UPOV

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COYD-GP ENHANCED DISTINCTNESS CRITERION FOR CROSS-POLLINATED AGRICULTURAL CROPS

Document prepared by an expert from the United Kingdom

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The annex to this document contains a copy of a presentation “COYD-GP Enhanced distinctness criterion for cross-pollinated agricultural crops”, to be made by an expert from the United Kingdom, at the third session of the TWM.

[Annex follows]

invite

COYD-GP

Enhanced distinctness criterion for cross-pollinated agricultural crops

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BioSS

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What is COYD?

Combined-Over-Years Distinctness criterion

- A long established method for assessing distinctness
- Main use for cross-pollinated varieties (can be used for other types in certain circumstances)
- Suitable for quantitative characteristics

COYD is based on the common statistical method, analysis of variance (ANOVA)

- Uses trial means for each variety over two or three growing cycles
- Calculates the overall means for each variety
- Compares this to a measure of precision using Student's t-test
- The method requires size of differences between varieties to be consistent between cycles

For more information

- TG/1: General Introduction to the Examination of DUS
  https://www.upov.int/edocs/tgdocs/en/tg001_03.pdf
- TGP/9: Examining Distinctness
  https://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf
- TGP/8: Trial Design and Techniques used in the Examination of DUS
  https://www.upov.int/edocs/tgpdocs/en/tgp_8.pdf

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Why is COYD-GP needed?

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

A view not held by all

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A view not held by all

Proposal made in BMT/19 targeted at crops like perennial ryegrass and lucerne

- BMT/19/6: vmDUS – Value-Molecular Linked Distinctness Determination
- https://www.upov.int/edocs/mdocs/upov/en/bmt_19/bmt_19_6.pdf
- Varieties that fail distinctness, but have improved performance would have a "distinctness" assessment based on markers

INVITE also led to another paper related to this concern

- Molecular markers enhance substantially the distinctness of alfalfa varieties for registration and protection, *Annicchiarico P. et al. (2024). DOI: 10.1002/tpg2.20556*
- More direct use of markers than with COYD-GP

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How was COYD-GP developed

European Horizon 2020 funded project INVITE gave BioSS the opportunity to look at reference collection management using markers and genomic prediction

- Included historical DUS field data for perennial ryegrass from Naktuinbouw
- Genetic markers provided by Teagasc (GBS with allele frequencies, 200k SNPs)
- TWM/2/4: Reference collection management using molecular markers: a new approach based on genomic prediction
  https://www.upov.int/edocs/mdocs/upov/en/twm_2/twm_2_4.pdf

During this study, BioSS was asked by a number of parties if we were looking at distinctness using markers

- Not an original aim of the project
- But data available ….

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What is COYD-GP?

COYD-GP is proposed as a new criterion for distinctness

- Designed for cross-pollinated agricultural crops with measured characteristics where COYD is used
- E.g. perennial ryegrass, alfalfa

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

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Why Genomic Prediction?

- Commonly used in plant-breeding to select breeding material
- Allows a better understanding of key traits, such as yield
  - Field data is always limited and variable
  - Augmenting field data with genetic data can give a better "prediction" of the trait
  - Genetics is used as a tool to better understand the trait
- Selection is still based on phenotype, not genotype

What is COYD-GP? Technical Details

COYD

- COYD uses standard 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
- Analysis of variance can be seen as a linear mixed model

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?

Initial study based on Perennial Ryegrass DUS data from Naktuinbouw, Netherlands

- Up to 13 years of trials
- 21 characteristics
- 119 diploids and 149 tetraploids
- 200k SNPs

- Applied long-term versions of COYD and COY-GP, using the whole data set.
- Counted number of differences with probability value 1%.

Note:

- 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	
	Distinct with COYD	Increase with COYD-GP	Distinct with COYD	Increase with COYD-GP
Plant: vegetative growth habit (without vernalization)	28.8%	1.8%	10.1%	1.3%
Leaf: intensity of green colour (without vernalization)	7.7%	2.2%	2.7%	0.5%
Plant: width (after vernalization)	25.3%	2.0%	15.3%	1.7%
Plant: vegetative growth habit (after vernalization)	37.0%	2.6%	21.2%	**6.6%**
Plant: height (after vernalization)	46.8%	2.0%	24.2%	**5.9%**
Leaf: intensity of green colour (after vernalization)	17.0%	1.5%	11.9%	**4.8%**
Plant: time of inflorescence emergence	77.5%	0.3%	70.0%	1.0%
Plant: natural height at inflorescence emergence	39.0%	2.2%	25.3%	**4.6%**
Plant: growth habit at inflorescence emergence	15.7%	**4.1%**	22.0%	**9.8%**
Flaf leaf: length	13.5%	2.2%	9.8%	3.5%
Flag leaf: width	39.8%	2.2%	28.0%	**5.0%**
Flag leaf: length/ width ratio	26.7%	2.9%	9.1%	3.4%
Plant: length of longest stem, inflorescence included (when fully expanded)	43.3%	2.9%	31.5%	**5.5%**
Plant: length of upper internode	17.6%	3.3%	6.4%	1.8%
Inflorescence: length	28.0%	0.8%	27.9%	3.4%
Inflorescence: number of spikelets	37.5%	1.3%	20.0%	2.5%
Inflorescence: density	29.7%	1.4%	29.4%	3.2%
Inflorescence: length of outer glume on basal spikelet	26.2%	1.5%	16.4%	3.5%
Inflorescence: length of basal spikelet excluding awn	22.2%	2.9%	16.8%	**4.1%**
Inflorescence: spikelet protuberance	22.4%	3.4%	15.7%	**5.4%**
Inflorescence: glume span	30.3%	3.0%	14.4%	2.9%

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Summary of results

Initial results quite promising

- Ryegrass historical data
- Up to 10% increase (absolute) in number of pairs of varieties distinguished in each characteristics, compared to COYD
  - Always increases
- More effective with tetraploids. Due to more varieties?
- Would need more testing using a more standard testing setup, i.e. 2 or 3 year cycles, with candidates

Summary

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

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 needed

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