





Training in organic breeding

Module 2: Phenomics: approaches and tools for genetic resources and breeding material characterization

Unit 2.4: Methods for phenotyping and selection of agronomic traits of interest in organic farming

Author: Pedro Mendes Moreira (PUC-ESAC, PT)





Co-funded by the European Union





CONTEXT: Training in LIVESEEDING project

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	Training Packages & Summer School	Info & Materials	Upcoming Sessions	Target Groups	
	Organic plant breeding	More info	Register here	breeders, researchers, students	
	Organic cultivar testing	More info	Register here	farmers, breeders, examination and certification offices, researchers, national/regional authorities, citizens/consumers	
	High-quality organic seed production	More info	Register here	farmers, seed producers and multipliers, seed savers, breeders, examination and certification offices, researchers	
	Regulatory and policy aspects of the organic seed market and organic seed databases	More info	Register here	farmers, seed producers and multipliers, seed traders, seed savers, breeders, examination and certification offices, expert groups, national/regional authorities, actors of long value chains, actors of local value chains, private and public procurement bodies/officers	
	Entrepreneurship in the organic seeds and breeding sector	More info	Register here	farmers, seed producers and multipliers, seed traders, actors of long value chains, actors of local value chains, private and public procurement bodies/officers	
	Embedding organic seed and cultivated diversity in city food policies	More info	Register here	farmers, seed producers, seed savers, researchers, national/regional authorities, private and public procurement bodies/officers, citizens/consumers, media, students	U ME
	Summer School	Mara info	Pogistor boro		





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
- **3. 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 2 – Phenomics: approaches and tools for genetic resources and breeding material characterisation

February 3rd 2025 - 9:00 to 17:30 CET

Unit 2.1: Main descriptors used worldwide in characterizing plant genetic resources

- 9:00-10:30 UPV (Adrian Rodríguez-Burruezo)
- 10:30-11:00 Break

Unit 2.2: Intro to ShineMas: a web tool dedicated to Seed Lots History, Phenotyping and Cultural Practices¹

- 11:00-12:30 INRAe (Yannick de Oliveira, Isabelle Goldringer)
- 12:30-14:00 Lunch Break

Unit 2.3: Guidelines and examples of good practices in data management

- 14:00-15:30 INRAe (Yannick de Oliveira, Isabelle Goldringer)
- 15:30-16:00 Break

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Unit 2.4: Methods for phenotyping and selection of agronomic traits of interest in organic farming
 16:00-17:30 - IPC (Pedro Mendes Moreira)

Unit 2.5: Methods for phenotyping and selection of added-value traits (e.g. taste and nutritional value)² ITAB (Solenne Jourdren)

1 - An extra practical session to use the tool with own data is scheduled for FEB 10th (9-12h) 2 - Unit 2.5 planned for the end of March 2025. Registrants will be invited for this extra training lesson





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T1.4 Training in Organic Breeding Module 2 – Phenomics: approaches and tools for genetic resources and breeding material characterization

Unit 2.4: Methods for phenotyping and selection of agronomic traits of interest in organic farming

Pedro Mendes-Moreira

Polytechnic University of Coimbra Agriculture School of Coimbra





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Module 2 – Phenomics: approaches and tools for genetic resources and breeding material characterization

Planned for today

DYNAMIC MIXTURE OF:

- 1. Presentation about main topics on Methods for **phenotyping** and selection of **agronomic traits** of interest in organic farming: utility, types, examples of descriptors, management of data, knowledge, additional material (50 min)
- 2. Fast quiz (about 10 min) ***

SEND TO BOTH:

- 3. Debate, Wrap up & Proposed homework (about 10-15 min) ***
- 4. QUESTIONS: THROUGH THE CHAT (Petra Jelincic will manage)

adrodbur@doctor.upv.es

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

Escola Superior Agrária

petra.jelincic@ips-konzalting.hr

Module 2 – Phenomics: approaches and tools for genetic resources and breeding material characterization

Where are the plant traits?

In U2.1 you have already contacted with the descriptors from BIOVERSITY, however... **Do all the traits have the same importance?... Never forget the phenology!**

Why we do not take all the data, described in the descriptors during all phases of a plant breeding program? More traits measured are needed for registration phase.

$P=G+E+S+G \times E \times S...$

Traits(n) x Plants/Plot x Plot x Locations x Managing Systems

Cost to measure

How can we be more effective with our resources?

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But why we care about Agronomic Traits for Organic?

Conventional varieties often lack traits essential for organic production!

Traditional breeding in high-input environments has led to:

traits with negative side-effects in organic settings, like reduced disease resistance and lower nutrient-use efficiency (<u>Bueren et al.</u>, <u>2011</u>).





But why we care about Agronomic Traits for Organic?

Conventional varieties often lack traits essential for organic production!

• Nutrient use efficiency from organic inputs,

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- Weed competitiveness (improving crop performance in organic systems and contributes to sustainable agriculture
- Pest and disease resistance (Bueren et al., 2011; Rempelos et al., 2023).



But why we care about Agronomic Traits for Organic?

Conventional varieties often lack traits essential for organic production!

- nutrient use efficiency from organic inputs,
 - Can enhance soil health, microbial biomass, and carbon content, potentially improving NUE over time (Toda et al., 2023).
 - A function of improved cultivars, best management practices, soil health factors (<u>Baligar et al., 2001</u>).





But why we care about Agronomic Traits for Organic?

Conventional varieties often lack traits essential for organic production!

- weed competitiveness (improving crop performance in organic systems and contributes to sustainable agriculture practices),
 - key traits for cereals, include plant height, early vigor, tillering capacity, and early maturity (<u>Mason et al., 2007</u>)
 - heterozygosity levels in faba beans (<u>Ghaouti et al., 2016</u>)
 - For soybeans, a direct selection system using crop species mixtures as competitors has been developed to screen for weed-tolerant genotypes (<u>Horneburg et al., 2017</u>).
 - o adaptation to specific environments, and PPB (Wolfe et al., 2008)
 - enhanced crop competitiveness on organic maize to sustain yield (<u>Ryan et</u> <u>al., 2009</u>).



But why we care about Agronomic Traits for Organic?

Conventional varieties often lack traits essential for organic production!

- **pest and disease resistance** (Bueren et al., 2011; Rempelos et al., 2023).
 - Organic management practices can enhance natural pest control by altering plant resistance to insects, mediated by rhizosphere microbial communities and salicylic acid accumulation (<u>Blundell et al., 2020</u>).
 - Arbuscular mycorrhizal fungi can induce plant disease resistance, suggesting their importance in organic plant breeding (<u>Hohmann and</u> <u>Messmer, 2027</u>).





But why we care about Agronomic Traits for Organic?

- Organic breeding programs should focus on traits prioritized by farmers, processors, and consumers (<u>Rempelos et al., 2023</u>).











The correspondence between descriptors by BIOVERSITY and Breeders indicate a GAP!

Knowing that fact <u>ECPGR EVA programs</u> are trying to fill that GAP

In 2011 an attempt was made to indicate some important traits

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Solibam

Strategies for Organic and Low-input integrated Breeding and Management Grant agreement number: FP7- KBBE-245058

Collaborative project (Large-scale integrating project)

SEVENTH FRAMEWORK PROGRAMME

Deliverable 1.5 Determination of traits most relevant for model species of cereals, grain legumes and vegetables

Due date: M12

Actual submission date: March, 11th 2011

Project start date: March 1st, 2010 Duration: 54 months

Workpackage concerned: 1

Concerned workpackage leader: Véronique CHABLE

Dissemination level: PU







3 - Traits of Maize (Zea mays L.)

List of characters to be scored, from Pedro Mendes-Moreira:

Characterisation to be carried out according to a randomized block design with replications. Data preferably to be collected on single plant basis (10-20 plants per genotype per each replicate) or per plot.

Traits	Pres	critive	Codes		Description
	Plot	PI/Ears			
Days-to-silk, nº †	1*		Fi	days	The beginning of days to silk (from planting until 50% of the plants in the plot begin silk emergence.
Days-to-silk, nº † end	1*		Ff	days	The end of days-to silk (from planting until 50% of the plants in the plot begin and finish silk emergence.
Days-to-anthesis, nº †	1*		Mi	days	The beginning of days-to anthesis, i.e., from planting until 50% of the plants in the plot begin anthesis
Days-to-anthesis, nº † end	1*		Mf	days	The end of days-to anthesis (from planting until 50% of the plants in the plot end silk emergence
Height	1		Ĥ	cm	Average plant height, from the stalk basis to the last leaf insertion before the tassel
Uniformity	1		U	score	 1 - minimum uniformity and 9 - maximum; 1-5 to populations and 6-9 to inbreds.
aNgle-leaf	1		N	score	Angle of the adaxial side of the leaf above the ear with the stalk ($5=45^{\circ}$, $<5=<45^{\circ}$ and $>5=>5=45^{\circ}C$)
Tassel	1		Т	score	Tassel branching. 1- absent tassel (Inbreeds and hybrids) 9- a much branched tassel (frequent in populations with abnormal fasciated ears).
Ear placement	1		E	score	5- indicates that the ear is located in the middle of the plant.
Root lodging %	1		R	%	Percentage of plants leaning more than 30º from vertical
Stalk lodging %	1		S	%	Percentage of plants broken at or below the primary ear node, related with the quality of the stalk and the stalk damage caused by some insect attack.
Yield	1		Yield	Mg ha⁻¹	Grain yield (Mg ha-1) 15% moisture, a1) hand harvest (Portugal), Grain yield = Ear weight x (Grain weight/Ear weight) five shelled ears are used for determination of this racio and for moisture content





- What do we search for our program?
- What is our context?





VASO 1.0 - Genesis reasons in 1984



To solve the problems of small Portuguese farmers with limited land, dense population, and an inadequate productivist model, where big corporations have little interest in this market.

The Majority of the germplasm that I work with is used for human consumption

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To respect local culture promoting the selection and breeding of genetic resources increasing the yield, maintaining the quality.

To keep germplasm evolving with time!









Yield maintaining quality?

Desirable stand?

Pest and diseases?

• • •

Can we know more about our populations flowering behavior?



Neutral marker

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What germplasm are you working in?

Do you have fasciation?



If your germplasm do not have fasciation your result will be always "1" so it is a neutral marker. But for us! You need to know what are your matherials and what you want from them! Case they have





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.







Yes! We can easily connect this traits with SEEEDLINKED







Unit 2.4

(A) - Immediately before the pollen shedding, detasseling all the undesirable plants (pest and disease susceptible, weakest and plants that do not fit the desirable ideotype);

(B) - **Before harvest**, besides selecting for the best ear size, the plants are foot kicked at their base (first visible internodes) to evaluate their root and stalk quality. Indirect measurement evaluation for pest and disease tolerance. If the plant breaks, it is eliminated. select prolific plants;

(C) - **At the storage** facilities, after harvest, selection is performed (normal and prolific ears) for ear length, kernel-row number, prolificacy, and the elimination of damaged/diseased ears. The selected ears are shelled and mixed together to form the next generation seed. The farmer selection pressure ranged from 1 to 5%

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c) Raíz a) Altura use dois sacos: C. No espigueiro (Armazém) Olhe para a espigal Comprimento Carreiras de grão Sanidade - (observa as bases do carolo!) Determinada vs indeterminada 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.

Olhe para as plantas! - Desbandeira as "doentes e anormais"

A. Antes da floração

B. A colheita (ou uma semana antes)

- 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)
- 3° Arguitetura Olhe para as plantas
 - b) Inserção da espiga
- c) Encamisamento
- d) Angulo das folhas

prolificas

normais

5° Elimine os dois extremos



of theoretical allogamy versus

✓ autogamy.

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Number of days



Unit 2.4: Methods for phenotyping and selection of $2^{(B-b)+(A-a)-|B-A|-|b-a|}$ agronomic traits of interest in organic farming

$$OI = \frac{(B - b) + (A - a) - |B - A| - |b - a|}{2(B - b)}$$

The number of days from planting, to the beginning (a) or end (A) of male flowering; or to the beginning (b) or end (B) of female flowering.

Evaluation of the overlapping index (OI) conditions:

OI is limited to 1 (100 %)

OI is either positive (some overlapping) or negative (overlapping does not occur).

Maize breeders always look for high functional allogamy, so the selection for negative values of OI is pursued.

The proposed evaluation method can be conducted in only one year time, based on 13 possible overlapping types (T1-T13) (Figure 1).

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Moreira, P. M., & Pêgo, S. (2003)

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• Verdial de Aperrela:

- ~19 % of no overlapping (OI <= 0, complete allogamy); ~15 % of complete overlapping (OI = 1, possible selfings all along the ear); ~66 % of partial overlapping (0 < OI < 1, possible selfings in specific parts of the ear).
- The population is represented in more than 50 % of the cases by partial overlapping of the type T11 (52.3 %). Even in this case (T11), our results show that 46.3 % of the kernels could have been selfed, while 53.7 % are hybrids.









- An unexpected amount of complete allogamy (OI = 19.16 %) constitutes a selection potential for increasing allogamy at the expenses of possible autogamy.
- Figures 4 and 5 present some differentiated peaks, showing that this population is not well recombined and suggest it to be composed by at least three sub-populations which almost do not overlap. This represents a potential for both recombination and further genetic diversity implementation.



Trials – What environments to use G + E + S + GxExS









Example 2 Trial in Organic and Conventional

Rank Cal	Organic - Caldeirão		Rank V	Conventional - Vagem		Rank diferences
	VA C0S0 19 - (Regadio 1 Lousada	4313,74	19 VA (C0S0 19 - (Regadio Lousada	6450,77	18
	2501 - (11-2019 <mark>2</mark> Caldeirão 2019	4252,3	21 250	1 - (11-2019 Caldeirão 2019	6344,97	19
	Bulk-Azores 2 - (110- 32019 Cald	4248,98	11 Bull	k-Azores 2 - (110-2019 Cald	7308,47	8
	2516 - (23-2019 4Caldeirão 2019	4235,95	30251	6 - (23-2019 Caldeirão 2019	5675,8	26
	2527 - (Caldeirão 52018)	4195,12	35252	7 - (Caldeirão 2018)	5575,57	30
	2499 - (9-2019 6Caldeirão 2019)	4127,95	5 <mark>249</mark>	9 - (9-2019 Caldeirão 2019)	7961,27	-1

What are the resources that you have?

Selecting for drought tolerance/resistance based on yield is a complicated task due to the complex nature of the trait and its low heritability. The task becomes particularly complex for participatory breeding approaches in which selection tools are to be applied directly by farmers.



Open Access Article

Maize Open-Pollinated Populations Physiological Improvement: Validating Tools for Drought Response Participatory Selection

by Susana T. Leitão ¹ ⊠ ⁽ⁱ⁾, Emanuel Ferreira ² ⊠, M. Catarina Bicho ^{1,3} ⊠, Mara L. Alves ¹ ⊠, Duarte Pintado ² ⊠, Daniela Santos ² ⊠, Pedro Mendes-Moreira ² ⊠ ⁽ⁱ⁾, Susana S. Araújo ¹ ⊠ ⁽ⁱ⁾, J. Miguel Costa ⁴ ⊠ and Maria Carlota Vaz Patto ^{1,*} ⊠

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Evaluation



https://www.facebook.com /ESAC.IPC/videos/4209739 37771190

LiveSeeding

Mister Tomate



LiveSeeding



Resultados da Escola da Agrária

Do we have the same results? https://www.facebook.com/ESAC.IPC/videos/420973937771190



Field Crops Research Volume 161, May 2014, Pages 75-86



Is ear value an effective indicator for maize yield evaluation?

Pedro M.R. Mendes-Moreira ^{a h i} ∧ ⊠, João Mendes-Moreira ^{b c} ⊠, António Fernandes ^d ⊠, Eugénio Andrade ^e, Arnel R. Hallauer ^f⊠, Silas E. Pêgo ^{g i}, M.C. Vaz Patto ^{h i} ⊠

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https://doi.org/10.1016/j.fcr.2014.02.015 7

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Highlights

- A set of maize traits that could help farmers on selection for yield was identified.
- A new ear value formula that better estimates the yield potential was obtained.
- A new instance ranking measure to be used for evaluating ear ranks is proposed.
- A new method to be used in participatory research and prebreeding is proposed.

PPB – from the farmer to the statistical tools - MARS, **RF**, CART



(1)

organic farming

(EV formula) was defined as:

 $\text{EV} = \frac{0.6 \times KW + 0.2 \times L + 0.15 \times R + 0.05 \times KN}{4}$

KW stands for kernel weight (grams) at15% moisture, L for ear length(centimeters), R for kernel row numberand KN for total number of kernels.

Not forgeting to feed local iniciatives that can help on PPB recognition

"Best Ear of Sousa Valley competition" can provide adequate measurements, indicate best traits for selection and prediction



 $EVA = mlr.varsEV = -7.030877 + 0.031605 \times KW + 0.387825$ $\times L + 0.337015 \times R12 - 0.008875 \times KN \quad (13 \text{ and } 14)$

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Table 1

Maize populations' characterization: kernel type, number of instances per population, origin and references.

Population	Kernel type	Data ^a	Origin	Area/plot, ears/plot	References
Nutica	Yellow dent	18	Synthetic Pt (80% USA germplasm)	9.6 m ² ; 20 ears/plot	Moreira (2006)
Fandango	Yellow dent	157	Synthetic Pt (80% USA germplasm)	9.6 m ² ; 20 ears/plot	Mendes-Moreira et al. (2009)
Pigarro	White flint	305	Populations, Pt	9.6 m ² ; 20 ears/plot	Mendes-Moreira et al. (2008)

^a The number of instances per population corresponds to the product of selection cycles (for 'Pigarro' and 'Fandango'), years, locations with three reps, with exclusion of instances that not have the complete set of traits.



PPB – from the farmer to the statistical tools



e 🔁		 						
				rf				
N15 N15 C N15 C N15 C N15 E2 E1 C2 C C C C M_EW 2 C 1		 0 0 0	0	rf 	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0	0	
andplha 2e3	0			R2 S2e3	o			
	0			DC4 DE4	0			

Searching for tools to help on the selection



Agronomic traits of interest in organic. Why?

• LIMITATION: there are many descriptors, but better to work with those which best encompass the diversity present on a crop and its relatives (comprehensive & discriminating ability)

• Characterization with the many descriptors of a crop variety is not enough, search for the traits that respond to agronomic questions, search for better correlations with complex traits such as yield

• Description better fit the varieties to organic environment (NUE, Weed competitiveness, pest and disease resistance) and focus on traits prioritized by farmers, processors, and consumers (<u>Rempelos et al., 2023</u>).

• Check the level of diversity you have

• Comparison to other and same accessions in other locations and actors (E + S and GXEXS) Live Seeding

PRACTICAL EXERCISES:

1. Guided visit to Overlap Index Maize

+ example with excel compiled data

1. Guided visit to Maize results

+ example of PUC-ESAC results with own data

SAME, but short, with seedlinked page?





Seedlinked Trials Results

Maize trial_Ensaio Milho: <u>https://app.seedlinked.com/trial/analytics/guest/MjA0Nw==</u>

Tomato degustation trial_Ensaio Tomate degustação: <u>https://app.seedlinked.com/trial/analytics/guest/MjYxMg==</u>



DEBATE

Revise the quiz in common

Own previous experiences

Other questions and doubts





WRAP UP



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, 🏓

What we have learned today?

Proposed homework: study case, consider a crop that you are working and identify the five top traits that you would use in a breeding programe

Send to : pmm@esac.pt and <u>petra.jelincic@ips-konzalting.hr</u> By next Monday 10th february



Additional available materials

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Additional available materials

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Further readings

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ORIGINAL RESEARCH article

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Setting Up Decision-Making Tools toward a Quality-Oriented Participatory Maize Breeding Program









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LiveSeeding

Thanks for your attention!



EUCARPIA OFFERENCE

on breeding to meet environmental & societal challenges

Instituto Politécnico de Coimbra - ESAC

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Tomato Evaluation - Choose your perfect tomato acording the shape and collour<u>https://www.facebook.com</u>/ESAC.IPC/videos/420973937771190

R: This question allowed to indicate that according to the group we can have different responses

In a breeding Program, you should measure all your accession with all the traits indicated by Bioversity

No – You should choose what are the most important traits to deal with

Are there any differences between Agronomic traits used for conventional and for organic?

Yes, for example, we are interested in measure competition or allelopathy between crop and weeds, which is not important in conventional where herbicides are applied

Nitrogen Use Efficiency (NUE) can be considered a simple trait.

No, because NUE comprises two main components: nitrogen uptake and utilization efficiency, each involving numerous physiological processes and biochemical pathways

LiveSeeding

Quiz U2.4 + homework

Consider the file "Degustação de Tomate - LIVESEEDING- ESAC 2024". What was the accession with higher acidity. Tomato degustation trial_Ensaio Tomate degustação:

https://app.seedlinked.com/trial/analytics/guest/MjYxMg==

R:631

How many overlapping index cases exist for maize ?https://esacptmy.sharepoint.com/:x:/g/personal/pmm_esac_pt/EbmeGIzGoKtGntb12adEWiMBKcRyL8KJk7-M1K_irL_a-g?e=4sdzCS

R:13

Proposed homework:

Study case, consider a crop that you are working and identify the five top traits that you would use in a breeding programe for organic.

R: open question depending on the chosen crop