

## Cypsela Diversity of the Genus *Inula* L. (Inuleae) and Its Taxonomic Implication

**Tulika Talukdar**

Department of Botany, Krishnagar Govt. College, University of Kalyani, Krishnagar, 741101, Nadia, West Bengal, India

### Abstract

In order to evaluate taxonomic application of cypselas features, detail macro as well as micro-morphology of cypsela of 5 species belonging to the genus *Inula* L. of the tribe Inuleae (Family Asteraceae) has been investigated under Light Microscope. Analysis revealed that in comparison to shape, size and colour of cypsela, surface features like hair type and distribution pattern, surface ornamentation, presence or absence of rib and their number, along with pappus diversity were taxonomically more significant characters. Detail carpodial features were also found to be diacritical for the genus *Inula* L. These cypsela features can be utilized in delimiting different species of *Inula* L. Finally, involving all these cypselas features an artificial key to the studied species is constructed. This could be used as reference key to identify taxa solely based on its cypsela.

**KEYWORDS:** Cypsela, carpodium, *Inula* L., pappus, trichome, taxonomy.

### Introduction

The tribe Inuleae of sunflower family (Asteraceae) is easily recognizable by the presence of tailed anthers in combination with two separated stigmatic surfaces. The first modern systematic review of the tribe was presented by Merxmuller et al. (1977) based on palynology, phytochemistry, cytology along with morphology. He recognized three sub-tribes- Athriniinae, Gnaphaliinae and Inulinae. Anderberg proposed Inuleae as a paraphyletic assemblage. In revised classification system, (Anderberg et al., 2005; Anderberg and Eldenas, 2007), Inuleae have been divided into two major sister clades or sub-tribes- Inuleae-Inulinae and Inuleae-Plucheinae. The sub-tribe Inulinae is predominantly European, North African, and Western Asian with its highest generic and morphological diversity around the Mediterranean (Anderberg, 2009). Recently, the key genus, *Inula* L. has been shown to be polyphyletic and many of its species have been transferred to other genera (Anderberg, 1991, 1996). Circumscriptions of cypsela in many tribes of Asteraceae have been proved to have enough taxonomic potential (Cassini, 1813). The morphology and anatomy of cypsela are often useful in identification of various taxa both at the generic and specific levels within the tribe Inuleae (Pandey et al., 1983; Abid & Qaiser, 2002; Abid & Zehra, 2007; Pandey & Kumari, 2007; Jana & Mukherjee, 2012).

The present investigation deals with detail morphological characterisation of cypsela in five species of type genus *Inula* L. with special emphasis on different cypsela parts like carpodium, stylopodium, pappus, and few micro-features like surface ornamentation, hair type, ribbing pattern etc. Finally, all these cypsela characters are utilized for separating the studied taxa at species level.

## Materials and Methods

Plant materials (cypselas) for the present investigation were obtained in the form of received herbarium specimens from the following herbaria of the world which are mentioned in Index Herbarium (Holmgren et al., 1981). The name of taxa with collection number is presented in Table 1.

DK	:	Hortus botanicus Hauniensis, Denmark.
Z	:	Botanischer Garten der Universitat Zurich, Zollikerstrasse 107, CH8008 Zurich, Switzerland.

## Macro-morphological studies of cypselas

In cases, where intact cypselas were available, the first and foremost step was to mark the posterior and anterior (abaxial) surface of the cypsel. Then 10 dry and 10 FAA preserved mature cypselas were randomly taken in glass slides and graphed slides and observed under Olympus stereo dissecting microscope (DM) and Olympus binocular microscope (No.-611062). Suitable images were taken using Zeiss Stemi DV4 camera equipped microscope.

Colour, shape, direction of cypselas was noted carefully. Length and width of the cypselas were measured visually by graphed slides, in few cases they were counted by ocular and stage micrometer. The length of the cypselas in the present study is defined as the length of the body of cypselas from basal meristematic zone (carpopodium) up to apical end excluding pappus. The width was measured at the widest part of the cypselas. Outline diagrams of complete cypselas and different parts were drawn by the Mirror type camera lucida.

## Micro-morphological studies of cypselas

Mature cypselas were dipped in 1-5% NaOH solution for 2-7 days depending upon the hardness. Then they were transferred into saturated chloral hydrate solution for few hours, repeatedly washed with water and properly stained in 0.2-0.5 % aqueous Safranin solution. After staining, specimens were placed in 70 % phenol glycerine solution and dissected carefully for studying different parts of cypselas. Suitable photographs were taken using Olympus C-310 zoom digital camera (3.2 Megapixel) and Zeiss-stereo microscope.

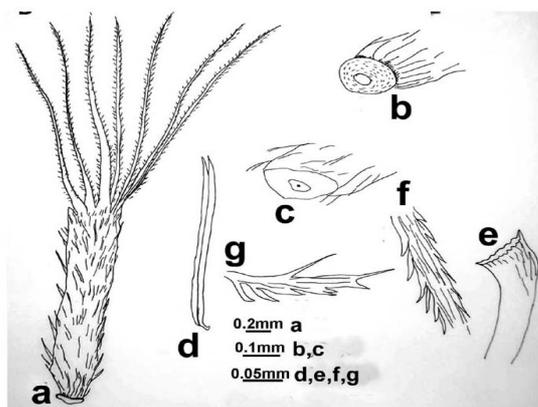
Nature of ribs, types, distribution and orientation of hairs, nature of surface cells, other epidermal structures, carpopodial cells etc. all were critically observed. Pappus characters such as nature of pappus bristles, their number, arrangement, apex organization etc. were also examined.

## Results

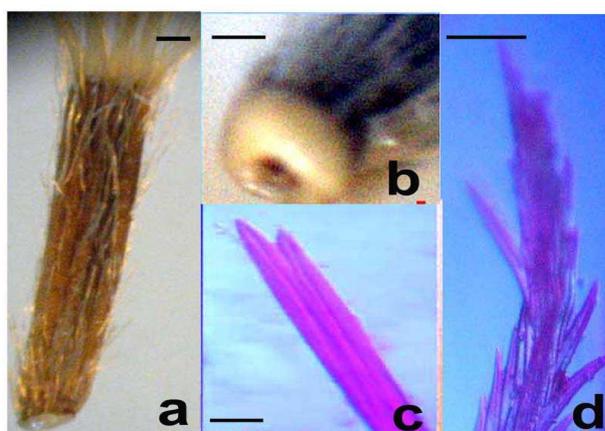
### *Inula candida* Cass. (Figure 1, 2)

Cypselas was homomorphic, 3.0-4.0 mm x 0.1-0.2 mm (excluding pappus) in size, deep brown, narrow oblong in shape with constricted apex and gradually tapering base, and straight in direction. A faintly dorsiventral differentiation was marked in cypselas. Cypselas surface was pubescent with pilose, densely distributed, twin hairs on both the sides. Prominent, straight, nine to ten ribs were noted on the cypselas surface. A well developed, tubular, solid stylopodium (style base) was noted at the apical

region of cypsela. At the base of cypsela, a symmetric triangular ring like carpodium was present. Carpodium cells were clearly distinguishable from the other body cells of cypsela, arranged in six to seven rows. They were thin-walled, parenchymatous, oval to rectangular and tangentially oriented. Diameter of carpodium was found to be wider than the base of the cypsela. Cypsela was papose at apex with many, persistent, barbellate bristles. Pappus bristles were 3.0-3.2 mm. long, unbranched, basally connate, each with two unequal sharply pointed apical cells.



**Figure 1:** Cypsela morphology of *Inula candida*. a-cypsela, b-base, c-apex, d-twin surface hair, e-basal part of pappus bristle, f-middle part of pappus bristle, g-apex of pappus bristle.



**Figure 2:** Digital images of different parts of cypsela in *Inula candida*. a-cypsela, b-base, c-twin surface hair, d-apical part of pappus bristle.[Bars = 20  $\mu$ ].

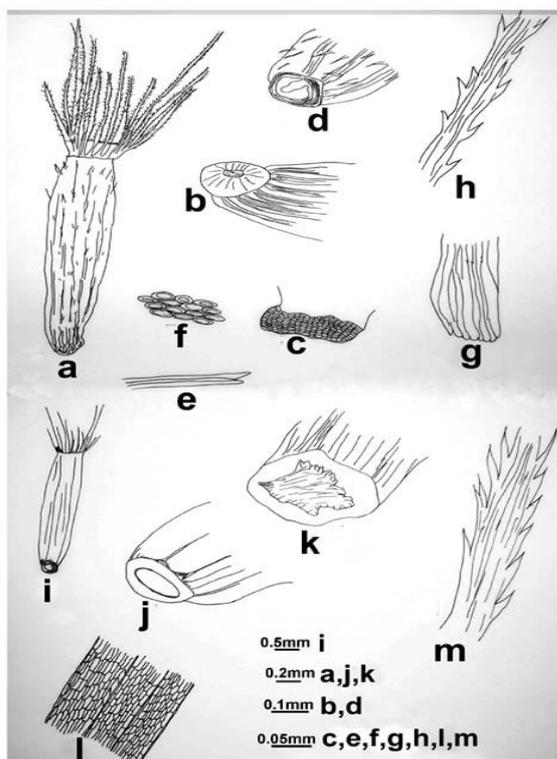
***Inula conyzae* (Griesselich) Meikle (Figure 3a-h, 4a-f)**

Cypsela was homomorphic, 2.5-3.6 mm x 0.2-0.5 mm (excluding pappus) in size, black, narrow oblong in shape with truncate apex and slightly narrowed base, and straight in direction. Cypsela was cylindrical without any dorsiventral differentiation. Cypsela surface was pubescent with pilose, sparsely distributed, bi-forked or twin hairs. Prominent, straight, 10-15 ribs were noted on cypsela surface and after cleaning, surface also showed ocellate markings. A well developed, tubular, solid stylopodium with rounded apex was noted at the apical region of cypsela. At the base of cypsela, a symmetric ring like carpodium was present. Carpodium cells were clearly distinguishable from the other body cells of cypsela, arranged in 10-12 rows.

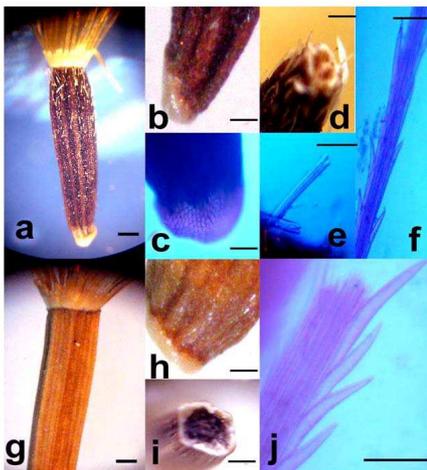
They were thin-walled, parenchymatous, oval to rectangular and tangentially oriented. Diameter of carpodium was found to be same as the base of cypsela. Cypsela was papose at apex with many, persistent, scabrous bristles. Pappus bristles were 1.3-2.3 mm. long, unbranched, basally connate.

***Inula helenium* L. (Figure 3i-m,4g-j)**

Cypsela was homomorphic, 5.0-5.5 mm x 0.8-1.0 mm (excluding pappus) in size, brown, narrow oblong in shape with, rounded apex and slightly narrowed base, and straight in direction. Cypsela was cylindrical without any dorsiventral differentiation. Cypsela surface was glabrous and ribbed with many prominent and straight ribs. After cleaning, cypsela surface showed lineate markings. Stylopodium was absent. At the base of cypsela, a symmetric ring like carpodium was present. Carpodial cells were clearly distinguishable from the other body cells of cypsela, arranged in 10-12 rows. They were oval to polygonal and tangentially oriented. Diameter of carpodium was found to be same as the base of cypsela. Cypsela was papose at apex with many, persistent, barbellate bristles. Pappus bristles were 0.83-1.66 mm. long, unbranched, basally free from one another.



**Figure 3:** Cypsela morphology of *Inula conyzae* (a-h) and *Inula helenium* (i-m). a,i-cypsela; b,j-base; c-carpodium; d,k-apex (after detachment of pappus); e-twin surface hair; f-surface cells; g-basal part of pappus bristle; h,m-middle part of pappus bristle; l-surface (after cleaning).



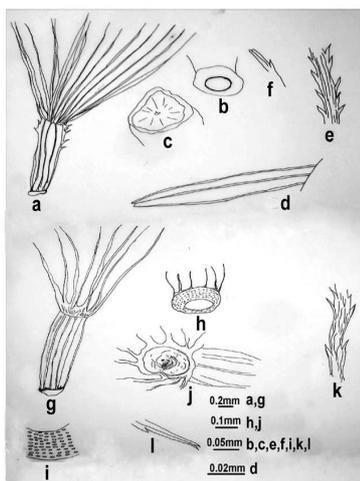
**Figure 4:** Digital images of different parts of cypselas in *Inula conyzae* (a-f) and *Inula helenium* (g-j). a,g-cypselas; b,h-base; c-carpodium; d,i-apex (after detachment of pappus); e-surface hair; f;j-part of pappus bristle. [Bars = 20  $\mu$ ].

#### ***Inula helvetica* Grauer (Figure 5a-f, 6a-d)**

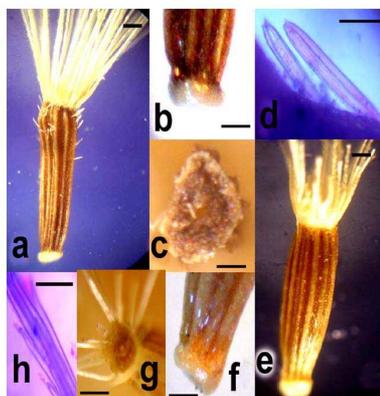
Cypselas was homomorphic, 1.5-1.8 mm x 0.1-0.2 mm (excluding pappus) in size, brown, narrow oblong in shape with truncate apex and base, and straight in direction. Cypselas was cylindrical without any dorsiventral differentiation. Surface was ribbed possessing 6 prominent ribs and glabrous only with few apical hairs. A well developed, tubular, solid stylopodium was noted at the apical region of cypselas. At the base of cypselas, a symmetric ring like carpodium was present. Carpodium cells were clearly distinguishable from the other body cells of cypselas, arranged in 10-12 rows. They were oval to rectangular and tangentially oriented. Diameter of carpodium was found to be same as the base of cypselas. Cypselas was pappose at apex with many, persistent, barbellate bristles. Pappus bristles were 2.7-3.0 mm. long, unbranched, basally free from one another with two, equal, blunt apical cells.

#### ***Inula salicina* L. (Figure 5g-l, 6e-h)**

Cypselas was homomorphic, 1.8-2.0 mm x 0.1-0.2 mm (excluding pappus) in size, yellowish brown, narrow oblong in shape with truncate apex and gradually tapered rounded base, and straight in direction. Cypselas was cylindrical without any dorsiventral differentiation. Surface was ribbed possessing numerous prominent ribs and glabrous. An inconspicuous stylopodium was noted at the apical region of cypselas. At the base of cypselas, a symmetric ring like carpodium was present. Carpodium cells were clearly distinguishable from the other body cells of cypselas, arranged in 10-12 rows. They were thin-walled, parenchymatous, oval and tangentially oriented. Diameter of carpodium was found to be same as the base of cypselas. Cypselas was pappose at apex with many, persistent, barbellate bristles. Pappus bristles were 2.0-2.5 mm. long, unbranched, basally connate with two, unequal, sharply pointed apical cells.



**Figure 5:** Cypselas morphology of *Inula helvetica* (a-f) and *Inula salicina* (g-l). a,g-cypselas; b,h-base; c,j-apex (after detachment of pappus); d-twin surface hair; e,k-middle part of pappus bristle; f,l-apex of pappus bristle;i-carpodial cells.



**Figure 6:** Digital images of different parts of cypselas in *Inula helvetica* (a-d) and *Inula salicina* (e-h). a,e-cypselas; b,f-base; c,g-apex (after detachment of pappus); d-surface hairs; h-part of pappus bristle. [Bars = 20  $\mu$ ].

## Discussion

Macro and micro-morphological features of studied cypselas were presented in Table 2. Cypselas of all the studied species were generally small, 1.5-5.5 mm long and brown. Black cypselas was observed in *Inula conyzae*. Mostly they were non-angular, cylindrical except in *Inula candida* where faintly, dorsiventrally compressed cypselas has been noticed. However, angular cypselas have been reported in *Pulicaria angustifolia* and *P. carnosa* of the tribe Inuleae by Abid and Quiser (2007), however they were not found in the present study. Variable number of ribs was observed on the cypselas surface in the studied species. Pubescent cypselas were prevalent in three species of *Inula* (*I. candida*, *I. conyzae* and *I. helvetica*), among which cypselas of *I. helvetica* were only apically pubescent. Hairs in all pubescent species were of non-glandular twin type with acute apex. Bicelled hairs were also found to be occurring in *Helichrysum umbraculigerum* of the tribe Inuleae by Mukherjee and Sarkar (1994). Presence of acute trichome in *Inula* is well supported by Anderberg (1989) who remarked that "hairs are either acute as in the Inulinae or obtuse as in Plucheinae and

Gnaphaliinae". After cleaning, cypsela surface showed ocellate markings in *I. conyza* and lineate markings in *I. helenium*.

Well developed, tubular stylopodium with enlarged base have been noticed in cypsela of all pubescent species, while in glabrous species, stylopodium was either inconspicuous or absent.

In all the investigated taxa symmetric triangular ring-like carpopodium was noticed. Carpopodium was well-developed; with oval to rectangular, thin-walled, tangentially oriented cells, arranged in variable number of rows. In most of studied species of *Inula*, carpopodial cells were arranged in 10-12 rows, except in *Inula candida*, where 6-7 rows of cells were noted with lesser vertical height of carpopodium. However, horizontal length of carpopodium in *I. candida* was higher than the other studied species.

Pappus features which were frequently utilized in the tribal taxonomy of Asteraceae, were also found to be dynamic for the present tribe. Cypselas of all the studied species were pappose, though epappose cypselas were also noted in *Carpesium* (Abid and Zehra, 2007; Mukherjee and Sarkar, 1994). Pappus were generally represented by many, uniseriate, capillary, barbellate bristles; usually connate but free in *I. helenium* and *I. helvetica*. In the contrary, biseriate pappus was also reported in few members of the tribe Inuleae (in *Pegolettia*) by Abid and Zehra (2007). The apical cells of pappus were generally acute in the studied species, though clavate blunt apical cells were noted in *I. helvetica*, also reported in few Australian species of *Waitzia* and *Helipterum* (Anderberg, 1989) and this character was first used by Hilliard and Burtl (1981). Bristles of *I. candida* and *I. salicina* were made up of two, unequal apical cells, which in *I. helvetica* were equal. Pappus features in *Inula* although showed an apparent uniformity but with all minor variations these features could be utilized as species delimiting factors.

Using all these macro as well as micro-morphological features of cypsela, an artificial key to the studied species was constructed.

### Key to the species

#### Genus- *Inula*

1. Ribs more than 15, surface glabrous; stylopodium either inconspicuous or absent. ----- **2**
1. Ribs not more than 15, surface pubescent; stylopodium well-developed. ----- **3**
2. Cypselar apex rounded; surface with lineate markings; pappus bristles basally free. ----- ***I. helenium***
2. Cypselar apex truncate; surface without markings; pappus bristles basally connate. ----- ***I. salicina***
3. Cypsela constricted at apex, faintly dorsiventrally compressed; hairs densely distributed; carpopodial cells arranged in 6-7 rows. ----- ***I. candida***

3. Cypsela truncate at apex, cylindrical; hairs sparsely distributed; carpopodial cells arranged in 10-12 rows. ----- 4
4. Ribs 10-15 in number; cypsela totally pubescent, with ocellate markings; pappus bristles basally connate. ----- *I. conyzae*
4. Ribs 6 in number; cypsela apically pubescent, without any surface markings; pappus bristles free. ----- *I. helvetica*

### Acknowledgements

Author is immensely grateful to University Grant Commission (UGC) for extending financial support for implementation of the project [Sanction No.F.PSW-068/11-12(ERO), Dated 3<sup>rd</sup> August, 2011]. Author is highly grateful to Prof. Sobhan Kumar Mukherjee, Dept. of Botany, University of Kalyani, for his valuable suggestions. Author extends special thanks to Dr. Hans Vilhelm Hansen, Curator, Denmark and to Dr. Peter Enz, Curator, Zurich for their active assistance in despatching the identified mature cypselas for studies.

### References

- Abid, R.D., and Qaiser, M. (2002) Cypsela morphology of *Inula* L. (*s. str.*) and its allied genera (Inuleae – Compositae) from Pakistan and Kashmir. Pak. J. Bot. 34(3), pp. 207 – 223
- Abid, R., and Qaiser, M. (2007) Cypsela morphology of the genus *Pulicaria* Gaertn., (Inuleae-Asteraceae) from Pakistan. Pak. J. Bot. 39(4), pp. 991- 997
- Abid, R., and Zehra, N. (2007) Micromorphology of cypsela and its taxonomic significance of some genera in the tribe Inuleae (Asteraceae) from Pakistan. Pak. J. Bot. 39(5), pp. 1407-1416
- Anderberg, A. (1989) Phylogeny and reclassification of the tribe Inuleae (Asteraceae). Canad. J. Bot. 67, pp. 2277-2296
- Anderberg, A. A. (1990) Taxonomy and phylogeny of the tribe Inuleae (Asteraceae). Pl. Syst. Evol. 176, pp. 75-123
- Anderberg, A. A. (1991) Taxonomy and phylogeny of the tribe Plucheeae (Asteraceae). Pl. Syst. Evol. 176, pp. 145-177
- Anderberg, A.A. (1994) Inuleae. In: Bremer, K. (Ed.), Asteraceae: Cladistics & Classification. Timber Press, Portland, pp. 273 – 291
- Anderberg, A. A. (2009) Inuleae. In : Funk, V.A., Susanna, A., Stuessy, T.F., and Bayer, R.J. (Eds.). Systematics, Evolution, and Biogeography of Compositae, Smithsonian Institution, Washington, D.C., U.S.A., pp. 667-680
- Anderberg, A. A., and Eldenas, P. 2007 [2006] Tribe Inuleae. In : Kadereit, J.W., and Jeffrey, C. (Eds.), The Families and Genera of Vascular Plants, vol. 8, Flowering Plants, Eudicots. Asterales, Springer, Berlin, pp. 374-391

Anderberg, A. A., Eldenas, P., Bayer, R. J., and Englund, M. (2005) Evolutionary relationships in the Asteraceae tribe Inuleae (incl. Plucheeae) evidenced by DNA sequences of *ndh F*; with notes on the systematic positions of some aberrant genera. *Organisms, Diversity and Evolution* 5, pp. 135-146

Cassini, H. (1813) Observations sur le style et le stigmati des Synanthérées. *Journal de Physique, de Chimie, d' Histoire Naturelle et des Arts.* 76, pp. 97 – 128

Hilliard, O. M., and Burtl, B. L. (1981) Some generic concepts in Compositae-Gnaphaliinae. *Bot. J. Linn. Soc.* 82, pp. 233 – 265

Holmgren, P. K., Keuken, W., and Schofield, E. K. (1981) *Index Herbariorum. Part I. The Herbaria of the World. Edition 7 (Haque : Regnum Vegetabile, 106)*

Jana, B.K., and Mukherjee, S.K. (2012) Cypselar morphology of some species of the family Compositae and their taxonomic significance. *IJPRBS.* 1(5), pp.463-484

Merxmüller, H., Leins, P., and Roessler, H. (1977) Inuleae- systematic review. In : Heywood, V. H., Harborne, J. B., and Turner, B. L.(eds.), *The Biology and Chemistry of the Compositae, Vol. 1, Academic Press, London, pp. 577 – 602*

Mukherjee, S. K., and Sarkar, A. K. (1994) Morpho-anatomical studies of cypselas in some members of the tribe Inuleae (Asteraceae) with the help of SEM. *J. Natl. Bot. Soc.* 48, pp. 19-39

Pandey, A.K., and Kumari, A.( 2007) Anatomical patterns of pericarp in Asteraceae. In: Chauhan, S.V.S., Rana, A., and Chauhan, S. (Ed.), *Plant Reproductive Biology and Biotechnology, Aavishkar Publishers, Distributors, Jaipur, pp. 64 – 77*

Pandey, A.K., Chopra, S., and Singh, R. P. (1983) Development and structure of seeds and fruits in Compositae, tribe Inuleae. *Proc. Indian Acad. Sci. (Pl. Sci.).* 92(6), pp. 467 – 471

Table 1: Source of materials with collection number.

Serial No.	Name of Taxa	Locality	Collection Number
1.	<i>Inula candida</i> Cass.	Z	Acc No.138 of 2006 Index Seminum
2.	<i>Inula conyzae</i> (Griess.)Meikle	DK	G E2426-0003
3.	<i>Inula helenium</i> L.	DK	G E2426-0004
4.	<i>Inula helvetica</i> Grauer	Z	CHOZ-20071220
5.	<i>Inula salicina</i> L.	DK	G E2426-0008C

**Table – 2**

Cypselar morphological features of different members of the tribe Inuleae.

Name of the Taxa	Type and Shape	Size excluding pappus (mm)	Colour	Shape at Base & Apex	Surface	Carpodium	Stylopodium	Pappus	General solid form
<i>Inula candida</i>	Homomorphic, narrow-oblong	3.0-4.0 x 0.1-0.2	Deep brown	Gradually tapered at base & constricted at apex	9-10 ribbed, pubescent	Symmetric, ring like, with 6-7 rows of cells. Diameter of carpodium wider than the base of the cypsel.	Well developed, tubular	Persistent, uniseriate, basally connate barbellate bristles. Each with 2, unequal pointed apical cells.	Faintly dorsiventrally compressed.
<i>Inula conyzae</i>	Homomorphic, narrow-oblong	2.5-3.6 x 0.2-0.5	Black	Slightly narrowed at base & truncate at apex	10-15 ribbed, pubescent with ocellate markings	Symmetric ring like, with 10-12 rows of cells. Diameter of carpodium same as the base of the cypsel.	Well developed, tubular	Persistent, uniseriate, basally connate scabrous bristles.	Cylindrical
<i>Inula helenium</i>	Homomorphic, narrow-oblong	5.0-5.5 x 0.8-1.0	Brown	Slightly narrowed at base & rounded at apex	Many ribbed, Glabrous with lineate markings	Symmetric, ring like, with 10-12 rows of cells. Diameter of carpodium same as the base of the cypsel.	Absent	Persistent, uniseriate, free, barbellate bristles.	Cylindrical
<i>Inula helvetica</i>	Homomorphic, narrow-oblong	1.5-1.8 x 0.1-0.2	Brown	Truncate at base & apex	6-ribbed, glabrous but apically	Symmetric, ring like, with 10-12 rows of cells. Diameter of carpodium same as	Well developed, tubular	Persistent, uniseriate, basally connate	Cylindrical

					pubescent	the base of the cypsela.		barbellate bristles. Each with 2, equal blunt apical cells.	
<i>Inula salicina</i>	Homo-morphic, narrow-oblong	1.8-2.0 x 0.1-0.2	Yellowish brown	Tapered and rounded at base & truncate at apex	Many-ribbed, glabrous	Symmetric, ring like, with 10-12 rows of cells. Diameter of carpodium same as the base of the cypsela.	Inconspicuous	Persistent, uniseriate, basally connate barbellate bristles. Each with 2, unequal pointed apical cells.	Cylindrical