MORPHINE CONTENT VARIATION IN *PAPAVER SOMNIFERUM* L. DURING PHENOLOGICAL DEVELOPMENT

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Abstract

In this sttuddy we analized dynamic of morfine content in different organs of five selected lines of *Papaver somniferum* L. during ontogenetic cycle. Five selected by self pollination lines (with pink, white, mauve, dark mauve and red petals) were use in our experiment. The collection of sample material and the analysis of the morphine content were conducted along five stages of vegetation corresponding to the the most important moments in the ontogenetic development of plants: rosette stage at 25 day after germination, bud initiation stage at 43 day after germination, bud dropping stage at 61 day after germination, flowering stage at 75 day after germination and lancing stage at 85 day after germination. The earlier stage of morphine detection was in rosette study only in the roots in the 0.001% d.w. The level of morphine in the leaves of all genotypes increased starting at the 43th day after germination and ending at the green capsule stage. During bud dropping stage (pendulous bud) the roots was recorded a higher morphine amount, which decreases to extinction in the lancing stage. The comparative analyze of morphine content of five different genotypes using in our experiment show that no many difference between morphine accumulation profiles during the life cycle.

Key words: *Papaver somniferum*, morphine, phenological stage

The opium poppy is an ancient medicinal plant and the only commercial source for the narcotic analgesics morphine and codeine. Despite the large significance of this specie, many basic aspects of morphynanic alkaloids metabolism are poorly understood.

Morphine is a major component of the alkaloid-rich latex in opium poppy, abundant in aerial organs. The accumulation of morphine and related secondary metabolites in the large, membranous vesicles of opium poppy latex has contributed to the long-standing assumption that alkaloids are synthesized in laticifers (Fairbairn and Wassel, 1964; Fairbairn et al., 1968; Wilson and Coscia, 1975; Roberts et al., 1983)

However, several key enzymes involved in morphine biosynthesis have not been detected in latex (Gerardy and Zenk, 1993), suggesting that although alkaloids accumulate in laticifers, their synthesis occurs elsewhere. Alkaloids can accumulate in the cell and tissue in which they are formed, but pathway intermediates and end products can also be transported to other locations for further elaboration or accumulation. Several different tissue types — epidermis, endodermis, laticifers, idioblasts, pericycle and cortex — have been implicated in the biosynthesis and/or accumulation of various alkaloids in plants (Facchini, 2001). Alkaloids generally accumulate in specific cell types because of their cytotoxicity and probable role in plant defense responses. In opium poppy, benzylisoquinoline alkaloid accumulation is restricted to laticifers, which are found adjacent or proximal to sieve elements of the phloem (Alcantara, Bird,. Franceschi and. Facchini, 2005).

It appears that the early stages of morphine biosynthesis, starting with the decarboxylation of amino acid L-tyrosine occur in the parenchyma cells surrounding laticifers. In contrast, the later stages of morphine biosynthesis occur in the laticifer, which is the storage site of morphine alkaloids thebaine, codeine and morphine (Kutchan, 2005).

However, much larger differences have been noted in alkaloid accumulation during the vegetation cycle.

In this sttuddy we analized dynamic of morfine content in different organs of five selected lines of papaver somniferum during ontogenetic cycle in order to obtain more data for elucidate the spatial localization of morphine biosinthesis.

MATERIAL AND METHOD

The plant material used in this study originated from seeds provided from some local biotopes and inbreeded by self pollination during 15 generation. During this period we selected some varieties with different petal colour, five of

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them (with pink, white, mauve, dark mauve and red petals) were use in our experiment. The seeds were open growth cultivated on the experimental field of Botanical Garden of Iasi.

The collection of sample material and the analysis of the morphine content were conducted along five stages of vegetation corresponding to the most important moments in the ontogenetic development of plants: rosette stage at 25 day after germination, bud initiation stage at 43 day after germination, bud dropping stage at 61 day after germination, flowering stage at 75 day after germination and lancing stage at 85 day after germination.

Five individual plants from each variant, was removed from the field and separated into root, leave and generative organ witch function of developmental stage was flower or green capsule and were analysed by the thin-layer chromatographic (NYMAN and HANSSON 1978). Quantitative determinations of the morphine content, were made according to the spectrophotometric method (NYMAN and HANSSON 1978). and morphine content was expressed in % of dry matter.

RESULTS AND DISCUSSIONS

In rosette stage (23 day after germination) we found the presence of small amounts of morphine only in the roots. Before this stage it was observed that seedlings do not accumulate any alkaloids in the radices (*fig.1*).

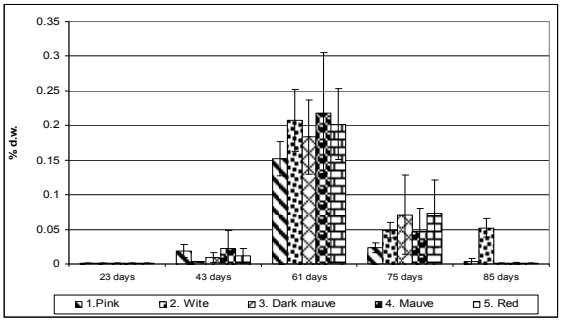

Figure1 **Dynamic of morphine content in roots of five varieties of *Papaver somniferum* during ontogenetic cycle**

From the anatomical point of view there is a structural possibility for the accumulation of alkaloids when the laticiferous vessels appear at the opening but, the accumulation starts two weeks later, after the formation of the leaves.

The results of our investigations support the theory that the formation of alkaloids is closely connected to the photosynthesis and they are synthesized only after assimilating tissue formation. At bud initiation stage morphine, was present in both roots and leaves, but the higher amount was detected in the leaves. As shown in *figure 2*, the level of morphine in the leaves of all cultivars increased starting at the 43th day after germination and ending at the green capsule stage.

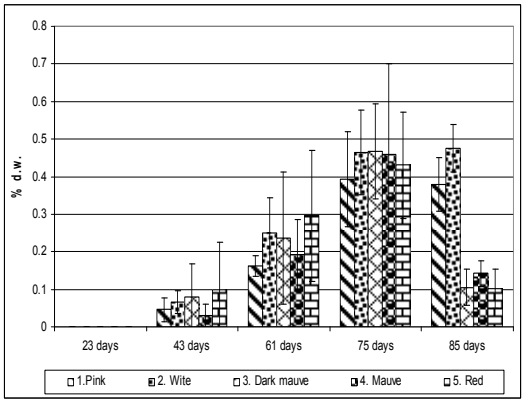

Figure 2 **Dynamic of morphine content in leaves of five varieties of *Papaver somniferum* during ontogenetic cycle**

During this period of the vegetation phase the live accumulation level of alkaloids increased 3–4 times. The proportion of morphine was in harmony with the findings of Fairbairn, J.W., Djote, M., and Paterson, A. (1968).

During bud dropping stage (pendulous bud) the sepals, petals and anthers had morphine in the similar concentration that we found in the lives around 0.3% d.w. In the same time the roots were recorded a pick of morphine amount (aprox. 0.2% d.w.) after this stage we was recorded a decrease of morphine content in the root to they disappearance in the lancing stage (*fig. 3*).

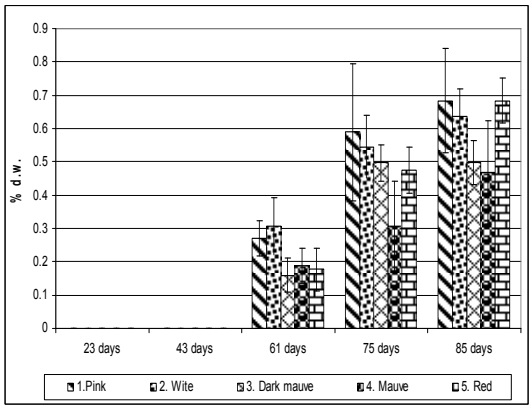

Figure 3 **Dynamic of morphine content in capsule of five varieties of *Papaver somniferum* during ontogenetic cycle**

According to Bunting et al. (1963) and Schröder (1965) (cited by Husain. and Sharma 1983), maximum morphine accumulation in the capsule was reached 42 days after flowering. There is a subsequent decrease in the alkaloid level which was explained in different ways, such as the effect of weather conditions, infection by fungi, enzymatic degradation, etc.

Alcantara, J. Bird, D.A. Franceschi V.R. and Facchini, P.J.,2005 studied the effect of leaching on the alkaloid content of capsules and concluded that in the absence of leaching the morphine percentage reaches its maximum 42 day after flowering and remains constant after there. The comparative analyze of morphine content of five different genotypes using in our experiment show that no many difference between morphine accumulation profiles during the life cycle.

Investigations concerning alkaloid accumulation in the capsule have both theoretical and practical importance. In general reproductive organs accumulate more of the alkaloids than other parts. In this experiment we can be seen that morphine content increased continuously during flowering and green capsule development (*Tab.1*).

Table1

Dynamic of morphine content (% d.w.) in the different organs of five varieties of *Papaver somniferum* during ontogenetic cycle

	1. Pink	2. Wite	3. Dark mauve	4. Mauve	5. Red
Roots					
23 days	0.001 ± 0.000	0.001 ±0.000	0.001 ±0.000	0.001 ±0.000	0.001 ±0.000
43 days	0.0184 ± 0.009	0.0032 ±0.001	0.0094 ±0.008	0.0224 ±0.025	0.0118 ±0.010
61 days	0.1526 ± 0.025	0.207 ±0.045	0.1834 ±0.05	0.2178 ±0.087	0.2016 ±0.051
75 days	0.0236 ± .007	0.0488 ±0.011	0.0712 ±0.057	0.0468 ±0.034	0.0728 ±0.049
85 days	0.0036 ± 0.005	0.052 ±0.014	0.001 ±0.000	0.0014 ±0.001	0.001 ±0.000
Leaves					
23 days	0.000 ±0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00
43 days	0.0456 ± 0.03	0.0656 ± 0.03	0.0802 ± 0.09	0.0308 ± 0.03	0.0986 ± 0.13
61 days	0.162 ± 0.03	0.2504 ± 0.09	0.236 ± 0.18	0.1918 ± 0.10	0.296 ± 0.17
75 days	0.3932 ± 0.13	0.465 ± 0.11	0.4682 ± 0.13	0.458 ± 0.24	0.4306 ± 0.14
85 days	0.3794 ± 0.07	0.4762 ± 0.06	0.1058 ± 0.05	0.1422 ± 0.04	0.1014 ± 0.05
Capsule					
23 days	0.00 0 ± 0.00	0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00
43 days	0.00 ±.000	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
61 days	0.27 ± 0.05	0.31 ± 0.09	0.16 ±0.05	0.19 ± 0.05	0.18 ± 0.06
75 days	0.59 ± 0.20	0.55 ± 0.09	0.50 ± 0.06	0.31 ± 0.13	0.47 ± 0.07
85 days	0.68 ± 0.16	0.64 ± 0.08	0.50 ± 0.07	0.47 ± 0.16	0.68 ± 0.07

Pink and white varieties maintained a high level of leave morphine even at lancing stage and white variety record a significant amount at the same stage in the root.

CONCLUSIONS

Summarizing results of the short term experiment results together with previously acquired experience following could be concluded:

The earlier stage of morphine detection was in rosette study only in the roots in the 0.001% d.w.

The level of morphine in the leaves of all genotypes increased starting at the 43th day after germination and ending at the green capsule stage.

During bud dropping stage (pendulous bud) the roots was recorded a higher morphine amount, which decreases to extinction in the lancing stage.

BIBLIOGRAPHY

Alcantara, J. Bird, D.A. Franceschi, V.R. and Facchini, P.J. , 2005 - *Sanguinarine biosynthesis is associated with the endoplasmic reticulum in cultured opium poppy cells after elicitor treatment,* Plant Physiol 138, pp. 173–183.

Bird, D.A. Franceschi, V.R. and Facchini, P.J.A, 2003 - *Tale of three cell-types: alkaloid biosynthesis is localized to sieve elements in opium poppy*, Plant Cell 15.

Facchini, P.J., 2001 - *Alkaloid biosynthesis in plants: Biochemistry, cell biology, molecular regulation, and metabolic engineering applications.* Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 29–66.

Fairbairn, J.W., and Wassel, G.,1964 - *The alkaloids of Papaver somniferum L.: Biosynthesis in isolated latex.* Phytochemistry 3, 583–585.

Fairbairn, J.W., Djote, M., and Paterson, A.,1968 - *The alkaloids of Papaver somniferum L. VII. Biosynthetic activity of the isolated latex.* Phytochemistry 7, 2111–2116.

Gerardy, R., and 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.

Griffing, L.R., and Nessler, C.L., 1989 - *Immunolocalization of the major latex proteins in developing laticifers of opium poppy (Papaver somniferum).* J. Plant Physiol. 134, 357–363.

Hosztfi, S., 1998 - *Chemistry-biochemistry of poppy. I*n: BernnathJ. (ed.), Poppy the Genus Papaver. Harwood Academic Publishers, Australia, pp. 105–153.

Husain, A. and Sharma, J.R., 1983 - *Opium Poppy: CIMAP.*Lucknow Publishing House, Lucknow.

Nyman , U. Hall, O., **1976** - *Some varieties of papaver somniferum L. with changed morphinane alkaloid* . Hereditas, 84, p69-76

Prajapati S., Bajpai, S., Singh, D., Luthra, R., Gupta, M.M. and Kumar, S., **2002 -** A*lkaloid profiles of the Indian landraces of the opium poppy Papaver somniferum L.* Genet. Resour.Crop Evol. 49(2): 183–188.

Roberts, M.F., McCarthy, D., Kutchan, T.M., and Coscia, C.J., **1983** - *Localization of enzymes and alkaloidal metabolites in Papaver latex.* Arch. Biochem. Biophys. 222, 599–609

Wilson, M.L., and Coscia, C.J., 1975 - *Studies on the early stages of Papaver alkaloid biogenesis*. J. Am. Chem. Soc. 97, 431–432.

Winkel B.S.J., 2004 - *Metabolic channeling in plants,* Annu Rev Plant Biol 55 , pp. 85–107.