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## Applications of Next-Generation Sequencing Technologies in Modern Plant Breeding

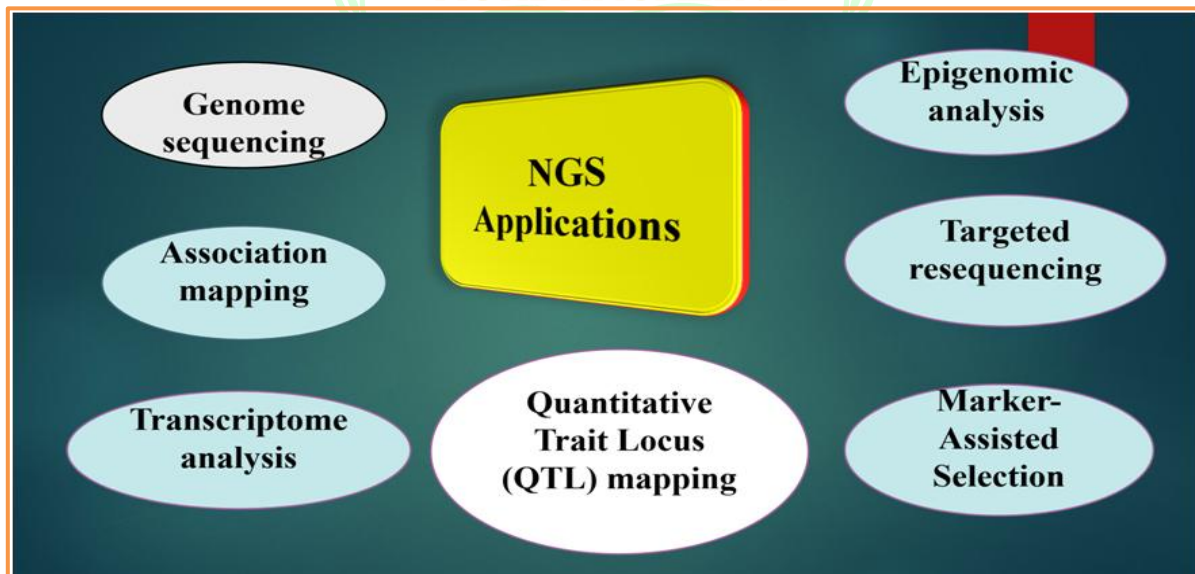
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**N**ext-Generation Sequencing (NGS) is a modern DNA sequencing technology that allows rapid, high-throughput sequencing of millions of DNA fragments simultaneously. NGS reads the sequence of nucleotides (A, T, G, C) in DNA or RNA much faster and cheaper than traditional methods like Sanger sequencing. It has transformed multiple fields, especially genetics, medicine, and plant breeding. NGS enables the identification of single nucleotide polymorphisms (SNPs), structural variations, and copy number variations, which are crucial for marker-assisted selection (MAS) and genomic selection (GS). In plant breeding, **Next-Generation Sequencing (NGS)** constitutes a transformative high-throughput genomic approach with extensive applications, including the systematic identification and characterisation of novel genes and molecular markers associated with desirable agronomic traits. Furthermore, NGS facilitates the development and deployment of robust marker systems that significantly enhance the accuracy, resolution, and efficiency of genotype-phenotype associations, thereby improving the precision of plant phenotyping and accelerating crop improvement programs. The application of **Next-Generation Sequencing (NGS)** in plant breeding enables the development of crop varieties with enhanced resistance to biotic stresses, improved tolerance to abiotic stresses, and superior nutritional quality and yield-related traits. By providing high-resolution genomic information, NGS facilitates precise and accelerated selection of desirable genotypes, thereby significantly enhancing the efficiency of breeding programs and contributing to the development of improved and high-performing crop varieties.

Following are applications of NGS in modern plant breeding



**1. Genome sequencing:** Genome sequencing, especially through Next-Generation Sequencing (NGS), has revolutionised molecular biology by enabling fast, accurate, and cost-effective analysis of an organism's DNA at a high level of detail. Unlike traditional sequencing methods, NGS can process millions of DNA fragments simultaneously, producing large volumes of genomic data efficiently. This advancement has had a profound impact on agriculture by enhancing crop productivity, accelerating breeding programs, and improving resistance to diseases. It also plays a key role in ecological research, including biodiversity evaluation and the study of microbial communities. In addition, NGS supports gene prediction and annotation, helps uncover mechanisms of abiotic stress tolerance in crops like sorghum, detects viral associations in plants such as chilli, and confirms the absence of transgenes in rice, thereby reinforcing its importance in modern genomics and crop improvement.

**2. Association mapping:** Association mapping, also referred to as linkage disequilibrium (LD) mapping, is an effective genetic strategy used to detect relationships between genetic variations and observable traits within natural populations. In contrast to traditional linkage mapping, which depends on pedigrees and controlled crosses, this approach examines genetically diverse and unrelated individuals by utilising existing natural variation. It involves genotyping numerous molecular markers, especially single-nucleotide polymorphisms (SNPs)-spread throughout the genome, followed by statistical analyses to identify significant marker–trait associations. This technique is widely applied to uncover loci governing complex traits such as disease resistance, yield, plant height, and stress tolerance. Owing to its high resolution and efficiency, association mapping has become a key tool in plant breeding, as evidenced by studies in spring wheat where genome-wide analyses have successfully identified quantitative trait loci (QTLs) and genomic regions linked to grain yield.

**3. Transcriptome analysis:** Transcriptome analysis is an advanced and versatile technique in molecular biology that allows for the complete examination of all RNA molecules expressed in a cell or tissue at a specific time, offering valuable insights into gene expression and regulatory mechanisms. By combining high-throughput sequencing technologies with sophisticated bioinformatics tools, it enables the discovery of new gene isoforms, alternative splicing patterns, and various non-coding RNAs. This approach is extensively used to investigate complex biological systems, identify potential biomarkers, and understand molecular pathways. In agriculture, transcriptome analysis significantly contributes to crop improvement by revealing mechanisms of stress tolerance, such as drought resistance in maize, cold tolerance in rapeseed, and efficient nitrogen use in wheat. It also plays an important role in exploring plant–microbe interactions, including the impact of biofertilizers on enhancing disease resistance in crops like lemon, thereby aiding in the development of robust and high-yielding crop varieties.

**4. Targeted resequencing:** Targeted resequencing is an efficient and economical genomic technique that concentrates on sequencing selected regions of the genome instead of the entire DNA sequence. Employing specially designed probes or primers, it isolates and enriches specific DNA segments of interest, allowing for higher coverage and precise identification of genetic variations, including rare mutations. This approach is particularly valuable for analysing known genes or genomic regions linked to specific traits, diseases, or biological functions. In agricultural research, targeted resequencing is widely used to detect important genetic variations and desirable traits in crops and livestock. It has been effectively applied in studies such as examining anthocyanin accumulation in grapes, enhancing pigment production in rice using CRISPR/Cas 9, identifying allelic variations associated with yield in rice, and targeting phenology-related genes in barley, thereby supporting crop improvement efforts.

**5. Quantitative Trait Locus (QTL) mapping:** Quantitative Trait Locus (QTL) mapping is an important genomic technique used to locate regions of the genome associated with complex traits that are influenced by multiple genes and environmental factors. By linking

phenotypic variation with molecular markers distributed across the genome, this method helps identify specific loci that contribute to traits such as yield, disease resistance, and stress tolerance. QTL mapping provides valuable insights into the genetic basis of complex traits and plays a key role in marker-assisted selection for crop improvement. It has been extensively applied in agriculture, including identifying genes that control plant architecture in maize, developing new yield-related alleles through CRISPR/Cas 9, and uncovering traits like spike extension in wheat, thereby improving breeding efficiency and overall crop performance.

**6. Marker-Assisted Selection:** Marker-assisted selection (MAS) is a modern breeding approach that uses molecular markers associated with desirable genes to achieve accurate and efficient trait selection in plants and animals. Unlike traditional breeding methods that rely only on observable characteristics, MAS enables the early detection of superior genotypes, thereby speeding up the breeding process and lowering both time and cost. It plays an important role in developing improved crop varieties with higher yield, better nutritional quality, enhanced disease resistance, and greater tolerance to environmental stresses. MAS has been extensively utilised in agriculture, for example, in increasing grain number and disease resistance in rice, selecting early-maturing soybean varieties suited to high-altitude regions, and developing drought-tolerant pearl millet, thus supporting sustainable crop improvement.

**7. Epigenomic analysis:** Epigenomic analysis is a sophisticated technique in molecular biology that explores genome-wide epigenetic changes, including DNA methylation and histone modifications, which influence gene expression without modifying the underlying DNA sequence. Through methods such as ChIP-seq, ATAC-seq, and bisulfite sequencing, it helps reveal how genetic and environmental factors interact to shape traits, development, and stress responses. This approach is widely used to study complex diseases, developmental mechanisms, and crop improvement. In agriculture, epigenomic analysis has played an important role in strengthening disease resistance, for example, in cassava, and in incorporating advanced tools like deep learning to enhance genomic research, thereby contributing to modern breeding strategies.

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