

Identification of Bitter Pit Markers in *Malus domestica*

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Bitter Pit

- Apple, pear, and quince disorder.
- Occurs at harvest and during storage.
- Thought to be caused by a lack of calcium.
 - Unable to accurately predict bitter pit incidence (high correlation with no predictive accuracy).



Why Bitter Pit Research is Important

- To improve marketable fruit quality
- Reduce fruit loss
- Reduce financial Loss

Objectives

- To investigate if the disorder bitter pit in apple is exclusively caused by a calcium deficiency.
- Using a proteomic approach, Identify proteins of interest which may serve as markers of bitter pit.



Known Studies

+100 Bitter Pit *and* foliar application articles

+13,500 Apple *and* genetics article (+1500 post-genome introduction)

1 Apple *and* bitter pit *and* genetics article

+100 Apple *and* protein articles (cancer, anti-inflammatory, Allergen)

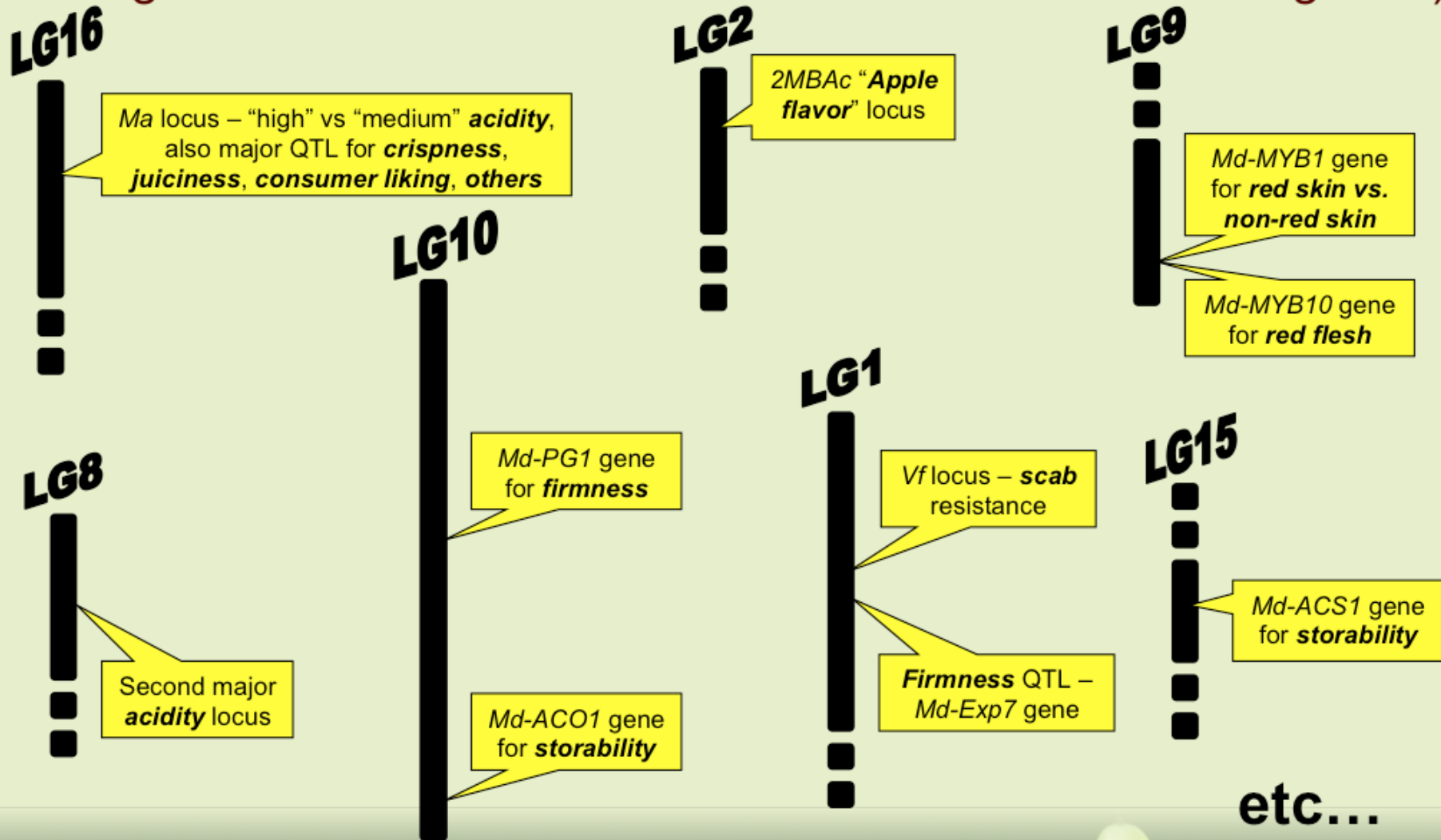
3 Apple *and* protein articles (MS, Databases, Gels)

1 Bitter pit *and* protein article

Val, J., Gracia, M.A., Blanco, A., Monge, E., Perez, M. 2006. Polypeptide Pattern of Apple Tissues Affected by Calcium-related Physiopathologies. Food Sci Technol Int, 12(5):417-421.

Some Apple QTLs Known Previously

(focusing on Mendelian Trait Loci and QTLs with candidate genes)



RosBREED

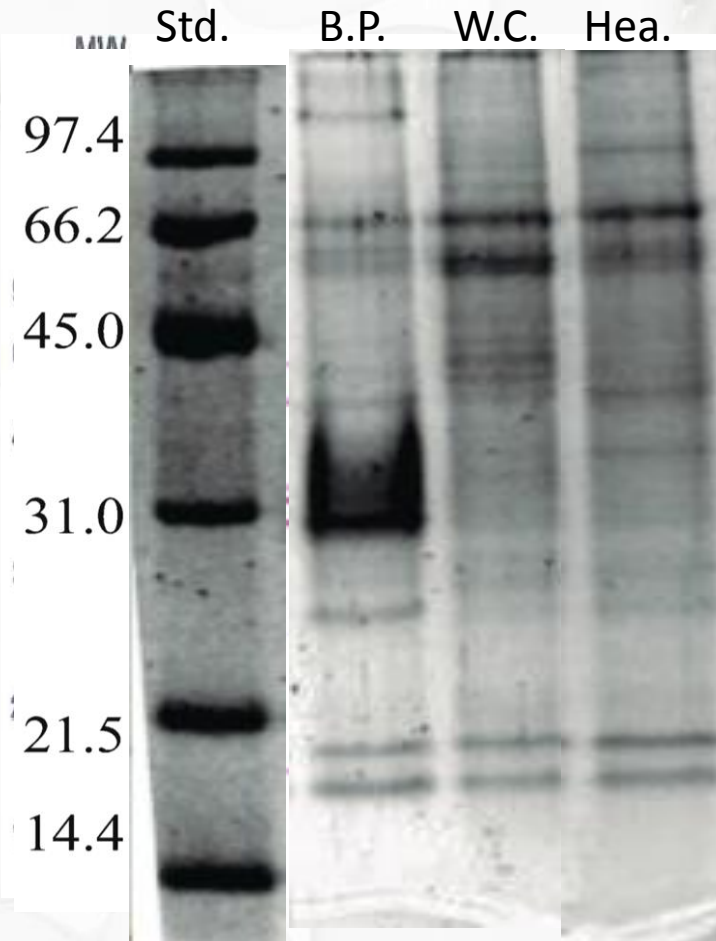
marker-assisted breeding in Rosaceae



www.rosbreed.org

Project Synopsis

- Personal gel electrophoresis data suggested apple samples (bitter pit, healthy, and water core) produced a 18 kDa protein band, a protein result previously reported to be found only in bitter pit.



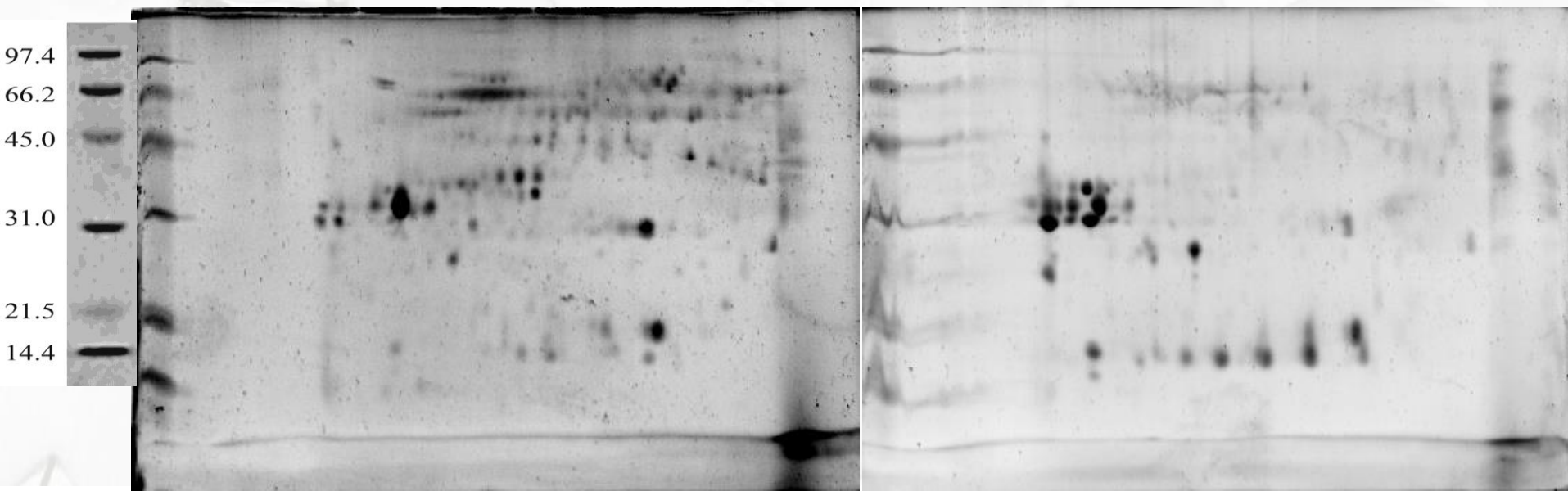
Val, J., Gracia, M.A., Blanco, A., Monge, E., Perez, M. 2006. Polypeptide Pattern of Apple Tissues Affected by Calcium-related Physiopathologies. *Food Sci Technol Int*, 12(5):417-421.

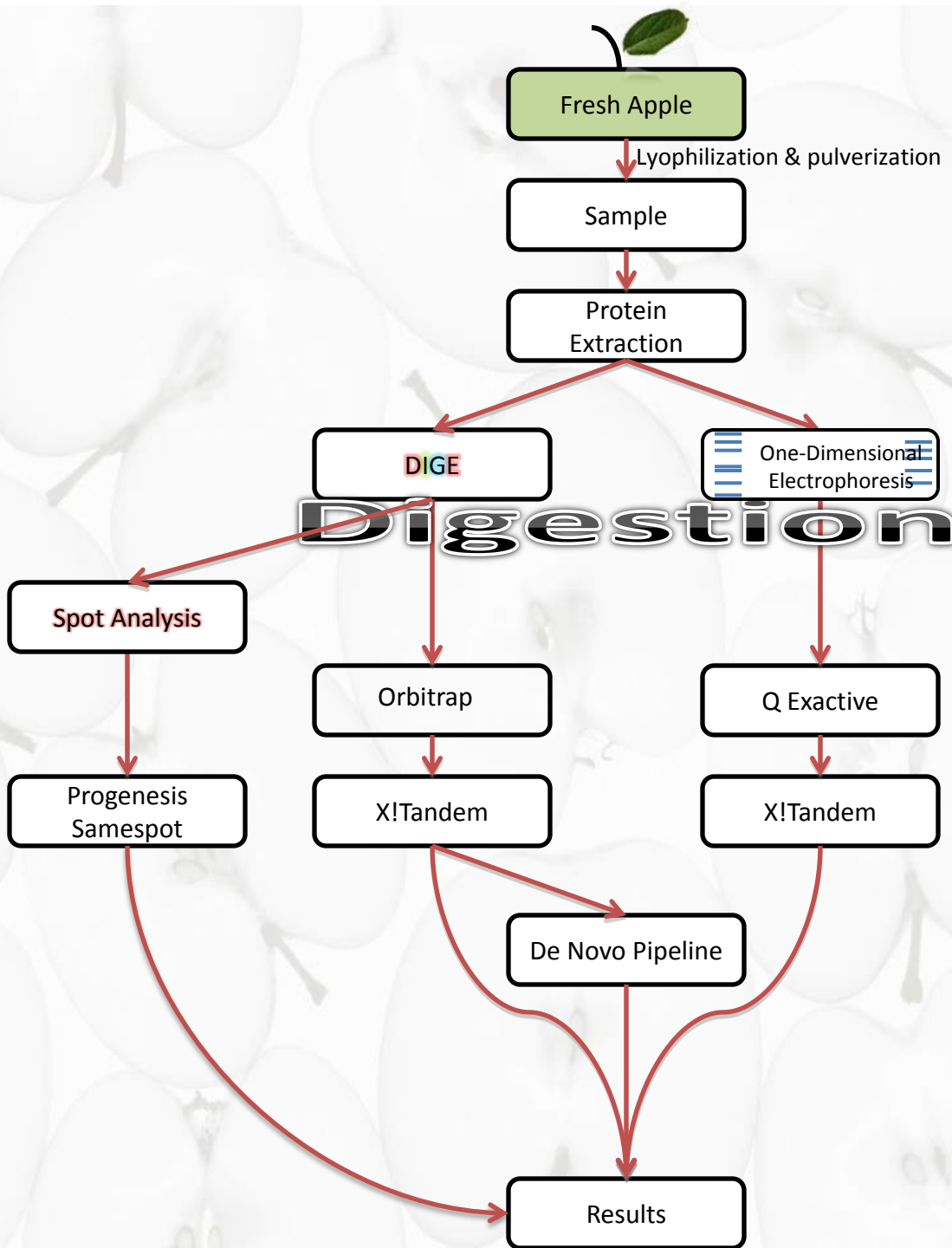
Project Synopsis cont.

- 2D gels (IPG: 3-10, 4-7) were infused with bitter pit and healthy extracted protein. Results expressed several distinct differences between 17 and 36 kDa.

Healthy

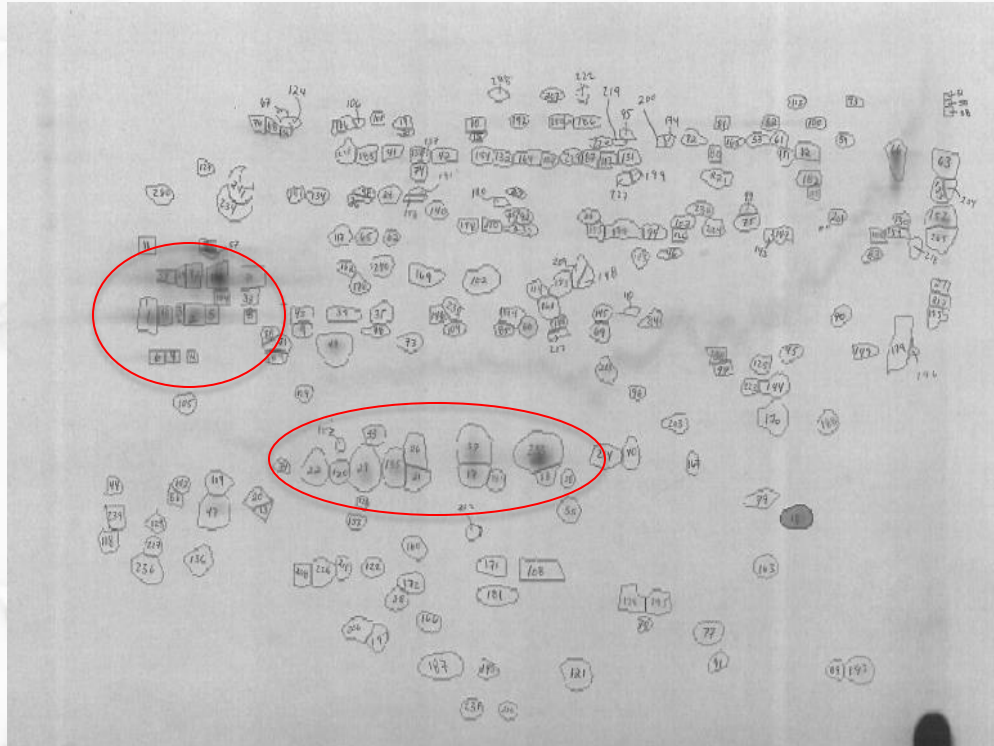
Bitter Pit





Project Synopsis cont.

- DIGE gel analysis (16%, IPG:4-7) was performed, eliminating gel to gel variation seen in 2D. Several spots were identified to be of interest.





DIGE Gel Scan at 600 PMT

100-200 spots cut

2 Gels

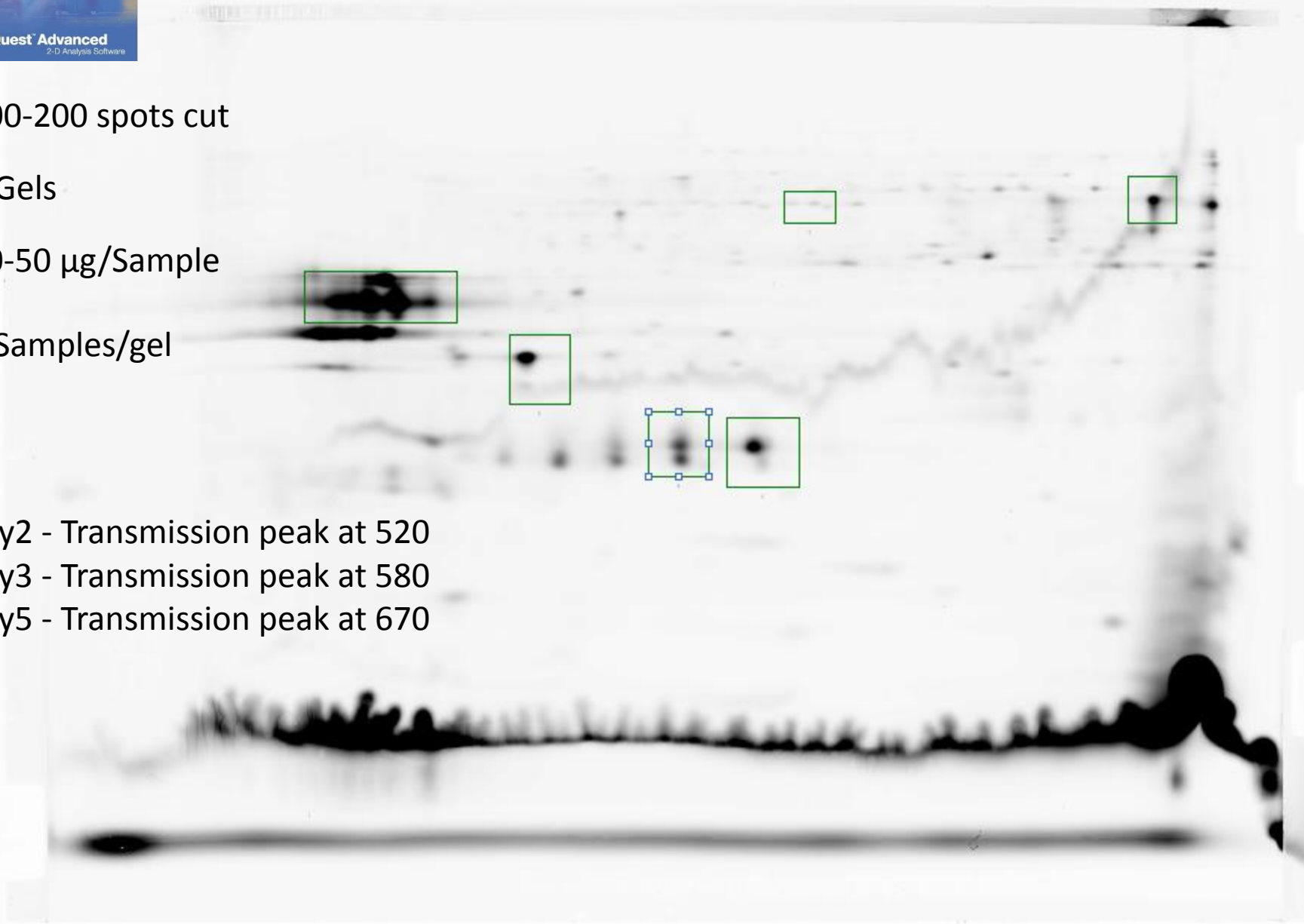
20-50 $\mu\text{g}/\text{Sample}$

3 Samples/gel

Cy2 - Transmission peak at 520

Cy3 - Transmission peak at 580


Cy5 - Transmission peak at 670

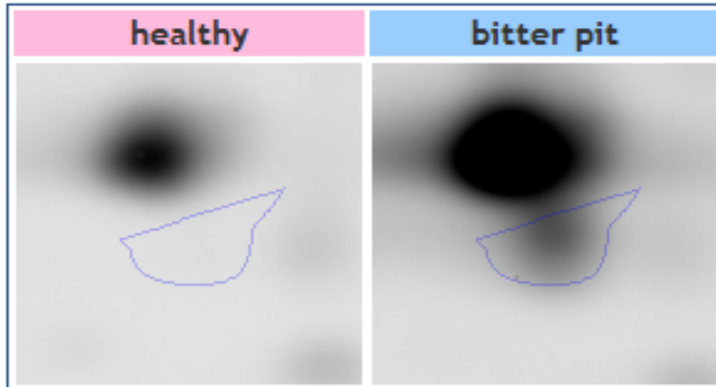


Mal d 2

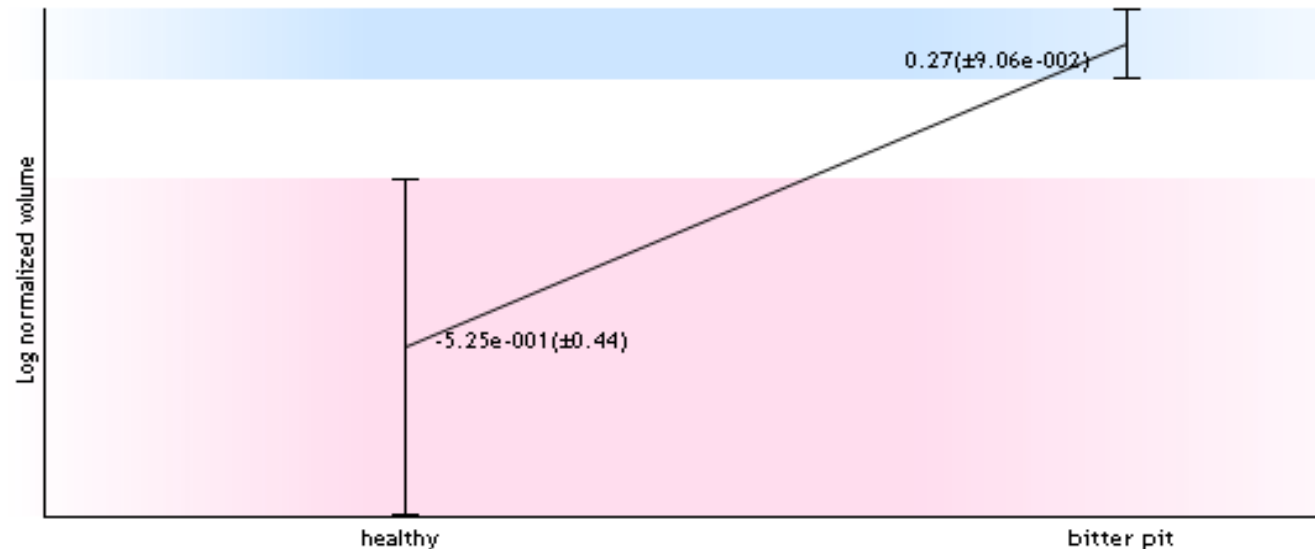
Position (1561, 1148)

Notes eID: 0159

 p<0.05

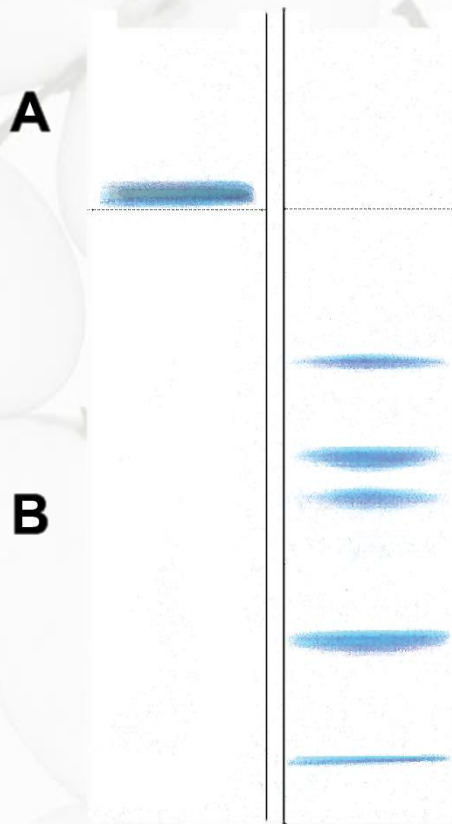


Mal d
Allerg
BetVI
2.4%



18.0%

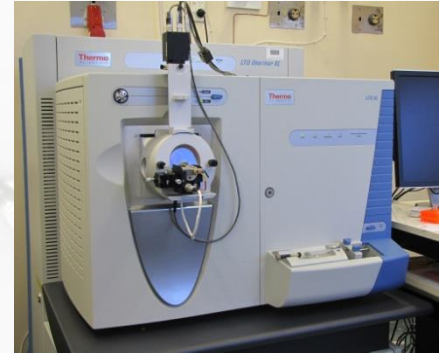
In-gel Digestion (Trypsin)



- 20 μL (10 μg) Protein were injected onto wells
- Short Migration gels were cut, digested, then injected into the Q-Exactive
- Proteins were analyzed with the most recent genomic database

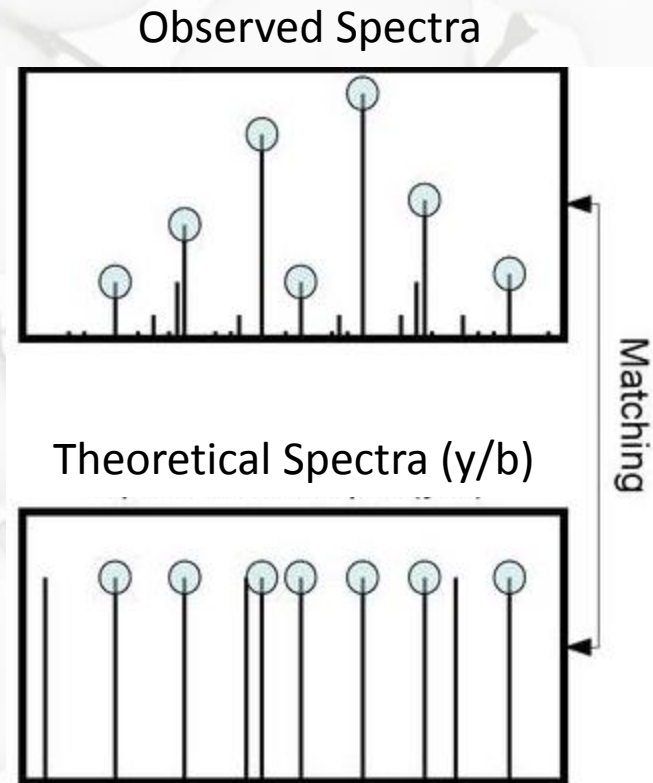
Equipment

- Equipment
 - LC-LTQ-Orbitrap
 - Acquired in 2008
 - LC-Q-Exactive
 - Acquired in 2010
 - Produces 3 to 4 times more identified protein results than Orbitrap



Data Interpretation

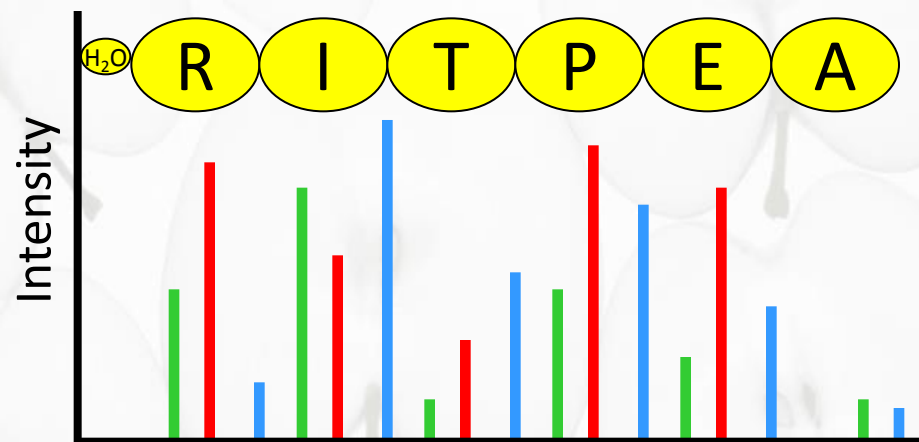
- Bioinformatics
 - X!Tandem
 - Protein mass matching
 - Matches the peaks between the observed and theoretical spectra. (produces a score)



Data Interpretation

– De Novo Pipeline

- Interprets the mass spectra (fragmentation).
- Produces an amino acid sequence tag.
- The sequence tags are used to find similarities in a heterologous database.



Database name	Source	# of proteins	Sequence type	Contents of Database
Uniprot_Malus_domestica	Uniprot.org	1,943	Direct Protein (annotated)	<i>Malus domestica</i> +allergens (incomplete)
Uniprot_Rosaceae	Uniprot.org	11,015	Direct Protein (annotated)	Malus, Pyrus, Rubus, Fragaria, Prunus, Rosa, etc.
IASMA (Italian) peptidic database	iasma.it	30,294	Direct Protein	<i>Malus domestica</i> (Complete Genome)
TGI_Malus_domestica	Harvard.edu	113,316	(t)-EST – Cluster 100	<i>Malus domestica</i>
Uniprot_Superkingom_Eukaryota	Uniprot.org	6,355,736	Direct Protein	All Eukaryota
UniprotKB/TrEMBL	Ebi.ac.uk	23,165,610	Direct Protein	Archaea, Bacteria, Eukaryota, Viruses

The background of the slide is a repeating pattern of white grape slices, showing the seeds and stems, arranged in a grid-like fashion.

Results

Results

Results

- Gel analysis

- DIGE

- 46 Spots were software identified as having $p < 0.05$

- 19 Spots (12 unique proteins) were identified utilizing the genomic database (Orbitrap):

- » Major Allergen Mal d 1 (3)

- » Thaumatin-like protein (TLP) (1)

- » PR-8 proteins (2)

- » 1-aminocyclopropane-1-carboxylate oxidase 1 (ACO) (2)

- » UTP-glucose-1-phosphate uridylyltransferase (UGPase) (2)

- » Chitinase (1)

- » Metal binding (1)

Results

- Short migration
 - **1543 Proteins identified** using Genome Database (Q-Exactive)
 - **170 Proteins identified** ($p < 0.05$) – For both Bitter Pit and Healthy
 - 32 Healthy Proteins identified with \geq Two-Fold Spectra and $P < 0.05$

Notable Proteins:

 - MDP0000937986/CAP160 repeat (15.5 Fold)
 - MDP0000193489/Metabolic Process Protein (7 Fold)
 - MDP0000193489/Binding Protein (5 Fold)
 - 43 Bitter Pit Proteins identified with \geq Two-Fold Spectra and $P < 0.05$

Notable Proteins:

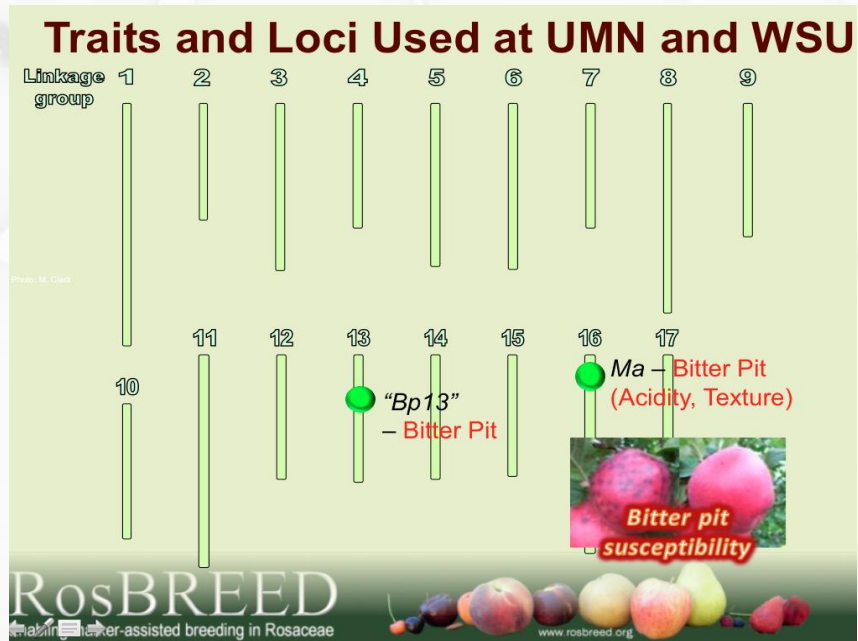
 - MDP0000287919/catalytic activity/ metabolic process Protein (100 Fold)
 - MDP0000137211/Oxidoreductase Protein (78 Fold) – 2 proteins
 - MDP0000287302/Thaumatococcus Protein (56 Fold)

Conclusions

- Twelve (12) unique proteins were identified in DIGE, the majority being Pathogenesis-Related (PR) proteins (Plant Defense).
- 170 Proteins ($p < 0.05$) were identified as quality markers for bitter pit

Conclusions

- Protein results are in the process of being compared with known genetic data from RosBREED.
- Proteomic data could assist plant breeders in creating bitter pit-free germplasm.



The background of the slide is a repeating pattern of white apples with green leaves, rendered in a light, semi-transparent style. The apples are arranged in a grid-like pattern, filling the entire frame.

Thank You!

Questions ?