Training in organic breeding

Module 4: Development and application of molecular methods in organic breeding

Unit 4.3: Application of marker-based selection in practical breeding

Intro

Authors: Barbara Pipan (KIS) & Mariateresa Lazzaro (FiBL)

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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).

UK Research and Innovation

MARCH 4th 2025 - 9:00 to 18:00 CET

Unit 4.1: Genome Wide Association and its potential for understanding the genetic basis of complex traits

- 9:00-11:00 - UPV (Neus Ortega Albero)
- 11:00-11:30 Break

Unit 4.2: Genomic Selection and its potential in organic breeding

- 11:30-13:00 - FiBL (Michael Schneider)
- 13:00-14:30 Lunch Break

Unit 4.3: Application of molecular marker-based selection in practical breeding

- 14:30-16:00 - KIS (Barbara Pipan) + FiBL (Lupins; Teresa Lazzaro)
- 16:00-16:30 Break

Unit 4.4: Overview on Experimental populations (e.g. NILs, RILs, introgression families, MAGIC) and their role in molecular breeding approaches

- 16:30-18:00 - UPV (Neus Ortega-Albero, Adrian Rodríguez-Burruezo) + KIS (Vladimir Meglic)

LiveSeeding

MODULE 3 – UNIT 3 – Marker Assisted Selection

Agenda

- Intro to MAS (14.35 – 14.50)
- Application case: bean breeding program in Slovenia (14.50 – 15.20)
- Application case: organic white lupin breeding program in Switzerland (15.20-15.40)
- Quiz (15.40-15.45)
- Discussion & Home work assignment (15.45-16.00)

How familiar are you with MAS?

www.menti.com

CODE **2251 7590**

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Marker Assisted Selection

- (DNA) markers to select for desired traits without directly measuring the trait itself
- can be used in combination with traditional phenotypic selection to enhance breeding outcomes

Photo: IRRI

LiveSeeding

Type of markers

- dominant/binary markers

>>They typically show the presence or absence of a specific DNA fragment, but cannot reveal if an individual carries one or two copies of the marker.

- Co-dominant markers

>>can distinguish between homozygous and heterozygous state

MAS vs phenotyping

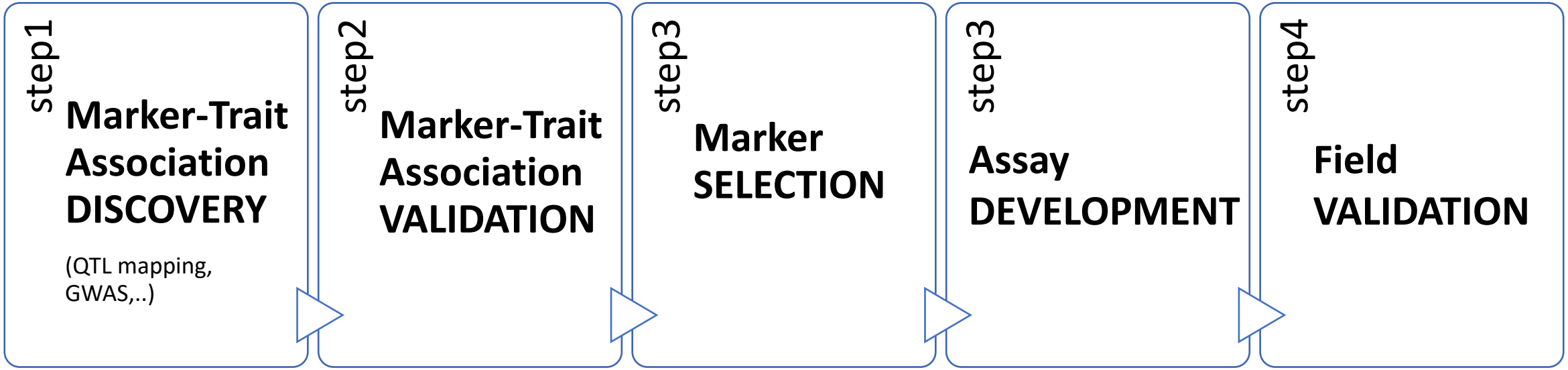
- generally more efficient for traits with low heritability,
  >> as heritability increases, the advantage over phenotypic selection decreases
- the higher the proportion of genetic variance explained by markers, the more effective relative to phenotypic selection

Photo: IRRI

MAS vs GS

- Effective for major genes: MAS is most efficient for traits controlled by a small number of genes with large effects
- GS is Effective for complex traits: GS considers all markers distributed throughout the genome, making it suitable for traits controlled by many genes with small effects

From marker-trait association to validated marker for MAS



Deployment in MAS

>> Use the validated marker(s) routinely in the breeding program to accelerate the development of improved varieties.

LiveSeeding

Example

Theoretical and Applied Genetics (2025) 138:52
https://doi.org/10.1007/s00122-024-04794-8

ORIGINAL ARTICLE

Check for updates

Identification by GWAS of marker haplotypes relevant to breed potato for *Globodera pallida* resistance

J. Leuenberger[1,2] · F. Esnault[1] · P. L. Lebas[1] · S. Fournet[3] · M. P. Cann[1] · S. Marhadour[1,4] · C. Prodhomme[3,4] · M. L. Pilet-Nayel[3] · M. C. Kerlan[1]

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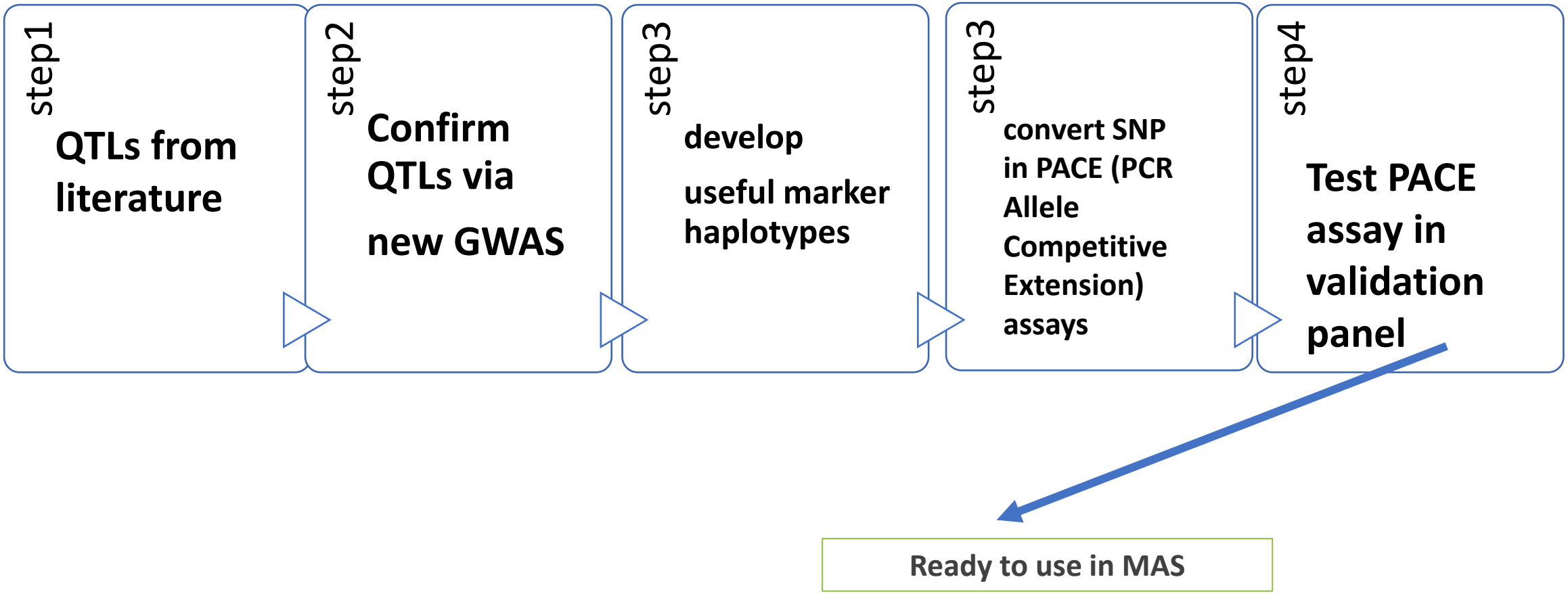
CROP: Potato TRAIT: resistance to cyst nematodes



LiveSeeding Leuenberger et al., 2025

Marker Assisted Selection in Organic Plant Breeding

Position paper of European Consortium for Organic Plant Breeding (ECO-PB) 2013
Ethical criteria

- Genome and cell is respected as indivisible entity, no technical/physical intervention (e.g. isolated DNA, GMO, NGTs)
- *Maintain reproducibility in species specific manner*
- *No legal or technical barriers to restrict breeders' privilege*
- *Natural crossing barriers are respected*

>>diagnostics only

Breeding strategies

- All steps in organic conditions. Selection in organic fields to account for plant-environment interaction

>> complementary tool only (does not substitute the field selection)

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Common bean example

Authors: Barbara Pipan (KIS)

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UK Research and Innovation

Common bean (*Phaseolus vulgaris* L.)

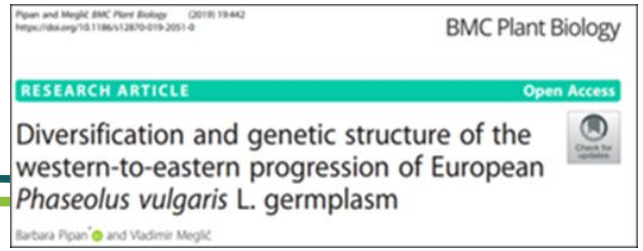
- Is one of the **most important edible legumes** for direct human consumption in the world, as it is a valuable source of **protein, carbohydrates, fibre** and is a rich source of other components with nutritional and health benefits.
- For the Slovenian production area, **common bean (CB) is known as a vegetable** of increasing agronomic interest.
- Our recently developed varieties are coming from **targeted hand-cross pollination to introduce** the genes which are associated with most **important traits of interest regarding breeding objectives** (dwarf and climbing bean for both dry bean and fresh pod consumption).
- It is a **self-pollinating** annual plant with a relatively **small genome of 587 Mbp (2n=22).**
- **Reference genome is available!**

Breeding objectives

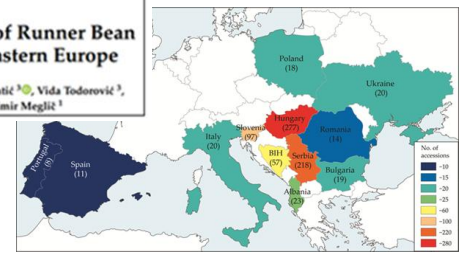
- **Tolerant to biotic** (BMMV/BCMNV, CBB, Anthracnose, bruchid) and **abiotic** (high T, water) **stresses**.
- **Earliness** (flowering before high temperatures occur and induce flower shedding).
- Tending to develop new **„maslenec“** type of varieties-> forms yellow/green long, flat and stringless pods (for climbing types).
- The **favourable position of the pods on the plant** (for dwarf types).
- **High-yielding** varieties.
- **Favourable nutritional composition** (↑proteins, Fe, Zn…; ↓phytic acid).

Identify/select the loci in the CB genome associated with traits of interest!

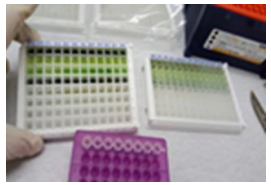
Selection of the DNA markers

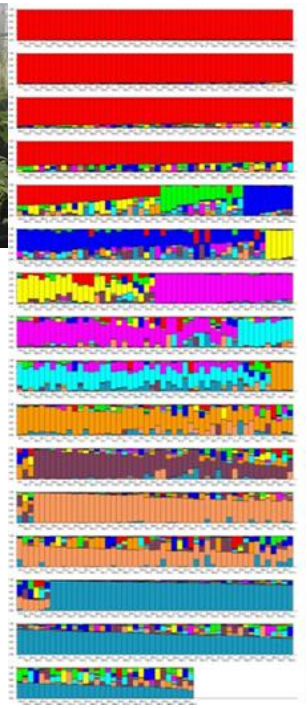


- Within project activities of SI national applicative project L4-7520 ***„Utilization of common bean genetic resources for sustainable crop improvement and healthy food“***



- The markers were identified/selected via **literature survey considering the main following issues**:
  - ❑ Species-specific (CB genome);
  - ❑ Covering the **traits of interest regarding breeding objectives** and **with background information about the trait inheritance relations and specificity** (quantitative, qualitative, dominant/recessive, level of heritability,…);
  - ❑ Proven to be **highly polymorphic**;
  - ❑ **Equally distributed** among CB genome;
  - ❑ Highly applicable in terms of **repeatability, reliability**, to be **quick and cost-effective for *in-house* use**.





- The **panel of selected trait-associated DNA markers was optimised, tested and validated on the core collection of CB (77 genotypes)-> to screen and cover highly diverse germplasm**; the CC was established from **782 ACCs from different 12 geographic origins** following western-to-eastern line in Europe.

- **The panel of 24 functional/trait-related DNA markers was established for routine use in common bean breeding programme in Slovenia.**

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Functional/Trait-related markers in MAS

Panel of **24 functional DNA markers** for MAS in common bean **/routine use in public breeding programme!**

- **different types of DNA markers: nSSRs, CAPS, locus-specific PCR markers, SCAR) -> binary and codominant genotyping**

Trait association
gene pool determination
BCMV_BCMNV resistance + earliness+yield
bean rust resistance
Antrachnose resistance
ALS (angular leaf spot)
Bean-pod weevil (bruchid resistance)
Fe+Zn+yield related markers
Low P uptake and favorable root morphology; low P toelrance
drought stress tolerance

Marker name	lab label	Gene related	Marker type/genotyping method	Trait/description	Reference	F sequence (5'-3')	R sequence (5'-3')	Expected size	M13 label
PvSHP1-A	x (ARRS I. faza)	SHATTERPROOF indel-spanning markers of Pv SHP1 gene	nSSR_ABI	**gene pool determination**	Nanni 2011	TTGAGGGTAGATTGGAGAAAGG	GGAAAATTTCATCAAAACATATCCA	232-251	FAM
PvSHP1-B	y (ARRS I. faza)	SHATTERPROOF indel-spanning markers of Pv SHP1 gene	nSSR_ABI	**gene pool determination**	Nanni 2011	GGAAATTGAGCTGCAAAACC	CACAGTGTTCCCTGCATCAT	211–221	NED
PvSHP1-C	w (ARRS I. faza)	SHATTERPROOF indel-spanning markers of Pv SHP1 gene	nSSR_ABI	**gene pool determination**	Nanni 2011	TTGAGGGTAGATTGGAGAAAGG	TTGGGTTTATAAGAAAACCTTCCA	141-159	HEX
Phs	si	Phaseolin (T and S alleles)	SCAR	Phaseolin (T and S alleles)+white mold (bela plesen)+CBB (common bacterial blight)		AGC ATA TTC TAG AGG CCT CC	GCT CAG TTC CTC AAT CTG TTC	multiple alleles	
za SNP ss751641188 (heterozigot A:A je rezistenten) designed CAPS marker BCMV_48289723_CAPS	ff (ARRS II. faza)	I gene position	CAPS marker_horiz. Agarose electrophoresis (I. in II. stopnja)	linked to I gene possition; **BCMV_BCMNV resistance**	Bello et al, 2014	AGGAGGAAGAACGGTGGTC	TTTGGTGGTAATTTGAAAATGG	I. 311 bp (uspešna amplifikacija mora biti pri vseh); II: the resistant allele is cleaved by TaqIgenerating two bands of 201 and 110 bp	
SW13!	ee (ARRS II. faza)	I gene	SCAR Two SCAR markers (SW13 and SBD5) were used in markeraided backcrossing for pyramiding the I and bc-12 genes, which provide host plant resistance to BCMV. Virus strains NL3 in NL6.	**BCMV resistance** (resistant genotype ima prisoten amplifikat); Linked to I BCMV resistance gene (Hegay, Bello, Melotto); linked to **earliness of the variety** (days to harvest as green pod, days to matunityand) and **yield** (seed lenght) regarding to Perez-Vega et al., 2010+ **HBB (halo bacterial blight)**	Hegay e tal., 2013; (za metodo glej Bello et al, 2014); original sekvenca Melotto et el,1996	CAC AGC GAC ATT AAT TTT CCT TTC	CAC AGC GAC AGG AGG AGC TTA TTA	690 bp	
ROC11!	eg (ARRS II. faza)	bc-3 I gene	SCAR_horiz. Agarose electrophoresis (sequence characterized amplified region)	**BCMNV resistance**; Linked to bc-3 BCMV resistance gene; (Hegay, Bello, Melotto); linked to **yield** (seed width and seed lenght) regarding to Perez-Vega et al., 2010	Hegay e tal., 2013; (za metodo glej Bello et al, 2014); original sekvenca Johnson et al, 1997	CCA ATT CTC TTT CAC TTG TAA CC	GCA TGT TCC AGC AAA CC	420 bp	
SG6	eh (ARRS II. faza)	bc-3 I gene	SCAR_horiz. Agarose electrophoresis	Linked to bc-I3 **BCMV/BCMNV resistance gene**	Hegay e tal., 2013; (za metodo glej Bello et al, 2014); original sekvenca Mukeshimana e tal.2005	GTG CCT AAC CGA GTT ATC TAG AGT	GTG CCT AAC CCT CCT AAA TGA CCT	595 bp	
SBD5	ef (ARRS II. faza)	bc-2 I gene	SCAR _horiz. Agarose electrophoresis Two SCAR markers (SW13 and SBD5) were used in markeraided backcrossing for pyramiding the I and bc-12 genes, which provide host plant resistance to BCMV. Virus strains NL3 in NL6.	Linked to bc-I2 **BCMV resistance gene**	Hegay e tal., 2013; (za metodo glej Bello et al, 2014); original sekvenca Melotto et el,1996	GTG CGG AGA GGC CAT CCA TTG GTG	GTG CGG AGA GTT TCA GTG TTG ACA	1250 bp	
EAACMCAC-1 (AFLP) to DESU-G1	ei (ARRS II. faza)	linked to Ur-3 loci contributing to rust resistance (*Uromyces appendiculatus*)	SCAR_horiz. Agarose electrophoresis	**Odpornost na fitokovo rjo (bean rust related resistance)** marker named DESU-G1 utilizing the amplified fragment length polymorphism (AFLP) methodology for this deleted region in the ur-3 rust susceptible mutants. This is the first attempt to develop AFLP markers	Melmaiee et al., 2013	CATGAAATTGTACGCCCAAA	CTCGACGGATGCAAACACTT	prbl. 1000 bp	
SW12	sa	Co-3 /Co-9	SCAR_horiz. Agarose electrophoresis	Ant resistance	Miklas et al., 2000c Singh et al. 2000 Rodriguez-Suárez et al., 2008	TGG GCA GAA GTT CTA GCA TGT GGC	TGG GCA GAA GCA CAG TAT GAT TTG	700 cis	
SAS13!	sb	Co-4[2]	SCAR_horiz. Agarose electrophoresis	Ant resistance	Young et al., 1998 *Kelly et al., 2003; Oliti et al., 2017	CAC GGA CCG AAT AAG CCA CCA ACA	CAC GGA CCG AGG ATA CAG TGA AAG	950 cis	
SAB3!	sc	Co-5	SCAR_horiz. Agarose electrophoresis	Ant resistance	Vallejo and Kelly, 2001 Campa et al., 2005; Oliti et al., 2017	TGG CGC ACA CAT AAG TTC TCA CGG	TGG CGC ACA CCA TCA AAA AAG GTT	400 cis	
SZ04	sd	Co-6	SCAR_horiz. Agarose electrophoresis	Ant resistance	Queiroz et al., 2004b *Kelly et al., 2003	GGC TGT GCT GAT TAA TTC TGG	TGC TCA TTT TAT AAT GGA GAA AAA	567 trans	
SF10	se	Co-10	SCAR_horiz. Agarose electrophoresis	Ant resistance	Correa et al., 2000 Alzate-Marin et al., 2003	GGA AGC TTG GTG AGC AAG GA	GGA AGC TTG GCT ATGATG GT	1072 cis	
SN02	sf	Phg-2	SCAR_horiz. Agarose electrophoresis	ALS-angular leaf spot (navadna pegavost fižola)	Nietsche et al., 2000 *Miklas, PC, 2002; Oliti et al., 2017	ACC AGG GGC ATT ATG AAC AG	ACC AGG GGC AAC ATA CTA TG	890 cis	
SBA16	sg	OuroNegro dominat gene	SCAR_horiz. Agarose electrophoresis	ALS-angular leaf spot (navadna pegavost fižola)	Queiroz et al., 2004a	TTC CAC GTC TAT TTT GCA TCA	CAC GCA TCA CGC AGA ACT	560 cis	
SW6-800R	sh	Agm J-117	SCAR_horiz. Agarose electrophoresis	Bean-pod weevil (ruchid resistance)	Blair et al., 2006	AGG CCC GAT GCC CCC TTA T	TGG AGT CGG TCA AAC CCA TGT T	520 cis	
BM165	c (ARRS II. faza)	Zn-AAS8c	nSSR_ABI	SW (seed weight)_yiled+(QTL) for iron (Fe) and zinc (Zn) concentrations or seed weight (SW) identiWed with composite interval mapping in the G21242 £ G21078 recombinant inbred line population (Andean germplasm)+ the closest marker position to ANT07.3 for joint Ant races (04, 38, 55); QTL Analysis of Adventitious Root Formation in Common Bean under Contrasting Phosphorus Availability	Blair et al., 2011	TCAAATCCCACACATGATCG	TTCTTTCATTCATATTATTCCGTTCA	(TA)3(CA)9	FAM
BM185	f (ARRS II. faza)	**QTL Fe-ICP7a**; the closest marker position **to ANT07.3 for joint Ant races (04, 38, 55)**; QTLHPSRL_**AdvGH.2**	nSSR_ABI		Blair et al., 2011; Ochoa et al., 2006	AAAGCAGGGATGTAGTTGC	AAGGAGGTTTCTACCTAATTCC	(CT)12	NED
BM239	d (ARRS II. faza)	QTL qSW_po11.1; QTLqF**e**Cont_poICP; QTLqZ**n**Cont_poAA; QTLqZnCont_poICP	nSSR_ABI	SW (seed weight)_yiled+Markers signifcantly associated with iron and zinc content (in g/seed) and seed size identiWed with single point regression analysis in the G14519 £ G4825 population (Mesoamerican germplasm)	Blair et al., 2010	CTGCTACTACTCCCACTACTTCA	ATGTAAGCCATTCCCTCTTC	(TC)12	HEX
BMd10	aa	QTL for P use efficiency; QTLSrtL.1	nSSR_ABI	QTL Analysis of Root Architecture Traits and Low Phosphorus Tolerance in an Andean Bean Population; QTLs for Root Architecture Traits Correlated with Phosphorus Acquisition in Common Bean	Beebe et al., 2006; Cichy et al., 2009	ATCTGAGAGCAGCGACATGGTAG	GCTCACGTACGAGTTGAATCTCAG	(GA)8	FAM
BMd27	ag	QTLTrd11.1+ QTLPSRL_AdvGH.1	nSSR_ABI	QTLs for Root Architecture Traits Correlated with Phosphorus Acquisition in Common Bean	Beebe et al., 2006;	GGACCCACCATCACCATAAC	TGGTGGAGGTGGAGATTTGT	(CCA)6	NED
BMb182	z	drought response+yield directly	nSSR_ABI	v bližini QTL regije za hitrost transporta elektronov (ETR_S1) glede na QTL ARRS projekt; na genski karti Galeano-Fernandez_2011 povezan s QTL regijami za dolžino in težo semena (Perez-Vega in sod., 2010), pridelek (Blair in sod., 2006, 2012) ter začetek in konec zrelosti strokov (Perez-Vega in sod., 2010; Blair in sod., 2012)	Matejina dd+Galeano-Fernandez_2011+(Perez-Vega in sod., 2010)+Blair in sod., 2006, 2012	AAATTCCAACCTCTGTGCTA	TTTGGCTGTGATTCTTCTTT	(TTA)11	HEX

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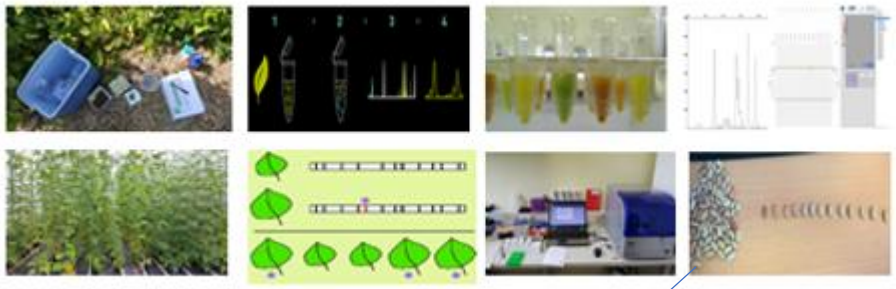
Breeding workflow_when apply MAS?



PARENT SELECTION

HAND-POLLINATION/TARGETED CROSSINGS

SELF POLLINATION AND SELECTION (PHENOTYPIC)+ MAS (F2)

SELF POLLINATION AND SELECTION (PHENOTYPIC)-6 CYCLES

FINAL SELECTION OF THE ELITE BREEDING LINES + *IN VITRO* TESTS+OPEN FIELD EVALUATIONS

REGISTRATION

The highest segregating diversity.

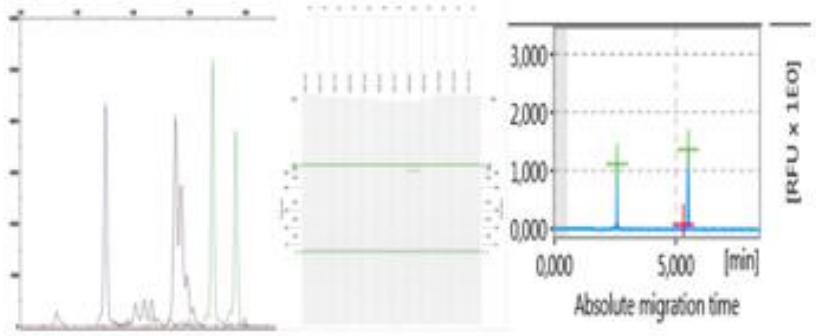
MAS workflow

- Sampling the leaf tissue (60-100mg) from F2 phenotypically (pre) selected plants/breeding lines.
- Homogenization of the plant sample.
- Extraction of gDNA (optimised method; automatable magnetic bead-based sample preparation technology using MagMax).
- DNA QC (qualitative on agarose-gel electrophoresis; quanitative using Qubit/Nanodrop)->prepare DNA templates and controls.
- (fast) PCR performance under optimised PCR reaction mixtures and temperature conditions/requirements for specific DNA marker (panel of 24).
- Visualisation of the results:

->binary scoring for dominant markers (HR agarose gel electrophoresis /QIAxcel):

->codominant scoring/allelic matrix for (EST) SSR (fragment reaction + fragment analysis): Sequencer/Genetic analyser (fluorescent detection)-> electropherograms

- Analysis of the molecular data/genotyping results.
- Highlight the most promising genotypes with the best genetic background according to the traits of interest.



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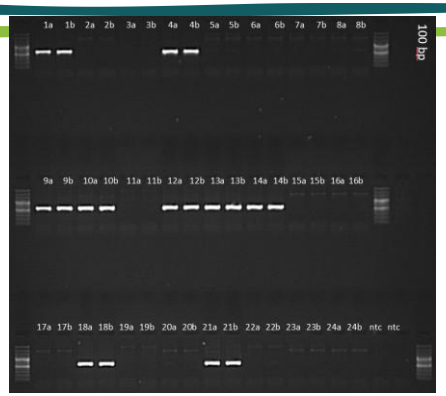
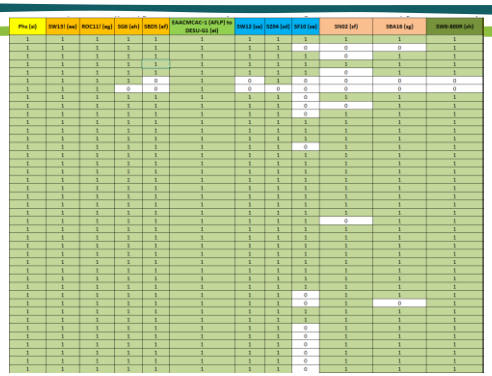
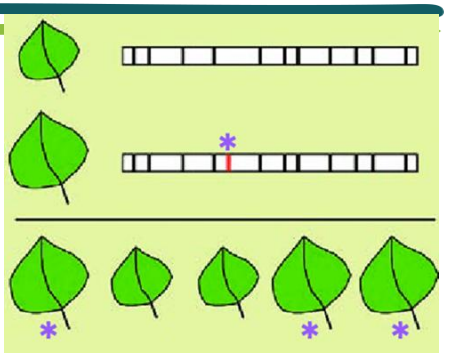
MAS workflow_Analysis of the results

- **Binary scoring** of the gel for dominant markers: presence/absence of the band is associated with the **presence/absence of the band** at the specific locus -> to identify the genotypes with the **presence of the alleles/multiple alleles/genes associated with the traits of interest.**

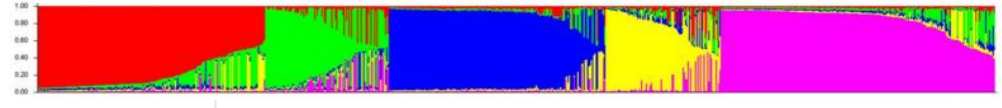


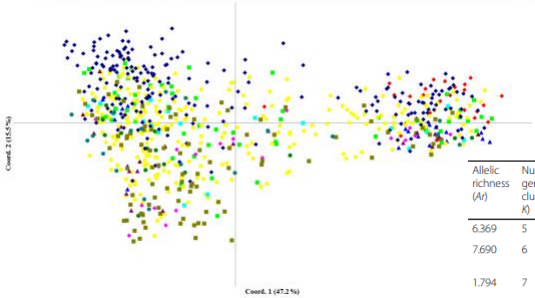




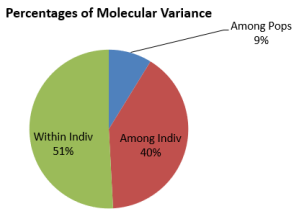
- For the results of **dominant and codominant genotyping** -> **Population genetics software** (*e.g. genAlEx, Structure, SH, Genetix, Arlequin, Populations, FreeTree, TreeView,... all in combination with the R programming environment using different software packages*) used to evaluate **genetic diversity, variability, genetic structure and clustering, marker informativeness, genetic distances and level of genetic distinguishing, including varietal authenticity**,... using various **population genetics parameters**.

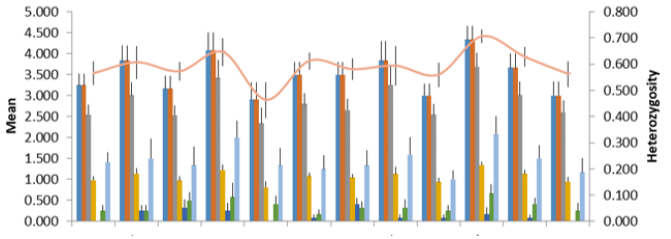
Locus	Expected heterozygosity (H_e)	Polymorphic information content	Allelic richness (Ar)	Number of effective alleles (Ne)	Shannon's information index (I)	Fixation index (F)
ATA003	0.879	0.867	27.709	8.212	2.446	0.198
ATA004	0.849	0.834	24.000	6.551	2.326	0.362
ATA005	0.843	0.828	30.132	6.362	2.392	−0.040
ATA007	0.804	0.779	24.845	5.080	2.040	0.663
ATA016	0.790	0.762	17.147	4.752	1.873	−0.0
GAT591	0.928	0.923	32.292	13.749	2.890	−0.0
ATA002	0.858	0.845	30.798	6.982	2.452	0.04
BM172	0.894	0.885	35.118	9.411	2.686	0.09
BMd001	0.923	0.917	43.451	12.801	2.944	0.01
ATA020	0.882	0.872	34.052	8.422	2.596	0.28
Pv-ag004	0.845	0.828	26.295	6.432	2.287	−0.1
SSR-IAC167	0.585	0.542	12.735	2.405	1.215	0.11
ATA010	0.825	0.802	14.617	5.690	1.986	0.43
BM155	0.741	0.694	9.139	3.847	1.447	−0.1
BM170	0.889	0.879	27.770	8.960	2.537	−0.0
BM183	0.724	0.682	14.747	3.610	1.601	−0.2
BM210	0.540	0.434	5.402	2.172	0.867	−0.3
BMd044	0.762	0.726	13.088	4.187	1.667	0.23
ATA009	0.901	0.893	23.516	10.083	2.593	0.17
ATA145	0.796	0.781	22.613	4.888	2.162	0.43
GA16	0.855	0.837	15.225	6.858	2.110	0.09
ATA006	0.912	0.905	23.074	11.346	2.642	0.08
BM157	0.813	0.788	14.464	5.345	1.938	0.46
BMd042	0.857	0.842	24.054	6.977	2.312	−0.1
ATA289	0.773	0.743	20.694	4.391	1.861	−0.239
PvSHP1-A	0.855	0.839	17.111	6.868	2.195	−0.138
PvSHP1-B	0.856	0.838	16.937	6.911	2.093	−0.132
PvSHP1C	0.849	0.833	16.159	6.615	2.173	−0.004
PvM04	0.734	0.705	14.745	3.752	1.727	0.359
PvM21	0.753	0.724	17.757	4.047	1.752	−0.279
PvM95	0.779	0.752	28.173	4.511	1.980	−0.208
SSR-IAC62	0.930	0.925	32.777	14.137	2.892	0.073
SSR-IAC66	0.895	0.885	40.677	9.449	2.671	−0.061
Mean values	0.822	0.800	22.767	6.842	2.162	0.042

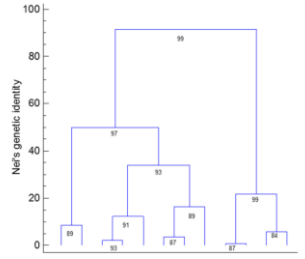




Allelic richness (Ar)	Number of genetic clusters (real K)	Expected heterozygosity (H_e) across clusters	Allele frequency ≥ 5%	Global unbiased expected heterozygosity of accessions	Principle coordinate analysis (first three axes) (%)	Mean polymorphic information content among all loci
6.369	5	0.676–0.681	3.515	0.626	67.7	0.569
7.690	6	0.739–0.805	4.939	0.783	71.0	0.746
1.794	7	0.778–0.779	5.788	0.794	69.3	0.724
16.653	3	0.747–0.764	5.242	0.815	68.5	0.815
4.699	11	0.808–0.809	6.061	0.815	65.6	0.761
4.066	11	0.805–0.807	6.273	0.816	63.6	0.761
1.791	9	0.745–0.747	5.909	0.791	68.2	0.683
6.330	5	0.754–0.755	5.091	0.746	62.8	0.725
16.678	9	0.804–0.809	5.121	0.797	73.5	0.769
10.405	5	0.788–0.774	5.152	0.816	69.0	0.788
					66.0	0.715
					63.7	0.723







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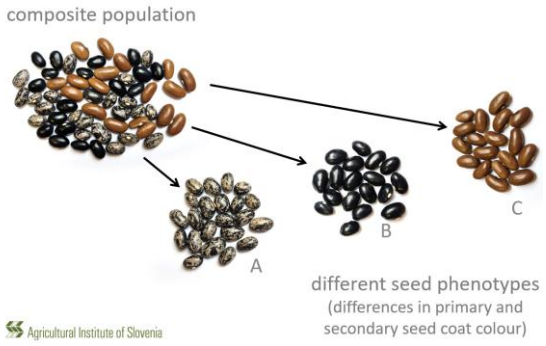
…and finally we are about to choose…

- Select the **most promising F2 breeding** lines with respect to the best genetic background according to the **results of the genetic analysis.**
- **Continue phenotypic selection** during subsequent generations of selfing to make the traits stable in homogenous…
- Allow us **to breed more effectively** during the process, maintaining the best breeding lines only with **a favourable genetic background for the traits of interest**.

...learn more about the research behind discovering trait-related markers...



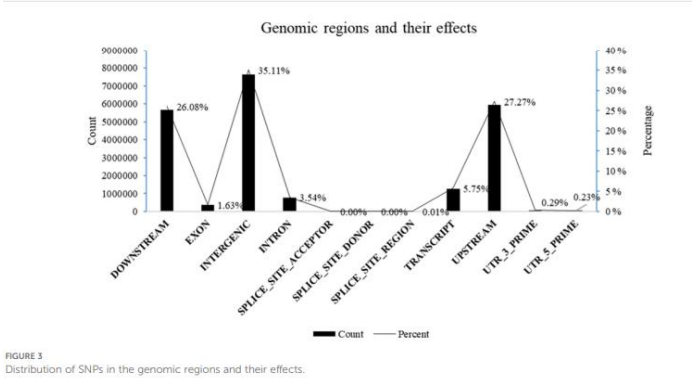


FIGURE 3
Distribution of SNPs in the genomic regions and their effects.

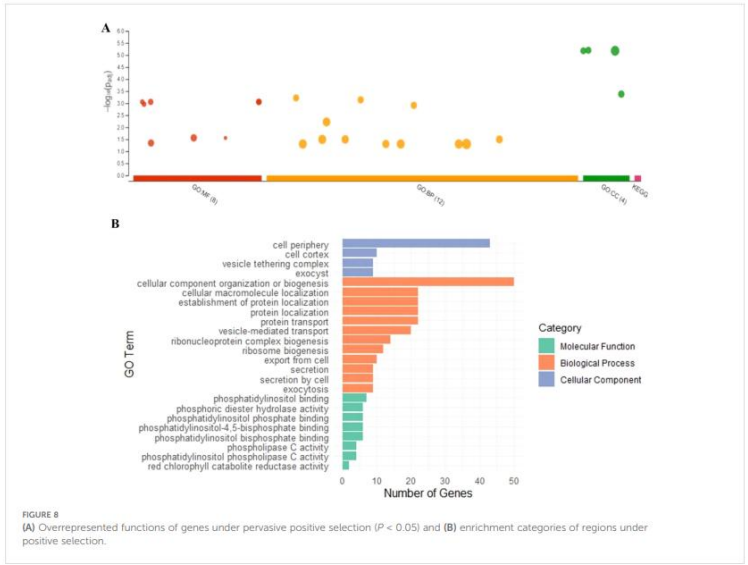


FIGURE 8
(A) Overrepresented functions of genes under pervasive positive selection ($P < 0.05$) and **(B)** enrichment categories of regions under positive selection.

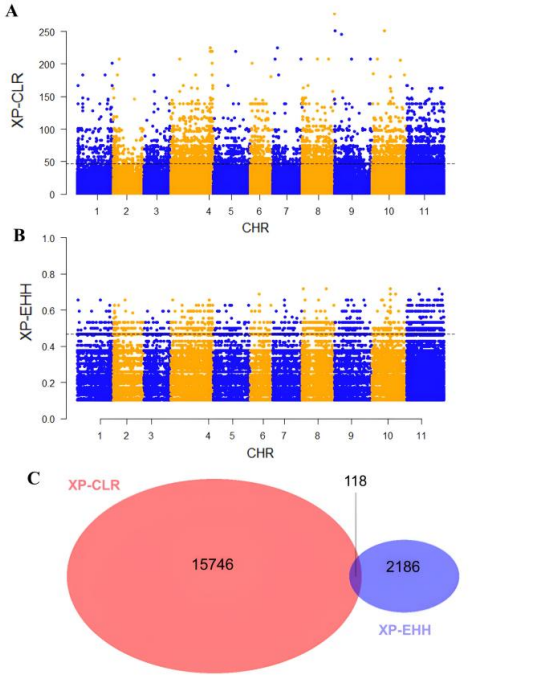


FIGURE 7
Manhattan plots of **(A)** pairwise XP-CLR and **(B)** XP-EHH showing the signatures of positive selection between populations with altered seed coat color and unaltered seed coat color. The top 1% of the empirical distribution of XP-CLR and XP-EHH scores are indicated by dotted lines, respectively. **(C)** Venn diagrams showing the overlap between the genomic regions identified by XP-CLR and XP-EHH tests.

- ❑ WGS identified 8.6 million SNPs, with chromosomes 4 and 1

having the highest SNP density (11% each), while chromosomes 3 and 6 had the lowest.

- ❑ Selective sweep analysis with XP-CLR and XP-EHH identified 118 significant regions associated with seed coat color change, with most regions located on chromosomes 4, 9, 10 and 11.
- ❑ Phosphatidylinositol signaling pathways were highly enriched in candidate regions, indicating that cellular transport mechanisms play a critical role in seed coat pigmentation.
- ❑ The identification of key genomic regions and pathways associated with seed pigmentation improves our understanding of the complex genetic interactions underlying this trait.

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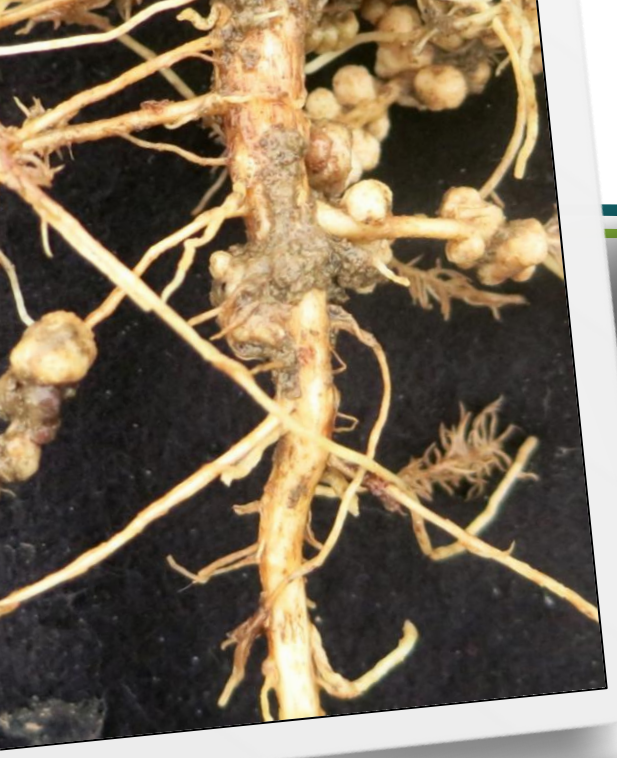
Wrap up: Why is MAS important in breeding?

- **Enhanced disease resistance:** Molecular breeding and MAS allow for the **precise identification and incorporation of genes responsible for resistance to various diseases**. This leads to the development of common bean varieties that are more resilient to, thereby reducing crop losses and improving yield stability.

- **Accelerated breeding process**: Traditional breeding methods can be time-consuming and labor-intensive. MAS significantly speeds up the breeding process by enabling the selection of desirable traits at the seedling stage, rather than waiting for the plants to mature. **This efficiency allows breeders to develop new, improved varieties more quickly.**

- **Improved trait selection**: MAS facilitates the selection of complex traits that are difficult to measure phenotypically, such as drought tolerance and nutrient use efficiency. By using molecular markers linked to these traits, breeders can more **effectively select and combine desirable characteristics, leading to the development of common bean varieties that are better adapted** to diverse environmental conditions.



Training in organic breeding

Module 4: Development and application of molecular methods in organic breeding

Unit 4.3: Application of marker-based selection in practical breeding

White lupin example

Authors: Mariateresa Lazzaro (FiBL, Switzerland)

CC BY SA

Co-funded by the European Union

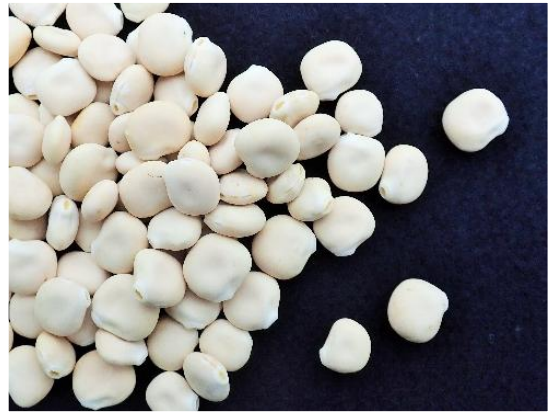
Funded by the European Union, the Swiss State Secretariat for Education, Research and Innovation (SERI) and UK Research and Innovation (UKRI).

UK Research and Innovation

White Lupin (*Lupinus albus*)

- 35 % (28% – 40%) protein content
- next best amino acid composition after soybean
- Legume family → N fixation
- Deep roots
- Soil structure improvement,
- P mobilization
- Pollinator attracting flowers (pollen / no nectar)
- frost tolerant summer crop
- drought tolerant

- Increasing demand:
  - feed
  - food: vegetarian / vegan trend



LiveSeeding

White Lupin (*Lupinus albus*)

Predominantly self-pollinating (but)

>>> up to 15% cross pollination possible

>>> isolation from insects needed for breeding

LiveSeeding

Breeding TARGET: anthracnose resistance

- fungal agent: *Colletotrichum lupini*
- transmission: seed, rain splash
- under wet conditions > 25°C
- **quantitative resistance**
- **very few resistance sources identified**

LiveSeeding

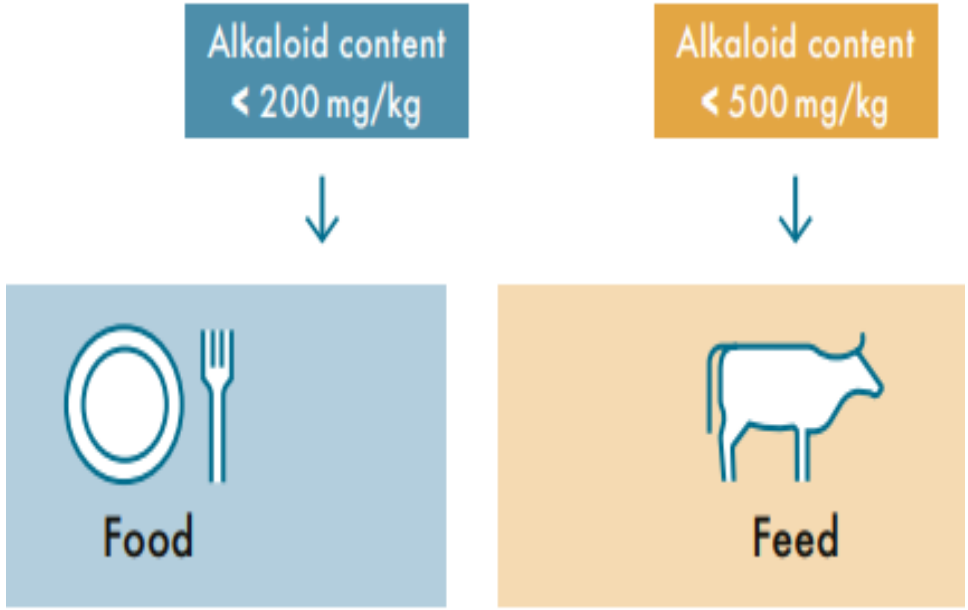
Breeding TARGET: low-alkaloid in seeds

Quinolizidine alkaloids (QAs):

- bitter & toxic

- Wild material and landraces are bitter
  - 2’000-4’000mg/kg in bitter cultivars
  - up to 127’000mg/kg in wild material
- «Sweet» varieties → low-alkaloid loci
- **controlled by multiple recessive loci,**

one major locus, *pauper* (Chr18, causal mutation published) and several other loci reported, but not genetically mapped)



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White Lupin Organic Breeding

Crossings

resistant (bitter, late)
X
sweet and/or early ripening

Phenotypic selection

ORGANIC

Direct Selection

in target environment, in organic certified fields (2 CH, 1 DE), following the farm crop rotation

LiveSeeding

Breeders: Christine Arncken (FiBL) & Miriam Kamp (gzpk)

How to integrate MAS in our organic breeding programme?

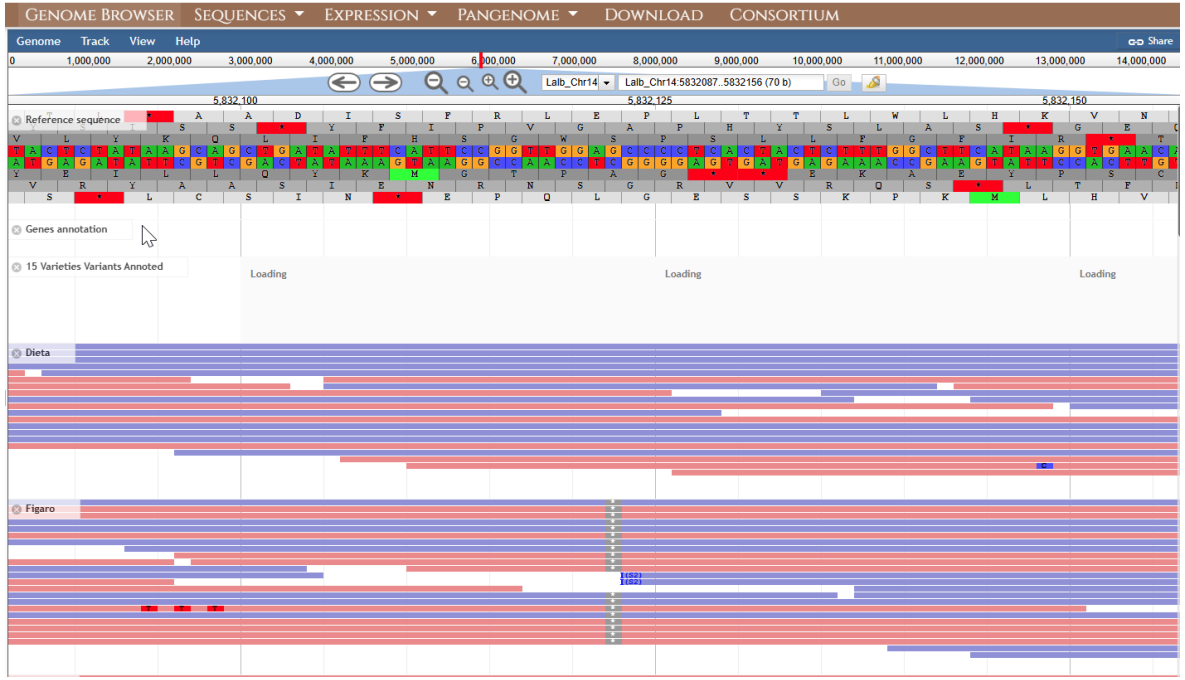
- advancing molecular breeding tools for this neglected crop
- development, validation and application of molecular markers associated to anthracnose resistance and alkaloid accumulation traits

Reference genome available

Genome size: Approximately 451-584 Mb

Chromosome number: 2n = 50 (25 pairs of chromosomes)

Total genes: ca 38k



LiveSeeding

Quinolizidine alkaloids and their determinants

At least 5 «sweetness» sources (**recessive**):

- *pauper* :
    - most market varieties
    - reduction to 200-500mg/kg
    - **causal SNP published** by Mancinotti et al. 2023 (SNP Lalb_Chr18_12359687)
- *mitis*, *reductus*, *exiguus, nutricius:* reduction to ≤1000mg/kg
    - loci not known

LiveSeeding

Is it possible to develop lines with very low and/or stable levels in different environments by stacking two low-alkaloids determinants?

Study

As only *pauper* is mapped, we needed to identify a second QTL to be able to respond to this question.

Determing stacked allele recombinant effect on alkaloid content

Apply in the breeding programme

LiveSeeding

Material

- Crossing: Dieta (PP**nn**) x Frieda(**pp**NN)
- F1: PpNn
- F2 (plants) in 2023:

7:9 sweet bitter

	PN	**Pn**	**pN**	**pn**
PN	PPNN	PPNn	PpNN	PpNn
Pn	PPNn	PPnn	PpNn	Ppnn
pN	PpNN	PpNn	ppNN	ppNn
pn	PpNn	Ppnn	ppNn	ppnn

Method

Genotyping F2 for *pauper*

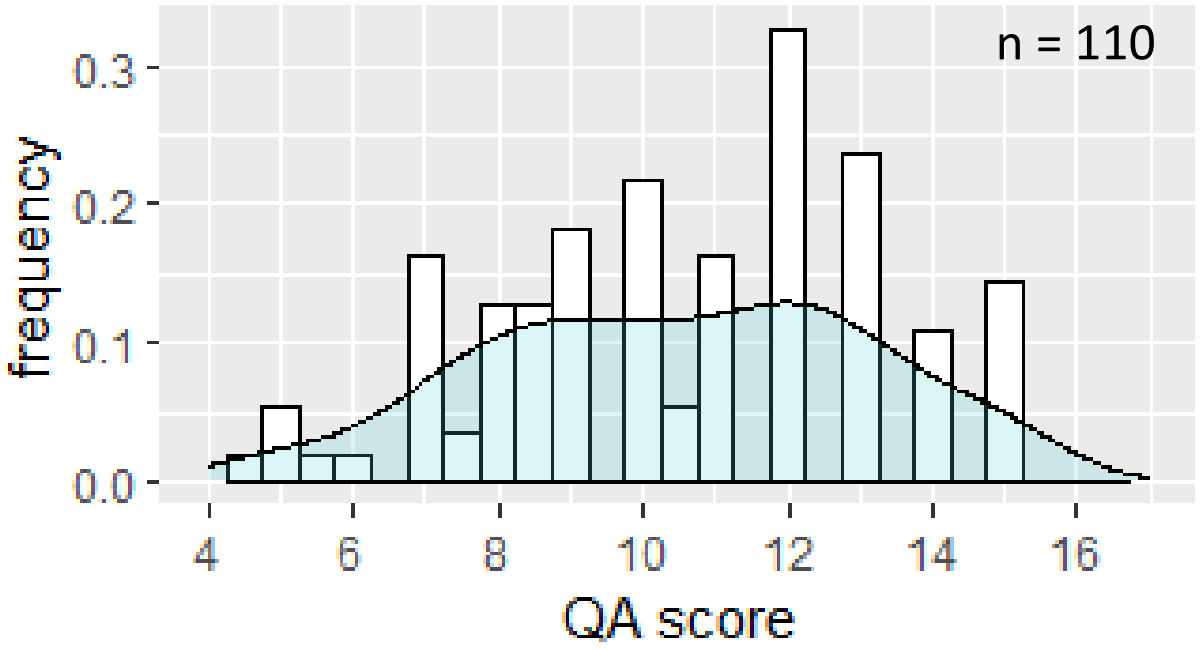
(Rough) phenotyping with Dragendorff & Tasting → calculation of combined QA score

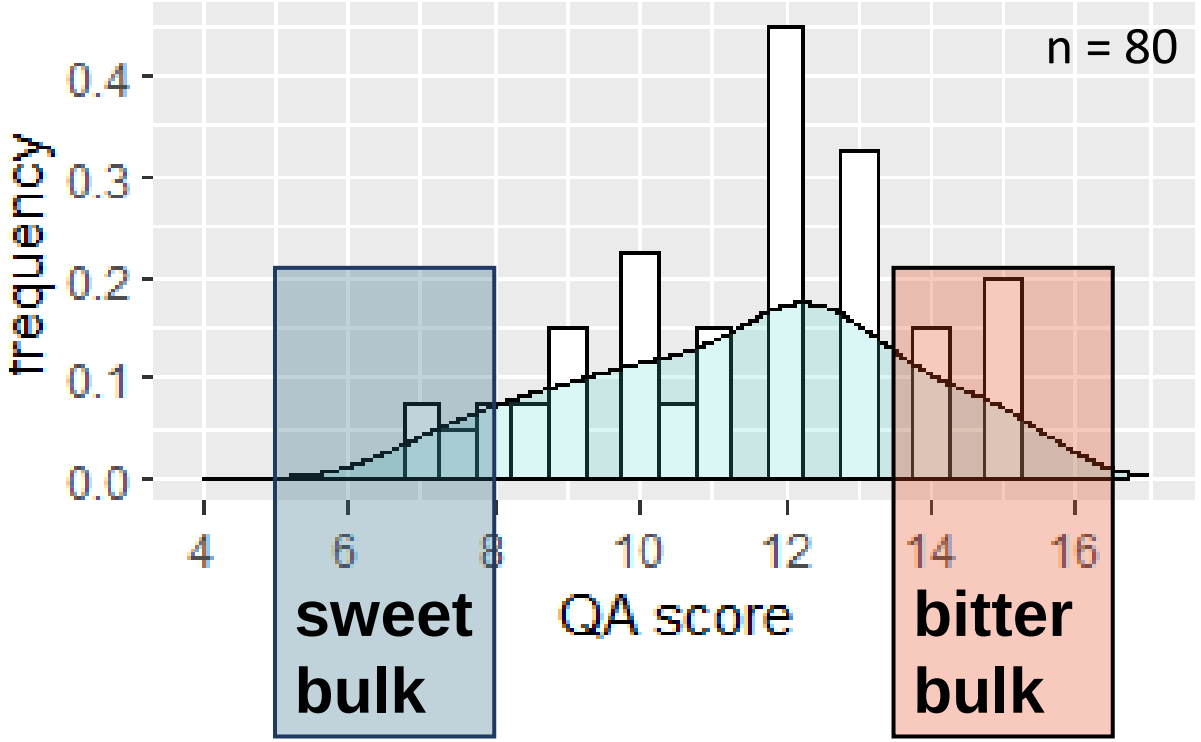
Bulked Segregant Analysis in F2 population

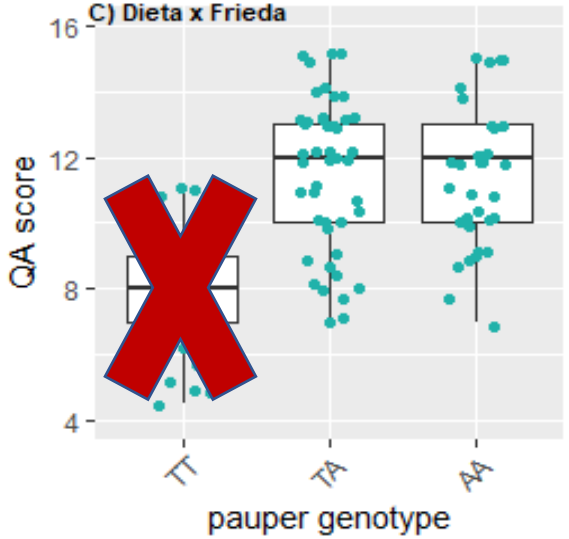
LiveSeeding

MSc thesis Miriam Kamp, WUR, 2023

Method: BSA







Dieta bulk

Frieda bulk

Bulk Segregant Analysis

LiveSeeding

Results BSA and marker for MAS

SUCCESS>>>>> **QTL on Chromosome 5**

SNPs choice in the QTL (4 selected)
>>
PACE assays development
>>
validation in a diversity panel (34 accession)
>>
1 PACE marker chosen
>>use in F3 population
>> identified individuals with stacked low alkaloid alleles with very low alkaloid content

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Next steps

Combine the MAS for low-alkaloid

with the selection (pheno and geno) for anthracnose resistance

- **quantitative resistance**
- **very few resistance sources identified**

LiveSeeding

Markers for anthracnose

- 3 loci from literature (Yang et al. 2010):
  - Kiev mutant x P27174 RIL
  - WANR1, WANR2, WANR3

→ not able to design KASP assay (WANR1&2),
n.s. in validation panel (WANR3)

- 3 SNPs from GWAS by Alkemade et al. 2022
  - Panel of 200 accessions of white lupin, 9940 SNPs, controlled conditions phenotyping
  - Lalb_Chr05_2957601, Lalb_Chr05_2957940, Lalb_Chr05_3706534

→ **n.s.** in validation panel

Yang, H., Lin, R., Renshaw, D. *et al.* Development of sequence-specific PCR markers associated with a polygenic controlled trait for marker-assisted selection using a modified selective genotyping strategy: a case study on anthracnose disease resistance in white lupin (*Lupinus albus* L.). *Mol Breeding* **25**, 239–249 (2010). https://doi.org/10.1007/s11032-009-9325-4
Alkemade, J. A., Nazzicari, N., Messmer, M. M., Annicchiarico, P., Ferrari, B., Voegele, R. T., Finckh, M. R., Arncken, C., & Hohmann, P. (2022). Genome-wide association study reveals white lupin candidate gene involved in anthracnose resistance. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik, 135*(3), 1011–1024. https://doi.org/10.1007/s00122-021-04014-7

Markers for anthracnose

- SNPs from dataset by Alkemade et al. 2022
  - **Genomic Prediction followed by marker selection** (predictive accuracy up to 0.79)
  - 50 loci selected for marker development

FiBL

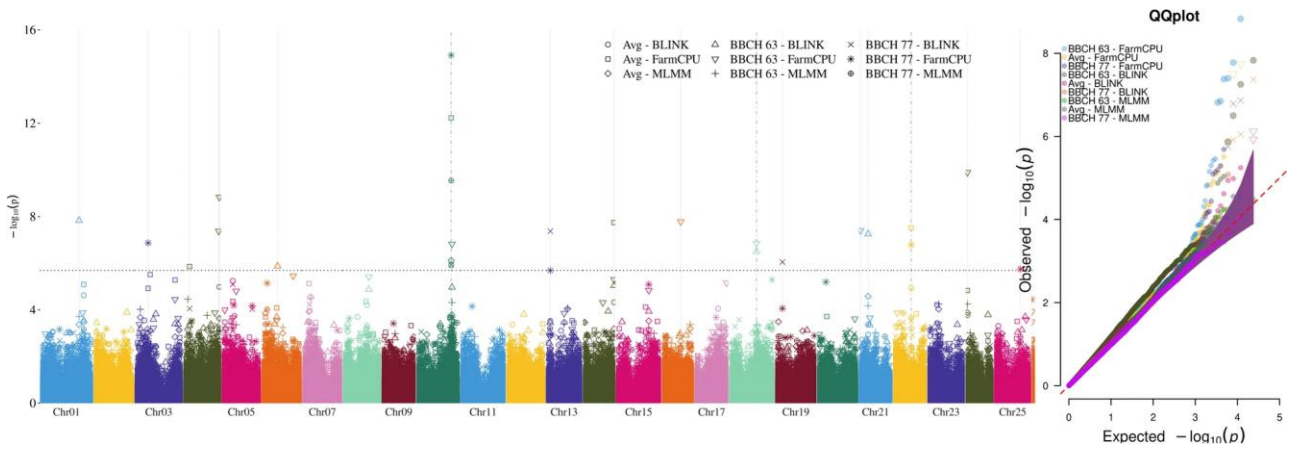
→ predictive ability dropped in validation panel,
further validation with field data ongoing

- SNPs NEWLY identified by GWAS and GS from data in Schwertfirm et al (2024)
  - 254 accessions analysed, 24'534 SNPs
  - phenotyping in field conditions

LfL

FiBL

→ **few markers confirmed** in validation panel, further validation with field data ongoing



Schwertfirm, G., Schneider, M., Haase, F. *et al.* Genome-wide association study revealed significant SNPs for anthracnose resistance, seed alkaloids and protein content in white lupin. *Theor Appl Genet* **137**, 155 (2024). https://doi.org/10.1007/s00122-024-04665-2

Genomic Prediction followed by marker selection

Genomic predition (based on high number of marker,

eg from WGS or GBS)

>>marker selection (to keep «good» predictive ability and have a number of markers that is affordable to genotype, eg 96 markers for Biomark X array)

Application of Genomic Selection based on the reduced number of markers

ipmorama.eu

LiveSeeding

GS in national project LuZIA

Quiz

www.menti.com

CODE **2251 7590**

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Homework

Present an example (real from your work or potential from literature) of Marker Assisted Selection in a crop of your interest

https://survey.fibl.org/index.php/281841?lang=en

Send answer via the link before by 11 March 2025

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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). 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.

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