

# A new method for estimating mixing ability: application to winter wheat variety mixtures and propositions for plant breeding



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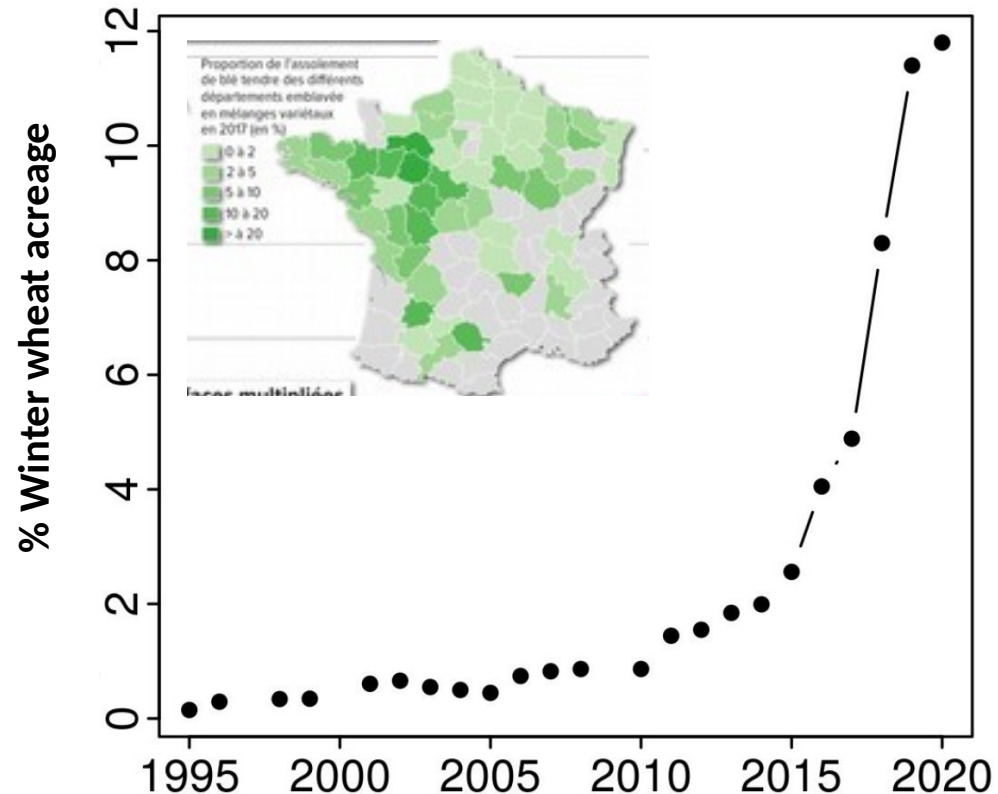
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# Context : growing interest in variety mixtures

Wheat variety mixtures in France



- Strong progression of wheat variety mixtures in France
- → Strong interest of farmers
- Recent evolution of regulation :  
→ sales of variety mixtures authorized (July 2018)

# Context : interest in variety mixtures

- **Farmers:** agroecological practice, easy to implement, possibility to design mixtures tailored for local context
- **Agronomy:** buffering of abiotic and biotic stresses → stabilization of production, reduction of foliar disease development (*Finckh and Mundt 1992*)
- **Ecology:** mobilization of species and genetic diversity → complementarity, compensation and synergy effects (*Tilman et al. 1997, Creissen et al. 2016*)
- Need to identify varieties adapted to this practice: lack of references due to evaluation in pure stand only (unpredictability of mixture performances)  
→ **estimation of mixing ability**

# Development of methods for estimating mixing ability

## Objectives:

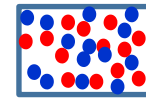
- Identify the best performers in mixture from a panel
- Screening of a large number of genotypes (! combinatorics)
- Propose the integration of these methods in breeding programs for mixtures

## Methodological approaches (Performance based – trait blind) :

I. Evaluation of a set of mixtures (analogy with combining ability)

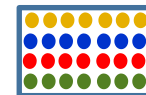
II. Evaluation of individual components in nursery trials (competitive ability)

Number of genotypes



$Y_{ij}$

	Var1	Var2	Var3	Var4	Var5
Var1					
Var2					
Var3					
Var4					
Var5					



$Y_{i(jj')} + Y_{j(ii')}$



# Development of methods for estimating mixing ability

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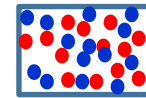
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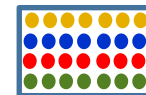
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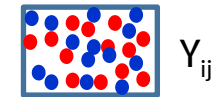
# I. Modeling mixing ability (following Griffing 1956)

Analogy with the concept of combining ability, evaluation of mixtures

	G1	G2	G3	G4	G5
G1	$Y_{11}$	$Y_{12}$	$Y_{13}$	$Y_{14}$	$Y_{15}$
G2		$Y_{22}$	$Y_{23}$	$Y_{24}$	$Y_{25}$
G3			$Y_{33}$	$Y_{34}$	$Y_{35}$
G4				$Y_{44}$	$Y_{45}$
G5					$Y_{55}$

→  $GMA_1$

Evaluation of a panel of genotypes in binary mixtures



- **Model (fixed effects):**  $Y_{12} = \mu + \frac{1}{2} (GMA_1 + GMA_2) + SMA_{12}$
- **General Mixing Ability:** Mean performance of a genotype in mixture

$$GMA_1 = (Y_{12} + Y_{13} + Y_{14} + Y_{15}) / 4 - \mu$$

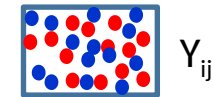
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G2	$Y_{21}$	$Y_{22}$	$Y_{23}$	$Y_{24}$	$Y_{25}$
G3	$Y_{31}$	$Y_{32}$	$Y_{33}$	$Y_{34}$	$Y_{35}$
G4	$Y_{41}$	$Y_{42}$	$Y_{43}$	$Y_{44}$	$Y_{45}$
G5	$Y_{51}$	$Y_{52}$	$Y_{53}$	$Y_{54}$	$Y_{55}$

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- **General Mixing Ability:** Mean performance of a genotype in mixture  

$$GMA_1 = (Y_{12} + Y_{13} + Y_{14} + Y_{15}) / 4 - \mu$$
- **Specific Mixing Ability:** Deviation of a mixture performance from the predicted value based on its components' **GMA** (interaction term):  

$$SMA_{12} = Y_{12} - \mu - \frac{1}{2} (GMA_1 + GMA_2)$$

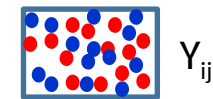
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G4	$Y_{41}$	$Y_{42}$	$Y_{43}$	$Y_{44}$	$Y_{45}$
G5	$Y_{51}$	$Y_{52}$	$Y_{53}$	$Y_{54}$	$Y_{55}$

Diagram description: A 5x5 matrix of yield values  $Y_{ij}$ . The first row (G1) is highlighted in blue. The cell  $Y_{12}$  is highlighted in purple. A blue arrow points from the  $Y_{12}$  cell to the label  $GMA_1$ .

Evaluation of a panel of genotypes in binary mixtures



- **Model (fixed effects):**  $Y_{12} = \mu + \frac{1}{2} (GMA_1 + GMA_2) + SMA_{12}$
- **General Mixing Ability:** Mean performance of a genotype in mixture  

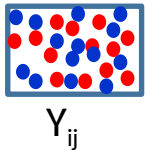
$$GMA_1 = (Y_{12} + Y_{13} + Y_{14} + Y_{15}) / 4 - \mu$$
- **Specific Mixing Ability:** Deviation of a mixture performance from the predicted value based on its components' **GMA** (interaction term):  

$$SMA_{12} = Y_{12} - \mu - \frac{1}{2} (GMA_1 + GMA_2)$$
- GMA and SMA are relative to the panel and the experimental conditions



# Broadening the GMA-SMA models

- Evaluation of a panel in all possible binary mixtures (diallel-like design)
- Limit: the curse of combinatorics (the number of possible mixtures increases with the size of the panel) -> ① incomplete designs



	Var1	Var2	Var3	Var4	Var5
Var1	■	■	■	■	■
Var2		■	■	■	■
Var3			■	■	■
Var4				■	■
Var5					■

Fig.1: Complete design

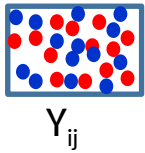


	Var1	Var2	Var3	Var4	Var5
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Fig.2: Incomplete design

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Var2		█	█	█	█
Var3			█	█	█
Var4				█	█
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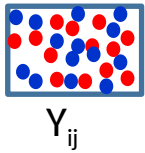
	Var1	Var2	Var3	Var4	Var5
Var1	█	█		█	
Var2		█	█		█
Var3			█		█
Var4				█	
Var5					█

Fig.2: Incomplete design

- Development of mixed models, variance components framework (ReML, BLUP):
  - ✓ Mixed model with  $GMA \sim N(0, \sigma_{GMA}^2)$ ,  $SMA \sim N(0, \sigma_{SMA}^2)$  and  $e \sim N(0, \sigma_e^2)$
  - ✓ Estimation of variances:  $\sigma_{GMA}^2$  and  $\sigma_{SMA}^2$  (ReML procedure),
  - ✓ Prediction of the GMA and SMA values (**BLUP**)

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Var5					█

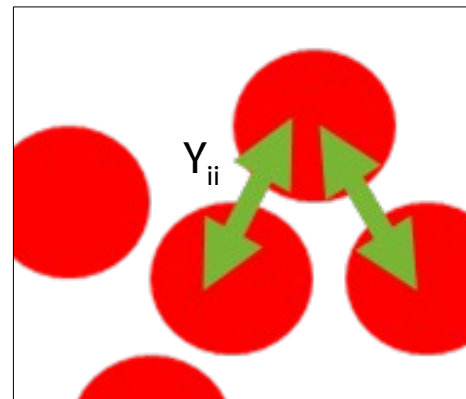
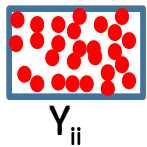
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	Var1	Var2	Var3	Var4	Var5
Var1	█	█		█	
Var2		█	█		█
Var3			█		█
Var4				█	
Var5					█

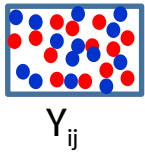
Fig.2: Incomplete design

- Development of **mixed models**, variance components framework (ReML, BLUP)
- Comparisons with pure stand performances: ② **inclusion of the pure stands in the analysis (SMA<sub>ii</sub>)**



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Var1	█	█	█	█	█
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Var3			█	█	█
Var4				█	█
Var5					█

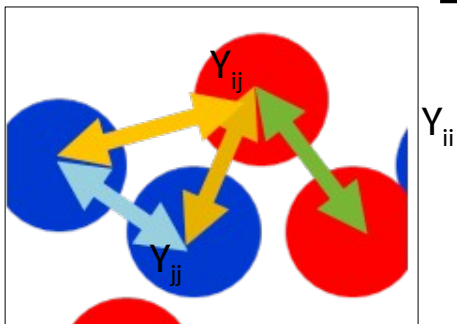
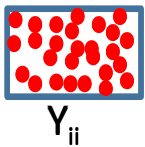
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	Var1	Var2	Var3	Var4	Var5
Var1	█	█		█	
Var2		█	█		█
Var3			█		█
Var4				█	
Var5					█

Fig.2: Incomplete design

- Development of **mixed models**, variance components framework (ReML, BLUP)
- Comparisons with pure stand performances: ② **inclusion of the pure stands in the analysis (SMA<sub>ii</sub>)**
- Accounting for genotypic frequencies: ③ **neighboring probabilities**  
→ Introduction of intra-genotypic interactions within mixtures for SMA modeling

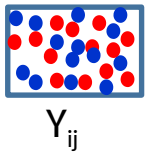


Example of proportions in binary mixture 50% A, 50% B

Interactions between pairs of plants:  $\frac{1}{4} AA + \frac{1}{2} AB + \frac{1}{4} BB$  (considering  $AB=BA$ )

# Broadening the GMA-SMA models

- Evaluation of a panel in all possible binary mixtures (diallel-like design)
- Limit: the curse of combinatorics (the number of possible mixtures increases with the size of the panel) -> ① **incomplete designs**



	Var1	Var2	Var3	Var4	Var5
Var1	Grey	Blue	Blue	Blue	Blue
Var2		Grey	Blue	Blue	Blue
Var3			Grey	Blue	Blue
Var4				Grey	Blue
Var5					Grey

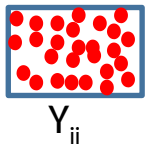
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	Var1	Var2	Var3	Var4	Var5
Var1	Grey	Blue		Blue	
Var2		Grey	Blue		Blue
Var3			Grey		Blue
Var4				Grey	
Var5					Grey

Fig.2: Incomplete design

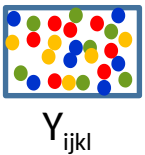
- Development of mixed models, variance components framework (ReML, BLUP)



- Comparisons with pure stand performances: ② **inclusion of the pure stands in the analysis (SMA<sub>ii</sub>)**

50% i, 50% j

- Accounting for genotypic frequencies: ③ **neighboring probabilities**



- ④ **Generalization to higher order mixtures (>2 components)**

$$Y_{nbr} = \mu + \alpha_b + \frac{1}{K(n)} \sum_{k=1}^{K(n)} GMA_{k(n)} + \frac{1}{K(n)^2} \sum_{k=1}^{K(n)} \sum_{k'=1}^{K(n)} SMA_{k(n)k'(n)} + e_{nbr}$$

Block b, replicate r, mixture n, and K the number of components

# Application: binary mixtures experimental design

Diversified panel:

Elites varieties, organic varieties, landraces, INRAE lines

**25 genotypes**

Mixtures design:



**75 binary mixtures** (/300 possible) + 25 pure stands

Each genotype observed in 6 different mixtures

Le Moulon (France), 2014-2015, 2 replicates of 7,5m<sup>2</sup> plots

Economic level of nitrogen fertilization

Response variables: yield and yield components

A

Genotype	A208	A22	A243	A398	Alauda	Altigo	Apache	Arezzo	Blé autrichien	Boregar	F236	F426	Grapelli	Hermes	Maxi	Midas	Renan	Ritter	Rouge de Bordeaux	Rouge du Roc	Saint Priest	Skerzzo	Sogood	Soissons	Trémie		
A208																											
A22																											
A243																											
A398																											
Alauda																											
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Saint Priest																											
Skerzzo																											
Sogood																											
Soissons																											
Trémie																											



# Results: binary mixtures experiment

Correlation between the mixtures and the mean of their pure stand components:

**0.51 for yield, 0.51 for the nb of spikes/m<sup>2</sup>, 0.42 for the grain nb/spike and 0.91 for TKW**

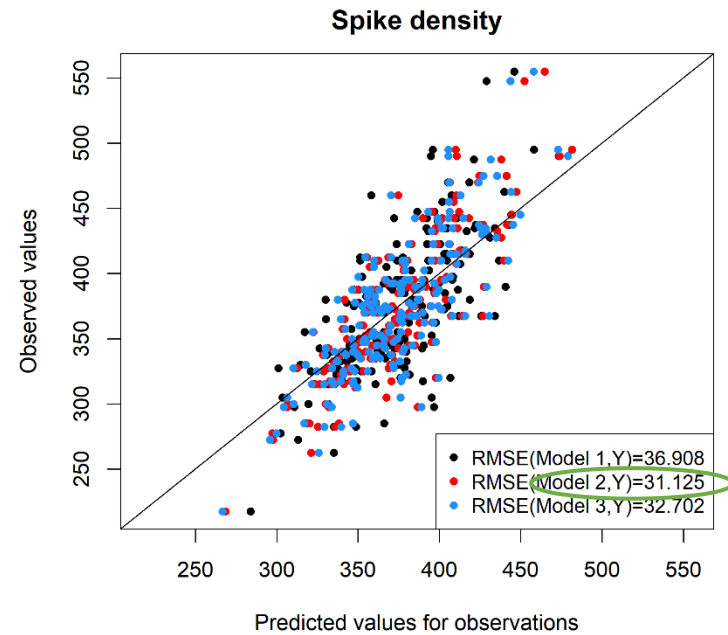
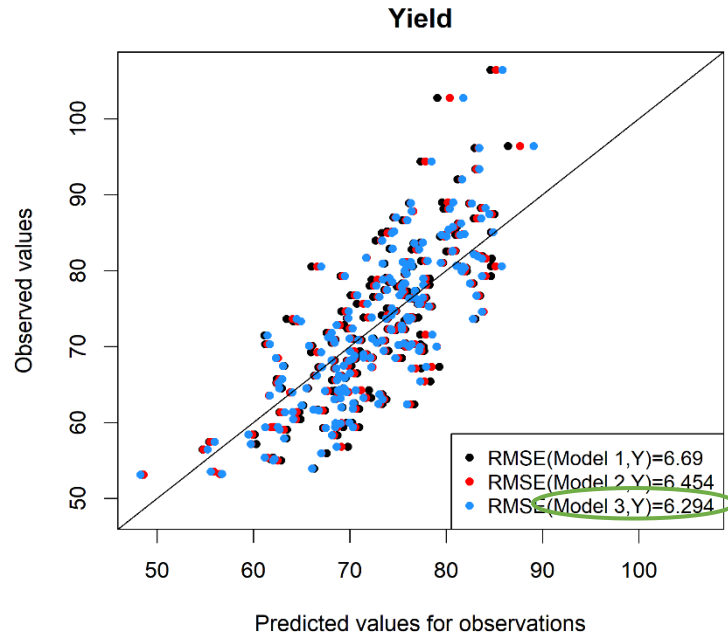
Importance of SMA effects (*model with neighboring probabilities*):

$$\sigma_{\text{SMA}}^2 / \sigma_{\text{GMA}}^2$$

Yield	0.27	
Spike density	<b>0.61</b>	→ Strong plasticity for tillering
Gr nb/spike	0.00	
TKW	0.07	

→ correlation between observed vs predicted (based on GMA-SMA) mixture yield: **0.88**

# Predictions : model comparison

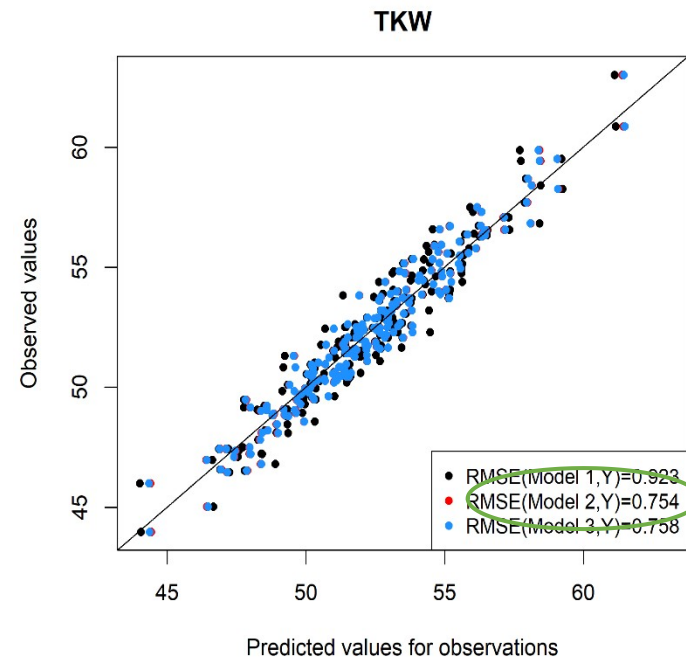


Model 1 = GMA only

Model 2 = GMA + SMA following Griffing (1956)

Model 3 = GMA + SMA including intra-genotypic interactions within mixtures

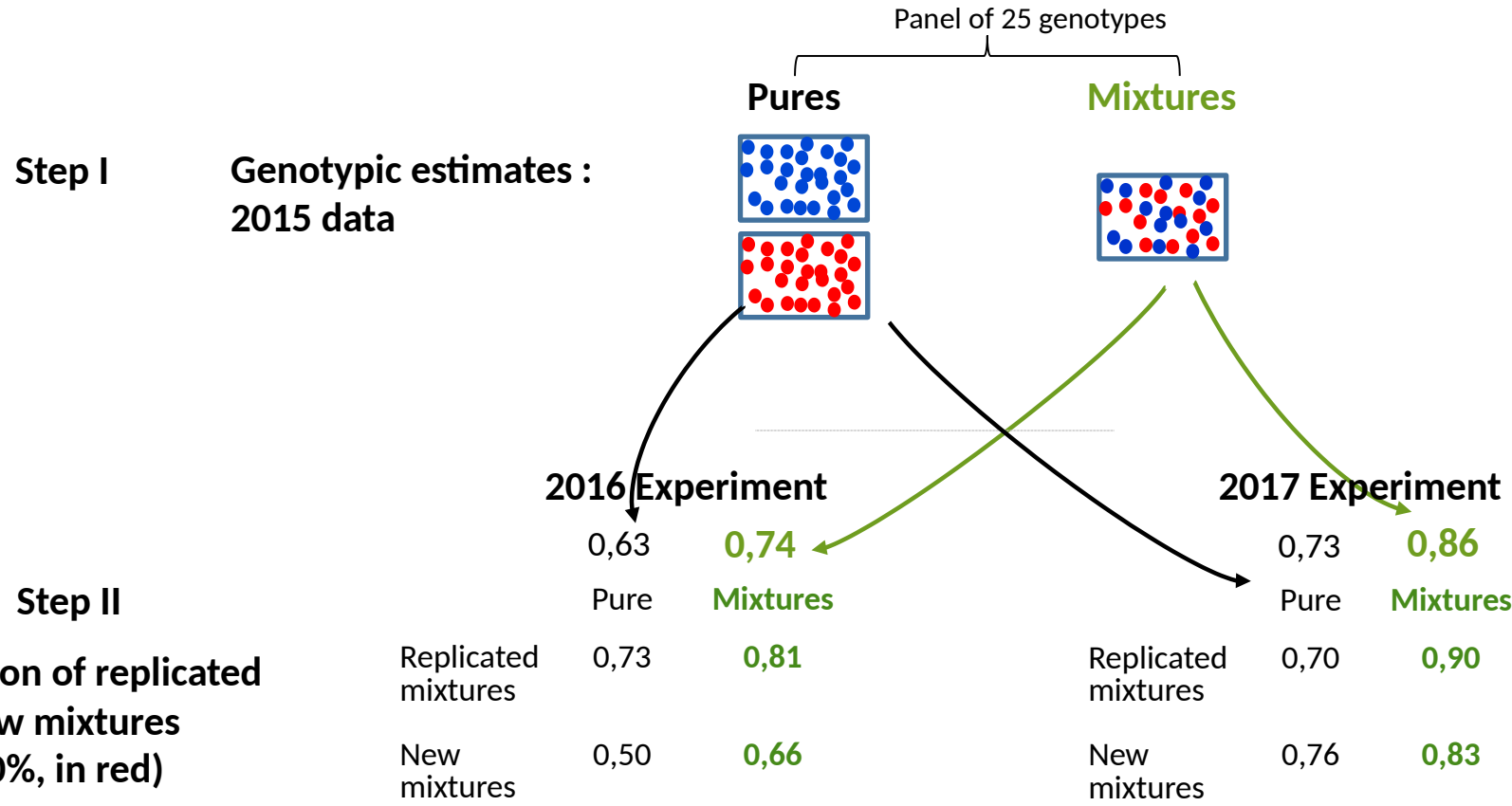
→ Only slight improvement with SMA models



TKW : thousand kernel weight



# Results: prediction of mixture performance

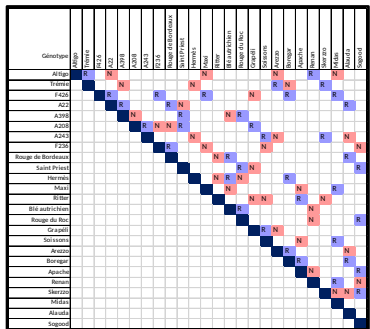


Prediction based on 2015 data :

—→ Pure

—→ Mixtures

**Step II**  
Prediction of replicated and new mixtures (50%, in red)



→ The GMA-SMA approach is more efficient in predicting mixture performance

# Development of methods for estimating mixing ability

## Objectives:

- Identify the best performers in mixture from a panel
- Screening of a large number of genotypes (! combinatorics)
- Propose the integration of these methods in breeding programs for mixtures

## Methodological approaches:

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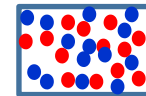
II. Evaluation of individual components in nursery trials (competitive ability)

Producer associate model → see Haug et al. 2021 (interspecific)

$$Y_{bi(jj')} = \mu + B_b + Pr_i + As_j + As_{j'} + E_{bi(jj')}$$

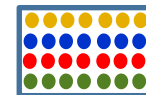
→ Identification of genotypes with both high Producer and Associate effects

Number of genotypes



$Y_{ij}$

	Var1	Var2	Var3	Var4	Var5
Var1					
Var2					
Var3					
Var4					
Var5					



$Y_{i(jj')} + Y_{j(ii')}$



# Integrating mixing ability in breeding programs

	Var1	Var2	Var3	Var4	Var5
Var1					
Var2					
Var3					
Var4					
Var5					

Objective

① GMA correlated with pure stand performance

Dev multi-purpose varieties for pure and mixed stands

Methodology

Few adaptations from a classical program:

- Evaluation **single-row plots**
- **Few mixtures/candidate genotype:** with a tester

② GMA poorly correlated with pure stand perf. + low SMA

Dev pool of varieties to combine or higher order mixtures

Focus on indiv components:

- Eval **single-row plots**
- Eval **mixtures** with testers or diallel-like design
- Eval mixtures with **sorted components**

③ important SMA

Dev 3-4 component mixtures

a) Structuring the panel:

- Eval structured single-row / diallels → determine the best **combination groups**, identify testers

b) Evaluate with testers

- > identify **best genotype combinations**

	Var1	Var2	Var3	Var4	Var5
Var1					
Var2					
Var3					
Var4					
Var5					

+ decentralized screening, on-farm evaluation

# Take-home messages

- **Broadening of the GMA-SMA models:** incomplete designs, for any number of components (including pure stands) and proportions, proposing a new modeling of SMA effects
- **Producer-Associate model:** informative on indirect effect of a genotype on its neighbors
- This statistical framework and experimental designs can be further integrated into **breeding programs for intra-specific or inter-specific mixtures**
- **Critical to link these mixing ability analyses with the description of underlying traits driving plant-plant interactions (ideotyping)**  
 → See the work of Haug et al. 2021 (H2020 Remix)

$GMA_{pea}$	$Pr_{pea}$	$As_{pea}$	Biological interaction-function (BIF) of pea trait	pattern
			Commensalism	+/0
			Commensalism	0/+
			Mutualism	+/+
			Antagonism	+/-
			Antagonism	-/+
			Neutralism	0/0
			Amensalism	0/-
			Amensalism	-/0
			Competition	-/-

# Thank you !

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Nathalie Galic

Sophie Pin

Pauline L'hote

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Christophe Ambroise

Tristan Mary-Huard

Inès Krissaane (M1)

GDEC Clermont-Ferrand :

Vincent Allard



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# Models comparison

**Model 1** = GMA only:

$$Y_{ij} = \mu + \alpha_i + \frac{1}{K(j)} \sum_{k=1}^{K(j)} GMA_{k(j)} + e_{ij}$$

**Model 2** = GMA + SMA following Griffing (1956):

$$Y_{ij} = \mu + \alpha_i + \frac{1}{K(j)} \sum_{k=1}^{K(j)} GMA_{k(j)} + \frac{1}{C_{K(j)}^2} \sum_{k=1}^{K(j)} \sum_{k'=1}^{K(j)} SMA_{kk'(j)} + e_{ij} \quad \begin{cases} k' \neq k \text{ in mixture} \\ k' = k \text{ in pure stand} \end{cases}$$

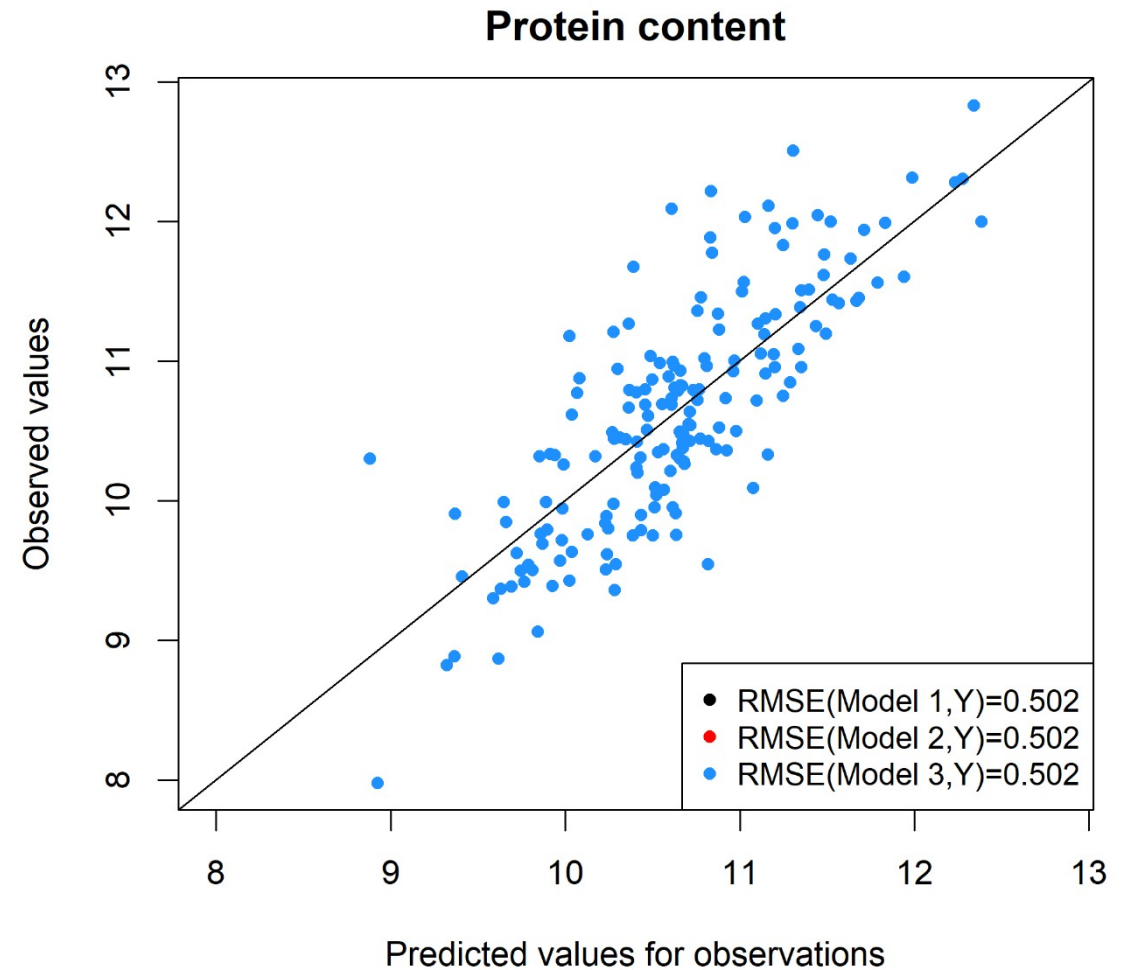
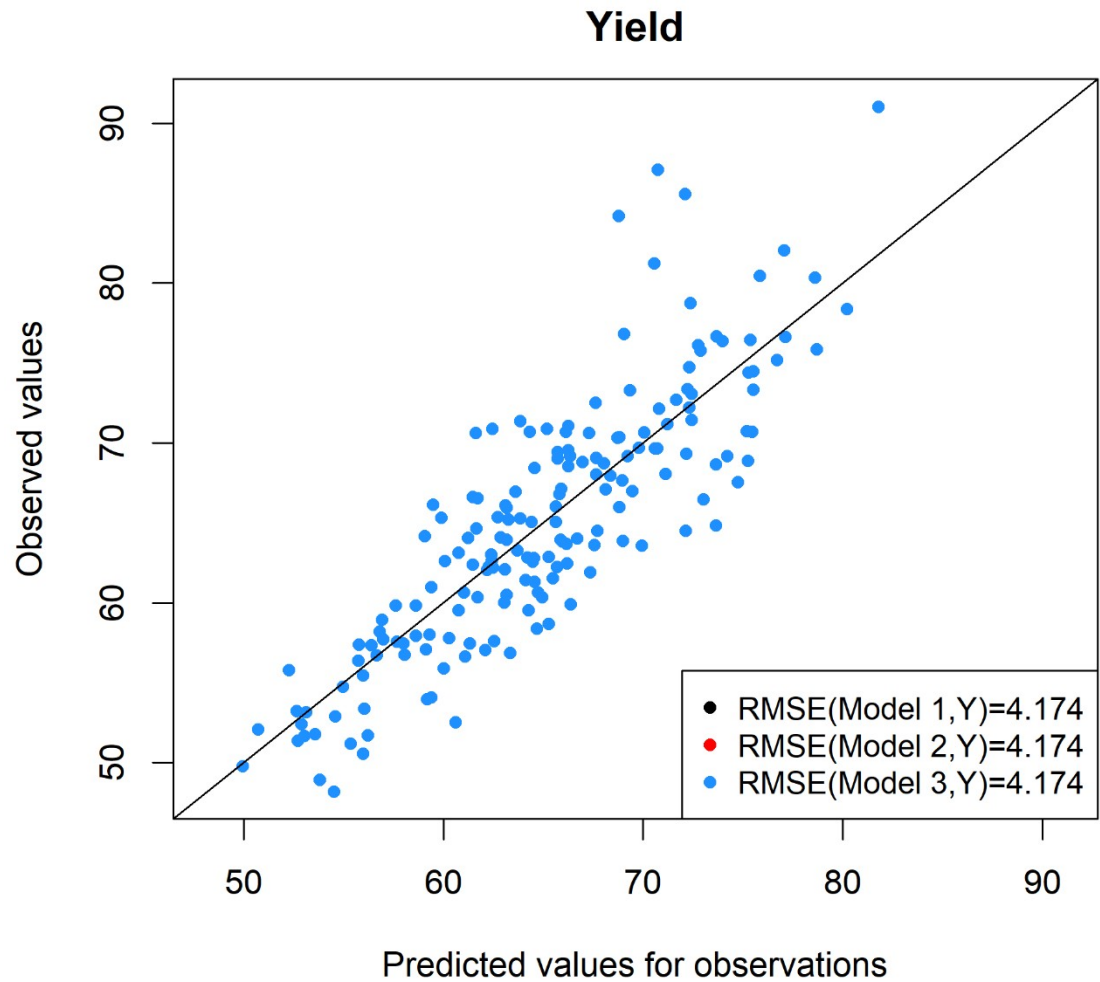
**Model 3** = GMA + SMA including intra-genotypic interactions within mixtures:

$$Y_{ij} = \mu + \alpha_i + \frac{1}{K(j)} \sum_{k=1}^{K(j)} GMA_{k(j)} + \frac{1}{K(j)^2} \sum_{k=1}^{K(j)} \sum_{k'=1}^{K(j)} SMA_{kk'(j)} + e_{ij}$$





# Predicted values in CF



# Correlation of the GMA between the two trials

