

A Contribution to the Pollen Morphology of *Indigofera* (Fabaceae) in Korea^{1*}

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韓國에서 生育하는 땅비싸리屬(콩科)의 花粉形態^{1*}
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ABSTRACT

The pollen morphology of six taxa of the genus *Indigofera* in Korea was investigated by light microscope(LM) and scanning electron microscope(SEM). The pollen grains of the genus were monads, prolate spheroidal or subprolate, and trizonocolporate. Sculpture patterns of mesocolpium area were densely microperforate(DMIP) or verrucate with pitted perforate striae(VPPS). Based on sculpture patterns in mesocolpium, apocolpium and colpus areas, three major pollen types of *Indigofera* were discernible; Decora, Kirilowii and Koreana. It seemed as if there was some relationship between chromosome numbers and mesocolpium sculpture patterns and the pollen types Decora and Kirilowii were branched out from the pollen type Koreana.

Key words: *Indigofera decora*, *I. kirilowii*, *I. koreana*, *pollen key*, *chromosome number*

요 약

본 연구는 한국에서 생육하는 땅비싸리속 6분류군의 화분형태를 광학현미경과 주사형전자현미경으로 관찰하였다. 본 속의 화분립은 단립이며, 적도면 입상은 약장구형 혹은 아장구형이고, 발아구는 삼공구형이고, 표면무늬는 DMIP상이거나 VPPS상이었다. 본 속은 mesocolpium, apocolpium 그리고 구구 주변의 표면무늬에 따라 Decora, Kirilowii, Koreana 화분형으로 구분되었다. 염색체 수와 mesocolpium 표면무늬에 연관성이 있고, Decora와 Kirilowii 화분형은 Koreana 화분형에서 분지된 듯 보인다.

INTRODUCTION

Pollen morphology has been useful for various research areas, such as taxonomy, genetic and evolutionary studies, allergy studies, forensic science, tracing plant geography, geology and climatic change studies. Erdtman(1969) and Wodehouse(1935) reported that pollen morphology had close contacts with plant taxonomic studies(Lee, S.T. 1979). It has been effectively applied in

several taxonomical problems(Erdtman 1969; Nair 1964). For example, *Tachigalia* and *Stahlia* of the Detarieae were identified as misplaced based on pollen data and now assigned to the tribe Caesalpinieae in Fabaceae(Graham and Barker, 1981). It has often been able to add indications concerning the groups to which such uncertain elements could be referred(Erdtman, 1969).

The tribe Indigofereae has the very large and distinct genus *Indigofera* and three small genera

¹ 接受 1999年 1月 21日 Received on January 21, 1999.

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* This paper was presented at the IUFRO Inter-Divisional Seoul Conference "Forest Ecosystem and Land Use in Mountain Areas" held in Seoul, Korea, October 1998.

Cyamopsis, *Phylloxylon* and *Rhynchotropis*(Polhill, 1981). The majority of *Indigofera* species(ca. 720 - 730 spp.) are distributed in the tropics and subtropics(Choi and Kim, 1977). The pollen of *Indigofera* has been studied and electron micrographs have been reported. Some differences in tectum and apertures of *Indigofera* in India(Vishnu-Mittre and Sharma, 1962) and descriptions of *Indigofera* taxa in Ethiopia(Bonnefille, 1971) were reported. Wu and Huang(1995) reported detailed 15 *Indigofera* taxa in Taiwan and classified them into five types. Ferguson and Strachan(1982) described and illustrated 76 *Indigofera* taxa along with eight other genera taxa in the Indigofereae. Therefore, pollen morphology was useful for systematic study of *Indigofera*(Ferguson and Skvar-

la, 1981).

In Korea, pollen morphology on a few taxa such as *Indigofera koreana* Ohwi, *I. kirilowii* Maxim. and *I. pseudotinctoria* Matsum. has been preliminarily reported(Lee, Y.H., 1979; Chang, 1986; Cho *et al.*, 1997). This study is extended to more taxa of *Indigofera* in Korea and presents detailed description of pollen morphology and pollen types of the genus to provide basic data for systematic consideration of the genus.

MATERIALS AND METHODS

Pollens of six taxa(Table 1); *Indigofera amblyantha* Craib., *I. decora* cv. Alba, *I. grandiflora* Choi et Cho *I. kirilowii* Maxim., *I. korea-*

Table 1. Pollen collection date and locality of *Indigofera* in Korea

Taxa	Date	Locality
<i>Indigofera amblyantha</i> Craib.	Jun 12, 1995	Chollipo Arboretum, Taean Choongnam
	Jun 12, 1995	Chollipo Arboretum, Taean Choongnam
	Jun 12, 1995	Chollipo Arboretum, Taean Choongnam
	Jun 12, 1995	Chollipo Arboretum, Taean Choongnam
	Jun 12, 1995	Chollipo Arboretum, Taean Choongnam
<i>I. decora</i> cv. Alba	Jun 6, 1997	Chollipo Arboretum, Taean Choongnam
	Jun 6, 1997	Chollipo Arboretum, Taean Choongnam
	Jun 6, 1997	Chollipo Arboretum, Taean Choongnam
	Jun 6, 1997	Chollipo Arboretum, Taean Choongnam
	Jun 6, 1997	Chollipo Arboretum, Taean Choongnam
<i>I. grandiflora</i> Choi et Cho	May 31, 1992	Eastern slope of Mt. Kaya, Songju Kyungbuk
	May 31, 1992	Eastern slope of Mt. Kaya, Songju Kyungbuk
	May 31, 1992	Eastern slope of Mt. Kaya, Songju Kyungbuk
	May 19, 1998	Eastern slope of Mt. Kaya, Songju Kyungbuk
	May 19, 1998	Eastern slope of Mt. Kaya, Songju Kyungbuk
<i>I. kirilowii</i> Maxim.	May 29, 1996	Chonbuk Forest Environmental Research Institute, Chonbuk
	May 26, 1997	Chonbuk Forest Environmental Research Institute, Chonbuk
	May 26, 1997	Chonbuk Forest Environmental Research Institute, Chonbuk
	May 26, 1997	Chonbuk Forest Environmental Research Institute, Chonbuk
	Jun 6, 1997	Chollipo Arboretum, Taean Choongnam
<i>I. koreana</i> Ohwi	May 17, 1996	Mt. Konji, Chonju Chonbuk
	May 18, 1996	Entrance of Mt. Baykoon, Chonnam
	May 20, 1997	Dasongli Hwangdeungmyon Iksan Chonbuk
	May 21, 1997	Agr. Bldg #1, Chonbuk Univ., Chonju Chonbuk
	May 21, 1997	Agr. Bldg #1, Chonbuk Univ., Chonju Chonbuk
<i>I. pseudotinctoria</i> Matsum.	Jun 10, 1996	Chonju Arboretum, Chonju Chonbuk
	Jun 10, 1996	Chonju Arboretum, Chonju Chonbuk
	Jun 22, 1996	Chonju Arboretum, Chonju Chonbuk
	Jun 22, 1996	Chonju Arboretum, Chonju Chonbuk
	Jun 22, 1996	Chonju Arboretum, Chonju Chonbuk

na Ohwi and *I. pseudotinctoria* Matsum. of the genus *Indigofera* were collected in Korea. Five different individuals per each taxon were collected. The pollens were treated by acetolysis modified by Livingstone from Erdtman's(Kim, 1982) and mounted in glycerine jelly on microscope slides for microscopic study by LM(Olympus BH). The five parameters ; PL(polar axis length), EW(equatorial width), CL(colpus length), CW (colpus width) and ET(exine thickness) of 100 pollen grains per a taxon, 600 grains in total were measured. For SEM study, surface pattern of the pollen grains($\times 15,000$) and the whole grain shapes($\times 2,000 - 3,500$) were observed by a scanning electron microscope(JEOL JSM - T330A).

Reitsma(1970)'s terminology following Erdtman's was used in this study.

RESULTS AND DISCUSSION

Pollen Morphological Characters

Pollen grain size of six taxa of *Indigofera* is 22.32 - 40.92 μm at PL, 18.60 - 32.00 μm at EW. CL is 13.02 - 27.90 μm and CW is 0.74 - 2.98 μm .

The P/E(PL/EW) value is 1.00 - 1.49 which means grains are either prolate spheroidal or subprolate at equatorial view. The grains of *I. decora* cv. Alba, *I. koreana* and *I. pseudotinctoria* fall in the former shape while those of *I. amblyantha*, *I. grandiflora* and *I. kirilowii* fit into the latter.

The sculpture patterns of the pollen grains of *Indigofera* are microperforate or verrucate with pitted perforate striae in monads(Plate 1, Figs 1 - 6). *I. decora* cv. Alba and *I. kirilowii* were characterized with two different sculpture patterns, psilate in the apocolpium area and verrucate with pitted perforate striae in the mesocolpium area. *I. kirilowii* is differentiated from *I. decora* cv. Alba. The former is psilate in colpus area while the latter is verrucate with pitted perforate striae in colpus area(Plate 1, Figs 3 - 6). This provides a key to define two different pollen types.

Pollen Key to *Indigofera*

Three pollen types could be recognized based

on sculpture patterns according to sculpture patterns of the areas on grains.

1. Densely microperforate in mesocolpium, apocolpium and colpus areas Type Koreana
1. Verrucate with pitted perforate striae in mesocolpium area and psilate in apocolpium area 2
2. Verrucate with pitted perforate striae in colpus area Type Decora
2. Psilate in colpus area Type Kirilowii

1. Type Decora : *Indigofera decora* cv. Alba

Pollen grains are prolate spheroidal(P/E=0.99 - (average 1.12) - 1.32) at equatorial view and trizonocolporate. PL is 23.06 - (25.66) - 29.76 μm , EW 19.72 - (22.98) - 27.53 μm , CL 13.02 - (15.56) - 18.97 μm , CW 1.49 - (1.78) - 2.23 μm and ET 1.49 - (1.72) - 2.23 μm . The sculpture pattern of this type is characterized with two different patterns according to the grain surface areas ; verrucate with pitted perforate striae in mesocolpium and colpus areas but psilate in apocolpium area(Plate 1, Figs 3 - 4).

2. Type Kirilowii

Pollen grain of this type is trizonocolporate. The grain shape at equatorial view is subprolate (P/E=1.05 - (1.21) - 1.41) which is different from that of type Decora. The pollen size is 23.44 - (27.50) - 40.92 μm at PL, 19.34 - (22.87) - 31.92 μm at EW, 15.25 - (18.07) - 27.90 μm at CL, 1.49 - (1.99) - 2.60 μm at CW and 1.49 - (1.73) - 2.60 μm at ET. The mesocolpium area is verrucate with pitted perforate striae but the apocolpium area is psilate. The sculpture pattern of this type is psilate in colpus area while the former type is verrucate with pitted perforate striae in the same area. *I. grandiflora* Choi et Cho and *I. kirilowii* Maxim. belong to this type.

Indigofera grandiflora Choi et Cho

Pollen grain size(Table 2) at almost every parameter of the present taxon is the biggest in the genus. PL is 27.90 - (33.16) - 40.92 μm , EW 23.43 - (28.74) - 31.92 μm , CL 18.23 - (22.19) - 27.90 μm , CW 1.86 - (2.11) - 2.60 μm and ET 1.49 - (2.08) - 2.60 μm . Trizonocolporate pollen grain at equatorial view is prolate spheroidal by the mean P/E value. Each grain is in the range of spherical and

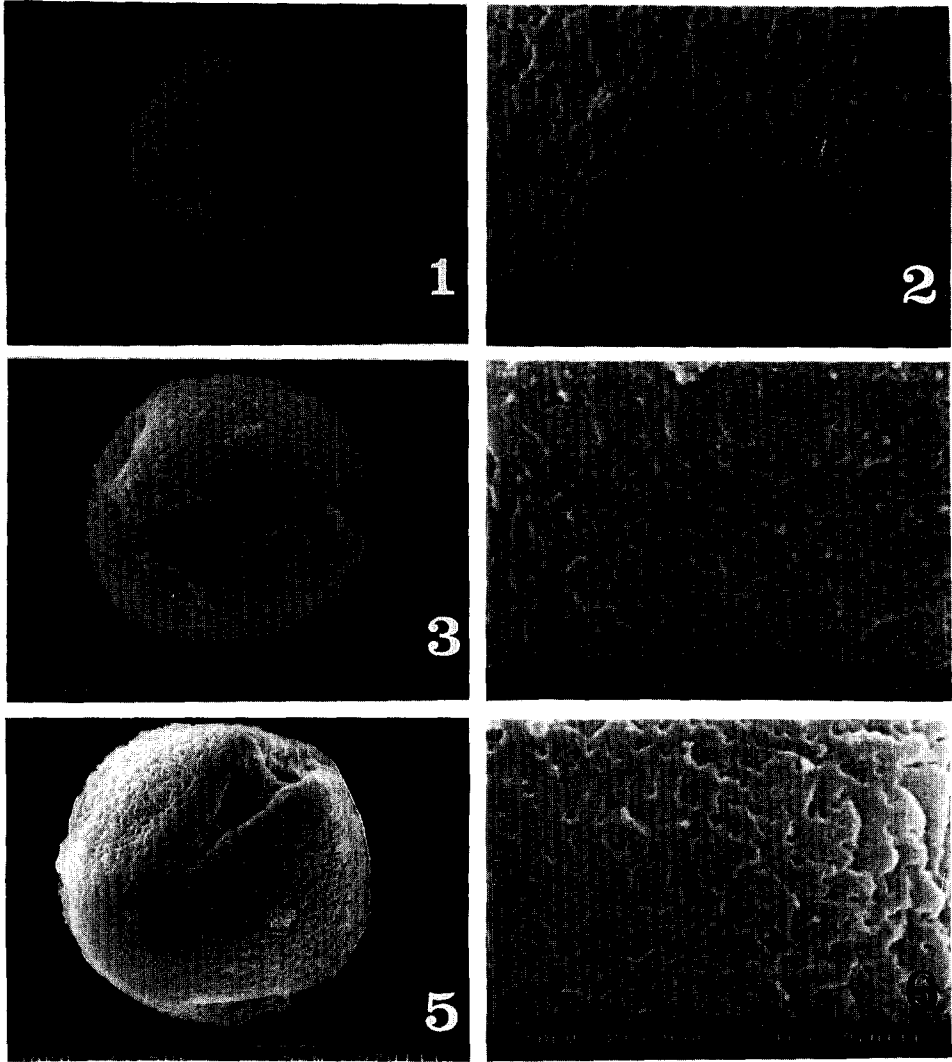


Plate 1. Scanning electron microscopy(SEM) of pollen grains of *Indigofera* in Korea.

Figs 1-2. *Indigofera koreana* Ohwi of type Koreana.

1. Pollen grain showing microperforate in mesocolpium, apocolpium and colpus areas($\times 2,000$)
2. Detailed microperforate sculpture pattern in mesocolpium area($\times 15,000$)

Figs 3-4. *Indigofera decora* cv. Alba of type Decora.

3. Pollen grain showing verrucate with pitted perforate striae in mesocolpium and colpus areas but psilate in apocolpium area($\times 3,500$)
4. Detailed sculpture pattern in mesocolpium area($\times 15,000$)

Figs 5-6. *Indigofera kirilowii* Maxim. of type Kirilowii.

5. Pollen grain showing verrucate with pitted perforate striae in mesocolpium but psilate in apocolpium and colpus areas($\times 3,500$)
6. Detailed sculpture pattern in mesocolpium area($\times 15,000$)

prolate by P/E value being 1.00 - (1.16) - 1.49. The sculpture pattern of this taxon is characterized with two different patterns according to the

areas being verrucate with pitted perforate striae in mesocolpium but psilate in the apocolpium and colpus areas.

Table 2. Pollen morphological data of *Indigofera* in Korea

Taxon	PL(μm)	EW(μm)	CL(μm)	CW(μm)	ET(μm)	P/E	Equatorial View	Aperture Type	Sculpture Pattern*	Pollen Type
<i>I. amblyantha</i>	27.19 + 1.90	22.96 + 1.61	18.36 ± 1.59	1.38 ± 0.36	1.67 ± 0.21	1.19 + 0.11	Subprolate	Tri-colporate	DMIP	Koreana
<i>I. decora</i> cv. Alba	25.66 ± 1.24	22.98 + 1.18	15.56 + 1.40	1.79 ± 0.26	1.72 ± 0.22	1.12 ± 0.07	Prolate spheroidal	Tri-colporate	VPPS	Decora
<i>I. grandiflora</i>	33.16 + 2.10	28.74 + 1.73	22.19 + 1.67	2.11 ± 0.21	2.08 ± 0.26	1.16 ± 0.08	Subprolate	Tri-colporate	VPPS	Kirilowii
<i>I. kirilowii</i>	27.50 + 1.58	22.87 + 1.20	18.07 + 1.24	1.99 ± 0.32	1.73 ± 0.24	1.21 ± 0.09	Subprolate	Tri-colporate	VPPS	Kirilowii
<i>I. koreana</i>	29.99 + 1.54	26.42 + 1.66	20.21 ± 1.46	2.04 ± 0.64	1.72 ± 0.40	1.14 ± 0.08	Prolate spheroidal	Tri-colporate	DMIP	Koreana
<i>I. pseudotinctoria</i>	23.87 ± 0.89	21.06 ± 0.95	14.71 ± 0.67	2.24 ± 0.24	2.08 ± 0.22	1.14 ± 0.07	Prolate spheroidal	Tri-colporate	DMIP	Koreana

* DMIP = Densely microperforate (with surface holding more than 10 small pits $\leq 1/10\mu\text{m}$ in diameter within $1\mu\text{m}^2$, VPPS = Verrucate (with wart-like sculpturing elements $> 1\mu\text{m}$ in diameter, usually broader than high) with pitted perforate striae.

Indigofera kirilowii Maxim.

Pollen grain of the present taxon is trizonocolporate. The grain shape at equatorial view is subprolate (P/E = 1.05 - (1.21) - 1.41). Each pollen grain falls into the range of prolate spheroidal and prolate. The pollen size is 23.44 - (27.50) - 31.62 μm at PL, 19.34 - (22.87) - 25.67 μm at EW, 15.25 - (18.07) - 21.95 μm at CL, 1.49 - (1.99) - 2.60 μm at CW and 1.49 - (1.73) - 2.23 μm at ET. The mesocolpium area is verrucate with pitted perforate striae while the apocolpium and colpus areas are psilate.

3. Type Koreana

The present type has prolate spheroidal or subprolate (P/E = 0.94 - (1.16) - 1.46) and trizonocolporate pollen grains. PL is 22.32 - (27.02) - 33.48 μm , EW 18.60 - (23.48) - 29.76 μm , CL 13.39 - (17.76) - 23.44 μm , CW 0.74 - (1.89) - 2.98 μm and ET 0.74 - (1.82) - 2.60 μm . The type has densely microperforate sculpture pattern in mesocolpium, apocolpium and colpus areas. Three taxa in the genus; *I. amblyantha* Craib., *I. koreana* Ohwi and *I. pseudotinctoria* Matsum. fall into this type.

Indigofera amblyantha Craib.

The pollen grain shape at equatorial view is subprolate (P/E = 1.00 - (1.19) - 1.46). This trizonocolporate pollen grain is 23.06 - (27.19) - 31.62 μm at PL, 18.60 - (22.96) - 27.90 μm at EW, 15.25 - (18.36) - 22.69 μm at CL, 0.74 - (1.38) - 1.86 μm at CW and 1.12 - (1.67) - 2.23 μm at ET. This taxon is densely microperforate in apocolpium, mesocolpium and colpus areas and this is quite similar to the sculpture pattern of *I. byobiensis* Hosokawa in Taiwan investigated by Wu and Huang (1995).

Indigofera koreana Ohwi

The grain size of this taxon is 26.78 - (29.99) - 33.48 μm at PL, 21.95 - (26.42) - 29.76 μm at EW, 15.62 - (20.21) - 23.44 μm at CL, 0.74 - (2.04) - 2.98 μm at CW and 0.74 - (1.72) - 2.60 μm at ET. The pollen grain shape at equatorial view is prolate spheroidal (P/E = 0.94 - (1.14) - 1.33) and the sculpture pattern is densely microperforate in mesocolpium, apocolpium and colpus areas. It is interesting to find the present taxon and two taxa; *I. glandulifera* Hayata and *I. pedicellata* Wight

et Arn. in Taiwan(Wu and Huang 1995) are alike in their sculpture patterns in those areas above.

Indigofera pseudotinctoria Matsum.

The present taxon has prolate spheroidal(P/E=1.00 - (1.14) - 1.29) and trizonocolporate pollen grains. Any pollen grain of this taxon does not show prolate pollen shape at equatorial view. The grain size is 22.32 - (23.87) - 26.41 μ m at PL, 18.60 - (21.06) - 23.44 μ m at EW, 13.39 - (14.71) - 16.74 μ m at CL, 1.86 - (2.24) - 2.60 μ m at CW and 1.86 - (2.08) - 2.60 μ m at ET. Again, this taxon is densely microperforate in apocolpium, mesocolpium and colpus areas.

The pollen in the tribe Indigofereae is varied and a little specialized. It is various in the tectum type, size of the endoaperture and wall stratification(Polhill, 1981). Some tendency towards thickening of the endexine and fractionation of the foot layer in Indigofereae might be a parallel with that in Desmodieae, Psoraleae and Phaseoleae. This also supports some of the chemical data of Bell(1981). The pantropical genus, *Indigofera*, is the third largest in the Fabales(Schrire and Sims, 1997). Recently, some pollen criteria played an meaningful part to identify *I. koreana* Ohwi from *I. kirilowii* Maxim. in Korea(Cho *et al.*, 1997). This suggested pollen morphology had some taxonomic value within *Indigofera*(Ferguson and Skvarla, 1981). Within the *Indigofera*, several taxa such as *I. kirilowii*, *I. kirilowii* var. *coreana*, *I. koreana*, and *I. pseudotinctoria* in Korea were studied and reported for their pollen morphology(Lee, Y.H., 1979 ; Chang 1986 ; Cho *et al.* 1997). However, the vouchers of other studies compared with the present study couldn't be checked. The pollen of *I. koreana* was the biggest(29.99 \times 26.42 μ m) and that of *I. pseudotinctoria* was the smallest(23.87 \times 21.06 μ m) in the present study. The pollen size(PL \times EW ; 23 - 32 \times 19 - 26 μ m) of *I. kirilowii* in the present study was similar to that(27 - 33 \times 28 - 31 μ m) of Lee, Y.H. (1979) in PL size but a bit smaller in EW value. The pollen size of this taxon was somewhat different from that(19 - 21 \times 20 - 23 μ m) of Chang(1986). He reported the pollen was oblate at equatorial view and the sculpture pattern was fine retic-

ulate. Also, Lee, Y.H.(1979) stated it had fine reticulate sculpture pattern which was different from the result of the present study. The pollen size of *I. pseudotinctoria* was quite different in PL and EW. It was 22 - 26 \times 19 - 23 μ m in the present study but 39 - 42 \times 20 - 22 μ m in Chang(1986)'s. It showed that PL from Chang(1986)'s was much bigger than that of this study. Again, he reported this taxon to be fine reticulate which was different from the current result(densely microperforate). Lee, Y.H.(1979) reported that *I. kirilowii* was tricolporate while Chang(1986) stated that *I. kirilowii* and *I. pseudotinctoria* were trizonocolporate. Cho *et al.*(1997) demonstrated that *I. kirilowii* and *I. koreana* were trizonocolporate supporting the result of the present study. The difference in sculpture patterns mentioned above may be developed because Lee, Y.H.(1979) and Chang(1986) did not include SEM works in their studies. However, Cho *et al.*(1997), who conducted SEM works in their study, reported that *I. kirilowii* was finely perforate with supracteal process and *I. koreana* was finely perforate in mesocolpium area. This was quite similar to the result of the present study. This strongly suggests that LM work is not enough for recognizing sculpture patterns. Since the tectum type, wall stratification and size of the endoaperture all differ in the genus *Indigofera*(Ferguson and Skvarla 1981), detailed TEM sections are recommended along with LM and SEM works to detect detailed subsurface features in this genus.

The noticeable trend in the pollen of Indigofereae is an increase in complexity of the exine stratification from a typical columellar infracteal structure to a complex lamellated foot layer or absent foot layer and granular infractum. A trend shows the tectum of the genus is changing from perforate to finely microperforate. However, there is no significant change in aperture type which is tricolporate(Ferguson and Strachan, 1982). Perhaps the nearest parallel in pollen morphology to the Indigofereae can be detected in the Desmodieae where more open tectum is associated with columellate infractum with a clear foot layer and endexine in *Dendrobium* and more closed perforate tectum with a rather complex

granular infratectum and loss of or fragmentation of the foot layer with a well developed endexine in *Desmodium*(Ferguson and Skvarla, 1979 ; 1981).

The alterations in the size of the pollen grains might be derived from various environmental constraints(McNeill and Crompton 1978 ; Ornduff 1978) or different acetolysis and mounting procedures(Moore *et al.* 1991). This implies that the size could be one of the useful criteria in the identification but can't be the only factor to depend on(Moore *et al.* 1991). From palynological point of view, the taxa in the genus were distinctive with three different types(*Decora*, *Kirilowii* and *Koreana*). It seemed as if the somatic chromosome numbers(Choi and Kim, 1997) were related with mesocolpium sculpture pattern which was the major criterion to group *Indigofera* taxa into pollen types. *I. decora* cv. *Alba*(Type *Decora*) with $2n=48$ was characterized with VPPS sculpture pattern while *I. koreana*(Type *Koreana*) with $2n=32$ was represented with DMIP sculpture pattern. In taxa(*I. grandiflora*, *I. kirilowii* and *I. pseudotinctoria*) with $2n=16$, both VPPS and DMIP patterns were observed. Among these three taxa, only *I. pseudotinctoria*, the outgroup taxon in ITS sequences(Choi and Kim, 1997), had DMIP pattern. Though *I. grandiflora* was believed to be similar to *I. koreana* in the ITS sequences(Choi and Kim, 1997), the pollen morphological data of the current study suggested *I. grandiflora* and *I. kirilowii* were close within the pollen type *Kirilowii*($2n=16$). Since *I. koreana*($2n=32$) and *I. pseudotinctoria*($2n=16$) were close within the pollen type *Koreana*, *I. koreana* seemed to be recently separated from a much more primitive species through polyploidization. Putting all accounts of pollen, chromosome numbers and ITS sequences together, the pollen type *Koreana* seemed to be the most primitive. And then from this type, the other two pollen types, *Kirilowii* and *Decora*, appeared to be branched out. Again, the quantitative data revealed by this pollen morphological work linked with cytological, anatomical or DNA studies would extend the data base for systematic consideration of the *Indigofera*.

For this study, Dr. Byoung-Hee Choi at Inha

University, Korea provided *Indigofera grandiflora* pollens and Dr. Chin-Sung Chang at Seoul Nat. University, Korea gave valuable comments. They were greatly appreciated.

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