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

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IMPERIAL BRANDS SCIENCE

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:

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 recommandations may be difficult to follow.

GENETIC IMPROVEMENT IS THE BEST WAY TO MANAGE PATHOGENS IMPACT

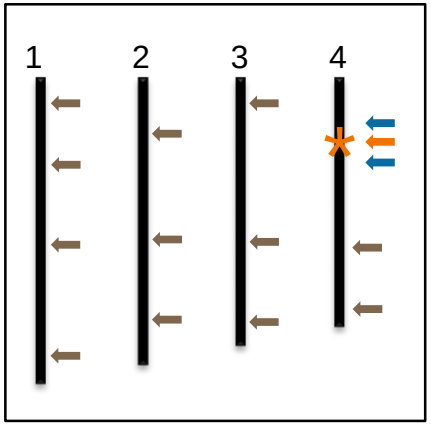
NEED FOR MARKERS/GENE IDENTIFICATION

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

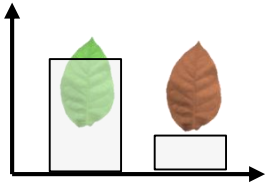
- Selection is carried out at the seedling stage
  - Save time, resources and effort (quick discarding of susceptible plants)
  - No need to fix for observing the phenotype with recessive genes
  - Able to identify heterozygous plants with codominant markers

- 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



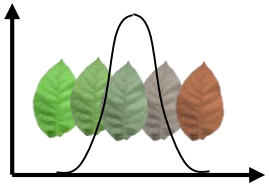
QUALITATIVE VS QUANTITATIVE RESISTANCE



+ 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, Phl)



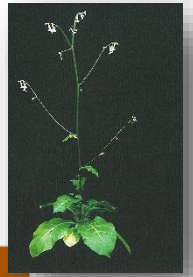
+ More often associated to several genes with small effect (QTLs)
+ Broad spectrum resistance
+ Tend to be more durable

- 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



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

D = dominant; R = recessive, P = polygenic

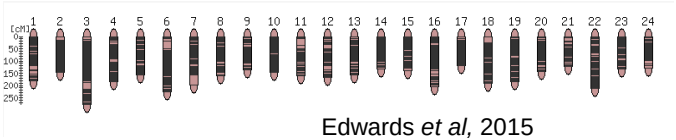
A RECENT SHIFT IN RESEARCH ON TOBACCO RESISTANCE

- 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

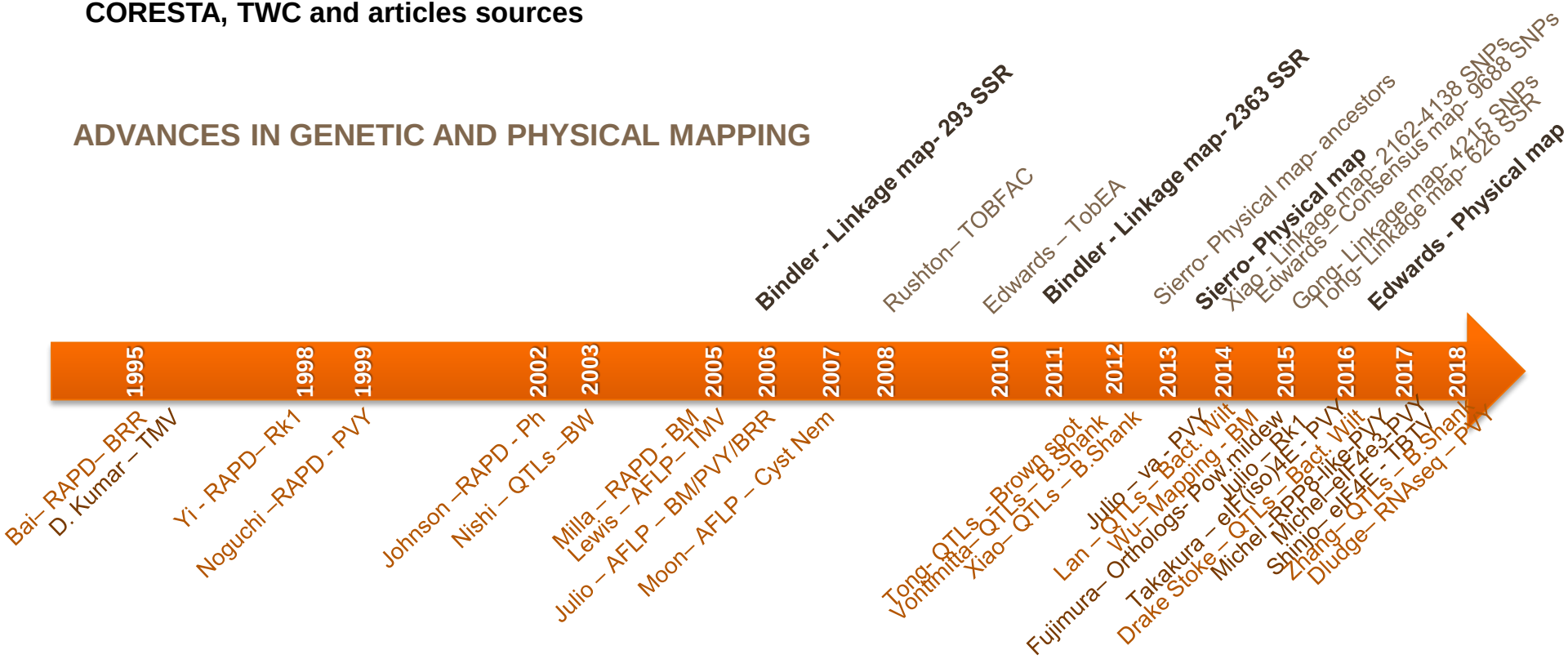


Edwards *et al,* 2015

AN ACCELERATION OF MARKERS/GENE DISCOVERY SINCE 2010

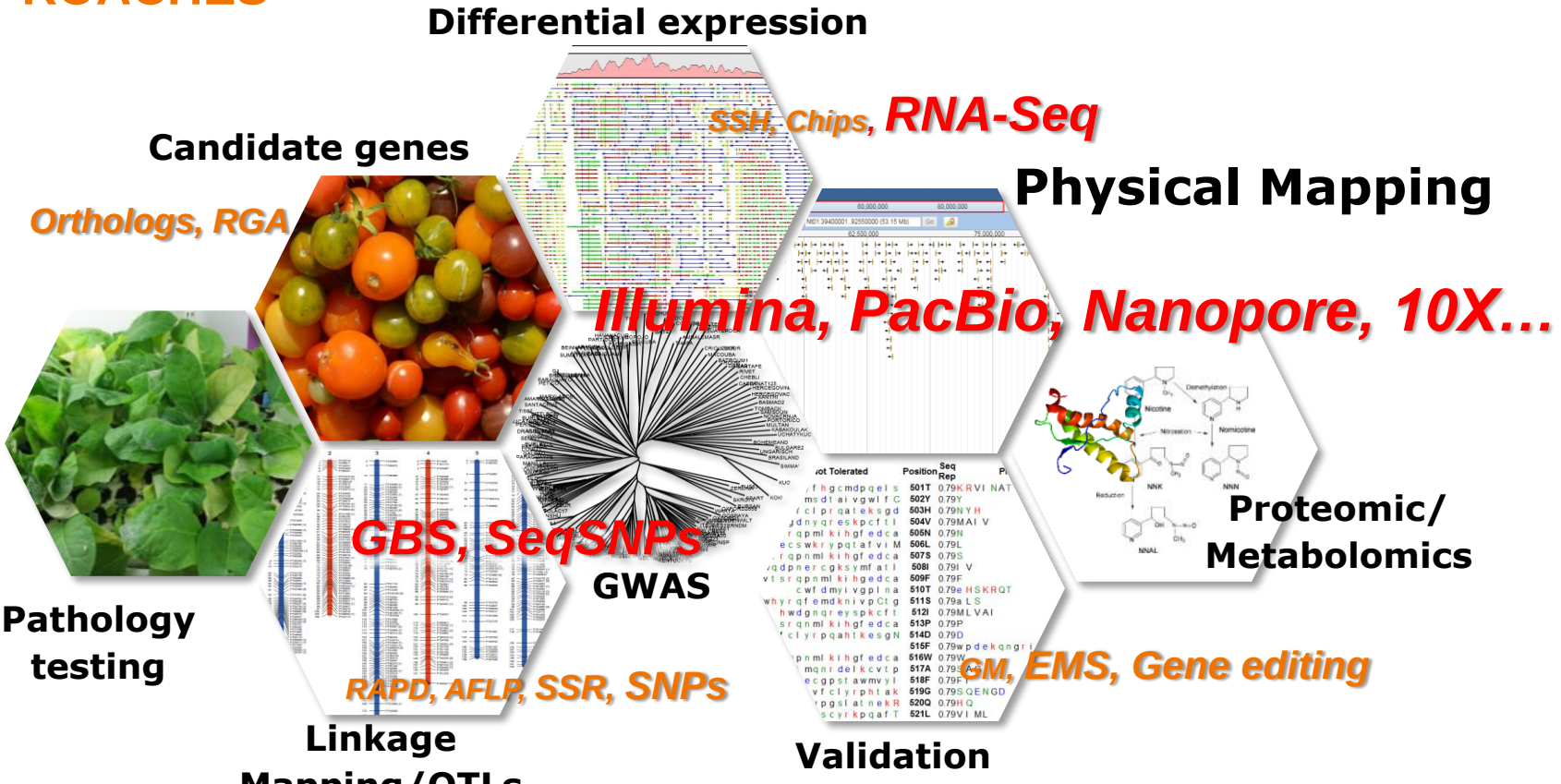
CORESTA, TWC and articles sources

ADVANCES IN GENETIC AND PHYSICAL MAPPING



ADVANCES IN RESISTANCE AND MARKERS DISCOVERIES

FINDING MARKERS/GENES: A COMBINATION OF SEVERAL APPROACHES

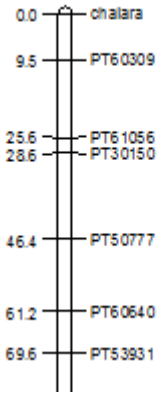


EXAMPLE OF BLACK ROOT ROT

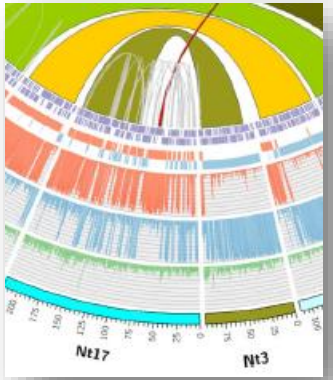
Full transfer of resistance from N. debneyi associated to undesirable traits potentially caused by linkage drag.

- 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



Synteny between chr 3 and 17



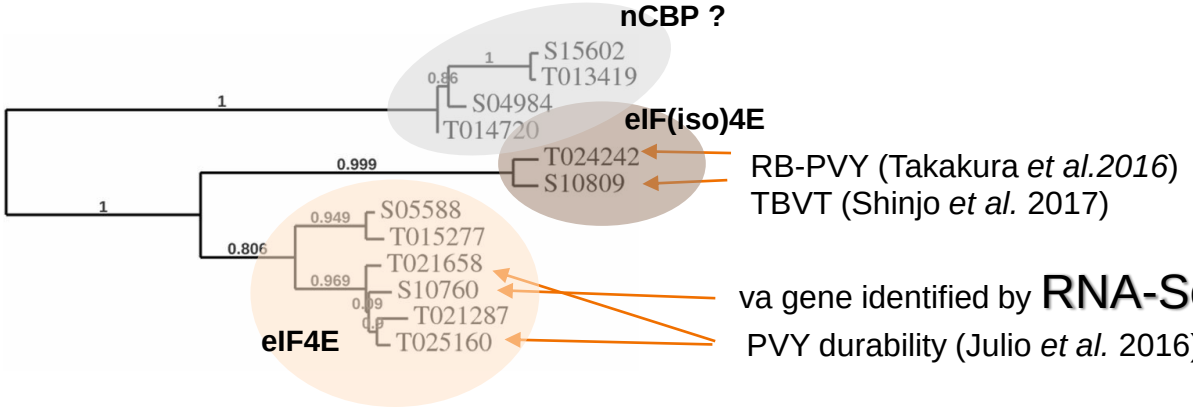
Edwards *et al,* 2017

⇨ Still some unresolved chromosomal complexity !

PVY : FROM 1999 TO 2018, THE NEVER ENDING STORY

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 ressources for viruses resistance

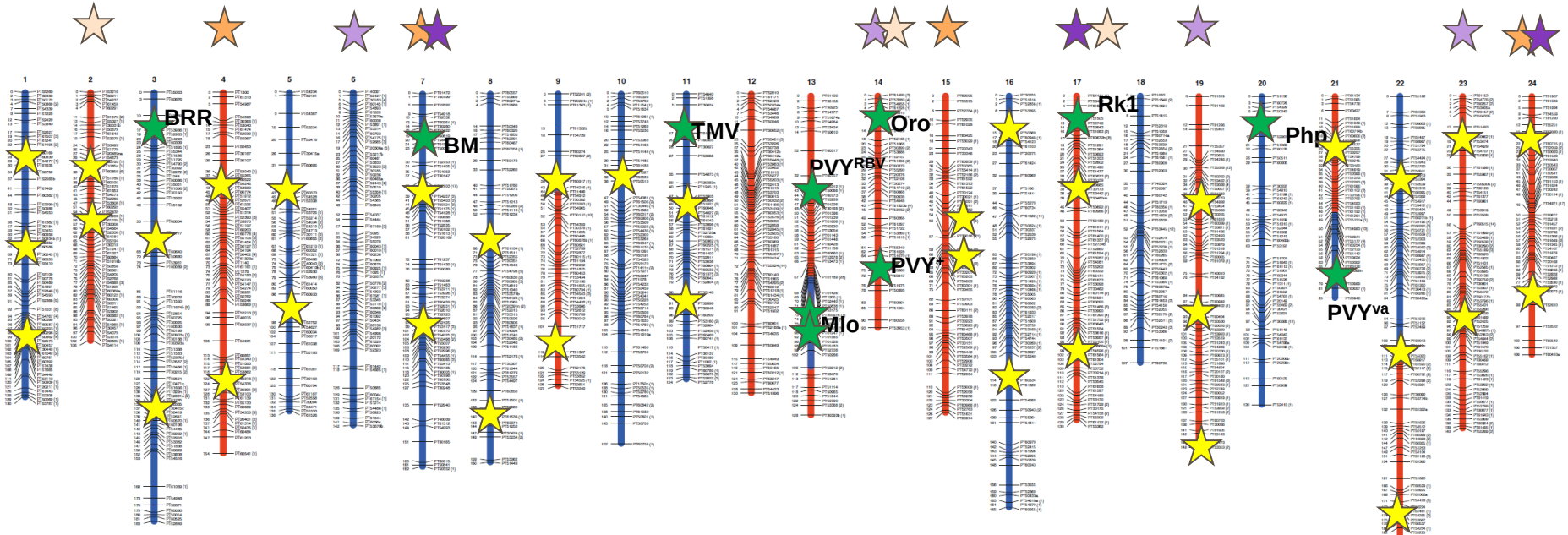
ACTUAL LIMITATIONS

- 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 QTLs

BS^B1000 = major QTL for Black shank from Beinhart1000
BS^Flo = major QTL for Black shank from Florida301

BS^Flo = minor QTL for Black shank

BW^Ch = major QTL for Bacterial wilt, chinese origin
BW^US = major QTL for Bacterial wilt, US origin

BW^US = minor QTL for Bacterial wilt, US origin

Pathogen related markers

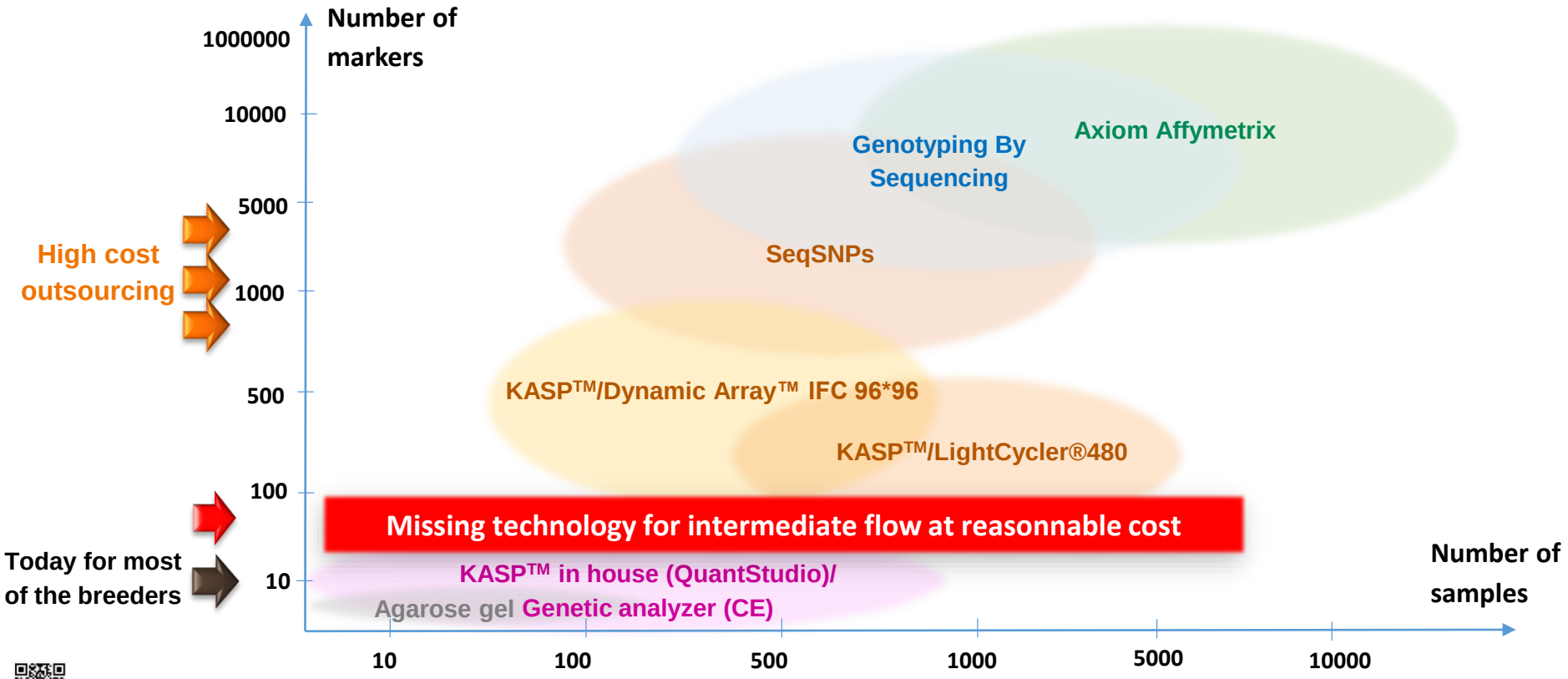
BRR= Black root rot
BM= Blue mold
TMV = Tobacco Mosaic Virus
Php = Black shank (N. plumbaginifolia)
Rk1= Root Knot nematode (M. incognita 1,3)

PVY= va gene for Potato Virus Y
PVY+=2nd locus for PVY resistance durability
PVY RBV = RPP8 gene for PVY resistant breaking variant
Mlo = mlo genes for recessive powdery mildew resistance
Oro = Orobanche

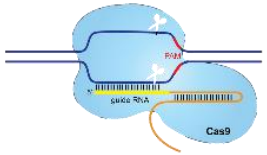
Others + background markers

Low nicotine, TSNA, Heavy metals, Yield, quality, flowering time, suckers…+ background markers !!!!

TECHNOLOGIES AVAILABLE: THE MARKERS/SAMPLES RATIO BOTTLENECK



ALTERNATIVE TO CONVENTIONNAL BREEDING: GM AND GENE EDITING APPLICATIONS



WITH R GENES

- SDN1 (Site Directed Nuclease) to disrupt susceptibilty genes, SDN3 to introduce resistance genes

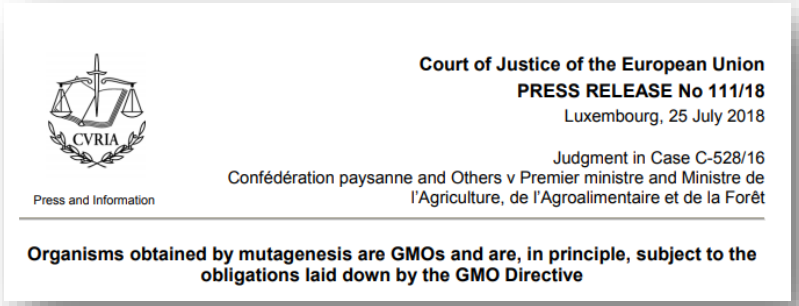
WITHOUT R GENES

- 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 ?



Press and Information

Court of Justice of the European Union
PRESS RELEASE No 111/18
Luxembourg, 25 July 2018

Judgment in Case C-528/16
Confédération paysanne and Others v Premier ministre and Ministre de l'Agriculture, de l'Agroalimentaire et de la Forêt

Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive

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.