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Inheritance of morphological and chemical characters in interspecific hybrids between *Papaver bracteatum* and *Papaver pseudo-orientale* *

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Summary. The alkaloid profiles and morphological traits of the capsules of *Papaver bracteatum*, *P. pseudo-orientale*, and their hybrids were studied. Dominance of the hexaploid parent *P. pseudo-orientale* was observed for various characters. A genetic model assuming allelic additive effects and polysomic inheritance was elaborated for the control of isothebaine content in the capsules. The distribution of thebaine content in the segregating generations, F_2 and BCF_1 was evidence of the transfer of genes from the diploid parent *P. bracteatum* in the gametes of the interspecific hybrid and their expression in its progenies. These findings indicate the potential use of interspecific hybrids between the *Oxytona* species in the breeding of cultivars for industrial or ornamental purposes.

Key words: *Papaver bracteatum* – *P. pseudo-orientale* – Interspecific hybrids – Alkaloids – Thebaine

Introduction

The *Papaver* species of the *Oxytona* section constitute a polyploid series including *P. bracteatum* (2n = 14), *P. orientale* (2n = 28), and *P. pseudo-orientale* (2n = 42) exhibiting a wide spectrum of alkaloids (Goldblatt 1974). The diploid species, *P. bracteatum*, is of considerable importance since it represents a safer substitute for the opium poppy, *P. somniferum*, in the pharmaceutical industry (Nyman and Bruhn 1979). The capsules and roots of *P. bracteatum* species contain substantial amounts of thebaine, which is the natural precursor of codeine and can be used also for the synthesis of morphine antagonists (McNicholas and Martin 1984). The *Oxytona* species have been characterized systematically and chemically (Goldblatt 1974; Theuns et al. 1987) and their phylogeny has been assessed (Milo et al. 1988). The alkaloid spectrum of various interspecific hybrids of *Papaver*, mostly with the opium poppy, has been determined (Böhm and Nixdorf 1983; Ojala and Rousi 1986), but the genetic control of the compounds of the *Oxytona* species was not investigated. The aim of the present study was to elucidate the inheritance of the major alkaloids, thebaine and isothebaine, present in *P. bracteatum* and *P. pseudo-orientale*, respectively. The implications of the results on the biosynthetic aspects of these alkaloids and the breeding of these species are discussed.

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Materials and methods

Seeds of one accession of *P. bracteatum* (P.I. 381442) and *P. pseudo-orientale* (P.I. 375952), originating from Iran, were sown in a greenhouse; the seedlings were transplanted to the field and reciprocal crosses were made between the two species. Three F_1 families obtained from crosses between different parental plants were grown in the field along with the parents; the parents and F_1 families were analyzed during the first growing season. The F_2 and backcross generations to the two parents were raised in the following season. Several plants (20–60) from each parent, F_1, F_2, and BCF_1 were analyzed during the second growing season, when flowering is profuse. Pollen stainability with acetocarmine and the chromosome number of each species and hybrid were examined in the pollen mother cells, as described previously (Milo et al. 1988). Capsules and roots from individual plants were collected separately, oven dried (50 °C) and, following alkaloid extraction (Fairbairn and Helliwell 1975), submitted to chemical analysis. In a few plants of the backcross generations that did not produce capsules, only the roots were analyzed.

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 Superspher RP-18 column (Merck, particle size 4 µm, 125 mm × 4 mm I.D.); the mobile phase was 5% 2-propanol, 40% acetonitrile, 55% water with 1% 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

Morphology

The flower phenotypes and chromosome number of the parents were characteristic of each species (Goldblatt 1974). *P. bracteatum* had numerous large floral bracts (four to eight) and dark red petals with black marks. *P. pseudo-orientale* had deep orange flowers, with black marks at the base of the petals, and a few bracts (one to four) on the flowering stem. The interspecific hybrids exhibited a flower phenotype intermediate between the parents and could be distinguished accordingly. The number of capsules per plant was similar in both species but capsule weight was much higher in the diploid *P. bracteatum* (Table 1). In the F_1 hybrid, dominance of the small-capsuled hexaploid parent was observed. Dominance of the polyploid parent was also reported in interspecific hybrids between *P. somniferum* and the Oxytona species (Ojala and Rousi 1986); some morphological characters of *P. somniferum* that were dominant in the F_1 hybrid with *P. bracteatum* were found to be recessive in the hybrid with the polyploid species.

The capsule weight is also affected by the fertility of the gametes and seed setting. Hence, the plants from the backcross to the diploid parent bearing large capsules were highly sterile and yielded smaller capsules than those from the backcross to the hexaploid parent (Table 1). Furthermore, capsules from unpollinated flowers of each species were smaller than normal-seeded ones.

Alkaloid profile

In *P. bracteatum* only thebaine was present both in the mature capsules and roots, whereas in *P. pseudo-orientale* a wider spectrum, including isothebaine as the major alkaloid and smaller amounts of thebaine, oripavine, and salutaridine, was found (Fig. 1, Table 2). The chemical spectra of the species were consistent with those reported in previous studies (Shafiee et al. 1975; Phillipson 1983; Theuns et al. 1987). A large variation in isothebaine content was found in the *P. pseudo-orientale* population (Fig. 2). The F_1 and F_2 generations had similar distributions, with averages of 0.21% and 0.19% isothebaine, respectively. The isothebaine content in the backcross to *P. bracteatum* decreased as expected; on the other hand, the plant distribution for isothebaine in the backcross to *P. pseudo-orientale* was similar to the F_1 and F_2 populations (Fig. 2).

The alkaloid spectra of the roots and capsules were similar in each plant for each species and hybrid examined; only quantitative differences in the various alkaloids were observed between the two parts of each plant (Milo et al. 1988). Significant correlation coefficients,

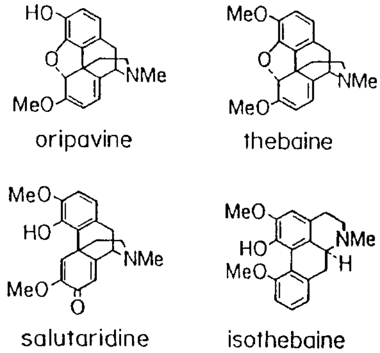

Fig. 1. Structures of the major alkaloids of *Papaver* section Oxytona. Me = methyl

Table 1. Pollen viability and capsule weight of *Papaver bracteatum* (PB), *Papaver pseudo-orientale* (PPO), and their interspecific hybrids

Species or hybrid	Chromosome no. (2n)	Pollen viability	No. of capsules per plant	Capsule wt. (g)
PB	14	96±0.2*	13.9±0.4	2.60±0.23
PPO	42	97±0.2	14.2±0.9	0.77±0.07
F_1	28	50±2.6	10.3±0.9	0.64±0.07
F_2	28	51±6.8	11.1±1.0	0.46±0.04
F_1 × PB	21	10±0.7	13.7±1.3	0.22±0.03
F_1 × PPO	35	62±5.2	9.8±1.0	0.79±0.05

* Mean ± standard deviation

Table 2. Content of the major alkaloids (% of dry weight) in capsules of *Papaver bracteatum* (PB), *Papaver pseudo-orientale* (PPO), and three different F_1 families at the first growing season

Species or hybrid	Thebaine	Isothebaine	Oripavine	Salutaridine
PB	2.15* (1.24–3.75)	–	–	–
PPO	0.007 (.001–.160)	0.716 (.008–1.38)	0.025 (.004–.038)	0.054 (.001–0.075)
F_1	0.382 (.170–1.92)	0.089 (.001–.217)	0.037 (.003–.120)	–
	0.280 (.142–.671)	0.197 (.007–.667)	0.085 (.016–.184)	0.014 (.006–.055)
	0.121 (.106–.153)	0.232 (.109–.398)	0.008 (.003–.022)	0.012 (.003–.018)

* Mean and range in parentheses

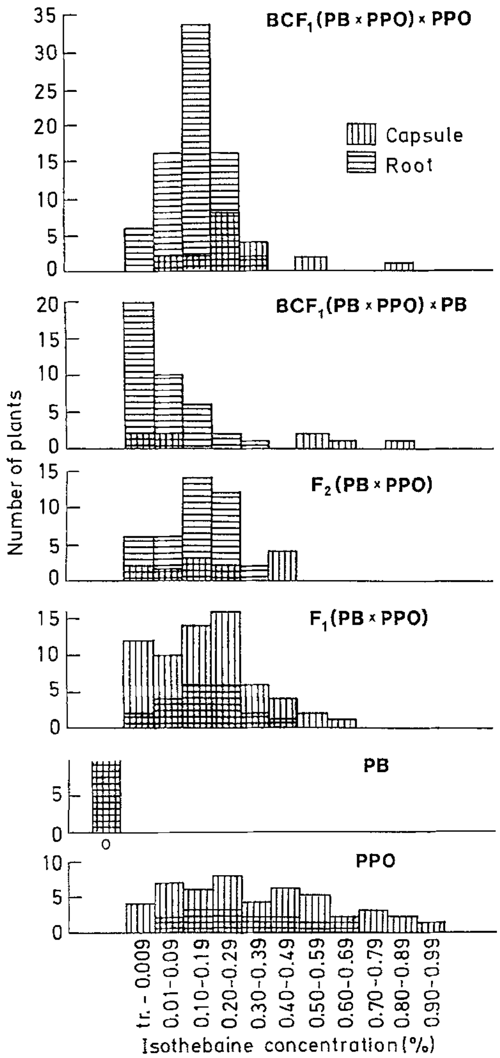

Fig. 2. Distribution of isothebaine content in roots and capsules of mature plants in the parental species, F_1 hybrids, F_2, and BCF_1 generations during the second growing season

−0.65 and 0.82 for isothebaine and thebaine, respectively, were found between the content of each major alkaloid in the capsules and in the roots for the bulked plant populations.

The F_1 hybrid had the alkaloid profile of the polyploid parent, with a higher thebaine content and reduced isothebaine concentration compared with *P. pseudo-orientale*. Marked differences were found in the content of the various alkaloids between the three families of this cross (Table 2). Such variability is expected, since the parental plants originated from natural populations where outcrossing is prevalent. The segregation patterns in the F_1 families and the variation for isothebaine content in the parents were used to elaborate a genetic model for the inheritance of this alkaloid. The results indicate that the isothebaine content is controlled by one gene

Table 3. Genetic model for the inheritance of isothebaine content in three different families of F_1 hybrids between *Papaver bracteatum* (PB) and *Papaver pseudo-orientale* (PPO)

Family no.	Parental genotype		F_1 hybrids		Expected ratio	Observed
	PPO	PB	Genotype	Content (%)		
1	Aaaaaa	aa	Aaaa	0.10–0.20	1	21
			aaaa	tr. –0.01	1	18
					$\chi^2 = 0.23$ $0.5 < P < 0.7$	
2	AAAaaa	aa	AAAa	0.45–0.60	1	1
			AAaa	0.25–0.40	9	13
			Aaaa	0.10–0.20	9	9
			aaaa	tr. –0.01	1	3
					$\chi^2 = 3.06$ $0.3 < P < 0.5$	
3	AAAAaa	aa	AAAa	0.45–0.60	1	2
			AAaa	0.25–0.40	3	8
			Aaaa	0.10–0.20	1	4
					$\chi^2 = 0.76$ $0.5 < P < 0.7$	

with allelic additive effects and polysomic inheritance (Table 3); each *A* allele contributes approximately 0.1–0.2% isothebaine in the dry capsules, whereas the *a* allele contributes only trace amounts of this alkaloid. In the hexaploid species, up to six *A* alleles can be present in the genotype, resulting in 1.2% isothebaine. The different parental genotypes and the segregation ratios of the three F_1 families gave a good fit to the expectations according to this model. Further evidence supporting polysomic inheritance and dosage effects in interspecific hybrids of *Oxytona* species was obtained from isozymes analysis; moreover, multivalent formation detected in the PMCs of the polyploids set the cytogenetic basis for the occurrence of polysomic inheritance (Milo et al. 1988). In order to validate more thoroughly the inheritance model for isothebaine, more crosses between defined genotypes within the *P. pseudo-orientale* species should be analyzed. The alkaloids of the *Oxytona* species are biosynthetically linked by the key precursor reticuline, from which branched pathways producing the various compounds evolve (Theuns et al. 1987). The major alkaloids of this section were detected in each of its species but their spectrum differs with the ontogenic stages of the plants (Böhm 1967; Theuns et al. 1987). This implies that the genes coding for the synthesis of all the alkaloids found in mature plants of *P. pseudo-orientale* are also present in *P. bracteatum*. Therefore, the alkaloid profile of the capsules and roots of the plant is determined mainly by regulatory genes; the gene controlling isothebaine synthesis identified in the present study is probably of this nature.

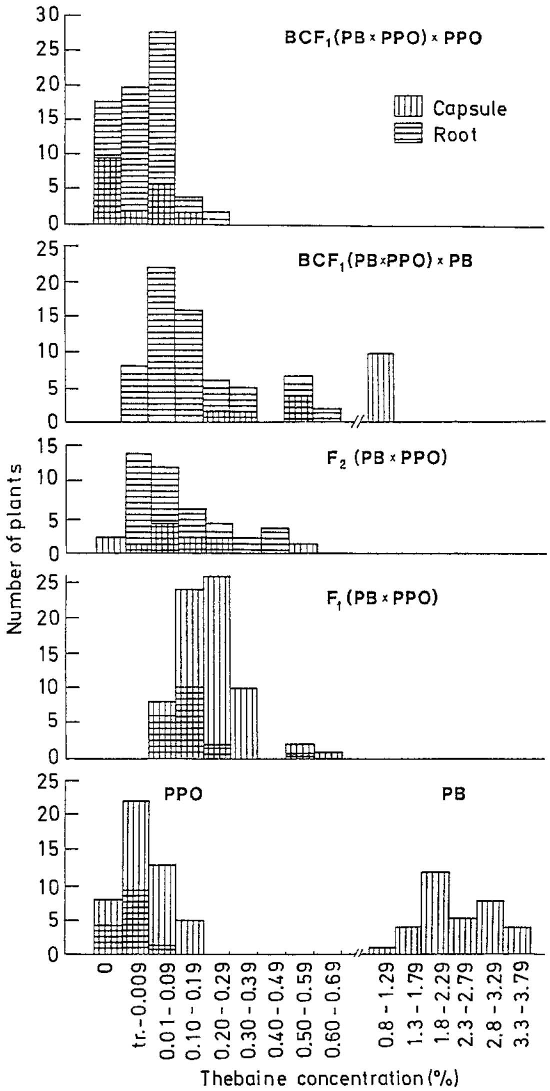

Fig. 3. Distribution of thebaine content in roots and capsules of mature plants in the parental species, F_1 hybrids, F_2, and BCF_1 generations during the second growing season

The alkaloid thebaine was found in the roots and capsules of *P. bracteatum* in much higher concentrations than in *P. pseudo-orientale* (Table 2, Fig. 3). Unlike the distribution of isothebaine, the thebaine content was reduced in the F_2 population compared with the F_1 generation. In both populations the thebaine distribution showed dominance of the polyploid parent. In some plants of the BCF_1 to *P. bracteatum*, the thebaine content was increased; likewise, the thebaine distribution in the BCF_1 to *P. pseudo-orientale* was similar to that of the recurrent parent.

The presence of thebaine in the segregating generations is indicative of the expression of the *P. bracteatum* genome in the interspecific hybrid. The comparison of the plant distributions for thebaine content in F_1, F_2, and BCF_1 demonstrates the transfer of genes and probably of chromosomes from the diploid *P. bracteatum* in the gametes of the F_1 hybrid.

These findings show that the *Oxytona* species can be used as a gene pool in breeding programs aimed at combining desirable chemical and agronomic characters from both species. Bidirectional selection could be practiced in the segregating generations of the interspecific hybrids for either ornamental or industrial purposes.

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References

Böhm H (1967) Über *Papaver bracteatum*. III. Mitteilung charakteristischer Veränderung des Alkaloidspektrums während der Pflanzenentwicklung. Planta Med 15:215–220

Böhm H, Nixdorf H (1983) Quality and quantity of morphinan alkaloids detectable in interspecific hybrids of the genus *Papaver*. Planta Med 48:193–204

Fairbairn JW, Helliwell K (1975) The determination of thebaine in *Papaver bracteatum* by gas liquid chromatography. J Pharm Pharmacol 27:217–221

Goldblatt P (1974) Biosystematic studies in *Papaver* section Oxytona. Ann Mo Bot Gard 61:264–296

McNicholas LF, Martin WR (1984) New and experimental therapeutic role for naloxone and related opioid antagonists. Drugs 27:81–93

Milo J, Levy A, Ladizinsky G, Palevitch D (1988) Phylogenetic and genetic studies in *Papaver* section Oxytona: cytogenetics, isozyme analysis, and chloroplast DNA variation. Theor Appl Genet 75:795–802

Milo J, Levy A, Palevitch D, Ladizinsky G (1989) High performance liquid chromatographic analysis of the alkaloid spectrum in the roots and capsules of the species and hybrids of *Papaver* section Oxytona. J Chromatogr 452:563–570

Nyman U, Bruhn JG (1979) *Papaver bracteatum* – a summary of current knowledge. Planta Med 35:97–117

Ojala A, Rousi A (1986) Interspecific hybridization in *Papaver*. I. F_1 hybrids of *Papaver somniferum* with perennial species of section Oxytona. Ann Bot Fenn 23:289–303

Phillipson JD (1983) Infraspecific variation and alkaloids of *Papaver* species. Planta Med 48:187–192

Shafiee A, Lalezari I, Nasseri-Nauri P, Asgharian R (1975) Alkaloids of *Papaver orientale* and *Papaver pseudo-orientale*. J Pharm Sci 64:1570–1572

Theuns HG, Janssen RHAM, Salemink CA (1987) The alkaloids of the *Papaver* section Oxytona Bernb. Herbs, spices, and medicinal plants. Recent Adv Bot Hortic Pharmacol 2:57–110