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Molecular relationships between *Papaver somniferum* L. and *P. setigerum* DC. (Papaveraceae)

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Riassunto. *Relazioni molecolari tra* Papaver somniferum *L. e* P. setigerum *DC. (Papaveraceae).*
È stato condotto uno studio sulla variabilità molecolare di *Papaver somniferum* L. e *P. setigerum* DC. Le sequenze degli Spaziatori Interni Trascritti del DNA ribosomale nucleare (ITS 1 and 2) sono risultati molto simili nelle due entità. Ad ogni modo, mentre i ceppi coltivati di *P. somniferum* sono quasi del tutto omogenei, i campioni selvatici di *P. somniferum* e *P. setigerum* presentano una sequenza maggiormente variabile. Un'analisi fenetica ha mostrato che gli esemplari di *P. setigerum* sono chiaramente separati, in termini di sequenza, da *P. somniferum*. I campioni selvatici di *P. somniferum* impiegati nello studio, benché molto più simili in termini di sequenza ai ceppi coltivati dello stesso taxon, si raggruppano alla base del gruppo di *P. setigerum*. L'osservazione dei siti paraloghi presenti nelle sequenze ha confermato sotto il profilo molecolare che le due entità ibridano e che le tracce di ibridazione sono più frequenti nelle località in cui i due taxa sono simpatrici.

Key words ITS, Molecular variation, *Papaver somniferum*, *P. setigerum*

INTRODUCTION

The genus *Papaver*, which includes 70 species (KADEREIT, 1986), has a range centered in South Western Asia, with some widespread species in North America and South Africa.

Among the eleven sections of the genus, sect. *Papaver* (KADEREIT, 1986) includes *P. somniferum* L., containing alkaloids of the morphinane group and therefore widely cultivated for opium extraction. In the same section, *P. setigerum* DC. is present, which, although often ascribed to a circumscription substantially coincident with that of *P. somni-*

ferum (FEDDE, 1909; MOWAT & WALTERS, 1964), has been equally often regarded as a distinct species (e.g., BERTOLONI, 1842; GUSSONE, 1854; PIGNATTI, 1982; GREUTER *et al.*, 1989). On the contrary, the various taxa in which *P. somniferum* has been subdivided in the past (e.g., *P. album* Miller, *P. hortense* Hussenot, *P. officinale* Gmelin, *P. somniferum* subsp. *nigrum* (DC.) Thell.) have been recently synonimized (GREUTER *et al.*, 1989). Previous literature (LA VALVA *et al.*, 1985) indicated that morphology and alkaloid chemistry of *P. setigerum* is different from those of *P. somniferum*. This study revealed that morphine codeine and thebaine, the major alkaloids of *P. somniferum* are absent in *P. setigerum*.

However, *P. setigerum* and the wild ecotypes of *P. somniferum* (formerly known as *P. somniferum* var. *nigrum*) show a similar habit, and frequently populations of *P. somniferum* are classified as *P. setigerum* and vice versa. Until now, no attempt to explore the range of molecular variation of the taxa, as well as its possible overlapping, has been carried out. This paper deals with a molecular investigation of *P. setigerum* and *P. somniferum*, aiming at understanding the relationships between the two taxa.

MATERIALS AND METHODS

The plants of *P. somniferum*, labeled "PALB", were grown from seeds at the Botanical Garden of Naples (Italy) and belonged to the "*album*" variety of *P. somniferum*; the plant labeled PCOS was collected in nature and belonged to the variety "*nigrum*". The plants of *P. setigerum* were collected in the wild. All the other accessions were grown from seed and used for extraction at the stage of seedlings (Tab. 1).

DNA was extracted from dried (0.05 - 1 g) or fresh (0.1 - 1 g) leaves. Extraction was carried out by using the protocol by CAPUTO *et al.* (1991) opportunely scaled and modified.

Table 1 - Acronyms and origins of the sampled specimens

PALB	*P. somniferum* "album"	Historically cultivated at the Botanical Garden of Naples
PCOS	*P. somniferum* "nigrum"	Neighborhood of Cosenza, Italy
PSET	*P. setigerum*	Posillipo, Naples, Italy
PVEN	*P. setigerum*	Ventotene, Pontian Islands, Italy
PVUL	*P. setigerum*	Vulcano, Aeolian Islands, Italy
P03	*P. somniferum*	Grown from seed at the Botanical Garden of Naples
P09	*P. somniferum*	Grown from seed at the Botanical Garden of Naples
P10	*P. somniferum*	Grown from seed at the Botanical Garden of Naples
P14	*P. somniferum*	Grown from seed at the Botanical Garden of Naples
P17C	*P. somniferum*	Grown from seed at the Botanical Garden of Naples
P22	*P. somniferum*	Grown from seed at the Botanical Garden of Naples

ITS1 and 2 were amplified by using primers and reaction conditions as reported in ACETO *et al.* (1999)

PCR fragments were then purified by using Microcon 100 micro-concentrators (Amicon) and double-strand sequenced in both directions by using a modification of the dideoxy method as implemented in a double strand DNA cycle sequencing system with fluorescent dyes. Sequence reactions were then loaded into a 373A Applied Biosystems Automated DNA sequencer (Applied Biosystems). Various sequencing experiments were repeated to solve all uncertainties.

The alignment was accomplished by using Clustal W ver. 1.6 (THOMPSON *et al.*, 1994) with default settings. Sequences were then reduced to only ITS1 and ITS2 by aligning them with the 3' termini of 18S and 5.8S and with the 5' termini of 5.8S and 26S of various sequences available in the literature. Clustal W was also used to produce the distance matrix, as well as the dendrogram of relationships.

RESULTS AND DISCUSSION

ITS1 length was 249 bp for all the investigated plants, and ITS2 length was 255 bp, except for the specimen from Ventotene, whose ITS2 is 254 bp long. ITS distances within *P. somniferum/P. setigerum* (Tab. 2) range from complete identity (between PALB, P14, PCOS,

P17C, P09) to 0.058 (between P22 and PVEN). The cultivated strains of *P. somniferum* are much more similar to each other than the three wild specimens of *P. setigerum* of different geographic origin.

Tab. 2 - Pairwise distances between the taxa and specimens employed in the present study

PALB	0.000	0.002	0.002	0.000	0.006	0.002	0.002	0.000	0.014	0.052	0.045
P10	0.002	0.000	0.006	0.002	0.012	0.008	0.008	0.006	0.020	0.056	0.049
P03	0.002	0.006	0.000	0.004	0.008	0.002	0.002	0.002	0.016	0.056	0.045
P14	0.000	0.002	0.004	0.000	0.008	0.002	0.002	0.002	0.016	0.052	0.043
P22	0.006	0.012	0.008	0.008	0.000	0.008	0.008	0.006	0.018	0.058	0.049
P09	0.002	0.008	0.002	0.002	0.008	0.000	0.000	0.000	0.014	0.054	0.043
P17C	0.002	0.008	0.002	0.002	0.008	0.000	0.000	0.000	0.014	0.055	0.043
PCOS	0.000	0.006	0.002	0.002	0.006	0.000	0.000	0.000	0.010	0.051	0.040
PSET	0.014	0.020	0.016	0.016	0.018	0.014	0.014	0.010	0.000	0.030	0.019
PVEN	0.052	0.056	0.056	0.052	0.058	0.054	0.055	0.051	0.030	0.000	0.008
PVUL	0.045	0.049	0.045	0.043	0.049	0.043	0.043	0.040	0.019	0.008	0.000

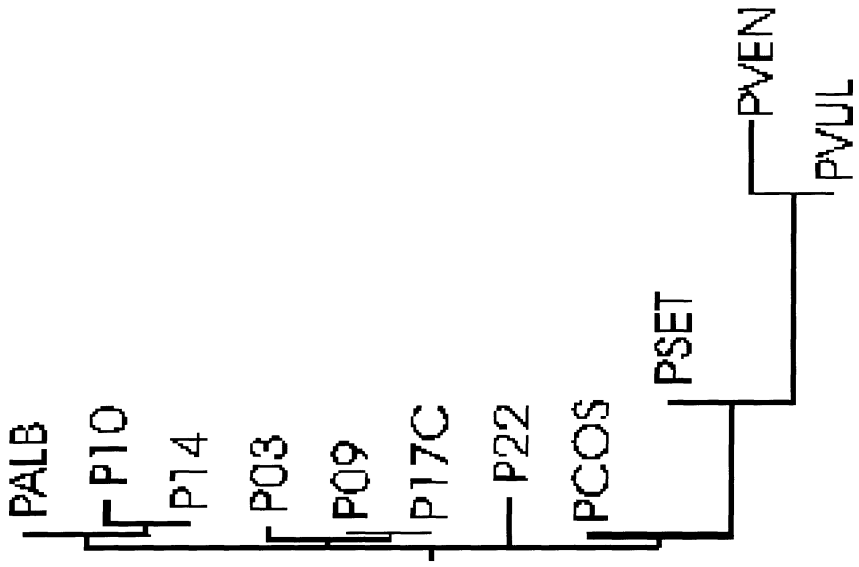

Fig. 1 - Dendrogram of the relationships among the taxa in study

The dendrogram of relationships (Fig. 1), in fact, shows that the various cultivated strains investigated are very similar to one another in terms of sequence. The three specimens which were investigated for *P. setigerum* were on the contrary clearly separate and make a distinct group. The wild *P. somniferum* (PCOS), although much closer in terms of sequence to the cultivated strains, groups at the base of the *P. setigerum* cluster.

The results obtained would indicate that *P. somniferum* and *P. setigerum* are two not very coherent groups on a biomolecular standpoint; leakage of one genome towards the other is quite abundant. The very close similarity among the cultivated strains (the maximum distance between which is 0.012) may be easily related to recent artificial selection starting from a pool of similar genotypes, whereas the relative distinctness of the wild *P. somniferum* may be related to independent evolution for the past few hundred or thousands years, after domestication of the wild plants. Interestingly enough, the two accessions of *P. setigerum* from Vulcano (Aeolian islands) and Ventotene (Pontian islands) are closer to each other than either is to the mainland accession from Naples (PSET). The latter, on the contrary, is closer, in terms of distance, to the wild specimen of *P. somniferum*.

Almost all sequences have several heterozygous positions (Tab. 3). The majority of these occur in the wild specimens of *P. setigerum* and *P. somniferum*, rather than in the cultivated ones; such result would only suggest that artificially propagated lines have been kept pure. For each specimen, several of these paralogous positions do not have additive counterparts in the other investigated plants, and this would indicate that not the entire range of molecular variation has been sampled. However, the positions which do have correspondence in other specimens are more frequent in the *P. setigerum* specimens from Naples and from Vulcano (seven such positions in the ITS's of PSET and PVUL) than in the *P. somniferum* specimen from Cosenza and in the *P. setigerum* specimen from Ventotene (two such positions in the ITS's of PCOS and PVEN).

Tab. 3 - Alignment of the sequences in study. An asterisk indicates identity. A dash indicates interspecific paralogies. Six unknown nucleotides "N's" have been placed downstream ITS1 with the purpose of separating ITS1 from ITS2

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PALB TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTGAATCCAAGTCCAGTGGTGGTGCA - 60
P10  TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTGAATCCAAGTCCAGTGGTGGTGCA - 60
P03  KTGAAACCTGCCCAGCAGAACGACCCGYGAACACRTGAATCCAAGTCCAGTGGTGGTGCA - 60
P14  TCGAAACCTGCCCAGCAGAACGACCCGYGAACACGTGAATCCAAGTCCAGTGGTGGTGCA - 60
P22  TCGAATCCTGCCCAGCAGAACGACCCGCGAACACGTGAATCCAAGTCCAGTGGTGGTGCA - 60
P09  TCGAAACCTGCCCAGCAGAACGACCCGTGAACACGTGAATCCAAGTCCAGTGGTGGTGCA - 60
P17C TCGAAACCTGCCCAGCAGAACGACCCGTGAACACGTGAATCCAAGTCCAGTGGTGGTGCA - 60
PCOS TCGAAASSTGCCCAGCASAACGACCCGYGAACAMGTGAATCCAAGTCCAGTGGTGGTGCA - 60
PSET TCGAAACCTGCCCAGCAGAACGACCCGYGAACACGTGAATCCAACTCCAATGGTGATGCA - 60
PVEN TCGAAACCTGCCCAGCAGAACGACCCGYGAACACGTGAATCCAACTCCAATGGTGATGCA - 60
PVUL TCGAAACCTGCCCAGCAGAACGACCCGTGAACACGTGAATCCAACWCSAATGGCGATGCA - 60
          ***    ********* ********  *****  *********  * * *** * **** - 60

PALB AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTTGGCTAACACCCTCTCT -120
P10  AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTTGGCTAACACCCTCTCT -120
P03  AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTTGGCTAACACCCTCTCT -120
P14  AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTTGGCTAACACCCTCTCT -120
P22  AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTTGGCTAACACCCTCTCT -120
P09  AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTTGGCTAACACCCTCTCT -120
P17C AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTTGGCTAACACCCTCTYT -120
PCOS AGTGGGGAGAGATCCCCCYTGCTCCACCGCTCGGTCGGGGAGKKGGSTAACACCCTCTCT -120
PSET AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTCGGTTAACACCCTCTCT -120
PVEN AGTGGGGAGAGATCCCCCTTGCTCCACCGCTCGGTCGGGGAGTCGGTTAACACCCTCTCT -120
PVUL AGTGGGGAGAGATCCCCCTTGCTCCRCCGCTCGGTCGGGGAGTCGGTTAACRCCCTCTCT -120
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PALB TTGTGCCGGAAAACGAACCCAAGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATGCTA-180
P10  TTGTGCCGGAAAACGAACCCAAGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATGCTA-180
P03  TTGTGCCGGAAAACGAACCCAAGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATGCTA-180
P14  TTGTGCCGGAAAACGAACCCAAGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATGCTA-180
P22  TTGTGCCGGAAAACGAACCCAGGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATGCTA-180
P09  TTGTGCCGGAAAACGAACCCAAGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATGCTA-180
P17C TTGTGCCGGAAAACRAACCCAAGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATGCTA-180
PCOS TTGTGCCGGAAAACRAACCCAAGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATGCTA-180
PSET YYGTGCCGGAAAACGAACCCAAGGCGCGGTGAGCGCCAAGGAAAAAACAAATGGATRCTA-180
PVEN CCGTGCCGAAAAACGAACCCAAGGCGCGGTAAGCGCCAAGGAAAAAACAAATGGATRCYA-180
PVUL CCGTGCCGRAAAACGAACCCAAGGCGCGGTAAGCGCCAAGGAAAAAACAAATGGATACYA-180
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PALB GCGGGCCTCTTCTCTTTCTCCTGCCTCGGTGGGAAAAATGCAGCGGTAGGTGTCGCGAAA -240
P10  GCGGGCCTCTTCTCTTTCTCCTGCCTCGGTGGGAAAAATGCAGCGGTAGGTGTCGCGAAA -240
P03  GCGGGCCTCTTCTCTTTCTCCTGCCTCGGTGGGAAAAATGCAGCGGTAGGTGTCGCGAAA -240
P14  GCGGGCCTCTTCTCTTTCTCCTGCCTCGGTGGGAAAAATGCAGCGGTAGGTGTCGCGAAA -240
P22  GCGCGCCTCTTCTCTTTCTCCTGCCTCGGTGGGAAAAATGCAGCGGTAGGTGTCGCGAAA -240
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P09  GCGGGCCTCTTCTCTTTCTCCTGCCTCGGTGGGAAAAATGCAGCGGTAGGTGTCGCGAAA -240
P17C GCGGGCCTCTTCTCTTTCTCCTGCCTCGGKGGGAAAAATGCAGCGGTAGGTGTCGCGAAA -240
PCOS GCGGGCCTCTTCTCTTTCTCCTGCCTCGGTGGGAAAAATGCAGCGGTAGGTGTCGCGAAA -240
PSET GCGTGCCTCTTCTCTTTYTCCTGCCTCGGTGGGARAAATGCAGCGGTAGGTGTCGCGAAA -240
PVEN GCGTGCCTCTTCTCTTTCTCCTGCCTCGGTGGGAGAAATGCAGCGGTAGGTGTCGCGAAA -240
PVUL GCGTGCCTCTTCTCTTTTTCCTGCCTCGGTGGGAGAAATGCAGCGGTAGGTGTCGCGAAA -240
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PALB TCCTATCTTNNNNNNACCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
P10  TCCTATCTTNNNNNNCCCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
P03  TCCTATCTTNNNNNNACCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
P14  TCCTATCTTNNNNNNACCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
P22  TCCTATCTTNNNNNNACCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
P09  TCCTATCTTNNNNNNACCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
P17C TCCTATYTTNNNNNNACCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
PCOS TCCTATCTTNNNNNNACCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
PSET TCCAATCTTNNNNNNACCGAGTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTCTGGCGA -300
PVEN TCCAATCTTNNNNNNACCGATTCTCCCCCTCCAACTCATGTCCTTGGCGCCTTC-GGCGA  -299
PVUL TCCRATCTTNNNNNNACCGATTCTCCCCCTCCAACTCATKTCCTTGGCGCCTTYTGGCGA -300
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PALB CATTGGCAYTGGGCAGTGAATGKGGAGGACATTGACCCCCCGTGCCTTTAAAGTGCGGTC -360
P10  CATTGGCACTGGGCAGTGAATGTGGAGGACATTGACCCCCCGTGCCTTTAAAGTGCGGTC -360
P03  CATTGGCATTGGGCAGTGAATGKGGAGGACATTGACCCCCCGTGCCTTTAAAGTGCGGTC -360
P14  CATTGGCACTGGGCAGTGAATGKGGAGGACATTGACCCCCCGTGCCTTTAAAGTGCGGTC -360
P22  CATTGGCATTGGGCAGTGAATGGGGAGGACATTGACCCCCCGTGCCTTTAAAGTGCGGTC -360
P09  CATTGGCATTGGGCAGTGAATGGGGAGGACATTGACCCCCCGTGCCTTTAAAGTGCGGTC -360
P17C CATTGGCATTGGGCAGTGAATGGGGAGGACATTGACCCCCCGTGCCTTTAAAGTGCGGTC -360
PCOS CATTGGCATTGGGCAGTGAATGGGGAGGACATTGACCCCCCGTGCCTTTAAAGTGCGGTC -360
PSET CATTGGCATTGGGCAGTGAATGGGGAGGAYATTGACCCCCCGTGCCTTKAAAGTGCGGTC -360
PVEN CATCGGCACTGGGCAGTGAACGGGGAGGATATTGACCCCCCGTGCCTTGAAAGTGCGGTC -360
PVUL CATYGGCAYTGGGCAGTGAAYGGGGAGGATATTGACCCCCCGTGCCTTKAAAGTGCGGTC -359
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PALB GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
P10  GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
P03  GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
P14  GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
P22  GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
P09  GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
P17C GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
PCOS GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
PSET GGTCTAAACACAGGCCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
PVEN GGTCTAAACACAGGTCCTGGGAGGCCAGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -420
PVUL GGTCTAAACACAGGTCCTGGGAGGCCGGCGTCACGATTCGTGGTGGTCGACACTCGTTGT -419
     ************** *********** ******************************** -420

PALB CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
P10  CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
P03  CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
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P14  CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
P22  CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
P09  CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
P17C CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
PCOS CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
PSET CTCTCTTCATTCCTGAATCCGTGTCTGCTGTGCTTACCGTGAAGGACCATAAGGAACCCA -480
PVEN CTCTCTTCATTCCTGAATTCGTGYCGGCTGTGCTTGCCGTGAAGGACCATGAGCAACCCA -479
PVUL CTCTCTTCATTCCTGAATTCGTGTCGGCTGTGCTTACCGTGAAGGACCATRAGCAACCCA -480
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PALB TCGGGCCATAAATATGGCACCCACTCTGCG  -510
P10  TCGGGCCATAAATATGGCACCCACTCTGCG  -510
P03  TCGGGCCATAAATATGGCACCCACTCTGCG  -510
P14  TCGGGCCATAAATATGGCACCCACTCTGCG  -510
P22  TCGGGCCATAAATATGGCACCCACTCTGCG  -510
P09  TCGGGCCATAAATATGGCACCCACTCTGCG  -510
P17C TCGGGCCATAAATATGGCACCCACTCTGCG  -510
PCOS TCGGGCCATAAATATGGCACCCACTCTGCG  -510
PSET TCGGGCCATAAATATGGCACCCACTCTGCG  -510
PVEN TCGGTCCATAAATATGGTACCCACTCTGCG  -509
PVUL TCGGTCCATAAATATGGTACCCACTCTGCG  -510
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By selecting only those positions for which nucleotides differ between *P. somniferum* and *P. setigerum*, the number of observed paralogies is lower. These positions (indicated with a dash in the alignment of Tab. 3) are seven in PSET, two in PCOS and PVEN and one in PVUL. As a consequence, it is possible to conclude that *P. somniferum* and *P. setigerum* can be distinguished in terms of sequence; however, the presence of shared paralogies indicates that they hybridize easily (this information is very well known from literature), and that hybridizations occurs more frequently when the two taxa grow sympatrically. In fact, the greater isolation of the insular specimens of *P. setigerum*, as well as the low number of paralogies shared with *P. somniferum*, may depend on the fact that in neither island *P. somniferum* is reported (FERRO & FURNARI, 1968; ANZALONE & CAPUTO, 1974-75). This species, on the contrary, grows in mainland Italy and the mainland Italian specimen of *P. setigerum* shows traces of this, in terms of frequent hybridization events, in its genome. In conclusion, the two entities, although distinguishable, appear to have a complex hybridization pattern which would make it preferable, as KADEREIT (1986) suggests, to treat them as infraspecific units.

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Abstract

A study on the molecular variation of *Papaver somniferum* L. and *P. setigerum* DC. has been undertaken. The sequences of the Internal Transcribed Spacers (ITS 1 and 2) of the nuclear ribosomal DNA are very similar in the two entities. The cultivated strains of *P. somniferum* are very homogeneous; the wild specimens of *P. somniferum* and *P. setigerum* have a more variable sequence. A phenetic analysis has shown that the specimens of *P. setigerum* are clearly separated in terms of sequence from *P. somniferum*. However, the wild accession of *P. somniferum* used in the study, although much closer in terms of sequence to the cultivated strains of the same taxon, groups at the base of the *P. setigerum* cluster. An investigation of the various paralogous sites present in the sequences, however, has confirmed on a molecular standpoint that the two entities hybridize, and that traces of hybridization in the genomes are more frequent where the two taxa live in sympatry.

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