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GENEVA

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POTATO VARIETY IDENTIFICATION (GENOTYPING). A BREEDER'S EXPERIENCE WITH AFLPS AND ISSRS (1998-2004)

Document prepared by an expert from France

For the period 1998-2003, Germicopa has been using AFLP markers[1] to fingerprint 56 commercial varieties and elite clones. Then, SSR markers have been used during years 2003 and 2004 to fingerprint 63 varieties[2].

Results:

For variety identification, complex DNA profiles consisting of 291 AFLP markers issued by 7 primer pairs, have been used to calculate genetic distances (Jaccard index). Data sets of the first three years (1998, 1999 and 2001) proved to be highly reproducible and powerful in discriminating varieties and clones. Data sets were cumulated and the relative values expressing the difference between the sampled units tested (Jaccard index) ranged from 25% to 65% in the case of clones known as being distinct, and from 0% to 5% in the case of true replicates within or between data sets.

[1] The AFLP work was contracted to AGROGENE (France);

[2] The SSR work was contracted to EUROFINS (France)

Interestingly, 3 genetically modified (GM) clones (cv 'Bintje') were included in these analyses and were also 0% or below 5% distance from the variety Bintje. Full-sib and half-sib varieties, which are representative of the smallest genetic distance in potato breeding, were above 25% difference.

A smaller set of 51 markers issued by 2 selected primer pairs proved to be powerful enough to check the variety identity in our quality control operations applied on pre-basic seed grown in the greenhouse, or on off-types grown in the field. The same 5% and 25% thresholds were clearly shown.

Therefore, the lack of values between 5% and 25% was considered to be reliable enough to set the practical decision rule to distinguish two potato varieties: "anything above 25% is for two distinct varieties, anything below 5% is for two samples of the same or essentially derived varieties (GM or mutated clones), anything in between 5% to 25% should be tested again".

Unfortunately, data sets obtained during the following years (2002 and 2003) were not as good as the first ones, resulting in a significant increase in similarity index above 10% for replicates, filling the gap between 5% and 25% and making this simple decision rule questionable. The reason for that was not clear (altered quality of the primer pairs or enzymes…?).

Then, DNA profiles consisting of 10 SSR markers (≈71 alleles) were investigated in 2003 and 2004. Although the number of markers was significantly lower than with AFLPs, repeatability and power of discrimination between varieties proved to be very high. Cumulated data sets resulted in differences in similarity index that ranked from 15% to 45%, in the case of distinct clones, and from 0% to less than 5% in the cases of replicates. Full-sib and half-sib varieties had distances between 15% and 25%. Still, a few replicated samples had values between 5% and 15% which raised questions about the repeatability within and between years. Then, a similar decision rule for variety identification as it was built after the first three years of AFLP experiments might be used, provided the repeatability will be improved …

Conclusions:

The most noteworthy result is the high degree of DNA polymorphism that has been revealed in a very limited private gene pool. It asserts that the genetic diversity used and created by private potato breeders is not as reduced as it has been reported from time to time either in the scientific literature or in recent newspapers. DNA polymorphism revealed by AFLP or SSR markers in potato seemed to be as high as polymorphism revealed in strawberry and sugarcane, and much higher than inbred species (tomato, wheat, inbred lines of corn).

Genetic proximity of varieties and clones, as calculated from our molecular data set, does not fit with phenotypic proximity which could be estimated for a few of the major traits of the varieties used (maturity group, yield, dry matter content, …). Thus, such neutral markers are unlikely to be predictive of the phenotypic traits in managing the reference collections for DUS experiments.

Out of three GM varieties analysed, none could be separated from the donor variety Bintje, although significant variation was observed in field for leaf morphology, plant vigor, tuber shape … . These results support the previous consideration and also raise the question

of essential derivation in potato which can be proved by molecular markers much more efficiently than by the phenotypic traits.

However, a noteworthy result is the minimum values of 25% and 15% for differences observed for two distinct potato varieties, including full-sibs and half-sibs, respectively with AFLP and SSR, which strongly sustains the interest of AFLP and SSR markers for clear-cut identification of a variety and supports the application in DUS reports for potato.

Nevertheless, breeders and molecular scientists have to be fully aware of the variability in DNA profiles, both from AFLP and SSR technologies, that results in a shift of the calculated genetic distance down to 5% in most of the cases in our data sets. This level might be acceptable for potato variety identification, provided that no higher values are observed.

In our experience, DNA profiles might be used for variety identification and essential derivation assessment in potato breeding, provided that:

- No one single current technology is selected. AFLPs and SSRs ought to be equally usable in potato. Technical efficiency and reliability prevail on the cost efficiency and on any other consideration. New emerging technologies (e.g. SNPs, …) should be given a chance and thoroughly evaluated.

- International sets of markers (one single set for each one of the agreed technology) are defined after international control of the repeatability over time and laboratories.

- Statistics applied on the data sets should be clearly defined, as genetic relationship may be modified according to the methodology used (data order for calculations, missing data, …);

- Similarity threshold index for identity and distinction are clearly defined for each one of the technologies and checked from time to time;

- Experts in genotyping should always be consulted along with experts in phenotyping and breeders for DUS assessment.

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[Annex follows]

Potato
Variety Identification
(Genotyping)

A breeder's experience with *AFLPs & ISSRs*
1998-2004

AFLPs

- ***Agrogène, Eurofins:*** **services companies**
- ***Publication:***

VOS P. & al. AFLP: a new technique for DNA fingerprinting.
Nucleic Acids Res 21: 4407-4414

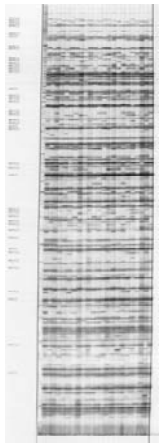
- ***European Patent*:**
- **EP 92402629.7**

AFLP
(1998-1999)

10 Primers Combinaisons

- **E32/M49: 32 markers**
- **E32/M51: 44 markers**
- **E32/M54: 51 markers**
- **E36/M47: 35 markers**
- **E36/M50: 40 markers**
- **E36/M59: 58 markers**
- **E39/M48: 61 markers**
- **E39/M49: 51 markers**
- **E39/M51: 37 markers**
- **E39/M61: 35 markers**

☞ 419 markers

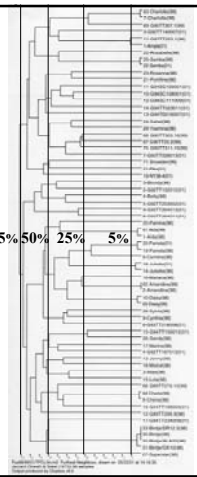


E36/M47
(ACC/CAA)
35 markers

22 variétés

Variety Identification:
AFLP
(2000-2004)

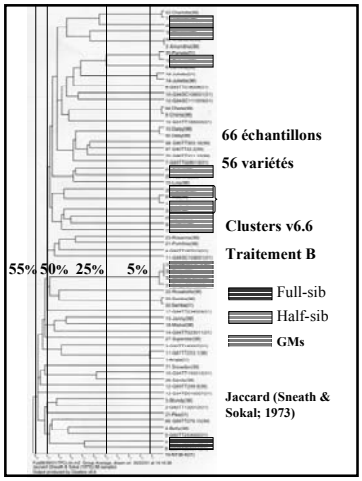
7 Primer Combinaisons:
E32/M49, E32/M51, E32/M54
E36/M47, E36/M59
E39/M48, E39/61
☞ 291 markers



66 échantillons
56 variétés

Clusters v6.6
Traitement A

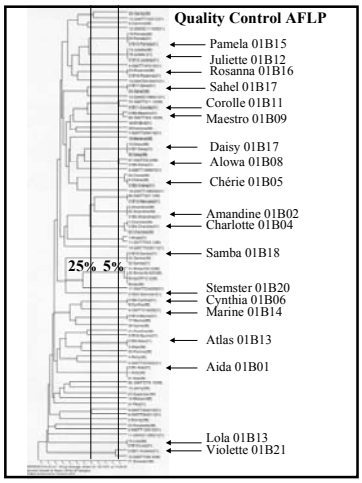
Jaccard (Sneath & Sokal; 1973)

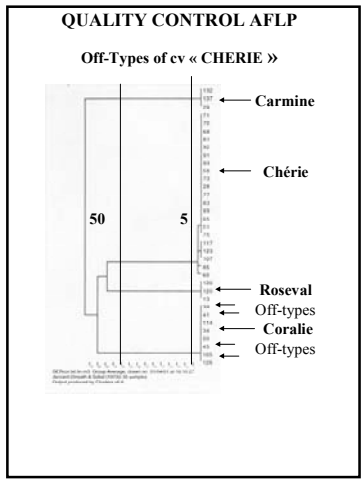


Quality Control
AFLP

2 Primer Combinaisons:
E32M49 et E36M59

☞ 91 markers
☞ ☞ 51 markers



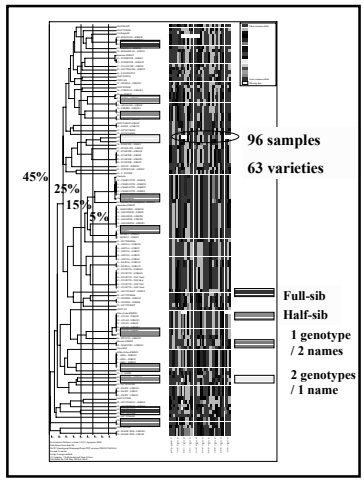


Variety Identification:
SSR
(2003-2004)

10 Primer Combinaisons

- LERNALX: 4 allels
- STM0037: 6 allels
- STM1100: 14 allels
- STM1105: 9 allels
- STM3012: 5 allels
- STM2020: 9 allels
- STM1064: 4 allels
- STM1003: 10 allels
- STM2005: 4 allels
- STM1097: 6 allels

☞ 71 allels



Quality Control
SSR

3 Primer Combinaisons:
STM0037, STM1003, STM1064
☞ 20 allels

☞ same variety discrimination

DISCUSSION

☞ **Both AFLP & SSR markers reveal a high degree of DNA polymorphism and genetic diversity within a limited potato gene pool;**

☞ **Genetic relationship based on randomly selected DNA markers reflects part of, but not all, the genealogy in potato breeding material;**

☞ **Genetic relationship based on randomly selected molecular markers does not help in predicting phenotypic relationship.**

Expectation 1

☞ **Improvement in repetability of the molecular fingerprints:**

↳ **reduce and stabilise the occurences of molecular artefacts below an agreed level (5% ?)**

Expectation 2

☞ **Availability and accreditation of a range of cost efficent, evolutive technologies and several sets of selected molecular markers to check :**

↳ **variety identity;**

↳ **essential derivation;**

Expectation 3

☞ **Tracing the genealogy of the potato varieties:**

↳ **Set molecular markers accordingly;**

↳ **Set statistic analyses accordingly;**

Conclusion

☞ **AFLP & SSR technologies have high potential to check Variety identification and Essential Derivation (GMOs).**

Intellectual Property Rights might use AFLP & SSR, along with phenotypic traits, in Potato.;

Final Conclusion

AFLPs and SSRs ought to be equally usable in Potato. New raising technologies (e.g. SNPs, …) should be given a chance and thoroughly evaluated. Technical efficiency and reliability prevail on the cost efficiency and on any other consideration.

☞ International sets of markers (one single set for each one of the agreed technology) should be defined after control of the repeatability over time and laboratories.

- Statistics applied on the data sets should be clearly defined as well, as genetic relationship may be modified according the methodology used (data order for calculations, missing datas, …);
- Similarity threshold index for identity and distinction are clearly defined for each one of the technology and checked from time to time;

Experts in genotyping should always be consulted along with experts in phenotyping and breeders for DUS assessment