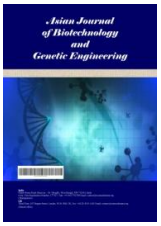

*Asian Journal of Biotechnology and Genetic Engineering*

*4(4): 14-26, 2021; Article no.AJBGE.76031*

# A Detailed Review of Conventional and Modern Breeding Technologies and Approaches of Field Crops

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

***Article Information***

Editor(s):
(1) Dr. S. Prabhu, Sri Venkateswara College of Engineering, Sriperumbudur, India.
Reviewers:
(1) Ivan Schuster, Brazil.
(2) Aibuedefe Osagie Eremwanarue, University of Benin, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/76031

***Review Article***

***Received 25 August 2021***
***Accepted 02 November 2021***
***Published 09 November 2021***

## ABSTRACT

The existence of genetic variability in plant material is considered to be the basis of crop improvement. A plant breeder finds this variability in various ways to create new crop varieties which are capable of withstanding different types of stresses including drought, temperature, diseases, insect pest attacks and unfavorable soil conditions. Breeders obtain their purpose and target varieties by using different breeding technologies, selection methods and improved approaches. This review presents an effective sketch of conventional and modern breeding technologies. Conventional breeding methods include the introduction, selection methods and hybridization. All these methods are categorized further into different types like mass selection, progeny selection, pure-line selection etc. Similarly, conventional breeding methods of hybridization include a pedigree method, bulk method and backcross breeding method. Among them, the backcross breeding method is being used widely to get better and fast results in a variety of development processes. Using conventional breeding methods, there are many varieties developed in the recent past like "Dirk" and "Penjamo-62" of wheat, "IR-6" variety of rice and "Beechar" of barley. The Final portion of this review explains modern breeding technologies which are being used widely all over the world. These methods are categorized into three different types. These

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include shuttle breeding, speed breeding and double haploid breeding technologies. Among them, speed breeding reduces the breeding cycle and accelerates the crop research process through rapid generation advancements. This can be carried out in many different ways. One of them includes the extension of the duration of the plant's daily light exposure. In short, we can easily get the targeted results in a short time duration by using modern breeding technologies and selection approaches. The whole concept gives an amazing figure to the new crop researchers in conventional and modern breeding technologies and selection methods.

*Keywords: Conventional and modern technologies; common breeding methods; breeding approaches of filed crops.*

## 1. INTRODUCTION

The primary purpose of plant breeding is the development of cultivars that have superior characteristics. For the development of successful cultivars plant breeders outline is to increase the chances of creation and identification of superior genotypes which would have all the required characteristics which are useful in the production system. Plant breeders create and manipulate the genetics of crop species so that they would obtain their desired recombination's. Plant breeding is playing the main role in for the development of human cultivation. The yield of crops is improved through conventional plant breeding by genetically manipulating characteristics which are agronomically important [1]. But conventional breeding has some drawbacks due to which it is dispraised like it doesn't fulfil the requirements of poor and marginal farmers and also it disregards the indigenous germplasm. A new term participatory plant breeding (PPB) is introduced in which conclusions are made for plant-breeding programmes through the involvement of creators and other investors. In 1996 working group on participatory plant breeding was established under the frame of a consultative group on international agricultural research (CGIAR) [1]. Conventional plant breeding is grown gradually with time and it creates a structure in which the crop performance is enhanced and also supports the production of food with nutritious and safe for consumption. In-plant breeding, there are numbers of plants and we select only some superior plants and this selection process is applied through different stages like trait mapping, trait introgression and then testing in the field [1]. Trait introgression main purpose is to carry the desired traits from the parent plant and introduce these characteristics into parental varieties germplasm. For the introduction of traits, two types of DNA markers are used by the plant breeders like the genome-wide markers from the commercial-track varieties trait-linked marker established from trait mapping [1]. Selection of living organism i.e., all plants, animals and microorganisms has been done or practiced since life was first formed [1]. There are two types of selection i.e., Natural selection and artificial selection. If we talk about natural selection then all the diversity in plants as well as in animals is the result of natural selection. It has the criteria as which is more fit for adaptation will survive and the living thing which is less fit for adaptation will become extinct. The main aim of plant breeding is the selection of desirable trait combinations that are best adapted and suited agricultural systems [1]. The breeder must have the ability to find out the variation in plants phenotypically and some of those variations must be genetic. A plant breeder has a proper work that what characters or traits are to be selected and at what stage selection will be applied. Selection is typically done on visual (marker-assisted selection) valuation of significant, highly heritable characters. Plant selection is always performed by plant breeder, for example, in the case of maize scattering and reproduction is ensured by man, hence it is also called pampered corn [1]**.** Hybridization is a very simple and versatile process. By reducing allelic effects, hybridization may help to move biological occupation. The hybridization processes increase the generation of novel adaptive phenotype or genotype and genetic variation. Hybridization is a method in which new varieties are produced by crossing two distinct genetically different crops. This method is applied to both cross and self-pollinated crops and its main objective is to develop variations [2]. According to a survey in 2015, the world population in2050 will rise up to 9.7 billion and as the population will increase there will be an increase in the demand of food which could be overcome by increasing the production of food by at least 70%. With the limited natural resources, it will be a great difficult task to increase food using conventional methods. Therefore, the present, all the attention has been governed to produce crops more

efficiently with the usage of the least resources [3]. Double haploid is one of these modern techniques which are recently getting studied to produce new crop varieties with more efficiency and within the least cost of time and increase the genetic gain [3]. One of the main problems in conventional breeding that most of the breeder's face is the high load of recessive mutations that occur in the genetic pools of CP crops especially maize. To overcome this problem by conventional methods, several inbreeding generations are required with great vigor plants but with the usage of in vivo haploid technology we can not only delete the recessive mutant alleles from the gene pool faster but it is also cheaper than other techniques because of complete homozygosity in just one generation [4]. The role of DH technique is not only limited to the complete homozygosity but it is also used in the selection of traits for using it as a variety in SP crops and as an inbred line in hybrid development. It has also been used to produce salt-resistance and drought resistance in various crops like rice and wheat. Nowadays, DH technique is getting used as a regular method for inbred development especially in maize because it offers great potential for the selection process [5]. With the increasing population in the world, the agricultural lands are now changing into industrial areas. This industrial land cause global change that leads to many biotic and biotic stresses. This stress lowers the yield of the crops badly. In this whole scenario, conventional breeding methods for crop improvement is not appreciated anymore. So, the world is now introducing some modern methods of breeding such as speed breeding, shuttle breeding and DH technology [6]. By using these methods, we can speed up the process of breeding. Speed breeding is the process in which almost 6 generations can be generated in one year with the help of controlled conditions. If there is drought or salinity stress in the world, the conventional method took almost 14 years to develop a resistant variety against stresses but with the help of speed breeding, this work can be done in 2-3 years [6]. Speed breeding is a potential tool to develop a variety in such a short period. Speed breeding is not a new concept but it came into existence a century ago. At that time scientists grow plants in a controlled environment providing artificial light. Years later, work has been done on the effect of different intensities of light on plants. After that different universities start their work on speed breeding [7]. Shuttle breeding is a modern crop improvement model in which a crop is grown in contrasting environments for the purpose to develop high yielding, adaptable and resistant varieties at a faster rate (4-6 generations) than conventional breeding methods [8]. This programme was launched by CIMMYT to develop high yielding, superior and adaptable wheat cultivars throughout the world and their large-scale production with rapid generation advancement, by combining wheat cultivars in high and low altitudinal areas of Mexico. Norman Borlaug is the pioneer of it [9]. In Japan, the shuttle breeding system was introduced to develop early maturing wheat cultivars by exploiting wide latitudinal variations. The early maturing wheat cultivar named 'Daichinominori' has been developed through this technique [10]. Disclosure of breeding material to diverse ecological conditions helps to identify adaptable and highly productive germplasm via selection for different biotic and abiotic stresses. The main objectives of shuttle breeding programme are:

- To speed up the process of breeding cultivars by shortening the time period.
- To determine effects of selected environments on the performance of a crop and genetic variations in inbred lines.
- To assess yield potential and genotype variability

## 2. CONVENTIONAL BREEDING METHODS

Conventional plant breeding is the production of hybrid varieties and it is continuous for hundreds of years. In conventional plant breeding by the use of tools plant genomes are manipulated for the improvement or development of cultivar. By the methods of selection, new plant varieties are produced in conventional breeding. Following are the methods of conventional plant breeding:

Plant Introduction
Selection
Hybridization

When any genotype is introduced into a new environment from the environment where it is typically grown is called plant introduction. It has further two types like the primary and secondary introduction. Selection is one of the oldest methods of plant breeding is selection. Selection is the choice of certain individuals from a mixed population for one or more desirable traits. It has further two types like natural and artificial selection [11]. When new crop varieties are

produced through the cross of distantly related varieties or species it's known as hybridization. It has types like intra-varietial hybridization, inter-varietial hybridization, interspecific hybridization and intergeneric hybridization. From the past few year hybrids produced through conventional breeding had a very remarkable effect on agriculture production. Besides advantages, conventional plant breeding also has its limitations as it can only occur between those plants that are able to sexually mate with each other and with the transfer of desirable traits undesirable traits are also transferred. By conventional methods, more time is required for the achievement of desired results [11].

## 2.1 Introduction

Introduction of genetic material in such an environment where it is never grown earlier. After this introduction of new genetic material proves its value then it will be released for the general cultivation of people. This introduction can also be used in the hybridization programmes for the transfer of characters. Before the release of this introduced material for general cultivation, it could be improved by artificial and natural selections [12]. Before the introduction to new environments also make sure that the material is safe and have no signs of diseases like viral and bacterial. There is also a need for adaptability in genetic material when introductions are made into different environments therefore it should be between areas of similar climatic conditions so there would be no issue of adaptation in new environments. If the introduced material is adapted to the new environment it is called acclimatization (14). Acclimatization depends upon the following factors:

- Cross-pollinated crops are more helpful in acclimatization due to the formation of new recombination's as compared to self-pollinated crops.
- Heterozygotes have more genetic variability or form more combinations after segregation that's why more adapted to new environments as compared to homozygotes because in homozygotes no new combinations formed due to the absence of the segregation process. Homozygotes have very few chances for adaptation to the new environment nor not.
- Annual crops have more probability of adaptation to new environments than perennial crops because many generations are produced by annual crops till perennial crop produces one generation so in every new generation of annual crops new combinations are formed which increases the chances of adaptation to the new environments as compared to perennial crops.
- A high mutation rate is also helpful in acclimatization in case the period of acclimatization is long because more mutants are produced when the mutation rate is high [13].

Introduction has following two types:

1. Primary introduction
2. Secondary introduction

### 2.1.1 Primary introduction

The introduced plant material can be released for general cultivation in case it has no issue in adaptation to the new environment then there is also no need to modify the genetics of the original genotype.

### 2.1.2 Secondary introduction

In case the introduced material is not adapted to the new environment then it is exposed to the selection process through which only superior plants are selected or there is another method in which hybridization process occurs between superior plants and local plants so that the specific characters can be transferred from the superior to the local one.

After the recognition of varieties desirable characteristics, these were combined by the process of hybridization. Through hybridization genotypes that have gene combinations superior to parents are selected from segregating populations.

From the historical point of view, international merchants called south East Asia which also include [12]. Pakistan golden sparrow because these areas have high production of cotton. Gossypium arboreum which is also known as Asian cotton and Gossypium herbaceum which is commonly called Levant cotton both are cultivated cotton. Another type of cotton called Gossypium hirsutum was also introduced in the eighteenth century because it has features like good quality. America, Peru, Brazil, Egypt and Sea Island introduced many cotton strains in the next few centuries. 95% of the cotton cultivated area is under American cotton. Likewise,

Sunflower, Maize and Soybean crops were also introduced from many other countries. In sixties a variety of wheat which has characteristic for short stature is introduced from Mexico. Now many hybridization programnmes are using wheat and producing various varieties with high yields. Our wheat adapted varieties are 'Yecora', 'Mexipark', 'Senora', 'Pitic' and 'W1-711' [12].

## 2.2 Selection

The selection method is an ancient and the simplest method of plant breeding. It is sometimes called as German Method as it was firstly used to improve certain crops like sugar beets, rye, wheat etc. We can define selection as the conservation of assured plants having required traits. It is the basic method of crop upgrading [13]. It ensures figuring out & breeding discrete genotypes and from the bulk population or segregating progenies. To make selection more effective there should be genetic diversity or genetic variability that can be recognized and different from ecological variations. As we know, many ancient or early varieties were genetically mixed so the selection was performed to isolate the genotype having high yielding capacity from those mixed genotypes [13]. There are two methods that are used in the selection method:

- Pure line Selection
- Mass Selection

### 2.2.1 Pure line selection

It is defined as the process of isolating the desired homozygous plants individually from the varied population. It is the descendants of a single homozygous self-pollinated crop, harvesting them one by one, assessing best progeny to release as a new variety. Hence, we also call pure line selection as individual plant or single plant selection. A variety developed by pure line selection is more accurate or uniform than mass selection. Its concept was first described in 1903 by a Danish botanist Johannsen during his work on beans variety "Princess". This strategy has also been used in many other breeding programs like coffee, beans, rice etc [13].

It has three steps:

- Selection of plants from a homozygous local variety but a variable population individually.
- Progeny test
- Yield trials [13]

### 2.2.2 Mass selection

In mass selection, a large number of plants are selected on the basis of their similar phenotypes and then their seeds are mixed together to develop a new variety. A cultivar developed by the mass selection method will be uniform phenotypically that can be recognized and used as the basis for selection [14]. In this method, plants are chosen on the basis of phenotype and seeds that are harvested are then composited without progeny test. It has two major applications in self-pollinated crops that are given below:

- For the improvement of desi variety or local variety
- For the purification of pre-existing pure lines

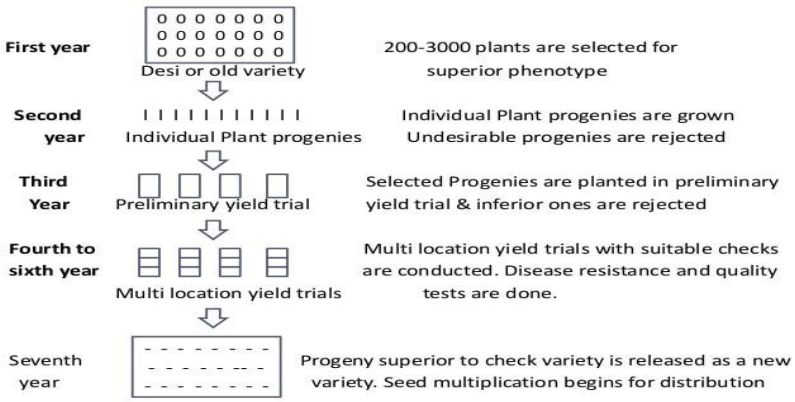


**Fig. 1. Pure line selection**

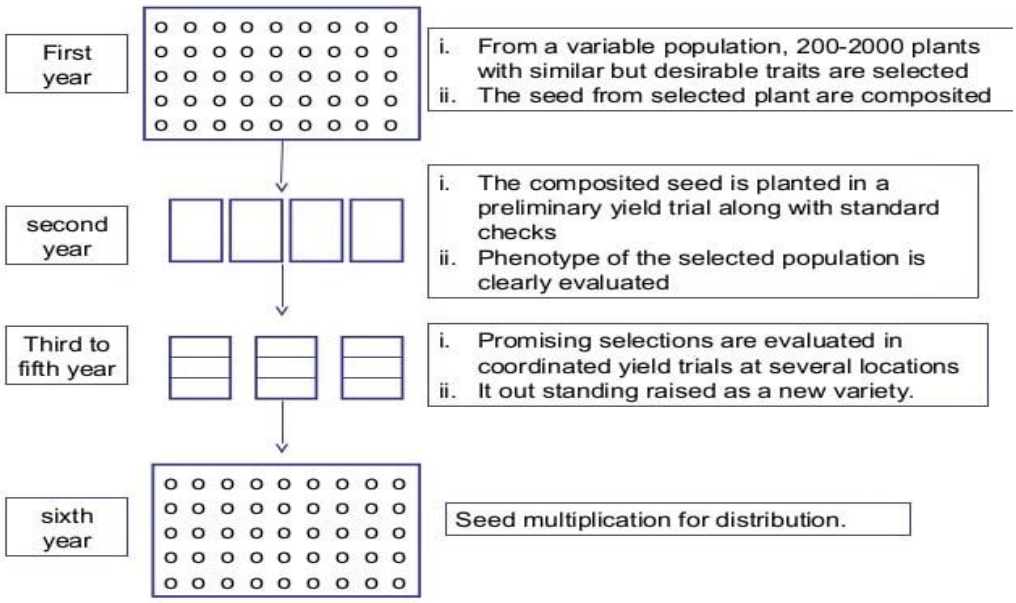


**Fig. 2. Mass selection**

In the mass selection method, open pollination takes place [14]. This method is an ancient easy to understand and simplest method of selection in which we select individual plants on the basis of their phenotypic performance. No change in the genotype of plants takes place. It is used in both cross and self-pollinated crops. In cross-pollinated crops, it is done for the improvement in population. On the other hand, self-pollinated crops, it is only used to maintain the purity of a cultivar. It is an effective method for landraces and only effective for highly heritable characters [14].

When mass selection is used to decontaminate an assorted cultivar, the examination may be dismissed and increase in seed rate initiated any time after it has been confirmed that the new strain does not vary in acclimatization and performance from the mixed variety and that it is superior to the mixed variety in uniformity. The genomic variation within a mass selection may stipulate some cushioning against varying environmental conditions [14].

## 2.3 Hybridization

The hybridization process has played a very vital role in the evolution of many heritages. Hybridization can have rapid phenotypical end results through the aspect of hybrid vigor (heterosis) [15]. It has taken a very long time to recognize it, it is defined as:

"The crossing of two genetically different structures of varieties (parents) to produce Hybrid."

For the production of hybrid, heterozygous genes are necessary without this segregation generation is not occur. And there are three methods to control segregation generation:

1. Pedigree Method
2. Bulk method
3. Backcross method [15]

### 2.3.1 Pedigree method

The pedigree method is considered the representative model of self-pollinated plant species. During the earlier 20$^{th}$ century, the pedigree method was first introduced in Sweden. The name of this method is according to its fact in which record mostly kept for the individual of all section, mating and performance evaluation. Mostly the plant breeders select two parents in which their first desire to extract the desirable traits and combine in desire attributes. For self-pollinated plants, the parents are supposed to be pure line or genetically fixed. the main aim of this method is to the extraction of desirable genes or alleles and combine them into a single genotype [16]. The concept of the pedigree method is basically derived from two words: Hybridization and pure-line selection. In a long period of time, plant breeders used the hybridization process

from the improvement of self-pollinated crops e.g.; wheat. During the late 19th century, William Farrer of Australia developed many important varieties of wheat by using the pedigree method that factor hybridization combine desirable genes or alleles of two cultivars [16].

**F1**: at least we grow emasculate 50 to 100 plants and from this, we get at least 10 to 30 seeds.
**F2:** Do space planting of these10 to 30 seeds of F1 in field to get maximum heathy f2 seeds in f2 so we space planting. Keep 5cm spacing from plant to plant.
In this point, no selection will be done all plants will be harvested in bulk quantity
**F3**: here we do selection at least select 100 to 500 superior plants. So that we grow 200 rows and then in which also select 100 superior rows. IN f3 individual plant selection done in every row almost grow 2 to 3 plants in every row.
**F4&F5:** here we select only superior rows
Individual plant selection will be done.
**F6&F7:** here we grow f5 to f6 plants in family rows, and then select superior plants from superior progeny
**F8**: here we grow f7 plants in preliminary yield trial and along with this we check and test for disease and quality.
**F9 to F11**: here we grow f8 and f9 superior plants in a Coordinated yield trail conducted in several locations to check the disease resistance and quality.
**F12**: here we multiply superior seeds for distribution [17].

### 2.3.2 Bulk Method

The bulking method is very easiest and conventional method of controlling segregation populations. The main step of this method is growing segregation generation as a breeding population after every year up to a reached adequate degree of homozygosity. The bulk method can be changed according to the requirement of facilities available, the objective of breeding [18]. In which in earlier segregation generation needs less work as compared to the pedigree method. It which necessary to grow large space planting to get desirable segregation when we made final selection made. Actually, bulk method is suitable for that crop which grow/planted in thick spacing for example the crops which contain small seeds foe which it is difficult to identify and separate in the field [18].

The main objectives of this breeding are natural selection by nature because which plants are selected by nature, they are strong to survive in every situation. In this method at least 14 years are required for natural selection [18].

Crossing over:

parent A x parent B

**F1**: Grow at least 50 to 100 plants in F1. Remove all plants which may occur due to self-pollination.
**F2:** Here we grow 2000 to 3000 plants and harvest in bulk amount from all plants at the time of harvesting.
**F3 &F4:** we grow seeds that collect in bulk harvest from the proceeding generation in 1/100- to 1/1000- hectares.
**F6:** Grow offspring/progeny in separate rows which collect from selected plants. Harvest 30 to 50 rows that have desirable characteristics of their parents. The superior rows which show segregation they again reselected for true-breeding.
**F7:** Grow in Preliminary yield trail.
**F8 to F10**: here we continue the yield trail as we do in the pedigree method
**F11 &F12:** here we multiple the seeds and distribute them to the farmers [18]

### 2.3.3 Backcross method

The first reference of backcross breeding was given by Harlen and the pope in 1922 who introduced that from which we can transfer genes from one individual to another. Backcross breeding involves the crossing of F1 our first aim to improve the variety or produce best lines by adding one or a few genes from the donor parents. This method does not work like the previous method but accompanies with them, in which we add one or a few genes for improvement of variety (mostly this method use to add disease resistance genes, herbicide resistance) [19]. This method is done for improvement of any variety. let suppose we have variety A which is very famous among farmers but it is disease suspectable this variety has no resistance genes and on the other side variety B is present it is not good and not popular among farmers but it has a resistance gene. So, we transfer desirable genes (resistance gene) from variety B to variety A to make it better by doing the Backcross method [19].

## 3. MODERN BREEDING METHODS

Modern Breeding Methods are explained as:

### 3.1 Shuttle Breeding

The shuttle breeding concept was firstly introduced by **CIMMYT (International Maize and Wheat Improvement Center).** Shuttle breeding uses multiple ecological environments to raise improved and highly adaptable varieties. In this approach, Subsequent generations of early breeding materials are grown under different environmental conditions. As the basic component of plant breeding is the development of new generations because it helps to develop **new recombinants** by meiosis, but the major concern in this regard is the time taken to get new segregating materials. Shuttle breeding is one of the classical forms to speed up the generation time [20]. This approach enables an extra generation to be advanced each year by using a different field location. It was proposed by **Dr. Norman Borlaug** in 1970 when Borlaug and his wheat improvement colleagues used back-to-back two distinct locations in **Mexico** to develop new wheat lines. One site was dry irrigated situated in Northwest Mexico and the other had a cool humid highland environment. Their main purpose was to speed up the generation time of stem rust-resistant wheat varieties. In this way, they were able to reduce the time to develop a wheat cultivar, from **10-12 years** to **5-6 years** [21]. Two more advantages were also observed during wheat breeding. When the breeding materials were disclosed in each distinct site to various soil types, disease spectra, multiple environmental conditions. This allowed selection of a wide range of diseases. Secondly, Photosensitive materials were excluded due to the requirement of photoperiod insensitivity at one location. It allowed **CIMMYT** semi-dwarf lines to expand throughout the world, indeed leading to the foundation of the **green** revolution whose basic output was semi-dwarf, high yielding, photoperiod insensitive and disease-resistant wheat cultivars which caused a revolutionary movement towards science-based agriculture in developing countries [22].

1. **Requirements for Shuttle breeding:** In this model, optimum testing and selection environments are a must in order to get high yield and input responsiveness. Segregating populations are required that are screened under diverse environments followed by multiple testing of advanced lined at distinct sites, and also for the identification of superior and improved genotypes which are resistant to diseases and other types of stresses [23].
2. By using this approach wheat germplasm adaptable to both low and high yielding conditions are significantly developed. Almost **500-800 lines** are considered as parents from which various distinct crosses are made having a wide range of cross combinations. Recently synthetic crosses have been made between Triticum durum and Triticum tauschi particularly for resistance to Helminthosporium leaf blight, heaf scab, Kernel bunt and Kernel size in the breeding population. Today spring wheat grown in developing countries are **CIMMYT lines** [24].
3. **Achievements:** Indian Council of Agricultural Research and International Rice Research Institute Philippines in collaboration have developed improved varieties of rice in rainfed lowland ecosystem in eastern India through shuttle breeding programme. Almost 632 rice varieties have been released in eastern India [24].
4. Parental materials shuttled at high and low altitudes have been used in breeding programme to improve the oil contents of rapeseed. High oil content cultivar **Qinyou 4** has been released by this method. Aluminium tolerant, high yielding wheat varieties have been developed through shuttle breeding [25].

### 3.2 Speed Breeding

Speed Breeding is a novel technique that shortens the harvest time and is used to achieve almost 6 generations per year at indoor conditions or in glasshouse conditions. . It is actually a time-saving technique. This technique is used mostly for spring wheat (*triticumaestivum*), barley (*Hordeum vulgare),* canola (*Brassica napus*) and chickpea (*Cicerarietinum*) [26]. Speed breeding in fully controlled conditions can accelerate research programs and studying phenotypes of traits, mutants and transformation. The applications for speed breeding is limited for short-day plants because it is directly influenced by extending photoperiod length. At first in 1980s, NASA's work on speed breeding encourage scientists in all over the world to work on it. In 2003, Scientists at the University of Queensland devised the name speed Breeding as a method used to accelerate the speed of wheat breeding. Currently, the speed breeding technique is used for several other crops [26]. The principles for speed breeding are optimum sunlight, optimum

temperature and adequate daytime length. The daytime length is necessary to increase the photosynthetic rate which stimulate the flowering and early maturation that ultimately shortens the generation time. Light intensity and wavelength of light are also very important for flowering [27], the conventional methods of breeding could not fulfil the requirements of the increasing population. The traditional methods took above 10 years to develop a new crop variety that is according to the demand of the breeder. On the other hand, the modern techniques of breeding took only 2 years to develop the variety. So by decreasing the breeding cycle per year, the rate of improvement will be increased [27], There are many methods to accelerate the speed breeding process. For a successful plant breeding program, a proper channel and set-up must be followed.

#### 3.2.1 Controlled environmental chamber speed breeding condition

This method is designed at the optimal temperature of 22°c during the photoperiod of 22 hours, the remaining 2 hours of darkness have the optimal temperature of 17°c. A photoperiod of 22 hours can be achieved with the help of white LED bars and red LED lamps. The light intensity must be between 360-380µmol. The pots are placed at bench height [28].

#### 3.2.2 Glasshouse speed breeding conditions

The controlled environment is adjusted in greenhouse. The optimal temperature is 17-22°c and photoperiod of 22 hours. Light intensity is maintained at 450-650µmol. The plants are adjusted at 45cm height [28].

#### 3.2.3 Homemade growth room design for low-cost speed breeding

A homemade structure of about 3cm ×3cm× 3cm with the installed sandwich and fitted lighting equipment of 7LB or 8LED lightboxes are used. Light intensity must be 210-260µmol at the height of 50 cm [28]. Speed breeding accelerates the breeding program. Markers assisted selection with speed breeding can give an enormous amount of the desired gene combating abiotic and biotic stress. Speed breeding can speed up the domestication process [29]. The domestication from speed breeding of various plant such as banana and peanut are recorded. It can also respond to the climate change faster than conventional breeding. Because it took a smaller time to develop the variety that has resistance against diseases and disastrous climatic conditions [29]. There are some advantages of speed breeding. It is one of the fastest methods to obtain fixed homozygous lines. There are many generations produced in one year. The selection of phenotypic traits can be done in early segregating generations. This technology permits the plant breeder to study flowering time and plant-pathogen interaction. Multi environmental trials can be done using this technology [30]. There are many varieties that are developed with the help of speed breeding technology.DS Faraday is a wheat variety contains high protein content and good milling quality, also resistant to pre-harvest sprouting. Scarlett is the barley variety in Argentina which have resistance against drought. This is developed in two years by making backcrossing methods in four elite lines. YNU 31-2-4 is a salt-tolerant rice variety that was developed by speed breeding. The gene for salt tolerance is inserted by using SNP markers [30].There are also some constraints of speed breeding. Most vegetables are long-day plant and some are day-neutral plant and they require continuous sunlight that accelerates genetic gain. Others are short-day plants that required limited photoperiod for genetic gain and accelerate the breeding program. The Speed breeding is more suitable for long day plants than that of short-day plants. The speed breeding process can speed up the process and early harvest of the immature seed that interfere with the phenotype of the traits. Speed breeding is carried out in closed chambers that cost high and expensive. Above all these, it is carried out in longer photoperiods that cause toxicity effects such as chlorosis, necrosis and yellowing due to deficiency and excess of various metals [30].

### 3.3 Double Haploid Technology

Usually, most plants contain two sets of chromosomes (one from each parent) and are considered diploid plants. However, sometimes a plant is created using a haploid pollen grain culture on an artificial medium and these plants are called double haploid plants [31]. Although double haploid plants also carry 2 sets of chromosomes the main difference between double haploid and diploid plants is that fertilization does not take place in double haploid plants and their culture is grown on a nutrient medium and their haploid genome is doubled using chemicals mostly colchicine. This produces a double haploid plant whose genome is fully

homozygous [31]. With one set of chromosomes, the haploid plant will be shorter and sterile and will not be able to produce its gametes (pollen or egg). That is the main reason to apply colchicine treatment to get fertile double haploid plants instead of sterile ones. This treatment not only gives a fertile plant but also make the plant homozygous at every locus due to the doubling of chromosomes [32]. Here are the applications of Double Haploid Technology:

- The only way to get full homozygosity in the genome of a plant is to make double haploid of that plant because of the doubling of chromosomes. Usually to produce a homozygous or pure line, a duration of seven to eight years is required for breeding cycles and even after that it is not 100% homozygous (nearer to it) but with the use of the double haploid technique 100% homozygosity can be attained and that also in just one generation of the breeding cycle which saves a lot of time [33].
- In self-pollinated crops, the plant made from the double haploid method can be used directly as a cultivar because there is no need of selfing it to get more homozygosity as it is already fully homozygous in just one generation. However, in cross-pollinated crops, double haploid plants can be used as an inbred lines and thus it is crossed with another inbred parent line to get a desirable hybrid.
- The conventional method used to get a desirable genotype is the backcrossing of the donor species with the recipient one and the crossing is made again and again to get a pure line of that trait but DH breeding programme has made the process much easier and the desirable trait can be attained in just one or two generations with full purity.
- Double haploids are used for the study of inheritance and for genetic studies in plant breeding. Due to being fully homozygous at all the loci in the genome, double haploids are used to identify and study the recessive mutants in plants. Otherwise in diploid plants, the study of recessive mutants become a difficult task because chances of homozygous recessive are less in diploid plants than in haploids [33].
- Quantitative trait locus analysis is the study of those loci which deals with the change in quantitative traits phenotypes in an organism. QTLs are mapped with the help of trait based molecular markers but the main limitation in this study is that due to the segregation phenomenon in the population the genes at each locus changes in each generation which makes it difficult to study and analyze. Double haploid plants due to being true breeding lines can be used to overcome this problem of diversity in genes because it is homozygous at each locu in all of its generations which makes it the best population for the analysis of quantitative traits.
- DH lines are considered to be the best and most effective way for the process of selection of desirable traits in the development of hybrid crops because there will be no variation in the genome of the variety due to being fully homozygous and it will also speed up the process of hybrid development.
- Construction of genetic map of a haploid plant is much easier than other plants as there will be no changes in the genome of the plant and mapping of genes that control the desirable economic trait, can be done easily with the help of different molecular markers based on the trait that has to be mapped [33].

### 3.3.1 Genomics in double haploid

One of the main things that differ double haploid plants from parental produced plants is its genomic. In normal breeding with alleles “A and a” there will be the probability of three genotypes (AA, Aa, aa) with frequency or probability of “1AA:2Aa:1aa”. However, in DH plants, it is different as there will be only two genotypes (AA, aa) with the frequency of “1AA:1aa” [34].

In diploid or other plants the segregation of alleles during gamete formation results in the formation of three genotypes. But in double haploids, the plant will either be homozygous dominant (AA) or homozygous (aa) recessive and it will never become a heterozygous one (Aa) because there will be no fertilization and as genes at all the loci will be doubled so the dominant alleles (H) will become homozygous dominant (AA) and recessive allele (a) will be doubled to dominant recessive (aa) [34].

### 3.3.2 Methods of double haploid technology

*In vitro* methods are mostly used to induce diploid hybrids in a plant. These methods include

androgenesis and gynogenesis reproduction. Androgenesis is a form of sexual reproduction in which male is the only source of genetic material in developing the embryo. It is mostly used to develop double haploid plants and there are further two culture techniques in androgenesis that are used to induce DHs [35].

**Pollen Culture:** Unopened flower buds are selected and sterilized and anthers are collected from these flower buds. Anthers are transferred to the nutrient medium (mostly Nitsch's medium is used) and pollen grains are then isolated from anthers by pressing anthers along the side of the beaker and then the pollen suspension is filtered to remove the anther tissue debris with the help of a centrifugal machine at low speed again and again until all the fine debris is discarded [36]. Then 0.5ml pollen suspension is placed on a petri dish and is covered with the lid. Petri dishes are incubated at 30-33$^{o}$C under the low intensity of light for 30 days. After 30 days young embryoids can be seen which will be developed into haploid plantlets which on maturation are transferred to the greenhouse [37].

**Anther culture:** The unopened flower buds are selected and then are sterilized using Mercuric chloride or sodium hypochlorite and then buds are washed with sterile distilled water. Anthers are then collected from those flower buds. Those anthers which are in the first meiotic division are preferably selected by using the acetocarmine test and are placed on an agar solidified medium (discard the damaged anthers). The culture is then incubated at 25-30$^{o}$C for 2-3 weeks in low light where anthers will grow into embryoids [38]. They are transferred to the rooting medium under 2500-3000 lux illumination where they will grow into plantlets. The plantlets are then transferred to the greenhouse in small pots with compost [39].

Gynogenesis is the production of haploid plants with the help of only female genetics (unfertilized ovary) and it has been widely used for DHs production.

The surface of the unfertilized ovary is sterilized and ovules are extracted and placed on the nutrient medium culture. Extraction of ovule could be an easy task as in large-seeded plants where only one ovule is present or it can be a difficult and time taken step if the seed are small seeds and it should not be damaged [40]. A filter paper is placed on the petri dish which contains the ovule and nutrient medium. It is then incubated at about 30-33$^{o}$C for about 4 to 5 days and produce better embryogenic simulation (in some cases) if followed by 4$^{o}$C. When the ovule is developed in to embryoids it is kept at about 25-30$^{o}$C for about 2 weeks until it will develop into plantlets [41].

After androgenesis of gynogenesis, the haploid plants are produced but they have only one set of chromosomes which make them sterile and they are unable to reproduce further. In order to restore their fertility, one set of chromosomes is doubled to get two sets of chromosomes which will give us a double haploid plant. Chemicals are used for the doubling of chromosomes like colchicine, trifluralin, oryzalin, etc [42].Out of the above-mentioned chemicals, colchicine is widely used for the doubling of chromosomes. It causes the doubling by acting as a spindle formation inhibitor during meiosis or mitosis which results in all the doubled chromosomes going in one cell and is also used to produce polyploidy plants. 0.5% colchicine solution is applied to the young plantlets of haploid plants for about 2 days. If haploid plantlets are matured then 4% colchicine treatment is used on the tips of leaves. After washing the plantlets with the colchicine solution, they are transferred to their medium [43].

## 4. CONCLUSION

This review allows estimating the state of a functional factor concerning the conventional and modern breeding technologies, selection methods and important approaches of field crops in a better way. All these technologies, approaches and methods have a common objective of yield and quality improvement and to produce new varieties with better results. Important progress in crop breeding has been made in the development of varieties of better quality that can withstand all types of stresses, diseases, insect pests and unfavorable soil conditions as well.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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