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POLYPLOIDY, NUCLEAR DNA AND HECOGENIN IN FOUR SPECIES OF *AGAVE*

SUCHITRA BANERJEE and A. K. SHARMA
Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India.

STEROIDAL compounds including sitosterol, diosgenin, hecogenin and tigogenin have been analysed in different species of *Agave*[1–14]. The compounds occur in different parts of the plants especially the leaves. Several species of *Agave* have recently been subjected to detailed cytological analysis in addition to the quantitation of the amount of DNA[15]. There are ample records in literature of variations in chemical content of the plant concomitant with polyploidy[16–19]. In view of the existence of diploid to polyploid species in this genus and the steroidal sapogenin so far recorded, the present study was undertaken.

Four species viz., *Agave angustifolia* Haw. var. *marginata* Hort., *A. americana* L. var. *marginata* Trelease, *A. sisalana* Perr. and *A. decipiens* were selected. For chromosome study, paradichlorobenzene pretreatment of root tips for usual schedule of orcein technique[20] was applied.

For estimation of *in situ* 4C nuclear DNA amounts, acetic acid: ethanol fixative and Feulgen staining technique was followed. Cytophotometric estimation of DNA from metaphase was carried out in a Reichert Zetopan microspectrophotometer following single wavelength (550 mm) method[20].

Sapogenins from all the four species were extracted from leaves following the usual procedure of drying, powdering acid hydrolysis followed by extraction with petroleum ether, the residue being dissolved in chloroform. For qualitative separation of sapogenin TLC techniques[21] were adopted comparing with standard sample under UV light and the purity of the hecogenin extract was confirmed through IR spectrophotometry.

For quantitative estimation, standard curves were made by both direct and preparative methods[22,23]. By comparing the two curves, the loss due to preparatory method could be calculated. Known amounts of the crude extract were applied to one spot on each plate for identification of hecogenin spot of the crude extract. The normal procedure for spectrophotometric quantitation was adopted at 390 nm. The concentration of hecogenin was worked out for each sample from the standard curve by extrapolation of values using the formula: $a = (c/d) \times b$, where a is the amount of hecogenin present in total crude sapogenin extract, b the total amount of the crude extract, c the concentration of hecogenin obtained from the standard curve and d the amount of crude extract applied in each plate. The percentage of hecogenin was calculated following the formula (a/e) 100 where e is the initial dry weight of the sample.

The four species revealed four different numbers viz. 60, 120, 150 and 180 chromosomes in the somatic cells indicating different degrees of polyploidy (table 1).

The amount of nuclear DNA differed to a certain extent among the four different species, the least amount i.e. 0.0993 units being recorded in the diploid species — *A. angustifolia* Haw. var. *marginata* Hort. ($2n = 60$) and the highest amount i.e. 0.1945 units in the hexaploid species — *A. decipiens* ($2n = 180$). Between the tetraploid and pentaploid species, the difference in the amount of DNA was rather negligible (table 1, figure 1), despite heavy difference in chromosome number and length between these two species. The absence of any direct multiplication of DNA amount in consonance with the multiplication of the chromosome number is an index of the differential DNA content in different species at the diploid level[24–26]. The structural alterations of chromosomes have also been recorded[27].

The presence of hecogenin was recorded at R_f 0.5/0.52 in the diploid, tetraploid and pentaploid species. In the hexaploid, the presence of hecogenin in trace amounts was noted.

Besides, tigogenin has been observed at R_f 0.74/0.76 in the diploid, tetraploid and pentaploid species, but the colour intensity of the spots was different in the different species. In the hexaploid species, *A. decipiens*, this spot was absent. E-sitosterol has been noted at R_f 0.8/0.83 in all the four

Table 1 *A comparative representation of different parameters with the hecogenin content of four different species of Agave*

Species	Total chromosome length (μm)	Amount of nuclear DNA (in arbitrary units)	Hecogenin content (%)
Agave angustifolia Haw. var. *marginata* Hort. ($2n = 60$)	129.98 ± 0.20	0.0993 ± 0.0024	0.192
A. americana L. var. *marginata* Trelease ($2n = 120$)	225.26 ± 0.12	0.1865 ± 0.0027	0.234
A. sissalana Perr. ($2n = 150$)	282.14 ± 0.12	0.1870 ± 0.0026	0.204
A. decipiens ($2n = 180$)	319.82 ± 0.10	0.1945 ± 0.0027	0.046

species studied while diosgenin was absent in all of them.

In *A. sissalana* and *A. decipiens*, a new spot could be observed at R_f 0.21–0.23, which was absent in *A. angustifolia* and *A. americana*. A perusal of the previous records does not indicate any such spot at this R_f value, with this specific solvent system. The chemical identity of this spot could not be worked out because of its presence in trace amounts. But as this spot was consistently obtained in all the sets, its constancy and genetic control are positively indicated. The occurrence of new compounds in polyploids through gene interactions has been reported in various plant species as well[16,19–21].

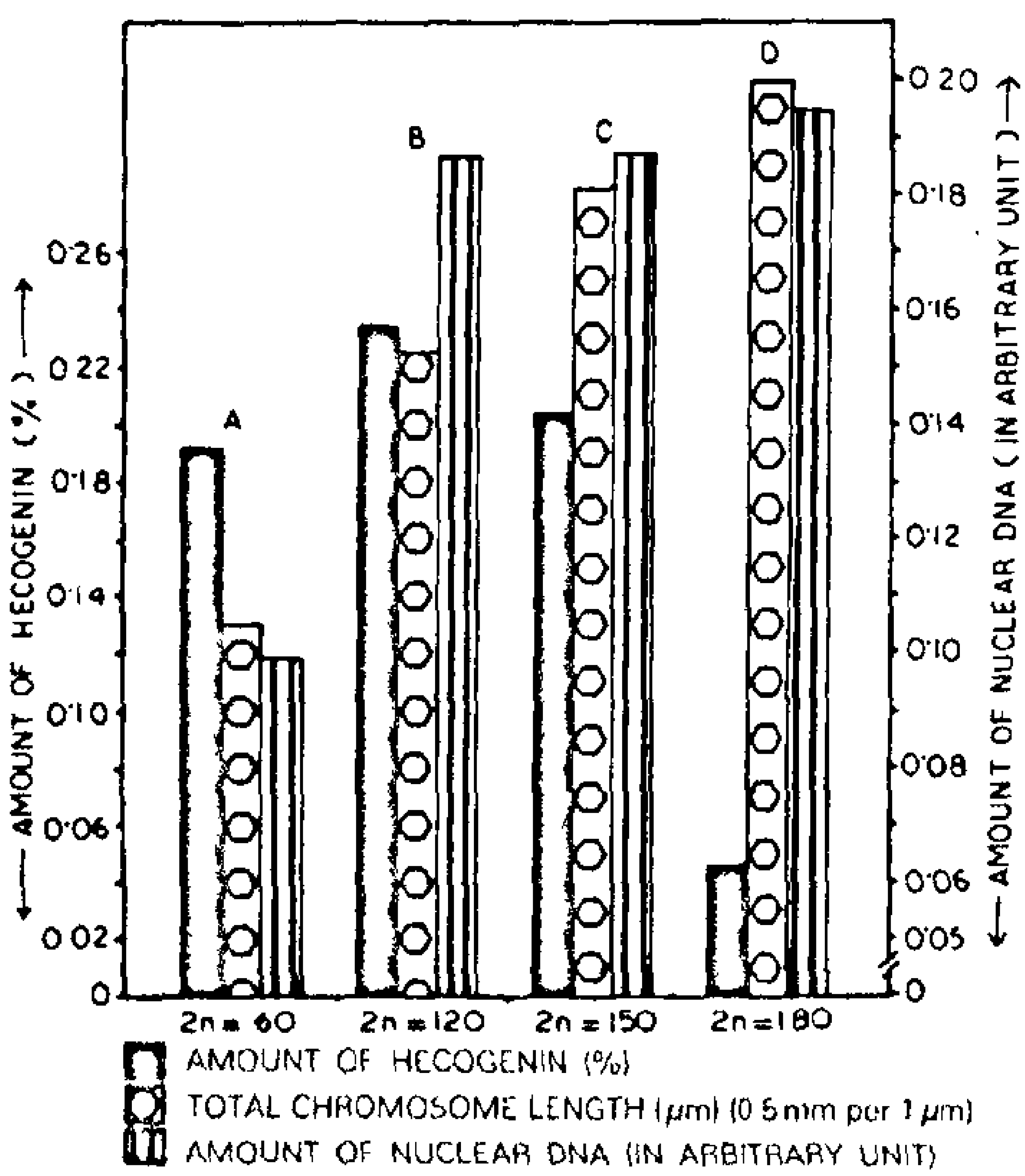

Figure 1. Comparative bar diagram representing the amount of hecogenin, total chromosome length and amount of nuclear DNA in four different species of *Agave*.

Hecogenin is reported in all species of *Agave*[9,11,12,28,29]. The amount of hecogenin in *A. angustifolia*, a diploid species, was comparatively lower (0.192%) than that in the tetraploid (0.234%) and pentaploid (0.204%). The hexaploid *A. decipiens* showed only 0.046% the least amount. The loss due to preparatory method was 6.5×10^{-6}. However, the increase or decrease in hecogenin content could not necessarily be attributed to the retarding or accelerating effect of the degree of ploidy (figure 1) as they belong to different species. However, genetic control of hecogenin content is clearly indicated by the consistency of the effect in all individuals.

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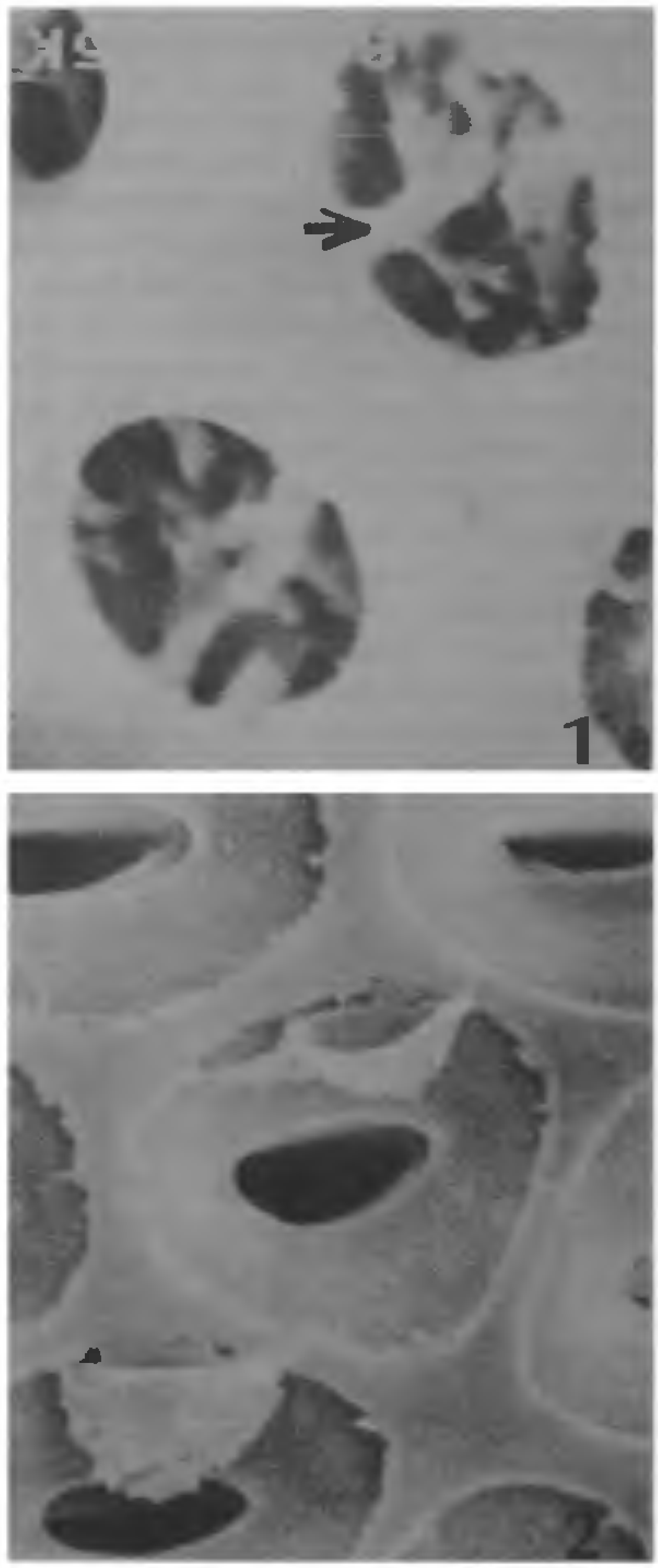
NON-VESTURED PITS OF *DELONIX ELATA* (L.) GAMBLE

K. RANJANI and K. V. KRISHNAMURTHY

Department of Botany, Bharathidasan University, Tiruchirapalli 620 024, India.

SINCE Bailey's[1] classical work on vestured pits, the Leguminosae (*Sensu lato*) was generally regarded as a family in which all taxa, with the exception of *Bauhinia* and *Cercis* (Tribe Cercideae) had vestured pits. Subsequently *Koompassia*[2–4], *Androcalymma*, *Apuleia*, *Dialium*, *Dicorynia*, *Distemonanthus*, *Martiodendron*, *Storckiella*, *Duparquetia*, *Labichea*, *Petalostylis* (all of Tribe Cercideae)[4] were also reported to lack vestured pits.

During a wood anatomical study of Caesalpiniaceae members, the pits of the two species of Delonix, *D. elata* and *D. regia* were found to be quite distinct from one another. In *D. regia*, all the pits had typical vestures which were dichotomously branching truncate structures arising from all sides of the roof of the pit chamber. They belong to type B form 1 vestures of Vliet[5] (figure 1). In contrast, all the pits of *D. elata* were non-vestured (figure 2). It is



Figures 1 and 2. 1. *Delonix regia*. SEM of vestured pits showing vestures (arrow) (× 7500); **2.** *Delonix elata*. SEM of non-vestured pits (× 5000).