

Genetic evidence for the conversion of the morphinan alkaloid thebaine to oripavine in interspecific hybrids between *Papaver bracteatum* and *Papaver orientale**

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The alkaloid profiles of ripe capsules of *Papaver bracteatum*, *P. orientale* and their interspecific hybrids (F_1 and BCF_1) were determined and compared. In the hybrids the thebaine content declined, while that of oripavine was higher than in the parental species. The results provided genetic evidence for the biosynthetic linkage between those alkaloids. A genetic model, assuming dosage effect interaction between the genomes of the two species was elaborated. The significance of the results for breeding purposes was considered.

INTRODUCTION

The species *Papaver bracteatum* and *P. orientale* of the section *Oxytona* are considered as potential plant sources of opiates. In *P. bracteatum* thebaine is found almost exclusively at a content of 1.5-3.8 per cent of the dry weight of the ripe capsules, depending on the plant population (Lalezari *et al.*, 1974; Levy *et al.*, 1979). This alkaloid can be used as a precursor for codeine production in the pharmaceutical industry. Oripavine is the major alkaloid of *P. orientale* (0.5-1.15 per cent in dry capsules) in all chemotypes having the characteristic chromosome number of the species ($2n=28$) and thebaine was reported as a minor alkaloid in this species (Shafiee *et al.*, 1975; 1977).

A similar pathway for thebaine biosynthesis was shown to operate in *P. somniferum*, *P. bracteatum* and *P. orientale* (Brochmann-Hanssen and Wunderly, 1978; Hodges *et al.*, 1977; Stermitz and Rapoport, 1961). The biosynthetic steps were determined using radioactive tracers and it was shown that the demethylation of the enol ether group of thebaine, which proceeds in *P. som-*

niferum to the production of codeine and morphine, is blocked in *P. bracteatum* (Horn *et al.*, 1978). Likewise in *P. orientale* thebaine is further metabolised to two phenolic compounds, one of which is presumed to be oripavine (Stermitz and Rapoport, 1961).

In the present study the alkaloid profiles and content of *P. bracteatum*, *P. orientale* and their interspecific hybrid were analysed. The variations in the relative concentrations of thebaine and oripavine in the various genotypes were compared in order to detect genomic interactions and biosynthetic linkage between the major alkaloids of the two species.

MATERIAL AND METHODS

Accessions of *P. bracteatum* ($2n=14$) and *P. orientale* ($2n=28$), originating from Iran, were sown in a greenhouse and the seedlings were transplanted to the field; reciprocal crosses were made between the two species and the backcross of the hybrid to *P. bracteatum* was performed in the following season. Three different F_1 and two BCF_1 families resulting from crosses between different parental plants were grown in the field along with the parents. The chromosome number of each

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species and hybrid was examined in the pollen mother cells as previously described (Milo *et al.*, 1988). Ten plants from each parent, F_1 and BCF_1 families were sampled randomly for chemical analysis. Capsules from individual plants were collected separately, oven dried (50°C) and, following alkaloid extraction, submitted to chemical analysis (Fairbairn and Helliwell, 1975).

A quantitative reversed-phase high pressure liquid chromatographic method was used for the analysis of the alkaloid spectrum. The separation was accomplished on a LiChrosorb Supersphere[®] RP-18 column (Merck, particle size $4\ \mu\text{m}$, $125\ \text{mm} \times 4\ \text{mm}$ I.D.); the mobile phase was 5 per cent 2-propanol, 40 per cent acetonitrile, 55 per cent water with 1 per cent ammonium carbonate. The solvent flow-rate was 1 ml/min and detection was at 280 nm. For a detailed description of the extraction and separation of the alkaloids, see Milo *et al.* (1989).

RESULTS AND DISCUSSION

Meiotic behaviour of the hybrid

The F_1 hybrid ($2n = 21$) between *P. bracteatum* and *P. orientale* had bivalents, univalents and a low frequency of trivalents at metaphase I (fig. 1(a)). Unequal migration of chromosomes at anaphase I was observed (fig. 1(b), (c)), resulting in unbalanced gametes and a high degree of sterility (8 per

cent pollen stainability). All the plants obtained from the backcross to the diploid parent were triploids ($2n = 21$, fig. 1(d)), indicating that the fertile gametes of the F_1 had 14 chromosomes. This feature should be borne in mind when elaborating a model for the interactions between the genomes of the two species.

Alkaloid spectrum

In the diploid species *P. bracteatum* ($2n = 14$), only thebaine was present. The alkaloid spectrum of the tetraploid species *P. orientale* ($2n = 28$) was wider and included thebaine, oripavine and alpinigenine at concentrations of 0.061, 0.086 and 0.080 per cent, respectively; as well as isothebaine and salutaridine as minor alkaloids (table 1, fig. 2). As expected for natural populations of out-crossing species, a large chemical variation between and within families was observed for the concentrations of the different alkaloids. The alkaloid spectrum of the hybrid between *P. bracteatum* and *P. orientale* was different from that of either parent and contained only thebaine and oripavine. The concentration of the latter compound was up to ten times higher in the hybrid than in *P. orientale*; likewise, its thebaine content was much lower than that of *P. bracteatum*. Moreover, the plants of the backcross generation to *P. bracteatum* had even higher oripavine and lower thebaine contents than the F_1 hybrid. Similar findings have been reported in interspecific hybrids between *P. somniferum* and *P. bracteatum*; in the hybrids, a higher codeine content and a lower thebaine content were found compared with *P. somniferum* (Bohm and Nixdorf, 1983). It is noteworthy that the minor alkaloids present in *P. orientale* are completely lacking in the ripe capsules of the hybrid. The minor non-morphinan alkaloids of *P. orientale* which disappeared in the interspecific hybrid belong to different branches of the biosynthetic pathway in these plants (Phillipson, 1983). Whether these alkaloids are not synthesized, or their formation occurs during the early stages of plant development and they are subsequently degraded, as in the case of *P. bracteatum* (Bohm, 1967; Nyman and Bruhn, 1979), remains to be determined. The alkaloid spectrum and especially the ratio between the morphinan and non-morphinan alkaloids were found to be affected by the ploidy level of the plants (Milo and Levy, 1989).

When no biosynthetic linkage between the compounds of the parents prevails, the hybrids exhibit generally an intermediate spectrum, includ-

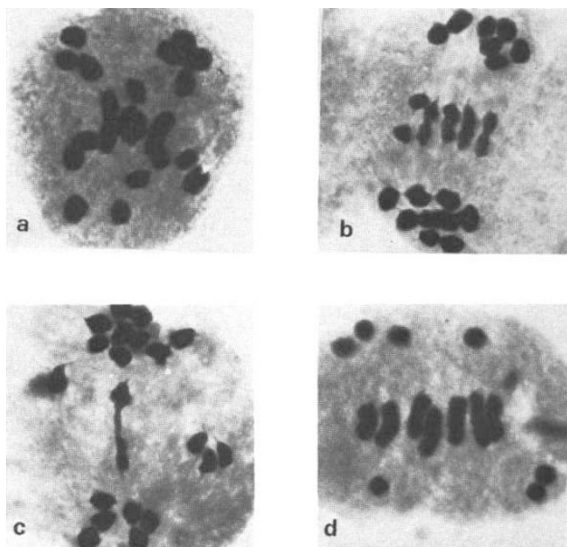
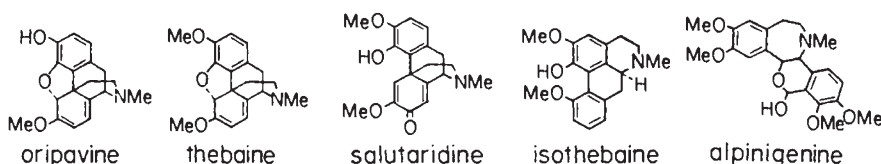


Figure 1 Chromosome associations at meiosis in F_1 (*Papaver bracteatum* \times *P. orientale*): (a) metaphase I; (b, c) anaphase I; and in BCF_1 (*P. bracteatum* \times *P. orientale*) \times *P. bracteatum*: (d) metaphase I.

Table 1 The alkaloid content (per cent dry weight; range given in parentheses) in capsules of *Papaver bracteatum* (PB), *P. orientale* (PO) and their interspecific hybrids.

Species or hybrid	Thebaine	Oripavine	Alpinigenine	Salutaridine	Isothebaine
PB	2.15 (1.24-3.75)				
PO	0.061 (0.020-0.130)	0.086 (0.020-0.125)	0.080 (0.032-0.128)	0.002 (0.001-0.004)	0.029 (0.001-0.085)
F ₁	0.193 (0.007-0.710)	0.522 (0.250-1.04)			
BCF ₁	0.129 (0.022-0.268)	0.779 (0.502-1.126)			

**Figure 2** Structures of the major alkaloids of *Papaver* section *Oxytona*. Me = methyl.

ing the compounds of both parents (Waller and Nowacki, 1978). In this study, the alkaloid profile of the interspecific hybrid can be explained if the conversion of thebaine to oripavine by the genome of *P. orientale* is considered. The high thebaine content, synthesized by the *P. bracteatum* genome, is further metabolized to oripavine by the enzymatic system of *P. orientale*.

A significant ($P < 0.05$) positive correlation ($r = 0.63$) was found in all the plant populations between the total morphinan alkaloid content and the ratio of oripavine to thebaine: when the total content is higher, the oripavine concentration increases at the expense of thebaine. Hence, in *P. orientale*, which has a low alkaloid concentration, thebaine and oripavine were present in about equal amounts, whereas in the hybrid containing a high alkaloid concentration, 59-97 per cent of the total

alkaloids were in the demethylated form of oripavine. These findings indicate that the efficiency of the enzymatic demethylation process increases at higher concentrations of the thebaine substrate.

Two genomic interactions may be responsible for this situation, as illustrated in table 2. In model I, additive effects of the parental genomes are assumed; hence, the expected morphinan alkaloid content in the hybrid is the arithmetic mean of the concentrations of the parents. In model II, a genomic interaction with dosage effect is considered between the two genomes of the parents. The hybrid has one set of chromosomes ($n = 7$) from the diploid parent and two sets of chromosomes from the tetraploid parent. In the backcross generation, two sets of chromosomes ($n = 14$) are transferred from the F₁ parent and one set ($n = 7$)

Table 2 Two models for estimation of the expected total morphinan alkaloids in hybrids of *Papaver bracteatum* (PB) and *P. orientale* (PO)

		Genomic constitution	Alkaloid content (per cent of dry wt.)
F ₁	Model I	1/2 (PB) + 1/2 (PO)	1.148 ± 0.197*
	Model II	1/3 (PB) + 2/3 (PO)	0.814 ± 0.172
	Observed		0.715 ± 0.074
BCF ₁	Model I	1/2 (F ₁) + 1/2 (PB)	1.432 ± 0.174
	Model II	2/3 (F ₁) + 1/3 (PB)	1.192 ± 0.140
	Observed		0.908 ± 0.053

* Mean ± S.E.

from *P. bracteatum*, as demonstrated by the cytological analysis. This ratio is retained in the calculation of the expected alkaloid content. The total alkaloid content of the F_1 and BCF_1 fits better with model II ($\chi^2 = 0.08$, $0.7 < P < 0.8$), assuming genetic interaction between the genomes of the two parental species. Dosage effects were also detected in these hybrids for various isozymes (Milo *et al.*, 1988), indicating further evidence for such an interaction between the genomes of these species. The genomal interaction between *P. orientale* and *P. bracteatum*, and the conversion of thebaine to oripavine in the hybrids, should be considered in breeding programs aiming at combining high thebaine content with desirable agronomic characters from both species. The genes controlling the demethylation of thebaine to oripavine should be eliminated by backcross to *P. bracteatum*: however, the differences in ploidy level between the two species might make this task difficult.

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