

Effects of gamma rays on ovary, ovule, megasporogenesis and megagametogenesis on *Solanum nigrum* L. complex

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Seeds of diploid, tetraploid and hexaploid *Solanum nigrum* L. exposed to different acute doses of gamma rays (Viz. 10, 20, 30, 40, 50, 60, and 70 k rads) were sown in experimental plots along with unirradiated seeds. Gynoecium in unirradiated material and in most of the treatment is bicarpellary. The ovary is syncarpous and bilocular having oblique septum. Tri- and tetra- carpellary gynoecia have also been seen in many cases of treatments. Ovules are tenuinucellate, unitegmatic and anatropous to campylotropous. Ovule sterility has been seen in several cases of treatments. In a few cases of tetraploids (50 k rad) complete female sterility has been observed. Development of the female gametophyte conforms to the *Polygonum*-type though a tendency towards bisporic type of development is occasionally observed. Organization of the cells in the female gametophyte is normal, but sometimes a few excessive nuclei are seen towards the egg apparatus before fertilization.

Key-words—Irradiation, Megasporogenesis, Megagametogenesis, *Solanum nigrum* L.

INTRODUCTION

VARIOUS aspects of embryology as affected by ionizing radiation have been initiated by Singh and Gunckel (1965), Chauhan (1968, 1969), Chopra and Singh (1974, 1976) and Chandra and Tewari (1978). But there is no record on embryological work in relation to ionizing radiation in any species complex. It is, therefore, important that such type of work is initiated on some aspects of embryology specially ovule, megasporogenesis and megagametogenesis in *Solanum nigrum* L. complex in response to different exposures of gamma rays.

MATERIAL AND METHOD

Dry (moisture content 11%) and soaked (moisture content 100%) seeds of diploid, tetraploid and hexaploid *Solanum nigrum* were irradiated at Radiation Laboratory of National Botanical Research Institute, Lucknow with the help of ⁶⁰Co source emitting 1000 Rads/75 seconds. The acute gamma-ray exposures used were 10, 20, 30, 40, 50, 60, and 70 k rads. 300 seeds were taken for each treatment. The irradiated seeds were sown in sterile petri-plates, pots and beds. At flowering time different stages of flower buds were fixed in formalin-acetic alcohol and stored in 70% ethanol. Material was

dehydrated in tertiary butyl alcohol series and embedded in paraffin wax in usual way. Serial microtome sections were cut at thickness ranging from 10 to 20 μ m. Safranin-fast green combination was used for staining.

OBSERVATIONS

Ovary—The gynoecium in control and many treated plants are bicarpellary in all the three ploidy groups of *Solanum nigrum*. The ovary is bilocular having obliquely oriented septum in relation to mother axis of flower. The placentation is axile and the placenta is swollen and bears numerous ovules (Text-fig. 1A). Variations in the number of carpels in a gynoecium occur in a good number of cases where it may be tricarpellary or tetracarpellary showing tri- or tetralocular ovaries respectively.

In a few cases of tetraploids of 50 k rad treatment one chamber developed normally showing ovules while the other chamber remained solid having parenchymatous cells with no indication of ovules.

Ovule—Numerous ovular primordia arise as small protuberances on the axile placenta. The ovule initial differentiates as a small bulge owing to periclinal division in the subepidermal layer. To keep pace with the activity of the subepidermal cells, the epidermal cells divide anticleinally. Initially, the ovular primordium is erect (Text-figs

1B,C) but very soon it becomes slightly curved. Subsequently, the ovule undergoes further curvature and attains nearly anatropous condition (Text-figs 1E, F, G), campylotropous conditions is attained in many cases afterwards. The vascular supply in the ovule terminates at the base of chalaza.

Megasporogenesis— Usually one archesporial cell differentiates in the hypodermal layer of the ovular primordium just below the apex (Text-figs 1B,C). The archesporial cell is larger than the surrounding cells and contains a distinct nucleus and dense cytoplasm. Differentiation of the archesporium takes place before the differentiation of the integument. The archesporial cell enlarges and directly functions as the megaspore mother cell (Text-fig. 1D). The integumentary primordium arises at the level of the archesporium (Text-fig. 1C). As the development proceeds, the integumentary initial grows and ultimately surrounds the nucellus leaving a narrow micropyle (Text-figs 1E,F,G).

Megasporogenesis in treatments, in general, was similar to the control in all the ploidy groups, but sometimes, the megaspore mother cell formed the dyad and one dyad cell degenerated while the other showed enlargement (Text-fig. 1E) suggesting a trend towards bisporic type of development.

Megagametogenesis— The nucleus of the functional megaspore enlarges and undergoes three successive mitotic divisions unaccompanied by wall formation to form 2, 4 and 8-nucleate female gametophyte in all the three ploidy groups (Text-figs 1I-N). At the 2-nucleate stage, a large central vacuole is formed resulting the two nuclei move apart towards the two poles of the developing female gametophyte (Text-fig. 1I). During the four-nucleate stage, the two nuclei at each pole, either lie side by side or one above the other (Text-figs 1J, K,L). The four nuclei divide and the orientation of the spindle is well-defined, based on the position of the synergids, egg, polars and antipodals. The two nuclei placed at the two extreme tips show spindles placed transversely to the long axis of the embryo sac, whereas the remaining two show orientation of the spindle parallel to the long axis of the embryo sac (Text-fig. 1M). The female gametophyte shows four nuclei in the micropylar portion and four in the chalazal region. Three of the four nuclei in the former region organize into egg apparatus and the fourth nucleus forms one of the polars. Out of the four nuclei in chalazal region, three form antipodal cells and the fourth nucleus forms the second polar nucleus. Two polar nuclei soon fuse to form the secondary nucleus which usually comes to lie close to the egg (Text-fig. 1P). The three antipodal cells are arranged either in a linear or in a triangular fashion. The egg apparatus consists of two synergids and an egg (Text-figs 1P-S). All these three cells are elongated structure.

In several ovules in tetraploids, presence of twin embryo sacs showing two-nucleate condition of development have been observed (Text-fig. 1T). Twin embryo sacs have not been seen in diploids and hexaploids.

Megasporogenesis in all the ploidy group is quite similar to control plants. However, in several treatments, it has been observed that antipodals organize earlier than egg apparatus (Text-fig. 1O). In one case of tetraploid treated with 50 k rad, the sequence of nuclei at mature embryo sac appears to be disturbed and a few excessive nuclei are seen towards micropylar side (Text-fig. 1C).

The length of the embryo sac is variable in different ploidy group, but under various treatments it remains uniform within the same ploidy group as in control. It has been recorded that the embryo sacs in the diploids are similar in length as compared to tetraploids and hexaploids. Tetraploid embryo sacs are, however, slightly longer than the hexaploids.

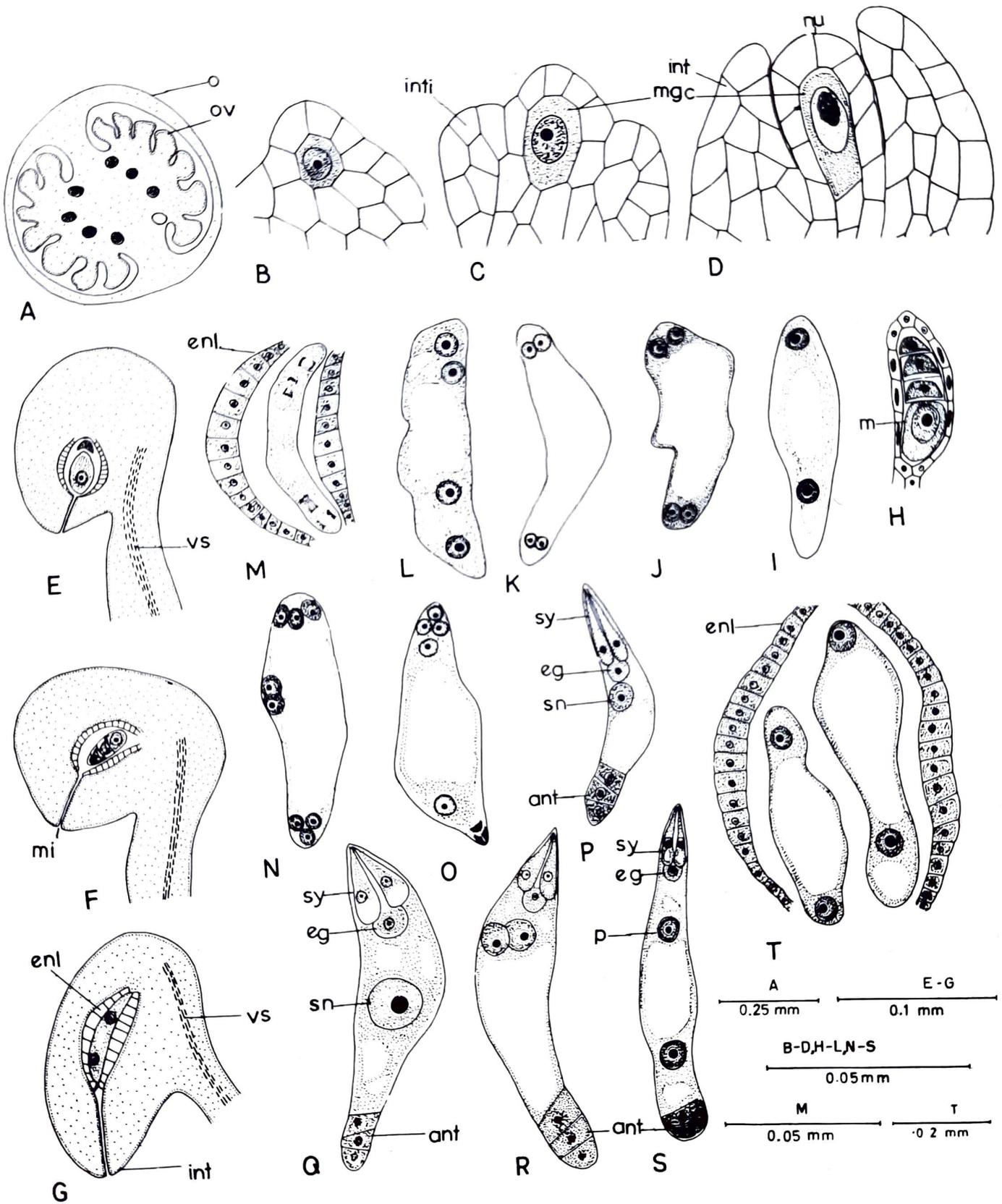
Ovular sterility— In a large number of cases under treatments, crumpling of ovules leading to the failure of seed development has been observed (Text-fig. 1D). The frequency of ovular sterility is greater in higher doses. In a number of cases, the degeneration starts from the megaspore mother cell stage or from the megaspore tetrad or even at 8-nucleate embryo sac stage. Such cases are greater in number at higher doses of treatment in all the ploidy groups.

DISCUSSION

The gynoecium in unirradiated and in most plants of treatments is bicarpellary, and the ovary is superior, syncarpous and bilocular having obliquely oriented septum. Contrary to this normal condition, variation in the number of carpels per gynoecium has been observed in all the three ploidy groups in treatments, specially under high doses. Gynoecium in such cases are either tri- or tetracarpellary with tri- or tetralocular condition of the syncarpous ovary. In a very limited number of cases one of the ovary chamber possesses ovules, while the other is solid with no ovules.

The ovules are unitegmic, tenuinucellate and anatropous to campylotropous in form in all the ploidy group in unirradiated and irradiated materials, and no variability in form has been seen. It is very likely that in *Solanum nigrum* complex no variability could be observed owing to overcrowding of ovules in the locules.

Ovular sterility has been frequently noted in higher doses of treatment in *Solanum nigrum* complex and was extremely high in tetraploid at 50 k rad. Ovular sterility in response to ionizing radiation has also been seen by Singh and Gunckel (1965), Monti (1967), and Chandra and Tewari (1978) in *Ricinus communis*, *Nicotiana tabacum* and Mung bean respectively. Some of these



Text-Figure 1

workers have suggested that ovular sterility is due to environmental factors. The present author is of the opinion that besides environmental factors, radiation has also played important role towards the induction of female sterility.

Female archesporial cell directly functions as the megaspore mother cell. Development of the female gametophyte conforms to the *Polygonum*-type. Sometimes there is a tendency towards bisporic type of development. Bisporic tendency was observed only in a few cases of treatments. Bisporic tendency of embryo sac development has also been reported by Singh and Gunckel (1965) in chronically irradiated *Ricinus communis*.

Organization of the female gametophyte is normal, but sometimes a few excessive nuclei are seen towards the egg apparatus. In this case the cells of the egg apparatus were not placed in the usual pattern and the number of cells in the embryo sac were more. This was probably because of disturbed polarity induced by gamma irradiation. It appears that gamma irradiation retards or exceeds divisions which results in the formation of fewer or excessive number of cells.

In a few cases of tetraploids, both in control and treatment, two embryo sacs in the same ovule have been seen. The occurrence of two embryo sacs in the same ovule may be due to presence of two archesporial cells or two megaspore cells of a tetrad developing further.

The irradiation has not affected the formation of vacuole at the two-nucleate stage and the movement of the two nuclei at the two poles. These two phenomena appear to be radio-resistant. Singh and Gunckel (1965) also pointed out the radio-resistant nature of these phenomena in chronically gamma irradiated *Ricinus*

communis. Likewise, the apparent cell structure of egg apparatus is also radio-resistant in most cases.

As regards the cause of degeneration, it is difficult to assign any one particular reason, but in certain cases, it can be presumed that degeneration of embryo sac may be due to abnormal meiosis or lack of pollination and fertilization. The other reason may be physiological for which gamma irradiation may also be responsible.

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