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BIOTECHNOLOGY FOR MANAGEMENT OF BIOTIC AND ABIOTIC STRESS IN SOYBEAN: A REVIEW

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ABSTRACT

Soybean (*Glycine max*) is an important crop globally, providing essential high-quality protein and oils, it is also called as the vegan protein. It contains roughly 15% soluble carbohydrates, 15% insoluble carbohydrates, 18% oil and 36% protein. Its protein contains all nine essential amino acids, which are required for human and animal body growth, therefore, it is considered as a vigorous protein. Though it is a very important crop, its production is greatly affected by biotic and abiotic stress factors. Stress is defined as an external condition that negatively affects the plant's growth, development, and productivity. Stress prompts changes in gene expression, cellular metabolism, and overall plant physiology, which potentially reduce crop yields, diminish growth, and even cause plant death when the stress crosses the plant's tolerance limits. These stresses can be biotic or abiotic in nature; biotic stresses include pathogens, pests, and weeds, while abiotic stresses encompass drought, salinity, extreme temperatures, and nutrient deficiencies. A comprehensive study of plant response against biotic and abiotic stress at the molecular level is essential for its effective management and control. Current advancements in the biotechnology field offer potential techniques to step down these stresses and enhance soybean stress tolerance. By leveraging these biotechnological tools, it is conceivable to improve soybean yield and stability, which ensures sustainable agricultural practices and food security in the aspect of shifting environmental conditions. In this review, we explore the up-to-date biotechnological strategies, including genetic engineering, Molecular breeding, CRISPR/Cas9 genome editing assisted with plant tissue culture, to develop stress-tolerant soybean.

***Keywords*:** Soybean, Genetic Engineering, Stress-resistant, Biotic stress, Abiotic stress , Biotechnology.

Introduction

Soybean crop (*Glycine max* L. Merr.) is a diploidized tetraploid (2n= 40) and has a genome size of 1.1–1.15 Gb. Soybean is recognized as the "golden bean" or the "miracle bean." (Meltzer *et al.,* 1993). It belongs to the family Leguminosae and subfamily Papilionaceae. The cultivated soybean has been proposed to be named correctly as *G. max* (L.) Merrill by Ricker and Morse in 1948. Originating from China, annual *G. max* is naturally self-pollinated and is usually less than 1% in the highly cross-pollinated, though it may sometimes reach up to 2–3%. The perennial species have been reported to have up to 60% out-crossing. This crop is grown under warm conditions in the tropics, subtropics and temperate climates; the ideal temperature range for growth and development lies between 18°C to 35°C. It is mainly a short-day plant, but response to day length varies with variety and temperature (Mishra *et al.*, 2024b, Mishra *et al.*, 2024c).

It is the world's most widely grown seed legume, providing an economical source of protein and vegetable oil for human feeding. It is important in sustainable agriculture because of its capacity to enrich

soil with nitrogen through biological nitrogen fixation. For a long time, interval, soybean has been used as a nutritious source of food like bread, meat, milk, cheese, and oil to the people of different nations, including China, Japan, Korea, and Indonesia. That is why they defined as a "cow of the field," or "gold from soil" by such nations (Meltzer *et al.*, 1993, Mishra *et al.*, 2024a).

India is one of the major producers as well as the consumers of oilseeds and edible oil in the world. India stands fifth in position for the production of soybean in the world (Mishra *et al.*, 2024b, Mishra *et al.*, 2024c). According to the Foreign Agricultural Service of United States Department of Agriculture report of 2024, the globally soybean production is about 395.12 million metric tons. Brazil is the leading producer of soybean in the world. Brazil contributes 39% of global production of soybean, second larger producer is USA it contributes 29% globally and India contributes 3% globally in the production of soybean. The area coverage of soybean cultivation in the world is about 139.79 million hectares, and in India, soybean is sown in 13.20 million hectares (Foreign Agricultural Service, 2024). In India, Madhya Pradesh stands as the largest soybean producer in India, it secretarial for nearly 50% of the total country's production, as reported by the Department of Agriculture & Farmers' Welfare, which has released progress of area coverage under kharif crops as on 8th July 2024.

In Madhya Pradesh, soybean cultivation is recognized due to its favorable climate, fertile soil, and advanced farming practices. Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, and Telangana are the major soybean-growing states. Soybean output is expected to reach 135.05 lakh tonnes in Kharif 2019-20, according to the first advance projections released by the Indian government. Though the soybean crop has a high utility, yield and production, it is highly susceptible to infestation by biotic (fungi) and abiotic (salinity) factors, and is highly prone to mechanical injury and damage stirring during post-harvest treatment. Among abiotic factors, salinity, flooding, drought, temperature, and pH are the principal factors that root cause of sluggishness in crop yield. In biotic stressors, diverse phytopathogens cause reductions in yield and production of soybean crops. Evidence indicates that biotic and abiotic stresses can cause 26.3% and 69.3% loss of soybean production, respectively (Mishra *et al.*, 2024c, Mishra *et al.*, 2025, Gautam *et al.*, 2025, Jhariya *et al.*, 2025).

Stress

Stress in plants refers to external conditions that adversely affect the growth, development, or productivity of plants. Hans Selye defined stress as "All agents can act as stressors, producing both stress and specific action, "and also mentioned that "There exist stressor-specific responses and non-specific general responses" (Lichtenthaler, 1998). The definition of plant stress is very wide sense, and this involves the formation of various joining concepts of plant stress. Some multiple hosted stressors with various modes of action often induce plant stress; during stress, plants cannot show the quick reformations of metabolic instabilities. Plants are acclimatized slowly and respond to the changes in cell metabolism rate and physiological accomplishments to respond to the changes in environmental conditions.

Two types of environmental stresses are affecting plants condition, which can be characterized as (Meltzer *et al.*, 1993) Abiotic stress and (Mishra *et al.*, 2024b) Biotic stress. The effect of abiotic stress lay down the major crop production; it includes drought, salinity, floods, excesses temperature, and heavy metals accumulation, etc., and biotic stress also severely affect the crop yield and it may occurs due to natural ecosystem disturbance and human interference, biotic stress effect spread or attacks by several pathogens such as fungi, bacteria, oomycetes, and nematodes etc. though both the stress condition are highly harmful and destructive for plant, but abiotic stress is more damaging as compare to biotic stress. By the use of signal mechanisms, plants can activate their rescue process from stress by stimulating appropriate cellular responses. They received stimuli from the stress sensors situated on the cell surface or cytoplasm, and the signal transferred to the transcriptional mechanism which is present in the nucleus, with the assistance of several signal transduction pathways. This leads to difference transcriptional changes occurs which make the plant tolerant against the stress.

Impact of Abiotic Stress on Soybean

Abiotic stresses are the most destructive of all major problems in crop production. These abiotic stresses are interrelated with each other and cause osmotic stress, ion distribution, and affect cell homeostasis. Soybean crop production is not only affected by environmental factors, such as drought, water submergence, salt, and heavy metals, but it also faces problems in adapting to non-traditional areas (Grainger and Rajcan, 2014). The factors of abiotic stress have a huge influence on world agriculture and result in more than 50% reduction in typical potential

yields for most major crops. The abiotic stresses that occur in plants during stress conditions are as follows.

Drought

Among the abiotic stresses, drought is considered the most devastating, affecting all the plant's growth and development stages and reducing the soybean yield. Drought is the main abiotic stress factor that affects crop productivity, and it is of major importance in soybean due to the susceptibility of this crop to drought, particularly during the reproductive growth stage. Often, the term "drought" is not defined based on the plant tissue hydration, but in soil changes and weather conditions. The most critical phase for the water deficit effect is during flowering or right after this period (Farooq *et al.*, 2017). In reduced conditions of available water, there is a shortening of the grain filling period, it decreases the transference of assimilates to the grain, which means that there is a reduction in the soybean grain weight.

The uneven rainfall occurrence could become a major initiator of drought stress. Such stresses occur simultaneously, limiting the growth and development of plants, thus compromising sustainable agriculture. Under stress conditions, plants reduce their growth of shoot length and reduce their metabolic loads; after that, plants produce defensive metabolites and mobilize metabolites for their osmotic adjustment. Lack of water decreases the soil water potential, stimulating the synthesis of the abscisic acid (ABA) hormone in the roots. From the roots, the ABA is translocated to leaves by endogenous signals that operate at long distances. These signals may be chemical (plant hormones and pH), hydraulic or electrical (Mishra *et al.*, 2024c, Osakabe *et al.*, 2014).

Flood and Waterlogging

Flood is the most recurrent type of natural calamity, and it occurs when excess runoff water immerses land that is usually dry and plain. It is often triggered by heavy rainfall, speedy ice melt, or cyclones in coastal areas. It can cause extensive destruction in a short time interval. Nowadays, the frequency and intensity of floods are increasing day by day due to changes in the climate and the weather cycle. Flooding affects about 10% of the global land area.

Waterlogging stress was mainly enacted at the time of anthesis and the seed filling stage. The flowering stage exhibited higher sensitivity, as compared to the seedling stage. The shorter the duration of stress, the shorter the adverse effect of waterlogging stress, and vice versa. The root system of soybeans also becomes shallow under waterlogged stress conditions, because of decreasing the tap root length, whereas the number of adventitious roots increases. Oxygen was markedly reduced under flooding conditions, and plants have to adapt to low oxygen status through increasing anaerobic energy production. In plants, oxygen is required for proper functioning, growth, and biological processes like cell division, transpiration, respiration, uptake of minerals, and transportation of nutrients. Chlorosis, necrosis, defoliation, growth reduction, reduced N fixation, yield loss, and plant death are effects of waterlogging stress in soybean plants, which often occur at various vegetative and reproductive stages (Manghwar *et al.*, 2024, Adegoye *et al.*, 2023).

Salinity

Salt stress is one of the severe abiotic stresses that significantly reduces the yield of soybean production. More than eight million hm^2 of land worldwide is affected by salinity, accounting for 6% of the world's total arable land area. It is expected that 30 percent of arable land will be lost in the next 25 years, and 50 percent by the middle of the 21st century (Ludwig *et al.*, 2018). Soil salinity is a worldwide threat to world agriculture by reducing the yield of crops; it decreases the growth and yield of crops in many ways. The two primary ways of affecting the crop plants are: osmotic stress and ion toxicity. It also affects the plants by assimilate production, inhibiting cell expansion and membrane function, as well as reducing the cytosolic metabolism. A vast collection of soybean genotypes was screened in order to identify genetic materials showing high tolerance to salt stress. Among various crops tested, legume crops have normally been found to be more sensitive to salinity stress.

Soil salinity has become one of the most environmentally problematic issues in the 21st century. Salt stress can be divided into two components. In the short-term, salt stress produced osmotic stress, whereas at long-term ion toxicity occurred due to the accumulation of phytotoxic ions, especially Na^+ and Cl^-. In addition to the osmotic and toxic effects, salt stress also induced oxidative stress; with all these factors contributing to the deleterious effects of salinity in plants (Acosta-Motos *et al.*, 2015). Salt stress also affects several physiological features of plant growth and development. Shoot growth and dry matter are reduced due to salinity, root: shoot ratio is increased. Salinity can affect seed germination by creating osmotic potential which prevent water uptake from root, and by making toxic effects of ions on embryo viability. Shoot growth was decreases under salinity stress due to the inhibitory effect of salt on cell

division and enlargement in the growing point (Atta *et al.*, 2023).

Cold

Cold stress is an environmental stress which has proved to be the foremost abiotic stresses that reduce the productivity of crop by disturbing the quality and their post-harvest life of crops. In most species, cold stress affected by several physiological processes such as: fluidity of membranes, water and nutrient absorption, the conformation of proteins and nucleic acids, modifications in cellular physiology by declining in the rate of metabolic processes of gene expression, free radical production. In soybean crop, cold stress has antagonist effects primarily on protein and cell membrane metabolism. Cold stress is an environmental stress that limits plant growth and development processes (Abdel *et al.*, 2016).

Soybean crop is sensitive to the temperature change during the course of the developing season, from emergence to maturity, where the temperature of 17–18 °C is considered lowest tolerating temperature and the optimum temperature for growth lies between 22–32 °C (Parent and Tardieu, 2012). When the temperature drop is prolonged below 15 °C, it slows the plant growth and inhibits leaf and shoot formation, while a drop below 10 °C disrupts the flowering process (Miransari, 2015). During the soybean growing period, there are two critical periods when plant shows high sensitivity against cold stress or low temperature. The first critical period occurs in between sowing to full emergence of plants. The second critical period of the particular sensitivity of soybean to cold occurs during the flowering phase (Staniak *et al.*, 2021). Plants have developed several strategies against the low-temperature or cold stress, by accumulating antioxidants and chaperones, activating the primary metabolism resulting in more energy production and altering the membrane structure which positively affects the osmotic balance of the cell (Ghosh and Xu, 2014).

Heat

The climate change is predictable to be more severe and drastic upsurge in the severity of heat waves and stress in plants and their impact on seed development during the current century (Huang *et al.*, 2019). Increase in temperature all over the world has become a great alarm, which not only affect the growth of plants but their productivity as well, especially in agricultural crops. The heat stress is estimated to increase in intensity in the upcoming phase, due to global warming and climate changes (Grossiord *et al.*, 2020). Climate change is expected to increase the incidence and severity of summer heat waves (Balfagón *et al.*, 2022). Heat stress or thermal stress is considered most drastic abiotic stresses which affect the crop growth and yield (Saleem *et al.*, 2020), hindrance to global food safety, and affected the huge area under crop cultivation (Rahi *et al.*, 2022). It is the most disturbing abiotic stress that directly affects plant growth, development, and yield (Nakagawa *et al.*, 2020). Several studies have publicized the effects of heat stress on soybean crop growth, yield, and quality (Cohen *et al.*, 2021).

In many studies it is found that, when soybean is exposed to short-term and long-term heat waves (Zandalinas *et al.*, 2020), it yields affect negatively and its seed germination extremely disturbed. As a result of poor seed germination (Haider *et al.*, 2022), its seed become vulnerable to pathogen attack, and reduce its economic value (Wang *et al.*, 2018). This heat stress alters the root growth of soybean crop and restricts the root elongation under stressed condition (Moraes and Gusmão, 2021). Heat stress imposes a direct effect on photosynthesis and transpiration, consequently affecting soybean yield. It also decreases carbon assimilation, which causes stomata closure and injured cell membrane, and slow down the action of several enzymes, which ruins the metabolic pathways, that costs in altered plant growth (Liu *et al.*, 2014).

A single degree increase in temperature decreases soybean yield by 16–17%. Schlenker and Roberts noticed the negative effect of temperature with a threshold level of 30°C for soybeans. Its yield started declining at temperatures higher than 26/20°C and if the temperature rises to 29/20 to 34/20°C during the seed-filling stage, there is a noteworthy decline in soybean seed yield. It has been predicted that by 2100, a 6°C increase in temperature might lead to a 49% decrease in soybean yield (Schlenker and Roberts, 2009).

Metal Toxicity

The metal toxicity in soil and water leads to the serious environmental stress that affect directly to the ecosystem. Metal toxicity is one of the major abiotic stresses foremost to have harmful health effects in animals and plants. Because of their high reactivity, they can straightly influence the growth, senescence, and energy synthesis processes. Metal toxicity in soybean crops, mostly from heavy metals like lead (Pb), cadmium (Cd), and arsenic (As), poses significant challenges to agricultural productivity and food safety. Once engaged, these metals in the soil it can disrupt essential physiological processes, including nutrient uptake, photosynthesis, and water balance,

ultimately reducing plant growth and yield (Ghorbani *et al.*, 2024).

For instance, lead contamination can severely affect root biomass and plant height, with the highest accumulation typically occurring in the roots (Blanco *et al.*, 2022). Additionally, heavy metals induce oxidative stress by generating reactive oxygen species, which damage cellular structures and impair metabolic functions (Baig *et al.*, 2018). Several research shown that soybean plants faces a lot by toxic metal/metalloids effects in the whole life cycle (from germination to maturity stage). Germination stage was seriously affected under different levels of cadmium (Cd), lead (Pb), and arsenic (As) stresses. The reduction in seedling growth under Cr stress due to which poor root growth, which impedes the transportation of water and nutrients to the shoot of the plants and the root nodulation was severely affected, and their number reduced drastically in Cd-treated soybean plants, which adversely affected the N fixation of plants Cd leads to oxidative stress, inhibition of respiration, increased production of ethylene (Chmielowska-Bak *et al.*, 2014). Aluminum (Al) metal stress caused noticeable reduction in root length, shoot height, and dry weight. Additionally, (Sundarmoorthy *et al.*, 2015) also reported the activity of antioxidant enzymes such as CAT and polyphenol oxidase decreased, whereas POX and SOD activities increased. Addressing metal toxicity in soybean crops requires integrated approaches, such as using nano-enabled agrochemicals and beneficial microbes, to mitigate the adverse effects and enhance crop resilience (Ghorbani *et al.*, 2024, Aksoy *et al.*, 2017).

Impact of biotic Stress on Soybean

Soybean is an important crop worldwide, it is a significant source of protein and oil content. However, its production is often hampered by various biotic stresses, which include pathogens, insect pests, and nematodes. These biotic factors can severely affect the health and yield of soybean crops, leading to substantial economic losses. Biotic stress is a serious factor limiting soybean growth and development. Biotic stress can turn out to be hazardous because of pre- and postharvest losses. The agents causing biotic stress directly divest their host from its nutrients that can lead to death of plants. Many biotic stresses affect mainly photosynthesis, as chewing insects decreasing the leaf area and virus infections reduce the rate of photosynthesis per leaf area. These biotic stress agents cause several types of diseases, infections and damage to crop plants and finally disturb the crop productivity. The biotic stresses in plants can be overwhelmed by the genetic tools.

Viral diseases

Some of the major viral infections of legumes are observed in peas. More than 20 different plant viruses have been identified in peas, and the pea seed-borne mosaic virus (PSbMV), pea/bean leaf roll virus (P/BLRV), and pea enation mosaic virus (PEMV) are of major economic importance. Infections can have a disastrous financial impact on the farmer. Soybean, a major economic commodity, is also affected by viral infections. The significant infections are caused by soybean mosaic virus (transmitted through the seeds) and tobacco ring spot virus. The virus, transmitted by the whitefly *Bemisia tabaci*, causes the appearance of irregular yellow and green patches on the plant, ultimately causing the total deterioration of the plant system (Chatzivassiliou, 2021, Jha *et al.*, 2023).

Other viral agents infecting leguminous plants include beet western yellow virus, subterranean clover red leaf virus, and other luteoviruses, cucumber mosaic virus, bud necrosis virus, and cowpea golden mosaic virus. Bacterial diseases and bacterial infections in legumes are relatively limited compared to viral and fungal infections. *Pseudomonas syringae* and *Xanthomonas campestris* have been associated with bacterial blight and bacterial pustule in soybean plants. Bacterial wilt is commonly observed in the groundnut; it is caused by the *R. solanacearum* and manifests as the damping and killing of seedlings. These bacterial infections may be spread by infected plant material, infected seeds, contaminated nutrient sources and water (Jones, 2012, Cun, 2022).

Fungal diseases

Due to the widespread geo-distribution of the legumes, a variety of different fungal species infect legumes according to their habitat. Several fungal species, including *Phakopsora pachyrhizi*, *Pythium ultimum*, *Fusarium oxysporum* and *Phytophthora sojae*, are established causes of infections in soybean (Wei *et al.*, 2016). Wet rust and rotting of seedlings and roots are the most important diseases, responsible for significant economic losses in soybean crops. Similarly, the important fungal diseases infecting lentils are dry root rot (*Macrophomina* spp.), vascular wilt (*Fusarium* spp.) and pythium root rot (*Phythium* spp.). The fungal diseases of groundnut include root diseases, foliar diseases, seed diseases and pod diseases attributable to at least 15 different species of fungi. Most of these fungal agents are soil-borne and tend to affect all the parts of the plant (Manathunga *et al.*, 2024, Hosseini *et al.*, 2023).

Nematode infestation

A number of worms and insects are associated with parasitic damage to legumes. Many nematodes, the most significant ones infecting legumes include root-knot nematode (*Meloidogyne* spp.), cyst nematode (*Heterodera* spp.), stem nematode (*Ditylenchus* spp.) and reniform nematode (*Rotylenchulus* spp.). Nematodes, in comparison to other biotic stresses, do not produce specific signs of infection; rather, their infection is exhibited as a delayed appearance of normal growth characteristics, decreased ability to fix nitrogen, and retardation of development. More than 70 insect species infect soybean crops. For example, *Spodoptera litura*, *Hedylepta indicata* and *Mylabris pustulata* have been implicated in causing crop losses of up to 50%. Other financially significant insects infecting legumes include aphid, hairy caterpillar, thrips, white fly (vector of the yellow mosaic virus), leaf hopper, army worm and green bug (Bahadur, 2022, Palomares-Rius *et al.*, 2017).

Management of Biotic and Abiotic Stress in Soybean by using Biotechnological tools

Soybean is an important and popular crop worldwide. It provides a main source of veg-protein and oil content but, its productivity is often hampered or disturbed by several biotic (living) and abiotic (non-living) stress factors. Biological and physical stress conditions are accountable for noteworthy losses in legumes crop. The conversational methods of stress control by using fungicides, pesticides and other hazardous chemicals for handling various biotic stress constraints, and molecular interventional tactics have helped in the accomplishment of tolerance against cold, herbicides, drought, salinity stress and high-intensity light. Biotechnological tools offer innovative solutions to manage these stresses effectively and managing biotic and abiotic stress in soybean crops using biotechnological tools.

Biotic stress management by biotechnological tools

Genetic Engineering

Genetic engineering in soybeans involves transforming the plant's DNA by introducing new traits or improving existing traits. The primary goals are to improve crop yield, increase resistance to pests and diseases, and enhance tolerance to herbicides. For example, genetically modified (GM) soybeans can be engineered to withstand harsh environmental conditions; genetic modifications can improve the nutritional content of soybeans, making them more beneficial for both farmers and consumers. Overall, genetic engineering holds great promise for the future of soybean cultivation, contributing to global food security and sustainable agriculture. The tools of genetic engineering used for sustaining soybean crop production are discussed below.

Genetic transformation: Genetic engineering has been used to improve the biotic stress-tolerant potential and enhance the protein quality of soybean by altering biosynthetic response pathways that increase lysine and sulfur-containing amino acids. Transgenic technology has been used to improve soybean agronomic traits, which include yield components, grain quality, and biotic and abiotic stress tolerance, as well as economic traits. The first transgenic herbicide-resistant soybean was commercialized in the year 1996, Genetically Modified (GM) soybean, particularly the GM Roundup Ready soybean resistant to glyphosate herbicides, has been cultivated in many countries including the United States, Argentina, and Brazil (Pagano and Miransari, 2016), which make it as important biotech crop. This soybean variety permits farmers to spray herbicides to kill any weeds in the field but not affect the soybean crop. The first genetically transformed soybean was developed in the late 1980s; the first fertile transgenic soybeans were developed by either regeneration of cotyledonary nodes by infecting with *Agrobacterium tumefaciens* or by particle bombardment/gene gun method using meristems of immature soybean seeds. Many types of genetically modified soybeans have been improved for the traits of oil content, like increasing oleic acid content, decreasing linoleic acid content, delaying the flowering time and increasing yield. Roundup Ready soybeans are a form of genetically modified crop (GMO) developed by Monsanto in 1996 to be resistant against glyphosate chemical. This specific trait allows the farmers to apply glyphosate to their fields to control weeds without damaging the soybean crop. The genetic modification involves inserting a gene from the bacterium *Agrobacterium tumefaciens* that produces an enzyme resistant to glyphosate. This enzyme, known as CP4 EPSPS, allows the soybean plants to survive applications of the herbicide. In 2016, Monsanto introduced Roundup Ready 2 Xtend soybeans, which are resistant to both glyphosate and dicamba, another herbicide. This newer variety offers even greater flexibility in weed control and has been widely adopted by farmers (Xu *et al.*, 2022, Dong *et al.*, 2024).

Trait improvements through forward and reverse genetic approaches in the last 5 years i.e., down regulation of the pyruvate dehydrogenase kinase gene GmPDHK through RNAi made an average of 42.2% protein content in seeds of transgenic plants, which is significantly increased compared with the non-transgenic control (Jones *et al.*, 2020). Soybean seeds

with linolenic acid content in excess of 50% of the total oil have been produced by increasing the expression of the FAD3 gene, which encodes the enzyme that converts linoleic acid to linolenic acid (Yeom *et al.*, 2020). Stable GmMYB14-overexpressing (GmMYB14-OE) transgenic soybean plants demonstrate semi-dwarfism and a compact plant architecture associated with decreased cell size, causing decreased plant height, internode length, leaf area, leaf petiole length, and leaf petiole angle, and improved yield in high density and drought tolerance under field conditions (Chen *et al.*, 2021). Overexpressing the GmmiR156b (Squamosa promoter-binding protein-like, SPL) gene in soybean and transgenic plants produced significantly increased numbers of long branches, nodes, and pods that exhibited increased 100-seed weight, resulting in a 46–63% increase in yield per plant (Sun *et al.*, 2019).

Resistance to soybean cyst nematode (SCN; *Heterodera glycines*) in stably transformed soybean plants is enhanced by downregulation of the HgY25 and HgPrp17 genes, which are related to reproduction and fitness (Tian *et al.*, 2019). Overexpression of GmDR1 [*Glycine max* disease resistance 1 (Glyma.10G094800)] led to enhanced resistance not only against *F. virguliforme* but also against spider mites (*Tetranychus urticae*, Koch), soybean aphids (*Aphis glycines*, Matsumura), and SCN (Ngaki *et al.*, 2021). Overexpression of PAC1 and GmKR3, a TIR–NBS–LRR-type R gene, can increase multiple virus resistance in transgenic soybean and, thus, provide an efficient control strategy against RNA viruses such as SMV, BCMV, WMV, and BPMV (Xun *et al.*, 2019). Many types of herbicide-resistant transgenic soybean, such as glyphosate-resistant, dicamba-resistant, and 2,4-D-resistant, are grown widely in the United States (Nandula, 2019). As of 2024, transgenic soybeans cover approximately 95.9 million hectares, which accounts for about 50% of the global transgenic crop area (Sandhu *et al.*, 2025). Therefore, a better soybean transformation system is the base for soybean improvement through transgenic technology.

RNA interference (RNAi): RNA interference (RNAi) or Post-Transcriptional Gene Silencing (PTGS) is a preserved natural biological response to double-stranded RNA (dsRNA) that facilitates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and controls the expression of protein-coding genes. RNA interference (RNAi) is an evolutionarily conserved mechanism that refers to a set of molecular processes in which small RNA (sRNA) molecules exert a silencing RNAi technology is a gene-silencing tool that uses small RNA molecules to inhibit the effect of complementary mRNAs (Ganbaatar *et al.*, 2017) and stop the expression of specific genes and subsequent inhibition of protein synthesis. The RNAi-based technology has recently been exploited to control insect pests of agriculturally important crops (Mao and Zeng, 2014). Some insects exhibit relatively low or inefficient RNAi responses, resulting in temporary gene silencing (Shukla *et al.*, 2016).

In late 1996, Fire and Mello discovered RNAi when they injected double-stranded RNA into the nematode *Caenorhabditis elegans*, leading to the selective silencing of specific genes and opening the way for a revolutionary gene regulation mechanism. In the first step, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into a short, interfering RNA (siRNA) of 21–23 base pairs (bp) by the RNase II enzymes Dicer (Zhao *et al.*, 2015) and Drosha. In the second step, siRNAs are loaded into the effector complex RNA-induced silencing complex (RISC). The siRNA is unwound during RISC assembly and the single-stranded RNA hybridizes with mRNA target. Gene silencing is a product of nucleolytic degradation of the targeted mRNA by the RNase H enzyme Argonaute2 (Slicer) (National Center for Biotechnology Information, 2017). Using siRNA as a guide, the RISC locates the target mRNA, which is then cleaved by Argonaute2, ultimately downregulating protein expression (Azlan *et al.*, 2016). If the siRNA/mRNA duplex contains mismatches, the mRNA is not cleaved.

Soybean pod borer (SPB; *Leguminivora glycinivorella* (Matsumura) (Lepidoptera: Tortricidae) is the most harmful soybean pest in Northeastern Asia, affecting annual yield losses that are estimated to exceed upto $10 million. The SPB shows a robust systemic RNAi response. The gene encoding SPB Spbtry1 (serine protease) can be silenced via the feeding of Spbtry1 dsRNA (Ran *et al.*, 2018).

Marker-Assisted Selection: Revolutionizing Breeding Programs

Marker-assisted selection (MAS) has revolutionized the field of breeding by providing a powerful tool for the precise and efficient selection of desirable traits. Marker-assisted selection has been widely applied in breeding programs for targeted transferring and pyramiding resistance loci in different crops. MAS is a breeding program in which the detection and selection of DNA markers are integrated (Kordrostami and Rashimi, 2015). MAS denotes a significant improvement in the field of genetics and breeding, and has obtained the linkages between

molecular markers and the desired traits, such as in biotic stress tolerance, pathogen and pest resistance, resistance to nematodes, and some quality and quantity parameters. With MAS, breeders can conduct multiple cycles of selection in a year, eliminate dependence on the natural occurrence of the pest or pathogen, and reduce the cost and labor associated with inoculum maintenance.

By the use of molecular markers, it allows breeders to select for desirable traits with superior exactness and efficiency genes. MAS is an effective technique to increase crop yield, which is free of the target environment. It can be used for indirect selection at the very first phase of the yield to find QTLs (Quantitative trait loci) in lines, varieties, and populations for breeding (Singh and Singh, 2015). MAS may be more effective for the selection of the first and second groups of resistant loci, whose functions depend on the specificities of encoding proteins. The key principle behind MAS is the linkage between markers and the genes of interest. It is based upon the establishment of a tight linkage between a molecular marker and the chromosomal location of the gene(s) governing the trait to be selected in a particular environment. This has totally transformed the standards of selection.

MAS can improve efficiency by using gene-specific or closely linked markers to avoid bringing undesired traits into an improved cultivar owing to linkage drag. In plant breeding, it is used to enhance traits such as disease resistance, drought tolerance, and yield. MAS has been used for the genetic improvement of various characters in different crops. Important characters that have been improved through MAS in different crops include disease resistance, insect resistance, salinity resistance, and shattering resistance. Molecular markers which have been widely used in MAS in different crops include Restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) or microsatellite. Other markers used in some crops include amplified fragment length polymorphism (AFLP), sequence tagged site (STS), expressed sequence tags (EST), and SCAR markers. Single-nucleotide polymorphism (SNP) is also used.

MAS applied in soybean disease resistance:

Soybean cyst nematode: (SCN, *Heterodera glycines* L.) is the most economically disturbing pathogen of soybean and causes damage in soybean-producing areas worldwide with no practical means of eradication. The two resistance genes for SCN, Rhg1 (Resistance to *H. glycines*) on Chromosome (Chr) 18 and Rhg4 on Chr 8, have been identified in soybean germplasm and have been presented as important sources of resistance in soybean cultivars. The Rhg1 locus, which is crucial for broad-spectrum resistance to SCN, has been identified in various resistance sources, such as PI 88788 and Peking. SCN resistance is a crucial improvement in breeding programs. Soybean cyst nematodes pose a serious problem, and most of the varieties are susceptible to this parasite. The resistant gene (rhg 1) is available. In soybean, nematode-resistant lines have been developed through MAS using the SSR marker (Sat 309). MAS is an effective and consistently preferred approach to develop SCN resistant soybean lines, representing the fastest, cost-effective, accurate, and reliable method. To arrange the major SCN resistance genes in breeding germplasm, several molecular markers have been developed and implemented in breeding programs by means of MAS. Although the use of molecular markers can potentially reduce resources and time needed for breeding disease resistance, Restriction fragment length polymorphisms (RFLPs) markers have been identified and reported to facilitate soybean breeding for both Rhg1 and Rhg4 loci. Simple sequence repeats (SSRs) were also discovered and applied in the marker-assisted selection of SCN resistance. SSRs and RFLPs are labor/time-consuming and cost-ineffective. Thus, they are very limited in being applied in breeding programs in a high-throughput setting (Zhang *et al.*, 2022, Peng *et al.*, 2021).

Root-knot nematodes: (*Meloidogyne* spp.) are major soybean pests that severely affect crop yield. Researchers have been escalating their plans to develop upgraded root-knot nematode resistant cultivars/varieties. In recent times, breeders have discovered the use of DNA marker tools instead of phenotypic screening. When the marker/QTL relationship has been recognized and confirmed, the resistant gene against the disease can be easily identified. The identification of molecular markers and subsequent application of MAS is a rapid and effective way to expedite a nematode resistance breeding programme. *Meloidogyne incognita* race 2 MAS is used in a breeding programme using markers Satt201, Satt358, Satt487, and Satt590, which were identified and validated for the disease-resistant genes (Yadav *et al.*, 2025, Khan *et al.*, 2023).

Abiotic stress in soybean management by biotechnological tools

Genomic Approach

A genomic approach in plants involves the use of modern and advanced genetic techniques for analysis

and manipulating the plant genome for many purposes, such as improving crop yield, disease resistance, and stress tolerance. The significant aspects of these genomic approaches include Whole-Genome Sequencing, Genomics-Assisted Breeding (GAB), Quantitative Trait Loci (QTL) mapping, Genome-Wide Association Studies (GWAS), and Genomics-Assisted Breeding (GAB). These genomic approaches are modernizing plant breeding and agriculture research, improving food security and sustainable agriculture in changing environmental conditions.

Quantitative Trait Loci (QTL) mapping in Soybean

Quantitative Trait Locus (QTL) mapping is a dominant tool used in plant genetics to identify the specific regions of the genome that are linked with certain quantitative traits, such as yield, disease resistance, or stress tolerance. A QTL is a genetic locus in which allelic variation affects variation in the observed phenotype. Usually, quantitative traits are influenced by numerous polymorphic genes and environmental conditions. The identification of the chromosomal regions where marker allelic and phenotype difference associates the place that shows the presence of a QTL. Each QTL recognizes the genomic location of a gene or the genes disturbing the trait of interest. Several genetic populations can be used for QTL mapping, including particular crosses of inbred strains and outbred strains. Various known markers, such as microsatellite DNA, restriction fragment length polymorphisms (RFLP), along with restriction enzymes and single-nucleotide polymorphisms (SNPs), can be used to map targeted genes (Kumawat *et al.*, 2016, Saini *et al.*, 2025).

QTL Mapping for Abiotic Stress Tolerance in Soybean

QTL mapping are marker based applications that have become more sophisticated with the availability of different genotyping platforms. Subsequently, several efforts have been made to identify QTL for abiotic stress tolerance in soybean. The use of molecular markers for improving breeding program was initially suggested in 1989. The two main methodologies, viz., linkage mapping and association mapping, are now being consistently used for identifying marker-trait associations in soybean. QTL mapping has been successful used for biotic stresses like water-logging, salt stress, manganese toxicity, iron deficiency chlorosis, aluminum tolerance and phosphorus deficiency soybean is the most effective legume crop where use of markers in breeding programs is routine and several improved lines/varieties for resistance to different SCN races. As like other biotic stress management several QTL are identified, drought tolerance QTL also Recognized in soybean. QTL of plant height and seed weight per plant were used as the indicators of drought tolerance and seed weight, measured as mass per seed, is an important yield component of soybean and is generally positively correlated with seed yield, for identifying QTL for drought tolerance two distinct cultivars were used, one was drought-sensitive cultivar ‘Zhonghuang 35’ and another was drought-tolerant cultivar ‘Jindou 21’ establish F6:9 recombinant inbred lines. Total of 23 QTL identified related to drought tolerance. Among them, seven QTL (qPH2, qPH6, qPH7, qPH17, qPH19-1, qPH19-2, and qPH19-3) on chromosomes 2, 6, 7, 17, and 19 were related to plant height, and five QTL (qSWPP2, qSWPP6, qSWPP13, qSWPP17, and qSWPP19) on chromosomes 2, 6, 13, 17, and 19 were related to seed weight and could be considered as the major QTL. In addition, three common QTL (qPH6/qSWPP6, qPH17/qSWPP17, and qPH19-3/qSWPP19) for both plant height and seed weight per plant were located in the same genomic regions on the same chromosomes. Three (qPH2, qPH17, and qPH19-2) and four novel QTL (qSWPP2, qSWPP13, qSWPP17, and qSWPP19) were identified for plant height and seed weight per plant, respectively. Two pairs of QTL (qPH2/qSWPP2 and qPH17/qSWPP17) were also common for both plant height and seed weight per plant. These QTL and closely linked SLAF markers can be used to speed up breeding program for drought tolerant cultivars via MAS in soybean crop (Kumawat *et al.*, 2016, Ren *et al.*, 2020, Deshmukh *et al.*, 2014).

In salt stress tolerance QTL mapping the targeted regions present on chromosomes that govern and salt stress tolerance in soybean. Cheongja 3 and IT162669 cultivars were analyzed and obtained the putative QTL for salt tolerance in soybean. Among the identified QTL, qST6 was detected on chromosome 6, and qST10 was detected on chromosome 10, which control the traits linked to ion toxicity under salinity stress. The region containing the QTL qST6 reported as QTL flood tolerance 4-1, where its blocks on chromosome 12 hold QTL related to iron deficiency chlorosis tolerance: Fe effic 4-3, 8-3, and 11-3 (Cho *et al.*, 2021). A main salt-tolerant QTL enclosed by SSR markers was identified on chromosome 3 by multiple intervals mapping analysis. The candidate gene for this QTL was Glyma03g32900 and would be the gene for salt tolerance in Jidou 12 (Shi *et al.*, 2018).

Soybean cultivars are sensitive to flooding stress and their seed yields are substantially reduced in response to the stress. Sixty recombinant inbred lines

derived from a cross between a relatively tolerant cv. 'Misuzudaizu' and a sensitive cv. 'Moshidou Gong 503' were grown Flooding tolerance was evaluated by dividing the seed weight of the treated plants by that of the control plants. Quantitative trait loci (QTL) analysis using 360 genetic markers revealed three QTLs for flooding tolerance, ft1 to ft3 in 2002. The ft1 (molecular linkage group C2) was reproducible and an additional four QTLs, ft4 to ft7, were found in 2003. A large QTL for days to flowering was consistently observed across treatments and years at a similar position to ft1 (Zhao *et al.*, 2018).

Identifying quantitative trait loci (QTLs) and understanding the inheritance of flooding tolerance will help in developing soybean cultivars with tolerance to flooding. The outcomes suggested that flooding tolerance is a complex trait, which is controlled by multiple QTLs and significantly affected by QTL × environment interactions. The QTL and marker information could be used to assist soybean breeders to develop flooding tolerant cultivars. Wide-ranging research studies using diverse mapping populations helping to identify the putative genomic regions controlling abiotic stress in soybean. Genotyping with molecular markers is a mutual requirement for findings quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS). Combination of these approaches aims to increase recognition power of genomic loci. By the analysis and combination of diverse germplasm followed by phenotyping and genotyping for identification of QTL hot spots for desired traits that can be used in development of new germplasm.

Genome-Wide Association Studies (GWAS) in Soybean

Genome-wide association studies (GWAS) in plants are a powerful tool used for determining the genetic variations associated with specific traits. These studies involve scanning the entire genome of different plant individuals to find genetic markers linked to phenotypic traits, such as disease resistance, yield, and stress tolerance. GWAS has several advantages over traditional gene mapping techniques: like as Higher Resolution, Diverse Populations and GWAS has been effectively applied to a wide range of plants, including both model species and crops (Alseekh *et al.*, 2021). These studies have led to significant discoveries in understanding plant genetics and improving crop breeding programs.

In contrast, to the genome-wide association study (GWAS) approach this provides opportunities to explore the tremendous allelic diversity existing in natural soybean germplasm. Mapping resolution of GWAS has been accumulated in the germplasm during evolution. GWAS is routinely being used in many plant species. GWAS in soybean is lagging behind compared to maize, mostly because of the slow linkage disequilibrium (LD) decay. GWAS for quantitative traits like abiotic stress tolerance are predictable to be affected by a confounding population. Recently, GWAS for *Sclerotinia sclerotiorum* resistance was performed using 7864 SNPs in soybean (Bastien *et al.*, 2014). In Chinese soybean cultivars 15 SNPs identified for germinating seed drought-tolerance by using of mixed-linear model of GWAS. It is notably, that three of these SNPs were commonly associated with two drought-tolerance indices. Two of these SNPs are positioned upstream of genes, and 11 of them are located in or near regions where QTLs (Liu *et al.*, 2020). 45 SNPs Salt tolerant identified by GWAS in soybean out of 45 SNPs nine genomic regions on chromosomes (Chr.) 2, 3, 7, 8, 10, 13, 14, 16, and 20 were significantly associated with both leaf chloride concentrations and leaf chlorophyll concentrations. The other significant SNPs represent seven putative novel QTLs for salt tolerance (Zeng *et al.*, 2017).

GWAS was performed with two contrasting models for Flood tolerance prospective in soybean, the Mixed Linear Model (MLM) and Multi-Locus Random-SNP-Effect Mixed Linear Model (mrMLM) for identify significant SNPs associated with electrical conductivity (EC), germination rate (GR), shoot length (ShL), and root length (RL) traits at germination stage in soybean. With MLM, a total of 20, 40, 4, and 9 SNPs associated with EC, GR, ShL and RL, respectively, whereas in the same order mrMLM detected 27, 17, 13, and 18 SNPs. Amongst these SNPs, two major SNPs, Gm_08_11971416, and Gm_08_46239716 were initiate to be consistently connected with seed-flooding tolerance related traits, namely EC and GR across two environments. Two SNPs, Gm_05_1000479 and Gm_01_53535790 linked to ShL and RL, respectively. These all SNPs associated with flood tolerance ability in soybean crop (Sharmin *et al.*, 2021). To improve the root trait in soybean genome-wide association study (GWAS) technology is used to determine the phenotypic relationships among root traits, elucidate the genetic bases, and identify significant SNPs associated with root trait-controlling genomic loci. A total of 112 significant SNP loci were detected for seven root traits, and identified 55 putative candidate genes considered to be the most promising (Kim *et al.*, 2023).

Transgenic and Genome Editing Approaches for Manage Abiotic Stress in Soybean

The transgenic and genome editing approaches are pivotal in managing abiotic stress in soybean, improving its resilience to environmental challenges such as drought, salinity, heat, and cold etc. Transgenic approaches contain introducing the foreign genes into the soybean genome to make plant stress tolerance. Transgenic plants have been developed by altering the expression of different genes responsible for a specific trait via different transformation methods. The major transgenic crops adopted by GMO-growing countries in 2019 were soybean, maize, cotton, and canola. According to the International Service for the Acquisition of Agro-Biotech Applications (International Service for the Acquisition of Agri-biotech Applications, 2019), the United States, Brazil, Argentina, Canada, and India are the top five countries that grow genetically engineered crops.

In these transgenic crops, genes often encode for proteins which help the plant manage against stress conditions. For example: Overexpression of stress-responsive genes like DREB (Dehydration-Responsive Element-Binding) and HSP (Heat Shock Proteins) are introduced to improve drought and heat tolerance. Genes encoding for enzymes like superoxide dismutase (SOD) and catalase (CAT) help in mitigating oxidative stress caused by abiotic factors (Esmaeili *et al.*, 2022). Genome editing tools using Zinc Fingers, TALEN and most advances CRISPR/Cas9 technology, allows exact modifications in the soybean genome to enhance stress tolerance, CRISPR/Cas9 tool can knock out or modify specific genes involved in stress responses. For example, editing of genes related to ABA (abscisic acid) signaling can develop drought tolerance ability in soybean (Ghosh and Dey, 2022).

These advanced techniques allow for more exact and efficient modifications, potentially leading to the development of soybean varieties with enhanced tolerance against multiple types of stresses (Ghosh and Dey, 2022). Combining of these both transgenic and genome editing approaches can lead to synergistic effects, providing robust results for handling abiotic stress in soybean. This unified approach can hasten the progress of robust soybean varieties, confirming sustainable agricultural productivity.

Transgenic approaches*:* In this overexpression of stress-responsive genes approaches, researchers are exploring transgenic methods. They aim to boost soybean crops' tolerance to abiotic stress by overexpressing stress-responsive genes. This may improve the crops' growth and yield. overexpression of AtNHX1 orthologs such as AtNHX5, OsNHX1, MdNHX1, TaNHX2, PgNHX1, and LeNHX2 also improved salt tolerance in crops including rice, eggplant, soybean, apple, and tomato. To improved salt stress tolerance in soybean transgenic plants overexpression of chloride channel protein genes GmCLC1 and GsCLC-c2 were identified (Wei *et al.*, 2016, Wei *et al.*, 2019), Overexpression of the dehydration responsive element binding protein (DREB) genes, GmDREB6 and OsDREB2A in soybean (Mishra *et al.*, 2024c, Nguyen *et al.*, 2019). In transgenic soybean overexpressing the proline biosynthetic gene P5CS that encodes the Δ′-pyrroline-5-carboxylate synthetase (P5CS) demonstrated increased salt tolerance with higher proline content (Zhang *et al.*, 2015). AtNCED3 in soybean (Molinari *et al.*, 2020) significantly improved drought tolerance in transgenic plants. The drought-responsive element (DRE)-binding proteins such as DREB1 and DREB2 are transcription factors that bind to the promoter region of dehydration-responsive genes such as RD29A and induce their expression in response to environmental stresses, including drought. The heat shock transcription factor gene, GmHsfA1, in soybean increased heat stress tolerance in transgenic plants.

Genome Editing Approaches: Genome editing includes the insertion, deletion, or modification of genomic DNA to improve a gene or to obtain a desirable trait (Gaj *et al.*, 2016). CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9), also identified as CRISPR-Cas9 is a commonly used for gene editing technique. In this technology a guide RNA (gRNA) is used, which guides Cas9 endonuclease towards the target DNA sequence, where Cas9 makes double-strand DNA breaks, inducing a DNA repair process during which particular genetic modifications occurs. CRISPR is an advanced genetic editing tool with transformative potential in agriculture, with considerable benefits for farmers, society, and the world (Ndudzo *et al.*, 2024). CRISPR technology also enables enhanced crop quality and have several benefits to using CRISPR in genome editing within the agriculture field, including: enhance beneficial traits in crops is now indispensable because of food insecurity due to increasing global population, climate change, increasing plant yield, quality, disease resistance and herbicide resistance, breeding and accelerated domestication. (CRISPR)/CRISPR-associated (Cas) technology, have been used for studying soybean genetics and commercial trait development (Xu *et al.*, 2020).

It was reported that the targeted mutation of GmAITR in soybean via the CRISPR/Cas9 gene editing strategy leads to improved salinity tolerance in mutants grown in the field. HD-Zip I genes in soybean. Here, a homeodomain-leucine zipper gene designated GmHdz4 was isolated. Chimeric soybean plants, GmHdz4 overexpressing (GmHdz4-oe), and gene-editing via CRISPR/Cas9 (gmhdz4) in hairy roots, HD-Zip gene plays a crucial role in plant development, secondary metabolism, and abiotic stress responses (Zhong *et al.*, 2022). Phospholipases A (PLAs) in plants plays a vital role in growth and development, as well as in response to biotic and abiotic stresses. The two paralog genes, GmpPLA-IIε & GmpPLA-IIζ, were knock-out by CRISPR/Cas9 technology to study their modulating role in soybean response to multiple abiotic stresses (Xiao *et al.*, 2021).

Conclusion

Biotechnological tools offer powerful solutions for managing biotic and abiotic stresses in soybean crops. By assimilating genetic engineering, marker-assisted selection, QTL mapping and GWAS technologies, researchers and farmers can develop resilient soybean varieties that thrive under diverse environmental conditions. These advancements not only enhance crop productivity but also contribute to sustainable agricultural practices. This review discusses the use of biotechnological tools to manage biotic and abiotic stresses in soybean crops. Soybean is an important crop globally, providing essential high-quality protein and oils, but its production is greatly affected by various biotic and abiotic stresses. This document explores the application of genetic engineering, marker-assisted selection (MAS), and genome editing techniques to develop stress-tolerant soybean varieties. For biotic stress management, the document discusses genetic transformation, RNA interference (RNAi), and MAS. Genetic transformation has been used to improve soybean's resistance to pests, diseases, and herbicides. RNAi technology has been exploited to control insect pests, and MAS has been effectively used to develop soybean lines resistant to soybean cyst nematode. For abiotic stress management, the document covers quantitative trait loci (QTL) mapping, genome-wide association studies (GWAS), and transgenic and genome editing approaches. QTL mapping and GWAS have been used to identify genomic regions associated with traits like drought tolerance, salt tolerance, and flooding tolerance. Transgenic and genome editing techniques, such as overexpression of stress-responsive genes and CRISPR-Cas9 editing, have been employed to enhance soybean's tolerance to various abiotic stresses. We discuss the highlights the potential of these biotechnological tools in improving soybean's resilience to environmental challenges, ensuring sustainable agricultural practices and food security.

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