WORKSHOP TOBACCO TECHNOLOGIES

APW07 - CONTRIBUTION OF GENOMIC RESEARCH TO DEVELOP DISEASE RESISTANCE IN TOBACCO

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TOBACCO PATHOGENS

13% of global crop yields are lost annually because of pathogens

In tobacco, 1-5% each year according to the location, can reach 25% to total losses

INRA e-phytia listed 52 pathogens on tobacco among which:

- **Fungal diseases**
  - Airborne Fungi (Blue mold, Frog-eye)
  - Soilborne Fungi (Black shank, Black root rot)
  - Vascular Fungi (Fusarium)

- **Viruses**
  - TMV, PVY, CMV, TSWV

- **Bacteria and Phytoplasma**
  - Bacterial wilt, Wild fire

- **Parasitic plants**
  - Orobanche

- **Nematodes**
  - Meloidogyne, Globodera
NEED FOR RESISTANT VARIETIES

• Plant pathogens are limiting factors in tobacco production and can have a severe impact on yield, but also on leaf quality and chemical composition.

• CPAs can provide effective protection but are not the best solution:
  – *Maybe not available*: viruses
  – *Have a high cost, particularly in developing countries*
  – *Using CPAs means selection of CPAs resistant pathogens (metalaxyl on blue mold)*
  – *Worldwide global concern to reduce their use*

• Agricultural recommendations may be difficult to follow.

GENETIC IMPROVEMENT IS THE BEST WAY TO MANAGE PATHOGENS IMPACT
NEED FOR MARKERS/GENE IDENTIFICATION

• It is easier than phenotypic screening
  – Pathogens maybe not available in biological testing (wait for field trials)
  – Variability of pathogen testing (environmental effects)

• Selection is carried out at the seedling stage
  – Save time, resources and effort (quick discarding of susceptible plants)
  – No need to fix for observing the phenotype with recessive genes
  – Able to identify heterozygous plants with codominant markers

• Markers can help minimizing the linkage drag
  – Flanking markers to reduce the introgression of genes linked to resistance and with a negative impact (ex: TMV, black root rot…)

• Markers can reduce the number of backcrosses
  – Background markers select against the donor genome
QUALITATIVE VS QUANTITATIVE RESISTANCE

+ Monogenic dominant (R genes) or monogenic recessive (S genes like va)
+ Complete or high level of resistance
+ Easier to find and to use
- More easily overcomed
- Potential yield cost

ex: PVY, TMV, black root rot, black shank (Php, Phil)

+ More often associated to several genes with small effect (QTLs)
+ Broad spectrum resistance
+ Tend to be more durable
- Often partial level of resistance
- More difficult to find and to use/introgress
- May be environment and background dependant

ex: bacterial wilt, black shank

THE BEST IS TO COMBINE BOTH TO INCREASE DURABILITY AND AVOID SHIFT IN POPULATION OF PATHOGENS (ex: PVY, black shank, nematodes)
## EXAMPLE OF RESISTANCE ORIGINS IN TOBACCO

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pathogen</th>
<th>Interspecific Source</th>
<th>Genes</th>
<th>N. tabacum Source</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black root rot</td>
<td>Chalara elegans</td>
<td>N. debneyi</td>
<td>D</td>
<td>TI 89, TI 87</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N. alata</td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVY</td>
<td>Potato Virus Y</td>
<td>N. africana</td>
<td>D</td>
<td>Virgin A Mutant</td>
<td>R (va)</td>
</tr>
<tr>
<td>TMV</td>
<td>Tobacco Mosaic Virus</td>
<td>N. glutinosa</td>
<td>D (N)</td>
<td>Ambalema</td>
<td></td>
</tr>
<tr>
<td>Blue mold</td>
<td>Peronospora tabacina</td>
<td>N. debneyi</td>
<td>D + modifiers</td>
<td>Chemical Mutant</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N. goodspeedii</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powdery mildew</td>
<td>Erysiphe cichoracearum</td>
<td>N. glutinosa</td>
<td>D</td>
<td>Kokubu, Kuo Fan</td>
<td>2 R (mlo)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N. tomentosiformis</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rootknot nematode</td>
<td>Meloidogyne incognita</td>
<td>N. tomentosa</td>
<td>D (Rk1, races 1 3)</td>
<td>Florida 301, Beinhart 1000</td>
<td>P P</td>
</tr>
<tr>
<td>Black shank</td>
<td>Phytophthora parasitica</td>
<td>N. longiflora</td>
<td>D (Phl, race 0)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N. plumbaginifolia</td>
<td>D (Php, race 0)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Bacterial wilt</td>
<td>Ralstonia solanacearum</td>
<td>X</td>
<td>X</td>
<td>TI 448A (Hatano, Xanthi)</td>
<td>P D? Rps, Rxa</td>
</tr>
</tbody>
</table>

D = dominant; R = recessive, P = polygenic
A RECENT SHIFT IN RESEARCH ON TOBACCO RESISTANCE

• Up to recent years, most of marker–trait association studies have focused on traits introgressed from other Nicotiana species.
  – Low polymorphism between *N. tabacum* cultivars has prevented research on *N. tabacum* resistance genes
  – Large introgression of interspecific DNA is « easier » to identify because it causes high polymorphism

• The development of complete high density SSRs and SNPs linkage maps have greatly stimulated research on polygenic and complex resistance from *N. tabacum* origin (bacterial wilt, black shank…).

Bindler *et al*, 2011

Xiao *et al*, 2015

Edwards *et al*, 2015
AN ACCELERATION OF MARKERS/GENE DISCOVERY SINCE 2010
CORESTA, TWC and articles sources

ADVANCES IN GENETIC AND PHYSICAL MAPPING

ADVANCES IN RESISTANCE AND MARKERS DISCOVERIES
FINDING MARKERS/GENES: A COMBINATION OF SEVERAL APPROACHES

- Candidate genes
  - Orthologs, RGA
  - Pathology testing
- Differential expression
  - SSH, Chips, RNA-Seq
- Physical Mapping
  - Illumina, PacBio, Nanopore, 10X...
- Linkage Mapping/QTLS
  - GBS, SeqSNPs
  - RAPD, AFLP, SSR, SNPs
- GWAS
- Validation
- Proteomic/Metabolomics
- GM, EMS, Gene editing
EXAMPLE OF BLACK ROOT ROT

Full transfer of resistance from N. debneyi associated to undesirable traits potentially caused by linkage drag.

- **RAPD** dominant markers (Bai *et al.* 1995)
- **AFLP**-derived SCAR dominant markers (Julio *et al.* 2006)
  - Mapped in 2012 on Nt.3 (not published)

- Use of **Genotyping By Sequencing** (GBS) to develop dCAPs codominant markers (Qin *et al.* 2018)
  - Localization on Nt.17

⇒ Still some unresolved chromosomnal complexity!
The main source of resistance comes from VAM deletion, but transferred resistance is not as strong as in the original variety and resistance can be broken by virus variants.

- **RAPD** (Noguchi et al. 1999) and **AFLP**-derived SCAR dominant markers (Julio et al. 2006)

- Large panel of sources of resistance to PVY and RB-PVY
- Still some potential resources for viruses resistance
ACTUAL LIMITATIONS

• Some regions of the reference genome are still not fully understood:
  
  – Homeolog regions and/or duplicated regions are still complex, creating confusion for locus identification: example of black root rot resistance
  – Low resolution assembly masking multicopy genes: example of the va gene, not identified on the reference genome available on Solgenomics (Edwards et al. 2017):

  ➞ Reference genome can still be improved with the help of third generation sequencing (10X Genomics, Nanopore…)

  ➞ De novo sequencing or re-sequencing of others varieties may help to understand complex resistances
AND NOW?

Pathogen related markers

BRR = Black root rot
BM = Blue mold
TMV = Tobacco Mosaic Virus
Php = Black shank (N. plumbaginifolia)
Rk1 = Root Knot nematode (M. incognita 1,3)
PVY = gene for Potato Virus Y
PVY<sup>RBV</sup> = gene for PVY resistant breaking variant
PVY<sup>+</sup> = second locus for PVY resistance
tolerance
PVY<sup>RAH</sup> = major QTL for Black shank from Beinhart1000
BS<sup>fl</sup> = major QTL for Black shank from Florida301
BS<sup>hm</sup> = minor QTL for Black shank
Mlo = mlo genes for recessive powdery mildew resistance
Oro = Orobanche

Others + background markers
Low nicotine, TSNA, Heavy metals,
Yield, quality, flowering time, suckers…+
background markers !!!!
TECHNOLOGIES AVAILABLE: THE MARKERS/SAMPLES RATIO BOTTLENECK

- **Number of samples**
  - 10
  - 100
  - 1000
  - 5000
  - 10000

- **Number of markers**
  - 10
  - 100
  - 1000
  - 5000
  - 10000
  - 100000
  - 1000000

- **KASP™/LightCycler®480**
- **KASP™/Dynamic Array™ IFC 96*96**
- **SeqSNPs**
- **Axiom Affymetrix**
- **Genotyping By Sequencing**

**Today for most of the breeders**

- **Missing technology for intermediate flow at reasonable cost**

**High cost outsourcing**

- **KASP™ in house (QuantStudio)**
- **Agarose gel Genetic analyzer (CE)**
ALTERNATIVE TO CONVENTIONNAL BREEDING: GM AND GENE EDITING APPLICATIONS

WITH R GENES

• SDN1 (Site Directed Nuclease) to disrupt susceptibility genes, SDN3 to introduce resistance genes

WITHOUT R GENES

• Transferring pattern recognition receptors (PRRs) between plant species (receptor kinase, receptor like proteins) to activate downstream defense signaling genes.
• Pathogen-derived resistance (PDR): expression of structural viral nucleic acid sequences
  - Useful when no source of resistance is identified (ex: Tobacco expressing CMV coat protein)
• Up or down regulation of regulating genes:
  - Downregulation of cellulose synthase increases Arabidopsis resistance to *Botrytis cinerea*
• Antimicrobial peptides (AMPs): use of defensin against fungal pathogens.
GM/GENE EDITING FOR RESISTANCE BREEDING: PROS AND CONS

+ Pool of potential useful genes extended
+ Reduced number of backcrosses = potential gain of time?
+ No linkage drag compared to classical breeding

- Techniques are not accessible for everyone
- Need transformation of multiple elite lines
- Cost of license for GE technologies
- Will it be worldwide usable?
PERSPECTIVE

• Recent advances in genomic research make resistance breeding in tobacco achievable by molecular markers or gene editing strategies, both with advantages and limitations.

• Compared to other domains, pathogen resistance gene/markers are published and freely available to breeders, which is encouraging to develop their use.

• There is still a lot to do for resistance gene discovery, but also to develop practical applications for breeders.
Thank you.