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MICROSATELLITE FINGERPRINTING TO DIFFERENTIATE *BRASSICA* VARIETIES

document prepared by experts from the United Kingdom

Microsatellite fingerprinting to differentiate *Brassica* varieties.

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Abstract

Varieties of *Brassica napus* L. *partim* (42 varieties), *Brassica rapa* L. var. *silvestris* (Lam.) Briggs (15 varieties), and *Brassica napus* L. *partim* synthetics (3 varieties) were screened by amplification using a set of eight microsatellite primers. The allele pattern of each of the 60 varieties, using all of the primers, was encoded as a binary matrix. These data were used to produce a distance matrix, and these distances were used in the production of a dendrogram showing the interrelationships of the varieties. Of the varieties screened, eleven varieties could not be differentiated using this method (two groups of four, and one group of three). In addition, the information from two sets of primers was dispensable without degrading the resolving power of the method. Information regarding contrasting results by individual and population sampling is also discussed.

Introduction

The use of DNA-based marker systems, in the estimation of relationships between plant varieties, is of especial interest in terms of development of methods for use in assigning plant breeders rights for new varieties. This group of techniques has the potential to contribute to measurement of Distinctness, Uniformity and Stability (DUS). The most examined area, in terms of published work, is in the use of molecular methods to assess D (e.g. Lee *et al*., 1996; Law *et al*., 1996).

We present in this paper an examination of the use of simple sequence repeats (SSR) in the application of a molecular fingerprinting method to *Brassica* spp. SSR, also known as microsatellites, are short regions of DNA consisting of tandemly repeated nucleotide units of between one and five bases. The variation in the number of repeats found in microsatellite regions provide the polymorphisms which have been used to differentiate many eukaryotic species (Weber and May, 1989) and these differences are inherited in a Mendelian fashion. We have used an existing method for SSR typing of *Brassica* spp. (Kresovich *et al*., 1995; Szewc-McFadden *et al*., 1996) to examine 60 varieties of Swede rape, Turnip rape and hybrid rape. The method used allowed resolution of the majority of varieties based on allele patterns.

Materials and Methods

Brassica Collection
The sixty varieties of *Brassica* used in this study are shown in Table 1. For convenience, the numbering used in Table 1 is used throughout the paper to refer to the seed stocks.

DNA isolation
Seed DNA was extracted using the method of Chee *et al*. (1995) using 0.1g seed material for each sample from 2 g macerated seed. For some samples, single seeds were extracted to compare to the bulk seed DNA.

PCR Amplification
The primers-pairs used were Bn6A1, Bn9A, Bn12A, Bn26A, Bn38A, Bn59A1, Bn72A and Bn92A1 which had been designed for use in *Brassica* spp. (Kresovich *et al*., 1995; Szewc-McFadden *et al*., 1996). The conditions used for amplification were as described by Szewc-

McFadden *et al*. (1996). PCR amplifications were performed on a MJ Research PTC-200 thermal cycler.

Post-PCR Analysis
PCR reactions were examined after electrophoresis on a PROTEAN II xi apparatus (Bio-Rad), through 8 % polyacrylamide gels (160 mm X 160 mm X 1 mm). After electrophoresis, the gels were silver stained (Wray *et al*., 1981) and dried between cellophane sheets.

Data Analysis
Allele patterns were scored as a binary table. Genetic distances were calculated by means of the program RESTDIST, part of the PHYLIP package, version 3.6 (Felsenstein, 1989). This program uses a modification of the distance method of Nei and Li (1979). Dendrograms were constructed using the NEIGHBOR program from PHYLIP.

Results & Discussion

Data collection
Each of the sixty *Brassica* seed lots was amplified with the eight primer-pairs, and the allele patterns were examined after gel electrophoresis and staining. The number of allele locations scored for each of the primer-pairs was as follows;

- Bn6A1 – 5 alleles
- Bn9A – 6 alleles
- Bn12A – 10 alleles
- Bn26A – 21 alleles
- Bn38A – 7 alleles
- Bn59A1 – 7 alleles
- Bn72A – 4 alleles
- Bn92A1 – 9 alleles

This added up to a total of 69 data points per sample, or 4140 data points in the final matrix (Appendix I).

Data Analysis
The genetic distances were estimated using the RESTDIST program, using a site length of 20 nucleotides (i.e. primer size). A matrix of genetic distances was then produced. This data set (Appendix II) was the basis for further calculations. The dendrogram produce from the full genetic distance matrix (Figure 1) shows that there are three unresolved clusters of *Brassica*. The first cluster was of *B. rapa* varieties (numbers 11, 12, 14 and 22), the second was *B. napus* (numbers 40, 47, 51 and 52) and the third was *B. napus* (numbers 58, 59 and 60). Eleven varieties could not be discriminated using the SSR method presented.

The informational content of each subset of data from individual primer-pairs was examined by its removal, in turn, from the set of data points, followed by dendrogram construction as before. The consequences of removal of data sets are shown in Table 2. The removal of data points can be extended to primer-pairs Bn6A2 and Bn72A with no degradation of variety resolution, or major change in tree topology (data not shown). However this must be regarded as exceptional; the general trend is that increasing the number of primer-pairs in the examination of the samples will increase the resolving power of the technique.

Bulked verses single seed extracts

A subset of the primer-pairs was used to examine the effect on allele patterns found in individual seed compared to the bulked seed for all varieties. A single individual from each seed lot was extracted and analysed by SSR in comparison to the bulked samples used previously. The findings from these experiments, an example of which is shown in Fig. 2, was that Turnip rape showed great deal of heterogeneity, with an average of approximately 50% common alleles when comparing single and bulked samples. The heterogeneity of Swede rape varieties was much lower, as might be expected from their breeding patterns.

Conclusions

The ability of the SSR technique to differentiate between varieties of *Brassica* has been shown, but problems remain. The resolving power needs to be increased to permit differentiation of all varieties, most simply by an increase in the number of primer-pairs used to examine this *Brassica* collection. Automation of the allele sizing techniques (Ziegle *et al*., 1992) would increase the number of samples which could be examined, but would, counter-intuitively, reduce the resolution of data obtained. In slab gels, samples of interest can be place in adjacent wells and allele correspondence can be ascertained with a high degree of certainty. However, when the same analysis is performed using automatic methods, a degree of inaccuracy is inevitable. The practice of “binning”, grouping together of alleles within a certain size range, is routinely utilised. The relative merits of automation verses older methods will only become apparent when the same techniques are compared on the two systems. However, there are indisputable advantages in the use of polyacrylamide gels, most obviously the relative cost and availability when compared to automatic systems.

The ultimate goal is the use of molecular methods in one, or more, elements of DUS. The survey presented here, using microsatellites, shows the need to conduct trials of methods on large collections of varieties. Problems may not become apparent if the testing is performed using small numbers.

The cut-off between utility as an identification method and a practical method for D testing is one which can only be determined empirically, and is likely to differ for each technique. To permit development of a method for D it may be necessary to decide upon one method to test across a number of groups, each testing a collection of varieties that have been granted PBR. Although microsatellites are a tried and tested identification system, a method with a higher resolving power might be required. Potential candidates include automated versions of AFLP (Vos *et al*., 1995) and ISTR (Rohde, 1996).

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TABLE 1. *BARSSICA* VARIETIES USED**.**

No.	Species	Crop	Type	Variety	Comments
1	*Brassica napus* L. *partim*	Swede Rape	Forage	Interval	-
2	*Brassica napus* L. *partim*	Swede Rape	Forage	Hobson	-
3	*Brassica napus* L. *partim*	Swede Rape	Forage	Hungry gap	-
4	*Brassica napus* L. *partim*	Swede Rape	Spring	BC95-106	-
5	*Brassica napus* L. *partim*	Swede Rape	Spring	Mars	-
6	*Brassica napus* L. *partim*	Swede Rape	Spring	SW02769	-
7	*Brassica napus* L. *partim*	Swede Rape	Spring	Corona	-
8	*Brassica napus* L. *partim*	Swede Rape	Spring	Acrobat	-
9	*Brassica napus* L. *partim*	Swede Rape	Spring	Aries	-
10	*Brassica napus* L. *partim*	Swede Rape	Spring	Canyon	-
11	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	Skye	-
12	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	SWA3152	-
13	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	Merit	-
14	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	SWB3156	-
15	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	SWA3157	-
16	*Brassica napus* L. *partim*	Swede Rape	Spring	Lila	-
17	*Brassica napus* L. *partim*	Swede Rape	Spring	Rebel	-
18	*Brassica napus* L. *partim*	Swede Rape	Spring	Sprinter	-
19	*Brassica napus* L. *partim*	Swede Rape	Spring	Maskot	-
20	*Brassica napus* L. *partim*	Swede Rape	Spring	SWB2777	-
21	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Winter	Triton	-
22	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Winter	Debut	-
23	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Winter	Fimbul	-
24	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Winter	Salut	-
25	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Winter	SW01622	-
26	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	Riina	-
27	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Winter	Debut	-
28	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	Kova	-
29	*Brassica napus* L. *partim*	Swede Rape	Winter	Commanche	Treated seed
30	*Brassica napus* L. *partim*	Swede Rape	Winter	Apex	Treated seed
31	*Brassica napus* L. *partim*	Swede Rape	Spring	Aries	-
32	*Brassica napus* L. *partim*	Swede Rape	Synthetic	Hyola 401	Treated seed
33	*Brassica napus* L. *partim*	Swede Rape	Synthetic	Hyola 38	Treated seed
34	*Brassica napus* L. *partim*	Swede Rape	Synthetic	Synergy	Treated seed
35	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	Agena	Treated seed
36	*Brassica rapa* L. var. *silvestris* (Lam.) Briggs	Turnip Rape	Spring	Kulta	-
37	*Brassica napus* L. *partim*	Swede Rape	Spring	Maskot	Treated seed
38	*Brassica napus* L. *partim*	Swede Rape	Winter	Cobra	Treated seed
39	*Brassica napus* L. *partim*	Swede Rape	Spring	Acrobat	Treated seed
40	*Brassica napus* L. *partim*	Swede Rape	Winter	Falcon	Treated seed
41	*Brassica napus* L. *partim*	Swede Rape	Spring	Solar	-
42	*Brassica napus* L. *partim*	Swede Rape	Winter	Express	Treated seed
43	*Brassica napus* L. *partim*	Swede Rape	Spring	Starlight	-
44	*Brassica napus* L. *partim*	Swede Rape	Winter	Inca	Treated seed
45	*Brassica napus* L. *partim*	Swede Rape	x	Corsair	Treated seed
46	*Brassica napus* L. *partim*	Swede Rape	x	Mlch58	-
47	*Brassica napus* L. *partim*	Swede Rape	Winter	Contact	-
48	*Brassica napus* L. *partim*	Swede Rape	x	Mlch56	-
49	*Brassica napus* L. *partim*	Swede Rape	Winter	Capitol	-
50	*Brassica napus* L. *partim*	Swede Rape	Spring	Briol	-
51	*Brassica napus* L. *partim*	Swede Rape	Winter	Hansen	Treated seed
52	*Brassica napus* L. *partim*	Swede Rape	Winter	Gazelle	Treated seed
53	*Brassica napus* L. *partim*	Swede Rape	Winter	Columbus	-
54	*Brassica napus* L. *partim*	Swede Rape	Winter	Bristol	-
55	*Brassica napus* L. *partim*	Swede Rape	Spring	CSH P003	-
56	*Brassica napus* L. *partim*	Swede Rape	x	Pactol	-
57	*Brassica napus* L. *partim*	Swede Rape	x	Cocktail	Treated seed
58	*Brassica napus* L. *partim*	Swede Rape	x	Superol	-
59	*Brassica napus* L. *partim*	Swede Rape	Spring	CSHP001	-
60	*Brassica napus* L. *partim*	Swede Rape	x	Concept	-

TABLE 2. EFFECT OF REMOVAL OF PRIMER-PAIR ALLELE DATA FROM THE DATA SET.

Primer-pair data removed	Number of unresolved varieties	Number of unresolved clusters
None	11	3
Bn6A2	11	3
Bn9A	13	4
Bn26A	17	6
Bn38A	15	4
Bn59A	12	3
Bn72A	11	3
Bn92A	14	4
Bn6A2 & Bn72A	11	3

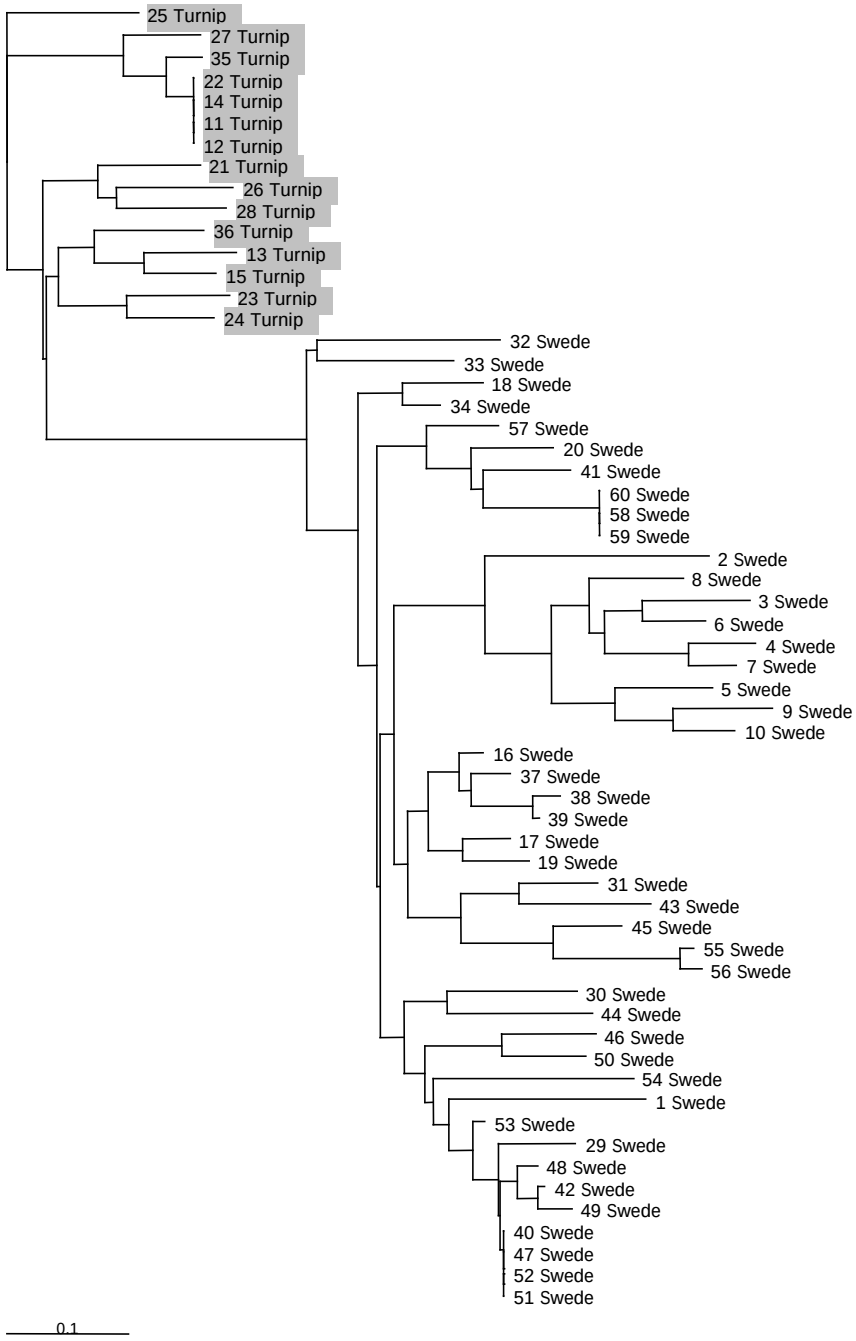

Figure 1. Dendrogram representing the comparison of bulked-seed allele patterns. Note Turnip Rape varieties are shaded to allow easy identification. See Table 1 for the variety numbers.

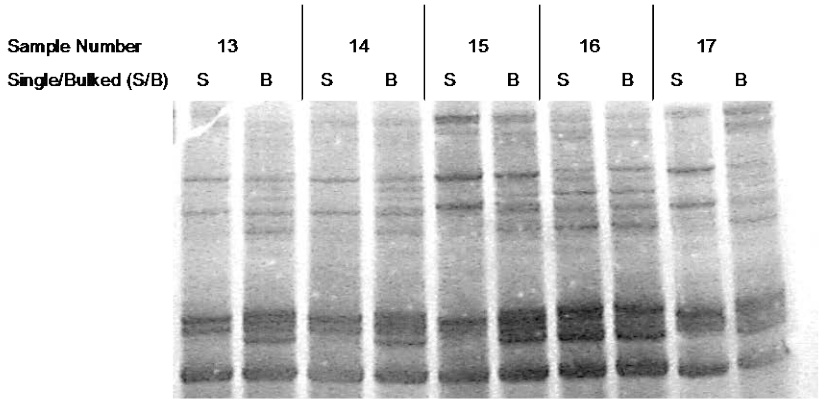

Figure 2. Silver stained polyacrylamide gel, showing allele patterns obtained from single and bulked seed extracts of *B. rapa* varieties, using primer-pair Bn38A1. Note the greater number of alleles in the bulked seed extracts.

Appendix I. Binary data set representing presence or absence of alleles for each variety, using all primer-pairs.

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1 Swede   -+++-+-+--+++---+-----+-----+---++------+--+-++++--++++++++++------
2 Swede   -+++-+-+--+++---+-----+-----+--+------++--+--+++---+--+++++++++------
3 Swede   -++-+-----+++---+-----+-----+---++----++--+--+++---+--+-+++++++------
4 Swede   -++-+-----+++---+---+-+-----+---+--+--++--+--+++---+--+-+++++++---+++
5 Swede   -++-+-----+++--+---+--+-+---++--+--+--++--+--+++---++++++++++---+++
6 Swede   -++-+-----+++---------+-----+--+++-+--++--+--+++---+--++++++++------
7 Swede   -++-+-----+++---+-----++----+---+--+--++--+--+++---+--++++++++---+++
8 Swede   -++-+-----+++---------+-----+--+++-+--++--+--+++--+++-++++++++---+++
9 Swede   -++-+--+--++---+---------+-++++-+--+--++--+--+++---++++++++++---+++
10 Swede  -++-+--+--+++--++---------+-+++-+--+--++--+--+++---++++++++++---+++
11 Turnip +++-+-----+++---------+-----+---++------+--+-++++-----++----------
12 Turnip +++-+-----+++---------+-----+---++------+--+-++++-----++----------
13 Turnip +++-+-----+++---------+-----+---++-------+--++++-----++--------+++--
14 Turnip +++-+-----+++---------+-----+---++------+--+-++++-----++----------
15 Turnip +++-+-----+++---------+-----+---++-------+--++++-----++----------
16 Swede  -+++-+-+--+++---------+-----+--+++-+-----+--++++---++++++++++---+++
17 Swede  -++--+-+--+++---------+-----+---+--+-----+--++++---++++++++++---+++
18 Swede  +++-+-----+++---------+-----+---+--+-----+--++++---++++++++++------
19 Swede  -+++---+--+++---------+-----+---+--+----++--++++---++++++++++---+++
20 Swede  ++++---+--+++--+--+-+-+-----+---+--+-----+--++++---++++++++++---+++
21 Turnip +++-+-----+++---------+-----+--+++-+----++-++++++---+-++--------+++--
22 Turnip +++-+-----+++---------+-----+---++------+--+-++++-----++----------
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25 Turnip ++++---+--+++---------+-----+---+--+----++-++++++-----++----------
26 Turnip -++-+--+--+++---------+-----+---+--+----++-++++++-----++--------+++--
27 Turnip +++-+-----+++---------+-----+---+--+----+--+-++++-----++----------
28 Turnip -++-+--+--+++---------+-----+---++------++-++++++---+-++-------+++---
29 Swede  -++----+--+++---------+-----+---++---+---+--++++---++++++++++------
30 Swede  -++----+--++---++-++--+-----+---++---+---+--++++---++++++++++------
31 Swede  +++-+--+--+++--+---+--+-+---++--++------+--++++---++++++++++---+++
32 synth. -++-+-----+++---+-----++----+---++------++-+++++++-++++++++++---+++
33 synth. -++-+--+--+++--+--+-+-+-----+--+++-+----++-+++++++-++++++++++------
34 synth. +++-+--+--+++---------+-----+--+++-+-----+--++++---++++++++++------
35 Turnip -++-+-----+++---------+-----+---++------+--+-++++-----++----------
36 Turnip -++-+-----+++---------+-----+--+++-+-----+--++++-----++----------
37 Swede  ++++-+-+--+++---------+-----+--+++-+-----+--++++---++++-+++++++---+++
38 Swede  ++++-+----+++---------+-----+--+++-+-----+--++++--+++-+++++++++---+++
39 Swede  ++++-+-+--+++---------+-----+--+++-+-----+--++++--+++-+++++++++---+++
40 Swede  -+++-+-+--+++---------+-----+---++---+---+--++++---++++++++++------
41 Swede  ++++-+----+++--+--+-+-+-----+--+++-+-----+--++++---++++-+++++++---+++
42 Swede  -+++-+-+--+++---------+-----+---++---+---+--++++---++++-+++++++------
43 Swede  ++-+++-+--+++--+---+--+-+---++-++-+-+----+--++++---++++++++++---+++
44 Swede  +++-+-+---+++--+--+-+-+-----+---++---+---+--++++---++++++++++------
45 Swede  -++-+-+---+++---------+-----+--++-+-+----+--++++---++++++++++---+++
46 Swede  -+++-+-+--+++--+--+-+-+-----+--++-+-+----+--++++---++++++++++------
47 Swede  -+++-+-+--+++---------+-----+---++---+---+--++++---++++++++++------
48 Swede  ++++-+-+--+++---------+-----+---++---+---+--++++---++++++++++------
49 Swede  ++++-+-+--+++---------+-----+---++---+---+--++++---++++-+++++++------
50 Swede  ++++-+-+--+++---------+-----+--++-+-+----+--++++---++++-+++++++------
51 Swede  -+++-+-+--+++---------+-----+---++---+---+--++++---++++++++++------
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54 Swede  -+++-+-+--+++++--+---+------+--+++-+-----+--++++---++++++++++------
55 Swede  -++-++----+++++--+---+------+--++-+-+----+--++++---++++++++++---+++
56 Swede  -++-+-----+++++--+---+------+--++-+-+----+--++++---++++++++++---+++
57 Swede  +++--+-+--+++--+--+-+-+-----+--+++-+-----+--++++---++++++++++------
58 Swede  +++--+-+--+++--++-+-+-++----+--+++-+----++-+++++---++++++++++---+++
59 Swede  +++--+-+--+++--++-+-+-++----+--+++-+----++-+++++---++++++++++---+++
60 Swede  +++--+-+--+++--++-+-+-++----+--+++-+----++-+++++---++++++++++---+++
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APPENDIX II. **GENETIC DISTANCE MATRIX DERIVED FROM THE RESTDIST PROGRAM**

1 swede																				
2 swede	0.012																			
3 swede	0.015	0.009																		
4 swede	0.022	0.013	0.006																	
5 swede	0.021	0.018	0.014	0.008																
6 swede	0.017	0.009	0.004	0.008	0.011															
7 swede	0.020	0.012	0.007	0.003	0.007	0.007														
8 swede	0.019	0.014	0.009	0.007	0.007	0.005	0.006													
9 swede	0.024	0.021	0.019	0.013	0.007	0.016	0.011	0.011												
10 swede	0.019	0.017	0.015	0.009	0.006	0.014	0.008	0.010	0.004											
11 turnip	0.022	0.040	0.028	0.038	0.039	0.026	0.034	0.033	0.049	0.045										
12 turnip	0.022	0.040	0.028	0.038	0.039	0.026	0.034	0.033	0.049	0.045	0.000									
13 turnip	0.034	0.044	0.032	0.037	0.038	0.030	0.034	0.032	0.048	0.044	0.011	0.011								
14 turnip	0.022	0.040	0.028	0.038	0.039	0.026	0.034	0.033	0.049	0.045	0.000	0.000	0.011							
15 turnip	0.030	0.040	0.028	0.038	0.039	0.026	0.034	0.033	0.049	0.045	0.006	0.006	0.004	0.006						
16 swede	0.011	0.015	0.021	0.017	0.014	0.015	0.015	0.010	0.016	0.015	0.038	0.038	0.029	0.038	0.030					
17 swede	0.012	0.017	0.021	0.014	0.011	0.017	0.012	0.012	0.014	0.012	0.039	0.039	0.030	0.039	0.030	0.003				
18 swede	0.014	0.020	0.014	0.016	0.013	0.011	0.014	0.014	0.018	0.017	0.026	0.026	0.022	0.026	0.019	0.010	0.007			
19 swede	0.011	0.018	0.022	0.015	0.012	0.018	0.013	0.013	0.015	0.013	0.036	0.036	0.031	0.036	0.031	0.004	0.003	0.008		
20 swede	0.016	0.021	0.025	0.015	0.013	0.021	0.016	0.015	0.015	0.014	0.039	0.039	0.031	0.039	0.031	0.006	0.005	0.009	0.004	
21 turnip	0.028	0.045	0.038	0.038	0.036	0.028	0.035	0.027	0.044	0.041	0.011	0.011	0.006	0.011	0.011	0.025	0.028	0.022	0.026	0.029
22 turnip	0.022	0.040	0.028	0.038	0.039	0.026	0.034	0.033	0.049	0.045	0.000	0.000	0.011	0.000	0.006	0.038	0.039	0.026	0.036	0.039
23 turnip	0.034	0.044	0.037	0.037	0.034	0.030	0.034	0.032	0.043	0.040	0.014	0.014	0.011	0.014	0.007	0.029	0.026	0.016	0.027	0.027
24 turnip	0.027	0.044	0.037	0.042	0.039	0.027	0.038	0.029	0.047	0.044	0.010	0.010	0.013	0.010	0.010	0.027	0.031	0.020	0.028	0.031
25 turnip	0.023	0.034	0.042	0.042	0.043	0.034	0.038	0.041	0.048	0.044	0.011	0.011	0.015	0.011	0.011	0.026	0.026	0.022	0.020	0.024
26 turnip	0.028	0.042	0.039	0.035	0.037	0.033	0.032	0.034	0.041	0.038	0.013	0.013	0.008	0.013	0.013	0.028	0.025	0.025	0.023	0.029
27 turnip	0.026	0.040	0.033	0.033	0.035	0.026	0.030	0.033	0.044	0.041	0.003	0.003	0.014	0.003	0.010	0.038	0.034	0.022	0.031	0.035
28 turnip	0.023	0.043	0.036	0.045	0.042	0.034	0.042	0.035	0.046	0.043	0.011	0.011	0.009	0.011	0.011	0.029	0.029	0.026	0.027	0.034
29 swede	0.009	0.017	0.014	0.022	0.018	0.014	0.020	0.016	0.021	0.019	0.031	0.031	0.026	0.031	0.022	0.008	0.007	0.006	0.008	0.011
30 swede	0.012	0.020	0.018	0.025	0.018	0.020	0.023	0.022	0.021	0.019	0.039	0.039	0.034	0.039	0.030	0.013	0.012	0.012	0.013	0.011
31 swede	0.017	0.025	0.020	0.018	0.008	0.019	0.017	0.014	0.014	0.012	0.032	0.032	0.025	0.032	0.025	0.009	0.008	0.010	0.009	0.008
32 hybrid	0.010	0.025	0.017	0.016	0.016	0.019	0.012	0.014	0.020	0.017	0.025	0.025	0.025	0.025	0.025	0.011	0.010	0.012	0.009	0.014
33 hybrid	0.011	0.023	0.021	0.022	0.019	0.015	0.023	0.017	0.021	0.020	0.026	0.026	0.029	0.026	0.026	0.012	0.013	0.011	0.012	0.010
34 hybrid	0.012	0.017	0.015	0.019	0.016	0.009	0.017	0.012	0.018	0.017	0.026	0.026	0.023	0.026	0.019	0.006	0.008	0.003	0.009	0.009
35 turnip	0.021	0.039	0.027	0.037	0.038	0.025	0.033	0.031	0.048	0.044	0.002	0.002	0.013	0.002	0.008	0.037	0.038	0.029	0.034	0.043
36 turnip	0.031	0.037	0.029	0.034	0.036	0.020	0.031	0.026	0.045	0.042	0.011	0.011	0.009	0.011	0.005	0.024	0.027	0.020	0.029	0.032
37 swede	0.013	0.017	0.021	0.017	0.016	0.017	0.018	0.012	0.019	0.017	0.038	0.038	0.029	0.038	0.030	0.002	0.005	0.010	0.005	0.006
38 swede	0.015	0.017	0.021	0.017	0.016	0.015	0.015	0.008	0.021	0.020	0.034	0.034	0.026	0.034	0.026	0.003	0.006	0.010	0.007	0.008
39 swede	0.014	0.016	0.022	0.018	0.017	0.016	0.016	0.009	0.020	0.018	0.035	0.035	0.027	0.035	0.027	0.003	0.005	0.011	0.006	0.007
40 swede	0.007	0.013	0.017	0.024	0.020	0.016	0.022	0.018	0.023	0.021	0.033	0.033	0.029	0.033	0.025	0.006	0.007	0.008	0.008	0.011
41 swede	0.017	0.022	0.023	0.016	0.016	0.019	0.019	0.014	0.020	0.019	0.041	0.041	0.032	0.041	0.032	0.005	0.008	0.012	0.009	0.004
42 swede	0.008	0.015	0.016	0.023	0.022	0.018	0.024	0.020	0.025	0.023	0.037	0.037	0.031	0.037	0.028	0.007	0.008	0.010	0.009	0.012
43 swede	0.020	0.022	0.029	0.024	0.012	0.025	0.022	0.019	0.018	0.017	0.044	0.044	0.035	0.044	0.035	0.009	0.011	0.015	0.012	0.010
44 swede	0.017	0.025	0.017	0.021	0.018	0.016	0.022	0.018	0.023	0.021	0.029	0.029	0.025	0.029	0.021	0.015	0.014	0.007	0.015	0.009
45 swede	0.019	0.022	0.020	0.016	0.013	0.016	0.014	0.011	0.018	0.016	0.037	0.037	0.028	0.037	0.029	0.008	0.008	0.009	0.008	0.011
46 swede	0.013	0.015	0.024	0.025	0.021	0.020	0.026	0.022	0.024	0.022	0.043	0.043	0.037	0.043	0.034	0.009	0.011	0.012	0.011	0.008
47 swede	0.007	0.013	0.017	0.024	0.020	0.016	0.022	0.018	0.023	0.021	0.033	0.033	0.029	0.033	0.025	0.006	0.007	0.008	0.008	0.011
48 swede	0.008	0.014	0.018	0.025	0.021	0.017	0.023	0.019	0.024	0.022	0.030	0.030	0.026	0.030	0.022	0.006	0.008	0.007	0.009	0.009
49 swede	0.009	0.016	0.017	0.024	0.023	0.019	0.025	0.021	0.026	0.024	0.033	0.033	0.029	0.033	0.025	0.008	0.009	0.008	0.010	0.011
50 swede	0.012	0.014	0.021	0.025	0.024	0.020	0.026	0.022	0.027	0.025	0.039	0.039	0.034	0.039	0.030	0.008	0.010	0.009	0.011	0.011
51 swede	0.007	0.013	0.017	0.024	0.020	0.016	0.022	0.018	0.023	0.021	0.033	0.033	0.029	0.033	0.025	0.006	0.007	0.008	0.008	0.011
52 swede	0.007	0.013	0.017	0.024	0.020	0.016	0.022	0.018	0.023	0.021	0.033	0.033	0.029	0.033	0.025	0.006	0.007	0.008	0.008	0.011
53 swede	0.006	0.012	0.016	0.023	0.019	0.015	0.021	0.017	0.022	0.020	0.032	0.032	0.027	0.032	0.024	0.005	0.006	0.007	0.007	0.010
54 swede	0.009	0.013	0.020	0.024	0.020	0.013	0.022	0.016	0.020	0.018	0.038	0.038	0.033	0.038	0.029	0.004	0.007	0.008	0.008	0.011
55 swede	0.018	0.021	0.022	0.017	0.015	0.018	0.016	0.013	0.017	0.015	0.040	0.040	0.031	0.040	0.031	0.007	0.007	0.010	0.010	0.012
56 swede	0.020	0.023	0.021	0.016	0.014	0.017	0.015	0.012	0.016	0.015	0.039	0.039	0.030	0.039	0.030	0.008	0.008	0.009	0.009	0.011
57 swede	0.013	0.017	0.021	0.022	0.019	0.015	0.023	0.017	0.021	0.020	0.034	0.034	0.029	0.034	0.026	0.007	0.008	0.008	0.011	0.006
58 swede	0.012	0.021	0.025	0.018	0.018	0.021	0.017	0.016	0.020	0.016	0.034	0.034	0.033	0.034	0.034	0.007	0.009	0.014	0.009	0.006
59 swede	0.012	0.021	0.025	0.018	0.018	0.021	0.017	0.016	0.020	0.016	0.034	0.034	0.033	0.034	0.034	0.007	0.009	0.014	0.009	0.006
60 swede	0.012	0.021	0.025	0.018	0.018	0.021	0.017	0.016	0.020	0.016	0.034	0.034	0.033	0.034	0.034	0.007	0.009	0.014	0.009	0.006

22 turnip	0.011																			
23 turnip	0.012	0.014																		
24 turnip	0.006	0.010	0.005																	
25 turnip	0.012	0.011	0.011	0.010																
26 turnip	0.006	0.013	0.014	0.013	0.008															
27 turnip	0.011	0.003	0.011	0.010	0.007	0.010														
28 turnip	0.007	0.011	0.015	0.011	0.012	0.006	0.015													
29 swede	0.028	0.031	0.026	0.027	0.026	0.029	0.035	0.023												
30 swede	0.035	0.039	0.034	0.034	0.034	0.036	0.044	0.029	0.005											
31 swede	0.027	0.032	0.028	0.029	0.032	0.030	0.036	0.028	0.010	0.010										
32 hybrid	0.021	0.025	0.028	0.023	0.028	0.024	0.028	0.022	0.012	0.015	0.010									
33 hybrid	0.019	0.026	0.026	0.018	0.023	0.022	0.026	0.020	0.011	0.011	0.013	0.009								
34 hybrid	0.019	0.026	0.019	0.018	0.023	0.025	0.026	0.023	0.005	0.010	0.008	0.012	0.007							
35 turnip	0.013	0.002	0.017	0.011	0.013	0.012	0.005	0.010	0.029	0.038	0.035	0.024	0.025	0.029						
36 turnip	0.009	0.011	0.009	0.008	0.012	0.011	0.011	0.013	0.024	0.031	0.029	0.026	0.021	0.017	0.010					
37 swede	0.025	0.038	0.029	0.027	0.026	0.032	0.038	0.033	0.010	0.015	0.009	0.013	0.014	0.006	0.042	0.027				
38 swede	0.022	0.034	0.026	0.024	0.026	0.032	0.034	0.033	0.012	0.018	0.011	0.013	0.016	0.008	0.037	0.024	0.003			
39 swede	0.023	0.035	0.027	0.025	0.024	0.029	0.035	0.030	0.011	0.016	0.010	0.014	0.014	0.007	0.038	0.025	0.003	0.001		
40 swede	0.031	0.033	0.029	0.029	0.025	0.031	0.038	0.025	0.002	0.007	0.011	0.014	0.012	0.007	0.032	0.026	0.008	0.010	0.008	
41 swede	0.027	0.041	0.032	0.029	0.032	0.038	0.041	0.039	0.014	0.015	0.010	0.014	0.011	0.010	0.044	0.029	0.003	0.005	0.006	0.011
42 swede	0.033	0.037	0.031	0.032	0.027	0.034	0.042	0.027	0.003	0.008	0.013	0.015	0.014	0.008	0.035	0.029	0.007	0.011	0.010	0.001
43 swede	0.033	0.044	0.035	0.035	0.035	0.037	0.044	0.038	0.017	0.017	0.006	0.017	0.018	0.013	0.048	0.037	0.009	0.011	0.010	0.014
44 swede	0.027	0.029	0.025	0.026	0.032	0.034	0.033	0.028	0.007	0.008	0.010	0.014	0.009	0.008	0.031	0.026	0.015	0.015	0.015	0.009
45 swede	0.027	0.037	0.028	0.029	0.036	0.031	0.037	0.032	0.011	0.017	0.010	0.010	0.015	0.010	0.036	0.026	0.010	0.010	0.011	0.013
46 swede	0.035	0.043	0.033	0.034	0.029	0.035	0.043	0.033	0.010	0.011	0.015	0.020	0.010	0.011	0.042	0.031	0.011	0.013	0.012	0.008
47 swede	0.031	0.033	0.029	0.029	0.025	0.031	0.038	0.025	0.002	0.007	0.011	0.014	0.012	0.007	0.032	0.026	0.008	0.010	0.008	0.000
48 swede	0.028	0.030	0.026	0.027	0.023	0.032	0.034	0.026	0.003	0.008	0.010	0.015	0.013	0.006	0.033	0.027	0.006	0.008	0.007	0.001
49 swede	0.031	0.033	0.029	0.029	0.025	0.035	0.038	0.028	0.004	0.009	0.011	0.016	0.015	0.007	0.037	0.030	0.006	0.010	0.008	0.002
50 swede	0.032	0.039	0.030	0.031	0.026	0.036	0.039	0.033	0.009	0.015	0.015	0.019	0.015	0.008	0.043	0.031	0.006	0.011	0.009	0.007
51 swede	0.031	0.033	0.029	0.029	0.025	0.031	0.038	0.025	0.002	0.007	0.011	0.014	0.012	0.007	0.032	0.026	0.008	0.010	0.008	0.000
52 swede	0.031	0.033	0.029	0.029	0.025	0.031	0.038	0.025	0.002	0.007	0.011	0.014	0.012	0.007	0.032	0.026	0.008	0.010	0.008	0.000
53 swede	0.029	0.032	0.027	0.028	0.024	0.030	0.037	0.024	0.003	0.008	0.011	0.013	0.011	0.006	0.031	0.025	0.007	0.009	0.008	0.001
54 swede	0.027	0.038	0.029	0.026	0.025	0.031	0.038	0.028	0.006	0.011	0.014	0.016	0.010	0.005	0.037	0.022	0.006	0.008	0.006	0.004
55 swede	0.029	0.040	0.031	0.032	0.039	0.033	0.040	0.034	0.013	0.018	0.011	0.011	0.016	0.011	0.039	0.029	0.009	0.009	0.010	0.012
56 swede	0.028	0.039	0.030	0.031	0.038	0.032	0.039	0.033	0.012	0.017	0.010	0.010	0.015	0.010	0.038	0.027	0.011	0.011	0.011	0.014
57 swede	0.025	0.034	0.026	0.024	0.026	0.032	0.034	0.029	0.008	0.008	0.011	0.017	0.006	0.005	0.037	0.024	0.007	0.009	0.008	0.008
58 swede	0.023	0.034	0.033	0.025	0.027	0.029	0.034	0.030	0.014	0.012	0.011	0.009	0.008	0.010	0.037	0.031	0.007	0.009	0.008	0.014
59 swede	0.023	0.034	0.033	0.025	0.027	0.029	0.034	0.030	0.014	0.012	0.011	0.009	0.008	0.010	0.037	0.031	0.007	0.009	0.008	0.014
60 swede	0.023	0.034	0.033	0.025	0.027	0.029	0.034	0.030	0.014	0.012	0.011	0.009	0.008	0.010	0.037	0.031	0.007	0.009	0.008	0.014

42 swede	0.011																		
43 swede	0.011	0.015																	
44 swede	0.010	0.010	0.017																
45 swede	0.012	0.015	0.009	0.011															
46 swede	0.009	0.009	0.009	0.010	0.010														
47 swede	0.011	0.001	0.014	0.009	0.013	0.008													
48 swede	0.010	0.002	0.013	0.008	0.014	0.008	0.001												
49 swede	0.009	0.001	0.014	0.009	0.016	0.010	0.002	0.001											
50 swede	0.010	0.006	0.009	0.014	0.010	0.005	0.007	0.006	0.005										
51 swede	0.011	0.001	0.014	0.009	0.013	0.008	0.000	0.001	0.002	0.007									
52 swede	0.011	0.001	0.014	0.009	0.013	0.008	0.000	0.001	0.002	0.007	0.000								
53 swede	0.011	0.002	0.013	0.010	0.012	0.007	0.001	0.002	0.003	0.006	0.001	0.001							
54 swede	0.009	0.005	0.014	0.013	0.013	0.008	0.004	0.005	0.006	0.007	0.004	0.004	0.003						
55 swede	0.011	0.014	0.008	0.015	0.003	0.009	0.012	0.013	0.015	0.009	0.012	0.012	0.011	0.010					
56 swede	0.012	0.015	0.009	0.014	0.002	0.011	0.014	0.015	0.016	0.010	0.014	0.014	0.013	0.011	0.001				
57 swede	0.005	0.009	0.013	0.006	0.015	0.005	0.008	0.006	0.008	0.008	0.008	0.008	0.007	0.006	0.014	0.015			
58 swede	0.005	0.015	0.013	0.012	0.014	0.011	0.014	0.012	0.014	0.014	0.014	0.014	0.013	0.012	0.013	0.014	0.006		
59 swede	0.005	0.015	0.013	0.012	0.014	0.011	0.014	0.012	0.014	0.014	0.014	0.014	0.013	0.012	0.013	0.014	0.006	0.000	
60 swede	0.005	0.015	0.013	0.012	0.014	0.011	0.014	0.012	0.014	0.014	0.014	0.014	0.013	0.012	0.013	0.014	0.006	0.000	0.000

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