Phyton (Horn, Austria)	**Vol. 41**	Fasc. 1	87–96	29. 6. 2001

Alkaloid Profile in Relation to Different Developmental Stages of *Papaver somniferum* L.

By

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With 2 figures

Received January 17, 2000

Accepted August 28, 2000

Key words: Alkaloid, *P. somniferum*, morphine, codeine, thebaine, noscapine, papaverine.

Summary

SHUKLA S. & SINGH S. P. 2001. Alkaloid profile in relation to different developmental stages of *Papaver somniferum* L. – Phyton (Horn, Austria) 41 (1): 87–96, 2 figures. – English with German summary.

The alkaloids variation and its synthesis were studied in two varieties (NBRI-1, NBRI-2) of opium poppy (*Papaver somniferum* L.) on fresh weight basis of different plant parts at different growth periods. In cotyledon stage (3–4 days after germination) only morphine was present. In roots of two leave stage, thebaine was observed beside morphine. At bud initiation stage morphine, codeine and thebaine were present during 1994–95 but in 1995–96 thebaine was absent. During bud dropping stage (pendulous bud) the sepals, petals and anthers had morphine. When pendulous bud straightened before flowering it has morphine, codeine and thebaine in all parts including ovary. In general reproductive organs accumulate more of the alkaloids than other parts. At lancing stage (green mature capsule) all the 3 alkaloids were found in traces in the roots and highest in capsules. Maximum morphine content in capsule reaches at maturity.

Zusammenfassung

SHUKLA S. & SINGH S. P. 2001. Das Alkaloidmuster von *Papaver* somniferum L. in Abhängigkeit vom Entwicklungsstadium. – Phyton (Horn, Austria) 41 (1): 87–96, mit 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Die Variation der Alkaloide und deren Synthese wurde an zwei Varietäten (NBRI-1, NBRI-2) des Schlafmohns (*Papaver somniferum* L.) auf Frischgewichtsbasis an verschiedenen Pflanzenteilen und unterschiedlichen Wachstumsstadien untersucht. Im Keimblattstadium (3–4 Tage nach der Keimung) konnte nur Morphin gefunden werden. In den Wurzeln des Zweiblattstadiums konnte außer Morphin auch Thebain beobachtet werden. Zum Zeitpunkt der Knospenanlage waren 1994–1995 Codein und Thebain zu finden, 1995–1996 jedoch fehlte Thebain. Während der Knospenkrümmung besaßen Kelchblätter, Kronblätter und Staubblätter Morphin. Sobald sich die hängenden Knospen vor der Blüte aufrichten, haben sie Morphin, Codein und Thebain in allen Teilen einschließlich des Fruchtknotens. Im allgemeinen akkumulieren reproduktive Organe mehr Alkaloide als andere Teile. Im Stadium der grünen reifen Kapsel wurden von allen 3 Alkaloiden in den Wurzeln Spuren gefunden, am meisten jedoch in den Kapseln. Am meisten Morphin findet man, sobald die Kapseln reif sind.

Introduction

Opium poppy (*Papaver somniferum* L.) has world wide demand in pharmaceutical industries due to its alkaloids viz. morphine, codeine, thebaine, noscapine and papaverine, morphine being the richest source. Whole plant has alkaloid content in it initially from cotyledon stage except seed. However, PETTITI & al. 1987 and PELDERS & ROS 1996 detected morphine and codeine in human urine who consumed poppy seeds. KARTNIG & al. 1993 also obtained morphine and codeine in different quantities among 33 different samples of poppy seeds collected from the different regions of the world. Several attempts have been made to study the alkaloid contents in different plant parts. AKSANOWSKI & al. 1962 have reported the presence of hydrophenantherene group of opium alkaloids in the reproductive organs. SARKANY & al. 1962 have reported codeine, thebaine, cotornoline, noscapine and papaverine in the roots at the early stage of development, but at the blossoming stage accumulation in the roots decreased and the reproductive organs contained the major amount of alkaloids. MICHALES 1966 has confirmed that during the flowering stages the reproductive organs accumulate most of the alkaloids. EL-KHEIR 1975 has done an elaborate study on the alkaloidal content of the stamens development, but no information is available on the different alkaloid synthesis at different stages of plant development at a time. The present investigation is an attempt to deal with the alkaloids variation and synthesis in various development/growth stages of plant on broader spectrum in opium poppy.

Material and Method

The pure seeds of two varieties developed were sown in experimental plot of National Botanical Research Institute, Lucknow in 2nd week of November [26°45 N, 80°53 E]. The spacing was 30 cm between rows and 10 cm between plants. Spacing within rows was maintained after second weeding. Normal cultural practices were followed through out the crop season. Collection of fresh samples were started from

sprouted cotyledons onwards up to mature capsule stage (Table 1) from the experiment conducted during 1994–95 and 1995–96. The samples were collected in forenoon at 10 A.M. by cutting the plant parts up till dryness of the plant in the year 1994–95 for one variety NBRI-1 and in 1995–96 for two varieties NBRI-1 and NBRI-2. In this way total 24 samples in 1994–95 and 27 samples each of NBRI-1 and NBRI-2 in 1995–96 were collected and kept in 10 ml Dimethyl Sulfoxide (DMSO). The culture tubes were weighed alongwith 10 ml DMSO priorily the collection of samples. The

Table 1. Alkaloid content (%) at different stages/part of plant in opium poppy.

Different Stages/parts of plant	days of sample collection (from sowing date)	1994-95					1995-96				
		M	C	T	N	P	M	C	T	N	P
1. Cotyledons	12										
(i)Upper portion (cu)		-	-	-	-	-	0.07 0	0.00	0.00	0.00	0.00
(ii)root (cr)		-	-	-	-	-	0.050	0.00	0.00	0.00	0.00
(iii)total plant (cp)		-	-	-	-	-	0.150	0.00	0.00	0.00	0.00
2. 2 leaves stage plant (2-l)	20										
(i)Leaves (L-2)		0.080	0.00	0.00	0.00	0.00	0.031	0.00	0.00	0.00	0.00
							(Traces)	(0.00)	(0.00)	(0.00)	(0.00)
(ii)root (RL-2)		-	-	-	-	-	0.083	0.009	0.004	0.00	0.00
							(0.020)	(0.00)	(0.267)	(0.00)	(0.00)
3. 4 leaves stage plants (4-l)	30										
(i)leaves (L-4)		0.047	0.028	0.019	0.00	0.00	-	-	-	-	-
							-	-	-	-	-
4. 8 leaves stage plants (8-l)	42										
(i)leaves (L8)		0.040	0.022	0.004	0.00	0.00	0.013	0.005	0.00	0.00	0.00
							(0.016)	(0.00)	(0.00)	(0.00)	(0.00)
(ii)root (RL-8)		-	-	-	-	-	0.040	0.00	0.053	0.00	0.00
							(0.029)	(0.029)	0.00	0.00	0.00
5. 16 leaves stage plants (16-l)	66										
(i)leaves (L-16)		0.015	0.004	0.002	0.00	0.00	Traces	0.00	0.00	0.00	0.00
							(0.007)	(0.00)	(0.00)	(0.00)	(0.00)
(ii)root (RL-16)		-	-	-	-	-	0.028	0.011	0.00	0.00	0.00
							(0.013)	(0.00)	(0.006)	(0.00)	(0.00)
(iii)stem top (STL-16)		0.005	0.00	0.00	0.00	0.00	-	-	-	-	-
							-	-	-	-	-
(iv)stem middle (SML-16)		0.009	0.005	0.002	0.00	0.00	0.017	0.002	0.00	0.00	0.00
							(0.022)	(0.00)	(0.003)	(0.005)	(0.00)
6.Bud initiation stage	90										
(i) leaves (Bl)		0.059	0.013	0.003	0.00	0.00	0.112	0.044	0.00	0.00	0.00
							(0.106)	(0.050)	(0.017)	(0.00)	(0.00)
(ii)stem upper (BSU)		0.011	0.003	0.00	0.00	0.00	0.010	0.010	0.00	0.00	0.00
							(0.220)	(0.00)	(0.015)	(0.00)	(0.00)
(iii)stem middle (BSM)		0.035	0.014	0.004	0.002	0.00	0.110	0.00	0.00	0.00	0.00
							(Traces)	(0.004)	(0.00)	(0.00)	(0.00)
(iv)root (BR)		-	-	-	-	-	0.013	0.00	0.00	0.00	0.00
							(0.012)	(0.012)	(0.00)	(0.00)	(0.00)
(v) bud (BB)		0.015	0.006	0.002	0.00	0.00	0.033	0.020	0.00	0.00	0.00
							(0.011)	(0.023)	(0.00)	(0.00)	(0.00)
7.Plant with bud dropping stage	96										
(i) sepals (BDS)		0.089	0.025	0.005	0.00	0.00	-	-	-	-	-
							-	-	-	-	-
(ii)petals (BDP)		0.099	0.146	0.009	0.003	0.00	-	-	-	-	-
							-	-	-	-	-
(iii)anthers (BDA)		0.093	0.020	0.016	0.023	0.00	-	-	-	-	-
							-	-	-	-	-
(iv)ovary (BDO)		0.025	0.00	0.00	0.00	0.00	-	-	-	-	-
							-	-	-	-	-
(v)peduncle (BDD)		0.147	0.038	0.007	0.00	0.00	-	-	-	-	-
							-	-	-	-	-

Contd.

8. Plants with erect bud stage	102										

(i)stem (EBS)		-	-	-	-	-	Traces (0.016)	Traces (0.039)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
(ii)peduncle (EBD)		-	-	-	-	-	0.046 (0.049)	0.018 (0.027)	0.009 (0.005)	0.00 (0.011)	0.00 (0.002)
(iii)root (EBR)		-	-	-	-	-	0.038 (0.053)	0.011 (0.011)	0.00 (0.011)	0.00 (0.00)	0.00 (0.00)
(iv)sepals (EBS)		0.114	0.025	0.006	0.00	0.00	0.086 (0.091)	0.029 (0.045)	0.00 (0.00)	0.00 (0.030)	0.00 (0.00)
(v)petals (EBP)		0.202	0.161	0.009	0.00	0.00	0.446 (0.298)	0.308 (0.226)	0.031 (0.021)	0.00 (0.021)	0.00 (0.00)
(vi)ovary (EBO)		0.151	0.018	0.015	0.00	0.00	0.028 (0.034)	0.014 (0.034)	0.007 (0.014)	0.014 (0.009)	0.00 (0.00)
(vii)anthers (EBA)		-	-	-	-	-	0.237 (0.287)	0.00 (0.198)	0.026 (0.049)	0.053 (0.019)	0.00 (0.00)
9.After flower droping	106										
(i) stem (FDS)		0.066	0.017	0.003	0.00	0.00	0.029 (0.038)	0.019 (0.019)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
(ii)root (FDR)		-	-	-	-	-	Traces (Traces)	Traces (Traces)	Traces (Traces)	0.00 (0.00)	0.00 (0.00)
(iii)peduncle (FDD)		-	-	-	-	-	0.135 (0.041)	0.052 (0.00)	0.021 (0.00)	0.417 (0.00)	0.00 (0.00)
(iv) leaf upper (FDLU)		0.155	0.032	0.007	0.00	0.00	0.144 (0.165)	0.066 (0.065)	0.018 (Traces)	0.042 (0.00)	0.00 (0.00)
(v)leaf middle (FDLM)		0.064	0.011	0.030	0.00	0.00	- -	- -	- -	- -	- -
(vi)leaf lower (FDLL)		0.116	0.024	0.012	0.00	0.00	- -	- -	- -	- -	- -
(vii)capsule (FDC)		0.337	0.135	0.026	0.00	0.00	0.837 (0.656)	0.216 (0.117)	0.012 (0.014)	0.504 (0.416)	0.00 (0.00)
10. Capsule late mature stage	139	0.486	0.124	0.014	0.224	0.00	- -	- -	- -	- -	- -
Mean		0.101	0.0363	0.008	0.0105	0.00	0.0995 (0.100)	0.0309 (0.0408)	0.0067 (0.0176)	0.0396 (0.0213)	- -
SE		0.022	0.005	0.0005	0.009	-	0.031 (0.027)	0.0114 (0.0112)	0.0011 (0.010)	0.020 (0.017)	- -
SD		0.1114	0.0245	0.0024	0.0456	-	0.1611 (0.1323)	0.0592 (0.0544)	0.0057 (0.0544)	0.1039 (0.0848)	- -

M = morphine, C=codeine, T=thebaine, N=noscapine
P = papaverine
- = data not recorded
values in parenthesis are of var. NBRI-2 in the year 1995-96
Abbreviations of each plant part under each stage are given in brackets.

weighs of the tubes were taken after the samples collection. By subtracting the two weighs, samples quantity was obtained. The fresh plant parts were kept in DMSO more than 3 months for complete extraction of alkaloids. The samples were filtered with ordinary filter using vacuo. The samples were run through micro filtration kit. The samples were analyzed by paired ion reverse phase chromatography (HPLC) following KHANNA & SHUKLA 1986. The injection volume was 10 µl. The mobile phase was constituted by methanol, glacial acetic acid and triple distilled water (40 : 1 : 59) to 1 liter of which 1-heptane sulphonic acid was added to get 3.5 pH of the solution. The flow rate was 2 ml/min. and attenuation 0.1 AUFS. The alkaloid contents were calibrated in reference to the standard curve.

Results and Discussion

The alkaloids (%) determined on fresh weight basis at different growth periods in opium poppy are presented in Table 1 and Figs. 1 & 2. The results revealed some important facts.

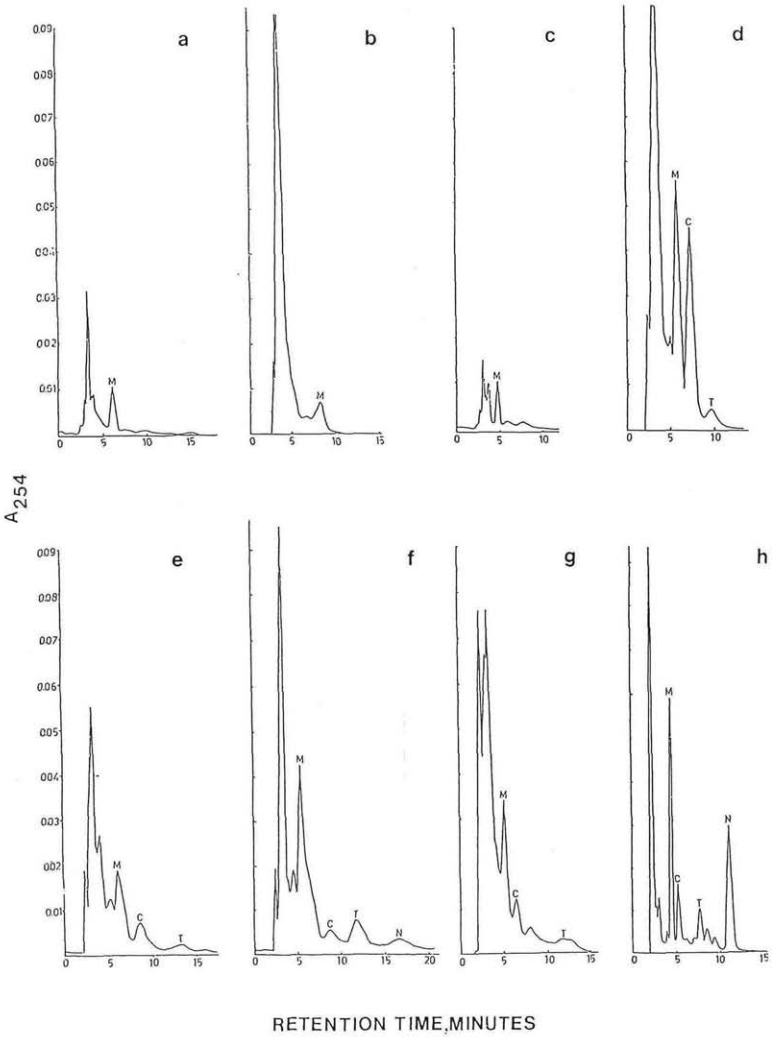

Fig. 1. Different stages of alkaloids profile (a) cotyledons; 2 leaves stage plants: (b) leaves (c) root; erect stage plants: (d) petals (e) peduncle (f) anthers with filaments (g) ovary (h) capsule.

In general variation in alkaloid content in different developmental stages was noticed not only for morphine but for all the alkaloids as earlier reported (BERNATH 1989). In cotyledon stage (3-4 days after seed sprout), the only morphine content was noticed in all the 3 parts viz. upper portion, root portion and whole cotyledon. In two leaves stage also only morphine was noticed in both the years while earlier the

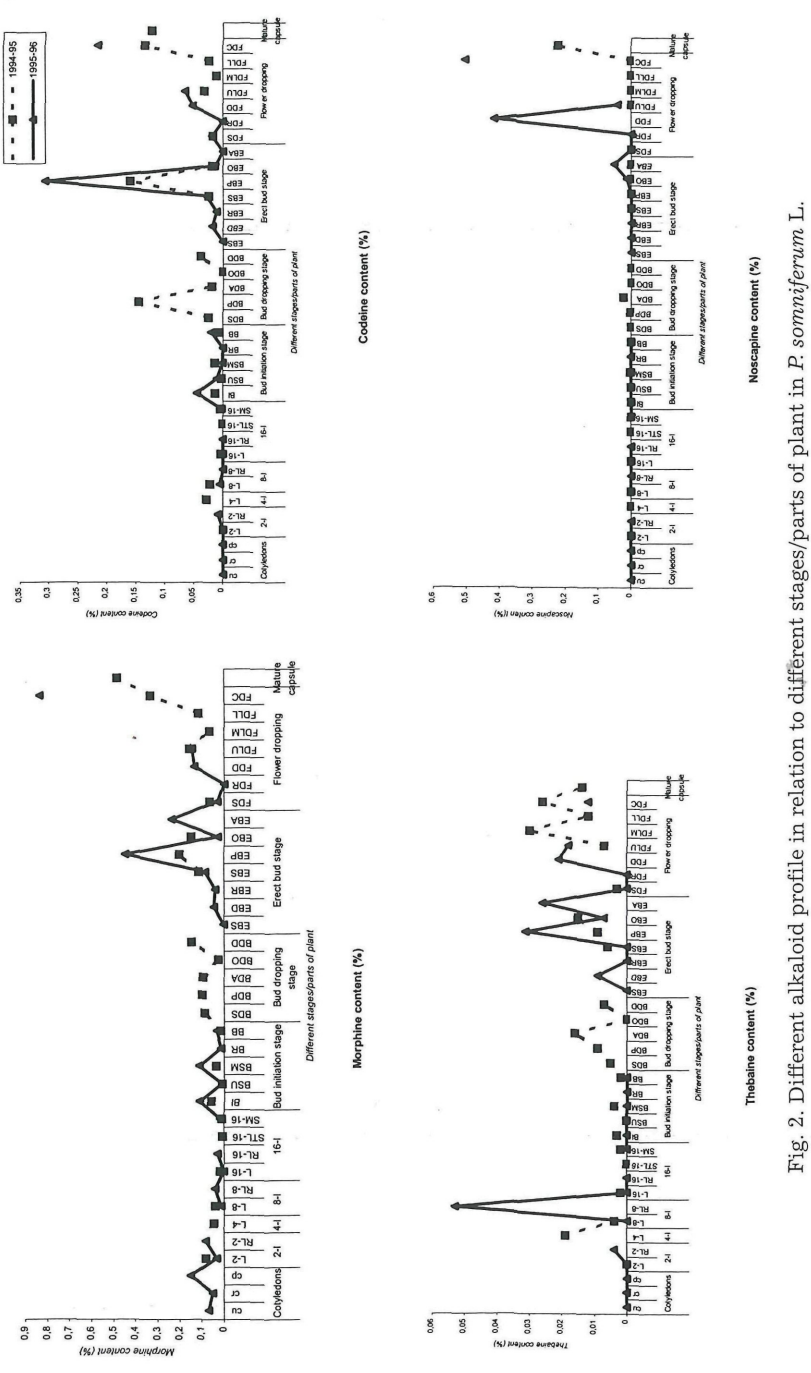

Fig. 2. Different alkaloid profile in relation to different stages/parts of plant in *P. somniferum* L.

presence of morphine was detected in plants from rosette stage onwards (FELKLOVA & al. 1976). The alkaloids are found in the roots in seedling stage and later on they increase in all the developing organs (SARKANY & al. 1970). Seed germination after one or two days gives rise to alkaloid appearance in seedlings as a result of release from bound form which was accumulated in the seeds during its development (FAIRBAIRN & EL-MASREY 1968).

Considering alkaloid biosynthesis in opium poppy plants noscapine is reported (FAROOQUI & CHANDRA 1983) to be the first alkaloid to appear 3 days after sprouting. Codeine, morphine and papaverine appear when the seedlings are around 7 cm tall but in present study, the only morphine content was detected. Probably, the noscapine synthesis may be started much earlier than the first sample collected in present study i.e. after sprouting of seeds. In roots of 2 leaves stage plants, all the morphinane alkaloids were present in both the varieties during 1995–96 except the absence of codeine in NBRI-2.

During 1994–95 all the morphinane alkaloids were noticed in leaves in the plants bearing 4, 6 and 8 leaves stages, while in 1995–96 only morphine was noticed at 8 and 16 leaves stage plants. In the roots of 8 and 16 leaves plants all the morphinane alkaloids (MCT) were present except thebaine in 8 leaves stage and codeine at 16 leaves stage in NBRI-2. Thebaine was also absent in NBRI-1 at 16 leaves stage. Besides morphinane alkaloids, noscapine was noticed in shoot part of NBRI-2 at 16 leaves stage. All the morphinane alkaloids were present at bud initiation during 1994–95 while thebaine was absent in 1995–96. This may be regarded as physiological explanation of the absence of alkaloids instead genetic block in the alkaloid biosynthesis at an early stage. Earlier SCHULZE 1989 also reported that there is possibility to obtain plants with blocked conversion of codeine to morphine.

At pendulous stage (bud dropping) all the 3 morphinane alkaloids (MCT) were present in sepals, petals and anthers while only morphine was present in ovary. Noscapine was present in sepals and petals. As soon as bud erects, all the morphinane alkaloids were noticed in all parts including ovary in bud. The alkaloid content was higher in petals followed by anthers in comparison to other parts of the plant. This indicates that reproductive organs accommodate most of the alkaloids than the vegetative parts of plant. EL KHEIR 1975 noticed high morphine in bud stage with maximum content in stamens of one day after flower bended. Noscapine was either absent or present in traces. Contrary to this noscapine was present in anthers of NBRI-1 and NBRI-2 in present investigation.

Alkaloids in different capsules, collected from just petal fall to full growth, revealed the presence of all the morphinane alkaloids in all the sizes of capsules. In general during this period all the morphinane alkaloids were present in all the parts of plant in varying quantities. Variation in alkaloid content at different periods was reported (FAIRBAIRN & WASSEL 1964 and HEYDENREICH & PFEIFER 1962). Diurnal variation of alkaloids on different period was noticed (SHUKLA & al. 1996, KINOSHITA 1966). However, ITENOV & al. 1999 reported that diurnal variation in alkaloids is mainly due to fluctuation of water content in latex.

Considering average mean of different alkaloids with different stages (Table 1) it was found that the morphine, codeine, thebaine and noscapine were significantly superior at fully developed capsule as well as late mature capsule stage. Morphine and codeine were also significantly superior in petals at erect bud stage in both the years. Morphine and thebaine in anthers at erect bud stage, morphine and codeine in upper leaf at flower dropping stage were significantly different in both the varieties during 1995–96. Noscapine in peduncle and thebaine in peduncle and upper leaf at flower dropping stage also had significant differences to general mean in NBRI-1. Morphine in stem at bud initiation stage and codeine in anthers at erect bud stage were also significant in NBRI-2.

At lancing stage of capsule, the alkaloids were found in traces in roots and maximum in capsule and peduncle in both the varieties. This indicates that the accumulation of alkaloids at the time of lancing remains maximum in capsule and peduncle than other parts of plant. AKSANOWSKI & al. 1962 and MICHALES 1966 also reported that at blossoming stage accumulation of alkaloid decreased in root and increased in the reproductive organs. Accumulation and translocation of alkaloids take place in the latex vessel and as the latex vessels are present in large number in the capsule, the highest accumulation of alkaloid occurs in the capsules. FAIRBAIRN & al. 1974 reported that alkaloids are accumulated in alkaloidal vesicle which are found in latex. Stem latex along with the alkaloidal vesicles are translocated into capsule during rapid expansion after petal falls. TOOKEY & al. 1976 studied that morphine content in the capsule rises very rapidly and lend off when the capsule reaches maturity as evidenced in present investigation (Table 1). Since morphine occurs as irreversible end product of sequence thebaine → codeine → morphine (STERMITZ & RAPOPORT 1961, SHUKLA & al. 1996), it decreased markedly at certain point. Morphine soon after its formation in latex is converted into non-alkaloidal substances and translocated from laticifers of capsule to other tissues and some reach to developing ovules (FAIRBAIRN & EL-MASRY 1967).

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Phyton (Horn, Austria) 41 (1): 96 (2001)

Recensio

STAINTON Adam 1997. Flowers of the Himalaya. A Supplement. – 8°, 86 Seiten, 128 Farbtafeln; kart. – Oxford University Press, Dehli, Calcutta, Chennai, Mumbai. – £ 9,99. – ISBN 0-19-564415-8.

Der „Hauptband" von Oleg POLUNIN & Adam STAINTON, Flowers of the Himalaya, erschien 1984; in ihm sind 1500 Arten enthalten, die durch kurze Beschreibungen im Text sowie durch 689 Farbfotos und 316 Strichzeichnungen dargestellt sind. Zu diesem Band erschien 1988 ein Supplement, das nun als neue Ausgabe von Oxford India Paperbacks vorliegt. Hier sind weitere 584 Arten durch Farbfotos dargestellt; die ca. 350 erstmals aufgenommenen Arten sind im Text – ähnlich wie im Hauptband – kurz beschrieben. Dazu kommen ca. 240 Arten, die zwar im Text des Hauptbandes schon enthalten , aber entweder gar nicht oder durch Strichzeichnungen illustriert sind; für diese Arten gibt es im vorliegenden Text nur die entsprechenden Verweise auf den Hauptband (dadurch, daß sich diese Hinweise nicht direkt bei den Abbildungen finden, wird das gemeinsame Benützen beider Werke unnötig kompliziert). Die Motive für die Artenauswahl sind im Vorwort erläutert; davon sei erwähnt, daß Pflanzen aus tieferen Lagen und Adventive stärker berücksichtigt worden sind. Für letztere , wie z.B. *Callistemon, Trichocereus peruvianus, Tagetes patula, Euphorbia pulcherrima* und *Grevillea robusta,* wären aus botanischer Sicht keine Farbfotos notwendig gewesen, doch das ist wohl das Zugeständnis an die Laien unter den Benutzern des Buches, die zwischen autochthon und eingebürgert natürlich nicht unterscheiden können.

Es ist jedenfalls erfreulich, Fotos so vieler weiterer Arten des Himalaya, auch von in Europa wenig bekannten Gattungen oder Familien, geboten zu bekommen. Interessant nicht nur seltene oder selten abgebildete Arten, auch von aus der Kultur so gut bekannten wie *Primula floribunda, Aeginetia indica* und *Coelogyne cristata* ist es schön, Fotos vom Standort zu sehen. Interessante Heilpflanzen sind ebenfalls unter den Abgebildeten, wie z.B. *Saussurea costus* (= *S. lappa,* Körbchen-Stand; durch Übernutzung gefährdet), *Mallotus philippensis* (zwei Fotos), *Coleus barbatus* und *Ephedra gerardiana* (zwei Fotos).

Besonders spannende Blüten haben unter den abgebildeten Arten: *Silene nigrescens, Hypericum hookerianum, Butea monosperma, Saxifraga nutans, Mussaenda roxburghii, Jurinea ceratocarpa, Codonopsis purpurea, Primula uniflora, Gentiana urnula, Maharanga emodi, Incarvillea younghusbandii, Cinnamomum tamala, Dendrophtoe falcata,* div. *Zingiberaceae* etc.

Bei der Beschriftung der beiden Abbildungen bei Nr. 366 ist offensichtlich ein Irrtum passiert. Manchmal weicht die tatsächliche Reihenfolge der Bilder erheblich von der Numerierung ab.

H. TEPPNER