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Genetic and Chemical Control of Alkaloid Biosynthesis in *Papaver bracteatum* and *Papaver somniferum*

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Papaver - Genetics

581.15

Papaver bracteatum

Papaver orientale

633.75

Papaver pseudo-orientale

Papaver somniferum

Poppy

Thebaine

Morphine

615.32

Seed shattering

581.135.35

Plants, effect of gibberellic acid on 581.134.1

$\frac{630.72}{BAR} : 581.15 : 633.75 : 615.32 :$
 $581.135.35 : 581.134.1$

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ABSTRACT

The genetic control of thebaine accumulation in the capsules, was studied in inter- and intra- specific hybrids between Papaver bracteatum and the other two species of the section Oxytona : P. orientale and P. pseudo orientale. The results indicated that a common genome is shared by the species and that the transfer of genetic material between them is possible. The alkaloid spectrum of the interspecific hybrids was qualitatively characteristic of the parents in the cross.

Induced triploid and tetraploid plants showed a significant increase in the thebaine content of the capsules as compared with the diploids.

Heterotic and reciprocal effects on the thebaine yield were detected in the hybrids between different clones of P. bracteatum; the thebaine content of the capsules showed a relatively high heritability and was not correlated to the content in the roots.

The breeding strategies for developing thebaine-rich cultivars, with desirable agronomical characters , were elaborated in light of the isolation of a shattering-resistant mutant and the genetic information accumulated during this project.

Various horticultural aspects of P. bracteatum were studied e.g. seed germination, thebaine accumulation during the maturation of the capsule, effect of gibberellin and reducing agents on the thebaine yield components and the exploitation of the roots for thebaine production; the findings were extended to practical applications for the cultural practices of the crop.

The biosynthesis of the morphine alkaloids was studied in tissue cultures and plants of P. somniferum. At least four isoenzymes were present in a partially purified polyphenol oxydase (PPO) isolated from suspension cultures; physical and chemical properties of the isoenzymes were determined and the involvement of PPO and other cofactors in the biosynthesis of morphine alkaloids was studied.

Reducing agents such as dithiothreitol, glutathione and β -mercaptoethanol altered the spectrum of the morphine alkaloids in isolated capsules and plants in the field of P. somniferum; the last two compounds reduced also the thebaine content in the capsules of P. bracteatum.

Differentiated cultures of P. somniferum were shown to recover the potential that was lost by undifferentiated cells to synthesize the morphine alkaloids ; labelling experiments with ^{14}C -codeine demonstrated that differentiated calli are able to synthesize thebaine, codeine, but not morphine.

The findings of the studies on the biosynthesis of the morphine alkaloids provide a chemical approach for the control of this process.

INTRODUCTION

The genus Papaver contains about 100 species among which Papaver somniferum L. and Papaver bracteatum Lindl. are the most important species for the pharmaceutical industry. The opium, morphine and codeine extracted from P. somniferum are indispensable medicines used for their analgesic and hypnotic effects. However opium, morphine and especially heroin derived from it, have narcotic and addicting effects which present serious world - wide social problems. P. bracteatum can be substituted for opium poppy as a source for codeine production ; thebaine is practically the only alkaloid produced in the capsules and roots of this plant and it is readily converted to codeine and to other drugs used experimentally against drug addiction.

The objectives of this research were to study the genetic control of the thebaine formation and accumulation in P. bracteatum, to develop appropriate cultural practices for increasing the thebaine yield of the plant and to investigate several potentially important steps in the biosynthesis of the morphine alkaloids in P. somniferum.

The findings of this research led to a better understanding of the genetic and horticultural factors affecting the thebaine accumulation and yield in P. bracteatum and to the identification of clones, and a shattering-resistant mutant which are used for breeding thebaine-rich cultivars with desirable agronomical characters.

The study of the various aspects of the biosynthesis of morphine alkaloids was accomplished by the U.S. scientists and led to an understanding of the formation of alkaloids and provided useful information concerning the control of morphine alkaloids biosynthesis in P. somniferum.

In this final report we present the findings and discuss them mainly in the form of research articles which were published or in press; the unpublished results are presented separately.

Characterization of the reproductive system of Papaver bracteatum.

Introduction

Contradictory results concerning the breeding system of this species were reported: Böhm (1970) was the first to suggest that self incompatibility prevails in P. bracteatum but Tétényi and Zambo (1978) reported the obtention of seeds after selfing of the flowers of the plants. We have demonstrated previously the existence of a self incompatibility system in this species (Palevitch and Levy, 1983) ; in order to characterize the system , cytological observations of the pollen germination and reciprocal crosses between different clones were made. The efficiency of various treatments in overcoming the self incompatibility was also studied.

Materials and methods

The germination of pollen on the stigmatic disc and the ovary was studied in selfed or cross pollinated flowers of different clones. Young buds of different clones were emasculated, pollinated at anthesis (4-5 days later) with self or foreign pollen and bagged to prevent contamination with pollen from unknown origin. The flowers were cut 24 hrs after pollination, fixed in ethanol 95% -acetic acid 1:1(v/v), stained with aniline blue and observed under a fluorescent microscope (kho and Baër, 1968).

Flowers from one clone (no.9) were used to study the efficiency of bud pollination and mentor pollen in setting seeds in comparison with self or open pollination; 10 capsules from different plants of the clone were used for each treatment. Bud pollination was performed on buds 4-5 days before anthesis by pollen from sister flowers at the begining of anthesis. Unviable pollen (heat treated at 90°C for 48 hrs) was used as mentor pollen in mixture with fresh viable pollen to pollinate flowers at anthesis of clone no.9 (De Nettancourt, 1977).

The capsules from the different treatments including controlled selfing, were bagged until maturity, collected individually and their seed set was determined.

Results and discussion

Three patterns of pollen germination were observed (Fig.1): in self pollinated flowers, no pollen tubes or very short ones were observed 24 hrs after pollination (Fig.1,A) whereas in the cross pollinated flowers, long tubes reaching the ovules were seen (Fig.1,B). In some cross pollinated flowers, half compatible pollen with germinating tubes and ungerminated pollen grains was observed (Fig.1,C), indicating the gametophytic nature of the self incompatibility in P. bracteatum.

Further evidence for this assessment came from the breakdown of the incompatibility observed in induced tetraploid plants and from the reciprocal differences found for seed set in crosses of a diallel between 10 different clones: in 10 combinations, seeds were obtained from only one way of the cross. These features are characteristic of self incompatibility of the gametophytic type (De Nettancourt, 1977). Moreover, gametophytic self incompatibility was identified in *Papaver rhoeas* (Lawrence, 1978) and generally, species of a family exhibit the same incompatibility system.

Fig.1. Photomicrographs of A = an incompatible, B = a fully compatible and C = a half compatible pollination.

Self pollinated capsules did not set seeds and the use of mentor pollen was most efficient in overcoming the incompatibility barrier : 90% of the capsules from this treatment set seeds in comparison with 20% obtained with bud pollination (Table 1).

Table 1. Effect of mentor pollen and bud pollination on the seed set of capsules from self pollinated flowers of P. bracteatum.

Pollination method	% of capsules with seeds	No. of seeds per capsule	Dry weight(g) per capsule
Self	0	0	1.3
Range	-	-	1.0 - 1.7
Bud	20.0	328.5	1.5
Range	-	232 - 425	1.2 - 1.8
Mentor	90.0	1110.3	1.9
Range	-	308 - 3170	1.6 - 2.3
Open	100.0	2053.6	2.1
Range	-	472 - 4437	1.8 - 2.6

The seed set with mentor pollen was higher than with bud pollination but both treatments gave fewer seeds per capsule than open pollination. It is noteworthy to mention the relationship between the decrease in the number of seeds per capsule and the decrease in the weight of the capsule. Because of its high efficiency in overcoming the self incompatibility and its easy use in the field, the mentor pollen method is the most appropriate for developing homozygous parental lines to be used in the production of hybrid cultivars of P. bracteatum.

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Combining ability of different clones of Papaver bracteatum, heritability, and repeatability of thebaine yield and its components.

Introduction

A large variation in the thebaine content of P. bracteatum was reported (Coffman & al., 1975; Levy & al., 1979); this variation could be used for improving the thebaine yield of this species. However, in order to establish on a sound basis the breeding strategy to be adopted, the inheritance of the yield components of the thebaine has to be studied. For that purpose, various genetic parameters were estimated from different parental clones and their diallel hybrids during two consecutive growing seasons; the significance of the findings for breeding thebaine-rich cultivars in this species is discussed.

Materials and methods

Root cuttings from 10 different plants were used to build clones; they were selected from the populations Arya I (Is) and Arya II (Is) (Levy & al., 1979) to represent the largest phenotypic variation in the thebaine yield and its components. The plants which were three years old, were dug out at the end of the dormancy period (October 1982) and 20-25 cuttings were made from the roots of each one. The cuttings of each clone were planted separately in a net-house in pots filled with a mixture of peat : vermiculite and a sandy loam soil, in 1:1:1 proportions ; two months later, when shoot formation occurred they were transplanted to the field at the experimental station near Jerusalem. At flowering, controlled crosses were performed in all the possible combinations, including reciprocals, between the ten clones; selfing of the clones was not performed because of the self incompatibility prevailing in P. bracteatum. Each bud used in the crosses was bagged until maturity to prevent contamination with foreign pollen and the seeds from each mature capsule were collected individually (July 1983). The seeds of each cross were sown separately in speedlings on September 83 and 25-30 seedlings from each combination were transplanted two months later in the field at the same experimental plot of the parental clones. The parents and F_1 transplants were planted in two rows, 70 cm apart on beds 1.2m width, and 35cm between plants within row. Five plants from each F_1 were randomly sampled at maturity of the capsules during the first (July 1984) and the second growing season (July 1985) and their thebaine yield components in the capsules were determined. A low flowering rate obtained in the parental

clones during the first growing season probably due to their late planting (Levy and Palevitch 1982); therefore, the few buds formed by each clone, were used only for the diallel crosses. At the second growing season, when flowering was profuse, five plants from each clone were used to analyze the thebaine yield components. The analytical methods used in this study were as previously described (Lavie & al., 1979, Levy & al., 1981).

Statistical procedures

Estimates of narrow sense heritability (h^2) were determined from the parent-offsprings' regression coefficients for the different characters studied (Falconer, 1961). The repeatability (R) of each character was estimated in the F_1 's population by the correlation coefficient between its value during the first and the second growing season. Phenotypic correlation coefficients between the thebaine yield and its components in the capsules and in the roots were estimated from the F_1 hybrids at the second growing season. Heterosis was estimated as the deviation of F_1 from the best parent in percent of the best performing parent. The t-test was used to detect significant reciprocal differences between F_1 's of the same cross (Falconer, 1961).

Results and discussion

Performance of the parental clones and their hybrids

Marked differences in the thebaine yield and its components from the capsules were obtained in the different clones (Table 1). Clone no. 42 had the highest thebaine yield (1368 mg per plant) and high yield components i.e. number and weight of the capsules and their thebaine content. Clone no. 40 had the highest thebaine content (3.4%) and also a relatively high yield. The clones no. 3 and no. 16 had the lowest thebaine yield (155mg per plant) and also the lowest yield components.

From the 90 F_1 combinations expected from the diallel crosses of the 10 clones, only 72 were obtained: in 10 combinations the reciprocal cross did not set seeds and the clones 28, 35, 40 and 17, 22 were incompatible and the reciprocal crosses within each group did not produce seeds.

The mean performance (general combining ability) of the different F_1 hybrids from each clone is given in Table 2. As already reported (Levy & al., 1981, Böhm, 1981) the thebaine yield of the plants was by far much

Table 1. Thebaine yield components from the capsules of the parental clones during the second growing season.

Clone No.	Capsules per plant		Weight (g) per capsule	Thebaine content	Thebaine yield mg per plant
	number	weight (g)			
3	8.6 (1.5)*	8.8 (1.3)	1.1 (0.1)	1.8 (0.1)	154.2 (22.8)
8	14.2 (0.7)	22.9 (2.6)	1.6 (0.1)	1.1 (0.1)	248.8 (26.9)
9	13.6 (2.0)	20.9 (2.7)	1.8 (0.1)	2.6 (0.2)	561.6 (107.0)
16	13.5 (1.2)	20.3 (2.3)	1.5 (0.1)	2.6 (0.1)	155.2 (13.4)
17	17.3 (3.1)	30.9 (4.8)	1.8 (0.1)	2.5 (0.5)	821.2 (177.0)
22	15.6 (1.1)	22.0 (2.3)	1.4 (0.1)	2.0 (0.1)	440.3 (42.2)
28	26.0 (3.5)	30.0 (4.1)	1.2 (0.1)	2.3 (0.3)	696.2 (131.4)
35	15.6 (1.7)	20.9 (2.4)	1.3 (0.1)	2.0 (0.2)	425.7 (96.7)
40	16.8 (2.6)	24.9 (5.2)	1.4 (0.1)	3.4 (0.1)	885.8 (190.6)
42	24.6 (1.9)	50.4 (4.5)	2.1 (0.1)	2.7 (0.2)	1368.0 (171.6)

*In parenthesis, the standard error of the means.

Table 2. Mean performance of F_1 's from the different clones during two consecutive growing seasons.

Clone No.	No. F_1 combinations	Capsules per plant				Weight (g)		Thebaine content		Thebaine yield	
		number		weight (g)		per capsule		% d.w of capsules		(mg) per plant	
		1*	2*	1	2	1	2	1	2	1	2
3	9	3.3 (0.3)	12.6 (0.9)	4.7 (0.7)	16.5 (1.5)	1.4 (0.1)	1.3 (0.1)	2.0 (0.2)	1.7 (0.2)	96.2 (16.8)	288.1 (39.9)
8	9	3.8 (0.3)	15.4 (0.9)	6.3 (0.7)	24.5 (2.0)	1.6 (0.1)	1.6 (0.1)	1.9 (0.1)	1.8 (0.1)	116.6 (15.6)	437.9 (57.5)
9	9	4.2 (0.3)	14.5 (0.8)	7.6 (0.9)	22.5 (1.4)	1.8 (0.1)	1.5 (0.2)	2.6 (0.1)	1.7 (0.1)	193.6 (20.4)	386.3 (23.2)
16	9	3.4 (0.2)	13.8 (1.0)	5.8 (0.4)	20.1 (2.0)	1.7 (0.1)	1.4 (0.1)	1.8 (0.2)	1.4 (0.1)	106.4 (17.1)	273.4 (36.5)
17	8	3.5 (0.3)	12.5 (0.6)	5.8 (0.6)	17.5 (1.2)	1.6 (0.1)	1.4 (0.1)	2.0 (0.2)	1.8 (0.1)	116.7 (20.1)	309.2 (39.2)
22	8	3.1 (0.4)	12.2 (0.9)	4.3 (0.6)	17.9 (2.3)	1.4 (0.1)	1.4 (0.1)	2.2 (0.2)	1.8 (0.1)	92.8 (16.1)	334.6 (56.3)
28	7	4.2 (0.3)	14.5 (0.8)	6.9 (0.6)	21.5 (2.0)	1.7 (0.1)	1.5 (0.1)	1.8 (0.2)	1.6 (0.1)	130.7 (22.1)	338.2 (42.6)
35	7	4.3 (0.4)	13.4 (0.6)	7.1 (0.9)	19.7 (1.3)	1.6 (0.1)	1.5 (0.1)	2.1 (0.1)	1.9 (0.1)	151.5 (28.6)	368.6 (21.0)
40	7	3.5 (0.2)	14.0 (1.2)	6.2 (0.6)	25.0 (2.6)	1.7 (0.1)	1.8 (0.1)	2.2 (0.1)	2.2 (0.1)	140.0 (20.1)	554.3 (70.2)
42	9	3.9 (0.4)	14.0 (0.9)	7.4 (1.0)	24.2 (1.9)	1.8 (0.1)	1.7 (0.1)	1.9 (0.1)	1.8 (0.1)	144.1 (22.3)	352.9 (62.6)
Mean		3.7 (0.1)	13.7 (0.3)	6.2 (0.3)	20.9 (1.0)	1.63 (0.04)	1.51 (0.05)	2.05 (0.08)	1.77 (0.07)	128.9 (9.6)	364.4 (26.0)

* 1 = First growing season, 2 = second growing season; the standard error of the means is given in parenthesis.

higher during the second growing season as compared with the first one. This was due mainly to the higher number of capsules formed by the plants and not to their thebaine content which was slightly reduced during the second season as compared with the first one. Clone no.40 had the highest general combining ability for the thebaine yield during the second growing season whereas clone no.9 performed best during the first one; this result is indicative of the low repeatability of the thebaine yield.

Heterosis, estimated as the deviation in percent from the best performing parent was detected in two F_1 combinations (Table 3): the F_1 from the cross 8x16 had a significant increase in the thebaine yield components and its thebaine yield was greatly higher (more than three times) than that of the best parent in the cross, clone no.8. A significant increase in the thebaine content of the F_1 from the cross 3x35 was obtained but its thebaine yield was similar to that of the best parent due to its low yield of capsules.

Table 3. Heterosis estimates in percent of the best parent for the thebaine yield and its components in the capsules.

Clones in cross	Capsules per plant		Thebaine per plant	
	number	weight	content	yield
3 x 35	-12.9	-41.6*	61.0*	6.4
8 x 16	63.4*	71.6*	100.0*	262.6*

*Significant at 5% level.

These results show that heterotic effects can be used in a breeding approach to increase the thebaine yield and / or its components in Papaver bracteatum. Moreover, this species is ideally suited for the production of hybrid seeds: large seed quantities are produced in each capsule and self-incompatibility can be used for that purpose. For an efficient exploitation of heterotic effects, compelled selfing of the parental lines for few generations should be conducted in order to increase the heterozygosity of the hybrids.

In six different F_1 combinations out of 31, significant differences in the thebaine yield were obtained in the F_1 plants from reciprocal crosses; both paternal and maternal effects were detected. The reciprocal differences in

thebaine yield stemmed mainly from differences in the yield of capsules per plant and in only one cross (22x40), a significant reciprocal difference in the thebaine content was found (Table 4). These findings point out to the need of testing reciprocal F_1 's when breeding hybrid cultivars in this species.

Table 4. F_1 hybrids with significant reciprocal differences in the thebaine yield during the first growing season.

Clones in cross male female	Capsules per plant		Thebaine in capsules	
	number	weight (g)	content (%)	yield (mg)
16 x 35	4.4*	8.8*	2.05	150.5*
Reciprocal	3.0	5.0	2.05	85.5
16 x 40	2.8	4.4	2.19	80.0
Reciprocal	4.0	6.9*	1.95	125.0*
17 x 35	4.0	4.9	1.96	86.0
Reciprocal	4.2	9.2*	2.10	174.0*
22 x 40	2.6	4.0	2.20	74.0
Reciprocal	5.8*	9.9*	2.80*	272.0*
28 x 42	4.6	9.0	2.30	196.0*
Reciprocal	4.4	7.6	1.80	135.0
40 x 42	2.8	5.5	1.90	97.0
Reciprocal	4.4*	9.6*	2.00	189.0*

*Significant at the 5% level

The narrow sense estimate of heritability (h^2) of the thebaine yield from capsules was very low but for the thebaine content and weight of the capsule, a higher heritability was found (Table 5). Since the thebaine yield per plant is the integrated result of its different components, the low heritability of one of the components such as the number of capsules, could cause the low heritability of the yield.

Table 5. Heritability (h^2) and repeatability (R) of the thebaine yield from capsules and its components.

	Capsules per plant		Weight per capsule	Thebaine content	Thebaine yield per plant
	number	weight			
h^2	0.11	0.27	0.37	0.37	0.12
R	0.61	0.65	0.60	0.37	0.39

These results confirm previous findings with other plant material of P. bracteatum (Levy et al., 1981) and are similar to those reported for other secondary metabolites (El Ballal, 1978; Murty & al., 1976). The higher heritability of the thebaine content indicates that this character is the most reliable for an efficient selection advance in the increase of thebaine as it was also established for P. bracteatum (Levy & al., 1981) and for the sapogenin yield of Dioscorea floribunda (Martin and Cabanillas, 1967).

The repeatability (R) of the different yield components was relatively high although not significant (Table 5); this was probably due to the large variation between the two growing seasons resulting from different environmental conditions during each season.

The thebaine yield from the capsules and from the roots were significantly correlated to each of the different yield components respectively but no significant correlation between the components was found (Table 6). A negative correlation was previously found between the number of capsules per plant and their thebaine content (Levy & al., 1979); this result was not confirmed in the present study probably due to the different genotypic backgrounds of the plants used in each study.

Table 6. Correlation coefficients between thebaine yield components in the capsules and roots during the second growing season.

Yield components	Capsules per plant number	weight	Thebaine in capsules content	yield	Roots weight per plant	Thebaine in roots content	yield
Capsules per plant no.		0.95**	0.24	0.78**	0.22	0.58	0.49
Capsules weight per plant			0.29	0.83**	0.34	0.52	0.56
Thebaine content in capsules				0.75**	0.04	0.14	0.07
Thebaine yield from capsules					0.28	0.41	0.45
Roots weight per plant						0.21	0.87*
Thebaine content in the roots							0.65*

*Significant at the 5% level

**Significant at the 1% level

Concluding remarks

The genetic information accumulated during this study indicates that various breeding approaches could be adopted for developing thebaine-rich cultivars of *P. bracteatum*. Mass selection, using the thebaine content as main criterium and some other important agronomic traits should be practiced for few generations in populations with a large genetic variation in this character. This method was successfully applied in original Arya I and Arya II populations of the species and resulted in a uniform flowering cultivar with a relatively high thebaine content (Levy, & al., 1981). The heterotic effects shown in F_1 hybrids should be exploited for a further improvement of the thebaine yield and content. Using the shattering-resistant mutant as a parent in the development of hybrid cultivars should combine rapidly this character with the high thebaine yield in such cultivars.

We have shown also that polyploidy is of potential value for increasing the thebaine content in this species and that the perspectives for the genetic stabilisation of the polyploids are good. At last, the fertility of the interspecific hybrids offers the possibility of transferring genetic material between the species of the section oxytona; this approach might be used for improving mainly some agronomic characters of *P. bracteatum* such as low flowering during the first year of growth and lodging susceptibility.

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B. HORTICULTURAL STUDIES

Seed dormancy and germination

Introduction

The light and temperature conditions needed for the germination of the seeds of P. bracteatum were studied previously (Bare & al., 1978); it was found that in the dark, germination occurs under a wide range of temperatures, 12° - 32°C. Seed dormancy was reported for the species (Böhm, 1981) but treatments for breaking the seed dormancy were not reported. In the present study, the effect of temperature and gibberellic acid on the germination of dormant seeds were investigated.

Materials and methods

Seeds of an early flowering cultivar were used (Levy and Palevitch, 1982). The germination of one year old and of freshly harvested mature seeds was studied in growth chambers with controlled conditions of temperature and light. The fresh seeds were collected at physiological maturity when the pores of dehiscence below the stigmatic disc of the capsule were opened and shedding of the seeds had started; their color was dark brown at harvest.

In a first experiment, the germination of fresh and of one year old seeds was compared in the dark under three temperature regimes: 25°C constant; 25° - 15°C and 20° - 30°C; each temperature was applied for 12h. Six replications of 25 seeds each in a petri dish with moistened filter paper were made for each treatment. The onset of germination (radicle emergence) was scored every 5-7 days after start of imbibition.

In a second experiment, the effect of various concentrations of gibberellic acid (GA₃) on the germination of dormant seeds was studied. The seeds were germinated in the dark at 15° - 25°C in petri dishes (6 replications x 25 seeds for each treatment) with filter paper moistened with the GA₃ solutions; the germination of the seeds was determined as for the first experiment.

Results and discussion

Marked differences between the germination of the different seeds were observed. A high percent germination was obtained seven days after the start of imbibition of the one year old seeds under the different temperatures (71-92%), whereas for the the fresh seeds, germination started only 30 days after imbibition and reached a low percent (17-32%) after 40 days (Table 1). Under constant temperature of 25°C, no germination occurred in the fresh seeds 40 days after imbibition.

These results confirm the existence of a dormancy period in mature seeds of *P. bracteatum* (Böhm, 1981). This dormancy period might delay early sowing of the species resulting thus in a reduced flowering rate of the plants (Levy and Palevitch, 1982).

Table 1. Percent germination of fresh and one year-old seeds under different temperatures, 7 and 40 days after imbibition*.

Temperature regime	Fresh seeds		one year-old seeds	
	7 days	40 days	7 days	40 days
25°C-constant	0	0 ^c	78.0 ^b	94.7 ^a
15° - 25°C	0	32.0 ^a	71.3 ^b	94.7 ^a
20° - 30°C	0	17.3 ^b	92.0 ^a	95.3 ^a

* within each column, values followed by different letters differ significantly at 5% level.

GA₃ was reported to break seed dormancy in sesame and other crops (Ashri and Palevitch, 1979); it was therefore experimented at various concentrations on dormant seeds of *P. bracteatum* (Table 2).

Table 2. Effect of treatments with GA₃ on the germination of dormant seeds 30 and 45 days after imbibition.

Concentration of GA ₃ (ppm)	Percent germination	
	30*	45*
0	13.3c	16.0c
50	38.7a	56.7a
100	32.7a	48.7a
200	37.3a	58.0a
500	22.7b	30.7 b

* Days after imbibition; values followed by different letters differ significantly at 5% level

The treatments with GA₃ enhanced the germination of the seeds; at 50 ppm the percent germination was increased from 13% in the control to 39%, 30 days after the start of imbibition. Increasing the concentration of GA₃ to 500 ppm, reduced slightly the seed germination in comparison with the lower concentrations. Gibberellin treatment could therefore be a valuable tool for breaking the dormancy of freshly harvested seeds.

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DESCRIPTION OF COOPERATION

The cooperation between the Israeli (Dr. Levy and Dr. Lavie) and the US (Dr. Bills and Dr. Hsu) scientists was a fruitful and a complementary one; it included mutual exchange of ideas, information, plant and chemical materials as well as scientists.

Taking advantage of the presence of Prof. Lavie in the U.S., thorough discussions were conducted with the group in Philadelphia and the research activities of this project were divided between the two groups: the genetic and horticultural studies as well as the analyses of the alkaloids were carried out in Israel; the enzymatic and biosynthetic aspects of the morphine alkaloids were investigated in Philadelphia. Subsequently, Prof. Lavie, during a stay in the U.S., visited Dr. Bill laboratory, reported on the research activities and discussed the results already obtained.

While visiting Israel, Dr. An-Fei Hsu got acquainted with the field experiments carried out in P. bracteatum and the methodology of the chemical and breeding work; her findings on the effect of reducing agents on the biosynthesis of morphine alkaloids were used to initiate the study of their effect on thebaine production of P. bracteatum.

Comprehensive discussions on the results and scientific approaches of the two groups were conducted with Dr. Hsu when Dr. Levy visited the USDA Research Center; new ideas and lines of research on Papaver alkaloids were also exchanged.

EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH RESPECT TO THE ORIGINAL RESEARCH PROPOSAL

In line with the original research proposal, most of the proposed objectives were covered during the course of this research. The genetic and horticultural studies on Papaver bracteatum provided basic information for the breeding and cultivation of the species.

The biosynthetic studies provided a better understanding of the formation of morphine alkaloids and revealed several chemicals which might be used for altering the spectrum of this alkaloids in P. somniferum.

BENEFIT TO AGRICULTURE

The genetic studies on Papaver bracteatum provided a sound basis for the development of thebaine-rich cultivars with desirable agronomical characters.

- The isolation of a shattering-resistant mutant is a major step forward the introduction of P. bracteatum as an alternative crop to P. somniferum for the codeine industry. A true breeding line for the mutation is being developed.
- Parental clones for the production of hybrid cultivars with a high thebaine yield were identified.
- The induced tetraploid and the derived triploid plants are used for the building of clones and lines with a high thebaine content.
- Following the findings of the horticultural studies, appropriate cultural practices for P. bracteatum were elaborated and commercial plantations of the crop have been initiated.
- Various chemicals which might be used in plantations of P. somniferum to alter the spectrum of morphine alkaloids were identified

LIST OF PUBLICATIONS

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