Stability and Variability of Alkaloid Accumulation in Poppy (*Papaver somniferum* L.) Induced by Crossing

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Abstract

Five poppy cultivars of high chemical diversity 'Kheops', 'A1', 'Tebona', 'Kék Gemona' and 'Przemko' were selfed and crossed in 2000. The hybrid generations (F1) were grown in 2001.

When the castrated alkaloid free cultivar 'Przemko' (accumulating only 0.01 mg/g morphine in capsules) was pollinated with cultivars rich in alkaloids, the morphine content of its capsules increased to 0.9-7.5 mg/g values, even in the year of the crossing. The same phenomenon was observed in the F1 generation, however the appearance of alkaloids became more characteristic showing intermediate type of inheritance (4.8-6.4 mg/g morphine was accumulated). Based on the well known biosynthetic background of poppy alkaloids, this can be explained by the promotion of (S)-norcoclaurine synthesis, which seems to be suppressed in alkaloid-free plants, but both tissues of the developing hybrid seeds (in a form of chemical metaxenia) and the genetic combination in the F1 can release this suppression.

In particular, the appearance of narcotine in narcotine-free cultivars 'Kheops', 'A1' and 'Przemko' (0.1-1.1 mg/g in the year of crossing and 2.4 mg/g in F1 generation) can be explained similarly. If the narcotine-type plants 'Kék Gemona' (accumulating 7.0-11.3 mg/g narcotine) were used for pollination the developing seed tissue, or the genetic combination in the F1 might contribute to the suppression of the activity of 1,2-dehydroreticuline reductase, which leads to the accumulation of narcotine at the cost of morphinanes.

INTRODUCTION

The cultivation of poppy is going on world wide for both pharmaceutical and nutritional purposes (Bernáth, 1998). The increased pharmaceutical demand is the consequence of the widening of the medical application of morphine and related compounds (Fürst and Hosztafi, 1998). The consumption of morphine since the second half of the 1980s has increased steadily (Anonyms, 1999). However, to limit drug abuse in European countries in parallel with the administrative regulations, a new strategy was accepted in plant production. As a part of this new strategy, the creation of new cultivars with especially high alkaloid content (15.0-25.0 mg/g morphine) and alkaloid-free cultivars for seed production (accumulating less than 0.1 mg/g morphine in capsules) has been intensified (Bernáth and Németh, 1999).

In accordance with the above mentioned strategies, new cultivars of high alkaloid content ('Monaco', 'Tebona'), and a cultivar accumulating large amount of narcotine ('Kék Gemona') were selected by the experts of our Department. More recently, good results in construction of a morphine-free cultivar were achieved (Németh et al., 2002).

The availability of poppy cultivars of a high chemical diversity offered a new challenge to clear up the regularity of the inheritance of alkaloid accumulation. This type of knowledge might contribute to the construction of new cultivars of high practical importance as well as provide a good scientific background to make regulations and put the regulations into effect.

MATERIALS AND METHODS

The experiments were conducted in 2000 and 2001 at the Research Station of the Department of Medicinal Plant Production in Soroksár, Hungary. The experimental field has light sandy soil; NO_3 - 10.1 mg/kg; P_2O_5 - 328 mg/kg; K_2O - 470 mg/kg; K_A<30; pH - 7.6; humus: 1.5%.

The plant materials involved in the crossing experiments were as follows: a) cultivar 'Kheops' of French origin, having about 12.0-14.0 mg/g morphine content, b) Hungarian cultivar 'A1' of at least 10.0 mg/g morphine content, or more (with traces of codeine and thebaine), c) cultivar 'Tebona' accumulating all members of morphinane group (morphine, codeine and thebaine) at about 5.0-9.0 mg/g values each, d) cultivar 'Kék Gemona' accumulating large amount of narcotine (8.0-10.0 mg/g) and all members of the morphinane group in lower quantities, e) cultivar 'Przemko', which is known world wide as an alkaloid-free plant (morphine below 0.2 mg/g).

The seeds of cultivars were sewn in mid-March 2000 and the seeds of cultivars and hybrids were sewn in mid-March 2001 were sown in the middle of March. 50 cm row distance was applied, using 10 m^2 plot size for each cultivar and hybrid. During the vegetation period, nutrition of 100-100 kg/ha N, P_2O_5 and K_2O was applied as a top-dressing. Weeds were controlled with continuous mechanical weeding, and plantings were irrigated twice during the growing season.

In 2000 selfing and reciprocal crossings were managed. The castrated flowers were pollinated by pollen mixture of 5 individuals of the partner cultivar. The pollination was made by hand. Both the selfed and crossed flowers were isolated individually. In each combinations 35-40 plant individuals were evaluated.

Both the selfed and cross-pollinated plants in 2000 as well as hybrids in 2001 were individually harvested at the stage of full ripening. Plants having less than 0.1 g of seeds (about 5% of individuals) were excluded from the evaluation. The alkaloid profiles of capsules were determined (Németh et al., 2002). Measurement was carried out by densitometric analysis (CHR-SCAN TR-541 equipment with a LabChrom™ Chromatographic Data processing System Version 5.2). The densitometric scanning profiles of four alkaloids (morphine, codeine, thebaine and narcotine) were calibrated against the corresponding standards.

To make biometrical evaluation SPSS 9.0 program and the Tukey B test were used.

RESULTS

Based on the results the alkaloid content and composition of capsules of the mother plants were affected by crossing even in the first year of experiment (Bernáth and Németh, 2003). Similar changes were also observed in the F1 generation.

The effect of hybridization on maternal alkaloid accumulation was the most evident when the alkaloid free cultivar 'Przemko' was used as a female partner (Table 1). As an effect of pollination, when either the morphine rich cultivar 'Kheops' or cultivars 'Tebona' and 'Kék Gemona', characterised by a much wider spectrum of alkaloids, were used as pollen donors, the former alkaloid free character of 'Przemko' disappeared. The cultivar 'Przemko', depending on the chemical character of the male partner, accumulated all the members of the morphinane group (morphine, codeine and thebaine) as well as narcotine in the first year of experiment. In the reciprocal combination, when the pollen of cultivar 'Przemko' was used to fertilize no effect on the alkaloid accumulation of the three other cultivars as female partners was observed.

When the morphine rich cultivar 'Kheops' (accumulating only codeine in small amount in 2001) was pollinated either by morphine-codeine-thebaine ('Tebona') or narcotine ('Kék Gemona') chemotypes, the similar changes occurred (Table 2). Based on the results, chemical metaxenia manifested itself in the firs year of investigation, while an intermediate type of inheritance was detected in F1 hybrids.

The selfing and reciprocal crossing of the 'A1' cultivar of high morphine content and the accumulating traces of codeine and thebaine, resulted in similar changes

concerning the alkaloid spectrum (Table 3). Both the high morphine content and the ability of side alkaloid accumulation appeared in the combinations fertilized by cultivars 'Tebona' and 'Kék Gemona'.

DISCUSSION

Based on the results, we must distinguish between changes occurring in the first and second year of experiment. In the first year of crossing the change of alkaloid profile can be explained by manifestation of chemical metaxenia. "Secondary xenia" or "metaxenia" was first described by Swingle (1928, cit. Nyéki, 1980). Metaxenia is the manifestation of the pollen genotype in maternal fruit tissues after crossing. This manifestation may be caused by hormonal effects (enzyme release) produced by the seeds or may be traced back to differences in growth and development of the embryo, endosperm or seeds (Denney, 1992). In case of poppy (*Papaver somniferum* L.) the existence of metaxenic effects was published by us at first (Bernáth and Németh, 2003).

In the second year of our experiment the alkaloid profile of F1 generations was regulated by hybrid nature of plants. The appearance and accumulation level of alkaloids show an intermediate type of inheritance.

Both the metaxenia, and the change of alkaloid pattern in F1 generation may be explained by the recent result concerning alkaloid biosynthesis (Psenák, 1998). With regard to the alkaloid free plants, we have to consider the statements of Battersby et al. (1965) and Roberts et al. (1987), that the precondition of alkaloid accumulation is the continuous and undiminished flow of precursors. From this point of view the precursor flow between shikimate and norcoclaurine biosynthesis has to be evaluated as a critical flow (Fig. 1). The transformation of leading to (S)-norcoclaurine in particular seems to have great importance. According to Nessler (1998) this step is regulated by the TyDC/DOCC gene family, which was recently isolated. In poppy plants having no or small amount of alkaloids, the activity of this gene family seems to be lacking or suppressed. Even Nessler (1998) suggested that, if sufficient suppression of the TyDC/DODC gene family is achieved in vitro, it may be possible to recover plants which produce very low alkaloid levels or no alkaloids at all. Contrarily, by the overexpression of tyrosine decarboxylase activity, the accumulation of different alkaloid groups might increase with a rise in the tyramine and dopamine pool size. In the first year of experiment, with high probability, the alkaloid biosynthesis of alkaloid free cultivar is enhanced by overexpressed tyrosine decarboxylase activity released from the developing seed tissues that own the gene pool of both alkaloid free cultivars and that of the pollen donor plants rich in alkaloids. In the F1 generation the same type of action expected, the hybrid plants are able to produce sufficient amount of tyrosine decarboxylase for the continuous precursor flow.

The appearance of narcotine in narcotine free cultivars as a result of pollination (in the first year) and hybridization (F1) can be explained by the known steps of biosynthesis (Fig. 1). Reticuline, which is a key intermediate in the biosynthesis of many groups of poppy alkaloids is formed from dopamine and tyramine (Psenak, 1998). The first product, (S)-reticuline, proved to be the immediate precursor of the phthalideisoquinoline alkaloids including narcotine and narcotoline (Hosztafi, 2000). For the biosynthesis of the members of the morphinane group (morphine, codeine, thebaine, etc.) the conversion of (S)-reticuline to (R)-reticuline has to be managed. According to the scheme the reduction of 1,2-dehydroreticulinium ion to (R)-reticuline is catalysed by an NADP-dependent 1,2-dehydroreticuline reductase (De-Eknamkul and Zenk, 1992). From a practical point of view it means that the activity of NADP-dependent 1,2-dehydroreticuline reductase plays a key role in the further formation of poppy alkaloids. By depression of the enzyme activity the formation of phthalideisoquinoline alkaloids is suppressed, while by the acceleration of conversion of (S)-reticuline to (R)-reticuline the biosynthesis and accumulation of morphinanes are promoted. This biochemical conversion could play an important role in metaxenia, as well as in alkaloid pattern of F1 generation. The developing seed tissue after pollination and hybrid generation which contains the gene

pool of narcotine type plants might contribute to the suppression of the activity of 1,2-dehydroreticuline reductase and the accumulation of narcotine starts at the cost of morphinanes.

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Tables

Table 1. Alkaloid spectrum measured in capsules of the alkaloid free 'Przemko' cultivar and its hybrids ferilized by pollen of different chemotypes (completed by data of F1 generation - Bernáth and Németh, 2003).

a) Crosses with a cultivar of high morphine content

Cultivar	Alkaloid (mg/g)							
	In the maternal capsules				F1			
	Mo.	Co.	Th.	Na.	Mo.	Co.	Th.	Na.
'Przemko' x 'Kheops'	2.9*	0.5*	1.7*	-	6.4*	1.4*	2.1*	-
'Kheops' x 'Przemko'	10.4	0.5*	0.3*	-	5.1	0.4*	0.2	-
'Przemko'	0.0	-	-	-	0.0	-	-	-
'Kheops'	10.4	-	-	-	13.3	-	-	-

b) Crosses with a cultivar of morphine-codeine-thebaine type

Cultivar	Alkaloid (mg/g)							
	In the maternal capsules				F1			
	Mo.	Co.	Th.	Na.	Mo.	Co.	Th.	Na.
'Przemko' x 'Tebona'	7.5*	4.6*	4.7*	-	4.8*	2.6*	4.9*	-
'Tebona' x 'Przemko'	5.1	5.7	4.8	0.1	10.1	4.2	3.6	-
'Przemko'	0.0	-	-	-	0.0	-	-	-
'Tebona'	5.7	4.4	6.5	0.1	8.0	6.1	4.1	-

c) Crosses with a cultivar accumulating morphinane alkaloids and narcotine

Cultivar	Alkaloid (mg/g)							
	In the maternal capsules				F1			
	Mo.	Co.	Th.	Na.	Mo.	Co.	Th.	Na.
'Przemko' x 'Kék Gemona'	0.9*	0.4*	0.2*	0.1*	5.6*	1.1*	2.8*	-
'Kék Gemona' x 'Przemko'	10.1	5.1	4.9	6.8*	8.5	1.5	1.0	7.0
'Przemko'	0.0	-	-	-	0.0	-	-	-
'Kék Gemona'	7.8	4.2	3.7	11.3	8.5	1.5	1.0	7.0

*Significance on 0.5% level proved by Tukey B test among each cultivar and its corresponding progeny

Table 2. Alkaloid spectrum measured in capsules of the morphine type cultivar 'Kheops' fertilized by pollen of different chemotypes (completed by data of F1 generation - Bernáth and Németh, 2003).

a) Crosses with a cultivar of morphine-codeine-thebaine type.

Cultivar	Alkaloid (mg/g)							
	In the maternal capsules				F1			
	Mo.	Co.	Th.	Na.	Mo.	Co.	Th.	Na.
'Kheops' x 'Thebona'	10.5	0.2*	0.5*	0.1*	8.2*	1.9	1.3*	-
'Tebona' x 'Kheops'	6.5	6.7	5.1	0.3	6.4	1.9	4.5	-
'Kheops'	10.4	-	-	-	13.3	2.1	-	-
'Tebona'	5.7	4.4	6.5	0.1	8.0	6.1	4.1	-

b) Crosses with a cultivar accumulating morphinane alkaloids and narcotine.

Cultivar	Alkaloid (mg/g)							
	In the maternal capsules				F1			
	Mo.	Co.	Th.	Na.	Mo.	Co.	Th.	Na.
'Kheops' x 'Kék Gemona'	11.9	0.5*	0.5*	1.1*	8.7*	1.9	2.4*	2.4*
'Kék Gemona' x 'Kheops'	10.2	4.1	3.2	6.8*	8.4	3.7	3.2	0.2*
'Kheops'	10.4	-	-	-	13.3	2.1	-	-
'Kék Gemona'	7.8	4.2	3.7	11.3	8.5	1.5	1.0	7.0

*Significance on 0.5% level proved by Tukey B test among each cultivar and its corresponding progeny

Table 3. Alkaloid spectrum measured in capsules of the 'A1' cultivar of high morphine content (with traces of codeine and thebaine) fertilized by pollen of different chemotypes (completed by data of F1 generation - Bernáth and Németh, 2003).

a) Crosses with a cultivar of morphine-codeine-thebaine type.

Cultivar	Alkaloid (mg/g)							
	In the maternal capsules				F1			
	Mo.	Co.	Th.	Na.	Mo.	Co.	Th.	Na.
'A1' x 'Tebona'	10.6	4.2*	2.3*	-	6.2*	4.1*	4.2*	-
'Tebona' x 'A1'	9.2*	6.0	2.2	0.2	12.7*	4.3	2.0	-
'A1'	9.5	0.5	0.1	-	10.2	2.7	0.5	-
'Tebona'	5.7	4.4	6.5	0.1	8.0	6.1	4.1	-

b) Crosses with a cultivar accumulating morphinane alkaloids and narcotine.

Cultivar	Alkaloid (mg/g)							
	In the maternal capsules				F1			
	Mo.	Co.	Th.	Na.	Mo.	Co.	Th.	Na.
'A1' x 'Kék Gemona'	10.2	5.6*	4.8*	-	X	X	X	X
'Kék Gemona' x 'A1'	7.9	1.9	2.6	5.9*	7.1	2.1	3.4	3.1*
'A1'	9.5	0.5	0.1	-	10.2	2.7	0.5	-
'Kék Gemona'	7.8	4.2	3.7	11.3	8.5	1.5	1.0	7.0

*Significance on 0.5% level proved by Tukey B test among each cultivar and its corresponding progeny

Figures

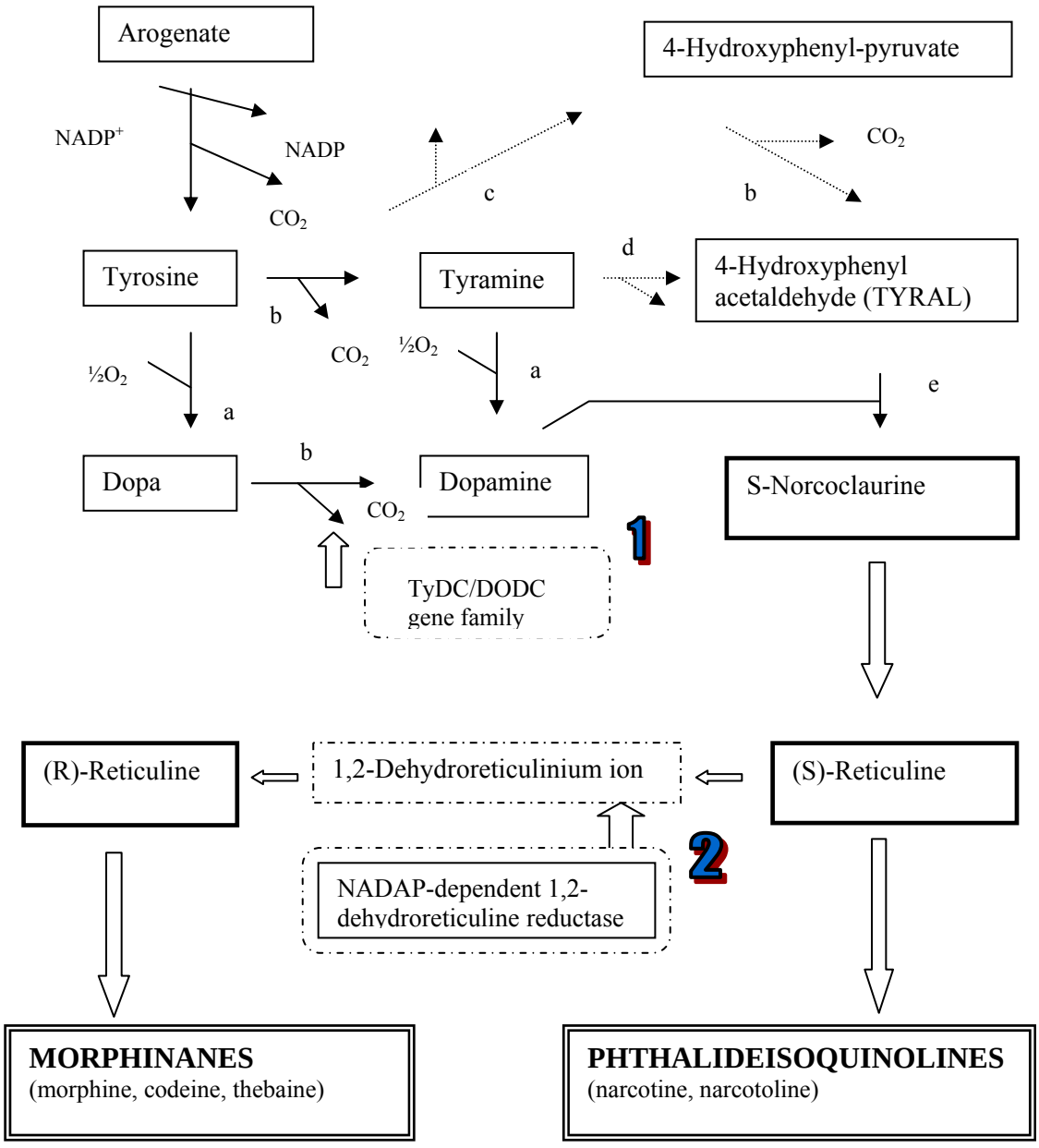

Fig. 1. Critical steps in biosynthesis of poppy alkaloids: 1. Decarboxylation step of biosynthesis which play an important role in formation of first alkaloid, S-Norcoclaurine. 2. Conversation of (S)-reticuline to (R)-reticuline having great importance in alternative, or parallel formation of morphinane and phthalideisoquinoline alkaloid groups. Enzymes participating in the reaction: a) phenoloxidase, b) decarboxylase, c) transaminase, d) amine oxidase, e) norcoclaurine synthase.