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# PALYNOLOGICAL STUDIES ON DIPLOID AND TETRAPLOID CLONES OF *HEVEA BRASILIENSIS* (WILLD. EX ADR. DE JUSS.) MUELL. ARG.[1]

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## Abstract

Palynological studies on diploid and induced tetraploid clone of *Hevea brasiliensis* (Willd. ex Adr. de Juss.) using light and scanning electron microscope brought out distinct variations in morphology and stainability. Pollen grains of diploid are 3-zonocolporate and stainability is 92.8%. In the tetraploid, pollen grains are tricolporate and tetracolporate and pollen stainability is 80%. In the diploid and tetraploid the pollen size is 35 x 29 $\mu$m and 48 x 36 $\mu$m, respectively. Exine thickness, ornamentations and ora diameter are also comparatively more for the tetraploid. The variation in genomic change has also manifested in the pollen characteristics.

**Key Words :** *Hevea*, diploid, tetraploid, pollen grains, exine, ornamentation.

*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. is a tree belonging to the family Euphorbiaceae and also the source of 99% of natural rubber produced in the world. It is monoecious; male flowers are numerous in comparison to the female flowers restricted to the tip of the main stalk and major branches of the panicle. Palynological studies are comparatively meagre in *Hevea* (Markose & Nair 1970, Saraswathy Amma et al. 1989). Pollen morphology of diploid and tetraploid clones of *Hevea brasiliensis* using light and scanning electron microscope is reported for the first time in this communication.

## Material and Methods

Mature male flowers of a diploid clone RRII 105 (2n = 2x = 36), and an induced tetraploid of RRII 105 (2n = 4x = 72) (Saraswathy Amma 1990) were collected just prior to anthesis and preserved in 70% alcohol. Pollen sterility was assessed by stainability test using 1 : 1 acetocarmine glycerol mixture. A total of 500 pollen grains were scored selecting 50 pollen each from 10 slides of different male flowers which were collected randomly.

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Acetolysis was done by the standard procedure (Erdtman 1952) and the terminology used was after Nair (1970). Pollen measurements like equatorial diameter, polar diameter, exine thickness and ora diameter were taken at a magnification of 400 x and by means of an ocular micrometer using light microscope and a total of fifty pollen grains-five each from ten slides were selected for the purpose.

SEM studies were carried out at the National Botanical Research Institute, Lucknow, for which the acetolysed pollen grains were placed on adhesive tape attached to an aluminium stub. The samples were coated with gold (200 A') JEOL ION sputter using a coater (JFC 1100) and observed with a Jeol JSM 35c scanning electron microscope. Photographs were taken at 1500 to 2000 and 6000 x. Five samples were observed from each cytotype.

## Observations

Pollen grains of *Hevea* are powdery yellow and sticky. There are noticeable differences between the diploid and tetraploid with regard to both pollen morphology and pollen stainability. In the diploid clone RRII 105, pollen grains are 3-zonocolporate and pollen stainability is 92.8% (range 90 to 95%). In the tetraploid there are tricolporate or tetracolporate pollen grains and their stainability is 80% (range 77 to 82%). Among the stainable pollen, 30% (range 28 to 33%) are tricolporate and 50% (range 47 to 52 %) are tetracolporate. The pollen characters of the diploid and tetraploids are given in Table 1.

TABLE 1 — MORPHOLOGICAL CHARACTERS OF STAINABLE POLLEN FROM DIPLOID, TETRAPLOID CLONES OF *H. BRASILIENSIS*

| PARAMETERS | DIPLOID MEAN ± SE | TETRAPLOID MEAN ± SE |
|---|---|---|
| Polar diameter (μm) | 34.98 ± 0.48 | 49.20 ± 3.26 |
| Range | (29.70 to 36.30) | (45.00 to 62.50) |
| Equatorial diameter (μm) | 28.61 ± 0.49 | 42.48 ± 2.37 |
| Range | (24.75 to 31.55) | (30.00 to 55.00) |
| Exine thickness (μm) | 3.00 ± 0.24 | 5.54 ± 0.41 |
| Ora diameter (μm) | 4.00 ± 0.31 | 5.82 ± 0.27 |

Compared to those of RRII 105, the pollen grains of the tetraploid are bigger. In diploid (RRII 105) the size of pollen grain is 35 x 29 μm, while in the tetraploid the size is 48 x 36 μm. The exine thickness and ora diameter are also more in pollen from the tetraploid compared to that of the diploid.

Scanning electron micrographs of the diploid and tetraploid are given in Fig. 1A-F. The polar view of pollen grains of diploid (RRII 105) and an enlarged view of the exine are shown in Fig. 1A,B respectively. The tricolporate and tetracolporate pollen grains of tetraploid are

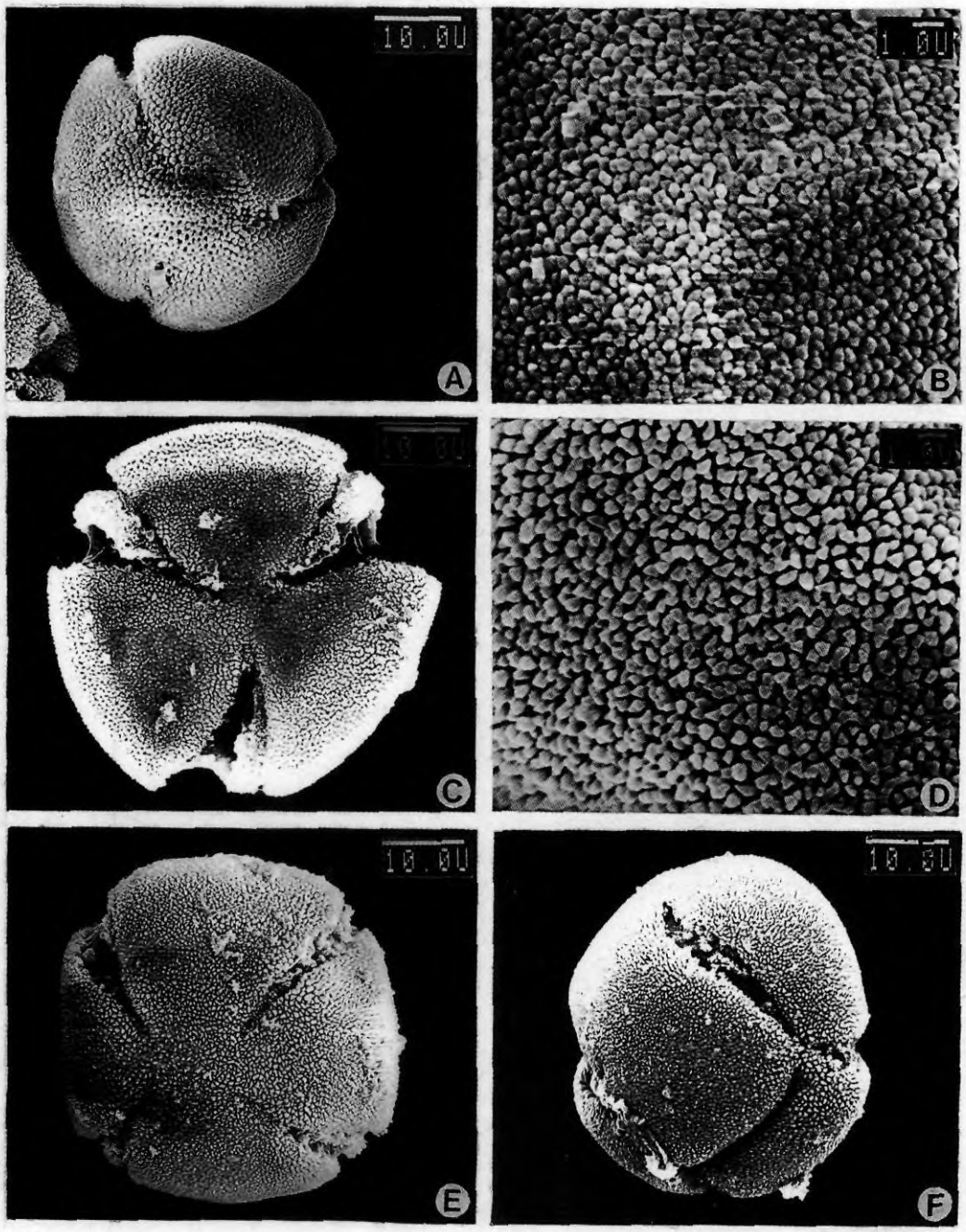


**Fig. 1A-F** — Scanning electron micrographs of pollen grains of diploid and tetraploid of *Hevea brasiliensis*. **A.** Polar view of the pollen grain of diploid (RRII 105). **B.** Portion of exine. **C.** Polar view of pollen grain of tetraploid with three pores. **D.** Portion of exine. **E.** Pollen grain of tetraploid showing four pores. **F.** Parasyncolpate pollen grain of tetraploid.

depicted in Fig. 1C,E. Parasyncolpate grain is shown in Fig. 1F. A portion of exine taken at a higher magnification is shown in Fig. 1D.

DIPLOID : SEM — Pollen grains are 3-zonocolporate; crustate island along the length of colpi is visible. Areoles rugulate, surface island conical and very close with narrow depression, areoles free or united island. There are furrow island with crustation. Furrow bridge is also seen.

TETRAPLOID : SEM — Pollen grains are parasyncolpate. Free areoles less than rugulate. There are three colporate and four colporate grains. Furrow islands are also seen. Areoles are united and they are of different size and shape.

Light microscopic observation had shown that there was distinct difference in size between the pollen from diploid as well as the tetraploid plant. In the diploid, all the pollen grains are 3-zonocolporate. But in the tetraploid, there is variation in the number of pores. Exine ornamentation units also showed larger size for the tetraploid. Exine thickness as well as ora diameter were also more for the tetraploid.

## Discussion

There is a general correlation between polyploid or chromosome number and the pollen size. The literature is replete with examples on this aspects (Kapadia & Gould 1964, Sreerengaswami & Raman 1973, Medius 1978). Taxonomists and cytologists have been using mature pollen size in sample from herbarium sheets as a method of surveying the extent of polyploidy within certain taxa (Stebbins 1950). Nair & Sharma (1966, 1967) observed the occurrence of varying degrees and combinations of apertural types in different cytotypes of *Sisymbrium irio* complex. The exine patterns of the diploid and octoploid taxa of *Gloriosa* are found to vary (Ravikumar & Nair 1985). In *H. brasiliensis* also there is an increase in size and number of pores in the pollen grains of tetraploid compared to those of diploid. The increase in pollen size, as observed for polar diameter and equatorial diameter as well as an increase in exine thickness and ora diameter in the tetraploid indicate an expression of genomic variation of the cytotypes in their pollen characteristics.

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