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13. Molecular Breeding and Its Current Scenario in Crop Improvement

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Abstract:

Increasing the global population and change in climatic conditions is a major threat to the food security. Rapid shrink in agricultural lands have been observed due to urbanization which is a matter of concern. Conventional plant breeding strategies will not be able to fulfill the food security for the world population due to its time-consuming process and molecular breeding strategies are the only source to produce optimum food in a limited period of time. In the current scenario, molecular breeding has gained widespread acceptance and adoption by plant breeders and researchers worldwide. It has aided in the creation of superior crop varieties that have higher yields, are resistant to pests and diseases, can withstand abiotic stressors, and have higher nutritional value. The application of molecular breeding techniques has contributed to the development of genetically diverse and adapted crop varieties, addressing the challenges of global food security and sustainable agriculture.

Keywords:

Climate Change, Global Population, Food Security, Crop improvement

13.1 Introduction:

The population of the world will top seven billion people by 2025. A annual drop in the amount of cultivable land and agricultural water available per person will occur in the interim as biotic and abiotic stressors increase. Food security, which is best described as having access to a healthy diet and safe drinking water on an economic, physical, and social level, will be jeopardized. To succeed in ending hunger, an integrated approach to dietary and non-nutritional elements is required. Science and technology have a significant impact on fostering and maintaining an Evergreen Revolution that boosts output over the long term without causing environmental harm.[1][2] Plant breeders can achieve their goals by combining traditional breeding with a variety of scientific advancements. [3] Plant breeding is getting faster, simpler, more effective, and efficient as molecular technologies evolve.[4]Plant breeders will have access to cutting-edge methods for identifying and/or creating genetic variation, defining the genetic characteristics of the genes related to the variation (position, function, and relationship with other genes and environments), understanding the structure of breeding populations, recombining novel alleles or allele combinations into specific cultivars or hybrids, and choosing the best individuals with desired genetic characteristics. The phrase molecular breeding refers to the interdisciplinary

area of contemporary plant breeding that blends molecular techniques and procedures with traditional approaches to crop development. The term "molecular breeding" (MB) refers to a number of contemporary breeding tactics that make use of molecular biology tools and techniques to enhance the efficacy and efficiency of conventional plant breeding programmes. Marker-assisted selection, marker-assisted backcrossing, marker-assisted recurrent selection, and genome-wide selection are all types of molecular breeding techniques that use molecular markers, or specific regions of DNA linked to desirable traits, to select individuals with the desired genetic characteristics.

The main goal of molecular breeding is to accelerate the breeding process by identifying and selecting individuals with desirable traits more accurately and efficiently. Traditional breeding methods rely on phenotypic observations, which can be time-consuming and influenced by environmental factors. Molecular breeding allows breeders to make selections based on the genetic makeup of individuals rather than relying solely on observed traits.

The process of molecular breeding typically involves the following steps:

1.Identification of Target Traits: Breeders determine the traits they want to improve, such as yield, disease resistance, or nutritional quality.

2.Marker Identification: Researchers identify molecular markers that are associated with the target traits. These markers can be DNA sequences, genes, or other genetic variations.

3.Marker Development: Specific markers are selected and developed into reliable and cost-effective tools for screening large populations. This may involve techniques such as polymerase chain reaction (PCR), DNA sequencing, or genotyping arrays.

4.Marker Screening: Large populations of plants or animals are screened using the developed markers to identify individuals that possess the desired traits.

5.Selection and Breeding: Individuals with the desired markers and traits are selected as parents for the next generation. By focusing on the molecular markers associated with the target traits, breeders can increase the likelihood of offspring inheriting those traits.

6.Evaluation and Validation: The selected individuals and their offspring are evaluated for the target traits through field trials, laboratory tests, or other relevant methods. This helps validate the effectiveness of the molecular markers in predicting trait performance.

7.Iterative Cycles: The breeding process continues through multiple cycles of marker-assisted selection, evaluation, and validation to further refine the breeding population and enhance the desired traits.

Numerous crops have successfully used molecular breeding, resulting in the creation of new varieties with improved attributes. It offers advantages such as increased breeding efficiency, more precise trait selection, and reduced time and resources required for traditional phenotypic-based breeding programs.

13.2 Molecular Breeding Tool:

Molecular Markers: Nucleotide sequences, which are particular portions of DNA, are known as molecular markers because they are easily recognized and connected to certain features. The polymorphism among the nucleotide patterns of various people allows for the investigation of these specific DNA regions. To "flag" the location of an identifiable gene or the genetic transmission of a particular or desired feature, all genetic markers possess specific genomic locations called "loci" inside the chromosome. Basis of these polymorphisms are insertion, deletion, point mutations duplication and translocation; however, they do not necessarily affect the activity of genes. With the advent of DNA-based molecular markers, the extensive genetic mapping of chromosomes became readily possible for a variety of species. An ideal DNA marker should be co-dominant, highly reproducible, evenly distributed throughout genome, and having ability to detect higher level of polymorphism. Knowing every nucleotide of a model or reference genome allows traits and genes from the reference genome to be tracked to other genomes, that's why DNA markers are widely used in agricultural breeding to help in identifying and improvement of desirable characteristics in plants.

1. Single Nucleotide Polymorphisms (SNPs): SNPs are the most common type of molecular marker used in crop breeding. They are single base pair differences in nucleotide sequences and can be easily identified using various techniques, such as DNA sequencing or genotyping arrays. SNPs are used for various applications, including marker-assisted selection (MAS), gene mapping, diversity analysis, and genomic selection.**SNP detection**: SNPs are detected by Oligonucleotide hybridization, DNA chip technology and Solution hybridization technique.

2. Simple Sequence Repeats (SSRs): SSRs, (microsatellites) are short tandemlyrepetitive DNA sequences found throughout the genome. Microsatellite loci are helpful as genetic markers because of their high amount of allelic variation, which is one of their most significant characteristics. They are highly polymorphic and often used for genetic mapping, cultivar identification, and assessing genetic diversity in crop populations.

3. Insertion/Deletion Polymorphisms (Indels): Indels are small insertions or deletions of DNA segments ranging from a few base pairs to several hundred base pairs. They can be easily detected using PCR-based techniques and are used for genetic mapping, linkage analysis, and marker-assisted selection.

4. Amplified Fragment Length Polymorphisms (AFLPs): AFLPs are PCR-based markers that detect variation in DNA fragment lengths. They provide high levels of polymorphism and have been extensively used for genetic diversity analysis, genetic mapping, and phylogenetic studies.

5. Restriction Fragment Length Polymorphisms (RFLPs): RFLPs are markers based on variations in DNA fragment sizes resulting from differences in restriction enzyme recognition sites. They were widely used in the past but have been largely replaced by other more efficient marker systems, such as SNPs and SSRs.

6. Sequence-Characterized Amplified Regions (SCARs): SCARs are PCR-based markers derived from specific DNA sequences associated with a particular trait or gene of interest. They are used to identify and select plants carrying desired traits in breeding programs.

The use of molecular markers in crop breeding allows breeders to make informed decisions during the selection process, leading to more efficient and targeted breeding programs. They help in identifying and selecting plants with desirable traits, tracking the inheritance of genes of interest, assessing genetic diversity, and accelerating the development of new improved varieties.

13.3 Strategies of Molecular Breeding:

1. Marker-Assisted Selection (MAS): MAS involves the use of molecular markers, which are specific DNA sequences associated with a particular trait of interest. By analyzing these markers, breeders can identify and select individuals with desired traits more efficiently. MAS can accelerate the breeding process by eliminating the need for time-consuming phenotypic evaluations. Marker-assisted selection enables selection at early stages of plant, even before the traits are fully expressed, accelerating the breeding process. Furthermore, MAS can be particularly useful for traits that are difficult to measure directly or influenced by multiple genes.

2. Marker-Assisted Backcrossing (MABC): Marker-assisted backcrossing (MAB) is a breeding technique used in plant breeding to transfer specific traits from one genotype to another while retaining the genetic background of the recipient genotype. It combines traditional backcrossing methods with the use of molecular markers to accelerate the breeding process. In traditional backcrossing, a desired trait from a donor genotype is introduced into a recurrent or recipient genotype through several rounds of crossing followed by selection of offspring with the desired trait.

However, this process can be time-consuming and labor-intensive, especially when the desired trait is controlled by multiple genes or is difficult to select visually. MABC is a variant of MAS that is commonly used in breeding programs for the transfer of a specific trait from one variety or species into another. It involves multiple rounds of backcrossing to introduce the desired trait while simultaneously selecting for the molecular marker associated with that trait.

3.Genomic Selection (GS): GS is a breeding strategy that uses high-throughput genotyping technologies to capture and analyze a large number of genetic markers throughout the genome. Genomic selection utilizes genomic information, specifically the analysis of an individual's DNA sequence or genetic markers spread throughout the genome. It allows breeders to identify and select individuals with desirable genetic variations associated with specific traits. The genetic markers used in genomic selection can be single nucleotide polymorphisms (SNPs), microsatellites, or other genetic variations. By correlating these markers with phenotypic data from a training population, breeders can predict the performance of individuals for various traits, even before they are phenotypically expressed. GS is particularly useful for complex traits controlled by multiple genes.

4. Genetic Engineering: By adding or changing certain genes, genetic engineering entails the direct modification of an organism's genetic material. This technique allows breeders to introduce novel traits or modify existing ones by inserting genes from unrelated species. The process of genetic engineering typically involves isolating and manipulating specific genes or DNA sequences from one organism and inserting them into another organism. This can be done using various techniques, such as recombinant DNA technology, gene editing tools like CRISPR-Cas9, or other methods that facilitate the transfer of genetic material. Genetic engineering has been widely used in crop improvement, such as introducing disease or pest resistance or enhancing nutritional content.

5. Genome Editing: Genome editing techniques, such as CRISPR-Cas9, enable precise modifications of specific genes within an organism's genome. Unlike genetic engineering, genome editing does not necessarily involve the introduction of foreign DNA. It allows for targeted changes in the DNA sequence, including gene knockout, gene insertion, or gene replacement, providing breeders with precise control over trait modification

13.4 Importance of Molecular Breeding:

Molecular breeding has a numerous advantage over the classical plant breeding technologies. Using DNA tags for characteristics whose phenotypes emerge in the subsequent phases of crop growth and development, reliable selection becomes feasible at the stage of seedling development. This reduces the expense of growing all the plants until the expression of phenotypic characteristics by allowing undesirable plants to be quickly identified and safely dismissed at the beginning of the seeding stage. It uses a unique sequence of DNA to identify crops with ideal qualities, thus it has an equivalent level of precision and efficiency whether the population is raised in the primary crop's growing season, off-season, outdoor environment, or protected green structures. Retaining a desirable trait dictated by a gene that is recessive in a backcross breeding programme takes a longer approach because each backcross requires an additional intermediate generation (self/test cross) for the recognition of cultivars having the appropriate allelic form. Co dominant DNA markers in MAB aid in detecting and selection of the desirable alleles that are recessive for a specific characteristic even in the heterozygous condition, allowing for continual backcrossing without self or test cross generation interruptions. As a result, the MB saves time and speeds up the breeding process. [5]

13.5 Current Scenario of Molecular Breeding in Crop Improvement:

A breeding approach called marker-aided selection employs genetic markers that are closely connected to the target trait(s) or gene(s) in order to indirectly select for that trait in segregating or non-segregating generations. When a marker is found to co-segregate with a major gene for a relevant behavior, it may be simpler and less expensive to screen for evidence of an associated allele connected to the gene than to evaluate the trait. Breeders need to find out how to combine as many advantageous alleles for the identified QTLs as is practical in order to produce more complicated, polygenically controlled characteristics. In this case, QTL-linked markers may be examined in the breeding material. Based on the results of this study, special crosses may be created to provide the optimum genotype by combining QTL alleles from different sources.[6]

For attributes that are challenging to choose, such as disease resistance, salt tolerance, drought tolerance, heat tolerance, and quality traits, marker assisted selection (MAS) is most advantageous. The method involves selecting crops with a single, large-scale marker-assisted selection while preserving as much allelic segregation in the population as is feasible, screening large populations, and selecting crops at early generations with a fixed, favorable genetic background at associated loci. No selection is made outside of the target genomic regions in order to preserve the maximum amount of Mendelian allelic segregation among the genotypes. Genetic diversity at unselected loci may enable breeders to create novel varieties and hybrids in response to breeding objectives after selection with DNA markers. The area of marker-assisted selection for polygenic trait improvement is poised to show spectacular results and is in a critical transitional period. The use of this technique in pulse breeding efforts has grown recently. [7][8] In marker aided backcross breeding, genes from a "donor" line are inserted into a "recipient" line's chromosomal background. Markers can be used to assess the presence of the introgressed gene ("foreground selection") when direct phenotypic evaluation is not feasible, is prohibitively costly, or is only feasible late in development. Additionally, markers can be used at additional loci to accelerate the return to the recipient parent genotype (this is referred to as "background selection"). The assumption that the introgressed gene can be recognised without ambiguity led to the theoretical analysis being restricted to background selection. Molecular markers have been successfully used in backcross processes for background selection, according to experiments. The use of molecular markers to locate and choose the genes affecting rice resistance is one of the most promising MABC strategies. This breeding technique has enhanced the rice variety "Maudamani."[9][10]

The genetic components known as quantitative trait loci (QTLs) are responsible for a portion of the observed phenotypic variation for a quantitative trait. Gelderman introduced the QTL concept. The attribute may theoretically be influenced by a single gene or a collection of genes that are connected to one another. The ability to discriminate between the impacts of quantitative trait loci (QTL) on the variation of a complex trait and the ease with which these QTL may be transferred into desired cultivars or lines are both made possible by the use of biological markers. In a recent study, QTL data was effectively used to replace the measured parameters in crop models, suggesting that QTL analysis can help to eliminate some of the random errors of observed model input parameters.[11] Using at least two different parental genotypes for the targeted trait is necessary to produce diverse mapping populations for QTL research. However, occasionally, populations for mapping are primarily developed for linkage map development but are also employed for QTL study. In these situations, the mapping population's parents are diverse but weren't selected for any particular attribute of interest. Another instance is the International Triticeae Mapping Population (ITMI pop) in wheat, which was developed to create genetic maps but later used for numerous QTL studies because the parent genotypes of ITMI were chosen to be diverse and as a result, the recombinant inbred lines (RILs) differentiated for a number of traits of economic importance, allowing QTL analysis for these traits.[12][13][14][15][16]

A cytoplasmic male sterility method is utilized to create hybrid seeds because it avoids the need for manual emasculation. The maternally transmissible characteristic known as CMS is characterized by the inability to generate viable pollen without affecting female fertility. It typically involves mutations, editing, and rearrangements of mitochondrial DNA. Several restorer loci have been identified in several crops using RAPD and STS, and DNA markers

linked to these loci allow for the molecular investigation of the CMS system. These co-dominant markers are useful in identifying the homozygous restorer genotypes following backcrossing to produce restoration lines. The restorer lines might be made in less time this way compared with traditional procedures. Pi2 gene-based TGMS rice lines have been successfully developed which provide resistance to Blast disease is one of the outstanding examples.[17]

The evaluation and preservation of genetic variety and biodiversity usually relies on comparative anatomy, morphology, embryology, physiology, and other domains that offer important information but have limited genetic resolution. Recent advances in molecular biology have created powerful genetic tools that can tackle genetic problems with speed and accuracy. It is necessary to develop a person's unique marker profile in order to do genotyping using molecular markers. Using a DNA identifier to identify crop varieties in an unmistakable pattern is referred to as "DNA Fingerprinting". The technique was developed by Alec Jeffery in 1985 for use with people, and Dallas used it for the first time in a crop (rice) in 1988 to identify cultivars. MtDNA RFLP may be performed to examine the diversity of pigeon peas. With low levels of polymorphism in crops and large levels in wild species, RAPD markers were utilised to produce DNA fingerprints for domesticated and wild pigeon pea accessions. To differentiate the molecular variety in lentils, new microsatellite markers were developed. To establish the genetic diversity among 18 black gramme cultivars, RAPD and IISR markers were used.[18][19][20][21]

It is expensive to assess hybrids in the field for heterosis or combining potential. Molecular markers have been used to correlate genetic diversity with heterosis in several cereal crops, such as rice, oat, and wheat. It has been proposed that the optimal hybrid pairings may be predicted using pedigree data and RFLP-based measures of similarity. However, it has been discovered that there are both little and substantial relationships between heterosis and DNA-based genetic distance. The association between heterosis and molecular (isozyme and RFLP) variability between parents was investigated in three soybean maturity groups. Inferring that yield heterosis may not be connected to genetic variation at the molecular level as determined by RFLPs, parental RFLP diversity was not significantly correlated with mid-parent and better-parent heterosis. Despite the fact that isozyme diversity in parents was linked to yield heterosis, soybean has a small number of assayable isozyme loci, hence its effects are negligible. [22]

Verification of the designated cross, the quantity of self-pollination between the female parents, and the appropriateness of the result are all necessary to verify the quality of hybrid seed. The sole method for determining the purity of hybrid seeds has historically been the grow-out test. The RAPD and RFLP markers are currently used to assess the purity of F1 hybrids. The tomato hybrids Pbc EC 538408, Pbc EC 520061, and H 86 EC 520061 have successfully passed the F1 purity test using SSR 218, SSR 306, and Ty2 gene CAPs gene markers.[23]

Gene pyramiding is a technique for finding and introducing many of genes that provide resistance to several insects or microbes or provide resistance to a single pest via various host pathways. One method for improving resistance persistence is the incorporation of many resistance genes into a single variety. It has been calculated that the resistance would last up to 50 years longer if the pyramided genes were never utilised as single genes.

The amount of resistance genes that were successfully pyramided throughout cultivar development is difficult to quantify. Plants with three resistance genes are just as resistant as those with one, even though the latter's resistance is probably more long-lasting. Soybean tolerance QTLs were pyramided for Phytophthora. A recombination inbred line population was produced by crossing two phytopthora-resistant soybean cultivars, Conrad and Hefeng 25, and it was discovered that the more QTLs there were, the higher the level of tolerance. [24] Another important area of research is the pyramiding of Bt genes. Bacillus thuringiensis (Bt) insecticidal cry (crystal) genes have been used as biopesticides and in transgenic plants to control insects. To prevent target insects from developing resistance, new insecticidal genes must be found. It is simpler to isolate novel cry and vip (vegetative insecticidal protein) gene types because of the diversity of Bt strains. In addition to other research techniques including RFLP, gene sequence analysis, electrophoretic, immunological, and chromatographic Cry protein analysis, and insect bioassays for toxicity assessment, PCR was employed to identify novel insecticidal proteins.

Finding a molecular marker close to the desired gene is the first step in map-based gene cloning. To find genes, a small mapping population is first employed, and to clone a gene, a highly saturated genetic map is needed. For an initial marker, there are several high-density markers in close proximity to the original molecular marker. To find a marker that seldom recombines with the target gene, a huge number of people are tested. The next stage is to look for clones that hybridise with previously discovered, closely related molecular markers in a large insert genomic collection (BAC or YAC). The target gene is located via chromosomal walking after two flanking markers that demonstrate linkage to the target gene have been identified. Finding clones with a set of flanking markers that co-segregate with the desired gene is the aim. These hypothetical clones are given to people who are deficient in the target gene. If a transgenic is demonstrated to reverse the mutant phenotype, a complete molecular and biochemical analysis is performed to characterise the newly cloned gene. A map-based cloning approach was used to clone the Pto (resistance to bacterial speck disease of tomato) gene. [25]

13.6 Future Prospects:

Crop improvement has adopted a rapid gain with the onset of molecular breeding as compared to the conventional plant breeding strategies. There is a tremendous growth in world population and issues related to climate change is increasing day by day. Also, the urbanization resulted in declining of the cultivable lands which is a major area of concern. To overcome these issues and food security aspects, molecular breeding technique is the only option to increase rapid yield growth and nutritional properties of crops in the upcoming days as it requires very less time compared to the conventional plant breeding.

13.7 Conclusion:

Wherever the minimal minimum of operational and human resources is available, molecular breeding is certainly a viable strategy by enhancing the genetic gain per crop cycle or by minimizing the number of selection cycles. It is important to note that the molecular breeding area is dynamic and quickly changing. The field of crop improvement is constantly changing due to new technology, methods, and research discoveries.

By giving breeders strong tools to select for desirable traits, boost genetic diversity, improve disease and stress tolerance, raise yield potential, and enhance quality features, molecular breeding has revolutionized crop development. It has accelerated up breeding, resulting in the creation of better crop varieties that support sustainable agriculture and tackle the issues of food security and climate change. There are several reasons to believe that MB will have a big influence on crop improvement, including the constantly falling cost of marker technologies, the introduction of platforms for accessing MB tools and support services, and the need-driven demand for better varieties to meet the global food crisis.

13.8 Reference:

1. Borlaug NE. 2001. Feeding the world in the 21st century: the role of agricultural science and technology. Speech given at Tuskegee University, April 2001. Available at: http://www.agbioworld.org/biotech-info/ topics/borlaug/borlaugspeech.html (accessed 17 November 2009).
2. Swaminathan MS. 2007. Can science and technology feed the world in 2025? *Field Crops Research* 104: 3–9.
3. Xu Y, Skinner DJ, Wu H, Palacios-Rojas N, Araus JL, Yan J, Gao S, Warburton ML and Crouch JH. 2009c. Advances in maize genomics and their value for enhancing genetic gains from breeding. International Journal of Plant Genomics Volume 2009, Article ID 957602, 30 pages. Available at: http://www.hindawi.com/journals/ijpg/2009/957602.html (accessed 21 December 2009).
4. Phillips RL. 2006. Genetic tools from nature and the nature of genetic tools. *Crop Science* 46: 2245–2252.
5. Singh NK, Joshi A, Sahoo S, Tufchi M and Rakshit S. 2023. Molecular breeding for improving yield in maize: Recent advances and future perspectives. QTL Mapping in Crop Improvement, 75-99.
6. Anonymous 2013. https://iipr.icar.gov.in/pdf/molecularbulletins2may13.pdf
7. Kumar J, Choudhary AK, Solanki RK and Pratap A. 2011. Towards marker-assisted selection in pulses: a review. *Plant Breeding*, 130(3): 297-313.
8. Ribaut JM and Hoisington D. 1998. Marker-assisted selection: new tools and strategies. *Trends in Plant Science*, 3(6): 236-239.
9. Hasan MM, Rafii MY, Ismail MR, Mahmood M, Rahim HA, Alam MA and Latif MA. 2015. Marker-assisted backcrossing: a useful method for rice improvement. *Biotechnology & Biotechnological Equipment*, 29(2): 237-254.
10. Pandit E, Pawar S, Barik SR, Mohanty SP, Meher J and Pradhan SK. 2021. Marker-assisted backcross breeding for improvement of submergence tolerance and grain yield in the popular rice variety 'Maudamani'. Agronomy, 11(7): 1263.
11. Yin X, Stam P, Kropff MJ and Schapendonk AH. 2003. Crop modeling, QTL mapping, and their complementary role in plant breeding. *Agronomy Journal*, 95(1), 90-98.
12. Gupta PK, Kulwal PL and Mir RR. 2013. QTL mapping: methodology and applications in cereal breeding. *Cereal genomics* II, 275-318.
13. Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P and Ganal MW. 1998. A microsatellite map of wheat. *Genetics*, 149(4),2007–2023. https://doi.org/10.1093/genetics/149.4.2007
14. Börner A, Schumann E, Fürste A, Cöster H, Leithold B, Röder M and Weber W. 2002. Mapping of quantitative trait loci determining agronomic important characters in

hexaploid wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, 105: 921-936.
15. Kulwal PL, Roy JK, Balyan HS and Gupta PK. 2003. QTL mapping for growth and leaf characters in bread wheat. *Plant science*, 164(2), 267-277.
16. Kulwal PL, Singh R, Balyan HS and Gupta PK. 2004. Genetic basis of pre-harvest sprouting tolerance using single-locus and two-locus QTL analyses in bread wheat. *Functional & Integrative Genomics*, 4: 94-101.
17. Jiang J, Mou T, Yu H and Zhou F. 2015. Molecular breeding of thermo-sensitive genic male sterile (TGMS) lines of rice for blast resistance using Pi2 gene. *Rice*, 8(1): 1-10.
18. Sivaramakrishnan S, Kannan S and Reddy LJ. 2002. Diversity in selected wild and cultivated species of pigeonpea using RFLP of mtDNA. *Euphytica*, 125: 21-28.
19. Ratnaparkhe MB, Gupta VS, Ven Murthy MA and Ranjekar PK. 1995. Genetic fingerprinting of pigeonpea [Cajanuscajan (L.) Millsp.] and its wild relatives using RAPD markers. *Theoretical and applied genetics*, 91, 893-898.
20. Hamwieh A, Udupa SM, Sarker A, Jung C and Baum M. 2009. Development of new microsatellite markers and their application in the analysis of genetic diversity in lentils. *Breeding Science*, 59(1): 77-86.
21. Souframanien J and Gopalakrishna T. 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theoretical and Applied Genetics*, 109: 1687-1693.
22. Cerna FJ, Cianzio SR, Rafalski A, Tingey S and Dyer D. 1997. Relationship between seed yield heterosis and molecular marker heterozygosity in soybean. *Theoretical and Applied Genetics*, 95: 460-467.
23. Kumar A, Datta D and Singh M. 2011. SSR polymorphism among ToLCV resistant and susceptible lines for genotype identification and F1 purity Testing. African J. Biotech.
24. Li X, Han Y, Teng W, Zhang S, Yu K, Poysa V and Li W. 2010. Pyramided QTL underlying tolerance to Phytophthora root rot in mega-environments from soybean cultivars 'Conrad'and 'Hefeng 25'. *Theoretical and applied genetics*, 121: 651-658.
25. Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R and Tanksley SD. 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science*, 262(5138), 1432-1436.