New trends in selection of poppy (Papaver somniferum L.)

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Summary: Since the isolation of morphine by Sertürner in 1805 more than 40 alkaloids have been isolated from the poppy *(Papaver somniferum L.)*. Some of them have high biological-pharmacological activity and economical importance, while others have none, or restricted ones. The increasing demand for poppy alkaloids is the consequence of the widening of the medical application of morphine and its related compounds: the quantity of morphine used for the treatment of pain reached a record level of 17.9 tonnes in 1997, compared with an annual average of 2.2 tonnes used during the period 1978-1983. However, the production of raw material (either opium, or dried capsule is produced) has to be re-evaluated taking into consideration the UN Convention signed in 1988 against Illicit Traffic in Narcotic Drugs and Psychotropic Substances. The countries were forced by the Convention to introduce new arrangements in poppy production including selection and introduction of new cultivars.

In the present work up to date results of poppy selection are reviewed explaining the biosynthetic and eco-physiological background of their alkaloid accumulation. The effectiveness and the possibilities of traditional selection methods as well as the probability of the application of biotransformation for producing cultivars accumulating low or high alkaloid content or plant material with special alkaloid spectrum (codeine, thebaine, narcotine) are discussed. The examples of Hungarian cultivars 'Monaco', 'Kék Gemona' and 'Tebona' are given in more detail.

Introduction

Poppy is one of the medicinal plant species which was cultivated, even in the prehistoric times (*Bernáth,* 1998). The utilisation of poppy was spread in Europe by the help of Roman Empire, and became an important alimentary and medical crop up to now. However, the opium was used by physicians all over the centuries the large scale manufacturing of morphine started in Europe in the 19th century, only. At the beginning, the industrial production was based on the raw opium of Turkish, Persian and Indian origin. In the first half of the 20th century a remarkable advance was made obtaining morphine and related compounds from the dried capsule (*Mórász,* 1979). The invention of the Hungarian pharmacist *Kabay* in 1928 opened a new perspective for the plant and for its industrial utilisation. His method resulted enormous changes in the world profile of opiate alkaloid production: the importance of countries characterised by traditional opium production decreased, and the dominance of the countries specialised for dry capsule production and processing became obvious, especially in the temperate zone (*Bryant,* 1988). Since the isolation of morphine by *Sertürner* in 1805 more than 40 alkaloids have been isolated from the plant. Some of them have high biological efficacy and economical importance, while others have none or restricted ones (*Fürst & Hosztafi, 1998*). The increasing demand for poppy alkaloids is the consequence of the widening of the medical application of morphine and related compounds. The consumption of morphine since the second half of the 1980s has been increased steadily (*Anonymus,* 1999). The quantity used for the treatment of pain reached a record level of 17.9 tonnes in 1997, compared with an annual average of 2.2 tonnes used during the period 1978-1983. The same tendency can be observed in consumption of a morphine related compound dihydrocodeine. Its global consumption is growing up steadily, too, passing over a new record level of 30 tonnes in 1997. The demand for many other opiates shows the same feature.

The production of poppy for both food and pharmaceutical purposes has to be re-evaluated taking into consideration the international efforts to limit drug abuse. Based on the United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances signed in 1988 a new strategy has been worked out by the countries interested in the production and processing of poppy and its products (*Anonymus,* 1995). This Convention forced the countries not only for controlling their cultivation methods and patrolling cultivation areas, but to build up a new strategy for making cultivars. According to the result of the last two decades the breeding of poppy was intensified in the main directions, which are as follows:

a) Selection of cultivars for seed and oil production, accumulating low level of alkaloids in the capsules (the amount of morphine is about 0.01% or less),

b) Selection of plant material for high opium yield for industrial extraction,

c) Creating cultivars accumulating high morphine (1.5–2.0%), or special alkaloid content (narcotine, codeine, thebaine e.t.c.) for industrial extraction,

d) Producing ornamental types with special flower or capsule form, accumulating restricted amount of alkaloids.

In the present work the biosynthetic and eco-physiological background, as well as the results of the above mentioned selection activity are reviewed.

Modification of the flow of primary precursors of benzylisoquinoline skeleton

It was proved, even by the early investigation (*Battersby et al.*, 1965) that the formation and accumulation of poppy alkaloids is depending on the activity of the primary processes. From point of view of the regulation of the alkaloid accumulation the pathway between shikimate and norcoclaurine biosynthesis has to be evaluated and reviewed at first (*Fig 1*). In this early stage of alkaloid biosynthesis the enzymatic transformation of tyrozine to dopamine and 4-Hydroxyphenil-acetaldehyd (TYRAL) seems to have a characteristic role.

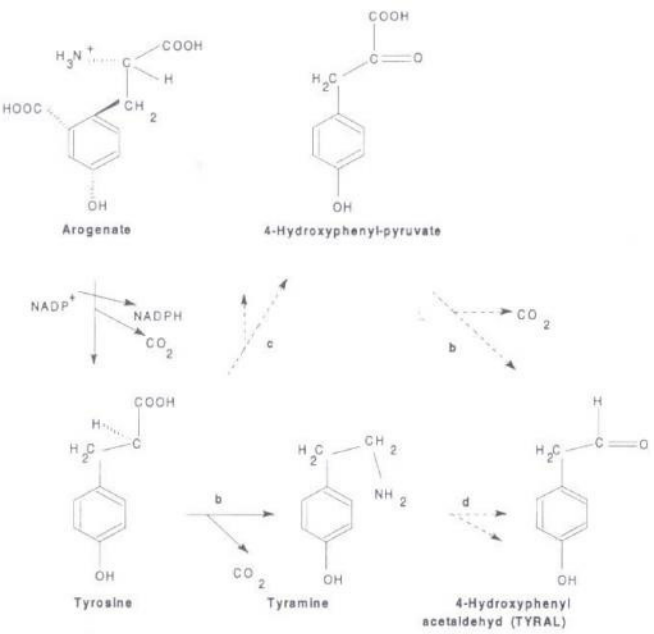

Figure 1 Biosynthesis of the primary precursors of poppy alkaloids with indication of biosynthetic step (tyrozine, tyramine decarboxylation) which can be the target of genetic modification (based on Psenak 1998)
Enzymes participating in the reaction: a) phenoloxidase, b) decarboxylase, c) transaminase, d) amine oxidase, e) norcoclaurine synthase

Nyman and *Hall* (1976) suggested the necessity of the modification of alkaloid biosynthesis for producing cultivars either with high or low alkaloid content at first. In accordance with the up to date biochemical and biotransformation results the genetic manipulation of poppy might be managed (*Nessler,* 1998). From this point of view the tyrosine transformation leading to (S)-norcoclaurine seems to have a great importance. The TyDC/DODC gene family, which was isolated by *Nessler* (1998) and his co-workers recently, is responsible for these steps. Experiments are currently underway in their laboratory to overexpress tyrosine decarboxylase in transgenic poppies to determine which subclass of alkaloid might increase with a rise in the tyramine and dopamine pool size. Overpressing tyrosine decarboxylase activity, the accumulation of different alkaloid groups might increase with a rise in the tyramine and dopamine pool size. In contrary, if sufficient suppression of the TyDC/DODC gene family is achieved it may even be possible to recover plants which produce very low alkaloid levels or no alkaloids at all.

It will be obviously a more sophisticated method in the development of cultivars with low alkaloid content, than the traditional ones, where the genetical point of intervention is unknown and therefore more uncertain. However, several efforts and also some results have been reported in this context in the last years (*Straka et al.*, 1993, *Dobos,* 1996). This selection procedure should be very time consuming, based on the polygenic heritability of this feature (*Nothangel et al.*, 1996). The procedure seemed to be more effective if individuals with modified tissue structure were selected. In the well known alkaloid poor Swedish cultivar 'Soma' the absence of alkaloids is the consequence of the degeneration of laticiferous vessel system, which is normally present in the capsules (*Straka et al.*, 1993). The absence of alkaloids in that case can not be explained by modification of alkaloid biosynthesis but the lack of special tissue-structures necessitated either for formation or accumulation of alkaloids (*Bernáth*, 1985).

Conversion of (S)-reticuline to (R)-reticuline and its importance in selection of cultivars with special alkaloid spectrum

Reticuline, which is a key intermediate in the biosynthesis of many groups of the poppy alkaloids, are formed from dopamine and TYRAL being catalysed by a sequence of five enzyme reactions (*Psenak,* 1998). As a result of the revision of the pre-reticuline pathway, some of the enzymes had to be renamed. The five enzymes involved into the conversation based on the up to date biochemical results are as follows, properly: (S)-norcoclaurine synthase, (S)-norcoclaurine-6-O-methyltransferase, tetrahydrobenzyl-isoquinoline-N-methyltransferase, polyphenoloxidase and (S)-coclaurine-N-methyltransferase.

The (S)-reticuline, which was isolated not only from poppy (*Zenk*, 1985) but other related species, like *Annona reticulata* (*Gopinath et al.*, 1959) and *Berberis* spp. (*Zenk*, 1985) is the starting product of this sequence of biosynthetic pathway (*Fig. 2*). (S)-reticuline proved to be the immediate precursor of the phtalideisoquinoline alkaloids, including narcotine and narcotoline (*Hosztafi*, 2000). For the biosynthesis of the members of morphinane group (morphine, codeine, thebaine e.t.c.) the conversion of (S)-reticuline to (R)-reticuline has to be managed. According to the shame given in *Fig 2.* 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). It is also an interesting fact that this enzymatic reaction is a very specific one being present in plants, only containing morphinane alkaloids (*Psenak*, 1998). From practical point of view it does mean 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 phtalideisoquinoline alkaloids, while by the acceleration of conversion of (S)-reticuline to (R)-reticuline the biosynthesis and accumulation of morphinanes are promoted.

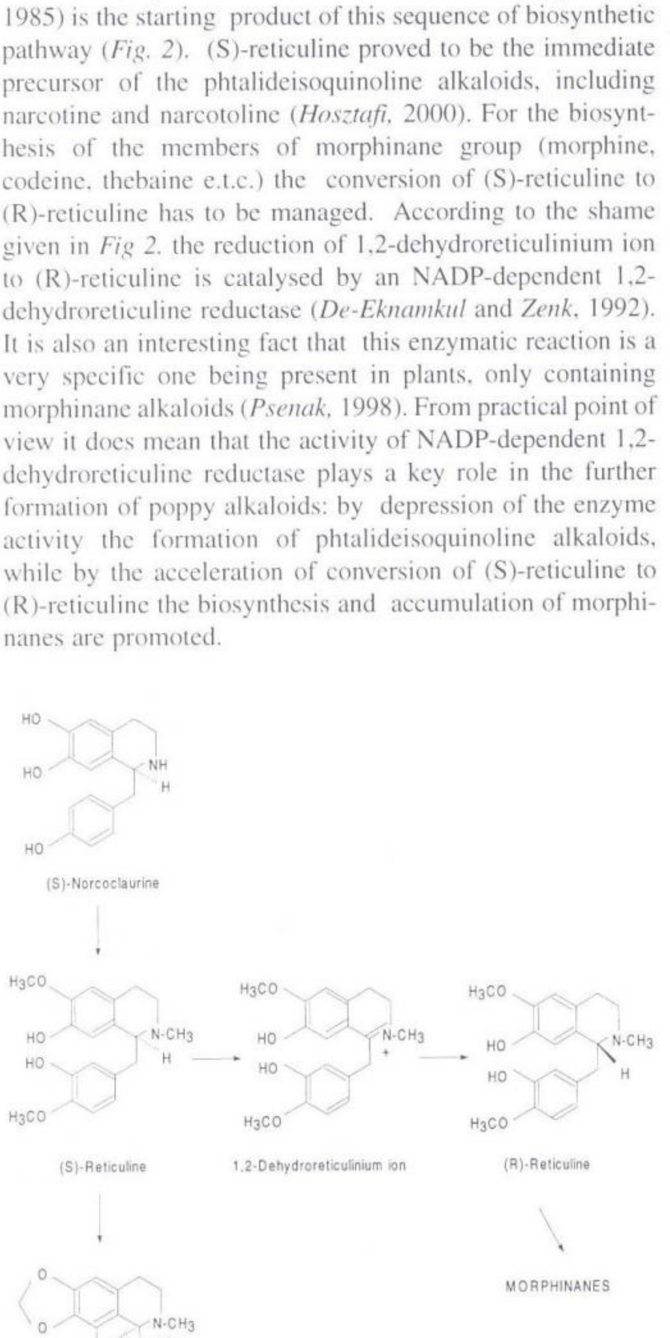

Figure 2 Conversation of (S)-norcoclaurine to (S)- and (R)-reticuline, being precursors of formation of phtalideisoquinoline and morphinane alkaloids

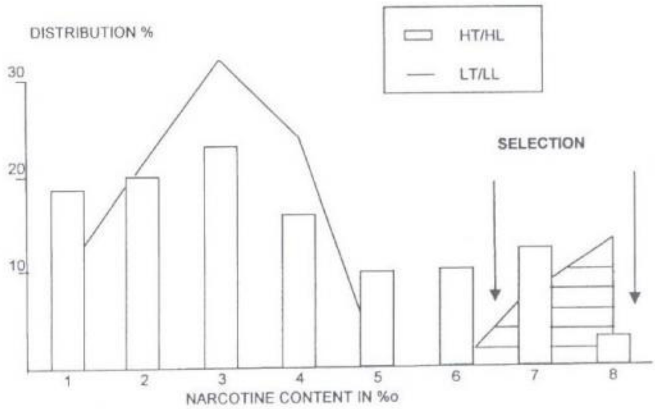

Figure 3 Changes of distribution of narcotine content affected by "cold" (LT/LL) and "warm" (HT/HL) programs in phytotron (*Bernáth* 1993)

Analysing the poppy collections from chemical point of view it became obvious that some population can be characterised by relative stable presence of phtalideisoquinoline alkaloids (*Tétényi*, 2000). In these populations the conversion of (S)-reticuline to (R)-reticuline going on partially, only. Based on this observation the selection of cultivars with special alkaloid spectra, accumulating narcotine as a main compound were started by us (*Bernáth*, 1993). However, it was obvious that the accumulation level of narcotine in the capsule changes from year to year. In the pre-selected population the accumulation of narcotine in mean of individual values was 3–5 ‰ in one vegetation cycle, while less than 1 ‰ in another one. Our intention was to stabilise the narcotine accumulation character. As a first step populations were tested under natural and two different conditions programised in phytotron. The narcotine distribution of individuals grown under two different phytotron programme is shown in *Fig 3.* By the data we have got continuous distribution of narcotine accumulation under appropriate ecological condition programming high light intensity and high temperature (HT/HL). As result of "cold and low illumination" (LT/LL) the population separated into the narcotine poor and narcotine rich sector with 2 ‰ and 8 ‰ maximum values. Taking into consideration the result of our earlier experiment (*Bernáth et al.*, 1988) and former crossings as model investigations (*Kálmán-Pál et al.*, 1987), plants with stable narcotine accumulation could be expected only by selecting individuals from the 6–8 ‰ intervals of LT/LL programme. Under this condition the low temperature and low illumination act against the overall alkaloid biosynthesis and accumulation (negative environmental pressure). Because of that fact the presence of narcotine postulates some kind of dominance. The selection process – in the course of 5 generations interrupting by another phytotron screening – resulted continuous increase and high stability in narcotine accumulation. The changes of quantitative values measured under natural conditions as a result of selection process are presented in *Fig 4.* It is obvious that the narcotine content rose from 6 ‰ up to 8.1–8,7 ‰. The changes of distribution of narcotine values – as a result of

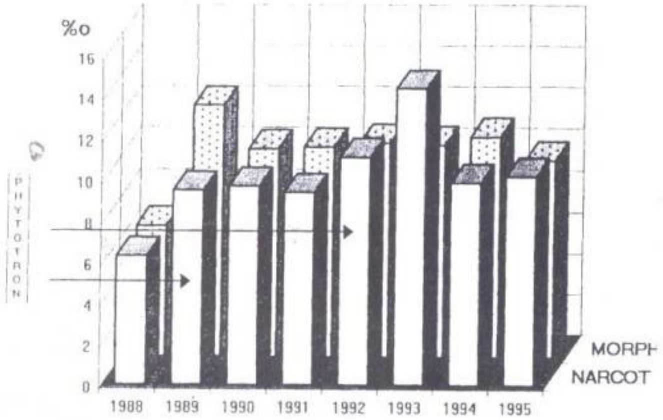

Figure 4 Changes of narcotine and morphine content as a result of selection process measured in plants grown under natural conditions MORPH=morphine, NARCOT=narcotine

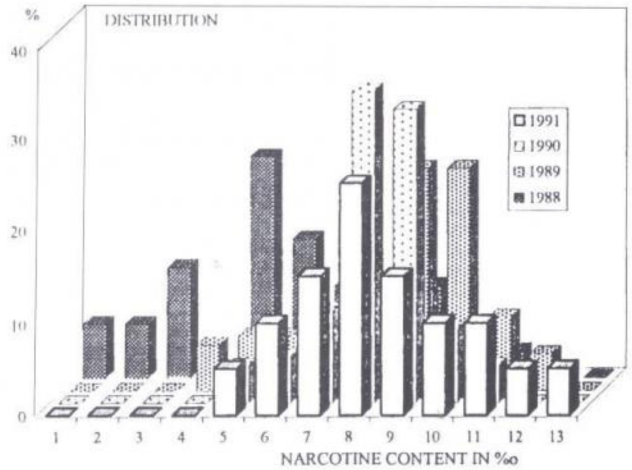

Figure 5 Changes of distribution of narcotine in poppy capsules as a result of the selection in the period 1988-1991 *(Bernáth 1993)*

selection in five generations – show a characteristic phenomenon, too. According to the data of *Fig 5,* the original narcotine content ranged between 1 and 11 ‰ under open field conditions. After the first selection process made in phytotron (1988) the amplitude of flexibility had been moderated showing 4 and 12 ‰ extremes. Till the final stage (1991) more advance had been achieved moving the distribution values to the direction of higher accumulation levels with acceptable limits (min 5 ‰, max 13 ‰). The population was registrated by Hungarian authorities as a cultivar under the name 'Kék Gemona' (Gemona =Genetically producing alkaloids morphine and narcotine).

Biosynthetic steps leading to the formation of thebaine the first representative of morphinane group

The conversation of (R)-reticuline to salutaridine (*Fig 6*), which is stated to be the first step of thebaine formation, is catalysed by salutaridine synthase (*Gerardy & Zenk,* 1993). Results of inhibitory studies indicated that salutaridine synthase is a cytochrome P-450 enzyme and its highest activity has been found in shoots and roots of three-month old poppy (*Psenak,* 1998). The reduction of salutaridine to 7-(S)-salutaridinol is the second step of the conversion. The last step of the conversion is the spontaneous transformation of 7-O-acetylsalutaridinol to thebaine, which is based on the previous acetyl-coA-salutaridinol acetyltransferase catalysed reaction (*Lenz & Zenk,* 1994).

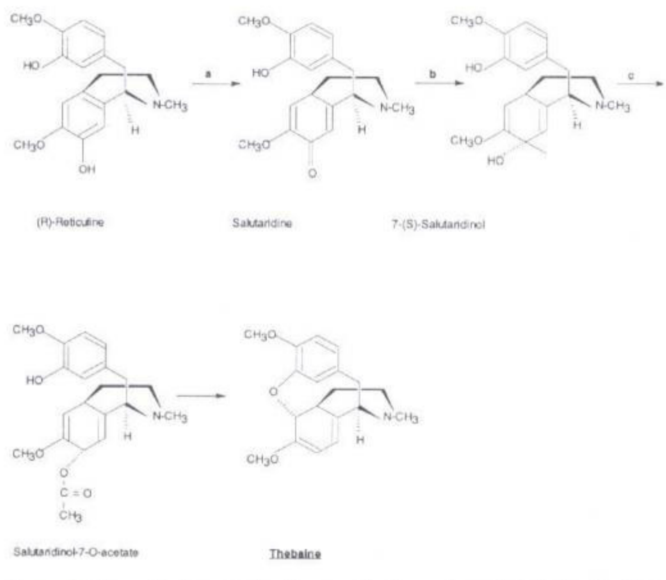

Figure 6 Biosynthetic steps leading to the formation of thebaine the first representative of morphinane group
Enzymes participating in the reaction: a) salutaridine synthase, b) salutaridine/NADP 7-oxidoreductase, c) acetyl-Co A/ salutaridinol 7-o-acetyltransferase

This special segment of biosynthetic route of poppy alkaloids, resulting thebaine accumulation, has a practical importance as well. World-wide efforts were invested during the 1970s to domesticate one member of genus *Papaver* (*Papaver bracteatum* Lindl.) in which thebaine was the end product. The chemical race of *P. bracteatum* ('Halle III.') which contained thebaine as predominant alkaloid (98 % of total alkaloids) was first reported by *Neubauer* (1965) who found a thebaine content in the roots of 0.7–1.3 % of dry matter. The thebaine was proved to be the end product in other *P. bracteatum* populations, too, grown in Alborz mountains in North Iran (*Shargi* and *Lalezari* 1967). In 1974, a populations called 'Arya II' with a thebaine content of 3.6 % of dry mater, was found in Western Iran by *Lalezari et al.* (1974) and drawn afterwards into the selection work. Based on their results the introduction of *P. bracteatum* in many countries in Europe, as well as in US was initiated.

In the case of *P. somniferum* no chemical race was found, accumulating thebaine as a main final product. In spite of this fact that pharmaceutical industry was interested in having such a cultivar (thebaine can be converted into codeine and morphine easily, *Hosztafi,* 1998). Motivating by this fact selection of population accumulating thebaine on a higher level were started by us *(Bernáth,* 1998). The selec-

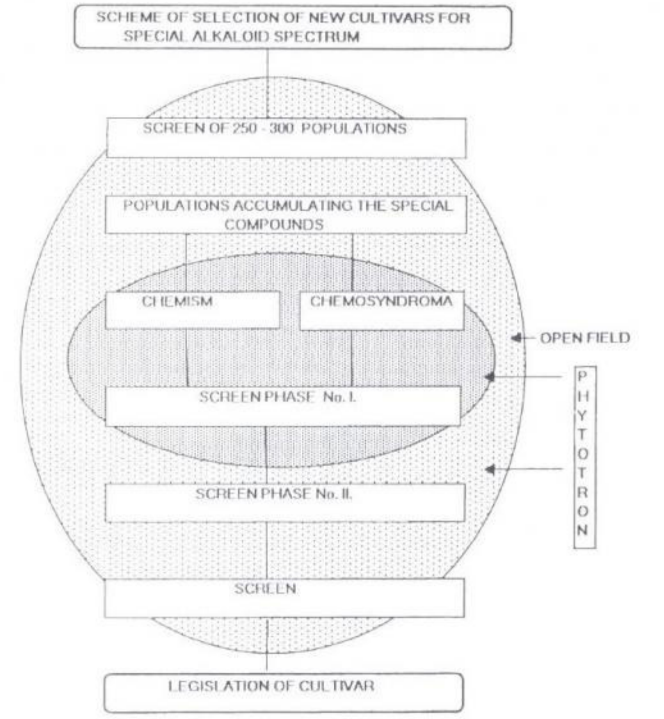

Figure 7 The selection shame applied for creation cultivars of special alkaloid spectra based on combination of open field selection as well as application of "environmental pressure" in phytotron

tion scheme, which was used similarly for getting narcotine type before *(Fig 7)*, was proved to be effective in the case of thebaine, too. As a result of the selection programme a new cultivar was given for the national registration process. The new cultivar characterised by intensive accumulation of thebaine is supposed to be registrated in 1999 in Hungary under the name 'Tebona'.

Biosynthetic background of morphine and codeine accumulation in capsules

Since the beginning of the industrial processing of poppy straw the increase of morphine content in the capsule stood in the centre of the interest of different working groups (*Mórász*, 1979). According to the latest results (*Fig 8*) thebaine is converted to morphine via neopinone, codeinon and codeine (*Battersby et al.*, 1967, *Blaschke et al.*, 1967, *Parker et al.*, 1972). Recently, the presence of the second alternative pathway from thebaine to morphine formation was proved via oripavine (*Brochmann-Hansen*, 1984). The in vitro enzymatic reduction of codeinone to codeine was first observed in crude enzyme extracts isolated from poppy cell cultures (*Furuya et al.*, 1978) and differentiated poppy plants (*Hodges & Rapoport*, 1980). The probability to increase the morphine level in the capsule, being the final product of conversion process leading from thebaine to morphine is supported by many theoretical and practical results. The effectiveness of the individual and mass selection is based on the well known chemical and morphological diversity of poppy populations (*Handera*, 1996). In spite of the former statements (*Kiskéri et al*,. 1977), that the traditional selection methods should fail in the future, the effectiveness of selection is proved by many authors, even nowadays (*Hörömpöli*, 1998, *Dobos*, 1996, *Dobos & Vetter*, 1997, *Kaicker*, 1985, *Bernáth & Zámboriné*, 1996). There are many publications indicating, that the accumulation of morphine can be accelerated by both intra- and interspecific crossings. The morphine accumulation level was increased by *Levy & Milo* (1998) using Pedigré-method, while *Lörinczné & Tétényi* (1970) reached this goal by interspecific reciprocal crossing of two species (*P. somniferum & P. orientale*). There are other publications reporting about the probability of producing hybrids (*Mórász*, 1979, *Patidar*, 1994, *Sharma et al.*, 1997) for increased morphine accumulation. However, because of the high expenses of hybrid propagation material the production of the synthetic cultivars is more frequent, especially in developing countries (*Khanna & Shukla*, 1989). By this method the production of propagation material is managed by selecting appropriate genotypes following the recurrent or reciprocal-recurrent crossing (*Heltmann & Silva*, 1978).

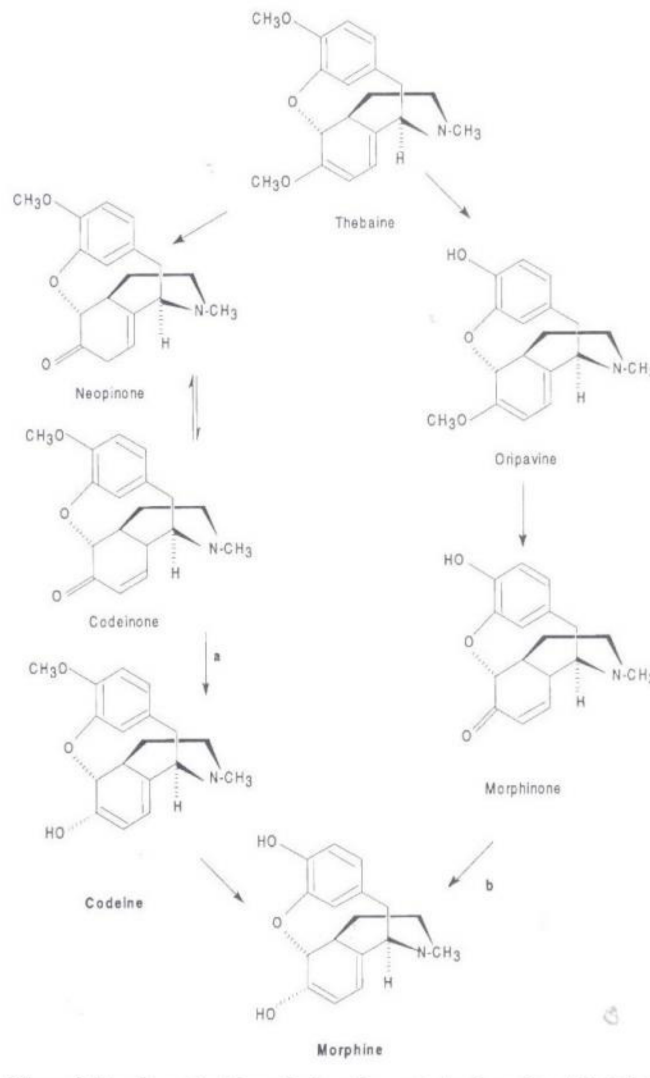

Figure 8 The alternative biosynthetic pathways in the formation of thebaine to morphine via neopinone and oripavine (based on Psenak 1998)
a) –b) codeinone reductase

Cultivars accumulating codeine, which alkaloid can be utilised by pharmaceutical industry without any conversion, came into the centre of interest, recently. However, the accumulation process of codeine seems to be a more sophisticated one comparing to the morphine accumulation. It can hardly be explained by the biosynthetic pathway, only, given in *Fig 8*. The presence or absence of the codeine in the capsule is depending on both the activity of enzymes responsible for demethylation as well as the intensity of the precursors' flow. This later phenomenon was justified by us investigating different poppy cultivars in phytotron experiments (*Bernáth et al.*, 1988). As it is demonstrated by the data of *Fig 9* the alkaloid accumulation in both Hungarian ('Kompolti M') and English ('Reading') cultivars is promoted by the more intensive light illumination. Whilst the increase of total alkaloid content as a result of increasing light intensity seems to be a general phenomenon, cultivar differences occur in the ratio of components. In the case of 'Kompolti M' – at the low light intensity level resulting low capsule weight and low total alkaloid content – the morphine provides 100% of the morphinanes. The first significant accumulation of codeine occurs at 16 klx, but more significant accumulation can be seen at 32 klx. The accumulation process in the English cultivar ('Reading') shows a different picture. The amount of morphine increases to a limited level, whilst the biosynthesis and accumulation of codeine occurs continuously. This phenomenon can be explained by the biosynthetic pathway of alkaloids. The acceleration of the total alkaloid formation under influence of high light intensity can be explained by the intensification of overall precursor flow and conversion processes. Similarly, the low alkaloid content at low light intensity seems to be interpretable by the absence of precursors and by the inhibition of methylation. The proportional change of components can be considered as a specific process being dependent on cultivars. The precursors of the morphine group are formed in a small amount in shortage of light and are able to demethylate without any difficulties almost up to morphine. On the contrary, a larger amount of precursors can be only partially demethylated when the overall alkaloid biosynthesis is promoted by light, and result in the accumulation of codein, as intermedier. In the case of certain cultivars these changes show different pictures which can be appreciated from chemotaxonomic point of view. At the same time it offers the genetical differences, which can be utilized in breeding.

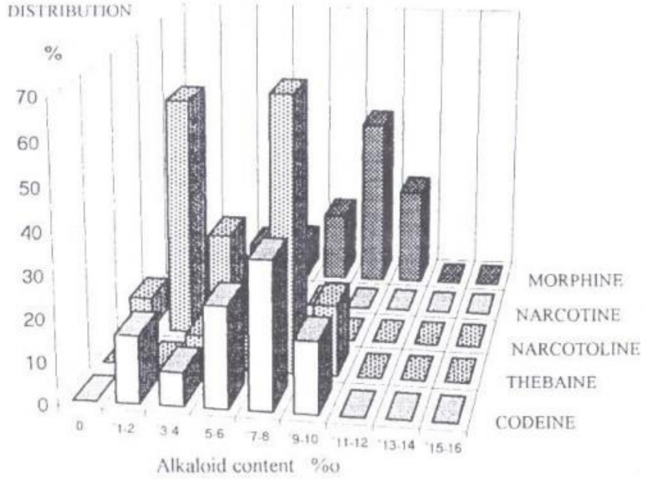

Figure 10 Characteristic alkaloid spectrum of cultivar 'Monaco' selected for intensive accumulation of codeine

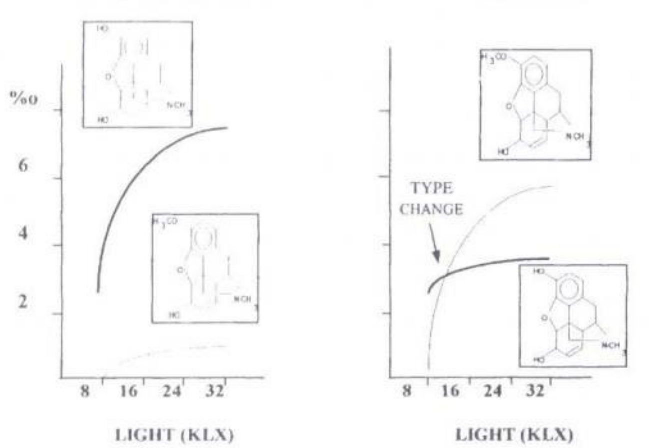

Figure 9 Ecological and chemotaxonomical differences in morphine and codeine saturation curve of cultivars 'Kompolti M' and 'Reading' affected by changing light conditions (*Bernáth* 1998)

The presence of the above mentioned chemotaxonomic races made our investigations hopeful to create a new cultivar with relative high accumulation of codeine. Combining the open field and phytotron selection method described in *Fig. 7* our efforts succeeded. Starting with about 250–330 population of UN origin a new cultivar accumulating relative high amount of codeine was selected and registrated by us under the name 'Monaco' (Monaco = accumulating Morphine, narcotoline (in small amount) and codeine). The cultivar, which alkaloid spectrum is demonstrated in *Fig. 10* is cultivated in Hungary on about 2000–3000 ha, yearly.

References

Anonymus (1995): Effectiveness of the international drug control treaties. Supplement to the Report of the International Narcotic Control Board for 1994. New York (Vienna), UN.

Anonymus (1999): Narcotic drugs. Estimated world requirements for 1999 – statistics for 1999. INCB, UN, New York

Battersby, A. R., Foulkes, D.M., Binks, R. (1965): Alkaloid biosynthesis, Part VIII. J.Chem Soc. 3323–3332.

Battersby, R.A., Martin, J.A., Brochmann-Hansen, E. (1967): Alkaloid biosynthesis, part X. Terminal steps in the biosynthesis of the morphine alkaloids. J.Chem.Soc. (C), 1785–1788.

Bernáth J. 1985: Speciális növényi anyagok produkció-biológiája. Doktori disszertáció, MTA, Budapest.

Bernáth J. 1993: Phytotron as a tool for speeding up selection of poppy (*Papaver somniferum* L.). Acta Horticulturae, 330, 177–184.

Bernáth J. (1998): Poppy – The Genus Papaver. Harwood Academic Publisher, Amsterdam, p. 352.

Bernáth J., Zámboriné Németh É. (1996): A mák *(Papaver somniferum* L.) populációk kemizmusának feltárása, mint a hatékony fajtanemesítés alapja. Növénynemesítési Tudományos Napok Összefoglalói, Jan. 22–23. Budapest, MTA, 20.

Bernáth J., Dános B.V., Veres T., Szántó J., Tétényi P. (1988): Variation in alkaloid production in poppy ecotypes: responses to different environments. Biochem. Syst. Ecol., 16, 171–178.

Blaschke, G., Parker, H.I., Rapoport, H. (1967): Codeinone as the intermediate in the biosynthetic conversion of thebaine to codeine. J.Am.Chem.Soc., 89, 1540–1541.

Brochmann-Hansen, E. (1984): A second pathway for the terminal steps in the biosynthesis of morphine. Planta Med., 50, 343–345.

Bryant, R.J. (1988): Fifth SCI process development symposium. Chemistry and Industry, Birmingham (7 March 1988), 146–153.

De-Eknamkul, W., Zenk, M.H. (1992): Purification and properties of 1,2-dehydroreticuline reductase from Papaver somniferum seedlings. Phytochemistry, 31, 813–821.

Dobos G. (1996): Winter poppy- a new genotype for seed production. Beitrage zur Züchtungforschung, 2, (1), 37–40.

Dobos G., Vetter S. (1997): Variation des Morphingehaltes bei Wintermohn-Herkünften *(Papaver somniferum* L.) Z. Arzn. Gew. Pfl., 2, 87–89.

Fürst Zs., Hosztafi S. (1998): Pharmacology of poppy alkaloids. in: Bernáth J. 1998: Poppy – The Genus Papaver. Harwood Academic Publisher, Amsterdam, 291–318.

Furuya, T., Nakano, M., Joshikawa, T. (1978): Biotransformation of (R,S)-reticuline and morphinane alkaloids by cell cultures of Papaver somniferum. Phytochemistry, 17, 891–893.

Gerardy, R., Zenk, M.H. (1993): Formation of salutaridine from (R)-reticuline by a membrane-bound cytochrome P-450 enzyme from Papaver somniferum. Phytochemistry, 32, 79–86.

Gopinath, K.W., Govindachari, T.R., Pai, B.R., Viswanathan N. (1959): The structure of reticuline, a new alkaloid from Annona reticulata, Chem. Ber., 92, 776–779.

Handera, D. (1996): Variability of quantitative characters in some land races of Papaver spp. Beitrage zur Züchtungsforschung, 2, (1) 127–129.

Heltmann H., Silva F. (1978): Zur Züchtung leistungfahiger Inzuchtlinien für eine synthetische Mohnsorte. Herba Hung., 17, (2) 55–60.

Hodges, C.C, Rapoport, H. (1980): Enzymatic reduction of codeinone in vitro cell-free systems from *Papaver somniferum* and *Papaver bracteatum*. Phytochemistry, 19, 1681–1684.

Hörömpöli T. (1998): Új, fehérmagvú mákfajta, a KP-Albakomp. Lippay J. és Vas K. Tudományos Ülésszak, Budapest, Szept. 16–17. Összefoglalók, 134–135.

Hosztafi S. (1998): Chemical structures of alkaloids, in Bernáth J.: Poppy – The Genus Papaver. Harwood Academic Publisher, Amsterdam, p. 352.

Hosztafi S. (2000): A mákalkaloidok bioszintézise. in: Sárkány S., Bernáth J. and Tétényi P. A mák. Magyarország Kulturflórája, Akadémiai Kiadó, Budapest (in press).

Kaicker, U.S. (1985): Opium and breeding research at Indian Agricultural Research Institute. Proceedings of Vth ISHS Symp. on Med. and Arom. Plants, Darjeeling, India, 75–81.

Kálmán-Pál Á., Bernáth J., Tétényi P. (1987): Phenotypic variability in the production and alkaloid spectrum of *Papaver somniferum* L. hybrid. Herba Hungarica 26, (2–3) 75–82.

Khanna, K.R., Shukla, S. (1989): Gene action in opium poppy *(Papaver somniferum)*. Indian J. of Agricult. Sci., 59, 124–126.

Kiskéri R-né, Mórász S., Bócsa I. (1977): Kísérletek tetraploid mák *(Papaver somniferum* L.) előállítására. Növénytermelés, 26, (5) 347–354.

Lalezari, I.A., Nasseri-Nouri, P., Asgharian R. (1974): *Papaver bracteatum population* Arya II. J. Pharm. Sci., 63, 1331.

Lenz, R., Zenk, M.H. (1994): Closure of the oxide bridge in morphine biosynthesis. Tetrahedron Lett., 35, 3897–3900.

Levy, A., Milo, J. (1998): Genetics and breeding of *Papaver somniferum*, in: Bernáth J. 1998: Poppy – The Genus Papaver. Harwood Academic Publisher, Amsterdam, 93–104.

Lőrincz Gy-né, Tétényi P. (1970): Távoli keresztezések módszerével végzett máknemesítések eredményeiről. Herba Hungarica, 9, (3) 79–85.

Mórász S. (1979): A mák termesztése. Mezőgazdasági Kiadó, Budapest, pp. 80.

Nessler, C. L. (1998): In vitro culture technologies. in: Bernáth J. 1998: Poppy – The Genus Papaver. Harwood Academic Publisher, Amsterdam, p. 209–217.

Neubauer D. (1965): Biosynthesis of thebaine from tyrosine in *Papaver bracteatum*. Arch. Pharm., 298, 747–741.

Nothangel, T., Straka, P., Schultze, W. (1996): Selection of low morphine poppy Papaver somniferum L. Beitrage zur Züchtungforschung, 2, (1) 120–123.

Nyman, V., Hall, O. (1976): Some varieties of *Papaver somniferum* L. with changed morphine alkaloids. Hereditas, 84. 69–76.

Parker, H. I., Blaschke, G., Rapoport, H. (1972): Biosynthetic conversion of thebaine to morphine. J. Am. Chem. Soc.,94, 1276–1282.

Patidar, H. (1994): Hybrid vigour in opium poppy. Indian J. of Genetics and Breeding, 54, (4) 395–397.

Psenak, M. (1998): Biosynthesis of morphinane alkaloids. in: Bernáth J. 1998: Poppy – The Genus Papaver. Harwood Academic Publisher, Amsterdam, 159–188.

Shargi, M., Lalezari, I. A. (1967): Papaver bracteatum Lindl. a highly rich source of thebaine. Nature, 213, 1244.

Sharma, J. R., Lal, R. K., Mishra, H. O., Lohia, R. S., Pant, V., Yadav, P. (1997): Economic heterosis for yield and feasibility of its exploitation in opium poppy *(Papaver somniferum* L.). J. Med. Arom. Plant. Sci., 19, 398–402.

Straka, P., Schultze, W., Nothnagel, T. (1993): Stand der Arbeiten zur Entwicklung morphinarmer Mohnformen. Vortr. Pflanzenzüchtung, 26. 36–41.

Tétényi P. (2000): A mák taxonómiája. in: Sárkány S., Bernáth J. and Tétényi P. A mák. Magyarország Kulturflórája, Akadémiai Kiadó, Budapest (in press).

Zenk, M. H. (1985): Enzymology of benzylizoquinoline alkaloid formation. In: Philipson, J.D., Roberts M.F and Zenk M.H. The chemistry and biology of isoquinoline alkaloids, Springer-Verlag, Berlin, 240–256.