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Molecular markers as a tool for the identification of hybrid plane trees

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Riassunto. *Sono state utilizzate le sequenze spaziatrici del DNA nucleare ribosomale per indagare la natura ibridogena di quattro putativi ibridi tra* Platanus occidentalis *e* P. orientalis*. Gli individui esaminati risultano possedere il DNA ribosomale di entrambe le specie parentali, confermando la loro natura ibridogena. Inoltre, le sequenze plastidiali confermano l'identificazione delle specie parentali, indicando che* P. orientalis *è la specie parentale coinvolta nella linea femminile dell'ibrido.*

Abstract. *The Internal Transcribed Spacer sequences of the nuclear ribosomal DNA (rDNA ITS 1) were used to verify hybrid origin of four putative hybrids between* Platanus occidentalis *and* P. orientalis*. The investigated specimens possessed ribosomal DNA from both the hypothesized parental species, confirming their hybrid nature. Also, chloroplast sequences (cpDNA) confirm the identification of one of the parental species, indicating that* P. orientalis *is the species involved in the maternal lineage.*

Key words*: Hybrids, London plane, Maternal inheritance,* Platanus

INTRODUCTION

One of the main aims of historical botany is the correct identification of the plant species growing in public or private gardens. Such identification can be useful in the restoration of ancient or historical gardens, when it is necessary to reintroduce the species that were originally growing there or, because of long absence of maintenance, are so seriously damaged (by fungi and other parasitic infections) that their substitution is recommended.

Even if, usually, a morphological approach is sufficient to correctly identify plant species, sometimes, especially in the cases of old, nearly forgotten cultivars or of seriously damaged specimens, correct identification may represent a hard task.

Among tree species, plane trees (*Platanus* spp.) often represent a problem in terms of correct identification. Genus *Platanus* includes six species, distributed from North America to Mexico and from Southern Europe to India. Planes are very large, long- lived deciduous trees, with buds hidden in the bases of the petioles and bark peeling in thin plates.

In Europe, *Platanus orientalis* L. colonized Mediterranean basin in the tertiary period, while *P. occidentalis* L. has been recently introduced from North America (in the XVII or XVIII century) and used as a garden tree or for road borders. As a consequence of this introduction, nowadays the distribution of the two species largely overlap in Europe, and putative hybrids have often been found in historical gardens, so causing problems in identification. *Platanus* x *hispanica* Muench, the London plane (= *P.* x *acerifolia* (Aiton) Willd. or *P.* x *hybrida* Brot.) is the hybrid

between the Eastern Plane and the American Plane, *P. orientalis* x *P. occidentalis*. This hybrid was probably first established in Spain or South France in about 1650 and has been planted in England (around 1680) at Ely and Barnes, Surrey (London). Today, it is quite common in towns and city streets, squares and parks in England, less common in North to mid-Scotland, and rare in the North to Rossshire and Ireland. In the last two centuries, this hybrid taxon has been also introduced in other European public and private gardens (including Italian ones), and at present, owing to its wide phenotypic variability, it is often misidentified with the parental species.

In this regard, in recent years molecular techniques are providing an useful tool for the correct identification of plant species, included hybrid taxa. In particular, DNA markers have been often used for hybrid characterization in higher plants (Doyle & Doyle, 1988; Harris & Ingram, 1992; Rieseberg & Brunsfield, 1992) and in tree species (Dumolin *et al*., 1995).

In the present study we focus on the development of useful molecular marker for the correct identification of hybrids among the two above mentioned plane species. The detection and quantification of gene flow and of natural hybridisation between different species and/or populations can be achieved through paternity analysis (comparison of genetic patterns of the offspring with their potential parents) (Streiff *et al.*, 1999) and through the use of maternally-inherited organelle genomes.

Recent phylogenetic studies carried out by using the sequences of the Internal Transcribed Spacers (ITS1 and ITS2) of nuclear ribosomal DNA have showed a high correlation between ITS nucleotide sequences and species for genus *Platanus* (Cafasso, unpublished data). Therefore, species-specific ITS sequences (and, as a consequence, species-specific restriction sites in these sequences) may be used as markers for the different species of the genus . For this reason, ITS's seem suitable to detect or confirm the parentage of plane tree hybrids. In fact, since nuclear markers are codominantly inherited, a putative hybrid plant must show the additive profile of the two parental sequences (Doyle & Doyle, 1988; Rieserberg *et al.*, 1990); otherwise, the plant is not a hybrid but simply a morphological variant of one parental species.

Together with nuclear markers, chloroplast DNA (cpDNA) is traditionally employed in order to detect maternal lineages in hybrid progenies (Palmer, 1985), because of its usually strict maternal inheritance in angiosperms (Harris & Ingram, 1992), in ferns (Gastony & Yatskieych, 1992; Guillon & Raquin, 2000) and in red algae (Zuccarello *et al.*, 1999).

In the present paper, the authors employ both these molecular markers to test the suitability of their application for the correct identification of hybrids between *P. occidentalis* and *P. orientalis.*

Material and Methods

Total DNA was extracted from 1 g of silica gel dried leaves according to the procedure described in Doyle & Doyle (1987) from four specimens of *Platanus* x *hispanica* (Kew Botanical Gardens) and from different samples of the two parental species (*P. occidentalis* from Canada and *P. orientalis* from Greece and from Southern Italy).

ITS 1 was amplified by polymerase

chain reaction (PCR) using specific primers (JK14: 5'- GGA GAA GTC GTA ACA AGG TTT CCG – 3'; JK11: 5' ATC CTG CAA TTC ACA CCA AGT ATC G – 3') which anneal in the 3' region of the 18S and in the 5' region of the 5.8S.

A chloroplast DNA region was amplified using specific primers (*Atp*B4: 5' – ATC CTT TAC TCA GTG AAT GAG – 3'; *rbc*L rev: 5' – GCT TTA GTC TCT GTT TGT GG - 3') which anneal in the 3' region of the β subunit of Atpase and in the 5' region of the Rubisco Large subunit.

All PCR reactions, with 10 ng of DNA as template (50 µl final volume), were conducted in a thermal cycler (Perkin Elmer 2700) for 30 cycles. Initial conditions were as follows: 30 sec denaturation at 94 °C, 1 min annealing at 55 °C, 45 sec extension at 72 °C; extension time was increased of 3 sec/cycle, and extension was further prolonged for 7 min at the end of the last cycle. PCR products were purified using Microcon microconcentrators MWCO 100,000 (Amicon, Beverly, MA, USA,) and cycle-sequenced using BigDye Terminator sequencing chemistry (ABI Applied Biosystems, Foster City, CA, USA). Sequences were then run on an ABI Prism 310 Genetic Analyzer. The Sequence Navigator software (ABI) was used to check electropherograms and to prepare sequence alignments.

Results and Discussion

The specimens of *Platanus* x *hispanica* showed morphological traits which were intermediate between the putative parental species (Fig. 1). Distinguishing features include: (1) leaves have deeper sinuses and (2) fruiting balls appear in pairs. Like its American parent, it typically grows as a single-trunk tree up to 75-100' (less frequently to 120') high with horizontal branching and a rounded habit. Trunk diameter typically ranges from 3' to 8'. The signature ornamental feature of this huge tree is its brown bark which exfoliates in irregular pieces to reveal a creamy white inner bark. Mature trees typically display mottled white bark that facilitates identification from great distances. The large 3-5 lobed medium to dark green leaves (4-9" wide) have coarse marginal teeth. In the fall, foliage typically turns an undistinguished yellow-brown. Male flowers are yellowish and female flowers are reddish. Female flowers give way to fuzzy, long-stalked, spherical fruiting balls (to 1 3/8" diameter) that ripen to brown in October and persist into early winter. Fruiting balls appear in pairs. Each fruiting

Fig. 1 - a) *Platanus orientalis* (Greece); b) *Platanus* x*hyspanica* (London); *Platanus occidentalis* (Canada).

ball consists of numerous, densely-packed, tiny seed-like fruits (achenes). Fruiting balls gradually disintegrate as fall progresses, dispersing their seeds, often in downy tufts, with the wind.

The Internal Transcribed Spacers (ITS I- II) of nuclear ribosomal DNA are among the suitable DNA regions which can be used to detect parental taxa since as nuclear markers they are co-dominantly inherited.

The ITS I of all sequenced specimens of *Platanus* were 340 bp long. In ITS I, sequence differences occur between *P. ori-*

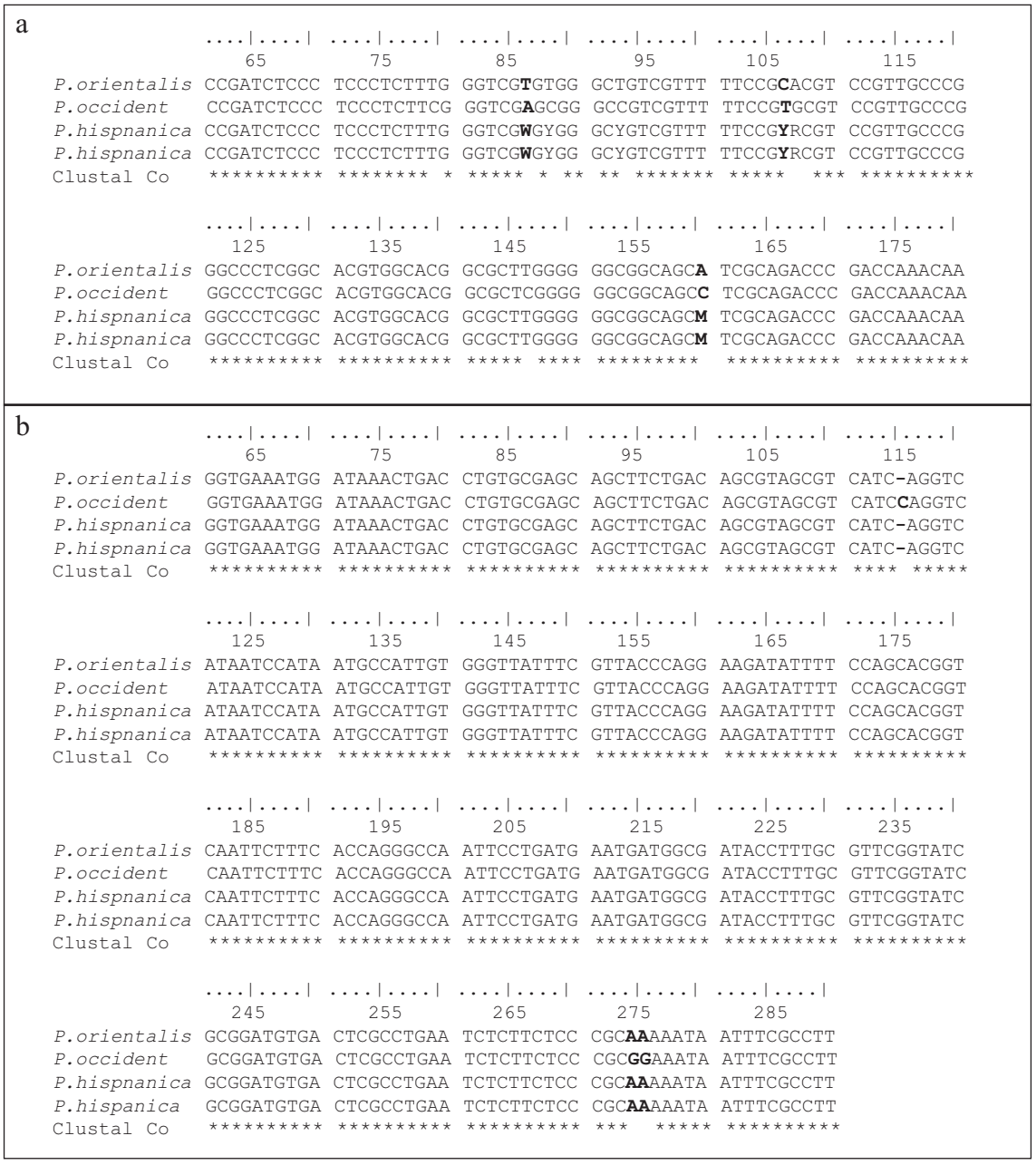

Fig. 2 - a) Partial ITS I allignment. ITS sequences showed heterozygote basis (marker) in the positions in which the two parental species are different in sequence. b) The alignment of the plastidial sequences where *Platanus* x *hispanica* has the identical sequence of *P. orientalis*.

entalis and *P. occidentalis* for 11 bases substitution (in positions 40, 48, 57, 86, 93, 106, 107, 160, 200, 227 and 280 respectively) (Fig. 2a). As expected for an hybrid, *Platanus* x *hispanica* ITS sequences showed heterozygote bases in the positions in which the two parental species are different in sequence (Fig. 2a).

The cpDNA spacer sequences of all examined specimens were about 600 bp long. The two parental species showed differences in sequence composition (*P. orientalis* vs. *P. occidentalis*). The alignment of the chloroplast sequences shows that all the examined samples of *Platanus* x *hispanica* have a sequence which is identical to that of *P. orientalis* (Fig. 2b).

Even if some morphological traits may help in hybrid recognition, a correct identification of a hybrid plant may often be difficult when it resembles more one parent than the other or when new morphological combinations of characters arise from recombination of distinct genotypes or when only few morphological traits are available for the analysis. In this regard, the molecular approach may be an useful tool for the assessment of hybrid status and for the correct identification of parental lineages. The molecular markers selected by us clearly identified the hybrid origin of the investigated specimens and proved to be an useful tool for the identification of doubtful specimens. From a molecular point of view, *Platanus* x *hispanica* presents conspicuous differences whit *P. orientalis* and *P. occidentalis.* Heterozygous positions, in ITS sequences, clearly show its hybrid nature. On the other hand, chloroplast DNA sequences (*atp*B-*rbc*L), confirming the identification of one of the parental species, and being maternally inherited, would indicate that probably only female specimens of *P. orientalis* are involved in the hybrid formation. This combination of data also excludes the possibility that any other species may be involved in the formation of this hybrid. Finally, it is interesting to note that the proposed molecular methods may be also employed for the identification of plant species by analyzing wood samples only. This opportunity further increases the usefulness of this method in the identification of historical plant samples from preserved materials.

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