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Karyological and Palynological Observations on *Amorphophallus titanum* (Becc.) Becc. ex Arcangeli (Araceae)

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Abstract — The karyotype of *Amorphophallus titanum* is reported. Cytological preparations were obtained by Feulgen technique. *A. titanum* showed *2n-26* (2msc+14m+6sm+3st+lsts) with chromosome length varying from 8.5 to 3.5 µm. The intrachromosomal and interchromosomal asymmetry indexes were calculated. The possible relationships with other species of the same genus are analysed and discussed together with some hypotheses on the general mechanism involved in the karyotypic evolution of the species for which the karyotype formulas are known in the literature.
Pollen and stigma were investigated for the first time by means of TEM and SEM. The pollen grains were also measured in water and glycerin under LO.

Key words: *Amorphophallus titanum,* karyology, palynology.

INTRODUCTION

In September 1994 and 1995 the Botanical Garden of Florence organized two expeditions to the Island of Sumatra to study *Amorphophallus titanum* (Becc.) Becc. ex Arcangeli and to collect its seeds and tubers. This plant (Figs. 1 and 2) is endemic to Sumatra and it produces the largest unbranched inflorescence in the world (up to 2.5 meters in height, CAMP 1937). Although *A. titanum* was discovered by the Florentine naturalist Odoardo Beccari in 1878 (BECCARI 1878), it has never been grown in the Botanical Garden of Florence or in other Italian botanical gardens (FABBRI 1994).

Also considering the rarity of this species, it seemed worthwhile to do a more thorough study of its karyotype and pollen.

MATERIALS AND METHODS

Karyology

The karyological analyses were carried out on mitotic metaphase plates of meristematic cells taken from root apices of germinating seeds. Root tips of seeds were pretreated with colchicine, fixed in ethylic alcohol-acetic acid 3:1 for 5 minutes and then stained according to the Feulgen technique. Karyograms were prepared on enlarged prints of the original micrographs. Measures and values were processed by an Excel worksheet in order to obtain chromosome ordering, recognition of homologues, karyotype formulas according to centromere position (LEVAN *et al.* 1964), intrachromosomal(A1) and interchromosomal (A_2) asymmetry indexes proposed by ROMERO ZARCO (1986).

Palynology

For SEM analyses the reproductive structures were fixed in 4% glutaraldehyde in phosphate buffer 0.1 M (pH 7.2). They were then dehydrated in aceton series, critical point-dried with liquid CO_2, mounted on aluminium stubs, coated with gold and observed by means of a Philips *XL* 20 Scanning Electron Microscope.

For TEM analyses the reproductive structures were fixed in 4% glutaraldehyde in phosphate buffer 0.1 M (pH 7.2) for 1 hour. Then they were post-fixed in OsO_4 at 2% in the same buffer for 3 hours. After a dehydration in alcohol series, they were embedded in Epon-Araldite. The sections obtained with LKB ultrotome IV and mounted on copper grids were stained with uranyle acetate and

Fig. 1. — Opened inflorescence *oiAmorphopballus titanum* in the forest of Sumatra.

lead cytrate. The electron micrographs were obtained with a Philips EM 201-C, working at 80 kW.

Due to the high sensibility of the pollen grains to the traditional acetolysis method (ERDTMAN 1960) for LM analyses, it was necessary to use a lighter acetolysis, keeping the pollen in the acetolysis fluid long enough to allow the temperature to reach from 65°C to 85°C (if the grains stay longer in the mixture they break down). Finally the pollen grains were measured in water and glycerina (50%); the measurements were done on a minimum of forty grains.

RESULT AND DISCUSSION

Karyology

The result of chromosome number was *2n=26* based on the monoploid number x=13 (Fig. 3). The chromosome length has a range from 8,5 µm to 3.5 µm with average width of 0.5 µ,m.

Here the karyotype formula is presented for the first time. As seen in Fig. 3 sixteen chromosomes are metacentric (number 3 and 4 with a secondary constriction), six are submetacen-

Fig. 2. — Inflorescence of *Amorphophallus titanum* in Sumatra.

trie and four are subtelocentric of which one bears a small satellite.

The karyotype formula is: 2n=2x=26: 2msc+ 14m+6sm+3 st+1 sts

In the previous literature (CHANDLER 1943; TJIO 1948) only the chromosome number was given. Our data confirm their results.

In the genus *Amorphophallus* two monoploid numbers have been identified: x=13 and x=14 (MARCHANT 1971; LARSEN and LARSEN 1974; RAMACHANDRAN 1977; CHAUHAN and BRANDHAM 1985). Most of the species are based on x=13 and can be diploids or triploids (2n=26,39); the species with x=14 are only diploids.

We analysed the possible relationships with other species of the same genus calculating the the intrachromosomal (A_x) and interchromosomal asymmetry indexes (A_2) of all the species reported below.

We compared only the species of which the karyotype formulas or the photos of the plates are known in the literature. The distribution of these species is shown in Fig. 4 and summarized here (HETTERSCHEID and ITTENBACH 1996):

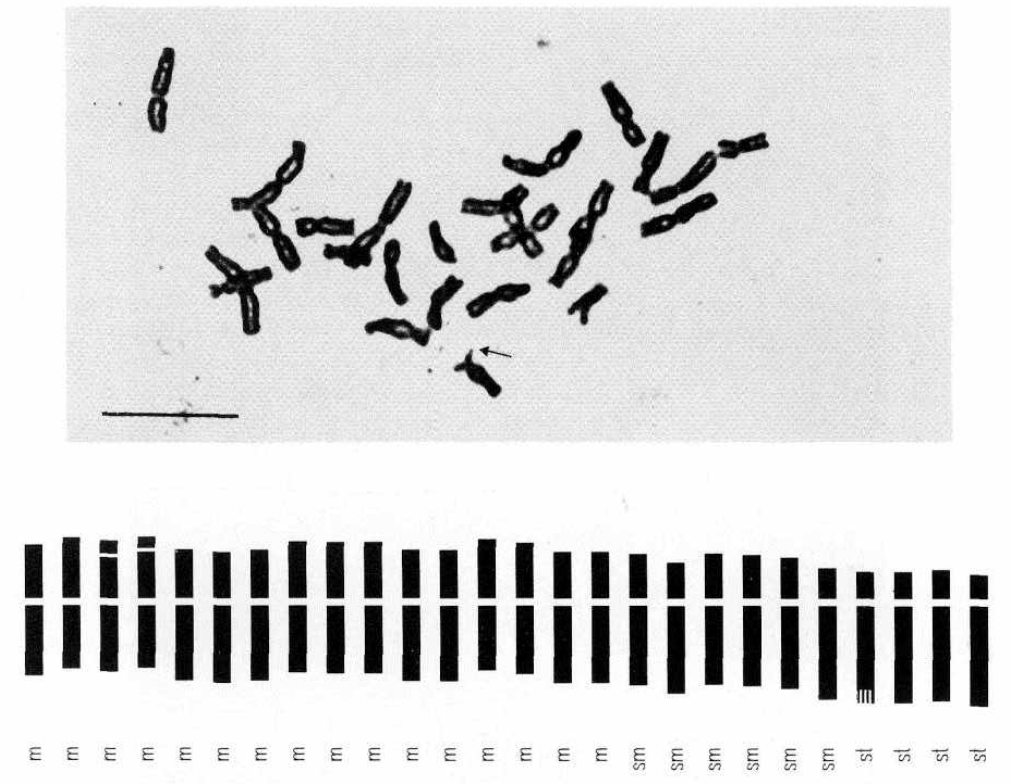

Fig. 3. — Mitotic metaphases and idiogram of *Amorphophallus titanum.* The satellite is indicated by the arrow. Bar = 10 μm.

A. abyssinicus (Rich.) N.E.Br.: Cameroon, Ivory Coast eastwards to Ethiopia, then southwards to Tanzania, Zambia, and the center of Zaire. *A. bulbifer* (Roxb) Bl.: Northern and eastern India, Bangladesh, Bhutan, Nepal. *A. dracontioides* (Engl.) N.E.Br.: Benin, IvoryCoast, Ghana, Niger, Nigeria, Togo, Central African Republic. *A. gallaensis* (Engl.) N.E.Br.: Ethiopia, Somalia, Kenya. *A. goetzei* (Engl.) N.E.Br.: Tanzania, Mozambique. *A. hildebrandtii* (Engl.) Engl. & Gerhm: Madagascar (endenic). *A. hohenackeri* (Schott) Engl. & Gerhm: Southern India. *A. konjac* K. Koch: Southern and southeastern China, Vietnam, Sabah. *A. krausei* Engl.: Northern Thailand, northern Myanmar, southern China. *A. lambii* Mayo & Widjaja: East Malaysia, Sabah, Central Kalimantan. *A. maximus* (Engl.) N.E.Br.: Kenya, Tanzania, Zimbabwe, Zambia, Somalia. *A. muelleri* Bl.: from the Adamans eastward through Myanmar into northern Thailand and southeastwards to Sumatra, Java, Flores and Timor. *A. paeonifolius* (Dennst.) Nicolson: Madagascar, eastwards via India to Malaysia, southern China, Indochina, Polynesia, northern Australia. *A. prainii* Hook. F.: Southern Thailand, the Malay Peninsula, Sumatra (eastern Kalimatan?). *A. titanum* (Becc.) Becc. ex Arcang.: Sumatra (endemic). A *variabilis Bl.:* Java, Kangean Archipelago. *A. yunnanensis* Engl.: China (Yunnan), northern Thailand, North Vietnam.

In order to obtain homogeneous and comparable data, it was necessary to recalculate the karyotype formulas of the species published by CHAUHAN and BRANDHAM (1985), since Levan's nomenclature was not used. The calculations were based on the photos of that article.

In MARCH ANT (1971) the karyotype formula was not calculated but since there is the photo of the plate it was also possible to recalculate the karyotype formula here (Table 1).

The diagram of the intrachromosomal (Aj) and interchromosomal asymmetry index (A_2) is reported in Fig. 5.

Our data can be used to show the affinities among the species and consequently to suggest the possible directions of evolution of the species of *Amorphophallus* analysed in this report.

TABLE 1.

Taxon	*x*	2*n*	Karyotype formula	A1**	A2**	L	References
A. abyssinicus	13	26	7m+4sm+2st*	0,36	0,178	3.6 - 6.5	Chauhan & Brandham 1985
A. bulbifer	13	39	1M+9m+3sm*	0,257	0,143	3.3 - 4.8	Chauhan & Brandham 1985
A. dracontioides	13	26	5m+4sm+2st+2t*	0,505	0,222	5.2 -12.2	Chauhan & Brandham 1985
A. gallaensis	13	26	9m+1sm+3t*	0,284	0,193	4.2 - 8.4	Chauhan & Brandham 1985
A. goetzei	13	26	9m+2sm+2st*	0,278	0,204	2.4 - 5.5	Chauhan & Brandham 1985
A. hildebrandtii	13	26	8m+3sm+2st*	0,317	0,202	2.5 - 5.7	Chauhan & Brandham 1985
A. hohenackeri	13	26	4M+8m+1sm	0,174	0,189	3.7 - 7.4	Ramachandran 1977
A. konjac	13	26	8m+4sm+1st*	0,134	0,235	2.6 - 5.2	Chauhan & Brandham 1985
A. krausei	13	26	8m+2sm+3st*	0,338	0,172	3.8 - 6.3	Chauhan & Brandham 1985
A. lambii	13	26	8m+2sm+3st*	0,368	0,169	3.1 - 5.5	Chauhan & Brandham 1985
A. maximus	13	26	9m+1sm+2st+1t**	0,314	0,211	3.7 - 8.5*	Marchant 1971
A. muelleri	13	39	6m+4sm+3st*	0,444	0,185	2.4 - 4.3	Chauhan & Brandham 1985
A. paeonifolius	14	28	7m+6sm+1st*	0,356	0,143	2.5 - 4.5	Chauhan & Brandham 1985
A. prainii	14	28	7m+5sm+2st*	0,371	0,149	1.8 - 3.5	Chauhan & Brandham 1985
A. titanum	13	26	1M+7m+3sm2st	0,381	0,229	3.5 - 8.5	Giordano
A. variabilis	13	26	7m+4sm+2st*	0,372	0,221	3.7 - 7.0	Chauhan & Brandham 1985
A. yunnanensis	13	26	12m+1sm*	0,197	0,163	2.6 - 3.9	Chauhan & Brandham 1985

*= The karyotype formula has been recalculated based on the author's photos in order to obtain homogeneous and comparable data.
**= Calculated by Giordano based on author's photos.

From the diagram and the map of distribution it can be seen that *A. titanum* has an intrachromosomal and interchromosomal indexes very similar to *A. variabilis*, a plant that lives in an area adjacent to Sumatra; consequently they seem to be phylogenetically close.

In the diagram a group of species with similar indexes can be distinguished, all of which are situated in the eastern part of Africa *(A. goetzei, A. maximus, A. hildebrandtii, A. gal-laensis,* and *A. abyssinicus).*

A. prainii and *A. paeonifolius,* of the group with x=14, have very close indexes. They are also the ones with the smallest chromosomes and little DNA value (CHAUHAN and BRAND-HAM 1985). They have an intermediate intrachromosomal index and the lowest interchromosomal index which are supposed to be a measure of karyotype evolution. MARCHANT (1971) suggested that species with a basic number of x=13 chromosomes are derived from ancestors with x=14. Since it is currently accepted that within a single basic chromosome number the taxa with low DNA values are usally less advanced than those with high DNA values (CHAUHAN and BRANDHAM 1985), CHAUHAN and BRANDHAM (1985) suggested that the ancestor with x=14 could be *A. prainii.* While CHAUHAN and BRANDHAM (1985) arrived at their conclusion based on a relatively small amount of data, this research confirms their thesis using more exhaustive data.

The two triploids *(A. bulbifer* and *A. muelleri)* have very different indexes, so they seem to be phylogenetically distant.

Palynology

The pollen grains are slightly ovoid with the diameter averaging 65 μm (57 μm minimum, 75 μm maximum) (Figs. 6a, 7a). For the first time the morphology of the pollen grains was also analysed by means of TEM and SEM.

The grain surface is quite psilate and inaperturate, both under LM and SEM, (Fig. 6b). The exina is without columellae and it does not have sculptural elements (Figs. 6b, 7a).

The pollen wall consists of three clearly distinguishable layers (Fig. 7a, b). The external one is the thickest (0.8 μm) with an irregular upper outline; it is formed by homogeneous matrix that becomes lamellar in its deepest part, the ectexine. The second layer, the endexine, 0,75. μm, thick, is darker than the former and has a lamellar matrix. The third layer which separates the endexine from the cytoplasm, is about

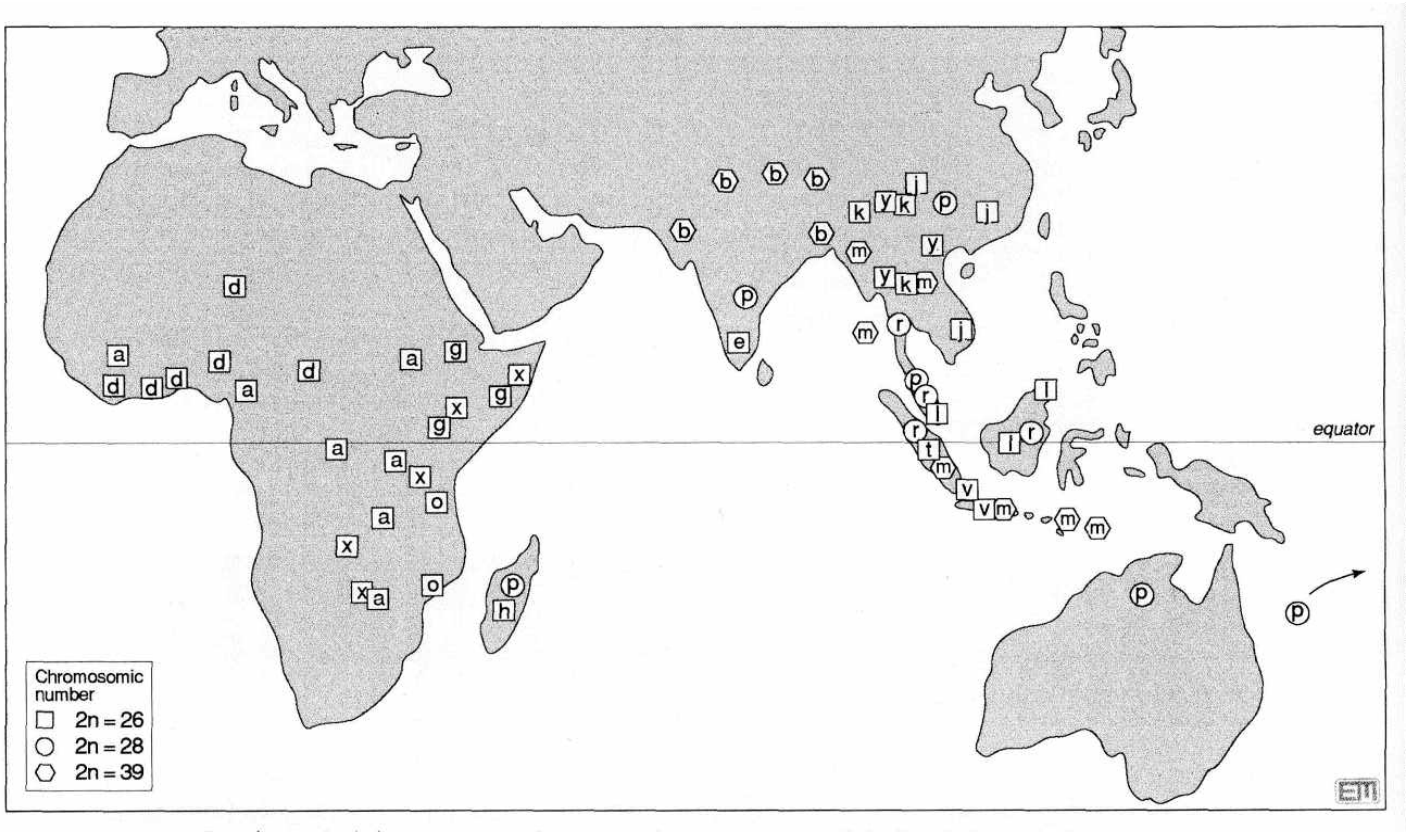

Fig. 4. — Distribution and chromosome numbers of: *A. abyssinicus* (a), *A. bulbifer* (b), *A. dracontioides* (d), *A. gallaensis* (g), *A. goetzei* (o), *A. hildebrandtii* (h), *A. hohenacken* (e), *A. konjac* (j), /I. *kmmei* (k), A. *lambii* (1), A *maximus* (x), A *muelleri* (m), A *paeontfolius* (p), A *prainii* (p), A *titanum* (t), A. *variabilis* (v), A. *yunnanensis* (y).

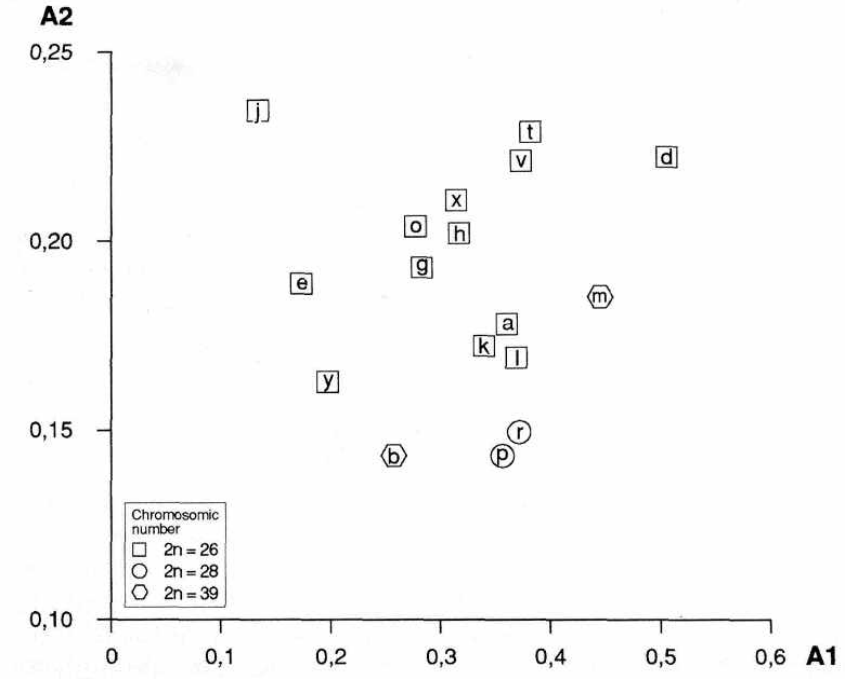

Fig. 5. — Two dimensional ordination of the species of *Amorphophallus* as a function of intra- (Aj) and interchromosomic (A_2) asymmetry indexes: A *abyssinicus* (a), A *bulbifer* (b), A *dracontioides* (d), A *gallaensis* (g), A *goetzei* (o), A *hildebrandtii* (h), A. *hohenackeri* (e), A *konjac* (j), A *krausei* (k), A *lambii* (1), A *maximus* (x), A *muelleri* (m), A. *paeonifolius* (p), A *prainii* (p), A /ti*tanum* (t), A. *variabilis* (v), A *yunnanemis* (y).

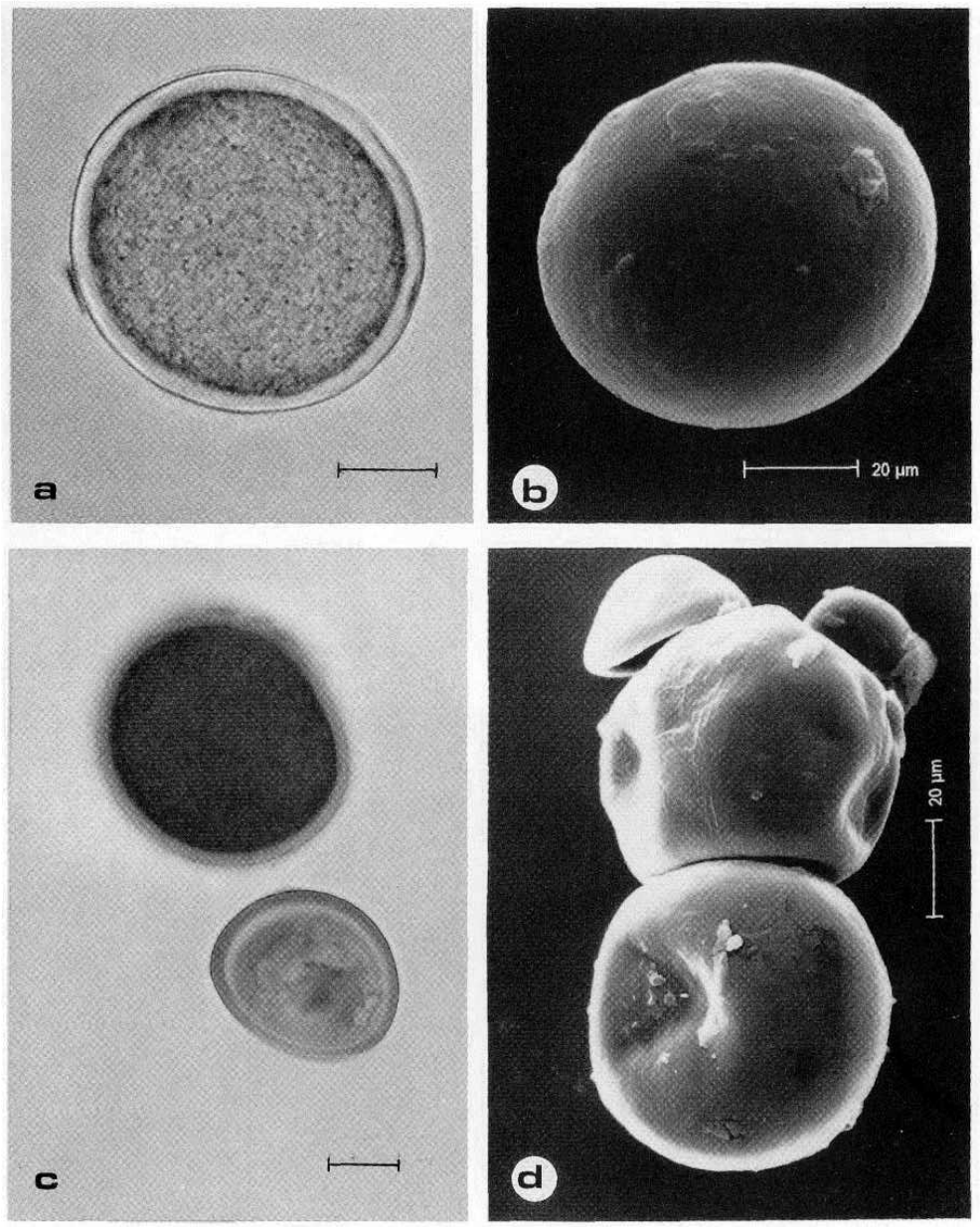

Fig. 6. —*Amorphophallus titanum*, pollen grains, a) LM; b) SEM micrograph; c) Hrishi coloration of abortive and normal pollen, LM; d) SEM micrograph of abortive and normal pollen. Bars: 20 µm.

0,5 µm thick and has irregular limits and a homogeneous matrix and can be considered intine.

Many abortive grains can be found interposed between the normal pollen grains (Fig. 6 c, d). They are smaller, 30-35 µm in diameter more or less collapsed. With the Hrishi's stain (HRISHI 1960) it was shown that these grains lack cytoplasm (Fig. 6c). These sterile grains are 30% of the total number of grains. It should be noted that the count must be done on untreated material because with whathever any kind of treatment used these grains remain in the supernatant.

The position of the abortive grains which remained attached to the normal grains, gives us cause to believe that the meiosis leads to the formation of T-shaped tetrads, as already noted in other plants of the same family (PACINI and JUNIPER 1983).

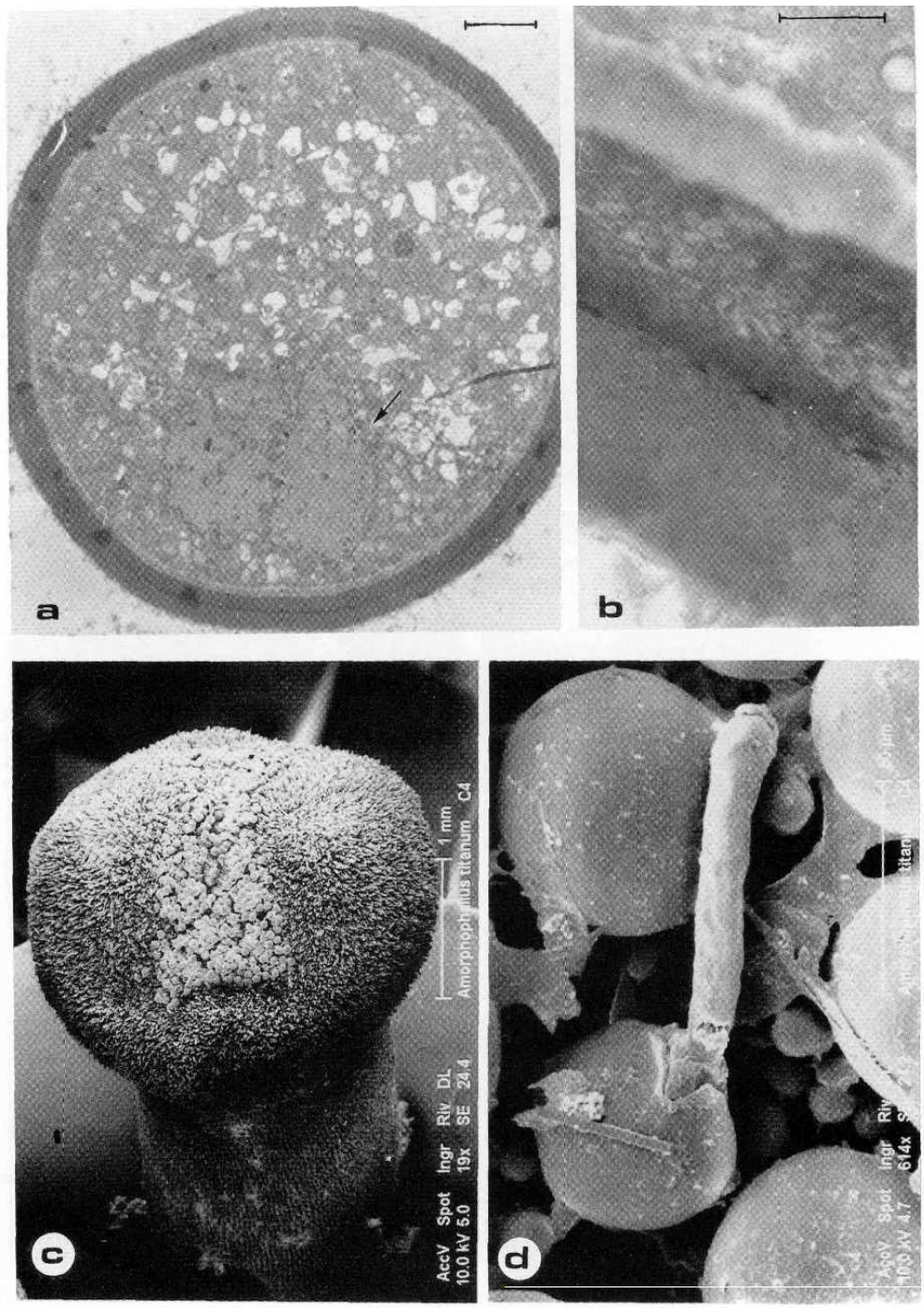

Fig. 7. — *Amorphophallus titanum:* a) section of pollen grain with vegetative nucleus indicated by the arrow, LM; b) section of the pollen wall, TEM; c) SEM micrograph of the stigma with pollen grains; d) SEM micrograph of germinating pollen. Bars: a 5 (Am, b 0,5 μm, c 1 mm, d 50μm.

The stigma is roughly ovoid in shape (Fig. 7c), considerably developed (about 3,5 cm. in diameter), generally bilobed and covered with large unicellular stigmatic papillae. These papillae have a cylindrical shape, round on the apex from 70 to 180 μm in length. They are about 15 μm in width, immersed in an exudate secretion that they produce. This kind of stigma is conse-

quently a "wet stigma" (HESLOP-HARRISON 1981).

The pollen tubes observed on the stigma have a diameter up to 12 µm (Fig. 7d).

The general aspect of the pollen is in accordance with what has been observed in most of the species of the tribe Amorphophallae, as THANIKAIMONI (1969) reported. It is inapertur-ate and in particular in the genus *Amorphophal-lus*, the exine is in most cases psilate as in *A.bulbifer, A. lambii, A. prainii, A. eichleri*, and *A. gliruroides* or striate as in *A. gallaemh, A. konjac, A. yunnanensis, A. krausei, A. chlorospathus, A. commutatus, A. purpurescens* and *A. variabilis* (THANIKAIMONI 1969; GRAYUM 1985).

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REFERENCES

BECCARI O., 1878. — *II Conophallus titanum Beccari.* Bull. R. Soc. Tosc. di Orticult., 3: 290-293. CAMP W.H., 1937. — *Notes on the physiology and morphology of Amorphophallus titanum.* Journ. N.Y. Bot. Gard., 38: 190-197. CHANDLER C., 1943. — *The number of chromosomes in two species of Amorphophallus.* Bull. Torrey Bot. Club, 70: 612-614.

CHAUHAN K.P.S. and BRANDHAM P.E., 1985. — *Chromosome andDNA variation in Amorphophallus.* Kew Bull., 40: 745-758.

ERDTMAN G., 1960. — *The acetolysis method. A revised description.* Svensk Bot. Tidskr. 54: 561-564.

FABBRI F., 1994. —*L'Amorphophallus titanum "unapianta maravigliosa" scoperta a Sumatra da Odoardo Beccari.* In G. Moggi, M. Maffioli and E. Sesti (Eds) "Fotografia e botanica fra ottocento e novecento", pp. 49-56. Alinari, Firenze.

GRAYUM M.H., 1985. — *Evolution and ecological significance of starch storage in pollen of the Araceae.* Amer. J. Bot., 72: 1565-1577.

HETTERSCHEID W.E. and ITTENBACH S., 1996. — *Everything you always wanted to know about Amorphophallus, but were afraid to stick your nose into!* Aroideana, 19: 7-132.

HESLOP-HARRISON Y., 1981. — *Stigma characteristics and Angiosperm Taxonomy.* Nord. J. Bot., 1: 401-420.

HRISHI NJ. and MUNTZING A., 1960 — *Structural heterozygos-ity in Secale kuprijanovii.* Hereditas, 46: 745-752.

LARSEN K. and LARSEN S.S., 1974. — *A new Amorphophallus from Thailand.* Reinwardtia, 9: 139-142.

LEVAN A., FREDGA K. and SANDBERG A.A., 1964. — *Nomenclature for centrometric positions on chromosomes.* Hereditas, 52:201-220.

MARCHANT C.J., 1971. — *Chromosome variation in Araceae: III Philodendreae to Pythonieae.* Kew Bull., 25: 323-329.

PACINI E. and JUNIPER B.E., 1983. — *The ultrastructure of the formation and development of the Ameboid Tapetum in Arum italicum Miller.* Protoplasma, 117: 116-129.

RAMACHANDRAN K., 1977. — *Karyological studies on four South Indian species of Amorphophallus.* Cytologia, 42: 645-652.

ROMERO ZARCO C.R., 1986. —*A new method for estimating karyotype asymmetry.* Taxon, 35: 526-530.

THANIKAIMONI G., 1969. — *Esquissepalinologique des Aracees.* Trav. Sect. Sci. Techn. Inst. Franc. Pondicherry, 11: 1-286.

TJIO J.H., 1948. — *The somatic chromosomes of some tropical plants.* Hereditas, 34: 135-146.

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