

Review Article

Role of pre-breeding in vegetable crop improvement - a review

ABSTRACT

Pre-breeding begins with the finding of beneficial genes from wild relatives, native species, and various other unadapted materials. These advantageous qualities are subsequently transferred to a moderate pool of resources so that breeders can create new varieties for farmers. With the process of discovering useful characteristics, preserving their genetic diversity, and incorporating these genes into a form that can be used, crop improvement innovation is generated. Connecting genetic diversity from wild relatives along with other uncontrolled sources is the main goal. Pre-breeding strategies aim to introduce genes for tolerance to environmental difficulties and resistance to major diseases and pests from wild relatives into cultivated crops using the application of introgression and integration procedures. Pre-breeding provides the base for commercially important techniques for plant breeding by broadening the diversity of germplasm and providing breeders with easily accessible resources to breeding beneficial characteristics, while adhering to the basic concepts of the field. The comprehensive discussion of pre-breeding is a valuable resource for scientists and researchers alike, addressing all aspects of this crucial phase in improving vegetable crops.

Keywords: Breeding, Cultivated, diversity Pre-breeding, Tolerance,

1. INTRODUCTION

India has made remarkable progress in vegetable production over the last thirty years, becoming the world's second-largest producer after China. However, to meet the demands of both local and global markets, there is a need for innovative methods in researching and breeding approved varieties and hybrids, as well as providing improved planting materials. Traditionally, crop development using plant genetic resources has relied on two techniques: introgression, which involves incorporating a few desirable genes into adapted stocks, and incorporation, which involves expanding locally adapted populations to enrich the genetic base of crops. While wild relatives offer genetic diversity for traits like tolerance to various stresses such as heat, drought, pests, and diseases, challenges like sexual incompatibility and hybridization barriers limit their use in crop improvement. The accumulation of 64,056 germplasm accessions comprising cultivated vegetable varieties and wild relatives from different regions in India between August 1976 and January 2019 showcases the nation's diverse vegetable genetic reservoir. 64,056 germplasm accessions of different vegetable genetic resources (VGR) have been collected from different locations across India during August 1976 and January 2019. Considerable numbers of cultivated vegetable germplasm (58,250) and wild relatives/wild vegetable resources (5,806) from different phytogeographical regions have been included in this collection. Cucurbitaceous vegetables (16,750), solanaceous vegetables (14,646), root/tuberous vegetables (8,298), bulbous vegetables (4,769), brassica/cole crops (1,776), leafy vegetables (2,084), leguminous vegetables (5,435), okra (4,235), and tree crops (257) had been the most common groups of cultivated crops collected (Pandey *et al.* 2019).

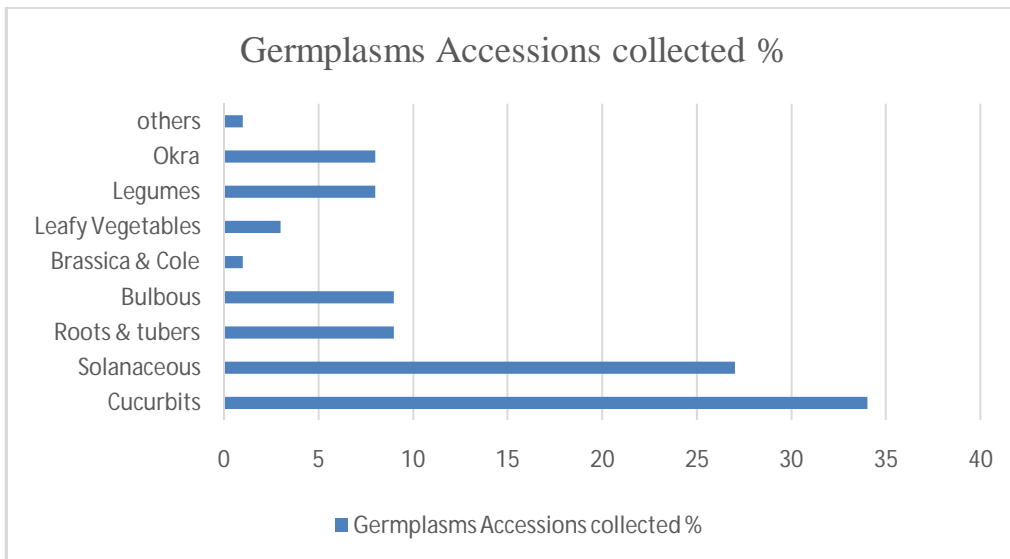


Fig 1. Germplasm accessions collected (per cent) in cultivated vegetable crop-groups.

2. Need for Pre-breeding

The enhancement of agricultural yields relies heavily on the presence of adequate genetic diversity that can be economically. The deficiency in reliability and diversity has hindered progress in breeding. The susceptibility to pests and diseases has increased due to the substitution of diverse native cultivars and landraces with genetically uniform modern types. Pre-breeding plays a pivotal role in initiatives focusing on germplasm diversity and plant breeding. Pre-breeding is instrumental in acclimating various germplasms to different genetic backgrounds and geographic regions. It serves to expand the genetic variability in crops to improve productivity, resistance to pests and diseases, and other desirable traits, thereby diminishing genetic homogeneity. Shimelis and Laing (2012) emphasize that the choice of pre-breeding relies on the anticipated success in integrating desired traits into cultivars. Given the limited genetic foundations, diminished biodiversity resulting from contemporary varieties, and heightened susceptibility to pests and diseases, pre-breeding emerges as a critical solution. It also addresses the challenges arising from climate change and new pest occurrences. Hence, pre-breeding is indispensable for fortifying crop resilience and supporting sustainable agriculture in response to evolving environmental and agricultural demands. Pre-breeding becomes necessary when the target genes are restricted to gene bank accessions unsuited to the target environments, closely linked wild species, and distant wild counterparts that are more conducive to cross-breeding, as posited by Kumar and Shukla (2014).

Pre-breeding work aims to achieve four goals

- 1) Using a broader collection of genetic material will help reduce genetic consistency in crops.
- 2) Identifying and transferring desirable characters and genes
- 2) Improving parental stocks for prompt usage in breeding applications; and
- 3) Finding potentially beneficial changes in a healthy-maintained and recognized gene bank.

Objective of pre-breeding

1. improved gene information and germplasm that improve resistance appearance and variety.
2. Utilizing a bigger pool of genetic material to boost production, resistance to diseases and pests, and additional beneficial features would reduce genetic uniformity in crops.
3. Finding advantageous features or genes and then transferring them to a good pair of parents to proceed with the selection procedure.
4. Improved methods for selection and better parental stocks that are easily useful to breeding operations.
5. Locate genes that may be supportive in a well maintained and recognized gene bank.
6. creating plans that result in stronger germplasm that has been collected for use in cultivar development.

3. Concept of Pre-breeding

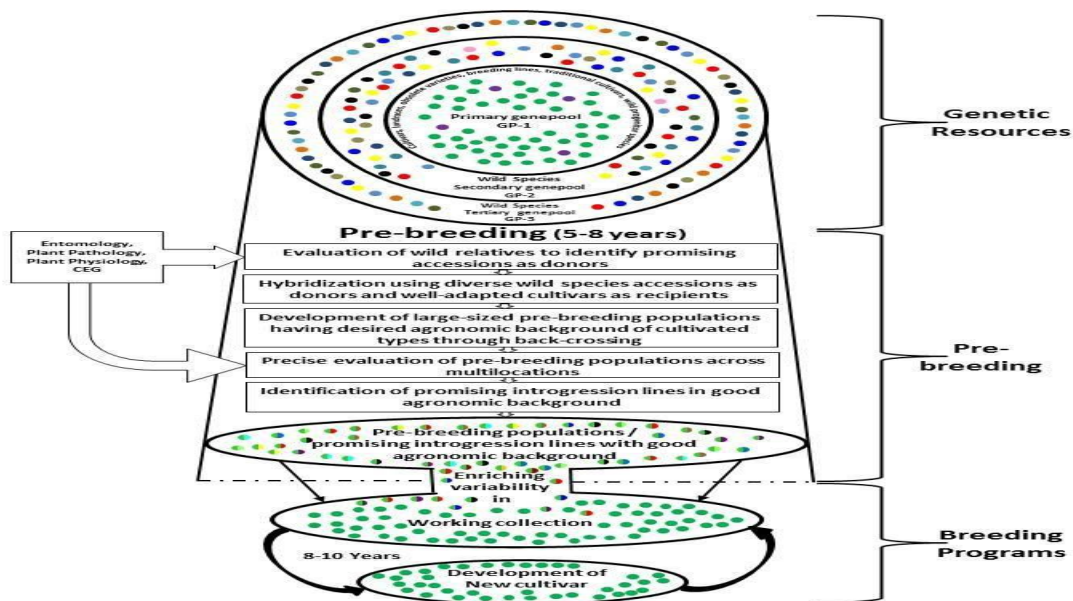


Fig 2. Pre-breeding as an association between crop growth and genetic resources.

To safeguard this unique genetic resource and fully utilize the abundant and easily accessible germplasm available in the country, thorough examination is essential. Pre-breeding provides a mechanism for incorporating specific genes from wild germplasm into genetic backgrounds to achieve desired traits. It serves as the starting point in the breeding process. Pre-breeding, as defined by Singh *et al.* (2019), constitutes a series of steps in plant breeding research that precede cultivar development, testing, and release. The connection between breeding efforts and genetic resources shows great potential. Materials developed through pre-breeding activities are considered valuable for integration into regular breeding procedures. The exotics used in pre-breeding encompass germplasm that has not been through adaptation selection and does not immediately display favourable characteristics (Hallauer *et al.*, 1988). Hence, races, populations, inbred lines, and similar groups may represent alien germplasms. Building on the work of Haussmann *et al.* (2004), pre-breeding utilizes a wider range of genetic materials to establish a new foundational population for the breeding program. To achieve outstanding outcomes, exotics require several generations of breeding to enable genetic recombination and mild selection. A minimum of five generations involving random mating under moderate selection pressure is necessary before beneficial recombinants can be identified (Lonnquist, 1974).

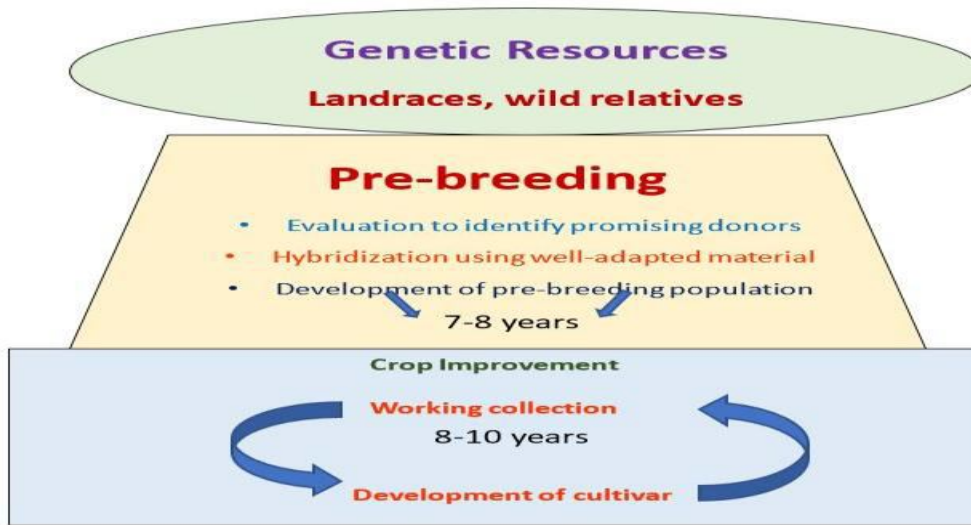


Fig 3. Pre-breeding as a bridge between genetic resources and crop improvement

4. The Gene Pool Concept

According to Haussmann *et al.* (2004). The term "gene pool" refers to the whole set of genes present in a species' reproductive population, including related species that are capable of interbreeding. The gene pool for crops includes botanical varieties, landraces, inbred lines, historic and modern landraces, related wild species, subspecies, and weedy companion species.

primary gene pool: The same species, both cultivated and wild.

Secondary gene pool: Species different from the cultivated one.

Tertiary gene pool: More distantly related species.

Quaternary gene pool: Unrelated plant species or other organisms.

5. Methods of Pre-breeding

Introgression involves transferring one or more genes from unadapted germplasm (the donor parent) to elite breeding material (the recurrent parent) through repeated backcrossing, using Dr. Edgar Anderson's concept. Three backcross strategies can optimize the recombination frequency between the parents.

5.1 Repeated Backcross: This method involves performing repeated backcrosses, usually six times, between the recurrent parent and the donor parent, with or without selection.

5.2 Inbred Backcross: This approach, first proposed by Wehrhahn and Allard in 1965, involves a small number of backcrosses, usually three, followed by multiple generations of selfing.

5.3 Congruity Backcross: In this method, backcrossing is conducted using both the donor and recurrent parents in different generations.

6. Approaches to pre-breeding

Under the field of agricultural development, numerous strategies propel advancements in crop breeding. Dr. Edgar Anderson invented introgression, which refers to the process used to introduce genes from wild or foreign stocks into breeding populations through strictly controlled crossings, most notably through backcrossing methods. Simmonds suggested integration to increase crop resilience and diversity by incorporating foreign genetic factors into populations that had already adapted locally. As demonstrated by the breeding of the potato wide crossings which break down species boundaries help to expand crop gene pools, especially for characteristics like disease resistance. In opposition to conventional procedures, Decentralized Participatory Plant Breeding is a cooperative effort between farmers and breeders that performs trials in farmers' fields, promoting more widespread use and local adaptation. Chromosome manipulation, such as the generation of Aneuploids and Polyploids, alters the gene pool to provide desired characteristics like disease resistance, improving the effectiveness of breeding. By mixing somatic cells, somatic hybridization avoids species boundaries and creates an avenue for hybridization across species that are taxonomically different. Advances in technology in plant breeding, including as biotechnology and molecular marker technologies, revolutionize trait selection and recognition by providing increased accuracy and efficiency. Pre-breeding efforts use wild species' genetic variety to improve agricultural varieties; multi-national programs like as LAMP serve as examples of this. Collectively, these methods encourage crop breeding innovation and sustainability, ensuring constant advances in agricultural resilience and manufacturing.

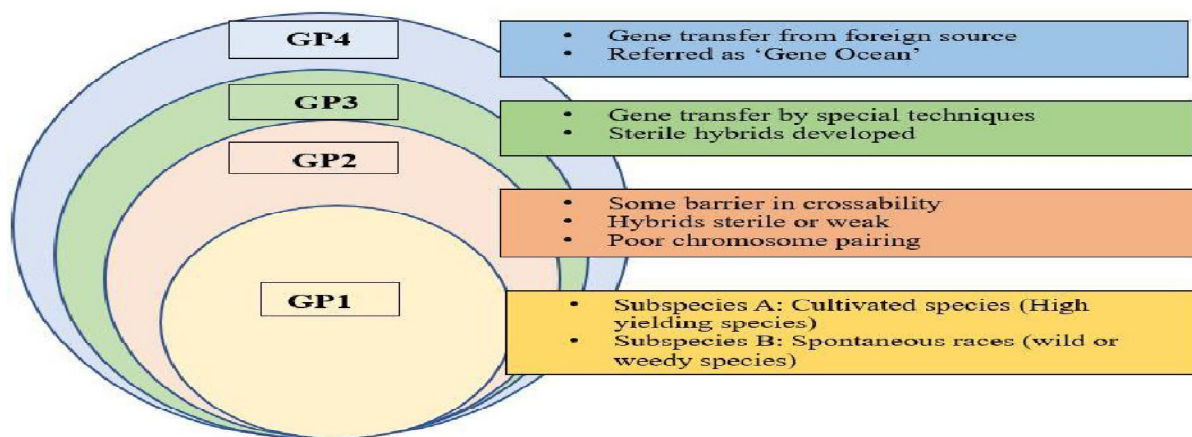


Fig 4. Modified gene pool concept in plants based on the hybridization study.

Table

S. no.	Crop	Findings	Reference
1	Tomato	Demonstrated that diploid plants from the	Mangat <i>et al.</i> 2021

1.

Important traits improved in vegetable crops through pre breeding approach.

		<p>offspring of monosomic alien addition lines (MAALs) of <i>S. lycopersicoides</i> may be identified for use as donors in tomato breeding to improve characteristics that are wanted. novel characteristics in the MDILs associated with drought tolerance that are not present in the fields of parents.</p>	
		<p>Divergent genes resistant to infections have been backcrossed and included in commercial hybrids produced with various natural resources. These genes are found on distinct tiny chromosomal regions that derive from various donor species. Fruit with an important gene introduced from a wild tomato species (<i>Lycopersicon pennellii</i> B.) raised by a factor of fifteen in pro-vitamin A attention to detail.</p>	Ronen <i>et al.</i> 2000
2	Brinjal	<p>It has been tried to pre-breed for bacterial wilt resistance.</p>	Neelambika <i>et al.</i> 2018
3	Potato	<p>In the genetically incompatibility species <i>S. bulbocastanum</i>, four cloned wide-spectrum genes (Rpi) resistant to the infection of <i>Phytophthora infestans</i> were discovered. Plants having each of the four-leaf blight-resistant genes had been chosen in trials performed in both greenhouse and outside environments. Somatic hybridization combined with gene-specific markers along with associated Avr effectors offers a successful approach of discovering and delivering late blight genes for resistance through the potato gene pool.</p>	Rakosy-Ticanet <i>et al.</i> 2020
		<p>Induced mutagenesis has been carried out on the potato using ethyl methane sulfonate (EMS) with the goal to expand the genetic potential of diploid potatoes for use as pre-breeding resource in both polyploid and diploid potato breeding.</p>	Somalrajuet <i>et al.</i> 2018
4	Cauliflower	<p>Interspecific asymmetric somatic hybrids of <i>Brassica oleracea</i> var. <i>botrytis</i> (cauliflower) and <i>Brassica nigra</i> (black mustard) were created by protoplast fusion, and the backcrossed (BC₃) and selfed (S₃) offspring were seen at.</p>	Wang <i>et al.</i> 2011
5	Asparagus	<p>Using crop wild relatives (CWR) such as <i>A. maritimus</i>, <i>A. pseudoscaber</i>, <i>A. brachyphyllus</i>, and <i>A. macrorrhizus</i> to produce novel plant material has widened the genetic base. The results demonstrate an increase in genetic variability, presenting new opportunities for improving asparagus.</p>	Wang <i>et al.</i> 2011
6	French Bean	<p>Wild relatives are a valuable source of novel alleles that can be utilized to enhance yield and other quantitative traits.</p>	Acosta-gallegos <i>et al.</i> 2007

Table 2. List of land races and their potential use in some of the vegetable cropsSource: Pandey *et al.*, 2005.

Crops	Traits	Germplasm conserved
Melon	Powdery mildew	PMR45, PMR450, PMR5, PMR6, PI124111
	Downy mildew	DMDR-1, DMDR-2
	Fruitfly	<i>Cucumis callosus</i>
	Nematode	<i>Cucumis metuliferus</i>
Watermelon	Whitefly	<i>Cucumis denterii</i> , <i>Cucumis dipsaceus</i> , <i>Cucumis sagittatus</i>
	Fusarium wilt	Summit, Conqueror, Charleston gray, Dixilee, Crimson sweet
	Anthraco nose	Charleston gray, Congo, PI189225
Bottle gourd	CMV, SqMV,	WMVPI271353
	Fusarium Wilt	Taiwan variety Renshi
Cucumber	Downy mildew, powdery mildew	Poinsette
	Anthraco nose	PI175111, PI175120, PI 179676, PI182445
	Powdery mildew	PI200818, <i>Cucumis hardwickii</i>
	CMV	WiscSMR-12, SMR-15, SMR-18
Pumpkin	Powdery Mildew and Viruses	<i>Cucurbita undelliana</i> , <i>Cucurbita martenezii</i>
	ZYMV, WMVC.	<i>Cucurbita ecuadorensis</i> , <i>Cucurbita faetidistima</i> , <i>Cucurbita martenezii</i>
Tomato	Bacterial wilt	EC467725-935, EC438314-317, EC182761-182874, EC26511-13
	Fusarium wilt	Pan American, Florida, PI79532
	Root knot Nematode	Nemared, VNF-8, Florida, Hawaii cross
	Heat tolerant lines	EC198416, EC501573-83, EC479027, 31, 34, 36, 139, 140, 141 and 143
Brinjal	Bacterial wilt	EC104107, Florida Market
	Phomopsis fruit rot	EC305069, 316274
	Tolerance to frost	Black torpedo, Long Tom '4'
	Tolerance to drought	Supreme, Violet round
Chilli	Cucumber mosaic virus	EC312342-312349
	PBNV mosaic virus	EC 121490
	Aphids	EC28, 30 and 34
Okra	YVMV	EC133408, EC169333, EC169334, Ghanared, <i>Abelmoschus manihot</i> ssp <i>tetraphyllus</i> , <i>Abelmoschus manihot</i> ssp <i>manihot</i>
	Jassids	EC305656, 305694, 305695
Cabbage	Blackrot	EC24855, EC28770, Cabbage Standby
Cauliflower	Blackrot	Aemel, Olympus, Lawyana
Onion	Purple blotch	EC328494, EC328492, EC328501, EC321463
Pea	Powdery mildew	EC342007
Muskmelon	Downey mildew, Powdery mildew, Anthracnose	Crimson sweet, shipper

Table 3. Registered germplasm of cucurbits with unique traits with the NBPGR, New Delhi (Rai *et al.*, 2008).

Crop	Line	Registered name	Trait associated
Pointed gourd	IIVRPG-105	INGR-03035	Parthenocarpic fruits
Bitter gourd	GY-63	INGR-03037	Gynoecious sex with high yield
Watermelon	RW-187-2	INGR-01037	High yield and yellow coloured flesh
	RW-177-2	INGR-01038	Leaf mutant with simple unlobed leaves
Bottle gourd	Androman-6	INGR-99009	Andromonoecious sex
	PBOG-54	INGR-99022	Segmented leaves
Cucumber	AHC-2	INGR-98017	High yield and long fruit
	AHC-13	INGR-98018	Small fruit, drought and temperature tolerant
<i>Cucumis melo</i> var. <i>callosus</i>	AHK-119	INGR-98013	High yield and drought tolerance
Round melon	HT-10	INGR-99038	Tolerant to downy mildew and root rot wilt
Snap melon	AHS-10	INGR-98015	High yield and drought tolerance
	AHS-82	INGR-98016	High yield and drought tolerance
	B-159	INGR-07044	Downy mildew resistance

6.1 Characterization of landrace populations

Landraces, extinct cultivars, wild relatives, advanced breeding lines, popular varieties, synthetic aneuploid and polyploid lines, and more have been included in the germplasm. These genetic lines had genes necessary for useful characters such as resistance to diseases and insects, early maturity, yield-related characteristics, and local adaptability (Prasanna, 2012). They are additionally useful to find new characters that are lacking in domesticated germplasm or to develop existing ones (Tables 1, 2 and 3). These lines may be defined using several markers (biochemical, physiological, morphological, and molecular) in the present-day context of genomics and proteomics to assist in breeding and assess genetic diversity (Podlich *et al.* 2004; Ortiz *et al.* 2008). Because of their farmer-preferred traits which have several applications—local varieties are usually grown in countries that are developing (Multau and Zelleke, 2002; Ceccarelli and Grando, 2007).

6.2 Marker assisted breeding

Breeding techniques which use DNA molecular marker patterns in addition to or instead of characteristic values have become more common. Plant breeders can select desirable and attractive crop includes more effectively due to this technique. When molecular markers that extremely co-segregate with candidate genes are available, marker-assisted selection (MAS) may enhance the effectiveness of selecting fundamental characteristics in conventional plant breeding programs (Knapp, 1998; Podlich *et al.*, 2004).

6.3 Tagging or mapping of gene/QTLs

Molecular mapping and gene tagging for many disease resistance genes have contributed to a serious increase in the use of wild material as a source of resistance. Gene cloning and QTL mapping are common methods currently. Numerous crops have had a variety of characteristics noticed and mapped, including plant height, male sterility, yield-related factors, biotic and abiotic stress-related traits, and a few quality attributes (Shen *et al.* 2007; Huang *et al.* 2003; Saxena *et al.* 2010).

6.4 Identification of novel allele

Significant variation for a single gene/QTL has been identified with the use of allele analysis methods. Several reverse techniques for breeding are currently employed to find new mutations that provide specific characteristics and can be utilized in plant breeding, including genome editing, site-directed mutagenesis, TILLING, Eco TILLING, and others. Materials derived from germplasm may help in understanding the nature of the causes of mutations that contributed to the evolutionary history of specific crops (Slade *et al.* 2005; Mejlhede *et al.* 2006).

6.5 Creating new parent populations

The selection of the most suitable parents with enhancing and desirable attributes are crucial to crop breeding success. Therefore, possible parent populations are continuously selected through breeders from a wide range of sources, including landraces, modern cultivars, obsolete or ancient cultivars, and wild or semi-wild species. To find parents with a significant amount of general or specific combining ability, progeny testing is frequently used. Breeders can evaluate the genetic potential of parents while determining the type of variation that is likely to be developed through progeny testing.

6.6 Somatic Hybridization

Sexual hybridization is limited in most of the crop. Species barriers thereby limit the usefulness of sexual hybridization for crop improvement. Somatic cell fusion leading to the formation of viable cell hybrids has been suggested as a method to overcome the species barriers to sexual hybridization. Plant protoplasts offer exciting possibilities in the fields of somatic cell genetics and crop improvement. The technique of hybrid production through the fusion of isolated somatic protoplasts under in vitro conditions and subsequent development of their product which is known as heterokaryon to a hybrid plant is known as somatic hybridization. It provides us with an opportunity to constructions hybrids between taxonomically distinct plant species beyond the limits of sexual crossability.

6.7 Creation of Aneuploids & Polyploids

The breeder could create a novel new variability through changing the number of chromosomes in a species, either by altering the basic chromosome set or addition or deletion of specific chromosome(s). Individuals with altered chromosome set (euploids) are developed by doubling the number of genome of a species or by crossing unrelated species followed by chromosome doubling of the inter-specific hybrid. Polyploids can be artificially induced by various means such as exposing plant materials to environmental shock (e.g. low or high temperature treatment, x-ray irradiation) or with chemicals (e.g. colchicine) that disrupt normal chromosome division [Brown and Caligar, 2008; Peiris *et al.*, 2008; Slepser and Poehlman, 2006]. Chromosome doubling of anther culture derived haploid plants from F₁ generates double haploids (DHs). The suitability of doubled haploid progenies for mapping project has been reviewed in by Lefebvre and coworker in pepper [1993]. *In vitro* production of haploid plants followed by doubling of somatic chromosomes is the quickest means to produce pure breeding doubled haploids (DHs) [Choo *et al.*, 1985; Donoughue and Bennet, 1994]. Haploids are produced through the method of anther culture [Henry *et al.*, 1990] or genome elimination following distant hybridization [Singh, 2001]. Selection is more efficient for oligogenic or polygenic traits in DHs because its ability to fix genes in a homozygous background, limiting dominance genetic variation and segregation [Choo *et al.*, 1995]. Therefore, double haploid derivatives could be selected for improved traits such as yield, earliness, plant height, nutritional quality and pest and disease resistance, in a fully homozygous state. Selected genotypes can be used as homogenous varieties or as breeding parents in the ensuing crosses and selection cycles in future.

7. Use of genomic approaches tools in pre-breeding

Genomics approaches are particularly useful when dealing with complex traits as these traits usually have a multigenic nature and an important environmental influence [Lu *et al.*, 2009; Brown *et al.*, 2006; Kottapalli *et al.*, 2006]. Genomic tools are thus facilitating the detect of QTL and the identification of existing favourable alleles of small effect which have frequently remained unnoticed and have not been included in the gene pool used for breeding [Frary *et al.*, 2005]. Recent technologies promise to provide an insight into the way gene(s) are expressed and regulated in cell and to unveil metabolic pathways involved in trait(s) of interest for breeders not only in model-/major- but even for under resourced crop species which were once considered "orphan" crops.

7.1 DNA based molecular markers and their applications

Molecular markers reveal genetic differences in the primary structure of DNA between individuals [Powell *et al.*, 1996; Horacek *et al.*, 2009]. Strategies like Marker assisted Selection, marker assisted backcrossing, marker assisted recurrent selection, marker assisted pyramiding and combined marker assisted selection can be utilized to assess the importance of wild relatives and to establish its relationship with cultivated improved cultivars. This will facilitate the identification of desirable characteristics or genes from unadapted plant genetic resources and transfer them to an intermediate product that breeder can manipulate to any kind of selection for improvement. MAS can assist for phenotypic screening by determining the allele of a DNA marker, plants that possess genes or quantitative trait loci (QTLs) may be identified based on their genotype rather than their phenotype. MAS has great advantage in early generation selections by eliminating undesirable gene combinations and retaining superior breeding line especially those that lack essential disease resistance genes [Eglinton *et al.*, 2004]. The relative efficiency of MAS is greatest for characters which has low heritability [Hiremath *et al.*, 2012]. Backcrossing is used in plant breeding to transfer favourable traits which is governed by few genes from a donor plant into an elite genotype (recurrent parent). While traditional backcrossing the donor segments attached to the target allele can remain relatively large, even after many backcrossing generations, so to minimize this linkage drag, marker assays could be a major advantage [Frisch *et al.*, 1999a]. With the use of markers, recurrent selection can be accelerated considerably, and several selection-cycles are possible within one year, accumulating favourable QTL alleles in the breeding population [Eglinton *et al.*, 2004]. To pyramid disease resistance genes that have similar phenotypic effects, and for which the matching races are often not available, MAS might even be the only practical method, especially where one gene mask the presence of other genes [Hiremath *et al.*, 2012, Sánchez *et al.*, 1993].

The strategic combination of MAS with phenotypic screening is known as 'combined MAS'. This may have merit over phenotypic screening or MAS alone to maximize genetic gain [Lande *et al.*, 1990]. Zhou *et al.* concluded that, MAS combined with phenotypic screening was more effective than phenotypic screening alone for a major QTL on chromosome 3BS for *Fusarium* head blight resistance in wheat [Zhou *et al.*, 2019]. The remarkable genetic gain through MARS is probably higher than that achievable through MABC [Ribaut *et al.*, 2007]. For a major resistance gene, marker based recurrent backcross programs are using frequently [Young *et al.*, 1993]. Tagging of gene in important vegetable crops has been made *viz.*, in tomato TMV resistance Tm-2 locus, nematode resistance, Mi gene, *Fusarium oxysporum* resistance gene, and powdery mildew resistance gene, etc. Huang *et al.* also make possible to tag powdery mildew resistance gene ol-1 on chromosome 6 of tomato using RAPD and SCAR markers [Huang *et al.*, 2003]. Many molecular

markers have been used today for DNA fingerprinting of cultivars and breeding lines in a number of vegetable crops viz., tomato, beans [Hamann *et al.*, 1995;], pepper [Prince *et al.*, 1995], and potato [McGregor *et al.*, 2000]. The fortuitous genetic linkage in tomato between the Aps-1 isozyme locus and the Mi locus that controls resistance to root knot nematode has been beneficial for developing nematode resistant tomato hybrids [Menda *et al.*, 2004]. Storage protein polymorphism in French bean (*Phaseolus vulgaris*L.) has been used to select for resistance to bean seed weevils which is very common in tropical and subtropical regions of the world.

7.2 SNP marker arrays or SNP chips approach

The discovery and labeling of novel genes have become significantly easier with the use of Genome-Wide Association Studies (GWAS) or QTL analysis. The advent of high-density SNP marker arrays has enabled cost-effective genome-wide association studies in wild populations. For instance, Zhou *et al.* (2019) utilized 775 tomato accessions (including wild accessions) and 2,316,117 SNPs from three GWAS panels to perform a meta-analysis GWAS. Their study identified 305 significant associations for sugars, acids, amino acids, and flavor-related volatiles.

7.3 Genotyping by sequencing (GBS) approach

Genotyping by sequencing (GBS) technique, combined with phenotyping, enables the discovery of QTLs influencing various traits. Genome-wide genotype-to-phenotype associations (GWAS) have gained popularity, thanks to SNP markers derived from whole-genome re-sequencing data and cost-effective automated genotyping systems.

7.4 Mechanical and precision phenotyping (Phenomics tools)

The rapid advancement of mechanical and precision phenotyping technologies has resulted in rapid development in germplasm assessment and dependable outcomes. For improved assessment, several direct and indirect methodologies are being developed. Many methodologies exist, such as NDVI estimation (Reynolds *et al.* 2007) phenotyping by drones (Holman *et al.* 2016) GIS-based phenotyping, non-destructive root phenotyping, and other techniques have made it possible to characterise germplasm in large quantities in a variety of crops (Hawkesford and Lorence, 2017).

7.5 Genome-wide selection

In addition to MARS, the genome-wide selection is another approach which can be utilized to pyramid favourable alleles for minor effect QTLs at whole genome level. GWS calculates the marker effects across the entire genome that explains entire phenotypic variation. The genome wide marker data available on the progeny lines, therefore, are used to calculate genomic estimated breeding values (GEBV). It is important to note that the GEBVs are calculated for individuals based on genotyping data using a model that was 'trained' from individuals having both phenotyping and genotyping data. These genomic estimated breeding values are then used to select the progeny lines for advancement in the breeding cycle. In summary, the GWS provides a strategy for selection of an individual without phenotypic data by using a model to predict the individual's breeding value [Poland *et al.*, 2012].

8 Challenges and Future Prospects

Inadequate character development, genetic diversity assessment, and funding pose hurdles to pre-breeding. Emphasizing the urgency of acquiring, characterizing, and documenting wild species, especially crop wild relatives, vital for conservation. Rising demand for specific genes in germplasm and genome banks aims to boost agriculture against biotic and abiotic challenges. Techniques such as genome mapping and genetic transformation offer avenues for crop improvement, enhancing stress resistance. To tackle complex traits, innovative breeding strategies and bioinformatics tools are essential, leveraging insights from genetic and genomics research.

Conclusion

Pre-breeding finds a significant characteristic in inadequate materials, "captures" its genetic diversity, and uses numerous methods to combine those genes into a form that is useful: There is adequate genetic variability in the form of landraces and wild relatives to enhance field crops, as they carry numerous valuable genes for cultivar improvement. Nevertheless, it demands time and resources for using these resources in breeding projects. Pre-breeding efforts should be undertaken to overcome this, utilizing promising landraces and wild relatives to create new genetic diversity that breeders may exploit in crop development efforts. Pre-breeding should not be done with the intention of increasing yield; rather, it should be done with a regular input of valuable variances into the breeding pipeline to create new high-yielding cultivars with a broad genetic basis. Pre-breeding is a time-consuming and challenging process, even if it is useful for improving cultivars by increase the fundamental gene pool.

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