



# Molecular Advancements in Soybean Seed Quality and Diversity: A Review

Ali Hassan <sup>a\*</sup>, Laiba Ashraf <sup>a</sup>, Menahil Riaz <sup>a</sup>,  
Saeed Ur Rehman <sup>a</sup>, Rafay Bin Mohiuddin <sup>a</sup>,  
Asfand Yar Rahim <sup>a</sup>, Asif Mehmood <sup>b</sup>,  
Azhar Mehmood <sup>b</sup> and Muntaha Sanaullah <sup>c</sup>

<sup>a</sup> Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

<sup>b</sup> Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.

<sup>c</sup> Department of Botany, University of Agriculture, Faisalabad, Pakistan.

## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

As a leguminous crop, soybean is an important to the economy. Progress in soybean genetics has been focused on producing types that are tolerant or resistant to different biotic and abiotic problems, increasing seed and oil output, and optimizing cropping system fit. Conventional breeding strategies have been employed by plant breeders to enhance these attributes in soybeans. Traditional breeding can be greatly accelerated by the use of genomic and molecular techniques. In soybean breeding, molecular markers have shown to be an invaluable new tool that may quickly and reliably increase selection efficiency. An overview of the molecular approaches is presented in this paper that has been recently used in "omics" research and marker assisted selection for the genetic enhancement of soybean seed quality standards.

\*Corresponding author: E-mail: hassanmuhammadali41@gmail.com;

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## 1. INTRODUCTION

Glycine max, or soybean, is a significant leguminous crop that yields 40 % of the world's edible oil and is a rich source of protein. Its effective symbiotic capacity to fix N<sub>2</sub> improves soil fertility. Additionally, the crop is promising since it may be used to produce anti-cancer compounds and biodegradable materials based on soybean protein [1]. Gaining an understanding of the genetic improvement process for soybeans is essential for developing superior soybean varieties in the future [2]. The yield of soybeans has increased globally due to technological advancements. As self-pollinating plants, soybeans naturally outcross at a rate of 0.5 - 0.1 % [3]. Because plants are autogamous, several methods have been utilized to enhance genetic quality attributes and produce high-yielding varieties of this important oil-seed crop, including population breeding, backcrossing, single pod descent, and pedigree. Precision in soybean breeding can be increased by combining conventional breeding methods with molecular technologies. The properties of soybean seeds, which are complex and challenging to control due to their multi-gene regulation, would benefit greatly from the application of molecular tools [4]. The most popular molecular technique for enhancing different crop species has been the application of molecular markers. Molecular markers are sections of the DNA sequence that, for different organisms, have a precisely defined nucleotide distribution and organization. DNA markers are more advantageous than standard morphological markers since the information they provide can be used to evaluate the target [5]. The numerous marker types that are employed to map and tag agriculturally significant genes are discussed in this section, along with the process of marker-assisted selection for these genes [6].

## 2. MARKERS

Three categories exist for these: morphological, biochemical, and genetic.

### 2.1 Morphological Markers

It is well recognized that morphological markers, like fruit form, stem length, awn type, colors of the blossoms, pods, hilum, seeds, awn type, seed coats, and the size and form of the

leaves, can be used to check the genetic association and establish the variety's genetic purity [7]. There are few morphological markers; environmental fluctuations frequently affect their expression; many of them are not strongly associated with economic features and may even be detrimental to the growth and development of plants [8]. On the other hand, morphological markers have been applied to several plant species to analyze diversity [9].

### 2.2 Biochemical Markers

Protein indicators are classified into two categories, storage proteins and functional proteins, often known as isozymes, which are biochemical markers. Isozymes are the most widely utilized protein markers. The nature of these markers is co-dominant however, their use is restricted because of their small population [10]. Biochemical markers are mostly used in soybeans to identify cultivars, test hybrid seeds, analyze divergence, and assess genetic purity and uniformity in seed production. Soybean cultivars can be distinguished from isozyme patterns by analyzing the banding pattern of storage proteins using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis or SDS PAGE [11]. Soybean genotypes exhibit significant regional variation; yet, variability among them can be successfully identified using seed storage protein polymorphism [12].

### 2.3 Dna Markers

DNA markers follow a basic Mendelian pattern of inheritance. Some DNA marker types are as follows: Restriction Fragment Length Polymorphism, or RFLP, marker sets: The first generation of molecular markers used in plant genome research are called RFLPs.[13]. The digestion of template DNA by restriction enzymes, or endonucleases, is necessary for RFLP. These enzymes cut DNA at various sequence-specific locations after recognizing small DNA fragments (3–6 base pairs) [14]. Restriction enzymes used in genomic DNA digestion produce polymorphism via DNA fragment length. Using a suitable probe and southern blotting, fragments of varying lengths between genotypes can be recognized following digestion [15]. There have been reports of using RFLP markers to assess genetic diversity in different species of legumes [16]. Randomly Amplified Polymorphic DNA or RAPD markers:

The first DNA markers created using PCR technique are called RAPD markers. A lot of work has been done on the genetic diversity of soybean germplasm using RAPD markers [17]. Using RAPD markers, the promiscuous nodulation trait in soybeans can be analyzed for genetic variation among Indian genotypes of the crop. Markers for simple sequence repeats, or SSRs: SSRs are single-locus markers that are typically 4 - 6 bp long and have a high acceptance and allelic variation [18]. In soybeans, the most frequent repeats are AT, ATT, TA, TAT, CT, and CTT. Soybeans were the first plant species to use SSRs in plant genome analysis. SSR markers are employed on large-scale molecular mapping in soybeans and have undergone ongoing development. Because SSRs have been applied so successfully, there are fewer reports on the development of Amplified Fragment Length Polymorphism (AFLP) markers in soybeans than in other plant species [19]. However, AFLP analysis was employed to examine the genetic relationships between 25 Thai and Japanese soybean types. Single Nucleotide Polymorphism or SNPs are variations in single bases of DNA that result from point mutations. Because SNPs make it possible to distinguish between different haplotypes, they have the potential to be valuable genetic markers [20]. In a study, the distribution of the 1006 SSR markers, 20 soybean chromosomes, and the 1536 SNP markers' associated linkage groups that make up the Universal Soy Linkage Panel 1.0 were mapped, and Kosambi centimorgans were used to scale the Consensus Map 4.0. Some soybean plant introduction lines have been successfully genotyped using the Illumina Infinium array (SoySNP50 KiSelectBeadChip) for \*50,000 SNPs [20]. This kind of technological advancement has ushered in a new era of genotyping by resequencing by making it feasible to resequencing hundreds of lines at a reasonable cost. Twenty-five SNPs linked to seed oil have been found in thirteen distinct genomic locations. Seven SNPs were identified among these markers that were linked to both oil and protein [21]. The SoySNP50 K BeadChip, equipped with over 50K SNPs, was utilized for genotyping these accessions. The collection had duplicate accessions and unique ancestry histories of soybeans from various geographical origins were noted [22]. Recently, functional SNP detection assays with high throughput have been conducted for oleic and linolenic acids in soybeans [23].

### 3. MARKER-ASSISTED SELECTION (MAS)

It may become more crucial to use molecular marker technologies like MAS in crop development initiatives to increase genetic advantages more quickly and precisely. It is yet unclear if MAS will live up to its promise of quickly and affordably enhancing polygenic characteristics [24]. A broader understanding exists that the ultimate goal of trait enhancement would not be served by merely proving that a complicated trait can be broken down into quantitative trait loci (QTL) and mapped to approximate chromosomal regions using DNA markers [25]. To optimize their influence in the face of the obstacle of improvising several lines for quantitative traits, MAS techniques employ DNA markers at a crucial selection stage. With the creation of locus-specific molecular markers and the availability of entire soybean genome sequences, marker-assisted breeding has advanced to a sophisticated level. Predicting genetic components and their evolution, distribution, and interactions is made possible by genomics. It has also helped identify trait-specific accessions [26]. Enhancing the application of genetic resources from soybeans for sustainable crop improvement is aided by it. The identification of alleles about agronomic ally significant traits and haplotype analysis is made easier by high-density markers across the entire genome being available. The following outlines an MAS application areas for enhancing the qualities of soybean seed quality Seed quality parameters [27].

### 4. SEED LONGEVITY

In subtropical hot and humid locations, soybean seed longevity is a significant hereditary problem that negatively impacts the quality of the seed that is sown. The seed reaches its peak vigor and germination ability at physiological maturity [28]. Numerous characteristics, such as the following characteristics of seeds: hull percentage, oil content, permeability, hardness, coat thickness, or size found to be hereditary. These characteristics have an impact on the longevity of soybean seeds. Breeding programs have taken advantage of these features to increase the lifetime and quality of soybean seeds [29]. Unfavorable weather conditions are common during soybean harvest, which leads to low-quality seed that quickly deteriorates in storage. Soybean seed lifetime may be influenced by the genome of the mother plant.

Between 6.3 % (Satt285) and 7.5 % (Sat\_434) of the trait's overall phenotypic variance could be explained by each of these markers alone [30]. SSR markers Satt371, Satt453, and Satt618 are possible markers for association with testa color and seed storability because of the specific bands they produce. In the past ten years, studies have also looked into quantitative trait loci linked to seed lifetime to deduce the genetic basis of seed deterioration [31]. As a complicated biological characteristic, seed aging is challenging to observe. In plant germplasm collections, these efforts have aided in the identification of beneficial longevity alleles for improved seed longevity prediction [32].

#### 4.1 Permeability of the Seed Coat

The porosity of the seed coat and the leaching of electrolytes are significant characteristics that have been adversely linked to the lifetime of soybean seeds [33]. The association between seed coat permeability and electrolyte leaching and four separate SSR markers, Satt434, Satt538, Satt281, and Satt598 have been described. This association explains 3.9 % (Satt434)–4.5 % (Satt538) of the overall phenotypic variation for seed coat permeability [34].

#### 4.2 Hardness of Seed

This is a quantitative characteristic of soybeans that impacts the quality, viability, and rate of germination of stored seeds. In soybeans, hard seededness is influenced by multiple main QTLs. breaking of seed coverup seed coat cracking is the term used to describe elliptical fissures in the soybean seed coat that open up the underlying parenchyma tissues and separate the epidermal and hypodermal layers [35]. It offers a pathway for the entry of pathogenic and unfavorable environmental elements to alter seed quality, thus affecting the seed's exterior appearance and decreasing its commercial value. Mature soybean seeds' appearance and quality are negatively impacted by the frequency of low temperatures during blooming [36,37]. The SoyPRPI gene for proline-rich cell wall protein is strongly linked to the SSR marker Satt264, which has a negligible impact on the average cracking index [38].

#### 4.3 Tocopherol Content

The function of tocopherol in seed lifetime has been determined, indicating a relationship

between the amount of degradation of Tocopherol and seed longevity. According to genetic research on soybeans, the amount of A-tocopherol in the seed is a highly heritable characteristic that can be simultaneously increased. Given their relatively greater tocopherol content values, the COSOYA2 and Ankur genotypes appear to be good donors [39]. The hybridization of cultivars with varying levels of tocopherol (20–30 %) and low levels (<10 %) revealed the presence of SSR markers Sat\_167 and Sat\_243 on MLG K. These markers were surrounded by a region containing genes that regulate a high concentration of tocopherol [40]. This implies that tocopherol and total tocopherol could have separate regulations. Compared to conventional methods, association analysis offers a reasonably high resolution for establishing the genomic site of a gene or QTL, making it a useful alternative for gene or QTL location detection. In 298 soybean germplasm accessions with a wide range of seed protein and oil content, genome-wide association research was conducted to discover the QTL regulating seed protein and oil content [41].

#### 4.4 Pod Shattering

Pod breaking in soybeans can result in production losses ranging from 34 % to 100 %, contingent on the length of time harvested after maturity. The degree of pod breaking is determined by the plant's morphological architecture, the anatomical structures of the pod, the chemical composition of the pod wall, the genetic makeup of the variety, and the environmental factors present at maturity [42]. SSR markers have reportedly been connected to seed properties in soybean LG Associate group Senior No. Seed durability Satt285, Satt434, J H. storability of seeds Satt371, Satt453, Satt618 [C2 B1 M]. Permeability of the seed coat SATT434, SATT538, SATT281, SATT598. H A2 C2 E. cracks in the seed coat Satt264 SoyPRPI K Nakamura et al. (2003) MLG K tocopherol 38:1645–1654 1649 123 Sat\_167, Sat\_243 C2 C2 D1b Satt376, Satt286 Satt266 c-TMT3 K Sat\_243. In soybeans, pod breaking is a strongly heritable characteristic with limited heritability. Pod breaking segregation is quite complicated and has a quantitative reaction. The primary QTL for pod shattering, designated as qPDH1, is between the Sat\_093 and Sat\_366 SSR markers [43]. Furthermore, the shattering resistance allele at qPDH1 was helpful at several sites and in a variety of genetic backgrounds [44].

## 5. USE OF MARKERS IN VARIETAL CHARACTERIZATION AND VERIFICATION

Plant varieties must have their genetic material recorded by the Stability, Distinctness, and Uniformity tests in addition to novelty, necessitating descriptors derived from the DNA fingerprinting, electrophoresis, and morphological features of different crop varieties. To keep agricultural production rising steadily, cultivar seeds must be preserved, multiplied, and certified [45]. This involves confirming the cultivar and evaluating purity. The use of molecular markers for DUS testing inside the variety registration system presents many important potential benefits and could provide a way to address the problem of safeguarding new soybean varieties. It has been demonstrated that a combination of SSR and morphological signals is the best way to look at genetic relationships, accurately categorize soybean varieties for their preservation and figure out the minimum genetic distances needed for distinctness [46]. The International Union for the Protection of New Varieties of Plants (UPOV) has recognized the value of descriptive phenotypic traits associated with molecular markers. A working committee was formed by the UPOV (2011) to look at the possible uses of molecular markers in the variety registration system. The group specializes in molecular and biochemical methods, including DNA profiling (BMT) [47,48]. Aiming to produce molecular data of superior quality for a range of applications, the thirteenth session of the BMT released "International guidelines on molecular methodologies" (BMT/13/ 13). While environmental factors also have a role, genetics controls the expression of hilum color in soybean seeds. Consequently, genetic variation is not always linked to variance or departure from normal expression [49]. A strong marker to differentiate soybean types based on hilum color is Satt070, which is present at B2. Using 16 microsatellite molecular markers, the UPOV working group assessed soybean seeds for variations in hilum color using varieties CD 222 (black hilum), CD 02RV 8444, and CD01RV-7618 (brown hilum) [50]. It was discovered that marker Satt020 at position B2 is appropriate for distinguishing purple and white flowered plants in population segregation. The results of discrimination tests indicated that the SSR was highly accurate in classifying features related to growth habit (95.8 %) and pubescence color (80.6 %) [51]. Leaflet size (73.5 %) and pod color (74.2 %) were compared with the SSR 1650. In

the current agricultural improvement landscape, new omics techniques are crucial, and several of them have been used to improve soybean advanced omics approaches in soybean [52]. A developing soybean seed's final protein, lipid, and carbohydrate balance is determined. There is still much to learn about the control of metabolic networks, and it is either unknown or difficult to characterize a large number of the genes and metabolites involved in seed metabolism [53]. Without making any presumptions regarding the composition of these networks, a global omics analysis can shed light on how seed metabolism is regulated. Below is a discussion of a few cutting-edge techniques that may be used to improve soybeans [54].

### 5.1 Transcriptomics

An affordable and high-throughput RNA-seq technology enables transcriptome study in crops with genomic sequence information, including soybeans. Compared to microarray technology, which limits the number of genes whose expression may be examined by designing probe sets based on available genetic information, it offers several advantages [55]. Gene information is not necessary for RNA-seq analysis, which can uncover previously unidentified new transcripts and study non-coding and differently spliced RNA. Rho danese and RPS4 most likely have a significant impact on photoperiod regulation, which affects when soybeans blossom [56]. The photoperiod was found to influence transcript-derived fragments recovered from soybeans [57].

### 5.2 Proteomics

The study of the structural and functional characteristics of every protein in an organism is known as proteomics. Among the most important quantification instruments in differential proteomic research is isotope utilization [58]. ITRAQ-based proteomics research was conducted to study a superior soybean variety and its progenitors to assess the parental contributions to the superior characteristics of the variety [59].

### 5.3 Metabolomics

Metabolism comprises the identification and measurement of the whole range of primary and secondary metabolisms, which contributes to a more thorough understanding of the molecular mechanisms underlying biological processes in

plants as well as the genes, transcripts, and proteins in those processes [60]. In one study, levels of 104 out of the 169 soybean metabolites identified in the cultivars under investigation by GC/MS and UHPLC-MS/MS differed considerably from one another. Metabolite markers were identified to aid in the identification of genetically related soybean cultivars, and significant relationships were established both within and between different metabolite categories [61].

#### 5.4 Economics

To guarantee higher crop production, fertilizers containing P and K are the two essential macronutrients [62]. Plants, however, need a wide range of additional components, and these are not evenly distributed across the many types of soil. Due to varying soil types, plants have evolved varied capacities for absorbing different elements at different places. This underlines the necessity of combining genomes and economics to investigate current genetic variations. The sensitivity and specificity of the plant have increased, allowing the element profile to represent several physiological states [63]. Bioinformatics Exploration of phenotypes at high throughput is the focus of phenomics. To identify any genetic system, precise phenotyping is essential. Plants can be identified by their meticulous phenotype, which can reveal biological statuses like infection, insect infestation, or physiological confusion. The degree to which a genetic marker and phenotype may be reliably linked determines the success of genomics[64]. Thus, the greatest potential for plant breeding lies in phenomics combined with other omics techniques. Additionally, precise characterization of two-dimensional leaf expansion with great temporal precision has been made possible using marker tracking techniques[65].

#### 6. CONCLUSION

Max Glycine (L.) Merr is a crop that is useful as food, feed, and bio-feedstock because of its balanced protein, fat, and carbohydrate content. The rapid development of molecular technology holds the possibility of realizing the forthcoming opportunities for the global cultivation of soybeans. Applications of various omics techniques have improved the precision of the selection process and the scientific treatment of population segregation. Alongside traditional breeding techniques, scientific advancements would make it easier to create novel types of

soybeans possessing the distinct qualities required to improve agronomic attributes in the domain of structural and functional plant genomics. Plant breeding in conjunction with biotechnology may undoubtedly bring about a "yellow revolution" in the production of oil seeds crops like soybean, simultaneously enhancing seed quality.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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