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THE VERIFICATION OF THE VARIETAL IDENTITY OF VCU SUBMISSIONS OF CEREAL CROPS USING BIOCHEMICAL METHODS

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THE VERIFICATION OF THE VARIETAL IDENTITY OF VCU SUBMISSIONS OF CEREAL CROPS USING BIOCHEMICAL METHODS.

Huw Jones, Carol Norris, John Law. NIAB, Cambridge UK

1. Introduction

In the United Kingdom, the variety testing authorities are under no legal obligation to authenticate (verify) the varietal identity/purity of the seed used in official VCU trials. However, it is clearly good practice to undertake such authentication, as a quality assurance measure, and to protect both the applicants and the authority against the risk of using incorrect seed in National List trials (NLT).

Previously, this authentication has been carried out by growing plots of the VCU submissions side-by-side and in the near vicinity of the DUS plots of the same varieties, and making visual assessments and comparisons of the plots (based on UPOV characteristics) throughout the growing season. Clearly there is both a cost and a time delay associated with this approach.

This paper reports the results of a project undertaken to examine the use of biochemical methods to replace the field authentication, thus reducing both costs and time.

2. Experimental approach

The project examined the use of gel electrophoresis of seed storage proteins as an alternative to visual assessments when verifying the varietal identity of VCU submissions of wheat and barley against reference samples.

Wheat candidates were assessed by two methods: firstly, the high molecular weight glutenin proteins were examined in accordance with the SDS PAGE method given in the UPOV Test Guidelines (document TG/3/11 + Corr.) and secondly the gliadins were analyzed using the ISTA (1989) acid PAGE standard reference method. All wheat gels were scored in accordance with the UPOV Test Guidelines or the method described in Cooke and Law (1998) and digital images recorded.

Barley candidates were assessed by analyzing the B- and C-hordein proteins in accordance with the acid PAGE method as in the UPOV Test Guidelines (document TG/19/10). All barley gels were scored as in the UPOV Test Guidelines and digital images recorded.

3. Comparative Results

There were 101 wheat candidates (NLT 1[1] & NLT 2[2]) and 111 barley candidates (NLT 1 & NLT 2) examined in 2002 in the course of the study, and in the majority of cases, the VCU and DUS stocks could not be distinguished from one another using gel electrophoresis, i.e. the VCU samples could be considered to be authentic. However, in a small number of cases, there were differences observed:

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[1] NLT 1 = First year of National List Trials
[2] NLT 2 = Second year of National List Trials

- In wheat, of the 101 varieties, the authenticity of six was called into question using one or other of the electrophoresis methods. Three of these varieties subsequently 'failed to authenticate' visually;

- In barley, the authenticity of two of the 111 varieties was questioned using electrophoresis. Neither of these barley varieties 'failed to authenticate' visually in the field.

Thus in summary, the electrophoresis methods failed to authenticate eight varieties in total, as follows:

		Not authentic by	
Crop	**Morphology**	**SDS PAGE**	**Acid PAGE**
Wheat	Var1	Var1	
Wheat		Var2	
Wheat			Var3
Wheat	Var4	Var4	Var4
Wheat	Var5	Var5	Var5
Wheat			Var6
Barley		---	Var7
Barley		---	Var8 (VCU 2 only)

On the basis of this, it was felt that electrophoresis alone might give rise to an unacceptable risk of 'failure to authenticate'. However, it was felt that timely electrophoresis analysis of VCU stocks in the period between receipt of seed at the test center and sowing of the VCU plots still had advantages and could be used as a screening tool. For instance, in 2002 the number of side-by-side visual assessments could have been reduced from the total of 202 to the 8 targeted cases where electrophoresis indicated a problem.

4. Operational System

Following this, a number of options were considered for a robust and cost-effective authentication scheme. The one implemented is a 'hybrid' one, with both electrophoresis and field grown plots, and is detailed below.

Step 1: All VCU candidates are screened by electrophoresis, using only those methods in the UPOV Test Guidelines (glutenins in wheat, hordeins in barley);

Step 2: Side-by-side field plot comparisons are carried out in those cases where protein electrophoresis calls the authenticity of a submission into question.

Under this scheme, a small (7 seeds per stock) sample of the VCU stocks would be examined shortly after receipt by the test center. Where authenticity is questioned, the VCU stock would be re-tested, taking a larger number of individual seeds (28 seeds per stock) to provide greater statistical confidence in the result. The applicant would be advised at this point. Where the authenticity remains in doubt, the authenticity of the VCU stock would be finally assessed against the DUS stock, using side-by-side field plot comparison.

5. Operational phase - results

Summary of operational results:

Test year	**Number of varieties tested in matched pairs**	**Number of matched pairs failed or required growing**
Winter 2004	Barley 48	Barley 1
	Wheat 120	Wheat 1
	Oats 4	Oats 0
Spring 2005	Barley 57	Barley 2
	Wheat 6	Wheat 0
	Oats 7	Oats 0
Winter 2005	Barley 48	Barley 0
	Wheat 112	Wheat 2
	Oats 3	Oats 0
Spring 2006	Barley 37	Barley 3
	Wheat 9	Wheat 0
	Oats 6	Oats 0
Winter 2006	Barley 42	Not completed
	Wheat 117	1
	Oats 2	Not completed

6. Future prospects

This work has shown that there are possible applications of biochemical and/or molecular methods which are related to the whole variety testing operation whilst not involving DUS testing *per se*. There are clear benefits to such applications, in terms of costs and time savings. However, in order to protect both the testing authority and the applicants, it is advisable to use methods that have been accepted at the international level, e.g are contained in UPOV Test Guidelines (such as the analysis of glutenins in wheat and hordeins in barley). Hence there is obvious potential to move on from gel electrophoresis of proteins to any suitable molecular marker system agreed by UPOV in the future.

7. References

Cooke R.J. and Law J.R (1998). The assessment of genetic diversity in winter wheat (*Triticum aestivum*) cultivars through gel electrophoresis and pedigree relationships.
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