

Training in organic breeding

Module 1: Plant Genetic Resources (PGRs): collection, conservation and exchange to support the increase of agrobiodiversity in farming systems

Unit 1.2: Prebreeding: from genetic resources characterisation to their use in breeding programmes

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UK Research
and Innovation

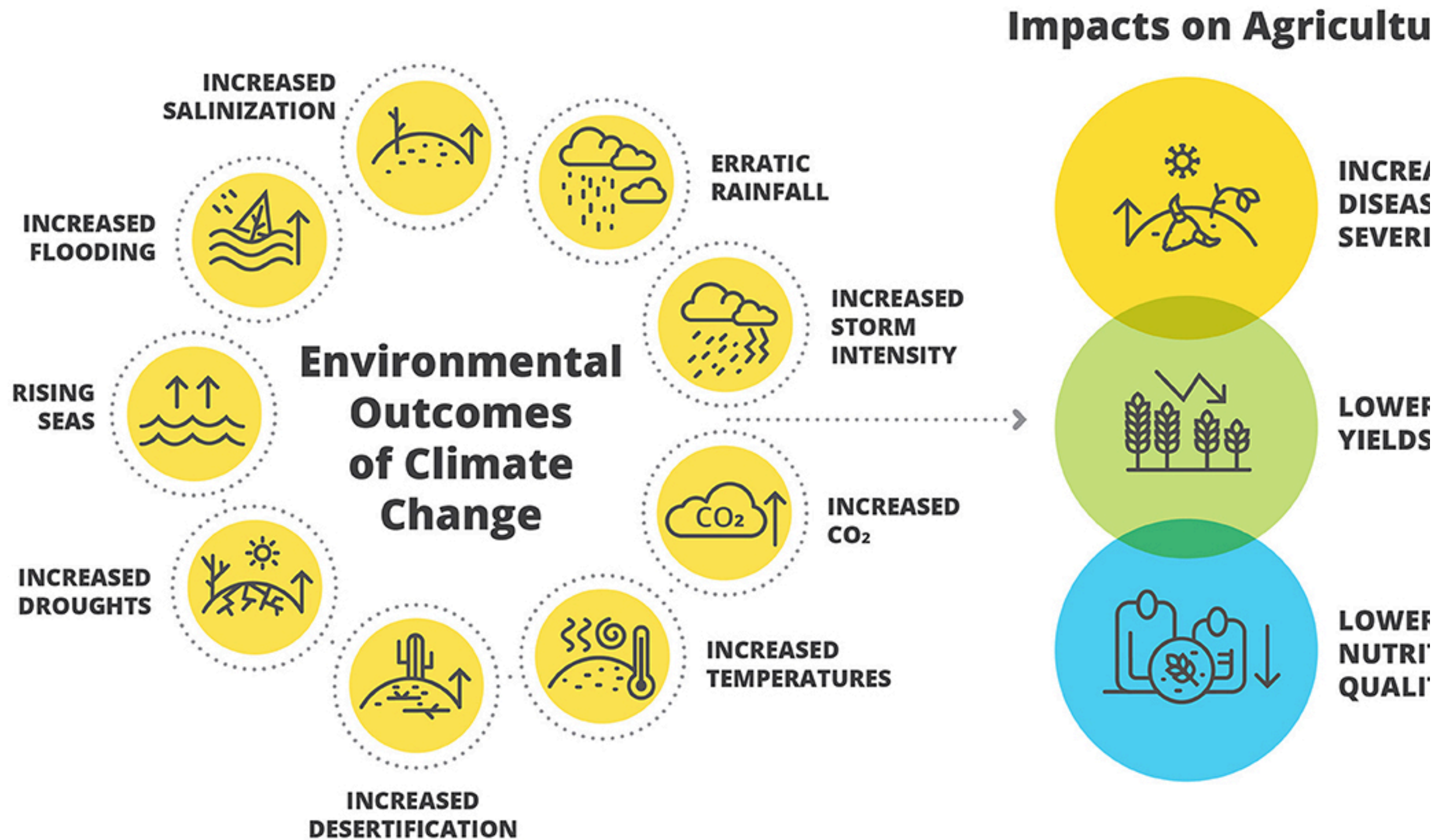
Module 1 – Unit 2 Pre-breeding

Planned for today

DYNAMIC MIXTURE OF:

1. *Presentation about main topics on Pre-breeding: intro, basics, examples, additional material (50 min)*
2. Fast quiz and discussion (about 20 min)
3. Wrap up & Proposed homework (about 10 min)

Module 1 – Unit 2 Pre-breeding



Module 1 – Unit 2 Pre-breeding

Plant stressors

Natural stressors:

WRITE TO US abiotic

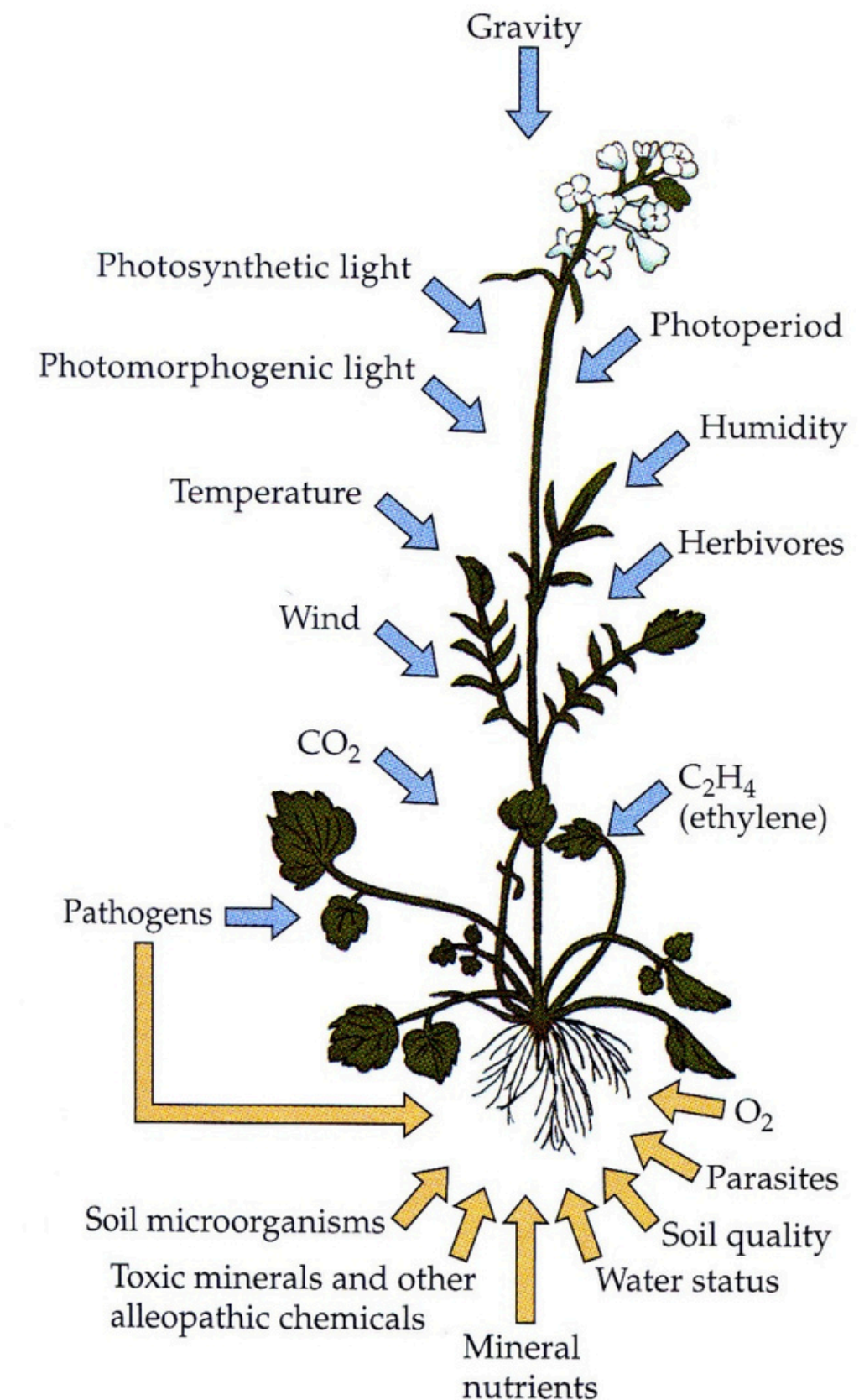
- drought
- flooding
- salinity
- cold
- heat
- mineral def.
- high light
- UV radiation
- oxidative stress

WRITE TO US biotic

- viruses
- bacteria
- fungi
- weeds
- herbivores
- insects
- competition
- allelopathy

Anthropogenic stressors:

- herbicides
- air pollution (SO_2 , NO_x , O_3)
- acid rain/fog/morning dew
- heavy metals



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Plant breeding

= human selection of the best plants from a diverse population



An increasing number of people, climate change, new plant diseases and pests have always demanded the creation of new, more resistant and more productive varieties of existing plant species, or the introduction of completely new (wild) species.

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The main goal of plant breeding is to create a new, more productive, more useful for humans and for the environment-friendly varieties.

The process of breeding consists of three basic parts :

- creating genetic diversity,
- selection or selection of the best individuals from genetically diverse populations,
- realization of the variety (testing and reproduction of selected individuals, stabilization of genetic diversity, validation, maintenance and distribution of the variety).

“

We are the first generation to feel the impact of climate change and the last generation that can do something about it

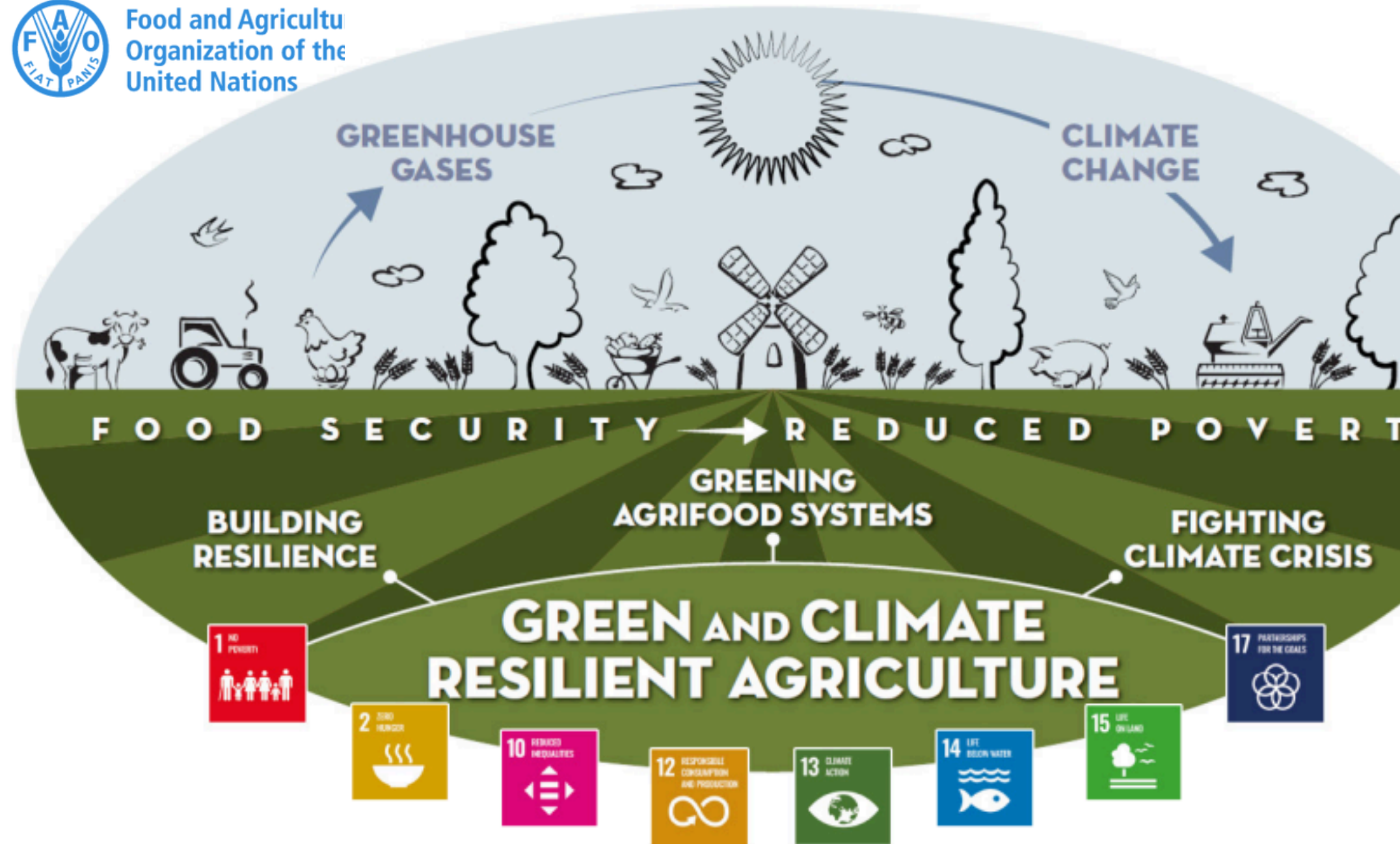
Barack Obama

”

The question arises: How can the pre-breeding serve as a solution to the specific demands of organic agriculture in the era of climate change?



Food and Agriculture
Organization of the
United Nations



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Since the beginning of the 20th century, plant breeders have methodically leveraged an ever-increasing set of knowledge, skills and tools to:

- add value to PGRFA , and
- hence make crops better for humankind

But, current crop varieties may be unsuited to the burgeoning set of challenges and opportunities. So,

- What are the alternatives?
- How to achieve genetic in the absence of easily exploitable traits (and genes)?
- How can genes be accessed to address this multiplicity of needs?

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Significant efforts and investments have resulted in the collection, characterization, evaluation, documentation, conservation and distribution of PGRFA.

Most of the world's important crops are in genebanks and these genebanks are the reservoirs for:

- different variations of the traits still used by farmers;
- traits that are no longer needed by farmers;
- traits that evolved but may never previously have been used by farmers; or
- traits that have not yet been discovered

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About 7.2 million accessions in over 1300 genebanks.

But these accessions are not used optimally in crop improvement because:

- Lack of documentation and adequate description of collections
- Insufficient evaluation of the collection
- Limited input by breeders during documentation to understand what information would be most valuable
- Accessions with limited environmental adaptability
- Accessible materials not always suited to agronomic needs
- Adequate quantities of seed are not available in a timely manner

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The narrow genetic base of cultivars coupled with low utilization of genetic resources is the major factor limiting production and productivity globally. To exploit this genetic diversity pre-breeding offers a unique opportunity by introgression of desirable genes from wild germplasm into cultivated backgrounds readily used with minimum linkage drag.

Pre-Breeding term was first coined by Rick in 1984.

It is an alternative term used for “genetic enhancement” and in recent times it has become an essential, planned part of all plant breeding activities. It refers to all activities designed to identify materials that cannot be used directly in breeding programmes, and further to transfer these traits to an intermediate set of materials that breeders can use further in producing new varieties for farmers.

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Pre-Breeding is defined as transferring of useful genes from exotics or wild (unadapted sources) types into agronomical acceptable background / breeding material (FAO, 1996). Further, the Global Crop Diversity Trust defined pre-breeding as 'the art of identifying desired traits, and incorporation of these into modern breeding materials. Pre-breeding is the transfer or introgression of genes or gene combinations from unadapted sources into breeding materials including those that, although adapted have been subjected to any kind of selection for improvement.

Pre-Breeding is focused 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 (Hausmann *et al.*, 2004).

Pre-breeding aims to generate new base population for breeding programme through the use of a wider pool of genetic material (Hausmann *et al.*, 2004). Pre-breeding has been used successfully in several crops (rice, tomato, soyabean, cotton, maize, wheat, barley, groundnut, chickpea, pigeon pea, sorghum, pearl millet) by transferring the genes from wild / exotic (unadapted) species into adapted material and improved many cultivated varieties for different qualitative and quantitative traits.

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Wild Species possesses the greater amount of genetic variation which can be exploited to improve the crops

Crop	Remark	Reference
Domestic Bean	Wild relatives are a potential source of novel alleles that can be exploited for the improvement of yield and other quantitative traits.	Acosta-gallegos <i>et al.</i> , 2007
Tomato	Different genes for disease resistance have been incorporated from various wild resources in commercial hybrids through recurrent back cross and each resides on a small independent chromosome segment from one of the diverse donor species. An important gene was introduced from the wild tomato species (<i>Lycopersicon pennellii</i> B.), which resulted into raised level of Pro-vitamin A in the fruit by more than 15 fold.	Ronen <i>et al.</i> , 2000

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Cotton	Pre-breeding has been attempted in cotton (since 1970) in different countries and several varieties were developed using wild species for resistance to biotic stress (sucking pest), Abiotic Stress (drought tolerance) and Quality improvement (fibre quality) Texas, USA.	Lokanathan <i>et al.</i> , 2003
Groundnut, Pigeon pea, chickpea,	Improved the existing cultivars using wild species for resistance to biotic stress and abiotic Stress and quality improvement	ICRISAT, 2004
mulberry	Crosses between cultivated (<i>M. indica</i>) and different wild species (<i>M. cathayana</i> , <i>M. pendulata</i> and <i>M. serrata</i>) for improve the quality	Tikader and Rao, 2002, Tikader and Thangavelu, 2002, Tikader and Dandin 2001, Tikader and Ananda 2003, Tikader and Dandin, 2007

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Objectives of pre-breeding

Improved germplasm and associated genetic knowledge that enhance resistance expression and diversity.

Reduce genetic uniformity in crops through the use of a wider pool of genetic material to increase yield, resistance to pests and diseases, and other quality traits.

Identification of desirable traits/genes and their subsequent transfer into a suitable set of parents for further selection.

Improved parental stocks which can be readily utilized within breeding programs and improved selection methodologies. Identify potentially useful genes in a well-organized and documented gene bank.

Designing strategies that lead to development of an improved germplasm that are ready to use in varietal development.

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Differences between pre breeding and traditional breeding

Sr. No.	Pre breeding	Traditional Breeding
1	Pre-breeding is also known as genetic enhancement.	Traditional breeding is also known as sustainable plant breeding.
2	It leads to genetic enhancement of germplasm.	It leads to development of productive cultivars/hybrids.
3	It leads to value addition.	It does not lead to value addition.
4	It leads to broadening the genetic base of the population.	It leads to development of improved cultivars with narrow genetic base.
5	The chief breeding method is backcross method.	All breeding methods such as introduction, selection, hybridization and mutation are used.
6	The end products are improved germplasm line.	The end product is cultivar or hybrid.
7	The end product is used as parent for developing improved cultivar, hybrid.	The end product is used for commercial cultivation.
8	It involves adapted and non-adapted genotypes in crossing programme	It includes only adapted genotype
9	It is a long term breeding programme.	It is a short or medium term breeding programme.
10	It is taken up by public sector plant breeding organizations.	It is taken up by both public and private sector organizations.

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Pre-breeding ‘Decision tree’

No pre-breeding needed if one of the following is available:

- Commercially-available adapted and acceptable varieties
- Advanced selections, well-adapted to the target environment
- Genebank accessions that are well-adapted to the target environment

Pre-breeding probably needed if only one of the following is available:

- Genebank accessions not well-adapted to the target environment
- Closely related wild species easily crossed with the crop species
- Wild species less closely related and more difficult to cross

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Classical approaches being used in crop improvement with the help of plant genetic resources (Cooper *et al.*, 2001) are:

Introgression

Introgression, also known as introgressive hybridization, in genetics is the movement of a gene (gene flow) from one species into the gene pool of another by the repeated backcrossing of an interspecific hybrid with one of its parent species. Purposeful introgression is a long-term process; it may take many hybrid generations before the backcrossing occurs. It is transfer of one or more genes from exotic/un-adapted/wild stock to adapted breeding material.

Incorporation Incorporation or broadening of genetic base refers to a large scale programme aiming to develop locally adapted population using exotic / un-adapted germplasm. The objective of incorporation is to produce new breeding populations that have very high proportions of unique, exotic-derived alleles in order to broaden substantially the crop's genetic base.

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Wide crosses A cross of two individuals belonging to different species or different genera is known as wide cross. Such a cross can be (rarely) realized in nature as it has to overcome barriers prohibiting the development of fertile offspring. Wide crossing has been used with considerable success in some crops viz., blight resistance in potato, rust resistance in wheat and insect resistance in rice.

Decentralized participatory plant breeding Plant breeding programs differ from each other in different aspects (in the crop, in the facilities and in the breeder) but they all have in common some major stages such as creation of variability, selection and testing of experimental cultivars. A decentralized-participatory plant breeding program also function with the same line provided with some differences like most of the process takes place in farmers' fields, the decisions are taken jointly by the farmers and the breeder and the process can be implemented at a number of locations involving a large number of farmers evaluating different breeding materials.

Marker assisted breeding Breeding methods based on DNA molecular marker patterns instead of, or in addition to, their trait values. When molecular markers are available, conveniently co-segregating with candidate genes, marker-assisted selection (MAS) or marker-aided selection may improve the efficiency of selections of simple traits in conventional plant breeding programs (Knapp, 1998; Podlich et al., 2004).

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Pre- Breeding act as an interface of conservation of PGR and breeding.

It is a multidisciplinary approach and requires the following:

Collection of underrepresented diversity, informed by gap analyses based on taxonomic designations and eco-geographic information along with detailed passport data to both conserve the breadth of diversity available in the wild and facilitate predictive trait mining based on eco-geographic data.

Coordinated evaluation and sharing of pre-breeding products across environmental conditions to better understand genotype–environment interactions;

Improved information, sharing of that information and feedback, especially with regards to genotypic and phenotypic data and the way they are linked between genetic resource conservationists, pre-breeders, breeders, and end users.

Enhanced coordination between basic and applied research communities.

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There are several challenges that are associated with genetic enhancement programmes particularly when genes are introgressed from wild species. Some challenges (Loknathan *et al.*, 2003) 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 and Insect and pests
- Exchange and accessibility of cultivated species germplasm material has become difficult due to legal restrictions like IPR.

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Pre-breeding is a highly multidisciplinary set of activities. At the core are the genebank manager and the plant breeder, and they are supported by a large group involving disciplines such as molecular biology, plant pathology, entomology, physiology, soil science, and others.

There is a greater likelihood of more complex issues for cross-pollination, such as timing of flowering, incompatibility, and irregular seed development, which need to be understood by the pre-breeder.

Pre-breeding is not a new science, but both the urgency for its use, as well as new opportunities for better identifying and using genes in exotic or wild materials, make it imperative to reinforce our ability to do effective, long-term pre-breeding.

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We can further understand pre-breeding in terms of **crop evolution** and the opportunities of **modern plant breeding**:

1. Pre-breeding in the framework of the evolutionary and agricultural background

With few exceptions, humans selected our food and industrial crops from their wild progenitors, in some cases as far back as 12,000 years ago.

The combined forces of human and natural selection produced a wide range of genetic diversity. This heritable diversity gave crops the ability to adapt to different climates, soil types, photoperiod, and to meet the requirements for different management systems, market-determined quality traits, etc.

Farmer and natural selection was responsible for nearly all the varieties of crops cultivated on the planet up to the early 20th century.

2. Pre-breeding in the framework of modern plant breeding

The situation changed with the rediscovery of Mendel's laws, and from that, the birth of the science of genetics. Modern plant breeding was born when scientists began to understand how genetic information could be applied to improving crops. Scientifically-based plant breeding came onto the scene and allowed dramatic changes in the ways that new varieties are produced.

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What genebank managers and plant breeders do

Genebank managers:

- assure that the widest possible diversity is held safely in ex situ collections under standardized protocols for the species in question;
- may also work to assure conservation in situ, but most don't have this responsibility;
- gather and organize the passport data, including any available information about the traits and performance of materials where collected, and the area itself through GIS technologies, (this activity might be carried out also by the collectors who supply the genebanks);
- manage the information to minimize errors and to maximize the utility of accessions,
- follow standards of regeneration and viability testing to minimize risk of loss;
- characterize the germplasm for those traits that are environmentally stable and serve to reliably aid in identifying an accession. This can involve whole-plant or molecular traits, which may or may not have any relationship to traits of economic importance.

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Plant breeders:

- Plant breeders evaluate a broad base of germplasm to identify sources of genes to combine into varieties that will be superior to existing ones in some significant way.
- The germplasm base of choice typically includes materials that are generally well-adapted to the target region for the breeding programme. The reason for this preference is that using a poorly-adapted parent will bring a set of undesirable genes into the breeding population, along with the gene or genes of interest.
- Then, the breeder will normally need to work for several additional generations to select out these undesirable genes while tracking and selecting for the desirable ones.
- After extensive testing, including input from farmers at all levels, breeders work with seed multiplication, certification and distribution systems to assure that farmers receive the intended benefits.

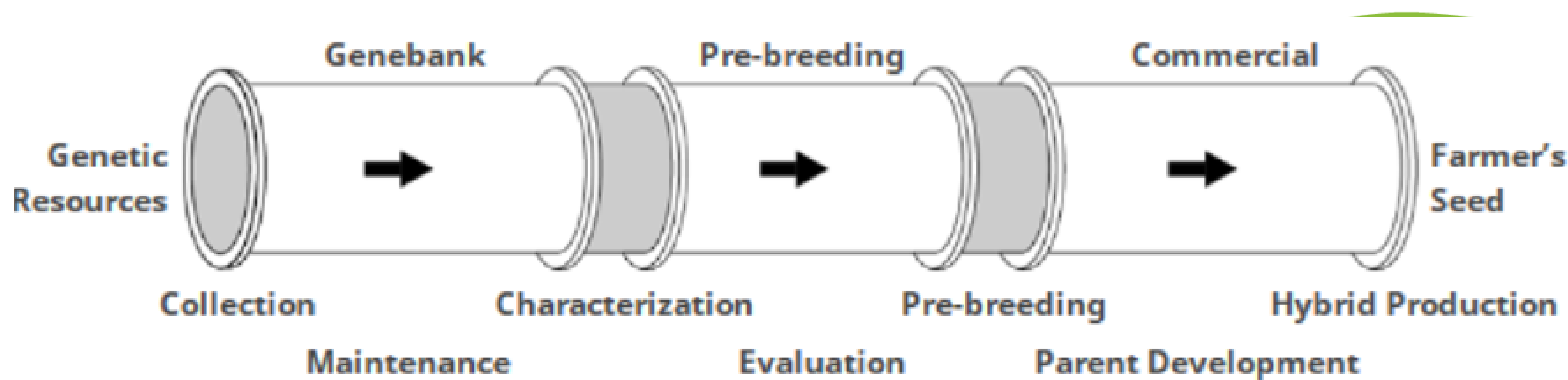
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Essential role of prebreeding is seen in cases when genes of interest cannot be found in adapted germplasm.

The **joint roles** for breeder and genebank manager (i.e. the pre-breeders) include:

1. evaluation for traits of importance;
2. information management;
3. study of reproductive biology;
4. additional multiplication to make materials available for crossing;
5. collaboration where crossing is difficult.

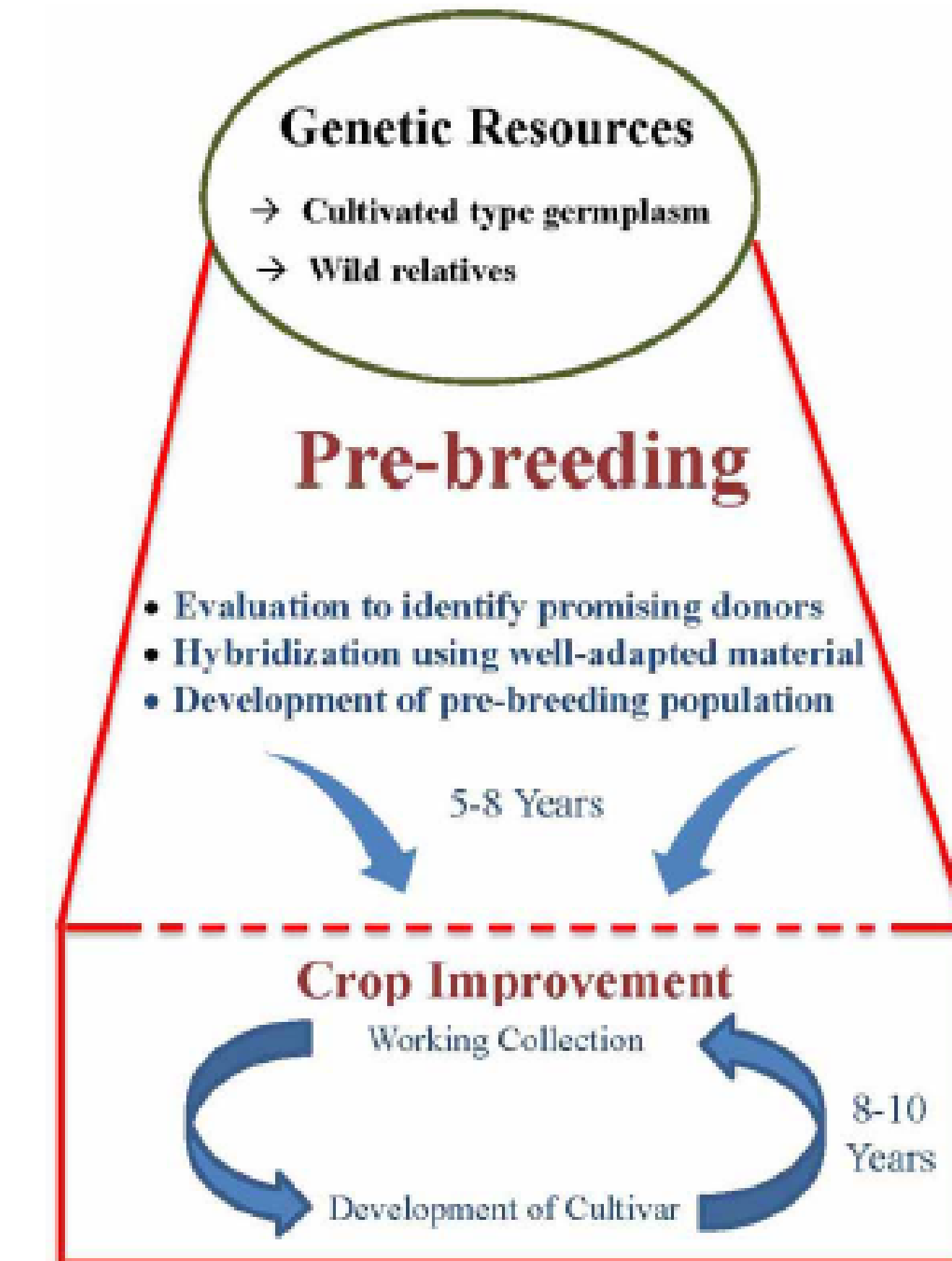
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At the beginning, genes are identified within unimproved genetic resources, and their value assessed (**valuation**). Then, these genes are introduced into better agronomic backgrounds, tested, selected, and tested some more (**Pre-breeding**). Finally, the desirable genes are incorporated into materials having promise of commercial value (**Breeding**). Often after 10 to 15 years or more, the finished product - a new variety - may emerge for farmers to use (more...).

Pre-breeding represents the middle segment of this pipeline, in those situations where extra time and resources are needed to bring the desired genes into a form that is more readily used by plant breeders to develop farmer-accepted varieties.

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

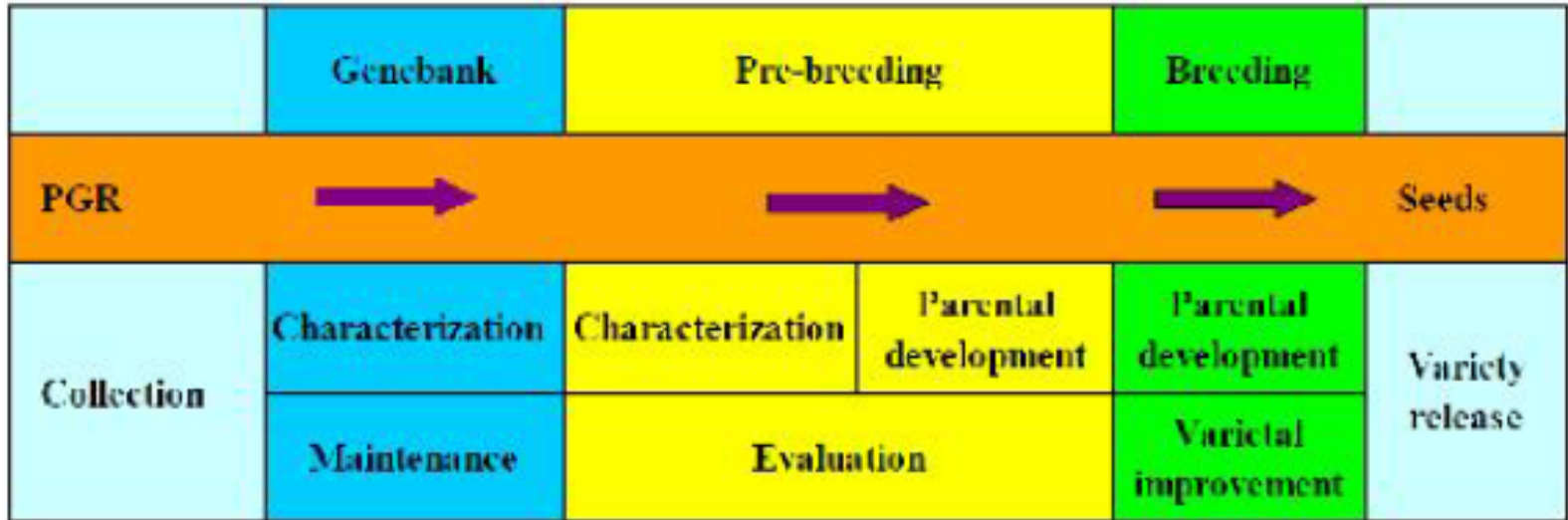
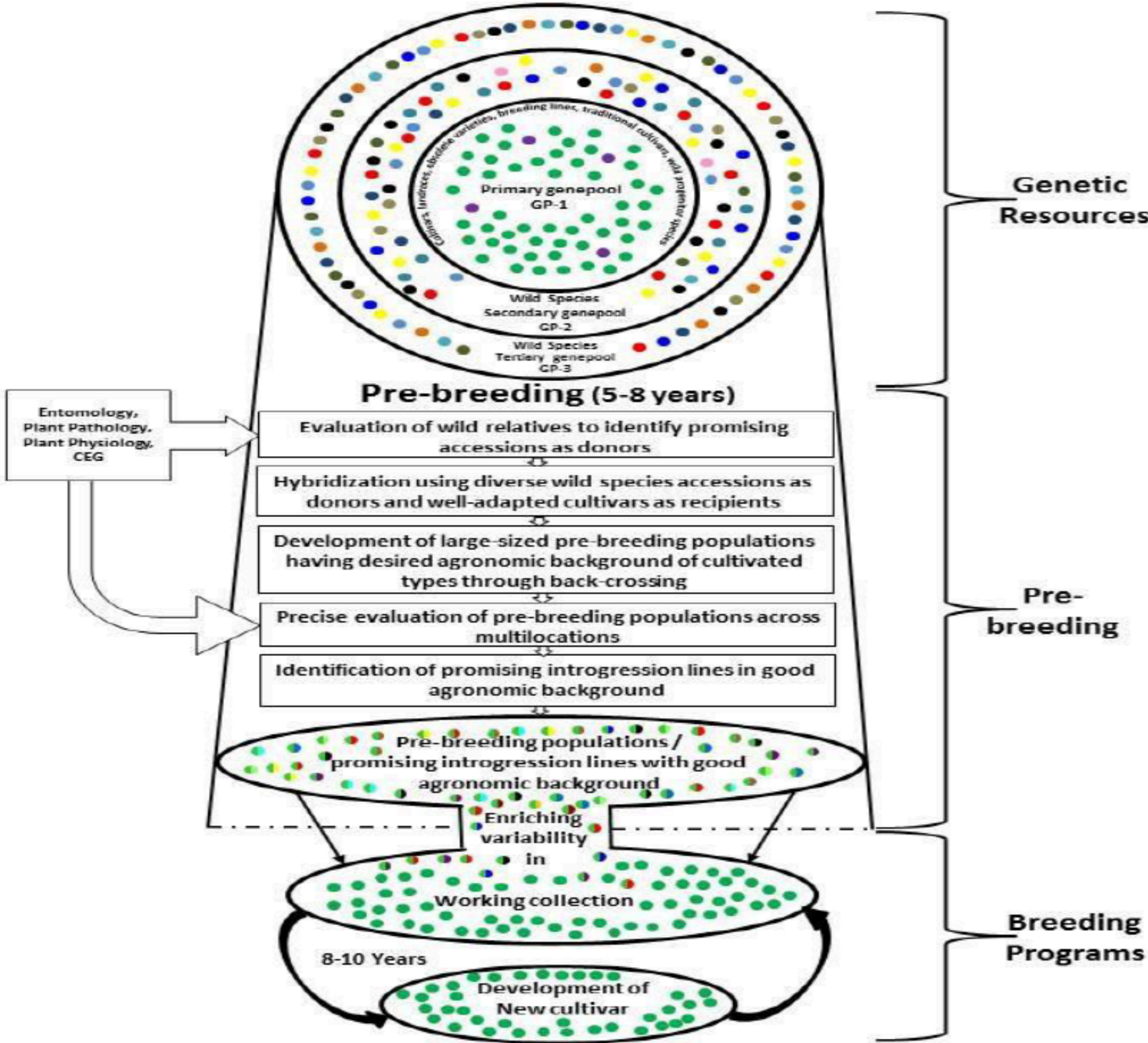


Figure adopted from: Crop improvement through pre-breeding technologies
Jitendra Kumar Meena¹ and Rahul Kumar ISSN-2321-7987

Integrating Crop Conservation and Pre-Breeding

"A Path to Sustainable Agriculture"

Conservation of Crops

Preserve and safeguard diverse crop varieties.
Prevent the loss of critical genetic resources.

Genetic Diversity Foundation

Genetic diversity is the foundation of resilient crops.
Conserved crops maintain a diverse gene pool.

Pre-Breeding Contribution

Identify and utilize beneficial traits for crop improvement. Methods include genomic approaches and marker-assisted selection.

Targeted Trait Enhancement

Focus on climate adaptation, disease resistance, and stress tolerance.
Transfer of specific traits from conserved crops to cultivated varieties.

Conservation provides raw material; pre-breeding refines it.

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Some examples

Pre-breeding of *Brassica oleracea*
&
Responses to Organic Agriculture Needs

**By Hajer Ben
AMMAR**

Research line 2

The effect of water stress on
the variation of the
biochemical profile of *Brassica
oleracea*

Research line 3

Evaluation of *Brassica oleracea*
based on agronomic trait:
Glucosinolates (GLSs) in relation
to water stress

Research Line 1

Genetic Diversity and Germplasm Characterization of *Brassica oleracea* L. complex species (n=9) core collection

Conclusion

Biodiversity

Morphotyping

The invaluable role of morphological traits in identifying and classifying the accessions studied

Genotyping

The selection of the most polymorphic markers Gi1, Gi5, Gi12, Gi30 and Gi38

Understanding these dynamics is crucial, as it informs conservation strategies and enables access to the valuable genetic reservoirs of *Brassica oleracea* to enhance crop breeding initiatives

Research Line 2

The effect of water stress on the variation of the biochemical profile of *Brassica oleracea*

Conclusion

water stress



The importance of **selecting cultivars** with an efficient antioxidant system, as this trait is associated with higher chances of survival and improved performance under **limited water conditions**.

Selection



By considering morphological and biochemical traits as indicators of abiotic stress response, wild species and the varieties **CR**(*B.o. gongylodes*), **CC** (*B.o. capitata*), **BH** (*B.o. acephala*), and **CI**(*B.o. incrocio*) were identified as the most water stress-tolerant among the studied cultivars.



Research Line 3

Evaluation of *Brassica oleracea* based on agronomic trait: Glucosinolates (GLSs)



Pre-breeding of *Brassica oleracea* & Responses to Organic Agriculture Needs

Genetic Diversity Exploration

1

A Comprehensive examination of *B. oleracea* gene pool, including wild relatives.
Unraveled phenotypic diversity and genetic relationships using 12 SSR markers delineated distinct groupings, enhancing understanding of evolutionary history.

Biochemical Responses to Water Deficiency

2

Discovery of specific biochemical markers linked to water stress tolerance.
Identification of genotypes with exceptional drought tolerance.
Useful information for domestication, and breeding and also for applications in food industries as potent natural antioxidants.

GLS Metabolism Exploration

3

Insights into how GLS production and functions are influenced by drought.
Explore the intricate network of GLS biosynthetic genes and their regulatory mechanisms.

Genome transfer of *Solanum lycopersicoides* into *L. esculentum* using introgression

Morphology: perennial – bush with hard fruits

Found in: Peru and Chile; found at 3600m

Self incompatible, cross pollinating sp.

Num. of chromosomes: 24 (2x)

Possibility to cross: F1 hybrid with tomato male sterile
and incompatible

- **Resistance to abiotic stress**

Low temperature

Frost

Drought

- **Tolerance to mechanical damage**

Fruit firmness

- **Resistance to biotic stress**

- **Viruses**

**Tomato Mosaic Virus (ToMV)

**Cucumber Mosaic Virus (CMV)

Curly Top Virus (CTV)

Tomato Yellow Leafcurl Virus (TYLCV)

- **Bacteria**

(*Xanthomonas campestris*)

(*Clavibacter michiganensis*)

- **Fungi**

**Phytophthora (*P. parasitica*, *P. capsici*)

**Siva plesen (*Botrytis cinerea*)

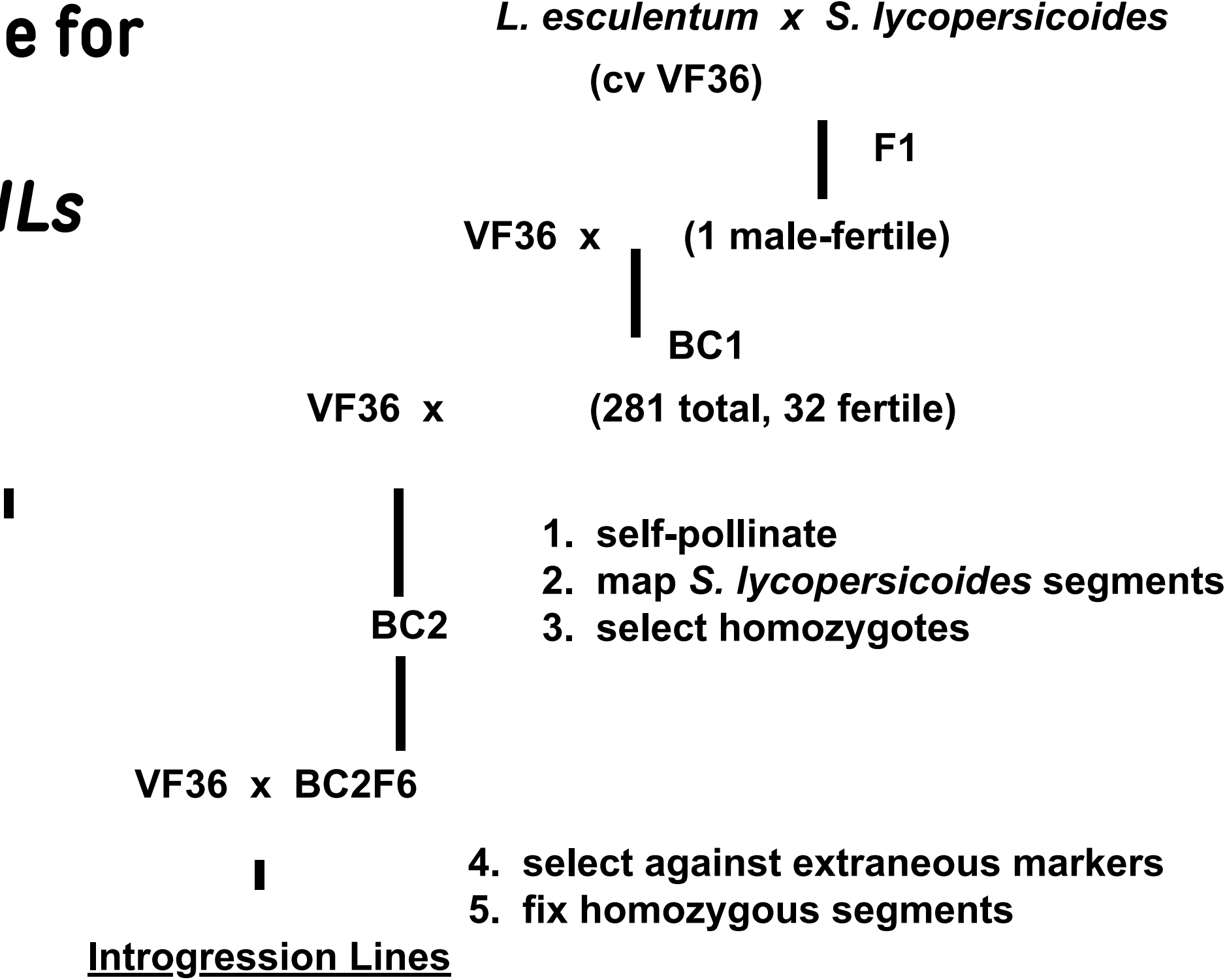
- **Insects**

On fruit (*Manduca sexta*, *Heliothis zea*)

Leaves (*Liriomyza trifolii*)

Hybridisation scheme for development of *S. lycopersicoides* ILs

Male fertility of F1 hybrids
(*L. esculentum* x *S. lycopersicoides*)



Module 1 – Unit 2 Pre-breeding 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 pre breeding is useful to enrich the primary gene pool for cultivar improvement, it is a time-consuming and difficult affair as well. Further, linkage drag associated with utilizing wild relatives makes the pre-breeding activities much more cumbersome. Genomic-assisted pre-breeding will help to overcome the linkage drag and will facilitate focused transfer of useful genes/segments from wild relatives for genetic enhancement.

Additional available materials

https://link.springer.com/chapter/10.1007/978-3-030-90673-3_25

<https://cwr.croptrust.org/project-components/pre-breeding-and-evaluation/>

<https://justagriculture.in/files/newsletter/nov/034.%20Pre-breeding.pdf>

<https://www.nordgen.org/media/qbadgkec/promoting-nordic-plant-breeding-for-the-future-ppp-public-private-partnership-for-pre-breeding.pdf>

<https://edepot.wur.nl/3667>

<https://www.cbd.int/doc/world/bt/bt-nbsap-v2-p9-en.pdf>

<https://www.cwrdiversity.org/project/pre-breeding/>



Additional available materials

<https://elearning.fao.org/course/view.php?id=487>



Pre-breeding for effective use of plant genetic resources
e-learning course



Sponsoring Organizations:



GLOBAL CROP DIVERSITY TRUST
A Foundation for Food Security



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Module 1 – Unit 2 Pre-breeding

- *What have we learned today?*

Pre-breeding is a highly multidisciplinary set of activities. At the core are the genebank manager and the plant breeder, and they are supported by a large group involving disciplines such as molecular biology, plant pathology, entomology, physiology, soil science, and others.

There is a greater likelihood of more complex issues for cross-pollination, such as timing of flowering, incompatibility, and irregular seed development, which need to be understood by the pre-breeder.

Pre-breeding is not a new science, but both the urgency for its use, as well as new opportunities for better identifying and using genes in exotic or wild materials, make it imperative to reinforce our ability to do effective, long-term pre-breeding.

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The importance of germplasm resources for crop improvement is widely recognized, but their effective use in breeding can be complex, risky, and expensive. On the other hand, it can have very large rewards in terms of making valuable traits more accessible to plant breeders, to address critical global issues such as food security in the face of climate change.

Pre-breeding is an opportunity to build a bridge that brings together the people who understand the scope and value of germplasm collections (genebank managers) with those who need to introduce new traits into their cultivars (plant breeders).

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FAST QUIZ

- *Question 1: Prebreeding normally results in intermediate products that require further crossings by the breeder. T or F*
- *Question 2: A prebreeding project helps in building bridges between germplasm collections and plant breeders T or F*
- *Question 3: The pipeline concept of plant breeding means that there is a constant flow of gradually improving germplasm through a series of stages of breeding. T or F*
- *Question 4: The risk factor for loss of genetic diversity is when breeders develop varieties that replace local landraces. T or F*
- *Question 5: One of the reasons for increasing needs to consider prebreeding approaches is a need to shorten breeding cycle. T or F*
- *Question 6: Prebreeding is a way to reduce the vulnerability of crops when they have a narrow genetic base. T or F*
- *Question 7: Prebreeder needs to know whether a gene of interest is found in the crop itself or in the wild species. T or F*

Module 1 – Unit 2 Pre-breeding

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Module 1 – Unit 2 Pre-breeding

- *Proposed homework:*

brief plan/pipeline on pre-breeding activities on species of your choice¹

¹ *send to vladimir.meglic@kis.si and petra.jelincic@ips-konzalting.hr*



LiveSeeding



This project has received funding from the European Union's Horizon Europe research and innovation programme, the Swiss State Secretariat for Education, Research and Innovation (SERI), and the United Kingdom Research and Innovation (UKRI)





LiveSeeding

