LIGHT AND ELECTRON MICROSCOPIC STUDY OF RADISH CYTOPLASMIC MALE STERILITY IN BRASSICA NAPUS.

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Amongst the different cytoplasmic male sterility systems that are known in rapeseed, the "Ogura" system is interesting since several male sterility inducing cytoplasms are now available. Originally found in radish by Ogura (1968) this cytoplasm provided a stable sterility that was transferred to *Brassica napus*. Unfortunately the plants were chlorophyllous deficient and produced too little nectar (Rousselle, 1982). Through protoplast fusion, male sterile cybrids with normal chlorophyll content and improved nectaries have been obtained (Pelletier *et al*,1983, these proceedings). The recombinant cytoplasms differred from the "Ogura" cytoplasm of radish if one considered their plastidial and mitochondrial genomes (Pelletier *et al*, 1983 ; Chétrit *et al*, 1985 ; Vedel *et al*, 1986) but also in the mechanism of restoration (Pellan-Delourme *et al*, these proceedings). The aim of the present cytological study was to compare microsporogenesis in normal fertile lines and different male sterile cybrids. It will be shown that, despite a broad diversity in stamens morphologies, the process of microspore degeneration is quite uniform in all these male sterile lines.

MATERIALS AND METHODS

Materials. *Brassica napus* lines 2405, Bienvenue and Rapol were mainly used to study the ontogeny of normal fertile pollen grains. A developmental series was also obtained for sterile stamens of 3.22.3.7 line and for the cybrids 27, 58, 77, 85 and 118, field-grown at the Station du Rheu, INRA (France) in 1986 and 1987.

Microscopy. After naked eye and binocular microscope study of flowers, some of the sampled stamens were mounted in Alexander's stain and observed with the light microscope (LM). Sterile and fertile, whole or sliced anthers were fixed in glutaraldehyde (2.5%, v/v) for 2 h at 4°C and post-fixed in osmium tetroxide (2%, w/v) for 1 h, both in 0.1 M-cacodylate buffer (pH 7.2). After dehydration through an ethanol series and propylene oxide, the samples were embedded in Epoxy resin. Untrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope operating at 80 kV (TEM). Semi-thin sections (1 µm thick) from each sample were stained with toluidine blue.

For scanning electron microscopy (SEM), both fertile and sterile flowers devoid of sepals and petals were fixed, dehydrated by the critical point method and then observed after gold sputtering.

RESULTS

The different and complementary methods we have used showed morphological, histological and cytological differences between normal flowers on one side and the abnormal stamens of cybrids and 3.22.3.7 on the other side.

Fertile lines of *Brassica*. The typical morphology of the six (4 + 2) stamens of Cruciferae is well known and easily depicted by scanning electron microscopy. When a submature stamen of *Brassica* was mounted within Alexander's stain, pollen grains escaped from the locules. Malachite green coloured their exine while fuchsin was fixed on the nuclei and cytoplasm. The thickenings of the cell-walls in the endothecium were prominent in whole mounts and in sections of epoxy embedded material. The latter technique gave the most precise data on the pollen grains.

The fertile pollen of *Brassica napus* appeared tricolpate and more rarely tetracolpate. Dry grains were prolate but wetted grains in aqueous fixatives were subspherical. The tectum was reticulate. The columellas were prominent. An almost continuous foot layer was present but the endexine was practically absent. Only a few "white lines" surrounded by a denser material was seen especially under the three or four apertures. So the endexine was extremely thin. It covered a normal intine and cytoplasmic membrane. The voids were filled with a prominent fibrous tryphin. The cytoplasm of the pollen grains was electron-dense because of the compactness of organelles and density of the hyaloplasm. Two sperm cells were found in the pollen grains, their diameter being approximately that of the nucleolus in the vegetative cell.

The ontogeny of rapeseed pollen grains and associated tapetum follows the same steps as those displayed in classical palynological papers (Heslop-Harrison, 1968 ; Dickinson, 1982).It has also been described by Grant *et al* (1986). So we only add some complements to the latter description. The tapetal cells were seen binucleate, with continuity of the external nuclear membrane of both. The plastids of the tapetal cells were crowded around the nuclei during microspore development. Their plastoglobules became very large as well as lipid bodies in the latest stages of tapetum existence. A thin peritapetal membrane was formed and persisted after tapetum disorganization.

Sterile stamens of cybrids 27, 58, 77, 85 and 118. Some of the cybrids that were studied (27, 58, 85) showed little difference in the morphology of their stamens. The six anthers in these cases, each contained four microsporanges -like in normal stamens- but here they were collapsed and dessicated, giving the locule a concave shape instead of convex profile. When sampled at a stage corresponding to release and mounted in Alexander's stain these stamens revealed four green bands each corresponding to a microsporangium derivative. Scanning microscopy and light observation of toluidine blue stained sections indicated that these bands mainly contained collapsed exines interspersed among cytoplasm and nuclear residuals. No pollen grain with cytoplasm and nuclei could ever be found.

A second group of abnormal stamens was observed, though a clear cut could not always be traced between them and the previous type. In cybrid 77 and more typically in cybrid 118 carpelloid bodies were found in place of stamens. These teratologic flowers parts possessed stigmatoid areas at their tip and more basally bore some ovules on their edges. They were bisexuate since they had differentiated some incomplete microsporanges. The latter contained collapsed exines but no pollen.

In both cases i.e. carpelloid and subnormal -though sterile- stamens of the first type, a true endothecium did not appear. The absence of wall thickenings could explain the indehiscence.

The study of resin embedded and sectioned material provided the opportunity of determining the moment of abortion during microsporogenesis. No abnormality was found before the vacuolate stage of microspores. moreover no pollen grain could be found in the locules. Degeneration affected the vacuolate microspores which often seemed to be bound to each other. Their cytoplasm and nucleus became more and more clear probably through autolysis of the protoplasm. The tapetal binucleate cells, before complete degeneration, were highly vacuolate and stuck to the exines.

Sterile stamens of *Brassica* 3.22.3.7 line. Morphologically these stamens were often intermediate between the two previously mentioned types of sterile flowers parts of cybrids. However, the process of degeneration was quite similar. These stamens were also indehiscent.

DISCUSSION AND CONCLUSIONS

Harmomegathy which is the change of shape of pollen grains upon hydration (Wodehouse, 1935) is especially important in prolate, colpate grains (Misset *et al*, 1982). It occurs in *Brassica* fertile pollen grains and cannot be prevented when stamens are fixed with aqueous fixatives. Wetting of the pollen also increases the volume of the tryphin. These remarks only concern the description of the fertile pollen grains.

The main conclusion of the present LM, SEM and TEM study is the uniformity of radish male sterility in rapeseed when examined at the cellular level. Despite the broad diversity in the morphologies of the stamens that vary from subnormal tetrasporangiate stamens in cybrids 27, 58 and 85, to carpelloid bisexuate bodies in cybrid 118, degeneration always occurs during the vacuolate stage of the microspores and follows the same pathway. Development does not go beyond this vacuolate stage. The stability of radish male sterility was already noticed by Ogura (1968), Bartkowiak-Broda *et al* (1979) and Pellan-Delourme (1986). On ultrastructural arguments we now conclude that this stability is maintained in the cybrids.

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