ISOLATION AND CHARACTERIZATION OF NUCLEAR MICROSATELLITE MARKERS IN DATE PALM (*PHOENIX DACTYLIFERA* L.)

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Summary

Simple Sequence Repeats were isolated from a genomic library of *Phoenix dactylifera* L. All primer pairs produced an amplification product of the expected size and detected high polymorphism among the analysed samples. SSR genetic markers are expected to be a very effective tool for evaluating genetic diversity in date palm germplasm. Cross-transferability in different species and genera was evaluated.

Keywords

molecular markers, fingerprinting, polymorphism, SSR, DNA

Introduction

Date palm (*Phoenix dactylifera* L.) is the major fruit crop of arid climate region, cultivated mainly in North Africa but also in South Asia, in USA and in Australia. It covers a surface of about 800.000 ha and it is important – directly or indirectly – for the life of about 100 millions of inhabitants.

Random amplified polymorphic DNA (RAPD) markers have been used for the characterization of *Phoenix dactylifera* L. cultivars [1], even if they require an accurate standardization of the analysis to give reproducible patterns and they can be difficult to interpret due to their dominant mode of inheritance. In comparison, simple sequence repeats (SSR) have the potential to provide a more reliable method for DNA fingerprinting because of their high degree of polymorphism, co-dominant inheritance and the high reproducibility of the analysis.

The present research reports the isolation and the characterization of microsatellite sequences in *Phoenix dactylifera* L. and the study of the cross-transferability of these markers in other palm species.

Materials and Methods

Total genomic DNA was extracted from frozen leaves using a modified method of Thomas *et al*. [2]. Date palm genomic DNA was digested with RsaI and then used to construct a (GA)n and a (GT)n microsatellite-enriched library. SSR-containing DNA fragments were sequenced on semi-automatic sequencers. A set of 14 polymerase chain reaction (PCR) primer pairs for microsatellite amplification was designed using the software Primer Express (Applied Biosystems - USA). The PCR amplification was performed on 28 DNA samples of *P. dactylifera* from two Algerian Oasis (Biskra and Golea) and the USDA-ARS National Germplasm Repository (California). Cross-species amplification was tested on individuals of 9 other *Phoenix*

species as well as of 10 other palm genera. The PCR products of each individual were analysed following Akkak *et al.* [3]. The PCR amplification was performed on 50ng of DNA in a 20 µl final volume of a mixture containing 0.5 U TaqDNA polymerase (AmpliTaq Gold, Applied Biosystems), with the supplied reaction buffer, 1.5 mM MgCl2, 0.5 µM of each primer and 200 µM of each dNTP. The PCR programme was an initial denaturation at 95°C for 9 min, 28 cycles of 95°C for 30 sec, 50-58 °C for 30 sec, 72 °C for 1 min and a final elongation at 72°C for 45 min.

Results and Conclusion

All DNA samples were amplified at the 14 loci and analysed on ABI PRISM 377. They showed at least 1 or 2 alleles per locus.

Across the genus *Phoenix*, all loci revealed clear SSR patterns and polymorphism within the expected allelic range in most species.

We evaluated in details 14 loci and 8 of them were particularly interesting (Table 1) considering their good polymorphism.

The isolation and the characterization of nuclear microsatellite markers in date palm and the study of their cross-transferability is still in progress.

The criteria followed to select the primers will allow to carry out multiplex analysis and, therefore, to reduce time and cost of the technique.

Tab. 1 - Parameters of 8 loci used to study 28 date palm cultivars

Locus	**Repeat type**	**Label**	**Allele size**	**Opt. T.a. (°C)**
PDCAT1	$(TC)_{21}$	6-FAM	100	54
PDCAT 5	$(AG)_{16}$	6-FAM	158	54
PDCAT6	$(CA)_{14}(GA)_{23}$	HEX	180	54
PDCAT8	$(TC)_{16}$	NED	230	54
PDCAT 10	$(TC)_{16}$	6-FAM	111	54
PDCAT 11	$(TC)_{7}(TC)_{20}$	6-FAM	146	54
PDCAT 13	$(TC)_{21}$	NED	101	54
PDCAT 14	$(TC)_{19}(TC)_{16}$	6-FAM	131	54

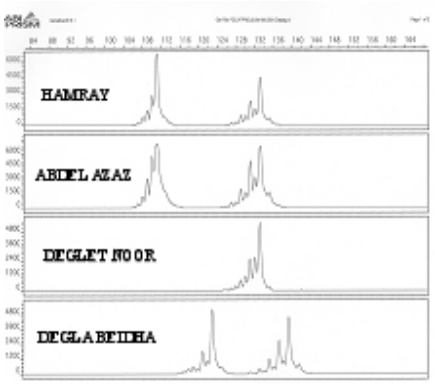

Fig. 1 – Polymorphism of PdCAT 1 locus and determination of a possible case of synonymy ('Hamray' and 'Abdel azaz')

References

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