





**Module 3: Breeding methods fundamentals** 

### Unit 3.2: Common methods and strategies in organic breeding

Authors: Pedro Mendes Moreira (PUC-ESAC, PT), Adrian Rodríguez-Burruezo (UPV), Barbara Pipan (KIS)





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### **CONTEXT: Training in LIVESEEDING project**

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### **Training in organic breeding organized in 5 Modules**

- Module 1 Plant Genetic Resources (PGRs): collection, conservation and exchange to support the increase of agrobiodiversity in farming systems
- 2. Module 2 Phenomics: approaches and tools for genetic resources and breeding material characterisation - FEBRUARY 3rd 2025, 9:00 to 17:30 CET
- Module 3 Breeding methods fundamentals FEBRUARY 13th 2025, 9:00 to 18:00 CET
- **4.** Module 4 Development and application of molecular methods in organic breeding MARCH 4th 2025, 9:00 to 18:00 CET
- 5. Module 5 Organic heterogeneous material (OHM) design and development MARCH 7th 2025, 9:00 to 18:00 CET

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Module 3 - Breeding methods fundamentals

#### February 13th 2025 - 9:00 to 18:00 CET

#### Unit 3.1: Generation of new diversity

- 9:00-10:30 UPV (Adrián Rodríguez-Burruezo, Neus Ortega Albero)
- 10:30-11:00 Break

#### Unit 3.2: Common methods and strategies in organic breeding

- 11:00-13:00 IPC (Pedro Mendes Moreira) + UPV (Adrian Rodríguez-Burruezo) + KIS (Barbara Pipan)
- 13:00-14:30 Lunch Break

#### Unit 3.3: Calculation and evaluation of key breeding parameters

- 14:30-16:00 UPV (Adrian Rodríguez-Burruezo) + KIS (Barbara Pipan)
- 16:00-16:30 Break

Live

#### Unit 3.4: Fundamentals in Participatory Plant Breeding

16:30-18:00 - IPC (Pedro Mendes Moreira) + INRAe (Véronique Chable)





### Planned for today

DYNAMIC MIXTURE OF:

- 1. Presentations about main topics on **Common methods and strategies on organic breeding,** i.e. basics and practical examples on 3 crops (90 min):
  - Basics A. Rodríguez-Burruezo (UPV, Spain)

@esac.pt

- Maize (allogamous) Pedro Mendes Moreira (IPC, Portugal)
- Beans (autogamous grain crop) Barbara Pipan (KIS, Slovenia)
- 1. Fast quiz (about 10-15 min) \*\*\*
- 2. Debate, Wrap up & Proposed homework (about 10-15 min) \*\*\*
- 3. QUESTIONS: THROUGH THE CHAT (Petra Jelincic will manage)

\*\* = IMPORTANT for CERTIFICATES (ALL THE UNITS!!!!)

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**\** drária

### Basics on methods and strategies. Fast review

REPRODUCTIVE SYSTEM defines the genetic structure of the populations.

- Autogamous equilibrium: homocigous (inbred lines mixtures)
- Allogamous equilibrium: mixtures of homocigous and heterozygous individuals (Hardy-Weinberg's law)
- Vegetatively reproduced: clones (one variety can be a unique clone or a mixture of clones)

Implications on inbreeding depression: No problem in autogamous Quite usual in allogamous Self-compatibility: Usual in autogamous Difficulties in allogamous (different mechanisms) Flower biology essential to decide the breeding method (in some cases, hybrids are not economically viable)





Individual selection

### Basics on methods and strategies. Fast review When the starting population is heterogeneous

Mass selection





### Basics on methods and strategies. Fast review

Creating diversity at the beginning by hybridization







Basics on methods and strategies. Fast review

As said.... The reproductive system determines the most accurate breeding method

In autogamous no problem on using selfing profusely

In allogamous selfings are limited





### Practical example: Maize (allogamous crop) Pedro Mendes Moreira (IPC, Portugal)







Allogamic plants = allogamous or cross-pollinating plants

Species that primarily reproduce through **cross-fertilization** rather than self-fertilization. This reproductive strategy is characterized by the **exchange of genetic material between different individuals**, which **enhances genetic diversity within populations**.

The genetic variability resulting from allogamous reproduction is crucial for the **adaptability and resilience** of plant species in changing environments.



## Some facts that we need to know about the plant we are working in

male inflorescence, the **tassel**, produces **25 million pollen grains** 

a pair of male spikelets with three anthers dangling from the upper floret in the pedicelled spikelet a single style, called silk, with adhering pollen grains, extending from one of the pistils in a female spikelet

a pair of young female spikelets and associated cupule

numerous styles forming the silks

female inflorescence, the ear on the tip of a side branch with up to 1000 ovules (i.e., potentional kernels)

Leaves of side branch forming husks

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**FIGURE 1** Morphology of the corn plant showing the physical structure of the corn plant and the placement of the male (tassel) and female (silk) flowers. (Drawing was by Walter G. Galinat and reproduced with his permission).

#### Some facts that we need to know about the plant we are working in - Morphology of maize The plant habit varies greatly with only one tiller shown here at the base pericarp and silk (2n, maternal aleurone and endosperm (3n) scutellum (2n, filial). shoot apex (2n, filial) embryo root apex (2n, filial) some nodes below the ear node develop rudimentary ears; one of these often produces an ear with a reduced grain set; in prolific strains grown in southern regions, several ears may develop primary and seminal roots supportive in the seedling stage the root system is mainly adventitious from the basal nodes FIGURE 1. plant showing the physical structure of the corn plant

and the placement of the male (tassel) female (silk) flowers. (Drawing was by Walter G. Galinat).

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**FIGURE 1** Morphology of the corn plant showing the physical structure of the corn plant and the placement of the male (tassel) and female (silk) flowers. (Drawing was by Walter G. Galinat and reproduced with his permission).

### **Flowering implications**



Flowering allows us to know and classify the

type of cycle. Early or late?

The homogeneity of the population

Studying possible diallel. Do we need to sow

on separate dates?







## On-farm trials

Stratified mass selection trials



## The ABC of phenotypic recurrent selection

## Two parental control mass selection:

### in the field

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- before pollen
  shedding
- before harvesting
- at the storing facilities

#### B. À colheita (ou uma semana antes) A. Antes da floração Olhe para as plantas! - Desbandeira as "doentes e anormais" 1 ° Produção - Olhe para a espiga! a) Tamanho b) Mais que uma? (Prolífica) 2º Sanidade - Olhe para as folhas e cale e...pontapé! a) Doenças (fungos) b) Pragas (insetos) c) Raíz 3° Arguitetura - Olhe para as plantas a) Altura b) Inserção da espiga c) Encamisamento d) Ângulo das folhas normais use dois sacos: prolificas C. No espigueiro (Armazém) Olhe para a espiga! Comprimento Carreiras de grão Sanidade - (observa as bases do carolo!) Determinada vs indeterminada André

**Figura 3.** Esquema de duas seleções de controle parental no campo, Fase A e B ao que se segue a Fase C no espigueiro ou armazém.

5° Elimine os dois extremos

Barata

### What context and requests?

### What is stratified selection? What traits did you define to target?(e.g. Ear height, stand)

Bordadura	Var 5	Var 1	Var 2	Bordadura
Bordadura	Var 4	Var 3	Var 4	Bordadura
Bordadura	Var 3	Var 5	Var 1	Bordadura
Bordadura	Var2	Var4	Var5	Bordadura
Bordadura	Varı	Var 2	Var 3	Bordadura
Bordadura	Bordadura	Bordadura	Bordadura	Bordadura







### Trials





### **On-station**



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### **Preparation for Polinations**





### **Pollinations**











#### Story of a synthetic population,

76 yellow elite inbred lines (dent and flint; 20% Portuguese and 80% North American

1	2		76
76		2	1

"NUTICA" - 1975-78

1983 - North Carolina Design 1 matting design (1 male crossed with 5 females)

'Fandango'- on farm introduction and mass selection until today

And other Populations

#### 'FANDANGO': LONG TERM ADAPTATION OF EXOTIC GERMPLASM TO A PORTUGUESE ON-FARM-CONSERVATION AND BREEDING PROJECT

P.M.M. Mendes-Moreira<sup>1,2,9,\*</sup>, M.C. Vaz Patto<sup>2,9</sup>, M. Mota<sup>3</sup>, J. Mendes-Moreira<sup>4,5</sup>, J.P.N. Santos<sup>1</sup>, J.P.P. Santos<sup>1</sup>, E. Andrade<sup>6</sup>, A.R. Hallauer<sup>7</sup>, S.E. Pego<sup>8,9</sup>

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 <sup>4</sup> Faculdade de Engenharia da Universidade do Porto, DEI, Portugal
 <sup>5</sup> LIAAD-INESC Porto L.A., Portugal
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 <sup>7</sup> Faculty of Agronomy, Iowa State University, Ames, IA 50010, USA
 <sup>8</sup> Fundação Bomfim. Rua da Boavista, 152-154, 4700-416 Braga, Portugal
 <sup>9</sup> Zea+, Portugal

Received July 6, 2009

**ABSTRACT** - Climatic change emphasize the importance

across cycles was done by the breeder (until cycle 5) and

Mendes-Moreira, P. M. M., Patto, M. V., Mota, M., Moreira, J. P. C. L. M., Santos, J. P. N., Santos, J. P. P., ... & Pego, S. E. (2009). "Fandango": long term adaptation of exotic germplasm to a Portuguese on-farm-conservation and breeding project.

#### POPULATION Co (SELECTION BY S<sub>2</sub> LINES) PIGARRO



**Our varieties are Irreverent!** 

In this photo you can see mostly hybrid maize and below our traditional varieties that have different vegetation habits.

Foto por Felipe Hanower

### **Module 3 - Breeding methods fundamentals**

**Broad diversity? Prebreeding Work** 

The HUNTERS (Hight, Uniformity, aNgle, Tassel, Ear, Root lodging and Stalk lodging) analysis was developed as a method of evaluation

1–9 scale (1: very low, 2: very low to low, 3: low, 4: low to intermedium, 5: intermedium, 6: intermedium to high, 7: high, 8: high to very high and 9: very high) defining an average value per plot (<u>Vaz Patto et al., 2007</u>; <u>Mendes</u> <u>Moreira et al., 2008</u>)

H: Plant high (m) from the stalk basis to the last leaf insertion before the tassel.

U: Uniformity, complementary to diversity, where 1 is the minimum of uniformity and 9 the maximum. In general 1–4 applies to populations and 5–9 to inbreeds.

N: Angle of the adaxial side of the leaf above the ear with the stalk. <5 - angles inferior to 45° and >5, angles higher than 45. Five = 45 angle.

T: Tassel branching. One - almost absent tassel common in inbreeds and hybrids, and 9 a very branched tassel, frequent in populations with abnormal fasciated ears.

E: Ear location. Five indicates that the ear is located in the middle of the plant.

R: Root lodging. It indicates the percentage of lodged plants and is related with the quality of the root system.

S: Stalk lodging. Percentage of broken plants. Is normally related with the quality of the stalk and the stalk damage caused by some insect attack.







### **Module 3 - Breeding methods fundamentals**

## Yes! We can easily connect this traits with SEEDLINKED







### Practical example: Common bean (autogamous crop) Barbara Pipan (KIS, Slovenia)







### Common bean (*Phaseolus vulgaris* L.)

- Is one of the most important edible legumes for direct human consumption in the world, as it is a valuable source of protein, carbohydrates, fibre and is a rich source of other components with nutritional and health benefits.
- For the Slovenian production area, common bean is known as a vegetable of increasing agronomic interest.
- In the past, four different varieties of common bean (Zorin, Klemen, Jabelski pisanec, Jabelski stročnik) were developed at KIS using individual selection procedures from autochthonous populations.
- In the last decade we started with hand cross-pollination of dwarf and climbing bean for both dry bean and fresh pod consumption.

Recently, we are introducing the organic breeding as well in parallel with conventional breeding

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## Plant breeding and ideotype



#### □ Plant breeding is the science and art of improving plants to produce desired traits.

- □ This involves selecting plants with favorable characteristics and breeding them to enhance qualities such as yield, disease resistance, drought tolerance, and nutritional value.
- The goal is to develop new plant varieties that are more productive, resilient, and beneficial for agriculture and horticulture.
- □ An <u>ideotype</u> refers to an **idealized model of a plant** that possesses a combination of traits aimed at achieving maximum yield and quality within a specific environment. This concept was first introduced by *Donald in 1968*.
- When breeding plants, the goal is to develop new varieties with desirable characteristics. An ideotype serves as a blueprint for these characteristics, guiding breeders in selecting and combining traits.

By targeting these traits, breeders can create plants that are more efficient in utilizing resources and better suited to their growing conditions.

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### **Breeding objectives**

- Tolerant to biotic (BMMV/BCMNV, CBB, Anthracnose, bruchid) and abiotic (high T, water) stresses.
- Earliness (flowering before high temperatures occur and induce flower shedding).
- Tending to develop new "maslenec" type of varieties-> forms <u>yellow</u>/green, long, flat and stringless pods (for climbing types).
- The favourable position of the pods on the plant (for dwarf types).
- High-yielding varieties.

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Favourable nutritional composition (proteins, Fe, Zn...; phytic acid).



### **Breeding workflow**





## Identification of the parental breeding lines and hand pollination

•Available germplasm

•Characterisation and evaluation of the common bean germplasm on different complementary levels /phenomics:

phenotypic: UPOV, CPVO, IBPGR, Phaselieu, internal

genetics and genomics data: DNA markers, SNPs -> trait-associated

tanscroptomics, metabolomics: associated with secondary metaboloites, nutritional

characteristsics

general agronomic performance





•Superior parental lines, with desired traits that we want to transfer on progenies via targeted hand –pollination with regards on the breeding objectives.

•Hand cross pollination of superior parental genotypes as described by Ivančič (2002).

•Every morning during flowering period of parental genotypes from June until the end of July under protected conditions.

•Each maternal plant was manually cross pollinated with different paternal genotypes. LiveSeeding



### Self-pollination, phenotypic selection, MAS and final selection

- **Under protected conditions, from F1 to F6.**
- Self-pollination and phenotypic selection regarding breeding objectives.
- □ MAS in F2 (a panel of 24 trait-related functional DNA markers).
- Biochemical and nutritional quality assessments (F6) for amino acids, macro/micro elements, fibers, fatty acids, phytic acid, proteins, TI content and activity, sugars (pods)...

Orga	noleptic	traits.
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Selection of the elite breeding lines for open field performance and *in vitro* tests.



### **Open-field performance, in vitro tests and registration**

•Randomised blocks, 3 rep., along with standard varieties /different locations

•*In vitro* tests for susceptibility to BCMNV, *Colletotrichum lindemuthianum* and *Pseudomonas phaseoli* 

•Final selection

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•Seed quality testing (ISTA accredited lab)

•*Submission to CPVO technical protocol:* CPVO/TQ-012/4.



### **Results\_New KIS varieties (3) and OHM (2)**



**Under registration process: 4 (different stages and years of DUS tests)** 



### **Results\_Scientific publications and additional materials**



### Main challenges for the future

**\***To find the **elite germplasm** of common bean.

To address the breeding objectives complementary with the agroecological changes/conditions.

**\***To employ **new breeding approaches** into the existing breeding programme.

- **\***To **accelerate** breeding procedure.
- **\***To **implement the organic breeding** in parallel with conventional breeding programme.
- **\***To find the **competent human resources**.
- **\***To implement **citizen science approach**?

After registration: Maintenance and marketing of the developed varieties.





### **Acknowledgements**

#### ICJ/Ptuj and OVR

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REPUBLIKA SLOVENIJA MINISTRSTVO ZA IZOBRAŽEVANJE, ZNANOST IN ŠPORT

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rotational crops resilient to environmental change ecobreed Anded by European Union Ration 3020 Grant agreement No. 771367 Javna služba

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### <u>COMMON QUIZ</u>

Question 1: Why stratified mass selection is preferably chosen for farmers selection than recurrent selection by S2 lines

- More complex/less complex
- Question 2: Should I follow the same methodology for conservation of inbreeds and maize populations, i.e., by selfing

□ Yes/no

- Question 3: Maize tassel produces circa 25 million grains of pollen
  - □ Yes/no
- Question 4: Can farmers produce population hybrid seed?
  - 🖵 Yes/no

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Question 5: What is the official name of the test needed for the registration of a new (common bean) variety?

Question 6: Using the example of common bean, how long does it take on average to develop a new registered variety (using traditional breeding methods allowed for organic breeding)?

*Question 7: Are the breeding objectives associated with the traits of interest to be improved through the common bean breeding programme?* 

Send to :

adrodbur@doctor.upv.es and petra.jelincic@ips-konzalting.hr

In 15 min





**COMMON OUIZ** 

•

### **DEBATE**

**Revise the quiz in common** 

□ What I know about allogamic plants? And about autogamous?

Own previous experiences

Other questions and doubts





### **Additional available materials**

- Mendes-Moreira, P., Satovic, Z., Mendes-Moreira, J., Santos, J. P., Nina Santos, J. P., Pego, S., & Vaz Patto, M. C. (2017). Maize participatory breeding in Portugal: Comparison of farmer's and breeder's on-farm selection. *Plant breeding*, 136(6), 861-871.
- Vaz Patto, M. C., Moreira, P. M., Carvalho, V., & Pego, S. (2007). Collecting maize (Zea mays L. convar. mays) with potential technological ability for bread making in Portugal. Genetic Resources and Crop Evolution, 54, 1555-1563.
- 3. <u>F. Rey, E. Flipon (Eds.)</u>; P. Riviere, F. Rey, I. Goldringer (auts), 2021. Selecting the appropriate methodology for organic on-farm cultivar trials: a technical guide for researchers and facilitators. LIVESEED project
- J.I. Cubero-Salmerón. 2013. Introducción a la Mejora Genética Vegetal. 3a edición. Libro completo. ISBN 978-84-8476-655-1. Ediciones Mundi-Prensa, Madrid, España. 602 p.
- 5. G. Acquaah. 2020. Principles of Plant Genetics and Breeding. 3rd edition. Book. Wiley, West Sussex, UK. FiBL manual on new breeding techniques?
- 6. **F.T. Lammerts v**an Bueren and J. Myers (editors). 2012. Organic crop breeding. Book. Wiley, West Sussex, UK. 312 p.
- 7. M. Messmer, K.P. Wilbois, C. Baier, F. Schäfer, C. Arncken, D. Drexler and I. Hildermann 2015. Plant Breeding Techniques: An assessment for organic farming. ISBN 978-3-03736-286-0. FiBL, Switzerland. 48 p.
- 8. H. C. Brookfield, Helen Parsons, Muriel Brookfield. 2003. Agrodiversity: Learning from farmers across the world. Book. United Nations University Press, New York, USA. 343 p.

### WRAP UP



Send to :



<u>pmm@esac.pt</u> and <u>petra.jelincic@ips-konzalting.hr</u> By next thursday 20<sup>th</sup> february



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Thanks for your attention!



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