REVIEW

The Problem of Charcoal Rot in Soybean, its Implications, and Approaches for Developing Resistant Varieties

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ABSTRACT: Soybean is an annual legume with edible seeds. The soybean’s charcoal rot is one of the serious challenges faced in its cultivation regions, which brings severe production and economic losses. Charcoal rot is the result of infection by the soil-borne fungus Macrophomina phaseolina. Though several researchers have made efforts to deal with soybean’s charcoal rot challenge, at present, there are no soybean varieties in the market that are resistant to charcoal rot. The pathogen is thought to infect plants in their roots from contaminated soil, using unknown toxin-mediated processes. Conventional integrated approaches for managing charcoal rot in soybeans have been implemented in the field, but their efficacy is limited. So, developing soybean durable resistant varieties against M. phaseolina is the only solution to rescuing this crop. The potential approach is identifying new genetic sources and quantitative trait loci (QTLs) associated with resistance to charcoal rot in the resistant soybean population and conducting genome-wide association studies to increase understanding of underlying resistance mechanisms. The discovery of the genetic markers associated with resistance will contribute to charcoal rot resistance genotype selection for breeding programs in the future.

KEYWORDS: Macrophomina phaseolina, genomics, charcoal rot, QTLs, genome selection

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1. Introduction

Soybean, scientifically known as Glycine max (L.) Merrill belongs to the family Leguminosae and the subfamily Papilionoidae (Pratap et al., 2012). It is believed that soybeans originated in Eastern Asia, namely in north and central China. It is widely accepted that the introduction of cultivated soybean cultivars to Korea and Japan occurred around 2000 years ago (Badole & Bodhankar, 2012). It has been reported that soybeans, as oil seeds, contain numerous essential components, such as protein, carbs, vitamins, and minerals. Dry
soybeans comprise 36% protein, 19% oil, 35% carbohydrates (with 17% dietary fiber), 5% minerals, and several additional constituents, such as vitamins (Liu & Liu, 1997). Soybean vegetable oil is a byproduct derived from the processing of soybean crops, which is extensively utilized in several industrial sectors. This oil comprises around 15.65% saturated fatty acids, 22.78% monounsaturated fatty acids, and 57.74% polyunsaturated fatty acids, including 7% linolenic acid and 54% linoleic acid (Wolke, 2007). In addition, soybeans possess a variety of bioactive chemicals (Figure 1), including isoflavones, peptides, flavonoids, phytic acid, soy lipids, soy phytoalexins, soyasaponins, lectins, hemagglutinin, soy toxins, and vitamins that exhibit numerous advantageous impacts on the health of both animals and humans (Young, 1991). Various commercially available non-fermented soy-based food products include soy milk, infant formulae, tofu (soybean curd), soy sauce, soybean cake, and tempeh are some of the main products (Kwon et al., 2010). Soybean derivatives have been found to have several beneficial impacts on human health, including but not limited to hypercholesterolemia, cardiovascular health, osteoporosis, menopause, hypotensive activity, insulin secretion, energy metabolism, blood pressure, endothelial function, platelet aggregation, and fibrinolytic activity (Ahn et al., 2000; Gilani & Anderson, 2002; Messina et al., 2006; Peterson & Barnes, 1991; C.-L. Sun et al., 2002). Although soybean eating positively impacts animal and human health, but some studies have reported few contentious effects. Soybean is known to possess various naturally occurring compounds that exhibit toxicity towards both humans and animals. These compounds include trypsin, a serine protease commonly found in digestive system inhibitors, phytic acid, toxic constituents like lectins and hemagglutinins, certain metalloproteins such as soyatoxin, and numerous other biologically active compounds associated with soyatoxin (Hogervorst et al., 2008; Sastry & Murray, 1987; Smith, 1977). So, it is imperative to conduct further comprehensive research to fully elucidate soybean consumption's positive and negative effects on human and animal health to make its use trustworthy.

Figure 1. Soyabean, a rich source of diverse bioactive molecules with nutritional and medicinal benefits.

2. Soybean global cultivation and production
Soybean is the king of beans, supplying a significant amount of the direct and indirect protein consumed globally. Soybean production has had a 15-fold rise since the 1950s and has transferred from Asia to the
United States, Brazil, and Argentina. These countries currently represent 80% of worldwide soybean production (Hannah Ritchie, 2021). The worldwide demand for soybeans is increasing, primarily driven by consumer desire for protein alternatives derived from animals and plants (Fraanje & Garnett, 2020). A significant number of people worldwide are employed in the soybean industry. The cultivation of soybean in the United States alone generates direct employment opportunities for over 280,000 farmers, while in 2016, nearly 240,000 farms in Brazil were engaged in soybean production (Bicudo et al., 2020). However, the global soybean production in 2021 exceeded initial estimates and reached 388,098 metric tons (Mt) (FAOSTAT, 2023). From 2016 onwards, Brazil, the United States of America, and Argentina have emerged as the leading nations in soybean exports, with respective export volumes of 86.1 Mt, 53.1 Mt, and 4.3 Mt in 2021. Conversely, China and the European Union have consistently maintained their position as the primary importers, importing approximately 95.5 Mt, 14.6 Mt, and 4.9 Mt in the same year (UNEC, 2022).

The International Grain Council (IGC) research indicates that the global soybean trade significantly increased from 156 million tons in the 2021/22 season to 172 million tons in the 2022/23 season. According to the IGC, the trade is projected to maintain a level of approximately 168 million tons throughout the 2023/24 season. According to the USDA's December 2023 report, soybean trade is projected to increase from 171 million tons in the 2022/23 season to around 170 million tons in the 2023/24 season. The global soybean trade ranking remained relatively stable in comparison to previous years. The leading producers also excel in terms of exports. In the 2022/23 season, Brazil, the USA, Argentina, Paraguay, and Canada collectively represented over 95% of global soybean exports. Brazil's export volume, which reached 95.5 million tons in the previous season, is projected to rise to 99.5 million tons in the coming season. During the same time frame, it is projected that the total exports of the United States of America will decline by 6.5 million tons, reaching a total of 47.7 million tons. China, the European Union, and Mexico are notable for their significant imports. China is projected to import 102 million tons of soybean in the 2023/24 season, making it the top importer with 100.8 million tons in the 2022/23 season. The European Union countries will maintain imports of approximately 13 million tons, while Mexico will continue to import around 6 million tons (IGC, 2023).

Soybean in Brazil holds a prominent position in the agricultural landscape, encompassing their cultivation area and production levels; however, this has not always been the case. In 1960, soybean cultivation was considered a subordinate crop, with production much lower than that of the United States of America (USA), which held the top position as the largest grower, and China, which ranked second. The United States of America remains the primary producer, with Brazil closely trailing after and Argentina securing the third position (Cattelan & Dall’Agnol, 2018). Soybean is typically sown in October in Brazil. However, soybeans are sown in the center-west region, particularly in Mato Grosso, as early as September. The harvesting of soybean takes...
place from early January to late April. This is often followed by the cultivation of a second crop of maize, which is planted in January-February (CONAB, 2022). The soybean supply chain has been critical in generating foreign exchange for the country to increase Brazilian international commercial trade. In half a century of cultivation, the area of soybean planted exceeded 36 million hectares, and its production for the 2020 harvest was over 122 million tons (Conab & das Safras, 2021). According to the Ministry of agriculture data, the gross value of agricultural production in 2019 reached more than BRL 600 billion (Mapa & e Abastecimento). For example, the national harvested area of soybean in 2020 was more than 37 million hectares (ha), higher than that of the United States of America, with approximately 33 million ha (Food & Nations, 2022). Between 2021 and 2050, Brazilian soybean production is expected to increase by 12 million hectares, with over 11 million hectares of newly planted acreage in the Cerrado expected to boost output by 27% during the next ten years (Alcock, Salt, Wilson, & Ramsden, 2022).

3. Soybean’s charcoal rot challenge
The soil-borne fungus *Macrophomina phaseolina* (*M. phaseolina*), belonging to the family Botryosphaeriaceae, is the source of charcoal rot disease, significantly lowering soybean yields (Tripathi et al., 2022). *M. phaseolina* is responsible for causing several pathological conditions in plants, including damping off, seedling blight, stem rot, charcoal rot, collar rot, basal stem rot, and root rot (Marquez, Giachero, Declerck, & Ducasse, 2021). The disease is called charcoal rot (CR) because of the large amount of minute black microsclerotia that turns the afflicted plant tissues black (Khan et al., 2022; Sarr et al., 2014). When charcoal rot was discovered for the first time in the USA in 1949, it was thought that two toxins, phaseolina and botryodiplodin, were to blame for the infection that *M. phaseolina* causes in crops (Ramezani et al., 2007). In the first step, *M. phaseolina* grows and multiplies under suitable environmental conditions in host plants, infecting the host's vascular system. In the second step, it blocks the flow of nutrients and water to the leaves, producing disease symptoms and causing early leaf loss (Gupta & Chauhan, 2005). After crop harvest, microsclerotia return to the soil and can survive for at least two years (Reis, Boaretto, & Danelli, 2014). Soybean crops have shown limited resistance to *M. phaseolina* (Coser et al., 2017). Breeding soybean cultivars resistant to charcoal rot is difficult because of polygenic inheritance (Coser et al., 2017). *M. phaseolina* is transmitted through the soil and infects over 500 plant species in more than 100 families; important host species majorly including soybean, peanut, cabbage, pepper, chickpea, sunflower, sweet potato, alfalfa, sesame, potato, sorghum, wheat, and corn (Kaur, Dhillon, Brar, & Chauhan, 2012). Since there are significant intraspecific differences in the phenotype of the isolates, the identification of *M. phaseolina* isolates is generally based on morphological variation, and they have been split into subspecies; however, morphological criteria are sometimes unreliable (Babu et al., 2007; Khan et al., 2022). The *M. phaseolina* impedes the movement of water and nutrients
to the upper sections of the plant by altering the fibrovascular system of its host's roots and basal internodes. Consequently, the manifestation of *M. phaseolina* infection is characterized by increasing wilting of plants, premature mortality, diminished vitality, and decreased crop productivity (S. N. Khan, 2007; Kumar & Dubey, 2023).

The *M. phaseolina* causes brown lesions on the hypocotyls or developing seedlings. Numerous other symptoms manifest during or after flowering, such as grey staining of the stem and taproots, shredding of plant tissue in the stem and top of the taproot, and hollowing of the stem (Fig. 2) (Sassenrath et al., 2019; Shoaib et al., 2022; Šućur Elez et al., 2023).

4. **Resistance to charcoal rot in soybean**

The precise mechanism used by *M. phaseolina* to invade plants remains undisclosed. However, there have been reports indicating that this fungus generates specific mycotoxins that could contribute to the infection of plants in their roots. The production of toxins in *M. phaseolina* depends on the isolate's origin because each isolate can create distinct toxins (Khambhati et al., 2023; Khambhati et al., 2020). An integrated management strategy incorporating crop rotation, tillage, irrigation, and seed treatments with fungicides and biological control is used to mitigate charcoal rot damage in soybeans (Twizeyimana et al., 2012).

However, these measures have not proven effective or generally embraced, and their ability to provide effective control has been limited (Hartman et al., 2015). Genetic resistance is the most practical and long-lasting approach to controlling charcoal rot.
(Mengistu et al., 2007). No plant species have been found to have complete resistance to *M. phaseolina*; however, partial resistance has been reported in soybeans, including moderately resistant cultivars like DT97-4290, which is used as a standard for checking the disease (Paris, Mengistu, Tyler, & Smith, 2006; Pawlowski, Hill, & Hartman, 2015; Twizeyimana et al., 2012). Nevertheless, extensive research has been lacking on the commercial germplasm and its overall resistance to the fungus. There is now no available control measure technique that is effective and economically feasible for managing charcoal rot under field situations.  

5. Genetic mapping and genomic selection

Finding genes inside a genome is known as "gene mapping." Scientists are typically very interested in the parts of a genome that directly encode proteins or protein-coding genes. Therefore, the location of every gene in a genome is frequently a top priority. Screening various genetic materials, such as pre-breeding lines, germplasms, and accessions, is highly significant in the quest for disease-resistance sources in agricultural fields and laboratory experiments. Several research organizations have made multiple attempts to evaluate soybean germplasm for the presence of resistance to fungal diseases. In a recent study, Nataraj et al. (Nataraj et al., 2020) assessed 225 different varieties of soybean and discovered five genotypes, namely EC 538828, EC 34372, EC 457254, AKSS 67, and Karune, that displayed a high level of resistance. This study also examined the genetic basis of anthracnose resistance in three F2 populations derived from the resistant parents EC 34372 × J.S. 95-60, EC 457254 × J.S. 95-60, and AKSS 67 J.S. 95-60. The results demonstrated that the resistance in all three resistant parents was governed by the interaction of two crucial genes in a complementary fashion. A study conducted by Sajeesh et al. (Sajeesh, Rao, & Shamarao, 2014) found that DSb 12 is a genotype resistant to anthracnose. Individuals originating from inter- or intraspecific crossing between two parents make up biparental mapping populations. These recombinant lines are mostly employed as pre-breeding sources for agricultural development, and they represent a powerful method for evaluating the genetic foundations of intricate crop attributes (Tripodi, 2021). Recently, Chanchu et al. (Chanchu et al., 2022) evaluated a recombinant inbred line (RIL) population consisting of 108 lines derived from a hybrid between a susceptible cultivar Sukhothai 2 (SKT2) and CM5 and revealed a single quantitative trait loci (QTL), qSBR18.1, for SBR resistance. Several researchers have used marker-assisted breeding to map soybeans' brown stem rot (BSR) resistance genes. Using 320 recombinant inbred lines (RIL) derived from a hybrid between (BSR) 101 and PI 437.654, Lewers et al. (Lewers et al., 1999) first mapped the Rbs3 gene. Klos et al. (Klos et al., 2000) used SSR markers to confirm the same study. Bachman et al. (2001) mapped the Rbs1 and Rbs2 genes on soybean chromosome 16 using SSR markers in a subsequent investigation. Furthermore, Perez et al. (2010) have discovered a few new origins of BSR resistance.

Genome-wide association studies link genetic variants throughout the genome to specific features, such as disease resistance, which are valuable for identifying key
genetic variations associated with phenotypes. These genome-wide association studies utilize markers with high density and a population of diverse individuals to achieve higher mapping accuracy than traditional methods. They help to predict or identify potential causal genes and reduce the time and cost required for the genetic analysis of traits (Iquira et al., 2015; Korte & Farlow, 2013; Song et al., 2013; J. Sun et al., 2014; Zhang et al., 2015). Genome-wide analysis has been used in soybeans to find genes linked to resistance against Phytophthora root rot, soybean cyst nematode, iron deficiency chlorosis, sudden death syndrome Sclerotinia stem rot, and soybean aphid (Bastien, Sonah, & Belzile, 2014; Mamidi, Lee, Goos, & McClean, 2014; Vuong et al., 2015; Zhao et al., 2015).

Genomic selection (GS) is a selection strategy that uses genetics to predict quantitative features. It is an alternative to marker-assisted selection. Genomic selection employs genetic and phenotypic data from a well-defined group of plant materials, known as the training set (TS), to predict the phenotypic values of upcoming plant materials, referred to as the prediction set (PS), using their genetic information. These connections are subsequently utilized to calculate a genomic estimated breeding value (GEBV) for each population structure (PS) individual. GS techniques often operate under the assumption that several genes influence the trait being studied, each having a minor impact. By assuming quantitative genetic control, GS models can accurately predict features that are complicated and highly quantitative (Heffner, Sorrells, & Jannink, 2009; Lorenz et al., 2011; Melchinger, Utz, & Schön, 1998; Meuwissen, Hayes, & Goddard, 2001)

6. Soybean resistant varieties development

The efficacy of fungicide treatment and biological control techniques in managing charcoal rot has been found to be limited. Therefore, cultivating soybean genotypes with high resistance against charcoal rot is the most effective and environmentally friendly approach to safeguarding optimal crop yields and seed quality. Nevertheless, there is currently a lack of soybean cultivars that possess resistance and are commercially available (Bellaloui et al., 2023). Combining QTL mapping with molecular marker-assisted selection is useful for characterizing complicated characteristics.

Resistant cultivars possess either vertical resistance, horizontal resistance, or both. Resistance genes (RG) for various diseases contribute to vertical resistance. R genes have been extensively utilized to provide absolute resistance against certain pathotypes of the disease. The R genes normally engage in a gene-for-gene interaction with the pathogen's equivalent avirulence (Avr) factors. Resistance is only achieved when the R gene and Avr factors are present (Whitham et al., 2016). Thus, R genes exhibit pathotype (race)-specificity, meaning they can provide total protection against certain pathotypes of the disease while being entirely susceptible to others. R genes are frequently not long-lasting and can be rapidly overcome due to the rapid change in pathogen populations. The recognition of peronospora parasitica genes (Rpp1 and Rpp3), which provided resistance to soybean rust, was overcome the year after the disease was first observed in Brazil in 2001 (Garcia et al., 2008;
Langenbach et al., 2016). In order to implement vertical resistance and ensure the long-term viability of the global soybean value chain, it is crucial to seek out new sources of resistant genes.

On the other hand, horizontal resistance, also known as partial resistance or tolerance, is a quantitative feature determined by several genes with minor effects and QTL. QTL is a statistical technique that connects phenotypic and genotypic data to identify the genetic factors underlying variation in complex traits. This method involves analyzing molecular markers (such as SNPs or microsatellites) in the species of interest to pinpoint specific locations in the genome that influence the phenotype of interest. Horizontal resistance, unlike vertical resistance, is limited to specific pathogens, is a type of resistance that is broadly effective against many soybean diseases. It is the only form of resistance that protects against various soybean diseases such as sudden death syndrome (SDS), Sclerotinia stem rot, root-knot nematode, and most Pythium species. Horizontal resistance in soybeans is typically regarded as non-specific to pathotypes, as shown by several studies. However, certain isolate-specific QTLs have also been discovered in soybeans, and as a result, horizontal resistance is regarded as longer-lasting (Karhoff et al., 2019; Lin et al., 2021; Mundt, 2014).

The conventional process of transferring resistance genes into susceptible cultivars can take over a decade, commencing with crossbreeding the recurrent and resistant donor parents. Fortunately, the advancement of molecular marker technology, particularly the sequencing of the soybean genome and the creation of cost-effective high-throughput genotyping methods like the BARCSoySNP6K and BARCSoySNP50K iSelect Bead-Chips, enables breeders to make selections with greater efficiency and precision (Song et al., 2013; Song et al., 2020). MAS has been demonstrated as the most effective method for selecting R genes or significant QTLs. The markers utilized for MAS have progressed from the low-efficiency restriction fragment length polymorphism (RFLP) markers to simple sequence repeat (SSR) markers and, presently, to more effective and cost-effective single nucleotide polymorphism (SNP) markers in contemporary soybean breeding programs. Nevertheless, studies have shown that GS surpasses marker- MAS in terms of accuracy and efficiency when it comes to minor effects on QTLs. An experiment was conducted where 282 soybean accessions were genotyped to determine their resistance to soybean cyst nematode (SCN) Heterodera glycines (HG) type 0. The results showed that employing a complete marker set for GS yielded much more accurate predictions than using only two rhg1-associated DNA markers for MAS. Another study examining soybean resistance to white mold found that the GS prediction accuracy was assessed to be 0.64, much more than that of MAS (0.47–0.51). However, MAS still showed a 24–26% improvement compared to using random SNPs (Bao et al., 2014; Ribaut & Hoisington, 1998). In addition, the advancement of new technologies like Genome-Wide Association studies (GWAS) has led to the discovery of several SNP markers that confer resistance to different diseases in soybeans, which hold the
potential for future deployment. Genome-editing technology, such as CRISPR/Cas9, enables plant breeders to precisely adjust gene regulation to enhance crop resilience to different diseases (Chang et al., 2016; Chen, Wang et al., 2019; Moellers et al., 2017; Vuong et al., 2015; Wen et al., 2014).

It is feasible to create disease-resistant cultivars by genetic engineering or conventional breeding techniques by incorporating resistance mechanisms derived from other plant species or pathogens (L. Sun et al., 2019).

Figure 3. Depiction of many approaches, incorporating cutting-edge breeding techniques, to create new soybean varieties with improved resistance to fungal diseases.
Traditional breeding methods can introduce natural resistance to plants without using genetic modification. One such approach is DNA-based MAS, which may involve multiple breeding cycles to combine disease resistance and desirable agronomic traits in a single plant genotype (Hammond-Kosack & Parker, 2003).

Progress in next-generation sequencing (NGS) and multi-omics technologies have enabled the acquisition of comprehensive genome sequence data, which can accelerate the discovery of several molecular markers employed in QTL mapping and GWAS (Roychowdhury et al., 2023). This data would be valuable for investigating the intricate genetic pathways involved in disease resistance. This allows for the identification of potential genes that are linked to features of disease resistance and their corresponding pathways. The found genes may be used in genetic engineering to create transgenic plants that display improved resistance, a crucial aspect of disease control. The term "transcriptome" refers to the entirety of ribonucleic acid (RNA) molecules found within an organism or a specific type of cell. This includes a wide range of RNA types, such as protein-coding messenger RNA (mRNA), non-coding RNAs like transfer RNA (tRNA), long non-coding RNA (lncRNA), ribosomal RNA (rRNA), primary microRNA (pri-miRNA), and small nuclear RNA (snRNA) (Amaro et al., 2016; Khan et al., 2021). Metabolomics is a scientific investigation of naturally occurring small, low-molecular-weight metabolites, including carbohydrates, fatty acids, amino acids, steroids, and lipids. These metabolites have specific functions in deciphering cellular biochemistry (Khan et al., 2023). Proteomics is a field of study that investigates the complete range of protein characteristics, including composition, structure, expression, modification status, connections, and interactions (Khan et al., 2022). Moreover, genome editing methods like CRISPR/Cas9 systems can swiftly enhance intricate disease-resistance characteristics by modifying either the genes responsible for vulnerability or the pathogen's genetic makeup (Mushtaq et al., 2019; Schäfer, 1994).

Nevertheless, the use of genome editing technologies to enhance fungal disease resistance in soybean plants is now in its nascent phase. Given their greater specificity, simplicity, and ability to be replicated, these techniques have immense promise to improve resistance to disease in soybean crops in the future. Moreover, novel techniques like host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) have developed as viable methods for crop protection that are both sustainable and eco-friendly (González et al., 2013). These methods use the capacity of dsRNA to suppress the expression of specific genes in plant diseases. Specifically, because SIGS does not need genetic modification, it may be effectively used for safeguarding soybean plants (Sciences et al., 2016). The integration of HIGS with the CRISPR/Cas9 technique will augment the ability of soybean plants to fight fungal diseases (McLaughlin et al., 2023). A large number of breeding techniques used to enhance fungal disease resistance soybean in crops is available currently (Figure 3).

7. Conclusion and Future Aspects
Genetic resistance is an extremely efficient and enduring method for managing diseases in soybeans, worldwide. It plays a crucial role in strengthening the global soybean value chain and ensuring food security. While several genomic areas that provide resistance to diverse infections have been documented in the literature, there are still many aspects of genetic resistance that have to be elucidated and continually studied.

Significant progress has been made in comprehending the dynamics of pathogen infections and the genetic controls that underlie them. The significant change and appearance of new and resistant strains and the establishment of pathogen races pose a danger to previously confirmed resistance genes.

Furthermore, the significance of resistance genes' pleiotropic effect and their interaction in terms of long-lasting, comprehensive resistance levels, yield cost, and environmental interactions is now of utmost importance due to the accessibility of large genomic data and the development of sophisticated analyzing algorithms.

Whole genome resequencing enabled the identification and analysis of many lines with exceptional haplotypes or alleles inside unknown germplasm. These findings may be used to implement long-lasting resilience in plant breeding programs.

Genomics-assisted breeding (GAB) is expected to dominate future breeding practices. This approach encompasses many techniques, such as marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MABC), haplotype-based breeding, and GS. NGS-based trait mapping and systems biology techniques would be used to map genes linked with traits.

However, the achievement of successful development of CRISPR/Cas9 transformants relies on the presence of an efficient genetic transformation mechanism. Regrettably, soybean poses challenges for plant transformation technology, making it difficult to engineer genetically. Furthermore, the majority of genetic engineering research on soybean is still in the early stages of development.

In summary, the early development of soybean research, the abundance of untapped genetic diversity in soybean accessions, the progress made in genomics, and analytics, the dynamic nature of the environment, pathogens, and host genetic background will greatly enhance the effectiveness and precision of global soybean breeding in the coming decades. This will guarantee the long-term viability and expansion of the cultivation of soybeans globally.

**Author Contribution:**
Q.K conceptualization, wrote the manuscript and contributed to all aspects of the paper. Q.Y review and suggestions. D.J.G review and editing. The Authors have read and approved the final manuscript for publication.

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