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Diurnal Variation of Major Alkaloids in *Papaver somniferum*

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With 2 Figures

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Summary

SHUKLA S., KHANNA K. R. & SINGH S. P. 1996. Diurnal variation of major alkaloids in *Papaver somniferum* L. – *Phyton* (Horn, Austria) 36 (1): 1-8, 2 figures. – English with German summary.

Biosynthetic variation of secondary plant products in opium poppy (*Papaver somniferum*) was studied during 1989-90 and 1990-91 on two varieties ('NBRI-1' & 'NBRI-2') at different lancing and opium collection period. A rapid marked variation in the content of major alkaloids in opium latex at different times suggests that the alkaloids play an active part in metabolism rather than accumulating. In general, morphine, codeine, thebaine and narcotine was high at 12 noon and 4 p.m. in Ist and IInd lancements in both the capsules of 'NBRI-1' and 'NBRI-2'. The papaverine content was noticed very low in both the varieties, however, it was noticed at 8 a.m. and 6 p.m. in 'NBRI-1' in both the years and only at 6 p.m. in 'NBRI-2' during 1989-90. Increase

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of morphinane alkaloids and decrease of papaverine or vice- versa suggests the different biosynthetic pathways.

Zusammenfassung

SHUKLA S., KHANNA K. R. & SINGH S. P. 1996. Tageszeitliche Änderungen in den Hauptalkaloiden von *Papaver somniferum*. - *Phyton* (Horn, Austria) 36 (1): 1-8, 2 Abbildungen. - Englisch mit deutscher Zusammenfassung.

Die biosynthetische Variation von sekundären Pflanzenstoffen wurde zu unterschiedlichen Tageszeiten an zwei Varietäten des Schlafmohns (*Papaver somniferum*, 'NBRI-1' und 'NBRI-2' zwischen 1989-90 und 1990-91 untersucht. Eine schnelle Änderung im Gehalt der Hauptalkaloide des Milchsaftes zu verschiedenen Zeiten läßt vermuten, daß die Alkaloide eher eine aktive Rolle im Stoffwechsel spielen, als daß sie bloß akkumulieren. Im allgemeinen ist der Gehalt an Morphin, Codein, Thepain und Narkotin um 12 und 16 Uhr hoch; dies betrifft sowohl beide Varietäten als auch die zwei untersuchten unterschiedlichen Schnittstellen. Die Menge an Papaverin war in beiden Varietäten sehr gering und konnte in beiden Jahren um 8 und 18 Uhr in 'NBRI-1' bzw. nur um 18 Uhr in 'NBRI-2' 1989-90 gefunden werden. Der Anstieg von Morphin-Alkaloiden und die Verminderung von Papaverin bzw. umgekehrt, deutet auf verschiedene Biosynthesewege.

Introduction

The opium poppy (*Papaver somniferum* L.) latex is a rich source of secondary metabolites (alkaloids) viz. morphine, codeine, thebaine, narcotine and papaverine, in which morphine, codeine and thebaine are extensively used in pharmaceutical industries. Secondary plant products in many crops show seasonal changes in the content at different stages of growth period. Thus, the appropriate plant part and optimum growth have to be determined. If optimum time of harvest is missed, the active principle may be metabolised, i.e. it may be transformed or degraded. If diurnal variation is not taken into consideration and samples for genetic analysis are collected at random during any time of the day, the data would be seriously biased. In opium poppy such studies have been limited, however, FAIRBAIRN & WASSEL 1964 observed marked variation in the content of alkaloids viz. morphine, codeine and thebaine at the interval of 1-3 hours, based on the paper chromatography. Keeping the practical aspects of alkaloids biosynthesis, the present investigation was undertaken to find out the proper time for latex collection and the period of maximum biosynthesis of particular alkaloid.

Materials and Methods

The genetically homozygous varieties of *Papaver somniferum* viz. 'NBRI-1' and 'NBRI-2' were selected. The crop was grown in the crop year 1989-90 and 1990-91. The cultural practices were the same as given earlier (KHANNA & GUPTA 1983, KHANNA & SHUKLA 1986a), the lancing was done in early March in both the years. The samples were collected from 8 a.m. to 6 p.m. at the interval of 2 hrs. A sample constituted fresh

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latex of five plants extracted in Dimethyl Sulphoxide (DMSO). Two successive lancing during 1989-90 and three in 1990-91 were made on two capsules i.e. Ist (terminal) and IInd (side) and latex were collected separately in both the years. The interval between successive lancements was 3-4 days. The fresh latex from the five plants of each line for each specific capsule position, lancing number and time were pooled and extracted in 5ml. DMSO. In this way, each line included 24 samples in 1989-90 and 36 samples in 1990-91.

The fresh latex weight was measured by subtracting the weight of culture tube + 5ml DMSO prior the collection and after the collection of latex. The opium samples triturated in 5ml DMSO were ordinary filtered and then runned the samples through water's classification kit. The injection volume was 5 μ lit. and attenuation was 0.05 AUFS. Other experimental details with regard to the analysis of the opium samples by HPLC are the same as given previously (KHANNA & SHUKLA 1986a).

Results and Discussion

A marked variation (Fig.1 & 2, Table 1) was noticed for five major alkaloids i.e. morphine, codeine, thebaine, narcotine and papaverine in both the varieties and years in different collecting period of samples in opium poppy (*Papaver somniferum* L.). In general alkaloid content was high at 12 noon and rapidly declines around 2 p.m. and further increases at 4 p.m. and 6 p.m. At 8 a.m., in some cases, an increasing trend of the alkaloids was observed, which indicates the possibility of the higher content in early hours, though it was not planned in the present experiment. In other parts of the day an irregular trend of alkaloids content was noticed.

The morphine content was high at 12 noon and 4 p.m. in Ist and IInd lancing of both the capsules of 'NBRI-1' and 'NBRI-2', in both the years except IInd lancing in 'NBRI-2' at 12 noon and in both the cultivars at 4 p.m. In third lancing too, the morphine peak was noticed at 12 noon and 6 p.m. in both the capsules in 1990-91 except the IInd capsule of 'NBRI-1'. Similarly, peaks of codeine, thebaine and narcotine were also noticed at 12 noon in Ist and IInd lancements except in IInd capsule of Ist lancing of 'NBRI-1' during 1989-90 and 'NBRI-2' during 1990-91 and in IInd lancing of IInd capsule during 1989-90 for codeine, in Ist and IInd lancing of Ist capsule in 1990-91 and IInd lancing of IInd capsule in both the years for thebaine in 'NBRI-2'. An irregular trend was also noticed for narcotine in 1990-91 for 'NBRI-2' in Ist and IInd capsules of both the lancements. At 4 p.m., though peaks were observed for codeine, thebaine and narcotine the trend was some what irregular. In general simultaneous increase and decrease of morphinane alkaloids in both the years and varieties were noticed. Contrary to this FAIRBAIRN & WASSEL 1964 observed irregular time of synthesis of morphine, codeine and thebaine. High morphine content in morning, codeine and thebaine at noon and further reported that rise in thebaine and codeine corresponded with marked fall in morphine content.

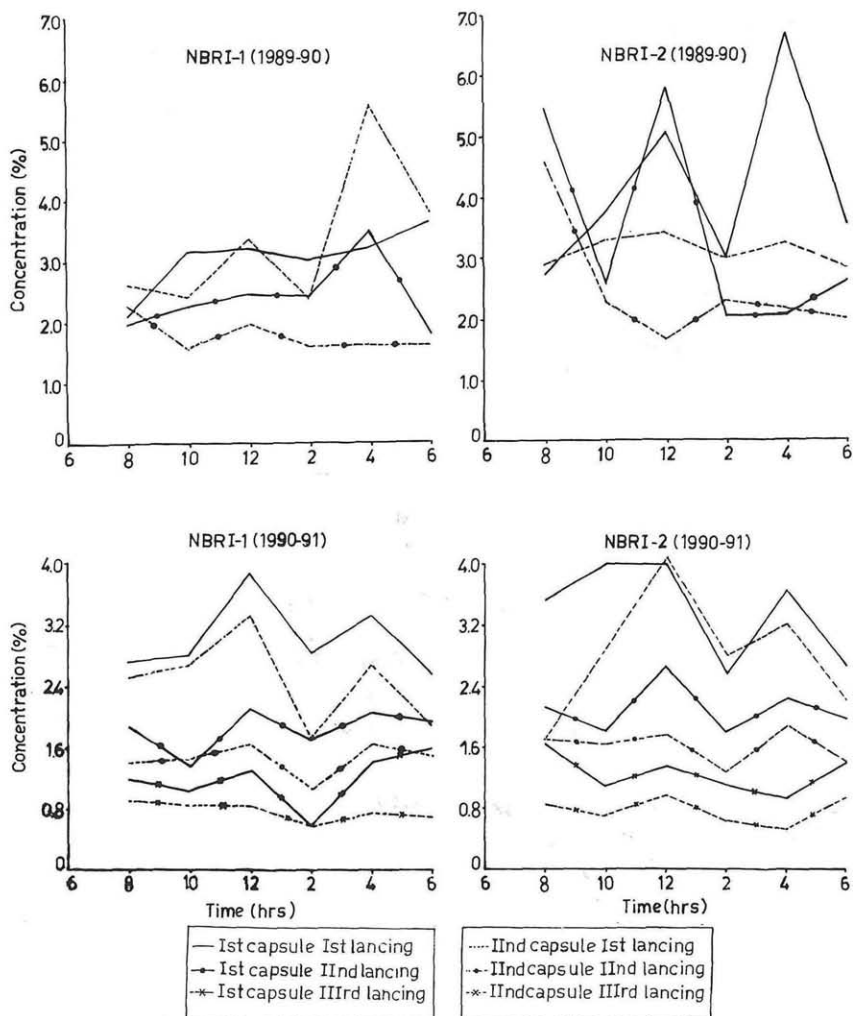


Fig. 1. Variation of morphine at different lancing period and capsule position in *P. somniferum* L.

The contradiction of this result may be due to temperate/tropical climatic conditions as well as the genetic material used.

Increase or decrease of morphine, codeine, thebaine and narcotine show single chain of biosynthesis. It is due to fact that at the above period initiation of principal component DOPA (dihydroxyphenylalanine) responsible for alkaloids biosynthesis takes rapidly by decarboxylation and condensation and forms norlaudanoline which after methylation gives

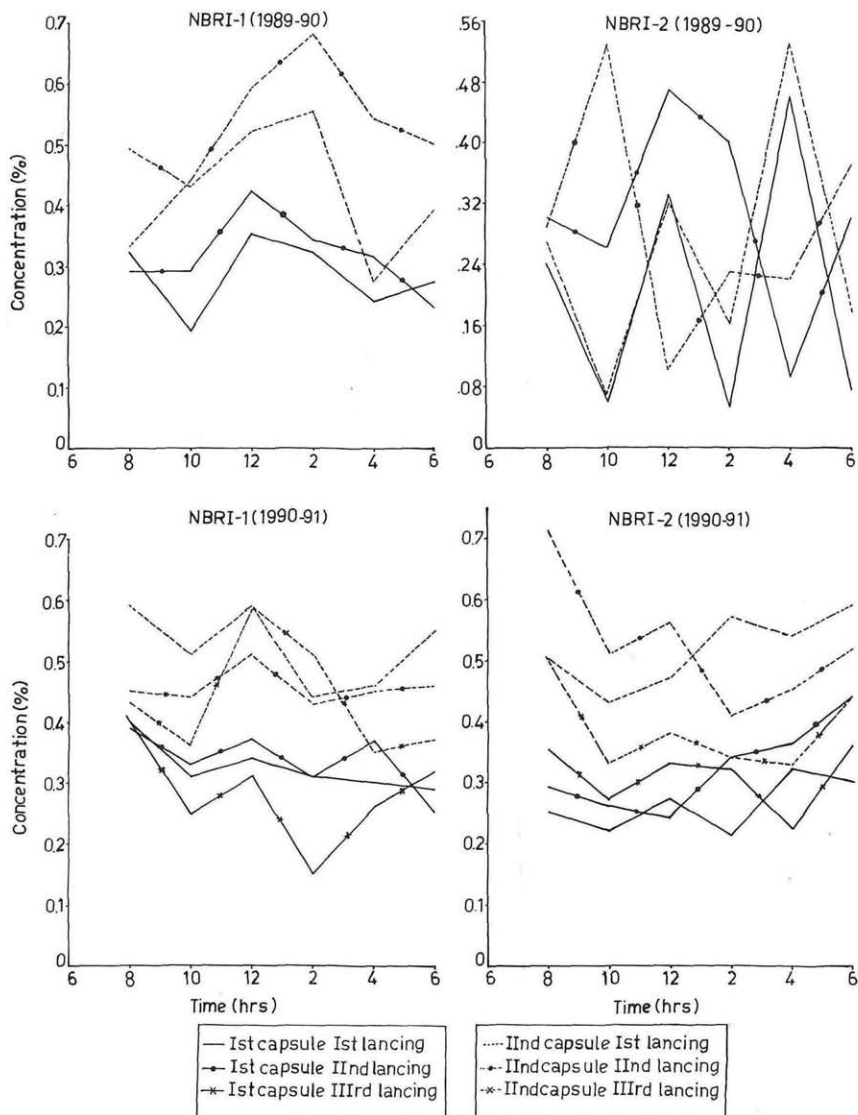


Fig. 2. Variation of codeine at different lancing period and capsule position in *P. somniferum* L.

an important compound, 'reticuline' involved in biosynthesis of morphine through thebaine and codeine at one hand and narcotine on the other. Since morphine occurs as irreversible 'end product' of the sequence thebaine → codeine → morphine (STERMITZ & RAPOPORT 1961), it decreased markedly at

Table 1: Peaks of major alkaloids at different time, lancing and Capsule position in opium poppy

I st Capsule												II nd Capsule											
I st lancing				II nd lancing				III rd lancing				I st lancing				II nd lancing				III rd lancing			
'NBRI-1'				'NBRI-2'				'NBRI-1'				'NBRI-2'				'NBRI-1'				'NBRI-2'			
Time (hrs)				Time (hrs)				Time (hrs)				Time (hrs)				Time (hrs)				Time (hrs)			
89-90	90-91	89-90	90-91	89-90	90-91	89-90	90-91	89-90	90-91	89-90	90-91	89-90	90-91	89-90	90-91	89-90	90-91	89-90	90-91	89-90	90-91		
90-91																							
8 am	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Irr.	-	-	-	-	-		
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	T	Irr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
10 am	P	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	T	Irr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
12 Noon	M	I	I	I	I	I	I	I	I	I	I	I	I	I	I	-	-	-	-	-	-		
	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	-	-	-	-	-	-		
	T	Irr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	N	I	I	I	I	I	I	I	I	I	I	I	I	I	I	-	-	-	-	-	-		
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
2 pm	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	T	Irr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
4 pm	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	T	Irr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
6 pm	M	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	T	Irr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	P	II	III	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

M = morphine, C = codeine, T = thebaine, N = narcotine, P = papaverine, I = Ist peak, II = IInd peak, III = IIIrd peak, Irr. = Irregular.

certain times. This suggests that morphine soon after its formation in latex is converted into non alkaloidal substances and translocated from laticifers of the capsule to other tissues and some reach to developing ovules (FAIRBAIRN & EL-MASRY 1967). The increase of papaverine and decrease of other alkaloids indicates another compound nor-orientaline responsible for papaverine synthesis. Formation of papaverine from nor-reticuline via nor-laudanidine and nor-laudanosine was reported (HEMA & al. 1975). In the present study, the papaverine content in both the cultivars is very low. However, in 'NBRI-1' papaverine content was noticed at 8 a.m., 2 p.m. and 6 p.m. in all the 3 lancings of both the capsules in 1990-91 and 8 a.m. and 6 p.m. during 1989-90. In 'NBRI-2', no peak was observed in 1990-91 but a peak at 6 p.m. in 1989-90 was observed in both the capsules and lancings. Variation in major alkaloids of opium poppy, is reported earlier as well. KHANNA & SHUKLA 1986a studied the inheritance of five major alkaloids viz. morphine, codeine, thebaine, narcotine and papaverine in interspecific cross of *P. somniferum* and *P. setigerum* and obtained heterotic increase in codeine and thebaine in F1 while in F2 with exception of codeine all the alkaloids showed transgressive segregation (for instance thebaine content in F2 was 14.67% while in parents, *P. somniferum* and *P. setigerum*, it was 1.2 and 2.27% respectively). KHANNA & GUPTA 1983 observed the decline of morphine content in successive lancings and from top to lower capsules. Further, KHANNA & SHUKLA 1986b extended this study for major alkaloids in two varieties viz. 'NBRI-1' and 'NBRI-2' and confirm the findings for morphine distribution while codeine and thebaine were maximum in IInd lancing of IInd capsule. The pattern of narcotine was irregular and papaverine was noticed only in the Ist lancing of Ist capsule. The present study involves diurnal variation of accumulation of the alkaloids, which is different from earlier studies (FAIRBAIRN & WASSEL 1964).

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