



Pre-Breeding's Role in Crop Genetic Improvement. A review

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ABSTRACT

The primary challenges limiting grain productivity and production globally are the narrow genetic bases of cultivars and the low exploitation of genetic resources. For genetically enhancing crop productivity, it is necessary to take advantage of new and diversified sources of variation. Important sources of genetic diversity for crop development can be found in wild relatives with increased levels of resilience to or tolerance of various stresses. Cross-incompatibility barriers, however, restrict their use for cultivar improvement and linkage drags. Pre-breeding provides a unique opportunity to solve these issues through the incorporation of desired genes from natural germplasm into genetic backgrounds that may be easily utilized by breeders with minimal linkage drag. The availability of molecular markers will play a significant role in lowering linkage drags and boosting introgression efficiency in pre-breeding programs. Basic research is done prior to breeding in order to achieve wide crosses and to make it easier to use exotic materials or wild relatives. Finding a desirable trait, capturing its genetic diversity, and transforming those genes into usable form are the steps in pre-breeding. Pre-breeding aims to increase the diversity of improved germplasm while also giving breeders a choice of ready-to-use materials with the traits they want. It is regarded as the first step in sustainable plant breeding and does not considerably diverge from the overall framework of plant breeding. The basis for the sustainable production of different types for current and upcoming challenges that result from the diverse biotic and abiotic stresses is genetic diversity. Pre-breeding offers a unique opportunity for making use of genetic diversity through the introduction of desirable characteristics or genes from wild crop relatives into cultivated backgrounds utilizing a variety of novel procedures. All plant breeding investigations that take place before cultivar development, testing, and release are referred to as pre-breeding. Pre-breeding is necessary because of the current genetic base's restrictions, the growth of genetically uniform modern varieties, the effects of climate change (better-adapted genes are needed), and the evolution of pest and pathogen populations (which call for new resistance genes). One of the pre-breeding activities that enables to adapt germplasm for immediate use in routine breeding programs is the characterization of landraces/accession, creation of new base population, creation of polyploidy, development of novel breeding procedures, wide cross, and creation of double haploids. In general, there are many opportunities to utilize the use of the pre-breeding benefit, even though pre-breeding adoption faces a variety of difficulties.

Keywords: Pre-breeding, Genetic base; Novel traits; Germplasm; Landraces; Wild relatives; Gene pool

1. Introduction

Pre-breeding is a broad term for all activities aimed at acquiring desirable traits and genes from unadapted materials that cannot be used directly in breeding populations and to transfer these traits to an intermediate set of materials that breeders can use in the future in order to create new varieties for farmers (Mohan *et al.*, 2021). The term "enhancement" was first used by Jones (1983), who described it as the introduction of valuable genes into an agronomically acceptable background from exotic or wild types. The introduction of genes or gene combinations from unadapted sources into breeding

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material is referred to as "genetic enhancement" or "pre-breeding." Pre-breeding is regarded as the first phase in sustainable plant breeding and does not significantly deviate from the fundamental principles of plant breeding. Choosing a valuable trait, capturing its genetic diversity, and transforming those genes into usable form comprise the pre-breeding procedure. Pre-breeding results in the germplasm's useful being increased (Nass, L.L and Paterniani, E, 2000). The primary goal of pre-breeding is to increase genetic diversity in the germplasm, and the improved germplasm is readily applicable to regular breeding programs for cultivar development (Lokanathan *et al.*, 2003).

Pre-breeding uses a larger pool of genetic material to generate a new base population for breeding programs (Haussmann *et al.*, 2004). By reintroducing genetic variation that has been forgotten, this pre-breeding aims to restore the genetic diversity of crops. Pre-breeding has actually been used to access genetic diversity that was previously unavailable because of genetic incompatibilities or because of non-overlapping regions of the globe (Ogbonnaya *et al.*, 2013). In order to develop beneficial cultivars and hybrids, it is crucial to use unsuitable and underutilized germplasm stocks and create huge genetic variability (Mohan *et al.*, 2021). Using the genes from wild (exotic (unadapted) species into adapted material, pre-breeding work has been successfully carried out in several crops (rice, tomato, soybean, cotton, maize, wheat, barley, groundnut, chickpea, pigeon pea, sorghum, pearl millet) and improved many cultivated varieties for different qualitative and quantitative traits (Sebolt *et al.*, 2000). Pre-breeding aimed at enhancing the germplasm and cultivar development are the two primary processes that make up plant breeding. Pre-breeding finds traits or trait combinations that can be used to improve grain quality or nutrition, protect it from biotic or abiotic stress, and increase yield.

Pre-breeding is "the art of identifying desired traits, and incorporating these into modern breeding materials," according to the Global Crop Diversity Trust. Pre-breeding is being done, and the materials that are produced are expected to be suitable of inclusion in regular breeding processes. Pre-breeding attempts to boost yield, pest and disease resistance, and other desirable traits in crops without reducing homogeneity in genes by using a larger pool of genetic material. Its purpose is base broadening or genetic enhancement, which is accomplished either by identifying the genes responsible for the desired characteristics or by changing these genes from an unadapted to an adapted background. It also plays a role significantly by genetically enhancing the performance of the yield and the germplasm's ability to withstand biotic, physiological, and agronomic stresses (Mohan *et al.*, 2021). The main factor limiting production and productivity globally is the limited genetic diversity of cultivars and the inadequate exploitation of genetic resources. Pre-breeding provides a special opportunity to take advantage of genetic variety by introducing desirable genes from wild germplasm into cultivated environments that may be used rapidly and with a minimum of linkage drag.

Choosing a valuable trait, capturing its genetic diversity, and transforming those genes into usable form comprise the pre-breeding process. Pre-breeding benefits include expanding the genetic base, which increases genetic variation, breaking yield barriers, developing donor sources conferring resistance against various abiotic and biotic stress factors, using wild relatives as rich sources of desirable traits that can be introduced into cultivars through crossing, and increasing variability for modifying different agronomic characters like earliness and non-shattering as well as to other yield characters. Pre-breeding is a term used to describe a number of plant breeding research efforts that must come before the stages of cultivar production, testing, and release (Singh *et al.*, 2019). It is the encouraging link between breeding programs and genetic resources. The pre-breeding process should produce materials that are suitable of inclusion in regular breeding activities. Any germplasm that lacks immediate usefulness without selection for adaptation is considered exotic for pre-breeding activities (Hallauer *et al.*, 1988).

Pre-breeding is becoming more significant in most crop development programs, including those for wheat (Valkoun, 2001), maize (Nass and Paterniani, 2000), and common bean (Acosta-Gallegos *et al.*, 2007), due to the limited genetic diversity present in cultivated germplasm. Currently, efforts are being made to improve bread wheat, kabuli chickpea, barley, and lentil through pre-breeding and genetic enhancement as part of the ICAR-ICARDA collaborative research. Pre-breeding activities are currently underway at ICRISAT for chickpea, groundnut, and pigeonpea using potential exotic landraces and wild species as donors and well-known varieties as recipients.

For essential agronomic and nutritional traits, as well as for resistance/tolerance to significant biotic/abiotic challenges, it aims to develop new gene pools with a higher proportion of valuable genes, wider adaptability, and a broader genetic basis. Ascochyta blight, Botrytis gray mold, and Helicoverpa

pod borer resistance in chickpea; sterility mosaic disease, Phytophthora blight, and pod borer resistance in pigeonpea; and LLS, peanut stem necrosis disease (PSND), and aflatoxin resistance in groundnut are all being prioritized as introgress genes. Due to its small gene pool, of the three SAT legumes, cultivated groundnut requires the most pre-breeding activities.

Pre-breeding can refer to both qualitative and quantitative traits, and it's not always easy to articulate the difference between introgression and incorporation. The major objective is to give breeders more "attractive" and appropriate plant genetic resources, such as sources of resistance with acceptable genetic background or inbreeding-tolerant variations of outcrossing species for hybrid breeding. In order to provide farmers and breeders with more sources of resistance from wild relatives, the Global Initiative on Late Blight (GILB) of the International Potato Center (CIP) has a resistant breeding program (Trognitz *et al.*, 2001). Commercial breeders use pre-breeding techniques to create "Base" or "Buffer" populations from genetically extremely varied materials in the sugar beet (*Beta vulgaris*) (Frese *et al.*, 2001; Frese, 2002). The objective of the paper was to understand the role of pre-breeding in crop improvement for desirable traits.

2. Role of Pre-Breeding in Crop Improvement

The most effective method of connecting breeding strategies and genetic resources collectively is pre-breeding. Pre-breeding is the term used to describe any method employed for identifying desirable traits and genes from unadapted (exotic or semi-exotic) materials, including those that, although adapted, went through any type of improvement-focused selection. The materials produced while pre-breeding is done are anticipated to be suitable of inclusion in regular breeding procedures. Although there are various definitions of exotics, Hallauer *et al.* (2010) consider any germplasm that lacks immediate use without selection for adaptation to a particular environment qualifies as an exotic for pre-breeding reasons.

In this respect, races, populations, inbred lines, etc. serve as representations of foreign germplasms. As a result, the products of crosses between adapted and exotic materials where various amounts of introgression are discovered and assessed have been referred to as semi-exotic materials. The most typical method for evaluating exotic germplasms, according to Hallauer *et al.* (1988), has been to use semi-exotic populations. Exotics have to reproduce for a few generations in order to allow for genetic recombination and mild selection, which are both important for obtaining encouraging results. It takes at least five generations of random mating under light selection pressure to select useful recombinants (Lonnquist, 1974).

Pre-breeding strategies may generate new basis populations for breeding initiatives and support the detection of heterotic patterns in hybrid programs. The biggest barrier to using landrace material and unadapted exotic lines from collections is the absence of a pre-breeding program. Through genetic improvement, it will also contribute to the value addition of various genotypes by raising their level of resilience to biotic and abiotic stress. Utilizing germplasm will increase the genetic diversity of crops and their genetic diversity.

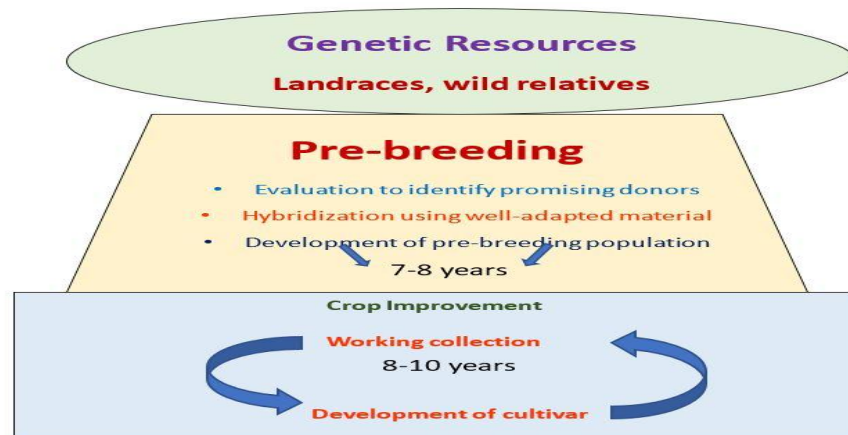


Fig. 1: Pre-breeding as a bridge between genetic resources and crop improvement

2.1. The purpose of pre-breeding

The current widening gap between the management of plant genetic resource collections and modern plant breeding could significantly impede the development of new cereal varieties that are required to address novel agronomic and environmental challenges. Pre-breeding, an approach based on characterizing genetic resources for desirable traits and then transferring these traits into acceptable, agronomically adapted genetic backgrounds, may address this gap.

Any crop development program's effectiveness is dependent on the availability of enough genetic diversity, but this diversity must be in a form that can be used in conventional agriculture. Breeding progress is hampered by a lack of variation. Increased genetic susceptibility to pests and diseases resulted from the substitution of genetically uniform new varieties with genetically differed local cultivars and landraces (Jain S K and Omprakash, 2019). Pre-breeding has evolved into a necessary, planned component of all plant breeding programs and germplasm diversification strategies. Broad ranges of germplasm varieties were adapted to new genetic environments and geographical regions through pre-breeding (Jain S K and Omprakash, 2019).

Using a larger pool of genetic material to improve yield, resistance to pests and diseases, and other quality characteristics, it aims to reduce the genetic uniformity in crops (Shimelis H and Laing M, 2012). Pre-breeding is being followed in the direction that will most effectively and efficiently transfer the intended characteristics into cultivars for farmers and source of desired gene. Pre-breeding is therefore necessary due to the restricted genetic basis, decline in biodiversity (modern varieties are genetically uniform and dominate), genetic uniformity that increases genetic susceptibility to pests and diseases, the effects of climate change, and the emergence of new pests.

If the target genes only exist in gene bank accessions that are poorly adapted to the target environment, closely related wild species, and farther-reaching wild species that are more challenging to cross, pre-breeding is required (Kumar V and Y M Shukla, 2018). Pre-breeding work typically focuses on the following four goals: 1) reduce genetic uniformity in crops by using a larger pool of genetic material, 2) identify desirable traits/genes and then transfer them, 3) improve parental stocks that can be easily used in breeding programs, and 4) identify potentially useful genes in a well-organized and documented gene bank.

2.1.1 Sustainable Use of Genetic Diversity

Because of this, gene banks frequently concentrate primarily on the immediate conservation aspects of efforts involving plant genetic resources. However, in order to ensure that the entire "Genetic resource-chain" operates properly, there is an urgent need for active engagement with all stakeholders to improve the usage of plant genetic resources. Small grain cereals have access to a variety of genetic resources, from collections of adapted varieties and genetic stocks carrying specific characteristics to land-races and wild crop relatives. However, this abundance of plant genetic variation is grossly underutilized, with smaller amount essential characteristics utilized and the potential value being lost to humankind.

If genetic resources are to play a significant role in climate change adaptation, then their conservation must be connected to their broadened and sustainable use. Lack of information at the genotypic and phenotypic levels, such as the requirement to assess for resistance to biotic and abiotic challenges, are bottlenecks that need to be addressed. To ensure the required degree of pre-breeding processes, data must be readily available in standardized and searchable electronic formats (Iqbal *et al.* 2017). This will allow strong networks linking conservation, evaluation, and plant breeding to be developed.

2.1.2 Identification of New Resistances

Identifying sources of resistance in the current germplasm and from other sources around the world constitutes the first phase. Resistance that is generally effective can be determined through testing over a number of years, in diverse environments, and at various growth stages. Upon the identification of potential resistant donors, breeding programs may use them directly, or they may be used for population development, the identification of genetic markers, and the mapping of novel resistance genes (Mohan *et al.*, 2009).

2.1.3 Abiotic Stresses - Adapting to Environmental Changes

Field crops have historically been able to deal with the common local stresses with an equilibrium of traits that were optimized to maximize economic yields in their environments. These particular abiotic stresses include tolerance to drought, cold, salinity, heat, water logging, and mineral toxicity. However, given the generally known impact caused by climate change, global warming is causing alterations to the occurrence of some abiotic stresses, with drought becoming more significant. Additionally, it is anticipated that levels of ozone in the troposphere will grow significantly in the next years, which will have an impact on how much UV radiation reaches Earth's surface and, in turn, cause an enormous increase in plant oxidative stress. The development of field crops that can tolerate such environmental changes is therefore urgently needed in order to boost or even just maintain existing production levels as it is anticipated that the agricultural areas of world are going to face enhanced or new abiotic stresses (Iqbal *et al.*, 2017).

2.1.4 Biotic Stress - Coping with Emerging Diseases

Plant diseases significantly reduce grain yields, lower crop quality, and impair food safety. Thus, disease control and prevention are essential for competitive field crop production and one of the most environmentally and economically beneficial methods to manage plant diseases is through the development of genetically resistant crops. The prevalence of various plant diseases is changing as a result of shifting environmental factors, including global climate change, as well as changes in agricultural production practices, such as planting larger areas with smaller or genetically uniform varieties, reducing crop rotation and soil tillage, losing biodiversity, changing pesticide usage, and trade across borders.

The emergence of a new strain of heat-tolerant wheat yellow rust and the escalating issues with *Fusarium* head blight and *Ramularia* leaf spot are examples of how plant diseases will inevitably appear due to the changing environmental conditions and economic context and threaten crop production in areas where they did not previously do so. The first step in reducing potential dangers posed by plant diseases is to have an understanding of the biology of the host-pathogen relationship. The basic strategy for controlling diseases in field crops is to incorporate long-lasting, race-specific and race-nonspecific, resistance into high-yielding genotypes. In the case of field crops, it will be necessary to look for new reliable and effective sources of resistance in landraces and in wild relatives of the crops (Iqbal *et al.*, 2017).

2.2 Major Applications of Pre-breeding in Crop Improvement

Pre-breeding has four primary applications: (1) broadening the genetic base for reducing vulnerability; (2) identifying traits in exotic materials and transferring those genes into material that breeders can more easily access; (3) introducing genes from wild species into breeding populations when doing so appears to be the most effective tactic; and (4) identifying and transferring novel genes from unrelated species using genetic transformation techniques. Pre-breeding is used to broaden access to and use of genetic variations kept in gene banks, which helps crop development initiatives, be more effective and efficient (Kumar and Shukala, 2014).

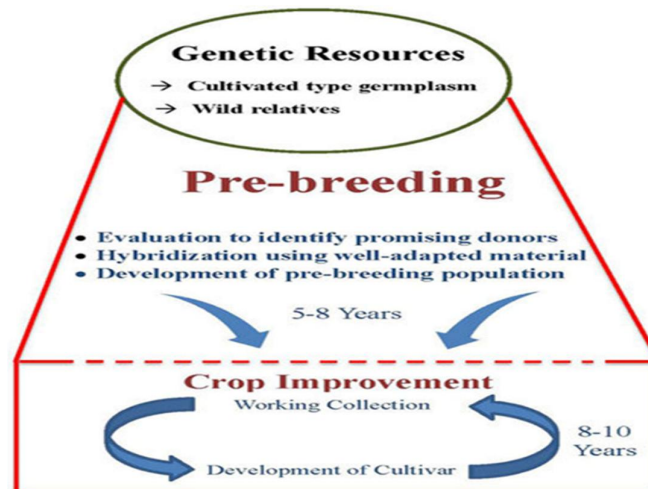


Fig. 2: Pre-breeding as a bridge between genetic resources and crop improvement

2.3. Pre-breeding Strategies

All the components that can be used to enhance a cultivated plant species are referred to as plant genetic resources. Gene banks are collections of diverse genetic material from wild species, relatives of domesticated species, and other wild species. The ultimate goal of gene banks is to provide pre-breeders and breeders with new genetic diversity that enhances the value of future varieties, so ensuring the long-term supply of crop germplasm to support agricultural production. Pre-breeding aids in bridging the gap between plant breeders, who add novel characteristics to their diversity, and gene bank managers, who are knowledgeable about the scope of germplasm collections. Plant genetic resources (gene bank managers) and breeding (plant breeders) are connected by pre-breeding. In order to efficiently use germplasm from gene banks to develop new varieties with the traits that the world needs, plant breeders and gene bank managers must find ways to make it easier.

Table 1: Traits grouped based on number of genes involved, trait heritability and selection methods-phenotypic and genetic used for pre-breeding

Trait grouping	
Simple traits	Complex traits
Major genes	Multiple genes with small effects
Traits with high heritability	Traits with relatively low heritability
Categorical traits	Quantitative traits
Easily measurable	Time consuming
Low $G \times E$	High $G \times E$
Eg. Flowering time, plant height, grain weight	Eg. Photosynthesis, grain yield, radiation use efficiency, water use efficiency
High heritability	Low heritability
Plant height, flowering time, maturity; grain size and color, spike size	Grain yield, most physiological traits, metabolites, spectral reflectance indices that are influenced by environmental fluxes
Methods used	
Marker assisted selections/marker assisted backcrossing	Trait-based selections, genomic selection
Fine mapping and cloning of genes possible	Cloning of genes not possible
Single plants can be measured	Need to measure multiple plants in the field

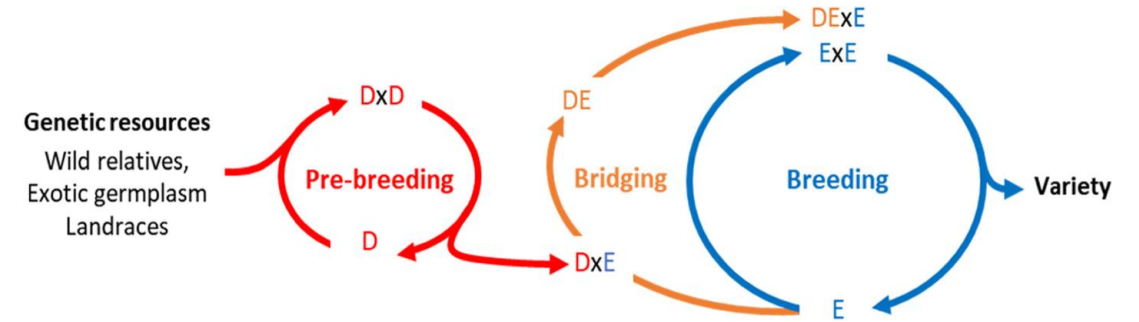


Fig. 3: Optimized breeding strategies to harness genetic resources with different performance levels

2.4. Major Approaches of Pre-breeding

2.4.1 Introgression

One or more genes are transferred from foreign, unadapted, or wild stock to breeding populations that are adapted. Crosses between the donor and the recurrent parent are performed to achieve it. Dr. Edgar Anderson developed the idea of introgression through backcross, which was originally depicted in cotton by Knight (1945). The main objective of pre-breeding is to incorporate yield boosting, yield protecting, or nutritional/quality trait(s) into elite backgrounds and to produce proof of ideas of their usefulness in suitable target situations by trialing. Here, breeding techniques vary depending on whether the traits are simple or complicated and whether molecular markers are available.

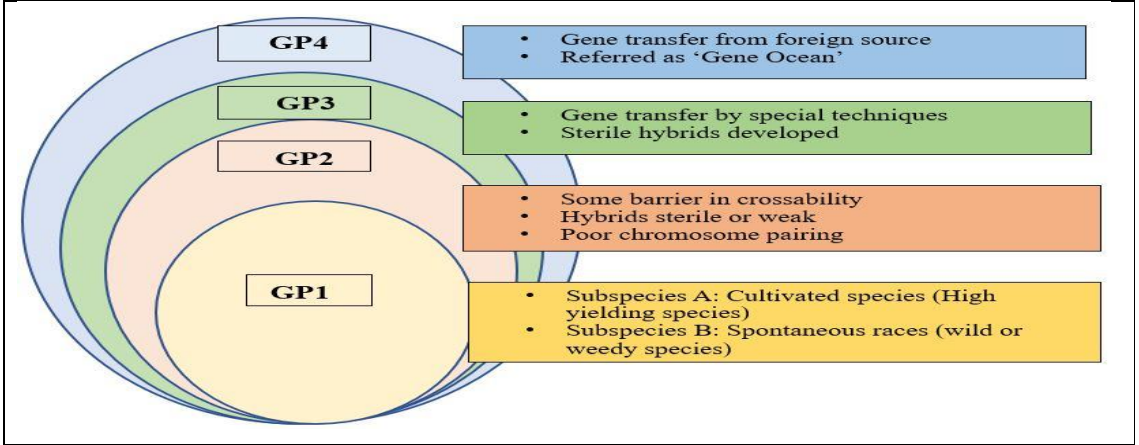


Fig. 4: Modified gene pool concept in plants based on the hybridization study

2.4.2. Incorporation

Through incorporating foreign or unadapted material, a large-scale program has the objective to develop a population that has become adapted to its environment.

Table 2: Utilization of genetic resources depends upon the pre-breeding step to allow commercial breeders to access the genetic diversity available in our gene banks in a genetic background that they can use

	Gene bank	Pre-breeding		Breeding	Seeds
PGR	Collection	Characterization	Parental development	Parental development	Variety release
	Maintenance	Evaluation		Varietal improvement	

2.5. Methods of Using Plant Genetic Resources in Crop Improvement

According to Cooper et al. (2001), there are three conventional techniques for harnessing plant genetic resources to improve crops: (1) Introgression (2) Incorporation or broadening of genetic base, and (3) Wide crosses: synthesis of new base populations. For the benefit of society, the tools of genome

research may ultimately unleash the genetic potential of our wild and domesticated germplasm resources (Tanksley and McCouch, 1997). In the context of utilizing plant genetic resources for crop improvement, the utility of molecular markers and genomic tools/techniques includes: (1) Diversity evaluation (2) Somatic hybridization (3) Anther culture (4) Embryo rescue (5) Marker assisted breeding (6) Mapping of quantitative trait loci (QTL) Association studies, (7) introgression libraries, and (8) genetic transformation.

2.6. Usefulness of Germplasm in Pre-Breeding

Crop cultivars using important characteristics can be improved with the use of germplasm. Broadening the genetic base, enhancing tolerance to biotic and abiotic stressors, enhancing yield and grain quality traits, enhancing biomass yield, and developing short-lived variety are among the traits targeted at main food crops.

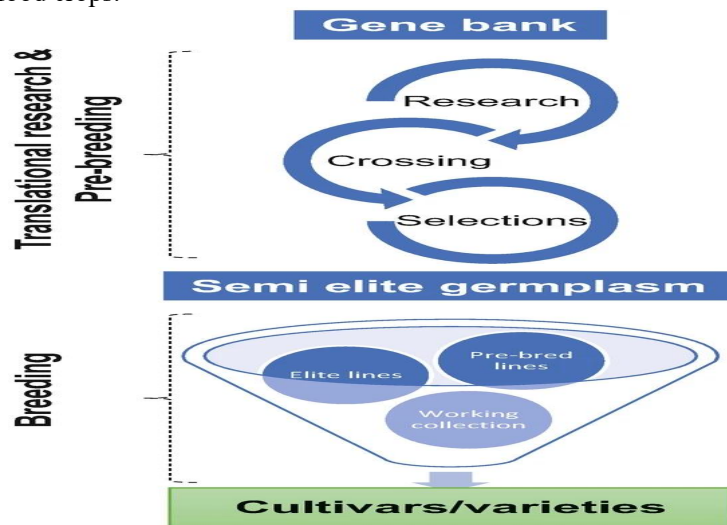


Fig.5: A general sequence of events from gene bank to varieties

2.7. Use of Exotic Germplasm for Crop Improvement

Exotic germplasm is all genetic material that is not immediately useful and has not undergone selection for environmental adaptation (Haullauer and Miranda, 1981). To be most useful in plant breeding, exotic germplasm must be transformed or pre-breeding. Due to its initial detrimental effects on elite breeding material, the majority of plant breeders are unwilling to use exotic or unadapted material (Kannenbergh and Falk, 1995). These are some major constraints on the direct use of exotic material in breeding programs: (1) Crosses with exotic material have the potential to disrupt co-adapted gene complexes in the elite material as well as introducing inferior alleles. (2) When introduced into a genetic background that has been locally acclimated, exotic germplasm might adversely impact adaptability. (3) Increased use of unadapted or exotic germplasm is severely hampered by the correlation of unfavorable genes with beneficial traits. This serves as a barrier to the flow of beneficial genes from diverse gene pools into the adapted gene pools. Exotic germplasm must go through "conversion or pre-breeding" in order to be used most effectively in plant breeding.

Despite greatest attempts, it has been observed that for the majority of crops, the genetic difference between elite gene pools that are adapted and exotic gene pools is widening with each breeding cycle. These barriers to gene flow might be solved with better understanding of the underlying genetic differences between adapted and alien germplasm. Studies on cotton have shown that there is a lot of room for genetic improvement. The number of alien land races being introgressed into elite germplasm in some crops, including wheat, is growing over time (Loknathan *et al.*, 2003). Exotic lines are those that lack immediate use without being selected for adaptation. In general, the direct use of exotic lines restricts breeding processes in breeding programs because: i) There is a greater risk of the introduction of inferior alleles; ii) This has an adverse effect on adaptability; and iii) There is a greater chance that undesirable genes will be linked to beneficial traits (linkage drag). The term "germplasm"

refers to both cultivated and wild species and relatives of crop plants, as well as land races, modern cultivars, obsolete cultivars, breeding stocks, wild forms, and wild species of cultivated crops.

2.8. Gene Pool Concept

The complete genetic diversity of a species' breeding population and closely related species that are capable of interbreeding with it is referred to as its gene pool. Botanical variations, landraces, inbred lines, obsolete and modern cultivars, related wild species, subspecies, and weedy companion plants all contribute to the gene pool of a crop (Hausmann *et al.*, 2004). As gene pools, three different forms of germplasm-primary, secondary, and tertiary are utilized. These genepool types each have their own significance and limitations. The primary gene pool was frequently used in the past to improve the genetics of various crops in order to increase genetic variability and the genetic diversity of the stock for breeding. Utilizing secondary and tertiary gene pools helps in generating a large amount of genetic variety for different traits. The utilization of secondary and tertiary gene pools in field crops has recently been more focused, and as a result, a great amount of genetic diversity has been produced for a variety of economically important characteristics, including resilience to biotic and abiotic stresses, plant type, and so on. The quaternary genepool is a novel genepool idea that has only recently emerged because of the development of biotechnology.

Uses of Gene pool

Harlan and deWet introduced the idea of a gene pool in 1971. All of an individual's genes and their alleles compose their gene pool. The three groupings of genes compose the gene pool, which was expanded by genetic engineering.

Primary gene pool

This refers to both domesticated and wild varieties of the crop. Through standard plant breeding techniques, crossing between group members can result in normal seed set, segregation, and recombination.

Secondary gene pool

Due to meiotic irregularities, the cross between the primary gene pool and secondary gene pool results in partially sterile hybrids.

Tertiary gene pool

Complete sterility is produced through gene pool hybrids. Specialized methods like embryo rescue, tissue culture, chromosomal doubling, and bridging species could get beyond these ability barriers.

Quaternary gene pool

The quaternary gene pool is also known as the "gene ocean," because using recombinant DNA technology; genes can be transferred from various organisms in addition to species or genera of the same organism.

2.9 Methods of Pre-breeding

Introgression and incorporation are two pre-breeding strategies that can be effective. Introgression is the term used to describe the transfer of one or more genes from unadapted germplasm (donor parent) to elite breeding material that has been adopted (recurrent parent). This can be done by repeatedly backcrossing and using Dr. Edger Anderson's concept of introgression through backcross. Three backcross strategies can maximize the frequency of recombination between the parents:

2.9.1. Repeated Backcross

This method entails repeatedly backcrossing (typically six times) of the cross between the recurrent parent and the donor parent, either with or without selection.

2.9.2. Inbred Backcross

Wehrhahn and Allard first proposed this approach in 1965. This technique requires a small number of backcrosses (typically three) and followed by several generations of selfing.

2.9.3 Congruity Backcross

Backcrossing is carried out using both donor and recurrent parents in different generations in this method.

3. Major Activities Involved in Pre-breeding

High cost and long-term return are characteristics of genetic resource-related activities. It is impossible to emphasize the importance of introduction and germplasm exchange, collection, characterization, evaluation, documentation, and conservation. For the bank to maintain genetic diversity and ensure germplasm, consumption there needs to be an appropriate synchronism between these activities. It is well acknowledged that genetic resources are important. Working with germplasm banks requires experienced researchers with a wide range of expertise. Utilizing the available accessions is a goal that is equally vital to preserving genetic diversity for the future. The lack of documentation and adequate collection descriptions, the lack of breeders' desired information, accessions with limited adaptability, a lack of plant breeders, particularly in developing nations, and a lack of collection evaluations are the main causes of the low utilization of plant genetic resources. Another barrier to their utilization is the low seed availability caused due to ineffective seed regeneration programs (Dowswell, 2019).

Landraces are native varieties that have been selected to grow in a particular agro-ecological and farming system, often known as farmer's varieties or cultivated native varieties. For crop breeding programs, landraces are a great source of genetic diversity because they are inevitably heterogeneous (Sleper and Poehlman, 2006). Genes for early maturity, yield potential, disease and insect resistance, and other beneficial traits are among the helpful genes found in landraces. The areas with the largest diversity are where landraces are most common. For breeding purposes and to assess the degree of genetic variation, they can be identified using a variety of markers (biochemical, physiological, morphological, and molecular markers). Landrace types are primarily cultivated in underdeveloped nations for the characteristics that farmers consider desirable (Ceccarelli and Grando, 2007).

3.1. Characterization of Landrace Populations

Landraces are cultivars that farmers nurtured and cultivated over many years using traditional methods of selection. According to (Arias *et al.*, 2007) and (Martnez-Castillo *et al.*, 2008), maize landrace populations in Mexico exhibited symptoms of genetic erosion and a decline in in-situ conservation, which led to the loss of novel, unusual, and advantageous features with potential for use in agriculture. In addition, factors that are making crop production difficult include drought and heat stress, low soil fertility, parasitic weeds, disease, and others (Nelimor *et al.*, 2020; Liu *et al.*, 2020). These factors indicate that plant breeders need consistent access to new genetic variation in order to develop crop varieties that can adapt to the changing climate.

This suggests the need for diversity research, characterization, and landrace conservation in order to be used in future breeding. Landraces are essential for enhancing abiotic stress resilience in current breeding programs, according to (Meseka *et al.*, 2013) and (Pineda Hidalgo *et al.*, 2013). Geographical, morphological, and molecular data are significant in landrace diversity research (Gonzalez *et al.*, 2013). The morphological descriptors are popular in poor nations because they are simple to record, affordable, and accurate at estimating heredity (Mengistu *et al.*, 2015). Phenotypic evaluation, according to (Sattler *et al.*, 2018) and (Tiwari *et al.*, 2019), has been successful in analyzing cereal diversity. However, because they are more reliable and less influenced by the environment, molecular markers are being used more frequently in the genetic research of plants. The landraces should be identified using one of the suggested markers and must be conserved for the advancement of breeding procedures.

3.2 Creation of New Parent Populations

The first step in crop breeding success is selecting parents with enhancing and desired traits. Due to these breeders, parent populations for future generations are continuously chosen from a variety of sources (landraces, modern cultivars, classical or ancient cultivars, species from the wild or semi-wild).

Through progeny testing, the parents with the best general or specific combining ability could be chosen. Breeders can identify the genetic potential of parents and suggest the type of variety that can be produced through progeny testing (Acquaah, 2009; Brown and Caligari, 2008).

3.3 Introgression of New Traits

It is the transfer of one or more desired genes into an intermediate variety that has excellent agronomic potential but lacking a specific trait from unrelated, exotic or semi-exotic, landrace, or related germplasm (Suarez-Gonzalez *et al.*, 2018, Simmonds, N.W., 1993). Both desired and considerable amounts of undesirable genetic material will be transferred to the progeny during interrogation; the undesirable genetic material must be removed through subsequent backcrossing and selection (Stam, 2003). An intermediate variety that has great agronomic potential but lacking a particular characteristic is produced by the plant breeder by transferring one or more desirable traits from unrelated, exotic or semi-exotic, landrace, or related germplasm (Simmonds, 1993). As a result, the new variety will be created using the unique gene(s) inserted into the genetic background. According to Hallauer and Miranda Filho (1988), exotic germplasm might include groups of races, populations, clones, inbred lines, and different kinds of genetic structure. A significant amount of undesired genetic material must be eliminated from the progeny through a succession of backcrosses to the recurrent parent when introducing genes from unrelated, exotic, primitive, or wild germplasm (Brown and Caligari, 2008).

3.4 Creation of novel traits

Mutations cause spontaneous genetic changes in people that are frequently heritable. Naturally, mutational events occur around 10^{-5} and 10^{-8} times per locus. In order to enhance the incidence of mutations and, as a result, to increase genetic variety, induced mutagenesis through the use of artificial mutagenic agents is a crucial strategy in plant breeding and functional genomics. Several crops have effectively utilized induced genetic variants to produce mutants that are valuable (Hohmann *et al.*, 2005). The method can be viewed as an effective choice for improving germplasm toward significant agronomic characteristics (Pozniak and Hucl, 2004). The novel mutational events may either occur directly as fundamentally derived varieties or novel genes can be incorporated into potential parents through a back cross program.

3.5 Creation of Polyploidy

Breeders can introduce additional variety by manipulating the number of chromosomes in a species, either by adding or deleting particular chromosomes or changing the basic chromosome set. Euploids are those who have an altered chromosomal set and are created by either doubling a species' genome or by mating two unrelated species and then doubling the chromosomes of the resulting inter-specific hybrid. Plant materials can be subjected to environmental shock (such as low or high temperature treatment, x-ray irradiation), or chemicals (such as colchicine) can be used to impair normal chromosomal division in order to artificially induce polyploidy (Sleeper and Poehlman, 2006). The masking of harmful recessive mutations should be possible with an increased number of alleles of a given gene in a polyploidy, protecting against performance loss (Gu *et al.*, 2003). According to (Birchler *et al.*, 2010), polyploidy facilitates heterosis development, which allows offspring to exhibit transgressive behavior when compared to their progenitor species. According to Adams K. L. and Wendel J. F. (2005) and Lynch M. and Walsh B. (2007), polyploidy may boost an organism's flexibility in how it responds to environmental change or allow it to enlarge its ecological niche.

3.6 Acquisition of new information on crop genetics

For improved nutritional traits, early maturity, high yield potential, and resistance to biotic and abiotic stress, breeders are continually looking for new genes from various sources. For successful transfer and to increase the effectiveness of selection in cultivar development, it is crucial to understand the candidate genes and the pattern of inheritance of the genes influencing these traits (Meneely, 2020).

3.7. Cultivar development

The development of cultivars includes specific breeding techniques with the objective of generating cultivars that are more appropriate to the crop's mating system. An established and relational

pre-breeding program must produce well-developed and elite breeding material for cultivar development. If available, regionally adapted and scrutinized germplasm makes an excellent base for cultivar development. To ensure that the best parental material is used that fits the demands of a defined set of agro-ecological zone and day-length requirements of a certain latitude, a plant breeder may spend many years in pre-breeding. Alternately, the breeder may use superior material (such as inbred lines) developed by other breeders, such as germplasm from the CGIAR centers that is not locally adapted.

A plant breeder will be able to introduce new candidate cultivars as a result, even if those cultivars are not yet adapted to the local agro-ecological and cropping system. Although the newly released cultivar may have some superior traits, local farmers frequently reject them since they were not grown to include the traits that farmer's desire. Given the broad spectrum and complexity of farmer trait preferences, total crop value is more significant than crop production alone (Witcombe *et al.*, 2001). Cooking quality, taste, market acceptability, storability, and the quantity of useable crop parts left after harvesting, processing, and storage are other characteristics that farmers seek (Mulatu and Zelleke, 2002).

Despite 25 years of sorghum breeding in Ethiopia, according to McGuire (2008), the majority of the cultivars that introduced were not well accepted by the small-scale farmers. Therefore, in order to increase farmers' adoption of a cultivar, breeders should strive for a balance between qualities that farmers value and ways to address production constraints. Crop breeding facilities with sufficient resources need to be handling two parallel programs simultaneously. First, a pre-breeding procedure that continually produces novel and enhanced parent materials with superior traits that are significant to commercialization.

3.8 Development of New Plant Breeding Techniques

New and modern breeding techniques, which improve selection response, include the development of more effective conventional selection procedures, biotechnology, molecular marker technologies and identification of markers linked to traits of interest, efficient gametocides, and cytoplasmic sterility systems with a desired genetic background (Lusser *et al.*, 2012). (Lusser *et al.*, 2012) described eight novel methods for plant breeding, including grafting on GM rootstocks, synthetic genomics, cisgenesis and intragenesis, oligonucleotide guided mutagenesis, and zinc finger nuclease technology. According to these researchers, cisgenesis and ODM are the most commonly utilized procedures, while grafting on GM rootstocks, RdDM, and the ZFN approach are less frequently used and still mostly used at the research level. The high technological potential and economic advantages associated with novel plant breeding methods are what drive their adoption (Lusser *et al.*, 2012). In order to speed up the breeding process and reduce production expenses, innovative plant breeding techniques are utilized.

3.9. Applications of doubled haploids in plant breeding

The simplest way to produce pure duplicated haploids (DH) is through in vitro haploid plant development followed by somatic chromosomal duplication.

3.10. Wide Crosses

It is a cross between two individuals from different species that gives plant breeders more appealing genetic resources for simpler utilization. Most frequently employed to transfer genes for resistance to biotic/abiotic stress, this method is typically utilized to increase the gene pool of crops in particular (Jain, S.K., and Omprakash, 2019). The main objective of the wide cross program is to introduce new characters to elite cultivar. This has been employed in various crops, including rice, blight-resistant potatoes, rust-resistant wheat, and insect-resistant rice, with great success.

4. Problems Associated with Genetic Enhancement

The introduction of genes from wild species into genetic improvement programs can cause a number of issues. The following are a few issues: (1) Cross incompatibility between different species. (2) The transfer of genes from wild species into domesticated ones has been hampered by stability barriers and chromosome pairing in hybrids. (3) Drag in links. (4) Sterility and hybrid inviability. (5) A population of inter-specific hybrids with a small sample size. (6) Limited genetic recombination in the population of hybrids. (7) There are not enough donors' traits, including as bollworm, pest, and disease

resistance. (8) Because of legal constraints like IPR, the exchange and accessibility of farmed species genetic material has become challenging (Loknathan *et al.*, 2003).

4.1. Limitations of Pre-breeding

Require a longer period of time (five to ten years longer), the exchange of germplasm is difficult due to intellectual property rights (IPR), new production practices are developed, cross incompatibility is created by widespread hybridization, linkage drag, and low levels of recombination in the hybrids, new pest or disease issues arise, and problems with climate change are needed to meet market demands.

5. Conclusion

Pre-breeding is a broad term for all activities designed to isolate desirable traits and/or genes from unadapted materials that cannot be used directly in breeding populations and to transfer these traits to an intermediate set of materials that breeders can use in the future in order to develop new varieties for farmers. Pre-breeding attempts to differentiate desired traits (such as disease resistance) from undesirable material like crop wild relatives and transfer them into breeding lines that are more easily able to be crossed with modern, elite varieties. Pre-breeding increases diversity in the elite gene pool by regaining losing desirable genetic variation. The genetic material of plants that has significance as a resource for both the present and the future is referred to as plant genetic resources, one of the key components of agro-biodiversity. The world's food and nutritional security, as well as the foundation of agriculture, are all dependent on plant genetic resources.

The effort to identify those desirable characteristics and create materials that breeders can use is known as pre-breeding. It is the crucial first step in the exploitation of variety resulting from wild relatives and other unimproved materials, and it is predicated on tight collaboration between gene bank curators and plant breeders. Wild species are a rich source of beneficial genes that can be effectively utilized to create genetic diversity after considerable hybridization. A vital stage in pre-breeding is to effectively transfer new genetic variation to the elite cultivar with little linkage drag and no alteration of gene balance for an elite genotype.

In order to speed genetic progress and enhance the nutritional value and resilience of modern crop varieties, it is important to boost pre-breeding programs, which will make sure that new genetic variability is regularly provided into the primary breeding programs. This is especially true today, when climate change has increased the urgency of using the wild to their maximum potential. While breeding can take some time, it is crucial to maximize the crop's biological potential when there is a need for discovery and translational research, as well as potential broad crossover with wild relatives or selective selection of progeny.

Any pre-breeding program's effectiveness mostly depends on different factors: These are identifying a potential donor who has excellent trait expression; the type of germplasm (cultivated/cross-compatible wild type/cross-incompatible wild type); and the donors' agronomic performance. In both cultivated and wild type germplasm, there are now new and varied sources of variation for traits related to crops and nutrition as well as resistant/tolerant sources for biotic/abiotic stresses that can be used to create new pre-breeding populations with greater variability for different traits. Climate variability is an increasing threat to crop production worldwide since it frequently causes droughts due to irregular rainfall, the predominance of high temperatures, water logging, increased soil salinity, and the appearance of new insect pests and diseases. A number of areas are now unsuitable for the production of traditional crops as a result of climate change. Breeding new crop cultivars with a wide genetic basis and the ability to cope with frequent climate fluctuates is necessary to deal with this predicament.

References

- Acosta-Gallegos, J.A., J.D. Kelly, and P. Gepts, 2007. Pre-breeding in common bean and use of genetic diversity from wild germplasm. *Crop Science*, **47**: S-44.
- Acquaah, G., 2009. Principles of plant genetics and breeding. John Wiley & Sons.
- Adams, K.L. and J.F. Wendel, 2005. Polyploidy and genome evolution in plants. *Current opinion in plant biology*, **8**(2):135-141.
- Arias, L.M., L. Latournerie, S. Montiel, and E. Sauri, 2007. Cambios recientes en la diversidad de maíces criollos de Yucatán, México. *Ecosistemas y Recursos Agropecuarios*, **23**(1).

- Birchler, J.A., H. Yao, S. Chudalayandi, D. Vaiman, and R.A. Veitia, 2010. Heterosis. *The Plant Cell*, 22(7):2105-2112.
- Brown, J and P. Caligari, 2008. *An introduction to plant breeding*. Blackwell Publishing Ltd, Oxford, UK.
- Ceccarelli, S., S. Grando, and M. Baum, 2007. Participatory plant breeding in water-limited environments. *Experimental Agriculture*, 43(4):411-435.
- Dowswell, C., 2019. *Maize in the third world*. CRC press.
- Frese, L., 2002. Combining static and dynamic management of PGR: a case study of Beta genetic resources. In *Managing plant genetic diversity. Proceedings of an international conference, Kuala Lumpur, Malaysia, 12-16 June 2000* (133-147). Wallingford UK: CABI Publishing.
- Frese, L., B. Desprez, and D. Ziegler, 2001. 17 Potential of Genetic Resources and Breeding Strategies for Base-broadening in Beta. *Broadening the genetic base of crop production*, 295.
- Gonzalez, C.M.E., R.N. Palacios, B.A. Espinoza, and S.C.A. Bedoya, 2013. Diversidad genética en maíces nativos mexicanos tropicales. *Rev. Fitotec. Mex.*, 36: 329- 338.
- Gu, Z., L.M. Steinmetz, X. Gu, C. Scharfe, R.W. Davis, and W.H. Li, 2003. Role of duplicate genes in genetic robustness against null mutations. *Nature*, 421(6918):63-66.
- Hallauer, A.R. and J.B. Miranda Filho, 1988. *Quantitative genetics in maize breeding*. Ames: Iowa University Press.
- Hallauer, A.R. and J.B. Miranda, 1981. *Quantitative genetics in maize breeding* Iowa State Univ. Press. Ames Iowa
- Hallauer, A.R., M.J. Carena, and J.D. Miranda Filho, 2010. *Quantitative genetics in maize breeding* (Vol. 6). Springer Science & Business Media.
- Hallauer, A.R., W.A. Russell, and K.R. Lamkey, 1988. Corn breeding. *Corn and corn improvement*, 18:463-564.
- Hallauer, A.R., W.A. Russell, and K.R. Lamkey, 1988. Corn breeding. *Corn and corn improvement*, 18:463-564.
- Hausmann, B.I.G., H.K. Parzies, T. Presterl, Z. Susic, and T. Miedaner, 2004. Plant genetic resources in crop improvement. *Plant genetic resources*, 2(1):3-21.
- Hohmann, U., G. Jacobs, and C. Jung, 2005. An EMS mutagenesis protocol for sugar beet and isolation of non-bolting mutants. *Plant breeding*, 124(4):317-321.
- Iqbal, A.M., A.A. Lone, S.A. Wani, S.H. Wani, and F.A. Nehvi, 2017. Pre-breeding and Population Improvement. *Inter Jour of Lif Scie* DOI: 10.5958/j.2319-1198.2.3.023.
- Jain, S.K. and Omprakash. 2019. Pre-breeding: A Bridge between Genetic Resources and Crop Improvement. *Int.J.Curr. Microbiol. App. Sci.* 8(02): 1998-2007.
- Jones, I.T., 1983. Transgressive segregation for enhanced level of adult plant resistance to mildew in the oat cross Mostyn x Maldwyn. *Euphytica*, 32:499-503.
- Kannenbergh, L.W. and D.E. Falk, 1995. Models for activation of plant genetic resources for crop breeding programs. *Canadian Journal of Plant Science*, 75(1):45-53.
- Knight, R.L., 1945. The theory and application of the backcross technique in cotton breeding. *Journal of Genetics*, 47(1):76-86.
- Kumar V, and Y.M. Shukla, 2018. Pre-breeding: its applications in crop improvement, *Double Helix Research*, 16:199-202.
- Kumar, V. and Y.M. Shukla, 2014. Pre-breeding: Its applications in crop improvement. *Double Helix Res*, 16:199-202.
- Liu, M., Y. Li, Y. Ma, Q. Zhao, J. Stiller, Q. Feng, Q. Tian, D. Liu, B. Han, and C. Liu, 2020. The draft genome of a wild barley genotype reveals its enrichment in genes related to biotic and abiotic stresses compared to cultivated barley. *Plant biotechnology journal*, 18(2):443-456.
- Lokanathan, T.R., P. Singh, D.K. Agarwal, P. Mohan, S.B. Singh, V. Gotmare, and V.V. Singh, 2003. Genetic enhancement in cotton. *Technical Bulletin from CICR Report*, (26).
- Lonnquist, J.H., 1974. Consideration and experiences with recombinations of exotic and Corn Belt maize germplasm. In *Proc. Annu. Corn Sorghum Res. Conf.*, 29: 102-117.
- Lusser, M., C. Parisi, D. Plan, and E. Rodríguez-Cerezo, 2012. Deployment of new biotechnologies in plant breeding. *Nature biotechnology*, 30(3):231-239.
- Lynch, M. and B. Walsh, 2007. *The origins of genome architecture* (Vol. 98). Sunderland, MA: Sinauer associates.

- Martínez-Castillo, J., M.P. Colunga-García, and D. Zizumbo- Villarreal, 2008. Genetic erosion and in situ conservation of Lima bean (*Phaseolus lunatus* L.) landraces in its Mesoamerican diversity center. *Genet Resour Crop Evol.*, 55: 1065–1077.
- McGuire, S.J., 2008. Path-dependency in plant breeding: Challenges facing participatory reforms in the Ethiopian Sorghum Improvement Program. *Agricultural Systems*, 96(1-3):139-149.
- Meneely, P., 2020. Genetic analysis: genes, genomes, and networks in eukaryotes. Oxford University Press, USA.
- Mengistu, D.K., A.Y. Kiros, and M.E. Pè, 2015. Phenotypic diversity in Ethiopian durum wheat (*Triticum turgidum* var. durum) landraces. *The Crop Journal*, 3(3):190-199.
- Meseka, S., M. Fakorede, S. Ajala, B. Badu-Apraku, and A. Menkir, 2013. Introgression of alleles from maize landraces to improve drought tolerance in an adapted germplasm. *Journal of Crop Improvement*, 27(1): 96-112.
- Mohan, A., N.P. Grant, W.F. Schillinger, and K.S. Gill, 2021. Characterizing reduced height wheat mutants for traits affecting abiotic stress and photosynthesis during seedling growth. *Physiologia Plantarum*, 172(1):233-246.
- Mohan, A., P. Kulwal, R. Singh, V. Kumar, R.R. Mir, J. Kumar, M. Prasad, H.S. Balyan, and P.K. Gupta, 2009. Genome-wide QTL analysis for pre-harvest sprouting tolerance in bread wheat. *Euphytica*, 168(3):319-329.
- Mulatu, E. and H. Zelleke, 2002. Farmers' highland maize (*Zea mays* L.) selection criteria: Implication for maize breeding for the Hararghe highlands of eastern Ethiopia. *Euphytica*, 127:11-30.
- Nass, L.L. and E. Paterniani, 2000. Pre-breeding: a link between genetic resources and maize breeding. *Scientia Agricola*, 57:581-587.
- Nelimor, C., B. Badu-Apraku, S.P. Nguetta, A.Y. Tetteh, and A.L. Garcia-Oliveira, 2020. Phenotypic characterization of maize landraces from Sahel and Coastal West Africa reveals marked diversity and potential for genetic improvement. *Journal of Crop Improvement*, 34(1): 122-138.
- Ogbonnaya, F.C., O. Abdalla, A. Mujeeb-Kazi, A.G. Kazi, S.S. Xu, N. Gosman, E.S. Lagudah, D. Bonnett, M.E. Sorrells, and H. Tsujimoto, 2013. Synthetic hexaploids: harnessing species of the primary gene pool for wheat improvement. *Plant Breed. Rev.*, 37:35-122.
- Pineda-Hidalgo, K.V., K.P. Méndez-Marroquín, E.V. Alvarez, J. Chávez-Ontiveros, P. Sánchez-Peña, J.A. Garzón-Tiznado, M.O. Vega-García, and J.A. López-Valenzuela, 2013. Microsatellite-based genetic diversity among accessions of maize landraces from Sinaloa in México. *Hereditas*, 150(4-6):53-59.
- Pozniak, C.J. and P.J. Hucl, 2004. Genetic analysis of imidazolinone resistance in mutation-derived lines of common wheat. *Crop Science*, 44(1):23-30.
- Sattler, F.T., M.D. Sanogo, I.A. Kassari, I.I. Angarawai, K.W. Gwadi, H. Dodo, and B.I.G. Haussmann, 2018. Characterization of West and Central African accessions from a pearl millet reference collection for agro-morphological traits and Striga resistance. *Plant Genetic Resources*, 16(3): 260-272.
- Sebolt, A.M., R.C. Shoemaker, and B.W. Diers, 2000. Analysis of a quantitative trait locus allele from wild soybean that increases seed protein concentration in soybean. *Crop Science*, 40(5):1438-1444.
- Shimelis, H. and M. Laing, 2012. Timelines in conventional crop improvement: pre-breeding and breeding procedures. *Australian Journal of Crop Science*, 6(11):1542-1549.
- Simmonds, N.W., 1993. Introgression and incorporation. *Strategies for the use of crop genetic resources*. Biological reviews, 68(4):539-562.
- Singh, K., S. Kumar, S.R. Kumar, M. Singh, and K. Gupta, 2019. Plant genetic resources management and pre-breeding in genomics era. *Indian J. Genet*, 79(1 Suppl 117):130.
- Sleper, D.A., and J.M. Poehlman, 2006. *BreedingField Crops*. Fifth Edition. Iowa State Press. Ames, USA.
- Stam, P., 2003. Marker-assisted introgression: speed at any cost. In *Eucarpia Leafy Vegetables*/Th. JL van Hintum, A. Lebeda, D. Pink, JW Schut (117- 124).
- Suarez-Gonzalez, A., C. Lexer, and Q.C. Cronk, 2018. Adaptive introgression: a plant perspective. *Biology letters*, 14(3):20170688.
- Tanksley, S.D. and S.R. McCouch, 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *science*, 277(5329):1063-1066.

- Tiwari, D.N., S.R. Tripathi, M.P. Tripathi, N. Khatri, and B.R. Bastola, 2019. Genetic variability and correlation coefficients of major traits in early maturing rice under rainfed lowland environments of Nepal. *Advances in Agriculture*.
- Trognitz, B.R., M. Bonierbale, J.A. Landeo, G. Forbes, J.E. Bradshaw, G.R. Mackay, R. Waugh, M.A. Huarte, and L. Colon, 2001. 24 Improving Potato Resistance to Disease under the Global Initiative on Late Blight. Broadening the genetic base of crop production, 385.
- Valkoun, J.J., 2001. Wheat pre-breeding using wild progenitors. *Euphytica*, 119:17-23.
- Witcombe, J.R., K.D. Joshi, R.B. Rana, and D.S. Virk, 2001. Increasing genetic diversity by participatory varietal selection in high potential production systems in Nepal and India. *Euphytica*, 122:575-588.