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DNA TECHNOLOGIES IN MODERN CROP IMPROVEMENT

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INTRODUCTION

DNA is a hereditary molecule present in the nucleus of a cell. The small steps of humankind to know the basics & importance of this molecule in the early 19th century have now much advanced in knowledge. It is a double helical structure made of a phosphodiester backbone with nitrogenous bases inside the backbone attached by hydrogen bonds, held in a staircase manner. DNA is called the hereditary molecule as it is the first important component of the central dogma of a cell, where it stores hereditary information of an organism. This stored information is transcribed to mRNA in the cell's nucleus, translating into different kinds of proteins performing important functions for an organism. Its hereditary property comes from the fact that it can generate its identical copy through replication. Processes like mitosis and meiosis maintain their number and quantity in an organism generation after generation. The knowledge about the molecule of DNA is exponentially increasing after humankind was able to sequence it in the early 1980s. Now, DNA based advanced technologies are available and are widely used, especially in the area of crop improvement. DNA markers are one of the important technologies impacting modern crop improvement through indirect selection. An ideal DNA-based marker must be reproducible, distributed throughout the genome, preferably codominant, and must have the ability to distinguish between individuals with maximization or polymorphic in nature. Apart from DNA markers, DNA is being used in many technologies that have become an essential part of modern crop improvement programmes (Agarwal *et al.,* 2008).

1. MOLECULAR CHARACTERIZATION

It is important to characterize an individual or group of individuals to classify them based on some shared characteristics. Before the DNA technological era, many morphological characteristics were used for classification purposes. However, knowing the genetic landscape of an organism has many added advantages, i.e., environmental independence. Nowadays, DNA markers are frequently being used in diversity analysis. Diversity analysis is very important for grouping, subgrouping, differentiating the plant species and phylogenetic studies (Ram *et al.,* 2007). It is particularly relevant to plant breeding; very different pools can be made based on molecular characterization. After that hybridization programme is formulated according to the short- and long-term goals, e.g., crossing between different heterotic pools in Maize crop to realize the heterosis phenomenon.

2. VARIETAL IDENTIFICATION

DNA based molecular profiling is now an essential component of the varietal registration process. DNA bands unique to a newly identified & released cultivar are essential for varietal characterization and identification. Simple sequence repeats (SSR) of the genome are the most frequently used genomic markers to characterize a newly identified cultivar for registration purposes (Nicholas *et al.,* 2012).

3. QTL DISCOVERY

Quantitative trait loci (QTL) are genomic regions that control a quantitative trait of interest. Due to the genomic abundance of DNA, markers like single nucleotide polymorphism (SNP) is widely used in identifying

QTLs for important traits like disease resistance, abiotic tolerance, grain quality, higher yields, etc. QTLs governing large phenotypic effects are of major interest to the plant breeders since their handling is comparatively easier compared to the QTLs with minor phenotypic effects. However, abundant DNA markers like SNPs have helped identify both major and minor types of QTLs. This is much relevant, especially in the era of genomic selection, where DNA technologies will greatly help accumulate major and minor QTLs in a single genotype.

4. GENE CHARACTERIZATION

Advancement in DNA sequencing technology has greatly benefitted gene characterization. The gene of interest can be isolated and sequenced on different sequencing platforms. The DNA sequences and mRNA sequences associated with the gene of interest can also be isolated and sequenced. Genetic sequence databases like GenBank at NCBI holds repository information for many such genes important in crop improvement of plant species. Computational biology and algorithms have helped further study the sequenced piece of the gene. Expressed parts of the genes (=exons) and the non-expressed portion of the genes (=introns) can be deciphered from the sequenced DNA. Methods based on DNA technology are now available, revealing the expression levels of a particular gene and the most abundant transcript (mRNA) belonging to the gene of interest with the help of real-time PCR.

5. GENETIC MAPPING

Genetic mapping is the localization of genes or features linearly or circularly corresponding to an organism's linear or circular chromosome. In the earlier days, when the idea was conceptualized in the times of T.H. Morgan and Alfred Sturtevant, the morphological features of the fruit fly (eye colour, wing type etc.) were placed on the linear chromosomes based on the results of various kinds of test crosses between two distinct parents. However, the number of such morphological features in a fruit fly or any other crop species is much less and hence the generated genetic map has limited improvement value. Thus, with the advent of the latest DNA technologies and the availability of high throughput DNA markers like SNPs, genome coverage is much magnified. Thus, even quantitative traits which were earlier very hard to explain genetically are now being pinned up at precise genetic locations.

6. GENOMIC SELECTION

The plant breeders often use conventional selection indices, mainly the correlated morphological traits, to improve a trait of interest. However, with the improvement in DNA based technologies, DNA markers on the genome of a crop species can be used for positive or negative selection of the individual plant by genomic selection. In genomic selection, the genetic estimated breeding value (GEBV) of an individual crop species is estimated based on its high throughput marker data, which predicts the phenotypic value for a trait of interest. The advantage of genomic selection in crop improvement is that it greatly saves field resources, reduces the breeding cycle, and ultimately enhances the genetic gain per unit of time.

7. GENE DELIVERY – RANDOM VS TARGETED

Conventional breeding utilizes the prowess of sexual hybridization for the introgression of genes of choice into a crop species. However, with the advent of novel DNA techniques, external pieces of DNA can now be injected into the organism to be modified. The targeted genes are delivered at a random or precise position in the genome of a crop species. Techniques like particle bombardment, Agrobacterium-mediated transformation patches the gene of interest at a random position in an organism's genome. Hence, after that, selecting the individual expressing the trait of interest in a desirable manner becomes imperative. However, the latest techniques like CRISPR-Cas can deliver the gene of interest at a predefined place in the genome.

8. TRANSGENICS

Modern DNA based technologies have pioneered the gateway for transgenic plants. Transgenics are genetically engineered plants containing foreign modified genes from different species or even a different kingdom. When the gene of interest (=trait of interest) is not available in the sexual hybridization pool (primary or secondary), genetic engineering techniques become useful in transferring such genes from an uncrossable species to the desired crop species. The added advantage is that a single gene and gene stacks can be combined

in a cassette or sequentially inserted into the crop species. Golden rice is the most relevant example of stacked genes, where genes are involved in the biosynthetic pathway of β-carotene formation, i.e., the precursor of Vitamin A in humans; are being inserted into the genome of rice plant from a plant daffodil (*psy* gene for phytoene synthase) and soil bacteria *Erwinia uredovora* (*crt1* gene for phytoene desaturase). Several traits like insect resistance, disease resistance, herbicide tolerance, abiotic stress tolerance (heat, drought, cold, etc.), improved yields and shelf life, etc., had been introduced through the transgenic approaches in different crop species.

9. BIOINFORMATICS

The world of DNA and its sequence information has opened novel frontiers in computational biology and bioinformatics. Bioinformatics is the field of science involved in interpreting biological data mainly of omics type (genomics, transcriptomics, metabolomics, proteomics) by employing interdisciplinary approaches like mathematics, statistics, computer science and information technology. Identification of novel candidate genes, their annotations & characterization, sequence alignment, multiple sequence comparison, genome assembly, predicting protein structure, protein-protein interactions, molecular docking etc., are some of the important uses of bioinformatics that are improving at a rapid pace (Varshney *et al.,* 2015).

10. COMPARATIVE BIOLOGY

Phylogenetic studies in the pre-DNA technology era largely depended on the morphological and geographical distinctions between the organisms for comparison. However, with the advancement of DNA marker-based techniques and whole-genome sequence information, evolutionary and phylogenetic studies have become more exhaustive with deeper interpretations (Gepts, 2006). DNA techniques have helped in studying orthologous genes, which might have originated from a single ancestral gene, but are present in different species due to speciation forces and perform more or less similar functions. Similarly, understanding the paralogous genes which are differentiated within a species due to events like intragenic duplication and performing different functions, had become more convenient with the advancement of technology. The use of chloroplast markers owing to their higher stability or mitochondrial genome for understanding maternal inheritance is adding new dimensions to comparative biology.

11. MARKER-ASSISTED SELECTION

DNA technologies like marker-assisted selection have greatly helped improve selection efficiency in a breeding programme. Robust DNA based genic markers or tightly linked markers are often used to improve a trait of interest in a crop species. Foreground selection is done to identify plants having genes of interest. In marker-assisted back cross-breeding, foreground selection plants with maximum background coverage are identified with the help of background selection. With the aid of marker-assisted selection, the breeder was able to breed improved varieties or hybrids like Vivek QPM9 (improved for lysine & tryptophan content), Improved Pusa Basmati (resistance against bacterial leaf blight), Improved Swarna (submergence tolerance) and Improved Samba Mahsuri (having submergence tolerance and bacterial leaf blight resistance).

12. GENE SPRAY

In modern crop improvement, futuristic advance researches are hinting towards the spray of desired segment of gene directly into the field crop for silencing a few key genes. A process called RNA interference (RNAi) is blocking the transcription or translation of a key gene with the help of small piece of matching RNA. This technique is highly contemplated by the researchers in public and large private companies to have biological value for protecting a crop species against number of biotic (viruses) and abiotic stresses. Moreover, in case of horticultural crops like flowers, genes involved in the colour biosynthesis can be targeted to generate flowers which are more appealing to the eye and hence having higher marketable price.

CONCLUSION

The advent of the latest developments in science revealed the genetic information in a cell, i.e., DNA. Later on, genome sequencing projects came up with whole-genome sequence information present in a particular organism. The genome sequence information has been utilized with advanced DNA technologies for various

crop improvement programmes. These technologies have accelerated the crop plants' genetic gains through indirect selection by utilizing DNA markers, varietal identification, gene, QTLs discovery, etc. Nowadays, various crop improvement programmes are underway nationally and globally to enhance crop plants' genetic yield, nutritional quality, biotic and abiotic stress tolerance utilizing these modern DNA technologies. These technologies would assist the conventional crop improvement programmes and ensure food and nutritional security at the national and global levels.

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