WG1: Vegetable rootstock breeding: genetic variation and selection strategies

Prof Andrew Thompson

19th September, Pula, Croatia

www.cranfield.ac.uk
Working group 1 – summary of activities

1. ~7 international meetings – breeders, experts in germplasm and molecular genetics => collaborations

2. Contribution to Veg. Grafting training school Catania: genetic resources and breeding (AT, Enza Zaden, Rijk Zwaan)

3. Visits to breeding companies with interests in vegetable rootstocks

4. Book chapters:

   Chapter 2: Genetic resources for rootstock breeding
   Belen Pico, Andrew Thompson, Carmina Gisbert, Halit Yetisir, Penelope Bebeli

   Chapter 3: Rootstock breeding: current practices and future technologies
   Andrew Thompson, Belen Pico, Halit Yetisir, Roni Cohen, Penelope Bebeli
WG1: Engagement with seed companies

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<tr>
<th>Company/Group1</th>
<th>Attended meetings</th>
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Market-leading tomato rootstocks Maxifort and Beaufort are coming home to EU, now German owned!
Rootstock traits

WP2
WP3
WP4

Book Chapt 3
Plant Breeding – What is it?

“...to develop superior cultivars.....adapted to specific environmental conditions and suitable for economic production in a commercial cropping system”

1. produce or identify genetically variable germplasm

2. select superior genotypes with specified characteristics (growers, processors and consumers must be happy)

3. stabilize and multiply for commercial release

1. Increase diversity

2. Decrease diversity
The impact of grafting on breeding strategies

Trait stacking is challenging:

1. Mathematics of independent assortment and problems of “linkage drag”

2. Epistatic interactions. The action of one gene may depend on a specific allele at another locus.

3. Pleiotropic effects: genes that affect rootstock may have negative effects on the shoot, if expressed in non-grafted plants.

4. Complex quantitative traits – large number of loci may have a small influence on a trait
The impact of grafting on breeding strategies

Trait stacking is challenging: BUT:
• grafting breaks down one big problem into two easier-to-solve problems

Which squirrel would you rather be?
The impact of grafting on breeding strategies

Grafting makes it easier to deliver improved root traits

1. Adding root traits to elite germplasm by crossing requires reselection of all shoot traits (~10 years). Faster to break one big problem down into two smaller problems with different objectives.

2. Roots traits interact strongly with soil conditions that can vary between fields. Quicker to deploy different rootstock × scion combinations than to locally adapt non-grafted cultivars. But not done in practice!

3. Rootstocks allow deployment of a lot of wild species DNA with far less chance of reducing fruit yield and quality.

4. Potential pleiotropic effects can be avoided.
Mendelian genetics: divide and conquer

Grafted, 2 + 2 genes

Total 32 combinations

$2 \times 4^{n/2}$

Non-grafted, 4 genes

Total 256 combinations

$4^n$

$n = \text{number of loci}$
Mendelian Genetics: Trait stacking – grafting vs non-grafting

- Assumes equal split of selected genes between scion and rootstock
- Linkage makes the problem much worse

![Graph showing the mean number of plants to screen to find homozygous individuals vs the number of genes. The graph includes two lines: one for whole plant (all genes) with a slope of \(4.3 \times 10^9\) and another for grafting with a slope of 131,072.]
Grafting can overcome pleiotropic effects

IL8-3 (S. pennellii single introgression line)

- IL8-3/M83 give 60% **less** yield than M82/M82
- M82/IL8-3 has 20-60% **more** yield than M82/M82.
- Non-grafted IL8-3 plants do worse than M82/M82.

**Benefits of IL8-3 can only be achieved when deployed as a rootstock**

Gur et al 2011
Rootstock breeding strategies

Genomics, mol. genetics

Marker discovery and validation

Marker assisted selection
Genome Editing

Collection of genetic resources

Diversity analysis and production of inbred lines

Land races

Wild species

Bred varieties

Core collection

Stage 1
Screening non-grafted accessions for rootstock traits

Stage 2
Enhancement of selected inbred lines
Production of F_1 hybrid seed
Testing for graft compatibility

Stage 3
Testing rootstock x scion x environment interactions: vigour, yield and quality
Registration, multiplication, testing

New rootstock cultivar release

Book Chapter 3
Post-genomic era: e.g. look up DNA sequence variation in 500 varieties of tomato on “genome browser”

Third generation sequence technologies are accelerating the pace, e.g.
• Hugely improved pepper genome using “10x Chromium technology”
• SL3.0 tomato reference genome to be released soon, PacBio based reference on its way.
• Understanding biological function limits progress, not genomics (see WP2, 3 and 4!)
Example 1: exploiting germplasm

Investigation of wild *Cucumis* species as cucurbit rootstocks to replace standard *Cucurbita maxima × Cucurbita moschata* F₁ hybrids

Parents tested as hybrids grafted to melon:
- *C. metuliferus* (M),
- *C. ficifolius* (F),
- *C. myriocarpus* (My),
- C. Zeyhery
- *C. anguria* (A)

*F x A* and *F x My* selected as:
- Cross-compatible
- Good graft compatibility with melon
- 100% *Fusarium* wilt resistance

Fusarium wilt resistance
Example 2: exploiting germplasm

**Screening Turkish Bottle Gourd (Lagenaria siceraria), germplasm for salinity resistance**

160 accessions were screened at 10 dS m\(^{-1}\) salinity in a hydroponic system (n = 3). Positions of two commercial rootstocks are given

A significant number of accessions showed less growth reduction than the commercial rootstocks
Example 3: searching for molecular markers

Fine mapping of a QTL for root vigour in tomato to an interval of eight genes (*S. lyc.* × *S. pennellii* introgression lines)

Chromosomes: parents and recombinants

*S. penn* M82 Rec1 Rec2

Root vigour in reciprocal grafting

N = 6, error bars = LSD 5%

Thompson et al
Conclusions

• WG1 has been beneficial for collaboration including with seed companies

• Book chapters 2 and 3 will be useful resources.

• Genomic technologies are running way ahead of biological understanding

• Public research and is vital to increase understanding of traits and to disseminate information on useful germplasm

Thanks!

Future?