Wandr lárynthe in the embryo sac of *Nigella damascena* Linn.

M R VIJAYARAGHAVAN, GAYATRI MISRA and VEDANTAM SUJATA*

Department of Botany, University of Delhi, Delhi 110 007, India

MS received 7 December 1987

Abstract. The embryo sac of *Nigella damascena*, with wall ingrowths over entire surface and distinctly formed wandlabyrinth at the poles, displays a well integrated model for short distance transport of nutrients. The degenerating nucellus creates an influx of metabolites into the embryo sac. The embryo sac wandlabyrinth play a dual role—initially aid with the influx of metabolites into the embryo sac, but later participate in endosperm wall formation. The two synergid apices exhibit prominent filiform apparatus which function differently. One of the synergids degenerates immediately after fertilization where filiform apparatus helps in receiving the pollen tube. The other undamaged synergid persists along with filiform apparatus upto the octant embryo stage and this filiform apparatus probably facilitates short distance metabolite transport into the synergid. The antipodal cells show 3 types of wall ingrowths—(i) wall ingrowths at the antipodal-nucellus interface which are long, branched and spread fan-like into the antipodal cytoplasm, indicating the flow of metabolites into the antipodes from the nucellus, (ii) inter-antipodal wall-ingrowths that are small, papillate and present on both sides of the wall indicating the exchange of metabolites between the antipodes and (iii) wall labyrinths at the antipode-central cell interface which are very small and are directed into the antipode cytoplasm.

Keywords. Embryo sac wall; synergids; antipodes; wall ingrowths.

1. Introduction

Since the publications of Gunning and Pate (1974) the occurrence of wandlabyrinthhe (wall ingrowths) have been reported in numerous plant tissues including the cells of female gametophyte (Schulz and Jensen 1968; Marinos 1970; Rifot 1973; Zhukova and Sokolovskaya 1977; Vijayaraghavan and Bhat 1984). A correlative histochemical study of the egg apparatus, the central cell and the antipodes is, however, lacking. *Nigella damascena* proved an ideal material for such a study. The present paper describes the presence of wandlabyrinthhe on the embryo sac wall and at the interfaces of both the antipodes and the synergids with the nucellus.

2. Materials and methods

Ovules of *N. damascena*, at progressive stages of development, were fixed in precooled 10% aqueous Acrolein for 24 h at 0°C. Dehydration, infiltration and embedding were carried out as per the method of Feder and O'Brien (1968). An AO Spencer Rotary microtome, fitted with a locally devised adaptor, was used to cut 2 μm thin, sections using glass knives. Periodic acid Schiff’s (PAS) reagent was used for the localization of insoluble polysaccharides (Jensen 1962). A few slides were counterstained with Aniline blue-black (Fisher 1968). Coomassie brilliant blue was also used to localize total proteins.

*Since deceased.
3. Results

The embryo sac of *N. damascena* is 8 nucleate, lodged in an anatropous ovule and is of the Polygonum type (Maheshwari 1950). The mature embryo sac shows an egg apparatus with two synergids and an egg at the micropylar end, two polar nuclei in the central cell and 3 antipodal cells at the chalazal end. After fertilization, the embryo sac wall elongates at the chalazal pole thus causing the antipodes to shift to a lateral position (figure 1f). The synergids, the central cell and the 3 antipodal cells show well-developed wall ingrowths both before and after fertilization.

3.1 Synergids

The synergid apices show well developed, elaborate filiform apparatus (FA) with extensive finger-like projections. They extend upto 1/3 the length of the synergid cytoplasm. The FA stains intensely with PAS reaction (figure 1a, b) but they are, however, protein negative. The synergid cytoplasm takes dense stain for proteins and polysaccharides. After fertilization, one of the synergids, along with the FA, degenerates, whereas the other synergid remains undamaged. The FA of the undamaged synergid persists upto the octant embryo stage.

3.2 Central cell

The central cell wall shows over its entire inner surface, before and after fertilization, prominent, intensely PAS positive wall ingrowths (figure 2a) that are always directed into the embryo sac. These wall ingrowths, before fertilization, are small and papillate (figure 2a) but after fertilization increase in length, branch and become labyrinth of wall material (figure 1c, d). The central cell cytoplasm is seen adpressed to the antipodal cells and shows numerous polysaccharide grains. At about the late preglobular embryo stage the wall ingrowths develop uniformly over the inner boundary of the embryo sac wall, extend into the coenocytic endosperm and divide the endosperm nuclei into compartments (figure 1e). These wall ingrowths traverse the embryo sac cavity from both sides and anastomose in the centre.

3.3 Antipodal cells

The antipodal cells, after fertilization, occupy a lateral position due to the elongation of the embryo sac at the chalazal end (figure 1f). A few nucellar cells positioned below

---

*Figure 1.* a, c and d. After PAS-staining. b. Coomassie brilliant blue stained. e and f. After PAS-aniline blue-black staining. a. Longisection of ovule showing the synergid cytoplasm and densely PAS-stained FA at the micropylar end of the embryo sac (×185). b. Longisection of the synergids with FA at the micropylar end of the embryo sac. The FA do not stain with Coomassie blue (×260). c. Intensely stained embryo sac wall projections (×290). d. Ovule showing embryo sac wall ingrowths. The nucellus is two layered and outer integument cells show lyses (×220). e. Longisection of ovule showing late proembryo and endosperm nuclei interspersed between embryo sac wall projections (×110). f. The embryo sac elongates at the chalazal end and results in lateral position of antipodes. The embryo sac wall also shows fibrillar wall ingrowths (×110).
Wandlabyrinthe in the embryo sac of *N. damascena*

**Figure 1.**
Figure 2. a–d and g. After PAS-aniline blue-black staining. e and f. After PAS staining.
a. Ripe unfertilized embryo sac with the central cell and antipodal cells. The antipodal cells show negligible wall projections (× 115). b. Part of the fertilized embryo sac showing the antipodal cells with small wall projections at the base (× 260). c. The antipodal cells at the 2-celled embryo stage, show small wall projections at the base of the antipodal cells. The walls separating the 3 antipodal cells show small papillate wall projections. The boundary wall of the antipodal cells also shows dense wall projections (× 330). d. Antipodal cells at 3-celled proembryo stage, are based on a small nucellar stalk and show prominent well branched wall projections at the base. The walls separating antipodal cells show thinner wall projections (× 460). e. Enlarged view of the antipodal cells showing the nucellar stalk with numerous PAS grains and the antipodal cells showing the wall projections at the base (× 610). f. The interantipodal walls thin down considerably and show a beaded appearance (× 610). g. Longisection of ovule showing the degenerating antipodal cells at the late proembryo stage. The degenerating antipodal cells show few wall projections (× 280).

(ant, Antipodal cells; cc, central cell; ea, egg apparatus; esw, embryo sac wall; n, nucleus; nu, nucellus; sy, synergid; ‡ wall ingrowths).
the antipodes invariably take dense stain for proteins and polysaccharides and are compactly arranged compared to the remaining nucellar cells (figure 1f). These cells, during further progressive stages, accumulate polysaccharide grains and present a stalk-like structure over which the 3 antipodes are perched. The antipode cytoplasm takes moderate stain with PAS reaction whereas the antipode walls stain well for polysaccharides (figure 2b–d). The antipodal nuclei show large quantity of distinctly oriented heterochromatin and large nucleoli with numerous vacuoles (figure 2a–c). Immediately after fertilization the antipodal cells also display numerous vacuoles which decrease during the progressive stages and finally disappear (figure 2d). The antipodal cell walls are endowed with 3 types of wall ingrowths— (i) those at the antipodal-nucellar interface which are long, branched that spread fan-like into the antipode cytoplasm. No wall ingrowths are present towards the nucellus (figure 2b–e), (ii) inter-antipodal walls that are small and papillate and present on both sides (figure 2b, c) and (iii) antipode-central cell interface shows very small wall ingrowths that are invariably directed into the antipode cytoplasm (figure 2b, c). Before fertilization, these wall ingrowths are poorly developed (figure 2a) but after fertilization many small protuberances of PAS positive wall material are observed at the antipode-nucellar interface (figure 2b). At the zygote stage, the inter-antipodal walls reveal maximum development after which they gradually thin down. The wall ingrowths at the antipode-central cell interface also gradually thin down during the progressive stages. The antipodal cells attain a large size and continue to increase in size until about the 4 celled proembryo stage when the wall ingrowths at the antipode-nucellar interface increase in length and density and traverse to about 1/4 the length into the antipode cytoplasm (figure 2e). These wall ingrowths are very well developed, branch considerably and stain intensely with PAS reaction. The inter-antipodal walls also thin down considerably and present a beaded appearance (figure 2f). By about the late proembryo stage, the antipodal cells begin to lyse and concomitantly the wall ingrowths also breakdown (figure 2g).

4. Discussion

The highly specialised cellular adaptations in which wandlabyrinthe increase the surface area of the plasma membrane are termed ‘wall membrane apparatus’ and such cells with these adaptations as ‘transfer cells’ (Gunning and Pate 1969, 1974). In this context, the central cell, synergids and antipodal cells of _N. damascena_ may be referred to as ‘transfer cells’. Such cell types with wall ingrowths were also reported in _Capsella bursa-pastoris_ (Schulz and Jensen 1968), _Linum usitatissimum_ (Vazart 1969), _Paspalum longifolium_ (Yu and Chao 1979), _Helianthus annuus_ (Newcomb and Steeves 1971), _Aquilegia vulgaris_ (Rifot 1971, 1973), _Conium maculatum_ (Dumas 1978) and _Iberis amara_ (Prabhakar and Vijayaraghavan 1983).

In _N. damascena_ the wall ingrowths are well integrated into the structure of the female gametophyte with its set of wall membrane apparatus at the micropylar and chalazal ends of the embryo sac and also in the central cell. These wall ingrowths in the embryo sac cells show a temporal relationship. The development of wall ingrowths initiated at the micropylar end with the formation of FA in the synergids, proceeds toward the central cell and finally, after fertilization, culminates in the massive growth of wall projections within the antipodal cells. In _N. damascena_, except for the central cell, the maximum development of wall ingrowths is always followed by
senescence, thereby implying the temporal importance for the development of wall ingrowths in the various cells of the gametophyte. Even spatially these wall ingrowths exhibit a set pattern. At the micropylar end, before and after fertilization, the FA consists of numerous wall ingrowths which are papillate and small. Similarly, the embryo sac wall ingrowths are fibrillar and short before fertilization, but after fertilization prolong excessively and participate in the endosperm compartmentation. In comparison, the wall ingrowths of the antipodal cells become massive and branched only after fertilization. Thus the set of wall membrane apparatus in the different cells of the embryo sac have strongly marked individual functions and aid in bringing about an influx of nutrients generated by the disintegrating nucellus into the embryo sac.

The FA of the two synergids in *N. damascena* function differently. While the FA of the degenerating synergid may have assisted in directing the pollen tube growth (Vijayaraghavan and Bhat 1984), the FA of the undamaged synergid persists until after fertilization and may facilitate short distance transport of nutrients into the synergids. The general absence of wall at the chalazal end of the synergids and the presence of cell wall contours at the micropylar end of synergids to form FA, endorses the role of synergids as transfer cells. The synergids due to their proximity to the egg cell may aid in nourishment.

The central cell wall ingrowths are present both before and after fertilization in *N. damascena* as also reported in *H. annuus* (Newcomb and Steeves 1971). In *C. bursa-pastoris* they develop only after fertilization and continue to increase in size even during the heart-shaped embryo stage (Schulz and Jensen 1968, 1969). In *Lobelia*, both the chalazal and micropylar ends show wall projections during embryogenesis (Torosian 1971). In *I. amara* wall ingrowths at the chalazal end become prominent only after the heart-shaped embryo stage (Prabakar and Vijayaraghavan 1983). In *N. damascena*, the wall ingrowths are found over the entire surface of the embryo sac wall. In *Pisum sativum* such wall projections are also found over the entire surface of the embryo sac wall but the quantum of elaboration is more near the micropylar end at which the growing embryo is located (Marinos 1970). In *H. annuus* embryo sac wall projections occur from a point approximately 1/3 the distance from the micropylar end of the synergids to roughly the level of the secondary nucleus (Newcomb and Steeves 1971). The embryo sac wall ingrowths in *N. damascena* play a dual role. In addition to absorbing the metabolites they also play an active and direct role in dividing the coenocytic endosperm into compartments. The involvement of central cell wall projections in compartmentalization of the endosperm has been suggested in *I. amara* and *Alyssum maritimum* (Prabakar and Vijayaraghavan 1983), in *H. annuus* (Newcomb 1973), *stellaria media* (Newcomb and Fowke 1973) and *Quercus gambellii* (Singh and Mogensen 1976). Mares *et al* (1977) also reported endosperm wall formation through the centripetal growth of wall projections in the central cell (see also Vijayaraghavan and Prabakar 1985).

Wall ingrowths are usually concentrated on those faces of the cell presumed to be most active in solute transfer (Gunning and Pate 1969). Applying this adage to the antipodal cells in *N. damascena* one can derive that the antipodal-nucellar interface is most active possessing the greatest degree of development of wall ingrowths. This is further emphasized by the distinctly developed nucellar cells at the bottom of the antipodal cells showing aggregation of proteins and polysaccharide grains. In *N. damascena* the inter-antipodal walls show well developed wall ingrowths whereas
Wandlabyrinthe in the embryo sac of N. damascena

267

The antipode-central cell interface reveal wall ingrowths only toward the interior of the antipodal cells suggesting that the antipodal cells absorb nutrients from the nucellus and act as a sink. It is also felt that the degree of wall ingrowth development may also be connected in some way with the high degree of polyploidy of these cells. On the whole, the antipodal cells present a model where the nutrients are absorbed from all sides and thus act as reservoir of metabolites.

The antipode-nucellar interface wall ingrowths are reported in Aquilegia vulgaris (Rifot 1973), Conium maculatum (Dumas 1978), Paspalum longifolium (Yu and Chao 1979) and Aconitum vulparia (Bohdanowicz and Turala-Szybowska 1985). In Linum usitatissimum (Vazart 1969) peg-like ingrowths were observed at the basal region of antipodal cells. In N. damascena the wall-ingrowths at the antipode-nucellar interface extend deep into the cytoplasm as proliferations of wall material extending upto 1/4 the antipode length. In Linum usitatissimum (Vazart 1969), Aquilegia vulgaris (Rifot 1971, 1973) and Aconitum napellus (Zhukova and Sokolovskaya 1977) and Scilla sibirica (Bhandari and Sachdeva 1985) both the antipode-nucellar interface and the inter-antipodal walls show wall ingrowths, whereas in Gasteria verrucosa (Willemsen and Kapil 1981) wall projections are prominent on chalazal wall and on the wall towards the central cell. This highly polarized distribution of ingrowths within the cells may be caused by a positive interaction with the neighbouring structures.

The structural features and functional potentialities described above, picture the cells in the embryo sac of N. damascena as a module, well equipped, to handle the transport of solutes between the symplast of the plant and its extra-cellular environment. The wall membrane apparatuses as seen in the embryo sac cells of N. damascena suggest that they may have developed only to cope with the nutrient flow into the gametophyte by increasing the surface area of the plasma membrane.

Acknowledgement

The award of a fellowship by the University to one of us (VS) is gratefully acknowledged.

References

Bhandari N N and Sachdeva A 1985 Some aspects of organization and histochemistry of the embryo sac of Scilla sibirica Sato; Protoplasma 116 170–178
Feter N and O'Brien T P 1968 Plant microtechnique: Some principles and new methods; Am. J. Bot. 55 123–142
Fisher D B 1968 Protein staining of ribboned epon sections for light microscopy; Histochemie 16 92–96
Gunning B E S and Pate J S 1969 “Transfer cells”—Plant cells with wall ingrowths, specialised in relation to short distance transport of solutes—their occurrence, structure and development; Protoplasma 68 107–113
Jensen W A 1962 Botanical histochemistry (San Francisco: W A Freeman)
Mares D J, Stone B A, Jeffrey C and Norstog K 1977 Early stages in the development of wheat endosperm. II. Ultrastructural observations on cell wall formation; Am. J. Bot. 25 599–613
Marinos N G 1970 Embryogenesis of the Pea (Pisum sativum). I. The cytological environment of the developing embryo; Protoplasma 70: 261–279
Newcomb W 1973 The development of the embryo sac of sunflower Helianthus annuus before fertilization; Can. J. Bot. 51: 863–878
Newcomb W and Steeves T A 1971 Helianthus annuus embryogenesis, embryo sac wall projections before and after fertilization; Bot. Gaz. 132: 367–371
Newcomb W and Fowke L C 1973 The fine structure of the change from free nuclear to cellular condition in the endosperm of chickweed Stellaria media; Bot. Gaz. 134: 236–241
Prabhakar Kumkum and Vijayaraghavan M R 1983 Embryo sac wall in Iberis amara—its ultrastructure and histochemistry; Phyton (Horn, Austria) 23: 31–38
Schulz S P and Jensen W A 1969 Capsella Embryogenesis: The suspensor and the basal cell; Protoplasma 67: 139–163
Singh A P and Mogensen H L 1976 Fine structure of early endosperm in Quercus gambelii; Cytologia 41: 345–361
Torosian C D 1971 Ultrastructural study of endosperm haustorial cell of Lobelia dunnii Greene (Campanulaceae, Lobelioideae); Am. J. Bot. 58: 456–457
Vijayaraghavan M R and Bhat U 1984 Synergids before and after fertilization; Phytomorphology 33: 74–84
Yu S H and Chao C H 1979 Histochemical studies of ovary tissues during the embryo sac development in Paspalum longifolium Roxb; Caryologia 32: 147–160
Zhukova G Y A and Sokolovskaya T B 1977 Ultrastructure of antipodes of Aconitum napellus (Ranunculaceae) embryo sac before fertilization; Bot. Zh. (Leningrad) 62: 1600–1611