Tropical Agricultural Research Vol. 10 1998, 254-263

Ultra Structural Features of Tea [*Camellia sinensis* (L.) O. Kuntz.] Pollen

S.K. Sathyapala, L.M. Guan[1], T. Adachi[1]

Tea Research Institute
Mid Country Station
Kandy, Sri Lanka

ABSTRACT. *Tea [Camellia sinensis (L). O. Kuntz.] is the major economic crop in Sri Lanka. Thus, there is an increasing demand for high yielding quality clones of tea. However, the present conventional breeding programmes of tea are unable to meet this demand. Application of advanced methods has been hindered due to the inadequacy of fundamental knowledge of male and female gametophyte of tea which is a prerequisite. Although in many plant species the fine structure of the pollen has been studied, no investigations are reported in genus Camellia, particularly in tea.*

The present study investigates the fine structure of the pollen (before and after anthesis) using the transmission electron microscopy and provides the details of pollen wall, vegetative cell and generative cell. The immature and mature pollen had different rER configurations, plastid formation and mitochondrial vesicles. Unlike in the mature pollen, the distribution of the chromatin in the nucleus of the immature pollen was not distinct.

The noteworthy feature of the mature pollen of tea was the absence of plastids or plastid like organelles in the generative cell cytoplasm, which produced evidence for the maternal inheritance of the plastid DNA of tea.

INTRODUCTION

In angiosperms, pollen grain represents the male gametophyte in sexual reproduction. The major function of the pollen grain is to deliver the male gametes through pollen tube for fertilization and eventual development of seed.

---

1 Laboratory of Applied Genetics and Plant Breeding, University of Miyazaki, Miyazaki, Japan.

With advancement of plant biotechnology use of pollen in gene transfer, cloning of genes and other applied breeding research has considerably increased. Thus, knowledge of pollen biology is crucial and an important prerequisite for application of novel techniques for crop breeding programmes. The development of transmission electron microscope together with appropriate preparation techniques have lead to a better understanding of internal anatomy of pollen in many plants (Cresti *et al.*, 1975, 1988; Schroder, 1984).

Tea [*Camellia sinensis* (L.) O. Kuntz.] is the main export crop in Sri Lanka. The production of high yielding quality clones is the main task in tea. Since it is a highly heterozygous, virtually self–incompatible perennial crop with a long breeding cycle, the production of homozygous lines is an immediate requirement in breeding programmes.

Studies on pollen biology of tea are indispensable to elucidate the self incompatibility, barriers for haploid production and breeding of tea. However, it has been largely ignored and the few publications that report on pollen biology are also limited to cytogenetical studies on meiosis through acetocarmine staining (Kato and Shimura, 1970), pollen grain morphology (Chen and Lin, 1991) and fluorescence microscopic studies on pollen development (Sathyapala and Adachi, 1995). The objective of this study was to demonstrate the fine structure of the pollen grain (before and after anthesis) of *C. sinensis*, which may facilitate access to future breeding programmes and finally crop improvement of tea.

MATERIALS AND METHODS

Immature pollen of late binucleate stage (six days before anthesis) and mature pollen were gathered from anthers of *C. sinensis* (L.) O. Kuntz. *cv.* Yabukita grown in the experimental field of Miyazaki University, Japan.

Fresh pollen grains were fixed directly in 2.5% gluteraldehyde and 1.5% paraformaldihyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature for 4 h. Glutaraldehyde–alcian blue (22.5% mixture) was used for improved visualization of the pollen grain. The samples were post fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) at 4°C for 3 h. Fixed samples were dehydrated in graded ethanol series and embedded in ERL 42–06 resin mixture. Ultra thin sections were cut on ultra microtome using glass knives. Silver and gold coloured sections were transferred to 0.05 mm diameter, 200 mesh formwar–coated copper grids. The specimens were

double stained using uranyl acetate and lead citrate and were examined under a Hitachi H–300MV transmission electron microscope at 75 kv.

RESULTS AND DISCUSSION

Pollen wall of the mature pollen grains of tea consists of two distinct layers (Figure 1). The outer layer (exine) is differentiated into sexine and nexine. The exine is composed essentially of electron dense sporopollenin both on the foot layer and in the space between the valleys that are formed by bacula. The inner layer (intine) formation was complete and consisted of a fibrillar material, which is completely condensed, and a thin layer of amorphous material. The intine was heterogeneous with an outer, electronlucent zone and inner zone that contains irregular, tubular, evaginations or plasma membrane (Figure 1). The vegetative cell contained very large and irregular shaped nucleus. In mature pollen, the chromatin in the nucleus was aggregated in several little masses with variable electron density (Figure 2).

The distribution of chromatin in immature pollen was not distinct as in mature pollen. In the vegetative cell cytoplasm, rough endoplasmic reticula (rER) and smooth endoplasmic reticula (sER) were observed in concentric and in stacked form. The presence of numerous number of rER in a stacked form in the vegetative cell cytoplasm has been reported in *Beta vulgaris* (Jensen *et al.*, 1974), *Lycopersicum peruvianum* (Cresti *et al.*, 1975) and *Linaria vulgaris* (Cresti *et al.*, 1988). Most of the sER was seen near the generative cell and vegetative nucleus while some single rER cistern, can be seen near the periphery of the cell (Figure 2, 4). In immature pollen the quantity of rER was less and the stacked form was rare in contrast to the mature pollen. Similar rER configurations have been reported in the inactive plant tissues (Shih and Rappaport, 1971) and suggest low rER activity in mature pollen of tea. Consequently the metabolism of the mature binucleate pollen of tea could be subdued. Accordingly, mature tea pollen grain is in a phase of stasis before pollination and pollen becomes active only after hydration. Jensen *et al.* (1974) reported that stacked rER forms the storage site of nutrient materials necessary for pollen tube growth. This further confirms the role of the vegetative cell as a storage organ.

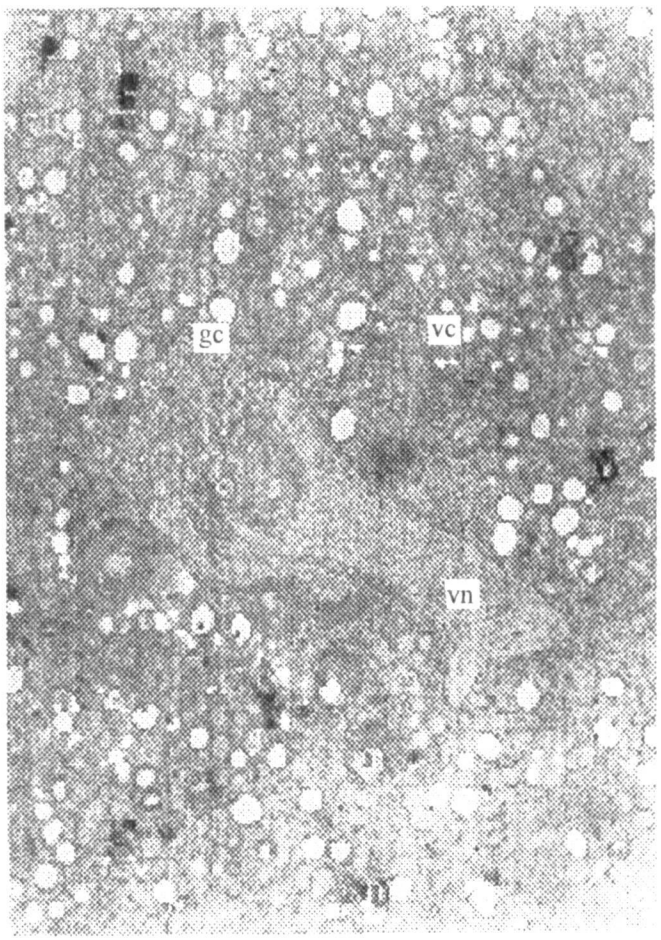

Figure 1. Ultra structure of the vegetative cell of the tea pollen at the early bi nucleate stage.

[Note: The nucleus of the vegetative cell is irregular in out line. Transverse section of the generative cell is present in this section of the pollen grain. *X* 8500. gc - generative cell, gn - generative nucleus, vn - vegetative cell nucleus.]

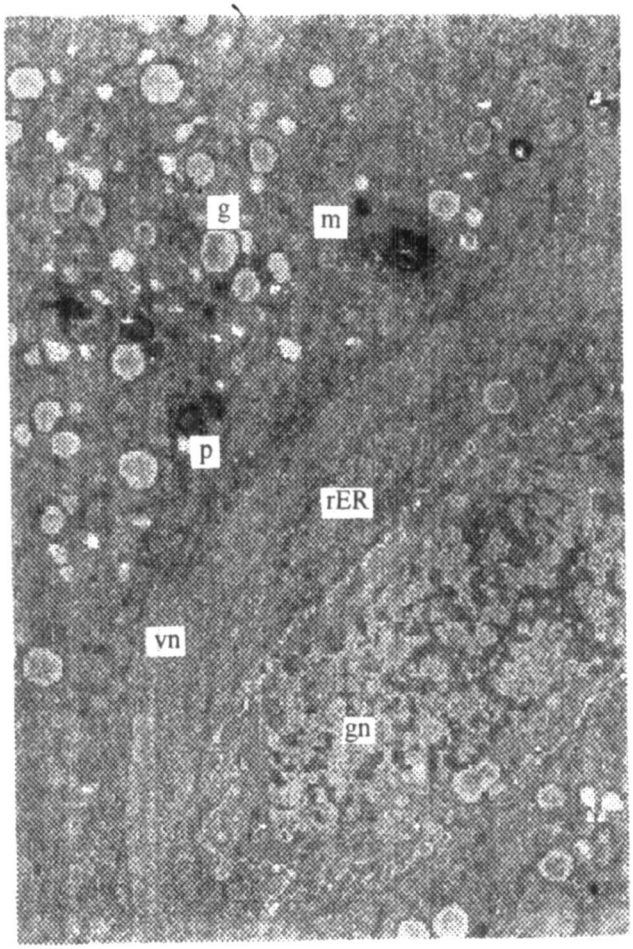

Figure 2. **Ultra structure of the immature pollen (early bi nucleate) of tea.**

[Note: Transverse section through the generative cell. *X* 20,000. gc - generative cell; gn - generative nucleus, vn - vegetative cell nucleus, m - mitochondria, rER - rough endoplasmic reticulum, vc - vegetative cell, p - plastids, g - golgi vesicle.]

Unlike in the mature pollen the vegetative cell cytoplasm of the immature pollen had large number of plastids with starch grains and osmiophilic globules. Both vegetative cell cytoplasms were abundant with randomly distributed spherical lipid bodies of varying sizes, large number of small vacuoles, numerous golgi vesicles and many cisternae of sER (Figure 3). The ribosomes were evenly distributed in the cytoplasm. Mitochondria observed in the cytoplasm had a few cristae and lacked dense matrices. Dictyosomes were occasional.

The generative cell was located in the central part of the pollen grain and delimited from the vegetative cell by conspicuously lobed plasma membrane. In both mature and immature pollen the generative cell was surrounded by the vegetative cell (Figures 1 and 4). The nucleus of the generative cell was large, lobed and assumed the shape of the cell (Figures 1 and 3). The generative nucleus of the mature pollen was highly heterochromatic with electron dense particles (Figures 3 and 4) and the aggregation was not distinct in the immature pollen (Figure 1). The generative cell cytoplasm was separated from the vegetative cell cytoplasm by a distinct wall with evaginations. The cytoplasm of the generative cell contained mitochondria vesicles, ribosomes, dictyosomes, and longitudinally arranged bundles of microtubules and small vacuoles. The mitochondria showed poorly developed matrices and had few cristae. The proplastids were not been observed in the generative cell cytoplasm.

In angiosperms, the mode of plastid inheritance depends on the mode of plastid distribution during the development of the male gametophyte. The unequal distribution or degeneration of plastids during the first pollen mitosis or during generative cell maturation may cause the absence of plastids in mature generative cell (Schroder and Hagman, 1985; Van Went, 1984). In genus *Camellia* the maternal inheritance of the plastids was reported by *in situ* detection of plastid DNA in generative cell (Correiveau and Coleman, 1988). The presence of plastids in vegetative cell cytoplasm and absence of these in generative cell cytoplasm elucidate the maternal inheritance of the plastid DNA in tea.

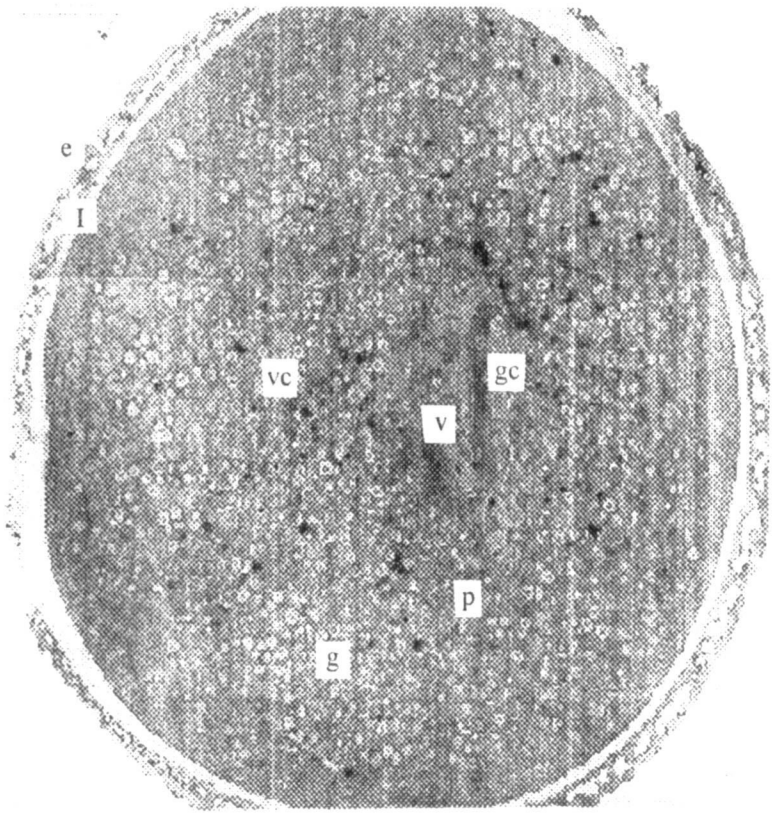

Figure 3. **Ultra structure of a mature pollen grain composed of two cells, a large vegetative cell and a smaller generative cell.**
[Note: A bi-layered wall, the outer exine and the inner intine enclose the vegetative cell. *X* 6500. e - exine, I - intine, gc - generative cell, vc - vegetative cell, p - plastids, g - golgi vesicle.]

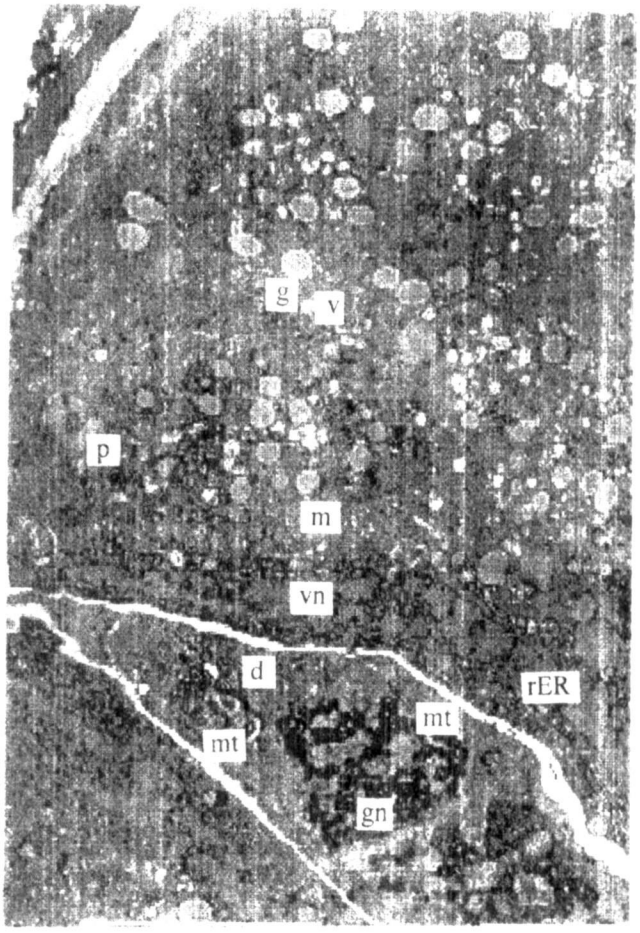

Figure 4. Enlarged portion of transverse section through the generative cell of tea at the mature pollen stage.

[Note: The two plasma membranes of the vegetative and the generative cell are clear. The cytoplasm of the generative cell contains less organelle, whereas the cytoplasm of the vegetative cell contains many inclusions. Stacked rER are clear in the vegetative cell cytoplasm. The generative nucleus is highly heterochromatic with many electron dense particles. *X* 22,000. v - vacuole, mt - microtubular, gc - generative cell, gn - generative nucleus, vn - vegetative cell nucleus, m - mitochondria, rER - rough endoplasmic reticulum, vc - vegetative cell, p - plastids, g - golgi vesicle.]

CONCLUSIONS

The study revealed the internal structure and the organelles of the tea pollen, which is a prerequisite for applied research in tea breeding and pollen biotechnology. In immature and mature pollen, different forms of rough and smooth ER were identified. High content of the stacked rER, which has a direct correlation to the metabolism, was observed in the mature tea pollen. Distribution of chromatin in the nucleus of immature pollen was not distinct. The observations on the fine structure of the pollen verified the absence of plastids in the generative cell cytoplasm and consequently the maternal inheritance of the plastid DNA in tea. This has been the first ultra structure study of pollen in the genus *Camellia*.

ACKNOWLEDGEMENTS

Financial assistance provided by the Japanese Government and the study leave granted by Tea Research Institute, Sri Lanka for the postgraduate study are gratefully acknowledged.

REFERENCES

Corrieveau, J.L. and Coleman, A.W. (1988). Rapid screening method to detect bi parental inheritance of plastid DNA and results for over 200 angiosperm species. Amer. J. Bot. 10: 1443-1458.

Chen, R. and Lin, H. (1991). The studies on pollen morphology of different tea varieties. Proc. Int. Symp. Tea Science Shizuoka, Japan.

Cresti, M., Pacini, E., Sarfatti, G. and Asimoncioli, A.C. (1975). Structural features and storage formation of *Lycopersicum peruvianum* pollen. pp. 19-38. *In*: Mulcahy, D.L.M. (Ed). Gamete Competition in Plants and Animals. North Holland Publ. Co., Amesterdam.

Cresti, M., Milansei, C., Tiezzi, A., Ciampolini, F. and Moscatelli, C. (1988). Ultrastructure of *Linaria vulgaris* pollen grains. Acuta. Bot. Neer. 37: 379-386.

Jensen, W.A., Ashton, M. and Heckared, L.R. (1974). Pollen of *Scrophulariaceae* contains stacked ER in cytoplasm. Bot. Gaz. 135: 210-218.

Kato, M. and Shimura, T. (1970). Cytological studies on *Camellia* species 1. The meiosis and gametogenesis of *C. wabiske* compared with *C. japonica* and *C. sinensis*. Jpn. J. Breed. 32: 276-277.

Sathyapala, S.K. and Adachi, T. (1995). Fluorescence microscopic studies on pollen of tea (*Camellia sinensis* L.). Proc. Int. Symp. Tea Science. Shanghai, China. pp. 90-102.

Schroder, M.B. (1984). Ultra structural studies on plastids on generative and vegetative cells in *Liliaceae*. Protoplasma. 124: 123-129.

Schroder, M.B. and Hagmann, R. (1985). Plastid content of generative cells in *Liliaceae*. pp. 52. *In*: Willimse, M.T.M. and Van Went J.L. (Eds). Proc. 8th Int. Symp. Sexual Reproduction in Seeds, Plants, Fern and Mosses. Wageningen, The Netherlands.

Shih, C.Y. and Rappaport. L. (1971). Regulation of bud rest in tubers of potato *Solanum tuberosum* L. VIII. Early effect of gibberellin and abscisic acid on ultrastructures. Plant Physiol. 48: 31-35.

Van Went, J.L. (1984). The ultrastructure of impatiens pollen. pp. 81-88. *In*: Linskens, H.F. (Ed). Fertilization in Higher Plants. North Holland, Amsterdam.