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CREATION OF AN SSR DATABASE FOR POTATO VARIETIES ON THE UK NATIONAL LIST

Document prepared by an expert from the United Kingdom

Introduction

The requirement for a rapid and reliable method for the identification of potato varieties is becoming increasingly more important. Recent articles (FSA, 2003, Davey, 2004) have shown that 'errors' can be made in variety identification especially when only part of the plant is available for morphological examination. The control of the recent ring rot outbreak in the UK also highlighted the necessity for a quick technique for variety identification. The evaluation of new varieties requires the maintenance of extensive living reference collections for comparative purposes. The UK National List currently comprises 122 varieties, a fraction of the 850 current listed in Europe, and, with the recent expansion of the EU this number has the potential to rise dramatically in the next few years. Owing to the increasingly large numbers of varieties it is clear that the maintenance of live reference collections for DUS testing is becoming impractical for both financial reasons and space constraints. Indeed 'paper' collections are becoming more common throughout Europe with comparisons made solely to morphological descriptions. Numerous molecular methods have been applied to the problem of fingerprinting potato varieties all with their own advantages and disadvantages. The use of SSRs (simple sequence repeats) is a logical choice as a large number of markers are readily available generated from genome mapping projects. However, it must be borne in

mind that there are limitations to the use of this technology in DUS testing, as only distinctness is realistically achievable at this moment. Prior to September 2002 SASA used a gel-based system, which was extremely labor intensive, difficult to interpret and allowed only a few samples to be run at one time. The purchase of a capillary system enabled higher throughput with greater accuracy, however it was necessary to screen a number of new markers better suited to this type of fragment separation. The following describes the process involved in choosing a core marker set and the establishment of a searchable database containing the 122 varieties on the UK National List.

Initial Screening

A total of 28 markers were screened from a database of over 200 obtained from the Scottish Crop Research Institute (Milbourne *et al*. 1998 and pers. comm.). This initial choice was made on the following criteria.

1. Products fell in the 100-500 base pair size range.
2. Microsatellites were generally not di-nucleotide repeats.
3. The primers had similar annealing temperatures.
4. Markers located on as many linkage groups as possible.

The primers for the 28 markers were then screened against 16 varieties and a further selection made.

1. Alleles were easy to score (i.e. good peak morphology and low stutter).
2. Markers displayed polymorphisms.
3. Alleles were stable over a range of annealing temperatures.

From the initial 28 markers tested 12 were chosen to screen the 122 varieties on the UK National List (Table 1).

Screening of the National List

DNA was extracted by a slightly modified protocol using GeneScan lysis buffer (Abgene). Where possible DNA was extracted in duplicate from tuber sprouts otherwise the extraction was from tuber material. Amplification was carried out using 10ng of DNA and Amplitaq Gold (Applied Biosystems) and the products separated on an ABI 3100. Alleles were scored as present or absent (1 or 0) and recorded in Excel. These binary data were imported into BioNumerics (Applied Maths) and each marker analyzed separately. Data from individual markers were then combined to find the best possible combination to differentiate the 122 varieties.

Results

With the exception of derived types such as King Edward and Red King Edward it was possible to differentiate all of the varieties with six markers STM1024, STM2022, STM2028, STM3012, S136 and S148 (Figure 1). Indeed it was possible to differentiate the National List with only four of these (STM2028, STM3012, S136 and S148) with the additional two markers included to give the system some ‘built in redundancy’. The data from the remaining

six markers also serve as a backup system should a situation arise where the core six markers cannot differentiate two varieties.

In addition to the accessions on the UK National List an additional 150 varieties have been added to the database, all of which can be differentiated using the six core markers.

Discussion

The primary reason for construction of the database was so it could act as an aid in the maintenance of the culture collection at SASA and it has proved to be of enormous use for this purpose. However, it has been put to additional uses, playing a crucial role in the recent ring rot outbreak in the UK and has been used for criminal investigations into breaches of plant breeder's rights in Scotland.

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References

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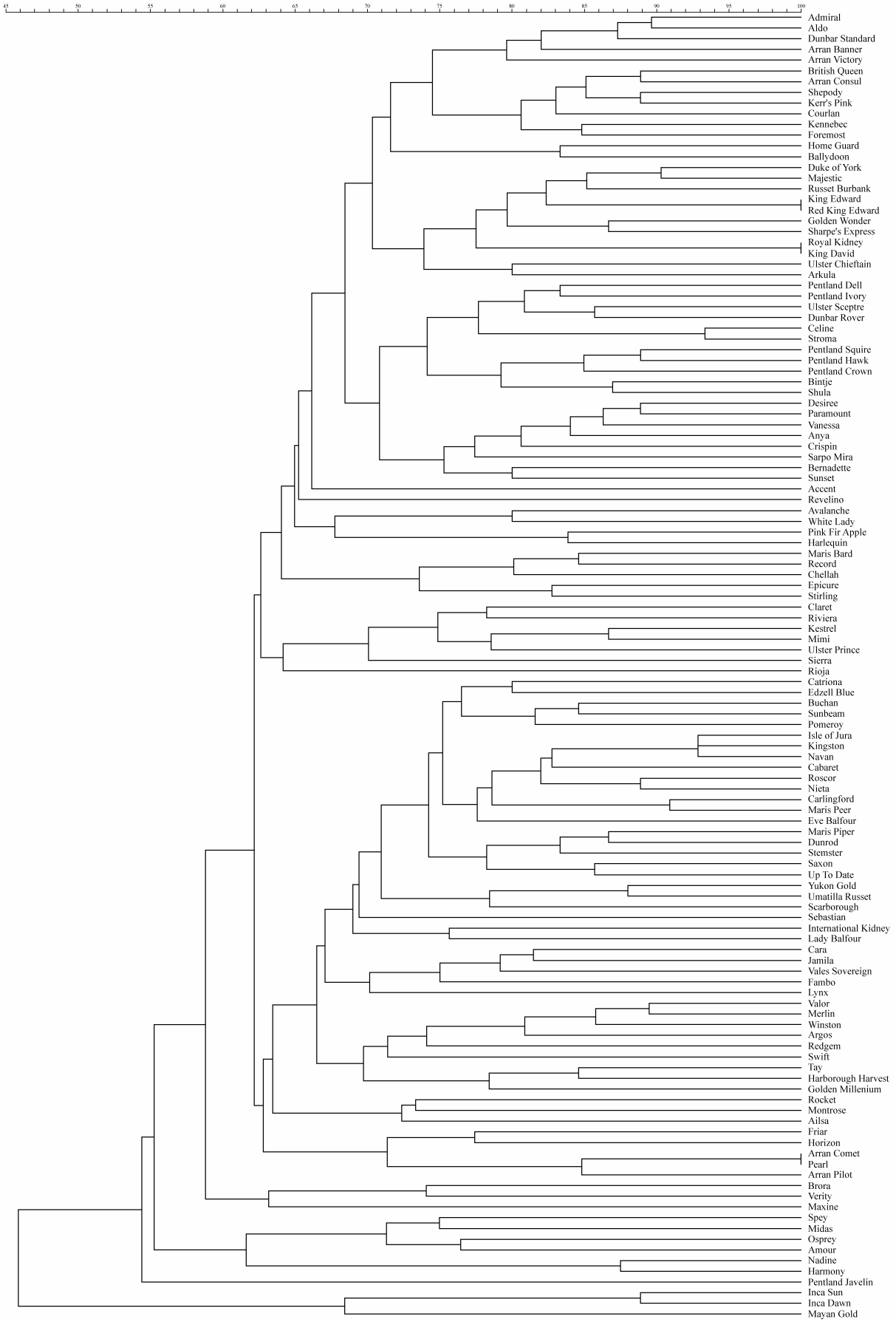
Table 1. SSR markers in use at SASA for potato variety identification.

Core markers	**Size range (bp)**	**Number alleles**	**Linkage group**	**Repeat**	**Allele PIC value**
STM1024	140-157	7	VIII	$(TTG)_6$	0.81
STM2022	169-236	7	II	$(CAA)_3..(CAA)_3$	0.64
STM2028	288-408	7	XII	$(TAC)_5..(TA)_3..(CAT)_3$	0.83
STM3012	166-211	7	IX	$(CT)_4..(CT)_8$	0.85
S136	219-256	9	I	$(AGA)_5$	0.90
S148	405-481	15	V	$(GAA)_{17}$	0.97
Other markers	**Size range (bp)**	**Number alleles**	**Linkage group**	**Repeat**	**Allele PIC value**
STM1104	168-186	9	VIII	$(TCT)_5$	0.84
STM1105	235-262	12	VIII	$(ACTC)_6$	0.90
STM1106	130-200	8	X	$(ATT)_{13}$	0.76
STM2005	152-195	6	XI	$(CTGTTG)_3$	0.84
STM3009	145-174	9	VII	$(TC)_{13}$	0.76
S127	243-277	7	I	$(TCT)_5$	0.87

Figure 1. UPGMA tree illustrating the differentiation of varieties on the UK National List.



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