Molecular marker application for tomato improvement in Thailand

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SEED CLUSTER

Suppliers
Core Activities
Producer → Trader → Grower

Service providers
Logistics
Finance
HR

Government Supporters
Min. Agriculture
Min. Industry
Min. Commerce
Min. Treadury
Min. Education
Min. S&T
Etc.

Privates
Industry council
Seed Assoc.
Seed trader Assoc.
PB Assoc.
Botanical Assoc.
Ppath Assoc.
Etc.

Program Seed Cluster
Resources/Capabilities/Capacities

NSTDA
Seed Cluster

NSTDA
Ministry of Science and Technology
Domestic consumption/Industry
Yield increase from 4-6 t/0.16 ha to 6-8 t/0.16 ha major diseases: BW, TYLCV, Nematode,

Export
From 245 MB to 434 MB

Germplasm collection & evaluation

KKU 377acc. TVRC 400 acc.

Evaluate
• Descriptor
• BW
• TYLCV
• Nematode
• Lycopene

Small fruit & Table type

Processing type

High Lycopene and vitamin

KKU

High Lycopene and vitamin

Disease resistance tomato
BW, TYLCV, Nematode

MAS for disease resistances

NILs for DR
• BW
• TYLCV (Ty2)
• Late blight (Ph3)
• Nematode
• TMV (Tm-2a)
• TSWV
• Powdery mildew (Lv)
• Bacterail speck (Pto)

NIL field trial ('07)

NILs field trial ('07-'08)

Breeding and Technology / basic knowledge

Company

DOA

KKU

UBU

KKU

DOA

BIOTEC

KKU

BIOTEC

Company

GMO

Late blight (Ph3)

TYLCV

Company

DOA

KKU

BIOTEC

KU
Disease resistance genes on tomato chromosomes

Modified from Lindhout (1996)
Disease Resistance

Tomato diseases in Thailand

1. Root-knot nematode
2. Fusarium wilt
3. Late blight
4. Tobacco Mosaic Virus
Backcross Scheme;

Target gene DR

Recurrent genome (%)  50:50  75%  87.5%  ...  99.22%
Chunwongse et al., 2005; Near Isogenic Lines (NIL) of P502 and SD3

Backcross; Disease Resistance

- **P502 x Donor\(^{DR}\)**
  - P502 x F\(_1\)^{DR}
  - P502 x BC\(_1\)^{DR}
  - P502 x BC\(_2\)^{DR}
  - BC\(_6\)
  - NIL P502 + DR

- **SD3 x Donor\(^{DR}\)**
  - SD3 x F\(_1\)^{DR}
  - SD3 x BC\(_1\)^{DR}
  - SD3 x BC\(_2\)^{DR}
  - BC\(_6\)
  - NIL SD3 + DR
Disease Resistance

1. NIL + Mi

2. NIL + I2

3. NIL + Tm2

4. NIL + Ph-3

Chunwongse et al., 2005
Marker Assisted Selection: MAS

- Indirect selection
- Quantitative Trait Loci (QTLs)
- Gene pyramiding

Positive marker assisted selection

Background marker assisted selection
1. Gene Pyramiding
(NIL SD3 + I2-Tm2-Ph-3-Mi)

2. Near isogenic lines
(NIL SD3 + brix9-2-5 -I2-Tm2-Ph-3-Mi)
1. Gene Pyramiding

NIL SD3+I2 (1)  
NIL SD3+Tm2 (2)  
NIL SD3+Ph3 (3)  
NIL SD3+Mi (4)

Heterozygote (1) (2)  
Heterozygote (3) (4)

Heterozygote (1,2) (3,4)

Self

Homozygote (1,2,3,4) (1,2,3,4)

Selection
Generation 1

Selection
Generation 2

Selection
Generation 3
<table>
<thead>
<tr>
<th>DNA markers</th>
<th>Resistance genes</th>
<th>Diseases</th>
<th>Type of DNA marker</th>
<th>Restriction enzyme</th>
<th>Size (S,R) bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>S20</td>
<td>l2</td>
<td>Fusarium wilt</td>
<td>CAPS</td>
<td>Dra I + Hha I</td>
<td>900, 835</td>
</tr>
<tr>
<td>TG207</td>
<td>Tm2</td>
<td>TMV</td>
<td>CAPS</td>
<td>Hinf I</td>
<td>384, 618</td>
</tr>
<tr>
<td>TG591</td>
<td>Ph-3</td>
<td>Late blight</td>
<td>CAPS</td>
<td>Hinf I</td>
<td>355, 255</td>
</tr>
<tr>
<td>Mi2</td>
<td>Mi</td>
<td>Root – knot nematode</td>
<td>CAPS</td>
<td>Hinf I</td>
<td>450, 360</td>
</tr>
</tbody>
</table>

The images below show the DNA profiles for each DNA marker:

- **l2**: 1 kb, 750 bp, 500 bp, 2000 bp, 1500 bp, 1000 bp, 750 bp, 500 bp
- **Tm2**: 1 kb, S, R
- **Ph-3**: 1 kb, S, R
- **Mi**: 1 kb, S, R
Results

Generation 2; Heterozygotes for $I_2$, $Tm_2$, $Ph\,-3$, $Mi$

- $I_2$
- $Tm_2$
- $Ph\,-3$
- $Mi$
Results

Generation 3,4;

Homozygous *I2, Tm2, Ph-3, Mi*

**I2**

**Tm2**

**Ph-3**

**Mi**

F4  Homozygous for 4 genes (*I2, Tm2, Ph-3, Mi*)
Results

NIL SD3 + *l2-Tm2-Ph-3-Mi* (NIL SD3+ 4DR)
Results

NIL SD3+ 4DR # 32-14
Late blight resistance gene (Ph-3) targeting and cloning
Late blight resistance genes

- *Ph* (Steven and Rick, 1986) or *Ph-1* (Moreau et al., 1998) chr 7 from WV106
- *Ph-2* (Turkensteen, 1973; Moreau et al., 1998) chr 10 from WV700
- Another source of resistance from *L. pimpinellifoilum* ‘L3708’->single gene dominant
Populations

- F2 from cross of CLN657-BC1F2-274-0-15-4 (susceptible) and L3708 (resistant)
- Introgressed lines (Ehsed and Zamir, 1994)
Bulk Segregant analysis

- 9 highly resistant --> R-pool
- 8 highly susceptible --> S-pool
- AFLP 120 primer pairs screening
Introgress lines (ILs)

Fig. 2. *L. pennellii* chromosome segments in the fifty tomato introgression lines (IL). Bars to the left side of the chromosome indicate the introgressed region in the lines.

Chromosome localization of Ph-3
Molecular mapping of the *Ph-3* gene for late blight resistance in tomato

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SUMMARY

Late blight, caused by *Phytophthora infestans* (Mont.) deBary, is a major disease of tomato (*Lycopersicon esculentum* Mill.) in cool, wet environments. In this study we report on the mapping of a gene in *L. pinninellifolium* (Just.) Mill. accession L3708 conditioning race-specific resistance to *P. infestans*. F₂ tomato plants from the cross CLN657 (susceptible) × L3708 (resistant) and segregating for late blight resistance were evaluated for resistance to *P. infestans* isolate Pi-16 that overcomes host resistance genes *Ph* and *Ph-2*. Resistant and susceptible DNA pools, created by bulking equal amounts of DNA from nine resistant F₂ plants (for R-pool) and eight susceptible F₂ plants (for S-pool), and the DNA pools were used to screen amplified fragment length polymorphic (AFLP) primers. Polymerase chain reaction (PCR) using 120 combinations of three-base selective nucleotide primer pairs revealed five DNA bands from five primer pairs unique to the resistant parent and R-pool, and a DNA band from one primer pair associated with the susceptible parent and S-pool. After DNA band isolation and cloning, two single-copied DNA clones associated with resistance were hybridized with 50 introgression lines (IL) derived from *L. esculentum* × *L. perennis*; IL9-3, containing an introgression from the long arm of chromosome 9, was the only polymorphic IL. Restriction fragment length polymorphism (RFLP) markers mapped to chromosome 9 and two AFLP-derived RFLP markers were used to probe a second F₂ population. A major quantitative trait locus (QTL) with an LOD score of 18.41 was found at RFLP marker TG591; it accounted for 71.4% of the variance. This gene, *Ph-3*, is non-allelic to tomato late blight resistance genes *Ph* (previously mapped to chromosome 7 or *Ph-2* (mapped to chromosome 10).
Candidate clones identification through map based gene cloning approach

1 Additional of new marker
DNA pool R&S → AFLP → New markers

2 Identification of recombination break points
F2 Pop. → recombinant screening → Recombinants (heterozygous) → Selfing & Selection → Recombinants (homozygous) → Candidate clones

3 Physical mapping
Recombinants (homozygous) → DNA sequencing, PCR Primer → Positive clones → Clone Fingerprint → HMWD contigs

4 High MW DNA library
L3708 → Cloning → 3 x BIBAC → Library screening → Positive clones → Clone Fingerprint → HMWD contigs

HMWD → 8 x pCLD

Heinz 1706 → High density filters → 15 x BeloBAC

Candidate clones identification through map based gene cloning approach
Genotype pooling for additional of L188 marker using AFLP primer pair GAT/ACC
R=Resistant parent S= Susceptible parent
PR=pool genotype similar to R parent
PS=pool genotype similar to S parent

(Boonruangrod et al., 2001)
<table>
<thead>
<tr>
<th>Marker</th>
<th>Value</th>
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<tbody>
<tr>
<td>TG390</td>
<td>4.0</td>
</tr>
<tr>
<td>TG551</td>
<td>2.8</td>
</tr>
<tr>
<td>TG404</td>
<td>1.9</td>
</tr>
<tr>
<td>TG186</td>
<td>2.0</td>
</tr>
<tr>
<td>TG429</td>
<td>1.0</td>
</tr>
<tr>
<td>TG348</td>
<td>3.0</td>
</tr>
<tr>
<td>TG248</td>
<td>1.0</td>
</tr>
<tr>
<td>GP94B</td>
<td>2.0</td>
</tr>
<tr>
<td>CT74</td>
<td>1.7</td>
</tr>
<tr>
<td>GP129</td>
<td>2.2</td>
</tr>
<tr>
<td>CT198</td>
<td>3.9</td>
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<tr>
<td>CT218</td>
<td>3.8</td>
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<td>TG421</td>
<td>5.5</td>
</tr>
<tr>
<td>TG424</td>
<td>5.9</td>
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<tr>
<td>GP101</td>
<td>4.5</td>
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<tr>
<td>CT112A</td>
<td>2.2</td>
</tr>
<tr>
<td>TG591A</td>
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<tr>
<td>CT71</td>
<td>2.7</td>
</tr>
<tr>
<td>CT220</td>
<td>Sw-5</td>
</tr>
</tbody>
</table>

Mapping of new additional markers to The long arm of chromosome 9
Graphical genotype of 19 homozygous recombinants with additional markers, 19S, 9S, 5T and 2S. Gray denotes DNA from L3708 (R) and black from CLN (S). Five informative RCs is depicted in the right panel.
Tomato BIBAC clones digested with *Not* I and separated on 1% agarose gel with pulsed condition as following: 1-40 sec switch time at 6 V/cm for 18 hours and 14°C.
Identification of TG591 hybridized BAC spots within grids. A) Scan images of hybridized filters showing hit spots for 165P17, 78E12 and 292I24. B) Enlarged images of the positive spots. C) Bioarray software generated dots within grid showing the duplicated clones in each grid.
Summary

- MAS for disease resistances
- Pyramiding of DR genes into public cultivars
- Late blight resistance gene (Ph-3) targeting/cloning
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