Development of Molecular Markers for MAS in Lettuce (*Lactuca sativa*)

Application of High-Resolution DNA Melting Analysis

Ivan Simko
Lettuce production

• Fresh vegetables in 2008 (mil. $):
  • Lettuce........... 1,979
  • Tomato............ 1,414
  • Onions............. 865
  • Cantaloupes….... 776
  • Peppers.......... 763
  • Sweet corn..... 753
  • Broccoli........... 708
  • Carrots.......... 600

Economic Research Service, USDA
Why do we need markers?

• Conventional breeding is time consuming and depends on environmental conditions.

• Breeders are interested in technologies that could make this procedure more efficient.

• Molecular marker technology offers improved selection strategies in breeding.
Marker-assisted selection

Phenotypic selection

Marker-assisted selection

Desired material
Segregating material
Not phenotyped accurately
Marker-assisted selection

is more efficient than selection based on phenotype

- If evaluation is:
  - Time consuming
  - Difficult
  - Costly
  - Performed off season

- If used for:
  - Gene pyramiding
  - Early stages of development
  - Several selections simultaneously
  - Low heritability traits
MAS in lettuce

- Downy mildew (*Dm3*)

*Dm3* is defeated by new races of *Bremia lactucae*
MAS in lettuce

- Corky root (*cor1*)

60-80% accurate
MAS in lettuce

- LMV ($mo1$)

alleles $mo1^1$ & $mo1^2$
Development of molecular markers for marker-assisted selection of dieback-resistant lettuce
Lettuce dieback

- Mottling
- Yellowing
- Necrosis

- Stunting
- Plant death
Lettuce dieback

- Stem
- Leaf
- Romaine
- Romaine

Healthy | Diseased
---------|----------
Healthy  | Diseased

Diseased

Healthy

Diseased
Material & Methods

- Combination of linkage and association mapping
- 192 RILs (Valmaine x Salinas 88) x Salinas - linkage mapping set
- 68 accessions from all cultivated types of lettuce - association mapping set
- 132 accessions from all cultivated types of lettuce - validation set
- EST-based SNP markers
- High resolution DNA melting curve analysis
- Sequencing
- 61 EST-SSR markers for assessment of population structure
- Replicated field trials
Workflow

- Linkage mapping set
  - 20 markers on LG2
  - 10-20 cM apart

- Linkage mapping set
  - 15 markers
  - Tvr1 region

- Association mapping set
  - Linked markers
  - Sequencing

- Development of High resolution DNA melting curve analysis markers

- Validation set
  - HRM markers

- Association mapping set
  - SNP-trait association

- Association mapping set
  - 61 EST-SSR
  - Population structure

- Markers ready for marker-assisted selection
• Linkage mapping set
• 20 markers on LG2
• 10-20 cM apart

Linkage mapping set
• 15 markers
• Tvr1 region

• Association mapping set
• Linkage markers
• Sequencing

• Development of High resolution DNA melting curve
  • Markers ready for marker-assisted selection

• Association mapping set
• 61 EST SSR
• Population structure
Linkage mapping of *Tvr1*

- Highly significant linkage (< 0.001) with nine markers.
- Each marker explains over 90% of the total phenotypic variation.
- A perfect match with two markers.
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- Markers ready for marker-assisted selection
Association mapping of Tvr1
Haplotypes of Cntg10192

L. sativa
L. sativa
L. serriola
L. sativa & L. serriola
L. saligna
L. virosa

Dissimilarity

0.010 0.008 0.006 0.004 0.002 0.000

SNP position

54 72 100 105 129 207 210 232 243 258 303
Res/Sus # of acc.

T T T G G C T A C C A Res 17
T T C G G C T A C C A Res 19
C T T G G C T A C C A Res 1
C C T G G C T A C C A Sus 33
T T T A G T C C T C G Res 1
C T T A C T C C C T A Res 2
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- **Development of High resolution DNA melting curve analysis markers**

- **Association mapping set**
  - SNP-trait association

- **Association mapping set**
  - 61 EST-SSR
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DNA Melting Curves
DNA Melting Curves
Workflow

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Markers ready for marker-assisted selection
Haplotypes of Cntg10192

Difference Curves

Δ Fluorescence

Temperature °C

R2
R1
R3
S1
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- Markers ready for marker-assisted selection
MAS in lettuce

- Dieback - TBSV (Tvr1)

Tested on 2,000+ accessions
...so far so good...
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