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BMT/7/5
ORIGINAL: English
DATE: November 21, 2001

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Seventh Session
Hanover, Germany, November 21 to 23, 2001

DEVELOPMENT OF MICROSATELLITE MARKERS FOR DUS TESTING IN WHEAT AND OILSEED RAPE

prepared by experts from the United Kingdom

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

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Work funded by DEFRA

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NIAB

Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

- **Two Projects - 'Development of Molecular Markers for DUS Testing in Oilseed Rape' and '….Wheat'**
- **Funded by DEFRA (MAFF)**
- **Started 1999 -**
- **Preliminary information given at sub-Group meetings (February/March 2001)**
- **Objective: '..to develop a test set of DNA microsatellite primer pairs for oilseed rape (wheat), to evaluate its application in DUS testing and to devise an operational system for its use..'**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

WORK PLAN (1)

- **obtain and optimise a 'suitable number' of SSR markers – the objective is to evaluate at least one marker per chromosome/linkage group**
- **test the polymorphism of these markers in a group of 10 varieties and select the 'best' SSRs**
- **assess the uniformity of these 10 varieties with regard to the selected SSRs, by analysis of a number (20-48) of individuals….**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

WORK PLAN (2)

- **develop a test set of SSR primer pairs that could be used in DUS testing, independent of detection platform**
- **evaluate this test set - analyse a larger number of current varieties (for both D and U), past and present candidate varieties (for both D and U), plus a parallel running exercise**
- **examine aspects of stability by analysing different seed lots of varieties.**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

RESULTS - WHEAT:

- **Builds on EU project**
- **55 primer pairs evaluated (10 varieties)**
- **23 chosen (good amplification, easily scored, PIC values >0.45, genome coverage)**
- **Used for initial uniformity analysis (10 varieties x 20 individuals)**
- **8 (+5) of these chosen for further study (low level of non-U, multiplexing) - D (x40 varieties) and U (x 48 individuals)**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

Selected Wheat SSRS

	Locus	Chromo-some	No. of alleles*	PIC*	Non-uniformity*	Comments
1	WMS 325	6DS	4	0.45	0.7 %	Bit faint, but good
2	WMS 261	2DS	3	0.58	2.7 %	U problem in one variety
3	WMS 155	3AL	3	0.50	0.7 %	Slightly stuttery, but OK
4	Taglgap	1BS	4	0.48	0.3 %	Null allele (multiplexing)
5	WMS 680	6B	2	0.48	2.3 %	OK
6	WMS 458	1DL	3	0.64	0 %	Good
7	WMS 161	3DS	3	0.60	0.5 %	Some stuttering, but OK
8	WMS 408	5BL	3	0.58	0 %	Good
	Reserves					
9	WMS 095	2AS	5	0.74	1.3 %	Stuttering, overlapping alleles
10	WMS 169	6AL	5	0.74	0.5 %	Stuttering & some smeariness
11	WMS 018	1BS	4	0.66	1 %	OK
12	WMS 102	2DS	4	0.66	0 %	Wide size range (120-180 bp) makes multiplexing difficult
13	WMS 186	5AL	4	0.66	0.6 %	U problem in one variety, stuttering, difficult to multiplex

* - 10 varieties

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Wheat SSRs: Distinctness – UK NL varieties

Locus	10 varieties PIC	10 varieties No. of alleles	40 varieties PIC	40 varieties No. of alleles
Taglgap	0.48	4	0.63	4
WMS 155	0.50	3	0.57	5
WMS 161	0.60	3	0.66	5
WMS 261	0.58	3	0.61	5
WMS 325	0.45	4	0.52	7
WMS 408	0.58	3	0.66	6
WMS 458	0.64	3	0.66	3
WMS 680	0.48	2	0.55	4

40 varieties can be ~discriminated using 8 SSRs

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Wheat SSRs - Uniformity (x48) - Preliminary data

Variety	Taglgap	155	458	161	680	408	325	261	% non-U
1	0	0	0	0	0	0	0	0	0.0
2	1	0	0	1	0	0	0	9	2.6
3	0	0	0	0	0	0	0	0	0.0
4	0	0	0	0	0	0	0	0	0.0
5	2	0	1	0	1	4	0	1	2.3
6	0	0	0	0	1	0	0	0	0.3
7	0	0	0	0	0	0	2	0	0.5
8	0	2	2	2	7	3	0	6	6.5
9	0	1	0	0	0	0	0	0	0.3
10	0	0	0	0	1	0	0	0	0.3
11	0	0	0	0	0	0	1	0	0.3
12	0	0	0	-	0	1	0	0	0.3
13	0	0	0	0	0	-	4	0	1.0
14	1	1	1	1	1	-	-	-	2.2
15	0	0	0	-	0	0	0	0	0.0
16	0	0	0	-	0	0	0	0	0.0
17	3	0	1	-	0	0	0	0	1.3
18	0	0	0	0	0	0	0	0	0.0
19	7	0	0	0	0	0	0	0	1.9
20	0	5	0	4	21	0	1	1	3.7
21	0	0	0	0	0	0	0	0	0.0
22	2	-	-	0	0	0	0	0	0.7
23	-	0	1	-	-	3	0	2	3.0
24	0	1	0	0	-	0	-	0	0.4
25	1	16	1	-	0	0	0	0	5.5
26	4	6	0	0	0	1	0	0	3.1
27	0	0	0	0	0	0	0	0	0.0
28	0	0	1	0	0	0	0	0	0.3
29	0	0	1	0	0	0	0	0	0.3
% variation at locus	1.7	2.6	0.8	0.8	2.7	1.0	0.3	1.5	
No. of U varieties	19	22	21	18	21	22	23	23	

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WHEAT - TYPES OF HETEROGENEITY

- **Uniform varieties - 15/39**
- **Varieties with 1 off-type at a single locus - 8/39**
- **Varieties with 1 off-type at two or more loci - 2/39**
- **Varieties with >1 off-type at a single locus - 5/39**
- **Varieties with off-types at more than one locus - 9/39**
- **Off-types can be homo- or heterozygous**
- **Could estimate uniformity of current varieties at selected SSR loci**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

WHEAT - FURTHER WORK

- **Complete U analyses (40 varieties x 48 individual x 8 SSRs) + candidates**
- **Examine aspects of Stability**
- **Compare results from use of SSRs and conventional tests**

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RESULTS - OILSEED RAPE:

- **NIAB and LARS involved**
- **50 primer pairs (BBSRC) evaluated (10 varieties)**
- **15 chosen (good amplification, easily scored in both labs, multiplexed, PIC values, genome coverage)**
- **Used for initial uniformity analysis (10 varieties x 48 individuals)**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

Selected Oilseed Rape SSRs

Locus	Multiplex	Core Motif	Linkage Group	Allele Size Range (bp)	No. of Bands	%*S*
1-Na12 EO2	1	TTG	-	124-129	2	53
2-Na12 AO8	1	GA	3, 6	162-318	8	66
3-Ra2 EO3	1	CT	19	262-294	5	38
4-Na12 AO2	1	CT	7, 10	161-197	7	82
5-Na12 AO7	1	GT	12	153-166	4	84
6-Ra2 A11	2	CT	9	243-245	2	80
7-Ol10 BO1	2	GA	17	168-188	4	51
8-Na12 F03	2	GA	17	254-314	6	89
9-Ol10 DO3	2	CT	-	91-142	6	97
10-Ra2 F11	2	CT	12, 13, 19	188-233	5	56
11-Na12 DO4	3	CA	6	282-288	2	20
12-Ni4 DO9	3	CT	9	167-207	6	91
13-Ra2 E11	3	CT	13	166-202	7	93
14-Ol10 F11	3	GGC	11	138-150	3	73
15-Ol10 HO2	3	GGC	12, 13	184-214	3	87

Data from LARS, from analysis of 10 varieties; %*S* = separation coefficient

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Oilseed Rape SSRs – Uniformity (x 48)

	A	B	C	E	F	G	H	L	M	N	Ave. non-U per locus
OI10-B01	4%	2%	2%	U	U	21%	U	54%	21%	2%	**11%**
Na12-E02	17%	2%	4%	U	U	25%	U	2%	21%	2%	**7%**
Ra2-F11	U	U	U	17%	U	U	U	U	U	2%	**2%**
Na12-A07	U	2%	32%	U	U	U	U	34%	19%	2%	**9%**
Na12-A02	4%	50%	27%	40%	U	10%	U	58%	U	65%	**25%**
Ra2-E03	6%	U	4%	U	U	U	35%	U	U	2%	**5%**
Ra2-E11	4%	6%	56%	U	6%	33%	U	58%	26%	69%	**26%**
OI10-D03	4%	21%	27%	U	4%	4%	U	67%	89%	72%	**29%**
Ra2-A11	8%	2%	4%	21%	U	U	U	U	U	U	**4%**
Ni4-D09	4%	U	15%	U	U	12%	U	21%	2%	44%	**10%**
OI10-F11	U	U	4%	U	U	8%	U	48%	6%	35%	**10%**
Na12-D04	U	U	U	U	U	U	U	58%	U	6%	**6%**
OI10-H02	U	U	6%	U	U	15%	U	U	24%	15%	**6%**
Na12-A08	U	4%	27%	U	U	52%	6%	48%	6%	23%	**17%**
Na12-F03	U	4%	30%	29%	U	10%	6%	23%	21%	73%	**20%**
Ave. non-U per variety	**3%**	**6%**	**16%**	**7%**	**1%**	**13%**	**3%**	**31%**	**16%**	**27%**	

Data from NIAB. U = uniform (i.e. all 48 individuals identical); % are deviations from the modal band pattern

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HETEROGENEITY IN OILSEED RAPE

- **No varieties were completely U at all SSR loci**
- **Levels of varietal non-uniformity (deviation from the modal pattern) ranged from 1 to 31%(average over all loci)**
- **Levels of heterogeneity at SSR loci ranged from 6 to 29% (average over all varieties)**
- **Could estimate uniformity of current varieties at selected SSR loci**

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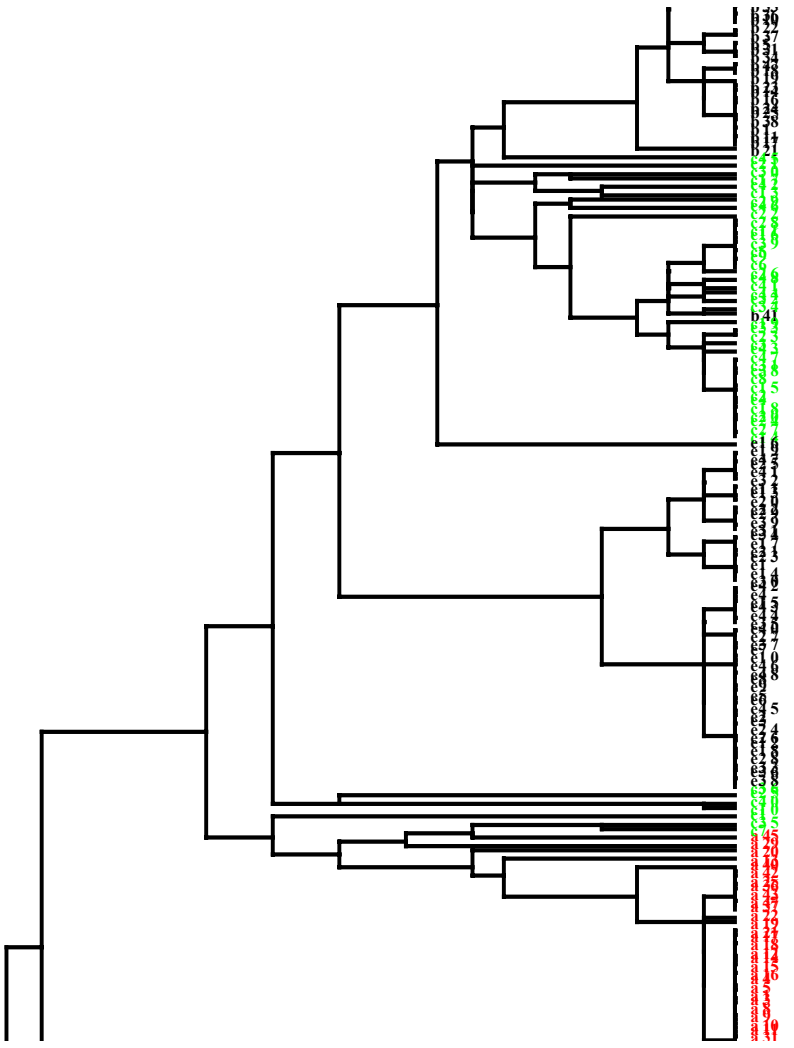
Oilseed Rape SSRs - Clustering based on analysis of individual plants.



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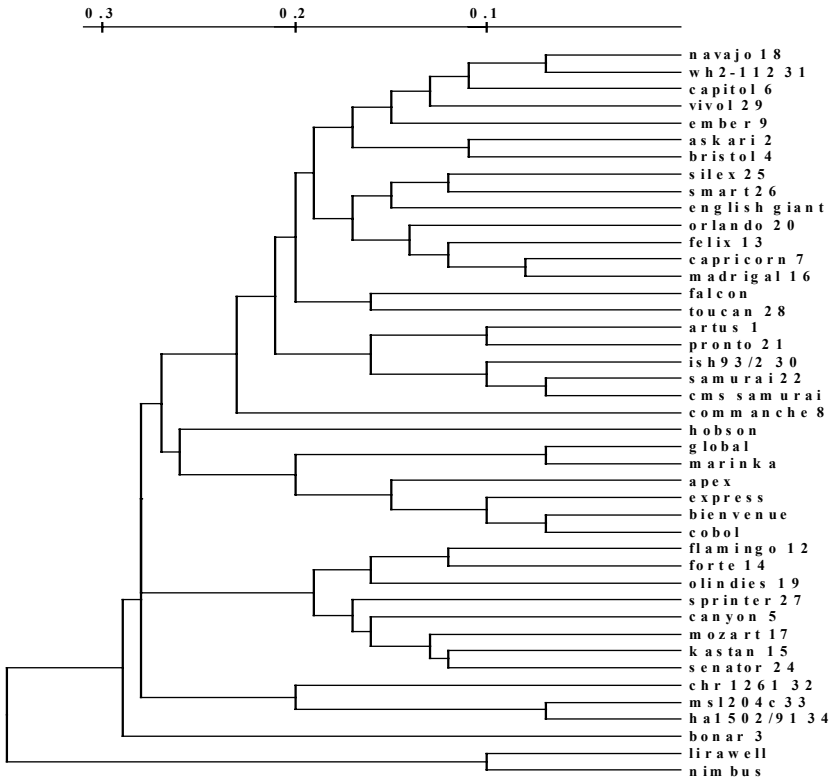
Oilseed Rape SSRs - Distinctness



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OILSEED RAPE - FURTHER WORK

- **Comparison of NIAB and LARS data showed that 5 of the 15 SSR primer pairs were not sufficiently robust to be useful independently of the detection platform - these need to be replaced**
- **Analyse more varieties (D and U) with the 'new' SSR set**
- **Analyse as many Common Catalogue varieties as possible**
- **Carry out 'parallel running' exercise**
- **Examine aspects of Stability**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

SUMMARY:

- **SSRs can readily discriminate between varieties (D?)**
- **Can select SSRs that are robust and repeatable between labs**
- **Can estimate 'uniformity' of varieties using SSRs (U?)**
- **Some varieties are more 'uniform' than others**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

SUMMARY (2):

- **Some SSR loci appear to be more 'uniform' than others**
- **Can select SSRs that show low levels of heterogeneity within varieties**
- **Can select SSRs that provide good levels of D and reflect current levels of uniformity (U?)**
- **S?**

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Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape.

- **A number of questions have been posed previously, including:**
  - Number of markers needed?
  - Mapped *vs* unmapped markers?
  - Distribution of markers important?
  - Markers related to expressed regions?
  - Distances?
- **Now should be looking at what happens next, i.e. an action plan - how to use markers?**

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