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From Wild genes to Elite varieties: Unlocking the power of Pre-breeding

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Abstract— **Pre-breeding, often termed genetic enhancement, is a vital strategy to connect vast genetic resources with the practical demands of plant breeding programs. It involves identifying, characterizing and transferring desirable traits from unadapted sources such as landraces, wild relatives and exotic germplasm into intermediate materials that breeders can readily use. Pre-breeding offers unique opportunity to exploit untapped genetic diversity rolling in nature. It serving as a collaborative bridge between gene bank curators and breeders. The transformative potential of pre-breeding in strengthening crop resilience observed in major crops such as rice, wheat, maize and in legumes such as mungbean and soybean. Despite its promise, pre-breeding faces several challenges, but it reconnects breeding programs with untapped variability, ensuring novel alleles and safeguarding future food production.**

Index Terms— **Pre-breeding, wild species and genetic resources.**

I. INTRODUCTION

Genetic diversity is the core foundation for development of new crop varieties, for present and future challenges which arises due to biotic (pest and pathogen) and abiotic (drought, flood, salinity and heat) stresses. In India, breeding programs in major crops reached to a point of saturation and without new genetic diversity, we face catastrophic reductions in productivity if the climate turns adverse. Therefore, 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 N.I. Vavilov. The conservation of CWR have been increased worldwide but 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 [1].

Most plant breeders fear in using exotic or unadapted material due to its initial detrimental effects on elite breeding material. It is a necessary first step to use diversity arising from wild relatives and other unimproved material. Pre-breeding attempts to reset the genetic diversity of crops by reintroducing genetic variation that has been left behind either due to genetic incompatibilities or geographical ranges [2].

II. ROOTS OF PRE-BREEDING

Harlan and De Wet (1971) introduced the idea of a gene pool. The gene pool encompasses the total genetic diversity within a species and its closely related species [3].

Jones (1983) was first used the term "enhancement" which can be defined as transferring useful genes from exotic or wild types into agronomically acceptable background [1].

Rick (1984) used the term "pre-breeding" or "developmental breeding" to describe the same activity [1].

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.

III. WHAT IS PRE-BREEDING?

Pre-breeding refers to all activities designed to identify desirable characteristics and genes from unadapted materials that cannot be used directly in breeding populations, to transfer those traits to an intermediate set of materials that breeders can use further in producing new varieties. It is the initial step in utilizing genetic diversity from wild relatives and other unimproved sources.

The pre-breeding germplasm are expected to have merit to be included in breeding programs for crop improvement. It plays an important role in the development of productive cultivars and hybrids. It focuses on enhancing grain quality, protecting against biotic and abiotic stress and increasing yield potential [4].

IV. WHY IT'S NECESSARY TO DO PRE-BREEDING?

1) **Limited genetic base:** The replacement of diverse local cultivars with genetically uniform modern varieties narrow down the genetic base of breeding population.
2) **Reduction of biodiversity:** Today, farmers grow only high yielding varieties and neglect landraces and wild species, which leads to the extinction of these species and a reduction in the biodiversity of crop species.
3) **Evolving pest and pathogen population:** Development of the boom and bust cycle occurs when in the boom phase, a resistant variety with a specific resistance gene is introduced and covers a large acreage due to widespread adoption by farmers. Over time, the pest or pathogen evolves and breaks the resistance, leading to an epidemic.
4) **Effect of climate change:** Environmental stresses such change in temperature, humidity, drought and excessive rainfall during critical stages of crop growth make it necessary to undertake pre-breeding to identify genes related to climate fluctuations.

5) **Non-availability of desired genes in gene bank:** In some cases, breeders are unable to identify the desired genes from available gene bank resources. therefore, it becomes essential to explore new sources of genetic variation.

All the above reasons are responsible for undertaking pre-breeding for future breeding programs.

V. OBJECTIVES OF PRE-BREEDING [5]

1) Improvement of germplasm and genetic knowledge to enhance resistance expression and genetic diversity.
2) Utilization of germplasm as parental lines which can be readily utilized in breeding programs.
3) Identification and documentation of potentially useful genes.
4) Exercising pre-breeding as a collaborative endeavour to fill the void between gene bank curators and plant breeders.

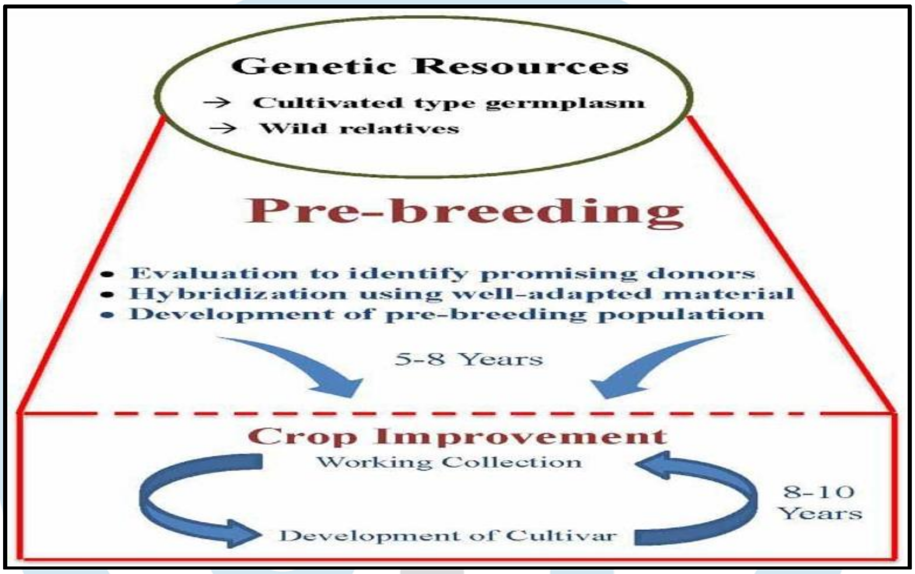

Figure 1: Utilization of genetic resources through pre-breeding [6]

VI. GENETIC RESOURCES FOR PRE-BREEDING

Gene pool is the total genetic variation in the breeding population of a species and its closely related species. The gene pool of a crop is made up of landraces, obsolete and modern varieties, breeding lines, genetic stocks, wild species and weedy companion [5]. Gene pool represents the entire genetic variability or diversity available in a crop species.

Landraces are primitive cultivars, which had evolved over centuries through both natural and artificial selection. These are selected and cultivated by the farmers for many generations without systematic breeding. Obsolete varieties were developed by systematic breeding effort, were once commercially cultivated, but are no more grown because of replacement by modern varieties.

Special genetic stocks include lines carrying gene mutation, chromosomal aberrations and markers genes. Wild forms are the wild species from which crop species were directly derived. They are easy to cross with the concerned crop species whereas wild relatives include all other species, which are related to the crop species by descent during their evolution. Weedy companions are plant species that grow naturally along with cultivated crops and share similar environments.

Types of Gene pool:

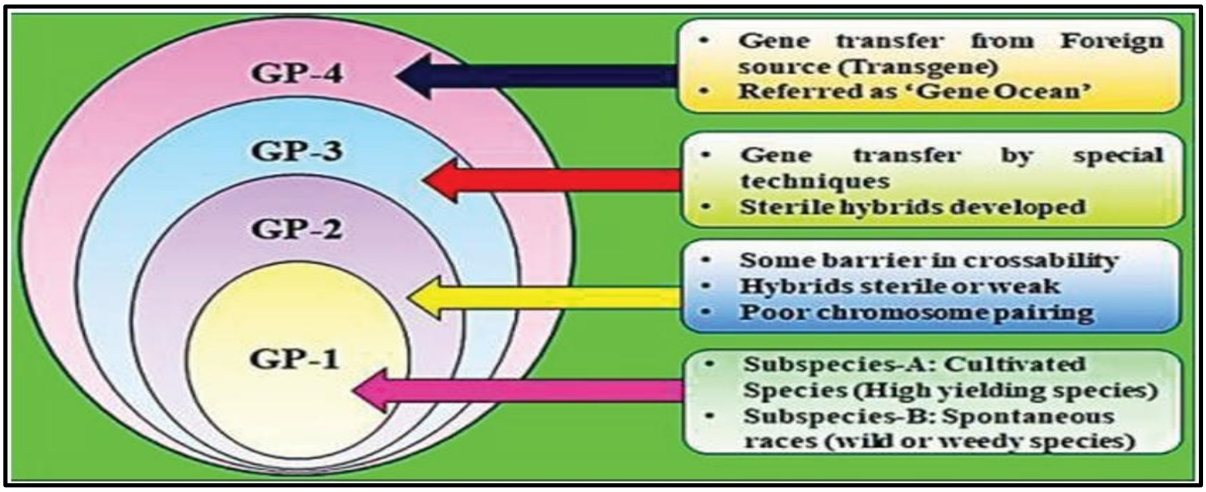

Figure 2: Modified gene pool concept in plants based on the hybridization study, a revised form of Harlan and De Wet 1971 [10]

1) **Primary gene pool (GP-1):** It includes all strains of the concerned crop species and may include its wild progenitors. Crossing between members of GP-1 is easy, the resulting hybrids are fertile and show normal meiotic chromosome pairing, recombination and segregation [7]. In wheat, GP-1 includes *Triticum aestivum*, *Triticum spelta*, *Triticum durum*, *Triticum monococcum* and *Aegilops tauschii* etc. *Triticum spelta* has provided valuable stripe and leaf rust Resistant genes including *Yr5*, *Lr44*, *Lr65* and *Lr71*. Wild emmer wheat (*Triticum dicoccoides*), contributing both major gene resistance loci and QTLs for complex traits such as Fusarium head blight (*Fusarium* species complex) and Septoria tritici blotch (*Zymoseptoria tritici*) [8].

2) **Secondary gene pool (GP-2):** It comprises of all those species that hybridize with the members of primary gene pool with some to considerable difficulty and the hybrids are at least partially fertile. The difficulty in hybridization is due to ploidy differences, chromosome alterations or genetic barriers. Gene transfers from GP-2 to GP-1 are possible, but usually difficult. Members of this group are often used in breeding programs [7]. In wheat, key species include in GP-2 are *Triticum timopheevii* (A^tA^tGG), *Aegilops ventricosa* ($D^VD^VN^VN^V$) whereas *Aegilops speltoides* (SS), which is closely related to the B genome of wheat. *Aegilops ventricosa* carries the *Yr17–Lr37–Sr38* resistance gene cluster and crossing modern cultivar leads to confers partial resistance to wheat blast (*Magnaporthe oryzae* pathotype *Triticum*). *Triticum timopheevii* has also served as a valuable source of disease resistance genes such as *Pm6*, *Lr50* and *Sr36* in wheat improvement programs [8].

3) **Tertiary gene pool (GP-3):** The species belonging to this group represent the extreme outer limit of the potential germplasm. They cross with the members of primary gene pool with considerable to great difficulty and hybrids are inviable or completely sterile. GP-3 is used occasionally in breeding programs, as group of researchers having the competence and the patience for tackling the problems associated with gene transfer [7]. Diploid and polyploid *Thinopyrum* species carrying subgenomes J, E, and St whereas rye (*Secale cereale* L.) carrying R genome are include in tertiary gene pool of wheat. Rye carries multiple resistance genes, including *Pm17*, *Pm8*, *Yr9*, *YrCN17*, *YrR2121* and *Lr26*. Resistance gene from *Thinopyrum ponticum*, introduced through a wheat-*Thinopyrum* translocation, which remains highly effective against the leaf rust and stem rust pathogens [8].

4) **Quaternary gene pool (GP-4):** It involves unrelated species or even organisms outside traditional plant breeding boundaries. Genes can be transferred using recombinant DNA technology, enabling genetic material to be introduced from diverse sources. The *Cry* gene (*Cry1Ac* and *cry2Ab*) was transferred from a bacterium (*Bacillus thuringiensis*) to cotton in order to develop Bt cotton which confer resistance against lepidopteran insect pests cotton bollworm (*Helicoverpa armigera*) and pink bollworm (*Pectinophora gossypiella*) expanding the gene pool beyond human imagination [9]. Modern science has thus made available a new form of gene pool (GP-4) that might be referred to as a "Gene Ocean" [10].

VII. FROM GERMPLASM TO IMPROVED LINES: PRE-BREEDING WORKFLOW [11]

1) Characterization of landraces
2) Acquisition of new genetic information
3) Creation of new parental lines
4) Development of wide crosses
5) Introgression of new traits
6) Development of pre-breeding germplasm

The developed pre-breeding germplasm utilized in breeding program for crop improvement.

VIII. CONTRIBUTION OF LANDRACES OR WILD SPECIES TO CROP IMPROVEMENT VIA PRE-BREEDING

Rice: Interspecific pre-breeding populations from crosses between the tolerant rice landrace GERVEX 2674 (*Oryza glaberrima*) and three sensitive *Oryza sativa* elite breeding lines. The three populations GERVEX 2674/ART27-79-1-3-B-B-B-2, GERVEX 2674/ART28-126-3-2-2-7 and GERVEX 2674/FAROX508-3-10 F44-2-1 were evaluated for stagnant flooding tolerance. They found best performing crosses exhibited moderate elongation under stagnant flooding and maintained high tillering compared to susceptible parents. The QTLs identified on chromosomes 1, 2, 3, 6, 10 and 12 were linked to days to 50% flowe ring, plant height and grain yield [12].

Maize: Pre-breeding done through wide hybridization between 69 inbred lines and their wild species *Zea mays* ssp. *Parvigluimis* (WS-5) and *Zea luxurians* (WS-1). They observed introduction of wild species will help in the creation of novel variation in the modern-day cultivars, as hybrids (IC0621166 × WS-5, IC0621634 × W-5 and IC522300 × WS-1) possess typical wild characteristics like tillering and prolificacy on main tillers and side tillers. The multiple tillering habit useful for fodder purposes [13].

Mungbean: The cross was conducted between BM-4 (a high yielding cultivar) and BWM-29, a wild accession of *Vigna radiata* var. *sublobata*, which is resistant to both pre-harvest sprouting (PHS) and fresh seed germination (FSG). Further, hybridization was carried out between the identified stable mungbean genotypes and commercially released mungbean cultivars, including BM 2002-1, Phule Chetak, Kopergaon, AKM 4, PKV Green Gold and BM-4 for development of 42 cross combination. The cross combinations exhibited PHS (<10%) were BM-4 × BWMCD-5, BM2002-1 × BWMCD-10, Kopergaon × BWMC-20-1 and Kopergaon × BWMC-30-2-1. PHS tolerant male parents BWMC-30-2-1, BWMC-20-1 and BWMCD-5 are recommended for direct utilization in future harnessed in mungbean improvement programs [14].

Soybean: Evaluation of 125 soybean accessions across five different locations along with specific checks. The accessions were characterized for eight morphological traits and screened for frog-eye leaf spot (FLS) and yellow mosaic virus (YMV) diseases. Twelve accessions (AGS 163B, AMS.MB 51-18, EC 251516, EC 393222, EC 457185, EC 456599, UPSL 470, JS 20-41, NRC 42, JS 89-24, RKS 52, and IMP-1) were found to be highly resistant to both FLS and YMV under field conditions. Therefore, accessions combining high yield with YMV and FLS resistance can be effectively utilized for developing widely adapted soybean genotypes in future hybridization programs [15].

Wheat: Thirty-two wheat landraces along with three commercial cultivars for various qualitative and quantitative traits. They observed several landraces exhibited strong glaucosity on the flag leaf and ear, which is a crucial trait especially under dry and hot cultivation conditions and is positively correlated with resistance to both biotic and abiotic stresses. It significantly reduces cuticle permeability and water loss through transpiration. Some landraces showed superiority in earliness, grain yield per plant and its components compared to the commercial cultivars. Therefore, these landraces could be hybridized with commercial wheat varieties to develop improved wheat genotypes [16].

Table 1: Pre-breeding work attempted in different crops

Crops	Trait	Landrace / source
Rice	Tolerance to stagnant flooding	GERVEX 2674 (*Oryza glaberrima* assession)
Maize	Prolificacy on main tillers and side tillers	*Zea mays* ssp. *Parvigluimis* and *Zea luxurians*
Mungbean	Tolerance to pre-harvest sprouting	BWM-29 (*Vigna radiata* var. *sublobata* accession)
Soybean	Resistant to frog-eye leaf spot and yellow mosaic virus	IMP-1, JS 20-41 and EC 393222
Wheat	Flag leaf and ear glaucosity	Wheat accession (2733)

IX. MAJOR CHALLENGES IN PRE-BREEDING [17]

1) Lack of characterization and evaluation data
2) Linkage drags, hybrid inviability and sterility
3) Inter-species relationships
4) Strong breeding programs and funding sources

X. FUTURE PROSPECTS

1) **Characterization and collection of wild species:** There is an urgent need to collect, characterize and document wild species and crop wild relatives, as they hold valuable genes that could enhance agricultural resilience to biotic and abiotic stresses.
2) **Genome mapping:** Advances in genome mapping allow for the identification of genes responsible for abiotic stress tolerance in crops, paving the way for targeted pre-breeding strategies.
3) **Tertiary and quaternary pool:** The tertiary and quaternary gene pools hold immense potential for the future of pre-breeding programs. These gene pools include distantly related species and even non-crossable organisms, which serve as reservoirs of novel and valuable genes for crop improvement.
4) **Bioinformatics tool:** Utilization of bioinformatics tools will help leverage genetic and genomic data to effectively tackle complex traits in crop improvement. Integration of classical breeding approaches with advanced molecular tools and techniques enhances the utilization of plant genetic resources for sustainable crop improvement, addressing global agricultural challenges and increasing food security.

XI. CONCLUSION

Pre-breeding is gaining importance in crop improvement due to limited genetic variability is available in cultivated germplasm. It has been playing great role in continuous supply of useful variability from promising landraces and wild relatives to the breeding pipelines.

Using crop wild relatives in crop improvement is much more difficult than breeding with domesticated varieties, but pre-breeding converts them in more readily crossable with modern and elite varieties. Linkage drags associated with exploiting of wild relative makes pre-breeding activities challenging. To overcome this and facilitate targeted transfer of useful genes for genetic enhancement, genome assisted pre-breeding will be useful. Generally, pre-breeding is fundamental for the success of plant breeders in developing crop varieties.

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