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Comparative morphology of pollen grains of some legumes from Punjab plains, NW India

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ABSTRACT

Pollen morphological studies have been carried out in 10 leguminous species (family papilionaceae) from the different habitats in the Patiala district of Punjab, NW India. Pollen grains are either 3-zonoporate, 3-zonocolpate or 3-zonocolporate with oblate spheroidal, suboblate or prolate spheroidal shape and psilate, faintly reticulate or reticulate exine. Key to identification of presently studied taxa has been evolved based on various palynological parameters like the range in size, shape, aperture, exine thickness, ornamentation, etc. This communication may help academicians, agronomists, environmentalists, biological scientists and researchers to identify and classify the commonly growing weeds.

KEY WORDS: LM, legume, pollen morphology, papilionaceae.

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INTRODUCTION

Papilionaceae the third largest family of the flowering plants (Mabberley 1997)[1] is a source of dyes, fibres, fuels, medicines, oils, pulses and timbers (Wojciechowski, 2003)[2]. Species of the family exhibit the formation of root-nodules with symbiotic bacteria to fix atmospheric nitrogen and thereby improving the soil fertility (Sprent and McKey 1994, Sprent 2001)[3,4]. Apart from economic importance, ecologically the family is important as the plant species of this family grow in a wide range of habitats from the tropical rain forests to deserts and alpine tundra. Most of the species of the family grow in wild as well as weeds in the vegetable and crop fields, pastures, orchids, lawns, etc.

Palynology, the science of pollen, spores, algal cysts and other microscopic plant bodies is a multi-disciplinary field with applications in the agriculture, environmental sciences, botany, zoology, entomology, immunology, forensic science, geology, archaeology and geography. The study of pollen is an important area of research as the pollen morphological characters such as the shape, apertural pattern and exine configuration are very conservative features for the taxonomic assessment of the plants (Perveen 2006, Bera *et al*. 2007, Keshavarzi *et al* 2012)[5,6,7].

This paper aims to analyse the pollen grains of some legumes using LM techniques. Various parameters as the shape, range in size, ornamentations, apertures, etc. have been of considerable helpful in identification of the taxon. Based on these features a key to identification of presently studied weed species has been evolved. The present study was undertaken to analyse palynology of the weed flora from the Punjab which is an agricultural state. The data generated will be helpful to agriculture scientists, botanists, taxonomists, etc. in identification of these species alongwith other morphological characters.

MATERIALS AND METHODS

Sample collection

For the present investigation plants at flowering stage were collected from various localities representing different habitats like boundries of fields, canals banks, crop fields, lawns, orchids and waste places in Patiala district of Punjab, NW India (Table 1).

Procedure

Pollen morphology was studied by acetolysis method suggested by Erdtman (1952)[8]. Anthers of fresh or dried material were crushed in 70% alcohol and divided into two parts in separate tubes, A and B in the ratio 1:2. Centrifuged the contents of tube A, added 5 ml of glacial acetic acid and 2-3 drops of 1% safranin to it. After keeping it for 15 minutes in the hot water bath the content was

washed with water, centrifuging at each step. Pollen grains obtained were mounted in glycerine and were stained red.

The part B was centrifuged and added 5 ml of acetolysis mixture. It was placed in the hot water bath for 3-5 minutes, then centrifuged and washed with water, 10ml of glacial acetic acid was added and dispersion was divided into two parts, C and D. Pollen grains from part C were mounted in glycerine and were brown coloured.

Contents of part D were subjected to chlorination by adding 2-3 drops of 1N HCl and 1-2 drops of saturated sodium chlorate solution and keeping it for half an hour. After washing with water few drops of methyl green were added and kept it for 2-3 minutes. Pollen grains were mounted in glycerine and were stained bluish-green.

Range in pollen size was determined by making observation on unacetolysed pollengrains. Pollen grain shape was determined with the formula $\frac{P}{E} \times 100$ where P stands for polar diameter and E for equatorial diameter.

Table 1: Investigated legumes with their common name, habitat, flowering period and accession number (PUN)

Name of the weed	Common name	Habitat	Flowering period	Accession number
Alysicarpus bupleurifolius DC.	Sweet alyce clover	Moist grassy areas, paddy fields, canal banks	August - October	33759, 33760
Alysicarpus vaginalis DC.	Alyce clover, Chauli	Paddy fields, lawns, waste places	August - October	33761, 33762
Desmodium triflorum (Linn.) DC.	Three flower beggar weed	Lawns, pastures	March - November	33765, 33766
Indigofera linifolia Retz.	Narrow leaf indigo, Torki	Boundaries of fields, lawns	April - October	32062
Lathyrus sativus Linn.	Grass pea, Latri	Cultivated fields	June - March	32028, 32029
Medicago denticulata Willd.	Bur clover	Cultivated fields, lawns	January - March	32050, 32051
Medicago lupulina Linn.	Black medic, Hop clover	Lawns	March -April	33769, 33770
Melilotus indica All.	indian sweet clover, Sengi methi	Cultivated fields, orchids	December - June	32026, 32027
Sesbania bispinosa (Jaq.) Fawcett. & Rendle.	Prickly sesban, Dhaincha	Maize, paddy, fodder crops	August - October	33773, 33774
Vicia sativa Linn.	Horse bean, Bakla	Sarson, gram, wheat, vegetable fields	January - April	32048, 32049

For Pollen terminology Erdtman (1952), Erdtman *et al*. (1961)[9], Nair (1965, 1966)[10,11], Reitsma (1970)[12] has been followed. Various parameters taken are the exine thickness, exine ornamentation, shape, aperture, colpi length and breadth, pore diameter, range in pollen size, etc. Voucher specimens have been deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

RESULTS AND DISCUSSION

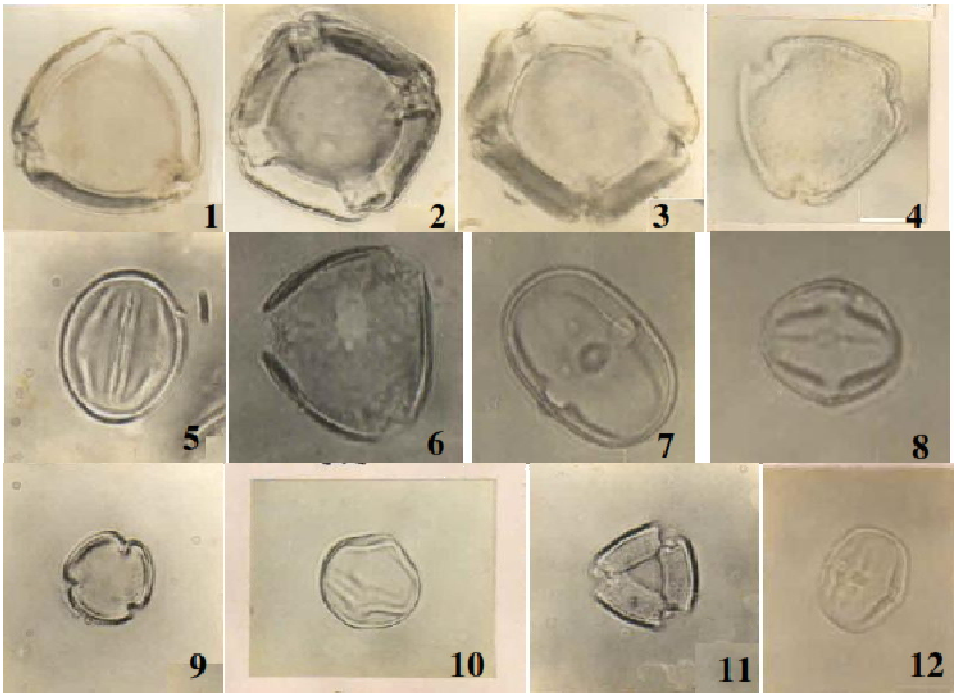
Data on pollen size, shape, aperture, exine and other pollen characters pertaining to 10 species of legumes has been provided in Table 2.

Table 2: Pollen morphological details of the investigated legumes

Name of species	Average size (µm)	Range in size (µm)	Shape	Aperture	Exine ornamentation	Exine thickness (µm)/ Figure
Alysicarpus bupleurifolius DC.	52.06 × 48.19	20.22-87.62 × 20.22-84.25	Prolate spheroidal	3-4 Zonoporate	Psilate	8.66/ 1-3
Alysicarpus vaginalis DC.	46.25 × 44.90	40.44-50.55 × 40.44-48.86	Oblate spheroidal	3-Zonocolporate	Faintly reticulate	2.86/ 4
Desmodium triflorum (Linn.) DC.	30.50 × 30.07	26.96-33.70 × 26.96-33.70	Prolate spheroidal	3-Zonocolpate	Reticulate	2.27/ 5
Indigofera linifolia Retz..	40.23× 37.38	35.00-45.00 × 30.00-40.00	Oblate spheroidal	3-Zonocolpate	Reticulate	4.04/ 6
Lathyrus sativus Linn.	31.66× 20.00	30.00-40.00 × 20.00	Sub oblate	3-Zonocolporate	Psilate	2.02/ 7
Medicago denticulata Willd.	25.00 × 23.12	20.00-30.00 × 20.00-30.00	Oblate spheroidal	3-Zonocolporate	Psilate	4.04/ 8
Medicago lupulina Linn.	22.32 × 20.30	20.22-23.59 × 16.85-23.59	Oblate spheroidal	3-Zonocolporate	Psilate	1.68/ 9
Melilotus indica All.	20.00 × 20.00	20.00 × 20.00	Oblate spheroidal	3-Zonocolporate	Reticulate	3.03/ 10
Sesbania bispinosa (Jaq.) Fawcett. & Rendle.	32.17 × 28.69	26.96-50.55× 20.22-50.55	Oblate spheroidal	3-Zonocolpate (Parasyncolpate)	Psilate	1.68/ 11
Vicia sativa Linn.	30.00 × 25.17	30.00-35.00 × 20.00-30.00	Sub oblate	3-Zonocolporate	Psilate	2.02/ 12

Presently studied species are characterized by the presence of 3- zonoporate, 3-zonocolpate and 3-zonocolporate pollen grains. Pollen grains are 3-5 zonoporate in 1 species : *Alysicarpus bupleurifolius*, 3-zonocolporate in 6 species : *Alysicarpus vaginalis, Lathyrus sativus, Medicago denticulate, Medicago lupulina, Melilotus indica* and *Vicia sativa* while 3 species : *Desmodium triflorum, Indigofera linifolia* and *Sesbania bispinosa* have 3-zonocolpate pollen grains. Average pollen size ranges from 20 × 20 µm in *M. indica* to 52 × 48 µm in *A. bupleurifolius*. Exine thickness

varies from 1.68 µm in *M. lupulina* and *S. bispinosa* to 8.66 µm in *A. bupleurifolius*. Exine is psilate in 6 species namely, *A. bupleurifolius, L. sativus, M. denticulate, M. lupulina, S. bispinosa* and *Vicia sativa*; reticulate in 3 species like *D. triflorum, I. linifolia* and *M. indica* and faintly reticulate in only 1 species i.e. *A. vaginalis.*



Figs.1-12. Photomicrographs of acetolysed pollen grains, 1-3. *Alysicarpus bupleurifolius,* 4. *A. vaginalis,* 5. *Desmodium triflorum,* 6. *Indigofera linifolia,* 7. *Lathyrus sativus,* 8. *Medicago denticulata,* 9. *Medicago lupulina , 10. Melilotus indica, 11. Sesbania bispinosa,* 12. *Vicia sativa.*

A. bupleurifolius, and *S. bispinosa* have psilate exine which is in conformity with previous reports of Kumari and Bir (1985)[13] whereas in *A. vaginalis* exine was reported psilate (Kumari and Bir 1985) but in present studies it is observed to be faintly reticulate. Pollen grain characters of *S. bispinosa* are in comformity with previous reports of Tewari and Nair (1979)[14] except exine ornamentation which was reported as faintly foveolate but in the present studies it is observed as psilate.

In *I. linifolia* pollen grains are oblate spheroidal in shape but Jain and Nanda (1966-67)[15] reported pollen grains for same species studied from Pilani to be prolate spheroidal.

For *M. indica* Jain and Nanda (1966-67) reported pollen grains as 3-colpate with prolate spheroidal shape but in present studies these are found to be 3-zonocolporate with oblate spheroidal shape.

Key to identification of investigated species

1a. Pollen grains 3-4 zonoporate……...*Alysicarpus bupleurifolius*

1b. Pollen grains 3-zonocolporate

2a. Exine reticulate

3a. Average pollen size 40.25 × 44.90 µm……*Alysicarpus vaginalis*

3b. Average pollen size 20.00 × 20.00 µm ……*Melilotus indica*

2b. Exine psilate

4a. Pollen sub oblate

5a. Pollen size ranges from 30-40 × 20 µm……*Lathyrus sativus*

5b. Pollen size ranges from 30-35 × 20-30 µm……*Vicia sativa*

4b. Pollen oblate sheroidal

6a. Exine 1.68 µm…… *Medicago lupulina*

6b. Exine 4.04 µm……..…….. *Medicago denticulata*

1c. Pollen grains 3-zonocolpate

7a. Exine psilate…….....……*Sesbania bispinosa*

7b. Exine reticulate

8a. Pollen prolate sheroidal…….....……..*Desmodium triflorum*

8b. Pollen oblate sheroidal…….....……..*Indigofera linifolia*

The presently investigated legume species show remarkable differences in the palynological characters which can be used to identify them alongwith other morphological features.

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