Endosperm responses to irradiated pollen in apples

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Accepted February 17, 1987
Communicated by H. F. Linskens and R. Riley

Summary. The cytological effects of pollen γ-irradiation at 50 and 100 krad on both embryo and endosperm development were studied in Malus × domestica. Fruit and seed set were reduced by increasing doses of pollen irradiation, while embryo sacs resulting from the treatments differed in number and morphology of endosperm nuclei and in the presence or absence of an embryo. Nuclear abnormalities, distinguished from normal nuclear behaviour in embryo sacs derived from unirradiated pollen, included enhanced numbers of polyploid restitution nuclei, bridges between nuclei, excluded metaphase chromosome fragments and disrupted mitotic synchrony. Generally, a high dose of pollen irradiation (100 krad) generated an all-or-nothing response in the embryo sac, either creating highly abnormal embryos and/or endosperms which aborted, or showing relatively normal development. Callus produced from excised cellular endosperm differed in average genome size, that derived from 100 krad pollen being smaller than that from unirradiated pollen.

Key words: Endosperm – Pollen irradiation – Malus – Fluorometry

Introduction

Pollen irradiation disturbs double fertilisation, and subsequently the development and interactions of embryo and endosperm, in a dose-related manner.

Genetic and developmental phenomena associated with irradiated pollen include mutational damage (Werner et al. 1984), selective gene transfer (Shizukuda et al. 1983; Borrino et al. 1985); “egg transformation” (via incorporation of fragments of male DNA after high pollen irradiation doses, Pandey 1978, but see Chyi and Sanford 1985); and the mentor pollen effect used to overcome incompatibility barriers (Wolf and van Tuyl 1984). The degree of matromorphy of the mature progeny is inferred from the frequency of transfer of marker genes carried by the irradiated pollen. In contrast to earlier descriptive work (Brewbaker and Emery 1962; Nishiyama and Uematsu 1957; Vassileva-Dryanovska 1966a, b, c) the cytological events after fertilisation of the egg and fusion nucleus have not been followed, and the endosperm largely ignored despite its potential as a matromorphic or transformed tissue.

This paper combines evidence from embryo sac dissection techniques (Allington 1985) and fluorescence microscopy to outline a new approach to the study of endosperm in exalbuminous seeds. Apple (Malus × domestica Borkh.) was chosen as the model system since it has 1) binucleate pollen (so irradiation occurs prior to division of the generative nucleus, Marcucci et al. 1984); 2) a large accessible embryo sac with a prolonged free nuclear stage of the endosperm; 3) seed parts, including endosperm, which are readily cultured in vitro (James et al. 1984) and 4) shown a possible ‘Hertwig’ response, as James et al. (1985) noted the reappearance of a small number of embryos after high pollen irradiation doses following a decline in embryo numbers at medium level irradiations.

Materials and methods

1 Pollen collection, irradiation and pollination

Pollen was collected from Malus × domestica cv. ‘Basketong’ which carries the dominant homozygous gene for anthocyanin production in all tissues (RR). Pollen was irradiated in Petri dishes using a 60Co source of γ-irradiation (Meat Research Institute, Langford) at a dose rate of 96.679 krad/h to give final doses of 0, 50 and 100 krad. Pollinations were carried out on orchard trees of ‘Cox’s Orange Pippin’ after emasculation of flowers and their reduction to four per cluster. Clusters were bagged after pollination to eliminate contamination by foreign pollen.
2 Fruit sampling and dissections

Fruitlets were sampled at 13–35 days after pollination when the endosperm is mostly free-nuclear, and at 57–65 days after pollination when it is cellular and the embryo is heart-shaped. At the first sampling, most swollen seeds were fixed in ethanol:acetic acid (3:1) for 24 h, then transferred to 70% ethanol for storage. Fixed or fresh embryo sacs were dissected out intact under a stereo dissecting microscope into 0.2 M phosphate buffer (pH 8). Seeds from fruitlets remaining 57–65 days after pollination were removed aseptically. The contents of each seed (nucellus, embryo and endosperm) were separated and placed next to one another on Linsmaier-Skoog hormone-free media (James et al. 1984). Cultures were incubated at 22°C in the dark, and subcultured every four weeks.

3 Staining and photometry

After incubation in phosphate buffer for 10 min fixed embryo sacs were stained with the DNA specific fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) (10 μg/ml in 0.2 M phosphate buffer, pH8) for 2 h in the dark. They were transferred to buffer alone for 10 min then gently mounted in glycerol with phenylenediamine to reduce dye fading, and the coverslip sealed with nail varnish. embryo sacs from ovules between 19 and 22 days after pollination were found to be most suitable for fluorometric evaluation of endosperm nuclear DNA contents. For this, each replicate consisted of one embryo sac from each pollen irradiation treatment, 0, 50 and 100 krad, taken from comparably sized ovules, and, when possible, a diploid tissue standard (apple root tips or integuments, fixed concurrently with embryo sacs). Relative DNA values of endosperm nuclei were measured using a Zeiss SF photometer attached to an epifluorescence microscope. Readings were taken on a transect of the embryo sac from the embryo zone to the chalazal end.

Relative DNA values of endosperm callus nuclei were also determined using DAPI fluorometry. Small pieces of callus were fixed overnight in 3:1 ethanol:acetic acid, hydrolysed for 5 min in 45% acetic acid at 60°C, squashed and then stained as above.

Results

Intact embryo sacs were isolated from ovules from about 13 to 35 days after pollination, before and after fixation, and their nuclei counted with their relative positions in the endosperm noted after DAPI staining (Fig. 1). The normal, untreated apple embryo sac is of the Polygonum type (Maheshwari 1950) and its three antipodal cells degenerate soon after fertilisation (Wanscher 1939). In this study, the free-nuclear stage of the endosperm of ‘Cox’ persisted for approximately 30 days after pollination, by which time waves of mitoses had produced up to 1,500 free endosperm nuclei and the embryo was globular. The embryo sac elongated with the ovule so it extended the length of ovule. Endosperm cell wall formation commenced at the micropylar end when the ovule was from 5–6 mm long. The haustorial (but unbranched) chalazal end of the endosperm did not become cellular but disintegrated.

Growth of fruitlets, ovules and embryo sacs derived from 0 krads and 50 krads pollen lagged behind that of tissues derived from 0 krads and 50 krads pollen but differences only became marked more than 16 days after pollination. The slower rate of ovule elongation was associated with a delayed onset of endosperm cell wall formation in irradiated endosperms derived from irradiated pollinations. Pollen irradiation effects on endosperm development were pronounced, but natural variation in nuclear number, size and morphology within and between normal endosperms derived from unirradiated pollen must be considered when examining treated endosperms from 50 krads and 100 krads pollen.

Number of endosperm nuclei

Numbers of nuclei in endosperms from ovules of comparable size and age differed within and between treatments (Table 1, Fig. 1). Endosperms resulting from 50 and 100 krads pollen irrigations contained, on average, less than half the number of nuclei of normal (0 krads) endosperms. However, the range in number was greatest after the 100 krads pollen dose than after 50 krads, as several sacs from the 100 krads treatment possessed fewer than 25 nuclei, and two contained more than 200, 20 days after pollination.

Morphology of endosperm nuclei

In normal embryo sacs up to eight large, lobed chalazal nuclei with diffuse chromatin were always observed, frequently densely packed together, making DNA determination difficult. These were not antipodal nuclei as they lay within the central cell without their own cell walls. These were never observed in division, but waves of mitosis were occasionally observed proceeding from the centre of the embryo sac towards the chalazal and micropylar ends, resulting in many endosperm nuclei in synchronous mitotic stages (Fig. 1). Spherical metaphase nuclei with highly condensed chromosomes and persistent (unstained) nucleoli were often observed in mitotically active sacs, in the chalazal zone, and nuclei of different ploidy levels were often found adjacent to one another. Their chromosome counts in one normal sac ranged from 36 to 108 (in apple, 2n = 34).

No nuclear abnormalities were observed in normal sacs as nuclei were regularly spaced and evenly sized, irregular lobing occurring only in the large chalazal nuclei. However, 50 krads endosperm generally contained highly lobed nuclei, chromatin bridges between nuclei at telophase and persisting into interphase, and acentric fragments excluded from metaphase plates (Figs. 1 and 2a, b, c, and Table 1). What were presumed to be nuclear fusions were more common in endosperms derived from 50 krads pollen than in those from 0 krads pollen (Fig. 2d). Embryo sacs from