

Morphological and Palynological Studies of Different Forms of *Datura metel* L. (Solanaceae)

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Abstract

Datura metel L. is one very interesting plant with medicinal properties. Several morphological variations are present in this species, which are generally referred as 'forms'. Previous authors studied different characters of these forms to establish relationship among them. Most of them had given emphasis only on external morphological characters. In the present study 27 morphological characters along with pollen morphology (light and SEM) have been made in four 'forms' of the species. To find out relationship among these four forms F-test and cluster analysis were performed. It showed that Form 2, 3 and 4 are considered here as closely related and Form 1 is very different. It is suggested to treat this form as a different variety under *Datura metel* L. or as a different species.

Key words: *Datura metel* L., Forms, SEM, RBD, F-DMRT, Cluster analysis, Duncan's test

INTRODUCTION

Datura L. (Solanaceae) is one of the most interesting genera of plants with high medicinal properties. The alkaloids contained in the plant have been in demand from the past and its role as a subject for botanical research is vast. Heiser (1969) has stated, "*Datura* is a genus of contrast – from smelly weeds to lovely ornamentals". Common names for *Datura* are numerous, common ones being raving night shade, thorn apple, sinkweed, devil's apple, Jimson weed and angel's trumpet (Heiser 1969; Avery 1959). Species of *Datura* are distributed throughout Asia, Europe and in America as either native or adventives and also in Africa and Australia. Among the 4 economically important *Datura* species, in India, *Datura metel* L. is most common in waste places, along roadsides throughout the warmer part. On the other hand, *Datura stramonium* is distributed from temperate Himalayan region in Kashmir to different tropical parts of India. Several morphological variations are present in *Datura metel*. Attempts have been made here to study different morphological features to get conclusive evidence whether these variations are strong enough to consider them as different species or varieties or forma. Stafford (1921) distinguished the varieties of *D. metel* on the basis of stem and flower color and the number of corolla tubes. Timmerman (1927 a, b) examined the leaves, stomatal frequency and seeds of different species of *Datura*. Bessis & Guyot (1976) attempted to use stomatal characters in systematics and phylogenetic studies of Solanaceae. Several taxonomic variants are present within the species differing in form and color of the corolla whorl ranging from single, double or triple form and white, purple or yellow (ornamental form) color. Hiraoka & Tabata (1983) reported that each of these variants should be considered as a variety or a form of *D. metel* based on the hybridization experiments of var. *metel* (single white corolla; *Me*), var. *rubra* (single purple, *Ru*), var. *fastuosa* (double or triple, purple; *Fa*) and forma "trumpet in trumpet". The color of the corolla is dominant in the following order: purple > white or yellow where as the order of dominance of corolla is: single > double > triple. Bhatt *et al.* (1984) dealt with morphometric analysis of four forms of *D. metel* considering all sorts of possible morphological characters. The results clearly indicate significant variations among the four forms deserving taxonomic circumscription. These earlier contributions stressed only on the morphological characters of the plant without giving any importance to the palynological characters. Study of pollen morphology is an important tool to find out relationship among different species, varieties or forms. In recent years the palynological criteria has been used to solve the taxonomic hierarchy of many angiosperm families, genera and species. Bernardello *et al.* (1997) studied pollen morphological variation within the tribe Lycieae (Solanaceae). Afterwards, Knapp *et al.* (1998) and Saleh Al-Quran (2004) palynologically characterized some solanaceous taxa. Keeping all these in mind present authors studied different forms of *Datura metel* to find out their relationship depending on 27 external morphological and pollen-morphological characters.

MATERIAL AND METHODS

Materials:

Fresh seeds of *Datura metel* L. were collected from medicinal plant units of Bidhan Chandra Krishi Viswavidyalaya, Dist. Nadia, West Bengal, India; medicinal plant garden, Ramkrishna Mission Ashram, Narendrapur, Dist. 24 Paraganas (S), West Bengal, India and Chandpara, Dist. 24 Paraganas (N), West Bengal, India.

Methods:

A. Study of external morphology:

Freshly collected seeds were air-dried and stored in airtight desiccators. Experiments were conducted in the medicinal plant garden of the Department of Botany, University of Kalyani, West Bengal, India. Seeds were soaked in water for 24 hours, washed thoroughly under flow of tap water and then spread over the soil on the trays so as to get light exposure evenly.

Plants were grown in the garden from the collected seeds using no plant protection measure and were studied for three consecutive years. Morphological data were collected from ten mature plants (6 months old) of each form of *Datura metel* each year.

B. Statistical analysis:

Statistical analysis was done with the help of SPSS software (version 7.0) under Window's 98 system. F-test and Duncan's (Duncan 1955) test were done to ascertain morphological characters in *D. metel*. Randomized block design layout was followed to compare the means of all morphological characters under study of four different forms of *D. metel* and statistical analysis for such design was followed. Here only F-statistics for treatment were included to adjudge the significance of treatment effect. Standard error of mean and critical difference (at 5% level of significance where necessary), values were also calculated. The analysis procedures were done for two years together over mean replications calculated under all forms and characters.

Duncan's test was also followed to compare the mean values for all characters found significant at 5% test of significance.

Hierarchical cluster analysis based upon distance matrix for morphological traits was followed on the basis of Euclidian distance matrix.

C. Study of pollen morphology:

Pollen viability test was done by acetocarmin method of Sharma & Sharma (1990). Four duplicates from each type were prepared and 6 slides from each were studied. Acetolysed (Erdtman 1956) pollen grains were studied under Leitg Laborlux-S microscope for pollen morphological characters. Four duplicates from each type were studied.

For scanning electron micrograph (SEM) pollen grains collected from fresh and healthy plants were washed in saline solution (0.9%). Then they were fixed in glutaraldehyde (3%) prepared in phosphate buffer (pH 7.0) for 24 hours and again fixed in Osmimum tetraoxide solution for 24 hours (pH 7.0). Then they were passed gradually through acetone at 50%, 70%, 90%, 100% concentrations respectively. After this they were fixed in a mixture of absolute acetone and isoamyl acetate 3:1, 2:2, 1:3 ratio respectively. In each case they were kept for 30 minutes. Then the material was passed through isoamyl acetate. After critical point, drying with liquid CO₂, pollens were mounted on aluminum stubs. Gold Sputtering was done and was made ready for study by Scanning Electron Microscopy (JEOL, JSM-5200). All photographs were taken with Nova NP22, 135-36 film.

RESULTS

Plants were grown for three consecutive years in the medicinal plant garden of Department of Botany, University of Kalyani and the qualitative characters were noted. It has been found that the selected qualitative characters (Table 1) are specific for each form and are very much stable. So,

Table 1: Different qualitative characters in *Datura metel* L.

Characters	Form 1	Form 2	Form 3	Form 4
Stem	Green	Green	Dark violet	Violet
Nodes	Purple	Green	Dark violet	Violet
Mature lamina	Green	Green	Upper surface green, lower surface faint violet spotted	Upper surface green, lower surface faint violet spotted
Calyx	Tubular with inflated base, green, tips some times violet blushing; collar like in fruit	Tubular gibbous at base, violet; collar like in fruit	Tubular with gibbous base, green; cupular in fruit	Tubular with gibbous base, violet; collar like in fruit
Corolla	Single whorl, yellowish- white, some times upper part purple blushing	Single whorl, white	Single whorl purple	2-3 whorled, purple
Fruit	Ovoid ellipsoid; prickles numerous erect strong, sharp green	Ovoid-orbicular; prickles many erect small, green	Ellipsoid orbicular; partially covered with scattered, blunt small violet prickles	Ovoid-ellipsoid, prickles many erect, long, violet

on the basis of qualitative characters plants are classified in four different forms. Table 1 represents the qualitative variations among these four forms.

Qualitative characters:

Study of qualitative characters revealed that every form has distinct qualitative and stable characters which can be used effectively for their identification. Forms 1, 2 and 3 have same floral formula and same floral diagram but Form 4 showed various types of floral formulas and floral diagrams. (Fig 3)

Floral Formulae:

Floral diagrams for Forms 1, 2 & 3 are similar but Form 4 shows much variations among its different populations.

Quantitative characters:

A. Results of F-test and Duncan's test for Pooled results of two year's mean of all morphological characters in *Datura metel* L.

F-test in Randomised Block Design (RBD) revealed that for all morphological characters there exists significant (5% level) difference among the four-pooled data due to four forms of *Datura metel* except adnate portion of stamen.

Then all four pooled data for each character was compared by Duncan's test at 5% level of significance and presented in the Table 2.

Cluster analysis showed that for morphological study three clusters were formed with cluster number (3, 4), 2 and 1. It is interesting to note here that cluster (3, 4) is closely related with cluster 2 but cluster 1 is distantly related with these clusters.

B. Pollen fertility:

Four forms showed following fertility sterility percentages:

C. Pollen morphology:

Pollen morphological investigation through light microscopy and SEM reveal the presence of following four different morphotypes as a whole within the four forms (1-4) of *Datura metel* L. :

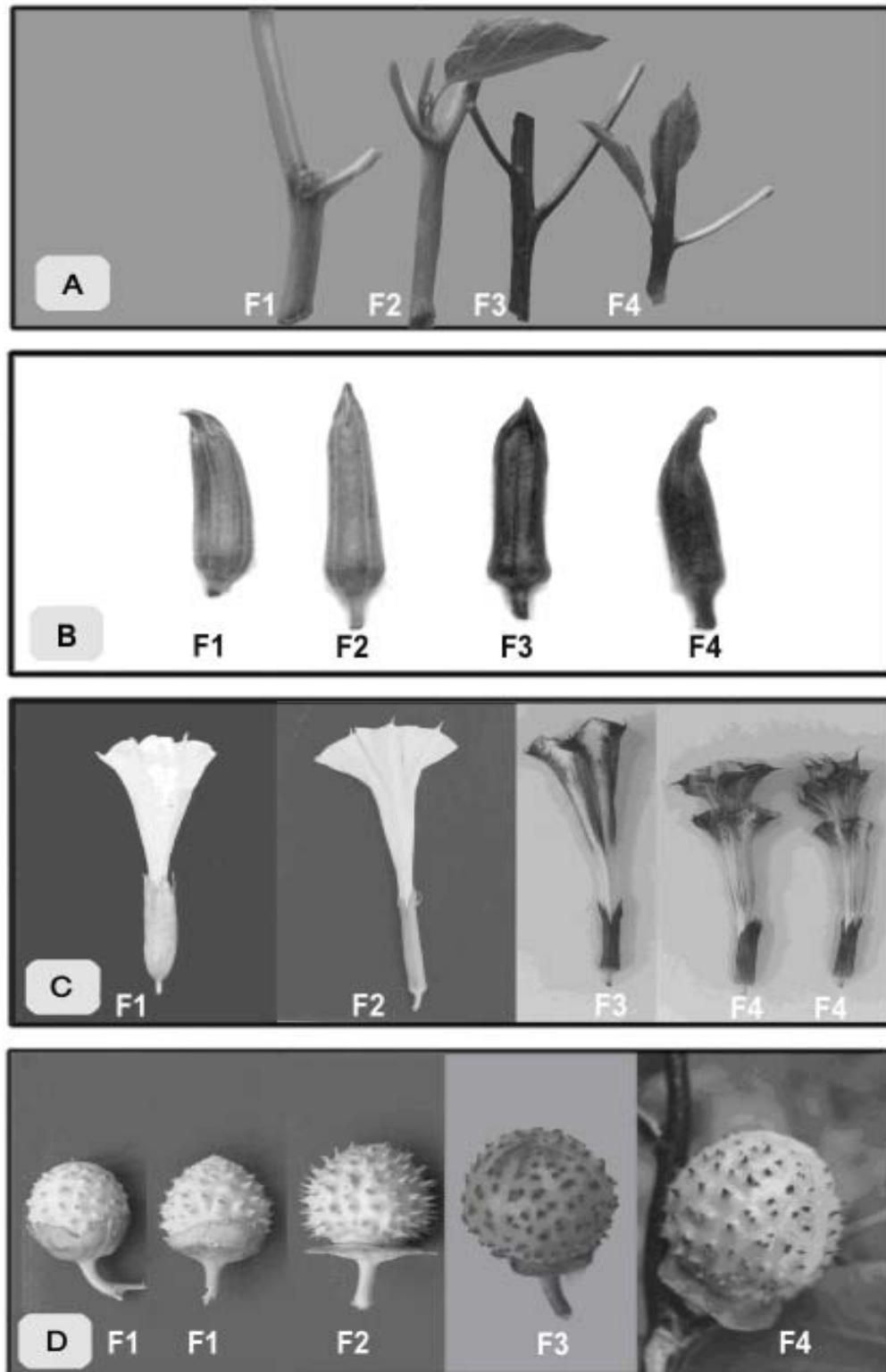


Fig. 1: Morphological variations in *Datura metel* L. A - Node, B - Bud, C - Flower, D - Fruit. F1 - Form 1, F2 - Form - 2, F3 - Form 3, F4 - Form 4.

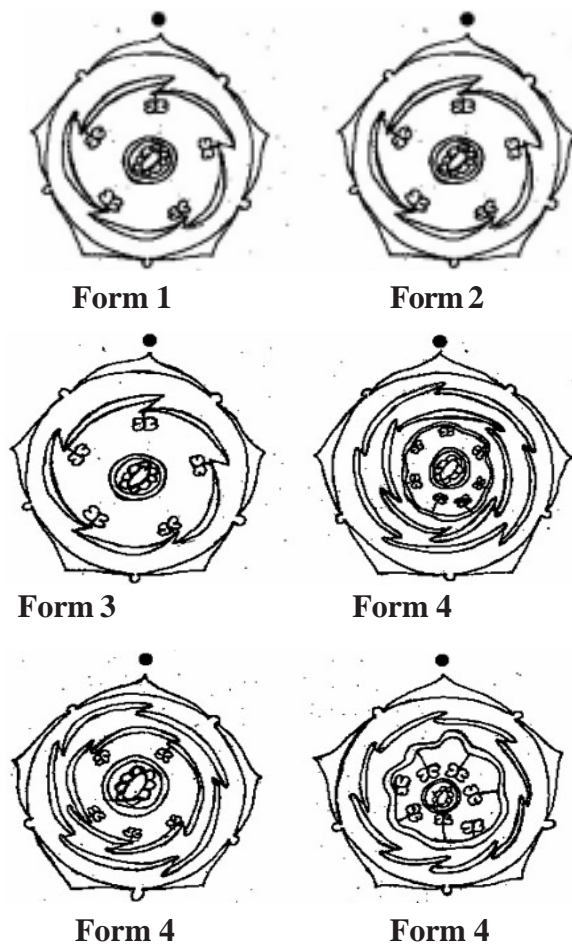


Fig. 2: Floral Diagrams for four different forms of *Datura metel* L.

medium in size, tenuimarginate, pores large, lalongate, margin thin. Exine thick, sexine much thicker than nexine, distinctly tectate-columellate. Columella thin, heads rounded, tectum thick, ruguli present on tectum, ruguli flattened, variously oriented, sometimes united to form pseudoreticulate sculpture.

Type 4

Pollen grains (Fig.6) circular to subcircular, $48-32 \mu\text{m} \times 38 \mu\text{m}$ in size, aperture indistinct, exine thick, distinctly tectate columellate, ornamentation verrucate, verrucae densely and closely set, rounded to polygonal to slightly elongated, some times verrucae joined together to form pseudoreticulate sculpture. Verrucae at polar area sparsely distributed. Aperture indistinct, possibly absent, sometimes a thin area is visible that presumably a germinal area.

These four types pollen grains are differentially distributed in four forms of *D. metel* as shown in Table 4. Pollen dimorphism has been observed in Form 1, 3, 4 while Form 2 represents only one type (unimorphic) of pollen grain.

Type 1

Pollen grains (Fig.5) circular to sub circular in polar view and sub triangular to sub-circular in equatorial view $50\mu\text{m}-50\mu\text{m} \times 65\mu\text{m}$ in size, tricolporate, brevicolpate, colpi margin thin, pores large, in mature pollen grains almost united to form a zonorate band. Exine thick, sexine much thicker than nexine, distinctly tectate columellate, surface ornamentation striato-reticulate, meshes polygonal and smaller at the polar regions, elongated and larger towards the periphery, striations discontinuous. Poles flattened to slightly rounded.

Type 2

This type of pollen grain (Fig.5) is almost similar in morphology to that of Type 1. However the pollen type 2 consists of elliptical to prolate-spheroidal pollen grains, poles tapering to rounded, tricolporate, colpi long, colpi margin thick, pores large, lalongate and in mature pollen grains form a zonorate band. Exine striato-reticulate except at polar area, striations much elongated and densely oriented than those found in Type 1. Polar area only with reticulate ornamentation, meshes uniform. In other parts of the pollen grain meshes heterobrochate.

Type 3

Pollen grains (Fig.6) circular to sub circular in polar view, spheroidal in equatorial view, poles flattened to slightly rounded, size $40 \mu\text{m} -72 \mu\text{m}$ in polar view, $42 \times 62 \mu\text{m}$ in equatorial view some times $51 \times 66 \mu\text{m}$, tricolporate, colpi

DISCUSSION

From the present study it is very clear that all qualitative morphological characters are very much stable and specific for each forms and it is very easy to isolate all four forms just on the basis of these characters (viz., Form 1, Form 2, Form 3 and Form 4). However, the variations in quantitative characters were also studied. Standard deviation (Table 2) presumed the mean and of 27 quantita-

tive morphological characters in four forms of *D. metel* and showed that the mean value of each quantitative character is specific to each form. F-test and Duncan’s test indicate that these features are true variations which are also reflected in their morphological characters. The statistical approaches applied on these features also indicate differences among these four forms as shown in the Table 2. Various sets of characters have been considered to study the similarities and differences among the four forms.

It is clear from Table 3 that Form 1 showed lowest pollen fertility (15.8%) and highest sterility (84.19%) followed by Form 4 (31.94% sterility and 68.06% fertility), Form 3 (10.06% sterility and 89.94% fertility) and Form 2 (7.0% sterility and 93.0% fertility) respectively. Such results clearly indicate that Form 2, 3 and 4 are closely related in pollen fertility (93% in Form 2, 89.94% in Form 3 and 68.06% in Form 4), where as in Form 1 fertility rate of pollen grains is very low *i.e.* 15.81%. Therefore this form may be placed in separate population.

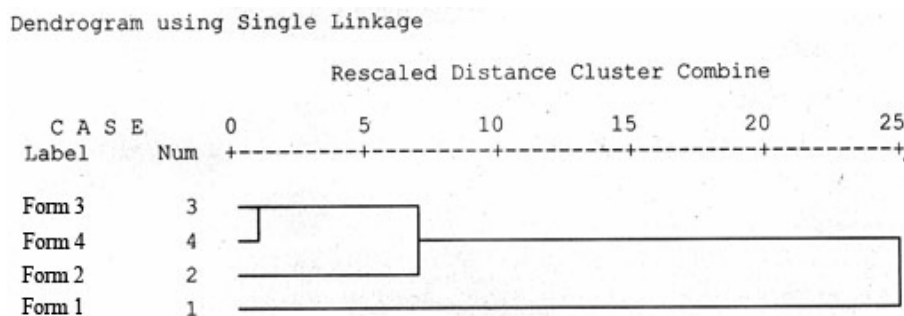


Fig. 4. Cluster analysis of four different forms of *Datura metel* L. depending on morphological characters.

Table 3: Pollen fertility and sterility percentage in different forms of *Datura metel* L.

Forms	% of fertility	% of sterility
1	15.81	84.19
2	93.0	7.0
3	89.94	10.06
4	68.06	31.94

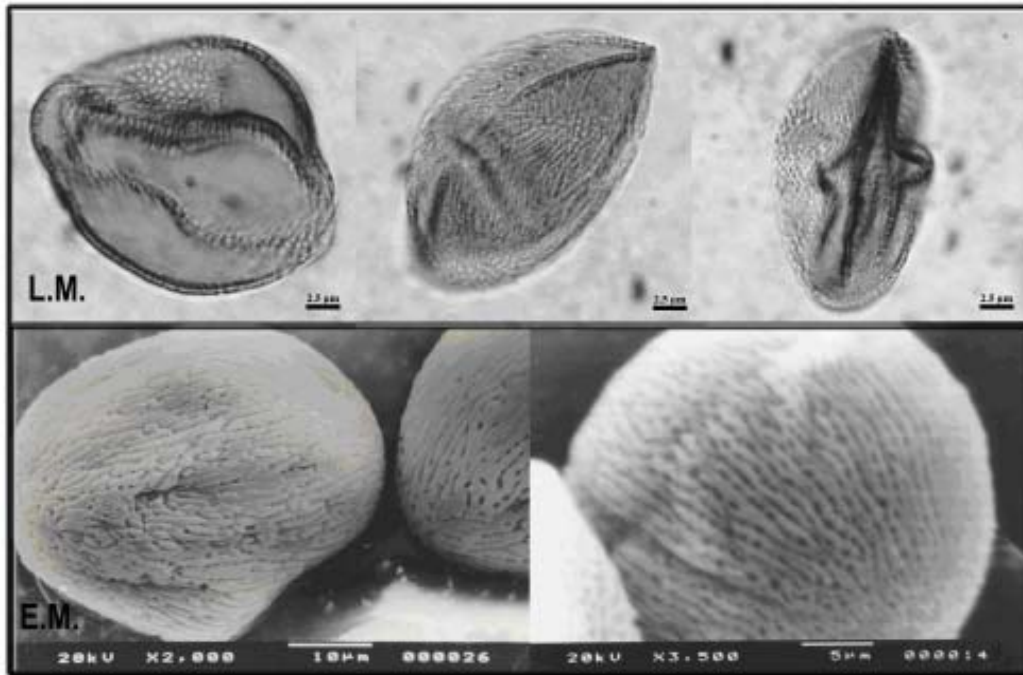
Table 4: Distribution of different pollen grain types in different forms of *D. metel* L.

Forms	Pollen Type 1	Pollen Type 2	Pollen Type 3	Pollen Type 4
1	75%	00	00	25%
2	00	100%	00	00
3	20%	80%	00	00
4	00	00	85%	15%

* 75% - 100% = major type, 1 - 25% = minor type of pollen grain.

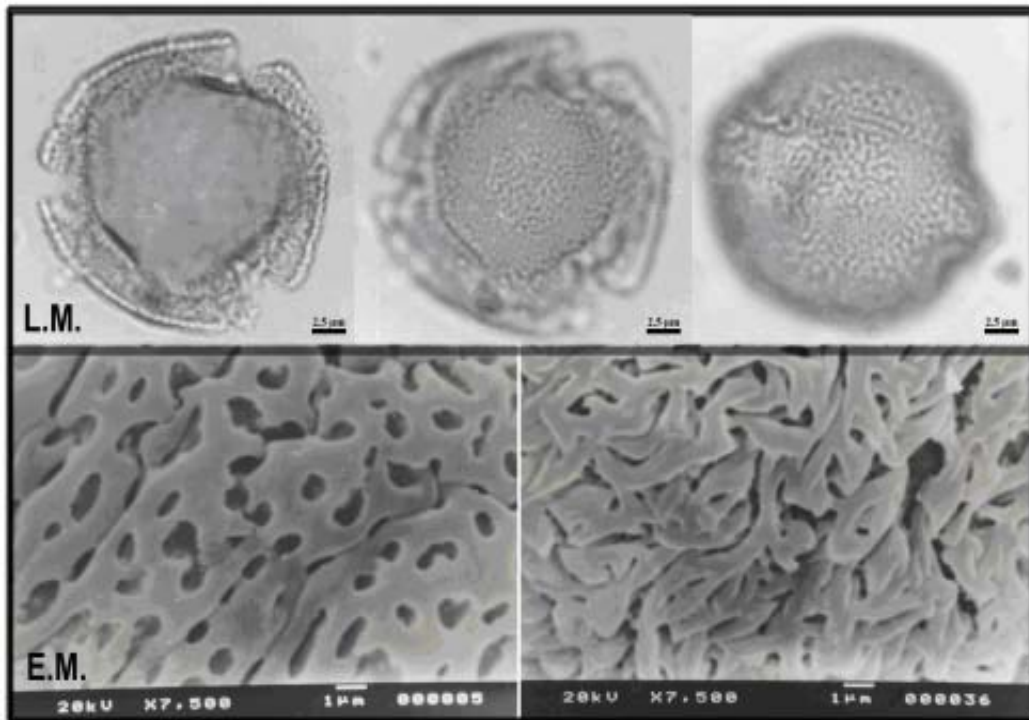


Pollen type 1

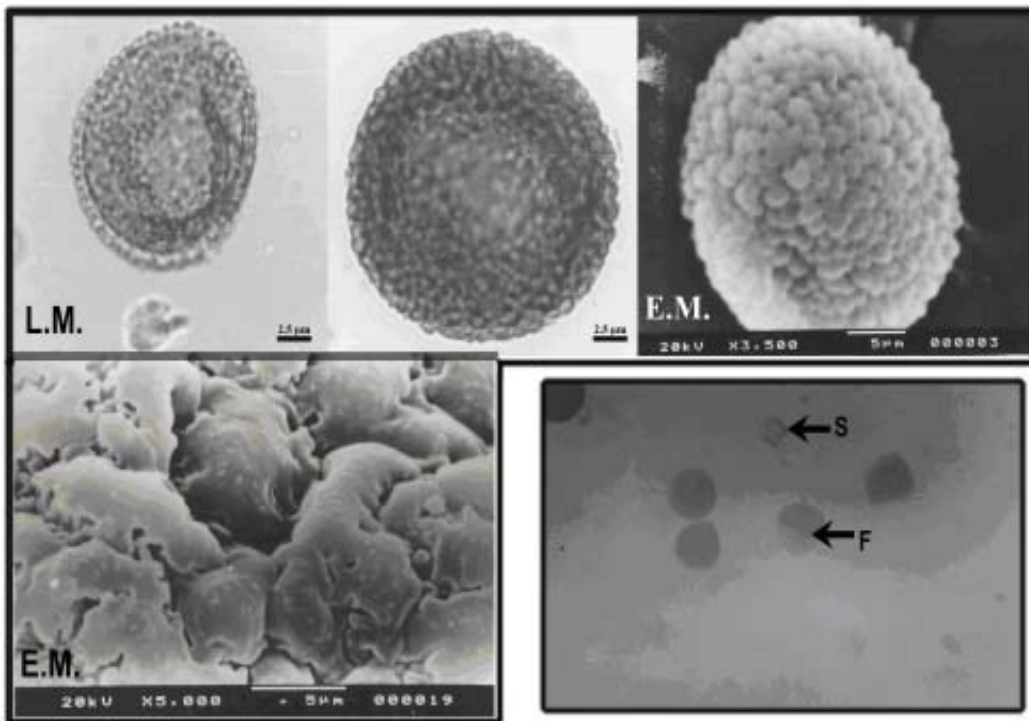


Pollen type 2

Fig. 5: Different types of pollen grains in *Datura metel* L.: L.M. - Light microscopic view; E.M. - Electromicroscopic view (arrow indicates zonorzta band).



Pollen type 3



Pollen type 4

Pollen viability

Fig. 6: Different types of pollen grains in *Datura metel* L.: L.M. - Light microscopic view; E.M. - Electromicroscopic view; S - Sterile pollen; F - Fertile pollen.

Morphological analysis of pollen grains of four forms revealed interesting findings. Among these four forms differences are very clear in light microscopic as well as in the electron microscopic studies. From the table 4 it is clear that four forms differ widely specially in their reference sexine sculpturing (Fig.2 and 3). Morphologically, four types of pollen grains have been recognized. Though Form 3 shows dimorphic palynological characters, it has close similarity with Form 2 regarding the percentage of major pollen type as indicated in Table 4. Major pollen type of Form 1 and Form 4 are different from that of Form 2 and Form 3. Besides, according to cluster analysis Form 3 and Form 4 are closely related, Form 2 shows some similarities where as Form 1 is very distantly related with the other forms. Additional supporting evidence comes from the leaf atropine content study and SDS-PAGE analysis of total leaf protein. Both of these study showed that Form 1 is very distantly related with the rest of the forms (manuscript under preparation).

Thus our investigation is in support of the earlier concepts (Bhatt *et al* 1984; Hiraoka & Tabata 1983) of classifying *Datura metel* L. in different forms. All the four forms studied here are different in the features exhibiting higher degree of diversity, which is reflected not only in their morphological characters but also in morphology of pollen grains. The palynological characters in support to the morphological variations to delineate the variations among the four Forms of *Datura metel* is considered here for the first time. F-test has been applied to see the relationship among the four forms with respect to each of the 27 morphological characters. Thus, on the basis of the information availed from the forgoing observations on pollen analysis, it is now clear that each Form has its own morphological status. However, Form 2, 3 and 4 show only little morphological variations among themselves. On the other hand Form 1 is very different from these three types by its morphological, palynological and biochemical aspects. Thus the character variations of Form 1 may be used to treat it as a variety under *Datura metel*, if not a different species. However, the close relationship of the four Forms suggests that they might have emerged from the same genetical stock sometimes in the remote past and diversified afterward. However further investigation is needed on DNA based work like RAPD, AFLP before delivering any conclusive remark on the species status of Form 1.

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