

Development and Degeneration of the Tapetum in Garlic, *Allium sativum* L.

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Introduction

Garlic, *Allium sativum* L., shows a wide range of variation on bolting. Usually many clones of garlic do not produce flower-stalks at all. While some clones produce them always, some other clones exert them partially. In the inflorescences on top of the flower-stalks, the bulbils are formed always, but the flower-buds are formed only in some limited clones. All these clones are, however, completely sterile, and the microspores degenerate prior to pollen mitosis²⁾.

The tapetum in the male sterile plants, especially in the cytoplasmic male sterile plants, has often been implicated in the direct or indirect cause of the abortion of pollen mother cells, or pollen grains⁴⁾. Degeneration and abnormal development of the tapetum have particularly been mentioned in relation to its presumed nutritive function during microsporogenesis.

For the reasons mentioned above, here in this paper the development and the degeneration of the tapetum in garlic were observed anatomically, or cytologically, in comparison with those in fertile Welsh onion, *A. fistulosum* L., and leek, *A. ampeloprasum* L.

Materials and Methods

The materials for paraffin-sectioning were cv. Shanhai-wase of garlic, and cv. Kujo of Welsh onion. Squash methods with aceto-carmin were applied to complement the observation. The materials for squash methods were cv. Shimonita-negi and a local variety from Nissato, Utsunomiya, of Welsh onion, and cv. American London of leek in addition to cv. Shanhai-wase and cv. Kujo. Leek is closely related to garlic among the cultivated *Alliums*¹²⁾. Four cultivars of Welsh onion and leek are fertile, though cv. American London of leek is tetraploid.

The flower-buds for paraffin-sectioning were fixed in Belling's modified Navashin fluid without saponin immediately after being cut off. The embedded materials were sectioned at a thickness of 9 μ and stained with Heidenhain's iron hematoxylin, modified Cajal basic fuchsin-indigocarmin stain, or Flemming's triple stain. Bordeaux red, Acid fuchsin, Light green, or Orange G was used to stain cytoplasm in the hematoxylin method, though hematoxylin was solely used at some occasions.

Observations

Development and degeneration of the tapetum in garlic

The anther-wall consists of four layers, and the innermost layer is tapetum. The tapetum was characterized by the densely staining protoplasts and prominent nuclei.

From the premeiotic stage to the zygotene or the pachytene, the tapetal cells showed regular arrangement, tightly connecting each other (Figs. 1–3). The tapetal cells were not so large or thick as those of the maximum sized ones observed at uninucleate pollen stage; and their inner surface toward the pollen mother cells were smooth. Their nuclei contained small chromatin granules and achromatin (Fig. 17).

At the pachytene or the diplotene, the tapetal cells and their nuclei began to increase in size, forming rough inner surface (Figs. 4, 5, 18, 19). After the diplotene or the diakinesis, the tapetal cells increased rapidly in size, and the chromatin granules in their nuclei enlarged (Figs. 6–9, 20, 21). The tapetal cells came to be easily separated from each other by squash methods, and their cytoplasm was stained darker after the interkinesis or the second division of meiosis (Fig. 22).

At the pollen-tetrad stage, the nuclei and the cytoplasm of tapetal cells increased their contents markedly (Figs. 10, 23). The sign of degeneration was wholly unobserved before the pollen-tetrad stage. After the microspores were released from the pollen tetrads into the anther sac, the microspore-wall began to thicken itself and developed into two layers. And then, the tapetal cells attained to their maximal development (Figs. 11, 24). At this stage, the inner surface of the tapetum was rough, but hypertrophy, periplasmodium, or pseudopodium-like incursions of the tapetum were not found here. The tapetal cells remained uninucleate.

After the maximal development, vacuoles, or vesicles looking like small bubbles, appeared in the cytoplasm of tapetal cells, and the tapetum began to degenerate (Fig. 25). At this stage, the cytoplasm of microspores was not stained well with aceto-carmin both in garlic and fertile Welsh onion. Moreover, the microspores at the same stage were diminished easily in size by paraffin methods. It might be vacuolate-microspore stage as Laser and Lersten⁴⁾ mentioned. Consequently, it was difficult to determine precisely which began to degenerate earlier of the tapetum and the microspores. Vacuolation in the tapetum, however, was supposed to have begun a little before the beginning of the degeneration of microspores.

The chromatin granules in the tapetal nucleus increased in size and decreased in number. The vacuoles in the tapetum enlarged, fusing together. Some tapetal nuclei split themselves during the tapetal degeneration (Figs. 26, 27). At this stage as shown in Fig. 26, the nuclei of the microspores were partly degenerated. The tapetum became to degenerate gradually into exhaustion, while the microspores degenerated to form empty pollen grains. Only the scattered remnants of the tapetal cells were observable when nearly two thirds of microspores were on the way of degeneration and the rest of them were made to be empty already (Fig. 29). The pollen mitosis in the degenerating microspores was observed quite rarely, though almost all the microspores had degenerated before that (Fig. 28). The tapetal cells, even the remnants, were not found when most of the degenerating microspores became empty (Fig. 30).

Development and degeneration of the tapetum in Welsh onion and leek

The tapetum of Welsh onion developed similarly to that of garlic until the pollen tetrads were formed (Figs. 13, 31). In Welsh onion, the tapetal development attained maximum at the same stage of microsporogenesis as that in garlic (Figs. 14, 32). The inner surface of the tapetum in Welsh onion seemed not to be so rough as that in garlic. And then, the tapetum got degenerated gradually (Fig. 15). Small vacuoles in the tapetal cytoplasm appeared before the pollen mitosis (Fig. 33) and enlarged during the pollen mitosis (Fig. 34). The tapetal nuclei had changed their shapes, decreasing in size until the early binucleate-pollen stage (Fig. 35). Fusion of the degenerating tapetal cells was observed sometimes at the advanced stage of degeneration. The endothecium

was developed well in contrast with the tapetal degeneration (Fig. 16). Only the remnants of the tapetum were sparsely observed at the advanced stage of pollen maturing (Fig. 36), and finally all the tapetum disappeared (Fig. 37). The microspores, or pollen grains, increased their contents during the tapetal degeneration. Differences in the tapetal development and degeneration were not seen among the three cultivars of Welsh onion by squash methods.

In leek, the tapetal development was similar to that in the other two species, excepting the larger nucleus of leek (Fig. 38). After the maximal development of the tapetum was reached at the uninucleate pollen stage (Fig. 39), vacuolation began (Fig. 40) and progressed prior to pollen mitosis (Fig. 41). The tapetal cells had been degenerated fairly at the pollen mitosis stage (Fig. 42). At the binucleate pollen stage as shown in Fig. 43, the tapetal cells exhibited only their scattered remnants. Vacuolation occurred in the cytoplasm and in the nucleus of the tapetum cells in leek simultaneously. On the other hand, in garlic and Welsh onion it occurred first in the cytoplasm, and their tapetal nuclei without cytoplasm were often observed at the advanced stage of tapetal degeneration by squash methods.

The behavior of chromosomes at the meiosis was normal in three cultivars of Welsh onion and in one cultivar of leek. Cv. Shanhai-wase of garlic, however, showed a chromosome configuration of $1_{\text{VIII}} + 4_{\text{II}}$ at metaphase-I as some other cultivars of garlic did¹⁾.

Discussion

The relationship between the developing microspores and the tapetum has been studied in many of the male sterile plants. The major abnormalities of the tapetum in cytoplasmic male sterile plants mentioned by Laser and Lersten⁴⁾ were as follows; early degeneration, periplasmodium, hypertrophy, vacuolation, persisting, or remaining intact.

In *Allium*, some male sterile plants were investigated on the relationship between the tapetum and the degenerating microspores. Monosmith (cited by Jones and Mann³⁾) found hypertrophy and abnormal degeneration of tapetum prior to the degeneration of microspores in the sterile onion. Tatebe¹⁰⁾ observed the tapetal degeneration in the fertile onion at the stage of pollen mitosis and ascertained vacuolation, periplasmodium and pseudopodium-like incursions in the sterile onion after the pollen-tetrad stage was reached. Nakamura⁶⁾ showed the tapetal disintegration in the fertile onion at the pollen-tetrad stage and the abnormal development of the tapetum in the sterile onion at the same stage. Nishi and Hiraoka⁷⁾ reported vacuolation and enlargement of the tapetal cells in the sterile Welsh onion immediately after the pollen-tetrad stage. Virnich¹¹⁾ noticed that the tapetum remained intact in the sterile onion after the pollen degeneration set in. Saini and Davis⁹⁾ reported hypertrophy and abnormal degeneration of the tapeta in the sterile *A. cepa* L. and in the sterile F₁ hybrids of *A. cepa* L. × *A. pskemense* B. Fedtsch.

Novak⁸⁾ observed hypertrophy of tapetum at the postmeiotic stage in *A. longicuspis* Regal and a cultivar of garlic; both of which showed normal chromosome configuration, 8_{II} , at metaphase-I of meiosis. *A. longicuspis* Regal is sterile, and it is supposed to be a wild ancestor of garlic⁵⁾. He concluded that the primary cause of the sterility in both species could be found "in the false function of tapetum as the sporogenous tissue expressing by its hypertrophy". However, hypertrophy was not found here, though the enlargement of the tapetal cells as shown in Fig. 11 was observed at the uninucleate pollen stage. The similar enlargement of the tapetal cells was observed also in the fertile Welsh onion, although the inner surface of tapetum was rougher in garlic than in Welsh onion. Moreover, Virnich¹¹⁾ showed a similar enlargement of the tapetal

cells and rough inner surface in the fertile onion at the pollen-tetrad stage.

In the present observation, the tapetum of garlic persisted until the uninucleate pollen stage, as that of the fertile Welsh onion did. The behavior of the tapetum in garlic was similar to that in the fertile Welsh onion. Hypertrophy, periplasmodium, or pseudopodium-like incursions of tapetum were not found in garlic. Vacuolation in the tapetum began at almost the same stage in the three species used. Consequently, no big difference between garlic and the two fertile species was found here in the development and degeneration of tapetum during microsporogenesis. It is probable that the sterility of cv. Shanhai-wase used here does not result from the histological abnormality of the tapetum, though it is uncertain whether the tapetum functions properly.

Summary

Development and degeneration of the tapetum in the sterile garlic were observed and compared with those of fertile Welsh onion and leek by paraffin and squash methods. The tapetum attained the maximal development at the uninucleate pollen stage in the three species. Vacuolation and degeneration of tapetum began at almost the same stage in the three species after the maximal development. Almost all the uninucleate pollen grains in garlic disintegrated to be empty prior to pollen mitosis. The tapetum of garlic degenerated during the uninucleate pollen stage, and even a remnant of the tapetum was not found at the time when most of the degenerating pollen grains became empty. On the other hand, the degenerating tapeta of Welsh onion and leek were observed during the pollen mitosis, and their scattered remnants of tapetum were seen at the advanced stage of binucleate pollen. Tapetal abnormality such as hypertrophy, periplasmodium, pseudopodium-like incursions were not found in both of the cultivars of garlic and of Welsh onion used here.

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Explanation of figures

Figs. 1–12. Tapetum of garlic observed by paraffin methods during microsporogenesis.

Fig. 1. Premeiotic stage. Fig. 2. Leptotene. Fig. 3. Zygotene. Fig. 4. Pachytene. Fig. 5. Diplotene. Fig. 6. Diakinesis. Fig. 7. Metaphase-I. Fig. 8. Interkinesis. Fig. 9. Telophase-II. Fig. 10. Pollen-tetrad stage. Fig. 11. Uninucleate pollen stage. Fig. 12. Degenerating tapetum at uninucleate pollen stage. Figs. 1, 3, 11. $\times 450$. Figs. 2, 4, 6–10, 12. $\times 310$. Fig. 5. $\times 400$. (Figs. 1–5, 8, 9, 11, 12: transverse sections. Figs. 6, 7, 10: longitudinal sections.)

Figs. 13–16. Tapetum of Welsh onion observed by paraffin methods after meiosis.

Fig. 13. Pollen-tetrad stage. Fig. 14. Uninucleate pollen stage. Fig. 15. Degenerating tapetum at uninucleate pollen stage. Fig. 16. Remnants of tapetum and well-developed endothecium at binucleate pollen stage. Figs. 13–16. $\times 220$. (transverse sections)

Figs. 17–30. Tapetum cells of garlic observed by squash methods during microsporogenesis.

Fig. 17. Zygotene. Fig. 18. Pachytene. Fig. 19. Diplotene. Fig. 20. Diakinesis. Fig. 21. Metaphase-I. Fig. 22. Telophase-I to metaphase-II. Fig. 23. Pollen-tetrad stage. Fig. 24. Uninucleate pollen stage. Fig. 25. Beginning of vacuolation in the tapetum at uninucleate pollen stage. Fig. 26. Degenerating tapetum with split nuclei at the advanced stage of vacuolation. Fig. 27. Degenerating nuclei of tapetum. All the pollen grains are disintegrating. Fig. 28. Scattered remnants of tapetum. Fig. 29. Exhausted tapetum cells. Fig. 30. Empty pollen grains and a degenerating pollen grain. Tapetum is not found. Figs. 17–29. $\times 300$. Fig. 30. $\times 150$.

Figs. 31–37. Tapetum cells of Welsh onion observed by squash methods after meiosis.

Fig. 31. Pollen-tetrad stage of cv. Nissato. Fig. 32. Uninucleate pollen stage of cv. Nissato. Fig. 33. Beginning of vacuolation in the tapetum of cv. Nissato at uninucleate pollen stage. Fig. 34. Enlarged vacuoles in the tapetum of cv. Shimonita-negi at the stage of pollen mitosis. Fig. 35. Degenerating tapetum of cv. Nissato at early binucleate pollen stage. Fig. 36. Scattered remnants of tapetum of cv. Nissato at binucleate pollen stage. Fig. 37. Maturing pollen grains of cv. Shimonita-negi. Tapetum has been disappeared. Figs. 31–36. $\times 300$. Fig. 37. $\times 150$.

Figs. 38–43. Tapetum cells of leek observed by squash methods after meiosis.

Fig. 38. Pollen-tetrad stage. Fig. 39. Uninucleate pollen stage. Fig. 40. Beginning of vacuolation in the tapetum at uninucleate pollen stage. Fig. 41. Vacuoles linked together in the tapetum at uninucleate pollen stage. Fig. 42. Exhausted cytoplasm and vacuolated nucleus of a tapetum cell at the stage of pollen mitosis. Fig. 43. Scattered remnants of tapetum at the late binucleate pollen stage. Figs. 38–42. $\times 300$. Fig. 43. $\times 810$.

e=endothecium, p=pollen grain, PMC=pollen mother cell, pt=pollen tetrad, t=tapetum, v=vacuole.







