

Production of morphine and variability of significant characters of *Papaver somniferum* L.

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ABSTRACT

Opium poppy (*Papaver somniferum* L.) is currently an important agricultural commodity, with the product being used in the food industry (seed) as well as in the pharmaceutical industry (morphine and other alkaloids). Intensive production of poppy leads to a specific direction of breeding with the aim to obtain highly productive cultivars of the so-called food industry or industrial type. The paper evaluates 57 genetic resources (mostly cultivars), comparing the groups of values representing the indicators of production-significant morphologic and agricultural characters (capsule size; morphine content in poppy straw; weight of dry, empty capsule) and content of morphine in the poppy straw, in relation to the ideotype of poppy, which in these indicators represents 100% of the value. On average lower values in the above indicators were achieved by cultivars with white-coloured seed, including morphine content; concrete data are specified in three cultivars with morphine content in the poppy straw above 0.40%. In blue-seed to grey-seed cultivars, except for the high-morphine cultivar Buddha (1.85% of morphine), we found only six materials with a minimum morphine content of 0.8% in the poppy straw (maximum of 0.92%). These genetic resources also achieved very good values in the morphological indicator and average value in the economic indicator. The results will be used in the selection and classification of suitable genetic resources of poppy in breeding of industrial forms.

Keywords: genetic resources; morphine content; economic and morphologic characters; poppy ideotype

The decisive factor of those that affect production and accumulation of pharmaceutically important alkaloid morphine (and other alkaloids) by *Papaver somniferum* L. is the cultivar with genetic fixation of alkaloid biosynthesis which is explained by Balážová and Pšenák (1998); the cultivar is therefore a limiting factor for economically beneficial harvest of this poppy product. Significant is the combination of efficiency of the cultivar and a climatically favourable vegetation period. It generally applies, that a warm vegetation period acts negatively on the formation of lipids due to negative correlation between lipid and protein contents with a subsequent increase of content of proteins – morphine precursors. A low effect

on the morphine content in the plant is that of soil conditions and agricultural technology. A drop of morphine content can be caused by diseases (especially fungal diseases), attack by plant pests and the effect of other unfavourable factors. There is for example water stress in germination (Mahdavi-Damghani et al. 2010) or influence of heavy metals (Lachman et al. 2006). In an effort to increase the plant efficiency, the influence of nitrogenous fertilization, application of morphologic regulators, fungicides, seed calibration etc. is checked. The main goal in Europe in the long run was to breed poppy of universal type, i.e. with high seed yield of suitable (usually blue) colour and concurrently with high morphine content in

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the wall of the ripe capsule, which was in line with the poppy ideotype. In such case, the product is used both in the food industry (seed) as well as the pharmaceutical industry (deseeded capsule). Intensive and targeted practical use of poppy leads to the specific direction in improvement with the intent to gain cultivars of certain production type. With regard to the economic efficiency, breeding, utilization and social reasons there is a tendency to differentiate the cultivars of *Papaver somniferum* L. into at least two types (directions), which also imply the tendencies in improvement. Though a clear definition has not been specified, the poppy cultivars are considered industrial if their ripe capsule walls contain about 1% (at least 0.8), mostly however more (approximately up to 2.5%) morphine, apart from minority alkaloids (codeine, thebaine, narcotine, papaverine and others); seed colour varies. The food industry poppies have lower alkaloid content, the seeds being most frequently blue or blue-grey or white. The morphine content, which is the limiting factor for economically beneficial collection of poppy straw is in the first place ensured by the cultivar. Exceptionally other cultivars with other major alkaloid were bred; Norman is a known cultivar with dominant thebaine in the alkaloid spectrum, representing one of the alternatives to morphine cultivars with regard to possible use in the pharmaceutical industry (Facchini et al. 2005). Fifty seven cultivars of *Papaver somniferum* L. were evaluated in the morphological and economic groups of characters in relation to morphine production; correlations between the morphological characters and chemical properties are purportedly very high (Tétényi 1997). The morphine content in various stages of phenological development was evaluated for instance by Shukla and Singh (2001), Chung (1982) and others.

MATERIAL AND METHODS

Plant materials and design. Seed material of 57 genetic resources of poppy (*Papaver somniferum* L.) was provided by the gene bank Oseva Pro – Research Institute of Oilseed Opava (Czech Republic), including cultivars currently cultivated as well as older ones, exceptionally newly bred cultivars (hereinafter referred to only as cultivars) of various origin: Albín – SK, Azur – CZ, Bernburg – DE, Böhmův bělosemenný – CZ, Buddha – HU, Dannerborg – DK, De Botosani – RO, Diospegi elit – HU, Drauseni – non-men-

tioned, Dubský stříbrošedý – CSK, Eckendorfský – DE, Erbachshofský – DE, Extaz – RO, Fertödi Suktuku Kek – HU, Freege Niebeski – PL, Goluboj jubilejnij – SUN, Hanácký modrý – CSK, Hatvani Zortan – HU, Hnědosemenný – CSK, Indra – SE, Kek Duna – HU, Kompolti M – HU, Lazur – PL, Legal – DK, Luna – SUN, Magurele – RO, Magyarský fialový – HU, Majak – SUN, Major – SK, Malsar – SK, Mansholt – NL, Maraton – SK, Marianne – NL, Nobel – DE, Nordstern – DE, Opál – CZ, SK, Opiferum Turci – TR, Parmo – DK, Pitvarosi Tef – HU, Prejmer – non-mentioned, R3 – FR, Rosemarie – NL, Ruský modrošedý – CSK, Rychetnikův Dubský – CSK, Schlandstadtzer – DE, Selecty stříbrošedý – CSK, Sokol – CZ, Solivarský – SK, Sonna – SE, Stupický bělosemenný – CSK, Sušický červenosemenný – CSK, Tatarstan – SUN, Tatranský – SK, Vahovecký – CSK, Vilmorin opiový – FR, Voschod – SUN, Zavolzskij – SUN (Country abbreviations according to ISO).

The experimental set of genetic resources was cultivated in the period from 2006 to 2008, always in two localities – on the demonstration and experimental land of the Czech University of Life Sciences Prague (CULS Prague) and on the experimental land of the Research Station of the CULS Prague in Červený Újezd. The size of experimental plots were 5–6 m², repetition is not necessary; manual seeding in the depth of 0.5 cm in the period from 4th to 14th April; singling in stage 3 and 4 of the right leaf, spacing of 25 × 12.5 cm; cultivation technology in line with large-area production and methodology of study of genetic resources (Dotlačil et al. 2004, Zehnálek and Šafaříková 2009). In the full ripeness stage 20 plants of each cultivar were harvested to obtain data, from which three-year average values were calculated. Equally the chemical analyses were conducted with the samples of cultivars of each locality and each year separately and average morphine-content values were calculated in the end.

Experimental sites. Experimental land of CULS Prague-Suchdol: altitude up to 300 m a.s.l., average annual temperature 8.4°C, normal precipitation 470 mm per year, moisture area according to Seljaninov 1,3-moderately dry, climatic area – warm, climatic district – dry, soil group – black soil, soil type – clay soil, C_{ox} content 3%, pH 6.99 (neutral).

Experimental land of CULS Prague-Červený Újezd: altitude up to 420 m a.s.l., average annual temperature 7.9°C, normal precipitation 526 mm per year, moisture area – moderately dry, climatic area – moderately warm, climatic district – mod-

Table 1. Evaluation of genetic resources in terms of industrial focus – characters and point scale

Feature and its significance (scoring)	Morphological features		Economic features		
	plant height (20%)	capsule shape (40%)	capsule size (40%)	morphine content in poppy straw (%) (70%)	weight of dry, empty capsule (g) (30%)
1	very small very high	other	very small very large	< 0.24	0.2
2		narrow-elliptical		0.25–0.3	0.21–0.5
3		reniform		0.31–0.4	0.51–0.8
4		elliptical		0.41–0.5	0.81–1.1
5	high small	cylindrical	small large	0.51–0.75	1.11–1.4
6		pear-shaped		0.76–0.8	1.41–1.7
7		cordiform		0.81–0.9	1.71–2.0
8		widely elliptical		0.91–1.0	2.1–2.3
9	medium high	oval ball-shaped	medium-sized	> 1.0	> 2.3

erately dry, soil group – brown soil, soil type – clay soil, C_{ox} content – moderate, pH neutral.

Evaluation of genetic resources. Concrete methodological procedures were specified on the basis of Framework Methodology of National Programme on Conservation and Utilization of Plant genetic Resources and Agro-biodiversity (Dotlačil et al. 2004). Evaluation of morphological and economic features in scales (Table 1) corresponding to the Poppy Descriptor List (Havel et al. 2008) and poppy ideotype (Vašák 2010). Each individual feature was assigned importance (%) with regard to industrial breeding direction on the basis of evaluation of their importance (Vašák 2010) regarding the production properties.

Statistical treatment. A mixed model procedure with a repeated statement for cultivars was used to analyse the content of morphine and characters in the poppy plants. Data from each part of plants were tested separately. The Saaty's method was used to determine significant differences (Saaty 1980). The method dealt with consistency of the pairwise comparison matrix. The graphic representation compares the groups of values representing the indicators of morphologic features (x axis) and economic features (y axis), the third variable is the indicator of morphine content in the poppy straw, which is expressed by

the diameter of the ring, characterizing the concrete cultivar; values of morphological and economic features are specified in Tables 2 and 3. The diagrams specify groups of these three values compared with theoretical 'ideotype cultivar' achieving 100% evaluation in all the three indicators; in compliance with Descriptor List (plant height; elliptical to oval capsule of medium size; dry and empty capsule at least 2.3 g weight and morphine content at least 1%).

Determination of morphine in poppy straw – high-performance liquid chromatography with mass detection. A powered ripe capsule without seeds was extracted with 5% acetic acid under sonication. The obtained suspension was centrifuged and the supernatant was purified by solid-phase extraction cartridges (Oasis MCX). The eluate was evaporated to dryness using rotary vacuum evaporator. The residue was dissolved in 50% aqueous methanol, filtered through a membrane PVDF filter (0.45 μ m) and analysed by HPLC-MS/MS.

Reversed phase chromatography (Hypurity Aquastar column) using gradient elution with A: (0.1% formic acid in methanol, v/v) and B: (0.1% formic acid in deionized water, v/v) as a mobile phase was employed. The detection was carried out using 3200 QTRAP detector with electrospray ionisation in positive ion mode.

Table 2. White-seed cultivars with morphine content in poppy straw higher than 0.4%

Cultivars	Morphine content (%)	Weight of dry, empty capsule (g)	Morphological features (%)	Economic features (%)
Böhmův bělosemenný	0.69	1.72	82	62
Sokol	0.64	1.55	82	59
Solivarský	0.43	1.42	96	51

Table 3. Blue-seed to grey-seed cultivars with morphine content in poppy straw higher than 0.8%

Cultivars	Morphine content (%)	Weight of dry, empty capsule (g)	Morphological features (%)	Economic features (%)
Buddha	1.85	1.62	91	90
Goluboj jubilenij	0.92	1.23	96	79
Danneborg	0.90	0.82	100	76
Magurele	0.90	1.24	82	79
Zavolskij	0.89	1.66	100	74
Pitvarosi Tef	0.83	1.28	73	71
R3'	0.82	1.42	91	74

RESULTS AND DISCUSSION

The evaluated cultivars of *P. somniferum* L. are further characterized on the basis of seed colour, within 5 groups, for the purpose of better clarity of the graphic presentation. Whereat, provable correlations were not found in the content of morphine in the capsule wall and the seed colour. Positive correlations were proved and confirmed between morphine content and the size of capsule (Yadav et al. 2004), further between the morphine content and weight of one thousand seeds (Harvest et al. 2009). Other morphological features with a certain effect on morphine production are the weight of the deseeded capsule, plant height etc. (Srivastava and Sharma 1987, Singh et al. 2003, Yadav et al. 2006). The traits of large collection of poppy were evaluated (except of alkaloids content) by Brezinova et al. (2009).

The graphic representation (Figure 1) clearly implies that even the best-evaluated white-seed cultivars Böhmv bělosemenný and Sokol achieved a lower level of the values of economic in the comparative context of the monitored genetic resources (62% and 59% of ideotype) as well as morphological feature (both 82% of ideotype). Other white-seed cultivars shifted away from the ideotype substantially in the economic indicator.

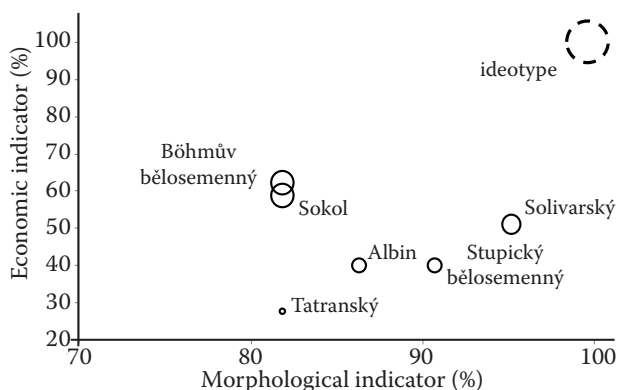


Figure 1. White-seed cultivars

Generally lower, inter alia with the economic yield, can be considered the values of morphine content that are relatively the highest also in the Böhmv bělosemenný and Sokol cultivars (Table 2).

A special position between the grey-seed to blue-seed genetic resource (Figure 2) is represented by the Buddha cultivar, one of the high-morphine cultivars, in which 1.85% of morphine was proved in the poppy straw (i.e. 100% of ideotype) with average weight of the capsule at 1.65 g; generally achieving 90% of ideotype in the economic and 91% of ideotype in the morphological indicator. The plants are relatively high (over 1 m), susceptible to creeping, its white crown petals have a violet stain on the base, the capsules are ball-shaped with widely cup-shaped stigma; seed colour is blue or bluish grey; vegetation period being 135 days. In the Lazur cultivar, also of industrial type, only 0.76% of morphine was found on average, which decreases the value of the economic indicator – 66% of ideotype (morphological indicator – 91% of ideotype). 0.92% of morphine was found in the poppy straw Goluboj jubilejnyj; the economic indicator being 79% and morphological indicator 96% of ideotype. A relatively high content of morphine in the poppy straw – 0.90% was proved also in the Danneborg cultivar with 76% of economic and 100% of morphologic indicator. The

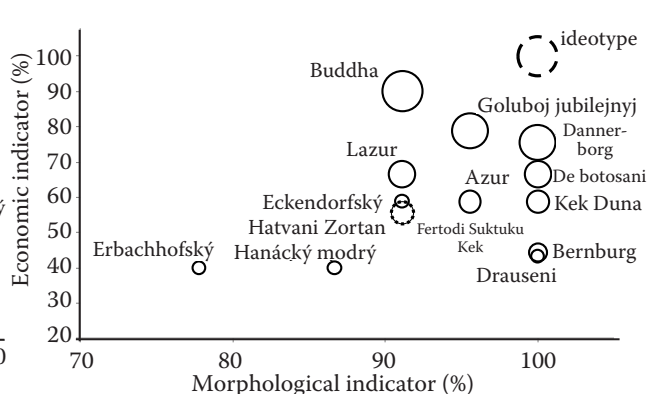


Figure 2. Blue-seed to grey-seed cultivars I

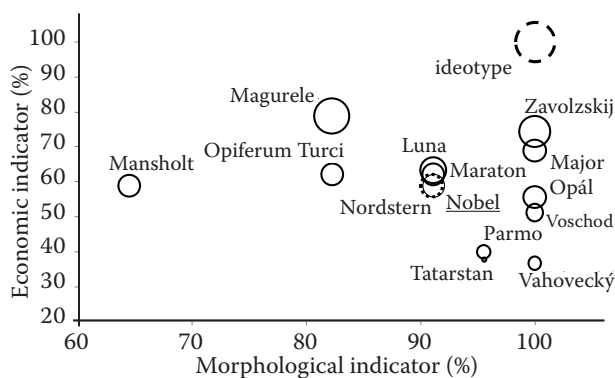


Figure 3. Blue-seed to grey-seed cultivars II

De Botosani cultivar, with only 0.76% of morphine in the poppy straw occupies practically the same position in the monitored indicators. The cultivars Fertodi Suktuku Kek and Hatvani Zortan overlap in the graphic representation, having approximately identical evaluation. Other cultivars can in terms of industrial usage be considered average, or possibly below-average with regard to lower production of alkaloids – Erbachshofský (0.40% of morphine), Hanácký modrý (0.35% of morphine) etc. Amongst other grey-blue seed or blue seed cultivars (Figure 3) the cultivars Magurele – 0.90% of morphine in the poppy straw (79% of economic ideotype and 82% of morphological indicator) and Zavolskij – 0.89% of morphine in the poppy straw (74% of economic ideotype and 100% of morphological indicator) corresponded most to the ideotype. Other specified cultivars are average (which does not exclude the good values of the individual features – Major (0.58% of morphine and 0.68% of economic indicator ideotype), Opal (0.55% of morphine; 56% of economic indicator) and Maraton (0.60% of morphine; 63% of economic indicator). In the field conditions (Vašák et al. 2010) the morphine content in these cultivars ranged from 0.31 to 0.65%. Nobel and Nordstern cultivars overlap in the graphical representation. The culti-

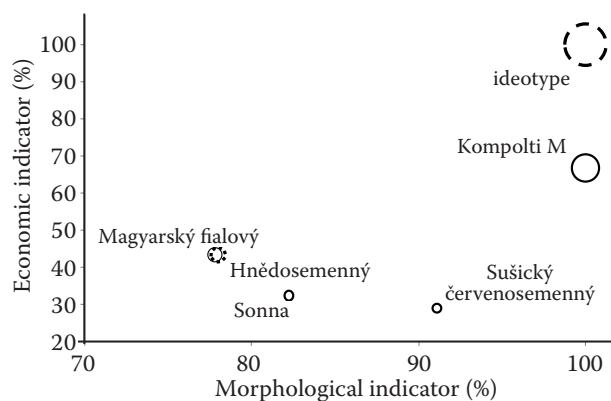


Figure 5. Cultivars with pink or even violet and almost black seed

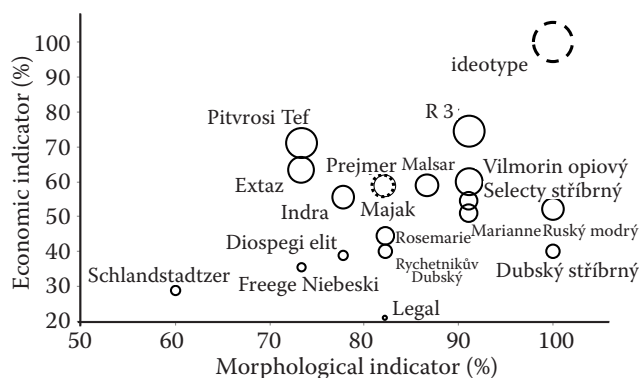


Figure 4. Grey-seed cultivars

vars with grey seed colour (Figure 4), i.e. cultivars less suitable for food industry use, the relatively highest values of ideotype were achieved by R3 with 91% of ideotype of morphological indicator, 74% of ideotype of economic indicator with 0.82% of morphine in the poppy straw, and further the cultivar Pitvarosi Tef – 0.83% of morphine, 73% of morphological ideotype, 71% of economic ideotype. Morphine content in other grey-seed cultivars did not achieve the level of 0.80%; in the Extaz cultivar on average 0.79% of morphine in the poppy straw (morphological indicator – 73%, economic indicator – 64%); in Malsar cultivar cultivated in field conditions, on average 0.49% of morphine was proved, which corresponds to values from field production (Vašák et al. 2010). Cultivars with pink or even violet seed (Sušický červenosemenný, Hnědosemenný, Magyarský fialový) and almost black (Kompolti M, Sonna) – Figure 5. The cultivar with the best values of all the three indicators was Kompolti M with 0.76% of morphine in the poppy straw, 100% of ideotype of morphological and 67% of economic indicator. The cultivars Magyarský fialový and Hnědosemenný overlap in the graphical representation. Content of morphine in the poppy straw in the range from 0.90 to 0.92% (Table 3) represents 89–90% of ideotype, in the range from 0.82 to 0.84% it is 78–80% of ideotype (in case of cultivar Buddha with 1.85% of morphine in the poppy straw it is 100% of ideotype).

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