

The ultrastructure of proplastids in the leaves of five species of Orchids

II. Intraplastid inclusions and DNA-containing areas*

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A b s t r a c t

In the stroma of leaves proplastids of all investigated species of orchids: *Paphiopedilum*, *Stanhopea*, *Cymbium*, *Coleogyne* and *Orchis* occur starch grains of different shape, size and electron density. The number of starch grains vary from one to several. The proplastids from cells of the vascular bundle sheath are completely filled with numerous starch grains and the stroma is strongly reduced. The formation of protein crystalloids was observed only in the proplastids of *Paphiopedilum*.

In the stroma of proplastids in investigated species the DNA-containing areas are present. A tendency of their peripheral situation and of the formation of contacts between DNA fibrils and the inner surrounding membrane or with rare thylakoid's membranes was observed. In these areas in propastids of *Stanhopea*, *Cymbidium*, *Coelogyne* and *Orchis* starch grains of different size, shape and electron density occur.

INTRODUCTION

The stroma of the proplastids of meristematic cells in the growth tips of roots and shoots and meristematic cells in leaves contains various kinds of inclusions.

A commonly met inclusion are granules of starch. Very young proplastids are devoid of starch which usually appears at later stages of the growth of these organoids (Mikulska 1964). Granules of starch may become smaller with the development of inner membranes in the stroma of the proplastids (Hinchman 1972).

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The proplastids of the buds of potato tubers contain membrane-bound inclusions with proteinaceous material (Marinos 1967). Electron-dense granules, usually in literature on the subject identified as phytoferritin, have been found in proplastids in the shoot tips of *Ginkgo biloba*, *Bryophyllum* and *Kalanchoë* (Gifford and Stewart 1967).

In this work particular attention has been devoted to the inclusions in the stroma of proplastids and also to DNA-containing areas.

MATERIAL AND METHODS

The plant material and fixation procedure were as described in the preceding part (Damsz 1975).

OBSERVATIONS AND DISCUSSION

The stroma of the proplastids of five investigated species of orchids was found to contain various types of inclusions. The most frequent were granules of starch. According to the observations of Mikulska (1964) the youngest stages of the proplastids were devoid of granules of starch which appeared in later developmental stages.

The number, size, shape and localization of the granules of starch in the investigated proplastids strongly differ, even within the same species. A cross-section of a proplastid usually shows one or two granules of starch which take up a considerable part of the section surface. The granules are usually located centrally. Sometimes the stroma surrounding the granules of starch form an electron transparent space termed a "halo". Some authors (Marinos 1967; Hinchman 1972) interpret this phenomenon as an artifact due to the differential shrinkage of the starch grain and stroma during dehydration.

The proplastids of the youngest parts of the leaves of *Paphiopedilum* contain single, large, spherical granules of starch, though sometimes several granules may be found (Plate I). These granules show varying grades of electron transparency from fully transparent to electron dense, coloured a dark grey. A large number of starch granules showing various electron density is also found in the proplastids of *Cymbidium* (Plate II, figs. 7—10). The granules can be small and lenticular (fig. 9), large and oval, being 3—4 in number (figs. 7, 10) or long and clavate (fig. 8). In the proplastids of the parenchyma cells surrounding the vascular bundle the granules of starch are so numerous and large that the membrane enveloping the proplastid is sometimes ruptured (Plate III, figs. 11, 12).

The plastids of the cells of the vascular bundle sheath are known to be able to accumulate a large number of granules of starch. This was observed by Laetsch (1968) in the leaves of some plants of the *Amaranthaceae* and *Chenopodiaceae*, by Dobichina (1972) in *Cyperus natalensis* and by Brangeon (1973) in *Zea mays*.

In *Coelogyne* proplastids the granules of starch occur very seldom. The granules are very small and their number ranges from 1 to 3 (Plate III, fig. 13).

A small number of granules of starch, one or two, are also observed in the proplastids of *Stanhopea* (Plate III, fig. 14). These granules are also featured by varying degrees of electron transparency.

In *Orchis* granules of starch are found in almost every proplastid and show a great diversity from small granules to very large ones. All the granules are electron transparent (Plate III, fig. 15; Plate IV fig. 16).

Literature on the subject has not yet elucidated the observed differences in the electron density of the starch granules. Dark belts or folds on the granules observed with electron microscope (Plate I, figs. 3—5; Plate II, figs. 7; Plate III, figs. 11, 12) are explained as artefacts caused by the contact the ultra-thin sections with water during staining (Gallant and Nougarede 1972).

In the investigated *Paphiopedilum* proplastids in the proximity of the starch granules occur lamella, which inside is filled with an electron dense substance (Plate I, figs. 1, 3, 4, 6; Plate V, fig. 26).

A number of authors have presented photographs from electron microscope showing various plastids in which the lamellae, vesicles and prolamellar bodies are in direct association with the granules of starch (Newcomb 1967; Marinos 1967; O'Brien and Thimann 1967; Weier and Brown 1970). According to these authors such an association is not accidental. The observed proximity of the lamella of the plastid and granule of starch suggests an functional cooperation. The membrane participates in the mobilization of starch. Hinchman (1972) suggests the presence of two enzymatic systems participating in the synthesis of starch granules in the plastids in the coleoptile of oats: one connected with the membranes and the other free in the stroma of the plastid. In proplastids, due to the absence of thylakoid membranes, the system responsible for the synthesis of starch is the system stroma-enzyme. This is confirmed by the appearance of the granules of starch which have a smooth surface as opposed to the irregular, spiny surface of an active membrane-enzyme system (Hinchman l.c.). In the light of these data the presence of the membrane near the granules of starch in the proplastid of the described orchids can be assumed to be accidental and not functional. This is confirmed by the smoothness of the surface of the granules.

In the proplastids of *Stanhopea*, *Cymbidium*, *Coelogyne* and *Orchis* the granules of starch are found in areas containing DNA (Plate II, fig.

10; Plate III, fig. 14; Plate VI, figs. 32, 33; Plate VII, fig. 34). Such location of the granules has also been described by other authors (Salema and Badenhuizen 1969; Mikulska and Odintsova 1970; Kowalik and Herrmann 1972; Salema and Abreu 1972; Mikulska et al. 1974).

Besides starch granules the stroma of the mentioned proplastids may also contain osmophilic globuli. In *Paphiopedilum* (Plate IV, fig. 17), *Stanhopea* (fig. 18), *Coelogyne* (fig. 21) and *Orchis* (fig. 22) they are not very numerous (2—3 in cross-section of plastid), whereas a cross-section of *Cymbidium* proplastid reveals from several to several score globuli (Plate IV, figs. 19, 20). They are featured by high electron-density and are irregularly distributed being single, in groups or rows along the periphery of the proplastid. The presence of osmophilic globuli in various types of plastids was observed by a number of investigators.

The stroma of the proplastids of *Paphiopedilum* contains structures not observed in the four species of orchids described above. These structures, which are electron-dense, occur in the form of a fine crystalline lattice (Plate V). Their structure shows them to be protein crystalloids without any definite shape — no clear-cut division between the edge of the crystalloid and stroma of the plastid is observed which allows the assumption that they are in a stage of development.

I was not able to find in the literature any data on the occurrence of protein crystalloids in proplastids, though they are known to be characteristic for the mature leuco- and chloroplasts of some plants of the *Orchidaceae* family. Behnke (1971, 1973) described protein crystalloids in the plastids of sieve cells from plants of the *Aristolochiaceae*, *Amaryllidaceae*, *Liliaceae* and *Pontederiaceae* families. Laser (1974) observed two kinds of protein crystalloids in plastids of the sieve cells of *Sorghum bicolor* (*Gramineae*). The author distinguished cuneate crystalloids composed of parallel filament subunits and crystalloids found in the proplastids of *Paphiopedilum* resemble the crystalloids composed of tubular subunits which Laser (l.c.) classified as type P₂. Larsson (1973) described the ultrastructure of protein crystalloids found in the stroma of isolated spinach chloroplasts incubated in a hypertonic medium and found them to be composed of units with dimensions 20—40 × 50—60 Å. Wrischer (1973), basing on the example of pea chloroplasts, argues that protein crystalloids occurs in dense regions of the stroma when the concentration of proteins reaches a certain specific level and is accompanied by loss of water. The stroma of the proplastids of *Paphiopedilum* is in fact the most electron dense of all the proplastids described in this paper and this may explain why protein crystalloids are observed within it and not in the proplastids of the other species.

Worth attention is the fact that *Paphiopedilum* proplastids contain besides protein crystalloids also granules of starch and osmophilic globuli (Plate V, fig. 24).

The stroma of the proplastids of all the investigated species contains electron transparent areas in which DNA fibrils are visible. In *Paphiopedilum* these areas on the cross-section of the proplastid are the least numerous and the least visible compared with similar areas in the proplastids of other species. Often they cannot be discerned at all. However, it is possible to find an electron transparent area, faintly delimited from the remaining stroma, containing very fine DNA filaments (Plate V, fig. 23, arrow; Plate VI, fig. 28, arrow).

The DNA-containing areas are clearly visible in the proplastids of *Stanhopea* (Plate VI, Figs. 29—33). Inside the electron transparent area some of the fibrils, probably artificially clumped, form a core-like structure giving off delicate fibrils. The areas in question are usually located on the periphery of the proplastid (Plate VI, figs. 29, 30). This fact enables observation of the association between DNA fibril and the surrounding membrane (Plate VI, figs. 30, arrow). If the area is near the inner single lamella of the proplastid sometimes an association between DNA and membrane of a thylakoid can be observed (Plate VI, fig. 31, arrow). Sometimes a cross-section of *Stanhopea* proplastids reveals so many DNA-containing areas that it is difficult to determine their number (Plate VI, fig. 32). This may, as suggested by Kowallik and Herrmann (1972), be a single, strongly ramified area.

In *Cymbidium* proplastids the shape of the DNA-containing areas resembles that in *Stanhopea* proplastids (Plate VII, figs. 34—36). They are visibly separated from the remaining part of the stroma. The areas may be close to each other (Plate VII, figs. 35, 36) or scattered in the stroma, often in the proximity of the membrane.

In *Coelogyne* a cross-section of a proplastid reveals several DNA-containing areas (Plate VII, figs. 37, 38). As in *Stanhopea* and *Cymbidium* they can occur close to each other and seem to be interconnected (Plate VII, figs. 37). Also in this case the peripheral localization allows observation of the association between DNA filaments and inner surrounding membrane (Plate VII, fig. 38).

Sometimes a connection between DNA filament and the still scarce thylakoids can be seen. However, it is difficult to follow the attachment of DNA to thylakoid membranes in the sections, because numerous dense ribosome particles, localized close to the membranes, mask it.

In *Orchis* the areas are difficult to distinguish due to the comparative electron transparency of the proplastid stroma. The areas are fairly large and DNA filaments very fine.

The shape of the DNA-containing areas in the observed orchid proplastids does not differ from the shape of similar areas described in leuco-

chloro- and chromoplasts. The stroma of such areas is always loose and contains a central body giving off in various directions fine DNA fibrils. In the proplastids of orchids with dense stroma the nucleoid-like areas are easily distinguished whereas such areas in proplastids with electron-transparent stroma are difficult to observe, hence the difficulty in determining their shape, number and distribution. According to Kowallik and Herrmann (1970) proplastids contain a single, central DNA-containing region.

The often observed peripheral arrangement of the nucleoid-like areas in the described orchid proplastids may be explained by the fact that part of the DNA fibrils is connected with the inner surrounding membrane. Bisalputra and Burton (1969, 1970) described a similar peripheral location of the area and connections between DNA fibrils and membranes in the plastids of *Sphacelaria* sp. cells. Sprey and Gietz (1973) present proof of the existence of a membrane-DNA complex in etioplasts isolated from the leaves of *Hordeum vulgare* L. Connections between DNA fibrils and the membranes of thylakoids in isolated pea chloroplasts have also been described by Mikulska et al. (1974).

The observed localization of starch granules in the nucleoid-like areas of orchid proplastids confirms the view of a number of investigators on the biological activity of DNA-containing areas in the accumulation of reserve substances in the plastids (Salema and Badenhuizen 1969; Mikulska et al. 1974).

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Ultrastruktura proplastydów liści pięciu gatunków storczyków

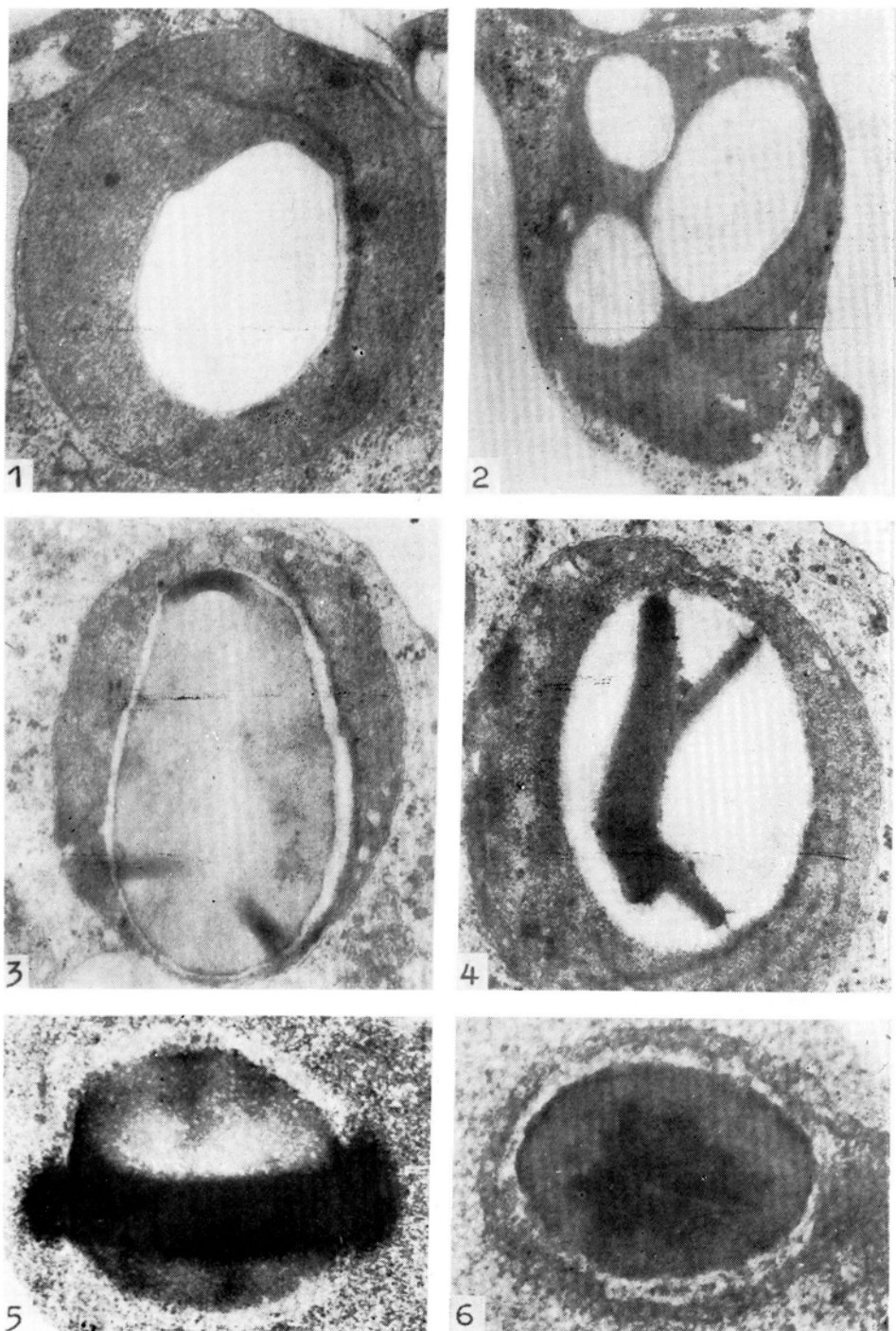
II. Inkluzje wewnątrz plastydowe i obszary zawierające DNA

Streszczenie

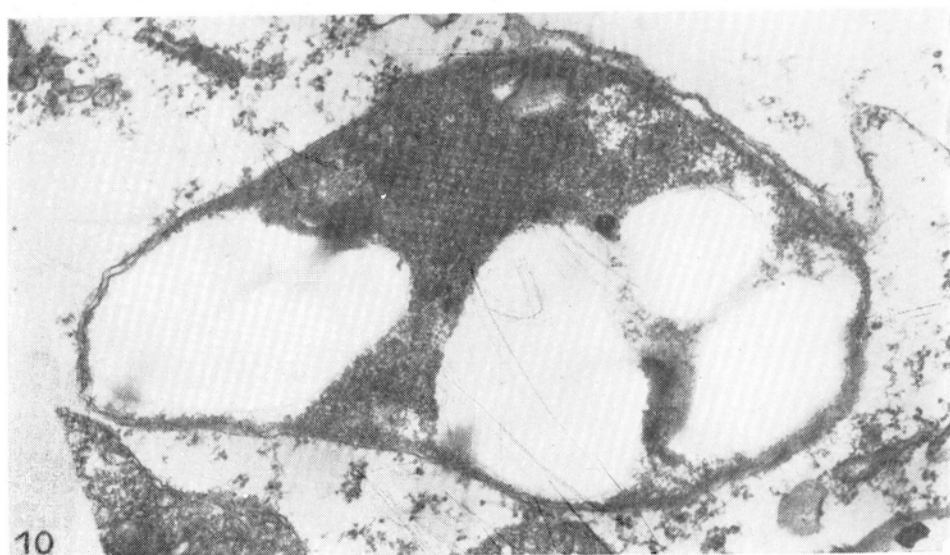
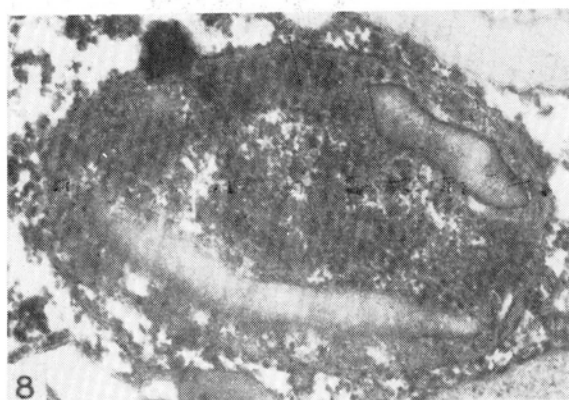
W stromie wszystkich proplastydów liści badanych storczyków: *Paphiopedilum*, *Stanhopea*, *Cymbidium*, *Coelogyne* i *Orchis* występują ziarna skrobi, różnego kształtu, wielkości i gęstości dla elektronów. Liczba ziaren skrobi może być różna, od jednego do kilku. Proplastydy komórek otaczających wiązki całkowicie wypełnione są licznymi ziarnami skrobi, a stroma jest bardzo zredukowana.

Tworzące się krystaloidy białka obserwowałem tylko w proplastydach *Paphiopedilum*.

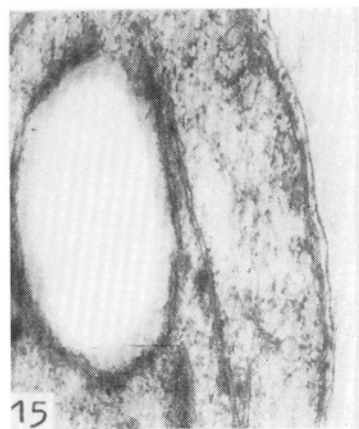
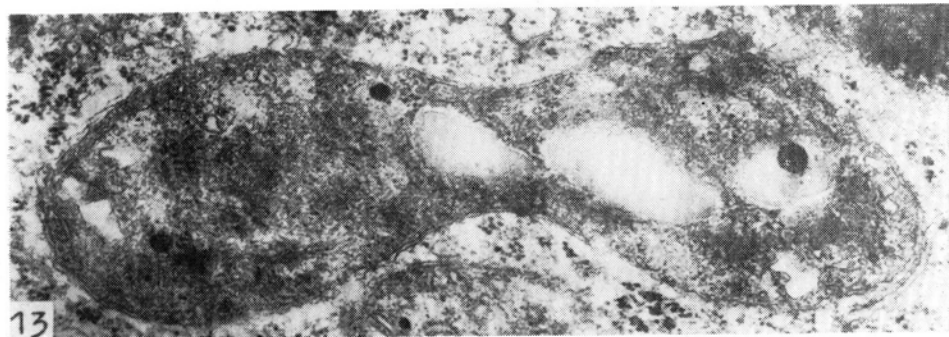
W stromie proplastydów wszystkich badanych gatunków storczyków znajdują się areale z DNA. Zaznacza się tendencja do peryferycznego ich ułożenia, a także do kontaktowania się fibryll DNA z wewnętrzną membraną otaczającą lub z membranami nielicznych tylakoidów. W arealach proplastydów *Stanhopea*, *Cymbidium*, *Coelogyne* i *Orchis* występują ziarna skrobi.



Figs. 1—6. Proplastids of the leaves of *Paphiopedilum mastersianum* showing granules of starch with varying electron-transparency. Near the granules visible lamellae (1: $\times 36\,400$, 2 and 3: $\times 25\,000$, 4 and 5: $\times 28\,000$, 6: $\times 84\,000$)



Figs. 7—10. Proplastids of the leaves of *Cymbidium insigne* with starch granules of different size, shape and electron density (7: $\times 25\,600$, 8: $\times 42\,000$, 9 and 10: $\times 27\,000$)



Figs. 11, 12. Proplastids of cells surrounding the vascular bundle in leaves of *Cymbidium insigne* showing numerous granules of starch (11: $\times 35\,000$, 12: $\times 26\,000$)
 Fig. 13. Proplastid of the leaf of *Coelogyne cristata* ($\times 30\,000$)
 Fig. 14. Proplastid of the leaf of *Stanhopea tigrina*. Visible granules of starch near the DNA-containing areas (arrows) ($\times 44\,000$). Fig. 15. Fragment of proplastid of the leaf of *Orchis latifolia* with small, electron-transparent granule of starch ($\times 31\,000$)

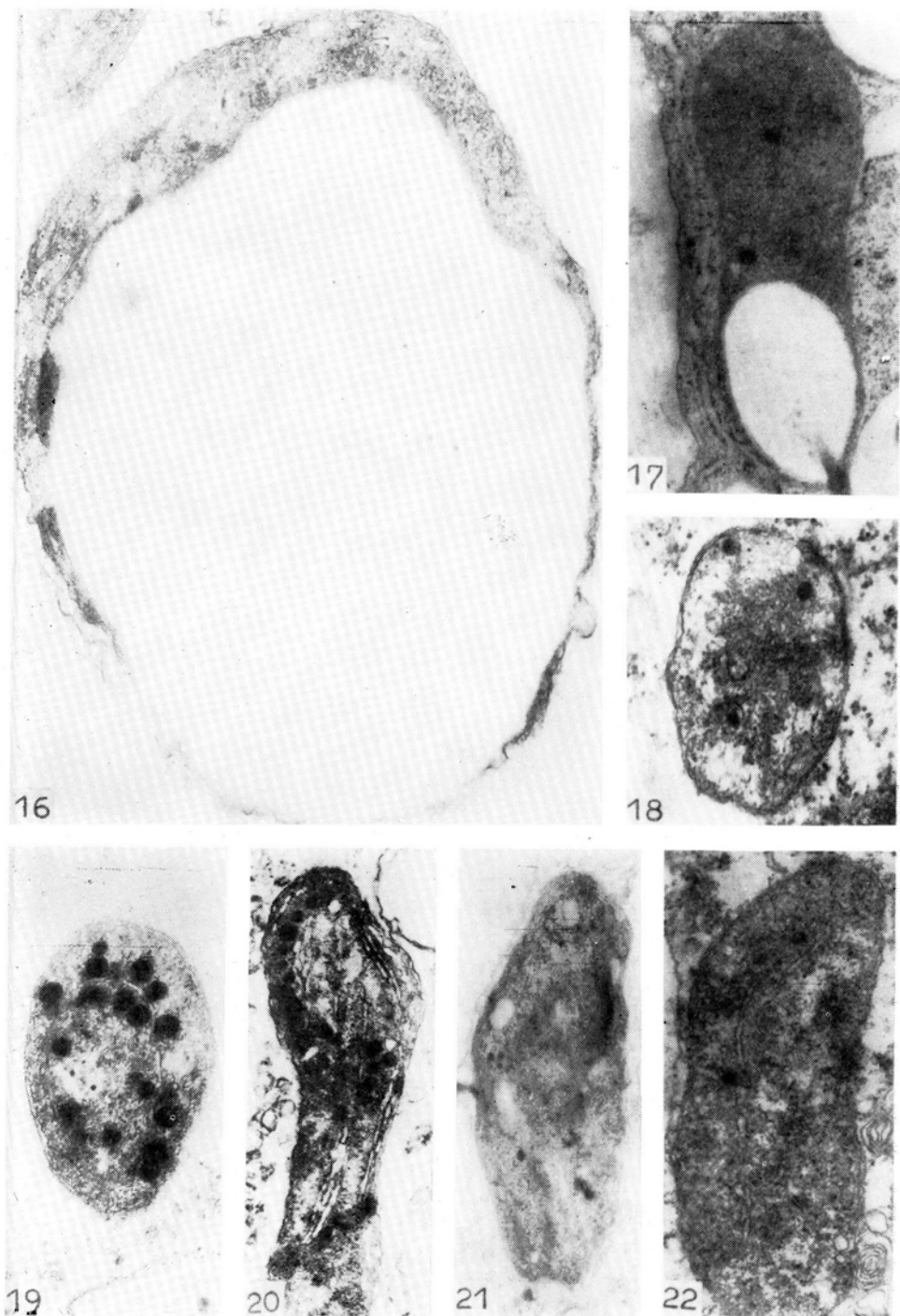
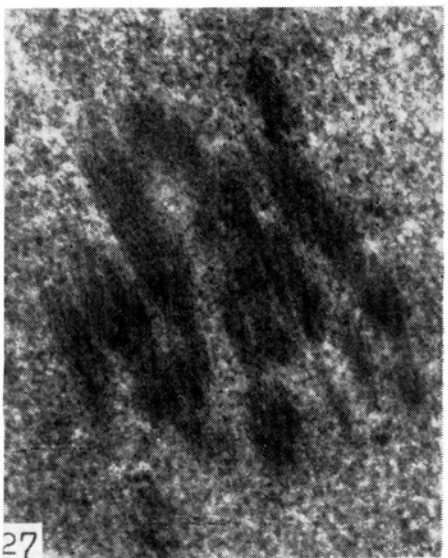
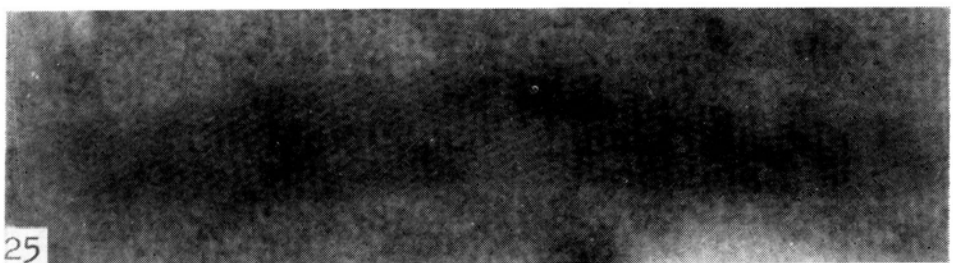
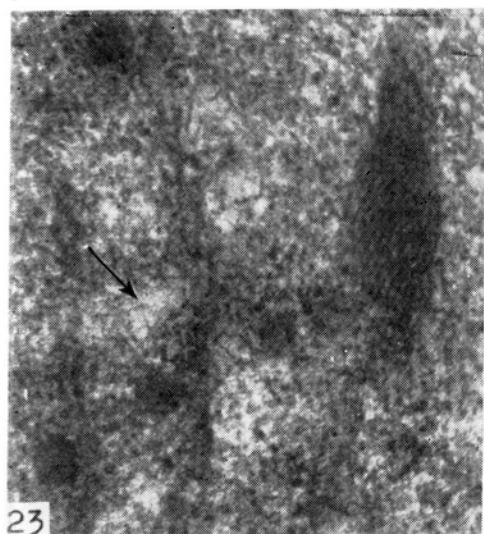


Fig. 16. Large granule of starch in proplastid of leaf of *Orchis latifolia* ($\times 26\,400$)
 Figs. 17—22. Osmophilic globules in stroma of proplastids of the leaves of orchids:
 17. *Paphiopedilum mastersianum*. ($\times 14\,400$), 18. *Stanhopea tigrina* ($\times 13\,000$), 19
 and 20. *Cymbidium insigne* ($\times 26\,000$), 21. *Coelogyne cristata* ($\times 26\,000$) 22. *Orchis*
latifolia ($\times 26\,000$)



Figs. 23—27. Different stages of the growth of protein crystalloids in the stroma of proplastids of the leaves of *Paphiopedilum mastersianum* (23: $\times 97\,000$, 24 and 25: $\times 79\,000$, 26: $\times 86\,800$, 27: $\times 76\,500$)

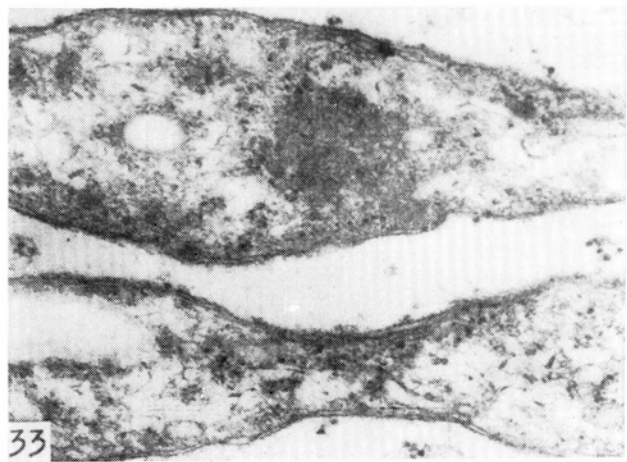
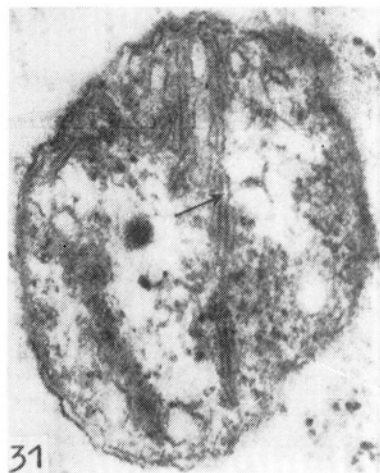
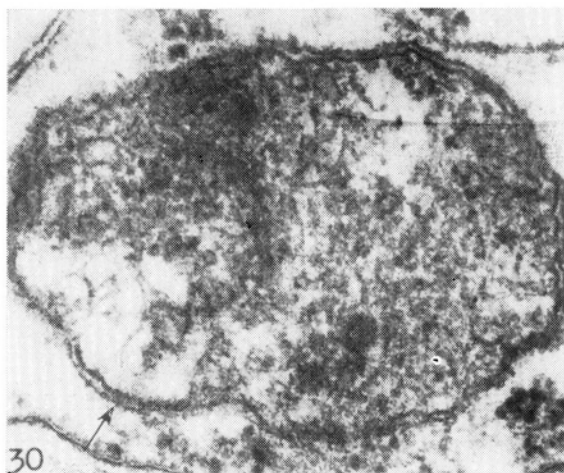
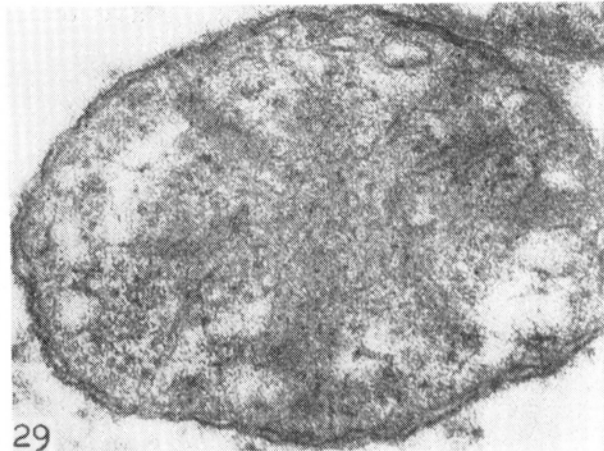
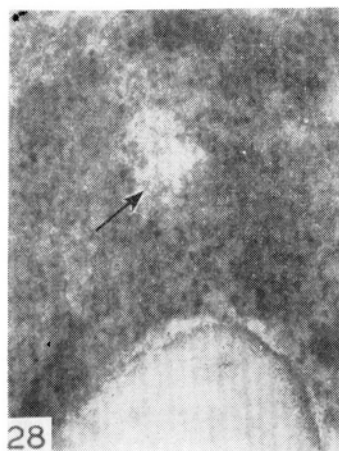
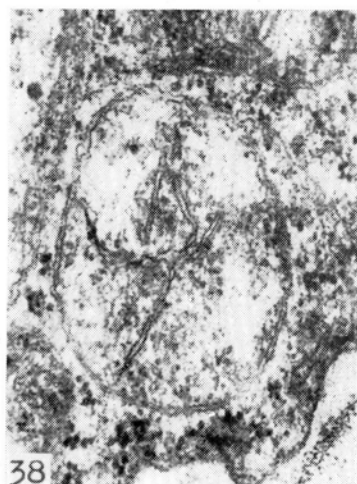
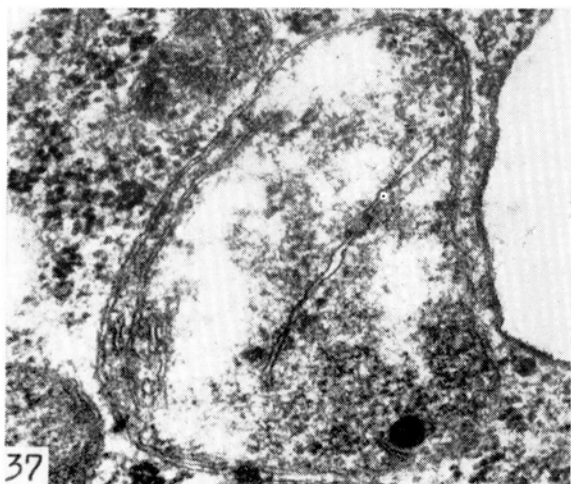
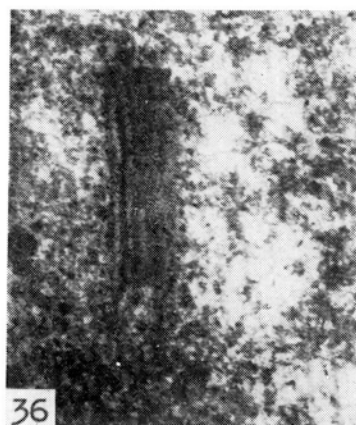
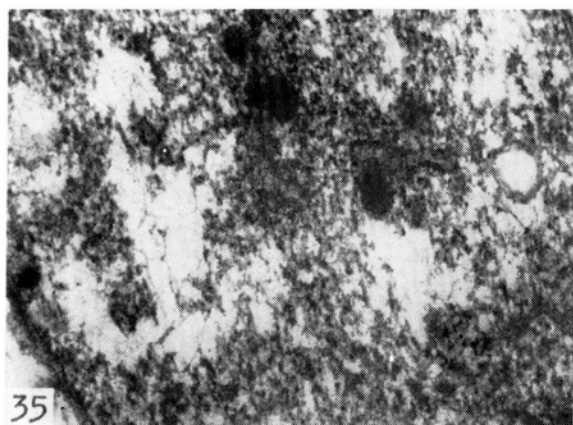
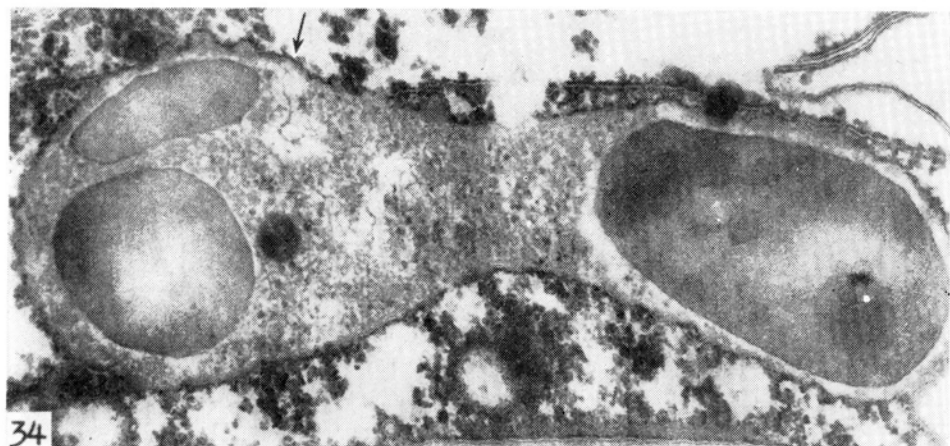


Fig. 28. Fragment of proplastid of leaf of *Paphiopedilum mastersianum* with DNA-containing area (arrow) ($\times 80\,500$). Figs. 29—33. Proplastids of the leaves of *Stanhopea tigrina* with DNA-containing areas. 29, peripheral arrangement of DNA-containing areas on cross-section of proplastid ($\times 84\,400$); 30 and 31, visible association between DNA fibrils and inner surrounding membrane or membrane of thylakoid (arrows) (30: $\times 90\,000$, 31: $\times 37\,000$), 32 and 33, DNA-containing areas and various stages in the formation of granules of starch ($\times 32\,000$)



Figs. 34—38. DNA-containing areas in the stroma of proplastids of orchid leaves: Figs. 34—36. *Cymbidium insigne*; visible association between DNA fibrils and inner surrounding membrane (arrow) (34: $\times 61\ 000$, 35: $\times 80\ 200$, 36: $\times 87\ 000$) Figs. 37, 38. *Coelogyne cristata*; Peripherally distributed DNA-containing areas (37: $\times 64\ 000$, 38: $\times 36\ 800$)