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BMT/13/7 Add.
ORIGINAL: English
DATE: December 8, 2011

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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

Thirteenth Session
Brasilia, November 22 to 24, 2011

ADDENDUM

USING SSR MARKERS FOR AUTHENTICATION OF SEED STOCKS IN WINTER OILSEED RAPE (WOSR)

Document prepared by an experts from the United Kingdom

Plant Science into practice

National Institute of Agricultural Botany

Presenter Name Carol Norris Date November 2011

Using SSR markers for the authentication of seed stocks in Winter Oilseed Rape (WOSR)

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Background

- Authentication of seed stocks in the UK DUS WOSR trial is currently carried out by field assessment
- Visual assessment of side by side plots
- DUS and VCU seed
- Replacement stocks
- Foreign varieties required for the reference collection (seed from the testing authority and breeder are checked)
- >100 extra plots

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Objectives

- To develop a test using SSR markers to authenticate seed stocks against the definitive DUS seed
- To test whether multiplexing of more than 3 markers was possible
- To produce a molecular test that is cheaper and more efficient than the field test
- Based on previous (Fera funded) work assessing SSR markers for authentication

Summary of previous work funded by Fera

- The set of SSR markers used could differentiate between varieties if the peak patterns were assessed visually
- Multiplexing of primers was successful for two or three primer pairs
- Discrepancies in the data were likely to be due to the change of equipment part way through the project
- The cost was not sufficiently low to warrant replacement of the field assessment

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Methods used

- 33 DUS seed stocks were tested against the VCU seed stocks of the same variety
- All samples were assessed in the field and in the laboratory
- 30 seedlings were collected in bulk for each DNA preparation
- DNA extracted using Qiagen DNeasy 96 plant extraction kits
- Fragments amplified using PCR
- Fragments visualised on ABI 3130 Genetic Analyser
- Data analysed on Genemapper v 3.7 software

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Methods used

- 12 markers selected with the greatest polymorphism

Marker No.	Marker	5' primer sequence	3' primer sequence	Chromosome	No. of Alleles*
M1	Ra2-E03	AGGTAGGCCCA TCTCTCTCC	CCAAAACTTGCT CAAAACCC	10	3
M2	BN12A	GCCGTTCTAGG GTTTGTGGGA	GAGGAAGTGAG AGCGGGAAATC A	13	2
M5	LS107	GTTAAGTGTGGC GTTAGAGG	CCTTGGTACATG CCACTGAA	Unknown	3
M6	MB5	AACATCTTTTTG CGTGATAT	AATAGCATTGA AGCCTTAC	Unknown	2
M8	Na10-H03	GAGCTGGCTCA TTCAACTCC	CACAATTTCTCA GACAAAACGG	Unknown	2
M9	Na10-E02	TCGGGCATGTA ATCAAAATC	TGTGACGCATCC GATCATAC	5	3
M14	Na14-H11	GGATGTTTTCAC AGACCCTG	CTTTGCAGGTAT GAACACGC	Unknown	4
M16	Ol10-B01	CCTCTTCAGTCG AGGTCTGG	AATTTGGAAAC AGAGTCGCC	17	4
M17	Ol10-BF11	TTTGGAACGTCC GTAGAAGG	CAGCTGACTTCG AAAGGTCC	11	2
M21	Ol12-F02	GGCCCATTGAT ATGGAGATG	CATTTCTCAATG ATGAATAGT	9	4
M22	Ol13-C12	AGAGGCCAACA AAGAACACC	GAAGCAGCACC AGTGACAAG	13	3
M25	Ra2-A11	GACCTATTTTAA TATGCTGTTTTA CG	ACCTCACCGGA GAGAAATCC	9	4

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Results using relative thresholding

- Bands of more than a third of the intensity of the major band peak for each marker were scored (relative thresholding)
- 14 of the 33 variety pairs were complete matches
- 12 varieties showed a difference at one allele, five showed a difference at two alleles
- One variety showed a difference at three alleles and one variety showed a difference at five alleles
- Two pairs of varieties could not be distinguished from each other

Results using visual assessment of traces

- Traces were compared by eye (no scoring threshold)
- 26 of the 33 varieties were complete matches
- 12 varieties showed a difference at one allele, five showed a difference at two alleles
- One variety showed a difference at three alleles
- Two pairs of varieties could not be distinguished from each other

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Results

VARIETY	NUMBER OF ALLELES WHERE DIFFERENT USING SCORING	MARKERS WHERE DIFFERENCES SEEN	NUMBER OF ALLELES WHERE DIFFERENT BY COMPARING TRACES	COMMENTS
1608	Complete match	None	Complete match	
2019	Complete match	None	Complete match	
20206	Complete match	None	Complete match	
2209	Complete match	None	Complete match	
2163	1	M8	Complete match	M8 didn't work well
2165	1	M16	Complete match	Trace shows complete match
2166	2	M9, M17	Complete match	Trace shows complete match
2174	Complete match	None	Complete match	
2175	1	M8	1	Trace shows 1 allele difference
2180	5	M1, M5, M9, M22, M25	3	Trace shows 3 differences (M5, M9, M22)
2232	Complete match	None	Complete match	
2187	Complete match	None	Complete match	
2188	1	M8	1	Trace shows 1 allele difference
2190	Complete match	None	Complete match	
2191	1	M9	Complete match	Trace shows complete match
2192	1	M1	Complete match	Trace shows complete match
2194	Complete match	None	Complete match	
2196	1	M8	Complete match	Trace shows complete match
2215	1	M5	Complete match	Trace shows complete match
2197	1	M5	Complete match	Trace shows complete match
2198	Complete match	None	Complete match	
2199	2	M2, M8	Complete match	Trace shows complete match
2201	2	M6, M16	1	Trace shows 1 allele difference (M6)
2202	2	M8, M9	1	Trace shows 1 allele difference (M8)
2209	Complete match	None	Complete match	
2210	1	M5	Complete match	Trace shows complete match
2216	Complete match	None	Complete match	
2220	1	M5	Complete match	Trace shows complete match
2221	Complete match	None	Complete match	
2227	2	M16, M25	2	VCU trace didn't work well for both markers
2231	Complete match	None	Complete match	
2240	3	M8, M9, M25	1	Trace shows 1 allele difference (M8)
2247	1	M5	Complete match	Trace shows complete match

Multiplexing PCR

- Allows simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers
- Primer pairs that could be combined for optimal amplification of all loci under the same conditions were selected
- Adjustments in cycling conditions and primer concentrations were made for the combined primer pairs
- The multiplexed PCR matched the PCR using individual primer pairs

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Conclusions

- Not all varieties had exactly the same molecular profiles between seed samples
- Differences between the duplicate samples show the heterogeneity of oilseed rape that needs to be taken into consideration
- There were fewer differences between DUS and VCU samples when traces were compared visually
- The relative response for each allele depends on the proportion of individuals within the bulked sample possessing that allele

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Conclusions

- Multiplexing of primers was successful for up to 4 primer pairs
- The molecular test is as accurate as the field assessment
- The molecular test is not influenced by the environment
- The cost of the molecular test is now less than that of the field assessment

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Implementation

- The system will be implemented this season (2011/12)
- Any duplicate seed samples showing a difference of more than two alleles will be sown in the field (in spring) for field assessment
- Cost!
- £35 per variety for molecular test/ £68 per variety for field test

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