Finalising the establishment of a DNA library of 10 chestnut varieties using fingerprinting techniques

John Stephen
Australian Genome
Research Facility Ltd

Project Number: CH04005

CH04005

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the chestnut industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the chestnut Industry.

ISBN 0 7341 1322 6

Published and distributed by:

Horticultural Australia Ltd

Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

E-Mail: horticulture@horticulture.com.au

ADELAIDE NODE
Plant Genomics Centre
Waite Building 22, University of Adelaide
PMB1 GLEN OSMOND SA 5064 Australia
Ph: 618 8303 7202 Fax: 618 8303 7124
Email: agrf@agrf.org.au
www.agrf.org.au

ABN 63 097 086 292

Finalising the establishment of a DNA library of 19 chestnut varieties using fingerprinting techniques

For: The Chestnut Growers of Australia Limited (CGA) and Horticulture Australia Limited (HAL)

John R. Stephen, Paul S. Gooding and Annette McGrath

Report date 4/7/06

Summary

The following report details progress towards generating a genetic fingerprint database of commercially grown chestnut varieties in Australia. The purpose of this study was to evaluate the possibility of establishing a fingerprinting protocol suitable for rapid and reproducible discrimination of nineteen selected cultivars, namely De Coppi Marone, Spanish Red, Orange Marone, Sassafras Red, Buffalo Queen, Morena (two accessions), Menzies, Mullion Range, Purtons Pride, Bouche de Betizac, Cusia, Early Marone, April Gold, Wandiligong Wonder, Mollisima, Sassafras Gold, Colossal and Knox Early. Nine cultivars were added to the database in this period. All nineteen cultivars can be discriminated by the approach used.

The current report is an update on the report submitted 22/9/05.

Next Steps

Should this project be continued, the need will arise to create a searchable index of DNA fingerprints for rapid comparison of test samples against the reference data generated thus far. Further additions of cultivars to the database can now be achieved as a routine procedure.

Communication / extension activities

None

Commercialisation and/or Intellectual Property issues

All data has been forwarded to Dr. Roberto Botta, University of Turin, as requested. The output is therefore presumably public domain and not amenable to IP generation. The database is suitable for commercial use as a Quality Assurance checkpoint.

ADELAIDE NODE
Plant Genomics Centre
Waite Building 22, University of Adelaide
PMB1 GLEN OSMOND SA 5064 Australia
Ph: 618 8303 7202 Fax: 618 8303 7124
Email: agrf@agrf.org.au
www.agrf.org.au

ABN 63 097 086 292

Detail of Methods and Results
Methods

Sampling

Triplicate leaf samples were provided from nine varieties by CGA members. DNA was extracted from leaf samples by freeze-drying, grinding and passage through a NucleoSpin Plant DNA extraction system (Machery-Nagel).

Polymerase chain reaction amplification of the nine microsatellite loci (CsCAT16, CsCat4, QpZag119, CsCAT17, CsCAT3, CsCAT6, EMCs15, QpZAG110, QrZAG96; Table 1) selected in the previous reporting period was conducted as before. The amplified fragments were sized by capillary electrophoresis on an Applied Biosystems 3730 Genetic Analyser system against a LIZ500 (-250) size standard.

Results:

Allele sizing of each sample submitted in 2005 in triplicate showed no intra-varietal variation. The sizing data was collated with the data generated in the previous reporting period (Table 2). Differences in allele sizes were found between each of the 18 varieties tested, and minor differences were also apparent between the two accessions of Morena (2004 and 2005).

Varietal differentiation

The sizes of these alleles for each marker and sample are given in Table 2 alongside the data generated in the previous reporting period.

A dendrogram using a maximum of two alleles at each marker data is presented in Figure 1. Please note that the discriminatory power of the dataset is greater than indicated in the dendrogram as less than 100% of the data could be included in this analysis. Scaling on the dendrogram is not an accurate reflection of the level of difference between cultivars, but clustering is an indication of similarities between cultivars at these loci.

Conclusions

The nine markers utilised in creation of this dataset discriminated all nineteen chestnut cultivars, with several differences being evident between any pair of cultivars. This set of nine markers can be considered suitable for the generation of an identity database of a larger number of chestnut

ADELAIDE NODE
Plant Genomics Centre
Waite Building 22, University of Adelaide
PMB1 GLEN OSMOND SA 5064 Australia
Ph: 618 8303 7202 Fax: 618 8303 7124
Email: agrf@agrf.org.au
www.agrf.org.au

ABN 63 097 086 292

cultivars. Further additions to this dataset can be made by AGRF at a cost of $240 per cultivar, assuming samples to be submitted in triplicate.

Fig. 1. Cluster diagram of ISSR analyses of 10 chestnut cultivars. The data generated by this technique demonstrated the existence of detectable differences between all ten cultivars. This procedure is not robust enough to be used as an identification tool, so a second approach was adopted utilizing highly reproducible microsatellite data.

ADELAIDE NODE
Plant Genomics Centre
Waite Building 22, University of Adelaide
PMB1 GLEN OSMOND SA 5064 Australia
Ph: 618 8303 7202 Fax: 618 8303 7124
Email: agrf@agrf.org.au
www.agrf.org.au

ABN 63 097 086 292

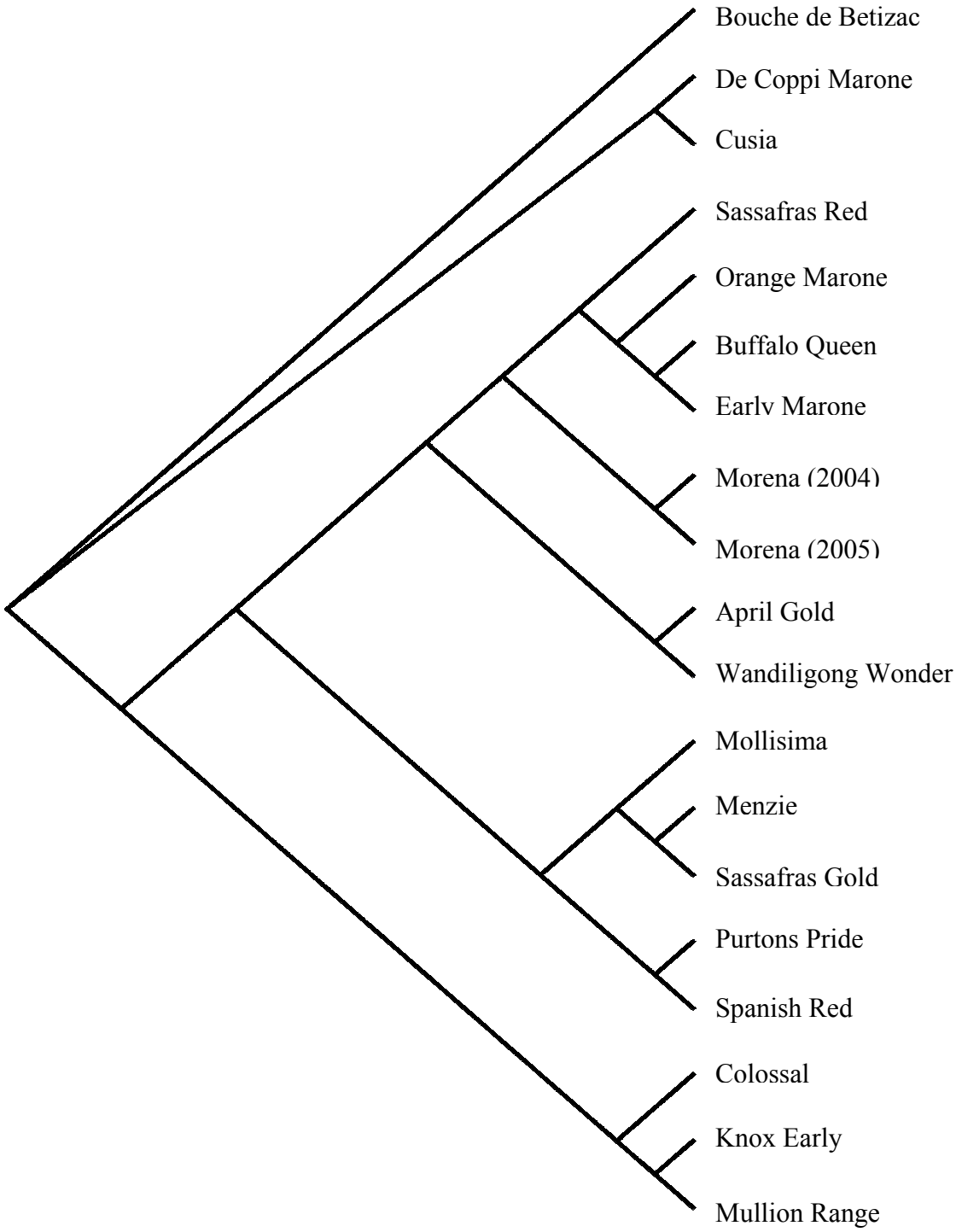

Fig. 1. Dendrogram showing clustering of cultivars based on differences in microsatellite fingerprints at a combination of 9 loci. Please note that branch lengths are not to scale.

ADELAIDE NODE
Plant Genomics Centre
Waite Building 22, University of Adelaide
PMB1 GLEN OSMOND SA 5064 Australia
Ph: 618 8303 7202 Fax: 618 8303 7124
Email: agrf@agrf.org.au
www.agrf.org.au

ABN 63 097 086 292

		(52 genotypes in 38 cultivars)			
	Linkage Group	N. alleles	He	Ho	**Comment**
CsCAT4	?	**6**	0.65	0.60	Scoring average good
CsCAT6	1	**14**	0.77	0.92	Scoring average good
QpZAG119	1	**10**	0.71	0.91	Good, but stutter bands, smaller size of alleles (64-92 bp)
CsCAT17	2	**9**	0.71	0.80	Scoring average good
CsCAT16	6	**7**	0.72	0.83	Scoring average good
QpZag110	7	**8**	0.65	0.62	Scoring average good. Requires "stronger" PCR conditions (>Mg, primers)
EMCs15	9	**7**	0.59	0.49	Difficult scoring after multiplex PCR
QrZAG96	10	**7**	0.60	0.76	Good scoring
CsCAT3	12	**14**	0.77	0.84	Easy to score and analyse

Table 1. Markers used and Dr. Botta's comments.

ADELAIDE NODE
Plant Genomics Centre
Waite Building 22, University of Adelaide
PMB1 GLEN OSMOND SA 5064 Australia
Ph: 618 8303 7202 Fax: 618 8303 7124
Email: agrf@agrf.org.au
www.agrf.org.au

ABN 63 097 086 292

Table 2. Allele sizes of nine microsatellite loci in nineteen chestnut cultivars

Variety	CsCAT16		CsCat4			QpZag119		CsCAT17	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 1	Allele 2
Bouche de Betizac	132		212	216		84		134	154
Buffalo Queen	154	156	220	222		60	62	134	146
De Coppi Marone	126	132	212			60	84	146	154
Orange Marone	140	142	220	234		60	62	142	146
Menzie	130		200	222		78	80	134	146
Morena (2004)	140	142	216	222		60	62	134	146
Morena (2005)		142	218	222	236	62		134	146
April Gold	126	144	236			62	84	138	146
Colossal	126		218	234		84		134	142
Cusia	126	132	212			62	84	146	154
Early Marone	144		220	236		62		142	146
Knox Early	156		218	222	236	84		134	146
Mollisima	130	146	224			48	82	132	134
Sassafras Gold	130		200	222		74		134	
Wandiligong Wonder	142	148	236			62	84	146	156
Mullion Range	144	156		234		74	84	134	146
Purtons Pride	142	144	222	228		102	106	134	138
Sassafras Red	130	144	220	234		62	90	146	154
Spanish Red	142	144	222	228		74		134	154

Allele names and sizes to the nearest base. Analysis performed on AB 3730 capillary electrophoresis platform

ADELAIDE NODE
Plant Genomics Centre
Waite Building 22, University of Adelaide
PMB1 GLEN OSMOND SA 5064 Australia
Ph: 618 8303 7202 Fax: 618 8303 7124
Email: agrf@agrf.org.au
www.agrf.org.au

ABN 63 097 086 292

CsCAT3		CsCAT6		EMCs15		QpZAG110		QrZAG96	
Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
212		174		78	88	217	219	155	157
224		160		78	84	207	219	157	159
226	240	160	174	90	92	207		153	155
224	226	160	184	86	92	207	211	153	155
204	232	195	198	78	80	211	225	159	173
208	226		160	78	88	217		153	157
	226	140	160	78	86	217		153	157
196		160	184	80	84	211		153	159
212		140		78	90	211	219	155	159
226		160	174	90		207		153	155
224		160	184	84	90	207	211	161	165
204		140	174	78	84	217	219	157	159
208		140	144	78		217	219	155	
196		140	174	78	84	217	225	159	
198		184		86	90	211	217	153	161
204	226	174		84	88	215	217	157	159
204		184		78	84	211	217	146	155
198	226	160	184	84	90	211	215	153	
212	234	158	184	78	84	211	219	155	165

Table 2. (Cont.) Allele sizes of nine microsatellite loci in nineteen chestnut cultivars

Allele names and sizes to the nearest base. Analysis performed on AB 3730 capillary electrophoresis platform