Review Article

Pre-Breeding in the Omics Era: A Review in Vegetable Crops

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Abstract

The main objective of plant breeding is to develop crop cultivars with improved genetic constitution in order to serve diverse human needs. Rapid progress in plant breeding activities has led most of the varieties developed so far in different crops coming from the same genetic background; this has led to narrow genetic base and hence vulnerable to biotic and abiotic stress. Therefore it is required to enhance the genetic variability in the germplasm collections and it can be possible through pre-breeding so that these genetically improved and untapped germplasm will be used in the breeding programme for cultivar development**.** India is considered as a primary centre of origin of many vegetable crops like brinjal, luffa, cucumber, pointed gourd, Indian spinach, dolichos bean etc. The introduction of new genetic information can result in increased resistance to insect pest, diseases tolerance to environmental condition, improved quality etc.

Pre-breeding is the most promising alternative to link genetic resources and breeding programs. Wild relatives with enhanced levels of resistance/tolerance to multiple stresses (Biotic and abiotic) provide important sources of genetic diversity for crop improvement in the future breeding programmes. However, exploitation of such useful germplasm for cultivar improvement is limited by cross-incompatibility barriers and linkage drags. Pre-breeding provides a unique opportunity, through the introgression of desirable genes from wild germplasm which is still untapped into genetic backgrounds which is adapted and readily used by the breeders with minimum linkage drag.

Pre-breeding activities using promising landraces, wild relatives, and popular cultivars have been initiated to develop new gene pools in crops with a high frequency of useful genes, wider adaptability, and a broad genetic base. Recent advances in molecular biology via Omics have given ample tools to handle the wild germplasm so that these can be efficiently used in general plant breeding programs.

Keywords: conventional breeding; cultivar development; pre-breeding; omics technologies

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Introduction

The narrow genetic base of crops today isalarming as it is threat to food security for growing population [1]. Current improved agricultural practices becomes the serious problem to our rich biodiversity, as the genetically uniform modern varieties has replaced the highly diverse local cultivars and landraces in traditional agro-ecosystems, resulted in increased genetic vulnerability of the cultivars for pests and diseases. In addition to this changing climate also demands for the search for new genes/traits for better adaptation. Therefore, necessitatesthe identification and utilization of diverse germplasm sources to develop new high-yielding cultivars with a broad genetic base [2]. These factors motivate the plant breeders to look for new sources of desirable genes in gene banks, which is the storehouse for the genetic diversity. Therefore there is need to increase the germplam stock of crops and their better

management.Plant germplasm management comprises two phases i.e. germplasm conservation (includes *exsitu* conservation via acquisition&maintenance and *in situ* protection) and germplasm utilization. Conservation is preserving its original genetic profile with maximum fidelity, monitoring its viability and health in storage or *in situ*, and maintaining associated passport information and other data.Pre-breeding is different from conservation it also includes genetic enhancement i.e. making particular genes more accessible and usable to breeders by adapting "exotic" germplasm to local environments without losing its essential exotic genetic profile, and/or introgressing high value traits from exotic germplasm into adapted varieties [3]. Simmonds [4] subdivided genetic enhancement into introgression i.e. backcrossing a few genes controlling desired characters into adapted stocks and incorporation i.e.the large-scale development of locally adapted populations good enough to enter the adapted genetic bases of the crops concerned.

Wild relatives with enhanced levels of resistance/tolerance to multiple stresses like heat, drought pest and diseases provide important sources of genetic variation for crop improvement. However, their exploitation for cultivar improvement is limited by different sexual incongruity and linkage drags.

India has made a significant progress in vegetable production in last three decades. It is the second largest vegetable producer next to China [69]. However, to meet the challenges in the domestic market as well as to compete in the international market, there is a need of evolving strategies for the development and breeding of suitable varieties/ hybrids and quality planting material to be provided to the growers. The introduction of new genetic information can result in increased resistance to insect pest, diseases tolerance to environmental condition, improved quality etc. India is bestowed with varied agro-climatic conditions which allow growing of all types of vegetables in one part of the country or other. The conservation of vegetable crop genetic resources is jointly managed by NBPGR along with National Active Germplasm Sites (NAGS). NBPGR has assembled a total of 1,678 exotic germplasm accessions comprising various vegetable crops namely tomato (934), brinjal (232), okra (273), vegetable pea (5), vegetable soybean (2), cabbage (56), radish (26), carrot (6), methi (28) and spinach (9) [70-71]. There is a need to undertake intensive research programme to make best use of available germplasm in the country and above all to conserve this valuable genetic resource. Pre-breeding provides a unique opportunity, through the introgression of desirable genes from wild germplasm into widely adopted genetic backgrounds for many desirable traits [5].

Concept of pre-breeding

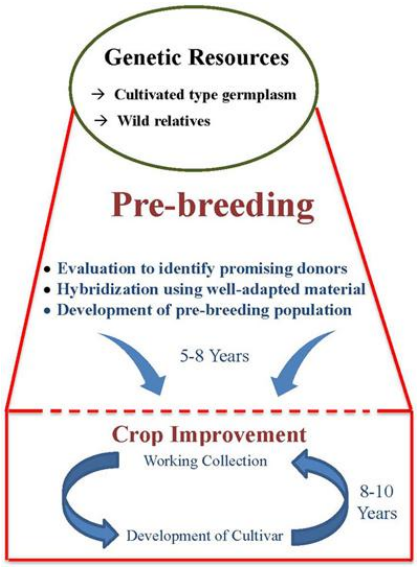

Figure 1 Pre-breeding as a bridge between germplasm and its end use.
Source: Sharma, S. *et al.*, 2013[9].

Pre-breeding refers to all activities designed to identify desirable characteristics and/or genes from un-adapted germplasm resources and transfer them to an intermediate product that breeder can manipulate to any kind of selection for improvement.The Global Crop Diversity Trust defined pre-breeding as 'the art of identifying desired traits, and incorporation of these into modern breeding materials'[6]. As pre-breeding is being carried out, the resulting materials will be suitable to included in ordinary breeding programs. Although there are some different

concepts of exotics, Hallauer & Miranda Filho [7] consider that exotics for pre-breeding purposes include any germplasm that does not have immediate usefulness without selection for adaptation for a given area. Overall, pre-breeding includes all activities directed at identification of desirable crop traits and their subsequent transfer into a suitable set of parents for further selection. Pre-breeding identifies useful character(s) or genes that can be exploited in cultivar development [8].

Characterization of Germplasms

Germplasmcomprises of landraces, obsolete cultivars, wild relatives, advanced breeding lines, popular varieties, synthetic aneuploids & polyploids lines etc. These unique genotypic lines harbor useful genes for early maturity, yield associated traits, local adaptability, disease and pest resistance and other desired traits [10]. They can also be used to define or generate a new trait not available in domesticated germplasm.In the present era of genomics and proteomics they can be characterized using various markers (biochemical, physiological, morphological and molecular markers) for breeding and to determine the level of genetic variation [11, 12]. In developing countries local varieties are predominantly grown for their farmers-preferred traits [13, 14] which may have many untapped use.

Molecular characterization

Genomics techniques such as DNA markers (RFLP, RAPD, SSRs etc.), Sequencing technology, isozyme marker etc. can be easily used nowadays to characterize germplasms. DNA fingerprinting of germplasm has become a daily routine of all centres dealing with plant germplasm conservation. Molecular diversity analysis and its use in grouping of germplasm is almost reliable nowadays with improved marker type; such as with the use of gene based, or functional markers [15-17].

Tagging or mapping of gene/QTLs

Molecular mapping and gene tagging for several disease resistance genes has enhanced the use of wild material as source of resistance. QTL mapping and gene cloning has a gameplay nowadays. There are many traits such as plant height, male sterility, yield associated traits, different biotic and abiotic stress related traits as well as several quality traits has been found and mapped easily in various crops [18-20].

Identification of novel allele

A lot of variation for a single gene/QTL can be now identified with the help of allele mining approaches. Several reverse breeding approaches, such as site-directed mutagenesis, genome editing, TILLING, EcoTILLING etc. are now being used to find novel mutation which can confer a particular trait and can be effectively be utilized in plant breeding. Germplasm materials can be used to decipher the causal mutation during due course of evolution which has led the popularity of a particular crop [21-22].

Mechanical and precision phenotyping (Phenomics tools)

Mechanical and precision phenotyping approaches has led to the fast progress in evaluation of germplasm and their reliable results. Several direct or indirect approaches are being developed for better use in evaluation. There are many techniques such as NDVI estimation [23] drone phenotyping [24] GIS based phenotyping, non-destructive root phenotyping etc, has given excellent opportunities to characterize germplasm at mass in several crops [25].

Introgression of new traits from germplasms

The plant breeder tries to transfers one or more desirable traits from unrelated, exotic or semi-exotic or related germplasm into an intermediate variety with good agronomic potential but lacks one or few desirable trait [4]. Therefore, by the introgression, new variety will be developed with novel gene(s) in the existing genetic background that was not available earlier. Exotic germplasm may constitute races, populations, clones, inbred lines, or other forms of genetic stock [7]. During conventional backcrosses while introgressing genes from unrelated, exotic, primitive or wild germplasm a considerable amount of undesirable genetic material is introduced into the progeny that has to be removed through a series of backcrosses to the recurrent parent [28]. This undesirable gene(s) that has passed to

recipient is termed as linkage drag. Removal of such linkage drag always seems to be cumbersome. Unmatchable success has been achieved in transferring the particular gene(s) with the help of molecular markers.

There are many successful achievement have been reported in tomato in transferring disease resistance genes from wild relatives into cultivated tomato. One of the first examples was the exploitation of *Cladosporium fulvum* resistance from *Solanum pimpinellifolium* in 1934 [29]. In tomato, several sets of Introgression lines have been developed from wild relatives of tomato. Among that *S. pennellii* [30], *S. Lycopersicoides, S. sitiens* [31-33] and *S. habrochaites* [34]. are of much importance.These introgression libraries have potential in breeding for quantitative and quality traits and can be pyramided into new breeding lines [35]. Thus, these pre breeding lines will offer tomato breeders a powerful tool to optimize the uses of genetic variation in nature by bringing together in one genotype alleles that maximize yield, resistance to different stressand also improve quality etc.

Identification and Creation of novel traits

Mutations can lead to spontaneous changes of the genetics of individuals that are often heritable. Induced mutagenesis is an important tool in plant breeding and functional genomics to increase the frequency of mutations and consequently to enhance genetic variation in crop species. This technique has the merit of overcoming genetic barriers such as cross-incompatibility, linkage, etc. In plant breeding strategies induced mutagenesis has become an effective way of supplementing the existing germplasm and improving new varieties [36]. The novel mutational events can either be directly developed as essentially derived varieties or novel genes introgressed into candidate parents through a backcross program.Resistance to bacterial wilt (*Ralstonia solanacearum*) in tomato has been developed with the use of induced mutation [37]. Cassava with high amylose content is preferred by diabetic patients because insulin lowers the insulin level in body, which prevents quick spikes in glucose contents. This quality trait improvement could possible with the help of mutation breeding in cassava [38]. Waycott and co worker [39] used EMS (mutagenic agent) to treatment of lettuce seeds to generate dwarf mutants and found that it is controlled by four dwarfing loci. These recessive lettuce dwarfs had reduced stature, shortened internodes, darker green leaves, and modified flower morphology as well as dwarf mutants have lost their ability either to produce active gibberellic acid (GA) or to respond to active GA that are involved in regulating lettuce stem elongation. Therefore these mutants could be used as a pre-breeding line in lettuce breeding program for resistance to premature bolting.

Table 1 List of land races and their potential use in some of the vegetable crops

Sl. No.	Crops	Traits	Germplasm conserved
1	Melon	Powdery mildew	PMR 45, PMR 450, PMR 5, PMR 6, PI 124111
		Downy mildew	DMDR-1, DMDR-2
		Fruitfly	*Cucumis callosus*
		Nematode	*Cucumis metuliferus*
		Whitefly	*Cucumis denteri, Cucumis dipsaceus, Cucumis sagittatus*
2	Watermelon	Fusarium wilt	Summit, Conqueror, Charleston gray, Dixilee, Crimson sweet
		Anthracnose	Charleston gray, Congo, PI 189225
3	Bottle gourd	CMV, SqMV,	WMVPI 271353
		Fusarium Wilt	Taiwan variety Renshi
4	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
5	Pumpkin	Powdery Mildew and Viruses	*Cucurbita lundelliana, Cucurbita martenezii*
		ZYMV, WMVC.	*Cucurbita ecuadorensis, Cucurbita faetidistima, Cucurbita martenezii*
6	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
7	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
8	Chilli	Cucumber mosaic virus	EC 312342-312349
		PBNV mosaic virus	EC 121490
		Aphids	EC 28, 30 and 34
		YVMV	EC 133408, EC169333, EC 169334, Ghana red, *Abelmoschus manihot ssp tetraphyllus, Abelmoschus manihot ssp manihot*
9	Okra	Jassids	EC 305656, 305694, 305695
10	Cabbage	Black rot	EC 24855, EC 28770, Cabbage Standby
11	Cauliflower	Black rot	Aemel, Olympus, Lawyana
12	Onion	Purple blotch	EC 328494, EC 328492, EC 328501, EC 321463
13	Pea	Powdery mildew	EC 342007
14	Muskmelon	Downey mildew, Powdery mildew, Anthracnose	Crimson sweet, shipper

Source: Modified from Pandey, P and co worker [26]

Table 2 Registered germplasm of cucurbits with unique traits with the NBPGR, New Delhi. [27]

Crop	Line	Registered name	Trait associated
1. Pointed gourd	IIVR PG- 105	INGR-03035	Parthenocarpic fruits
2. Bitter gourd	GY-63	INGR-03037	Gynoecious sex with high yield
3. Water melon	RW-187-2	INGR-01037	High yield and yellow coloured flesh
	RW-177-2	INGR-01038	Leaf mutant with simple unlobed leaves
4. Bottel gourd	Androman-6	INGR-99009	Andromonoecious sex
	PBOG-54	INGR-99022	Segmented leaves
5. Cucumber	AHC-2	INGR-98017	High yield and long fruit
	AHC-13	INGR-98018	Small fruit, drought and temperature tolerant
6. *Cucumismelo*var. *callosus*	AHK-119	INGR-98013	High yield and drought tolerance
7. Round melon	HT-10	INGR-99038	Tolerant to downy mildew and root rot wilt
8. 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

New and modern breeding techniques can assist in improving selection response. These include development of moreefficient conventional selection procedures, biotechnology, molecular marker technologies and identification of markerslinked to traits of interest, effective gametocides and cytomlasmic sterility systems with a desired genetic background [28, 40, 41].

Approaches to pre breeding

Introgression

Introgression is the transfer of one or more genes from exotic/un-adapted / wild stock to adapted breeding populations. This can be achieved by making crosses between the donor and the recurrent parent. The concept of introgression through crop breeding techniques like backcrossing was evolved by Dr. Edgar Anderson.

Incorporation

Incorporation aims to develop locally adapted population using exotic / un-adapted germplasm. This was first suggested by Simmonds [4]. In contanary to introgression, incorporation aims at indexing the crop genetic base.
The following are the genetic principles of incorporation.

- Use of material covering wide range of variability
- Use of un-adapted introduced material
- The process is complementary to conventional breeding
- The breeding methods will depend on the biology of the crop, its breeding system and reproduction behaviour
- Maximizing recombination through cyclic or recurrent crossing.
- Testing for adaptability under diverse agro-climatic conditions
- Local genetic adaptation - horizontal resistance (HR) to disease
- The outcome of an effective base-broadening programme will be enhanced genetic variance in economic characters and either good materials*per s*e or good parents for crossing into established programmes.

Therefore genetic base broadening results in the development of potential parents either from adapted stocks through the use of unadapted stocks. E.g. day length adaptation, disease resistance and quality improvement.

Other Breeding Approaches

It includes (i) convergent improvement, (ii) modified convergent improvement, (iii) decentralized breeding, and (iv) participatory plant breeding

Use of Omics tools in Pre-breeding

Genomics approaches are particularly useful when dealing with complex traits as these traits usually have a multi-genic nature and an important environmental influence [42-44].Genomic tools are thus facilitating the detect of QTL and the identification of existing favorable alleles of small effect which have frequently remained unnoticed and have not been included in the gene pool used for breeding [45].

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.

DNA based molecular markers and their applications

Molecular markers reveal genetic differences in the primary structure of DNA between individuals [46, 47]. 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 particular 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. [48]. The relative efficiency of MAS is greatest for characters which has low heritability [49]. 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, soin order to minimize this linkage drag, marker assays could be a major advantage [50]. 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 [48]. In order 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 masks the presence of other genes [49, 51]. The strategic combination of MAS

with phenotypic screening is known as 'combined MAS'. This may have merit over phenotypic screening or MAS alone in order to maximize genetic gain [52].

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 [53]. The remarkable genetic gain through MARS is probably higher than that achievable through MABC [54]. For a major resistance gene, marker based recurrent backcross programs are using frequently [55].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 [19]. A large number of molecular markers have been used today for DNA fingerprinting of cultivars and breeding lines in a number of vegetable crops viz., tomato [56], beans [57], pepper [58], and potato [59]. The fortuitous genetic linkage in tomato between the *Aps-1* isozyme locus and the *Mi* locus that controls resistance to rootknot nematode has been beneficial for developing nematode resistant tomato hybrids [60, 61]. 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.

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 somaticprotoplasts 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.

Creation of Aneuploids & Polyploids

The breeder could create a novel new variability through changing the number of chromosomes in a species, either by altering thebasic 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 bychromosome doubling of the inter-specific hybrid. Polyploids can be artificially induced by various means such as exposingplant 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 [28, 37, 62]. Chromosome doubling of anther culture derived haploid plants from F_1generates double haploids (DHs). The suitability of doubled haploid progenies for mapping project has been reviewed in by Lefebvre and co worker in pepper [63]. In vitro production of haploid plants followed by doubling of somatic chromosomes is the quickest means to produce pure breeding doubled haploids (DHs) [64, 65]. Haploids are produced through the method of anther culture [66] or genome elimination following distant hybridization [67]. Selection is more efficient for oligogenic or polygenic traits in DHs because itsability to fix genes in a homozygous background, limiting dominance genetic variation and segregation [64]. 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.

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 [68].

Conclusions

This paper summarizes the importance of wild relatives or untapped germplasm for future use in vegetable crop breeding. Pre-breeding is an essential part of germplasm diversification strategies. It is the most promising alternative to link genetic resources and breeding programmes. By exercising the pre-breeding procedure in crop improvement programme, the genetic vulnerability due to uniformity can be avoided in the population. Breeders need to develop novel cultivars of each crop to be specifically adapted to each and every agro-ecological systems. Marker-assisted selection (MAS) should be integrated with traditional breeding methods to enhance the efficiency of cultivar development in vegetable crops. The application of MAS is currently limited to Mendelian traits, whereas it is less efficient for complex quantitative traits. The scale of pre-breeding that is needed, and the timescales of the pre-breeding operations that have to be followed, before the actual breeding or cultivar development can take place. Several new advancements has been made in the area of application of molecular tools in genotyping and precision phenotyping but the cost of application of these novel techniques is very high and country like India can not afford. Infuture breeder will give emphasis on reduced cost of genotyping and phenotyping so that plant breeding can be accelerated at a greater pace and crop diversification may be achieved at last.

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