Tradescantia bracteata pollen in vitro: pollen tubes development and mitosis*

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Abstract

About 90 per cent of *Tradescantia bracteata* pollen germinates *in vitro* after 15 min. Mitosis starts in the pollen tube after about 3 h. The mitotic transformations of chromosomes within the generative nucleus are not synchronized. They involve successively the linearly arranged chromosomes in the elongated generative nucleus. In metaphase the chromosomes are arranged tandem-like linearly along the pollen tube. The chromatids translocate in anaphase from various distances to the poles in a plane parallel to the metaphase plate. This suggests that chromosomes have individual mitotic spindles and that coordination of the chromosome transformations in the generative cell is much less strict than in a typical somatic mitosis.

Starch is the storage material of pollen grains. In the vegetative cytoplasm of mature pollen grains minute reddish-orange vesicular structures are visible after staining with neutral red. They do not fuse with the vacuoles proper arising in germinating pollen grains to form the vacuolar system of the pollen tube.

INTRODUCTION

Pollen germination and growth of pollen tubes *in vitro* have been described in detail in many plant (for review see Golubiński, 1947).

Germination usually starts soon after planting the pollen on medium. Tube growth lasts several up to a dozen or so hours, and usually ends before the tube reaches dimensions seen *in vivo*.

Mature *Tradescantia* pollen grains are two-celled. The generative nucleus divides mitotically to two sperm nuclei in the pollen tube several hours after its germination.

Several authors report that in species with large chromosomes and pollen forming a tube of small diameter, the mitotic figures are atypical,

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this refers particularly to the metaphase plate and the chromosome arrangement in anaphase (Eigsti, 1940; Sax and O'Mara, 1941; Uppcott, 1936).

The present paper is a microscopic analysis of cytomorphological changes and *in vitro* development of the pollen tube during pollen grains germination of *Tradescantia bracteata*. Particular attention was devoted to the identification of the storage substance in maturing pollen grains, the development of the vacuolar system during germination and pollen tube growth, the structure of the vegetative and generative nucleus during their translocation to the pollen tube and the course of mitosis in the generative cell.

MATERIAL AND METHODS

The observations on pollen grain germination and pollen tube development were performed on a clone of *Tradescantia bracteata* Small. The pollen tubes were cultured on liquid medium by the standing drop method of Kwack and Kim (1967) (distilled water containing 10 per cent sucrose and H_3BO_3 — 0.01%, $\text{Ca}(\text{NO}_3)_2$ — 0.03%, MgSO_4 — 0.02%, KNO_3 — 0.01%). The medium pH was adjusted with 1 per cent KOH to 8.3. The cultures were run at 22—23°C.

In vivo observations were made in the medium alone and in medium with neutral red as well as on permanent preparations after fixation and staining with acetoorcein.

Moreover, the preparations were stained with Lugol solution and by the PAS reaction according to two variants:

- a) after previous hydrolysis with α -amylase
- b) without enzymatic hydrolysis.

Starch hydrolysis was run with α -amylase in acetate buffer, pH 5.6 at 35°C for 24 h.

Callose was identified by the method of Eschrich and Currier (1964).

RESULTS

In maturing pollen grains of *Tradescantia* (24 h before pollen shedding) the generative nucleus is spindle-shaped and has a structure corresponding to that of the nucleus in prophase. The vegetative nucleus does not stain as strongly as the generative one and is amoeboidal in shape. Several hours before pollen shedding the structure of the generative nucleus becomes much less distinct and is similar to that of the nucleus in interphase. Such a state of the generative nucleus was also

observed in mature pollen grains and at the time of translocation of the generative cell in the pollen tube (Photos 1 and 4).

There are, in the vegetative cytoplasm of maturing pollen grains, minute granules strongly refracting light, similar to those in young *Tradescantia* microspores. They also occur in most mature pollen grains. It has been demonstrated that they are PAS-positive and stain with JKJ to red-brown. After digestion with α -amylase these grains undergo hydrolysis and JKJ and PAS staining give negative results. Thus these are "red starch". In mature pollen grains, after staining with neutral red, numerous minute vesicles are visible in the vegetative cytoplasm, staining metachromatically to orange-red (Photo 2).

After transfer of dry pollen grains to liquid medium they immediately swell and Brownian movements are observed in the cytoplasm. A few minutes after swelling, a zone of homogeneous cytoplasm forms at both ends of the grain (Photo 17). There appear in the granular cytoplasm limited circulatory movements, the generative nucleus takes on an irregularly rolled up shape.

Usually more than 50 per cent of the grains germinate after 10 min. After 15 min of culture about 80 per cent of pollen grains have germinated.

The pollen tube arises as a rule at one end of the long axis of the grain, that is at the edge of the elongated aperture situated at the distal pole of the pollen grain (Photos 4 and 17).

Sporadically 2 pollen tubes emerge, only one of which develops. At first only homogeneous cytoplasm enters the pollen tube (Photo 17).

About 10 per cent of pollen grains produce only very short (10—20 μ m) initial pollen tubes. Two kinds of such pollen grains were observed: in one kind "vacuoles" staining orange-red with neutral red form, probably as the result of merging of metachromatic vesicles (Photo 7), in the second kind the true vacuoles stain light pink.

In most normally germinating pollen grains after 20 min the pollen tubes reach a length of 20—30 μ m.

At this time callose appears in the pollen tube wall with the exception of its apical part. In the cytoplasm of the pollen tube there occurs an intensive streaming movement, except for the immobile homogeneous apical zone (Photo 22). The movement in the cytoplasm of the pollen grain continues to be circulatory.

In the part of the grain lying opposite to the pollen tube numerous minute vacuoles form, increasing in size and fusing with one another (Photos 18 and 19). These vacuoles stain pink with neutral red. A little later similar pink vacuoles appear in the remaining part of the cytoplasm pollen grain and the basal part of the tube where they occur independently of the red-orange vesicles (Photos 5, 6 and 20, 21).

The expansion of the central vacuole in normally germinating pollen

grains and the flow of cytoplasm to the pollen tube lead after 40 min to the translocation of both nuclei to the tube inlet (Photo 21). Soon after this, when the tube length is about 260 μm the nuclei start passing from the pollen grain into the tube. Usually, in about 80 per cent of cases the vegetative nucleus is the first to penetrate into the pollen tube (Photo 8). The vegetative nucleus elongates considerably as it enters the tube and becomes similar in structure to the generative nucleus. Both nuclei take in the pollen tube frequently the shape of a corkscrew and reach a length of about 100 μm (Photo 9). After some time these nuclei lose their screw-like shape and still elongate somewhat. From this moment the differences in structure between the vegetative and the generative nucleus become again more pronounced. The generative nucleus settles at a distance about 100 μm from the tube apex, whereas the vegetative nucleus shifts further towards the latter.

During the first three hours of growth the pollen tubes reach a length of 2500 μm , then their growth is gradually inhibited. In the basal part of the pollen tube incomplete callose plugs begin to form. Vacuolized cytoplasm is present in the pollen grain and in the zones of the pollen tube partly separated by callose rings. This cytoplasm exhibits a circulatory movement, but in the subapical part of the tube the streaming movement continues.

After 3 h of culture there appears again in the generative nucleus a distinct prophase structure which was already seen in maturing pollen grain (Photo 10).

In the meantime the vegetative nucleus undergoes a marked contraction lying close to the tube apex. Prophase chromosomes become so short that it is easy to distinguish the chromatide pairs lying in rows (Fig. 1). The particular chromosome become condensed usually successively, beginning from one end of the prophase nucleus (Photo 11). In prometaphase they move along the pollen tube so that the metaphase plate is composed of 6 chromosomes one following the other along the tube (Photo 12). This plate is by 30-50 per cent longer than the prophase nucleus.

Anaphase begins after about 6 h of pollen tube growth.

The centromeres of the chromosomes in tandem position divide successively beginning with one of the marginal ones. The sister chromatides shift in respect to one another and migrate to the opposite poles of the plate (Photo 13) where telophase groupings form (Photo 14). Anaphase chromosomes are usually straight, erect, less frequently bent with the centromere oriented towards the pole. In spite of the non-simultaneous segregation of chromosomes in anaphase the transformations of telephase chromosomes occur almost simultaneously.

Eleven hours after the beginning of pollen grain germination sperm nuclei are already formed (8 h after visualization of the prophase

structure) (Photos 15 and 16). In cultures *in vitro* the growth of *Tradescantia* pollen tubes is ended during mitosis in the generative cell. The whole tube wall together with its apex is lined with callose.

Fig. 1. Chromosome arrangement in prophase of mitosis in the generative cell of *Tradescantia* (after slight drawing apart of the chromosomes thus arranged in vivo ca. 3000X)

Cytomorphological changes in the course of pollen grain germination and pollen tube development in *Tradescantia bracteata* are shown in Fig. 2.

	time	
	1'	Brownian motion (agitation)
	10'	circulation in pollen grain reverse-fountain streaming in pollen tube
	20'	circulation and rotation in pollen grain reverse-fountain streaming in pollen tube
	40'	initiation of vacuoles development in pollen tube
	60'	protoplasmic streaming
	3 h	starch grains
		densely staining small vesicles
		vacuoles
	6 h	generative nucleus
		vegetative nucleus
		sperm nucleus
	11 h	

Fig. 2. Changes of cytoplasmic streaming and vacuolation. The nuclei in the pollen grain and pollen tube of *Tradescantia bracteata* in the period from pollen grain imbibition to sperm nuclei formation

DISCUSSION

Cytochemical tests and α -amylase digestion demonstrated that red starch is the storage material of microspores and pollen grains in *T. bracteata*. Thus, the present results contradict the data of Sax and Edmonds (1933) and those of Schmitt (1938) quoted later by other authors (La Cour, 1949; Rodkiewicz, 1973) who claim a protein character of these granules. The nontypical staining of these granules with Logol solution is due to the lower degree of polymerization and is characteristic for storage starch in pollen grain of many plant species (Dexheimer, 1970).

In mature pollen grains of *Tradescantia* minute strongly metachromatically staining vesicles were observed after staining with neutral red. These vesicles are capable of coalescing and during germination and pollen tube growth they translocate with the cytoplasm current into the pollen tube. These vesicles are distinct from vacuoles proper forming at the pollen grain pole opposite to the germinating pollen tube, staining orthochromatically with neutral red and forming the system of vacuoles of the germinating pollen grain and tube.

The present observations agree with those of Dexheimer (1970) in the light microscope and those of earlier authors, for instance of Plantefol (1933) and Dangeard (1957) performed on pollen grains of species other than *Tradescantia*. Dexheimer (1970) demonstrated in the electron microscope in *Hippeastrum* and other plants in the pollen grain and tubes stained *in vivo* with neutral red and fixed thereafter with KMnO_4 the presence of vesicles with electron-dense contents, surrounded with a single membrane. In pollen grains and tubes previously not stained, however, he did find the counterparts of these structures. According to the above named author, the vegetative cell in a mature pollen grain accumulates the dye in the vesicle-like structures, the origin of which he was unable to establish. The latter structures correspond to the minute "vacuoles" strongly staining with neutral red in mature pollen grains seen in the light microscope. He considered the possibility of their arising both *de novo* and from earlier existing element (dictyosomes or reticulum cisternae).

The cytoplasm movement in germinating pollen grains and tubes has been analysed in detail in *Lilium* by Iwanami (1952, 1953) and Kamiya (1959). In *Tradescantia* the starting time of this movement and its character are almost the same as in *Lilium*. A dozen or so seconds after placing mature pollen grains on the liquid medium there appear in the vegetative cytoplasm Brownian movements, and, after a further dozen or so seconds, circulatory movements. In the apical part of the pollen tube streaming is noted up to the end of growth of the tube *in vitro*. Contrary to *Lilium*, however, many cytoplasmic bridges were

found in the elongated pollen tubes of *Tradescantia*, beside the cytoplasm layer adjacent to the wall in which rotational movement occurs. Cytoplasm translocates along these bridges in various directions. The movement in the full-grown pollen tube is thus of rotational-circulatory character.

Homogeneous cytoplasm is present at the pollen tube apex during its growth. It is known from electron-microscopic studies (Rosen and Gawlik, 1966; Dashek and Rosen, 1966, in *Lilium*) that the apical part of the pollen tube is deprived of cell organelles. It only contains endoplasmic reticulum and abundant Golgi vesicles which contribute to the building of plasmalemma and of the pollen tube wall. It was observed in *Tradescantia* pollen grains that, before the tubular, out growth is formed segregation of granules occurs from the region of the vegetative cytoplasm which translocates to the arising pollen tube.

The mechanism of translocation of the vegetative nucleus and the generative cell into the tube is not clear. Woycicki (1926) considered that translocation of the generative cell depends on the growth of vacuoles in the pollen grain and the movement of the cytoplasm. Navashin (1969) also believed that the nuclei are passively translocated by the cytoplasmic streaming. Venema and Koopmans (1962) are of the opinion that the direction of translocation of the nuclei depends on the resultant of the mechanical forces of the ascending and descending current in the pollen tube.

It was observed in *Tradescantia* that in 80 per cent of cases the vegetative nucleus is the first to enter the tube. In an inverse situation the vegetative nucleus overtakes the generative one in the tube and finally the former lies always closer to the tube apex. This as well as the fact that during translocation both nuclei greatly elongate and frequently take the shape of a corkscrew seem to suggest that not only cytoplasm flow and development of the vacuolar system decide of the translocation of the nuclei to the tube, but also the active movement of the nuclei themselves.

The development of two-celled pollen grains in the anther of *T. bracteata* lasts about 7 days. DNA synthesis in the generative nucleus starts as early as 24 h after the end of mitosis in the microspore (Charzyńska and Maleszka, in preparation; Moses and Taylor, 1955 in *T. paludosa*). The G_1 period of the generative cell is, thus, relatively short. It is not known how long period G_2 lasts. The opinion prevails to date represented among others by Vazart (1958) that mitosis in the generative nucleus starts, also in plants in which pollen matures in two-celled form, during maturation of the pollen and that the generative nucleus in mature pollen is in prophase (frequently referred to as "arrested prophase").

We confirm the fact that the structure of the generative nucleus in maturing pollen grains of *T. bracteata* corresponds to the picture of prophase transformations, however, on the last day before pollen shedding, the chromosomes of the generative nucleus are strongly despiralized in pollen grains before emergence and in mature shed grains the structure is similar to that of an interphase nucleus. The generative nucleus exhibits such a "non-prophase" structure during translocation to the pollen tube and in the tube at least for 3 h after tube germination. Recession of the prophase transformations was also observed in the generative nucleus of mature pollen grains of *Eremurus* (Charzyńska et al., 1977). We believe that the starting of prophase transformations in maturing pollen grains makes possible regrouping of the chromosomes of the generative nucleus into a parallel arrangement making translocation of the nucleus to the pollen tube and further mitotic transformations in the narrow tube easier.

Mitosis in the generative nucleus in the *T. bracteata* pollen tube has a similar course as in other species with large chromosomes and narrow tubes (Sax and O'Mara, 1941; Venema and Koopmans 1962; Kwack and Kim, 1967). The chromosome arrangement different than in somatic cells is conditioned by the length of the nucleus and the disproportion between the chromosome size and the tube diameter. In extreme cases as for instance in *T. bracteata* the chromosomes in the metaphase plate are arranged linearly and parallelly to the long axis of the pollen tube.

It is interesting that the mitotic transformations in the generative nucleus of *Tradescantia* are beginning with prophase, initiated by the chromosomes situated at one end of the long nucleus (exceptionally by chromosomes at both ends). Centromere division and segregation of chromatids to the poles occur also successively from one of the ends of the tandem arrangement in metaphase. Moreover, each chromatide of the same chromosome, translocating to the pole parallelly to one another cover different distances. The arrangement of metaphase chromosomes and the course of segregation of sister chromatids in the pollen tube of *T. bracteata* indicate that each chromosome has a separate individually functioning spindle. Similarly as in somatic mitosis, substances impairing the karyokinetic spindle produce disturbances in the normal movement of anaphase chromosomes during mitosis in the pollen tube (Tarkowska and Matuszewska, 1975).

It would seem, therefore, that in somatic mitosis, in contrast to that in the pollen tube, mechanisms operate ensuring simultaneous or almost simultaneous shortening of chromosomes in prophase and their separation in anaphase. The common spindle and simultaneous segregation of non-sister chromatids to the poles of this spindle in usual somatic mitosis

are due to strict structural and functional coordination of the individual chromosome spindles.

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Pyłek Tradescantia bracteata in vitro, rozwój łagiewek i mitoza

Streszczenie

In vitro 80—90% pyłku *Tradescantia bracteata* kielkuje po 15 minutach. W większości łagiewek po 3 godzinach rozpoczyna się mitoza. Całość przemian mitotycznych w jądrze generatywnym ma charakter asynchroniczny. W znacznie wydłużonym, profazowym jądrze generatywnym chromosomy są rozmieszczone równolegle do siebie i jednocześnie są względem siebie poprzesuwane. W profazie chromosomy skracają się w określonej kolejności począwszy od jednego z krańców jądra. W metafazie chromosomy tworzą tandemowy układ liniowy i ułożone są wzdłuż łagiewki. Podział centromerów oraz rozdział chromatyd w anafazie jest inicjowany zwykle od jednego chromosomu znajdującego się na końcu jądra generatywnego, pozostałe chromosomy dzielą się sukcesywnie. Dwie chromatydy z jednego chromosomu pokonują drogi różnej długości.

Sugeruje to, że chromosomy mają indywidualne wrzeciona mitotyczne oraz, że kordynacja przemian chromosomowych w komórce generatywnej jest znacznie słabsza niż w typowej mitozie somatycznej.

Zapasowym materiałem w ziarnach pyłku *Tradescantia* są drobne ziarna skrobi, barwiące się jodem na czerwono-brązowo (red starch). Zapasowych ciał białkowych nie ma.

W cytoplazmie ziarna pyłku i łagiewki są drobne pęcherzyki, barwiące się *in vivo* czerwienią obojętną metachromatycznie na pomarańczowo-czerwono.

Właściwe wakuole powstają w kielkujących ziarnach pyłku *de novo*, są zabarwione ortochromatycznie na różowo i nie zlewają się z metachromatycznymi pęcherzykami.

Po ca 50 min. jądro wegetatywne i komórka generatywna przemieszczają się do łagiewki.

W 80% wypadków pierwsze do łagiewki wchodzi jądro wegetatywne. W pozostałych przypadkach jądro wegetatywne wyprzedza potem komórkę generatywną, przeciskając się wzdłuż niej w łagiewce.

Zmiany kształtu jąder oraz wyprzedzanie komórki generatywnej przez opóźnione jądro wegetatywne w łagiewce wskazują na aktywny udział jąder w przemieszczaniu się ich w łagiewce.

PLATE I

Mature and germinating *Tradescantia bracteata* pollen grains

- 1—3. Mature pollen grain of *T. bracteata*
 - 1 — stained with acetoorcein
 - 2 — stained with neutral red
 - 3 — after PAS reaction
- 4—5. Germinating pollen grains
 - 4 — stained with acetoorcein
 - 5 — stained with neutral red
6. Pollen grain with pollen tube stained, with neutral red
7. Pollen grain with pollen tube inhibited in growth, stained with neutral red
8. Translocation of vegetative nucleus and generative cell to pollen tube stained with acetoorcein

×1100

PLATE II

Mitosis in generative cell of *Tradescantia bracteata*

9. Generative nucleus soon after entering pollen tube
10. Early prophase in generative nucleus
11. Prometaphase in generative nucleus — on left 3 separate chromosomes, 3 others in compact prophase arrangement
12. Metaphase in generative nucleus
13. Anaphase — on right 4 anaphase chromosomes, in middle — 2 pairs of chromatides, on left — group of 4 chromosomes
14. Telophase
15. Two sperm nuclei in pollen tube
16. Two sperm nuclei (right and middle), on left vegetative nucleus

Acetoorcein staining, ×1100

PLATE III

Development of vacuole system in pollen grain of *Tradescantia bracteata* during pollen tube germination

17. Pollen tube germination (10 min) — homogeneous cytoplasm is visible at the site of pollen tube formation
18. Network of vacuoles in vegetative cytoplasm in pollen grain
- 19, 20, 21. Successive steps of pollen grain vacuolization
22. Subapical part of pollen tube (tube length 250 μm , 50 min of tube growth) with dense cytoplasm, homogeneous cytoplasm is visible at tube apex

Intravital observations in medium, ×1100

PLATE I