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A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm

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Summary

Several DNA marker systems and associated techniques are available today for fingerprinting plant germplasm but information on their relative usefulness in particular crops is limited. The study investigated PCR based DNA fingerprinting in a set of 39 potato cultivars using RAPDs (20 primers), ISSRs (6 primers), AFLPs (2 primers) and SSRs (5 primer pairs). Results show that each of the four techniques can on their own, individually identify each cultivar, but that techniques differ in the mean number of profiles generated per primer (or primer pair) per cultivar, referred to as Genotype Index (GI). The order of merit based on this criterium and in this material was AFLPs (GI = 1.0), a multi-locus SSR (GI = 0.77), RAPDs (GI = 0.53), ISSRs (GI = 0.47) and single locus SSRs (GI = 0.36). Problems in relating banding patterns to individual loci and alleles for polyploid genomes, using these techniques as they are currently employed, are also discussed.

Introduction

DNA fingerprinting for cultivar or varietal identification has become an important tool for genetic identification in plant breeding and germplasm management (Jondle, 1992; Smith, 1998). When planning DNA fingerprinting, one of the most important decisions is the marker system and technique to be used. Various systems and their related techniques are currently available, and those based on the polymerase chain reaction (PCR) are most commonly used. PCR based techniques are generally quick and straightforward to perform, and the fact that PCR requires only small amounts of DNA, makes it especially useful when dealing with *in vitro* plantlets.

Several different PCR based techniques have been developed during the last decade, each with specific advantages and disadvantages. The Random Amplified Polymorphic DNA (RAPD) marker technique (Welsh & McClelland, 1990; Williams et al., 1990) is quick, easy and requires no prior sequence information. A single random 10-mer primer is used to specify the sequence that is to be amplified. Polymorphism is then observed and scored as the presence or absence of a fragment and relates to sequence variation due to nucleotide insertion, deletion or substitution. The homozygous presence of a fragment is not distinguishable from its heterozygote, and as such RAPDs are dominant markers. The technique has been used for identification purposes in many crops (Khandka et al., 1996; Iqbal & Rayburn, 1994; Golembiewski et al., 1997; He & Prakash, 1997), including potato (Demeke et al., 1996; Hosaka et al., 1994; Milbourne et al., 1997; Sosinski & Douches, 1996).

Another marker system developed recently is the Amplified Fragment Length Polymorphism (AFLP) technique (Vos et al., 1995). This makes use of restriction enzyme digestions followed by a method of selective amplification of fragments involving ligation of terminal adaptor sequences, and PCR primers

modified by adding two or three selective nucleotides. The basis of the observed polymorphism in terms of nucleotide variation may be mutation in restriction site sequences or in sequences complementary to the adaptor and selector nucleotides (Matthes et al., 1998). As in the case of RAPDs, AFLPs are dominant markers but technical refinements to distinguish homozygous and heterozygous genotypes have recently been mooted (Vos & Kuiper, 1998). The technique is more reliable than the RAPD technique (Vos et al., 1995; Jones et al., 1997), but also more laborious and time consuming (Milbourne et al., 1997; Powell et al., 1996). The technique has been widely applied in mapping (Lin et al., 1996; Mohan et al., 1997), DNA fingerprinting (Powell et al., 1996), analyses of genetic relationships (Milbourne et al., 1997) and genetic diversity (Russell et al., 1997).

A potentially powerful technique for DNA fingerprinting has followed on successful PCR amplification of tandemly repeated sequences, which have long been known to be polymorphic and widespread in plant genomes, referred to as Simple Sequence Repeat (SSR) or microsatellite polymorphism (Cregan, 1992; Morgante & Olivieri, 1993). The fragment polymorphism here relates to total sequence length as determined by the number of repeat units, and the heterozygote for different fragments in diploid genomes can generally be distinguished. Individual loci corresponding to specific primer pairs are therefore co-dominant and can be multi-allelic. The products generated have been found to be highly reproducible (Jones et al., 1997) and although these markers are usually species specific, costly to develop, and prior sequence information is a requirement, once the primers have been developed the system becomes relatively inexpensive. Microsatellite markers have been developed for various important crops, including maize (Taramino & Tingey, 1996), soybean (Akkaya et al., 1992; Powell et al., 1996), wheat (Devos et al., 1995), barley (Russell et al., 1997; Becker & Heun, 1995), potato (Provan et al., 1996a), and others.

Zietkiewics et al. (1994) and Kantety et al. (1995) described a marker system now referred to as Inter-Simple Sequence Repeat (ISSR) amplification. This makes use of anchored primers to amplify simple sequence repeats without the requirement for prior sequence information. According to Hantula et al. (1996), Charters et al. (1996) and Zietkiewics et al. (1994) the technique is more reliable than the RAPD technique and generates larger numbers of polymorphisms per primer. Theoretically, polymorphisms should be easier to detect because variable regions in the genome are targeted. The technique is quicker and more straightforward than AFLPs and does not require the high development cost of conventional SSRs. Although the ISSR technique also yields dominant markers, it has been reported that a longer 5′-anchor can yield markers which are codominant (Fisher et al., 1996). Provan et al. (1996b) were able to differentiate between twelve *S. tuberosum* cultivars using two 5′-anchored repeat primers.

It is clearly necessary to establish a common basis for assessing the effectiveness of the various marker systems currently available for DNA fingerprinting and several published reports on a variety of plant species have addressed this matter (Liu & Furnier, 1993; Powell et al., 1996; Vogel et al., 1996; Milbourne et al., 1997; Russell et al., 1997). The analysis commonly involves experimental fingerprinting of a representative sample of the source germplasm. Marker systems have invariably differed in the number of 'assay units' employed for fragment amplification, i.e., primers for RAPDs, primer pairs for SSRs, primer combinations for AFLPs and anchored primers for ISSRs. One is then interested in the experimental identification of the marker system that yields the maximum polymorphism for the particular germplasm sampled in terms of (i) the number of fragments amplified per assay unit, (ii) the frequency (%) of polymorphic fragments per assay unit and (iii) the number of unique profiles generated. Further refinement in establishing a common basis for comparative assessment is possible when an observed fragment polymorphism can be unambiguously interpreted in terms of alleles of a common locus, as might be verifiable through pedigree or segregation analysis. Estimates of allele frequencies can then be computed from the band data (Liu & Furnier, 1993; Powell et al., 1996; Russell et al., 1997), and the polymorphism expressed in terms of expected mean heterozygosity averaged over all loci, the standard measure of genetic diversity in population genetics (Marshal & Allard, 1970; Nei, 1973). Problems in relating observed fragment polymorphisms to alleles of specific loci for the various marker systems, particularly in polyploid germplasm, are looked at in depth in the discussion.

In this study we assess the value of the RAPD, SSR, AFLP and the potentially useful ISSR marker systems for their ability to distinguish polyploid potato cultivars, and we propose appropriate criteria for assessment and comparison based on fragment polymorphism results obtained.

Table 1. Potato cultivars used in this study and their country of origin (G = Germany, N = Netherlands, NI = Northern Ireland, SA = South Africa, SC = Scotland, USA = United States of America)

Cultivar	Cultivar	Cultivar	Cultivar
Adora (N)	Carlingford (NI)	Kimberley Choice (SA)	Rowane (SA)
Agria (G)	Charlie (SA)	Lady Rosetta (N)	Sandvelder (SA)
Alto (SA)	Crebella (N)	Late Harvest (SA)	Saturna (N)
Aviva (SA)	Darius (SA)	Mnandi (SA)	Sebago (USA)
Baku (G)	Dawn (SA)	Mondial (N)	Serenade (N)
Baroc (SA)	Devlin (SA)	Navan (NI)	Spunta (N)
Bravo (SA)	Erntestoltz (G)	Pimpernell (N)	Up-to-Date (SC)
Bright (N)	Felsina (N)	R100 (SA)	Van Gogh (N)
Calibra (SA)	Herta (N)	Ronn (SA)	Vanderplank (SA)
Caren (SA)	Hoëvelder (SA)	Rotharo (SA)	

Materials and methods

Plant material

In vitro potato (*Solanum tuberosum* L.) plants were supplied by the South African national cultivar collection of the ARC Roodeplaat potato gene bank. Leaf material from 39 cultivars commonly planted in South Africa (Table 1) were used for the DNA isolations.

DNA isolation

DNA was isolated from leaf material by a modified version of the method described by Edwards et al. (1991). Two leaf discs were collected in a microfuge tube using the lid as a punch. After adding Carborundum (400 grit) the discs were ground with a glass grinder and incubated at 60 °C for 30 minutes in 400 μl prewarmed Supaquick buffer (200 mM Tris-Cl [pH 7.5]; 250 mM NaCl; 25 mM EDTA; 0.5% SDS). An equal volume of chloroform:isoamyl alcohol was added and after mixing, it was centrifuged at 10 000 × g for 10 minutes at room temperature. 0.6 Volumes ice-cold isopropanol was added to the aqueous phase. The DNA was left to precipitate for 30 minutes at –20 °C, centrifuged (10 000 × g; 10 minutes; 4 °C) and then washed with 70% ethanol. Finally, the DNA was dissolved in TE (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) and stored at –20 °C until use. DNA concentrations were determined with a digital fluorometer (Sequoia-Turner, model 450). Parallel DNA extractions were performed on sixteen of the cultivars to test the reproducibility of the extractions.

RAPD analysis

A total of 21 primers were used in this study of which 20 (Table 2) gave scorable results. Primer kits OPA and OPH were acquired from Operon Technologies (Alameda, Calif., USA). The RAPD reactions were performed in a volume of 10 μl and contained 10 mM Tris-Cl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 0.1 mM of each dNTP, 1 μM primer, 0.75 U TaKaRa Taq (Takara Shuzo Co. Ltd., Otsu, Japan) and 10 ng template DNA. The reaction mixture was covered with 30 μl liquid paraffin to prevent evaporation. Amplifications were performed in an automated cycler (PTC-100, MJ Research, Watertown, MA) programmed for a 10 min. denaturation step before 35 cycles of 30 sec. at 95 °C, 45 sec. at 37 °C, 120 sec. at 72 °C as well as a final elongation cycle of 7 min. at 72 °C.

The RAPD fragments were separated according to size in a 2% MetaPhor agarose (FMC BioProducts, Maine, USA) gel run in 1 × TAE (40 mM Tris-acetate, 1 mM EDTA [pH 8.0]) buffer. A 1 kb DNA ladder (Gibco BRL) was also loaded on the gel as size standard. The gel was stained with ethidium bromide and visualised under UV light. The first 20 RAPD primers that yielded scorable results were used.

ISSR analysis

Six 5′-anchored primers (Table 2) described by Hantula et al. (1996) and Charters et al. (1996) were used. For the VHV-$(GT)_7$G primer, two additional (GT) repeats were added to the primer (VHV-$(GT)_5$G) described by Hantula et al. (1996). Some of these

Table 2. Primer sequences used in this study

Primer	Sequence (5′→3′)	Primer	Sequence (5′→3′)
RAPD		ISSR	
OPH-01	GGTCGGAGAA	ACA[a]	BDB-$(ACA)_5$
OPH-02	TCGGACGTGA	CCA[a]	DD-$(CCA)_5$
OPH-03	AGACGTCCAC	CGA[a]	DHB-$(CGA)_5$
OPH-04	GGAAGTCGCC	GT[a]	VHV-$(GT)_7$G
OPH-05	AGTCGTCCCC	AC[b]	DBD-$(AC)_7$
OPH-06	ACGCATCGCA	CAC[b]	BDB-$(CAC)_5$
OPH-07	CTGCATCGTG	SSR	
OPH-08	GAAACACCCC	STIIKA[c]	TTCGTTGCTTACCTACTA
OPH-09	TGTAGCTGGG		CCCAAGATTACCACATTC
OPH-10	CCTACGTCAG	STWIN12G[c]	TGTTGATTGTGGTGATAA
OPH-11	CTTCCGCAGT		TGTTGGACGTGACTTGTA
OPH-12	ACGCGCATGT	STGBSS[c]	AATCGGTGATAAATGTGAATGC
OPH-13	GACGCCACAC		ATGCTTGCCATGTGATGTGT
OPH-14	ACCAGGTTGG	STS 1[d]	TCTCTTGACACGTGTCACTGAAAC
OPH-15	AATGGCGCAG	STS 2[d]	TCACCGATTACAGTAGGCAAGAGA
OPH-16	TCTCAGCTGG	STS 3[d]	TTGCCATGTGATGTGTGGTCTAGAA
OPH-17	CACTCTCCTC	AFLP	
OPH-18	GAATCGGCCA	EcoRI 1	E-AAC
OPH-19	CTGACCAGCC	MseI 1	M-CAG
OPA-10	GTGATCGCAG	EcoRI 2	E-ACA
		MseI 2	M-CAC

where B = G, T or C; D = G, A or T; H = A, T or C; and V = G, A or C.
[a] Hantula et al. (1996).
[b] Charters et al. (1996).
[c] Provan et al. (1996a).
[d] Kawchuk et al. (1996).

primers were also used in combination. The ISSR reactions were performed in a volume of 12.5 μl and contained 10 mM Tris-Cl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 50 μM of each dNTP, 0.4 μM primer, 1.25 U TaKaRa Taq (Takara Shuzo Co. Ltd., Otsu, Japan), and 12 ng template DNA. The reaction mixture was covered with 30 μl liquid paraffin to prevent evaporation. Amplifications were performed in an automated cycler (PTC-100, MJ Research, Watertown, MA) programmed for a 10 min. denaturation step before 35 cycles of 30 sec. at 95 °C, 45 sec. at 60 °C (except for primers BDB-$(ACA)_5$ and DBD-$(AC)_7$, where an annealing temperature of 58 °C was used), 120 sec. at 72 °C as well as a final elongation cycle of 7 min. at 72 °C.

The amplification products were separated by electrophoresis in a 2% agarose MP (Boehringer Mannheim, Germany) gel in 1 × TAE (40 mM Tris-acetate, 1 mM EDTA [pH 8.0]) buffer. A 1 kb DNA ladder (Gibco BRL) was used as a molecular size marker. The gel was stained with ethidium bromide and visualised under UV light.

SSR analysis

Five SSR primers (Table 2) described by Provan et al. (1996a) and Kawchuk et al. (1996) were used in this study. The 12.5 μl reaction volume contained 20 ng of template DNA, 0.5 pmol of the labelled primer (γ^{32}P), 10 pmol of each of the unlabelled forward and reverse primer, 400 μM of each dNTP, 10 mM Tris-Cl [pH 8.3], 50 mM KCl, 0.001% (w/v) gelatin, 0.4 μM primer, 0.75 U TaKaRa Taq (Takara Shuzo Co. Ltd., Otsu, Japan), and 2.0–2.5 $MgCl_2$. PCR samples were overlaid with a drop of mineral oil and subjected to the following temperature profile, (PTC-100, MJ Research, Watertown, MA) 10 min. denaturation step before 35 cycles of 30 sec. at 95 °C, 45 sec. at annealing temperature, 120 sec. at 72 °C as well as a final elongation cycle of 7 min. at 72 °C.

Samples were mixed with gel-loading buffer, denatured and loaded on a 6% denaturing polyacrylamide gel using a vertical gel apparatus (Gibco BRL Life Technologies, Inc). Gels were electrophoresed for 2–3 hours (depending on the size of the amplified products) at a constant current of approximately 30 mA, dried and exposed to X-ray films (Cronex). Sequencing ladders of M13mp18 DNA templates were used to size the PCR products.

AFLP analysis

The Life Technologies AFLP^{TM} Analysis system I, AFLP Starter Primer Kit (Gibco BRL) was used in this study. Five hundred and fifty nanograms of genomic DNA were restriction digested with *EcoRI* and *MseI*, followed by the ligation of the *MseI* and *EcoRI* double-stranded (ds) adaptors.

The pre-amplification reaction volume of 50.3 μl contained 5 μl of the diluted ligation reaction, 40 μl of the pre-amp. primer mix, 1 $\times$ PCR buffer and 1.67 units of Taq DNA polymerase (Gibco, BRL). Temperature cycles for the pre-amplification and selective amplification was obtained from the Perkin-Elmer Applied Biosystems manual. Amplification was carried out on a Perkin-Elmer GeneAmp PCR system 2400. To determine whether the pre-amplification succeeded, products were electrophoresed on a 1% agarose gel (FMC BioProducts, Rockland, ME) in 0.5 $\times$ TBE buffer (0.45 M Tris, 45 M Boric Acid, 0.01 M EDTA) and visualised by ethidium bromide staining. The selective amplification reaction was carried out in a total volume of 20 μl containing 5 μl diluted pre-amplification mixture, 1 pmol of fluorescently labelled *EcoRI* primer (Perkin-Elmer), 4.5 pmol *MseI* primer (Table 2) and 10 μl of mix 1 (6.9 μl AFLP grade H_2O, 1 μl of 10 $\times$ PCR buffer, 1 μl 1% W1 and 0.5 units *Taq* DNA polymerase) (Gibco, BRL).

AFLP selective PCR reactions were denatured together with the ABI GENESCAN 500 ROX internal lane size standard for 2 min at 90 °C before being loaded on a 36 cm denaturing 5% Long Ranger gel (FMC BioProducts, Rockland, ME). The samples were electrophoresed for 3.5 hours at a limiting parameter of 3000 V on an ABI 377 automated DNA sequencer. Product size was determined with the ABI GENESCAN 2.1 using the Local Southern method. All of the PCR products were thus detected as a peak height at a particular size (bp), which was rounded to the nearest 1. The dye amplitude thresholds were set to 75 and all peak sizes, except the primer peak area, were subsequently selected for the size calling range. For data analysis only the peaks with sizes between 100 and 350 were selected which limited the maximum number of bands scored for the two primer combinations to 248 bands.

Band scoring and data analysis

Primers used for the different assays were chosen on the basis of availability alone and with no previous screening in this plant material. RAPD, ISSR and SSR reactions were repeated at least three times to test reproducibility. Due to cost considerations and the relatively large amount of DNA required, the AFLP analyses were only repeated on 30 of the 39 cultivars. Bands that were not found to be reproducible in all three reactions were counted and not used for the comparative analysis of the techniques. The percentage reproducibility was determined by dividing the number or reproducible bands by the total number of bands observed. The intensity of banding was not taken into account for reproducibility and for general scoring. Profiles for each cultivar and marker system were constructed by scoring 0 or 1 for absence of fragments respectively and the final data sets included both polymorphic and monomorphic fragments. This also applied to the AFLP fluorescence-based analysis where the scoring was for the presence or absence of a peak.

Profile data was then summarised by (i) the mean number of fragments per assay-unit (A_A), (ii) the mean number of fragments per cultivar (AC), (iii) the number of polymorphic fragments per assay-unit (P), (iv) the frequency of polymorphic fragments (%P), (v) the mean number of polymorphic fragments per assay-unit (P_{AV}) and (vi) the mean number of genetic profiles per assay-unit (G_{AV}). Assay unit (A) is defined as one reaction of a specific technique, e.g., one primer for RAPDs and one primer pair for SSRs. The mean number of profiles generated per assay-unit for a marker system is then expressed as a fraction of the number of entities typed (39 in this study) for comparative purposes and is referred to here as the genotype index (GI).

Results and discussion

Although the four profiling techniques are all based on DNA amplification by PCR, it was expected from the nature of the primers and reactions that the techniques

would differ in the specific sequences targeted and in the number of fragments amplified. Interest then revolves around the comparison of techniques with respect to the number of unique groups of cultivars identified in each case, and in the formulation of some kind of common index reflecting the degree or level of DNA polymorphism generated in each case.

Distinguishing between cultivars

All four marker systems could successfully distinguish the 39 potato cultivars. The minimum number of assay-units per system needed to distinguish between all cultivars, was two for RAPD and ISSR primers. Two SSR primers (STIIKA, STS 1+2) could identify all cultivars except Late Harvest and Ronn. These two could be distinguished using either STWIN12G or STGBSS. It was possible to distinguish between all 39 cultivars with use of either one of the two AFLP primer combinations. These results are similar to other observations in tetraploid potato (Demeke et al., 1996; Hosaka et al., 1994; Provan et al., 1996a, 1996b; Milbourne et al., 1997).

RAPD primer OPH-10 yielded 38 different genotypes and only the cultivars Late Harvest and Navan could not be distinguished from each other. One primer, OPH-06, yielded only monomorphic fragments. The ISSR primer DBD-$(AC)_7$ yielded 36 genotypes and was the most useful ISSR primer. When some of the ISSR primers were used in combination, the total number of fragments amplified did not increase. On the contrary, more polymorphic fragments were detected with primers DBD-$(CGA)_5$ and DBD-$(AC)_7$ separately than when these primers were used in combination with primer DD-$(CCA)_5$. This supports the idea that the number of fragments seen, when amplification is possible at a large number of sites, is probably a result of competition (Rafalski et al., 1991). The ISSR technique also showed a high percentage polymorphism (78.8%). This was not unexpected, because the technique amplifies microsatellite areas that are potentially highly polymorphic, and concurs with previous studies on fungi, oilseed rape and redcurrant cultivars (Hantula et al., 1996; Charters et al., 1996; Lanham & Brennan, 1998). However, the fact that RAPDs produced nearly the same percentage polymorphism (73.7%) suggests that this expectation is not necessarily valid. Here it should be stressed that the ISSR technique actually amplifies at least two microsatellite regions as well as the regions in between. It is also doubtful that the often small sized variations of the repeated regions will be scorable on agarose gels. It would therefore seem that the majority of the polymorphisms observed is probably caused in the same way as RAPD polymorphism, and is not always representative of the polymorphisms in repeat regions.

Microsatellite primers STS 1+2, STS 1+3, STGBSS and STWIN12G amplified single locus microsatellites, but STIIKA gave rise to complex fragment patterns. True to their tetraploid nature, each cultivar entry had any number from one to four different alleles for the single locus SSRs. With the use of STIIKA, eleven different alleles were amplified and each of the cultivar entries attained three to eight of these alleles. This SSR marker produced 30 different genotypes (24 cultivars had unique profiles and six genotypes represented the other 15 cultivars), while the most useful single-locus primer pair, STS 1+2, yielded 19 profiles. The two AFLP primer combinations produced a total of 244 polymorphic fragments or 122 polymorphic fragments per assay. This number is higher than that reported previously by Milbourne et al. (1997) and can probably be ascribed to the detection method used in this study.

The results generated by the SSR and AFLP techniques were found to be highly reproducible. The reproducibility of the SSR method was found to be 100%, while only one fragment was found not to be reproducible for AFLPs (% reproducibility = 99.6). In contrast, the reproducibility of RAPDs and ISSRs were 84.3% and 87% respectively. The results for the RAPD technique were in general agreement with other reports (Jones et al., 1997), but for the ISSRs the results were contrary to previous reports by Hantula et al. (1996), Charters et al. (1996) and Zietkiewics et al. (1994). We postulate that competition, which is the probable cause of low reproducibility of RAPDs (Halldén et al., 1996), may also be the cause of the low reproducibility of ISSRs. No variation was observed between different DNA isolations of the same cultivar for any of the techniques. Only reproducible fragments were selected for data analyses.

Technique evaluation

As mentioned in the introduction, different DNA profiling techniques can be compared using the principles of population genetics, and this has been the approach in several previous studies. The approach was initially adopted in the present study but was subsequently abandoned for reasons clarified briefly as follows.

Mean heterozygosity, $\overline{H}$, averaged over all loci analyzed, is commonly used as a measure of genetic diversity, as first proposed by Nei (1973). A more general definition of $\overline{H}$ is the probability that two randomly drawn alleles at a locus will be different. Within this conceptual context, $\overline{H}$ is used for comparisons of different populations within and across species, for populations under different mating systems, and genomes of different ploidy (Crow, 1986; Hartl, 1988). In general, practical conditions for computing expected heterozygosity for the comparison of various marker systems are (i) unambiguous identification of genotypes at each locus in terms of allelic fragments, and (ii) estimates of allele frequencies computed from the data for each locus separately.

In the case of recessive alleles in diploid genomes where the heterozygote and dominant homozygote are not distinguishable, as for example in RAPDs, the condition is unambiguous identification of the recessive homozygote at the locus. Then, provided Hardy-Weinberg frequencies prevail in the population sampled (implying random mating), the allele frequency is estimated by the square root of the frequency of the recessive homozygote. Liu & Furnier (1993) used this devise to assess RAPD markers in aspen forest trees, scoring the absence of a band as the recessive homozygote at a locus, and the presence of a band as the dominant homozygote or heterozygote. The standard statistical test for Hardy-Weinberg equilibrium on codominant isozyme and RFLP marker data on the same trees verified random mating and thereby justified the procedure. Thus, in the case of dominant markers, the generality of $\overline{H}$ as a measure of genetic diversity no longer holds unless random mating can be otherwise verified or safely assumed.

In the case of diploid inbred lines, the allele frequency is simply the frequency of the corresponding homozygote in the sample profiled, and dominance in the system is then no problem in the estimation of genetic diversity as defined above. This was essentially the approach followed by Powell et al. (1996) and Vogel et al. (1996) in a comparison of various techniques in inbred lines of diploid soybean, and by Russell et al. (1997) in inbred lines of diploid barley. In addition to expected genetic diversity, these authors base the comparison of alternative techniques on the total heterozygosity (ΣH) which they refer to as the Marker Index (*MI*).

What is problematic in profiling work of this nature is the occurrence of multiple fragments for a given assay-unit, generally interpreted as amplified fragments of separate loci differing in total sequence length and used as such in the computation of expected diversity. We feel inclined to doubt this generality, in awareness of the fact that fragment length polymorphism may arise from insertions, deletions and duplications in a nucleotide sequence and may therefore be allelic fragments of one and the same locus. It seems that nothing short of pedigree or segregation analysis, or total DNA sequencing of fragments isolated in an appropriate way, can resolve this issue. For similar reasons, fragments of the same length in different sample entities profiled may be wrongly construed to be identical alleles of the same locus while actually relating to different loci of the same sequence length but not necessarily of the same base pair content. In the case of the codominantt SSRs, fragment polymorphism directly relates to specific loci and alleles, with the exception of known multi-locus SSRs (for example STIIKA; Provan et al., 1996a).

Further limitations arise in measuring genetic diversity for assessing marker techniques in polyploid genomes, such as tetraploid potatoes used in this study. Even if one can identify a specific locus, as with single SSR primers, determining the exact allele dosages (monoallelic, biallelic, triallelic, etc.) of individual alleles remains problematic. In the light of the above possible difficulties in relating fragment patterns to specific loci and genotypes we have in this study therefore stopped short of attempting to estimate heterozygosity for single loci, accepting only that an individual profile for a given marker assay-unit relates to a unique complex polyploid genotype, and that observed profile polymorphisms might involve multiple loci and multiple alles. We have then followed the example of Liu & Furnier (1993) who also compared allozyme, RFLP and RAPD systems in terms of the number of fragments amplified per assay and germplasm entity, as well as the number, average number and frequency (%) of polymorphic fragments produced and the number of unique genotypes generated which we express as a fraction of the number of entities typed (GI), as presented in Table 3.

It is evident that AFLPs generated the largest number of fragments, as well as the largest number of polymorphic fragments. Only one multi-locus SSR was examined, and it was the only assay for which the frequency of polymorphic fragments (%P) was 100. Others varied from 98.4 (AFLPs) to 96.4 (single locus SSRs), and 78.8 and 73.7 for ISSRs and RAPDs respectively. When the number of different genotypes generated per assay was compared, AFLPs (GI =

Table 3. Summary of the data generated by this study: (i) the mean number of fragments per assay-unit (A_A), (ii) the mean number of fragments per cultivar (A_c), (iii) the number of polymorphic fragments per assay-unit (P), (iv) the frequency of polymorphic fragments (%P), (v) the mean number of polymorphic fragments per assay-unit (P_{AV}) and (vi) the mean number of genetic profiles per assay-unit (G_{AV}), and the genotype index (GI)

Profiling technique	No. of assays	No. of fragments	A_A	A_c	P	%P	P_{AV}	G_{AV}	GI
RAPD	20	209	10.45	7.43	154	73.7	7.7	20.75	0.53
ISSR	8	80	10	6.70	63	78.8	7.9	18.25	0.47
SSR (all)	5	39	7.8	2.93	38	97.4	7.6	17.2	0.44
Single-locus SSR	4	28	7	2.27	27	96.4	6.8	14	0.36
Multi-locus SSR	1	11	11	5.56	11	100	11	30	0.77
AFLP	2	248	124	37.22	244	98.4	122	39	1.0

1) had the best discriminatory power, followed by the multi-locus SSR (STIIKA), RAPDs, ISSRs and single-locus SSRs (Table 3). When the 5 SSR markers were considered as a group, the GI-value was similar to that of ISSRs. Using this method, the AFLP technique proved to be the most powerful technique for DNA fingerprinting of potato cultivars and the only one that could distinguish all 39 cultivars using either one of the two primer combinations. However, it is predictable that significantly higher GI values would be found for SSRs if it were possible to score allele dosage with greater certainty.

These results are in broad agreement with those of the three other studies on tetraploid potatoes, soybean and barley already referred to (Milbourne et al., 1997, Powell et al., 1996; Russell et al., 1997) where the comparison was in terms of the Marker Index (MI). In these studies it was found that the AFLP technique scored the highest MI, SSRs scored higher than RAPDs in soybean, but not in potato. This suggests that the choice of method may be dependent on the crop investigated. Vogel et al. (1996) also included the RFLP marker technique in this study and this scored the lowest MI of all. Results obtained by Russell et al. (1997) in barley based on the diversity index are similar in that the AFLPs scored highest, followed in order by SSRs, RAPDs and RFLPs.

Marker informativeness is an important element when comparing different assay systems, but other factors such as cost per assay, level of skills required, reliability and reproducibility of assays should also be considered (Karp & Edwards, 1997). Fluorescently-based detection methods are normally more expensive than other methods, but the added advantages that these systems offer, make them very attractive. We regard methods employing internal size standards for automatic size determination to be far more reliable than methods where numerous monomorphic and polymorphic fragments, discernible only by eye, have to be score manually over several gel lanes. The results of this study confirm that the problems experienced with reproducibility, plus the lower informativeness compared to the multi-locus SSR and AFLP systems, limits the use of RAPD and ISSR markers in DNA fingerprinting. However, they will remain useful where costs exclude the use of AFLPs and SSRs. With the current technology problems prevail in relating fragment polymorphism to specific loci and alleles, particularly in the case of dominant markers and polyploid germplasm. In continuing research on DNA fingerprinting, methods of unambiguously identifying all possible allele combinations of single amplified loci would seem to deserve high priority.

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