Application Note

Potato variety testing and certification – improved method of seed potato testing in France using SSR Markers and QIAxcel® capillary gel electrophoresis system

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Introduction

The potato is a versatile, nutritious staple food that has cultural significance in many societies. It thrives in diverse conditions, supporting food security and local economies worldwide (1).

Good management and certification of seed potato varieties is crucial to preserve the quality of potato production areas and guarantee a maximum yield to the producers (2).

With over 21,000 hectares of seed potato production area, 700,000 tons of seed potato produced in 2023 and 400 varieties reproduced each year, France ranks 2nd worldwide for seed potato production (3).

Varietal identification is one of the requirements in the certification process, which also guarantees sanitary quality of seed potatoes. In France, varietal and sanitary inspections and controls are carried out by 65 inspectors and 3 approved and accredited specialized laboratories coordinated by the FN3PT throughout the production process (4) under the umbrella of SEMAE/DQCO, who is the competent authority (5). These laboratories include Bretagne Plants, Comité Nord and Comité Centre et Sud. Two other laboratories are members of the network: one is in charge of the Biological Resource Center BrACySol (lab of INRAE/UMR Igepp) and the other one is in charge of the official tests on ware potatoes (Service Commun des Laboratoires) (6).

Implemented in the early 2000s, this laboratory network has established a microsatellite (SSR) markers-based fingerprinting method and built the IdeAle reference internal database (7). These genetic fingerprints are used, together with field inspection, to check the varietal conformity of plants during the vegetative propagation stages and to characterize new varieties. Moreover, fingerprints are used to describe new hybrids to protect breeders' rights to test consumer batches and to manage genetic resources. The IdeAle database now holds more than 5,000 profiles. Nine ring tests have been organized since 2003 to check the ability of the partners on the method, the power of the kit of markers and the reliability of the database (8).

This paper presents an adaptation to the official method carried out during the IdEvol research project, which was initiated in 2019 and concluded in 2023. This project aimed to assess the suitability of the QIAxcel capillary ▷

gel electrophoresis system to replace the polyacrylamide gel-based method for testing the SSR markers used in the official procedure.

The project's objectives were to simplify and reduce manual tasks, decrease overall analysis time, minimize exposure to hazardous substances for laboratory personnel and reduce waste treatment costs. An additional objective was to identify new SSR markers suitable to the workflow with the QIAxcel system (9). One of the main objectives was to examine if the QIAxcel DNA High Resolution Kit (10), with its resolution of 3–5 bp, was as resolutive as polyacrylamide gels to separate the marker alleles. This would guarantee continued use of the database.

Materials and methods

Input material / sample description

As a first step, inov3PT tested QIAxcel system run conditions in one laboratory. Over 24 potato varieties were used to identify the best analysis method to start with. Thereafter, a panel of 12 varieties was used in three additional laboratories to test run conditions on the QIAxcel system and several PCR conditions, such as concentration of the primers and quantity of DNA to be amplified. Each of the six partners participating in the project further genotyped a specific batch of up to a maximum of 75 varieties. A panel of 8 to12 varieties was systematically used as controls during the experiments in all laboratories.

The DNA of the varieties was obtained from tubers using a magnetic bead-based extraction kit. The protocol was performed either in manual or automated conditions, as recommended by the supplier. DNA concentrations were determined, and samples were diluted to 5–10 ng/µL. During the project, the RNase step was shown to negatively impact DNA yield on potato samples, so this step was skipped during subsequent experiments.

PCR / SSR assay

The initial procedure included eight SSR markers described in Table 1.

Table 1. Characteristics of the SSR markers used in the official procedure

Markers	SSR repeat	Chromosomal location	Range of molecular weight (bp)
SSR1 (11)	(TCAC)n	8	200–230
STM2005 (12)	(CTGTTG)n	11	150–200
LEMALX (12)	(ATT)n	5	120–140
STM1097 (12)	(CGTTT)n	7*	230–280
STM2020 (12)	(TAA)n	1	160–200
STGBSS (13)	(TCT)n	8	120–160
STM5136 (14)	(AGA)5	1	210–250
STM5140 (15)	(AAT)n	4	180–219

* Updated from MOISAN-THIÉRY *et al.*, 2005 (7).

All of the markers, except STM2020 and STGBSS, could finally be used on the QIAxcel system. Three additional markers were identified during the project that can be used on the QIAxcel system instead: STI028 (16), STM2028 and STM5148 (14).

PCR conditions were adapted to standardize signal intensities (RFU) across SSR markers and to limit primer–dimer formation, which improved the automated assignment of the alignment marker in the QIAxcel ScreenGel® Software and facilitated SSR marker sizing. Initial conditions include the following: 4 µL of a DNA suspension (10 to 20 ng/µL) were amplified using 0.03 units of GoTaq® G2 Hot Start Taq Polymerase (Promega Corporation) for all the markers, except for LEMALX, wherein 0.03 units of Taq DNA Polymerase (MP Biomedicals) were used. The concentrations of the primers were 0.6 µM and the concentration of dNTPs was 0.15 mM with a final PCR volume of 17 µL. At the end of the optimization step, the final concentration of the primers ranged from 0.15 to 0.6 µM, depending on the markers.

PCR conditions were as follows: 95°C, 5 minutes, 30 cycles of (95°C, 1 minute; 54°C, 45 seconds; 72°C, 1 minute), 72°C, 10 minutes.

Capillary Gel Electrophoresis System

QIAxcel system and run parameters used in the study are described in Table 2 below.

Table 2. QIAxcel system and run parameters

Instrument	**QIAxcel Advanced***
Supplier	**QIAGEN**
Software	**ScreenGel software version 1.6**
Run parameters	
Kit used	QIAxcel DNA High Resolution Kit
Method (customized)	OM1300 with additional separation time per run (at least 40 seconds): • Alignment marker injection at 4 kV for 10 sec • Sample injection at 5 kV for 10 sec • Separation at 2 kV for 1300 sec
Alignment Marker	15–600 bp
Size Marker	25–500 bp

* Superseded by the QIAxcel Connect system.

Analysis

Analysis of results was done with the QIAxcel ScreenGel Software Version 1.6. Correct assignments of the lower and upper alignment marker peaks were visually confirmed in the electropherogram profile of each sample, as primer–dimer peaks could falsely be assigned to the 15 bp alignment marker peak. Electropherogram profiles of the control varieties were examined to verify amplification of all expected alleles. The threshold was increased to get rid of the background noise (when observed). Lastly, each electropherogram profile was examined, and the "superimpose view" function of the ScreenGel Software was used to overlay the peak pattern with the peak pattern of some of the control varieties to manually assign the alleles.

Results

The official procedure includes eight markers (Table 1). All of them were tested on the QIAxcel system in four laboratories. Analysis of the control varieties common to all labs showed the overall reproducibility of the markers analyzed on the QIAxcel system. Differences in signal intensities were observed between laboratories, which likely comes from variations in PCR efficiency. Still, despite these differences, varietal analysis was not affected (Figure 1).

Analysis of the 326 varieties spread between the partners showed all but two markers (STM2020 and STGBSS) could be efficiently scored on the QIAxcel system. These two markers showed alleles that could not be resolved under the new conditions, mainly because they are not easily distinguishable on the electropherograms.

For the six remaining markers that can be detected on the QIAxcel system, concordance between the manual scoring of the profiles on the QIAxcel system and the reference profiles stored in the IdeAle database was excellent, with concordance rates from 94% to 97%. An example is given in Figure 2 for marker STM1097. Discordant profiles were found at low frequencies and were mostly linked to misidentification of the samples, as the profiles obtained with the other markers were also completely different from the reference ones. Unclear profiles during the initial steps of the project were subsequently mostly solved with additional experience when examining the profiles on the QIAxcel system. Specifically, for marker STM1097, one of the alleles was not easily scored at the beginning (Figure 3).

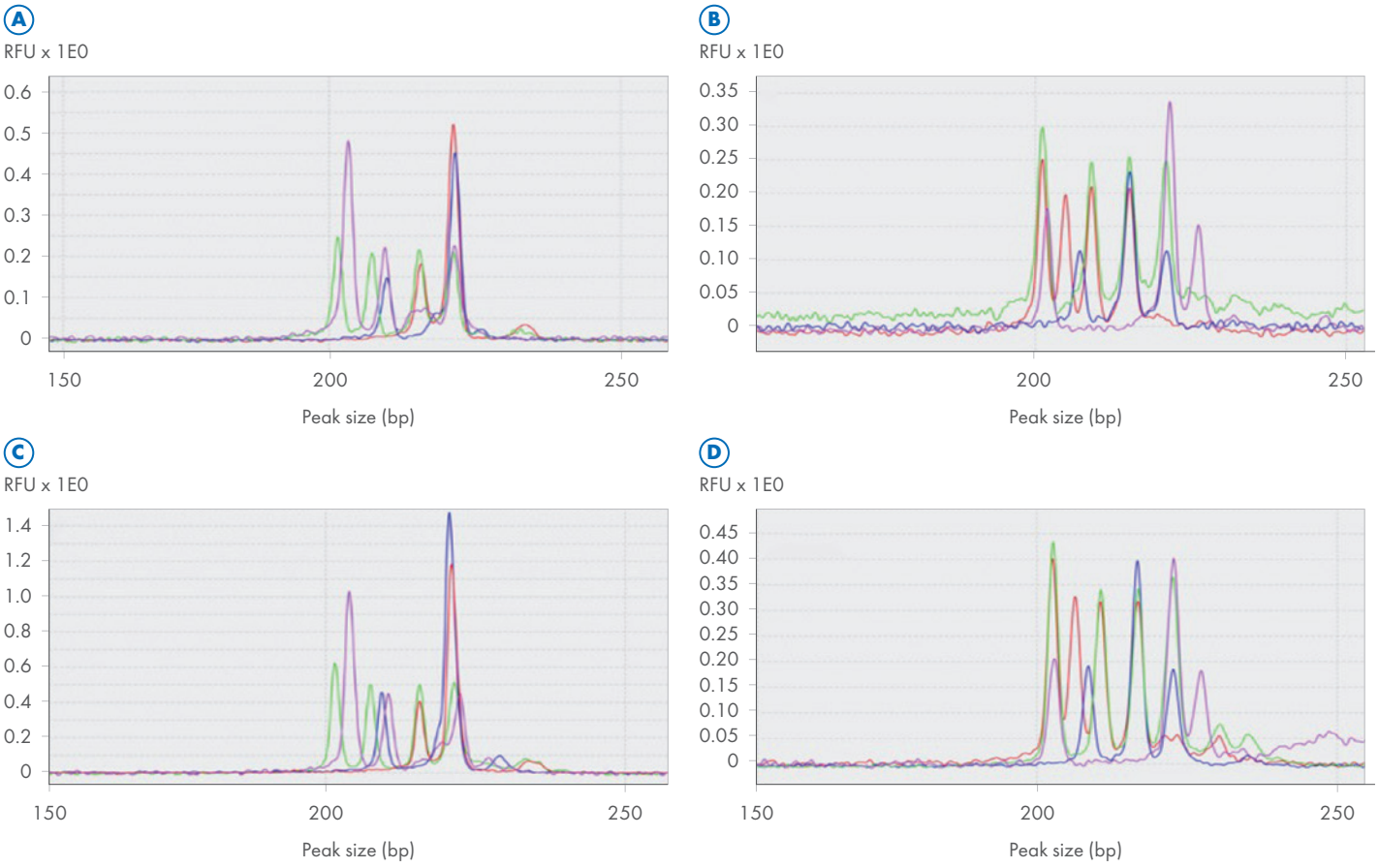

Figure 1. Electropherogram profiles were obtained using marker SSR1. A and **C** are the same four varieties of seed potato amplified in two different laboratories. On the other hand, **B** and **D** include another batch of four seed potato varieties amplified in two other laboratories.

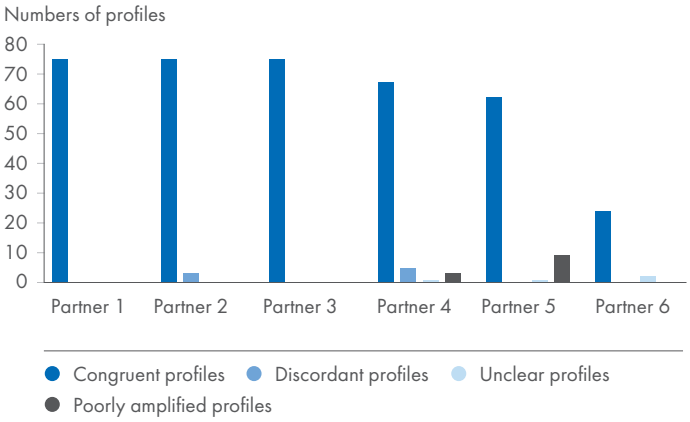

Figure 2. For marker STM1097: results of the comparison between the electropherogram profiles obtained on the QIAxcel system and the reference profiles contained in the database IdeAle using polyacrylamide gel and silver staining. The number of profiles obtained by each partner (1 to 6) on different batches of seed potato varieties were qualified as congruent profiles (no difference between QIAxcel system and reference profile), discordant profiles (clear difference in allelic composition), unclear profiles (difficult to read the profile on the QIAxcel system) and poorly amplified profiles (Inefficient PCR).

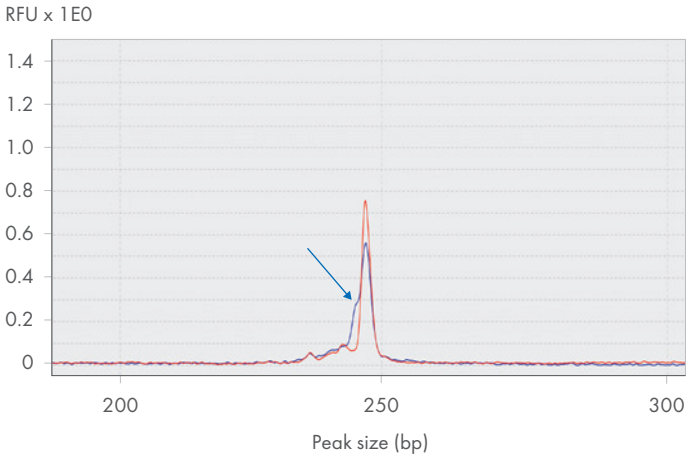

Figure 3. Marker STM1097: example of the difficulty solved during the project when examining the electropherogram profiles. One allele was only represented by a shoulder on the left portion of the main peak (arrow). In this example, the allele is present in the sample shown in blue and is absent in the sample shown in red. This shoulder was reproducible between laboratories.

Another objective of the project was to identify new markers that can be directly used on the QIAxcel system that can be implemented into the process. A total of 15 SSR markers used by Reid et al. 2011(17) and Esnault et al. (18) were chosen to be tested on a panel with 24 varieties using previous in-house experience (19, 20) and unpublished results. These tests were done by inov3PT in a single laboratory (data not shown).

Four new markers were found to be suitable in meeting the conditions required to use the QIAxcel High Resolution DNA Kit on the QIAxcel system. The suitability of the markers was evaluated using characteristics such as molecular weight, number of alleles obtained on the 24 varieties included in preliminary tests, gaps between alleles and overall shape of the electropherogram profiles. Unfortunately, one marker failed the first inter-laboratory testing. As a consequence, the list was shortened to three potential markers: STM2028, STI028 and STM5148. These three new markers were used to genotype all 326 varieties already depicted in the first part of the project, along with controls in all laboratories (Figure 4).

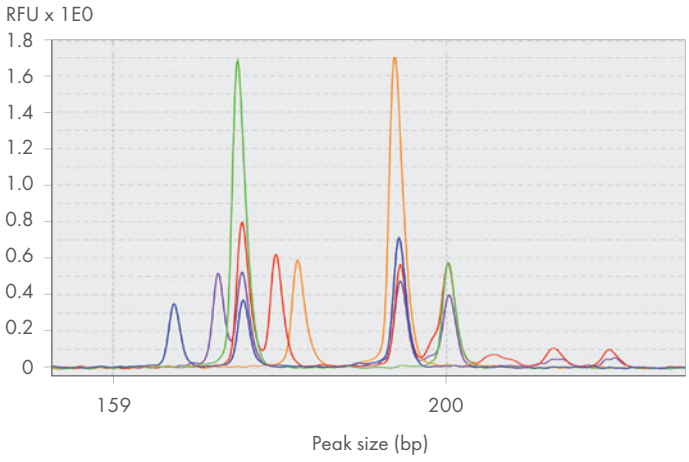

Figure 4. Example of allelic variability observed in one laboratory using STI028 on five varieties.

Discussion

One of the main objectives was to examine if the QIAxcel system, when run using the QIAxcel DNA High Resolution Kit, was resolutive enough to separate the alleles of the eight markers in the official procedure. This would guarantee continued use of the database. Results showed that six of the markers could be used on the QIAxcel system. Profiles obtained in the laboratories of the six partners using the QIAxcel system were reproducible with minor changes in PCR conditions.

For the remaining two markers, the resolution of the QIAxcel system did not allow us to obtain enough concordance between the profiles obtained on the QIAxcel system and the reference profiles stored in the database. This can be linked to varying conditions of electrophoresis in both systems. The official procedure mentions using 6% polyacrylamide gel containing 8M urea buffered with 1X Tris Borate EDTA. Electrophoresis lasts for three hours under high-voltage (60W) conditions (7). These conditions lead to very high resolutive power, which is different from the most resolutive conditions on the QIAxcel system. For the marker STM2020, this result was congruent with results that were previously obtained when trying to transfer the markers onto a capillary sequencing system (21).

Therefore, it is possible to use the database on the QIAxcel system for these six markers. The possibility of non-transferable markers was anticipated as some limitations were reached when using all eight markers. As a consequence, another objective of the project was to identify new markers specifically adapted to the new system characteristics. Bibliographic data and in-house data were used to test 15 markers chosen from a set with already proven SSRs (17,18). At the end of the process, three markers can be confidently used on the QIAxcel system. As compared with previous implementations of new markers in the procedure, the use of the QIAxcel system allowed faster testing and rapid generation of large amounts of data. Other markers could be extracted from the originally identified 15 markers, with minor adaptations of PCR conditions.

Conclusion

During the IdEvol research project, the QIAxcel system has been successfully evaluated for potato variety testing of microsatellite markers. The QIAxcel system adapted well to the medium-scale needs of the lab due to its throughput and size of the investment needed. With minor adaptations of PCR conditions and based on the genotyping of 326 varieties by six partner laboratories, it was deemed possible to confidently score six of the eight SSR markers already used in the official procedures of potato seed testing on the QIAxcel system. Moreover, three additional SSR markers could be identified and were implemented in the official procedure. New profiles could be rapidly generated for the same 326 potato varieties. The QIAxcel system has proven to be adaptable to the project objectives to simplify and reduce manual tasks, decrease overall analysis time, minimize exposure to hazardous substances for laboratory personnel and reduce waste treatment costs.

Acknowledgments

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Ordering Information

Product	Contents	Cat. no.
QIAxcel Connect	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9003110
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX Alignment Marker 15 bp/600 bp (1.5 mL)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25-500 bp (50 µL) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/µL	929559

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