

REDUCED AND UNREDUCED EMBRYO SACS IN *SOLIDAGO PURPUREA* LINN.

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In *Solidago purpurea* Linn. the ovary is unilocular with anatropous unitegmic and tenuinucellate ovule. Female gametophyte development is of reduced and unreduced (apomictic) type. In the former the development is of monosporic, eight nucleate *Polygonum* type with antipodal variations. The unreduced embryo sac development follows the *Taraxacum* type of aneuspory (Battaglia, 1963) or the recurrent type of apomixis of Maheshwari (1950). In this type the embryo sac always remains diploid due to the failure of normal meiosis. Endothelium is organized in both the types of embryo sacs.

Key words : *Solidago purpurea*, Apomixis, Aneuspory.

Compositae show several interesting embryological features such as periplasmodium formation, 3 nucleate pollen grains, monosporic, bisporic and tetrasporic embryo sacs in the various species of the same genus or even in the same species; multiplication of antipodals, haustorial nature of antipodal cells, Asterad type of embryo development, polyembryony, diploid parthenogenesis etc. (see Davis, 1966). Embryology of *Solidago canadensis* has been investigated by Pullaiah (1978). He reported normal *Polygonum* type of embryo sac with antipodal variations and probably development of antipodal embryo parthenogenetically. Harling (1951) investigated three species of the genus *Solidago* viz. *S. lanceolata*, *S. latifolia*, *S. shortii*. In *S. lanceolata* and *S. shortii* the embryo sac development is monosporic, normal type, but in *S. latifolia* the apical or subapical megaspore gives rise to the embryo sac and the development is undoubtedly monosporic. *Solidago purpurea* Linn. is an ornamental plant, which does not set seeds, which led to the present embryological investigation on the female gametophyte.

MATERIALS AND METHODS

The inflorescence at various stages of development were collected and fixed in formalin-acetic-alcohol. Customary methods of dehydration and embedding were followed. The serial microtome sections were cut at 10-12 micron thick and stained with Iron-alum-haematoxylin using the normal procedure. The stigmas were freshly mounted in cotton blue in lactophenol to observe fresh germinating pollen grains. Pollen viability is counted by staining the pollen grains in acetocarmine.

OBSERVATIONS

Ovary and ovule - The ovary is inferior, bicarpellary, syncarpous and unilocular with a single basal, anatropous, unitegmic and tenuinucellate ovule

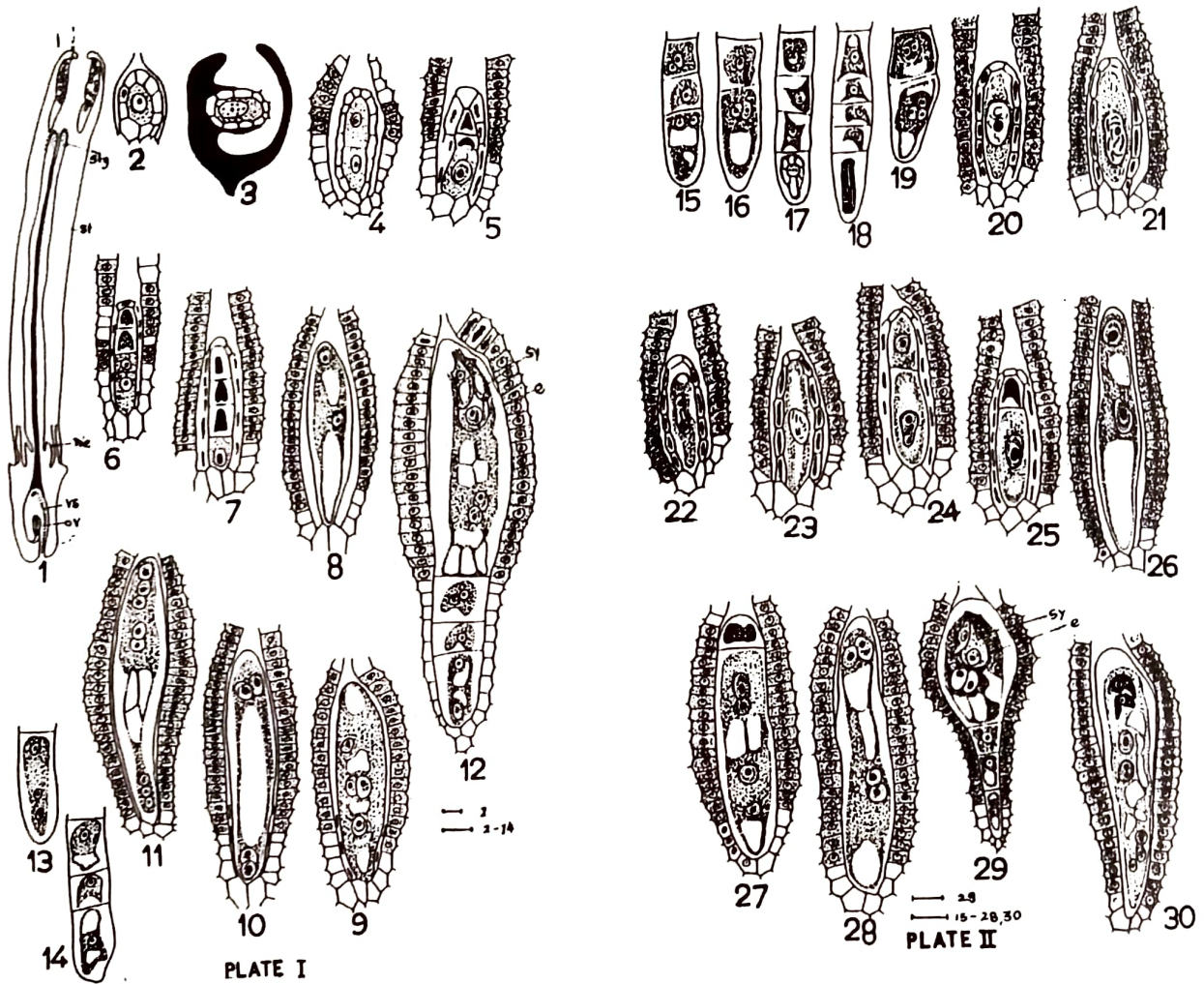
(Fig.1). Vascular strand enters through the funiculus and reaches up to the base of the ovule (Fig. 1).

A well developed integumentary tapetum is differentiated from the time of megaspore mother cell stage onwards in the apomictic embryo sac (Figs. 20, 21, 22), while in a normal sexual embryo sac it starts differentiating at dyad stage (Fig. 4).

Reduced Embryo Sac

Megasporogenesis - The female archesporium is unicellular (Fig. 2). It directly functions as a megaspore mother cell. It is rich in cytoplasm. By the time the growth of the integument takes place the megaspore mother cell reaches its full size and its nucleus enters the prophase I (Fig. 3). Meiosis I takes place in the megaspore mother cell to form a dyad of two equal cells (Fig. 4). The upper dyad divides transversely, but the lower dyad undergoes a linear elongation to form a triad (Fig. 5). Normally two meiotic divisions give rise to a linear tetrad (Fig. 6). The products of upper dyad degenerate early while those of lower dyad are seen healthy (Fig. 6). Ultimately upper three megaspores degenerate and the chalazal is functional (Fig. 7). The breakdown of the contents of the nucellar epidermis is apparent at the chalazal end of the nucellus and proceeds towards the apex (Figs. 6, 7).

Femal gametophyte - The single functional megaspore increases in size and takes part in the further development of the embryo sac, therefore, it is of the monosporic type. The first mitosis of the megaspore nuclei results into two nuclei. There is a large vacuole extending from middle of the embryo sac to the chalazal end (Fig. 8). This is a 2-nucleate embryo sac. During the female gametogenesis both the nuclei of the two nucleate embryo sac undergo second mitosis of the female gametogenesis. It results into a four nucleate embryo sac (Figs. 9, 10). The third and the final mitosis



Figs. 1-14: *Solidago purpurea* Linn. Development of reduced embryo sac. Fig. 1: V.S. of disc floret, showing an anatropous unitegmic ovule. Note the vascular supply (VS) and nectary (ne), with stigma (Stg) and style (St). Fig. 2: L.S. nucellus showing unicellular female archesporium. Fig. 3: The same as above showing megaspore mother cell at prophase stage. Fig. 4: A dyad. Fig. 5: A triad. Fig. 6: A linear tetrad showing upper two megaspores degenerating. Fig. 7: The same as above with upper three megaspores degenerating. Fig. 8-11: 2, 4, 8-nucleate embryo sac. Fig. 12: Mature embryo sac. Fig. 13-14: Antipodal variations.

Figs. 15-30: *Solidago purpurea* Linn. (Antipodal variations and development of unreduced Apomictic embryo sac.) Figs. 15-19: Antipodal variations. Fig. 20: L.S. of nucellus showing megaspore mother cell. Fig. 21: Same as above showing prophase. Fig. 22: Formation of dumbbell shaped restitution nucleus. Fig. 23: The same as above with a rounded restitution nucleus. Fig. 24: A dyad. Fig. 25: Upper dyad degenerating. Figs. 26-28: 2 and 4 nucleate embryo sacs. Fig. 29: An organised embryo sac with egg (e) and synergid (sy). Fig. 30: An unorganised embryo sac.

takes place simultaneously in the four nuclei and an eight nucleate unorganized embryo sac is formed (Fig. 11). It organizes into an egg apparatus consisting of an egg and two synergids, two polars and three antipodals (Fig. 12). Thus the development of the embryo sac is of monosporic Polygonum type and is surrounded by an integumentary tapetum.

Antipodal variations - Initially in an unorganized embryo sac, all the three antipodal nuclei are enclosed in a common cell wall as cytokinesis fails and it gives rise to a trinucleate antipodal cell (Fig. 13). When the cytokinesis occurs there are three uninucleate antipodal cells in a row (Fig. 14). Further in these uninucleate antipodal cells the nuclear division may

Table 1: Comparative study of the size of embryo sac and nucleus in *Solidago purpurea* Linn.

Stage	Reduced embryo sac				Unreduced embryo sac			
	Embryo sac		Nucleus		Embryo sac		Nucleus	
	Length (in micron)	Breadth	Length (in micron)	Breadth	Length (in micron)	Breadth	Length (in micron)	Breadth
M.M.C. Stage	25.0	7.5	0.54	0.5	40	12.5	15	7.5
Dyad cells	37.5	8.0	2.5	2.5	50	20.0	5/10	3/5
2 nu. embryo sac	72.5	25.0	5.0	5.0	80	28.0	10/12.5	7.5
4 nu. embryo sac	87.5	27.5	6.25	6.25	92	30.0	7.5	7.5
8 nu. embryo sac	125.0	27.5	5.0	5.0	112.5	40	5/6.5	5/6.5
Organized mature embryo sac and egg nucleus	137.5	30	5.0	5.0	125	67.5	5/6.5	5/6.5

take place, so that the micropylar and chalazal one become bi or trinucleate, while the middle one remains uninucleate (Fig. 15). At times the lower two antipodal nuclei do not undergo cytokinesis, so they form a binucleate antipodal cell and one uninucleate antipodal is cut off on the upper side (Fig. 16). On further observation of the antipodal variation, it is seen that even four or five antipodals are present in a row (Figs. 17, 18). The presence of two antipodals shows an interesting variation. The lower antipodal is conical and is three nucleate, while the upper one is uninucleate, with a very big nucleus and two nucleoli. It has probably arisen due to fusion of two antipodal nuclei (Fig. 19). There is possibility of obtaining many more variations.

Unreduced embryo sac : In unreduced embryo sac the female archesporium directly functions as a megaspore mother cell. It undergoes linear expansion. Its nucleus undergoes prophase which is marked by an intense hydration of the cytoplasm (Fig. 20). The chromosomes do not exhibit either the pairing or the marked contraction characteristic of meiosis (Fig. 21). After mitosis I the restitution nucleus is formed. This nucleus is somewhat dumb-bell shaped in the beginning (Fig. 22) but soon it becomes rounded (Fig. 23). The restitution nucleus undergoes mitosis II, and cytokinesis takes place between the two nuclei and a dyad of unequal cells are formed (Fig. 24). The chalazal dyad is functional which develops into a diploid embryo sac. The upper dyad degenerates (Fig. 25). The nucleus of the lower dyad undergoes the first mitosis to form a two nucleate embryo sac (Fig. 26). The position of the two nuclei are not at the poles as in a sexually developed reduced embryo sac. The two nuclei are also bigger in size and there is difference in vacuolation in the embryo sac. The second mitosis gives rise to a 4 nucleate embryo sac the upper dyad

is seen degenerating at this stage (Fig. 27). At times the position of the four nuclei are very much similar to that seen in sexually produced embryo sac. The only difference noted is in the size of the nucleus and size of the embryo sac of reduced and unreduced embryo sac (see Table I. & Figs. 27, 28). The third mitosis produces an eight nucleate embryo sac. The eight nucleate embryo sac rarely organizes into a mature embryo sac (Fig. 29). However, in one instance, an embryo sac showed an egg apparatus with an egg and degenerating synergids, two polars and three antipodals in a row (Fig. 29). Majority of the micropreparations showed an unorganized embryo sac with 3-nuclei at the antipodal end and probably a secondary nucleus in the centre. At the micropylar end there are two nuclei surrounded by darkly stained cytoplasm; and there is a nucleus above the secondary nucleus. This is probably an unorganized egg nucleus. The embryo sac is surrounded by a single layer of endothelium as in a normal embryo sac (Fig. 30).

Since no chromosome count could be done, our interpretation are based on the comparative size of the nucleus and embryo sac.

Further, in a reduced embryo sac nucellar epidermis is intact at megaspore mother cell stage (Fig. 3) and starts degenerating from triad or tetrad stage (Figs. 5, 6). But in the unreduced embryo sac nucellar epidermis starts degenerating at megaspore mother cell stage as it is a resting stage (Figs. 20, 21).

In sexually developing embryo sac the nuclei are small in size as compared to the unreduced embryo sacs. Unreduced embryo sacs showed disturbed polarity of nucleus (Fig. 27).

There is a difference in size of the embryo sac and nucleus of the reduced and unreduced embryo sac which is illustrated in Table I. The sexually produced

embryo sacs are elongated while the apomictic embryo sacs are broad.

The development of female unreduced gametophyte follows the *Taraxacum* type of aneuspory (Battaglia, 1963) or the recurrent type of apomixis of Maheshwari (1950). In this type the embryo sac always remain diploid as described above due to the failure of normal meiosis.

Fertilization: No pollen grains are seen to germinate on the stigma, as it showed about 98% of pollen sterility and the reduced sexual embryo sacs degenerated. As the pollination impact is eliminated, no apomictic embryos are seen, and the unreduced embryo sacs also degenerated.

DISCUSSION

The occurrence of anatropous, unitegmic and tenuinucellate ovule and monosporic Polygonum type of embryo sac is similar to the observations in various species of Compositae (see, Harling, 1954; Davis, 1966; Khan and Sharma, 1969; Deshpande, 1970).

Pullaiah (1978) studied *Solidago canadensis* and reported monosporic Polygonum type of embryo sac. Harling (1951) investigated three species of the genus *Solidago* viz. *S. lanceolata*, *S. latifolia*, *S. shortii*. In *S. latifolia* apical or subapical megaspore gives rise to the mature embryo sac while in remaining two species it is the chalazal megaspore give rise to embryo sac of monosporic type. In the present study sexual (reduced) as well as apomictic (unreduced) embryo sac development have been observed. Both the types occur side by side in the different florets. The common occurrence of diploid embryo sac in the present study may be defined as *Taraxacum* type of aneuspory (Battaglia, 1963) or under recurrent apomixis (Maheshwari, 1950). This is also called diploid parthenogenesis by Rosenberg (1930). Occurrence of haploid parthenogenesis is reported by Sharma and Murthy (1977) in *Brachycome iberidifolia*.

In the reduced embryo sac antipodal variations has been seen, but most of the unreduced embryo sac do not show antipodal variation. It is quite common in Compositae and it has been of special interest due to their secondary multiplication and haustorial nature (see Maheshwari Devi, 1963; Davis, 1966; Maheshwari Devi and Pullaiah, 1977). Davis (1964) observed as many as 20 antipodal cells in *Brachycome ciliaris* and reported about their peristant nature. In *B. iberidifolia* (Sharma and Murthy, 1977) there are as many as 35

antipodal cells and some of them are binucleate. In the present study some of the antipodal cells are trinucleate and chalazal antipodal becomes elongated to serve as haustoria. Pollen sterility led to collapse of the embryo sacs as fertilization failed and thus seeds become non-viable.

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