The Verification of the Varietal Identity of VCU Submissions of Cereal Crops using Biochemical Methods

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Purpose and background

- BMT informed in 2003 of future implementation of EP in UK
- Provides details of successful implementation since 2003
- Represents a 'peripheral' use of markers
- Possible model for other markers

Electrophoresis

- UPOV allows use as additional characteristics
  - Wheat - HMW glutenins
  - Barley - hordeins
  - Maize - isoenzymes

Electrophoresis

- Accepted for use in variety ID
  - Used by breeders
  - Used by seed trade
  - Used in commodity trading

Electrophoresis

- Limited use in testing at national level
  - GAIA: isoenzymes in OSR and maize DUS (in parallel with phenotypic characteristics)
  - Suggested as a grouping character in wheat
  - Authentication of VCU test material

Background

- DUS seed represents "the definitive stock"
- VCU test seed (VCU year 1 & 2) verified against DUS stock
- Before 2003 in the UK authentication was carried out by comparison of side by side field plots
- Data not available until VCU trial is nearing harvest
- Expensive

Project Objectives

- Examine the use of protein electrophoresis (EP) as an alternative to visual field assessments
- Assess the risk to applicants and testing authority of implementing new method
- Assess the relative costs of lab vs field testing

Methods used - EP

- Wheat:
  - SDS PAGE method (UPOV TG/3/11) for HMW glutenins
  - Acid PAGE method for gliadins ISTA (1989)
- Barley:
  - Acid PAGE (UPOV TG/3/11) for B – and C-hordeins
- Results recorded as digitised gel images and database of scores

Methods used-morphology

- All wheat and barley VCU candidate varieties assessed in field plots in 2002
- Visual comparison of side by side plots
- Comparisons made from growth stage 30 until full harvest maturity

Comparative results (2002)

- 101 wheat candidates (NL1 & NL2)
- 6 samples failed EP authentication
- 3 of these failed visual authentication

- 111 barley candidates (NL1 & NL2)
- 2 samples failed EP authentication
- Both also failed visual authentication

Operational System

- 7 individual seeds per sample
  - (7 x DUS & 7 x VCU)
- Re-test with 28 seeds if not authentic
  - Low levels of admixture
  - Biotypes (?^{2} analysis)
  - VCU not authentic
- Applicant informed
- Side by side field plot comparison

Biotypes:
acceptance criteria using χ^2 statistics

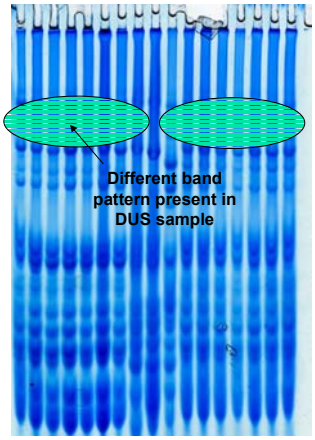
Pattern	*A*	*B*
DUS	30	5
VCU	28	7

☺ ACCEPT

Pattern	*A*	*B*
DUS	30	5
VCU	7	28

☹ REJECT

- Compare with critical value of χ^2 from tables



Barley DUS and VCU samples not authentic by EP

Summary of operational results

Year	No. of pairs tested by EP	No. of pairs failed by EP	Result of authentication in field
Winter 2004	Barley 48	Barley 1	√
	Wheat 120	Wheat 1	√
Spring 2005	Barley 57	Barley 2	√
	Wheat 6	Wheat 0	
Winter 2005	Barley 48	Barley 0	
	Wheat 112	Wheat 2	X failed DUS
Spring 2006	Barley 37	Barley 3	√
	Wheat 9	Wheat 0	
Winter 2006	Barley 42	Barley 0	
	Wheat 117	Wheat 0	
TOTAL	596	9	2 failures

Advantages of the current system

- Cost reduction
- Rapid: applicant has option to replace VCU stock or request side by side test within the same growing cycle
- Data can be retained digitally

Cost savings (2002)

- Total cost of visual assessment = £18,000
- Total cost of EP = £9,000
- Possible ~50% cost savings

Conclusions

- Protein EP useful for DUS-related testing issues
- Higher failure rate of EP would improve protection of the VCU system from 'non authentic' seed
- A three-tier system protects the applicant from higher failure rate
- Cost savings

Any questions?

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