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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

Fourteenth Session
Seoul, Republic of Korea, November 10 to 13, 2014

ADDENDUM TO DOCUMENT BMT/14/9

DETERMINING A THRESHOLD FOR GENETIC CONFORMITY IN POTATO SEEDLINGS

Document prepared by experts from the United Kingdom

The Annex to this document contains a copy of a presentation “Determining a Threshold for Genetic Conformity in Potato Seedlings” made at the fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

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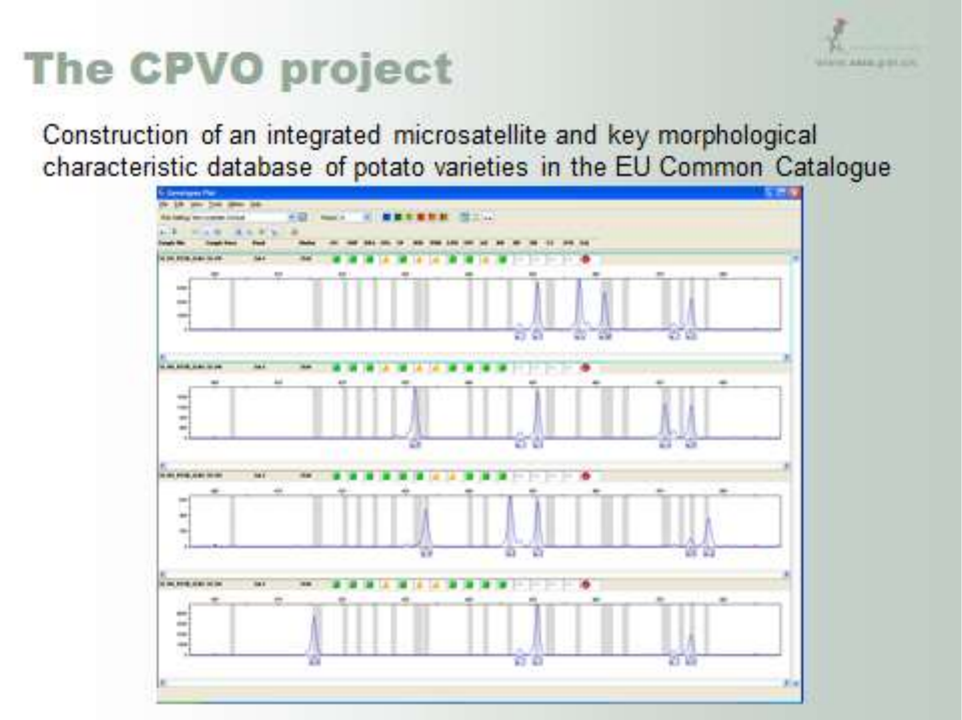
Abbreviations used in the Annex;

CPVO	Community Plant Variety Office of the European Union
EU	European Union
ESA	European Seed Association

[Annex follows]

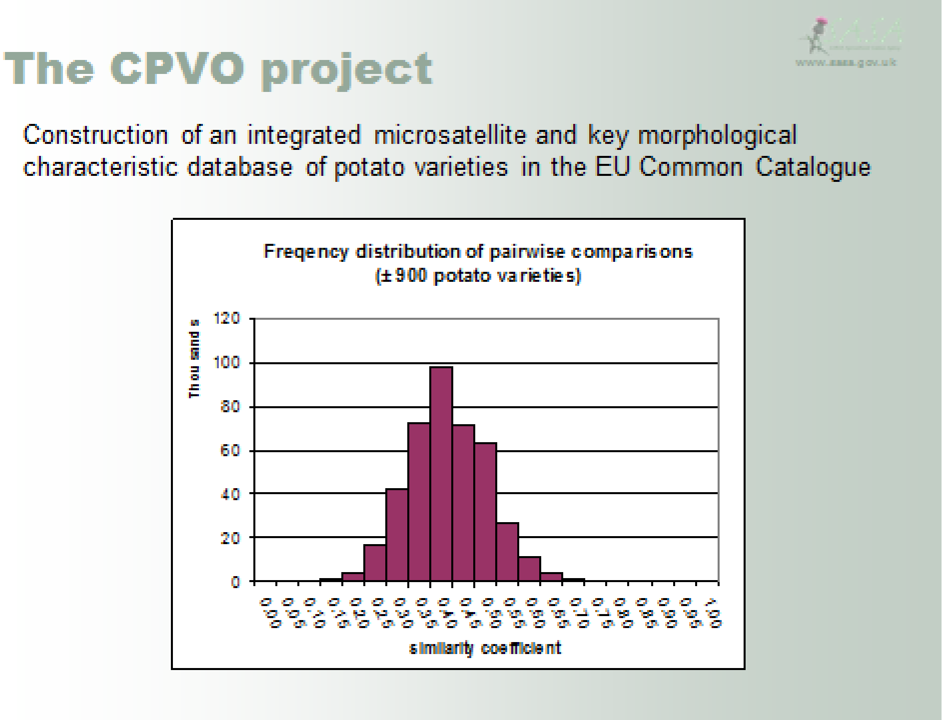
SASA

Determining a threshold for genetic conformity in potato seedlings



The CPVO project

Construction of an integrated microsatellite and key morphological characteristic database of potato varieties in the EU Common Catalogue



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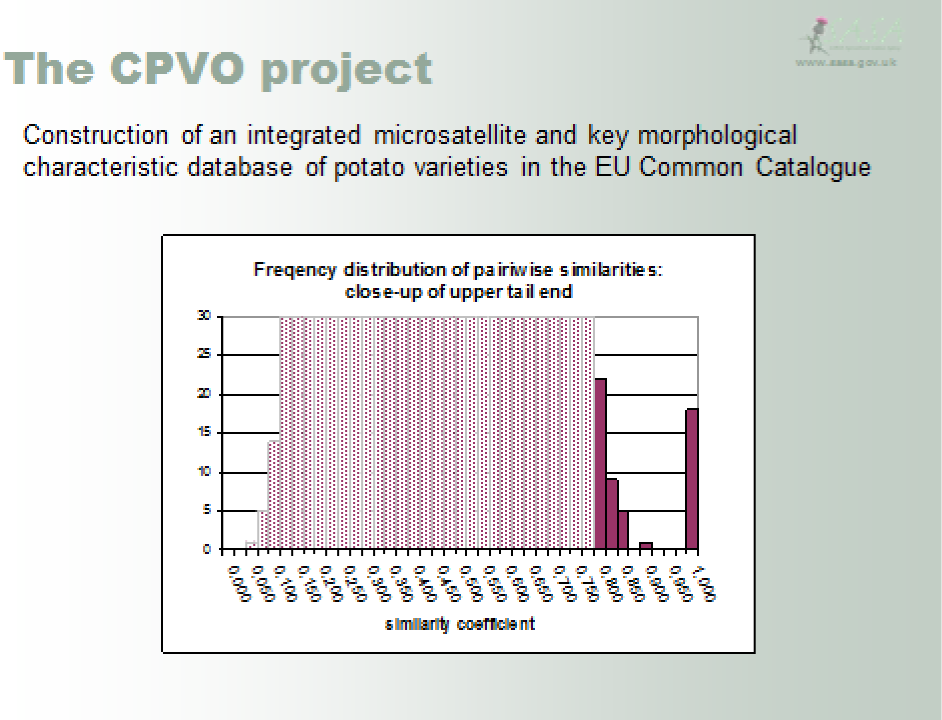

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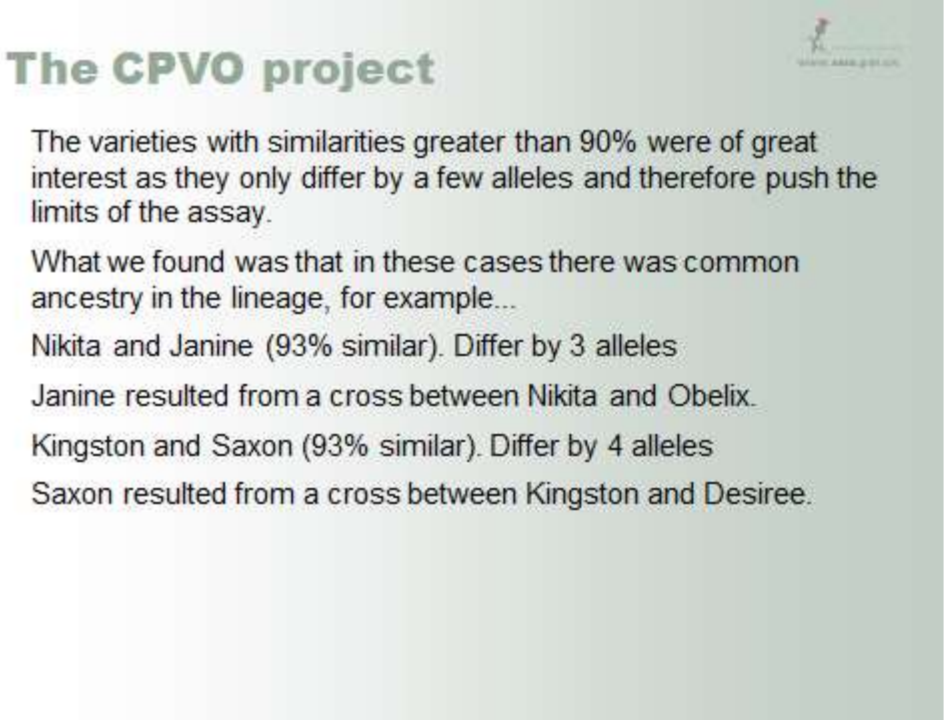


The CPVO project

The varieties with similarities greater than 90% were of great interest as they only differ by a few alleles and therefore push the limits of the assay.

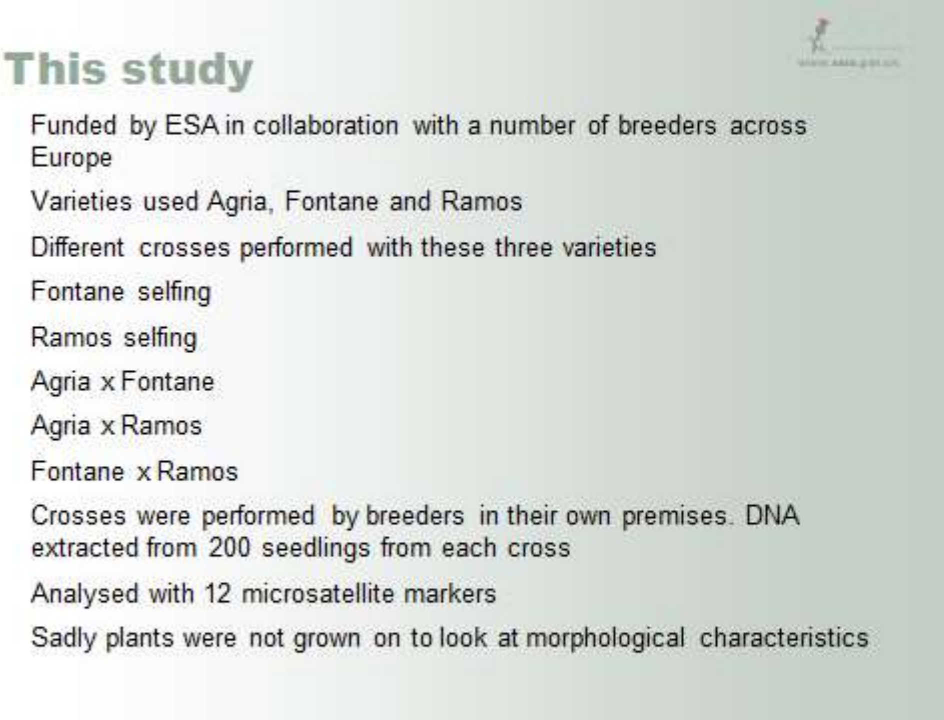
What we found was that in these cases there was common ancestry in the lineage, for example...

Nikita and Janine (93% similar). Differ by 3 alleles

Janine resulted from a cross between Nikita and Obelix.

Kingston and Saxon (93% similar). Differ by 4 alleles

Saxon resulted from a cross between Kingston and Desiree.



This study

Funded by ESA in collaboration with a number of breeders across Europe

Varieties used Agria, Fontane and Ramos

Different crosses performed with these three varieties

Fontane selfing

Ramos selfing

Agria x Fontane

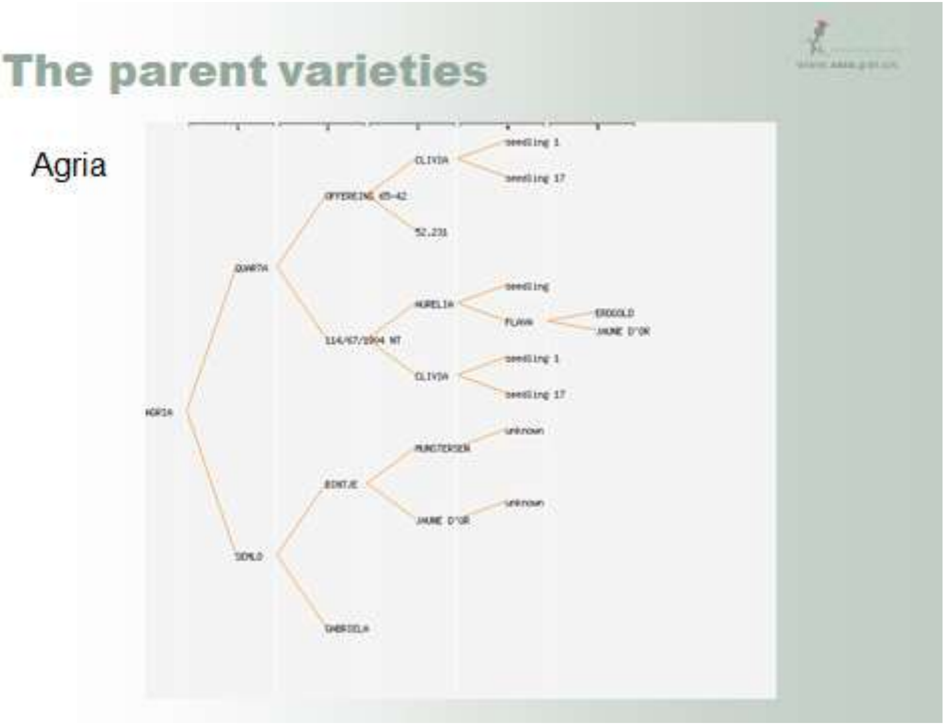
Agria x Ramos

Fontane x Ramos

Crosses were performed by breeders in their own premises. DNA extracted from 200 seedlings from each cross

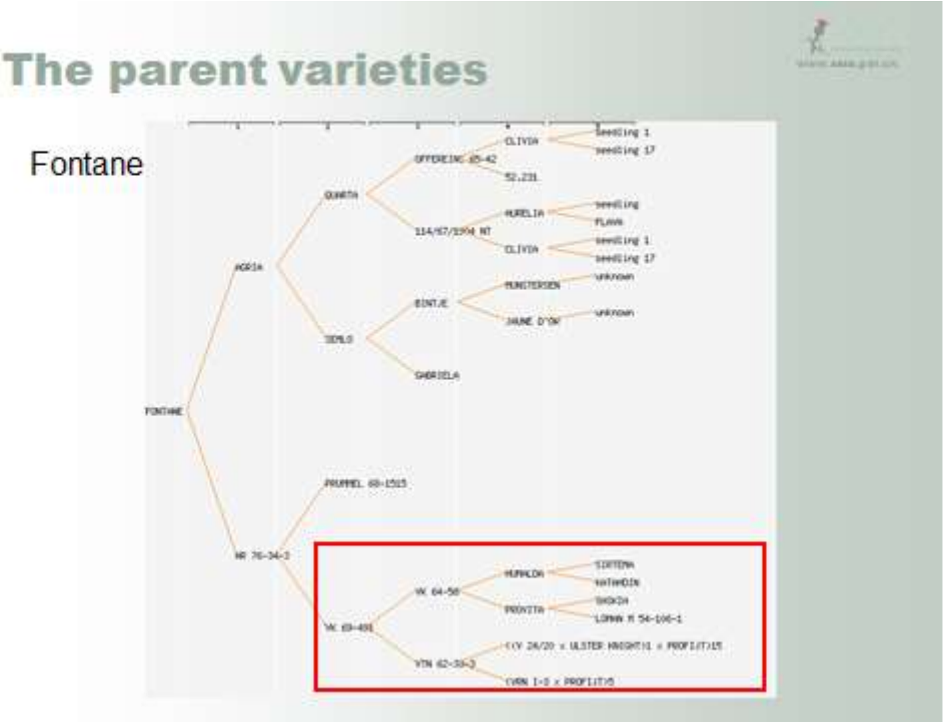
Analysed with 12 microsatellite markers

Sadly plants were not grown on to look at morphological characteristics



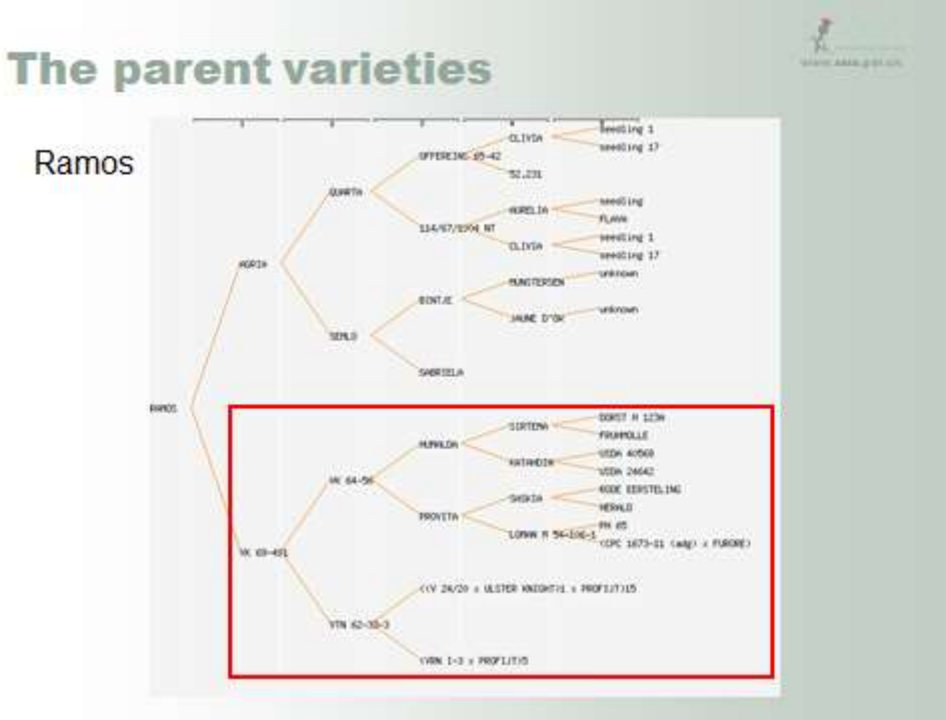
The parent varieties

Agria



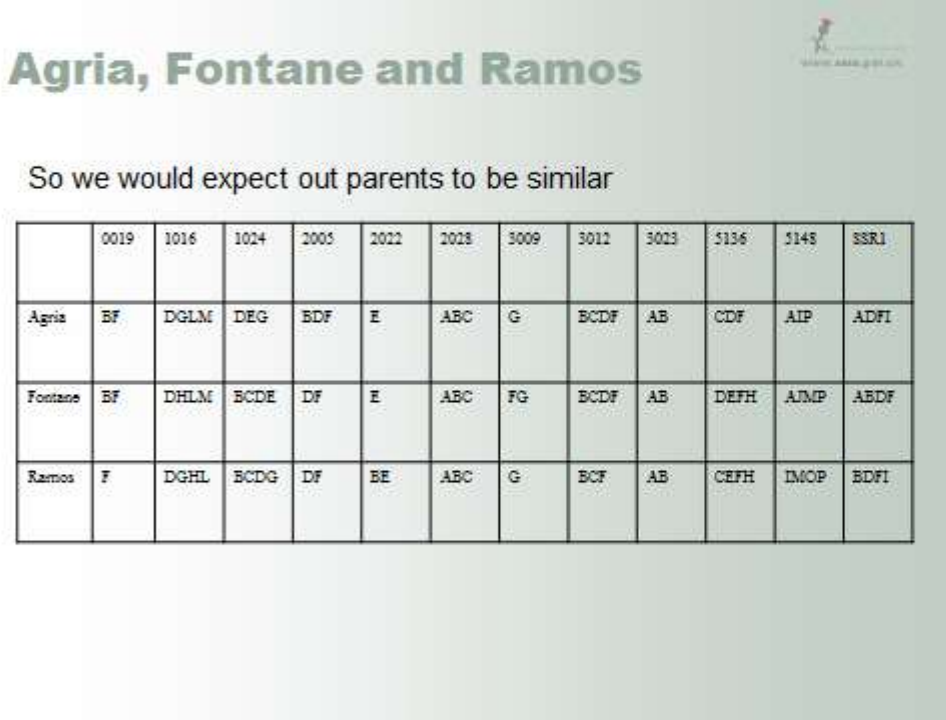
The parent varieties

Fontane



The parent varieties

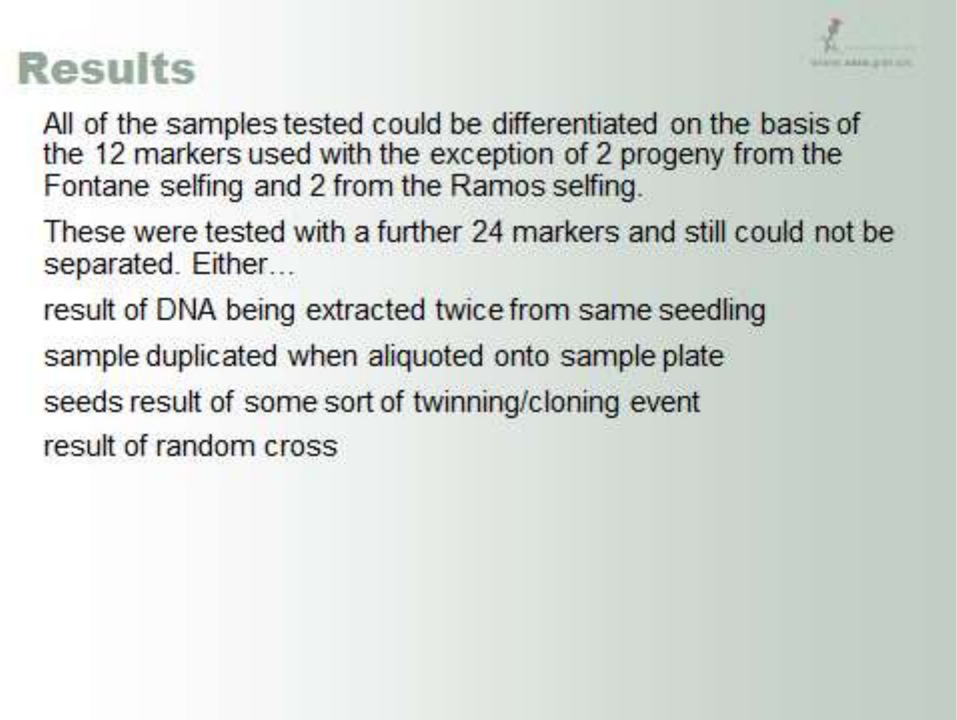
Ramos



Agria, Fontane and Ramos

So we would expect out parents to be similar

	0019	1016	1024	2005	2022	2028	3009	3012	3023	5136	5148	SSR1
Agria	BF	DGLM	DEG	BDF	E	ABC	G	BCDF	AB	CDF	AIP	ADFI
Fontane	BF	DHLM	BCDE	DF	E	ABC	FG	BCDF	AB	DEFH	AJMP	ABDF
Ramos	F	DGHL	BCDG	DF	BE	ABC	G	BCF	AB	CEFH	IMOP	BDFI



Results

All of the samples tested could be differentiated on the basis of the 12 markers used with the exception of 2 progeny from the Fontane selfing and 2 from the Ramos selfing.

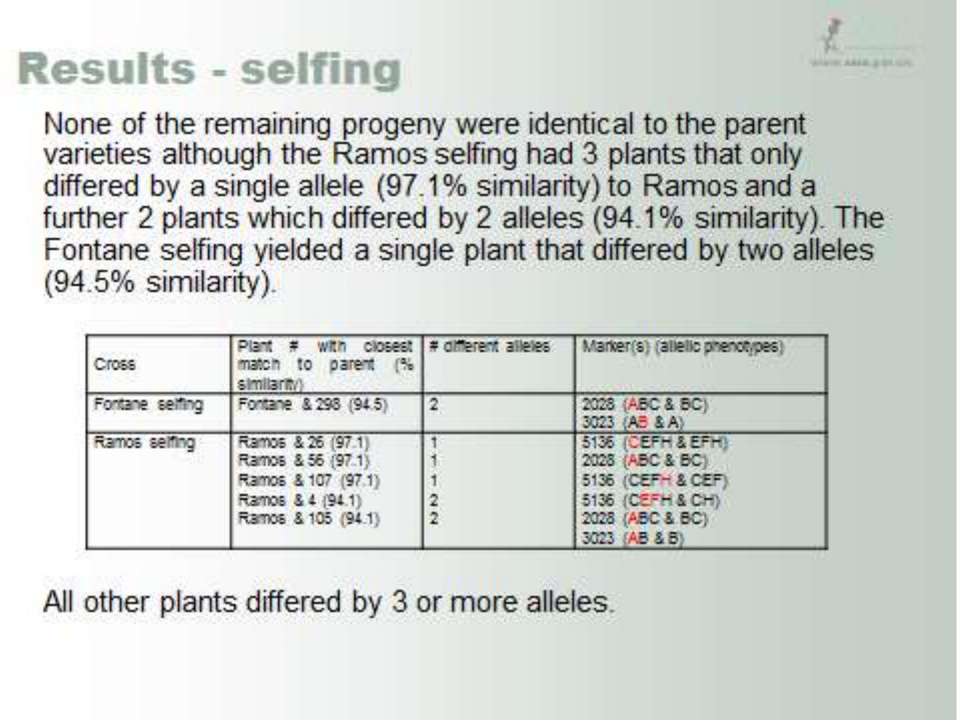
These were tested with a further 24 markers and still could not be separated. Either…

result of DNA being extracted twice from same seedling

sample duplicated when aliquoted onto sample plate

seeds result of some sort of twinning/cloning event

result of random cross

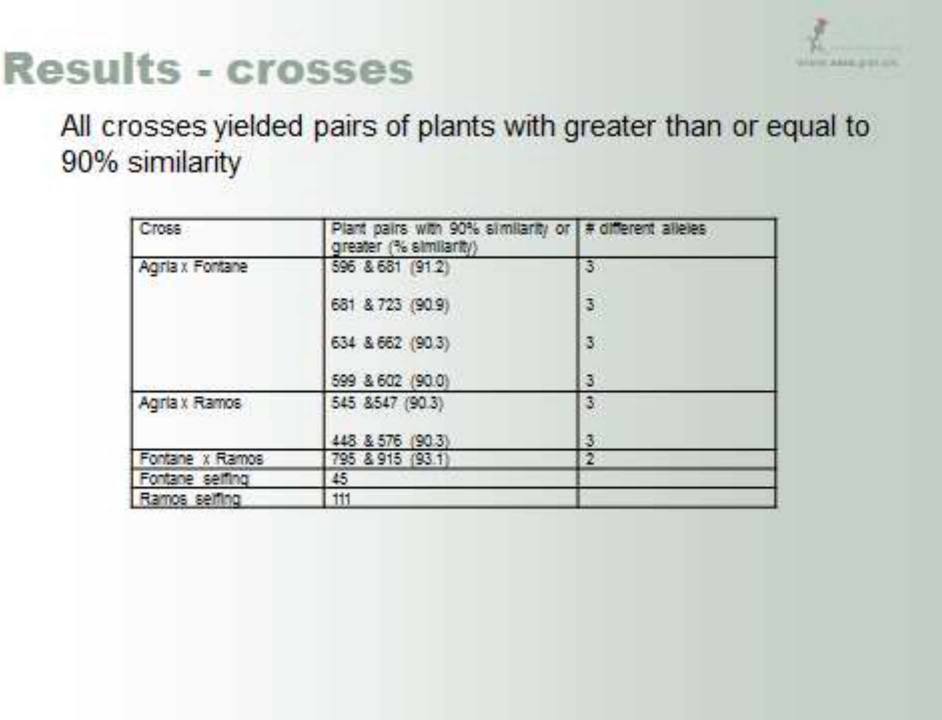


Results - selfing

None of the remaining progeny were identical to the parent varieties although the Ramos selfing had 3 plants that only differed by a single allele (97.1% similarity) to Ramos and a further 2 plants which differed by 2 alleles (94.1% similarity). The Fontane selfing yielded a single plant that differed by two alleles (94.5% similarity).

Cross	Plant # with closest match to parent (% similarity)	# different alleles	Marker(s) (allelic phenotypes)
Fontane selfing	Fontane & 298 (94.5)	2	2028 (ABC & BC) 3023 (AB & A)
Ramos selfing	Ramos & 26 (97.1) Ramos & 56 (97.1) Ramos & 107 (97.1) Ramos & 4 (94.1) Ramos & 105 (94.1)	1 1 1 2 2	5136 (CEFH & EFH) 2028 (ABC & BC) 5136 (CEFH & CEF) 5136 (CEFH & CH) 2028 (ABC & BC) 3023 (AB & B)

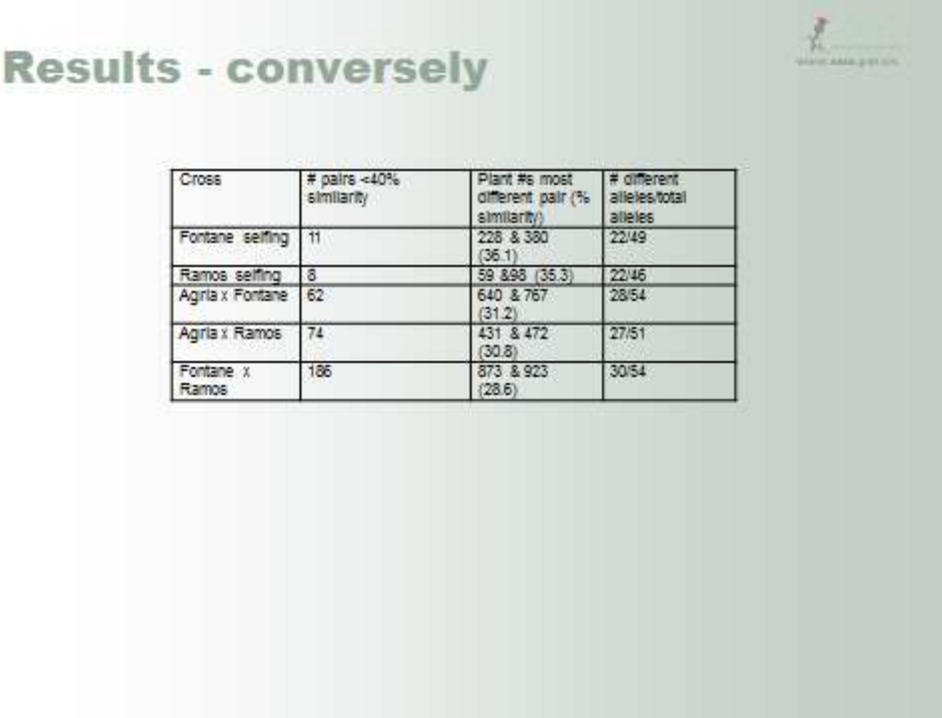
All other plants differed by 3 or more alleles.



Results - crosses

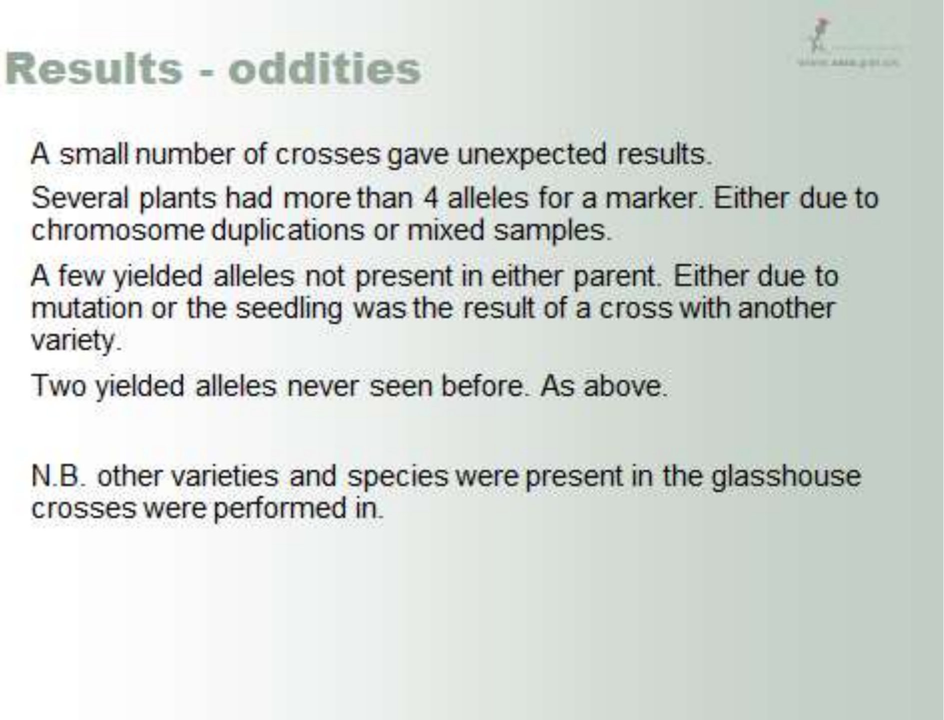
All crosses yielded pairs of plants with greater than or equal to 90% similarity

Cross	Plant pairs with 90% similarity or greater (% similarity)	# different alleles
Agria x Fontane	596 & 681 (91.2) 681 & 723 (90.9) 634 & 662 (90.3) 599 & 602 (90.0)	3 3 3 3
Agria x Ramos	545 &547 (90.3) 448 & 576 (90.3)	3 3
Fontane x Ramos	795 & 915 (93.1)	2
Fontane selfing	45	
Ramos selfing	111	



Results - conversely

Cross	# pairs <40% similarity	Plant #s most different pair (% similarity)	# different alleles/total alleles
Fontane selfing	11	228 & 380 (36.1)	22/49
Ramos selfing	8	59 &98 (35.3)	22/46
Agria x Fontane	62	640 & 767 (31.2)	28/54
Agria x Ramos	74	431 & 472 (30.8)	27/51
Fontane x Ramos	186	873 & 923 (28.6)	30/54



Results - oddities

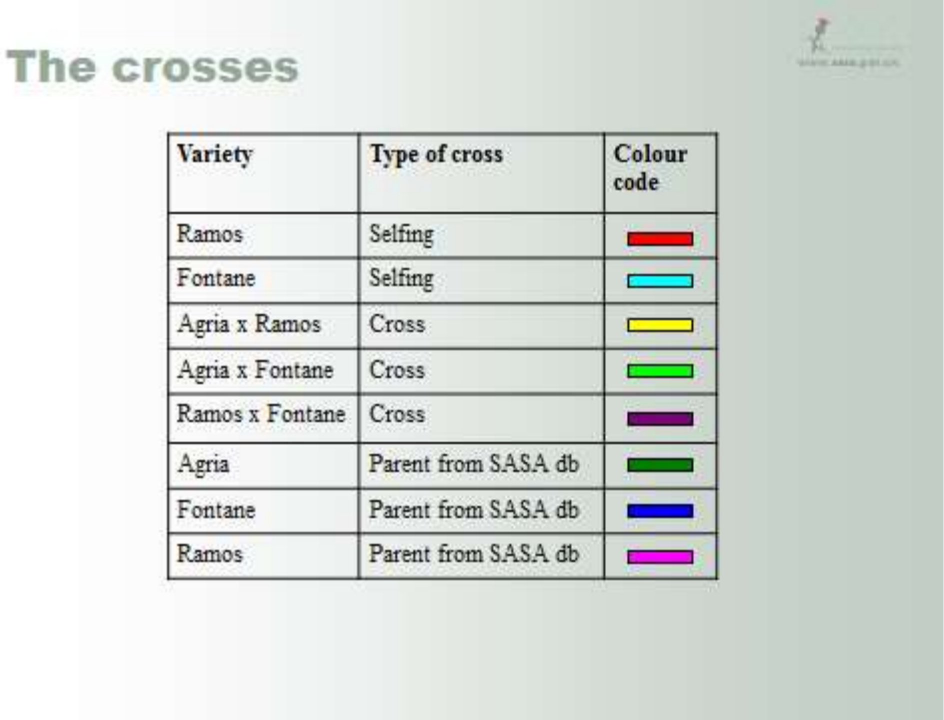
A small number of crosses gave unexpected results.

Several plants had more than 4 alleles for a marker. Either due to chromosome duplications or mixed samples.

A few yielded alleles not present in either parent. Either due to mutation or the seedling was the result of a cross with another variety.

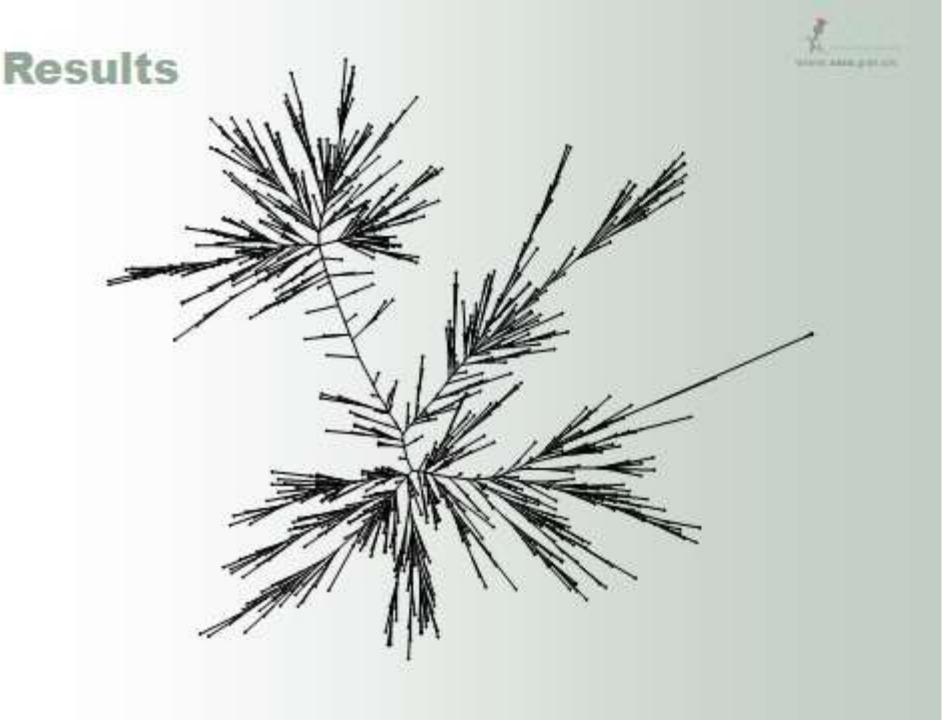
Two yielded alleles never seen before. As above.

N.B. other varieties and species were present in the glasshouse crosses were performed in.

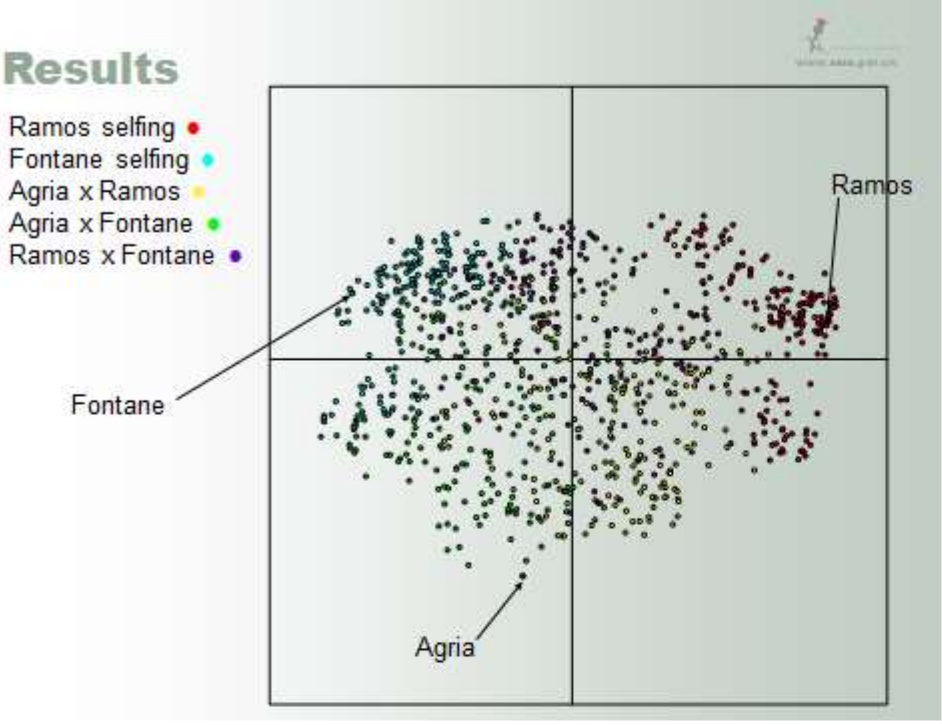


The crosses

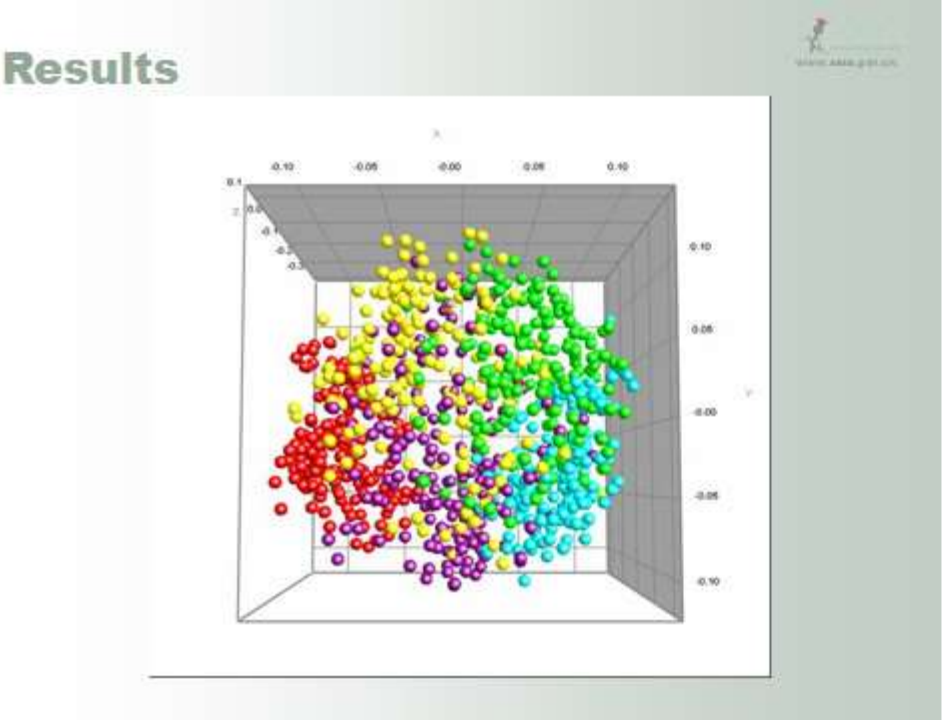
Variety	**Type of cross**	**Colour code**
Ramos	Selfing	🟥
Fontane	Selfing	🟦
Agria x Ramos	Cross	🟨
Agria x Fontane	Cross	🟩
Ramos x Fontane	Cross	🟪
Agria	Parent from SASA db	🟩
Fontane	Parent from SASA db	🟦
Ramos	Parent from SASA db	🟪



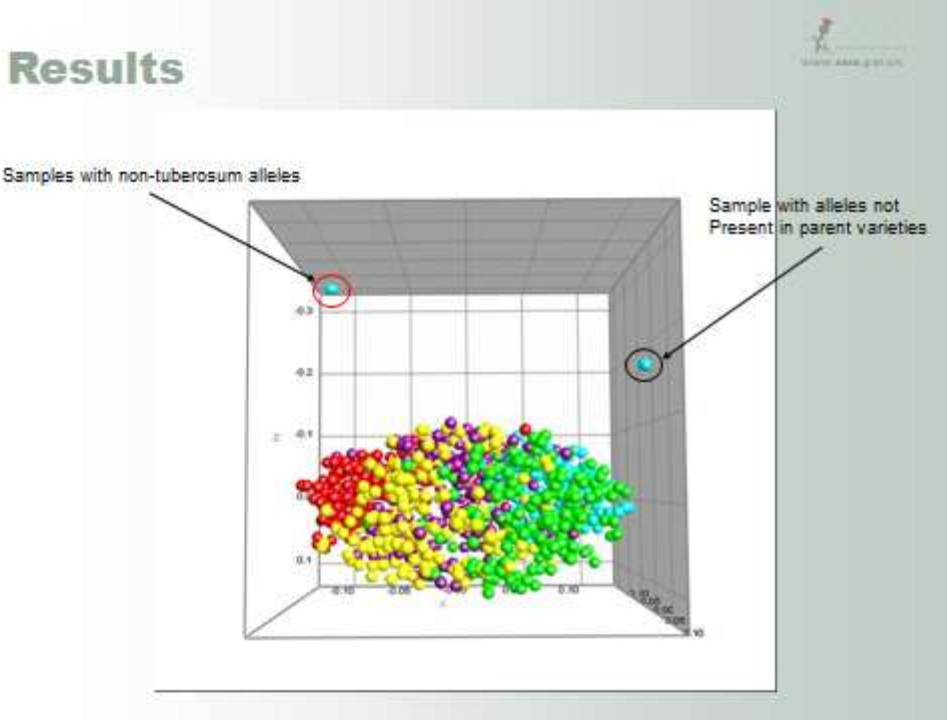
Results



Results



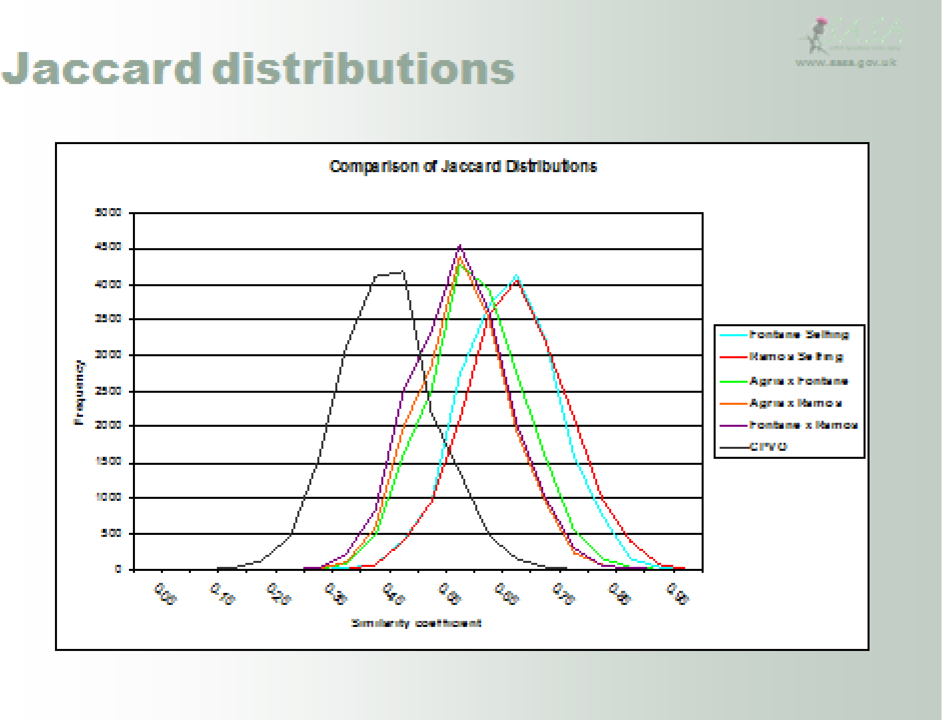
Results



Results

Samples with non-tuberosum alleles

Sample with alleles not Present in parent varieties



Jaccard distributions

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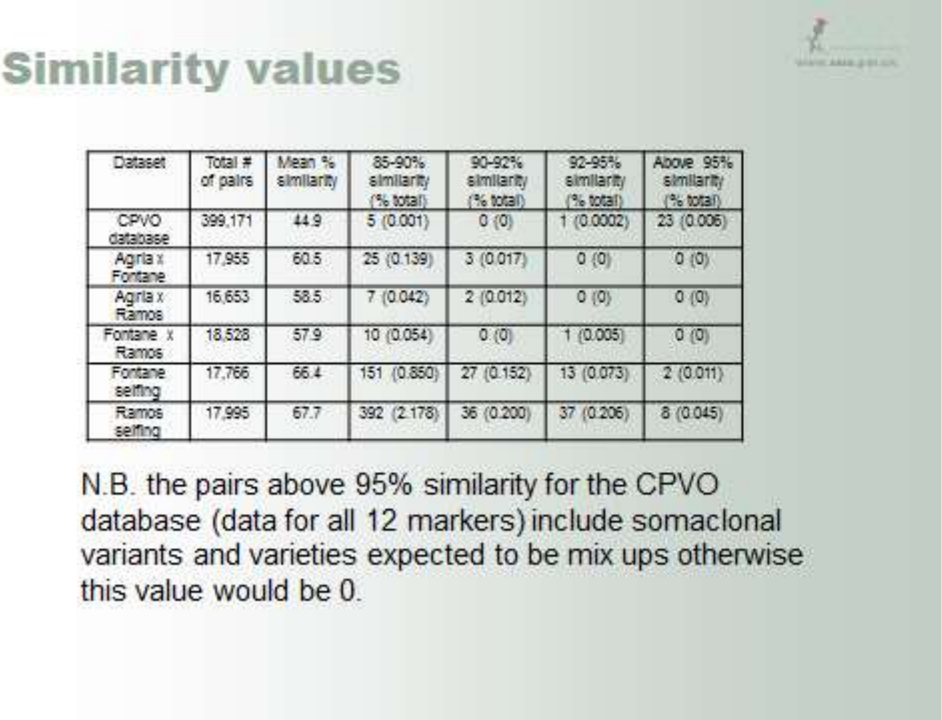

Similarity values

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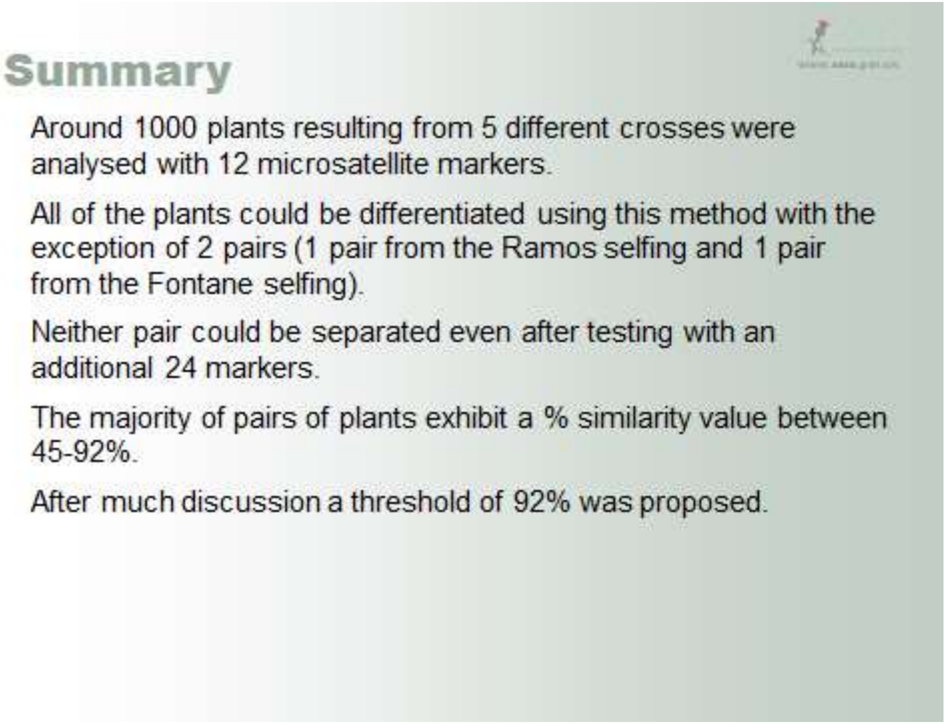
Dataset	Total # of pairs	Mean % similarity	85-90% similarity (% total)	90-92% similarity (% total)	92-95% similarity (% total)	Above 95% similarity (% total)
CPVO database	399,171	44.9	5 (0.001)	0 (0)	1 (0.0002)	23 (0.006)
Agria x Fontane	17,955	60.5	25 (0.139)	3 (0.017)	0 (0)	0 (0)
Agria x Ramos	16,653	58.5	7 (0.042)	2 (0.012)	0 (0)	0 (0)
Fontane x Ramos	18,528	57.9	10 (0.054)	0 (0)	1 (0.005)	0 (0)
Fontane selfing	17,766	66.4	151 (0.850)	27 (0.152)	13 (0.073)	2 (0.011)
Ramos selfing	17,995	67.7	392 (2.178)	36 (0.200)	37 (0.206)	8 (0.045)

N.B. the pairs above 95% similarity for the CPVO database (data for all 12 markers) include somaclonal variants and varieties expected to be mix ups otherwise this value would be 0.



Summary

Around 1000 plants resulting from 5 different crosses were analysed with 12 microsatellite markers.

All of the plants could be differentiated using this method with the exception of 2 pairs (1 pair from the Ramos selfing and 1 pair from the Fontane selfing).

Neither pair could be separated even after testing with an additional 24 markers.

The majority of pairs of plants exhibit a % similarity value between 45-92%.

After much discussion a threshold of 92% was proposed.

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