Original Research Article

GENOMIC ASSISTED CROP BREEDING APPROACHES FOR DESIGNING FUTURE CROPS TO COMBAT FOOD PRODUCTION CHALLENGES

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Abstract: *Improvement of crop species has been a fundamental goal of mankind since the dawn of agriculture. The key to increasing agricultural productivity and improving other attributes has been plant breeding. The foundations of conventional breeding are the utilization of diversity, which occurs naturally in the form of land races and wild relatives, and the development of diverse selection and breeding techniques. Selection, which was the first strategy identified and is currently utilized by the majority of breeding programs, is the most fundamental aspect of plant breeding. There is a need to boost global food production in order to meet the rising demand of a growing population as a result of a growing human population and a changing environment, which have both heightened concerns about global food security. Conventional breeding methods are inadequate to supply this rising need. In the past few decades, numerous advancements in genetic engineering and molecular biology have led to the emergence of novel approaches that rely on phenotypic characterization. Now, the wide availability of molecular markers has facilitated the identification of variation sources and selection. Specifically, genomics played a crucial part in the revolution of plant breeding. Because genomics enabled the extended study of genotype and its relationship to phenotype for multigenic characteristics, allowing for a greater understanding of genotype and phenotype. In this overview, we will address conventional breeding methods and contemporary genomics techniques, and their function in crop improvement.*

Keywords: plant breeding, selection, breeding methods, genomics

Introduction

Improvement of crop species has been a basic objective of humans form the very beginning of cultivation about 9,000 years ago. Fertile Crescent of Middle East is believed to be the place where agriculture is believed to be started (Ceccarelli 2009). Plant breeding has been the key for enhancing crop yield and improving other characteristics. Plant breeding is a gradual process and it can be thought as evolutionary process that took place along with evolution of mankind. Human brought changes in plants and planting methods and as a result of this new characteristics appeared in these plants (Breseghello and Coelho 2013). The basics of conventional breeding are the use of diversity, which is naturally present in the form of land races and wild relatives, and developing various approaches for fruitful selection and breeding (Prohens 2011). The most basic technique and core of plant breeding is selection which was the first one to be discovered and is still used in most of breeding programs. In that era, those plants were selected that were most useful and productive for human requirements. Humans, thus, developed such cultivars that give more yield and resistant to insects. This resistance was incorporated in the plant from their wild relatives and this strategy is still used these days for developing resistance in plants cultivars from various stresses (Hallauer 2011). Crop improvement in the previous century is the result if both Mendel's work, molecular and genetic improvements that have resulted in
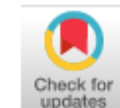
unparalleled productivity increase (Brown and Fedoroff 2004). In 19th century, another event that also contributed much to the modern-day crop varieties of rice and wheat was the Green Revolution which was result of the efforts of Borlaug who averted the expected famines. This event resulted in such crop varieties having genes which were dwarf and more fertilizer responsive (Khush 2001, Hedden 2003).

The increasing human population and a changing environment have raised significant concern for global food security (Scheben et al. 2016) and there is need to improve the global production of food in order to meet the increasing demand of growing population. Conventional breeding approaches are not sufficient to meet this increasing demand (Araus et al. 2008). Fortunately, during the last decades many developments have been made in the fields of genetic engineering and molecular biology which have resulted in the new techniques which rely on phenotypic characterization. Now, the availability of a vast range of molecular markers has assisted in identifying the variation sources and selection specially for those traits which are multigenic, in other words complex, and were previously difficult to characterize (Hallauer 2011). With the beginning of twentieth century where developments were made in in every field of science revolution also took place in plant breeding due to genetics. To be specific genomics played the vital role in the revolution in of plant breeding. Because it is genomics which allowed the extended study of genotype and its relationship with phenotype for multigenic characters (Tester and Langridge 2010). In this review, we will discuss about the conventional breeding approaches, modern genomics tools and their role in crop improvements and why these are necessary to be used in development of crop varieties.

Evolution of crops and breeding methods

Crop domestication took place when agricultural practices were started. Many wild species were transformed into crop plants as a result of which changes took place which were genetic in nature. With the passage of time some breeding methods were developed for crop improvements which mainly depend on the apparent selection. With the assistance of these methods, improvements were made in yield and other characteristics and a large number of varieties have been developed using these traditional practices. Where evolution has been a huge contributor in improving crops and adjusting them to the changing climatic conditions, it has also increased genetic complexity. For example, *Triticum*was diploid in the beginning then it became tetraploid naturally further got transformed into modern day hexaploidy wheat (Reif et al. 2005). Still diploid and tetraploid forms of wheat exist naturally. Similar changes occurred in rice, cabbage, tomatoes and oranges (Fedoroff 2010). Compared to animals, transposable elements and repeats are more in plants which are major factors in reshaping genomes. It is clear that plant genomes have more repetitive sequences and show more polyploidy (Morrell et al. 2012). Polyploidy, increase in gene content by whole genome duplication or chromosome doubling in result of inter-specific and inter-genic hybridization, also poses difficulty in locating doubled genes in crops. With the advent of time, where most of the plants have evolved and adjusted to the changing environments, there are also wild ancestors of some plants in their natural habitats and germplasm centers have been set up worldwide to conserve these valuable resources in the form of seed banks. These facilities have been of much use in crop improvement by acting as a source of useful traits such as increased yield and resistant to biotic and abiotic stresses. Improved crop attributes rely on the stability and uniformity of the traits which are transferred (Adlak et al. 2019). In past farmers also faced many problems like attack of insects and pests, lodging of crops with tall and thin stem, yield loss due to various abiotic stresses and shattering of ripened seeds. With the passage of time and improvement in breeding practices, many of these problems are under control. One of the practices used for this purpose is use of wild relatives of plants. Wild relatives of our cultivated crops are naturally resistant to various biotic and abiotic stresses and this characteristic is utilized by crossing the cultivars with their wild relatives. In result, we get a variety of offspring some of which have desirable characters and are selected for further varietal improvement. Various breeding methods used for crop improvement are mass selection, pure line selection, pedigree method, bulk population method, single seed decent method, back cross method, marker assisted selection and recurrent selection method (Heffner et al. 2010; Moose and Mumm 2008;Nakaya and Isobe 2012). In these methods, plants are selected on the basis of their morphological characters in other words phenotype.

Various breeding methods, their advantages, disadvantages and time required for variety development are shown in table 1 (Bisognin 2002; Acquaah 2009).

In addition to these, there are other methods which are developed by modifications in these methods like half-sib and full-sib family selection with or without progeny testing and test cross, ear to row method.

Along with many benefits traditional breeding approaches also have some short comings. For instance, conventional breeding methods require hybridization of plants, variety improvement procedures take seven to twelve years and when two parent plants are crossed along with useful traits some others which are undesirable also get transferred because of linkage phenomenon. This can be overcome by repeated back crosses with donor parent in case of characters which are controlled by single gene. This is the simplest possible case and a person can be successful if he is as lucky as Mendel was. In other cases when a single gene is involved in more than one function or multiple genes control one trait then it becomes complex to get the expected result. In addition to pleiotropy there are some other phenomena which affect the process of crop improvement by traditional approaches like epistasis and some others (Cao et al. 2001;Chai et al. 2018).

With some improvement and modification some other methods were developed including heterosis breeding, polyploidy breeding, mutation breeding and ultimately leading to molecular breeding (Wu et al. 2004;Wu et al. 2010;Sattler et al. 2016; Oladosu et al. 2016). Indeed, conventional breeding methods are labor intensive, time consuming, less efficient and environment dependent.

Genomic approaches in crop improvement

Continuous varietal improvement through conventional breeding needs biotechnology to maximize the chances of success. Modern breeding methods have resulted in consistent crop improvement by manipulating variation within cultivated gene pool. Population level genotyping has provided opportunities to identify the widespread genomic variation within specie (Huang and Han 2014). In case of multigenic traits, those genotypes are selected from germplasm that show transgressive segregation that is foundation for breeding programs. Moose et al. 2008emphasize how the application of molecular plant breeding is now contributing to discoveries of new genes and their functions. Watson et al. (2018)focused on the integration of speed

breeding with other modern crop breeding technologies including high throughput genotyping, genome editing and genomic selection. In plant breeding, biotechnology deals with problems in every aspect of agricultural production. This includes increased and stabilized yields, enhanced resistance to pests, diseases and biotic and abiotic stresses like drought, salinity and cold, and to improve the nutritional quality of foods.
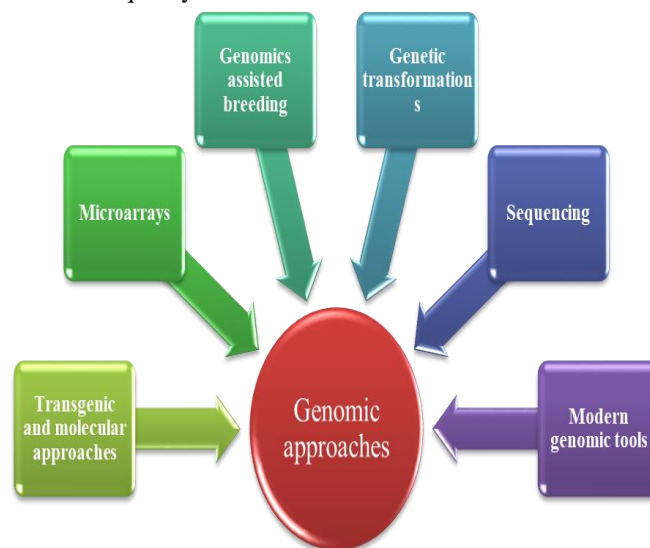

Figure 1. Various genomic methods for crop improvement

Transgenic and molecular approaches

The major aspects of biotechnology utilized in plant breeding are transgenic approaches and molecular breeding methods. The most utilized method for crop improvement is molecular method, where we use DNA markers and improve variety by marker assisted selection (Leng et al. 2017; Tester and Langridge, 2010) that is faster and better approach to select better phenotypes of plants. Talking about MAS, which is important tool for indirect selection of various characters at early stage before progressing to next generation thus making variety improvement quicker. Emerging from the advancements in genomics there are tools which are used in identification or markers which are linked to genes and this approach has made possible the development of near isogenic lines, recombinant inbred lines, chromosome segment substitution lines (Prohens 2011).

Table 1: Traditional breeding methods and their attributes

Sr.No	Name of method	Crop Developed	Time for variety development	Advantages	Disadvantages
1	Mass Selection	Sunflower	3 years	Simple and rapid breeding method Inexpensive to conduct	Needs progeny testing, less phenotypic uniformity, selection is phenotype dependent
2	Pure line selection	Wheat	10 years	Rapid method Inexpensive to conduct High uniformity achieved	Purity of cultivar may alter, Cultivar developed has narrow genetic base, new genotype is not created rather improvement occurs.
3	Pedigree selection	Cucumber	10-12 years	Genetic information of traits available, selection is based upon both genotype and phenotype, selection of only progeny lines can be done, high genetic purity	Record keeping high slow, tedious, expensive and time consuming, less suitable for quantitative traits, long procedure, selection is done in F_2 which is less effective.
4	Bulk population method	Cotton	9-11 years	Simple and convenient method, less labor intensive, large amount of segregating population can be handled.	Superior genotypes may be lost in early generation, genetic characterizations are difficult, lengthy procedure.
5	Single seed descent method	Tomato	9 years	Easy and rapid way to attain homozygosity, can be conducted in green house as small place is required, duration of program can be decreased.	Phenotypic selection so no progeny performance involved, the selected seed may not germinate later.
6	Back cross breeding	Rice	11-13 years or more	Less field testing needed, repeatable, does not allow new recombination to occur, applicable for bath self- and cross-pollinated species.	Not effective for quantitative traits, undesirable linkages may occur, more time consuming in case of recessive traits.
7	Recurrent selection	Soybean	Variable	Repeated intercrossing can break linkage, applicable for both monocots and dicots.	Extensive crossing required, sufficient seed may not be available after intercrossing, desirable linkages may also break.

MAS have been of much importance in maize improvement. Maize is also helpful for genetic and molecular studies as many tools have been developed, including large-scale collections of mutants as a result of chemical mutagenesis and transposon additions (Takeda and Matsuoka 2008). More improvement has been done in barley as compared to wheat because of its simple genetic makeup (Varshney et al. 2006).

Microarrays

Another genomic tool used for studying the expression is microarrays (Rensink and Buell 2005) which give the opportunity to study the expression of thousands of genes at same time in conditions when plant is under certain stress or other conditions. DNA or proteins are arrayed on solid surface that can be a glass slide, nylon membrane or any other material and then expression is calculated by fluorescent or chemiluminescent signals produced as a result of target and probe hybridization (Aharoni and Vorst 2001). DNA microarrays have been constructed from cotton fibers (Stan and Difazio, 2003; Wullschleger and Difazio 2003), maize and soybean (McGonigleet al. 2000).

Genomics assisted breeding

With advancements in the field of breeding, the focus has diverted from phenotype-based selection to genotype-based selection. A whole new aspect of breeding aided by genomic approaches, known as genomics assisted breeding (GAB), has proven of much assistance in the development of new cultivars which are better as compared to the varieties developed by traditional breeding approaches. GAB allows breeders to begin with a large population of only those offspring which are genotypically characterized and then use only a selected subset for more expensive phenotypic evaluation (Leng et al. 2017). Thus, it saves time, resources and energy. Shattering is one of the major problems which cause the loss in yield of canola. Resistance to shattering has produced in canola by the application of genomics assisted breeding and Bayer has released some new cultivars which are shattering resistant (Lambert et al. 2015). In addition to this, various other multinational companies have developed and released soybean, sunflower and maize varieties with the help of marker assisted breeding (Eathington et al. 2007)

Genetic transformations

After the Green Revolution in 1960s, efforts have been made for developments of methods to produce transgenic organisms and it has been achieved in many species. With the discovery of plasmid in bacteria, a self-replicating chromosome, the idea generated that these plasmids can be converted into recombinant plasmids containing foreign gene, encoding a useful trait. Efforts were made which resulted into the production of many copies of gene of interest as the bacteria hosting the gene multiplied. This cloning technique is the foundation of modern day's genomic revolution (Federoff, 2010). Using this principle many other methods were developed including use of *Agrobacterium tumefaciens* as vector for transforming genes into plants (Gelvin 2006). *Bacillus thuringiensis* (Bt) toxin genes and herbicide tolerant genes have been introduced in several crops like cotton, corn (Vaeck et al. 1987), soybean (Koch et al. 2015) and some others using transformation. Peanut has been transformed with Bt using microprojectile bombardment (Singsit et al. 1997). All Bt crops, most used are cotton and corn (Abbas 2018).

Sequencing

Sequencing is also one of the techniques used to detect the presence and function of new alleles. This is done either by directly knowing the sequence or by comparing it with already available reference sequences (Varshney et al. 2009). Sequencing is of much assistance in improving a crop's genetic makeup. Sequencing by Sanger's method is one of the earliest used methods for whole genome sequencing but is costly and time requiring that has limited its use in large populations. Because of rapidly developed high throughput sequencing techniques, a number of genetic maps have already been developed by using single nucleotide polymorphism (LIU et al. 2020). Next generation sequencing (Singsit et al.1997) technologies have covered the flaws of previous techniques and are now used widely in crop breeding field (Ray and Satya 2014). Techniques based on NGS are used in discovery of Single nucleotide polymorphism (SNP) and genotyping at the same time accelerating the sequencing process. By using the next generation sequencing many crop genomes have been sequenced like soybean (Valliyodanet al. 2017; Yanget al. 2015) and barley (Takahagiet al. 2016).

Modern genomic tools

As plant traits like yield, plant height, disease resistance, stress resistance and many others are controlled by genes and for improving these characteristics genome editing. Firstly, Zinc finger nucleases (ZNFs) were used for this purpose. ZNFS are proteins which can cut DNA sequences at specific site. These facilitate gene editing by creating DNA Double Stranded Breaks (DSBs) and target gene is replaced (Carroll 2011). After ZNFs, Transcription Activator-Like Effector Nucleases (TALENS) emerged and were used as their alternative. TALENS differ from ZNFS is this aspect that they contain non-specific endonucleases while those in ZNFs are specific to highly conserved repeats of DNA (Mohanta, Bashir et al. 2017). Then, CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic
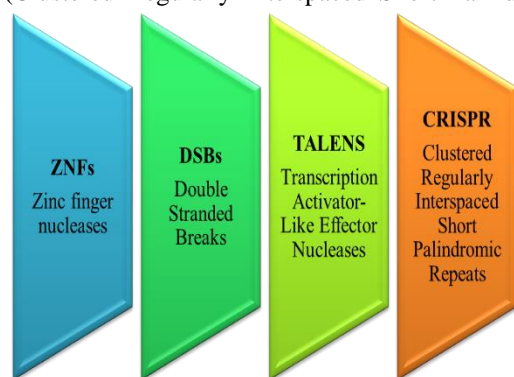
Repeats) technique, which is widely used these days, brought revolution in the field of genome editing. Cas9 is an enzyme used as molecular scissors to cut DNA strands. All that is needed is guiding RNA and Cas9 protein (Redman et al. 2016). Various studies have been under gone describing the function of Cas9 in a number of plants like wheat (Upadhyay et al. 2013), Arabidopsis, rice, tobacco (Jiang et al. 2013; Nekrasov et al. 2013). In short, genomics-assisted breeding is thought to be the best option to meet the challenges and ensuring a sustainable enhancement of food production by adapting available crops to various stresses and breeding novel crops (Batley and Edwards 2016). Countless efforts have been made and are undergoing in order to cope with various stresses and to increase yield to fulfill the demand of increasing population.

Fig: 2 Modern genomic tools

Conclusion

Plant breeding is a vast field and enormous development and improvement has been made during the last century. A lot of varieties have been developed using traditional approaches but with the passage of time it became difficult to meet the demands of increasing population. With the incorporation of biotechnology in breeding it has become easy to develop plants with desirable characteristics within short span of time thus covering the flaws of conventional breeding approaches. And now the advancement in genomics is letting the addition of new tools and techniques to cope with confronting challenges in agriculture.

Conflict of interest

The authors declared absence of conflict of interest.

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