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Review Article

Pre-breeding is a Bridge between Wild Species and Improved Genotypes-a Review

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Abstract

Pre-breeding is an alternative term used for 'genetic enhancement' and in recent times it has become an essential, planned part of all plant breeding activities. This article discusses the various activities involved in a pre-breeding program targeting disease resistance with recent outcomes of genetic enhancement for adult plant resistance. Therefore, pre-breeding is a promising alternative to link genetic resources and breeding programs. Several aspects of pre-breeding are discussed such as concept, objectives, application, importance and methods. Problems and challenges to be investigated and suggestions are also presented.

Keywords: Pre-breeding, genetic enhancement, genetic resources

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Introduction

Crop domestication and improvement can be described as a process of successive rounds of selection that ultimately results in the isolation of genetic diversity valuable to agriculture from ancestral wild species. These successive rounds of selection have resulted in the crops that feed the world today, but at the cost of reducing their genetic variation, leaving them with less allelic diversity than their wild progenitors and other crop wild relatives (CWR) (Ross-Ibarra *et al*., 2007; van Heerwarden *et al*., 2011). This is often referred to as "domestication bottleneck" (Hammer, 1984; Tanksley and Mc Couch, 1997).

In India, breeding programs in major crops reached to a point of diminishing returns and it is feared that unless new diversity is infused into the breeding germplasm, we face catastrophic reductions in productivity if the climate turns adverse. Wild species and exotic cultivated germplasm holds a wealth of alleles. If we can able to find them (alleles of interest) then it can be helpful to break yield barriers and enhance tolerance to stresses and stability. It is worthwhile to highlight that hybridization between diverse germplasm followed by selection may result in cultivars with improved stress tolerance, better adaptation, quality traits and yield (Arunita *et al*., 2010).

Crop wild relatives (CWR) have a high level of genetic diversity that enabled them to survive in natural and adverse environments. The value of CWR was recognised by the Russian botanist NI Vavilov and the geographical centres of origin and diversity of crops were identified in 1920s. Since then CWR have been used to increase adaptability of several crops, but largely for disease and pest resistance. Though efforts to conserve CWR worldwide have increased, their use in breeding has not kept pace largely because of undesirable linkage drag and the long time taken to release varieties when CWR are used. The integration of molecular marker techniques in breeding has the potential to increase the efficiency of transfer of useful alleles from wild species and accelerate crop improvement. This is valid for food, fodder, fibre or commercial crops. (Dowswell *et al*., 1996).

Most plant breeders fear in using exotic or un-adapted material due to its initial detrimental effects on elite breeding material (Kannenberg and Falk, 1995). It is a necessary first step in the use of diversity arising from wild relatives and other unimproved materials. An enhanced exploitation of cereal crop genetic diversity through novel pre-breeding strategies and a fundamental reinforcement of the entire plant breeding chain is a vital part of a sustainable system for global food security (Nass *et al*., 1993). Taking the necessary time for breeding processes into account, action is urgently required. Pre-breeding refers to all activities designed to identify desirable characteristics and genes from unadapted materials that cannot be used directly in breeding populations and to transfer these traits to an intermediate set of materials that breeders can use further in producing new varieties for farmers (Mohan *et al.*, 2009).

The term "enhancement" was first used by Jones (1983) which according to him can be defined as transferring useful genes from exotic or wild types into agronomically acceptable background. Rick (1984) used the term pre-breeding or developmental breeding to describe the same activity. Thus "genetic enhancement" or "pre-breeding" refers to the transfer or introgression of genes or gene combinations from unadapted sources into breeding materials. It is an emerging concept emphasizing the use of plant genetic resources. Pre-breeding does not differ significantly from general framework of plant breeding and is considered as prior step of sustainable plant breeding. The pre-breeding consists of identifying a useful character, capturing its genetic diversity and putting those genes into usable form. Pre-breeding leads to value addition in the germplasm (Nass *et al*., 1993). Pre-breeding deals mainly to enhance genetic variability in the germplasm and the improved germplasm can be readily used in regular breeding programme for cultivar development (Lokanathan *et al*., 2003). Pre-breeding aims to generate new base population for breeding programme, through the use of a wider pool of genetic material (Haussmann *et al*., 2004).

This “prebreeding” attempts to reset the genetic diversity of crops by reintroducing genetic variation that has been left behind. In fact, prebreeding has also been employed to use genetic diversity that was not previously accessible either due to genetic incompatibilities or non over lapping geographic ranges (Cooper *et al*., 2001; Dwivedi *et al*., 2008; Ogbonnaya *et al*., 2013).

With the renewed interest and emphasis in Plant Genetic Resources (PGR) activities, it has become increasingly important to utilize the collected genetic diversity. Genetic enhancement plays an important role in utilizing unadapted and unutilized germplasm collections and creating vast genetic variability for development of productive cultivars and hybrids (Mohan *et al.,* 2009). Pre-breeding work was successfully done in several crops (rice, tomato, soyabean, cotton, maize, wheat, barley, groundnut, chickpea, pigeon pea, sorghum, pearl millet) by using the genes from wild / exotic (unadapted) species into adapted material and improved many cultivated varieties for different qualitative and quantitative traits (Plunkett *et al*., 1987, Eshed and Zamir, 1996, Iqbal *et al*., 2001, Wang *et al*., 1999, Sebolt *et al*., 2000 and Seetharam, 2007).

Concept of Pre-breeding

Pre-breeding is the most promising alternative to link genetic resources and breeding programs. Pre-breeding refers to all activities designed to identify desirable characteristics and/or genes from unadapted (exotic or semi-exotic) materials, including those that, although adapted have been subjected to any kind of selection for improvement. As pre-breeding is being carried out, the resulting materials are expected to have merit to be included in ordinary breeding programs. Although there are some different concepts of exotics, Hallauer & Miranda Filho (1988) consider that exotics for pre-breeding purposes include any germplasm that does not have immediate usefulness without selection for adaptation for a given area. In this sense, exotic germplasms are represented by races, populations, inbred lines, etc. Consequently, the results of crosses between adapted and exotic materials, where different proportions of introgression are obtained and evaluated, have been denominated as semi-exotic materials. According to Hallauer (1978), the utilization of semi-exotic populations has been the most common procedure to evaluate exotic germplasms. In order to obtain promising results with exotics their reproduction is necessary for a few generations in order to allow genetic recombination accompanied by mild selection. Before useful recombinants can be selected a minimum of five generations of random mating with mild selection pressure is indicated (Lonnquist, 1974).

Urgent need of pre-breeding

There is currently a major gulf between the operations of plant genetic resource collections and modern plant breeding that is potentially a major restriction in the development of cereal varieties that are needed to meet novel agronomic and environmental challenges. This disconnect can be bridged through a process known as pre-breeding that is based upon the characterization of genetic resources for traits of interest and then transferring these traits into suitable, agronomically adapted genetic backgrounds. Pre-breeding in field crops for India for long-term goals must be performed in close collaboration between advanced and applied research institutes, gene banks as well as plant breeding entities in order to be sustainable and successful. Such partnerships will ensure that targets are chosen to meet the demands for climate adaptation and environmental policies – changed crop production systems, extended cultivation areas, improved water and nutrient use efficiency, improved resistance to pests and pathogens, *etc*. and also the demands on increased production in combination with specific quality requirements of the market. Such partnerships will also help to develop the capacity building for breeding that is needed to counteract the competence erosion that has resulted from structural changes and low priority given to this area in recent years. Such capacity will be of paramount importance to balance the challenges that future agriculture has to meet, both in India and in developing countries. (Arunita *et al*., 2010)

The main objectives of pre-breeding

- Improved germplasm and associated genetic knowledge that enhance resistance expression and diversity
- Improved parental stocks which can be readily utilized within breeding programs and
- Improved selection methodologies (Shankar, *et al* 2012)
- Identify potentially useful genes in a well-organized and documented gene bank
- Design strategies that lead to development of an improved germplasm that are ready to use in varietal development
- Pre-breeding is a collaborative endeavor, that is buttressed by communication, between gene bank curators and breeders.

Sustainable Use of Genetic Diversity

Gene banks have often, through necessity, focused mainly on the immediate conservation aspects of plant genetic resource activities. However, there is an urgent need for active engagement with all stakeholders to enhance the utilization of plant genetic resources in order to assure the functionality of the entire “Genetic resource-chain”. There are considerable genetic resources for small grain cereals including collections of adapted varieties and genetic stocks carrying defined traits through to land-races and wild crop relatives. However, this richness of plant genetic diversity is greatly under-utilized, with less exploitation of important traits and the potential value not being channeled back into societal benefit. The conservation of genetic resources must be linked to their increased and sustainable use if they play a key role in climate change adaptation. Bottlenecks that need to be addressed include lack of information at genotypic and phenotypic level, e.g., need for evaluation for resistance to biotic and abiotic stresses. Data need to be easily accessible in standardized and searchable electronic formats enabling strong networks linking conservation, evaluation and plant breeding to be established to secure the necessary level of pre-breeding activities. (Iqbal *et al* 2017)

Identification of new resistances

The first step includes identification of sources of resistance within the existing germplasm and from various resources worldwide. Testing over multiple years, in various environments and at different growth stages can identify resistance as broadly effective. Potential resistant donors identified can then be directly incorporated into breeding programs or can be used for population development, identification of molecular markers and mapping of novel resistance genes (Mohan *et al.*, 2009).

Abiotic stresses – adapting to environmental changes

Tolerance to particular abiotic stresses such as drought, cold, salinity, heat, water logging, nutrient use efficiency and mineral toxicity has traditionally enabled field crops to cope with the prevalent local stresses with a balance of traits that were fine-tuned to optimize economic yields in their environments. However, global warming is producing shifts in the prevalence of some abiotic stresses with drought being increasingly important, given the widely acknowledged effect of climate change. In addition, ozone level in the troposphere are expected to rise notably in the near future, affecting the amount of UV radiation that reaches the Earth surface in turn causing large increase of plant oxidative stress. Therefore, it is expected that the agricultural areas of India will experience enhanced or novel abiotic stresses making it increasingly urgent to develop field crops that can withstand such environmental changes, in order to increase or even just to maintain current yield levels.(ECPGR, 2009, Iqbal *et al* 2017)

Biotic stress – coping with emerging diseases

Plant pathogens cause considerable yield losses in cereal production, reducing crop quality and threatening food safety. Disease prevention and control is thus a prerequisite for competitive field crop production with the breeding of genetically disease resistant crops being one of the most environmentally and economically desirable ways to manage plant diseases. The prevalence of different plant diseases is changing due to changing environmental conditions, including global climate change, but also changes in agricultural production practice like planting of larger area with few or genetically more uniform varieties, reduced crop rotation and soil tillage, loss of biodiversity, change in use of pesticides, and global trade. Due to the changing environmental condition and economical context, plant diseases will inevitably appear and compromise crop production in regions where they did not represent a problem before, as is already being seen with the emergence of a new strain of heat tolerant wheat yellow rust and increasing problems with Fusarium head blight and Ramularia leaf spot .Understanding the host-pathogen biology is the first step towards minimizing the risks represented by plant diseases. Durable, both race non-specific and race-specific, resistance incorporated into high-yielding genotypes are the main method to manage diseases of field crops. New durable and efficient sources of resistance will have to be sought, in the case of field crops, from landraces and from wild relatives of the crops. **(**ECPGR, 2009, Iqbal et al 2017)

Use of Gene pool for Crop Improvement

The gene pool is the total genetic variation in the breeding population of a species and closely related species capable of crossing with it'. The gene pool of a crop is made up of botanical varieties, landraces, inbred lines, ancient landraces, obsolete and modern cultivars, related wild species, subspecies, and weedy companion species (Haussmann et al., 2004).

Three types of germplasm namely, primary, secondary and tertiary are used as gene pools. Each of these categories of genepool has its own significance and limitation. In the past, primary gene pool was extensively used for genetic improvement of different crops with a view to create vast genetic variability and broadening the genetic base of breeding material. The use of secondary and tertiary gene pool helps in creating vast genetic variability for various traits. Recently, the work on use of secondary and tertiary gene pool in field crops has been intensified and as a result vast genetic variability has been created for various economic characters such as resistance to biotic and abiotic stresses, and plant type *etc*. Quite recently, the advent of biotechnology has given rise to a new genepool concept, the quaternary genepool.

Use of Exotic Germplasm for Crop Improvement

Exotic germplasm refers to all the germplasm that do not have immediate usefulness without selection for adaptation in a given environment (Haullauer and Miranda, 1981). Exotic germplasm has to undergo conversion or pre-breeding to find its best use in plant breeding. Most of the plant breeders fear in using exotic or un-adapted material due to its initial detrimental effects on elite breeding material (Kannenberg and Falk, 1995). There are some major constraints in the direct use of exotic material in the breeding programmes which are given below:

- Crosses with exotic material can result in the concurrent introduction of inferior alleles and disruption of co-adapted gene complexes in the elite material.
- Exotic germplasm can negatively affect adaptiveness when introduced in locally adapted genetic base.
- The linkage of undesirable genes with desirable traits is a major constraint to increased utilization of un-adapted or exotic germplasm. This acts as a barrier in promoting useful gene flow from exotic to the adapted gene pools.

Exotic germplasm has to undergo "conversion or pre-breeding" to find its best use in plant breeding. In spite of best efforts, it is realized that for most of the crops the genetic gap between elite adapted gene pools and exotic pools is growing larger with each breeding cycle.

Better knowledge of underlying genetic differences between adapted and exotic germplasm could help to overcome such barriers to gene flow. Studies on cotton have indicated an ample scope for genetic enhancement. In some crops like wheat the number of exotic land races introgressed in to elite germplasm is increasing overtime **(**Loknathan *et a.,l* 2003).

Pre-breeding: a new requirement in plant breeding

Activities related to genetic resources are characterized by high cost and long-term return. Introduction and germplasm exchange, collection, characterization, evaluation, documentation and conservation are essential steps that cannot be over emphasized. An appropriate synchronism among these activities is required for the bank to be effective in maintaining genetic variability and to assure germplasm utilization. The importance of genetic resources is widely recognized. Activities in germplasm banks demand qualified researchers with knowledge in several areas. Besides the conservation of genetic variability for the future, the actual utilization of available accessions is another important goal. However, the low utilization of germplasm banks is a rule worldwide but it is not restricted to India or to developing countries (Nass *et al*., 1993). The main factors responsible for the low utilization of plant genetic resources are lack of documentation and adequate description of collections, lack of the desired information by breeders, accessions with restricted adaptability, insufficient plant breeders, particularly in developing countries and lack of collection evaluations. Low seed availability due to inadequate seed regeneration programmes is another barrier to their use (Dowswell *et al*., 1996).

Various activities involved in Pre-breeding (Shimelis and Laing, 2012)

Characterization of landrace populations :

Landraces are often referred as 'farmer's varieties' or 'cultivated native varieties' that are adapted to a specific agro-ecological and farming system without any scientific form of selection. Landraces are invariably heterogeneous and an excellent source of genetic variation for crop breeding programs (Sleper and Poehlman, 2006). Landraces harbor useful genes such as genes for early maturity, yield potential, disease and pest resistance and other desired traits. Landraces are most prevalent in the centers of diversity. They can be characterized using various markers (biochemical, physiological, morphological and molecular markers) for breeding and to determine the level of genetic variation (Podlich *et al*., 2004; Ortiz *et al*., 2008). In developing countries landrace varieties are predominantly grown for their farmers-preferred traits (Mulatu and Zelleke, 2002; Ceccarelli and Grando, 2007).

Creation of new parent populations

The success of a crop breeding program relies on choice of the best parents possessing complementary and desired traits. Thus, breeders continuously select potential parent populations from diverse sources including landraces, modern cultivars, obsolete or primitive cultivars, wild or semi-wild species. Parents with high specific or general combining abilities are selected via progeny testing through well-designed recombination. Progeny testing is performed in a set of target and representative environments with half-sibs, full-sibs, test crosses or recombinant inbred (Acquaah, 2007; Brown and Caligari, 2008).

Introgression of new traits from other useful sources

The plant breeder transfers one or more desirable traits from unrelated, exotic or semi-exotic, landrace or related germplasm into an intermediate variety with good agronomic potential but lacking a specific trait (Simmonds, 1993). Thus, the new variety will be developed with the introduced novel gene(s) in the existing genetic background. Exotic germplasm may constitute races, populations, clones, inbred lines, or other forms of genetic structure (Hallauer and Miranda Filho, 1988). When introgressing genes from unrelated, exotic, primitive or wild germplasm, both the desired gene(s) and 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 (Brown and Caligari, 2008).

Creation of novel traits

Mutations lead to spontaneous changes of the genetics of individuals that are often heritable. Naturally, mutational events occur at low frequencies, i.e., 10^{-5} to 10^{-8} per locus. Induced mutagenesis through the use of artificial mutagenic agents is an important tool in plant breeding and functional genomics to increase the frequency of mutations and consequently to broaden genetic variation. Induced genetic variations have been used successfully in several crops to create useful mutants (Ahloowalia *et al*. 2001; Pozniak and Hucl, 2004; Hohmann *et al*., 2005). The technique can be regarded as an efficient option for germplasm enhancement towards important agronomic traits (Pozniak and Hucl, 2004). The novel mutational events can either be directly developed as essentially derived varieties or novel genes introgressed into candidate parents through a back cross program.

Creation of polyploidy

The breeder may create 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. Polyploidy 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. (Sleper and Poehlman, 2006; Acquaah, 2007; Brown and Caligari, 2008).

Acquisition of new information on crop genetics

The breeder constantly looks new genes from diverse sources for enhanced nutritional qualities, early maturity, high yield potential and biotic and abiotic stress tolerance. Understanding the candidate genes and the pattern of inheritance of the genes in controlling these characters is profoundly significant for effective transfer and to improve the efficiency of selection in cultivar development (Ortiz *et al*., 2008; Meneely, 2009).

Development of new plant breeding techniques

New and modern breeding techniques can assist in improving selection response. These include development of more efficient conventional selection procedures, biotechnology, molecular marker technologies and identification of markers linked to traits of interest, effective gametocides and cytoplasmic sterility systems with a desired genetic background (Acquaah, 2007; Brown and Caligari, 2008; Lusser *et al*., 2012).

Cultivar development

Cultivar development embraces well-defined breeding procedures directed at the production of improved cultivars with respect to the mating system of the crop. Cultivar development requires well-developed and elite breeding material, generated from an established and relational pre-breeding program. If present, locally screened and adapted germplasm are an ideal starting material in cultivar development. A plant breeder can spend many years on pre-breeding to ensure that locally adapted and the best available parental material is used to meet the demands of a defined set of agro-ecological zone and day-length requirements of a given latitude. Alternatively, the breeder may use elite material (e.g. inbred lines) that other breeders have developed, such as the germplasm available from the CGIAR centers that is not locally adapted. This will allow a plant breeder to release new candidate cultivars, even if they are not adapted to the local agro-ecological and cropping system into which they are to be released. The released cultivar may have some superior traits but the local farmers often reject the new cultivars because they are not bred to include farmers preferred traits. Farmers' trait preferences are diverse and complex such that the total crop value is more important than absolute crop yield (Witcombe and Virk, 1997). Other farmers-preferred traits include: cooking quality, taste, market acceptability, storability (Tripp et al., 1997) and the quantity of utilizable parts of the crop left after harvesting, processing and storage (Mulatu and Zelleke, 2002). McGuire (2008) indicated that despite 25 years of sorghum breeding in Ethiopia most of the released cultivars had been poorly adopted by the small-scale farmers. Thus, a balance between farmers-preferred traits and solutions to production constraints should be the breeder's goal in order to enhance cultivar uptake by farmers. Crop breeding units with adequate resources should run with two parallel programs concurrently. Firstly, pre-breeding program to continually develop new and improved parent materials with superior traits of commercial importance.

Applications of doubled haploids in plant breeding

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 various means, such as anther culture (Henry and De Buyser, 1990), or genome elimination following distant hybridization (Barclay, 1975; Matzk and Mahn, 1994; Singh *et al.*, 2001). During wide crosses the entire genome of one of the parents are lost from a hybrid embryo and endosperm in the early cell divisions. In wheat breeding, genome elimination, in particular the use of wheat by maize cross is usually more reliable than anther culture (Kisana *et al.*, 1993; MuJeeb-Kazi *et al*., 1995). The doubled haploid method has several advantages in crop breeding programs.

Prebreeding and wide crosses (Haussmann et al., 2004)

Prebreeding includes basic research to achieve wide crosses, and activities that facilitate the use of exotic materials or wild relatives. It can refer to both qualitative and quantitative traits and the distinction between prebreeding, introgression and incorporation is not always clear. The main objective is to provide breeders with more 'attractive' PGR that are easier to use, i.e. resistance sources in acceptable genetic background; or inbreeding tolerant forms of out crossing species for hybrid breeding. An example is the resistance breeding programme of the International Potato Center (CIP), within its Global Initiative on Late Blight (GILB) (Trognitz *et al*., 2001) whose aim is to provide breeders and farmers with new sources of resistance from wild relatives. In sugar beet (*Beta vulgaris*), a number of commercial breeders are involved in prebreeding activities, i.e. in the development of 'Base' or 'Buffer' populations from genetically extremely diverse materials (Frese *et al*., 2001; Frese, 2002).

DNA based molecular markers and their applications in plant breeding

Molecular markers reveals the genetic differences in the primary structure of DNA between individuals. Compared to protein markers, DNA based polymorphisms are more stable, and can reveal subtle changes in the genomic DNA (Powell et al., 1996; Horacek et al., 2009). Different DNA based marker techniques have been successfully used such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) (Powell et al., 1996; Lusser et al., 2012). Molecular markers are 'landmarks' on chromosomes that serve as reference points to the location of other genes when a genetic map becomes available. If genetic maps are constructed, then the plant breeder establishes association between markers and desirable phenotypic traits. The trait of interest is then selected by indirectly selecting for the marker which is readily accessed or observed (Podlich *et al*., 2004; Goodman, 2004). In plant breeding, markers are used to locate the chromosomal positions of candidate genes, to determine genomic organization among different gene pools and to conduct marker-assisted breeding. Identification of DNA markers associated with traits of interest may be facilitated by comparative mapping, i.e., by cross-referencing to the maps of model crop species, owing to gene synetny. These markers may facilitate inter-generic gene transfers and help to minimize linkage drag (Podlich *et al*., 2004).

Major applications of pre-breeding in crop improvement:

There are major four applications of pre-breeding:

- Broadening the genetic base, to reduce vulnerability
- Identifying traits in exotic materials and moving those genes into material which are more readily accessed by breeders
- Introgression genes from wild species into breeding populations
- Identification and transfer of novel genes from unrelated species using genetic transformation techniques.

The adoption of pre-breeding facilitates the efficiency and effectiveness of crop improvement programmes by enabling increased access to and use of genetic variations conserved in gene banks (Kumar and Shukala, 2014).

Methods of Utilization Wild species Through Pre- breeding

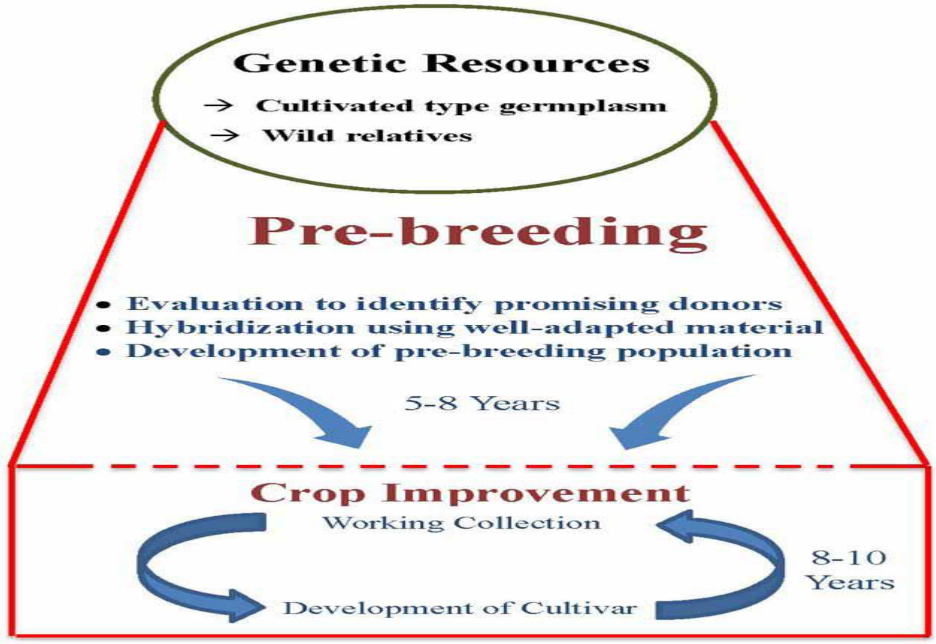

Figure 1 Pre-breeding as a bridge between genetic resources and crop improvement. (Sharma *et al*., 2013)

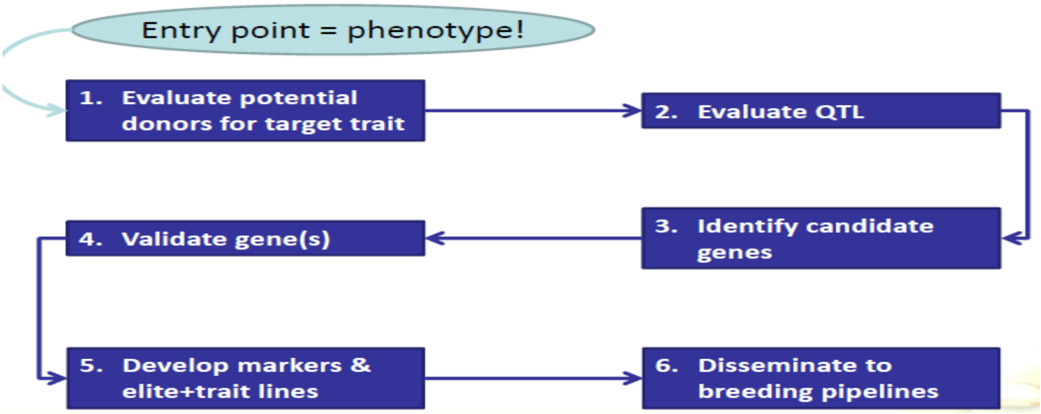

Figure 2 Pre-breeding for molecular breeding: Gene discovery & trait development (Hamilton)

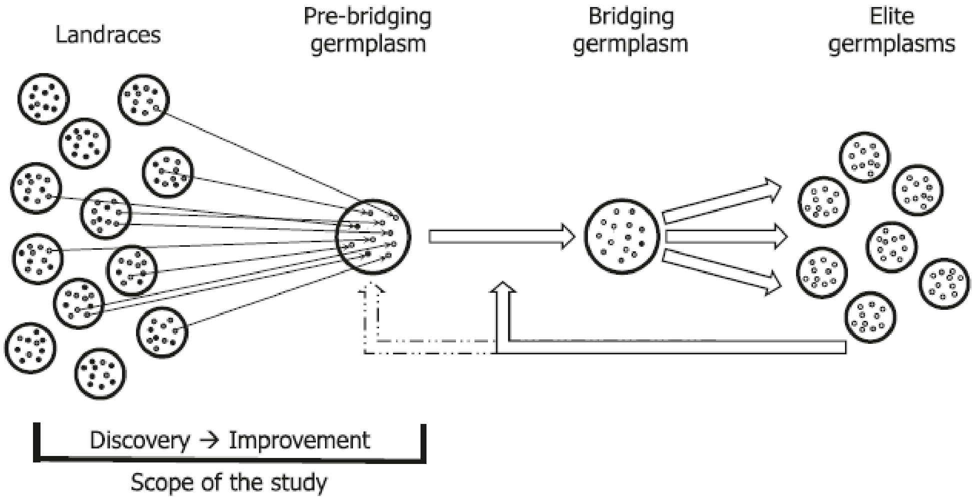

Figure 3 Scheme of a pre-breeding program in the Seeds of Discovery (Seed) initiative (Gorjanc, *et al*., 2016)

Problems associated with Genetic Enhancement

There are several problems that are associated with genetic enhancement programmes particularly when genes are introgressed from wild species. Some problems are listed below:

- Cross incompatibility in inter-specific crosses.
- Stability barriers and chromosome pairing in hybrids have restricted the access to genes from wild species into cultivated ones.
- Linkage drag.
- Hybrid inviability and sterility.
- Small sample size of inter-specific hybrid population.
- Restricted genetic recombination in the hybrid population.
- Lack of availability of donors for specific traits viz. resistance to diseases, pests and bollworm.
- Exchange and accessibility of cultivated species germplasm material has become difficult due to legal restrictions like IPR. (Loknathan *et al.,* 2003)

Challenges

- Lack of characterization and evaluation data
- Knowledge of the genetic diversity
- Inter species relationship and
- Strong breeding program and funding sources.

The use of gene bank accessions in breeding programmes is limited by the high degree of difficulty and length of time often associated with separating the desirable genes from the undesirable ones. (Kumar and Shukala, 2014)

Conclusion

For field crops improvement, sufficient genetic diversity exists in the form of landraces and wild relatives, which carry several useful genes for cultivar improvement. However, utilization of these resources in breeding programs is time-consuming and resource demanding. To overcome this, pre-breeding activities should be initiated to generate new genetic variability using promising landraces and wild relatives for use by the breeders in crop improvement programs. Pre-breeding should focus on the continuous supply of useful variability into the breeding pipeline to develop new high-yielding cultivars with a broad genetic base, pre-breeding should not focus on increasing yield. Though prebreeding is useful to enrich the primary gene pool for cultivar improvement, it is a time-consuming and difficult affair as well.

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