Genomic Prediction for DUS

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BioSS

INVITE

HORIZON 2020 Project

Innovations in plant Varlety Testing in Europe to foster the introduction of new varieties better adapted to varying biotic and abiotic conditions and to more sustainable crop management practices

- 1 July 2019 – 31 December 2024
- 29 Partners – 13 Countries
- 10 example crops
- *h2020-invite.eu*

BioSS role in INVITE

Our focus on improved statistical methods for variety assessment, including use of DNA markers

- DUS ←
- VCU/Performance (perennial ryegrass)

+ DUS examination offices: Austria, Germany, Hungary, Italy, Netherlands, UK

Overview

- DUS testing
- Genetic markers in DUS testing
- Genomic Prediction
- More efficient planning of trials using genetic markers
- Distinctness assessment with help from genetic markers

DUS Testing

DUS Testing

- Required for plant breeders rights (IP) and for national listing in EU and UK

- UPOV system *www.upov.int:* over 90 states around the world

- New varieties must be assessed as **D**istinct, **U**niform and **S**table = DUS
  - Distinct from any other variety whose existence is a matter of common knowledge ✓
  - Uniform – individuals of a variety are sufficient similar (new method for uniformity with markers)
  - Stable – over repeated propagations

UPOV (2002) General introduction to the examination of distinctness, uniformity and stability. UPOV document TG/1/3. Published by UPOV, Geneva. https://www.upov.int/resource/en/introduction_dus.html

DUS Testing - Distinctness

- **Distinctness is often assessed by field trials:**
  - Often in one location over two or three years
  - Comparing with existing varieties
  - Using several phenotypic traits (mainly morphological)
    - Specified in crop guidelines
  - Only needs to be distinct in one trait
    - But needs to be distinct from all existing varieties

- **For established agricultural crops:**
  - Large trials
  - Lots of measurements at multiple time points
  - Very expensive

Genetic markers in DUS testing

Markers in DUS testing

Currently: two main use cases for genetic markers (related to distinctness):

- Direct assessment of distinctness

- For management of DUS trials

Markers cannot be used on their own to establish distinctness

But they can be used to predict phenotype in terms of DUS traits

UPOV FAQS Does UPOV allow molecular techniques (DNA profiles) in the examination of Distinctness, Uniformity and Stability ("DUS")? Published by UPOV, Geneva. https://www.upov.int/about/en/faq.html#QB80

Markers in DUS testing

Direct assessment of distinctness

- Markers must be apply to predict trait precisely (~100%)
  - Examples include herbicide resistance and disease resistance
  - Requires simple genetics
- Often found by GWAS or by prior knowledge (or both)
- Use for quantitative traits by this approach unlikely (as described currently)

For **trial management**

- Selection of varieties (to order trial or to plant)
- Different approaches for different types of crops, e.g. cross-pollinated versus self-pollinated

Markers in DUS testing

For **trial management:** cross-pollinated crops

e.g. oilseed rape, perennial ryegrass

- Most traits quantitative
- Measurements are made for individual plants or plots, in SI units, e.g. millimetres
- Candidates compared with all varieties in reference collection
  - *A priori* selection difficult
  - Very large trials for established crops

Can we use markers to preselect which reference varieties to plant?

⇨ smaller trial

- Use markers to identify which varieties are likely to be phenotypically distinct from the candidate
- Reduce trial size

Markers in DUS testing

For **trial management:** self-pollinated crops and hybrids

e.g. wheat, maize

- A mix of types of DUS traits: quantitative, qualitative, pseudo-qualitative
- Measurements are mainly made visually, and on a 1-9 scale
- In first year, candidates are planted alone (or with few comparators) to identify close reference varieties
- In second year, candidates are planted next to close reference varieties
  - Sometimes further years need to establish distinctness

With markers, can we identify close reference varieties for the first year?

- Use markers to identify phenotypically similar varieties
- Improves chance of finding distinctness within 2 years (or even 1)
- Therefore reducing costs, and improves process for applicant

Markers in DUS testing

Existing UPOV application models for use of genetic markers

Approach A (for distinctness and trial management)

Use markers that link closely to genes controlling expression of a trait

Approach B (for trial management)

Use relationship between overall phenotypic distance & genetic distance

- To predict pairs of varieties clearly distinct or close phenotypically
- Requires a reasonable correlation to be worthwhile

UPOV (2020a) Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS). UPOV document TGP/15/3. Published by UPOV, Geneva. https://www.upov.int/edocs/tgpdocs/en/tgp_15.pdf

Markers in DUS testing

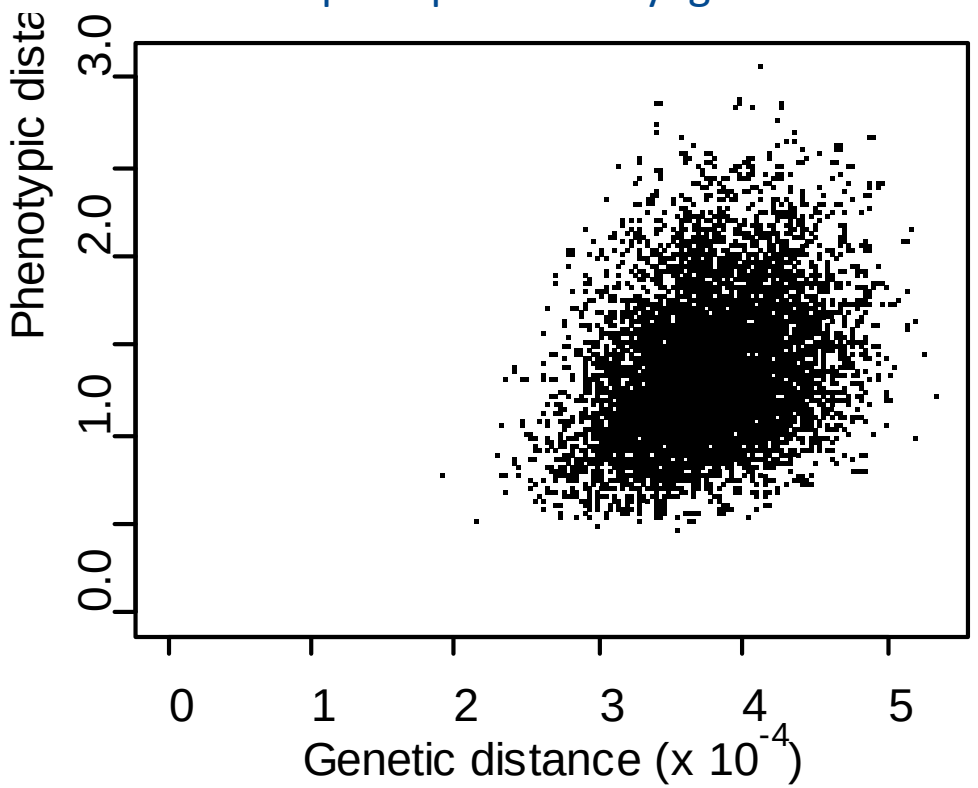
Our proposal is to use genomic prediction:

- To widen the number of cases where markers can be used to assess distinctness directly (extension of approach A)

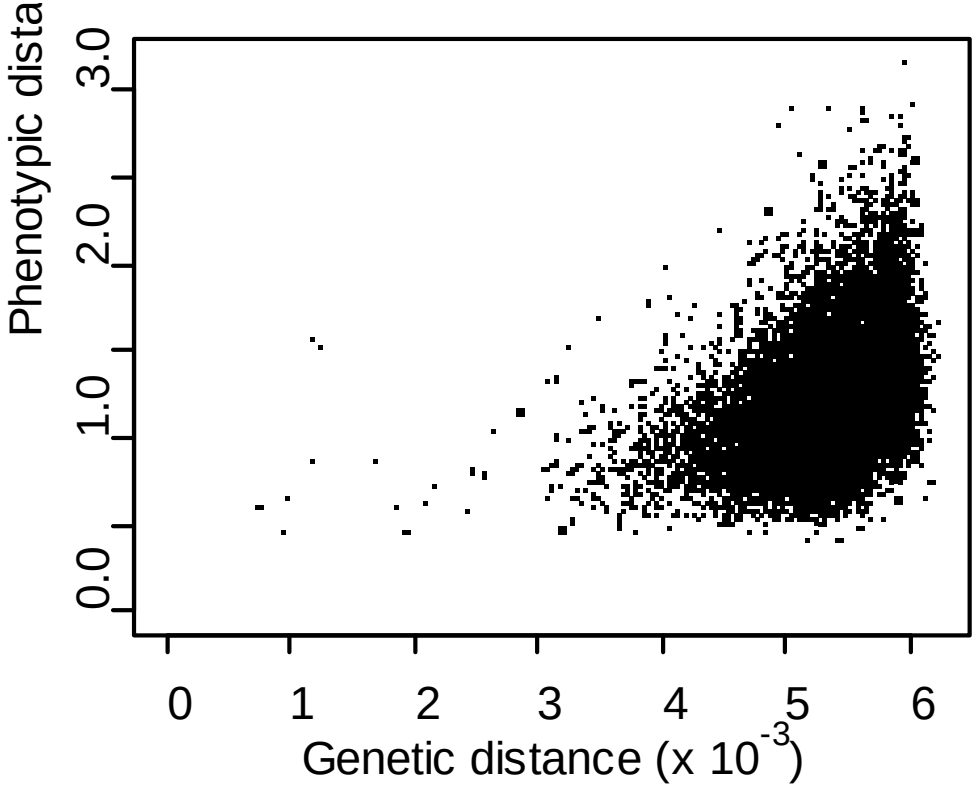
- To improve on current use of markers for trial management (under approach B)
  - Motivation – poor correlations between genetic and phenotypic distance
    - Note – phenotypic distance is a composite distance over all traits

Markers in DUS testing

Diploid perennial ryegrass



Wheat



Markers in DUS testing

Unsurprising that correlations between genetic and phenotypic distance are poor

Also, distinctness assessment is trait-by-trait

We can do better by predicting differences in individual traits using markers

Genomic Prediction

We propose the use of genomic prediction

- For planning trials
- For improving distinctness assessment in some cases

Genomic prediction uses genetic information e.g. from SNP markers to:

- Improve estimates of traits for existing genotypes
- To predict traits for new genotypes

The approach mirrors current DUS practice:

- Applied trait by trait

Genomic Prediction

There are large numbers of GP methods

- Statistical: ridge regression, GBLUP, Bayesian alphabet, ….
- Machine learning: including random forests, deep learning

Best method depends …

- Data set size and structure
- Genetic architecture
- Response type
- Computational concerns

Lourenço V, Ogutu J, Rodrigues R, Psekany A, Piepho H-P (2024) Genomic prediction using machine learning: a comparison of the performance of regularized regression, ensemble, instance-based and deep learning methods on synthetic and empirical data. BMC Genomics 25:152. https://doi.org/10.1186/s12864-023-09933-x

For illustration here, focus on GBLUP (genomic best linear unbiased prediction)

- Can be framed as a linear mixed model
- Also a variant that adds in specific SNPs as fixed effects
  - Found to be linked to QTLs by GWAS

Genomic Prediction

BLUP:
Fixed effects:
Random effects: year + variety

GBLUP:
Fixed effects:
Random effects: year + vm(variety, GRM)
With a variance structure for variety setting the correlation between the variety effects – based on the genetic relationship between markers

GBLUP + QTL:
Fixed effects: marker1 + marker2 + marker 3 …
Random effects: year + vm(variety, GRM)
with marker i indicating the SNPs found to be associated with the trait by GWAS

More efficient planning of trials using markers

Planning of trials

We claim that genomic prediction can outperform the current UPOV approach

- Separate predictions for each trait rather than a composite phenotype
- Informed by modelling the phenotype-genotype relationship

Genomic prediction can

- Predict the size of the difference in trait between two varieties
- Deliver an associated measure of precision (depends on method)
  - Helps to inform decisions

Planning of trials: framework

1. Fit a genomic prediction model to existing data

2. Predict the difference between a candidate and the reference variety
   - Reference varieties have genetic data + historic phenotypic data
   - Candidate variety has genetic data only

3. Assess whether the difference is significant

Planning of trials

To assess performance of GP method

- Use leave-one-**variety-**out cross-validation
- Historical data set
- Simulates real-life usage

Compare cross-validated predictions of differences with BLUPs (using all data)

- If ANOVA (=COYD) used to assess distinctness, we can compare decisions from COYD and cross-validated GBLUP

Planning of trials

Cross-validation scheme:

1. Take each variety in turn and treat as candidate
   - try to predict its over-year mean (like COYD)
   - use only its genetic data – as if it hadn't been trialled yet

2. Predict differences between "candidate" and reference variety
   - Reference based on phenotype data (and genetic data)
   - Candidate based on genetic data (no phenotypic data) – LOO

3. Assess whether this GBLUP predicted difference is significant at 1%

4. Compare with actual differences in phenotypic means

Data sets

Perennial ryegrass

DUS data from Naktuinbouw

- Up to 13 years 2007-2019
- 21 characteristics – measured in SI units

Marker data develop by Teagasc

- 187,000 SNPs with allelic frequencies, after quality screen

Varieties with both DUS data and markers

- Diploids: 119
- Tetraploid: 149
- Combine for analysis (268), taking into account ploidy, then separate for decisions
- Note permission only gained to access about 50% of collection

Compare gBLUP to long-term COYD at 1%

Wheat

DUS data from Austria, Germany, Hungary, Italy, and the UK

- Just one data point per variety per country
- Traits scored on 1-9 scale
- 27 characteristics -> 19 ordinal analysed

Marker data by NIAB

- '*Triticum aestivum* Next Generation' (TaNG) 43k Axiom array

Varieties with both DUS data and markers

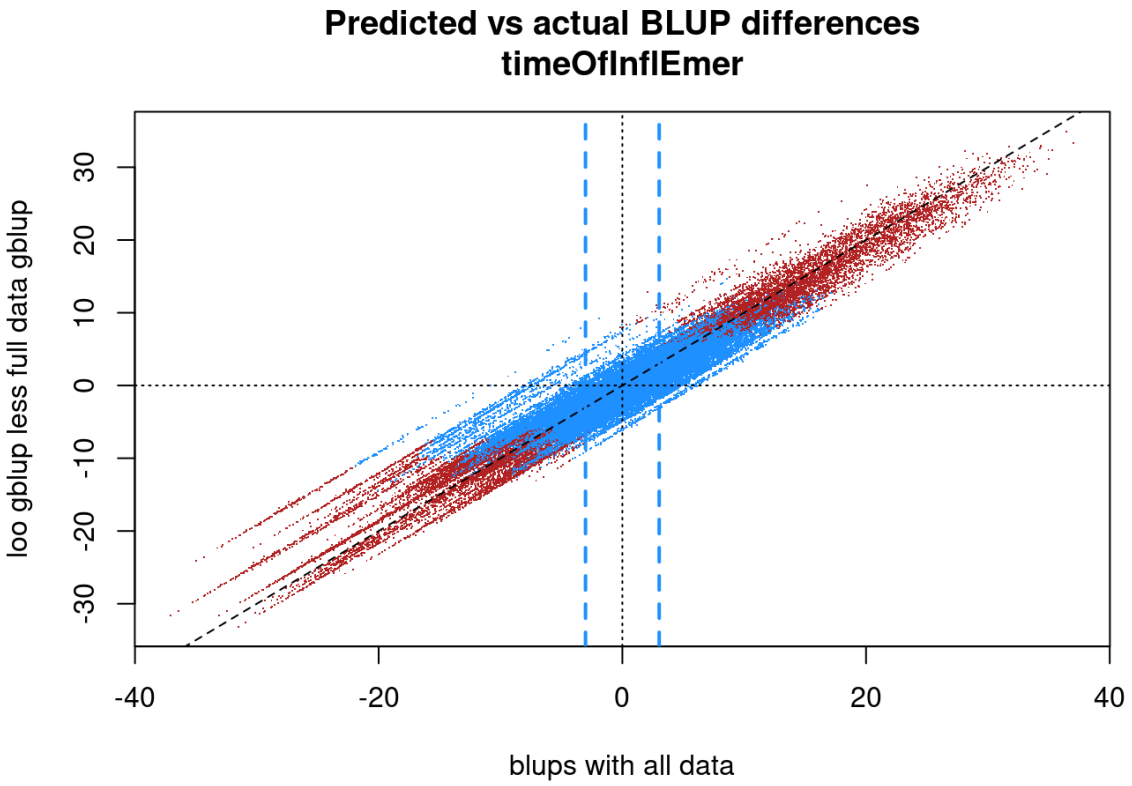
- 423 varieties, with 17 being sampled from more than one country

Compare gBLUP to "variety descriptions"

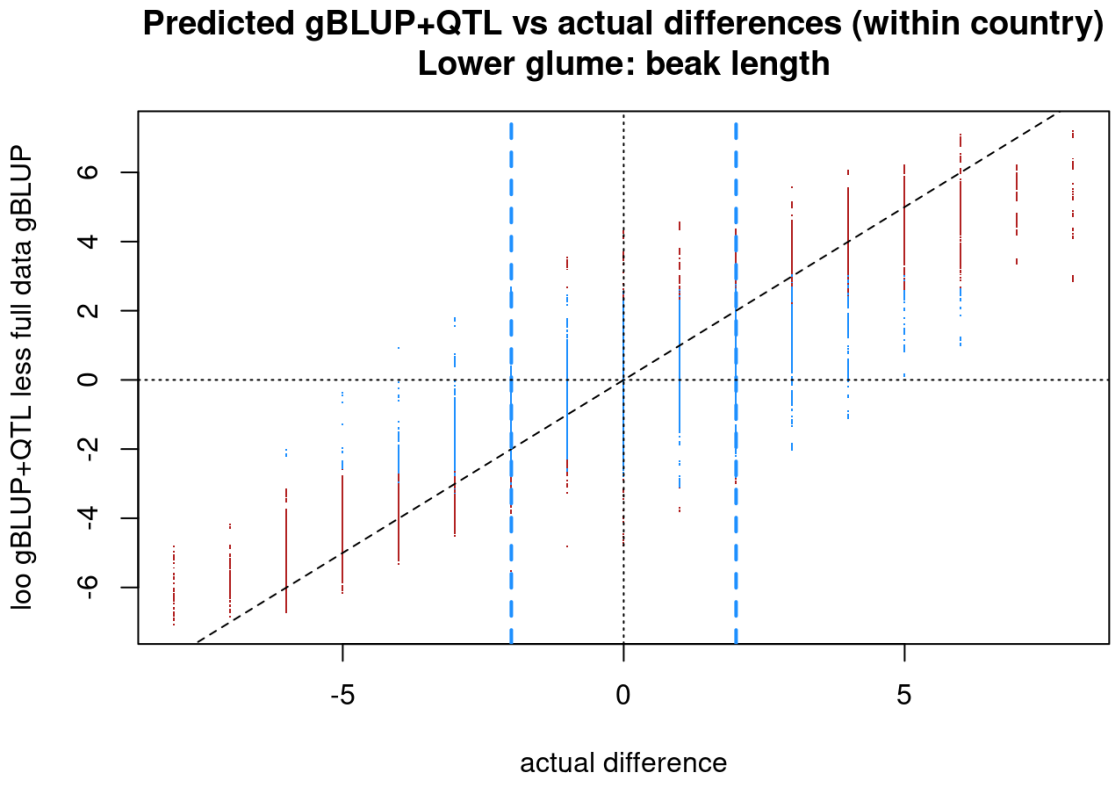
- Cannot sensibly compare varieties tested in different countries

Cross-validation performance

Ryegrass: timing of inflorescence emergence



Wheat: lower glume beak length



Ryegrass: Proportion of varieties distinguished

	diploid		tetraploid	
Characteristic	gBLUP	gBLUP+QTL	gBLUP	gBLUP+QTL
Plant: vegetative growth habit (without vernalization)	2.4%		0.4%	
Leaf: intensity of green colour (without vernalization)	0.3%		0.1%	
Plant: width (after vernalization)	0.2%	1.0%	0.7%	2.9%
Plant: vegetative growth habit (after vernalization)	7.2%	9.4%	6.5%	9.0%
Plant: height (after vernalization)	11.0%	13.2%	6.5%	8.8%
Leaf: intensity of green colour (after vernalization)	1.2%		4.1%	
Plant: time of inflorescence emergence	24.4%	38.6%	27.0%	39.0%
Plant: natural height at inflorescence emergence	5.3%		4.8%	
Plant: growth habit at inflorescence emergence	2.9%		12.5%	
Flag leaf: length	0.7%		1.0%	
Flag leaf: width	6.5%		9.6%	
Flag leaf: length/ width ratio	3.7%	5.1%	1.1%	2.4%
Plant: length of longest stem, inflorescence included (when fully expanded)	7.7%		9.2%	
Plant: length of upper internode	1.1%		0.6%	
Inflorescence: length	0.7%		4.8%	
Inflorescence: number of spikelets	2.6%		1.4%	
Inflorescence: density	2.1%		5.6%	
Inflorescence: length of outer glume on basal spikelet	2.0%		0.1%	
Inflorescence: length of basal spikelet excluding awn	2.2%		4.1%	
Inflorescence: spikelet protuberance	1.6%		3.8%	
Inflorescence: glume span	4.4%		2.0%	

Wheat: Proportion of varieties distinguished

Characteristic	gBLUP	gBLUP+QTL
Seed: colouration with phenol	5.8%	16.3%
Coleoptile: anthocyanin colouration	1.7%	5.9%
Growth habit	1.4%	
Frequency of plants with recurved flag leaves	7.1%	
Ear emergence	2.6%	
Flag leaf: glaucosity of sheath	1.0%	
Flag leaf: glaucosity of blade	3.8%	
Ear: glaucosity	1.6%	
Culm: glaucosity of neck	1.1%	
Plant: length	4.2%	
Ear density	2.3%	2.9%
Ear length	3.7%	
Awn or scur length	11.9%	23.3%
Area of hairiness on convex surface	1.1%	
Lower glume: shoulder width	1.3%	
Lower glume: shoulder shape	0.1%	
Lower glume: beak length	13.8%	19.8%
Lower glume: beak shape	0.9%	
Area of hairiness on internal surface	2.5%	4.5%

Over characteristics – success rates

	Proportion distinct	
	GBLUP	GBUP+QTL
PRG - Diploid	41%	52%
PRG -Tetraploid	41%	51%
Wheat	38%	55%

Recall – only need distinctness in one trait
Wheat – would be higher proportion if all traits included?

How useful might this be?

Perennial ryegrass (cross-pollinated)

- All about trial size reduction
  - Reference varieties need to be found different from all candidates to be removed from trial
- Reduces effectiveness, even if candidates tend to be more similar than random
- Competes against cyclic planting – gives 1/3 reduction
- Something more radical? Would more varieties improve results?

Wheat (self-pollinated)

- About selecting similar varieties
- A number of predictable traits – combined with traits with simpler genetics
- UK Government enthusiastic about GP (especially if labelled AI)

Comments on GP

Success depends on:

- Genetics of crop
- Genetics of traits
- Quality of field data (wheat vs ryegrass)
- Number of genotypes (artificially reduced for INVITE studies)
- Appropriate methodology

Methodology

- GBLUP often works as well as other methods, especially when data “small”
  - Computationally cheap cf Bayesian and ML
- Many traits are scored 1-9 for DUS
- Need software for ordinal mixed models with variance structures
  - BioSS reviewing possibilities
  - NIAB used random forests for barley
- Data set structures simple
  - trial effects, or just one number per variety
  - May wish to embed genetic structures if data set small – eg ploidy or hybrid/lines

Distinctness assessment

Distinctness assessment

Motivation:

Concern by some that in some cases distinctness is hard to achieve despite improved performance of the new varieties

- Crops like perennial ryegrass, lucerne
- Large reference collections
- Population varieties
- Many measured characteristics

This view is not held by all

- But lobbied to include in INVITE work
- Previous proposals have not followed UPOV principles (pre-eminence of phenotype)

Distinctness assessment

Proposed new method called COYD-GP

- Designed for cross-pollinated agricultural crops with measured characteristics where COYD is used

COYD-GP uses genomic prediction to improve estimates of variety means from the 2 or 3 years of trials

- Still phenotype focussed, but markers help to improve estimates

COYD versus COYD-GP

COYD

- Combined-Over-Years Distinctness criterion is a **standard** UPOV method

  UPOV (2022) Trial design and techniques used in the examination of distinctness, uniformity and stability. UPOV document TGP/8/5. Published by UPOV, Geneva. https://www.upov.int/edocs/tgpdocs/en/tgp_8.pdf
- COYD uses analysis of variance as a basis for assessing distinctness, using the t-test for comparing two varieties
- This is generally applied when the same varieties are compared over two or three cycles

COYD-GP

- In COYD-GP, variety is treated as a random effect, with correlations between effect levels
- Correlations come from a genetic relationship matrix (kinship matrix) calculated from the genetic markers ➔ gBLUP
- Everything else is the same
  - Differences in variety means compared to a measure of precision (t-test)

How well does COYD-GP work

Use perennial ryegrass data set

- Long-term versions of COYD and COYD-GP, using whole data set
- Compare number of difference (prob value 0.01)

Notes:

- It would be better to look at 3 year cycles as normal practice
- But this was an initial evaluation, and the data set had fewer varieties
- A much reduced marker set would work

Ryegrass DUS Characteristic	Diploid		Tetraploid	
	COYD	COYD-GP	COYD	COYD-GP
Plant: vegetative growth habit (without vernalization)	28.8%	30.6%	10.1%	11.5%
Leaf: intensity of green colour (without vernalization)	7.7%	9.9%	2.7%	3.2%
Plant: width (after vernalization)	25.3%	27.3%	15.3%	17.0%
Plant: vegetative growth habit (after vernalization)	37.0%	39.6%	21.2%	27.8%
Plant: height (after vernalization)	46.8%	48.8%	24.2%	30.1%
Leaf: intensity of green colour (after vernalization)	17.0%	18.5%	11.9%	16.8%
Plant: time of inflorescence emergence	77.5%	77.8%	70.0%	70.9%
Plant: natural height at inflorescence emergence	39.0%	41.2%	25.3%	29.9%
Plant: growth habit at inflorescence emergence	15.7%	19.8%	22.0%	31.8%
Flag leaf: length	13.5%	15.7%	9.8%	13.3%
Flag leaf: width	39.8%	41.9%	28.0%	33.0%
Flag leaf: length/ width ratio	26.7%	29.6%	9.1%	12.4%
Plant: length of longest stem, inflorescence included (when fully expanded)	43.3%	46.2%	31.5%	37.0%
Plant: length of upper internode	17.6%	20.9%	6.4%	8.3%
Inflorescence: length	28.0%	28.7%	27.9%	31.3%
Inflorescence: number of spikelets	37.5%	38.8%	20.0%	22.4%
Inflorescence: density	29.7%	31.0%	29.4%	32.6%
Inflorescence: length of outer glume on basal spikelet	26.2%	27.8%	16.4%	20.0%
Inflorescence: length of basal spikelet excluding awn	22.2%	25.1%	16.8%	20.9%
Inflorescence: spikelet protuberance	22.4%	25.8%	15.7%	21.1%
Inflorescence: glume span	30.3%	33.3%	14.4%	17.3%

Summary for COYD-GP

New method for distinctness proposed: COYD-GP

- Designed for cross-pollinated agricultural crops with measured characteristics where COYD is used
  - E.g. perennial ryegrass, alfalfa/lucerne
- Works like COYD
- Still phenotype focussed, but markers help to improve estimates
- Initial results quite promising but needs more testing
  - Up to 10% increase in varieties distinguished – more effective for tetraploids
  - Needs to be evaluated under more real conditions

Next Steps

- How would this work within UPOV?
- Further evaluation of performance:
  - Compared to COYD in normal cycles
  - Complete DUS data
  - Test in relevant crops
  - How many markers to use

Any questions?