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THE USE OF SSRs FOR DUS TESTING OF WHEAT

1. UNIFORMITY AND STABILITY OF VARIETIES

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The Use of SSRs for DUS Testing of Wheat

1. Uniformity & Stability of Varieties

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1. Introduction

We have reported at previous BMT meetings progress in a Defra-funded research programme into the development of a "test set" of DNA microsatellite (simple sequence repeat, SSR) primer pairs in wheat. A preliminary screen of 55 markers (mostly from the IPK programme) produced an initial set of 23 SSR primer pairs, which were used to analyse 20 individuals from each of 10 varieties. A sub-set of 8 markers, chosen for their ease and reliability of scoring as well as their PIC values and levels of "uniformity", was then used to analyse 48 individuals from a further 30 varieties. We now report (i) the outcome of this uniformity assessment, (ii) a comparison with storage protein and phenotypic data, (iii) an analysis of successive generations of varieties (i.e. an examination of aspects of stability), and (iv) a comparison with some field-based observations.

2. Distinctness

The 40 varieties (a mixture of spring and winter, feed and bread-making types from a range of breeders) were analysed using the 8 selected SSRs, and also using gliadins and HMW glutenins. The discrimination between the varieties was compared to that achieved using the current 26 UPOV characteristics, in terms of the separation coefficient (the number of variety pair-wise comparisons that are different). The results (see summary in Table 1) showed that whilst the UPOV characteristics when considered as a whole could distinguish between all 40 varieties, the 8 SSRs were able to discriminate between the 40 varieties, apart from two pairs, and the storage proteins were slightly less powerful. However, this needs to be set against the time taken – a whole season for the phenotypic approach from start to finish, compared to weeks (SSRs) or days (proteins).

3. Uniformity

48 individuals from each of the 40 varieties were analysed using the 8 SSRs (note that actually variety 24 was omitted from the results, due to technical difficulties). The results are summarised in Table 2, and need to be interpreted carefully. Given the numbers of individuals and loci examined, an overall level of U of c. 97% within a variety is equivalent to 1 off-type (the precise percentage depends on the number of scorable results, as it was not possible to score 48 individuals reliably in all cases) – see for example varieties 10 and 11, whilst c. 95% = 2 off-types (or one at two loci) etc. However, this can be somewhat misleading, since in some cases, an off-type which varies at several loci can be found (e.g. variety 14) which gives the same overall U level as an off-type at a single locus (e.g. variety 10). The former almost certainly represents a variety admixture, whereas the latter can be attributed to a low level of residual segregation at a particular locus. Again, in some cases, notably variety 21, an apparently low level of overall U is driven almost entirely by data from a single (presumably segregating) locus (325 in this case). Variety 20 is a hybrid and thus can

probably be ignored to some degree, since other issues (such as hybrid purity of the seed lot analysed) will also be involved in the U assessment. Thus the way in which uniformity is defined and measured needs some consideration. Notwithstanding that, 17 of the varieties were completely U at all loci, and a further 9 had no more than 2 off-types. Thus 26 out of 38 varieties (excluding variety 20) would satisfy the current "phenotypic" U standards, with no additional effort on the part of the breeders. The mean level of overall varietal uniformity was 91.9% (93% if variety 20 omitted).

The SSR loci showed differing levels of heterogeneity, with Taglgap being the most homogeneous (only 4 individuals within the 40 varieties were found to carry different alleles from the majority at this locus).

4. Stability

Aspects of the stability of wheat varieties at SSR loci were examined by analysing the uniformity (x24 individuals) of a selection of different seed lots of 9 varieties. The seed lots (28 in total) had been produced under certification standards and represented situations both where the lots were related (seed produced from different generations) and effectively unrelated (no record of shared provenance). The results are summarised in Table 3 and again the same caution needs to be exercised as for the uniformity data in interpretation (e.g. first seed lot of variety 32 – the apparent non-U is caused by 1 individual which is an off-type at 3 loci, and therefore probably represents mechanical admixture). Nonetheless, these data show that there are no obvious stability problems when varieties that are largely uniform are examined at SSR loci.

5. Comparison With Field Observations

A number of particular varieties were examined in an attempt to correlate results observed in field plots (primarily cases of non-uniformity) with those from SSR analysis. A number of different instances of non-U varieties were chosen, including varieties varying for height and glaucosity, and for leaf colour (chlorosis). In a number of these cases, it was possible to demonstrate that the SSR markers confirmed the results seen in the field. Some of these cases will be discussed in more detail in the presentation.

6. Conclusions

This project has shown that D, U and S of wheat varieties can be assessed using a carefully evaluated set of DNA microsatellite markers and that such assessment can be carried out using the same approach as is currently used for morphological characteristics. Levels of D are very high and compare well with storage protein analysis. Contrary to some assertions, it is not possible to distinguish every variety uniquely and easily using markers – a finding which is confirmed by the results from an EU-funded project (Röder *et al*., Theor & Appl Genet 106, 67, 2002). Uniformity of varieties can also be determined. The current results are broadly in line with previous studies arising from the same EU-funded project (Cooke *et al.,* Euphytica, in press), where in a less extensive study of the uniformity of 25 UK NL varieties at 8 loci, 15 were found to be sufficiently U using the current phenotypic standards. It needs to be remembered that this is achieved with no additional effort on the part of the breeder and is applying the standards used for phenotypic examination, which may be neither useful nor appropriate to apply directly to markers. Variety stability levels at SSR loci appear to present no problems.

On this basis, there would seem to be no reason why such well characterised and tested SSRs could not be used for DUS testing. It would be possible to use the SSRs as additional characteristics, but this would not be an entirely logical course of action, as it does not make best use of the markers, especially in terms of distinctness. We have already suggested a revised approach to DUS testing in wheat using SSRs (an Option 3 approach – see BMT Review Group meeting, April 2002), which could offer reductions in time and costs and/or form the basis of a means of managing large reference collections more effectively, without undermining current levels of protection. Moreover, the current results specifically address the assertion made by some members of the Review Group that U and S aspects were not being examined. However, given the current view of the BMT Review Group that there are fewer difficulties with Option 2 approaches, we have also extended the number and range of SSRs used to analyse this set of varieties, and this work is described in a separate paper for this meeting.

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Table 1 – Separation coefficients of different characteristics on a set of 40 wheat varieties

MICROSATELLITES									
	Taglgap	155	161	261	325	408	458	680	Overall
Chromosome	1BS	3AL	3DS	2DS	6DS	5BL	1DL	6B	--
No of Pairs	780	780	780	780	780	780	780	780	--
Separation coefficient %	55.6	62.3	67.6	62.1	69.5	72.8	71.3	56.0	**99.7**
	HMW GLUTENINS			GLIADINS					Overall
Chromosome	1AL	1BL	1DL	1DS	1AS	1BS	1BS	6AS/DS	
No of Pairs	780	780	780	780	780	780	780	780	
Separation coefficient %	46.5	79.2	57.6	14.6	35.8	61.9	5.0	78.1	**92.1 – Glu** **95.7 - Gli**
UPOV CHARACTERISTICS									
	1	2	3	4	5	6	7	8	9
Chromosome	?	?	?	?	?	?	?	?	?
No of Pairs	780	780	780	780	780	780	780	780	
Separation coefficient %	73.8	40.0	13.8	67.7	41.5	46.5	52.7	47.4	47.9
UPOV CHARACTERISTICS									
	10G	11	12	13	14G	15	16G	17	18
Chromosome	?	?	?	?	?	?	?	?	?
No of Pairs	780	780	780	780	780	780	780	780	
Separation coefficient %	34.7	0.0	45.4	14.5	9.7	50.3	0.0	56.8	31.2
UPOV CHARACTERISTICS									
	19	20	21	22	23	24	25	26G	Overall
Chromosome	?	?	?	?	?	?	?	?	
No of Pairs	780	780	780	780	780	780	780	780	
Separation coefficient %	55.3	20.5	24.9	42.2	56.4	5.0	68.7	30.8	**100.0**

Note: for storage proteins and SSRs, a one allele difference was considered sufficient for distinctness; for UPOV characteristics, the current routinely applied standards were used for each characteristic.

Table 2 – Summary of uniformity data for 40 (39) wheat varieties at 8 SSR loci

Variety	**Taglgap**	**155**	**161**	**261**	**325**	**408**	**458**	**680**	**Overall U at all loci (%)**
1	U	U	non U	non U	U	U	U	U	84.4
2	U	non U	non U	non U	U	non U	non U	U	84.2
3	U	U	U	U	U	U	U	U	100.0
4	U	U	U	U	U	U	U	U	100.0
5	U	U	U	U	U	U	U	non U	97.4
6	U	U	U	U	non U	U	U	U	97.7
7	non U	U	U	non U	U	non U	non U	non U	85.0
8	U	U	U	U	U	U	U	U	100.0
9	U	non U	U	U	U	U	U	non U	92.0
10	U	U	U	U	U	U	U	non U	97.5
11	U	U	non U	U	U	U	U	U	97.4
12	U	U	U	U	U	U	U	U	100.0
13	U	U	U	U	non U	U	U	U	87.8
14	non U	non U	non U	non U	U	non U	non U	non U	97.5
15	U	U	U	U	U	U	U	U	100.0
16	U	U	U	U	U	U	U	U	100.0
17	non U	U	non U	U	U	U	non U	U	94.7
18	U	U	U	U	U	U	U	U	100.0
19	U	U	U	U	U	U	U	U	100.0
20	U	non U	non U	non U	U	U	U	non U	47.2
21	U	U	U	non U	non U	U	U	U	48.8
22	U	U	U	U	U	U	U	U	100.0
23	U	U	non U	non U	U	non U	U	non U	86.7
25	non U	non U	U	U	U	U	non U	U	73.8
26	U	non U	U	U	U	non U	U	U	88.9
27	U	U	U	U	U	U	U	U	100.0
28	U	U	U	U	U	U	U	U	100.0
29	U	U	U	U	U	U	non U	U	93.3
30	U	U	U	U	U	U	U	U	100.0
31	U	U	U	U	U	U	U	U	100.0
32	U	U	U	U	U	U	non U	U	93.0
33	U	U	U	U	U	U	U	U	100.0
34	U	U	U	U	U	U	U	U	100.0
35	U	U	U	U	U	U	U	U	100.0
36	U	non U	non U	non U	non U	U	non U	non U	70.6
37	U	U	U	U	non U	U	U	non U	84.8
38	U	non U	non U	U	non U	U	non U	non U	90.5
39	U	U	U	U	U	U	U	U	100.0
40	U	U	non U	non U	non U	U	U	U	88.6
									mean U of varieties = 91.9
Homogeneity at locus (%)	99.7	98.0	98.9	97.7	96.0	99.4	99.2	97.9	mean H of loci = 98.4
No of U varieties	35	31	29	30	32	34	30	30	No of varieties U at all loci = 17

Note: U = 100% of individuals carry the same allele; non U = some variation noted

Table 3 – Stability of some wheat varieties at 8 SSR loci

Time period	Variety	Taglgap	155	161	261	325	408	458	680	Overall U at all loci (%)
1960s	11	U	U	non U	U	U	U	U	U	95.7
	11	non U	non U	non U	U	non U	U	non U	U	82.6
	11	U	U	U	U	U	U	U	U	100.0
	13	U	U	U	U	non U	non U	U	U	77.3
	13	U	U	U	non U	non U	non U	U	non U	68.2
1979	12	U	U	U	U	U	U	U	U	100.0
	12	U	U	U	U	U	non U	U	U	95.5
	12	U	U	U	U	U	U	U	U	100.0
1989	7	U	U	U	U	U	U	U	U	100.0
	7	U	U	U	U	U	U	U	U	100.0
	7	U	U	U	U	U	U	U	U	100.0
	7	U	U	U	U	U	U	U	U	100.0
1990s	27	U	U	U	U	U	U	U	U	100.0
	27	U	U	U	U	U	U	U	U	100.0
	27	U	U	U	U	U	U	U	U	100.0
	27	U	U	U	U	U	U	U	U	100.0
	6	U	U	U	U	U	U	U	U	100.0
	6	U	U	U	U	U	U	U	U	100.0
	6	U	U	U	U	U	U	U	U	100.0
	6	U	U	U	U	U	U	U	U	100.0
	32	non U	U	U	non U	U	U	U	non U	95.7
	32	U	U	U	U	U	U	U	U	100.0
	32	U	U	U	U	U	U	U	U	100.0
	34	U	U	U	U	non U	U	U	U	91.7
	34	U	U	U	U	U	U	U	U	100.0
	34	U	U	U	U	U	U	U	U	100.0
	35	U	U	U	U	U	U	U	U	100.0
	35	U	U	U	U	U	U	U	non U	95.0
	Homogeneity at locus (%)	99.7	99.8	99.5	99.7	98.3	99.2	99.8	99.3	mean U of varieties = 96.5% mean H of loci = 99.4%

Note: U = 100% of individuals carry the same allele; non U = some variation noted

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