

Improved evaluation method for reliable distinction of poppy (*Papaver somniferum* L.) cultivars

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Summary: Investigating seven Hungarian standard cultivars (i.e. 'A1', 'Kék Gemoná', 'BC-2', 'Kék Duna', 'Kompolti M', 'Gödi N', 'Kozmosz') it was proved that for the reliable distinction between cultivars a complex analysis is necessitated. Because of the large morpho-phenological and production-biological plasticity of the species qualitative and quantitative features as well as alkaloid accumulation characters have to be evaluated simultaneously applying discriminant analysis.

It was proved by the investigations that some of the qualitative and quantitative characters included into the DUS evaluation procedure have slight importance for reliable distinction. As an example the majority of cultivars show high inhomogeneity in such characters like the shape of stigmatic disc, the number of stigmatic lobes etc. Furthermore these characters never had been chosen for the goal of any practical selection and are modified by the weather conditions as well.

In the evaluation process the alkaloid accumulation characteristics (both qualitative and quantitative ones) have to be evaluated on a higher rank. For instance the high accumulation level of morphine in cv. 'A1' (10.5 ‰), exceptionally high narcotine accumulation in 'Kék Gemoná' (7.8 ‰), or the low morphine accumulation level in cv. 'Kozmosz' (1.1 ‰) proved to be proper characters for their distinction, alone. However, at the same time the weather conditions existing at the time of experiment have to be taken into consideration. From practical point of view, the years rich in precipitation and poor in sunshine are less suitable for distinction of cultivars selected for higher alkaloid accumulation, especially for production of codeine and thebaine characterized by a higher methylation level. Based on the example of 'Kék Gemoná', the accumulation level of morphine decreases from 7.4 ‰ to 4.1 ‰ under such conditions, while thebaine and codeine disappear, practically.

Introduction

Poppy seems to be one of the few species which was utilised, and cultivated since prehistoric times (Tétényi, 1997). The narcotic and nutritive values of its products were recognised by Greeks, Egypt and Romans. Hippocrates (460–377 BC) was one of the first who emphasised the medical advantages of the poppy and its preparations. The nutritive property of the seeds was also recognised by him. Poppy spread over from its Central Asian gene centre through the Roman Empire, later its cultivation became very common in Europe. At present, as a result of the large genetic, morphological and physiological plasticity of poppy, the species is cultivated up to the Arctic Circle as well as under tropical conditions (Bernáth, 1998).

The reason of the wide distribution of the poppy can be explained by the well known narcotic, pharmacological and nutritive value of its products. The utilization of opiate alkaloids (either taken from the opium or extracted from the dry capsule) are important, even nowadays. Analgesics of morphine origin are used mainly to control severe pain and for their anti-diarrhoea and sedative effects. Codeine and to a lesser extent pholcodine, ethyl morphine and narcotine are

utilised as cough depressant agents. Apomorphine hydrochloride can be used as an emetic in small quantities and its anti-Parkinson efficacy has been recognised and tested. At the same time poppy seed is utilised for human consumption, industrial processing and in the manufacture of animal feeds.

However, large amount of poppy is cultivated in the world for other as official use. Referring to the data of Bryant (1988) around 40 000 tonnes of opium is produced world-wide and only 5% of it is used officially, as a raw material of industrial production, or source of seed. This contradictory situation was recognised even at the beginning of the twentieth century and international struggle was started against drug abuse and illicit traffic. As a result of the international harmonisation a special board was brought into existence for checking and advising global tendencies (Bernáth, 1998). In European countries, parallelly with the administrative regulations, a new strategy was accepted by the governments interested in the large scale cultivation of poppy. Utilising the large biological diversity of the species the selection work had been intensified into three main directions (Németh, 1999):

- Cultivars with especially high alkaloid content (1.5–2.5% morphine) are reserved for industrial

utilization, only. This genetic pool can be utilised under strictly controlled agricultural areas.

- For the seed production selection of cultivars with low morphine content (less than 0.1% morphine) are in progress. These cultivars are suggested for garden and large-scale production in any agricultural region.
- Recently, selection of cultivars was started for producing ornamentals, showing special morphological characteristics of flower and capsule. These types of cultivars, because of their low morphine content and small cultivation area can be cultivated without severe restriction.

The appearance of the new cultivars, both in Europe and other part of the world made necessary to revise the traditional system of evaluation of poppy cultivars. By the help of the Hungarian experts (Köck, 1995), including specialists of Hungarian Agricultural Quality Control Organisation, a new DUS evaluation system was developed (Anonymus, 1997) and registered (Hoberg, 1998). In the present work our intention was to clear up the importance of the quantitative and qualitative features in distinction among cultivars and to apply biometrical analysis to make the evaluation process more accurate. At the same time the phenotypic plasticity of poppy cultivars generated by weather conditions was checked by us comparing DUS characteristics of cv. 'Kék Gemona' in two successive years.

Material and methods

The experiments were carried out in 1997 and 1998 at the Research Station of the Department of Medicinal Plant Production at Soroksár. The experimental field has light sandy soil with main characteristics, which are as follows:

NO_3 – 10.1mg/kg; P_2O_5 – 328 mg/kg; K_2O – 470 mg/kg; K_A < 30; pH – 7.6; Humus 1.5%

The cultivars were chosen from the spectrum of Hungarian registered plant materials (OMMI 1998).

- a) cultivar with 1% morphine content, or more: 'A1'
- b) cultivar with 0.7% narcotine content or more: 'Kék Gemona' (Fig. 1)
- c) cultivars with moderate morphine content: 'BC-2', 'Kék Duna', 'Kompolti M', 'Gödi N'
- d) autumn sown cultivar with low morphine content: 'Kozmosz'



Figure 1 Flower of the cultivar 'Kék Gemona' selected for the high narcotine production

The seed of the cultivars were taken from the "super elite" (stock) collections of the breeders being responsible for the reservation and maintenance of each cultivar.

In both years the seed of the cultivars was sown in 4th of April, equally. 50 cm row distance was applied, using 28m² plot size in 4 replications. During the vegetation cycle nutrition of 100–100 kg/ha N, P_2O_5 and K_2O were given in form of top-dressing. Continuous mechanical weed control and two irrigations were managed. The characteristic data of weather conditions existing during the growing season of both years (1997 and 1998) are given in Table 1.

Table 1 Weather conditions of the growing seasons characterised by mean values of decades (1997-1998)

	1997			1998		
	Temperature (oC)	Duration of sunshine (h)	Precipitation* (mm)	Temperature (oC)	Duration of sunshine (h)	Precipitation* (mm)
March	6.9	8.5	10	7.3	4.2	8
	5.4	6.2		2.5	7.3	
	4.4	6.2		4.2	8.0	
April	7.4	10.0	11	12.3	5.3	94
	5.2	6.3		9.1	4.3	
	9.9	6.7		14.0	6.2	
May	16.2	8.8	49	15.3	8.3	88
	19.9	11.9		15.6	6.0	
	13.5	7.2		16.4	10.2	
June	16.9	7.1	45	23.3	12.3	61
	20.9	8.1		17.2	6.9	
	22.1	12.3		22.5	9.1	
July	20.1	7.8	54	18.0	6.1	59
	19.1	5.5		21.4	10.7	
	21.4	9.6		26.3	12.3	

* total monthly amount of precipitation

The evaluation of the cultivars according to their qualitative, quantitative and the alkaloid accumulation properties (according to the DUS description) is shown in the *Table 2*.

Table 2 Selected morphological and production characteristics used for evaluation of cultivars

Qualitative characters*	Quantitative characters	Alkaloid accumulation**
Shape of capsule Shape of stigmatic disc	Plant height Number of capsules Length of main capsule Width of main capsule Mass of main capsule Number of stigmatic lobes Total plant mass	Morphine Codeine Thebaine Narcotine

* Characterised by DUS numeric values

**measured in the main capsules

The plant material was harvested at the stage of full ripening. After the evaluation of yield, the alkaloid content and alkaloid composition of the capsules were analysed using the modified DESAGA TLC method (Kraus, 1992).

To make biometrical evaluation Statgraph 5 program was used.

Results

Evaluation of characteristics of the standard cultivars

The main characteristics of seven cultivars are given in *Table 3*, *4* and *5*.

It is obvious from the data presented in *Table 3* that the shape of the capsules and that of the stigmatic disc are homogenous in the case of some cultivars, only. The occurrence of decimal values is the consequence of the intermediate morphological forms being observed in neighbouring groups. For instance, cv. 'A1' and 'BC2' have rectangular, 'Kék Duna' elliptic, 'Gödi N' circular, while cv. 'Kozmosz' pear-shaped capsule form. The stigmatic disc shows similar morphological inhomogeneity and seems to be stable in the case of four cultivars, only.

Table 3 Qualitative differences of seven Hungarian cultivars characterized by two essential morphological properties

Cultivar	Mean of DUS numeric values	
	Shape of capsule	Shape of stigmatic disc
'A1'	2.0	2.3
'Gödi N'	3.0	1.5
'Kompolti M'	2.5	4.0
'Kék Duna'	1.0	1.0
'Kozmosz'	6.0	3.0
'Kék Gemona'	2.3	1.3
'BC2'	2.0	4.0

Among quantitative morphological and production characteristics (*Table 4*) the height of the plants, capsule number and number of stigmatic lobes seems to be valuable

characters for evaluation. Concerning these features cv. 'A1' and 'Kék Gemona' show restricted growth, 'Kompolti M' and 'Kozmosz' relatively high capsule number, while both 'A1' and 'Kozmosz' excel in rather low number of stigmatic lobes.

Table 4 Quantitative differences of seven Hungarian cultivars characterized by morphological and production-biological values

Cultivar	Plant height (cm)	Number of capsules (pieces)	Main capsule			Mass per plot		Number of stigmatic lobes (pieces)
			mass (g)	length (cm)	width (cm)	capsule (kg)	seed (kg)	
'A1'	85.5	1.5	1.7	4.5	2.6	0.95	1.10	10.1
'Gödi N'	93.6	1.0	2.1	4.6	3.2	0.75	0.45	14.6
'Kompolti M'	88.0	3.0	2.1	3.9	2.9	0.60	0.75	14.8
'Kék Duna'	98.7	1.0	2.5	4.1	3.8	0.70	0.75	14.8
'Kozmosz'	86.1	2.0	0.6	4.9	2.9	1.05	1.10	10.8
'Kék Gemona'	82.3	1.0	0.9	3.2	3.3	0.81	0.60	14.8
'BC2'	87.0	1.0	0.7	4.4	3.1	0.60	0.40	14.8

The alkaloid content and composition of the plants (*Table 5* and *Fig. 2*) show much similarity with the features which had been defined in the original description of the cultivars. Cultivar 'A1' accumulates large amount of morphine, while 'Kék Gemona' accumulates considerable amounts of narcotine. The traditional Hungarian cultivars ('Gödi N', 'Kompolti M', 'Kék Duna' and 'BC2') can be characterised by moderate morphine accumulation. The situation of cv. 'Kozmosz' is an exceptional one. This autumn sown type cultivar could not adapt well to the spring vegetation cycle, which was reflected in the reduction of both dry-matter production and alkaloid accumulation.

Table 5 Differences of seven Hungarian cultivars characterized by their alkaloid accumulation properties

Cultivar	Alkaloid content (‰)			
	Morphine	Codeine	Thebaine	Narcotine
'A1'	10.5	0.8	0.1	0.0
'Gödi N'	5.0	0.2	0.1	0.1
'Kompolti M'	7.8	0.1	0.1	0.0
'Kék Duna'	3.5	0.0	0.0	0.0
'Kozmosz'	1.1	0.0	0.0	0.0
'Kék Gemona'	6.2	1.5	1.8	7.8
'BC2'	3.8	0.2	0.1	0.0

To make the evaluation and comparison of the cultivars more accurate biometrical analysis was applied. The different groups of characters were analysed by the discriminant function, step by step. At first, the characters were analysed separately and afterwards in combined form. The results are summarised in *Table 6* and *Fig 2*. It was proved by the analysis that neither the qualitative and quantitative characters, nor the alkaloid accumulation features, alone can facilitate accurate distinction of cultivars. However, hundred percent separation is possible when all the characteristics are taken into consideration and analysed,

Table 6 Distinction between standard cultivars analysing different groups of their characters by discriminant function

Actual groups of cultivars	Distribution of individuals in the predicted groups calculated by discriminant function (%)						
	1.	2.	3.	4.	5.	6.	7.
Qualitative characters							
‘A1’	100,0	–	–	–	–	–	–
‘Gödi N’	50,0	25,0	–	25,0	–	–	–
‘Kompolti M’	–	–	75,0	–	–	–	25,0
‘Kék Duna’	–	–	–	100,0	–	–	–
‘Kozmosz’	–	–	–	–	100,0	–	–
‘Kék Gemona’	25,0	25,0	–	50,0	–	–	–
‘BC2’	–	–	–	–	–	–	100,0
Quantitative characters							
‘A1’	75,0	–	–	–	25,0	–	–
‘Gödi N’	–	75,0	–	–	–	–	25,0
‘Kompolti M’	–	–	100,0	–	–	–	–
‘Kék Duna’	–	25,0	–	75,0	–	–	–
‘Kozmosz’	–	–	–	–	100,0	–	–
‘Kék Gemona’	–	–	–	–	–	100,0	–
‘BC2’	–	25,0	–	–	–	–	75,0
Alkaloid spectra							
‘A1’	75,0	–	25,0	–	–	–	–
‘Gödi N’	–	25,0	25,0	25,0	–	–	25,0
‘Kompolti M’	25,0	–	25,0	–	–	–	25,0
‘Kék Duna’	–	–	–	75,0	25,0	–	–
‘Kozmosz’	–	–	–	–	100,0	–	–
‘Kék Gemona’	–	–	–	–	–	100,0	–
‘BC2’	–	25,0	–	–	25,0	–	50,0
Analysis of all characteristics							
‘A1’	100,0	–	–	–	–	–	–
‘Gödi N’	–	100,0	–	–	–	–	–
‘Kompolti M’	–	–	100,0	–	–	–	–
‘Kék Duna’	–	–	–	100,0	–	–	–
‘Kozmosz’	–	–	–	–	100,0	–	–
‘Kék Gemona’	–	–	–	–	–	100,0	–
‘BC2’	–	–	–	–	–	–	100,0

simultaneously. In this case the actual and predicted groups of cultivars, calculated by discriminant analysis coincide, absolutely (Fig.3).

Seasonal variation of characteristics of cultivar ‘Kék Gemona’

It is well known either from scientific publications (Bernáth and Tétényi, 1982, Németh 1998), or from practical observations (Morász, 1979), that poppy shows high morphological and production-biological flexibility under the pressure of the environment. To establish the flexibility of features used for evaluation of standard cultivars,

population of ‘Kék Gemona’ was tested in two successive years.

Based on the data of qualitative morphological features both the shape of the capsules and that of the stigmatic disc change seasonally (Table 7). As an effect of 1998, which can be characterised by high precipitation and low number of sunny hours, the rectangular capsule form of the cultivar turns to transverse elliptic and obovate type. At the same time the vessel-like appearance of stigmatic disc tended to get dish-like form.

The overall dry-matter production of the cultivar ‘Kék Gemona’ was accelerated by the higher precipitation of

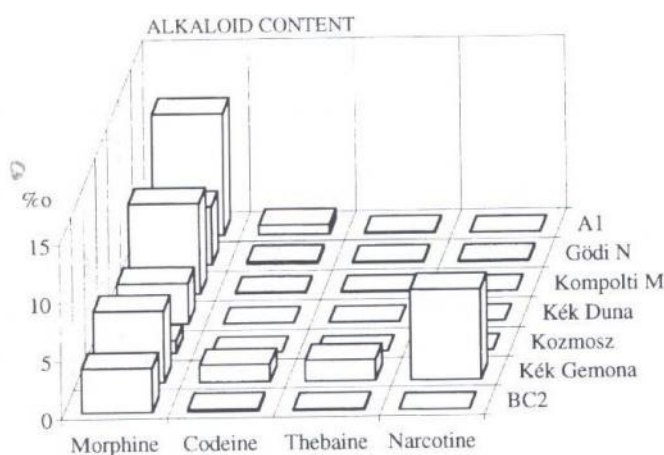


Figure 2 Alkaloid content and composition of the cultivars drawn into the investigation (Soroksár 1997)

1998 as well. It was reflected in increased plant height, larger size and number of capsules (Table 7). However, it was an interesting phenomenon that the number of stigmatic lobes decreased, parallelly.

Both the level and composition of alkaloids were modified by the different conditions of the two vegetation cycles. In 1998 when the higher precipitation was associated with shortage of sunshine the intensity of the alkaloid accumulation was restricted and the higher methylated compounds of morphinane group (codeine and thebaine) practically disappeared. At the same time the accumulation level of narcotine did not changed.

Table 7 Qualitative and qualitative morphological and production characteristics of 'Kék Gemona' cultivar grown in two successive vegetation periods (1997–1998)

Characters	Year of investigation	
	1997	1998
Qualitative*		
Shape of capsule	2.2	4.1
Shape of stigmatic disc	1.2	1.5
Quantitative		
Plant height (cm)	86.5	124.7
Number of capsules (pieces)	1.4	2.1
Main capsule mass (g)	2.3	4.2
Main capsule width (cm)	3.2	2.9
Main capsule length (cm)	3.5	4.3
Number of stigmatic lobes (pieces)	15.4	14.0
Alkaloid accumulation		
Morphine	7.4	4.1
Codeine	0.6	0.1
Thebaine	1.6	—
Narcotine	7.3	6.0

* Characterised by DUS numeric values

The mathematical comparison of the characters measured in two successive years prove, that the morphological and production-biological flexibility of the poppy is rather high (Table 8). Especially, analysing the complex system of characteristics, hundred percent

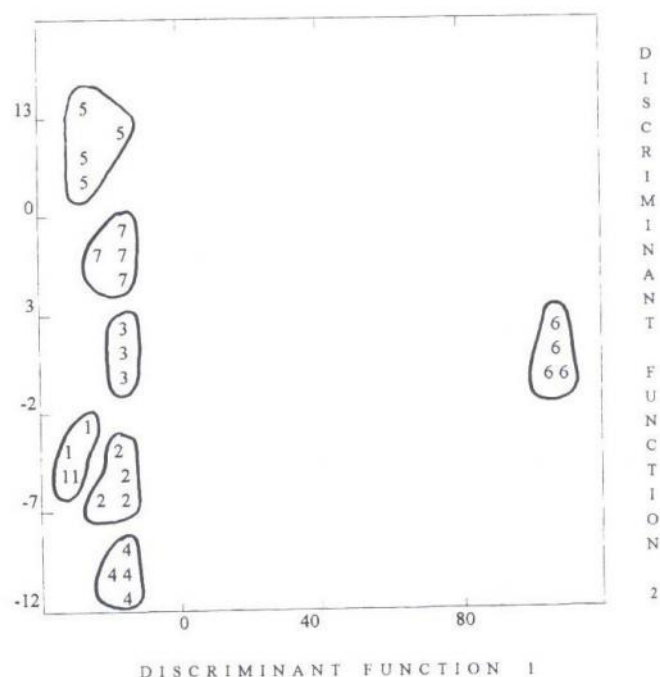


Figure 3 Distinction of cultivars by discriminant analysis, taking into consideration all of the qualitative, quantitative and alkaloid accumulation characteristics

1. 'A1'; 2. 'Gödi N'; 3. Kompolti M'; 4. 'Kék Duna'; 5. 'Kozmosz'; 6. 'Kék Gemona'; 7. 'BC-2'.

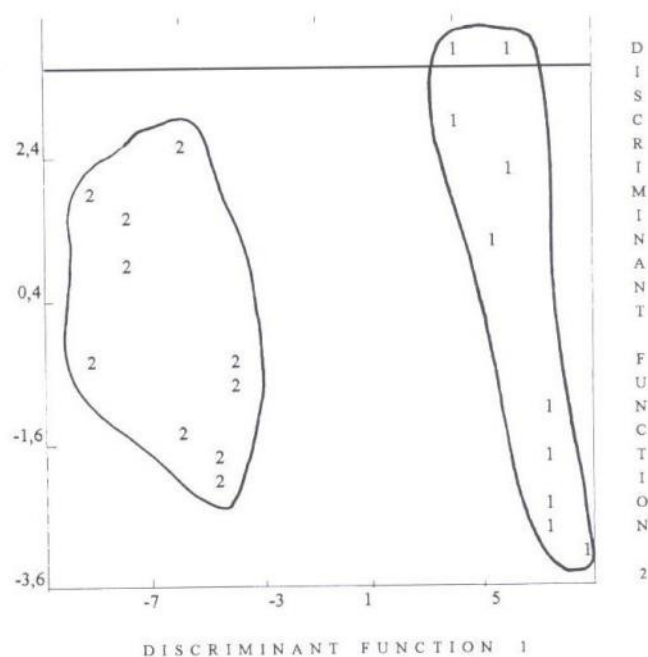


Figure 4 Distinction of individuals of c.v. 'Kék Gemona' by discriminant analysis, grown in two successive years, taking into consideration all of the qualitative, quantitative and alkaloid accumulation characteristics

1. Population grown in 1997, 2. Population grown in 1998

discrimination of the individuals grown in different vegetation cycles could have been justified (Fig 4). It does mean, that the validity of any measured characters and its expenditure for comparative analysis of cultivars is limited to the actual year and conditions of the experiment.

Table 8 Distinction between populations of 'Kék Gemoná' cultivar grown in two successive vegetation periods (1997–1998) by discriminant function

Actual groups of cultivars	Distribution of individuals in the predicted groups calculated by discriminant function (%)	
	1.	2.
Qualitative characters		
Plants grown in 1997	60.0	40.0
Plants grown in 1998	20.0	80.0
Quantitative characters		
Plants grown in 1997	100.0	–
Plants grown in 1998	–	100.0
Alkaloid spectra		
Plants grown in 1997	80.0	20.0
Plants grown in 1998	–	100.0
Analysis of all characteristics		
Plants grown in 1997	100.0	–
Plants grown in 1998	–	100.0

Discussion

By our investigations, in accordance with earlier statements (Bernáth, 1998), the high morpho-phenological and production-biological plasticity of the species had been justified. However, the majority of the qualitative and quantitative characters as well as the alkaloid accumulation process may vary on a large scale, strict cultivar dependency was proved in some cases. For instance, the shape of capsule and stigmatic disc of cultivar 'BC2', or low number of stigmatic lobes in the case of cultivars 'A1' and 'Kozmosz' seem to have identical value. Similarly, the high accumulation level of morphine in cv. 'A1', or the exceptionally high narcotine accumulation in cv. 'Kék Gemoná' proved to be a proper character for their distinction. In spite of these exceptional cases for the reliable distinction between larger groups of cultivars a complex analysis is necessitated. Qualitative and quantitative features as well as alkaloid accumulation characters have to be evaluated simultaneously applying biometrical model. The effectiveness of discriminant analysis can be increased by taking more plant characters involved into the comparison.

In harmony with our earlier investigations (Bernáth, 1998) the morphological and production biological characters of plants are modified at a great deal by weather conditions. On the example of cv. 'Kék Gemoná' our conclusions are as follows:

- The habit of the plants becomes more robust, universally, if frequent precipitation and satisfactory temperature regime exist at the time of the rosette and shoot development stages (actually it has happened in April–May of 1998). It manifests itself in increased plant height, formation of larger and more capsules, etc.

- The reduction of alkaloid accumulation processes as well as the simplification of the alkaloid spectrum of cultivars are the consequence of the shorter duration of sunshine affected by frequent precipitation in 1998. In harmony with our earlier investigations (Bernáth and Tétényi, 1979) the lack of illumination decreases the accumulation of thebaine and codeine more characteristically, being the highly methylated compounds of morphine group.
- The lower alkaloid accumulation level observed in 1998 may be explained by leaching effect of frequent precipitation as well. Its probability has been proved earlier (Laughlin, 1977, Bernáth, 1989).

Based on the above mentioned conclusions to make reliable distinction between cultivars the weather conditions existing at the time of experiment have to be taken into consideration. We have to consider that the frequent precipitation and the adequate temperature promote the growth and dry-mass accumulation of the cultivars. On the opposite, warm and sunny growing period accelerates the "secondary" alkaloid accumulation features. From technical point of view, the years rich in precipitation and poor in sunshine are less suitable for distinction of cultivars selected for higher alkaloid accumulation, especially for production of codeine and thebaine characterized by a higher methylation level.

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