New Results of Poppy (Papaver somniferum L.) Breeding for Low Alkaloid Content in Hungary

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Abstract

Until the last few years, breeding work of poppy in Hungary has been focused on increasing the production and alkaloid level of the capsules and having a special spectrum of alkaloids for the pharmaceutical processing. Recently, (parallel with the reconstruction of the production system), special cultivars of low alkaloid content are required for food products. Our work was focused on establishing new cultivars fulfilling the criteria of DUS and up-to-date requirements for high yield and quality of seeds, possessing low (below 0.01%) alkaloid level. Beside the practical aims, we wanted to obtain scientific information on the variability of several morphological traits and alkaloid pattern.

Reciprocal crossings of 3 cultivars ('Óriás kék', Przemko', Libra') followed by individual plant selection has been done. The majority of F1 strains were not uniform. The registered plant characteristics showed an independent combination with each other, the progenies consisted several new recombinants. Over-dominance has been observed for plant height, number of stigmatic rays, and size of the capsules, although not in each combination. Petal colour is supposed to be of inheritance, however, seed colour shows more complex inheritance, possibly a polygenic one. Maternal effects have been proved for leaf incision, capsule form, anthocyanin coloration of calyces. By the selection procedure, morphological homogeneity of some strains increased, while other accessions showed a considerable variability even in the third generation. For morphine, codeine and narcotine, intermediate level of F1 strains were comparable with their parents. In combination 'P' x 'L' and in case of thebaine, however, heterosis effects can be supposed. During selection, a successful lowering of total alkaloid contents could be achieved. In F3 'L' x 'P' crossings, the majority of individuals contained less than 0.001% of total alkaloids. Main compound is morphine.

INTRODUCTION

Production of poppy (Papaver somniferum L.), both the capsules for morphinane alkaloids as well as the seeds for culinary purposes, has a great economic significance in Hungary. Recently, 5-8 thousand hectares has been cultivated as yearly average. Today, 11 cultivars are registered, all of them results of Hungarian breeding. Until the last few years, breeding work has been focused on increasing the production and alkaloid level of the capsules assuring special spectra of alkaloids for the pharmaceutical processing. Recently (parallel with the reconstruction of the production system), special cultivars of low alkaloid content are required for food products having the proper production and nutritive value without the danger of alkaloid abuse. Today, breeding has been oriented into three main directions: creating cultivars accumulating high morphine (1.5-2.0%) or special alkaloid content (narcotine, codeine, thebaine etc.) for industrial extraction; selection of cultivars for seed and oil production, accumulating low level of alkaloids in the capsules (0.01% or less); producing ornamental types with special flower or capsule form, accumulating restricted amount of alkaloids (Bernáth and Németh, 1999).

Breeding activity in achieving varieties of low alkaloid content has been strengthened in the last decades thorough in the worlds. However, first data are dated back to the 1970s. Nyman (1979) studied several populations and screened 60 thousand
individuals in order to find low alkaloid mutants. Mutation frequency for low alkaloid production proved to be very low. Although the aim of these studies was a theoretical one, it assured useful information for further works. In the 1980’s, the first low alkaloid (0.01-0.05%) varieties 'Soma'; 'Przenko' has been appeared (Szymanowska et al., 1994). In the 1990’s, Nothnagel et al. (1996) selected a new strain containing only 0.007% alkaloids however stabilising it seemed to be difficult because of the presumed polygenic inheritance of the accumulation level. Sharma et al. (1999) provided information on an Indian variety ('Sujata') established by mutation breeding, which is free from latex and is therefore said containing no alkaloids.

The lack or very low level of alkaloids seems to be the result either of a genetic block in the biosynthetic pathway or as a consequence of the lack of laticifers and latex. The genetic block is supposed to exist at the protoalkaloid level, before formation of norcoclaurin, possessing a monogenic recessive inheritance feature (Nyman, 1980). Other authors conclude a polygenic, quantitative characteristics in inheritance of alkaloids. It is most likely, that both regulation systems are present in the genom of poppy, however, working at different levels of biosynthesis (Németh, 2001).

Our work was focused on establishing new cultivars fulfilling the criteria of DUS (distinctness, uniformity, stability) and up-to-date requirements for high yield and quality of seeds, possessing low alkaloid level. Beside the practical aims, we wanted to obtain scientific information on the variability of several morphological traits and alkaloid pattern.

MATERIAL AND METHODS

The experiments were done in open field plots at the Experimental Station of the Faculty of Horticultural Sciences in Budapest. Highest temperatures during vegetation period of poppy (March- July) regularly exceeds 30 °C. The natural precipitation is low, 150-250 mm in this period, which was completed by another 50-80 mm irrigation. The sum of sunshine during this period was 1000-1100 hours. The soil is sandy-clay, humus content and nitrogen amounts are low (0.65% and 8.7 mg/kg respectively), phosphorous content moderate (170 mg/kg) and potassium sufficient (72 mg/kg).

Breeding of low alkaloid variety was started in 1997, at our department by reciprocal crossing of three cultivars 'Óriás kék' (hereafter O), 'Przemko' (hereafter P) and 'Libra' (hereafter L). Seeds were obtained from the gene-bank of the Department of Medicinal and Aromatic Plants of the SZI University. In the successive years F1, F2 and F3 progenies were developed by self pollination of individuals. During development of progenies, selection was done first for low alkaloid content (criterion was a total alkaloid level below 0.1%). As another criterion, the material should comply with the DUS requirements. The selected lines were therefore checked for several morphological traits. Plant height, leaf incision, presence of hairs and anthocyain coloration on the calyces, colour of the petals were described before and during flowering, respectively. Number, dimension and shape of capsules, shape of stigmatic disk and number of stigmatic rays, colour of the seeds were measured after harvesting. Quantitative traits were measured on 10-25 individuals/strain, while qualitative characteristics had been evaluated by classification of individuals according to the DUS guidelines.

Measurement of alkaloid content was done on capsules of 10-25 individuals/strain by modified Desaga TLC method. For the extraction 0.2 g of pulverised capsule and 20-25 ml solvent chloroform- methanol (4:1) was used in Soxhlet apparatus. After vacuum distillation and repeated solution of samples in 1 ml solvent, the separation procedure was done in horizontal chambers (H-Trennkammer Desaga Nr. 120150). The procedure consisted of toluol- ethyl-acetate- diethyl-amin (7:2:1) forming the mobile phase on Silica gel plates 60 F254 (Merck). Evaluation was done 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.
RESULTS AND DISCUSSION

In spite of the autogamous behaviour and theoretical homozygotic structure of the poppy genotypes - the majority of F1 strains did not prove to be uniform. The registered plant characteristics showed an independent combination with each other, the progenies consisted several new recombinant individuals.

Appearance of the studied morphological characteristics in F1 showed different nature. Among the quantitative characteristics, inheritance of plant height exhibited over-dominance or heterosis in combinations of variety 'Ó'. However, it showed intermediate values in case of crossing of L x P (Table 1.). Reciprocal combinations were also different in each crossing. In former publications heterosis for inheritance of height was mentioned by several authors, like Kálmán et al. (1987), Heltmann and Silva (1978) or Sharma et al. (1997), however, other opinions are also known (Singh and Khanna, 1991).

An opposite tendency was observed for inheritance of the number of stigmatic rays, for which no previous literature data are known. It proved to be intermediate in crossings of 'Oriás Kék' and showed heterosis in progenies of L and P combinations (Table 1.). The number of capsules/plant showed high variability, most likely dependent not only genetic factors. Nevertheless, in each crossing combinations of variety 'O' less capsules developed, then in accessions of the other two parents, reflecting the characteristics of the Hungarian variety of few branches (Table 1). As for the size of the capsules, it showed a slight heterosis in each combination (Table 1), where also maternal effect could be observed similarly to studies of Sharma et al., (1988).

At description and classification of qualitative traits proved to be difficult, because of several transition forms and neocombinants had been detected. Leaf incision showed definite maternal effect. In progenies of 'O' a weak incision was always observable, while progenies of 'L' as maternal parent exhibited heavily incised leaves. Antociane coloration of the calyces below capsules is a characteristics of variety 'Ó', which can only very rarely found in 'L' and 'P'. In each crossings, where variety 'O' was present, this coloration of stems was observable in varying proportion of individuals. No special segregation pattern could be established in the formation of stigmatic disks of F1 strains. In each combination, shapes similar to both parents as well as transition forms could also be detected. Neither of the strains was homogenous for this trait. Presence of hairs on the stem below capsule is not a characteristic feature for either of the parent varieties. In some progeny strains, however, this feature appeared in considerable proportion (up to 20%), especially in accessions of variety 'L'. In case of the capsule form, a considerable maternal effect was registered. Among the combinations, the "pear" form of variety 'L' proved to be dominant in most cases (Table 1). In the majority of combinations, forms divergent from those of the parents were also observable. Colour of petals is considerably varying. In case of 'Ó' the flowers show a special reddish colour, while in the other two parent varieties it is white with a light violet spot at the base of the petals. The majority of F1 strains was uniform concerning the petal colour. In combinations with the variety 'O' a light reddish-dark pink coloration was observable, however, only in combinations where the mentioned variety of reddish flowers was the maternal partner. In all of the other combinations, the white petals with violet basic spot appeared. Data support the idea of Kálmán et al. (1987) that petal colour is of intermediate inheritance and does not suit to the opinion of Patra et al. (1992) on dominance row of multiple alleles regulating petal colour. Inheritance of seed colour showed dominance of grey and greyish coloration over the blue ones. In progenies of combinations of variety 'O' the greyish shade of seed colour of this cultivar is the most abundant, however it appears even in accessions of the crossings 'P' x 'L' (Table 1). Seed colours divergent from the parental ones has been observed, too. These results contradict to findings of Kálmán et al., (1987), who described inheritance of seed colour as an intermediate characteristics.

By the selection procedure, morphological homogeneity of some strains increased, while other accessions showed a considerable variability even in the third generation. No
connection seems to exist among the inheritance of the studied morphological features. Some neocombinant variants (divergent from the parents) became stable, too. Some examples are listed below:

- **Ó x L**: reddish petals (Ó), without anthociane coloration of the calyces (L);
- **L x Ó**: white petals (L), upwards shape of stigmatic disk (Ó);
- **P x Ó**: pink colour of petals (Ó), globular shape of capsules (P).

In the selected F3 generation, highest proportion of homogenous strains was found among the accessions of combination 'Ó x L'.

As for the total alkaloid content, in F1 generations, varying values had been obtained (Fig. 1). In each combination, the width of variation extended from 0.01-0.06 % till 7.50-9.20 %. The only exception was the progeny of crossing 'L x P', where even the maximum values did not exceed 0.2 %. In combinations of variety 'Ó', with considerable deviations, the mean values showed an intermediate level of total alkaloid content in F1 strains compared with their parents. In combination 'P x L', however, heterosis effects can be supposed, F1 individuals showing higher levels of alkaloids than the mean of the parents. Reciprocal combinations proved to be different, reflecting maternal effect.

Concerning the level of individual alkaloids, heterosis may be supposed only in case of thebaine, while the other three components show intermediate contents (Table 2). These finding on inheritance partly coincides with former results (Dános, 1965; Morice and Louarn, 1971, Nyman, 1979), however contradict to some other ones who found considerable heterosis or dominance of total alkaloid content in crossing of different cultivars (Heltmann and Silva, 1978; Kálmán et al., 1987; Krenn et al. 1998). The contradictory results may arise from the two levels of genetic regulation of alkaloid content: regulation during proto-alkaloid synthesis (before norcoclaurine) and regulation during further steps, e.g. morphinane conversions (Bernáth and Németh, 1999). The genetic diversity of the parent varieties concerning these loci as well as the possible polygenic feature of regulation may explain also the mentioned large variation of F1 progenies.

Based on the finding, in the first generations, further selection had been focused on accessions of crossings 'L1 x P' and 'L x Ó'. During selection, a successful decrease of total alkaloid contents could be achieved. In F3, especially in progenies of 'L x P' crossings, the majority of individuals contained less than 0.001 % of total alkaloids. However, in the progenies of 'L x Ó' a great number of individuals fulfilled the breeding aim, too. According to the findings, also other accessions may be promising: in progenies of 'Ó' as maternal partner a characteristic segregation has been observed: beside a group of individuals following a normal distribution in domain over 0.1 % total morphinane content, another segment became obvious in domain below 0.001 %, which promises results also by selection of these individuals. It might be especially important in progenies, showing advantageous morphological or production characteristics.

As result of the selection process, it could be established, that (based on the studied 12 morphological features) each of the F3 progenies of different maternal varieties and the three parent varieties represented well distinguished groups. According to the discriminant analysis, especially the characteristics height, seed, and petal colour, leaf incision (Discriminant function 1) furthermore shape of stigmatic disc and number of stigmatic rays (Discriminant function 2) played considerable role in differentiation of populations (Figure 2.). It means, that each of the accessions (maternal combinations) are distinct from any other ones (criterion of DUS). It also shows, that reciprocal combinations could not be well separated if taking consideration all of the studied morphological features, even if strong maternal effects were demonstrated in case of some characteristics.

Based on the contents of the measured four alkaloids, the parents and their progenies are more closely to each other, than based on the morphological features. Only variety 'Ó' and progenies of 'Ó' as maternal parent are separated, representing higher contents or a slightly divergent pattern of alkaloid. When evaluating morphological and chemical features together by the same method, plot segregation shows a similar feature
than that using only morphological traits. In this case 'Ó' and 'L' x 'Ó', accessions form the most diverse groups, while the two parent species ('P' and 'L') became close to each other.

ACKNOWLEDGEMENTS

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Literature Cited

Nyman, U. 1980 Alkaloid content in the F1 and F2 generations obtained from crosses between different chemoprovarieties in *Papaver somniferum* L. Hereditas 93:115-119.
### Table 1. Some morphological features of the parent varieties and their F1 strains

<table>
<thead>
<tr>
<th>Cultivars and crossings</th>
<th>Height (cm)</th>
<th>Number of capsules/plant</th>
<th>Length of capsules (cm)</th>
<th>Width of capsules (cm)</th>
<th>No. of stigmatic rays</th>
<th>Form of capsules (%)</th>
<th>Seed colour (blue: grey)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Óriás</td>
<td>121</td>
<td>1.5</td>
<td>6.25</td>
<td>4.06</td>
<td>16.2</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Libra</td>
<td>122</td>
<td>2.1</td>
<td>3.75</td>
<td>2.98</td>
<td>12.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Przemko</td>
<td>114</td>
<td>2.8</td>
<td>3.58</td>
<td>2.95</td>
<td>12.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Óriás x Libra</td>
<td>129</td>
<td>1.7-4.6</td>
<td>6.00</td>
<td>4.01</td>
<td>15.0</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>Libra x Óriás</td>
<td>142</td>
<td>2.3-5.0</td>
<td>5.52</td>
<td>4.13</td>
<td>14.9</td>
<td>16</td>
<td>79</td>
</tr>
<tr>
<td>Óriás x Przemko</td>
<td>126</td>
<td>1.5-5.6</td>
<td>5.83</td>
<td>3.83</td>
<td>15.3</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Przemko x Óriás</td>
<td>146</td>
<td>1.4-4.8</td>
<td>5.41</td>
<td>4.04</td>
<td>14.3</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Przemko x Libra</td>
<td>109</td>
<td>2.9-3.8</td>
<td>4.86</td>
<td>3.14</td>
<td>14.0</td>
<td>-</td>
<td>47</td>
</tr>
<tr>
<td>Libra x Przemko</td>
<td>123</td>
<td>3.5-5.0</td>
<td>5.58</td>
<td>4.06</td>
<td>14.0</td>
<td>-</td>
<td>83</td>
</tr>
</tbody>
</table>

### Table 2. Mean alkaloid contents of the dried capsules in F1 strains and their parents (%o)

<table>
<thead>
<tr>
<th>Cultivars and crossings</th>
<th>Morphine</th>
<th>Codeine</th>
<th>Thebaine</th>
<th>Narcotine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Óriás</td>
<td>3.67</td>
<td>1.06</td>
<td>0.40</td>
<td>0.95</td>
</tr>
<tr>
<td>Libra</td>
<td>0.43</td>
<td>0.10</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Przemko</td>
<td>0.32</td>
<td>0.05</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Óriás x Libra</td>
<td>1.48</td>
<td>0.65</td>
<td>0.64</td>
<td>0.24</td>
</tr>
<tr>
<td>Libra x Óriás</td>
<td>0.39</td>
<td>0.12</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Óriás x Przemko</td>
<td>1.23</td>
<td>0.44</td>
<td>0.39</td>
<td>0.07</td>
</tr>
<tr>
<td>Przemko x Óriás</td>
<td>0.85</td>
<td>0.55</td>
<td>0.52</td>
<td>0.01</td>
</tr>
<tr>
<td>Przemko x Libra</td>
<td>1.24</td>
<td>0.86</td>
<td>0.35</td>
<td>0.01</td>
</tr>
<tr>
<td>Libra x Przemko</td>
<td>0.06</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Fig. 1. Total alkaloid content of the F1 generations compared with the parents (min.. max.. mean)
Fig. 2. Distribution of the studied cultivars and populations in the co-ordinate system of 7 morphological features.