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Biotechnological Strategies for Enhancing Yield, Stress Resistance, and Nutritional Quality in Black Gram *(Vigna mungo L. Hepper)*

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Abstract: Black gram (Vigna mungo L. Hepper) is a very significant pulse crop that is produced a lot in South Asia. It is valued for its high protein content, essential amino acids, and health benefits. Black gram output is still low, even though it is important for food and nutrition security. This is because it is sensitive to biotic and abiotic stresses, there isn't much genetic variability, and conventional breeding methods have their own problems. The tissue culture technique has become a practical biotechnological approach for overcoming these limitations and accelerating crop growth. Cotyledonary nodes, shoot tips, hypocotyls, and cotyledons have been successfully utilised to establish in vitro regeneration systems for black gram. Cotyledonary node explants have exhibited significant regeneration potential when cultivated on Murashige and Skoog (MS) media enriched with suitable concentrations of cytokinin's, including 6-benzylaminopurine (BAP) and kinetin, frequently in conjunction with auxins such as naphthalene acetic acid. These regeneration methods are great for micropropagation, genetic transformation, and quickly multiplying the best genotypes. Tissue culture techniques may also assist create superior cultivars that can better handle biotic stresses like virus infections and insect pests, as well as abiotic stresses like drought and salt. Also, methods based on tissue culture make it possible to save germplasm and create soma clonal variation, which may subsequently be utilised to choose superior plant lines with better nutritional and agronomic qualities. Using tissue culture, contemporary molecular breeding methods, and genome editing technology together provides a lot of potential for making black gram varieties that yield a lot, can handle stress, and are better for you. Consequently, tissue culture techniques are essential elements of contemporary crop improvement programs, significantly enhancing sustainable agriculture and food security.

Key Words: Black gram (Vigna mungo L. Hepper), tissue culture, in vitro regeneration, cotyledonary node explants, micropropagation, plant growth regulators, genetic transformation, stress tolerance, somatic variation, nutritional enhancement, and crop improvement are some of the things that come to mind.

1. Introduction

One of the most significant pulse crops grown in India and other regions of South Asia is black gram (Vigna mungo L. Hepper). Because it has a lot of protein, vitamins, minerals, and amino acids, a lot of people eat it. Black gram is not only good for you, but it also helps the earth stay fertile by fixing nitrogen in the soil (Graham and Vance, 2003; Singh et al., 2016).

Even though it is important, black gram doesn't produce as much as other pulses. Mungbean yellow mosaic virus (MYMV), powdery mildew, and insect pests like bruchids are only a few of the biotic stressors that can hurt the crop. These pests cause big production losses in key growing areas (Varma and Malathi, 2003; Basu et al., 2009). At the same time, abiotic stresses, including drought, salinity, and heat stress, also adversely affect crop growth, yield, and seed quality (Singh et al., 2015; Tripathy et al., 2017). Conventional breeding techniques have achieved limited advancements in the development of enhanced black gram varieties; yet, the restricted genetic diversity and prolonged breeding cycles hinder the efficacy of traditional methods (Souframanien and Gopalakrishna, 2004; Gupta et al., 2019).

Recent progress in molecular biology and plant biotechnology has opened up new ways to get around these problems. Techniques including marker-assisted selection, genetic transformation, and genomic analysis are being employed more and more to improve the yield, stress tolerance, and nutritional quality of black gram (Gupta et al., 2013; Souframanien et al., 2016). Molecular markers like SSR, RAPD, and AFLP have been widely used to find trait-linked markers and measure genetic diversity in Vigna mungo. This can help molecular breeding strategies make better cultivars (Gupta and Gopalakrishna, 2010; Souframanien and Gopalakrishna, 2006). In addition, advances in genomics and transcriptomics have enabled researchers to identify candidate genes associated with disease resistance, stress tolerance, and seed quality traits, thereby accelerating crop improvement programs in black gram (Kaushal et al., 2019; Pandiyan et al., 2018).

So, for sustainable crop production, it is important to have a full awareness of the latest advances in genetic improvement and biotechnological treatments in black gram. Many research has shown that new molecular markers, genomics, and current breeding methods can speed up the creation of high-yielding and stress-tolerant varieties of Vigna mungo. Nevertheless, the existing data is dispersed throughout several research and need careful aggregation.

The current study seeks to synthesise and critically evaluate the latest advancements in genetic enhancement, molecular breeding, and biotechnological methodologies in black gram. It emphasises the significance of contemporary molecular instruments, genetic assets, and sophisticated breeding methodologies in improving production, disease resistance, and stress resilience. This review will help researchers, plant breeders, and biotechnologists who are trying to enhance pulse crops and will help them come up with ways to increase black gram output that are good for the environment.

2. Marker-Assisted Selection and Marker-Assisted Backcross Breeding

Molecular marker technologies have significantly improved the efficiency of plant breeding programs by enabling the precise identification of genetic variation associated with important agronomic traits. Marker-assisted selection (MAS) allows breeders to identify and select plants carrying desirable genes at an early developmental stage without relying solely on phenotypic evaluation, thereby increasing the accuracy and speed of crop improvement programs (Collard and Mackill, 2008; Xu and Crouch, 2008).

In black gram (Vigna mungo), molecular markers such as simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and single nucleotide polymorphism (SNP) markers have been widely used to analyze genetic diversity and identify genes associated with disease resistance, yield components, and other agronomic traits (Souframanien and Gopalakrishna, 2004; Gupta and Gopalakrishna, 2010). These molecular tools have significantly contributed to the identification of quantitative trait loci (QTLs) linked to economically important traits in pulse crops. Marker-assisted selection has been particularly effective in transferring resistance genes against Mungbean Yellow Mosaic Disease (MYMD), one of the most devastating viral diseases affecting black gram cultivation across South Asia (Varma and Malathi, 2003; Basu et al., 2009).

Marker-assisted backcross breeding (MABC) is another important strategy used to transfer specific resistance genes from donor parents into elite cultivars while retaining their desirable agronomic characteristics (Hospital, 2005). This method enables breeders to recover the recurrent parent genome rapidly while incorporating target genes for disease resistance or stress tolerance. The integration of MAS and MABC in black gram breeding programs has helped shorten breeding cycles, increase the efficiency of selection, and accelerate the development of improved cultivars with enhanced yield potential and resistance to biotic and abiotic stresses (Gupta et al., 2013; Pandiyan et al., 2018).

3. In Vitro Regeneration and Tissue Culture Techniques

Efficient plant regeneration systems are essential for successful genetic transformation and large-scale propagation of improved cultivars. Tissue culture techniques provide a controlled environment for the regeneration of plants from various explants and play a critical role in modern crop improvement programs. Black gram (Vigna mungo L. Hepper) has traditionally been considered a recalcitrant species in tissue culture due to its genotype-dependent response and low regeneration frequency. However, several studies have developed improved protocols to overcome these limitations and enhance regeneration efficiency (Somers et al., 2003; Popelka et al., 2004).

Various explants such as cotyledonary nodes, hypocotyls, shoot tips, and cotyledons have been successfully used for plant regeneration in black gram. Among these, cotyledonary node explants have shown particularly promising results for multiple shoot induction and regeneration under in vitro conditions (Muruganantham et al., 2007; Ignacimuthu and Franklin, 1999). The application of optimized plant growth regulators, especially cytokinins such as **6-**benzylaminopurine (BAP**)** in combination with auxins like naphthalene acetic acid (NAA**)** or indole-3-butyric acid (IBA), has significantly improved shoot regeneration and root formation in cultured explants (Saini and Jaiwal, 2005; Franklin et al., 2000).

These tissue culture techniques not only facilitate genetic transformation but also enable the rapid multiplication of elite genotypes and the production of disease-free planting material. Furthermore, in vitro regeneration systems provide a platform for advanced biotechnological applications such as somatic embryogenesis, mutation breeding, and genetic engineering in black gram (Sahoo et al., 2016; Tiwari et al., 2018). Therefore, the development of efficient regeneration protocols is a crucial step toward the successful application of modern biotechnology for the improvement of black gram cultivars.

4. Genetic Engineering and Transgenic Approaches

Genetic engineering offers the possibility of introducing novel genes into crops to improve resistance to pests, diseases, and environmental stresses. Unlike conventional breeding methods, genetic transformation allows the direct transfer of specific genes from unrelated organisms into plant genomes, thereby expanding the genetic variability available for crop improvement (Gelvin, 2003; Christou, 2013). In black gram (Vigna mungo L. Hepper), Agrobacterium-mediated transformation has been widely used as an effective method for gene transfer due to its relatively high transformation efficiency and stable gene integration (Somers et al., 2003; Popelka et al., 2004).

Transgenic plants have been developed with genes that confer resistance to insect pests and viral pathogens. For instance, genes encoding Bacillus thuringiensis (BT) toxins have been successfully used in several crops to protect against insect pests, while antiviral genes and pathogen-derived resistance strategies have been explored to combat viral diseases such as mungbean yellow mosaic virus (MYMV) in legumes (Gatehouse, 2008; Varma and Malathi, 2003). Similarly, genes involved in stress tolerance pathways, including those associated with Osmo protectants, transcription factors, and antioxidant systems, can be overexpressed to improve plant resilience against drought, salinity, and temperature stress (Bhatnagar-Mathur et al., 2008; Umezawa et al., 2006).

Although the development of transgenic black gram varieties is still limited due to challenges such as genotype dependence, low transformation efficiency, and regulatory constraints, genetic engineering approaches hold significant promise for future crop improvement programs. The integration of transgenic technologies with molecular breeding and genomics can accelerate the development of high-yielding black gram cultivars with improved resistance to biotic and abiotic stresses (Popelka et al., 2004; Tiwari et al., 2018).

5. Genomics and Transcriptomics in Black Gram Improvement

Recent breakthroughs in genome sequencing technology have substantially sped up research on pulse crops by giving scientists a lot of information about genome structure, gene organisation, and functional elements. Next-generation sequencing (NGS) technologies have made it possible to get high-quality genomic data for a number of legume species. Chromosome-level genome assemblies for black gram (Vigna mungo L. Hepper) have given us important information about gene organisation, genome evolution, and functional genomics. This has helped programs for molecular breeding and crop improvement (Kang et al., 2014; Yang et al., 2015).

Transcriptomic analysis has become an effective method for examining gene expression patterns in response to various biotic and abiotic stressors. RNA sequencing (RNA-Seq) allows for the discovery of differentially expressed genes associated with physiological responses to stressors, including drought, salt, and pathogen infection. These investigations help find genes that are linked to stress tolerance, disease resistance, and features that affect yield. These genes may then be used in programs to enhance crops (Wang et al., 2009; Garg et al., 2016).

Molecular markers, quantitative trait loci (QTLs), and gene expression databases are examples of genomic resources that are useful for current breeding tactics. These tools make it easier to find genomic areas that are linked to critical agronomic features and make it possible to use marker-assisted selection (MAS) and genomic-assisted breeding. The combination of genomics, transcriptomics, and bioinformatics has made breeding operations much more effective in creating black gram varieties that produce a lot of food and can handle stress (Varshney et al., 2013; Pandiyan et al., 2018).

6. Biofortification and Nutritional Enhancement

Black gram (Vigna mungo L. Hepper) is widely recognized as a rich source of plant protein and essential nutrients, making it an important component of the human diet, particularly in South Asian countries. The seeds contain significant amounts of protein, carbohydrates, dietary fiber, vitamins, and minerals. However, there is increasing interest in further improving its micronutrient composition and health-promoting compounds to address global nutritional challenges (Graham et al., 2007; Bouis and Welch, 2010).

Biofortification strategies aim to increase the concentration of essential micronutrients such as iron (Fe) and zinc (Zn) in edible plant parts through breeding and biotechnological approaches. These strategies are particularly important in combating micronutrient deficiencies, often referred to as "hidden hunger," which affects millions of people in developing countries (White and Broadley, 2009; Saltzman et al., 2013). In black gram, conventional breeding combined with molecular breeding techniques is being explored to develop nutrient-rich varieties with improved mineral content.

Biotechnological approaches, including marker-assisted selection, genomics-assisted breeding, and genetic engineering, are being utilized to enhance micronutrient accumulation in black gram seeds. These techniques enable the identification and manipulation of genes involved in nutrient uptake, transport, and storage within plant tissues (Velu et al., 2014; Garg et al., 2018). In addition to mineral biofortification, research efforts are also focused on improving the antioxidant capacity and phenolic content of black gram seeds, which contribute to their potential health benefits such as reducing oxidative stress and preventing chronic diseases (Xu and Chang, 2008; Singh et al., 2017).

Improving the nutritional quality of black gram can play a significant role in addressing micronutrient deficiencies and malnutrition, especially in developing countries where pulses serve as an affordable and major source of dietary protein and minerals. Therefore, integrating biofortification strategies with modern breeding and biotechnological tools will contribute to the development of nutritionally enhanced black gram varieties for sustainable food and nutritional security (Bouis and Saltzman, 2017).

7. Future Prospects

The integration of modern biotechnological tools with conventional breeding strategies has great potential to accelerate the genetic improvement of black gram (Vigna mungo L. Hepper). Recent advances in plant genomics and molecular biology have opened new opportunities for developing improved cultivars with enhanced productivity and stress tolerance. Emerging technologies such as CRISPR/Cas genome editing, genomic selection, and high-throughput phenotyping are expected to play an important role in future crop improvement programs (Jaganathan et al., 2018; Varshney et al., 2021).

Genome editing technologies, particularly CRISPR/Cas systems, allow precise modification of specific genes associated with disease resistance, yield, and stress tolerance. These tools offer a faster and more efficient approach compared to traditional breeding methods (Chen et al., 2019). In addition, genomic selection and high-throughput phenotyping enable breeders to analyze large populations and identify superior genotypes based on genomic data and phenotypic traits (Crossa et al., 2017).

Developing climate-resilient, high-yielding, and nutritionally enhanced black gram varieties will be crucial for ensuring global food and nutritional security in the face of increasing population growth and climate change. Therefore, integrating genomics, bioinformatics, and biotechnology with conventional breeding approaches will play a significant role in future black gram improvement programs (Varshney et al., 2019).

8. Conclusion

Black gram (Vigna mungo L. Hepper) is a nutritionally important pulse crop with significant potential to contribute to sustainable agriculture and food security, particularly in developing countries. The crop serves as an important source of dietary protein, essential amino acids, and micronutrients for millions of people. However, its productivity is constrained by several biotic stresses such as viral diseases and insect pests, as well as abiotic stresses including drought, salinity, and temperature extremes (Basu et al., 2009; Singh et al., 2016).

Recent advances in plant biotechnology have provided powerful tools for accelerating the genetic improvement of black gram. Approaches such as marker-assisted selection, genetic transformation, tissue culture techniques, and genomic analysis enable the identification and incorporation of desirable traits more efficiently than conventional breeding methods (Collard and Mackill, 2008; Varshney et al., 2013). These technologies facilitate the development of improved cultivars with enhanced yield potential, disease resistance, and environmental adaptability.

Furthermore, biofortification strategies aimed at increasing micronutrient content and antioxidant compounds can significantly improve the nutritional quality of black gram and help address micronutrient deficiencies in human populations. Continued research integrating genomics, biotechnology, and modern breeding strategies will be essential for developing improved black gram cultivars capable of meeting the growing demands of future agriculture and ensuring long-term food and nutritional security (Bouis and Saltzman, 2017).

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