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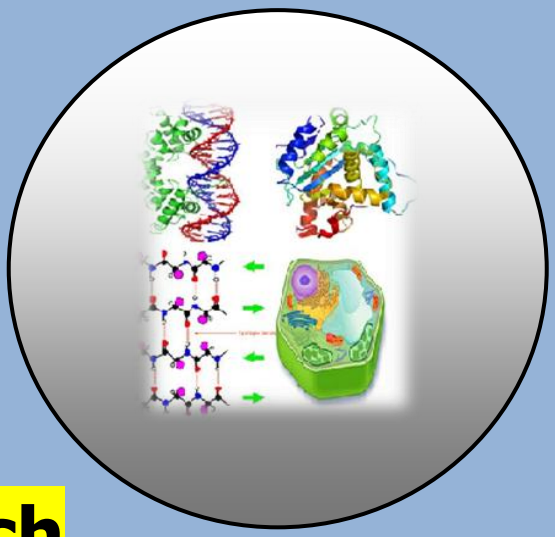
ISSN 0970-4973 Print
ISSN 2319-3077 Online/Electronic

Global Impact factor of Journal: 0.756
Scientific Journals Impact Factor: 3.285
Index Copernicus International Value
IC Value of Journal 6.01 Poland, Europe

J. Biol. Chem. Research
Volume 32 (2) 2015 Pages No. 449-454



Journal of Biological and Chemical Research

An International Peer reviewed Journal of Life Sciences and Chemistry

Indexed Abstracted and Cited in about 25 different Scientific Databases around the World

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 32, No. 2: 449-454, 2015
(An International Peer reviewed Journal of Life Sciences and Chemistry)
Ms 32/2/01/2015

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

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RESEARCH PAPER
Received: 30/03/2015 Revised: 24/04/2015 Accepted: 10/05/2015

Marker Gene (s) for Pollen Morphology in Autotetraploid Fenugreek (*Trigonella foenum-graecum L*)

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ABSTRACT

Although tetraploids in general can be distinguished from their diploid progenators by various morphological traits. However, chromosomal status finally stabilized the level of ploidy. But colchitetraploids of four varieties (selections) of Trigonella showed various types of pollen morphology, studied from newly induced tetraploid to meiotically established highly advanced (C_{14}) generation. This is a stable heritable trait. Their pollen morphology cannot be categorized into definite shape classes because every form has its intermediate type. It is concluded that application of colchicine changed the expression of gene (S). It may be due to interaction among more genes responsible for the same trait in autotetraploids. This ultimately caused disturbed pollen polarity which is responsible of various pollen morphology. Changed morphology of pollens can be used as marker trait for polyploidy.
Key words: Trigonella foenum-graecum L, Marker Gene, Autotetraploid, Pollen Morphology and Genome.

INTRODUCTION

Trigonella foenum-graecum, commonly known as fenugreek (methi) is extensively cultivated in India for use as nutritious green vegetable, food flavourant, fodder and green manure. Seeds are an important source of an alkaloid known as diosgenin used as a starting chemical for the manufacture of cortisone and different sex hormones.

During *Trigonella* breeding programme for economic excellence, germ plasm was collected from different locations in India and 4 varieties (selections) were selected on the basis of

different morphological traits. Colchitetraploids were raised and judicious selection was carried out over succeeding generations. It is observed that inspite of luxuriant vegetative growth and almost regular meiotic behavior; fertility did not improve beyond a certain limit. Therefore, detailed pollen study was carried out to find out the cause of poor pollen fertility at tetraploid (4x) level. Studies were carried out under light as well as scanning electron microscope (SEM) following standard procedure.

MATERIAL AND METHODS

For morphological studies both unacetolysed and acetolysed pollen grains were studied in all the four varieties (selections 1, 2, 3 & 4) at both the polidy levels. For morphological studies pollens were primarily prepared by the acetolysis method as described by Erdtman (1952). Unacetolysed grains were studied by the method of Woodhouse (1935). For scanning electron microscopy, unacetolysed pollens were dried in 100% ehanol, sputter coated with gold and studied under Jeol-JSM-35C Scanning electron microscope at 10KV. Pollen fertility was studied after staining with 2% acetocarmine.

RESULTS

Pollens of diploid (2x) Plants

In all the 4 selections pollen grains are radiosymmetrical, bipolar, 3 colporate and prolate. P/E ratio of acetolysed grains ranged from 24.19 to 24.26 mμ. Colpi thin, long ranging from 176 mμ. to 320 mμ. in length, ends acute, ora circular, lolongate or lalongate. P/E ratio of ora ranged from .75 to 1.67 mμ. Exine is 16 mμ in thickness, Sexine thicker than nexine. Sexine pattern reticulate and tegillate (Plate I).

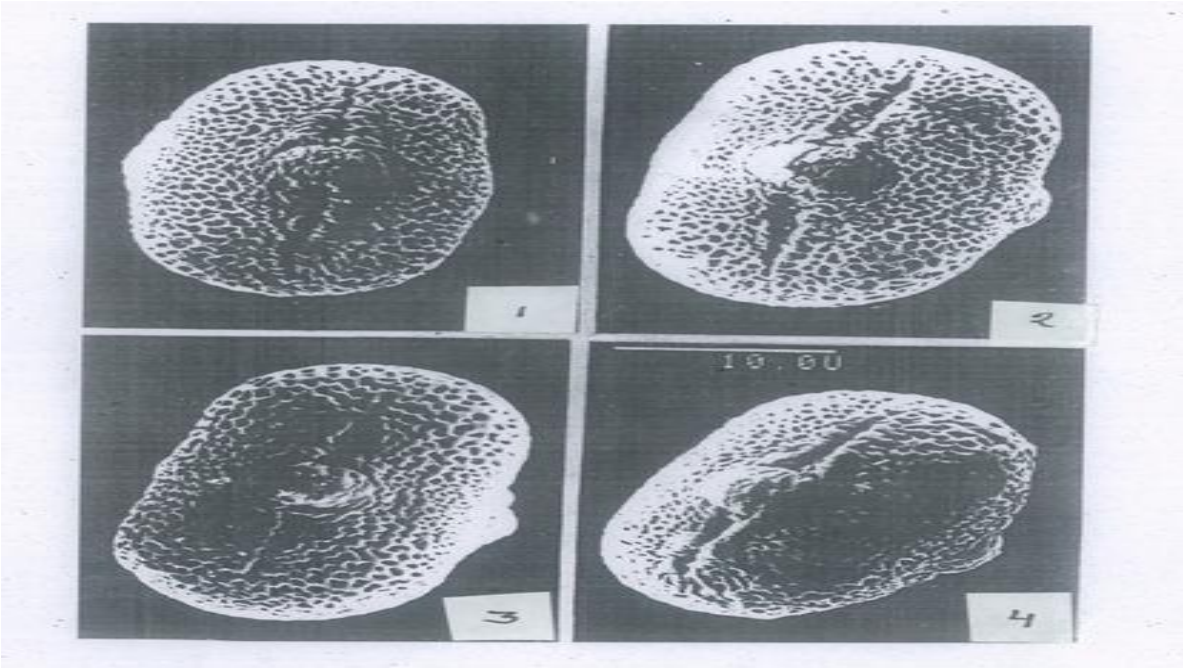

PLATE I. Pollen grains of different sets (1, 2, 3 & 4) of 2x *Trigonella* as seen by SEM (X3000).

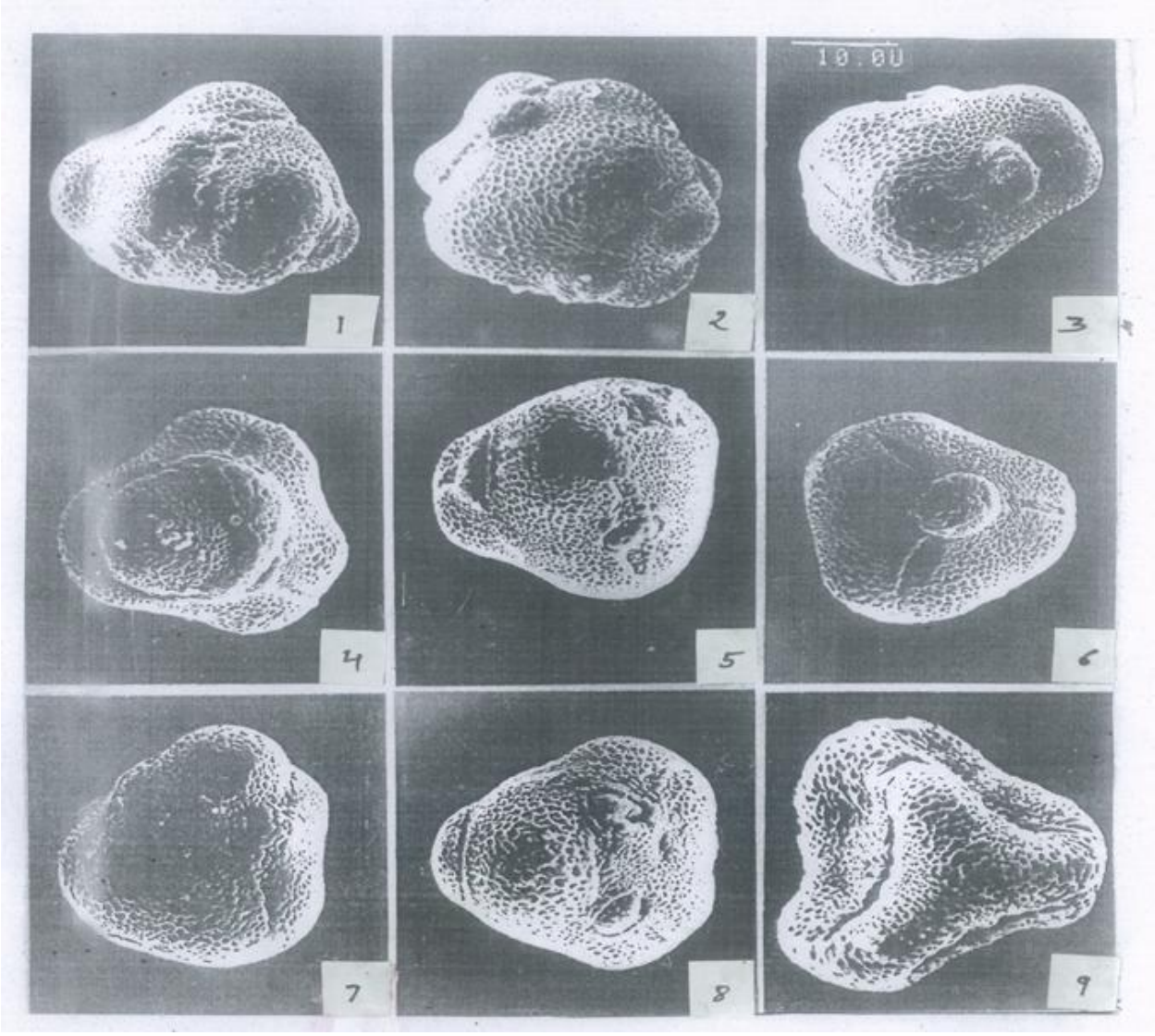

PLATE II. Pollens of 4x *Trigonella* as seen by SEM (X2000).

Pollens of tetraploid (4x) Plants

In all the four 4x selections both normal and abnormal pollen grains of various shapes are seen. The normal grains were observed at 4x level are similar to diploids in morphology but slightly bigger in size. The morphology of pollens is described under following sub-headings:

Shape and Size

The abnormal grains are asymmetrical, non-fixiform and of various sizes and shapes. They are oval, elliptical, oblong, rounded, trilobbed, tetra-, penta- or multilobbed having arms in various planes. The poles of arms are mostly rounded but in a few tapering also. P/E ratio of 4x grains ranged from 12.8 mμ to 26.6 mμ in different varieties. It is interesting to note that pollen shapes of 4x cannot be categorized into definite pattern because every form has its intermediate type (Plate II).

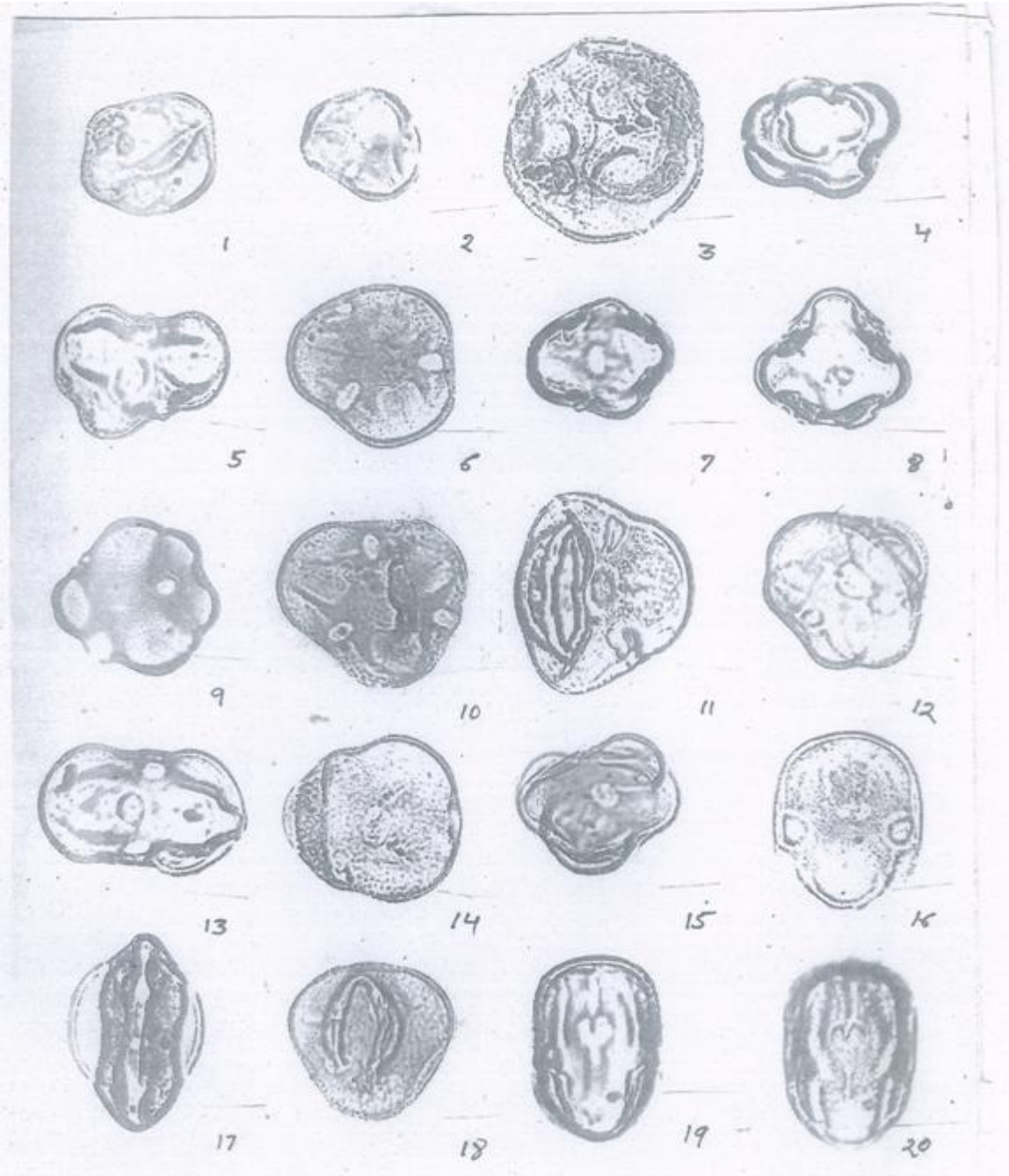

PLATE III. Pollens of 4x and 2x *Trigonella* as seen by light microscope (oil immersion).

a. **1 – 18 – abnormal pollens of 4x.**
b. **19 – normal pollen of 2x**
c. **20 – Normal pollen of 4x.**

Colpi and apertures (ora)

Colpi 3-6 in number. In some grains the colpi ends get united in various ways. In others they altogether get united forming syncolpus. Sometimes Colpi with apertures was observed. In 4x grains syncolpus with one or many ora was also observed. Colpi length with free ends ranged from 160-416 mμ with tapering or actute ends. Shape of endocolpi (apertures or ora) was either circular or lalongate or lolongate having P/E ratio 9.6 to 10 mμ (Plate III).

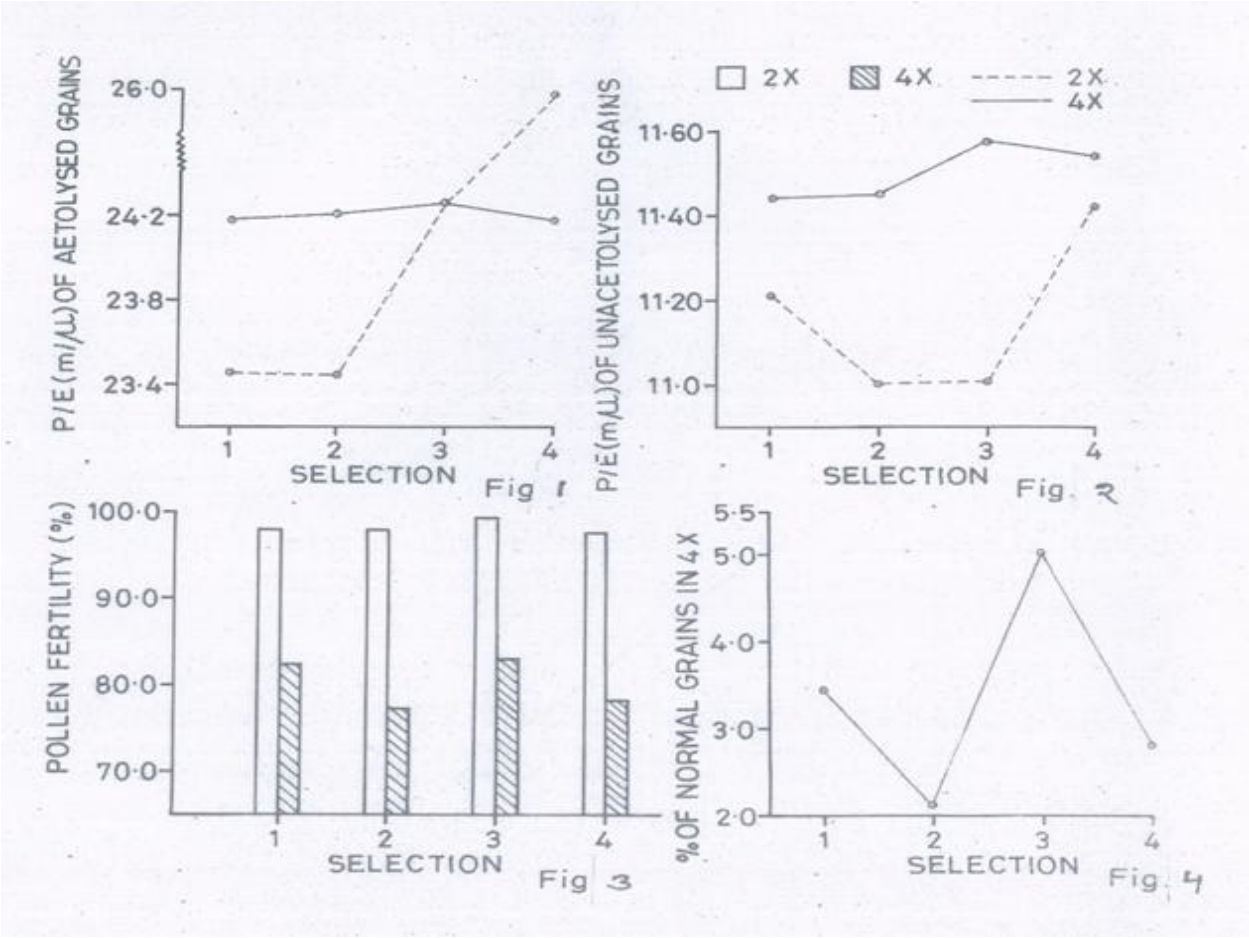

PLATE IV.

(1) P/E ratio (mμ) of acetolysed pollens in different selections of 2x and 4x *Trigonella.*
(2) P/E ratio (mμ) of unacetolysed pollens of 2x and 4x
(3) Pollen fertility (%) of 2x and 4x.
(4) % of normal pollens among abnormal pollens of 4x.

Exine

The exine pattern, as seen by SEM is similar to diploids. Exine thickness is also 16 mμ. It is tegillate and reticulates but the size of lumina is slightly bigger in 4x grains. Lumina are triangular in shape being slightly smaller towards colpi margins.

Pollen fertility and % of normal grains in 4x

Pollen fertility in different selections of diploid plants ranged from 98.1% to 99.4%. In tetraploids it ranged from 77.59 to 82.98% in different selections. Percentage of normal grains among abnormal grains in tetraploid selections ranged from 2% to 5.2% (Plate IV).

DISCUSSION

It is observed by several workers that physio-chemical treatment is responsible for pollen shape variability. Colchicine not only induces polyploidy but causes pollen shape variability in umbelliferous plants (Raghuvanshi *et.al,.* 1965, 67). Pollen variability can also be induced by physical mutagen like gamme-rays (Raghuvanshi, *et.al.*, 1967). But in both the cases there are two main differences from the case of tetraploid *trigonella.*

(a) While in umbelliferous autotetraploids the abnormal grains have some definite shapes and can be classified into various shape classes. But in *Trigonella* they cannot be categorized into shape – classes because every shape types had its intermediate form.

(b) In umbelliferous plants the frequency of abnormal pollens were reduced in subsequent generations but in the case of tetraploid *Trigonella*, frequency is stabilized even in highly advanced C_{14} generation.

Thus, it is clear from the present study that pollen variability is associated to the ploidy level. Previously it was thought that it is the effect of colchicine treatment which changed the expression of gene (S) controlling the polarity of pollens. But shape variability is also observed in naturally occurring 4x plants found among cultivated diploid crops.

Hence it has been concluded that pollen shape variability in tetraploid plants is due to the pollen polarity disturbances. It may be due to interaction among genes (S) due to duplication of genome. Pollen variability is thus a Genetic marker trait (S).

ACKNOWLEDGEMENTS

The author is grateful to Prof. S.S. Raghuvanshi, Plant Genetics Unit, Botany Department, Lucknow University, Lucknow, India for his guidance.

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