Mondstuk van die Suid-Afrikaanse aartappelbedryf • Mouthpiece of the South African potato industry

CHIPS

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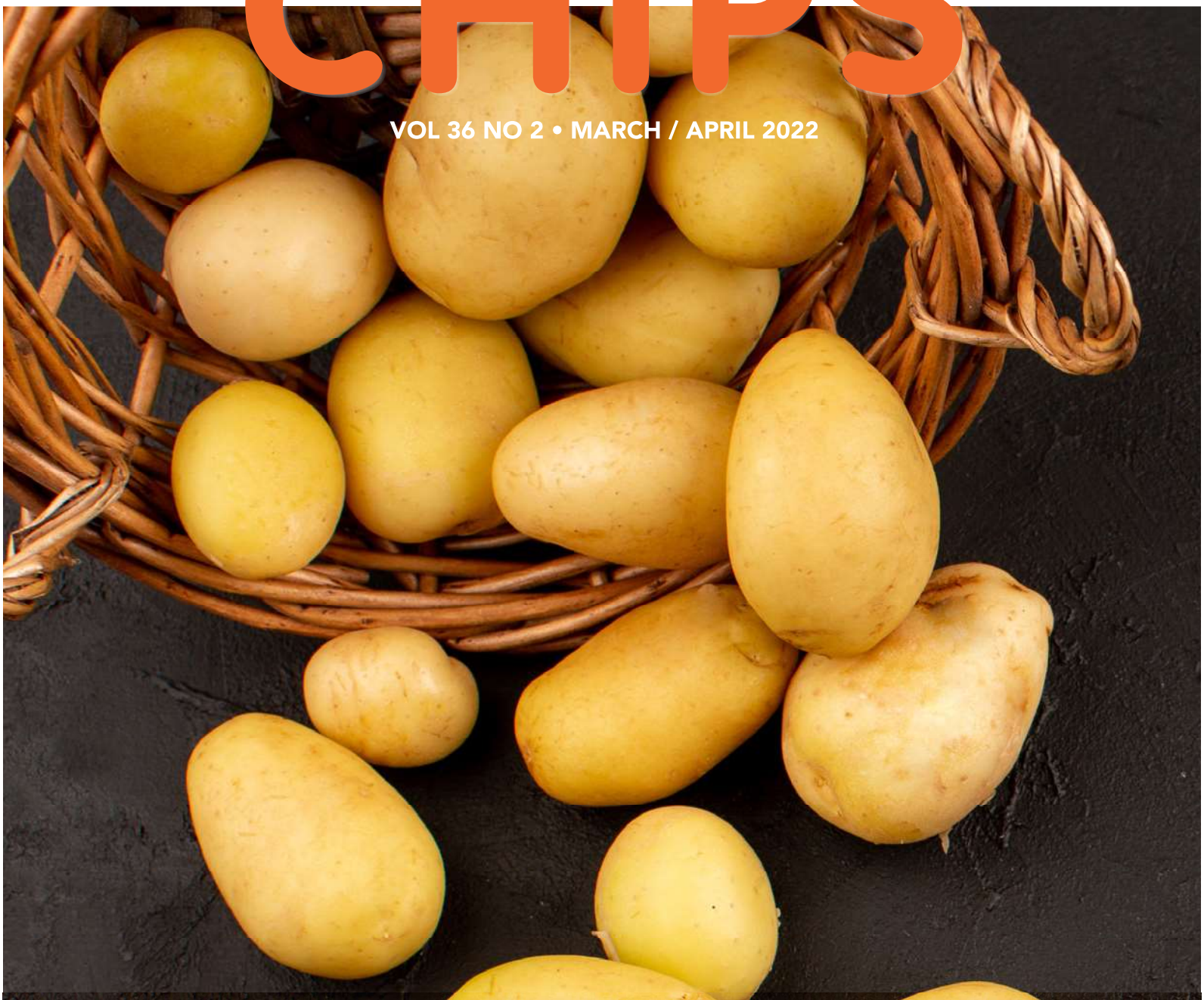

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New technology available for fingerprinting of potato cultivars in South Africa

By Dr Inge Gazendam, researcher: Plant Breeding, ARC-VIMP

Trueness-to-type information of cultivars is essential for seed potato growers to provide true varieties to the potato industry. DNA fingerprinting is a molecular technique applied in the identification of genetic differences between cultivars or lines (clonal identity) and used for genetic purity testing (trueness-to-type).

The advantage of potato DNA fingerprinting is that it can be done at very early developmental stages, such as on mini-tuber or in vitro leaf material, and it is less resource intensive than morphological methods. Accidental mix-ups can therefore be identified at an early stage, before in vitro multiplication, to prevent costly mistakes later on. Cultivar genetic identity is important in the protection of plant breeders' rights.

The in vitro laboratory of the ARC-VIMP (Vegetable, Industrial and Medicinal Plants) is also dependent on DNA fingerprinting, and the laboratory cannot release material to the industry unless trueness-to-type is confirmed.

SSR fingerprinting method

The DNA fingerprinting method previously applied at the ARC-VIMP is based on the polymerase chain reaction (PCR) of simple sequence repeat (SSR) markers, and the resulting mixtures of DNA fragments are separated according to their size by denaturing polyacrylamide gel electrophoresis (PAGE) on a large gel system.

The fingerprint fragments are then stained and scored for their presence and size (*Figure 1*). Differences are observed as the presence or absence of a particular fragment. The results are also visually compared with a known cultivar fingerprint as reference.

The SSR fingerprinting method has low throughput, is labour intensive, incurs a high cost per data point, and scoring is highly subjective due to the indirect method of determining fragment sizes.

SNP genotyping technology

Recently, single nucleotide polymorphism (SNP) became the marker of choice for applications in plant breeding and genetics as they are abundant, stable, amenable to automation, efficient, and increasingly cost-effective.

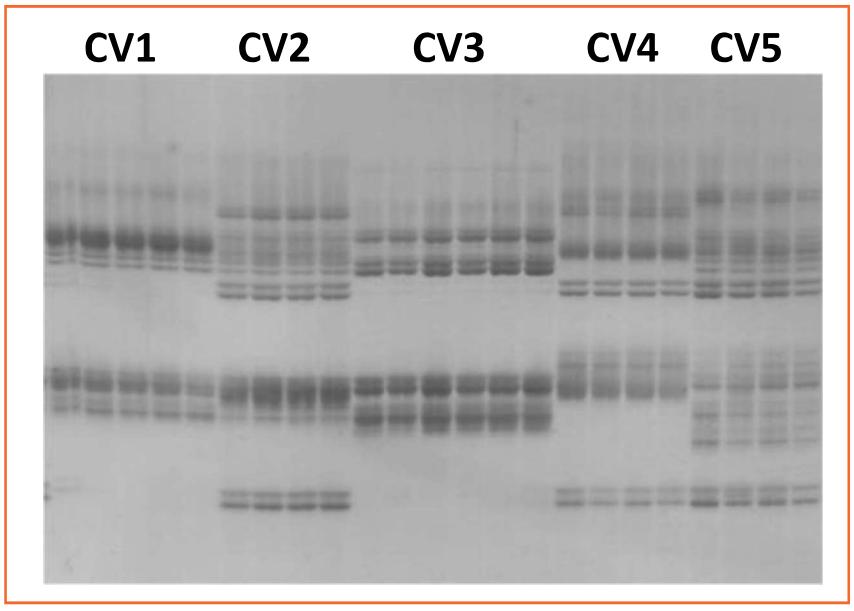
A new protocol was therefore developed for potato fingerprinting, using SNP genotyping. An SNP is the variation in a single nucleotide that occurs at a specific position in the genome of any organism. SNPs are highly abundant in plants and are spread out evenly over the genome. In the potato genome, large numbers of SNPs have been identified, and one SNP is found on average in every 20 base pairs (bp).

Due to advances in SNP genotyping technology, it has become easier and more affordable to use SNP assays than ever before. Panels of SNP markers are therefore employed instead of SSRs for varietal identification at the institute.

An SNP panel for local cultivars

An SNP panel was developed and optimised to fingerprint potato cultivars locally, using highly efficient competitive allele-specific PCR (KASP) assays. The purpose of

Figure 1: DNA fragments generated by PCR of SSR sequences, separated on a large PAGE gel and stained with silver staining.



** The SSR fingerprints of five cultivars, indicated by CV 1 to 5, are presented for the same SSR marker.*

this development was to replace the outdated SSR markers currently being used. The aim is to better serve the South African potato industry through the deployment of an efficient, reliable and cost-effective genetic fingerprinting method for clonal identification and trueness-to-type determination of potato cultivars.

All commercial potato cultivars have four copies of each chromosome. A specific potato cultivar can therefore have one of five SNP genotypes, indicated by the number of times one of the SNP alleles occurs, also referred to as the dosage number. For example, if 'A' and 'B' are the different alleles of a given SNP and 'A' is the reference allele, then the allele dosage classes are 0 (BBBB), 1 (ABBB), 2 (AABB), 3 (AAAB) or 4 (AAAA), as shown in *Figure 2*.

Dissimilarity between potato varieties can be caused by one dosage difference, suggesting that allele dosage is useful for variety identification. SNP markers are able to estimate allele dosage, therefore the nucleotide genotype as well as the copy number can be determined in a polyploid genome. In contrast, it is nearly impossible to determine the copy number of a fragment produced using an SSR marker.

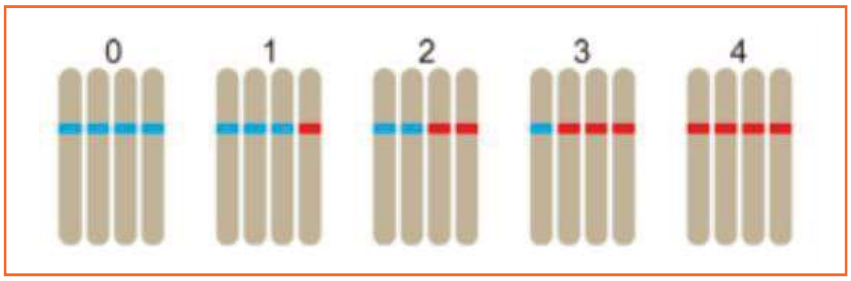
Method development

Initially, during method development, a collection of 190 potato cultivars and breeding lines were genotyped at 500 SNP sites using SeqSNP from LGC Genomics. All major potato varieties planted during the 2018/19 growing season were included in this study. An optimal panel of 25 informative SNP markers that can discriminate the 173 unique potato cultivars on a genetic allele dosage basis were identified.

SNPs were carefully selected to have a balanced allele frequency, resulting in a high polymorphism information content. The SNPs in this small panel were then validated with KASP assays in the laboratory.

Results were plotted to see how the genotypes cluster into the five dosage classes (*Figure 3*). Only two of the 25 SNPs failed to cluster into the five dosage classes (results not shown). The final selected and successfully validated panel of 21 SNPs was able to distinguish among all 173 unique potato cultivars used during method development, including those currently important in South Africa.

Figure 2: Dosage of alleles in tetraploid potato. (Adapted from Bourke *et al.*, 2018)

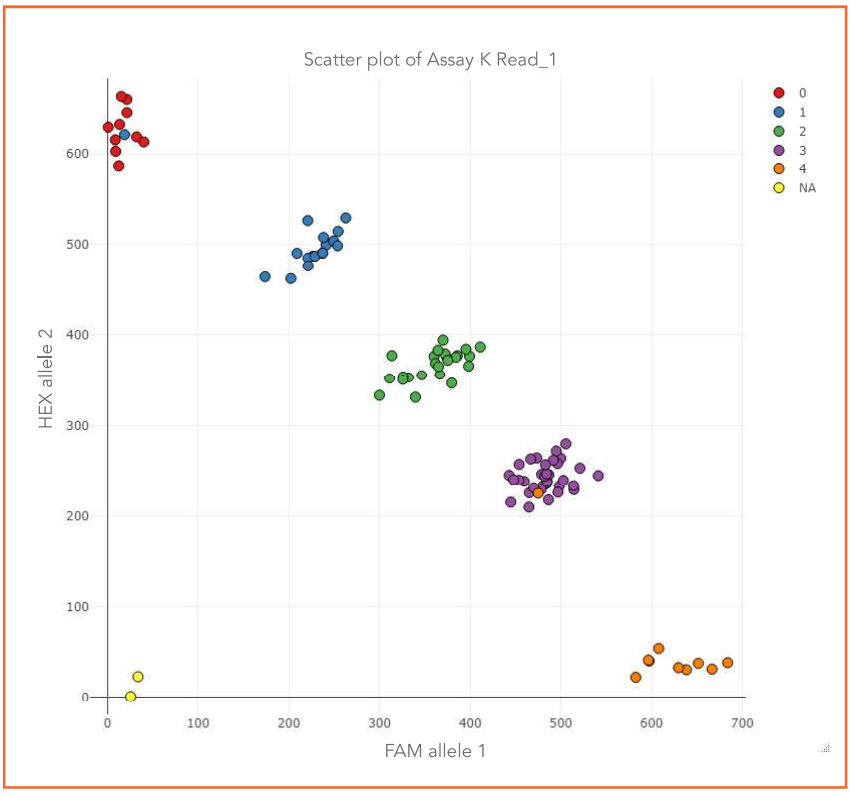


** In a tetraploid species, five distinct allele dosage classes are possible at a bi-allelic marker position, ranging from 0 to 4 copies of the reference allele. Here, the reference allele is coloured red, with the alternative allele coloured blue.*

Output and application

One of the most important outputs of the project has been setting up an SNP genotype database.

Figure 3: Scatter plot constructed with KASP data points in the colour of the expected SeqSNP allele dosage.



** An example of a successful KASP SNP assay using marker 'K', which was able to cluster potato genotypes into the five gene dosage classes (colours according to the expected dosage). The yellow samples at the origin are no-template or water controls.*

The subjective and sometimes inaccurate sizing of the SSR allele database makes comparisons of fragments from different potato profiles difficult. On the other hand, SNP genotypes simplify the germplasm genotype database, enabling automatic comparisons to determine the suggested identity of an unknown cultivar.

The SNP panel has been validated and is ready for application to potato samples submitted for fingerprinting or purity requests. All private farmer customers and public or private laboratories will have access to the service.

Purity tests

Purity tests can be performed objectively and with higher throughput using KASP SNP assays to resolve identity or mixing issues of potato production stakeholders. If two cultivars are suspected to be mixed, a minimum number of appropriate SNPs can be applied to confidently discriminate between them.

A flexible selection of the most appropriate SNPs can also be made when a list of cultivars need to be distinguished. This minimises the analysis costs. When three SNP markers are applied for scientific robustness, the price break-even point of SNP fingerprinting compared to the current SSR prices is at four samples, and almost half of the current price for fingerprinting twelve samples.

Identification of samples

The probable identity of an unknown sample can be determined by comparing the SNP genotypes of the individual to the germplasm SNP genotype database. Possible hits with the lowest genetic differences will be identified and reported to the client.

Genotyping new cultivars

If a new cultivar needs to be added to the SNP genotype database, it needs to be genotyped with all the 21 successful KASP SNP assays. There is a slight possibility that the KASP SNP panel cannot discriminate new cultivars from the ones used during method development. However, this panel of SNPs were chosen for their high polymorphism information content, and they should therefore be useful to discriminate between wider sets of potato germplasm.

Previously, five SSR markers were traditionally used to genotype a new cultivar to be added to the database. In reality we cannot size the SSR alleles anymore due to the discontinuation of the sizing ladder. For the full SNP panel, the price break-even point compared to the current SSR prices is at twelve samples. So rather than submitting one or two samples at a time, it is advisable to submit twelve or more samples at a time to expand the database.

Mixed samples

Mixes of plant material cannot be tested, since DNA fingerprinting tools are designed to treat one sample as one individual, and to assure the identity of an individual and not a group of individuals. Each individual has a unique genetic signature and has to be assayed separately. Any fingerprinting technique will simply try to find a match to the complex mix of genotypes.

One could find a match to the database, but you wouldn't be able to say if it was from one or more cultivars. Therefore, pooling of samples cannot be used as a cost saving approach. It is recommended to randomly select a set of individual plants to identify the identity of a cultivar within a varietal mix.

Conclusion

This new method enables the ARC-VIMP to continue to serve the South African potato industry by providing a more effective and accurate genetic fingerprinting service for the purpose of clonal identification and trueness-to-type determination of potato cultivars.

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