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UNIVERSITÀ DEGLI STUDI DI
MODENA E REGGIO EMILIA

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IL SEME DELLA RICERCA

NOVEL BREEDING APPROACHES FOR THE IMPROVEMENT OF PROFESSIONAL SEED PRODUCTION IN HORTICULTURAL CROPS

University of Modena and Reggio Emilia, Research Doctorate in
Agri-food sciences, technologies and biotechnologies

Academic tutor: Prof. Enrico Francia

Academic co-tutor: Dr. Justyna Anna Milc

Company tutor: Dr. Massimiliano Ballardini

Industrial Ph.D Candidate: Dr. Marco Martelli

Cycle – XXXV

Ph.D Workshop - 2021, December 17th

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GENES AND QTLs AGAINTS CHOSEN PEPPER PATHOGENS

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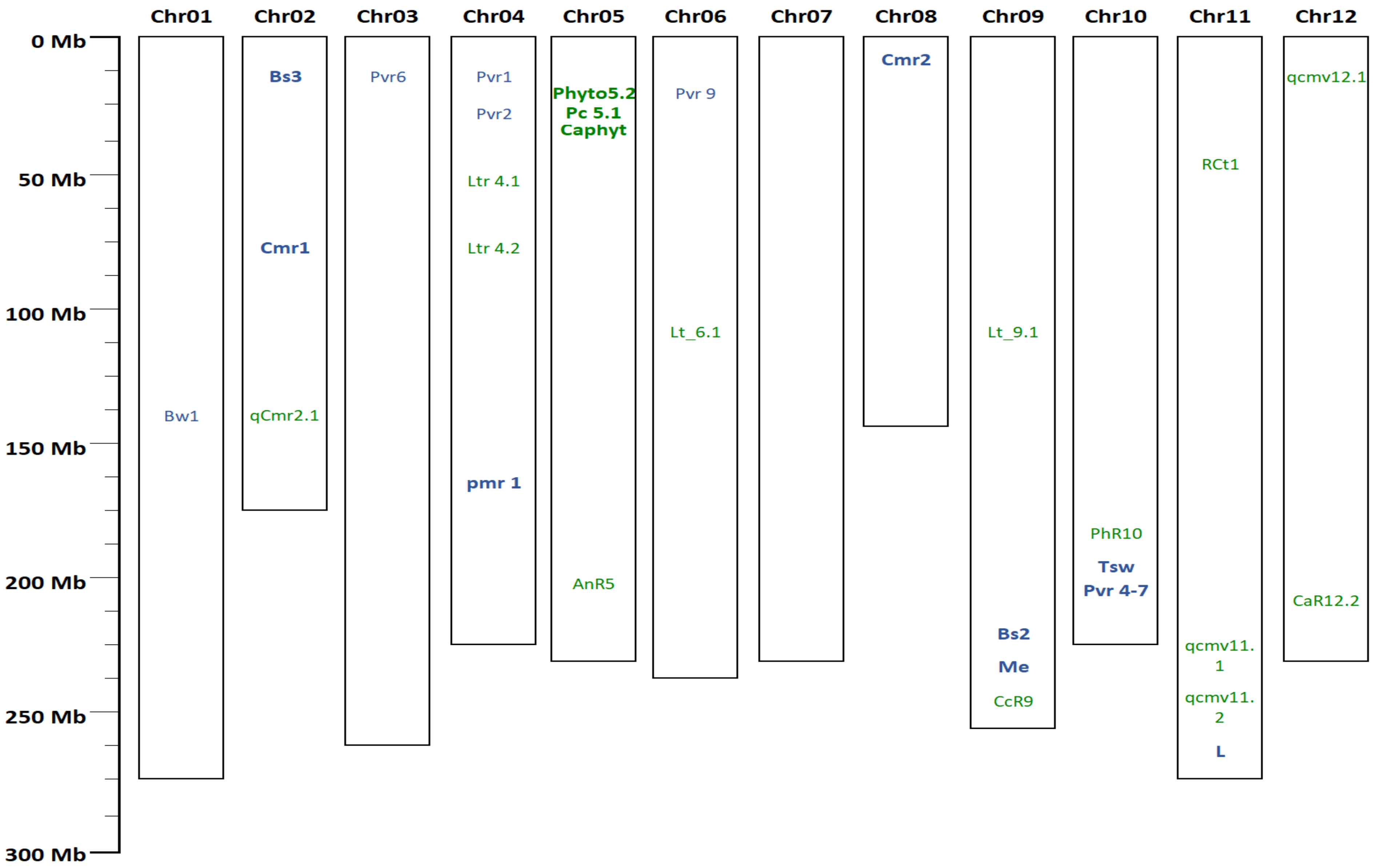
Physical genome map of pepper

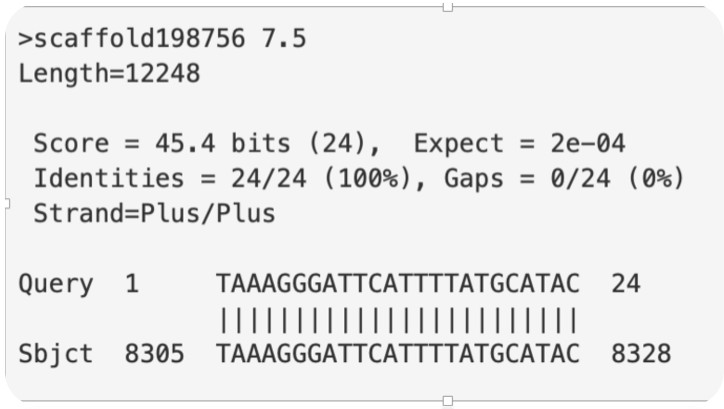


GENES

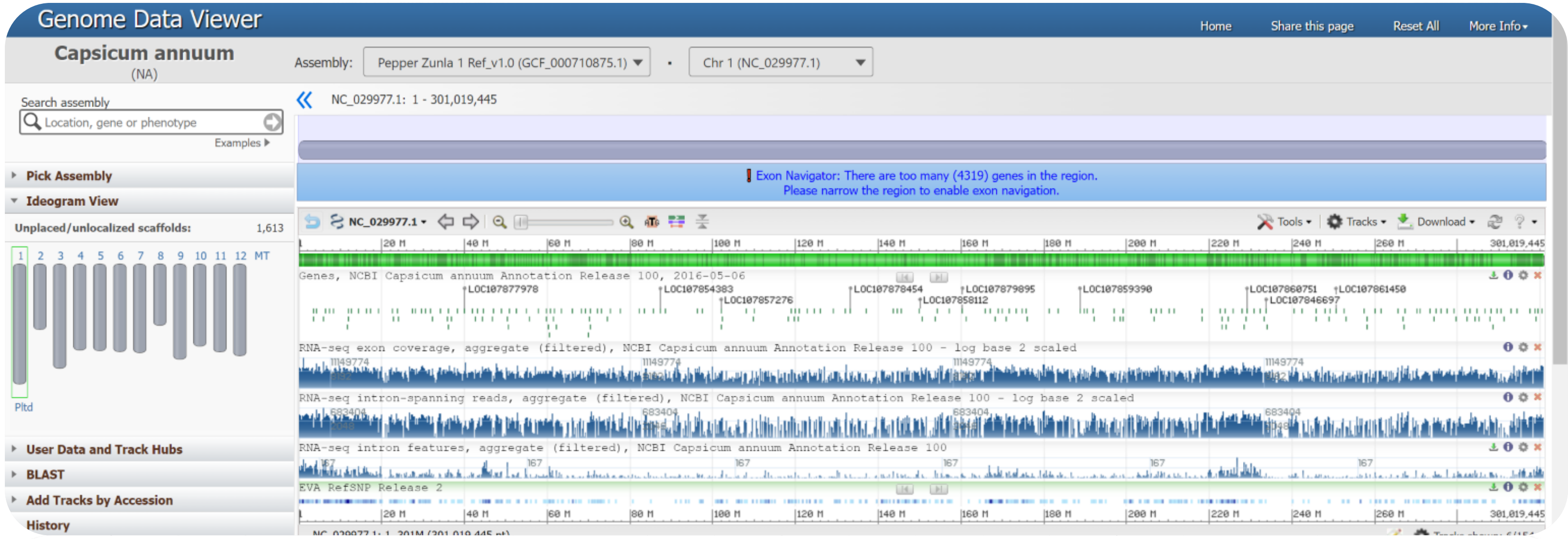
QTLs

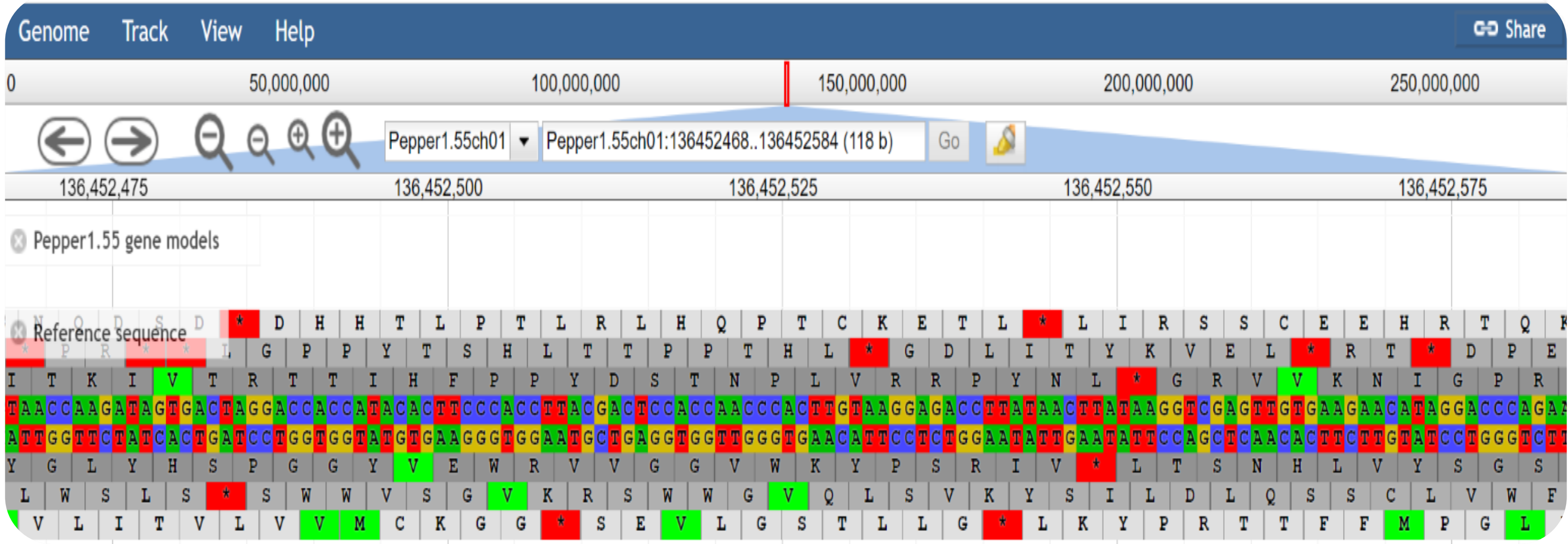
ASSOCIATED MOLECULAR MARKER IDENTIFICATION

- ✓ Blast searches for nucleotide sequence homology
- ✓ Primer retrieved from literature were aligned to genome sequence to confirm their correspondence



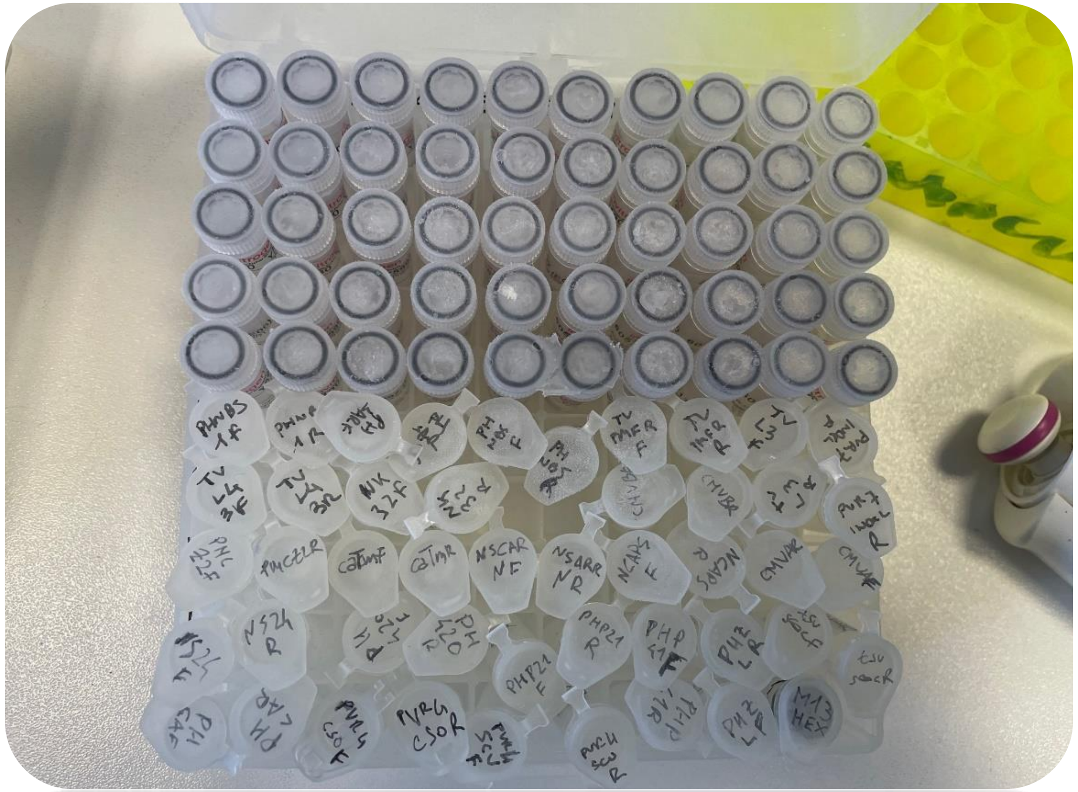
```
>scaffold198756 7.5
Length=12248

 Score = 45.4 bits (24),  Expect = 2e-04
 Identities = 24/24 (100%), Gaps = 0/24 (0%)
 Strand=Plus/Plus

Query  1     TAAAGGGATTCATTTTATGCATAC  24
             ||||||||||||||||||||||||
Sbjct  8305  TAAAGGGATTCATTTTATGCATAC  8328
```





RETRIEVED PRIMER PAIRS

- ✓ 42 primer pairs whose sequence was confirmed were chosen for the preliminary analysis of parental genotypes



gene/qtl	marker name	marker type	primer F(for)R(Rev)	label name	primer sequence
Phyto.5.2 (QTL)	CAMS420	ssr	PhCAMS420F	Ph420F	CACGACGTTGTAAAACGACCAGCGTTCTATCGTCTCAAATG
			PhCAMS420R	Ph420R	TTGACAAACCAGAAATTGATCG
Phyto.5.1 (QTL)	P5-SNAP	caps	PhP5-SNAPF1	PhP5F1	TCATGAGGTTGCTATTAAGATTGGTCCTGTTATATA
			PhP5-SNAPF2	PhP5F2	GAGGTTGCTATTAAGATTGGTCCTGTTATCCG
			PhP5-SNAPR	PhP5R	CATAGAAAGGGATATCATCTGGTACATGCAGAAA
Phyto.5.1 (QTL)	Phyto5NBS1	snp	Phyto5NBS1F	PhNBS1F	CATACAGCCAAAGTTAGAGC
			Phyto5NBS1R	PhNBS1R	GCATAGAGTTCTCCCATTTC
Phyto.5.1 (QTL)	Phyto5SAR	snp	Phyto5SARF	PhSARF	GGGCAGAAAGATTACAATGTC
			Phyto5SARR	PhSARR	TTTTATTCTCACACCATACACG
PhR10 (dominant gene, Phyto.5.2 QTL)	P52-11-21	ssr	PhP52-11-21F	PhP21F	CACGACGTTGTAAAACGACCAATCCAAACAAGTCCTAAG
			PhP52-11-21R	PhP21R	GGTGCAATTGAAAATCTAAG
PhR10 (dominant gene, Phyto.5.2 QTL)	P52-11-41	ssr	PhP52-11-41F	PhP41F	CACGACGTTGTAAAACGACTTGATGAGATGGGAAGTAAA
			PhP52-11-41R	PhP41R	CACCAACAATAATAGAACTACA
Phyto.5.2 (QTL)	ZL6726	ssr	PhZL6726F	PhZLF	CACGACGTTGTAAAACGACTCCAGCCATCCATTATTTCAT
			PhZL6726R	PhZLR	ATCCCGAACTGCCAATAATTA
Phyto.5.2 (QTL)	CA524065	ssr	PhCA524065F	PhCAF	CACGACGTTGTAAAACGACTCTCTCTCTACATCTCTCCGTTG
			PhCA524065R	PhCAR	TGTCGTTCGTCGACGTACTC
Phyto.5.1 (QTL)	NBS1-CAPS	caps	PhNBS1-CAPSF	PhNBSF	AGGACTTTGATAAGGTTTC
			PhNBS1-CAPSR	PhNBSR	TGCAATATAGAGCTTCTGCTG
L3	PMFR11	scar	TVPMFR11F	TVPMFRF	CTGCAGAACAACAATGGCACG
			TVPMFR11R	TVPMFRR	GGACTGCAGAGGAGGAAGC
L3	L3-SCAR	scar	TVL3-SCARF	TVL3F	AACAATTTACAAATAATACACAAGGC
			TVL3-SCARR	TVL3R	TTGGGAAGGAAAGACATCAT
L3	A339-NK	scar	TVA339-NKF	TVA339F	TCTCGGTAGGCCATTTTGCT
			TVA339-NKR	TVA339R	GTAAGTTGCTATGCCCACCA

WORKFLOW

COLLECTION SET UP

160 genotypes choice

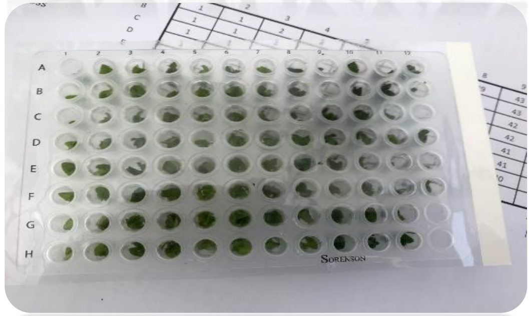
CHECKING RELIABILITY

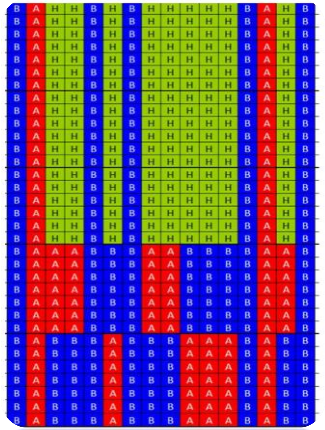
pathology and fingerprinting

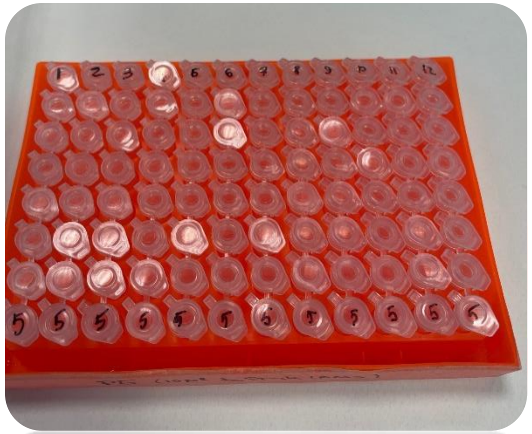
GENOMIC DNA EXTRACTION and STORAGE

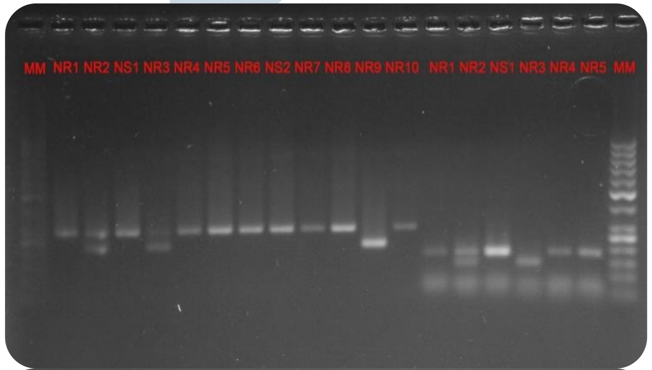
5 plates

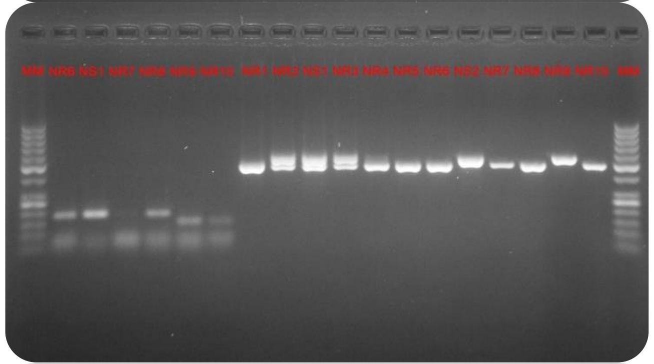
PCR OPTIMIZATION PROTOCOL











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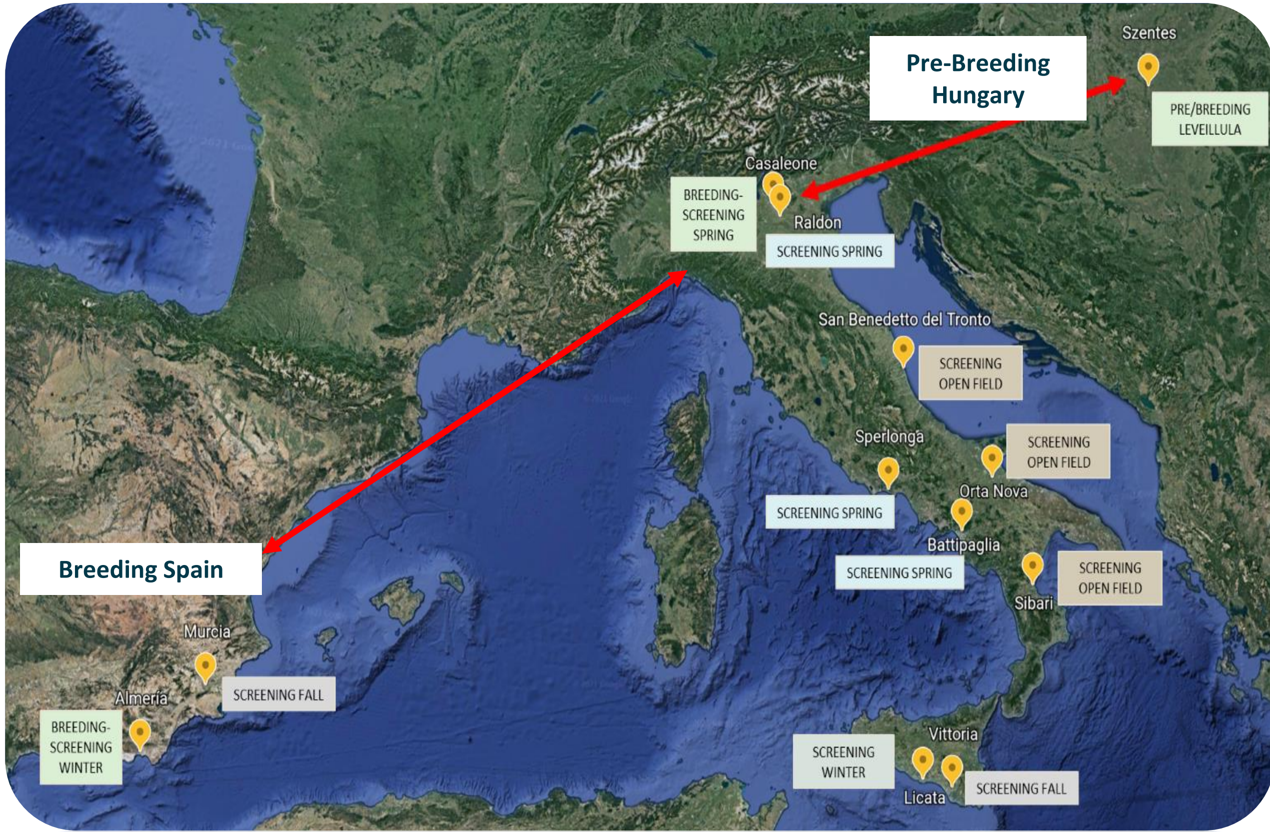
PRE-BREEDING ACTIVITIES

- ✓ **PRE-BREED CROSSES**: performed ca. 100 new combinations for gene introgression and variability expansion.
- ✓ **MULTILOCATION FIELD TRIAL**: evaluation in selective envinroments



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CONCLUSION and FUTURE PERSPECTIVE

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WP1
- GENES AND QTLS AGAINTS CHOSEN PEPPER PATHOGENS

WP2
- ASSOCIATED MOLECULAR MARKER IDENTIFICATION

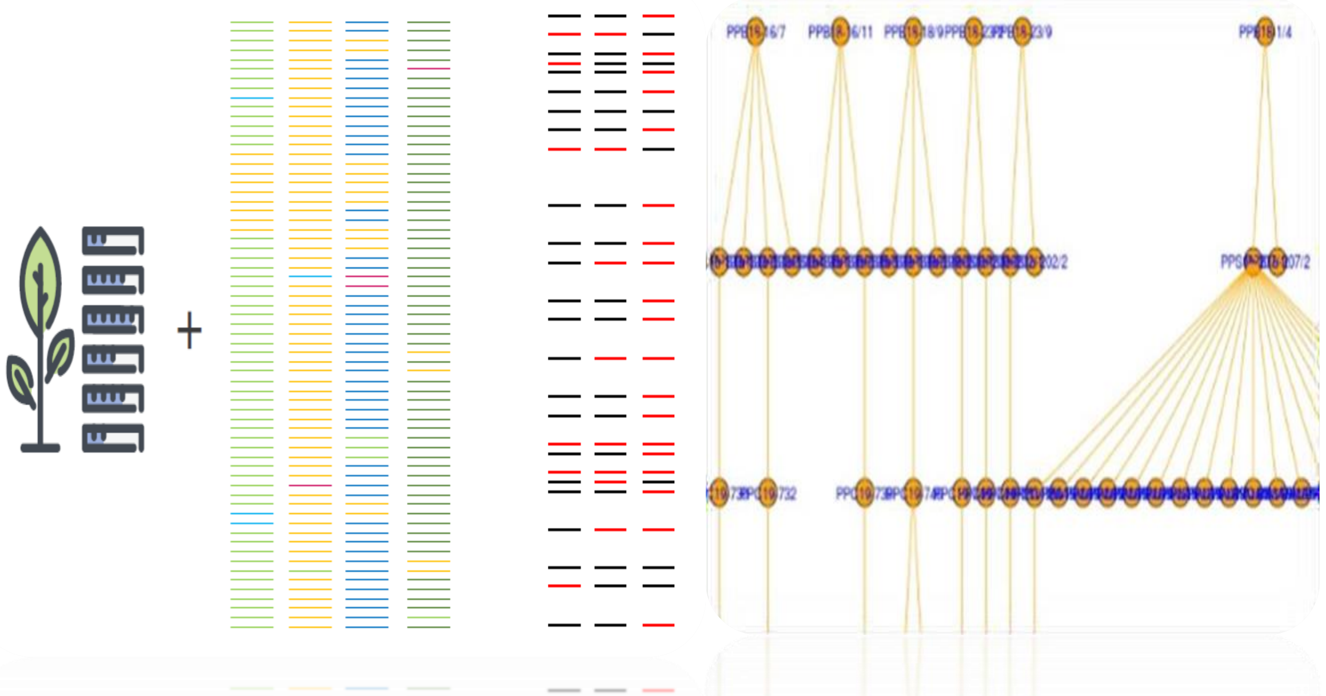
WP3
- RETRIEVED PRIMER PAIRS

WP4
- COLLECTION SET UP for MARKER EVALUATION

WP5
- PRE-BREEDING ACTIVITIES

WP6
- NEW BREEDING SCHEME FOR MULTI-GENE PYRAMIDING

WP7
- EXPLORING ALTERNATIVE RESISTANCES GENE POOL



PREDICTIVE INFORMATION DERIVED FROM MULTI-GENE INVESTIGATION IS PERMITTING A FIRST IMPROVEMENT OF SELECTION PROCESS WITH RELATED GENE PYRAMIDING TIME REDUCTION

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Thank you for your attention!