



➤ Benefits and limits of the use of molecular markers to support the assessment of Distinctness in certain cross-pollinated species

Bernadette Julier

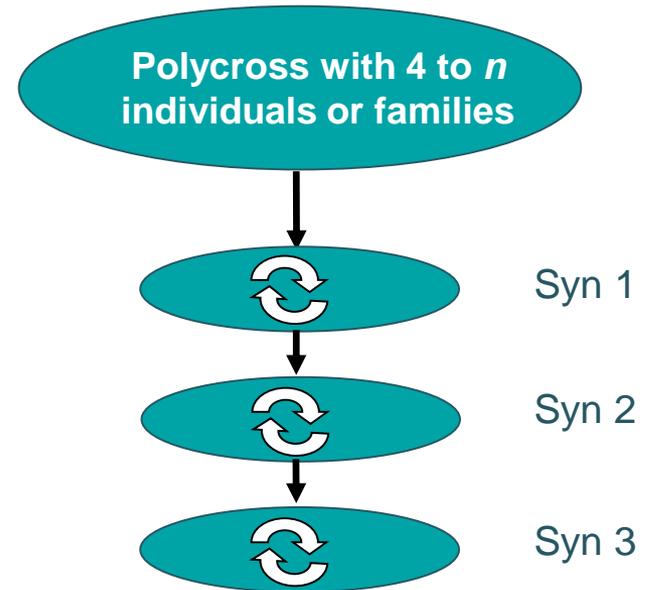
Unité de recherche pluridisciplinaire Prairies et Plantes Fourragères
Lusignan, France

➤ Cross-pollinated species

Forage and turf species



Synthetic varieties



Families of related individuals

2x, 4x, 6x, auto or allo-polyploid



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Use of molecular markers to support the assessment of Distinctness in certain cross-pollinated species

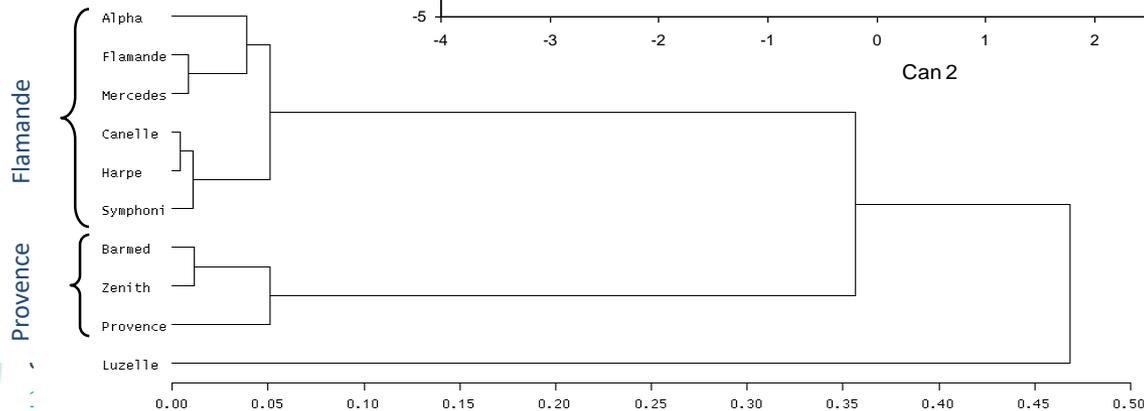
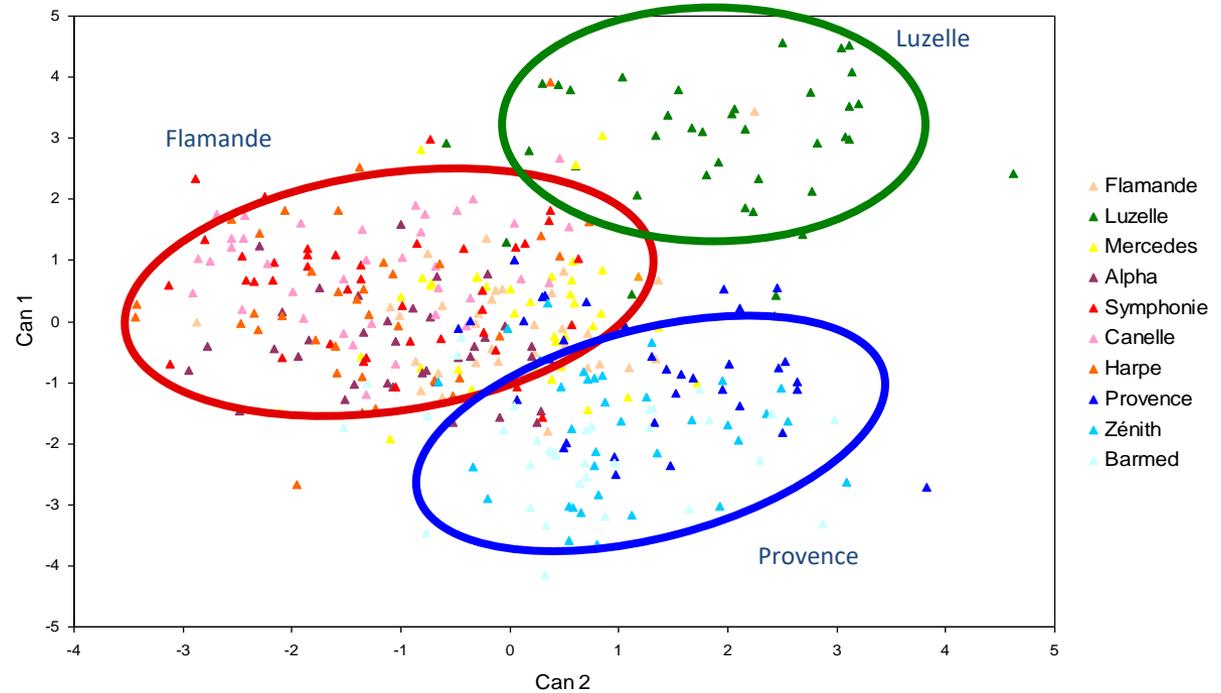
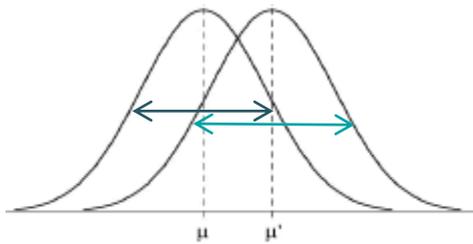
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➤ Distinctness

A rising problem, since the very early times of forage catalogues

Why?

Distribution of 40 individuals/variety, 10 varieties
Phenotypic traits (lucerne)

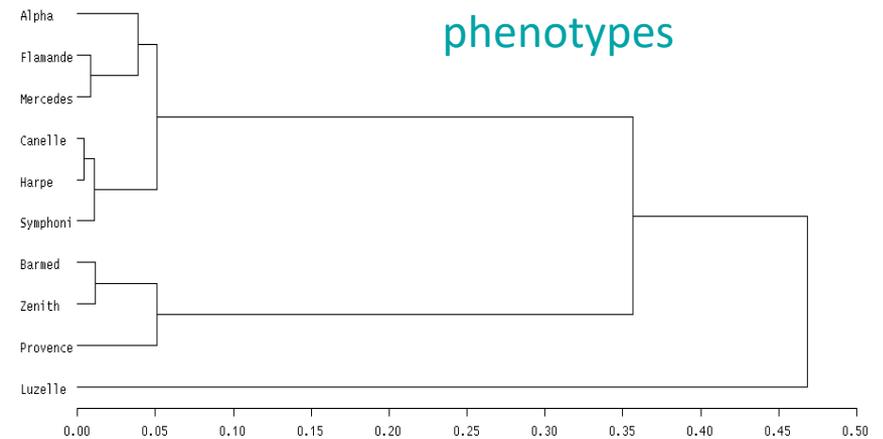
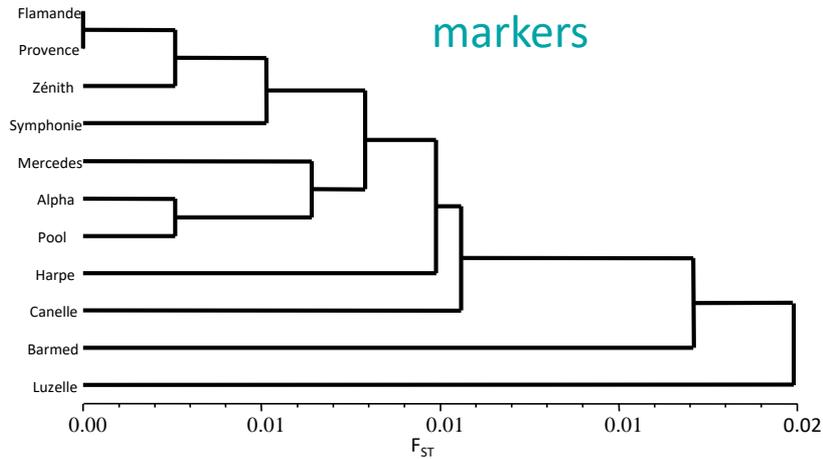




➤ Use of markers

20 SSR, $F_{ST} = 0.013$ ***

AMOVA	Effect	DF	Variance	% total variance
	Among-variety	9	0.021***	0.28
	Within-variety	390	7.56	99.72



Huge within-variety variation

Weak difference among varieties

Herrmann et al. (2018) Genetic Resources and Crop Evolution 65, 527-540
doi:10.1007/s10722-017-0551-z

➤ Context of variety registration

Lucerne

Reference collection ~600 varieties,
with seeds ~230 varieties

Registered varieties:

Europe: 555

USA: 167

Canada: 280

China: 77

Argentina: 715

...



➤ Use of « many » molecular markers?

A key study on lucerne

An emerging technology :
Genotyping by Sequencing (GBS)



Elshire et al. 2011

Used on bulks of heterozygous individuals
(= synthetic variety)



Raineri et al. 2012

Encouraging results on perennial ryegrass
Reasonable costs



Byrne et al. 2013



> Questions

Lucerne

Method: is the evaluation of allele frequency on bulks of individuals accurate ?

On 3 varieties, comparison of allelic frequencies on bulks and on 40 individuals that were separately genotyped

Structure of genetic diversity

Is it possible to differentiate among 20 varieties?

Is the genetic distance correlated to the phenotypic distance?

Julier et al. (2018) *Molecular Breeding* 38:133 doi 10.1007/s11032-018-0891-1



> List of 20 varieties

Name	Flower Colour				Dormancy	Verticillium	Ditylenchus	Colletotrichum	Type	Breeder	Registration year
	C1	C2	C3	C4							
Arpège	1	8	2	1	4	7	7	4	N	Florimond Desprez	2004
Artémis	1	8	2	1	4	6	8	9	N	Barenbrug	2010
Barmed *	1	8	2	2	7	5	5	8	S	Barenbrug	2002
Capri	1	7	2	1	4	7	7	4	N	Florimond Desprez	1995
Daphné	1	7	2	2	4	5	7	4	N	Florimond Desprez	1996
Dorine	1	7	3	1	6	3	5	3	S	Barenbrug	2001
Europe	1	8	2	1	4	5	3	RT	N	Florimond Desprez	1961
Fado	1	8	2	1	4	6	8	6	N	Florimond Desprez	2012
Félicia *	1	7	2	2	4	7	8	7	N	GIE Grass	2009
Franken Neu	1	5	1	6	4	3	4	RT	N	Schmitz Ernst	1980
Galaxie *	1	7	2	2	4	5	7	7	N	Semunion	2007
Greenmed	1	7	2	2	1	4	7	RT	N	AgriObtentions	2011
Juurlu	5	1	1	4	no	no	no	RT	N	Estonian Crop Research Institute	1993
Luzelle	1	6	2	3	3	3	6	RT	N	INRA	1993
Meldor	1	6	2	2	6	4	5	4	S	INRA	1995
Midi	1	6	2	2	5	4	5	RT	S	Tourneur	2002
Milky Max	1	7	2	2	4	7	8	6	N	GIE Grass	2015
Orca	1	1	8	1	4	2	4	RT	N	Carneau	1966
Verdor	1	7	2	2	6	5	5	3	S	Barenbrug	2011
Vernal	1	4	4	4	2	no	no	RT	N	Wisconsin and Utah Exp. Stn.	1953

* 3 varieties studied at the individual level

2 very close: Félicia et Galaxie, 1 different: Barmed



> GBS sequencing

From sowing to genotyping

Sowing, leaflet sampling

DNA extraction on individual plants and bulks

Building of libraries

Sequencing

Alignment on a reference genome (*M. truncatula*)

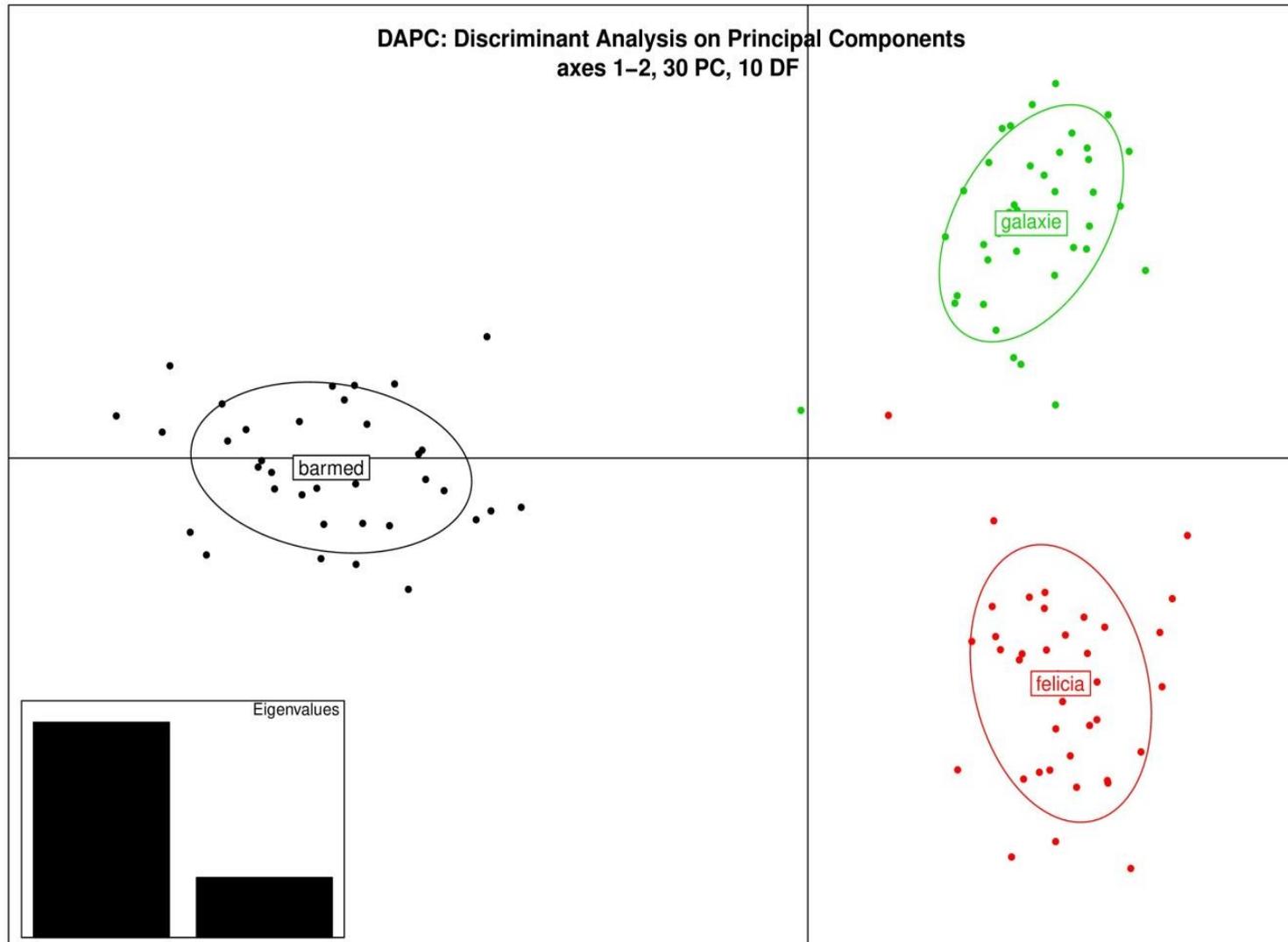
SNP calling

for individuals: 0 / 1 / 2 / 3 / 4

for bulks: 0 to 100%



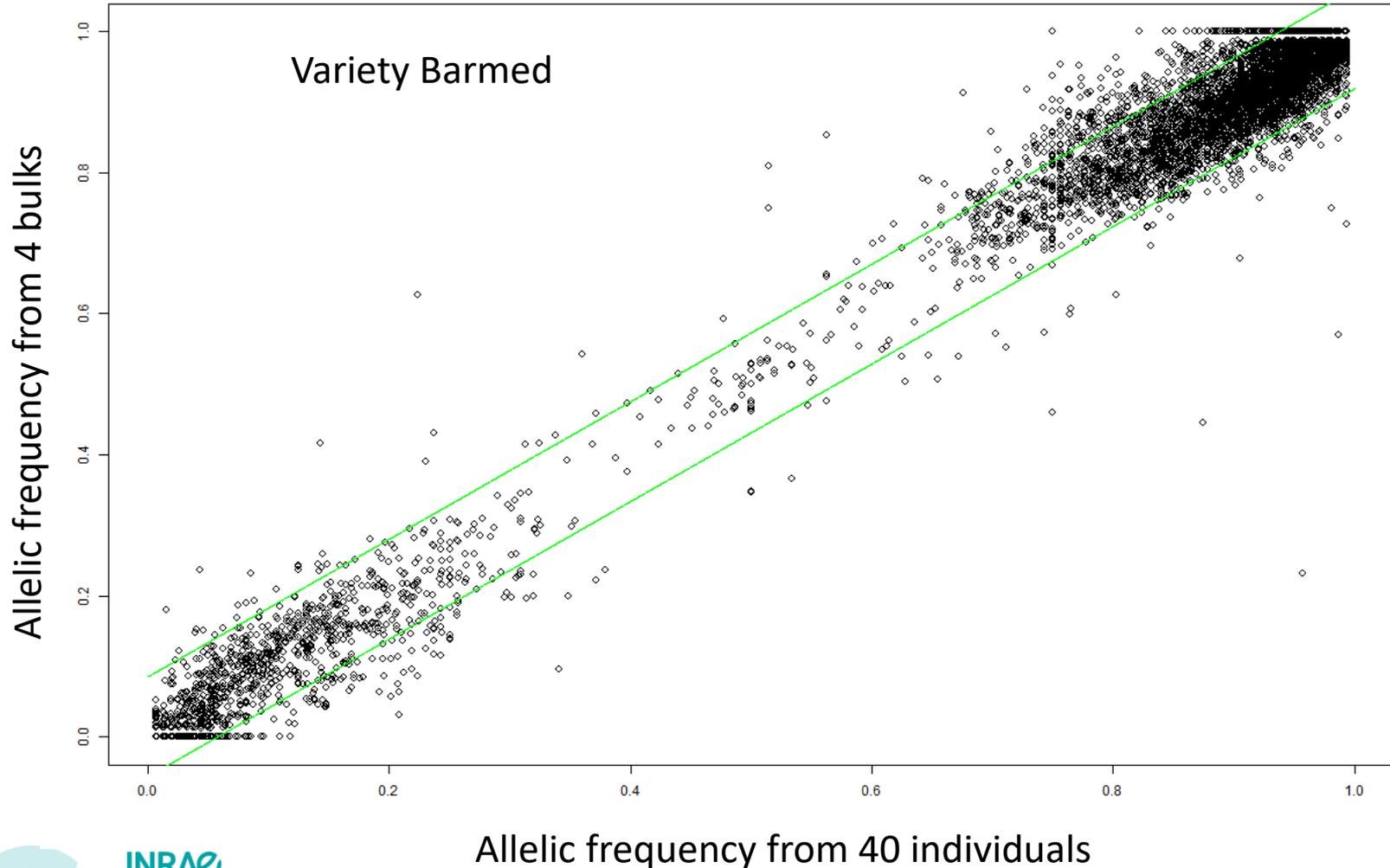
➤ Distinction among varieties with information at the individual level



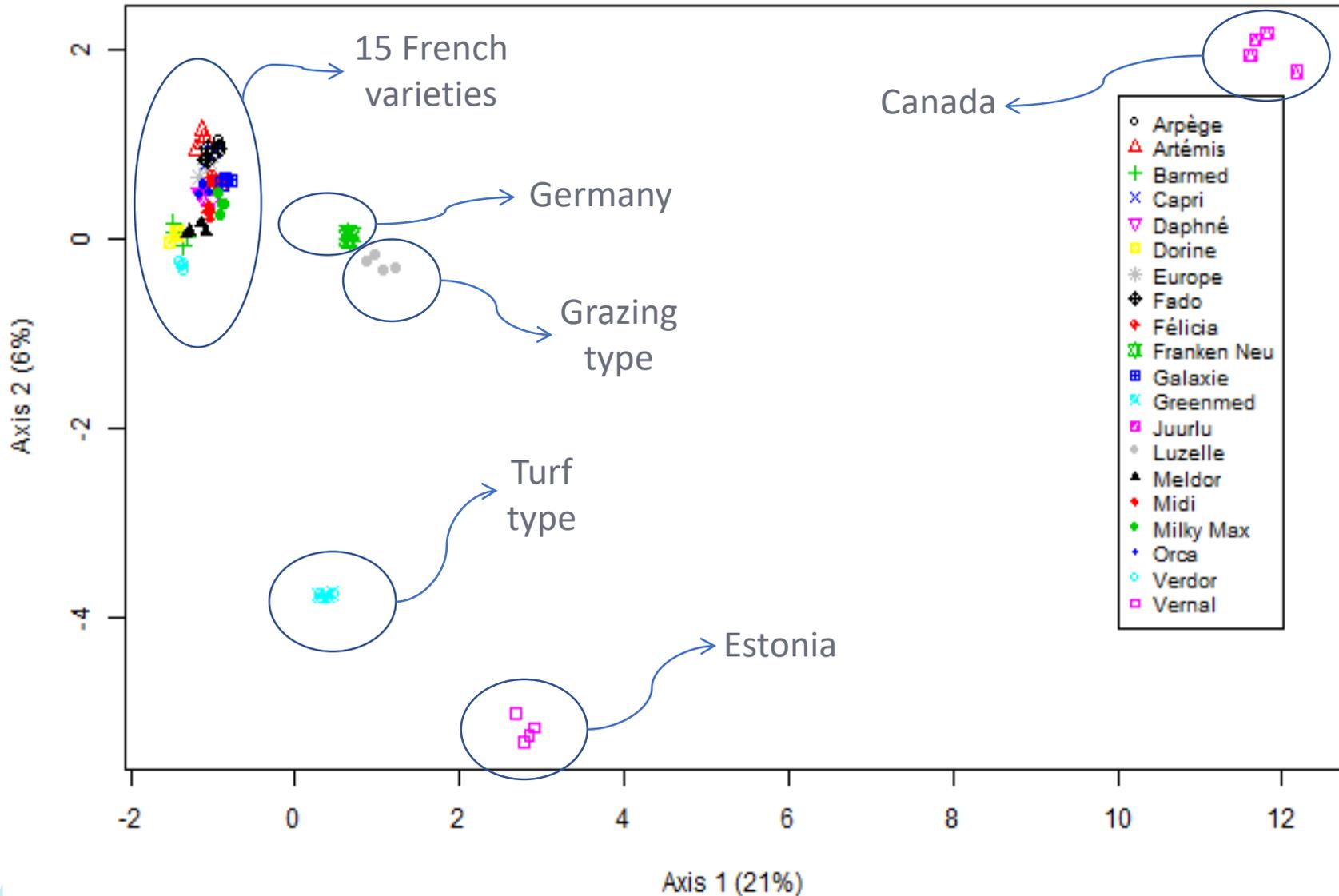
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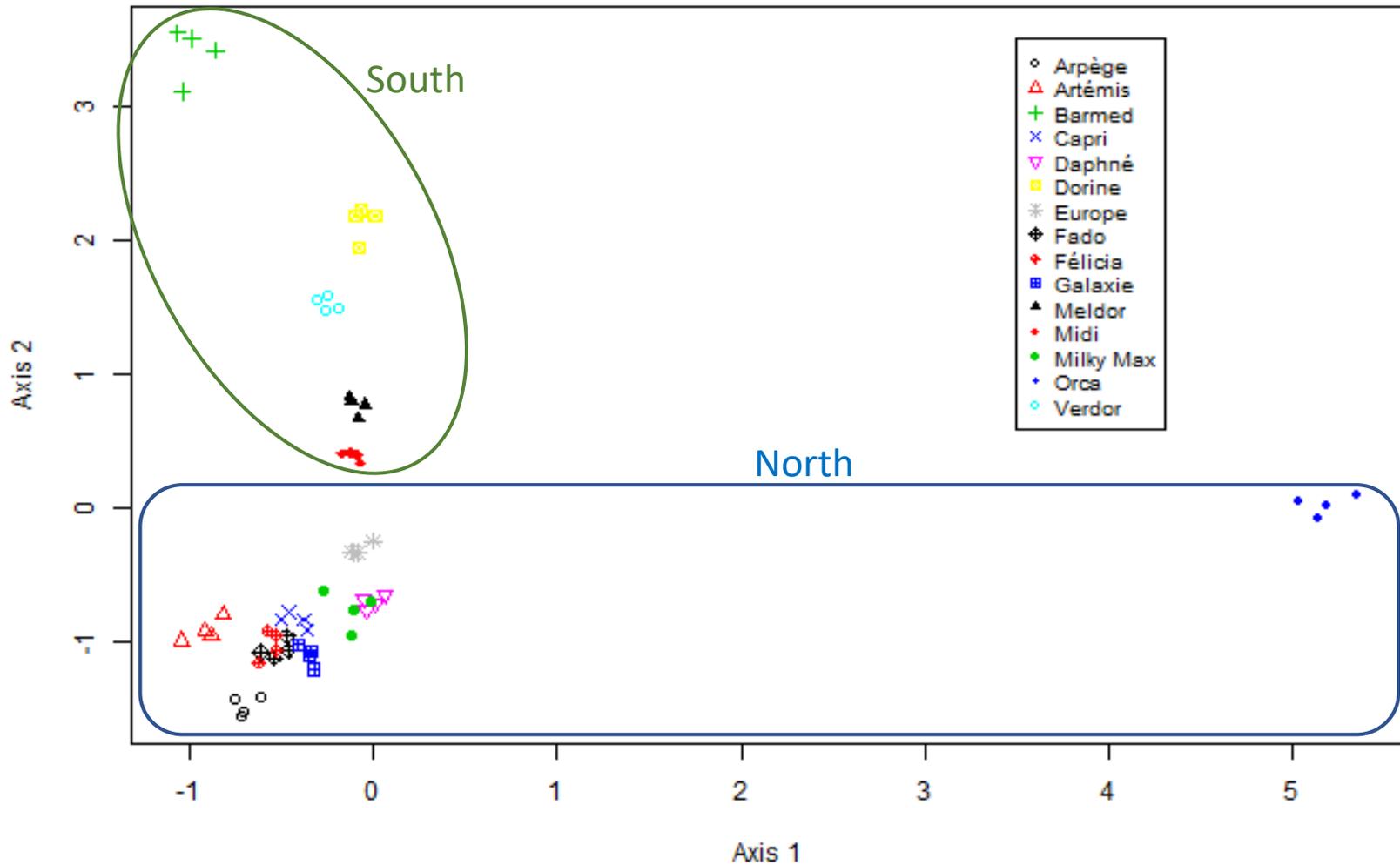
➤ Comparison of allelic frequency calculated on the average of 40 individual genotypes and on bulks



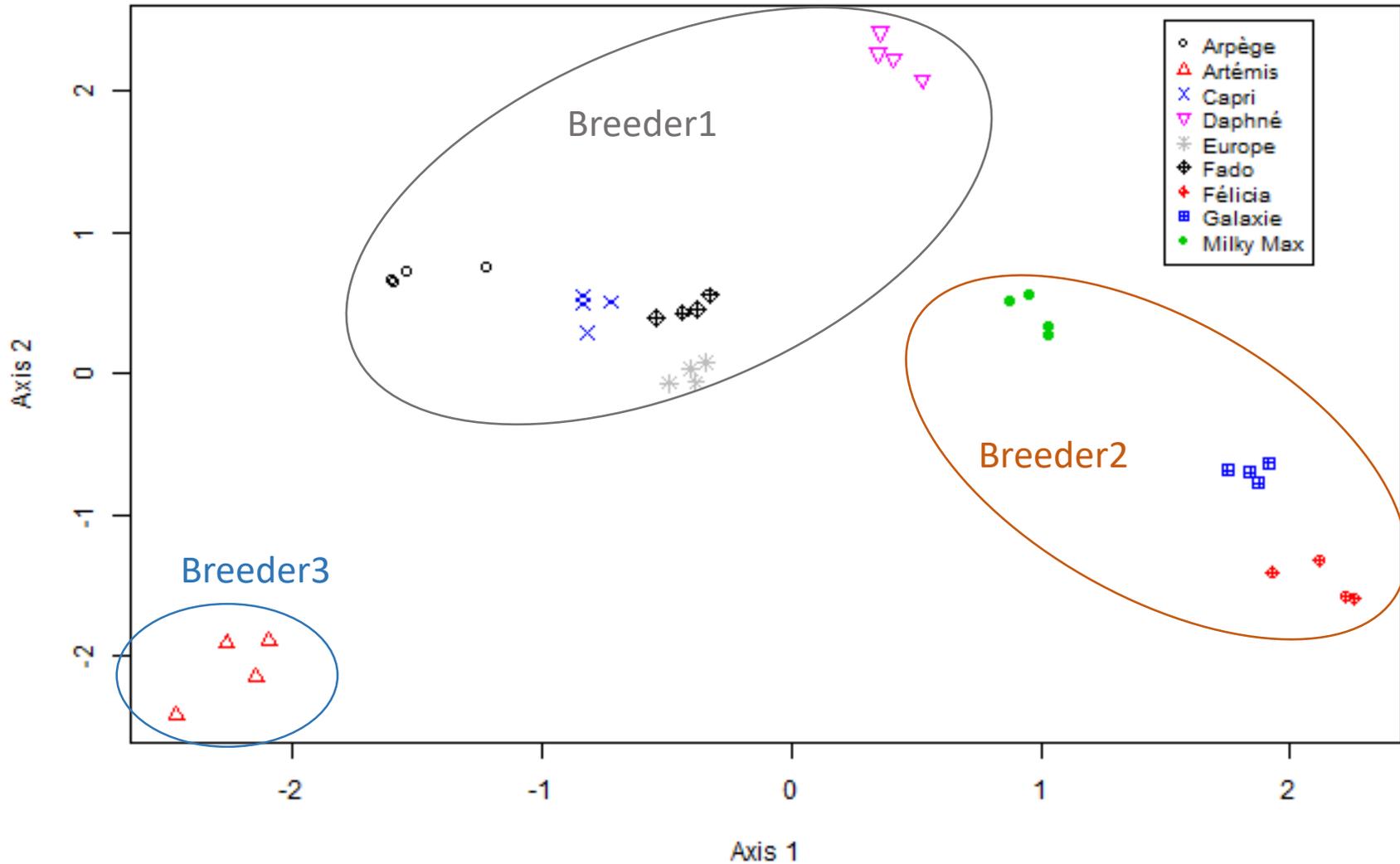
➤ Distinctness of 20 varieties for which 4 bulks have been genotyped



➤ Zoom on the 15 French varieties



➤ Zoom on the 9 North varieties



➤ Test of difference between pairs of varieties

AMOVA and F_{ST}

AMOVA between pairs of varieties: significant P values [0.025 to 0.033]

F_{ST} : all significant at $P = 0.05$

from

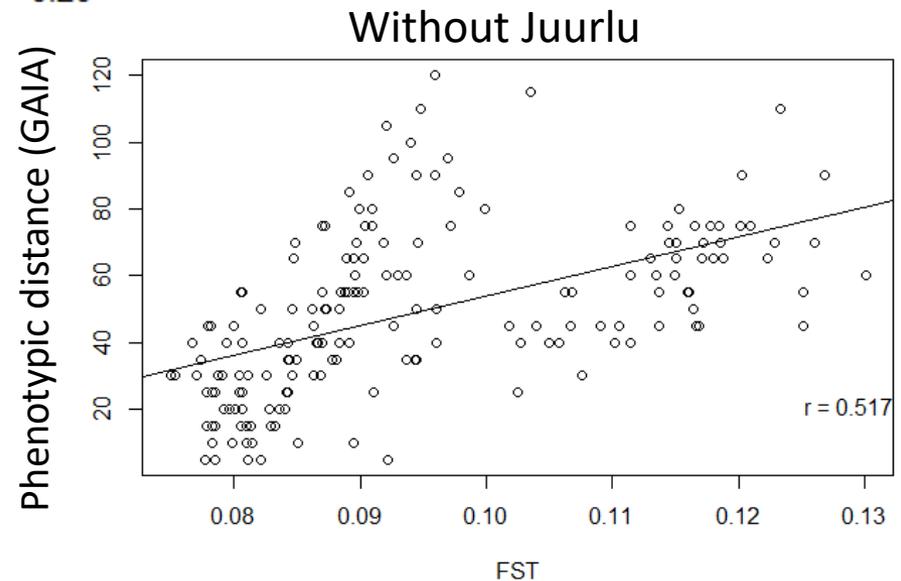
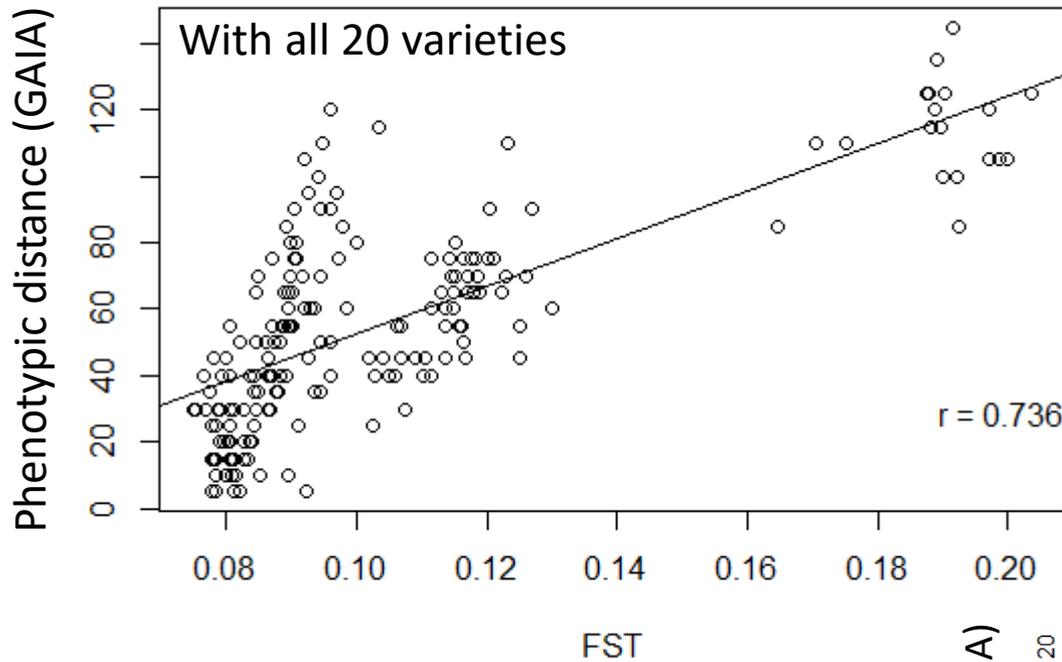
0.075 (between Europe and Capri, both bred by Florimond Desprez)

to

0.203 (between Juurlu and Barmed, the two extreme varieties for autumn dormancy).



➤ Correlation of genetic distances (F_{ST}) with phenotypic distances



> Conclusion

At the technical level

Efficiency of GBS to genotype individuals or bulks on a heterozygous tetraploid species

Accuracy of the estimation of allelic frequencies on bulks

At the scientific level

Significant difference between each pair of varieties

Structure of the diversity is consistent to our knowledge of the varieties

Good correlation between genetic distance and phenotypic distances



> Conclusion

Use of GBS markers to help DUS testing?

To structure the collection of varieties

- A candidate variety is tested phenotypically with the varieties with small genetic distance

- Less field plots / experiments

- More precise trials

Use of molecular markers only

- Good correlation with phenotypic traits

- No effect of environment

- No phenotypic description

A mixed use of phenotypic data and markers?



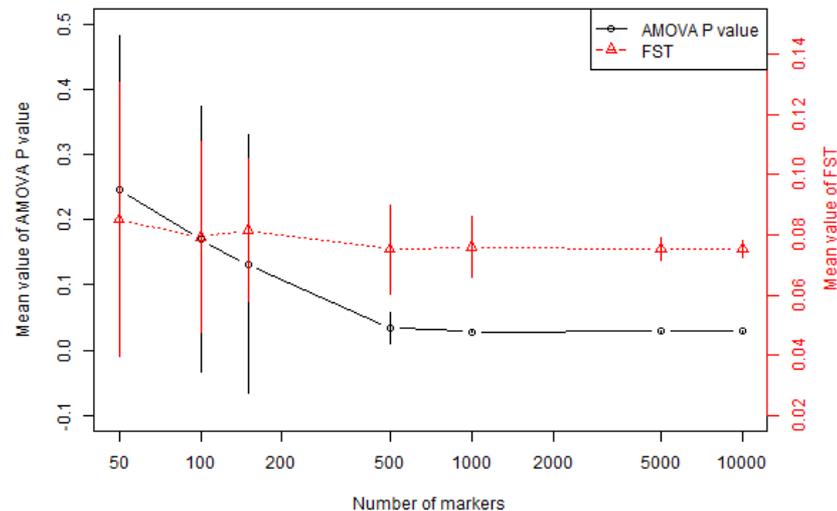
➤ Conclusion

More studies needed

Development of license-free, cost-efficient markers

SNP based on capture (project INVITE, H2020, 2019-2023)

Number of markers



To estimate genetic distances

between candidate varieties that were not distinct in DUS tests,
between seed lots of the same variety, etc.



➤ Thanks

 **INRA**
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ACVF

 **BARENBRUG**

 **JD**
ECO PERFORMANCE

**ASSOCIATION DES
CRÉATEURS DE
VARIÉTÉS FOURRAGÈRES**

 **FLORIMOND
DESPREZ**

 **FRAGT**
2n



Project Amediluze,
C-2014/05,
2014-2017

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