





Training in organic breeding

Module 3: Breeding methods fundamentals

Unit 3.3: Calculation and evaluation of key breeding parameters

Authors: Adrian Rodríguez-Burruezo (UPV), Barbara Pipan (KIS)





Co-funded by the European Union Funded by the European Union, the Swiss State Secretariat for Education, Research and Innovation (SERI) and UK Research and Innovation (UKRI).



CONTEXT: Training in LIVESEEDING project

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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
Summer School	More info	Register here	

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

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

Unit 3.4: Fundamentals in Participatory Plant Breeding

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







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Module 3: Breeding methods fundamentals

Unit 3.3: Calculation and evaluation of key breeding parameters

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Planned for today

MIXTURE OF:

- 1. Presentations about main topics on *Kind of traits, ways and parameters to analyse* (60 min):
 - 1.1. Qualitative vs Quantitative A. Rodríguez-Burruezo (UPV, Spain)
 - 1.2. Qualitative traits analysis A. Rodríguez-Burruezo
 - 1.3. Quantitative traits analysis A. Rodríguez-Burruezo: Basics, heritability, correlation

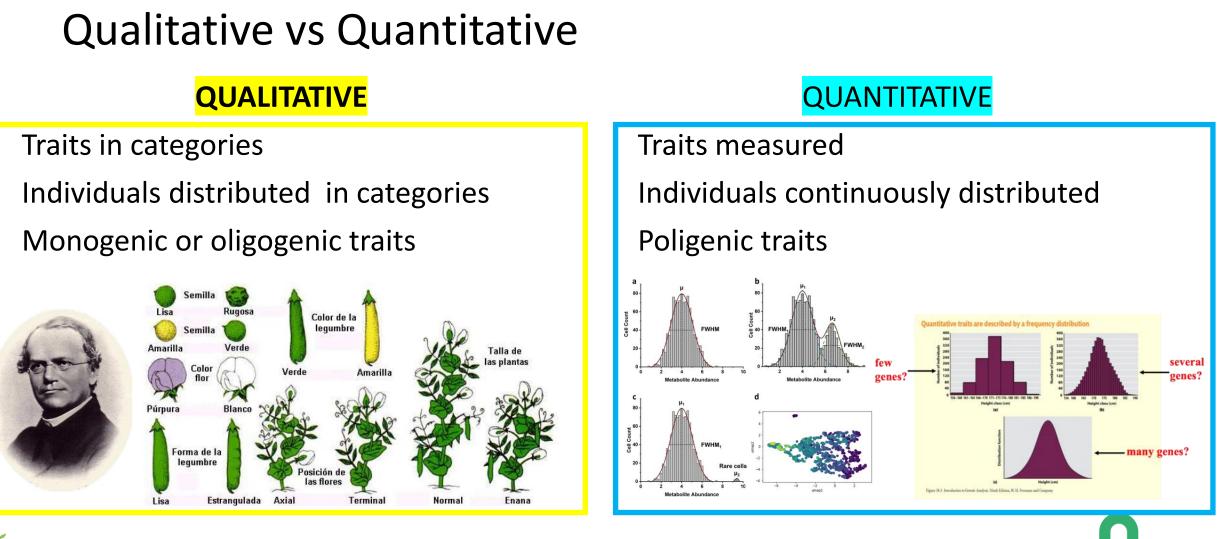
LiveSeeding SEND TO: <u>barbara.pipan@kis.si</u> <u>adrodbur@upv.es</u> <u>petra.jelincic@ips-konzalting.</u>ł

- Other parameters & traits of interest during breeding workflow Barbara Pipan (KIS, Slovenia)
- 2. Fast quiz (about 15 min) ***
- 3. Debate, Wrap up & Proposed homework (about 5-10 min) ***

QUESTIONS: THROUGH THE CHAT (Petra Jelincic will manage)

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

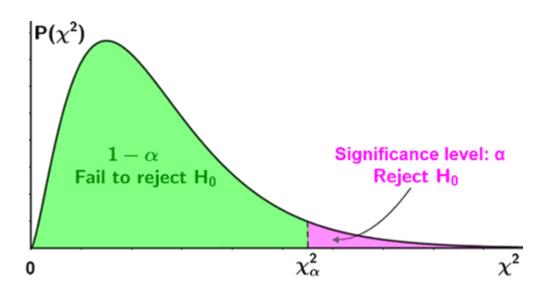
Agrária



Escola Superior Agrária

Qualitative traits analysis

- Working with number of individuals per category
- > Check inheritance models statistically with the frequencies of each category
- > Chi square (X2) and binomial functions



Binomial Distribution Formula

$$P(x) = \binom{n}{x} p^{x} q^{n-x} = \frac{n!}{(n-x)! x!} p^{x} q^{n-x}$$

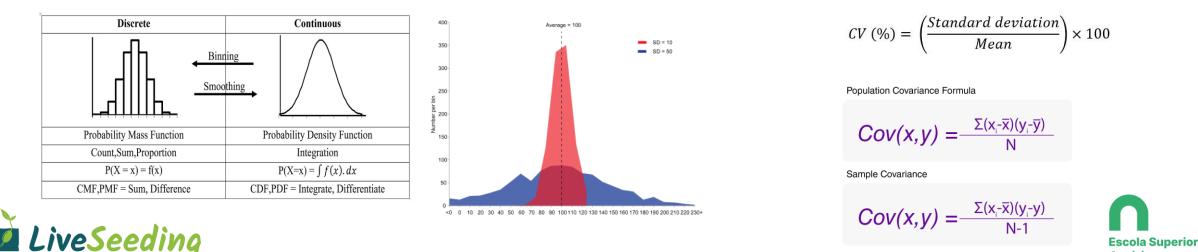
where

- n = the number of trials (or the number being sampled)
- x = the number of successes desired
- p = probability of getting a success in one trial
- q = 1 p = the probability of getting a failure in one trial



Quantitative traits analysis

- > As traits are measured, no natural categories possible
- Also, as many genes can be involved, environment (E) may modulate the phenotypic expression (P), creating "mirages" and hiding the gene function (G)
- > Basic parameters to represent the population: mean (μ) and variance (V or σ^2)
- > Manage info for breeders and geneticists with biometrics & quantitative parameters
- > Coefficients of variations, covariances, correlations, heritability, GxE interaction



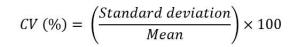
Agrária Politécnico de Coimbra

r = -

 $\overline{y} = me$

Quantitative traits analysis

Coefficients of variation, covariances, correlations



Population Covariance Formula

$$Cov(x,y) = \frac{\sum(x_i - \overline{x})(y_i - \overline{y})}{N}$$

Sample Covariance

$$Cov(x,y) = \frac{\sum(x_i - \overline{x})(y_i - y)}{N-1}$$



$$r = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar$$

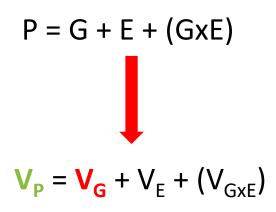


Quantitative traits analysis

 \geq

HERITABILITY (H²)

On the whole, it explains to which extent the phenotypic differences you see and record in one population are based in genetic differences.... And will be inherited in next generation



LiveSeeding

$$H_{bs} = V_G / V_P = V_G / [V_G + V_E (+V_{GxE})]$$

CONSIDERS: all the genotype pass from one generation to the next

 $H_{ns}^{2} = V_{A}/V_{P} = V_{A}/[V_{G}+V_{E}(+V_{GxE})] = V_{A}/[V_{A}+V_{D}+V_{E}(+V_{GxE})]$

CONSIDERS: only the additive part pass from one generation to the next (dominant and other genetic interactions do not pass)

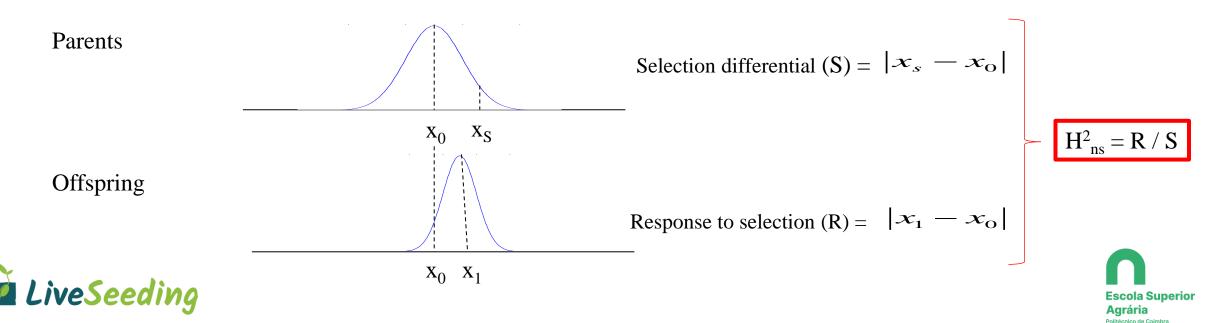


Quantitative traits analysis

HERITABILITY

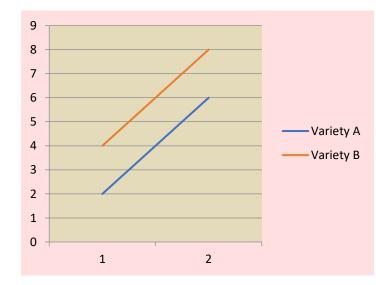
 H^2_{bs} mainly for clone selection or selection of inbred/autogamous lines

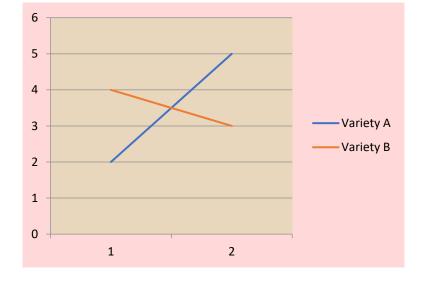
 H^2_{ns} mainly for sexually reproduced species, whose populations are allogamous and/or open pollinated

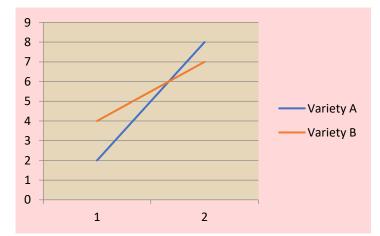


Quantitative traits analysis

G x E interaction









Practical: parameters & traits during breeding workflow

Barbara Pipan (Agricultural Institute of Slovenia, KIS)



Agricultural Institute of Slovenia

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Introduction to key breeding parameters

- Key breeding parameters are essential metrics and concepts used in plant breeding to evaluate and improve the (genetic) traits (of interest).
- Some key breeding parameters: Heritability, Genetic Variance, Estimated Breeding Value, Selection Differential, Response to Selection, Genetic Correlation, Inbreeding Coefficient, Best Linear Unbiased Predictic

...but first we need to observe and record different types of data for our breeding material in terms of characterisation at different levels on separate filial generations (phenotypic, genetic, genomic (other omics data if relevant), biochemical, nutritional, in vitro tests, seed quality parameters,...)-> from laboratories, fields, screenhouses,...-> case-by-case with respect to plant species -> following the descriptors / specific traits

These parameters are crucial for making informed decisions in breeding programs to achieve desired genetic improvements -> to select the elite breeding lines for registration.



Characterisation of common bean (breeding material)->traits of interest

...when calculating and evaluating key breeding parameters, the traits of interest to be improved by the breeding programme, should be recorded and tracked first...



Towards the Development, Maintenance, and Standardized Phenotypic Characterization of Single-Seed-Descent Genetic Resources for Common Bean

Gaia Cortinovis,¹ Markus Oppermann,² Kerstin Neumann,² Andreas Graner,² Tania Gioia,³ Marco Marsella,⁴ Saleh Alseckh,^{5,6} Alisdair R. Fernie,^{5,6} Roberto Papa,¹ Elisa Bellucci,^{1,7} and Elena Bitocchi^{1,}

¹Department of Agricultural, Food and Environmential Sciences, Polytechnic University of Marche, Accous, Italy ²Research Group Genebank Decumentation, Leibniz Institute of Plant Genetics and Crop Plant Research (Plast Genebank, Science), Science (SAFE), University of Basilicata, Pherrar, Ibly

International Teetty on Plant Genetic Resources for Food and Agriculture (FAO), Rome, Italy ⁵Department of Molecular Physiology, Max Planck Institute of Molecular Plant

⁷Department of Molecular Physiology, Max Planck Institute of Molecular Plan Physiology, Potsdam-Golm, Germany ⁶Center for Plant Systems Biology, Plovdiv, Bulgaria ⁷Corresponding authors: *ebellacci@univmm.it.ebitocchi@univmm.it*

The optimal use of legume genetic resources represents a key prerequisite fo coping with current agriculture-related societal challenges, including conser vation of agrobiodiversity, agricultural sustainability, food security, and human health. Among legumes, the common bean (Phaseolus vulgaris) is the most omically important for human consumption, and its evolutionary traie ries as a species have been crucial to determining the structure and level of its present and available genetic diversity. Genomic advances are considerably enhancing the characterization and assessment of important genetic variants. For this purpose, the development and availability of, and access to, well-described and efficiently managed genetic resource collections that comprise pure lines derived by single-seed-descent cycles will be paramount for the use of the reservoir of common bean variability and for the advanced breeding of legume crops. This is one of the main aims of the new and challenging European project INCREASE, which is the implementation of Intelligent Collections with appropriate standardized protocols that must be characterized, maintained, and made available, along with the related data, to users such as breeders and researcher © 2021 The Authors, Current Protocols published by Wiley Periodicals

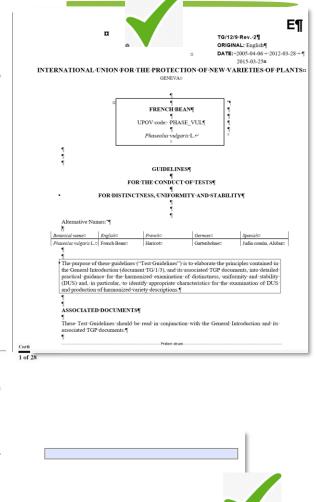
Basic Protocol 1: Characterizing common bean seeds for seed trait descriptors Basic Protocol 2: Bean seed imaging Basic Protocol 3: Characterizing bean lines for plant trait descriptors specific for common bean Primary Seed Increase

Keywords: common bean • genetic diversity • genetic resources • intelligent collections • single-seed-descent line • standardized phenotyping protocols

CPVO



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Technical questionnaire

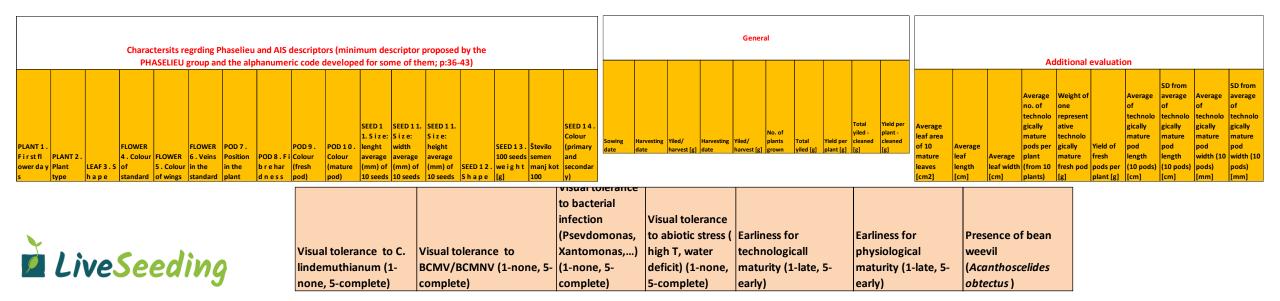
French bean CPVO/TQ-012/4 Mandatory fields or sections are marked with an asterisk (*)

Breeder's Re

Characterisation of common bean breeding material_KIS example - Morfo data+photo material

During different F generations: in a screenhouse, on the field

Obv	ezno	izpolr	niti za										onds regai cal Proto						2-012/4 2	27/2/201	3 (the nu	mber in	Breeder's
								sponum									10/4/20/	237					score
					05.03.		05.04.														05.11.		
					Dwarf		Climbing				05.05.										Time of		
			05 . 01.02		beans		beans				Pod:				05.07.				05.09.	05.10.	flowering		
	05.01.01		. Flower:		only:		only:		05.04.01		shape of		05.06.01		Pod:				Seed:	Seed:	(50% of		
	. Leaf:	05.01.02	size of	05.02.	Pod:		Pod:		. Pod:		cross	05.06.	. Pod:	05.06.02	stringines		Please	05.08.	main	predomin	the plants	Please	
05.01.	intensity	. Flower:	bract (15)	Flower:	length	Please	length	Please	width at	Please	section	Pod:	intensity	. Pod:	s on	05.07.01	indicate	Seed:	colour	ant	with at	indicate	
Plant:	of green	size of	* (width x	colour of	(excludin	indicate	(excludin	indicate	maximu	indicate	(through	ground	of ground	secondar	ventral	. Seed:	the 1000	number	(largest	secondary	least one	an	
growth	colour (9)	bract (15)	length -	standard	g beak)	length in	g beak)	length in	m point	width in	seed) (22)	colour	colour	y colour	suture	weight	seeds	of colours	area) (42)	colour	flower)	example	
type (2)*	*	*	mm)	(16) (G) *	(18) *	cm	(19) *	cm	(20) *	mm	(G) *	(24) (G) *	(25)	(26) *	(29) (G) *	(37) *	weight	(43) (G) *	(G) *	(45) (G) *	(48) *	variety	



Characterisation of common bean breeding material_KIS example – genetic background

During F2 generation: in the lab

Lab label		ee		eg	eh		ef	ei	sa	sb	sd	se	sf	sg	sh
Marker		SW13		ROC11	SG6	arliness+yield BCMV BCMNV	SBD5	DESU-G1	SW12	SAS13	SZO4	SF10	SN02	SBA16	SW6-800R
Lastnost	BCININ_BCININA LE	sstance+earliness+yleid	BCININ_BCININN	resistance+earliness+yield	BCIVIV_BCIVIIVV resistance+e		resistance+earnness+yleid	bean rust resistance	anthrachhose resistance	anthrachnose resis	tance anthrachnose resistant	anthrachnose resistance	ALS resistance	e ALS resistance	bean-pod weevil resistance
	Lab label	si										Trait assoc	intion		
	Marker	Phs										gene pool	ation		
		phaseolin + wl	hite mold	+ CBB								determinat BCMV_BC			
			ndean / h			Mesoamerican						resistance earliness+y	•		
			nacan y n	, on a		mesoumeneum									
		ff										bean rust resistance			
		BCMV_48289										Antrachnos resistance			
	Lastnost	RCWA RCWN	V resistan	ce+earliness+yie	ld										
						T						ALS (angula	r leaf		
												spot)			
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												low P toelr	ance		
Ø	live	Seedi	no									drought str	ess		
~		CCCUT	· · 9									tolerance			

Characterisation of common bean breeding material_KIS example – pathogen resistance

During F5-F7 generation: in the lab (in-vitro tests according to CPVO requiremnents)

BCMNV, Colletotrichum lindemuthianum, Pseudomonas phaseoli, Xantomonas pv. phaseoli

	rase glive Colletotrichum lindemuthianum										
križanci fižola	23	131	55	103							
385 x 425	S (9 [*])	S (9)	S (9)	S (9)							
359 x 417	R/S (4-5)	S (6-7)	R/S (5)	S (9)							

id_leto	Vzorci_ID	Številka zapisnika	Rezultati gojišč	Rezultat PCR na izolatih
2022	57	359x417	neg	ni bilo sumljivih izolatov
2022	58	385x425	neg	ni bilo sumljivih izolatov
2022	59	428xČEŠ F6	neg	ni bilo sumljivih izolatov





Characterisation of common bean breeding material_KIS example – biochem and organoleptic data

During F5-F7 generation: in the lab

sugar	% inhibition of trypsin	TUI / mg
Bugui	70 millionion or d'ypom	sample

			Macroel	ement (g kg ⁻¹)			
N		K	Р		S N	Лg	Ca
			Protein (%)				
			24,21	1			
			23,31				
			23,59				
				nent (mg kg ⁻¹)			
Fe	Zn	Mn	Na	Cu	Mo	Cr	Co





Characterisation of common bean breeding material_KIS example – Seed testing lab results

Germination rate, vigor, ATM



					št. semen	ENERGI	JA kalitve			KALIVOST			DATUM		
OZNAKA vzorca	Rastlinska VF SORTA		ANALIZ	ZNA št.	danih na kalivost	št.dni	normalne klice (%)	št.dni	normalne klice (%)	nenorm. klice (%)	trdo seme (%)	mrtvo seme (%)	zaključka analize	METODA	
vzorec 2, 1900g	Nizki fižol 3	59×417	1991	/2021	2x50	6	96	6	96	2	0	2	25.10.2021	S 20-30°C	
OZNAKA vzorca		inska VRSTA in ANALIZNA št.		7N14 č+	ABSOLUTNA masa									zaključka	
	SORT	A			(0	J)			met	toda			analize		
vzorec 2, 1900g	Nizki fižol 3	59×417	1991	/2021	47(0,0	,0 štetje 8 ponovitev po 100 semen								

Characterisation of common bean breeding material_KIS example – data analysis workflow I.

During different F generations and before registration process -> select the elite breeding line (s) along with parental lines and /or standard varieties

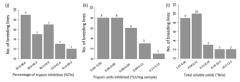


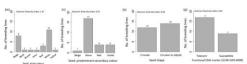
- □ **To evaluate the variability** within and between the common bean breeding materials, data on **agromorphological-biochemical-molecular traits are analyzed using the R-programming** environment->different packages.
- Descriptive statistics are used to illustrate variations in both the quantitative and qualitative traits->including the coefficient of variation (CV), standard error (Se), maximum (Max) and minimum (Min) values, and mean for quantitative traits.
- Population genetics analysis using the algorithms for molecular data -> more in Module 4
- Determine the frequency distribution and estimate diversity levels using the Shannon-Weaver diversity index (h') (Shannon & Weaver, 1949) for all traits.
- **Ο** To calculate the **Spearman coefficient** (ρ) to assess **pairwise patterns of associations** between traits.
- To calculate the **Gower distance** (Gower, 1971) to estimate the **similarity between pairwise breeding materials based on all traits analyzed**.

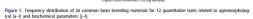
Table 2. Summary of descriptive statistics for 12 quantitative traits of the common bean breeding material studied.

	Part of the								
Group of parameters	plant	Trait	Unit	Min	Max	Mean	SE	CV (%)	H
Agromorphological	Flower	GP	days	74.00	106.00	96.54	1.70	9.04	1.31
parameters		TF	days	28.00	48.00	36.31	0.90	12.11	1.37
	Pod ATMPL ATMPW Seed SSLA	cm	10.40	28.20	15.18	0.90	29.70	1.21	
		ATMPW	mm	9.23	22.95	15.02	0.60	22.02	1.37
	Seed	SSLA	mm	11.13	15.99	13.89	0.30	10.51	1.53
		SSHA	mm	5.71	8.38	7.29	0.10	8.87	0.76
		SSWA	mm	3.98	6.86	5.39	0.10	13.45	1.30
		SW100	g	20.81	50.90	33.58	1.70	25.98	1.54
		TY	g	0.58	15.82	6.63	0.90	67.04	1.51
Biochemical parameters	Pod	INH	%TIn	30.89	68.29	44.79	2.10	23.95	1.48
		TUI	TUI/mg sample	0.04	0.12	0.07	0.02	28.24	1.43
		TSS	°Brix	1.97	11.33	5.52	0.50	42.29	1.37

SE: standard error; CV: coefficient of variation; H²: Shannon-Weaver diversity index; GP: growing period; TF: time of flowering; ATMPL: technologically mature pod length; ATMPW: technologically mature pod width; SSLA: seed size length; SSHA: seed size height; SSWA: seed size width; SW100: 100 seeds weight; TY: seed yield per plant; INH: percentage of trypsin inhibition; TUI: trypsin units inhibited; TSS: total soluble solids.

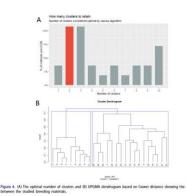






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Characterisation of common bean breeding material_KIS example – data analysis workflow II

During different F generations and before registration process -> select the elite breeding line (s) along with parental lines and /or standard varieties



- **To Factor analysis of mixed data (FAMD) to** investigate the differentiation patterns between breeding materials and to identify the main factors responsible for this differentiation.
- Employed hierarchical clustering based on principal components (HCPC), which is commonly used as a complement to factor analysis, to further categorize breeding materials into more specific groups.
- Then we are using a comprehensive multi-criteria decision analysis (MCDA) to evaluate and rank breeding materials based on their suitability for the final selection and registration of new varieties.

(Our analysis included all qualitative and quantitative traits to ensure a thorough evaluation of the performance of each breeding material. First, we normalized the quantitative traits to a common scale of 0 to 1 using a min-max normalization technique to ensure consistent treatment of all quantitative criteria. In addition, qualitative weights reflecting the relative importance of each categorical trait and quantitative weights representing the importance of numerical measurements were provided. To integrate these weights into the decision-making process, they were normalized to allow fair comparisons between the different criteria. We then calculated the weighted sum of the normalized feature values for each alternative, considering the preference direction for each quantitative feature.)

This resulted in composite scores reflecting the overall performance of each breeding material - Composite performance index; CPI

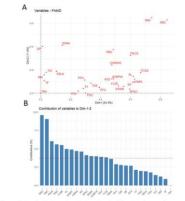
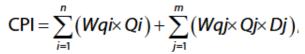


Figure 5. The contribution of qualitative and quantitative traits to the differentiation between the common bean breeding materials studied

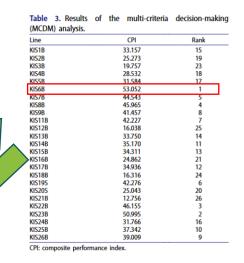


where n is the number of qualitative characteristics, m is the number of quantitative characteristics, Wqi and Qi are the normalized weight and normalized value of qualitative trait i, Wqj and Qj are the normalized weight and normalized value to quantitative trait j and Dj is the direction of preference for the quantitative characteristic j, where 1 means that higher values are preferred and 0 means that lower values are preferred.

Finally, the alternatives are ranked based on their CPI, with a score indicating better suitability.

LiveSeeding





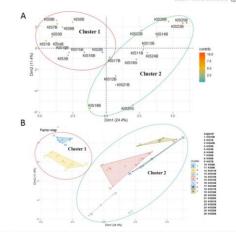


Figure 6. (A) Factor analysis of mixed data (FAMD) biplot and (B) subsequent hierarchical clustering of principal components (HCPC) biplot showing differentiation between common bean breeding materials based on 27 agromorphological-biochemical-molecular traits.

Further reading and available materials





Question 1: Differences between Qualitative and quantitative traits Question 2: Components of phenotypic variation Question 3: Why are correlations useful for breeders? Question 4: Explain the concept of heritability Question 5: How can G x E interaction exploded by breeders?

Send to :

adrodbur@doctor.upv.es and petra.jelincic@ips-konzalting.hr iveSeeding In 15 min

Wrap up/Main challenges for the future

- To calculate and evaluate key breeding parameters is highly complex covering different types of data and results from different years within breeding procedure.
- □ Key breeding parameters are **crucial for final selection** in breeding programms.
- Our main challenge is to make the breeding process, breeding materials, data and the results digitalised and to make key breeding parameters automatically calculated from the data within breeding workflow-> platform KISDigi Fižol is being established.



WRAP UP



What we have learned today?

Proposed homework: make a search and list at least 5 traits with high heritability and 5 traits with low heritability. Include the literature you are supported for the list

Send to :



<u>adrodbur@doctor.upv.es</u> and <u>petra.jelincic@ips-konzalting.hr</u> By next thursday 20th february



Thank you for your attention



https://www.kis.si/en/ https://www.kis.si/en/Associates PSGZ/dr. BARBARA PIPAN 2/ https://vrtnarstvo.javnasluzba.si/ https://www.kis.si/en/Genetic laboratory/







Funded by the European Union, the Swiss State Secretariat for Education, Research and Innovation (SERI) and UK Research and Innovation (UKRI). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or REA, nor SERI or UKRI.









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).

