Systematic implications of pollen morphology in ten taxa of the genus *Iris*

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The genus *Iris* with its many species is conveniently divided into several sections. One among them is section *Iris* that contains more than 40 taxa which have some taxonomic problems. Such taxonomic riddles can also be tackled by the pollen morphological data. The palynological studies indicate that there existed a correlation between the pollen size, shape and ploidy among the ten taxa currently investigated. The diploid taxa exhibited large oblate pollen whereas tetraploid taxa show very large-sized prolate spheroidal pollen. The discontinuity thus developed between diploids and tetraploids was ascertained in taxonomic groupings. Among the diploids and tetraploids, the individual taxon exhibited its own intrinsic characters.

Key-words—*Iris*, Diploids, Tetraploids, Pollen morphology.

INTRODUCTION

THE petaloid monocot family Iridaceae comprises of 60 genera (Goldblatt 1990, 1991) and belongs to the superorder Liliiflorae and order Liliales (Dahlgreen *et al*. 1985). *Iris* is the largest genus of the family and in northern hemisphere has wide distribution with a centre in Asia. Members of Iridaceae are typically characterized by the possession of isobilateral, equitant leaves, styloid crystals, inferior ovaries and flowers, each with three stamens (Reeves *et al*. 2001).

The genus *Iris* with its many species was divided into many sections (Dykes 1913, Lawrence 1953). The section *Iris* of Mathew (1981) is one among them which bears pivotal position in the genus. Due to natural and conventional hybridization followed by polyploidization and migration to new geographical regions, the species problem became more complicated. Due to species distribution in varied geographical regions the identification has become a difficult question in this taxon. In view of the polyploidization, intraspecific variation and overlapping boundaries between different species, interrelationships in the section of *Iris* are not fully understood.

Several attempts were made to tackle the species problem in section *Iris* from various aspects such as cytological, phytochemical, anatomical and seed coat sculptural images along with palynological studies and observed that pollen grain characters in various groups are useful for the determination of phylogenetic relationships (Nabli 1976, Husain & Heywood 1982). Pollen morphology has become increasingly useful in considerations of interrelaionships of plants of various taxonomic levels (Panchami *et al*. 1998).

Information on the pollen morphology of various species of section *Iris* and other sections is rather meager except for limited findings of Dykes (1909), Iverson and Troels-Smith (1950), Zaklinskaya (1950), Paperburg (1952), Rodionenko (1961), Beug (1961) Schulze (1964, 1971), Karihaloo et al. (1984), Cauneau (1988), Colasante *et al*. (1989), Davis and Jury (1990). Ratnam (2001a) carried out an analysis of some members of Iridaceae and particularly pollen grains of *Iris*.

The brearded *Iris* entities, mentioned in this paper have not been fully examined previously except for a few samples from Rome.

MATERIAL AND METHOD

The anthers of 10 dependable taxa of *Iris* viz *I. aphylla* L., *I. attica* Boiss & Heldr, *I. croatica* Horvat *I. florentina* L., *I. germanica* L., *I. kashmiriana* Baker, *I. lutescens* Lam., *I. marsica* Ricci & Cola, *I. pallida* Lam and *I. variegata* L. were procured from Baladou, France (genetic resource). The anthers were subjected to revised acetolysis treatment of Erdtman (1969). The methodology employed here is similar to Dykes (1909), Claussen (1962), Schulze (1964; 1971), Radulescu (1968), Reitsma (1969) and Karihaloo et al. (1984). Both chlorinated and non chlorinated grains were used in the present study. Care was taken to avoid abortive grains from the randomly selected ones. The pollen slides were scanned under light microscope. Dimensions were measured with oculometer and stage-micrometer. The type, size, shape and exine pattern were recorded and data were presented in tables for comparison.

Various parameters such as polar axis (P) equatorial axis (E) and equatorial index and polar equatorial index (PEI) were calculated by using formula PAE = A/E X 100 (Faegri & Iverson 1964). The range, mean, standard deviation, standard error, were employed for the comparison of ten taxa following the procedure adopted by Snedecor and Cochran (1967). The pollen shape Oblate 75-100μ, Prolate spheriodal 100-113μ, Subprolate 113-133μ are based on Erdtman (1952, 1969).

OBSERVATION

The pollen morphological descriptions of ten taxa of section *Iris* comprises the following aspects namely, colour of the grains, aperture shape, size, columella, pollen shape, size, polar axis, equatorial axis, polar equatorial index value, exine thickness, etc. are of diagnostic value and some time even specific in occurrence (Table-3).

Colour : The colour of the pollen grains in majority of the species observed are light yellow (six out of ten species), whereas two species displayed yellow colour (*I. aphylla* and I. *marsica*) and other two displayed cream colour (*I. attica* and *I. germanica*).

Aperture shape : The aperture shape in majority of the species (six out of ten) are elliptic (tapering ends) and 4 species show spherical shape.

Colpi : Interestingly, all the ten taxa investigated exhibit monocolpate pollen grains (Plates 1 & 2).

Colpi margins : Colpi are both tenuimarginate and crassimarginate. The crassimarginate type is present in *I. aphylla,* and *I. florentina, I. kashmiriana, I. lutescens, I. pallida* and *I. croatica.* While, tenuimarginate type of columella is present in *I. attica, I. germanica, I. marsica* and *i. variegata.* Though *I. germanica, I. marsica* are tetraploid but, exhibit tenuimarginate type unlike those of crassimarginate tetraploid plants. These two forms being amphidiploids exhibit one of the diploid parent's characters.

Columella : The columella forms polygonal patterns on the pollen surface which are referred to as reticulations. When the exine is free, it gives a wavy appearance. This type of wavy appearance is noticed in *I. aphylla; I. croatica; I. germanica; I. lutescense* and *I. marsica.* Whereas, in *I. pallida;* and in *I. variegata,* the columella forms luminar areas but in *I. florentina; I. kashmiriana* the columella is fused towards apex and in *I. attica* the columella is closely placed giving a homogeneous appearance (Table 3).

Pollen shape and size : The pollen shape and size are shown in (Table 2). The diploid species such as *I. attica, I. pallida* and *I. variegata* composed of ob-

PLATE 1

Pollen grains with monocolpate aperture

A. *I. kashmiriana* Baker

B. *I. lutescens* Lam

C. *I. lutescens* Lam

D. *I. marsica* Ricci & Cola

E. *I. variegata* L

F. *I. variegata* L

Bar : 30μm

→

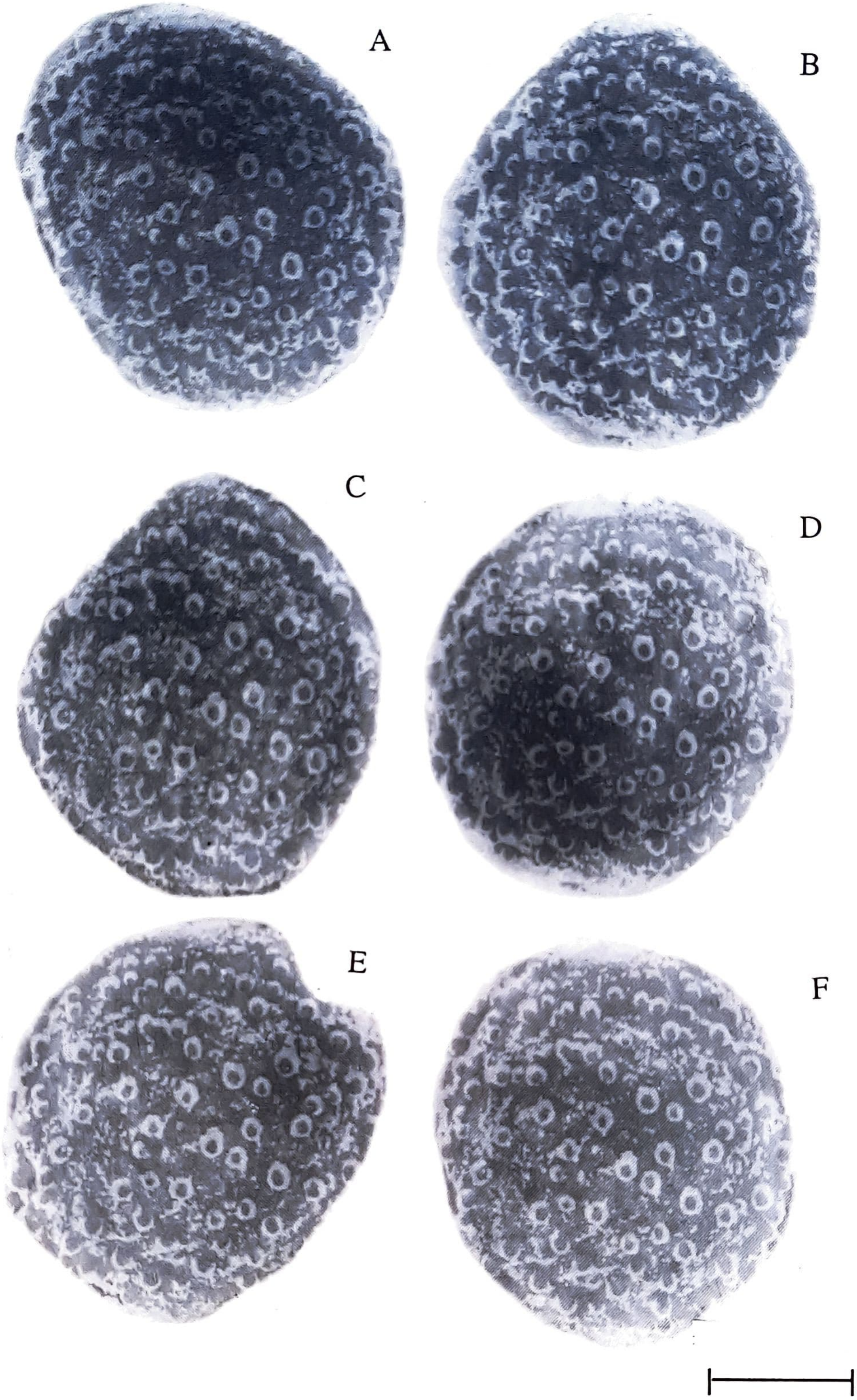

PLATE 1

late-shaped pollen and are large in size, whereas, *I. florentina, I. croatica, I. germanica, I. kashmiriana, I. lutescens, I. marsica,* and *I. aphylla* which are tetraploids exhibit prolate spheroidal shape and possess very large pollen (Table 3).

Polar axis : The polar axis obtained for the ten taxa ranges between 100.15± 0.40 and 143.00 ± 0.54. The highest value for polar axis has been noticed in *I. kashmiriana* (143.00 ± 0.54) followed by *I. variegata* (140.46 ± 0.54). The lowest values for polar axis have been observed in *I. croatica* (100.15±0.40) followed by *I. pallida* (117.60 ± 0.48) (Table 1).

Equatorial axis: The equatorial axis studied for the ten species ranges between 93.28 ± 0.43 (*I. croatica)* and 140.16 ± 0.71 (*I. variegata).* Among others, the highest values were displayed by *I. kashmiriana* (126.30 ± 0.71) followed by *I. florentina* (123.00 ± 0.87), *I. aphylla* (122.54 ± 0.79) and *I. lutescens* (122.04 ± 0.93). Interestingly, in the five taxa such as *I. attica, I. aphylla, I. lutescens, I. florentina* and *I. kashmiriana,* the equatorial values are very similar (Table 1).

Polar equatorial index: Polar equatorial index ranges between 98.74 ± 0.54 (*I. attica)* and 113.22 ± 0.43 (*I. kashmiriana).* The four species, viz., *I. attica, I. pallida, I. variegata* and *I. florentina* exhibited almost similar polar equatorial index values, (98-100) whereas, *I. aphylla, I. germanica, I. croatica* and *I. lutescens* exhibit medium sized equatorial index values (106-109) as shown in Table 1.

Exine thickness : The exine thickness ranges between 3.57 ± 0.02 (*I. kashmiriana*) and 6.41 ± 0.03 *(I. aphylla).* The other taxa with thin exine are in *I. pallida* (3.72 ± 0.03*)* and *I. variegata.* (3.78 ± 0.02), whereas *I. crotica, I. lutescens, I marsica, I. florentina* exhibit a medium range (4.36 ± 0.34, 4.47 ± 0.62, 4.53 ± 0.01 and 4.91 ± 0.22) while exine thickness is relatively high in *I. attica* (5.01 ± 0.02) and *I. germanica* (5.04 ± 0.03) (Table 1).

Aperture size : The aperture size of the pollen ranges between 29-70µm. The pollen aperture values are of similar in size *I. germanica* (48-60µm), *I. kashmiriana* (40-60µm.), *I. marsica* (40-60µm), *I attica* (45-65µm), whereas the pollen aperture values among *I. lutescens* (30-70µm), *I. florentina* (38-70µm) and *I. variegate* (30-40µm) exhibit almost uniform values. While in two species *(I. pallida* and *I. aphylla)* the aperture size is relatively high (50-70µm).

Correlation between pollen size, shape and chromosome number

Tables 1 and 3 depict the correlation between pollen grains size, shape and chromosome number. It is noticed that the pollen grains diploid species namely *I. attica, I. pallida* and *I. veriegata* the exhibit oblate shape in large pollen. As the ploidy is doubled the pollen shape and size virtually changed. Seven out of ten species, which are tetraploids exhibit prolate spheroidal shape with very large pollen grains.

Similarly, the diploid species viz. *I attica, I. pallida, I. variegata* display relatively low polar equatorial index value, while all other seven tetraploid species exhibit higher polar equatorial index values.

Aperture shape: Diploid species *I. pallida* and *I. variegata* display spherical aperture whereas, polyploids exhibit elliptic aperture except in *I. germanica*, *I. germanica* being a amphidiploid exhibits one of its diploid parent characters.

The pollen of the ten taxa of section *Iris* composed of only monocolpate pollen grains.

DISCUSSION

The important morphological characters relating to pollen grain size, shape, the form and type of germ pore; exine strata and exine ornamentation are of value in taxonomy and evolution. Of these characters, aperture may be considered as primary, exine surface patterns as secondary and other as tertiary in

PLATE 2 →

Pollen grains with monocolpate aperture
A. *I. aphylla* L.
B. *I. attica* Boiss & Heldr.
C. *I. germanica* L.
D. *I. pallida* Lam.
E. *I. croatica* Harvat
F. *I. florentina* L.
Bar : 30 µm

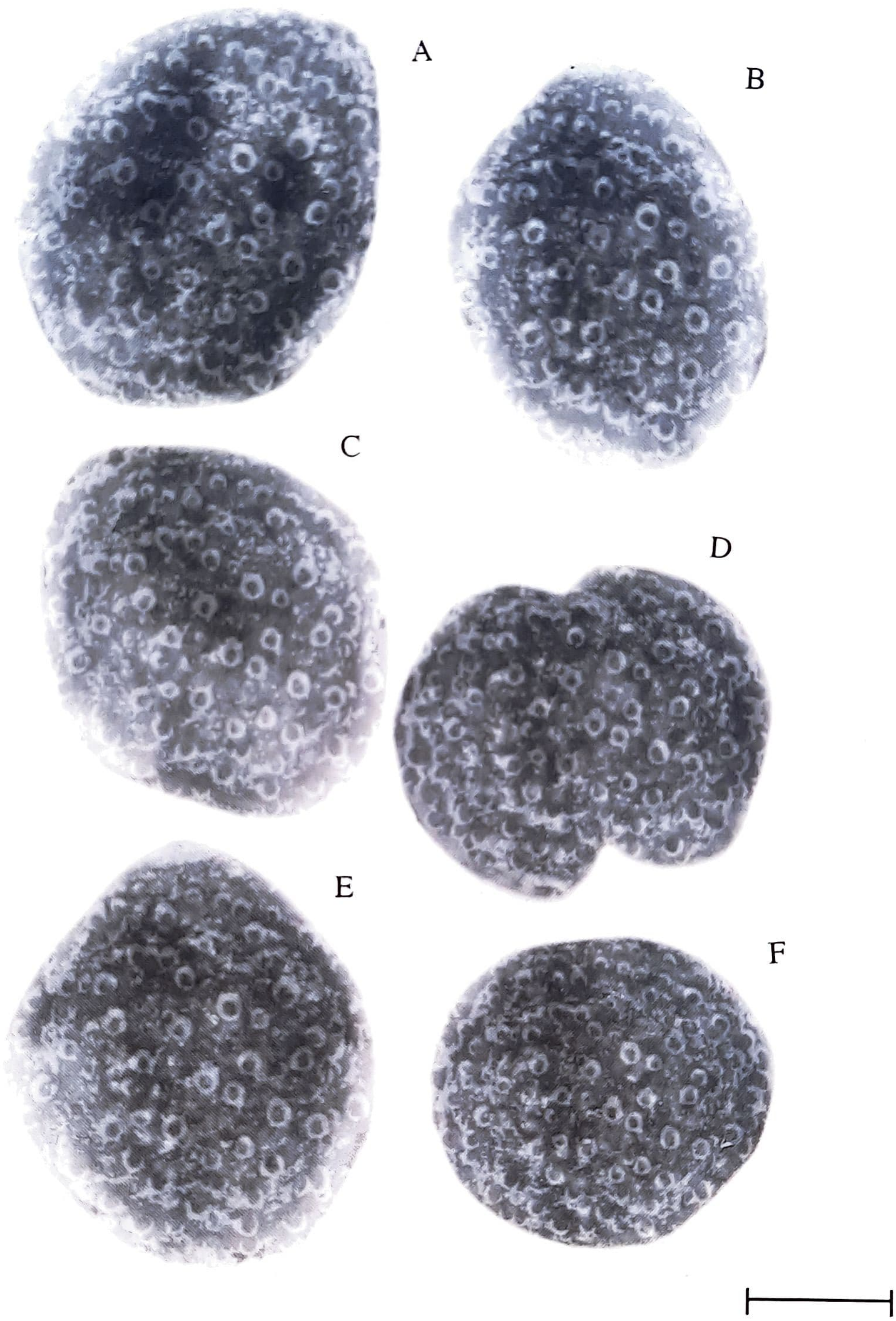

PLATE 2

their degree of importance. But Kuprianova (1948) considered the form of aperture alone for evaluating the significance of pollen morphology in the evolution of monocotyledons.

Ten taxa presently investigated exhibit monocolpate grains, similar type of monocolpate grains were also observed by Karihaloo et al. (1984) in 13 taxa that were randomly selected from different

Table 1. Mean frequency for pollen characters of some taxa of section *Iris* (pollen morphological characters) and their chromosome numbers

Sl. No.	Name of the Species	Chromosome no.	Polar axis (P)	Equatorial axis (E)	Polar Equatorial Index size	Exine thickness (U)
1.	*Iris aphylla* L.	2n = 48	130.00 ± 0.71 (108 – 144)	122.54 ± 0.79 (96 – 144)	106.00 ± 0.33 (89.47 – 121.05)	6.41 ± 0.03 (2.70 – 8.10)
2.	*I. attica* Boiss & Heldr	2n = 16	120.00 ± 1.11 (60 – 180)	121.53 ± 1.11 (48 – 168)	98.74 ± 0.54 (80 – 125)	5.01 ± 0.02 (3.96 – 6.10)
3	*I. croatica* Horvat	2n = 48	100.15 ± 0.40 (84 – 144)	93.28 ± 0.43 (78 – 114)	107.365±0.41 (86.67 – 123.07)	4.36±0.34 (3.58 – 5.17)
4.	*I. florentina* L.	2n = 44	123.92 ± 1.18 (60 – 180)	123.00 ± 0.87 (48 – 168)	100.75 ± 0.34 (66.6 – 125)	4.91 ± 0.22 (3.98 – 5.92)
5.	*I. germanica* L.	2n = 36, 44	119.00 ± 0.42 (96 – 132)	111.36 ± 0.42 (96 – 132)	106.86 ± 0.35 (88.23 – 118.75)	5.04 ± 0.03 (4.16 – 5.73)
6.	*I. kashmiriana* Baker.	2n = 44, 48	143.00 ± 0.54 (96 – 150)	126.30 ± 0.71 (90 – 144)	113.22 ± 0.43 (87.7 – 121.057)	3.57 ± 0.02 (3.00 – 4.58)
7.	*I. lutescens* Lam	2n = 40	133.12 ± 0.97 (96 – 168)	122.04 ± 0.93 (90 – 174)	109.07 ± 0.52 (93.10 – 126.66)	4.47 ± 0.62 (3.00 – 5.63)
8.	*I. marsica* Ricci & Cola	2n = 40	128.78 ± 0.71 (96 – 156)	114.00 ± 0.62 (90 – 162)	112.96 ± 0.55 (84.21 – 144.44)	4.53 ± 0.01 (3.71 – 5.69)
9.	*I. pallida* Lam	2n = 24	117.00 ± 0.48 (108 – 144)	118.00 ± 0.52 (96 – 144)	99.15 ± 0.45 (83.33 – 121.05)	3.72 ± 0.03 (2.70 – 5.10)
10.	*I. variegate* L	2n = 24	140.46 ± 0.54 (138 – 180)	140.16 ± 0.71 (114 – 168)	100.20 ± 0.55 (104.16 – 126.31)	3.78 ± 0.02 (3.4 – 7.6)

sections of the genus *Iris*. Pollen size is considered to be an important diagnostic character in the investigated taxa of section *Iris* as evidenced by significant differences between diploid and tetraploid species in the present study.

There existed a correlation between polyploid or chromosome number and pollen size in the investigated taxa of section *Iris* (Kapadia & Gould, 1964; Nair & Sharma 1966; 1967; Sree Rangaswamy & Raman 1973; Saraswathi Amma & Sethu Raju 1992). The very large size of pollen grains provides a reliable index for the detection of tetraploids of the investigated taxa. Based upon the size of the pollen grains, the taxa are classified into two types : oblate (75-100μ) and prolate spheroidal (100-113μ). In polar view, the shape markedly varies with species and ploidy is considered to be valuable in this taxon as was also observed by Muller (1969) in *Sonneratia* species. With the formula of Erdtman (1952), the investigated pollen grains of 10 taxa of section *Iris* are classified into (i) large monocolpate oblate shaped pollen and (ii) very large monocolpate prolate spheroidal grains (Table 2). The pollen grains of 13 taxa studied by Karihaloo *et al.* (1984) from different sections of genus *Iris* show radiosymmetric grains ranging from 68-167μ along the polar axis and 55-160.5 along the equatorial axis with prolate spheroidal to subprolate spheroidal variation (113-130). Dykes (1909) examined the pollen of certain species of *Iris* under light microscope and classified them into four groups and correlated with morphological groupings (12 sections) which were not tallied, unfortunately. Among the diploid taxa, the size variation was clearly noticed as in *I. pallida* 2n = 24 (99.45 ± 0.45) and in *I. variegata* 2n = 24 (100.20 ± 0.55) and the taxa exhibit delimiting variation, whereas in tetraploids the pollen size ranges between 100.75 ± 0.34 to 113.22 ± 0.43µ. The equatorial size varies in tetraploids : *I. florentina* 2n = 44 (100.75 ± 0.34), *I. croatica* 2n = 44, (107.36 ± 0.41), *I. aphylla* 2n = 48 (106.00 ± 0.33), *I. germanica* 2n = 44, 48 (106.86 ± 0.35), *I. kashmiriana* 2n = 48 (113.22 ± 0.43µ). The species such as *I. luterscens, I. marsica* are marshy forms but *I. kashmiriana* used to exist in foot hills where soils and moistures are abundantly made available, hence they exhibited the degree of larger sizes and two species are amphidiploids, which were said to possess the perfect homologous chromosomal pairing and increased size pollen grains (Ratnam 2001a) whereas,

Table 2. Chromosome number correlation with pollen grain size and shape classification

Sl. No.	Name of the Species	Chromosome no.	Polar Equatorial index size	Shape Description
1.	*Iris attica* Boiss & Heldr	2n = 16	98.74 ± 0.54	Oblate
2.	*I. pallida* Lam	2n = 24	99.15 ± 0.45	Oblate
3.	*I. variegata* L.	2n = 24	100.20 ± 0.55	Oblate
4.	*I. florentina* L.	2n = 44	100.75 ± 0.34	Prolate spheroidal
5.	*I. croatica* Horvat	2n = 48	107.36 ± 0.41	Prolate spheroidal
6.	*I. aphylla* L.	2n = 48	106.00 ± 0.33	Prolate spheroidal
7.	*I. germanica* L.	2n = 44, 48	106.86 ± 0.35	Prolate spheroidal
8.	*I. lutescens* Lam	2n = 40	109.07 ± 0.52	Prolate spheroidal
9.	*I. marsica* Ricci & Cola	2n = 40	112.96 ± 0.55	Prolate spheroidal
10.	*I. kashmiriana* Baker	2n = 48	113.22 ± 0.43	Prolate spheroidal

Table 3. Pollen morphological features of some taxa of section *Iris*

S. No.	Name of the Secies	Colour	Apeture Size	Apeture	Colpate shape	Colpi Margin	Columella appearance	Pollen size
1.	*I. aphylla* L.	Yellow	50-70µ	Elliptic	Mono	Crassi marginate	Wavy	Very large
2.	*I. attica* Bois & Heldr	Cream	45-65µ	Elliptic	Mono	Tenui marginate	Homogenous	Large
3.	*I. croatica* Horvat	Light Yellow	40-60µ	Elliptic	Mono	Crassi marginate	Wavy	Very large
4.	*I. florentina* L.	Light	38-70µ Yellow	Elliptic	Mono	Crassi marginate	Fused towards apex	Very large
5.	*I. germanica* L.	Cream	48-60µ	Spherical	Mono	Tenui marginate	Wavy	Very large
6.	*I. kashmiriana* Baker	Light Yellow	48-60µ	Elliptic	Mono	Crassi marginate	Fused with Exine	Very large
7.	*I. lutescens* Lam.	Light Yellow	30-70µ	Elliptic	Mono	Crassi marginate	Wavy	Very large
8.	*I. marsica* Ricci & Cola	Yellow	40-60µ	Spherical	Mono	Tenui marginate	Wavy	Very large
9.	*I. pallida* Lam.	Light Yellow	50-70µ	Spherical	Mono	Crassi marginate	Form luminar areas	Large
10.	*I. variegata* L.	Light Yellow	30-40µ	Spherical	Mono	Tenui marginate	Form small luminnar areas	Large

I. kashmiriana though autopolyploids, exhibit very large sized pollen grain, because of soil fertility and double number of harmonious chromosomes. These autopolyploids were formed long back in *I. kashmiriana*. The double number of chromosomes in these autopolyploids developed acquaintance among themselves which led to harmonious pairing and increased size of pollen grains that were boosted by fertile soils (Ratnam 2001b). Though there was no correlation between the chromosome number and pollen size it is probable that the pollen with lower chromosome number have large sized pollen with less variation within the diploids and the tetraploid taxa exhibit still higher equatorial index values with very large sized pollen grains.

Ten taxa which were palynologically investgated revealed that there is a degree of intrinsic variation among the diploid species and tetraploid species in relation to equatorial index values. Thus, the pollen grain characters display phylogenetic relationship, taxonomic similarity and intrinsic dissimilarity. The discontinuity developed among the diploids and tetraploids was shown in terms of equatorial index values, size and shape of the pollen. Hence, the palynological studies had taxonomic implications to set boundaries among diploids and polyploids and even among the taxa of both ploids. Thus, pollen grain characters in this groups are useful for the determination of phylogenetic relationship and to sort out taxonomic problems.

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