

THE SEED ATLAS OF PAKISTAN-V. BALSAMINACEAE

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Abstract

Seed morphology of 11 taxa of the genus *Impatiens* L. belonging to the family Balsaminaceae, was investigated using light and scanning electron microscopy (SEM). The variation in macro and micro morphological characters in seeds was found quite significant for the taxonomic delimitation at specific and infraspecific levels.

Introduction

The family Balsaminaceae comprises 2 genera and 1000 species mostly distributed in tropics and rarely in temperate regions (Mabberley, 2008). The family is represented in Pakistan by a single genus, *Impatiens* L. with 12 species and 9 infra specific taxa (Nasir, 1980). Seed morphological studies particularly the seed coat patterns have been used as an important tool for solving various taxonomic problems such as, tracing the evolutionary relationship (Akbari & Azizian, 2006), solving classificatory problems (Tantawy *et al.*, 2004; Utami & Shimizu, 2005) or the delimitation of genera and species (Ather *et al.*, 2009; Abid & Ali, 2010, Rajbhandary & Shrestha, 2010). Previously, Lu & Chen (1991) have studied the seed morphology of 12 species of *Impatiens* and seed characters were found very significant for the specific delimitation. Song *et al.* (2005) studied 38 species of *Impatiens* from south western China, and recognized four seed types based on seed coat micro morphology. These seed types provided additional evidences for tracing taxonomic and phylogenetic relationships. Similarly, Utami & Shimizu (2005) studied the seed coat morphology of 65 species belonging to 10 sections of the genus *Impatiens* and utilized seed coat characteristic for distinguishing groups or aggregate species. Most of the above studied species do not occur in Pakistan. No information on the seed morphology of various species of *Impatiens* occurring in Pakistan is available. The purpose of the present study is two fold. First to provide the seed micromorphological information of the *Impatiens* species belonging to Pakistan and secondarily to utilize this information as an additional evidence for the specific delimitation of *Impatiens*.

Material and Methods

Mature and healthy seeds of 11 taxa of the genus *Impatiens* were collected from herbarium specimens. Mostly 10 plants/species and 10 seeds/plant were studied (Appendix 1) and examined under stereomicroscope (Nikon XN Model), compound microscope (Nikon type 102) and scanning electron microscope (JSM-6380A). For scanning electron microscopy dry seeds were directly mounted on metallic stub using double adhesive tape and coated with gold for a period of 6 minutes in sputtering chamber and observed under SEM. The terminology used is in accordance to Lawrence (1970), Radford *et al.* (1974) and Stearn (1983) with slight modifications. The characters of seed viz., size, shape, colour, surface, and hilum were studied.

Observations

General seed characters of the family Balsaminaceae: Seeds 2.3-3.8 x 1-2.5mm, ovate, subglobose, narrowly elliptic or broadly elliptic, brown with or without light brown or yellow patches or blackish brown. Surface pattern varies from reticulate, foveated colliculate with pointed or rounded ends, foveated appressedly colliculate, alveolate, foveated alveolate, areolate, rugosely areolate, rugosely ruminant, tuberculate, granulate or egranulate, hilum basal. (Table 1; Fig. 1. A-L; Fig. 2. A-J).

Represented by single genus *Impatiens* with 11 taxa viz., *Impatiens brachycentra* Kar. & Kir. var. *jacquemontii* Hook.f., *I. bicolor* Royle ssp. *bicolor*, *I. bicolor* Royle ssp. *pseudo-bicolor* (Grey-Wilson) Y. Nasir., *I. edgeworthii* Hook .f., *I. flemingii* Hook.f., *I. glandulifera* Royle, *I. lemannii* Hook.f. ssp. *lemannii*, *I. lemannii* Hook.f. ssp. *kurramensis* Grey-Wilson, *I. scabrida* DC., *I. sulcata* Wall. and *I. thomsonii* Hook.f.

Key to the species of *Impatiens* L.

- 1+ Seeds elliptic 3
 - Seeds ovate or subglobose..... 2
- 2+ Seeds brown, granulate..... *I. sulcata*
 - Seeds blackish brown, egranulate..... *I. glandulifera*
- 3+ Seeds reticulate, colliculate, rugosely ruminant..... 4
 - Seeds areolate, rugosely foveate or alveolate and tuberculate..... 8
- 4+ Seeds narrowly elliptic..... *I. thomsonii*
 - Seeds broadly elliptic..... 5

- 5+ Seeds brown without yellow or brown patches6
 - Seeds brown with yellow or brown patches..... 7
 6+ Seeds surface reticulate..... *I. bicolor*
 - Seeds surface foveated apressedly colliculate with rounded ends..... *I. flemingii*
 7+ Seeds surface colliculate with pointed ends..... *I. brachycentra* var. *jacquemontii*
 - Seeds surface colliculate with rounded ends *I. edgeworthii*
 8+ Seeds granulate..... *I. lemannii*
 - Seeds egranulate..... *I. scabrida*

Key to the subspecies of *I. bicolor* Royle

- 1+ Seeds granulate..... *I. bicolor* ssp. *bicolor*
 - Seeds egranulate..... *I. bicolor* ssp. *pseudo-bicolor*

Key to the subspecies of *I. lemannii* Hook.f.

- 1+ Seeds tuberculate..... *I. lemannii* ssp. *kurramensis*
 - Seeds not tuberculate *I. lemannii* ssp. *lemannii*

Results and Discussion

The genus *Impatiens* has shown much diversity in its seed morphological characters, particularly seed coat patterns have been proved very rewarding for taxonomic and phylogenetic delimitation (Lu & Chen, 1991; Song *etal.*, 2005; Utami & Shimizu, 2005). The micro and macro morphological characters of seeds provide additional taxonomic characters in the intricate genus *Impatiens* where character differences are not much and these are difficult to delimit. Previously, on the basis of seed micromorphological characters species of *Impatiens* were divided in to various types. Lu & Chen (1991) recognized two types viz. laevigate and scabrous type. Song *etal.* (2005) distinguished four morphological types viz., laevigate, granulate, reticulate and protrusive type. Similarly, Utami & Shimizu (2005) recognized several types of seed coats for grouping species however, seeds micromorphology alone did not provide universally applicable key characters for identification although it was well correlated with other morphological characters to form groups or aggregate species. In the present study the specific or infra specific delimitation of the genus *Impatiens* is well correlated with seed morphology such as, *I. glandulifera*, *I. sulcata*, *I. lemannii* and *I. thomsonii* are grouped together by having nodding capsule. These species are further divided in 2 sub groups by having robust plant with reddish flowers in *I. glandulifera* and *I. sulcata*. While, *I. lemannii* and *I. thomsonii* are characterized by having slender plants with pink, white and yellow flowers (Nasir, 1980). Similarly, these species are also differentiated from each other by having exclusive seed characters such as, *I. glandulifera* and *I. sulcata* are characterized due to the presence of subglobose and ovate seeds respectively. While remaining two species are coupled together by having elliptic seeds but both remain distinct with different seed colours and surface patterns. The two subspecies of *I. lemannii* viz., *I. lemannii* ssp. *lemannii* and *I. lemannii* ssp. *kurramensis*

can be differentiated by having specific seed surface patterns such as, rugosely foveate surface pattern and alveolate and tuberculate surface pattern respectively. While, rest of the species viz., *I. bicolor*, *I. flemingii*, *I. brachycentra* var. *jacquemontii*, *I. edgeworthii* and *I. scabrida* are grouped by having erect capsule (Nasir, 1980). Amongst these species *I. brachycentra* var. *jacquemontii* is characterized by having spurless and white flower along with brown patched seeds and foveated colliculae with pointed ends surface pattern. The remaining species of this group have well developed spur and with distinct flower colours. From these, *I. flemingii* remains distinct by having pink-white flowers (Nasir, 1980) and seeds with foveated and apressedly colliculate surface. The findings are also supported by the pollen morphology where, suboblate pollen were reported, while in rest of the species oblate pollen were found (Perveen & Qaiser, 2001). Similarly, *I. edgeworthii* has yellow flowers (Nasir, 1980) with yellow patched seeds and foveated colliculae with rounded ends. The remaining two species *I. bicolor* and *I. scabrida* are quite dissimilar in gross morphology as well as in seed characters such as *I. scabrida* remains distinct by having areolate surface pattern while in *I. bicolor* reticulate surface pattern is reported. However, the present finding are in contrast to that of the finding of Song *etal.* (2005) where reticulate seed surface was observed in *I. scabrida*.

The seed morphological data also support the taxonomic decision of Nasir, (1980) for recognizing two subspecies of *I. bicolor* viz., *I. bicolor* ssp. *bicolor* and *I. bicolor* ssp. *pseudo-bicolor*, as granulated seed surface was observed in type subspecies while, other subspecies is characterized by having non granulated seed. In contrast to the pollen characters (Perveen & Qaiser, 2001), seed characters are significant enough to correlate the taxonomic delimitation of the genus *Impatiens* both at specific and infra specific levels.

Table 1. Seeds morphological characters of the family Balsaminaceae.

Name of taxa	Size mm	Shape	Colour	Surface	Hilum
<i>Impatiens brachycentra</i> var. <i>jacquemontii</i>	2.5-2.8 x 1.2-1.6	Broadly elliptic	Brown with light brown patches	Foveated colliculate with pointed ends, granulate	Basal
<i>I. bicolor</i> ssp. <i>bicolor</i>	3-3.2 x 1.5-1.6	Broadly elliptic	Brown	Reticulate, granulate	Basal
<i>I. bicolor</i> ssp. <i>pseudo-bicolor</i>	2.4-2.5 x 1.2-1.3	Broadly elliptic	Brown	Reticulate, egranulate	Basal
<i>I. edgeworthii</i>	3.3-3.6 x 1.8-2	Broadly elliptic	Brown with yellow patches	Foveated colliculate with rounded ends, granulate	Basal
<i>I. flemingii</i>	2.4-2.5 x 1.1-1.2	Broadly elliptic	Brown	Foveated appressedly colliculate with rounded ends, granulate	Basal
<i>I. glandulifera</i>	3.5-3.8 x 2.4-2.5	Subglobose	Blackish brown	Rugosely areolate, egranulate	Basal
<i>I. lemarii</i> ssp. <i>kurrarnensis</i>	2.3-2.5 x 1-1.2	Broadly elliptic	Brown	Alveolate and tuberculate, granulate	Basal
<i>I. lemarii</i> ssp. <i>lemarii</i>	3.3-3.4 x 1.5-1.6	Narrowly elliptic	Brown	Rugosely foveate, granulate	Basal
<i>I. scabrida</i>	2.3-2.8 x 1.1-1.2	Narrowly elliptic	Brown	Areolate, egranulate	Basal
<i>I. sulcata</i>	3.2-3.5 x 1.3-1.4	Ovate	Brown	Foveated alveolate, granulate	Basal
<i>I. thomsonii</i>	3-3.3 x 1.4-1.5	Narrowly elliptic	Brown with light brown patches	Rugosely ruminata, granulate	Basal

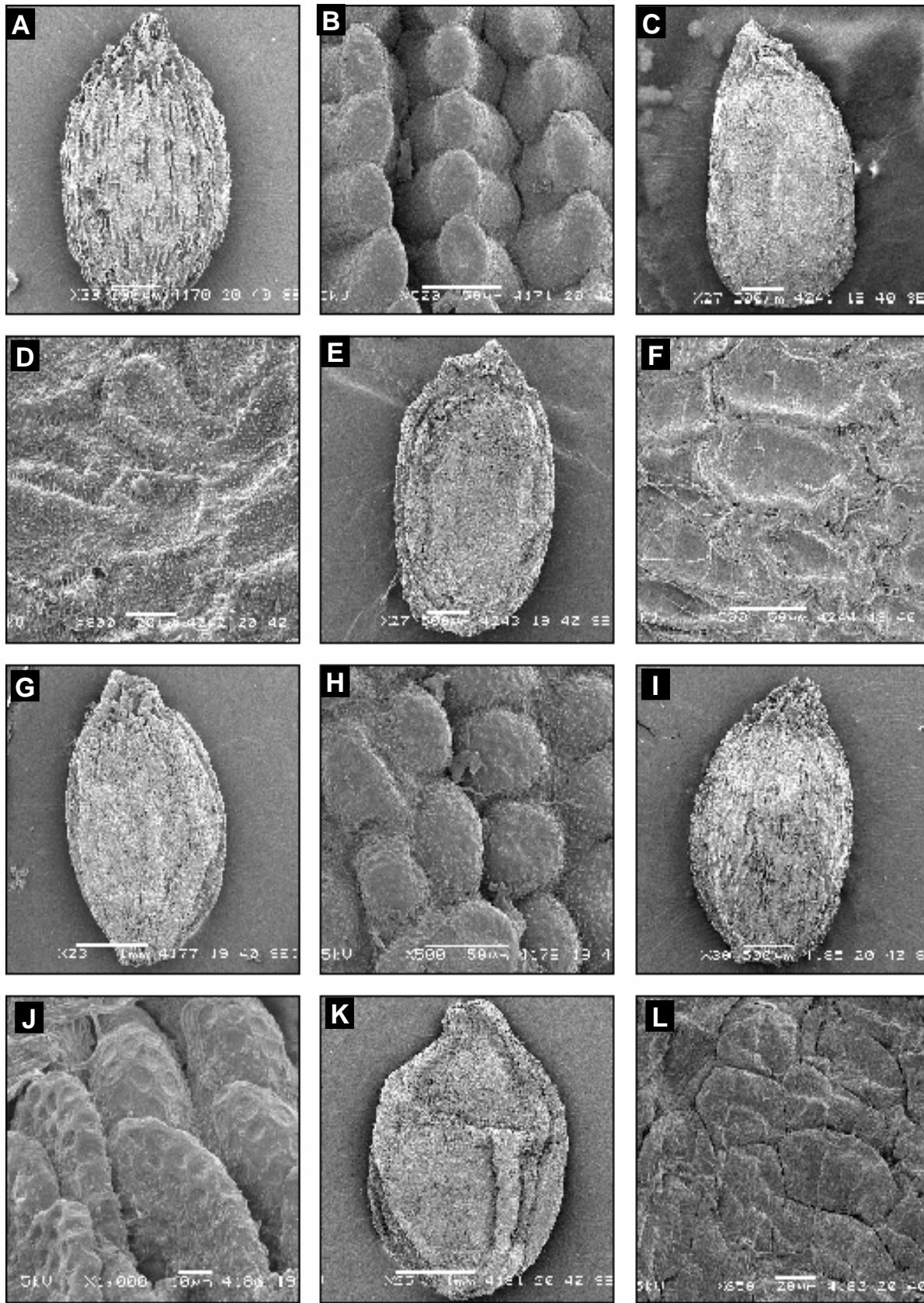


Fig. 1. Scanning electron micrographs. *Impatiens brachycentra* var. *jacquemontii*: A, seed; B, surface. *I. bicolor* ssp. *bicolor*: C, seed; D, surface. *I. bicolor* ssp. *pseudo-bicolor*: E, seed; F, surface. *I. edgeworthii*: G, seed; H, surface. *I. flemingii*: I, seed; J, surface. *I. glandulifera*: K, seed; L, surface. (Scale bar: G, K = 1mm; A, C, E, I = 500 μ m; B, F, H = 50 μ m; D, L = 20 μ m; J = 10 μ m).

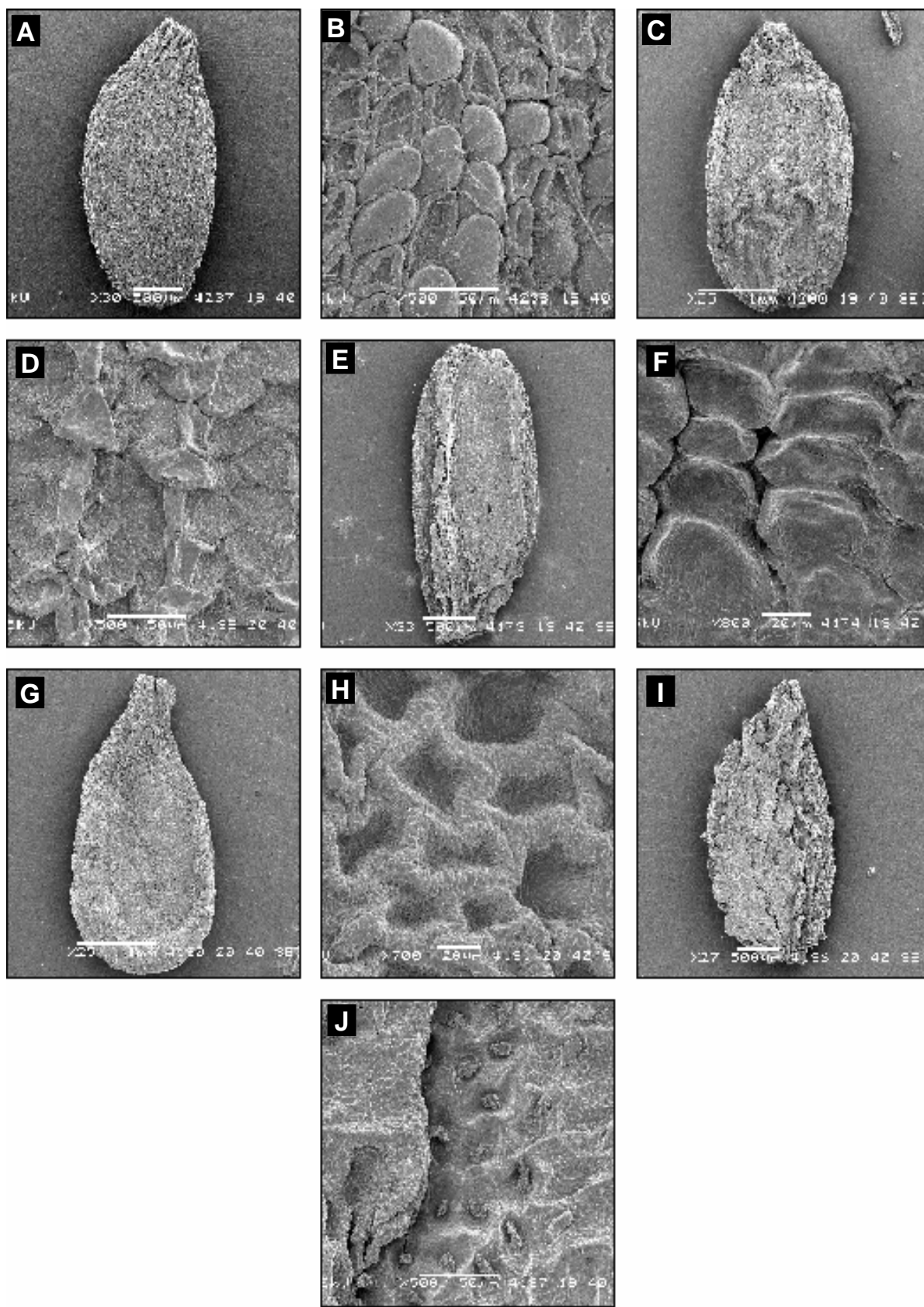


Fig. 2. Scanning electron micrographs. *I. lemarii* ssp. *kurrumensis*: A, seed; B, surface. *I. lemarii* ssp. *lemarii*: C, seed; D, surface. *I. scabrada*: E, seed; F, surface. *I. sulcata*: G, seed; H, surface. *I. thomsonii*: I, seed; J, surface. (Scale bar: A, G = 1mm; C, E, I = 500µm; B, D, J = 50µm; F, H = 20µm).

Appendix-1. List of voucher specimens.

Taxa	Collector, number and herbarium
<i>Impatiens brachycentra</i> var. <i>jacquemontii</i>	M. Qaiser & A. Ghafoor 1802 (KUH); Farrukh Hussain 7059, 7089 (RAW); Jafri 33 (KUH); M. A. Siddiqui 12290 (RAW); Sultanul Abedin 1959 (KUH); Y. Nasir & Nazir 8472 (RAW); B. Datta s.n. (RAW).
<i>I. bicolor</i> ssp. <i>bicolor</i>	Y. Nasir & Nazir 8507 (RAW); S. A. Farooqi & M. Qaiser 3227 (KUH); Sultanul Abedin & M. Qaiser 9047 (KUH); R. R. Stewart 24417 (RAW); R. R. Stewart & A. Rehman 25123a (RAW); Nasir 8468 (RAW);
<i>I. bicolor</i> ssp. <i>pseudo-bicolor</i>	R. R. & I. D. Stewart 6086 (RAW); Saida Qureshi 100 (KUH); R. R. Stewart 24758 (RAW); E. Nasir & M. A. Siddiqui 1021 (RAW)
<i>I. edgeworthii</i>	R. R. Stewart et al 1373 (RAW); M. A. Farooqi & M. Qaiser 3420 (KUH); E. Nasir & M. A. Siddiqui 451, 1021 (RAW); Sultanul Abedin 8307 (KUH); J. Mohd s. n. (KUH); R. R. & I. D. Stewart 19246, 6086 (RAW); M. Hanif 51 (RAW); Y. Nasir & Nazir 8480 (RAW); R. R. Stewart s.n., 7717 (RAW).
<i>I. flemingii</i>	R. R. Stewart 24416 (RAW); R. R. Stewart & A. Rahman 25152 (RAW); E. Nasir & M. A. Siddiqui 1087 (RAW); S. A. Farooqi & M. Qaiser 3117, 3226 (KUH); R. R. Stewart & E. Nasir 23785 (RAW); Sultanul Abedin & A. Ghafoor 8761 (KUH); R. R. & I. D. Stewart s. n. (RAW); Y. Nasir 6168 (RAW); E. Nasir 3618 (RAW).
<i>I. glandulifera</i>	R. R. Stewart 24658, 2775 (RAW); E. Nasir 2954 (RAW); Y. Nasir & Nazir 8496, 8501 (RAW); M. A. Siddiqui & Y. Nasir 6518 (RAW); R. R. Stewart 7704, s. n. (RAW); R. R. & I. D. Stewart 17627 (RAW); Ch. Shukat Ali 86 (RAW).
<i>I. lemannii</i> ssp. <i>lemannii</i>	M. Qaiser & A. Ghafoor 1898 (KUH); M. A. Siddiqui & Y. Nasir 6504 (RAW); Sultanul Abedin 8119 (KUH); Hassandin 553 (RAW).
<i>I. lemannii</i> ssp. <i>kurramensis</i>	M. A. Siddiqui & Y. Nasir 6505 (RAW); R. R. Stewart 28042 (RAW).
<i>I. scabrida</i>	M. Qaiser & A. Ghafoor 5134 (KUH); R. R. Stewart 12420 (RAW).
<i>I. sulcata</i>	R. R. Stewart 26300, 26458, 20960, 28869, s.n. (RAW); Sultanul Abedin & M. Qaiser 9152 (KUH); E. Nasir 2930 (RAW); A. Rahman 131 (RAW); Saida Qureshi 181 (KUH).
<i>I. thomsonii</i>	R. R. Stewart 20901 (RAW); M. A. Siddiqui & A. Rahman 26800 (RAW); R. R. Stewart 19739 (RAW).

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