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REVIEW ARTICLE

Pre-breeding approaches towards vegetable improvement: A review

Anuj Sohi[1], Balbir Singh Dogra[2], Shivali Dhiman[2*], Rahul Pathania[1*], Ankush Chaudhary[1], Prakash Singathiya[3], Aman Deep Ranga[1] & Anjali Kumari[2]

[1]Department of Vegetable Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Solan 173 230, Himachal Pradesh, India

[2]Department of Vegetable Science, College of Horticulture and Forestry, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Hamirpur 177 001, Himachal Pradesh, India

[3]Division of Vegetable Science, Indian Institute of Horticultural Research, Bengaluru 560 089, Karnataka, India

*Email: shivalidhiman26@gmail.com; rahulpathania008@gmail.com

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Abstract

The current genetic framework in agriculture poses risks as it undermines global food security. The shift from diverse landraces and local cultivars to genetically uniform modern varieties in traditional agroecosystems has increased the susceptibility of cultivars to pests and diseases. This poses a significant challenge to our valuable biodiversity. In response to changing environmental conditions, there is a need to discover novel genes and traits that enhance adaptability. To create high-yielding cultivars with broader genetic diversity, plant breeders must explore various germplasm sources. Gene banks play an important role in preserving beneficial genes and maintaining genetic variation. The urgent need to expand agricultural germplasm stocks and enhance crop management is beneficial for global food security. Plant genetic resources play a vital role in expanding germplasm stock and improving crop management systems. Pre-breeding delivers the unique advantage of incorporating desired genes from wild germplasm into cultivated varieties, minimizing linkage drag. It focuses on increasing genetic variability among germplasm so that it may be employed in normal genetic improvement programmes for cultivar development. Pre-breeding serves as a beneficial bridge between breeding efforts and genetic resources especially when non-adapted materials are involved. Despite limited utilization of plant genetic resources in crop development programs, pre-breeding can build new genomic technologies to enhance genetic diversity analysis and manipulation with an ultimate approach to create improved cultivars with desirable traits.

Keywords

crop improvement; plant genetic resources; pre-breeding; vegetables; wild relatives

Introduction

The current genetic framework in agriculture poses risks as it undermines global food security. The shift from local diverse cultivars and landraces to genetically uniform modern varieties in traditional agroecosystems has increased the susceptibility of cultivars to pests and diseases (1). The substitution of old cultivars and landraces with modern variants results in genetic vulnerability to pests and diseases. This poses a serious challenge to our rich biodiversity. Additionally, the changing environment necessitates the search for novel genes and traits to enhance adaptability (2).

Plant breeders are motivated to explore gene banks for valuable genes, which act as repositories of genetic diversity. Consequently, expanding agricultural germplasm stocks and improving management practices become essential.

Germplasm management involves both ex-situ conservation (acquisition and maintenance) and in-situ preservation. Conservation involves preserving the original genetic profile to the greatest extent possible, monitoring the viability and health of specimens (whether stored or in their natural habitat) and maintaining detailed records, including passport information and related data. Pre-breeding is different from conservation in which pre-breeding incorporates genetic improvement, which includes adapting "exotic" germplasm to local settings without losing its exotic and core genetic profile and introgression of improved characters from exotic germplasm into an adapted variety (3).

According to Simmonds, genetic improvement is divided into two categories: introgression which includes the backcrossing of a few genes to regulate desirable characters into adapted stocks and incorporation which incorporates the major improvement of locally adapted populations that is good enough to enter the adapted genetic base of concerned crops (4). Wild relatives with increased resistance to various stresses like drought, heat, pests and diseases are hazardous for crop diversity. However, their use in breeding is hindered by sexual incompatibility and genetic linkage issues. Over the past three decades, India has significantly advanced in vegetable production, becoming the second largest producer after China (5). Addressing domestic market challenges and competing globally complicates the development of breeding techniques to produce appropriate varieties and hybrids, as well as the provision of high-quality planting materials to producers. Integrating new genetic information will result in increased resistance to insect pests, enhanced disease tolerance, superior quality and various other benefits.

The incorporation of novel genetic information will enhance resistance to insect pests, improve disease tolerance, elevate quality and provide additional benefits. India's diverse agro-climatic conditions enable the cultivation of various vegetable types across different regions of the country. To maximize the use of the country's rich germplasm and protect this unique genetic resource, intensive research efforts are essential. Pre-breeding delivers a unique opportunity to incorporate desirable genes from wild germplasm into widely used genetic backgrounds, enhancing various desired traits (6).

Pre-breeding

Pre-breeding involves locating beneficial genes from non-domesticated sources and incorporating them into a transitional set of materials. These materials can then be utilized by breeders to create new plant varieties (7, 8). Pre-breeding is the initial phase in the breeding process. Pre-breeding comprises a series of actions in plant breeding research that must take place before the testing, development and release of cultivars (9). The plant breeding process is divided into three key stages. It starts with collecting germplasm, including genetic resources, landraces and crop wild relatives. The next stage, pre-breeding, which spans 6-8 years, involves evaluating and hybridizing selected accessions, followed by backcrossing to create pre-breeding populations with desirable traits. The final stage, breeding programs, focuses on crop improvement, maintaining a working collection and developing new cultivars (Fig. 1). This process is beneficial for producing new and high-quality plant varieties. Pre-breeding serves as a bridge between genetic resources and breeding programs. The materials produced through pre-breeding are considered valuable enough to be integrated into standard breeding practices. Exotic germplasm used in pre-breeding includes any genetic material that is not directly advantageous and has not been subjected to adaptation selection (10). Thus, foreign germplasms can include races, populations, inbred lines and similar categories. Pre-breeding attempts to produce a new base population for the breeding program by utilizing a larger pool of genetic material (11). Exotic germplasms need to be propagated over several generations to facilitate genetic recombination and moderate selection, aiming for promising results. Random mating of at least five generations with mild selection pressure is necessary before valuable recombinants can be identified (12).

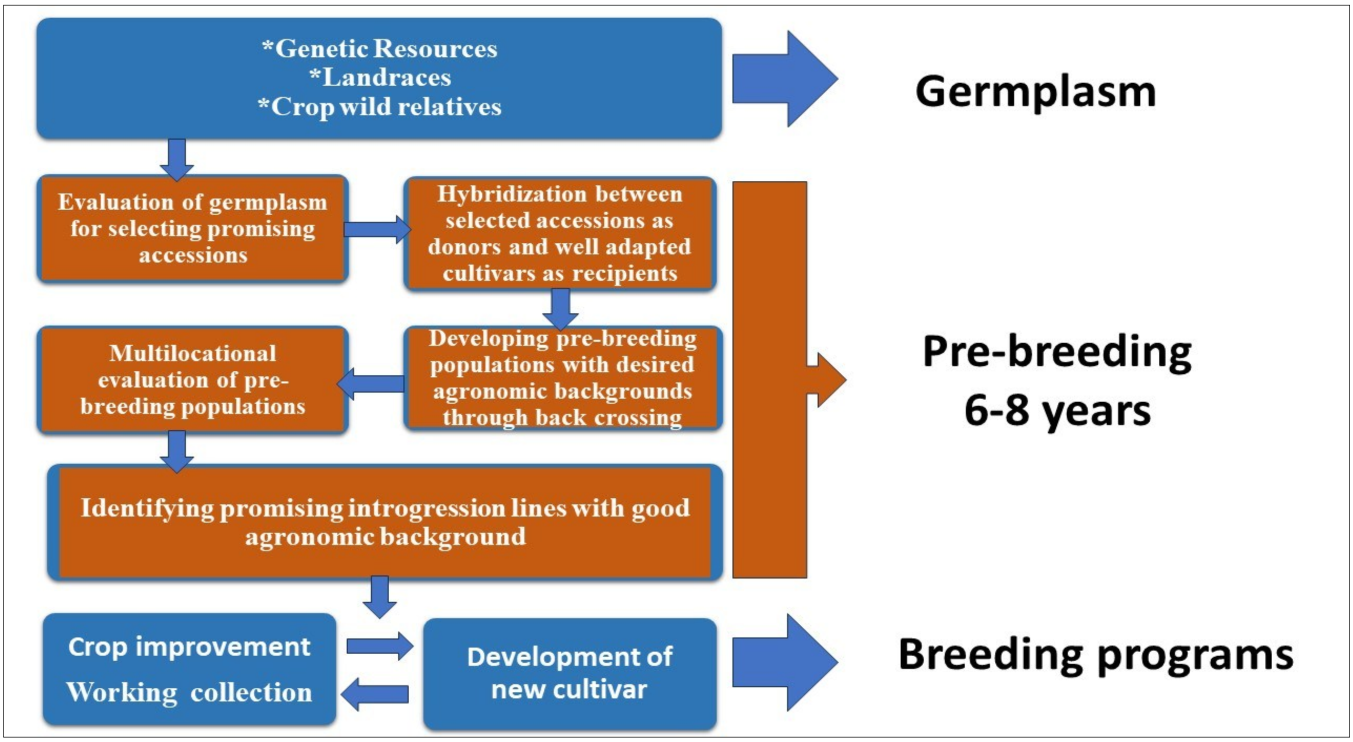

Fig. 1. Pre-breeding as a bridge between genetic resources and crop improvement.

Need for pre-breeding

While having sufficient genetic diversity is essential for successful crop improvement, the variability must be in a form that can be conventionally utilized. The progress in breeding is limited due to a lack of variability and diversity. The replacement of highly diverse landraces and local cultivars with modern and genetically uniform varieties has more susceptibility to various pests and diseases. Nowadays, pre-breeding is an important component of plant breeding and germplasm diversification. Pre-breeding has been used to adapt different types of germplasm to new genetic backgrounds and geographic regions (13). Pre-breeding aims to reduce genetic uniformity in crops by utilizing a broader range of genetic materials to enhance yield, resistance to pests and diseases and other quality traits (7). The inclusion of pre-breeding is determined by its anticipated efficiency, outcomes and effectiveness in transferring desired traits into cultivars for farmers and sourcing the necessary genes. Pre-breeding is crucial due to a limited genetic base, decreased biodiversity (with genetically uniform modern varieties dominating), genetic uniformity that enhances susceptibility to pests and diseases, climate change and the emergence of new pests. It is important only when target genes are found in gene bank accessions that are not well-adapted to the target environment, closely related wild species, or more distantly related wild species face difficulty while crossing the program (8).

Pre-breeding objectives

- To enhance resistance expression and variability through improved germplasm and genetic insights (14).
- To improve resistance to pests and disease, increasing yield and other quality traits by utilizing a broader genetic pool to reduce crop genetic uniformity (15).
- To identify and transfer desirable traits or genes to suitable parent groups for further selection (16).
- To develop better parental stocks for easier use in breeding programs and to refine selection procedures (17).
- To identify valuable genes within a well-organized and documented gene bank (18).
- To develop and design methodologies for creating enhanced germplasm for use in varietal development (19).

Activities involved in pre-breeding

Characterization of landrace populations: Germplasm includes wild relatives, obsolete cultivars, landraces, advanced breeding lines, synthetic aneuploids, popular varieties, polyploid lines and more. These genotypic lines possess important genes for early maturity, local adaptation, disease and insect resistance, yield-related traits and other desirable characteristics (20). They can also be utilized for identifying and developing new traits that are absent in domesticated germplasm. The catalog of the specimens in the National Herbarium of Cultivated Plants was specified in a study (Table 1) in which landraces of specific importance and their potential use in different vegetable crops (21). These traits can be characterized by using various markers (morphological, physiological, biochemical and molecular) for breeding and assessing genetic diversity in the modern era of

Table 1. List of germplasm conserved and their potential use (traits) in different vegetable crops (21)

Crop	Traits	Germplasm conserved
Melon	Powdery mildew	PMR 45, PMR 5, PMR 6, PMR 450, PI 124111
	Downy mildew	DMDR-1, DMDR-2
	Fruitfly	*Cucumis callosus*
	Nematode	*Cucumis metuliferus*
	Whitefly	*Cucumis dipsaceus, Cucumis denteri, Cucumis sagittatus*
Muskmelon	Downey mildew, Powdery mildew, Anthracnose	Crimson Sweet, Shipper
Watermelon	Fusarium wilt	Summit, Conqueror, Charleston Gray, Dixilee, Crimson Sweet
	Anthracnose	Charleston Gray, Congo, PI 189225
Bottle gourd	CMV, SqMV,	WMVPI 271353
	Fusarium Wilt	Taiwan variety Renshi
Cucumber	Downy mildew, powdery mildew	Poinsette
	Anthracnose	PI 175111, PI 175120, PI 179676, PI 182445
	Powdery mildew	PI 200818, *Cucumis hardwikkii*
	CMV	Wisc SMR-12, SMR-15, SMR-18
Pumpkin	Powdery Mildew and Viruses,	*Cucurbita lundelliana, Cucurbita martenezii*
	ZYMV, WMVC.	*Cucurbita faetidistima, Cucurbita ecuadorensis, Cucurbita martenezii*
Tomato	Bacterial wilt	EC 467725-935, EC 438314-317, EC 182761-182874, EC 26511-13
	Fusarium wilt	Pan American, Florida, PI 79532
	Root knot Nematode	Nemared, VNF-8, Florida, Hawaii cross
	Heat tolerant lines	EC 198416, EC 501573-83, EC 479027, 31, 34, 36, 139, 140, 141 and 143
Brinjal	Bacterial wilt	EC 104107, Florida Market
	Phomopsis fruit rot	EC 305069, 316274
	Tolerance to frost	Black torpedo, Long Tom '4'
	Tolerance to drought	Supreme, Violette round
Chilli	Cucumber mosaic virus	EC 312342-312349
	PBNV mosaic virus	EC 121490
	Aphids	EC 28, 30 and 34
	YVMV	EC 133408, EC 169334, EC169333, Ghana red, *Abelmoschus manihot* ssp *Manihot, Abelmoschus manihot* ssp *tetraphyllus*
Cabbage	Black rot	EC 24855, EC 28770, Cabbage Standby
Cauliflower	Black rot	Aemel, Lawyana, Olympus
Onion	Purple Blotch	EC 328501, EC 328492, EC 321463, EC 328494
Pea	Powdery Mildew	EC 342007
Okra	Jassids	305695, EC 305656, 305694

genomics and proteomics (22, 23). Local varieties are mostly produced in developing nations for their farmer-preferred features (24, 25), which may have a wide range of applications.

Marker-assisted breeding: The methods of breeding that utilize patterns of DNA molecular markers can be used to identify valuable traits that can help plant breeders more effectively select desirable crop characteristics. Marker-assisted selection (MAS) can increase the efficiency of selecting simple traits in traditional plant breeding programs when molecular markers that co-segregate with candidate genes are available (22, 26).

Tagging or mapping of gene/quantitative trait locus (QTLs): The use of wild material for resistance has increased significantly due to gene tagging and molecular mapping for various genes' resistance to diseases. Currently, QTL mapping and gene cloning are widely used (27). Numerous traits such as male sterility, plant height, yield-related factors, abiotic and biotic stress-related traits and multiple quality characteristics have been identified and mapped in various crops (28, 29).

Identification of novel allele: Allele mining tools now allow for the detection of significant variation within a single gene or QTL. Various reverse breeding techniques, such as genome editing, site-directed mutagenesis, Eco-TILLING, TILLING and other techniques are being used to identify new mutations that confer specific traits to be utilized in plant breeding programs. Germplasm materials can help interpret the causative mutations that contributed to the popularity of certain crops throughout evolution (30, 31). Exploration of various onion gene pools to find desirable genes and identifying markers that are functionally linked with these genes (Fig. 2) can speed up onion breeding programs leading to the development of improved cultivars (32).

Creating a new parent population: The effectiveness of crop breeding relies on selecting the best parents with desirable traits. Therefore, breeders continuously select potential parent populations from obsolete or primitive cultivars, diverse sources including landraces, wild or semi-wild species and modern cultivars. Progeny testing can be employed to identify parents with high specific and general combining abilities. This testing will help breeders to determine the genetic potential of the parents and the variation to be developed. The National Bureau of Plant Genetic Resources (NBPGR) and National Active Germplasm Sites (NAGS) worked together to safeguard vegetable crop genetic resources. From August 1976 to January 2019, they accumulated 64,056 germplasm accessions of diverse vegetable and genetic resources from various regions of India (33). This collection included 58,250 accessions of important cultivated vegetable germplasm and 5,806 accessions of wild relatives and wild vegetable resources. The major cultivated crop diversity gathered comprised solanaceous vegetables (14,646), cucurbitaceous vegetables (16,750), root and tuberous vegetables (8,298), brassica/cole crops (1,776), bulbous vegetables (4,769), leafy vegetables (2,084), okra (4,235), leguminous vegetables (5,435) and tree crops (257), respectively (Fig. 3).

Genome-wide association studies (GWAS): Genome-wide association studies utilize recombination in various association panels to identify genes that are linked to phenotypic traits with greater accuracy. With a broader range of genetic resources available for marker-trait associations and no limitations on marker availability, the GWAS technique transformed into a powerful tool for QTL mapping in plants. With the help of GWAS,

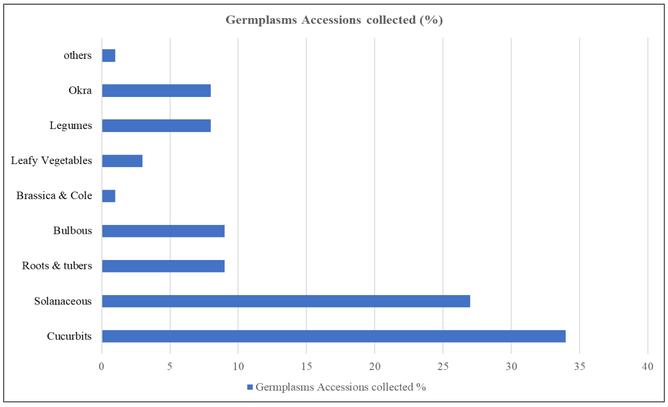

Fig. 3. According to NBPGR, the germplasm accessions collected (%) in groups of cultivated vegetable crops (33).

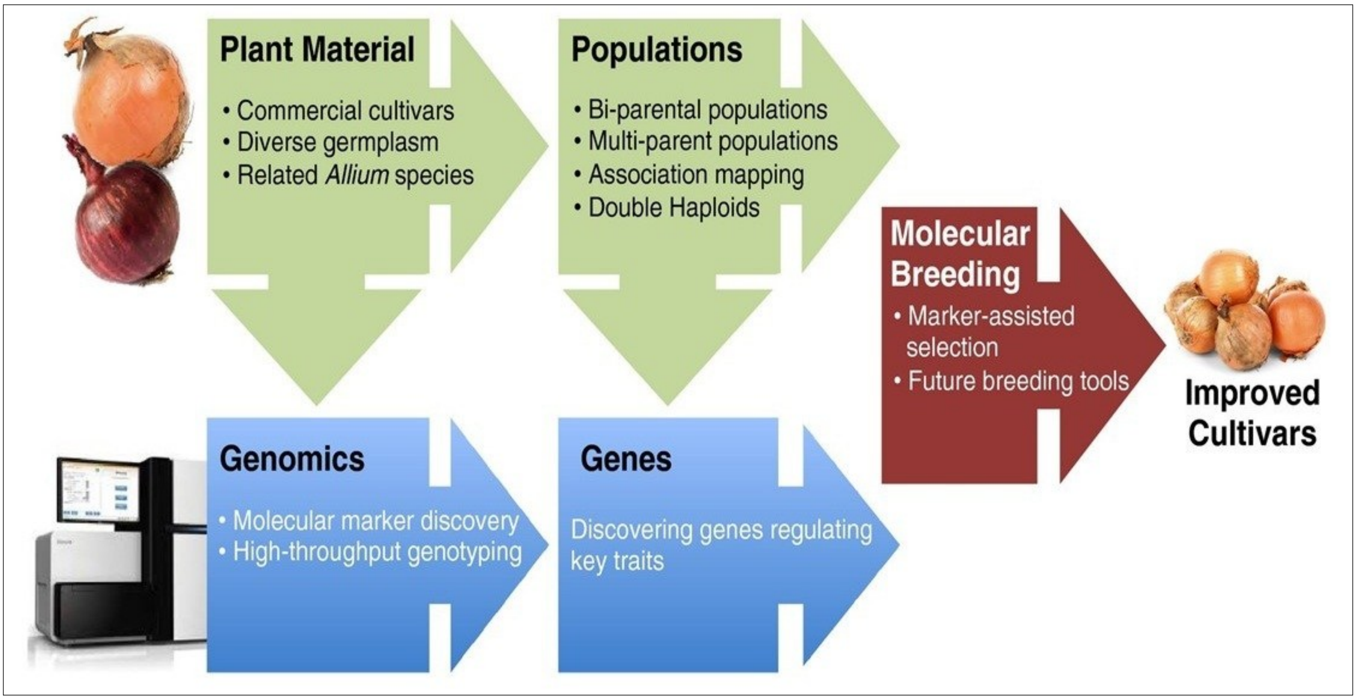

Fig. 2. The genomic based breeding approach in bulb onion.

the selection of individuals has been done without phenotypic data by using a model to estimate their breeding value (34). Genome-wide association studies can be utilized by various approaches which includes the following:

Single nucleotide polymorphism (SNP) marker arrays or SNP chips approach: Genome-wide association studies and QTL analysis have significantly simplified the labeling of novel genes. The creation of high-density SNP marker arrays has enabled cost -effective GWAS in wild populations. According to Zhao and his coworkers, they used 775 tomato accessions (including wild accessions) and 2,316,117 SNPs from three GWAS panels to conduct a meta-analysis GWAS and found 305 significant relationships for acids, amino acids, sugars and flavor-related volatiles (35).

Genotyping by sequencing (GBS) approach: The discovery of QTLs influencing diverse attributes may be done by using GBS technique in conjunction with phenotyping. Genome-wide association studies are now very popular because SNP markers from whole genome re-sequencing and affordable automated genotyping systems are readily available (36).

Mechanical and precision phenotyping (phenomics tools): The fast development of mechanical and precision phenotyping technologies has greatly improved germplasm assessment, providing reliable results (37). Various direct and indirect methodologies are being developed for better assessment. These include Normalized Difference Vegetation Index (NDVI) estimation, drone-based phenotyping, non-destructive root phenotyping, GIS-based phenotyping and other techniques, enabling large-scale germplasm characterization across various crops (38, 39).

Pre-breeding approaches

Introgression: Introgressive hybridization in genetics refers to the transfer of one or more beneficial genes from exotic, semi-exotic, unrelated, landrace, or related germplasm into an intermediate variety with a strong agronomical potential but lacking in a specific trait (40). Numerous successful attempts have been made to transfer disease-resistance genes from wild relatives into cultivated tomatoes. One of the earliest examples, involved using *Cladosporium fulvum* resistance from *Solanum pimpinellifolium* (41).

Several introgression lines have been developed in tomatoes from wild relatives which include *S. pennellii* (42), *S. lycopersicoides* (43), *S. sitiens* (44) and *S. habrochaites* (45). These introgression libraries can be combined into new breeding lines for quantitative and quality traits (46). Consequently, these pre-breeding lines offer tomato breeders a valuable tool to optimize genetic variation by combining alleles that enhance yield, stress resistance and quality into a single genotype (47).

Incorporation: A big initiative to develop locally adapted populations by using foreign or unadapted genotypes is known as genetic base incorporation or broadening. It aims to create a locally adapted population by utilizing unadapted or exotic materials. This concept was originally proposed by Simmonds (4). Unlike introgression, incorporation seeks to expand the genetic base of the crop.

The principles of genetics of incorporation include using material with a wide range of diversity and newly introduced, unmodified content, alongside traditional breeding methods. The breeding techniques will depend on the crop's biology, breeding system and reproductive behavior. This involves increasing recombination through cyclic or recurring crossing, assessing adaptation across various agro-climatic conditions and achieving horizontal resistance (HR) to diseases as a form of local genetic adaptation. A successful base-broadening program will enhance genetic diversity in economically important traits and provide suitable materials or parents for integration into existing breeding programs.

Wide crosses: It involves crossing individuals from different species to provide breeders with more attractive and user-friendly plant genetic resources. This method is typically used to expand a gene pool of crops and is commonly employed to transfer genes for abiotic and biotic stress tolerance (13). The primary aim of the extensive cross-program is to introduce new traits into superior cultivars. This approach has been used in crops like potatoes for blight resistance.

Decentralized participatory plant breeding: Plant breeding programs vary widely in terms of crops, facilities and breeders, but they all follow the same fundamental steps: creating diversity, selecting experimental cultivars and testing them. A decentralized participatory plant breeding program adheres to these principles with some differences, as most processes occur in the fields of farmers and decisions are made collaboratively by breeders and farmers.

Creation of aneuploids & polyploids: Changing the number of chromosomes in a species, either by modifying the basic set or by adding/removing individual chromosomes, can create unique varieties. Euploids include individuals with a different set of chromosomes which are created by doubling genomes of species, crossing unrelated species and then doubling the chromosomes of interspecific hybrid (48). Polyploids can be intentionally produced by exposing plant materials to environmental shocks (e.g., x-ray irradiation and extreme temperatures) and using chemicals (e.g., colchicine) that disrupt normal chromosomal division (49, 50). Double haploids (DHs) are generated by doubling the chromosomes of anther culture-derived haploid plant from F1. Lefebvre studied the utility of doubled haploid progenies for mapping projects in pepper (51).

The simplest method to produce pure breeding DHs is to grow haploid plants *in vitro* and then perform somatic chromosomal doubling (52, 53). Haploids can also be produced through anther culture (54) or genome deletion after distant hybridization (55). Double haploids possess genes in a homozygous background by reducing segregation and dominant genetic variation making selection more efficient for oligogenic or polygenic traits (52). Consequently, double haploid derivatives in a completely homozygous state can be selected for improved traits such as earliness, nutritional quality, plant height, yield and pest and disease resistance. In subsequent crossings and selection cycles, these selected genotypes can be used as parental material and uniform varieties.

Somatic hybridization: In many crops, sexual hybridization is limited by species barriers, restricting its use for crop development. Somatic cell fusion has been proposed to overcome these limitations by producing viable cell hybrids. Plant protoplasts show great potential in somatic cell genetics and agricultural development. Somatic hybridization involves fusing isolated somatic protoplasts under in vitro conditions to create a heterokaryon, which is then developed into a hybrid plant. This method allows the creation of hybrids between distinct plant species (taxonomically), surpassing the limitations of sexual cross-ability.

Development of new plant breeding techniques: The modern breeding techniques that enhance selection response include more efficient conventional selection methods, molecular marker technologies, biotechnology and identifying markers linked to traits of interest as well as effective cytoplasmic sterility systems and gametocides with the desired genetic background (56). Eight innovative plant breeding strategies: oligonucleotide-directed mutagenesis (ODM), Zinc finger nucleases (ZFN) technology, RNA-dependent DNA methylation (RdDM), cisgenesis and intragenesis, synthetic genomics and grafting on genetically modified (GM) rootstocks have been discussed in an article. Among these, cisgenesis and ODM are the most widely used, while RdDM, ZFN and GM rootstock grafting are less common and primarily used in research (57). The high technological potential and economic benefits of these novel techniques drive their adoption, accelerating the breeding process and reducing production costs.

Pan genomics: The pangenome concept was first introduced to describe a bacterial species (58). A pangenome includes all genes of a species, rather than depending on a singular reference sample, so offering a more precise depiction of diversity by reducing sampling tendency (59). Pangenomics is based on genomic structural diversity and can differentiate genomes based on the presence or absence of sequences (presence/absence variants) and variations in copy numbers (copy number variants) (60). The identification of links between variations in copy number and key traits in crops has advanced pre-breeding projects (36). Pangenomes for responses to biotic stressors are available for multiple species, including muskmelon (61) and various crops such as soybean (62).

Pre-breeding work done in different vegetable crops

Wild species possess greater genetic diversity than domesticated species, which can be utilized to enhance crop diversity (63). The Latin American Maize Project (LAMP), involving 12 countries (Argentina, Brazil, Bolivia, Chile, Colombia, U.S., Guatemala, Paraguay, Mexico, Peru, Venezuela and Uruguay) is a prime example of a pre-breeding program. In its initial phase, LAMP evaluated 15,000 applications through strong collaboration between the public and private sectors. The findings and research conducted on pre-breeding have been tried in vegetable crops (Table 2).

Tomato: The segregating diploid plants derived from the progeny of monosomic alien addition lines (MAALs) of *Solanum lycopersicoides* were analyzed to assess their potential as donors for enhancing specific traits in tomato breeding. The study focused on identifying and characterizing novel traits that could be beneficial for tomato improvement. One significant finding was the identification of novel drought-tolerant phenotypes in the MAALs which were not present in the cultivated parent tomato varieties. These drought-tolerant phenotypes in the MAALs demonstrate the potential of *S. lycopersicoides* as a valuable genetic resource for introducing and enhancing drought tolerance in cultivated tomatoes, thereby contributing to the development of more resilient tomato varieties (64). The recurrent backcrossing with multiple wild sources has been essential for integrating diverse disease-resistance genes into commercial tomato hybrids, as evidenced by a study in which each introduced gene is strategically positioned on separate chromosomal segments from different wild donor species, thereby ensuring a broad spectrum of resistance traits (65). A significant achievement highlighted in the study is the integration of a key gene from the wild tomato species *Lycopersicon pennellii*. This gene has been successfully incorporated into commercial hybrids, leading to a remarkable 15-fold increase in Pro-vitamin A levels in the fruit. This enhancement not only improves the nutritional value of the tomatoes but also showcases the potential of using wild species in breeding programs to achieve substantial improvements in crop quality and resilience (65). The success of this gene integration underscores the importance of wild genetic resources in developing superior cultivars with enhanced disease resistance and improved nutritional profiles.

Table 2. Pre-breeding work done by various workers as mentioned in different vegetable crops

Crop	Findings	Reference
Tomato	Analyzed segregating diploid plants derived from the progeny of MAALs of *S. lycopersicoides*, highlighting their potential as donors for enhancing specific traits in tomato breeding. Identified novel drought-tolerant phenotypes in the MDILs, which are absent in the cultivated parent varieties.	(64)
	Various disease-resistance genes have been incorporated into commercial hybrids through recurrent backcrossing with multiple wild sources, each gene located on a distinct chromosomal segment from different donor species. A crucial gene from the wild tomato species *Lycopersicon pennellii* B. was introduced, leading to a 15-fold increase in Pro-vitamin A levels in the fruit.	(65)
Brinjal	Pre-breeding has been attempted for resistance to bacterial wilt.	(66)
Potato	Four broad-spectrum resistance genes (Rpi) against *Phytophthora infestans* were identified and cloned from the sexually incompatible species *S. bulbocastanum*. Greenhouse and field studies selected plants with all four genes for their resistance to leaf blight. Combining somatic hybridization with gene-specific markers and related Avr effectors is an effective method for discovering and introgressing late blight resistance genes into the potato gene pool.	(67)
	Ethyl methane sulfonate (EMS) induced mutagenesis was applied to diploid potatoes to increase genetic variability, making them valuable pre-breeding materials for both polyploid and diploid potato breeding programs.	(68)
Cauliflower	Asymmetric interspecific somatic hybrids of *Brassica oleracea var. botrytis* (cauliflower) and *Brassica nigra* (black mustard) were created using protoplast fusion and their backcrossed (BC_3) and selfed (S_3) progeny were analyzed.	(69)
Asparagus	Developing new plant material to broaden the genetic base using crop wild relatives (CWR) such as *A. maritimus, A. pseudoscaber, A. brachyphyllus* and *A. macrorrhizus.* The findings indicate an increase in genetic variability, presenting new opportunities for improvement in asparagus.	(70)
French Bean	Wild relatives offer a valuable source of novel alleles that can enhance yield and other quantitative traits.	(71)

Brinjal: A study was conducted to develop brinjal (eggplant) variants that are resistant to bacterial wilt. The study focused on ten F4 generation green long brinjal lines, derived from a cross between IIHR-3 and Green Long along with their parent lines and control varieties (Arka Kusumakar and Arka Anand). These lines were evaluated for resistance to bacterial wilt through artificial inoculation and assessed for desirable horticultural traits (66). The findings revealed that some F4 lines exhibited moderate resistance to bacterial wilt, while others showed full resistance. Specifically, lines 12-36-164-7, 12-36-164-10, 12-36-164-11 and 12 -36-164-14 demonstrated moderate resistance. In contrast, lines 12-36-46-3, 12-36-46-6, 12-36-164-1, 12-36-170-9, 12-36-170-11 and 12-36-170-19 were resistant. The study emphasized the need to further advance these generations to achieve homozygosity and identified superior green round brinjal plants with good horticultural traits and resistance to bacterial wilt from the F3:4 segregating population.

Potato: The Identification and cloning of four broad-spectrum resistance genes (Rpi) from the sexually incompatible species *Solanum bulbocastanum* was performed in a study (67). These genes designated Rpi-blb1, Rpi-bt1, Rpi-blb3 and Rpi-blb2, confer resistance against *Phytophthora infestans*, the causative agent of late blight in potatoes. The study included both greenhouse and field trials, demonstrating that plants carrying these genes exhibited significant resistance to leaf blight, highlighting their effectiveness in providing broad-spectrum resistance (67). To introduce these resistance genes into the potato gene pool, researchers utilized somatic hybridization and gene-specific markers. The process was further refined with the use of Avr effectors, which interact with Rpi genes to trigger resistance responses. This approach proved highly effective in identifying and integrating late blight resistance genes into cultivated potato varieties.

The use of ethyl methane sulfonate (EMS) induced mutagenesis to enhance genetic diversity in diploid potatoes was investigated in a study (68). This method aimed to create valuable pre-breeding materials for both diploid and polyploid potato breeding programs. In the study, true potato seeds were treated with 1.2% EMS for 4-6 hours, resulting in a wide range of phenotypic variations. These variations included differences in germination rates, plant morphology, flower and tuber characteristics and yield potential. EMS-treated plants exhibited abnormal phenotypes such as chlorotic leaves, twisted stems, variations in stem color and weak stems with lateral growth habits. There were also changes in flower color and tuber color, shape and size (68). The induced genetic variability in these diploid potatoes provides a valuable resource for breeding programs aimed at developing new varieties with desirable traits. This mutagenesis method is especially effective in expanding the genetic base of cultivated potatoes, which are typically autotetraploid and possess limited genetic diversity. The study highlights the efficacy of EMS-induced mutagenesis in generating diverse pre-breeding materials, thereby significantly enhancing potato breeding efforts.

Cauliflower: A study was conducted to create asymmetric interspecific somatic hybrids between *Brassica nigra* (black mustard) and *Brassica oleracea* var. *botrytis* (cauliflower) using protoplast fusion (69). This technique effectively overcame the sexual incompatibility between the two species. The resulting hybrids were backcrossed (BC3) and selfed (S3) to produce progeny, which was thoroughly analyzed. The study found that backcrossed progeny gradually eliminated *B. nigra* chromosomes, leading to plants resembling cauliflower, while selfed progeny retained more characteristics of *B. nigra*. Cytological analysis revealed asynchronous and abnormal chromosome behavior during meiosis in the hybrid progenies, resulting in varied fertility levels. Genetic analysis using sequence -related amplified polymorphism (SRAP) indicated that the hybrids possessed DNA banding patterns from both parent species, with some alterations. Additionally, analysis using cleaved amplified polymorphic sequences (CAPS) and restriction fragment length polymorphisms (RFLP) revealed that all somatic hybrids contained chloroplast DNA from *B. nigra*, while mitochondrial DNA showed recombination and variations. Notably, three BC3 plants derived from specific somatic hybrids exhibited morphology similar to cauliflower (69) and demonstrated enhanced resistance to black rot, caused by *Xanthomonas compestris* pv. *Compestris.* These plants are identified as promising bridging material for further breeding programs. This study highlights the potential of somatic hybridization as a gene transfer method from wild relatives to cultivated crops, offering a valuable approach for enhancing genetic diversity and disease resistance in cauliflower.

Asparagus: The integration of crop wild relatives (CWR) such as *A. pseudoscaber*, *Asparagus maritimus*, *A. macrorrhizus* and *A. brachyphyllus* has been utilized to enhance the genetic diversity of *Asparagus*. The objective of this study was to develop new plant material to broaden the genetic base of cultivated asparagus (70). The findings revealed that incorporating these wild relatives significantly increased genetic variability. This expanded genetic pool offers new opportunities for improving asparagus, particularly in terms of disease resistance, environmental adaptability and overall crop performance. By harnessing the genetic traits of these wild species, breeders can develop more resilient and productive asparagus varieties, addressing some of the limitations of current cultivars.

French bean: Wild relatives hold immense potential for enhancing crop performance. These relatives contain novel alleles essential for improving yield and other quantitative traits in cultivated crops. Incorporating these alleles into breeding programs allows breeders to reintroduce beneficial traits that may have been lost or diminished during domestication (71). The study highlights that wild relatives often possess genetic diversity lacking in modern cultivars. This diversity can be utilized to enhance various agronomic traits, such as stress tolerance, yield and disease resistance. Integrating these novel alleles can result in the development of more resilient and high-performing crop varieties, better suited to cope with changing environmental conditions and increasing food demands.

Challenges in Implementing Pre-Breeding Strategies

There are some important challenges to adopting pre-breeding as a tool for a new era of vegetable improvement in breeding programs (Fig. 4).

Poor documentation and data gaps: The use of accessions in pre-breeding programs faces significant challenges due to data gaps, including insufficient characterization, inadequate genetic diversity evaluation and poor documentation (72). These issues hinder the comprehensive understanding and effective utilization of genetic material. To overcome these challenges, it is

Fig. 4. Pre breeding challenges while adopting new era in vegetable improvement.

essential to urgently collect and meticulously document wild species. This proactive strategy is necessary to prevent the extinction of valuable genetic resources and fill knowledge gaps, thereby enhancing the effectiveness of pre-breeding efforts and promoting genetic diversity.

Incompatibility in crossing: In pre-breeding, interspecies cross incompatibility is a major hurdle in transferring valuable genes between species. This issue arises from genetic and reproductive barriers that block successful fertilization or the development of viable offspring when species are crossed. Barriers such as differences in chromosome numbers, structural chromosomal variations and genetic incompatibilities pose significant challenges (73). These issues restrict the integration of desirable traits from one species to another, limiting genetic variability and potential breeding program improvements. Advanced techniques and strategies, including the use of bridge species, somatic hybridization, or biotechnological interventions, are needed to overcome these barriers and enhance genetic diversity in cultivated crops.

Linkage Drag: In pre-breeding, linkage drag is a major challenge in isolating beneficial genes from undesirable ones. This problem arises because genes close to each other on a chromosome are often inherited together, forming a linkage group. As a result, when selecting beneficial traits, undesirable genes can also be inherited, complicating the breeding process (74). Separating these linked genes requires extensive backcrossing, MAS and other advanced genomic techniques. This process is both labour-intensive and time-consuming, requiring precise identification and segregation of linked genes to ensure only desirable traits are retained while eliminating harmful ones. Overcoming linkage drag is essential for efficiently developing improved crop varieties with enhanced traits while maintaining genetic diversity.

Legal constraints: Legal constraints, especially intellectual property rights (IPR), significantly impede the exchange and access to genetic material from cultivated species in pre-breeding. While these IPR laws safeguard the rights of breeders and corporations over their plant varieties and genetic resources, they also erect barriers that can stifle collaborative research and retard scientific progress. The complexities and financial burdens of navigating these legal restrictions make it challenging to obtain essential genetic resources. Balancing the protection of intellectual property with the necessity for freely accessible genetic resources is crucial for fostering agricultural innovation and maintaining genetic diversity (17).

Prospects

Given the increasing risk of extinction for narrowly adapted and endemic species, the future of pre-breeding demands a stronger emphasis on documenting, collecting and characterizing wild species, especially CWR (75). This is essential for preserving genetic diversity and ensuring the availability of new genes to bolster agriculture's resilience to biotic and abiotic stresses. The growing need for novel genes in germplasm collections is driven by the necessity to develop crops that can withstand various environmental challenges.

Effective germplasm screening for traits like quality attributes and biofortification is critical, as it can identify genetic resources that enhance nutritional quality and stress tolerance. Advanced genomic techniques, such as genome mapping and synteny studies of wheat and barley genes known for abiotic stress tolerance, have the potential to improve vegetable crop programs (76). These methods can help identify and transfer beneficial genes to enhance vegetable crop performance under adverse conditions. Genetic transformation techniques also offer a promising route for pre-breeding, enabling the transfer of desirable genes from or beyond the tertiary gene pool.

These approaches can introduce new traits unavailable in the primary gene pool, broadening the genetic base and improving crop development efforts. To effectively utilize genetic and genomic analyses for managing complex traits, developing new breeding strategies and bioinformatics tools is necessary. These tools can integrate extensive genetic data, streamline the breeding process and improve the precision and efficiency of selecting desired traits. Ultimately, the future of pre-breeding lies in adopting advanced technologies and methodologies that enable the effective use of genetic resources. Embracing these innovations will help develop resilient, high-yielding and nutritionally enhanced crop varieties to meet the demands of a growing population and a changing climate.

Conclusion

Germplasm diversification strategies should integrate pre-breeding, as it successfully connects genetic resources with breeding initiatives. Pre-breeding can reduce genetic susceptibility caused by population homogeneity in crop development. Breeders need to develop new cultivars tailored to specific agroecological environments. To enhance the efficiency of developing vegetable crop varieties, MAS should complement traditional breeding methods. Currently, MAS is only effective for Mendelian traits and not for complex quantitative traits. The scope and timelines for pre-breeding activities must be established before true-breeding or cultivar development can proceed. Although significant advancements have been made in using molecular markers for precision phenotyping and genotyping. The high costs of these technologies are a barrier for developing countries like India. In the future, breeders will prioritize reducing genotyping and phenotyping costs to accelerate plant breeding and ultimately achieve agricultural diversity.

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Authors' Contributions

AS, BSD, SD carried out conceiving, designing and conducting extensive literature searches. RP and AC originated the study and contributed to its design and coordination. AK, PS and ADR reviewed and drafted the manuscript. AS, SD and RP analyzed and synthesized the gathered information. AS and AK revised the manuscript. All authors reviewed and endorsed the final manuscript.

Compliance with Ethical Standards

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