Conventional Plant Breeding to Modern Biotechnological Approaches in Crop Improvement

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Abstract

In an era of climate change along with increasing world population, the food security can be ensured by developing climate resilient crop varieties having good nutritional quality. Improved crop varieties can be developed through importation of desirable features which encompass the discrimination of available variability through introduction and selection as primitive method. Conventional breeding methods take more time in developing and delivering crop varieties. In the last two decades, modern biotechnological tools have been developed which led to the better understanding of the genetics of traits and are able to assist conventional breeding to release new cultivars in a shorter span of time. Thus, integrating modern biotechnological tools into the conventional breeding offers new opportunities to breed crop cultivars with enhanced quality, quantity, and tolerance to abiotic and biotic stresses. Besides, locally adapted, several landraces can be developed through plant domestication, farmer's selection, and self- and cross-pollinated methods. In this chapter, conventional breeding methods, modern and advanced biotechnological tools, viz., plant tissue culture, genetic transformation, hybrids generation, TILLING, RNAi, genome editing, and nano-biotechnology along with their importance will be discussed which would aid in transferring knowledge from scientist to farmer fields.

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1.1 Introduction

Due to the increasing human population and declining area under cultivation, crop improvement through plant breeding and biotechnological tools are one of the major approaches to meet out the present and future food demand. In past, domestication, introduction and selection of crop varieties led to the increased production and productivity in several agricultural crops (Chahal and Gosal 2002). The movement of new crops and varieties from its origin to different regions where it can adopt with desirable characteristics played a significant role to augment food demand in every part of the globe. Conventional breeding methods were followed for creation and discrimination of variability and development of new cultivars with superior yield and quality traits (Allard 1960; Fehr 1987; Acquaah 2012). The agricultural crop improvement methods are being continuously practiced from thousands of years to introduce the desired traits for the development of nutritionally enriched and high yielding food crops (Poehlman and Sleper 1999) (Fig. 1.1). The twentieth century is evident that crop production developed through conventional plant breeding methods has played an important role in ensuring food security and production (Tester and Langridge 2010; Shiferaw et al. 2013). However, with a rapid increase of world's population and challenges of climate change, these approaches have become insufficient for plant genome enhancement and development of nutritionally enriched crops, within a short period of time. Due to continuous demand for plant-based products and high annual crop yield, integration of conventional methods with modern biotechnological tools has provided an extensive range of innovation and achievements in plant breeding (Varshney et al. 2006; Li et al. 2018).

In the era of advanced biotechnological tools and deciphering the molecular mechanism of the desirable traits, it becomes possible to integrate modern breeding methods with the conventional approaches for the development of elite cultivars. Phenotypic selection made by crop breeder involves focused desirable trait selection. Ever since 1990s, molecular markers and modern plant breeding approaches were practiced for the selection of superior breeding lines based on the genetic makeup of the plant. Several conventional and modern breeding methods combined with genome studies can be used to enhance the accuracy of breeding practices within a

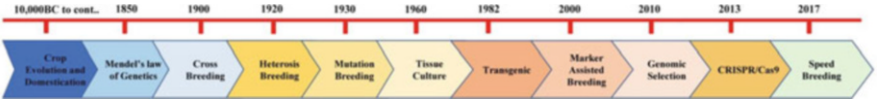

Fig. 1.1 Chronological development of crop evolution, breeding methods, and modern breeding tools and improving crops

short time span (Doust and Diao 2017). There are numerous examples to show that biotechnology plays a crucial role in understanding crop genetics and improving of food crops. The industrial use of many crops has been met through various biotechnological approaches. The ever-increasing demand for food has exerted pressure on the agriculture sector to speed up the breeding process. There is a requirement for highly productive resistant varieties with minimum inputs. One potential solution for these demands is the integration of biotechnological tools with breeding programs (Gosal et al. 2010; Beddington et al. 2012). Gene discovery has been accelerated through genomics, proteomics, and other biotechnological approaches. Identification of novel genes and introducing those genes into the desired species for plant breeding programs is of immense importance to crop improvement. To increase crop yield, quality improvement, resistance to insect pests, crop adaptability to adverse climate conditions, harvesting quality are the major target in the crop improvement program. The main objective of this chapter is to present the role of different plant breeding and biotechnological approaches—plant tissue culture, genetic transformation, hybrids generation, TILLING, RNAi, genome editing, and nano-biotechnology have been discussed which represents the enhanced methods of crop improvement.

1.2 Definition of Plant Breeding and Conventional Breeding

Plant breeding is a purposeful effort by humans to change certain traits of crop plants (Acquaah 2012). The process of evolution is also often linkened to plant breeding (Zohary 1988). The artificial process (orchestrated by humans) of selection is a relatively quick process rather than the evolution of crop spp. (Gepts 2002). The crop adaptability is increased due to crop evolution, whereas the aim of the plant breeder is specific toward population improvement and predetermined goal (Borojevic 1990).

An organized and thought-out method for developing high-quality cultivars with high predictability is now being practiced in plant breeding instead of the traditional approach of crossing the best with the best and hoping for the best. Major category of plant breeding approaches can be divided into two types, i.e., conventional and unconventional breeding programs, which keep changing with advanced methodology and technology (Chahal and Gosal 2002; Acquaah 2012). Conventional breeding methods employed in the development of new cultivars, which is averse to the newer, more advanced, and sometimes revolutionary tools of molecular plant breeding (Jain and Kharkwal 2004). By molecular breeding, sometimes manipulation of the genes in a plant may desecrate due to natural biological barriers, perhaps the ability to transfer desirable genes from alien species (Acquaah 2012).

1.3 Origins of Conventional Plant Breeding

Since the humans started to feed themselves, cultivation started with whatever plants they found edible. More than 10,000 years ago, farmers have been changing the genetic architecture of the available crops and selected the best-looking plants that grow well and used them for the upcoming season. This was the phase to start the era of domestication of plants (Gepts 2002). Then, the selection process is started with the availability of natural biological diversity and gradually increases the utility of desirable traits of the plants. Domesticated plants become more valuable and advantageous to the farmer concerning wild progenitors (Zohary 1988). Selection is the ancient and most widely used method in plant breeding for crop improvement. It consists mostly of phenotypes with a mental picture of the focus traits. Plant breeder discriminates, identifies, and selects the desirable plants among the available variability. Crop improvement techniques by the farmers were followed by the seed selection of best-looking and superior plants from their crop and using to next year's crop. The development of seed by this technique is called landraces (Allard 1960). Dramatic changes were observed in plant species as compared to their wild relatives when the selection process was faster with plant features like, crop duration, high productivity, resistance to insect and pest, seed quality, etc. In modern plant breeding, a rich source of variability is provided by the landraces (Harlan 1975).

1.3.1 Conventional Plant Breeding Forward with Science

Once the knowledge abounds, methodology advances, and science of genetics becomes better understood, plant breeders are gradually dependent on science and reduce the selection process (Chahal and Gosal 2002). However, conventional breeding still relies to some extent on instinct, judgment, and skill of the breeder (breeder's eye), in co-occurrence with plant breeder knowledge (Allard 1960). With the advancement of plant breeding science in the twentieth century, plant breeders created new and improved cultivars for different crops, increasing their efficiency.

The conventional plant breeding process has improved with technique over time, creating a productive framework for the enhancement of crop performance. During this process, the plant breeder decides which parents are to be used for pollination and which are to produce advanced plants. In plant breeding, creating a very large population is a benefit, unlike animal breeding. As a consequence, only a small proportion of the plants are selected for advance breeding program and the vast majority are discarded with undesirable characteristics. This process is applied during many stages to select few individuals from the large population.

1.4 Breeding Methods Based on Observed Variation

1.4.1 Origin of Crops-Plant Domestication

In modern cultivated plants, genetic variability tends to be decrease than in their wild relatives because of the *founder effect* in crop domestication (Breseghello and Coelho 2013). Wild progenitors of crop species were domesticated primarily before 10,000 years ago into the major crops worldwide (Doebley et al. 2006). The domestication process by ancient humans led to selection of rare mutants for adaptation of cultivation, resulting in most of the crop variations in the populations of the cultivated forms. So that many desirable genes related to insect and pest resistance were eliminated from the cultivated gene pool (Zamir 2001).

In the course of the development of crop domestication or breeding program, primitive landraces developed intermediate domestication levels, whereas in modern breeding, cultivars or varieties were developed, and that was the beginning of ideotype breeding. The ideotype concept was evolved to maximize the genetic yield potential and enhancement of germplasm (Donald 1968). Through the domestication and breeding steps within time, germplasm pools were extensively changed and move forward to the narrowing genetic diversity (Tanksley and McCouch 1997). In some examples, broader phenotypic diversity was observed by domestication which is exemplified by the shape, size, and color in tomato (Paran and Van Der Knaap 2007; Rodrigues 2011). In the case of maize, cultivars have developed with narrower phenotypic diversity as compared to their less domesticated landraces (Goodman and Brown 1988; Troyer 1999). Domestication and breeding programs demonstrate the historically useful and parallel domestication syndrome in various crop species. The most challenging task in modern plant breeding is the incorporation of desirable genes into the modern cultivars with the help of applications of molecular tools in the recent breeding program.

1.4.2 Landraces: Inherent Farmer Selection

Landraces are the region-specific popularize population and have been cultivated for many generations, which is resistant to biotic and abiotic stresses, seed handling, and developed on eating habitat. They are continually changing dynamic genetic entities either by the natural or artificial directional selection process and other mechanical impurities. Landraces are keeping their identity by stabilizing selection and also lead to the slow adaptation to environmental changes in a given region. In other cases, landraces quickly change their genetic architecture by the introduction into the different regions or due to the outcrossing with nearly cultivated original landraces. Although modern plant breeding methods are used to develop modern cultivars, landraces can be developed when farmers plant modern cultivars to create landraces. Several key characteristics of landraces have been identified (Zeven 1998): (a) high genetic diversity within populations with limited variation between individuals, (b) greater adaptability in different climate conditions with resistance to insects,

(c) more valuable edible parts picked by local populations, and (d) stable yield yields under normal climate conditions.

A farmer's selection is integral to shaping the varieties to a particular area and environment, based on local preference, well-serving and production being consumed locally. However, unintentional selection by farmer's for a desirable trait may lead to undesirable changes in landraces due to genetic correlations between them. The selection of landraces is based on edible parts such as panicles, ears, spikes in cereals, presenting the low harvest index and they are normally tall plants and prone to lodging. Landraces are one of the most beneficial genetic resources for breeding programs and used for germplasm conservation under "ex situ" conditions. The majority of germplasm conversion regulators encourage local commodities by financially incentivizing them to keep growing their landraces in their "in situ" conservation. This type of conservation will also take advantage of scientific communities for modern crop breeding. In terms of landrace breeding, it refers to initiatives that allow developing varieties that evolved through natural selection in the local cropping system, with or without mass selection. They are developed by using closely related crossing parents in a breeding program.

1.5 Self- and Cross-Pollinated Cultivars

Self-pollinated cultivars are developed either by a single plant seed or a mixture of plant seeds (Briggs and Knowles 1967). The two most common methods for breeding self-pollinated species are hybridization (crossing of pants) and selection (Acquaah 2012). A single plant or a mixture can be made into a final product. At present, several types of cultivar development methods are being used, such as pedigree, single seed descent, pure line, bulk, backcross, etc. Mass selection, synthetic, composite, and recurring selection methods can be used for a plant mixture.

1.5.1 Mass Selection

It is one of the methods that we can use for both self-pollinated and crossbred crop species with different genetic consequences (Allard 1960). It is also a population improvement method that increases the average performance of the base population by increasing the gene frequencies after the selection of desirable genes. This can use to maintain genetic purity, selection for lasting resistance, and wide adaptation to an area of production. When the heritability of the traits of interest is high, the mass selection is more effective (Brown and Caligari 2008).

1.5.2 Pure Line Selection

The varieties of pure lineages are genetically homozygous and homogeneous (Allard 1960). The uniformity is the main feature of such cultivars for the trait of interest with a narrow genetics base. Selection is followed; from a variable population to repeated self pollination until there is no segregation in the progeny (Poehlman and Sleper 1999). This was proposed by Louis de Vilmorin (1856) in self-pollinated crops, though the use of his principles in the nineteenth century by certain farmers (Allard 1999). Until now, homogeneity has prevailed and has been considered into breeding and agriculture, after the realization of this paradigm. In self-pollinated species, landraces are mainly of a mixture of pure lines, due to the low frequency (heterozygous individuals) of cross-pollination. In this case, the crossing of selected single plants develops progenies with higher productivity than their original landraces. However, these superior progenies have some disadvantages: they are less stable than the base population in terms of stress resistance and short-term adaptation under growing conditions. Pedigree, selection, bulk population breeding, single-seed-descent, backcross breeding, multiline breeding, cultivar blends and composites methods in the self-pollinated crop for crop enhancement.

1.6 Plant Breeding Methods Controlled by Mating

The ideotype breeding has been designed to improve gain for quantitative traits, especially in terms of productivity. It is a simple and straightforward method for complex traits by changing other simpler traits that are positively correlated with each other (Donald 1968). The main advantage of this method is, once the underlined hypothesis proves, that could encourage a significant gain for the product, even with a small or smart breeding program. Ideotype breeding can change the variation to the current plant type, beyond the boundaries of elite germplasm. Once the ideotype is assembled with the desired trait and inserted into an elite genetic pool, these lines can be transferred to the advanced breeding program.

The population breeding, a method designed to enhance overall phenotypic performance by increasing the frequency of favourable alleles which is dominant trait of interest in intermating population. Mass selection, is one of the simplest method of population breeding in cross-pollinated species, has developed a cultivar directly as enhanced population. Increasing the value of the working population from source lines is the main objective in modern population breeding improvement programs. Over-performing individuals are selected from average quality improved lines; with variations within those lines to be preserved. These outperforming lines can be used as release cultivars or parents of hybrids in case of self and cross-pollinated species, respectively.

In hybrid cultivars, which are measuring by heterosis, with superiority of individuals over their inbred parents (Shull 1948). Hybrid vigour, deteriorates abruptly because of inbreeding, indicating the presence of heterozygous loci. For this reason, maize breeding programs are currently focused on competitive F_1

hybrids development. In the plant kingdom, heterosis is more prominent in cross-pollinated species rather than self-pollinated ones. With increasing parental genetics distance, heterosis increase significantly (Springer and Stupar 2007).

The major challenges in hybrid breeding are: (a) extend of heterosis exhibited in crop plant species, (b) efficient pollination control mechanism, (c) seed rate of the crop plant and (d) efficient hybrid seed production methods to reduce the cost of seed. In Brassica, a high level of heterosis was found in spring and winter type of *B. napus* for seed yield, up to 40% heterosis has been reported in summer rape for yield and 60–70% for winter form (Lefort-Buson et al. 1982; Sernyk and Stefansson 1983; Erickson et al. 1986). Hybrids have a great advantage irrespective of business purpose, despite using mechanical emasculation ("detasselling" in maize) to avoid self-pollination in high yield for seed production. Due to the segregation of thousands of genes in genetically heterogeneous hybrid seeds, resulting in wide variations in agronomic traits, crop architecture, maturity duration, hereby reducing seed quality and overall yield if they planted in the next generation. So that, farmers have to buy fresh seed every year for the production of hybrid seed.

1.7 Speed Breeding: A Time Saving Tool

Researcher from the University of Queensland has coined the new term "speed breeding" as a method to accelerate the breeding speed in wheat crop. Now, it is has been developed in many crops (Watson et al. 2018; Ghosh et al. 2018). It does not require any specific equipment or technique like doubled haploids (DH), for the production of homozygous lines (Slama-Ayed et al. 2019). In a speed breeding program, using the optimum light intensity, temperature, and controlled day-length, increase the photosynthesis rate which is directly stimulates flowering and shortens to harvesting time (Watson et al. 2018; Chiurugwi et al. 2019). The wavelength and intensity of light are directly proportional to the regulation of flowering in plants. Early and late flowering genotypes were developed in peas, chickpeas, lupins, and faba beans under different light spectrum (Croser et al. 2016). Positive correlation between red:infrared proportion was observed in these species (R:FR) (Moe and Heins 1990; Ribalta et al. 2017). Reduces stem elongation and increases lateral branching by utilizing light with high R:FR and light with low R:FR, enhance stem elongation but reduces flowering and lateral branching. Speed breeding under controlled condition in greenhouse strategies increase the generation cycles by extended photoperiod (Lionneton et al. 2004; Ochatt and Sangwan 2008; Yao et al. 2020). Until now, speed breeding has introduced in some crop species, i.e., Wheat (*Triticum aestivum* L.), Chickpea (*Cicer arietinum* L.), sunflower (*Helianthus annuus*), pepper (*Capsicum annum*), radish (*Raphanus sativus*), and Amaranth (*Amaranthus spp*.) by extending photoperiods to shorten the crop duration (Stetter et al. 2016; Ghosh et al. 2018; Chiurugwi et al. 2019). International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) has also worked on crops such as pigeon pea, sorghum, and millet for developing a protocol for short-day plants. Another success story was reported in speed breeding of peanuts (*Arachis*

hypogaea). Induction of rapid crop development and early flowering is one of the main goal by improving protocol and required conditions (Chiurugwi et al. 2019). In conclusion, at present, cutting-edge genomics, modern plant breeding practicies, and innovative agronomic strategies have contributed to the development of improved cultivars that have led to remarkable agriculture outcomes (Ghosh et al. 2018).

1.8 Biotechnological Approaches

1.8.1 Plant Tissue Culture (PTC)

Plant tissue culture is an in vitro method of culturing cells, tissues, organs, single cells, protoplasts, embryos or whole plants (explants) on nutrient media under aseptic controlled conditions. Due to its high reliability and efficiency, PTC has played a crucial role in agricultural research and crop improvement during the past century. Various tissue culture methods have been used for crop improvement such as micro-propagation, shoot apex culture, germplasm conservation, haploid/diploid culture, embryo rescue technique, somaclonal variation, protoplast fusion, etc.

1.8.1.1 Micro-Propagation

Micro-propagation involves clonal propagation from small plant tissues and ensures rapid mass multiplication of genetically identical plants. Tiny parts of leaves, stems, buds, etc. are cultured on a synthetic medium in suitable containers such as glass jars. Later on, these are transferred onto the soil under greenhouse conditions. It is one of the successful commercial applications of PTC and has developed rapidly during the past five decades. It allows large scale production of plants in a smaller duration of time in the laboratory under controlled conditions. Many crop plants such as potato, sugarcane, sweet potato, yam, taro yam, turmeric, ginger, and cassava are multiplied through vegetative micro-propagation (Ahloowalia 2003). Micro-propagation has various advantages as compared to the traditional propagation methods such as rapid multiplication of true-to-type plants, production of disease-free plants (Brown and Thorpe 1995), and in some cases, increase in yield had been observed (Hussain et al. 2012).

Somaclonal Variation

Inheritable genetic or epigenetic variation is sometimes observed under in vitro conditions in callus cultures, isolated protoplasts or undifferentiated cells as compared to their donor plants and is termed as somaclonal variation (Larkin and Scowcroft 1981). The variation is usually due to chromosomal variation. Novel crop genotypes resistant to biotic and abiotic stresses, high and low pH, various diseases, salinity, herbicides could be obtained through somaclonal variation. Somaclonal variants had been reported in sugarcane (Heinz and Mee 1971), potato (Sharma et al. 2007), bananas (Sahijram et al. 2003), tomato (Bhatia et al. 2005), oats (Molnar et al. 2011), etc. and have improved traits. Notable commercial examples of

some clones include Bio-13 variety of medicinal plants *Citronella java* and Super tomatoes (Bhatia 2015).

1.8.1.2 Somatic Embryogenesis

In somatic embryogenesis, somatic cells or tissues (cells other than gametes) are induced to differentiate into embryos (without fertilization) and subsequently develop into whole plants. It is a valuable genetic manipulation tool in crop plants. It was used for the development of resistant varieties in cotton (Sun et al. 2003; Han et al. 2009). In vitro cultures of mature and immature embryos are possible for recovering plants from interspecies and intergeneric crosses.

1.8.1.3 Doubled Haploids

Anther or Pollen culture has been used for doubled haploid production. Under normal conditions, it takes around 8–10 years to have desirable gene combinations in homozygous forms. With haploid culture, homozygous true breeding lines can be obtained instantly. Anthers/pollens from an unopened flower bud are cultured into a nutrient medium to form embryoids or callus (haploid plantlets). These are treated with colchicine to obtain homozygous doubled haploid plants that can be used for field testing and selection (Gosal et al. 2010). From F_1 progeny in rice plants, doubled haploids have been produced in less than one year in a study (Davey 2009). Doubled haploids have been produced in wheat, maize, mustard, rapeseed, tomato, and pigeonpea (Guzmán and Zapata Arias 2000; Croser et al. 2006; Seguí-Simarro and Nuez 2007).

1.8.1.4 Genetic Transformation

It is one of the aspects of tissue culture where gene(s) of interest are transferred to the host plants and transgenic plants are obtained. Genetic transformation has a high potency of crop improvement by modifying its genetics and introducing various agronomically important traits. It can be achieved by vector-mediated indirect or vector-less direct gene transfer methods. *Agrobacterium* based vector-mediated method is widely used for gene transformation. DNA and RNA virus-based vector methods are also available for efficient genetic transformation of target regions. *Agrobacterium tumefaciens* are gram-negative soil born bacterium that causes crown gall disease in host plants. Naturally, they infect the wounded plant by transferring T-DNA located on their Ti (tumor inducing) plasmid which integrates into the plant genome (Tisser and Bourgeois 2001; Gelvin 2003). Using this method, plants were regenerated from shoot apices in Jatropha (Purkayastha et al. 2010) and new grass peas varieties have been developed (Girma 2010). Herbicide tolerance in cotton, canola, maize, and soyabean, virus resistance in papaya and squash, insect resistance in wheat, cotton, rice, potato and maize, abiotic stress tolerance in rice, beta carotene enriched potato, bacterial wilt resistance in banana, etc. had also been achieved (Gelvin 2003).

1.8.1.5 Protoplast Fusion

Protoplasts (cells without cell walls) from two different species or genera are fused to form new living entities and the process is termed protoplast fusion or parasexual hybridization. It is one of the best methods to overcome sexual barriers and pre- or post-fertilization barriers. Moreover, nuclear and organellar genomes from distant species could be combined through protoplast fusion. Fusions of protoplasts could be symmetric where whole genomes from both parents combine or asymmetric where partial genome from a donor is transferred to the recipient (Wang et al. 2013). It had been used for the production of somatic hybrids and cytoplasmic hybrids (Downey and Rimmer 1993). Protoplast fusion can be induced by using chemical (polyethylene glycol (PEG) with sucrose and calcium chloride), mechanical, and electrical methods. This method had been used widely in the polyploid *Brassica* species. In the past, various protoplast hybrids were made between different species such as Brassica and Arabidopsis (Arabido brassica) (Hoffmann and Adachi 1981; Bauer-Weston et al. 1993), Brassica and *Sinapis turgida* (Toriyama et al. 1987), *Eruca sativa* and *B. juncea* (Erussica) (Sikdar et al. 1990), *Diplotaxis muralis* and *B. juncea* (Chatterjee et al. 1988), *B. nigra* and *B. oleracea* (Narasimhulu et al. 1992), *B. juncea* and *B. oleracea* (Lian et al. 2011), *Raphanus sativus* and *B. oleracea* (Yamanaka et al. 1992), common wheat and *Agropyron elongatum* (Xia 2009), seedless citrus hybrids (Grosser and Gmitter 2011), cotton hybrids (Sun et al. 2011), etc.

1.8.2 Germplasm Conservation

Endangered genotypes could be conserved with in vitro cultures. Clones can also be preserved with tissue culture methods to conserve the genetic background of a crop. Moreover, the plant species that do not produce seeds or have recalcitrant seeds could be preserved via in vitro methods for long periods in gene banks. In vitro methods are coupled with cryopreservation.

1.8.3 Genomics and Transcriptomics

Genomes and transcriptomes are sequenced at a mass scale with next-generation sequencing (NGS) technologies. These produce a large amount of genomic information that allows the discovery of new genes and regulators. The molecular basis of the traits can be studied with this information which could be used further in marker-assisted selection for the development of crop varieties. Association mapping is one of the advanced genomic technologies for studying genetics of the desirable traits. Genomic technologies are very useful for studying the genetics of complex traits of multi-gene nature in polyploid crops (such as wheat and Brassica) where more than one type of genome is present. These tools allow the detection of QTLs (quantitative trait loci) and alleles with small effects. Various crops have large and complex genomes and their sequencing is a challenging task. Thus, transcriptome sequencing

is an alternative tool to understand expression behavior of the genes. Their is another important DNA sequencing technique - whole genome resequencing that allows the genome-wide discovery of markers such as SNPs (single nucleotide polymorphism) and the construction of high-density genetic maps (Bentley 2006).

1.8.4 TILLING and EcoTILLING

TILLING (target induced local lesions in genomes) allows screening of allelic variants in the target loci by screening mutant and germplasm collections. It is a reverse genetics approach that allows rapid and efficient detection of induced point mutations in mutagenized population. It is one of the powerful tools for crop improvement. In the original method, chemically (for example EMS) induced mutagenesis of the plant population is carried out. The seeds are treated with the mutagen and grown to produce M_1 plants which are self-fertilized to generate the M_2 population. DNA is extracted from leaf tissues of M_2 plants, followed by pooling and amplifying the region of interest. It creates heteroduplexes in the pooled DNA and then denaturing high performance liquid chromatography is performed to detect basepair changes (McCallum et al. 2000). EMS is the most widely used mutagen in TILLING and it produces transitions (G/C: A/T). DNA samples are normalized to avoid any biasing and then pooled together into 96 well microtiter plates, pooled samples are amplified for the target region of interest using fluorescently labeled forward and reverse primers (5′ end labeled with IRD700 and IRD800 dye). Heteroduplexes are formed during the denaturation and annealing cycles. An endonuclease CEL1 (isolated from celery) is applied for a short incubation period which recognizes the mismatch and cleaves the DNA at 3′ end of the mismatch. The digested fragments are separated on a denaturing polyacrylamide gel attached to an LI-COR 4300 DNA analysis system. Homo and hetero duplexes can be detected and the location of the mutation can also be estimated in the DNA analysis system. The detected mutation is later on validated by sequencing of that particular region (Till et al. 2003; Comai and Henikoff 2006; Barkley and Wang 2008). TILLING has been widely used in the model plant *Arabidopsis thaliana* (Till et al. 2003), *Lotus japonicus* (Horst et al. 2007), Pea (Triques et al. 2007), maize (Till et al. 2004), Barley (Caldwell et al. 2004), wheat (Slade et al. 2005), rice (Wu et al. 2005), soybean (Cooper et al. 2008), rapeseed (Wells et al. 2014), etc.

EcoTILLING is a type of TILLING where natural genetic variation in the population is analyzed instead of induced variation. It is useful in species where it is not possible to induce mutations. Naturally occurring SNPs can be quickly screened in a population with this method (Barkley and Wang 2008). This approach had been used in various studies including Arabidopsis (Comai et al. 2004), black cottonwood (Gilchrist et al. 2006), Barley (Mejlhede et al. 2006), melon (Nieto et al. 2007), mung bean (Fery 2002), etc.

1.8.5 Genome Editing

Genome editing is an advanced molecular biology method that allows the targeted alteration of an organism's genome in a precise, robust, and efficient manner. It is used to elucidate the gene functions and thus, contributing to crop improvement. This technique uses sequence specific nucleases that recognize specific DNA sequences and generate double stranded breaks. The plant's endogenous repair mechanism heals this double stranded lesion by homologous recombination or non-homologous end joining. It can lead to gene replacements, insertion or deletions, thereby generating gene knockouts (Gao et al. 2015). Originally, genome editing used zinc-finder nucleases (ZFNs) (Kim et al. 1996) and transcription activator-like effect or nucleases (TALENs) (Christian et al. 2010). Recent advances in genome editing employs the use of CRISPER/Cas (Clustered regularly interspaced short palindromic repeats/CRISPER associated system) technology that offers a simple and efficient method of targeted gene editing (Jinek et al. 2012; Cong et al. 2013). It has been used in various important crops such as wheat, rice, rapeseed, soybean, potato, tomato, cotton, barley, soybean, apple, oranges, watermelon, grapes, etc. (Zhang et al. 2018). Examples of genome editing used in various crop improvement programs include tiller spreading phenotype in rice (Miao et al. 2013), enhanced grain number and size in rice (Li et al. 2016), increased shelf life of soybean oil (Haun et al. 2014), increased oleic acid and decreased polyunsaturated fatty acids in *Camelina sativa* (Jiang et al. 2017), high amylopectin maize (Pioneer 2016), waxy potatoes (Andersson et al. 2017), browning resistant mushrooms (Waltz 2016), fragrant rice (Shan et al. 2015), purple tomatoes (Čermák et al. 2015), etc. Resistant genotypes had also been developed in wheat, rice, tomatoes, grapefruits, and cucumber (Zhou et al. 2015; Nekrasov et al. 2017; Zhang et al. 2017, 2018; Ortigosa et al. 2019; Zaidi et al. 2020). Genome editing has the potential to manipulate multiple genes simultaneously and thus, allowing stacking of genes. With this approach, a trait having complex genetics can also be manipulated.

1.8.6 RNAi

RNA interference (RNAi) is a powerful tool in molecular biology and genetic engineering. RNAi is based on the naturally occurring conserved defense mechanism against double stranded RNA of cellular and viral mRNAs. In this process, small non-coding RNAs (micro RNA and small interfering RNA) interfere with target mRNA translation and thus leads to transcriptional or translational repression and hence suppress the expression of the gene. These small RNAs in association with RISC (RNA-induced silencing complex), Argonaute and other effector proteins lead to the phenomenon of RNAi. RNAi construct is designed in a way that self-complementary sequence is homologous to the target gene and forms a hairpin RNA (Redfern et al. 2013; Wilson and Doudna 2013; Saurabh et al. 2014). RNAi has great potential for crop improvement in the fields such as improving the quality, reducing

the toxic substances, providing the resistance against various biotic and abiotic stresses, altering phenotype, in therapeutics, allergen and toxin elimination, and many more (Saurabh et al. 2014). Examples of RNAi for crop improvement include increased shelf life of tomato (Xiong et al. 2005; Meli et al. 2010), seedless watermelons (Varoquaux et al. 2000), restoring fertility in male sterile tobacco plants (Nizampatnam and Dinesh Kumar 2011), biofortification of tomatoes with antioxidants and essential elements (Niggeweg et al. 2004), flavor enhancement in canola seeds (Hüsken et al. 2005), lowering the allergen content in peanut (Dodo et al. 2008).

1.8.7 Nano-Biotechnology

Nano-biotechnology is one of the most promising technologies of the twenty-first century for sustainable agriculture practice. It combines the use of nanotechnology or nano-engineering in biology. It has high potential in crop improvement by increasing production, assuring sustainability, and lowering crop losses. Nanotools (nanobiosensors) helps in the precise, controlled and efficient management of agrochemicals such as fertilizers, pesticides and herbicides (Shang et al. 2019). For the detection of environmental stress and enhancing the plant's potential against diseases, nanosensors have high potential (Kwak et al. 2017). Agrochemicals are usually applied to the crops by spraying which results in ultralow levels reaching the target sites as there are losses by degradation or leaching. Nanotechnology offers controlled delivery techniques by nanoparticles that would release ideal amount of the agrochemical over the period and also, lower the harmful effects of over application (Nair et al. 2010; Pandey 2018; Rashid et al. 2018). Potassium nitrate was capsulated in graphene oxide films that slowly release the fertilizer (Zhang et al. 2014). The use of porous nanomaterials (zeolite, chitosan or clay) reduced the nitrogen loss by regulating the release on demand. They also increase the solubility of phosphate minerals, thus increasing the availability and hence uptake by the plants (Abdel-Aziz et al. 2016; Dwivedi et al. 2016). Nanotubes were used for extended release of active ingredients of pesticides, better control, and minimum environmental effect (Dwivedi et al. 2016). For foreign DNA and chemical deliveries inside the cell to manipulate the target gene(s), nanoparticles, nanocapsules, and nanofibers could be used (Torney et al. 2007). For instance, silica nanoparticles were used to deliver DNA inserts into tobacco and corn plants (Galbraith 2007). In the particle bombardment method of genetic transformation, nanoparticles delivery systems had been used (Vijayakumar et al. 2010). Nanoparticles based delivery had also been used for CRISPER/Cas9 technology (Mout et al. 2017).

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