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Connection of frost tolerance and alkaloid accumulation potential in poppy (*Papaver somniferum* L.)

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Summary

In the Central-European region, a growing interest for winter poppy varieties both for industrial and culinary purposes can be observed. In connection with breeding, we studied if winter hardiness and alkaloid accumulation capacity are linked in some way. Experimental plots sown in autumn and thinned (selected) due to winter frosts were compared with spring sown ones of the same genotypes during 2009-2010. We found, that the overwintered populations exhibited significantly lower total alkaloid contents (1.065%) than the ones from spring sowing (1.479%). This tendency was proved for each of the individual alkaloid compounds morphine, codeine, thebaine and narcotine, too. At the same time, maximum values in some individual samples of the overwintered plants reached similar or higher contents than the spring sown ones. Therefore it is supposed that even if gene frequency in direction for enhanced alkaloid synthesis might be larger in frost sensitive ecotypes, the potential for breeding high alkaloid accumulating cultivars exists also among frost tolerant genotypes.

Introduction

In Hungary, poppy (*Papaver somniferum* L.) is an important industrial crop which is cultivated on large scale thorough the country, on more than ten thousand hectares. Besides, poppy is an essential culinary crop producing delicious, fatty oil containing seeds both for food industry and the households. In the Central-European region, especially in Hungary and Austria, poppy cultivation may take place either as winter or a spring crop, similarly to cereals. Winter poppy ecotypes possess enhanced frost tolerance in leaf rosette stage, while spring poppy is generally killed or severely damaged by frosts under average winter conditions. Cultivation of winter poppy in our region obtained enhanced significance in the last decade, because of the higher and more stable yields among the changing and varying climatic conditions.

Until recently, breeding has not been focused on this aspect. In the Hungarian list of varieties, only five cultivars belong to the winter poppy group while 14 ones are registered as spring poppy cultivars. Breeding has now started to establish new, frost tolerant genotypes which possess also high capsule and seed yields and can be used either as industrial varieties of high alkaloid contents or as culinary ones with very low alkaloid contents in coincidence with the 162/2003. (X. 16.) Government Decree of Hungary.

Unfortunately, there is practically no scientific background information on the genetical determination of frost tolerance in poppy. Not even the physiological or morphological protection mechanisms against low temperatures are fully ascertained. Our recent results suggested, that proline and soluble sugars (glucose, fructose, sucrose) might contribute to the osmotic protection mechanism against cold stress, however, presumably it is not the only and main factor in it (Jászberényi and Németh, 2011a). Dobos (2010) observed that winter poppy is a morphologically adapted genotype with greyish coloured and horizontally laying leaf rosette, violet coloured petals and shorter vegetation cycle compared with spring types. However, these traits may only marginally stay in real connection with winter hardiness.

Information on the inheritance of opiate alkaloids is much more abundant (Bernáth and Németh, 2009; Németh et al., 2011). However, it has been proved, that accumulation of individual alkaloids is regulated in a complex, metabolon like system including multigene effects, enzymatic interactions and compartmentation. Therefore, till now practical results in breeding have mainly been achieved by individual selection.

In the practice it seems to be accepted that winter tolerant genotypes are not able to accumulate high levels of alkaloids. However, as no scientific information and experimental results exist on the linkage of the winter hardiness and alkaloid content, it can be presumed as a statement based on limited information and experience. Therefore, in our work we studied the question if there is any well established connection between alkaloid accumulation and the overwintering potential of poppy genotypes. Using several breeding strains – because of the varieties of original spring ecotype usually killed by frost – and sowing them both in autumn and spring, we examined if the selection by winter frosts resulted in any shift in alkaloid accumulation level and/or pattern of the capsules of the same genotypes.

Materials and methods

Our examination was carried out at the research field of the Department of Medicinal and Aromatic Plants of Corvinus University of Budapest, in 2009-2010. The experimental area is located in the south-eastern part of Budapest, at a geographical elevation of 100-150m. The soil is light sand, the organic matter content is low (0.2-0.4%), humus content is 1.5%, nitrogen and phosphorous levels medium, potassium levels show good supply. Fig. 1 and 2. illustrate the precipitation and the temperature values during the examination period.

Different crossed F2 and F3 breeding strains were used for this experiment. Its reason was that based on the earlier experiences the varieties belonged to spring ecotype are often damaged or killed by frost in winter, contrary to the winter poppy varieties which are able to overwinter. The experimental population propagated by selfings afterwards (Jászberényi and Németh, 2011b). The parental combinations were as follows: '*Medea*' x 'No.67', '*Minoán*' x '*Kozmosz*', '*Medea*' x '*Kozmosz*', '*Korona*' x 'No.67', '*Korona*' x '*Kozmosz*', '*Ametiszt*' x '*Leila*', 'No.1/172' x '*Leila*'. The names in italics show registered varieties, while the ones indicated by numbers were selected and stabilized breeding strains. They included both high and low alkaloid accumulating genotypes. Reciprocal crossings were handled jointly. Each progeny represented the full genotypic constitution without previous selection into any direction.

Sowing of each seed plot was carried out at the end of September 2009 and at the end of March 2010 by hand into open field. The plots were $10 m^2$ in 3 replications. During the vegetation period, the usual poppy agrotechnics had been applied. To avoid the effects of metaxenia (Bernáth et al., 2003), isolation of individual flowers was carried out before anthesis with removing the isolation after

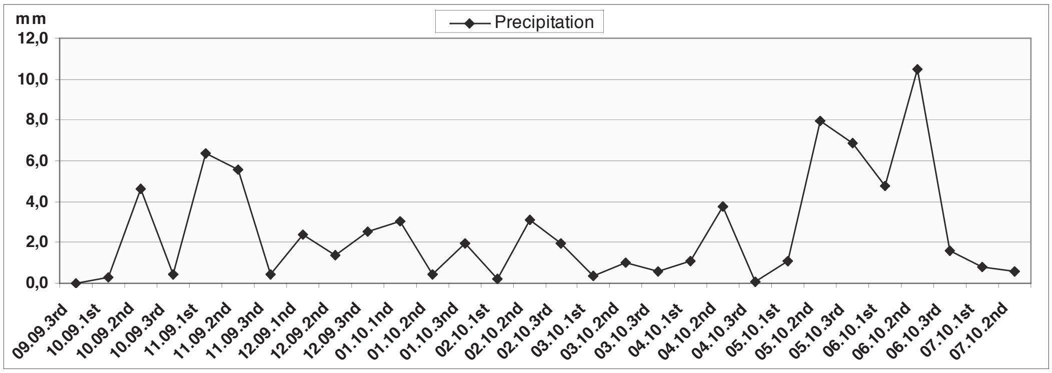

Fig. 1: Precipitation amount at the experimental field in 2009/2010 from the time of sowing to harvesting by decadal (mm)

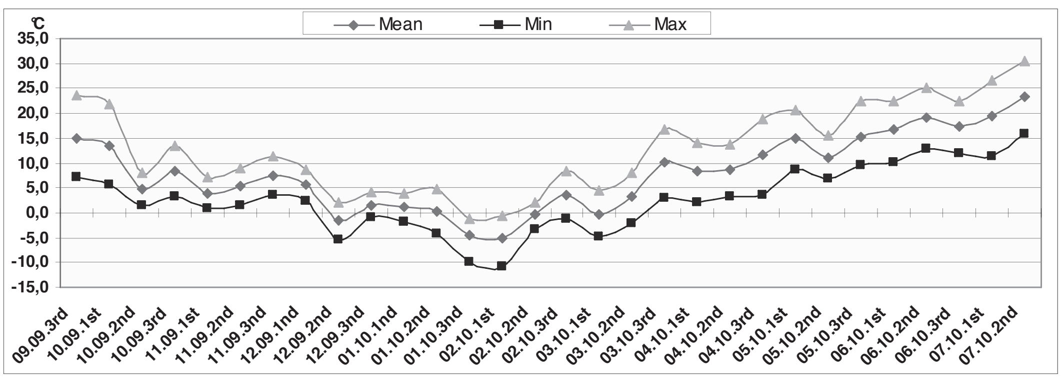

Fig. 2: The temperature values at the experimental field in 2009/2010 from the time of sowing to harvesting by decadal (°C)

fertilisation. The capsules were harvested on 7th July (autumn sown plots) and the 13th of July (spring sown plots).

Analysis of alkaloids was carried out by TLC method in 12 replications per population (NÉMETH et al., 2011). After extraction of pulverized capsules in chloroform- methanol (4:1) solvent in Soxhlet apparatus, for separation horizontal TLC chambers (H-Trennkammer Desaga Nr. 120150) were used. Evaluation was carried out by densitometric analysis (CHR-SCAN TR-541 equipment with a LabChrom™ Chromatographic Data processing System Version 5.2). The densitometric scanning profiles of four alkaloids (morphine, codeine, thebaine and narcotine) were calibrated against the corresponding standards. The accuracy of the measurements is the level of 0.0001%, coefficient of variation of parallel measurements is 3.19%. In the following, alkaloid data refer to the content of ripe capsules in % dry mass.

Biometric evaluation of data (descriptive statistics, distributions fitting, t-test) was carried out by PASW Statistics 18 software.

Results

Based on field evaluation at the end of November and beginning of April, density of the overwintered F2 and F3 populations decreased by 30-50%. It proves the intended natural selection by winter frosts and shows in general a medium level frost tolerance.

Alkaloid content of the experimental populations

Because of the different levels, the evaluation of the total alkaloid content of the experimental population were at first carried out in two groups: combinations of low alkaloid containing varieties and that of high alkaloid containing cultivars. In both categories, samples from autumn sown plots are inferior (0.37% and 1.27%, respectively) compared to the spring sown ones (0.57% and 1.76%, respectively). *Standard deviations* of data in autumn sown and spring sown plants are practically the same (0.66; 0.68) in the high alkaloid group, while it is somewhat different in the low alkaloid group (0.26; 0.48).

In Fig. 3. distributions of the studied samples are illustrated together with the fitted normal equations whose peaks show the mean values of the populations. The negative shift in the alkaloid content of the overwintered plots is obvious.

Evaluation has been carried out also according hybrid strains, separately. Comparing the *total alkaloid values* of the same genotypes originating from autumn or spring sowing, it could be established that in the majority of cases there are significant deviations between them (Tab. 1). The presence of this difference seems to be independent of the genetic combination or of the fact if it is an F2 or F3 generation.

Not only means but also the *marginal values* show the difference among the experimental populations. Except the combinations '*Korona*' x '*Kozmosz*', '*Ametiszt*' x '*Leila*', 'No.1/172' x '*Leila*', the *minimum values* of the strains are lower in autumn sown plots than in the spring sown ones in each case. The lowest *individual value* in the populations is 0.10% both in case of autumn hybrids or the population not being selected by frost (Tab. 1.).

The *maximum values* are even more interesting. Existence of some single plants in the frost selected population reaching similar values than the ones in the non-selected ones may indicate the potential of winter hardy genotypes for high alkaloid accumulation. In the recent study both similar ('*Medea*' x No.67 F2, '*Medea*' x '*Kozmosz*' F3) and higher ('*Minoán*' x '*Kozmosz*' F2, '*Korona*' x No. 67 F3) *individual values* had been found in the autumn sown plots compared

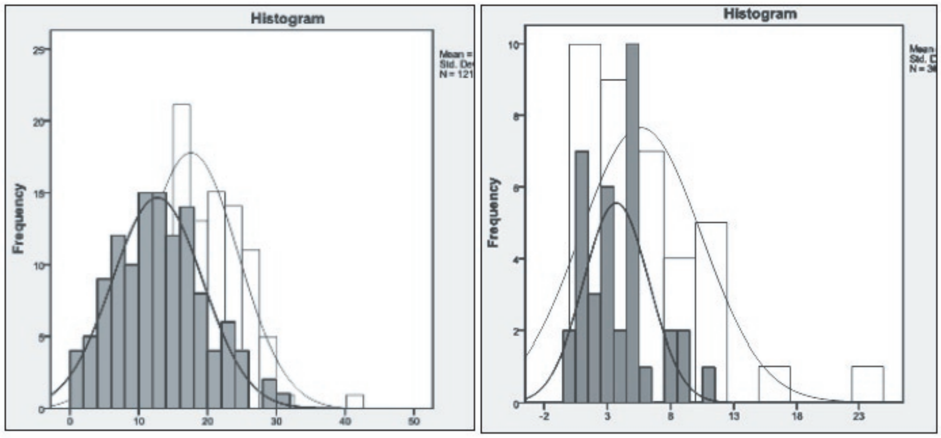

Fig. 3: Distributions of experimental data from autumn sown (dark) and spring sown (light) plots. Left: high alkaloid containing group; Right: low alkaloid containing group of hybrids.

Tab. 1: Total alkaloid content values of the experimental populations in autumn and spring sown (selected by frosts) plots

		Total alkaloid content (%)							
		Autumn sowing				**Spring sowing**			
		Min.	**Max.**	**Mean**	**Std. Dev.**	**Min.**	**Max.**	**Mean**	**Std. Dev.**
F2	**'*Medea*' x No. 67**	0.10	2.90	1.54 A	0.94	0.60	2.90	1.90 A	0.63
	'*Minoán*' x '*Kozmosz*'	0.15	2.20	1.24 A	0.48	0.70	2.05	1.39 A	0.47
	'*Medea*' x '*Kozmosz*'	0.40	2.30	1.43 **A**	0.61	1.15	2.60	1.91 **B**	0.46
	'*Korona*' x No. 67	0.40	2.20	1.28 **A**	0.54	1.10	4.20	2.18 **B**	0.90
	'*Korona*' x '*Kozmosz*'	0.30	1.70	0.88 **A**	0.44	0.30	2.50	1.59 **B**	0.80
	'*Ametiszt*' x '*Leila*'	0.05	1.05	0.48 A	0.30	0.00	1.20	0.51 A	0.41
	'No. 1/172' x '*Leila*'	0.00	0.85	0.36 A	0.22	0.00	2.30	0.58 A	0.72
Mean		**0.27**	**2.26**	**1.04 A**	**0.69**	**0.77**	**2.85**	**1.43 B**	**0.88**
F3	**'*Medea*' x No. 67**	1.05	2.40	1.73 **A**	0.43	1.70	3.20	2.37 **B**	0.44
	'*Minoán*' x '*Kozmosz*'	0.20	2.30	1.26 **A**	0.64	0.85	2.95	1.84 **B**	0.64
	'*Medea*' x '*Kozmosz*'	0.00	2.50	1.23 A	0.60	0.60	2.60	1.56 A	0.54
	'*Korona*' x No. 67	0.25	3.10	1.33 A	0.90	0.55	2.10	1.36 A	0.47
	'*Korona*' x '*Kozmosz*'	0.10	1.65	0.78 **A**	0.38	0.50	2.70	1.52 **B**	0.74
	'*Ametiszt*' x '*Leila*'	0.00	0.75	0.28 A	0.23	0.25	1.00	0.61 B	0.25
Mean		**0.32**	**2.39**	**1.10 A**	**0.72**	**0.84**	**2.71**	**1.54 B**	**0.74**
Total mean				**1.07 A**	0.70			**1.48 B**	0.82

with the spring sown ones. Nevertheless, in majority of cases, the populations' maximum values were higher in the non-frost selected, spring sown plots (1.00%-4.20%) (Tab. 1.).

The *total mean standard deviation* of the tested combinations in autumn and spring sowing plots are 0.70 and 0.82 respectively. It indicates similar order of magnitude and no obvious connection with the experimental treatments.

Alkaloid spectrum of the experimental combinations

Fig. 4 shows the levels of four measured *alkaloid compounds: morphine, codeine, thebaine* and *narcotine*, separately. (As any other alkaloids are present only in traces, the total values are identical with the mean values of the total alkaloid levels in Tab. 1.). It seems that in the majority of strains each alkaloid compound was reduced in the samples from overwintered plants, although a significant difference could be proved only for *thebaine*. Opposite tendencies were found only in two cases: for *codeine* content in F2 population of the cross '*Medea*' x No.67 and for narcotine content in F3 population of the cross '*Korona*' x No. 67.

Mean values for *morphine, codeine, thebaine* and *narcotine* in samples of overwintered plants are 0.68, 0.06, 0.16 and 0.16%, respectively. The same compounds in samples of spring sown plants were 0.84, 0.10, 0.34 and 0.21%, respectively.

Maximum values of alkaloids in the overwintered populations were 1.12, 0.29, 0.50 and 0.53 for *morphine, codeine, thebaine* and *narcotine*, respectively, while they reached 1.26, 0.49, 0.93 and 0.60% for these four compounds, respectively.

Discussion

The study focused on the question if alkaloid accumulation may be limited to winter hardy genotypes of poppy as frequently anticipated in the practice.

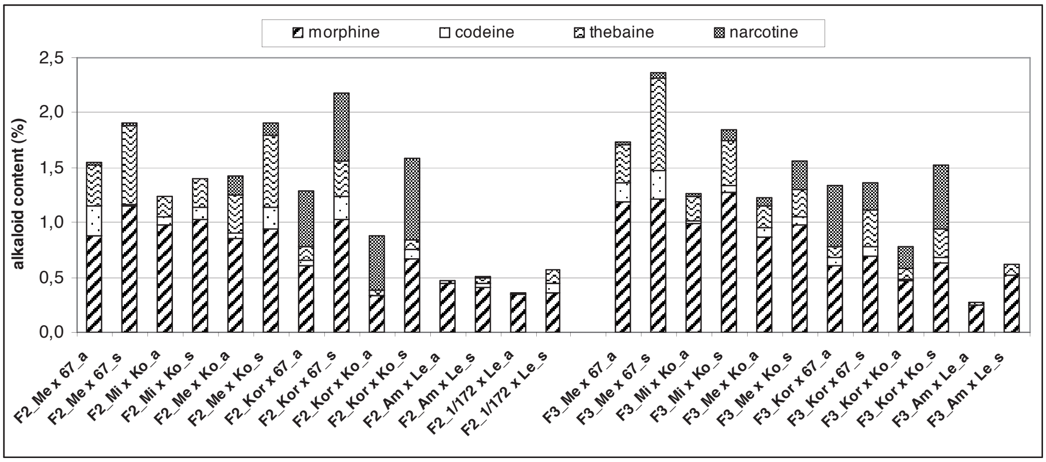

Fig. 4: Alkaloid spectrum of the experimental populations in autumn ('a') and spring ('s') sown (selected by frosts) plots

The experimental plots thinned due to winter frosts exhibited significantly lower *total alkaloid contents* than the samples from spring sown plots of the same populations. Although in open field, weather conditions – especially summer rain – may contribute to alkaloid accumulation and eventual losses (BERNÁTH and TÉTÉNYI, 1981), our findings ascertained the old practical observations. The lower level in overwintered plants was proved for each of the individual alkaloid compounds *morphine, codeine, thebaine* and *narcotine,* too.
The genes which might be in linkage with those regulating frost tolerance seem to be more likely present at the primarly level of alkaloid biosynthesis. These regulate the synthesis of general precursors and not the characteristic opiate alkaloid compounds of the poppy capsule formed at the final stages of biosynthesis (ZIEGLER et al., 2009). Clarification of this question needs further research.
However, at the same time, *maximum values* in some individual samples of the overwintered plants reached similar or higher contents than the spring sown ones. Therefore it is supposed that even if gene frequency in direction for high alkaloid level might be larger in frost sensitive ecotype, the potential for breeding high alkaloid accumulating cultivars exists also among frost tolerant genotypes.

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