 2015). LOS5/ABA3 gene in soybean could increase drought tolerance and also switch on many stress-up-regulated genes that produce many biochemical and physiological resistant responses in soybean (Li et al., 2013).

Flooding/ excess water stress

Worldwide 27% of cultivated land is affected by excess water stress, causing over $371 billion of economic losses to crop production. Timing, duration, and cultivar susceptibility are crucial factors that determine the extent of damage to crops by flooding. Flooding proved to be devastating in the early reproductive stage in soybean, 17%-43% yield loss occur at vegetative stage and 50%-56% yield loss at the pod filling stage when flooding stress is applied (Pasley et al., 2020). Excess water stress mainly affects the plant by decreasing its biomass and by affecting the processes like photosynthesis. Nodulation, stomatal conductance, nitrogen fixation, and nutrient uptake also induces disease in plant and cause death. Plants show tolerance against short-term excess water stresses by shifting mechanisms from aerobic to anaerobic, increasing soluble sugars, aerenchyma formation, enhanced the activity of glycolysis and fermentation, some plants also form adventitious roots including soybean. Wild soybean accessions (*G. soja*) performed excellent flooding tolerance as compared to exotic lines, through genetic mapping QTL against flooding in soybean has been identified on Chro.18 (Valliyodan et al., 2017). There are dozens of QTLs linked with flooding tolerance in soybean located on Chromosomes no 4, 9,10,12,13, and 14. The flooding tolerance gene qWT-Gm03 is also found in soybean. Proteomics studies have shown that chromosomes no 5, 10, 11, and 13 carry maximum flooding tolerance genes (Wang and Komatsu, 2020). A combination of two lines, tolerant exotic cultivar P1 408105A and susceptible elite cultivar S99-2281 was analyzed, and four QTLs on chromosomes no 11 and 13 are found for flooding tolerance and pathogen *Phytophthorasojae* in soybean (Mustroph, 2018). Xyloglucanendotransglycosylases/hydrolases (XTHs) belong to a class of enzymes linked with organ elongation. GmXTHs genes have the potential against flooding stress in soybean (Song et al., 2018).

Temperature

High-temperature stress is a crucial environmental factor that highly affects the soybean by interfering with pollen formation and pod setting. In an, in vitro experiment, it was observed that seed germination was reduced by 22.7% due to high-temperature stress, and pod set was reduced by 35.2% (Djanaguiraman et al., 2013). Soybean requires a specific temperature range for their proper growth and development, the reproductive stage of soybean is extremely temperature-sensitive. High-temperature stress has marked effects on male reproductive structures rather than female reproductive structures, anther indehiscence, and abandonment of pollen are also due to HT stress (Ding et al., 2021). The high temperature at the flowering stage limits the seed number in soybean. when the temperature rises above 30°C it negatively affects the seed vigor and levels of stachyose and phytic acid decrease posing difficulties in membrane biogenesis and germination (Katam et al., 2020). GmHSFA2 genes in arabidopsis help in the expression of HT protective genes i.e.

HSP20, and also improve HT tolerance at the time of flowering in soybean CMS-based F1 (Ding et al., 2020). GmSBH1 is a homeobox gene isolated in soybean, it expresses itself differently in different tissues of soybean against high temperature and humidity stress, GmSBH1 mainly shows tolerance against pre-harvest deterioration caused by high temperature and humidity stress (Shu et al., 2015).

BIOTIC RESISTANCE

Viruses

Viruses are an important biotic factor in soybean production. More than 50 different viruses affect the soybean crop but few have economic importance (Lal et al., 2005). These include soybean mosaic virus (SMV),soybean dwarf virus and bean pod mottle virus (BPMV) (Widyasari et al., 2020). SMV is the most common among all the viruses and can cause a serious threat to the crop (Cho and Goodman, 1979). SMV belongs to the genus potyvirus. SMV can infect soybean plants in 3 ways: Firstly, It can be transmitted through infected seeds. Secondly, it can be transmitted through different aphid species, Thirdly, SMV can enter the plant at the site of tissue damage. SMV has 7 stains represented as G1-G7. Their virulence increase as the stain no. increases (Ross et al., 2021). Four nuclear genes control the resistance of SMV in soybean. *Rsv1, Rsv3, Rsv4 and Rsv5* are SMV resistant loci against various stains. Most resistant genotypes carry one dominant gene (Usovsky et al., 2022). BPMV is also a viral disease in soybean and it causes chlorosis, delayed maturity, and severe leaf mottling. This virus is reported to be transmitted through seed. It decreases the yield by 10-40% but when both diseases (BPMV and SMV) present at the same time, yield losses can be more than 65% (Zheng et al., 2005). Resistance against viruses can be improved through conventional breeding as well as transgenic approaches. Conventional Breeding for disease resistance is not a good choice as there is improvement in biotechnology and it is easily achieved through transgenesis. Pathogen Derived Resistance method was used to improve resistance against SMV (Rahman et al., 2022). So, the most effective method for viral diseases is genetic engineering (Zheng et al., 2005).

Insects

The soybean crop is attacked by many insects i.e. bean beetles, leaf beetles, caterpillars, pod borers, and stink bugs. These insects damage the crop badly if not controlled timely (Johnson et al., 1967). Insect injury is categorized into two types. (i) insects according to their feeding e.g. leaf feeding, pod feeding etc. (ii) based on the effect of injury on plant physiology e.g. photosynthesis rate reduction, water imbalance, leaf mass reduction, plant death, etc. For insect pest management, identification of plant response to injury is important. The most efficient concept is the insect guild. Guild is the sum of insect species that attack the same part of the plant and produce the same response. In this way, more species can be handled at the same time rather than handling one specie (Higley, 1994). Cutworm (*Spodoptera litura* Fabricius) is an important insect in soybean and 2 QTL has been identified for its resistance. 23 QTLs have also been detected against other leaf-eating insects. Improvement in genetic studies helps to develop the elite soybean cultivars which are high-yielding and insect resistant (Komatsu et al., 2010). Aphid is also a major insect in soybean which can cause significant yield loss. Six aphid resistance genes have been identified named *Rag1, rag1c, Rag2, Rag3, rag4, and Rag5.* The first aphid-resistant cultivar of soybean with Rag1 was biotype 2. SSR markers are used to identify resistant genes and resistant cultivars are developed through marker-assisted selection (Hill et al., 2012). With progress in biotechnology, resistant cultivars are developed through transgenesis. The best example of it is Bt Soybean (Rahman et al., 2022).

Nematodes

Nematodes cause major losses in agriculture if not managed properly. 100 species of nematodes in 50 genera associated with soybean crop (Lima et al., 2017). Common nematodes affecting the soybean crops are Root-knot nematodes (*Meloidogyne spp., M. incognita, M. javanica*), Soybean cyst nematodes (*Heterodera glycines*), Root lesion nematodes (*Pratylenchus brachyurus*), Reniform nematodes, spiral nematodes, Lance nematodes, and sting nematodes. Soybean cyst nematodes are most damaging and cause severe yield loss. It can be found in the roots of the plant in the form of an adult female or the form of a cyst. It also lowers the nitrogen-fixing ability of the plant. It can be controlled by crop rotation with non-host crops and using resistant varieties (Lima et al., 2017). *Rhg1* and *Rhg4* genes are SCN resistant in soybean. If plant roots have the *Rhg* gene, the juvenile can penetrate but when the feed cells, it will degenerate and cause the death of nematodes before the adult stage (Liu et al., 2012). Root lesion nematode is an important parasite of soybean in tropics and subtropics with a broad host range. Nematodes penetrate roots by the stylet. They attack mostly in sandy soils when conditions are favorable and easily leave the field when the environment is unfavorable. Seed treatment with nematicide is mostly recommended to control this. Infected plants of Root-knot nematode show wilting and chlorotic patches. Galling is found on roots and inside the galls female nematodes and eggs are present. Aldicarb is used against RKN attack but the most effective way is using resistant varieties of soybean. Its population is decreased in the dry season and increases in moisture (Lima et al., 2017).

DEVELOPMENT OF TRANGENIC SOYBEAN

Soybean is a good source of oil and protein. Their contents can be increased by genetic modifications. Soybean oil is a mixture of oleic acid, stearic acid, linoleic acid, palmitic acid, and linolenic acid. All these fatty acids have different melting points, stability, and chemical functions. So, genetic engineering techniques are used to enhance one or more fatty acid contents. For example, conventional soybean has 25% oleic acid while this is increased up to 80% in transgenic soybean (Cahoon, 2003). This can be achieved by decreasing the expression of the FAD2 gene. FAD2 gene converts oleic acid into linoleic acid. Recently, oleic content of more than 85% has been achieved by decreasing FAD2 genes and controlling the genes that affect palmitic acid. Transgenic soybean has a high level of oleic acid (90%) and a low level of linoleic and linolenic acid while conventional soybean has a high level of linoleic acid and a low level of oleic acid. The oil with high oleic contents has more health benefits and oxidative stability (Cahoon, 2003). *Cry* protein of *Bt* toxin is a biological insecticide. *Bt cry* gene expression controls the insect pests in soybean. For soybean mosaic virus resistance, overexpression of coat protein gene and 3UTR region from SMV is reported. Drought stress is major abiotic stress. Transgenic soybean which expresses P5CR encodes L-Δ1-pyrroline-5-carboxylate reductase. It catalyzes the last step of proline biosynthesis under the control of a promoter. This type of transgenic soybean shows more tolerance to drought and temperature than conventional soybean. Overexpression of endogenous gene delay leaf senescence during drought conditions. The most successful introduction of a transgenic trait in soybean is herbicide resistance. A glyphosate-tolerant EPSPS was inserted in soybean which can bear a high level of glyphosate (Yamada et al., 2012). New plant breeding technologies made it easier to improve any crop fast and efficiently. These methods cause variation in genes or genes. Different plant genomes have been edited by using NPBTs. Due to these technologies, the desired mutation can be obtained (Rahman et al., 2022). There are two most common methods of genetic engineering in soybean (i) Particle bombardment and (ii) Agrobacterium-mediated genetic transformation. In

Agrobacterium transformation, cotyledonary nodes are used as plant material for reproducible transformation (Lee et al., 2013). It is the best method to transfer DNA into tissues of explant. Three factors are important in developing transformation through this method. 1: Soybean cultivar which is needed to transform should be susceptible to Agrobacterium transformation. 2: Soybean cotyledons develop regeneration responses. 3: Enrichment of transformed tissue by kanamycin selection (Hinchee et al., 1988). Agrobacterium is an organism that is used to develop a transgenic plant in natural conditions. It allows bacterial TDNA to enter the host cell and integrated with DNA of interest causing genetic manipulation in the host. Zinc finger nucleases (ZFNs) are another tool for genetic modification as they can cut the specific DNA sequence. There is a disadvantage of ZFNs is that it is not successful and has a high failure rate. To overcome this problem, DNA binding domain was identified. Transcription activator-like effectors (TALEs) are produced by *Xanthomonas* by pathogens and their gene induction causes developmental changes in the plant. When TALEs enter the nucleus and bind with a specific sequence of DNA, they activate gene expression (Lee et al., 2013). One most efficient and rapid method is CRISPR Cas9. CRISPR Cas 9 acts for the immunity of prokaryotes against viruses. There are three types of systems of CRISPR Cas. 1 & 3 CRISPR Cas system has many associated proteins for degrading viral nucleic acid while 2 has only one protein, Cas9 which degrades the viral genome. CRISPR Cas9 system can cut the specific sequences and can also target many genes at a time. This made it easy to insert a single nucleotide into the plant genome precisely (Rahman et al., 2022). The transformation process has been used in many genotypes of soybean. Following these techniques, breeders made transgenic soybean with improved traits such as high yielding, high oil, and protein contents, multiple stress resistance etc. It is also helpful to study the genes function(Yamada et al., 2012).

NUTRITIONAL ENHANCEMENT OF SOYBEAN

Carotenoids

Carotenoids are among the most abundant natural colors, having 600 distinct compounds, with β -carotene being the most prominent. Carotenoids has importance since several epidemiological studies have found that eating more carotenoids is associated with a lowering the risk of ophthalmological disease, cardiovascular disease, and cancer. The preventative effects have been linked to antioxidant activity, which protects cells and tissues from oxidative damage (Stahl and Sies, 2003). A seed-specific gene from *Pantoeaananatis* (pineapple fruitlet rot) called phytoene synthase is used to create transgenic soya beans with increased levels of β -carotene accumulation. Beta-carotene changes into vitamin A in the body (retinol) (Schmidt et al., 2015). The seed-specific overexpression of Capsicum phytoene synthase, and Pantoea carotene desaturase (both are carotenoid biosynthetic genes) resulted in a high-carotene soybean. This nutritional modification of soybean seeds through increased provitamin A content to make biofortified food may have potential practical health benefits in both humans and livestock (Kim et al., 2012). Antioxidants called tocopherols are lipophilic and are produced by plants in their plastids. Because of an eight-fold rise in -tocopherol levels, which went from making up 10% of total vitamin E to over 95% in transgenic soybeans, there was a large increase in the quantity of vitamin E activity overall (five times greater than in wild plants) (Zhu et al., 2007). It is now possible to enhance the monounsaturated fatty acid (oleic acid), provitamin A (beta-carotene) and the seed protein contents of the soybean by expression of PSY gene (Garg et al., 2018). The transformation of three bacterial genes *crtB, crtW, and bkt1* to soybean enhanced the levels of provitamin A (canthaxanthin) in the transformed soybean plants (Pierce et al., 2015).

Protein

Soybean cultivated worldwide due to their high nutritional value, literature was reviewed to depict the proper understanding of different properties of soybean especially their oil and protein contents (Medic et al., 2014). Soybean is a good source of protein, but its quality is not as good as animal protein. Methionine content in soybean is 43%, it should be almost double to provide protein equal to egg protein (Burton, 2022). Seeds of soybean comprise 40-41% protein, and this protein is categorized into four classes according to its role, these are, metabolic enzymes, structural, membrane, and storage protein. storage protein contributes 65-80% to total protein, the most important storage proteins are glycinin and b-conglycinin (1). Soybean seed storage proteins are encrypted by a small number of conserved gene families most of them belong to the cupin superfamily (glegumins and vicilins) (Schmidt et al., 2011). Major QTLs for soybean protein has identified and located on chromosomes No 20 and 15. Soybean seed storage protein is a highly complicated feature as it is influenced by environment and genotype-environment interaction, enhancing seed storage protein is negatively correlated with oil content and yield (Patil et al., 2017). Protein quality depends on the Amino acid composition of that protein, in soybean amino acid composition is not upto the mark. The storage protein glycinin is sulfur-rich while b- conglycinin is sulfur-poor. If the plant is subjected to low sulfur availability, then sulfur deficient or poor protein b-conglycinin is accumulated more than sulfur-rich glycinin. Through transgenesis or other breeding technologies, one can improve soybean's protein quality by increasing sulfur-rich- protein (Fujiwara et al., 1992). Soybean also contains vegetative storage proteins (VSP) having properties of storage proteins. These are specific for nitrogen storage available to plants throughout their growth. The expression of these proteins is controlled by many different stimuli like removal of seed pod, and elevate nitrogen nutrition. Soybean production and nutritional quality can be improved by targeting VSP genes through Genetic engineering (GE) and other metabolic activity (Staswick, 1990).

Isoflavonoids

With around 5000 members, flavonoids are one of the biggest groups of plant phenolics. Isoflavonoids are a special subclass of these chemicals. Isoflavonoids are chemicals found in many legumes, although they are mostly obtained by the human diet from soybeans and other foods made with soybeans. The glycosides of genistein and daidzein are the main isoflavonoids found in soybeans (Hodgson et al., 1996). In this sense, it is possible to establish the amount of isoflavonoid. The amount of phenolics, flavonoids, and isoflavonoids in soybean seeds that have been produced organically is greatly influenced by biofertilizers. In comparison to inorganic fertilizer or organic fertilizer treated with various compost levels alone, adding multi-biofertilizers, such as Bacillus megaterium var. phosphaticum, Azospirillum spp., Pseudomonas spp., and Bradyrhizobium japonicum to 50 or 75 percent compost had a significant enhancement effect on total phenolics, total flavonoids, protocatechuic acid, p- The phenolic acids, quercetin, and genistein showed the greatest enhancing impact (Taie et al., 2008). Because soybean isoflavonoids' molecular structures mirror those of the hormone estrogen and because they may interact with the estrogen receptor, they are frequently referred to as "phytoestrogens." The isoflavonoid concentration of soybeans ranges from 0.14 to 1.53 mg/g, and it is between 1.3 and 1.98 mg/g in soy flour. According to estimates, Japanese people take 25–100 mg of isoflavonoids per day. 39 mg of isoflavonoids are thought to be consumed daily by Chinese women. Less than 1 mg/day in the US and UK, isoflavonoids are consumed significantly less often in Western diets (McCue and Shetty, 2004). Isoflavonoids and other phenolic chemicals often exist as glycones, which are glucoside-bound moieties. However, the physiologically active form of isoflavonoids is the aglycone (glucoside-free) form. The physiologically active,

health-promoting aglyconeisoflavonoids are released into the body after absorption by probiotic bacterial enzymes in the colon. Aglycone phenolic compounds have been reported to have stronger antioxidant activity than their glucosidebound counterparts and to absorb more quickly in the intestines. It's interesting to note that because of microbial bioprocessing during fermentation, meals made from fermented soy may be high in isoflavonoidaglycones. Aglyconegenistein, which is physiologically active, enters the bloodstream and goes to the liver where it is changed into an inactive glucuronide. Genistein's biological action cannot occur until the glucuronide moiety has been removed by cellular glucuronidases. Isoflavonoids have undergone extensive research and exhibit a wide range of biological functions. For instance, topoisomerase II, tyrosine kinase, NF-B, cancer cell proliferation, and non-oxidative pentose-phosphate pathway ribose synthesis in cancer cells are all inhibited by genistein. The capacity of phenolics to act as antioxidants has been related to several of the health-promoting properties of isoflavonoids (McCue and Shetty, 2004). Numerous biological actions of soybean isoflavonoids appear to maintain cellular health in healthy cells while concurrently promoting apoptosis in pathological cells. Other health advantages are still being identified, but research on soybean isoflavonoids has shown that they protect against health issues related to menopause, cancer, and cardiovascular disease (McCue and Shetty, 2004).

Oleic acid and linoleic acid

Comestible oil is mostly obtained from seed storage lipids. Mostly unsaturated fatty acids like oleic acid, linoleic acid and linolenic acids determined the quality of comestible oil. Oleic acid and linolenic acid negatively correlate with each other, high oleic acid content is preferred and this oil is used for human consumption while high linolenic acid content deteriorates the quality of oil and makes it unfit for human consumption because of its unstable flavor. Furthermore, high oleic acid brings down the number of many coronary heart diseases by lowering the low-density lipoprotein cholesterol in the blood (Rahman et al., 1994). High linolenic and linoleic acid content in soybean oil, make it produce an unpleasant smell and flavor, low oxidation and frying capability. Hydrogenation is referred to control the above problems and lowered PUFA contents in soybean oil but hydrogenation is not the final solution as it produces 10-40% trans fatty acids which poses obesity and heart problems (Pham et al., 2012). Combining mutants FAD2-1B andFAD2-1A genes produce the high oleic contents in soybean. It has only 2-4% linoleic acid contents but these are enough for producing oxidative instability in oil. So, there is a need to further reduce linoleic acid contents in soybean. Oleic acid contents are increased by a mutation in FAD2-1B or FAD2-1A genes by 27-50% (Pham et al., 2012). Mutation in FAD3A was induced by introducing TALENs to the soybean plant. Through this mutation, linoleic acid contents are decreased. FAD3 enzymes convert linoleic acid into linolenic acid. It includes FAD3A, FAD3B, and FAD3C genes of this family (Demorest et al., 2016). Its expression is high in developing seed and FAD3A greatly control the linolenic content in oil. If any two of these mutations combine, it cause <3% linoleic acid in soybean oil (Demorest et al., 2016).

ANTINUTRATIONAL FACTORS IN SOYBEAN

Soybean includes a variety of substances that harm the protein's nutritional value. Protease inhibitors and lectins are among the substances that are eliminated by heat treatment. Protease inhibitors restrict growth by promoting pancreatic hypertrophy, which is how they achieve their antinutritional impact. By preventing nutrients from being absorbed, the lectin prevents growth by binding to glycoprotein receptors on the epithelial cells lining the intestinal mucosa. The antinutritional effects caused by moderately heat stable substances such as goitrogens, tannins etc, that cause flatulence, saponins, and phytate are less

significant (Liener, 1994). Antinutritional factors in Soybeans are; protease inhibitors, saponins, lectins, tannins, estrogens, goitrogens, antivitamins, lysinoalanine, allergens, phytate, Flatulence factors etc. The nutritional value of soybeans is impacted by several anti-nutritional substances, namely raffinose. Since raffinose cannot be digested by humans or animals, these substances are hazardous when eaten in food. Raffinose content in soybean has been reduced by manipulation. Using RNA interference, the raffinose gene 2 (RS2) in soybeans was silenced. In addition to being allergies, the -conglycinin subunits in soybeans also have antinutritional properties. Low abundance proteins include several allergens, such as the Gly m Bd 30 K protein. To lessen the allergenicity of soybean, the gene encoding the Gly m Bd 30 K protein might be knocked down (Rahman et al., 2022). Anti-nutritional elements such as protease inhibitors, lectins, cyanogens, anti-vitamin factors, phytic acid, goitrogens, saponins, and estrogens are present in raw, mature soybean seeds. If the cost of processing were decreased by eliminating anti-nutritional elements using conventional plant breeding techniques, soybean products would be less expensive. In comparison to soybeans that have the Kunitz trypsin inhibitor, soybeans without it exhibit around 50% less trypsin inhibitor activity per gram of material (Hymowitz, 2022). Among the components of soybean seed are a group of glycoproteins that cause the agglutination of certain red blood cells. These glycoproteins are called lectins or phytohaemagglutinin. Soybeans contain many anti-vitamin factors such as A, B12, and D3. Saponins are glucosides characterized by their foaming in aqueous solutions and hemolyzing red blood cells (Hymowitz, 2022).To improve the safety and quality of edible grains, traditional and cutting-edge food processing techniques have been used to eliminate anti-nutritional factors (ANFs), Microwave processing stands out among them as a quick, reliable, safe, effective, and environmentally friendly method of lowering ANFs. To improve the safety and quality of edible grains, traditional and cutting-edge food processing techniques have been used to eliminate anti-nutritional factors (ANFs) such phytic acid, trypsin inhibitors, tannins, saponins, and oxalate. Microwave processing stands out among them as a quick, reliable, safe, effective, and environmentally friendly method of lowering ANFs (Suhag et al., 2021). Due to its high nutritional value, great overall seed composition, and favorable protein content, soybean is a key protein and oilseed crop for the manufacture of food and livestock feed. It is being used more often in the food sector. However, several of the components of soybean seeds pose various dangers to food safety, which might lower the value of soy-food products. As a result, the goal of the current research was to assess potential methods for improving soybean genetics to create food-grade soybeans with an emphasis on features related to food safety (Watanabe et al., 2018). Too far, important genetic diversity in soybean germplasm collections and breeding materials has been identified for fatty acid composition relevant to food safety, hazardous heavy metal buildup, and protein components such as allergens or antinutritional factors. Genetic markers are now available to help in the introduction of important food safety traits into breeding populations as a result of advancements in genomic research, and the genetic pathways underlying specific food safety features have been elucidated (Watanabe et al., 2018). Additionally, for confirming selection response and tracking quality traits across genotypes, analytical techniques from the disciplines of proteomics or ionomics are useful. Plant breeding techniques are becoming more significant because they can supply the food sector with high-quality soybean raw materials as customer demand for food safety constantly rises. However, it appears that coordinated action between plant breeding and genetic research, food processing, and product marketing has to be created in order to implement greater food safety at the consumer level (Watanabe et al., 2018).

USE OF NEW PLANT BREEDING TECHNOLOGIES (NPBTs) IN SOYBEAN IMPROVEMENT

Improvement in soybean through new technological methods in different traits (stresses) to meet the consumption demand of market (Rahman et al., 2022). The emergence of advanced molecular techniques in form of NPBTs has reduced difficulties in many crops because these are fast and efficient (Liu et al., 2017). NPBTs increase crop yield and reduced the use of inputs; Crops acclimatize to climate and more nutritious foods (Bailey-Serres et al., 2019). These are efficient techniques used for the improving different traits in crops (Osakabe et al., 2010). The desired mutation could be generated by using NPBTs and can choose transgene-free in segregating generations (Curtin et al., 2012). In this section, we, quickly depict a couple of specific parts of NPBTs before looking at significant imitating objectives and mechanical risks in coming about portions. In NPBTs, the most economic and efficient tool, CRISPR is tool used to edit crop genome, the most useful method for breeding crops in crop biotechnology (Kim and Choi, 2021). CRISPR/ Cas 9 and TALENs have been broadly utilized lately, in which changes are presented in two exceptionally rationed ways (i) non-homologous end-joining (NHEJ) (ii) homologous recombination (HR) (Wyman and Kanaar, 2006). CRISPR-Cas provides immunity framework against unknown nucleic acid trespassers in bacteria and archaea (Koonin and Makarova, 2009; Wiedenheft et al., 2012). The CRISPR/ Cas (type II) directs the Cas nuclease to incite site for DNA cleavage at specific site by utilizing non-coding RNAs as a layout in prokaryotic adaptive immune response system use for crop improvement (Hsu et al., 2014). A single guided RNA ties to recombinant Cas protein , comparison of a crRNA sequence intended for the DNA target, and a tracer RNA grouping collaborates with Cas 9 protein. The resultant complex will divide DNA at specific target. Then cleavage effectiveness of sgRNA will be tried (Siddique, 2022). Then parts are conveyed to cells. There are two strategies for genome editing/altering through CRISPR Cas9. 1st is steadily communicate sgRNA/Cas9 DNA by developing CRISPR Cas9 plasmid and by using agrobacterium method or bombardment of articles send plasmid to plant genome (Ma et al., 2015). Mature crRNA that contain both crRNA and trans-RNA can be super cede in the research center with sgRNA (Jinek et al., 2012). Thus, Cas9 and sgRNA are only supposed to edit genome efficiently. The CRISPR-Cas9 technique is frequently used in genetic study of prokaryotes and eukaryotes in the past (Hsu et al., 2014). The 2nd method is transient articulation arrangement of CRISPR Cas9. In this method, collected sgRNA/Cas9 RNPs/ in vitro records (IVT) are conveyed to the cell by molecule assault without editing/altering of DNA genome (Woo et al., 2015; Zhang et al., 2016). The CRISPR-Cas9 system is proficient tool to make transgenic plants with maximum transformation (Belhaj et al., 2013). CRISPER/Cas9 can be helpful for mutagenesis in soybean and important for research purpose particularly for root nodules (Sun et al., 2015). Nonetheless, research in soybean utilizing the CRISPR Cas9 system is as yet uncommon, because the way that soybean change is as yet quite difficult for most exploration groups. Besides, the majority of the objectives of the effective utilization of the CRISPR Cas9 system in gene-altering in soybean were monogenes (Cai et al., 2018; Li et al., 2015). Four SPL9 qualities in soybean are targeted through CRISPR Cas-based different gene-editing systems. T4-age soybean mutants plants conveying various mixes of transformations displayed various modified qualities in plant engineering (Bao et al., 2019).This technique empowers an extensive variety of altering uses which include inclusions, deletions, insertions, and point changes without donor DNA templates(Anzalone et al., 2019).ZFNs have recently been replaced by TALENs for targeted DSB introduction and genome editing. Such a DSB stimulates the cell's two primary repair processes, (i) homologous recombination (HR) (ii) non-homologous end joining (NHEJ). The majority of time, NHEJ is inaccurate, due to this there is insertion or deletion at the repair site and, frequently, so gene sequence changes that cause mutation and it can't perform its function. Real gene targeting is possible through HR in which the DSB is repaired by a homologous template (Sprink et al., 2015). Similar to

ZFNs, TALENs are composed of a general FokI nuclease domain linked to a programmable DNA-binding domain. Highly conserved repeats from transcription activator-like effectors make up this DNA-binding domain (TALEs) (Joung and Sander, 2013). TALEs are made by the genus Xanthomonas plant pathogens, which use the type III secretion route to transfer the proteins to plant cells while infected (Bogdanove et al., 2010). Arrays of 33-35 highly conserved amino acid repeats mediate TALEs, and the amino- and carboxy-terminal ends of the array have additional TALE-derived domains bordering the repetitions (Christian et al., 2010). According to (Boch and Bonas, 2010), Each repeat's amino acid composition is largely constant, with the exception of two adjacent amino acids (the repeat variable residuum, or RVD), which distinguish TALE proteins from one another and ensure target sequence specificity (Boch and Bonas, 2010). When repeats with various RVDs recognize various DNA base pairs, there is a one-to-one correspondence between the nucleotides in the target DNA sequence and the RVDs in the repeat domain.

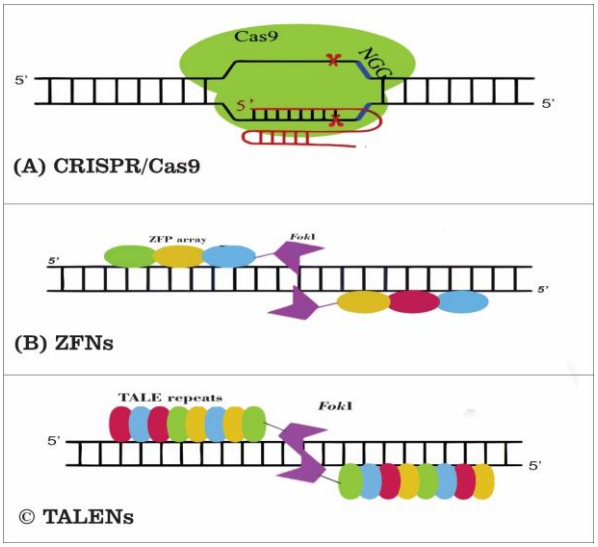

Figure 1. (A) Cas9 and sgRNA are the two components of the CRSPR/Cas9system. (B)Two ZFN modules make up the ZFNs system, and they are coupled to DNA sequences in the reverse direction. (C) Two TALEN modules are often used in TALEN systems, which are coupled to DNA sequences in the reverse direction. TALE protein repeats make up each TALEN module.

This produces a straightforward cypher. With the aid of this cypher, targets of novel TALEs have been successfully predicted, and useful targets for TALEs composed of randomly generated repeats have been produced (Moscou and Bogdanove, 2009). Based on the identities of these two hypervariable residues (RVD) identified at domain positions 12 and 13, each TALE repeat in an array designates a single DNA nucleotide. This modular DNA-binding property of TALE repeats has spurred the development of custom-designed TALE repeats for gene editing. Experimentally or computationally, the preferred nucleotide recognition of the most popular RVDs has been identified (Miller et al., 2015). Large deletions, inversions, or translocations may occur when many loci in a cell are concurrently targeted by two pairs of TALENs. When evaluating the effects of substantial genome rearrangements, huge non-coding RNAs which don't respond to frame-shift mutations, microRNAs, gene clusters, or any other of these entities, this approach offers several advantages(Wright et al., 2014).Engineering TALENs is significantly simpler and less expensive than ZFNs since each TALE domain's activity is limited to a single nucleotide and has no impact on the binding specificity of nearby TALEs. The creation of TALENs and associated TALE technology is progressing quickly. As indicated in Table 1, TALENs and

CRISPR Cas9 have been employed to improve a variety of soybean properties. The user-friendly layout and accessibility of public resources have already changed the view of DNA-binding domain engineering from one of difficulty to one of ease, attracting a larger spectrum of researchers. As a result, a substantial quantity of data showing the efficiency of various TALE-based strategies and TALEN-mediated genome altering in many species has been made public. Miller et al. (2011) identified TALE truncation variants that effectively cleaved. Human NTF3 and CCR5 endogenous alterations or minor deletions with up to 25% efficiency (Miller et al., 2011). Arabidopsis knockout mutations have been created using TALENs. (Cermak et al., 2011),rice (Li et al., 2012),tobacco (Zhang et al., 2013) wheat (*Triticum aestivum*) (Wang et al., 2014) and soybean (Haun et al., 2014), Compared to ZFNs, ALENs are more frequently used for targeted Gene editing, However, they still require a successful method to create tandem repetitions that will bind to the desired DNA region. Furthermore, the enormous size and repetitive nature pose serious obstacles to the effective dissemination of TALENs (Razzaq et al., 2019).

Table 1. Improved traits in soybean (Glycine max) through NPBTs

Target gene(s)	Function of targeted gene (s)	Delivery method/ transformation method	Technique/system used	References
GmFT2a (Glyma.16G150700) and GmFT4 (Glyma.08G363100)	Flower control	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Cai et al., 2020a)
GmPRR37	Flower control	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Wang, L. et al., 2020)
GmFT2a	Flowering Induction	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Cai et al., 2018)
GmFT2a, GmFT5a	Flowering Induction	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Cai et al., 2020b)
GmNAC8	Drought stress	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Yang et al., 2020)
FAD2	Fatty acid synthesis	Soybean protoplast were used	CRISPR/Cpf1	(Kim et al., 2017)
Glyma07g14530, Glyma01g38150, Glyma11g07220, miR1509, miR1514	Endogenous genes	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Jacobs et al., 2015)
GmFE12, GmSHR	Endogenous genes	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Cai et al., 2015)

Glyma.20g148400, **Glyma.03g163500,** **Glyma.19g164900**	Seed storage proteins	*Agrobacterium rhizogenes*-mediated transformation	CRISPR Cas9	(Li et al., 2019)
GmPRR3b^{H6}	Circadian Gene	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Li et al., 2020)
GmLCLal, **LCLa2, LC Lbl, and LCLb2**	Circadian Gene	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Wang, Y. et al., 2020)
GmFAD21A, GmFAD-2A	Fatty acid Desaturase	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Wu et al., 2020)
GmF3H1, GmF3H2 and GmFNSII-1	Isoflavenoids	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Zhang et al., 2020)
CPR5	Trichome development	Biolistic transformation	CRISPR Cas9	(Campbell et al., 2019)
ALSI	Acetolactate synthase for resistance to herbicides	Biolistic transformation	CRISPR Cas9	(Li et al., 2015)
LATE ELONGATED HYPOCOTYL (LHY)	Plant height and internode distance	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Cheng et al., 2019)
Glyma.10G244400 (GmPPD1), Glyma.20G150000 (GmPPD2)	Transcriptional regulator of cell division	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Kanazashi et al., 2018)
GmDrb2a (Glyma.12g075700) and GmDrb2b (Glyma.11g145900)	Drought and salt resistance	*Agrobacterium rhizogenes*-mediated transformation	CRISPR Cas9 and TALENs	(Curtin et al., 2018)
FAD2-1A, FAD2-1B*	Fatty acid desaturase 2 (oil improvement)	*Agrobacterium rhizogenes*-mediated transformation	TALENs	(Haun et al., 2014)
DCL1a (Glyma03g42290), **DCL1b (Glyma19g45060), DCL4*a(Glyma17g11240), DCL4b (Glyma13g22450),**	DICER-LIKE soybean genes involved in gene silencing	*Agrobacterium rhizogenes*-mediated transformation	ZFNs	(Curtin et al., 2011)

RDR6a(Glyma04g07150), RDR6b (Glyma06g07250), HEN1a (Glyma08g08650)				
GmFAD2-1A and GmFAD2-1B	Fatty acid desaturase 2 synthesis	Both *Agrobacterium rhizogenes*-mediated transformation (Transient)	CRISPR Cas9	(Do et al., 2019)
GmFAD2-1A	Fatty acid desaturase 2 synthesis	Poly ethylene glycol (PEG) Induced transient expression	CRISPR Cas9	(Al Amin et al., 2018)
FAD2-1A FAD2-1B and FAD3A	*Fatty acid desaturase* (Oil Improvement)	Biolistic transformation	TALENs	(Demorest et al., 2016)
GmSPL9	Squamosa Promoter binding protein-like (SPL) transcription factor	*Agrobacterium tumefaciens*-mediated transformation for	CRISPR Cas9	(Bao et al., 2019)
Glyma.11G253000 (GmPDS11), **Glyma,18G003900 (GmPDS18)**	Phytoene desaturase enzyme involved in carotenoid biosynthsesis pathway	*Agrobacterium rhizogenes*-mediated transformation for transient	CRISPR Cas9 and TALENs	(Du et al., 2016)
Glyma03g36470, Glyma14g04180 and Glyma06g136900	To study the effect of GmU6 promoters	*Agrobacterium rhizogenes*-mediated transformation	CRISPR Cas9	(Di et al., 2019)
FAD2-1a	Fatty acid desaturase	Biolistic transformation	ZFNs	(Bonawitz et al., 2019)
Gmric and Gmrdn	Involved in nodulation	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Bai et al., 2020)
(Glyma.18g041100(GSI) **Glyma.20g241500 (CHI20)**	Glutamine synthase	*Agrobacterium rhizogenes*-mediated transformation	CRISPR Cas9	(Michno et al., 2015)
GmAGO7a **(Glyma.01G053100) and GmAGO7b** **(Glyma.02G111600)**	Regulator in transacting small interfering RNAs (ta-siRNA) pathway.	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Zheng et al., 2020)
Glyma06g14180, Glyma08g02290, Glyma12g37050	Targeted 102 genes in soybean with various function	*Agrobacterium rhizogenes*-mediated transformation	CRISPR Cas9	(Sun et al., 2015)

Dc14a and Dc14b	Dicers		ZFNs	(Sander et al., 2011)
Rj4 (Glyma.01G165800, Glyma.01G165800-D)	Involves in roots nodulation	*Agrobacterium rhizogenes*-mediated transformation	CRISPR Cas9	(Kim et al., 2017)
GmLax1(Glyma.13g347600), GmLax2 (Glyma.13g347500), and GmLax3 (Glyma.15g026300)	Induced beany flavor which is undesirable for human consumption	*Agrobacterium tumefaciens*-mediated transformation	CRISPR Cas9	(Wang, J. et al., 2020)

For genome editing the initially established tool is ZFNs system. ZFN module contain Cys2-His2(C2H2) zinc finger protein array that was specially developed and common nuclease. Due to the DNA binding domain capability of the ZFP array, particular DNA locations were cut to create DSBs and identify specific DNA sequences. (Urnov et al., 2005). Preserved C2H2 residues with zinc ion form stable bba structure through chelation in an individual having approximate 30 amino acids (Pavletich and Pabo, 1991). ZFP's a-helix side chain and codons coexist in a DNA sequence. ZFPs have the ability to alter the selectivity of codons, and when three or more of them are combined, they can create a ZFP structure that aids in identifying a 9–12 bp long target sequence(Urnov et al., 2005). Folk's endonuclease activity is only active in a dimer form, thus a ZFN module is from my fusing folk nuclease and ZFP array (Bitinaite et al., 1998). For fabrication of DSB in a genome two upside-down (with a 5-7 bp gap between them) ZFN modules are compulsory (Wright et al., 2006). Normally a cell repair DNA by NHEJ which cause insertion and deletion at DSB, Nonetheless, if a sequence identical to the DSB sequence is present, the aforementioned function may be carried out utilizing HR without having to deal with DNA replacement. (Bleuyard et al., 2006). A plant cell has difficulties with transduction of genome editors and HR donor also directional integration of DNA with low frequency is a problem, in contrast NHEJ- mediated genome editing is much accessible or smooth in pants (Baltes et al., 2014).

CURRENT APPLICATION OF ADVANCED TECHNOLOGIES OF GENETIC ENGINEERING

Transgenesis and gene silencing are examples of genetic engineering (GE) which serve to decrease risks and boost soybean adaptability. In recent decades, a significant increase in the use of particle bombardment and agrobacterium-mediated transformation has seen to create transgenic soybean (Bao et al., 2020). Recently emerged NPBTs such as ZFNs, TALENs and CRISPR Cas9 have, paving the door for improved soybean genetic manipulation. These NPBTs could enhance traits of soybean through specific genome engineering and functional characterization of genes (Rahman et al., 2022).Transgenic soybean can be produced by using ZFNs via NHEJ-mediated targeted insertions of multigene donors at an endogenous genomic locus. Curtin et al. (2011) used whole-plant transformation to introduce a ZFN into soybean, resulting the paralogous genes DCL4a and DCL4b underwent distinct mutation. The ZFN-induced mutated gene was efficiently inherited and transmitted in the subsequent generation with the dcl4b mutation. Results show that ZFN-based mutagenesis is a good way to create mutations in duplicate genes that are normally challenging to study due to

redundancy (Curtin et al., 2011). Several DNA donors were delivered to the endogenous soybeanFAD2-1a locus by Bonawitz et al., 2019using one of the ZFNs (potent of generating DSBs at this locus). The largest donor provided had four transgenes, was successfully transmitted to primary transformant progeny, and had no remarkable resemblance to the FAD2-1atarget region. These results shows that NHEJ mediate integration of donor. NHEJ eliminate the gene (targeted) by insertion or deletion but HR replace or integrate targeted genes (Bonawitz et al., 2019). Additionally, Curtin created single (DCL1a) and double mutants (DCL1b) of the soybean genes by using ZFN-based mutagenesis. The main purpose is to find their function in soybean miRNA system (Curtin et al., 2011). In 2014, TALENs is new class of nucleases that detect and cleave conserved DNA sequences, were developed for both genes. Mutations in FAD2-1AandFAD2-1B were found in DNA isolated from leaf tissue of four of the 19 transgenic soybean lines expressing the TALENs; three of these four lines effectively produced a high oleic acid soybean variety by passing heritable FAD2-1 mutations to the next generation (Haun et al., 2014). CRISPR has been widely used to modify the genome of soybeans. The CRISPR Cas9 system accurately mutates DNA sequences in a variety of organisms. In 2015, Jacobs et al. demonstrated that homologous genes were successfully targeted both individually and collectively, indicating that CRISPR Cas9 may target members of gene families both selectively and broadly (Jacobs et al., 2015). Another study published in 2019 employed the genome-editing tool CRISPR Cas9 to find mutant alleles of the two primary storage proteins, Conglycinins and Glycinins, that account for more than 70% of the total protein in soy seeds and are expressed by a small family of genes. The entire study aimed to evaluate sgRNAs that were targeted to nine storage protein genes in soybean roots, and they discovered DNA alterations in three of them. These findings will contribute significantly to the development of a novel and helpful resource for breeders looking to build and generate varieties with such mutations (Banerjee et al., 2021). Another study published in 2019 looked at genome editing technology. Different studies in 2019 that examined genome editing techniques. Other cutting-edge genome editing techniques, like the CRISPR Cas12a system, BE systems, and other CRISPR Cas variations, are used in many other crops, but to our knowledge, they aren't very common in soybean. We believe that these techniques will be used in the near future despite the fact that substantial effort may be needed to use them given the enormous potential of genome editing and the economic importance of soybean. Because of their technique complexity, high cost, and lack of flexibility in use, ZFNs and TALENs have had limited use in soybean. ZFNs and TALENs have only been used sporadically in soybean due to their difficult technology, high cost, and rigid application. However, the most widely used technique is the CRISPR system, which has been extensively utilized in soybean for functional genomic research and trait enhancement because it is cost effective, less time consuming, and allows for effective targeted gene editing.

CONCLUSION

Crop improvement by traditional breeding is a labor- and time-intensive technique. but it’s more widely acknowledged than transgenic approaches. For more than two decades, conventional breeding has coexisted with transgenic techniques in the soybean industry.GE can be useful when the genes are known which we need to alter, such as when providing quick and long-lasting pest resistance. Because of their lack of transgenes, speed, and cost-effectiveness in the current regulatory environment, NPBTs are growing in favor for the continuous improvement of soybean because transgenic soybean has poor public approval because of ethical restrictions such as trans-genes. TALENs, ZFNs, and CRISPR Cas9 systems are among the NPBTs. Different nutritional traits in soybeans can be enhanced. In recent years, it has become more crucial than ever to target disease susceptibility (S) genes in order to give stable resistance to both biotic and abiotic environment as well as nutritional

advancements. In the future, food science and the animal feed industry will benefit from extensive studies of anti-nutritional genes in soybean using CRISPR. Identifying and knocking down genes that control anti-nutritional chemicals will be a crucial step in improving nutritional quality. Using germline-based promoters, the number of mutants in transformed soybean is controlled. Using these promoters to improve the efficiency of NPBTs in soybeans could be crucial. Cas9 delivery into plants has several drawbacks, not the least of which is public acceptance. In the future, it will be beneficial to find novel delivery techniques for soybeans, such as nanoparticle-based delivery. While NPBTs now face many obstacles in the soybean agriculture, there are also many chances for NPBTs to increase soybean yield and productivity. There is no doubt that NPBTs have become entrenched in soybean and will continue to be crucial to the crop's future. With its high ability to site-guided modification, genome editing technology has now replaced traditional mutagenic methods and is projected to radically transform breeding selection, even though its use in breeding of soybean still faces some challenges. The intricacy of the soybean genome makes gene functional analysis difficult. The absence of plentiful gene resources is a key stumbling block to adopting genetic manipulation technologies to improve agronomic qualities, including genome editing technology. Because earlier genome editing applications in soybeans mostly concentrated on modifying a restricted number of known genes, more decoded genomic resources are a requirement for applying genome editing technology in soybean breeding Furthermore, the adoption of gene editing techniques in combination with A. rhizogenic-mediated transformation allowed for functional evaluation of soybean genes. It is extremely difficult to build a soybean mutant library that is genome-wide, but it is doable to create a smaller mutant library that is more narrowly focused on a particular activity. Building a small-scale mutant library that is relevant to specific functions is doable, despite the fact that creating a soybean genome-wide mutant library is extremely challenging. Even if crossing is doable to achieve gene editing for crop varieties that are very resistant to genetic transformation and cannot supply gene editing tools through recurrent marker assisted backcrossing, the procedure is still exceedingly labor-intensive and time-consuming. By using Gemini virus-based replicons to introduce SSNs such as ZFNs, TALENs, and CRISPR Cas9 in plants, gene targeting frequencies have increased. In conclusion, a powerful toolset for genome editing that includes ZFNs, TALENs, CRISPR Cas9, CRISPR Cas12a, BEs, and additional CRISPR Cas variants will advance future work on genetic analysis and genetic improvement in soybeans. Recent studies have revealed the active participation of micro-RNAs (miRNAs) in many aspects of plant development and the response to numerous environmental stress conditions. A list of genes considered prospective candidates for the future development of drought- and cadmium-tolerant soybean has been identified. Instead of transgenic entire plants in soybean, it is also feasible to use Agrobacterium Rhizobium rhizogenic to stimulate the synthesis of transformed hairy roots and produce composite plants. MiRNA-based biotechnology offers a lot of potential for improving crop tolerance to abiotic stressors since plant miRNAs play a role in stress responses and adaptive mechanisms.

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author Kamran Arshad designed the study and wrote the protocol. All the graphics and tables in whole manuscript were designed by Kamran Arshad. The critical study of CRISPR Cas9, ZFNs, and TALENs was also carried by Kamran Arshad. Authors Maham Sajid and Tayyaba Sajid covered the portion of stress resistance. Authors Faiza Mubarak and Mehrab Ijaz managed the literature searches and contributed a lot in nutrients enhancement section of manuscript. The final part of manuscript that is related to the transgenic soybean and it’s new breeding

technologies was written by Umar Azam and Ali Haider. References and citations were managed by Kamran Arshad. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors have declared that no conflict of interest exists.

ETHICS APPROVAL

Not applicable

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