Short Communications

The use of Simple Sequence Repeats (SSRs) to identify commercially important potato (*Solanum tuberosum* L.) cultivars in South Africa

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Accepted 20 June 2000

The morphological traits that are traditionally used to identify potato cultivars are not always reliable, especially when dealing with *in vitro* plants. Various molecular marker techniques have been described for cultivar identification, but the Simple Sequence Repeat (SSR) technique has certain qualities that render it highly suitable for this application. In this study five SSR primer sets were used to characterize twenty-four commercially important potato cultivars. All profiles were found to be reliable and no variation was found between clones of the same cultivar. The number of polymorphisms detected with each of the markers varied, but none of the primers yielded only monomorphic fragments. The multilocus primer, STIIKA produced unique profiles for each of the 24 primer sets. However, the complicated nature of the profiles limits its use for routine diagnostic purposes. The use of the markers STS and STU 69633 in combination yields unique profiles for 20 cultivars and the fact that they can be analysed simultaneously on one gel, saves time and costs. The present study thus confirms that SSRs can be employed in a routine profiling system to provide highly discriminative and standardized profiles for South African potato cultivars.

Keywords: Cultivar identification, *Solanum tuberosum*, SSRs.

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Potatoes are an important vegetable crop in South Africa. In the 1997 season 163 million 10 kg pockets were produced in 14 production regions (Report on the South African Potato Industry, 1996/1997). Sixty five percent of these potatoes were used in the local commercial market (fresh and processed), while 6% were exported and 15% were put to use as seed potatoes. The rest were used in direct trade or on the farms.

The availability of different potato cultivars is important for optimum production since specific cultivars are adapted to specific environmental conditions and end uses (fresh vs. processed). Worldwide, thousands of potato cultivars have been released (Hosaka, Mori & Ogawa, 1994) and in South Africa more than 300 cultivars are available (Sanette Thiart, ARC Roodeplaat, personal communication). The identification of these cultivars is important for plant breeders, producers of table and seed potatoes, processors and consumers alike. This has traditionally been carried out with the aid of morphological characteristics such as tuber type, leaf type, flower color and sprout appearance. However, the identification of cultivars using these traits is limited because they can be influenced by environmental factors and are seldom all available simultaneously (Sosinski & Douches, 1996). The problems are compounded by the use of *in vitro* propagation because identification based on morphological traits is practically impossible during these procedures.

Since molecular technologies have become available, numerous groups have used various DNA profiling techniques for the identification of potato cultivars (Milbourne *et al*., 1997; Prevost & Wilkinson, 1999; Demeke, Kawchuk & Lynch, 1993; Kawchuk *et al*., 1996; Sosinski & Douches, 1996; Schneider & Douches 1997; Provan, Powell & Waugh, 1996). Weising *et al.* (1995) listed several desirable properties for techniques to be used for profiling, and although the various methods currently employed all have their specific advantages, there is not a single technique that meets all these criteria. However, their co-dominant behavior, high reproducibility, marked polymorphic content, and frequent occurrence in the genome probably makes Simple Sequence Repeat (SSR) (Cregan, 1992) markers currently the method of choice in many laboratories (Schneider & Douches, 1997; Morell *et al*., 1995; Jones *et al*., 1997). With this technique simple sequence length polymorphisms, comprising of tandem copies of 1–4 basepair units, are revealed by amplifying genomic DNA with two unique oligonucleotide primers that flank the specific locus.

The aim of this study was to use the SSR technique to fingerprint the commercially most important potato cultivars grown in South Africa. These cultivar fingerprints can be used by, and to the benefit of, all role-players in the potato industry to aid with the identification of cultivars.

The choice of cultivars was simplified by the fact that 15 cultivars are responsible for >99% of all the potatoes produced in South Africa (Report on the South African Potato Industry, 1996/1997). In conjunction with Potatoes South Africa, 24 potato cultivars were selected for this study (Table 1).

In vitro plants of each cultivar (3 to 5 different clones per cultivar) were obtained from the South African National Cultivar Collection of the ARC Roodeplaat potato gene bank. The DNA was isolated from leaf material as described by McGregor *et al.* (2000).

Five SSR markers were used in this study: STS (amplifying repeat $[TCAC]_n$, and associated with the starch synthase gene) described by Kawchuk *et al.* (1996), STIIKA, STWIN12G, and STGBSS described by Provan *et al.* (1996) and STU69633 (Warnich, unpublished).

Table 1 The commercially important potato cultivars selected in conjunction with Potatoes South Africa for this study

Cultivar	Cultivar	Cultivar	Cultivar
890/20	Buffelspoort	Hoëvelder	Ropedi
Agria	Calibra	Mnandi	Sandvelder
Alto	Caren	Mondial	Sebago
Astid	Crebella	Morene	Spunta
Aviva	Darius	Pimpernel	Up-to-Date
BP_1	Herta	Ronn	Vanderplank

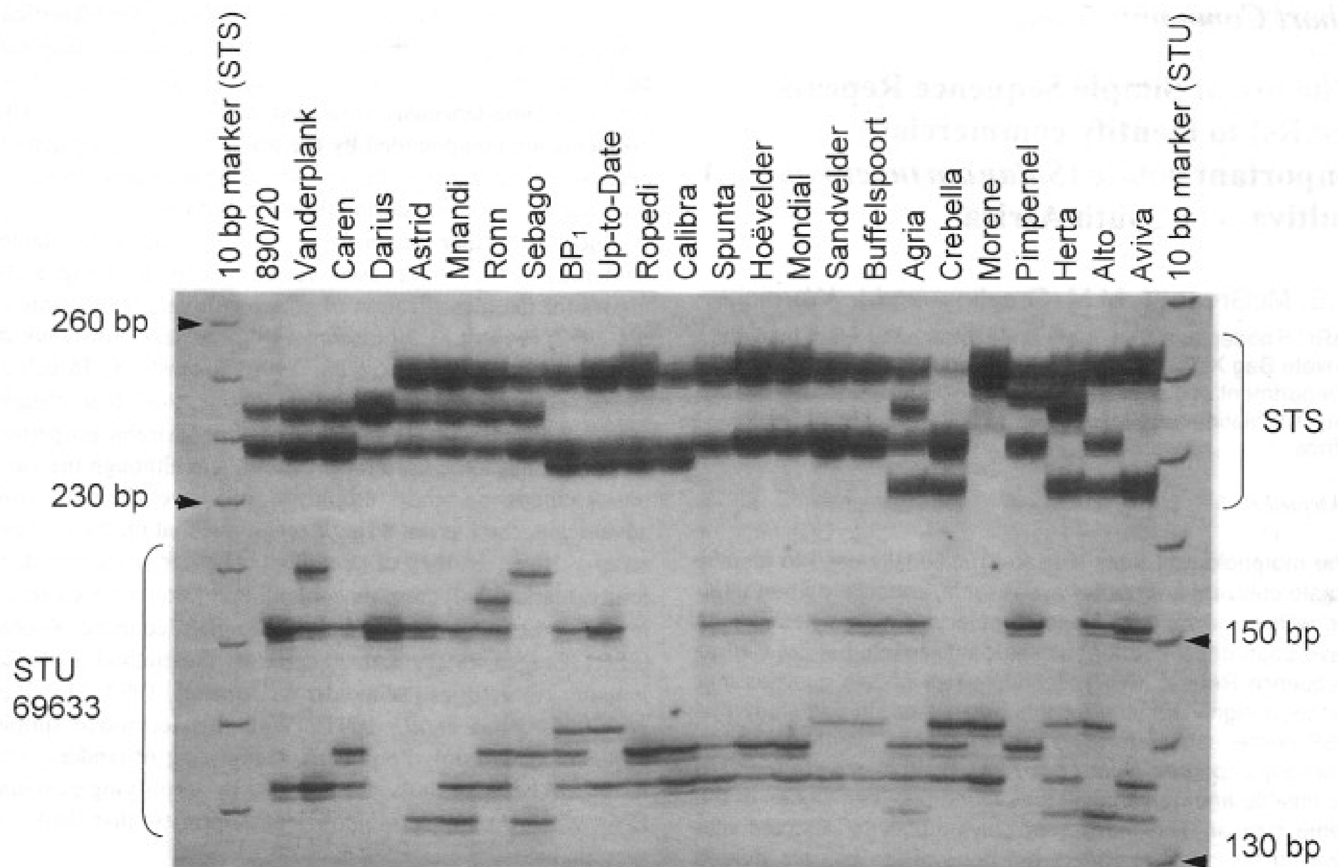

Figure 1 Primer pair STS and STU69633 run simultaneously on a 6% denaturing PAGE gel. The larger STS fragments (together with a 10bp marker) are loaded prior to the smaller fragments (with its' 10bp marker) produced by the STU69633 primers.

PCR amplifications were carried out in a total volume of 12.5 µl, containing 10 mM Tris-Cl [pH 8.3], 50 mM KCl, 3.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 40 µM of each dNTP, 0.24 µM of each 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 followed by 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. The annealing temperatures ranged from 50 to 55 °C depending on the sequence of the primers.

The PCR products were analysed on 6% (m/v) polyacrylamide sequencing gels for between 1.5 and 3 hours at 40 to 60 mA depending on the size of the fragments. The gels were silver stained according to the protocol recommended by the manufacturer (Promega Silver Staining Kit, Cat# Q 4132).

Over the past two decades cultivar identification in South Africa was based on morphological characteristics. Since 1995 the utilization of molecular markers, (RAPD analysis), has become increasingly popular. Although this method offered an improvement on the previously adapted methods, it also had some disadvantages associated with its use (Jones *et al.*, 1997). It was thus necessary to look into other identification methods.

SSR markers have been used to characterize potato germplasm/cultivars in a number of reports (Kawchuk *et al.*, 1996; Schneider & Douches, 1997; Provan *et al.*, 1996). In the present study the identification of 24 cultivars commonly grown in South Africa, using five SSR markers, is described. All the SSR markers used in this study, except STIIKA, were tri- or tetranucleotied type repeats. When compared to dinucleotide markers, these markers allow more accurate sizing of amplified alleles and are much less prone to forming stutter-bands, which can complicate analysis. Our data showed that the profiles are repeatable and the results obtained are very reliable. Furthermore, no variation was found between clones of the same cultivar with any of the markers.

None of the SSR markers used in this study were monomorphic for all 24 cultivars, but some markers were much more informative that others. Markers STGBSS and STWIN12G could only differentiate one and two cultivars respectively. Markers STS and STU69633 produced unique fingerprints for seven and 13 cultivars respectively, while the multilocus STIIKA could identify all 24 cultivars. Although STIIKA produced unique fingerprints for all cultivars, its use in a routine cultivar identification set-up is hampered because of the complicated nature of the profiles. Eighteen out of the twenty-four cultivars could be identified by using markers STS and STU69633 in combination (Figure 1). A further advantage of this option is that the cost of the silver staining procedure can be halved by loading the two markers on the same PAGE gel. The PCR products produced by marker STS are all between 230 and 260 bp in size, while the products produced by STU69633 are between 130 and 160 bp. We could obtain well defined profiles for both markers in a single lane by loading the STS reactions 50 minutes prior to the

STU69633 reactions, and running the gel until the xylene cyanol (associated with the STU69633 reactions) are approximately 3 cm from the bottom of the gel (± 2 hours at 60 mA, gel length = 38 cm). The cultivars that cannot be uniquely identified with these two markers are 'Ropedi', 'Calibra', 'Hoëvelder', 'Spunta', 'Sandvelder', and 'Buffelspoort'. Cultivars Ropedi, Calibra, Hoëvelder and Spunta can be identified with marker STG, but only STIIKA can be used to distinguish 'Sandvelder' from 'Buffelspoort'. Intensity differences caused by the effect of different allele dosages were not taken into account, since it complicates the procedure for routine use.

While the cultivars included in this study are the major contributors to the total potato production in South Africa, many more cultivars are currently planted. However, it should not be a problem to distinguish more cultivars, even if additional SSR markers have to be included in the assay. With numerous SSR primers now available for potatoes (Milbourne *et al.*, 1998) it should be feasible to include more primer sets that will also be compatible with a multiple-loading strategy on a single gel. Although our results imply that a relatively small number of SSRs might be needed to distinguish all cultivars, the use of more markers can provide the high level of confidence that is sometimes required for high standard DNA identification. SSR analysis can also be automated when fluorescent detection methods are employed, allowing for both high precision results and high throughput (Kijas, Fowler & Thomas, 1995).

The fingerprints generated in this study have already been used in several cultivar disputes with excellent results. The data thus confirm that (1) SSRs can provide highly discriminative and standardized profiles for South African potato cultivars and (2) the technology, as implemented in this study, is also robust and technically simple enough to employ for routine profiling purposes.

Acknowledgements

The authors would like to thank the ARC, PSA and the NRF for financial support.

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