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Optimizing the genetic resources of a *Capsicum* spp. (peppers) proprietary collection through effective pre-breeding approaches

Marco Martelli[1,2], Giovanni Caccialupi[1], Enrico Francia[1], and Justyna Anna Milc[1]

[1]Department of Life Sciences, University of Modena, and Reggio Emilia, Via Amendola 2, Pad. Besta, 42122 Reggio Emilia, Italy
[2]ESASEM SpA a Socio Unico, Via G. Marconi 56, 37052 Casaleone (Verona) – Italy

Objectives

Genomically profile the **proprietary pepper collection** to support breeding decisions.

Validate molecular markers linked to resistance genes/QTL retrieved from literature.

Resequencing **elite genotypes** to discover informative SNP/InDel for MAS and varietal fingerprinting.

Propose and test a **selection scheme** to pyramid resistance genes and improve environmental adaptability.

Materials & Methods

Germplasm collection genotyping & phenotyping

- Panel of **128 genotypes** from ESASEM proprietary collection
- Multi-site trials (EMEA) and controlled **bioassays** for: **Tobamovirus** (**TMV/ToMV/PMMoV**), **Potyvirus** (**PVY**), **TSWV**, **CMV**, **bacterial spot** (*Xanthomonas* spp), **powdery mildew** (*Leveillula* spp), **Phytophthora capsici**, and **root-knot nematodes** (*Meloidogyne* spp.).
- Phenotypic assignment of resistance classes; survivors advanced to retain target alleles.
- **KASP genotyping (collection profiling)**
- **48-SNP panel** screened across **128 genotypes** (hybrids + inbred lines).
- SNP informativeness assessed; **genetic similarity** (PAST) and clustering by **fruit type/market segment**.

Resistance marker screening (PCR validation)

- Literature review/mining → in-silico primer check → **PCR optimization** → validation on reference set (RR/SS/heterozygotes)
- Marker types: **SCAR**, **CAPS**, **SNAP**, **InDel**.

Whole-genome resequencing (elite set)

- Five elite genotypes: **PM, TSW, L3, L4, CM**. Platform: Illumina NovaSeq (PE150).
- Variant calling (**SNP/InDel** in coding/intronic/intergenic regions) → **genetic distances** and **private SNPs** (emphasis on non-synonymous and stop-gain/loss).

Selection scheme (multi-environment)

- **Top crosses (TC-F1)** among elites evaluated in **four environments**: **W** (winter GH), **S** (spring GH), **F** (autumn GH), **O** (open field).
- Selection from **F1→F4** combining **integrative MAS** (e.g., *L4*, *Tsw*, *Pmr1*) with phenotypic choice; environment-specific pedigree advancement.

Key results

1) Collection structure

- Clustering matches **fruit type** (e.g., blocky/lamuyo, charleston, cayenne) and **market destination** (Western/Eastern EU; extra-EU).
- A concise **molecular pattern** enables rapid type identification and parent selection.

2) Panel of markers associated with resistant genes validated on the proprietary collection (Table 1) – Targeted diseases:

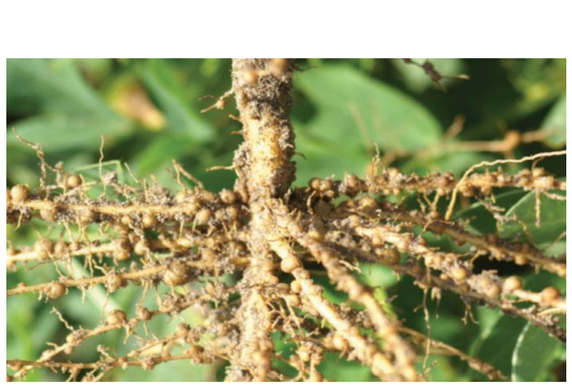
- **Powdery mildew -** *Pmr1* on chromosome 4
- **Tobamovirus -** *L4* on chromosome 4
- **Bacterial spot (*Xanthomonas* spp) -** *Bs2* on chromosome 9
- **Root-knot Nematodes –** *Me1 (M1/Me3/Me4/Me7)* on chromosome 9
- **Tomato spotted wilt virus (TSWV)** - *Tsw* on chromosome 10
- **Potyvirus -** *Pvr4/Pvr7* on chromosome 10
- **Cucumber mosaic virus (CMV) -** *Cmr1/Cmr2* on chromosome 2

3) Elite resequencing

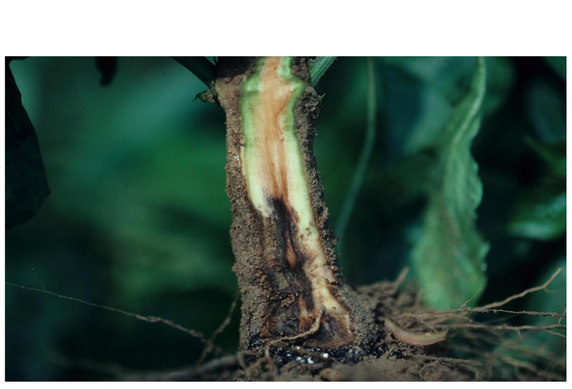
- **>1M SNP/genome** with overall **narrow genetic base** among elites.
- **A set of 8–10 private SNPs/genotype** with stop-gain/loss → candidates for **varietal fingerprinting** and IP protection; immediate utility for purity control.

4) Selection scheme

- **3 four-way top crosses** × **~200 plants/environment**; early MAS on *L4/Tsw/Pmr1*.
- **F2** (~**2000 seeds**) split across the four environments; integrative MAS retaining heterozygotes; **F3** bulks; **F4** single-plant derivation → **21 F4 populations**.
- Expected outcome: **Fn elites** with enhanced **plasticity** (environment-typed WINTER, SPRING, FALL, OPEN FIELD) and **pyramided resistance**.



Root-knot nematodes

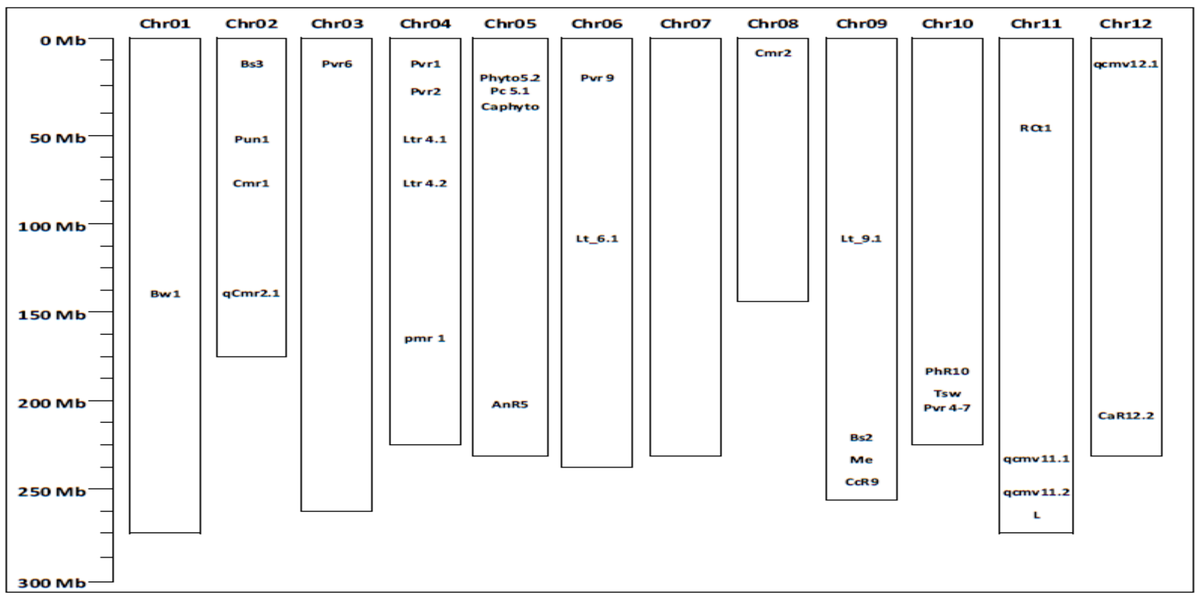


Phytophthora capsici root rot

Tomato spotted wilt orthotospovirus

Tobamovirus

Cucumber mosaic virus (CMV)



Position of disease resistance genes or QTLs on pepper genome

Target gene	Marker name	Marker type	Restriction enzyme	Marker result	Source
Pmr1	ZL1_1826	Scar	-	Codominant	Jo et al., 2017
L4	L-V0-4	Scar	-	Codominant	Lee et al., 2012
Bs2	14F/14R	Scar	-	Dominant	Troung et al., 2011
Me-1	16880-1-V2	Scar	-	Codominant	Wang et al., 2018
Tsw	SCAC-568	Caps	XbaI	Dominant	Di Dato et al., 2015; Özkaynak et al., 2014; Moury et al., 2000
Pvr-4	CSO	Caps	CaiI (AlwNI)	Codominant	Caranta et al., 1999; Özkaynak et al., 2014
Cmr 1	CAPS A	Caps	XbaI	Codominant	Kang et al., 2010

Table 1. Promising markers identified during screening activity.

Take-home messages

48-SNP KASP panel: maps collection structure and fruit-type classes; useful for QA/QC and parental choice.

Validated marker set for key resistance genes (Tobamovirus, TSWV, PVY, CMV, bacterial spot, powdery mildew, nematodes) ready for **routine MAS**.

Elite WGS: immediate resources for **diagnostic markers** and **fingerprinting** via non-synonymous/stop-codon private SNPs.

Selection scheme: an operational framework to combine **G×E** learning with **gene pyramiding**, scalable in industrial pipelines