

Barley soil borne mosaic viruses: Identification of predominant viruses affecting yield and malting quality, in order to orientate breeding towards a sustainable resistance

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Objectives

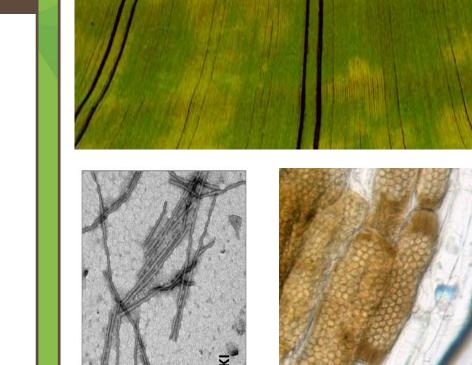
Prior to the year 2000, barley yellow mosaic disease was responsible in France for high yield losses due to the prevalence of the bymovirus **Barley yellow** mosaic virus Y1 (BaYMV Y1), sometimes associated with Barley mild mosaic virus (BaMMV) (Hariri et al, 2000). Genetic resistance is the only way to control these viruses transmitted by the soil-borne protist *Polymyxa graminis*.

Recessive resistance gene rym4 was widely deployed in winter malting barley cultivars, providing a good control. Since 2009, new significant mosaïc damage has been observed in malting barley in a widening area, indicating the possible overcoming of rym4 resistance.

The objectives of this CASDAR project (2013-2016) were to:

- 1) identify and map viruses or pathotypes causing mosaic disease in barley crops and develop new detection tools
- 2) confirm the efficiency of resistance genes cited in literature (rym 1 to 18) and assess current cultivar resistance (rym4/rym5)

3) quantify the impact of viruses on yield and malting quality.



Material and methods

1. Experimental designs & Plant material

4 types of experimental designs:

Design A.Differential third O I:

2. Methods

Table 1: Molecular tools used for the detection and

Design1: samples collected for geographic and genetic diversity A complete viral inventory was carried out during 4 years, mainly in France by collecting 914 infected samples, coming from 230 trials in 108 single sites.

Design2: Healthy/infected farmer plots to measure impact of viruses on yield & quality

> 2013 2014 2015 2016 Total Design 2 Nb pairs of plots (infected/healthy) 11 11 10 5 37

Design3: Healthy/infected variety trials, to measure impact of viruses on yield & quality in relation to resistance cultivar

5 trials (4 in France, 1 in Germany) of 12 winter barley cultivars, with current genetic (*rym4, rym5*), in 2014 & 2015.

Design4: Differential trials to confirm efficiency of resistance genes (rym 1 to 18) & mosaic detection by serological & MM tools

Design 4: Differentials	2014	2015	2016	Total
Nb infected sites	6	12	3	21

Design 4:Differential trial & list



/ariety	Resistance genes	BaMMV	BaYMV-1	BaYMV-2
Plaisant	Sensible	S	S	S
Orelie (2rg)	Sensible	S	S	S
Mihori Hadaka 3	rym2 (7H)	R	R	R
Ea 52	rym3 (5H)	S	R	R
KATHLEEN	rym3 (5H) ?		R	R
Esterel	rym4 (3H)	R	R	S
Vanessa (2 rg)	?	R	t	S
Arturio	rym4?	R	R	S
Platine (2 rg)	?	R	t	S
Paisha Tayeh	rym?			
ҮОКОНАМА	rym ?		R	R
MARNE	rym4 + ?(ry2)	R	R	R
Super Champenois	rym4 (3H) + rym11 (4H) ?	R	R	R
Mosaïc	rym5 (3H)	R	R	R
Malice (2rg)	rym5 (3H)	R	R	R
οπο	rym 5 (3H)	R	R	R
Chikurin Ibaraki 1	rym3+rym15			
Mokusekko 3	rym1+5	R	R	R
10247	rym8 (4H)	R	R	S
Bulgarian 347	rym9 (4H)	R	S	S
HIBERNA (2rg)	rym10 (3H)	S	R	R
Russia 57 (2rg)	rym11 (4H)	R	R	R
Muju Covered 2	rym12 (4H)	R	R	R
Taihoku A	rym13 (5H)	R	R	R
PK23-2 (Pakistan)	Rym17+ rym 18		R	R

Molecular tools used or developped to analyse viruses

and phylogenetic analyses

- ✤ Usual tools : cf table 1
- **New Genome Sequencing** was used to :
- Provide sequence data to help development of typing assay
- Identify new viruses on atypical samples
- Analyze the diversity of BaMMV & BaYMV
- **2.** Varietal resistance assessment:

Visual scoring of symptomatic leaves : yellowing, and/or chlorotic blotches, confirmed by TaqMan Real -time PCR and Elisa tests.

- 3. Measurements of yield components and malting quality on healthy & infected pairs
- Barley : Yield, nb spikes&kernels/m², TKW, Proteins, sieving
- Malts (μ-malting according to CTPS diagram)
- Pilot brew: study the techno-functional impact on the beer

identification of Bymoviruses and other barley viruses

Targeted viruses	PCR method	Reference
BaMMV and BaYMV	real-time PCR	Mumford <i>et al.</i> 2004
WDV and BSMV	PCR	Tao et al. 2012
B/CYDVs (PAV, MAV, RPV)	PCR	Deb <i>et al.</i> 2008
BaMMV	PCR	Achon et al. 2003
BaYMV	PCR + Sanger sequencing	Vaïanopoulos <i>et al.</i> 2007

visual scoring:

0: no symptoms 1: doubt (symptoms unusual) 2: a few mosaic plants

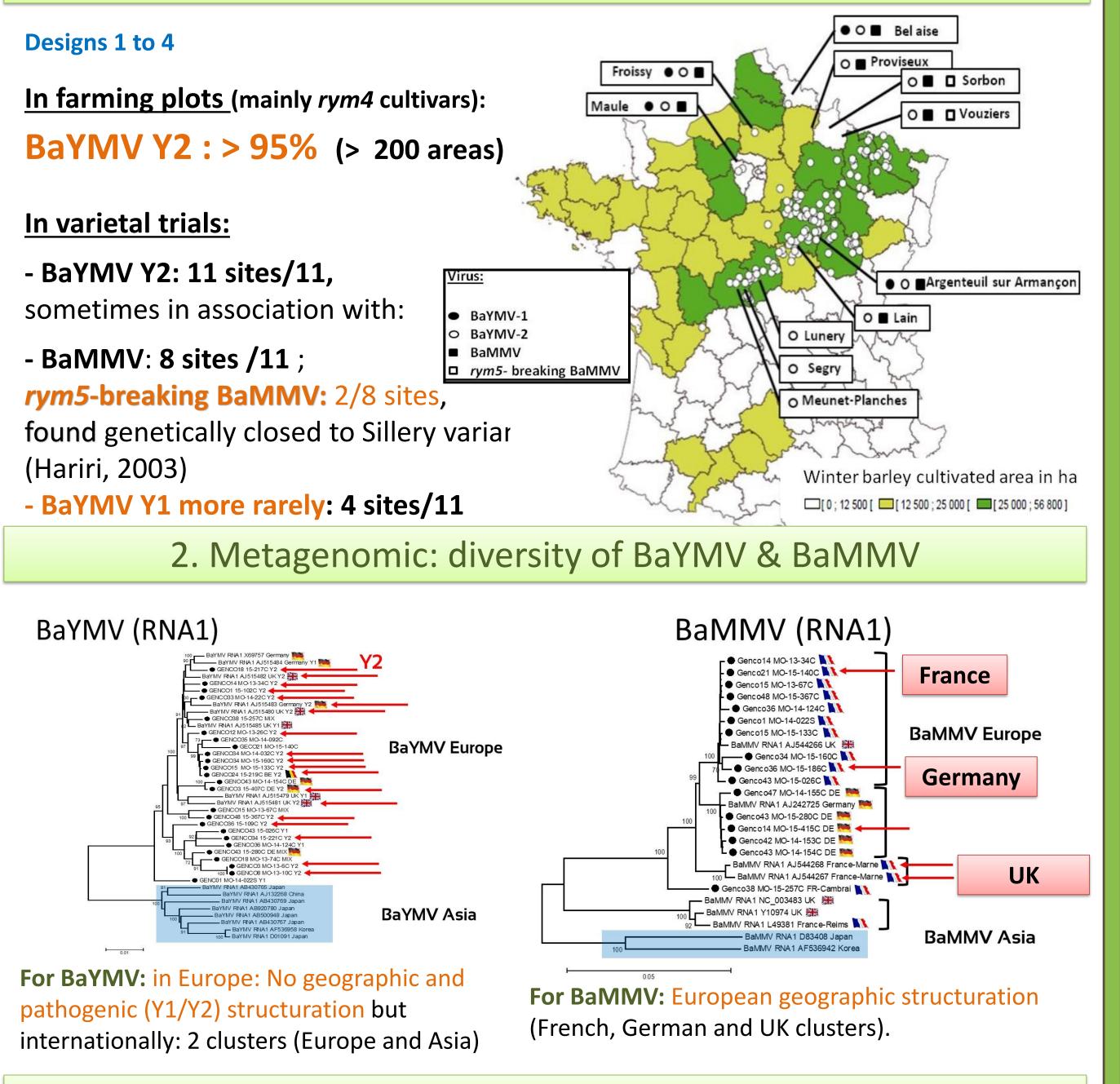


3: intermediate contamination: no uniformity or medium intensity of symptoms 4: rough high uniform contamination 5: high contamination on the whole plot Scale of

Results

1. Prevalence of BaYMV 2 in France

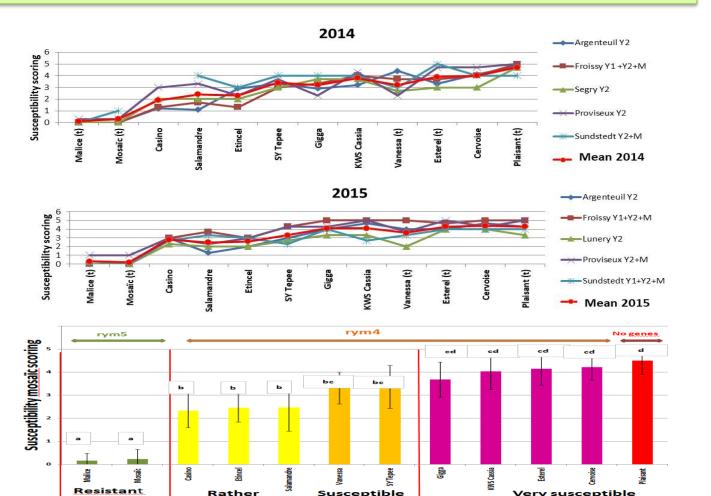
Designs 1 to 4



4. Resistance assessment of current resistance (rym4/rym5)

Design 3

Stability of cultivar resistance: No interaction between Cultivar-Year-Site-Complex of viruses (BaYMV Y2/BaYMV Y2+BaMMV/BaYMV Y2+Y1 +BaMMV)



3. A new molecular tool: dCAPS to differentiate BaYMV Y1 and Y2

- dCAPS: "derived Cleaved Amplified Polymorphic Sequence", used to discriminate BaYMV Y1/Y2 (Villemot & Rolland, Journ. of Virol. meth., 2016)

Electrophoresis profiles after

- Gradient of susceptibility inside rym4 cultivars, leading to the hypothesis of the existence of other factors.

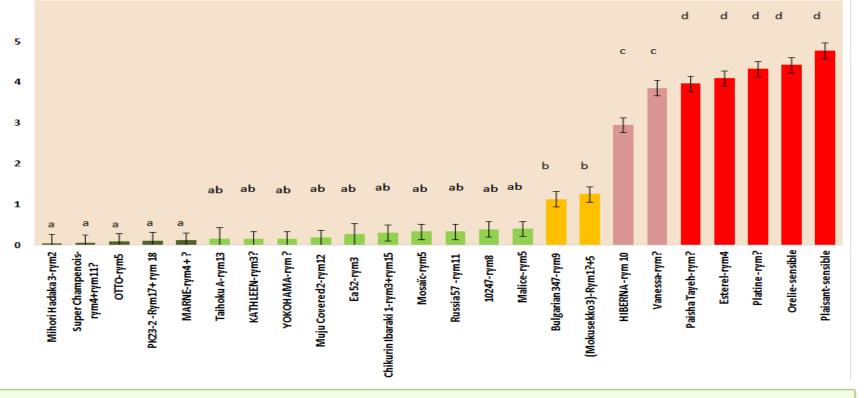
- Confirmation: rym5 is still efficient against BaYMV **Y2**

5. Efficiency of resistance genes against BaYMV 2 & BaMMV in France

Design 4

- 10 major resistance genes, efficient alone or in association: rym2, rym3, rym5, rym8, Rym17+rym18, rym3+rym15, rym11 rym12, rym13, gène Yokohama - Polygenic résistance

in French varietal populations: Marne, Super Champenois

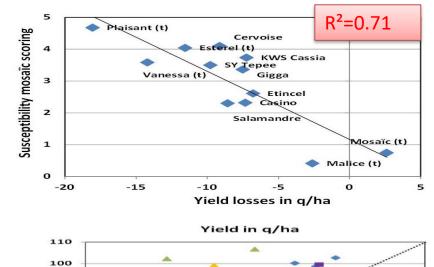


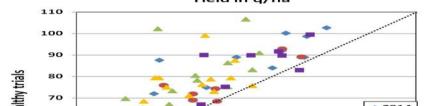
6. Mosaic impact on yield & malting quality in healthy/infected areas

- Yield : 1)
- Correlation between yield losses and cultivar **resistance** to BaYMV Y2/BaMMV (Design 3) High correlation R²=0.71

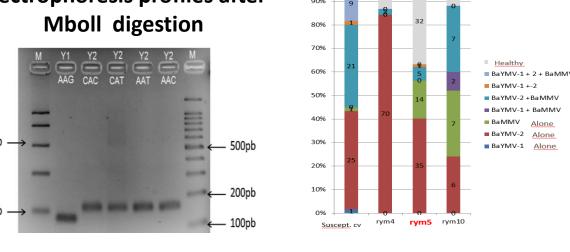
b) Yield components impacted (Designs 2&3):

- Yield : losses of 1.1 t/ha (-17%)
- Nb of spikes (-16%) and kernels (-13%),





A restriction enzyme was chosen to digest specifically BaYMV Y1 VPg PCR product: only 1 codon for BaYMV Y1 and 4 possible codons for Y2, resulting in one aa change (position 1307).



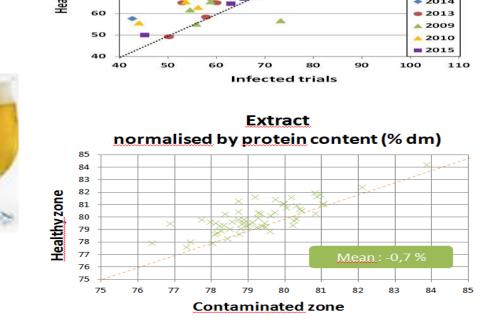
dCAPS is available for the typing of BaYMV isolates but is not suitable for confirming genetic resistance: BaYMV 2 is detected by dCAPS in rym5 cultivars, in the absence of symptoms, contrary to Real time PCR (Mumford, 2004)

Sieving & hectoliter mass

2) Malting quality (Designs 2&3):

- μ-malting: from 2013-2016 on 73 healthy/infected pairs

- **Extract:** losses of 0.7%
- Y Proteins: 0.4%
- Pilot production (500Kg): No effect of mosaïc on the quality of the beer. An even better effect with a more rapid filtration.



Conclusion

- Viral mapping showed the **predominence of BaYMV Y2 in France**, overcoming *rym4* resistance. BaMMV is also present while BaYMV 1 is still locally present.
- * Two sites were found with *rym5***-breaking BaMMV** in the east of France.
- * The absence of geographic and pathotype structuration for BaYMV strongly suggests that **mutation from BaYMV Y1 to** Y2 has independendly occured on multiple occasions.
- A new tool, dCAPS was developped for BaYMV Y1 and Y2 typing in routine and offered to the seed sector.
- * rym5 gene appears to be still efficient against BaYMV Y2. A gradient of resistance was revealed for rym4 cultivars, without interaction with sites or years.
- * Varietal tests confirmed the efficiency of 10 major resistance genes and of polygenic resistance against BaYMV Y2 & BaMMV, providing hope for sustainable resistance (diversification or pyramiding of sources).
- * Impact of BaYMV Y2 on yield is significant: 1,1t/ha, impacting mainly the number of spikes and kernels, the sieving and hectoliter mass, the protein content and the extract.
- The quality of the beer does not seem to be affected.

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