

18MBO22C-U5

**Paper – V – Anatomy and Embryology
Unit – 5**

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Polyembryony

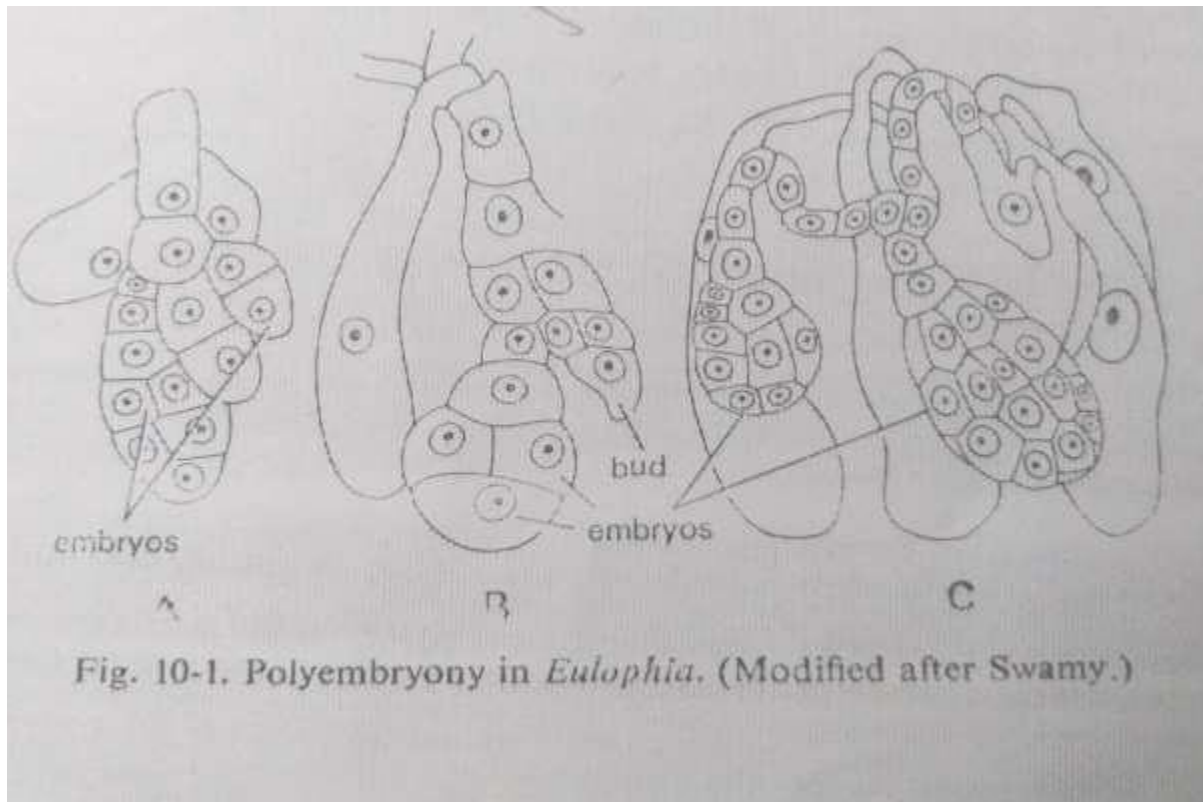
The term polyembryony is commonly related to presence of two or more than two embryos in one seed. When there is a situation of polyembryony, some of the embryos may be diploid and others haploid. The embryos developed from the diploid cells of nucellus, integuments and zygote, are diploid whereas those arising from the haploid cells of the embryo sac such as Synergids and antipodals are haploid. During apomixis several embryos appear along with the normal zygotic embryo. In Citrus ovule thirteen embryos, including one zygotic embryo, have been reported. The phenomenon of polyembryony is more frequent in gymnosperms than angiosperms. Leeuwenhock (1719) was first to report two embryos in a seed of orange. It was a unique discovery which led a large number of embryologists to study several other plants. It was reported that the development of many embryos in an ovule was much occasional but was found in many plants. According to an older classification, the polyembryony may be true or false. In true polyembryony, several embryos appear within one embryo sac developing from its different cells or nuclei or sometimes the developing embryos may penetrate into the embryo sac from outside. In false polyembryony, adventive embryos develop from many embryo sacs present in the same or different nucellus. Later Gustafsson (1946) suggested that only those cases, where two or more nucelli with their embryo sacs fuse during their early development, should be considered under false embryony. However, those cases where several embryos develop within a nucellus of an ovule should be included under true polyembryony. In angiosperms, the polyembryony arises in the following manner:

- (1) Cleavage polyembryony.
- (2) Cells of embryo (excluding egg) developing into embryo sacs
- (3) Cells of nucellus and integuments developing into embryos.
- (4) Many embryo sacs of an ovule giving rise to embryos.
- (5) Special cases of polyembryony.

[1] Cleavage polyembryony

Cleavage polyembryony is the simplest method by which the embryos increase in their number. The splitting or cleavage occurs in zygote or proembryo into more than one unit. Each such unit develops into an embryo. All the developed embryos are identical. This type of polyembryony is more frequent in gymnosperms but is occasional in angiosperms. Jeffrey (1895) described cleavage polyembryony in *Erythronium americanum* where the synergids disintegrate soon after the fertilization and the zygote divides to give rise to a small group of cells, irregularly arranged. Later two to four outgrowths develop from the lower end of this group of cells, each developing into an independent embryo.

Cleavage polyembryony is more frequent in Orchidaceae, Swamy (1943) has pointed out the following variations in the methods of embryo formation in *Eulophia eplendrea* (Fig. 10-1),



- (1) The zygote divides in an irregular manner to form a mass of cells, of which those lying towards the chalazal end grow simultaneously and give rise to several embryos.
- (2) The proembryo gives out small buds or outgrowths which themselves function as embryos.
- (3) The filamentous proembryo becomes branched, and each branch develops into an embryo.

[II] Cells of embryo sac (excluding egg)

developing into embryos

In some plants additional embryos may develop from one or both the synergids. The synergids become egg-like and get fertilized with the male gamete released by the additional pollen tube, which makes its entry within the embryo sac as in *Sagittaria graminea*, *Aristolochia bracteata*, etc. The unfertilized synergids have been also reported developing into embryos, e.g. *Argemone mexicana*. In the former cases the embryo is diploid whereas in the latter it is haploid. These embryos, developed from the synergids, are in addition to the diploid zygotic embryo. Although formation of the embryo from the antipodals is rare, Shattuck (1905) observed the antipodals becoming like in *Ulmus americana* and developing into embryo-like structures. However, fully developed embryo could not be observed.

(III) Cells of nucellus and integuments

developing into embryos

Polyembryony is also brought about due to the development of embryos from the cells of nucellus and integuments. Although taking their origin outside the embryo sac, these embryos later penetrate into the embryo sac to be nourished by the nutritive tissue of the endosperm. Such embryos, developed from the female sporophytic tissue, are termed as adventive embryos. The nucellar embryos develop in Citrus, Mangifera, Opuntia, etc. Opuntia dillenii represents an interesting example of adventive embryony where all the nuclei of the embryo sac, i.e. egg, synergids, antipodals, and polar nuclei disintegrate and the nucellar cells develop many embryos (Bhataagar and Bhojwani, 1974). Both nucellar and integumentary embryos occur in Eugenia.

[IV] Many embryo sacs of an ovule

giving rise to embryos

Sometimes polyembryony is due to the presence of more than one embryo sac within an ovule. Multiple embryo sacs may arise :

- (1) From the derivatives of the same megaspore mother cell.
- (2) From the derivatives of two or more megaspore mother cells, or
- (3) From the nucellar cells.

Citrus may be quoted as most common example where more than one embryo sac is found in an ovule. Usually twin embryo sacs in Citrus ovule give rise to twin embryos.

[V] Special cases of polyembryony

Allium odorum may be cited as an interesting example where polyembryony is caused due to the operation of more than one of the methods described above, at the same time. In this plant a total of five embryos develop, i.e. one from zygote, one from synergid, two from antipodals, and one from integument.

Polyembryony has been suggested as a primitive feature of the taxon in which it is prevalent. Although it is more common in gymnosperms, but from the foregoing account it is evident that angiosperms, to some extent, are also not much lacking behind. Simple reason behind this fact is that in the former, there being several archegonia, there are chances of fertilization of more than one egg. However, in the latter, there is only one egg present in most of the cases which after fertilization, gives rise to one zygotic embryo.

It has been further observed that in most of the cases when plural embryos are produced, it is only the zygotic embryo which attains maturity within the seed and the rest disorganize, sooner or later. There are also several examples where due to polyembryony multiple seedlings develop. Adventive embryos give rise to parental type of similar and uniform seedlings. The nucellar embryos of Citrus, sometimes also called as adventive embryos, are utilized for developing improved clones.

Embryoids

Possibility of growing the entire plant has been demonstrated by several workers from a group of cells obtained from an embryo or a plant. Small discs, obtained from the region of secondary phloem of carrot root, have been found developing embryo-like structure, called embryoids, when grown on liquid medium containing milk of coconut. These embryo-like structures resembled too much, with the normal embryos. In wild carrot, the embryo (diploid) was allowed to proliferate and separate into free cells. These free cells were later very easily induced to develop the embryoids when grown on a suitable medium. It has been discovered that practically every cell of an embryo is capable of developing into an embryoid. The pollen grains (haploid), at certain stage of their development, also develop embryoids as in Datura (Guha and Maheshwari, 1964, 1966) and Nicotiana (Nitsch and Nitsch, 1969). Several embryoids can be easily obtained from the tobacco anther (at certain stage of its growth) when grown on a suitable medium containing sucrose. The discovery of embryoids is significant as it shows that a cell obtained from any source, whether haploid or diploid, develops like normal zygote, if provided suitable conditions. It has been thus experimentally proved that not only the cells of an embryo behave like the zygotes but they even mature and differentiate in a normal manner.

Apomixis

Normally, diploid embryo results after the sexual union, i.e., fusion of haploid male and female gametes but sometimes this normal process of sexual reproduction is not at all essential for the development of embryo. Thus, in some plants the process of sexual reproduction (amphimixis) is replaced by asexual reproduction. Winkler (1908) called it apomixis and defined it as a process of asexual reproduction during which there is no nuclear fusion of the male and female gametes. The sexual reproduction is very well substituted by apomixis. Four types of apomixis have been distinguished by Maheshwari (1950). i.e., 1. non-recurrent apomixis, 2. recurrent apomixis: 3. adventive embryony and 4 vegetative reproduction.

[1] Non-recurrent apomixis

Regular meiotic divisions take place in the megaspore mother cell and a haploid embryo sac develops. The new embryo either develops from the unfertilized egg or from any other haploid cell of embryo sac (female gametophyte). The above two methods of formation of the embryo are called haploid parthenogenesis and haploid apogamy, respectively. The plants produced by haploid parthenogenesis and haploid apogamy contain only one set of the chromosomes and are sterile. This process is not repeated from one to another generation.

Haploid parthenogenesis. The haploid egg cell remains unfertilized and develops into a haploid embryo. Sometimes one of the male gametes, released from the pollen tube enters into the egg but fails to fuse with the egg nucleus and disorganizes. It is believed that the egg gets stimulated with the presence of this male nucleus and the embryo develops parthenogenetically having only one set of chromosomes. Haploid parthenogenesis has been reported in *Solanum nigrum* and *Nicotiana*.

According to Maheshwari (1950), the egg cell remains unfertilized due to : absence of the pollen tube, male nuclei are not discharged from the pollen tube, feeble attraction between male and female nuclei, earlier disintegration of the male gametes or the egg cell being not mature during the penetration of the male gamete into the embryo sac. Haploid apogamy. In haploid apogamy there is no sexual union of the gametes. The embryo develops from any other cells of the embryo sac such as synergids or antipodals or from both of these. Such embryos are haploid and sporophytes produced from these embryos contain only one set of the chromosomes, thus are usually sterile.

[II] Recurrent apomixis

When the embryo sac develops from its sporogenous cell (archesporium), it is called generative apospory and diplospory. In case it develops from other cells of the nucellus or integuments, it is known as somatic apospory as in *Parthenium* and *Ranunculus*, respectively. As these embryo sacs develop from those cells which have not gone under reduction in the number of the chromosomes, they are always diploid and possess all diploid cells like the egg, synergids, antipodals, etc. Such embryo sacs have a disturbed polarity. Their poles are not well organized with the proper nuclei. Mostly these embryo sacs are non-functional. However, when the embryo develops in such cases, it either develops from the diploid egg cell or from any other diploid cell of the embryo sac without the act of sexual union and is known as diploid parthenogenesis or diploid apogamy, respectively.

[III] Adventive embryony

In this type of apomixis the embryo does not develop from any of the cells of the embryo sac but from a diploid cell of the integument or the nucellus. Adventive embryony is sometimes also called as sporophytic budding because the cells from which the embryos develop belong to the sporophyte and are diploid. The initial cells from which embryos are going to develop, first become rich in the protoplasm and later divide actively to produce small groups of cells, which ultimately push their way into the embryo sac and develop the embryos. Four to five adventive embryos are commonly found in the seeds of *Citrus* but sometimes as many as thirteen viable embryos have been counted along with a zygotic embryo (Webber and Batchelor, 1943). The zygotic embryo also develops along with the adventive embryos, and may be easily differentiated from the latter by its lateral position (Fig. 9-1). Adventive embryony is independent of pollination and fertilization but sometimes may be stimulated with either process or both.

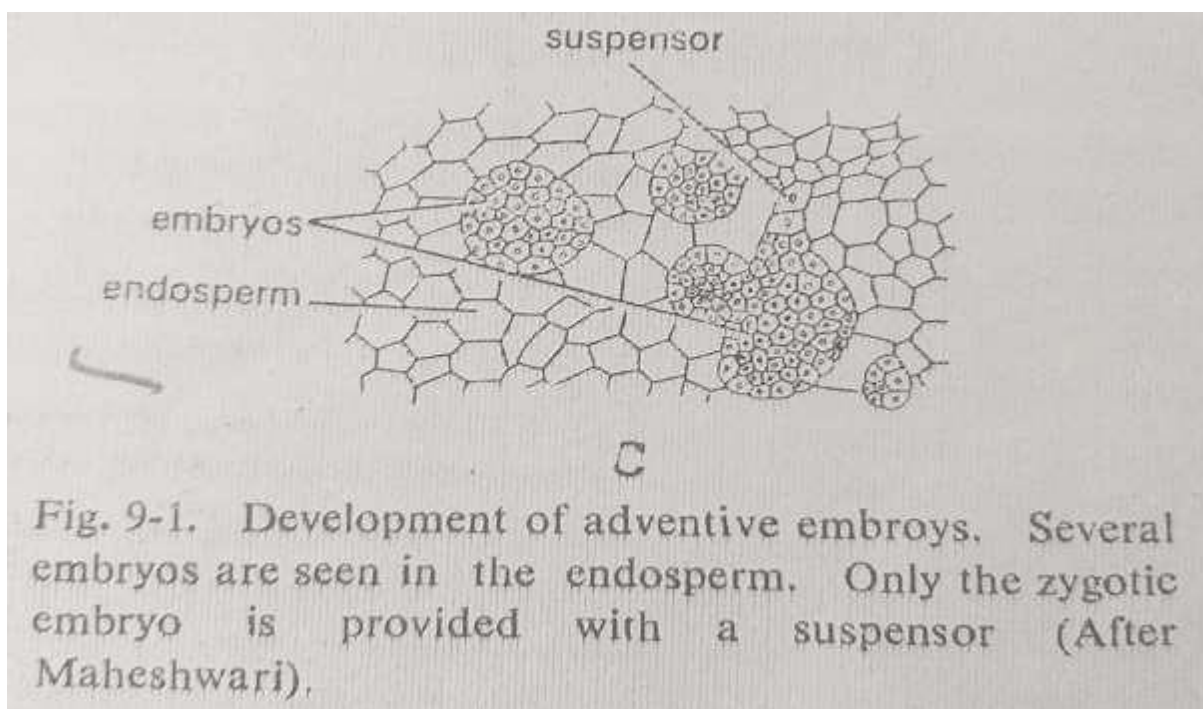


Fig. 9-1. Development of adventive embryos. Several embryos are seen in the endosperm. Only the zygotic embryo is provided with a suspensor (After Maheshwari).

[IV] Vegetative reproduction

In the vegetative reproduction the flower or its parts are replaced by bulbils. Sometimes other vegetative propagules like bulb, runner, sucker, etc. take up the work of vegetative reproduction. These vegetative propagules may germinate while still on the parent plant.

Formation of seed and fruit

After the fertilization the fertilized egg and the triple fused nucleus start developing into an embryo and endosperm, respectively. As a result of this internal growth of the new tissues, the ovule increases in size. In many dicotyledonous plants the food material present in the endosperm is completely absorbed by the cotyledons. The endosperm in such plants gets completely obliterated and the cotyledons become thick and fleshy. However, in some dicotyledonous plants the food material present in the endosperm is not absorbed by the cotyledons and it persists within the seed. In such plants the cotyledons remain thin and leaf-like. Thus two types of seeds develop in the angiosperms. The seeds without the endosperm are called non-endospermic and those with the endosperm as endospermic seeds.

When the fertilized ovule is being converted into a seed, the two integuments gradually become devoid of their water content, ultimately becoming dry and woody. In bitegmic ovules, it is the outer integument which greatly modifies and gets converted into a hard, woody and shell-like testa. The inner integument generally remains thin, forming the tegmen. However, a part of nucellus, not distinguishable from the inner integument, also gets in a combination and form the seed coat to come seeds both the integuments develop thick coverings. Sometimes the integuments develop such a thin seed coat that it becomes difficult to differentiate it from the pericarp. In those ovules where a third integument (aril) is found, it develops an additional covering over the seed. Some seeds possess a fleshy and spongy outgrowth near the micropyle, called caruncle. Its function is to absorb

water during the seed germination. Generally, the synergids and antipodals in the fertilized ovule, disorganized. The nucellus may or may not disintegrate. When it is present in the seed, it is termed as perisperm. The funicle becomes converted into the stalk of seed. A minute scar present on the seed surface represents the hilum. It is the place where the ovule remains attached with the placenta. With the above post-fertilization changes taking place in the fertilized ovule, the ovule gradually gets converted into a seed.

As the seeds are being developed from the fertilized ovules, the ovary also gets ripened into a fruit. The wall of ovary gets modified and converted into a complicated wall of the fruit or pericarp. The pericarp is much variable. It may be fleshy or dry in nature. When it is fleshy, it may be differentiated into three distinct layers-outer pericarp, middle mesocarp and inner endocarp in drupes, the epicarp forms the leathery skin of the fruits, mesocarp the pulp or

libres as in *Mangifera indica* (mango) and *Cocos nucifera* (nariyal), respectively and the endocarp forms a hard and stony covering around the seed. In berries, the pericarp becomes juicy. The dry and stony fruits have woody and stony pericarp.

In most of the cases the floral parts such as the sepals, petals and thalamus do not take part in the formation of fruit and get separated sooner or later but there are exceptions too. In *Physalis peruviana* (rasbhari) the sepals develop an outer papery covering in which the fruit is completely enclosed. The thalamus becomes fleshy and forms the edible part of the fruit. *Pyrus communis* (pear), *Pyrus mahas* (apple) and many species of *Ficus*. In *Artocarpus integrifolia* jack fruit the entire inflorescence takes part in the formation of one large fruit.

PARTHENOCARPY

Parthenocarpy is of widespread occurrence, especially among species which have a large number of ovules per ovary, such as banana, pineapple, tomato, melons and figs. According to Noll (1902), who introduced the term, parthenocarpy means the development of fruits without pollination or any other stimulus. Since then the definition of parthenocarpy has undergone slight modification and, according to the present concept, it refers to "the formation of fruits without fertilization" (Nitsch, 1965). The parthenocarpic development of fruit may require the pollination stimulus (stimulative parthenocarpy) or it may occur in unpollinated flowers (vegetative parthenocarpy).

"Seedless fruits" should not be considered synonymous to "parthenocarpic fruits" because in a seedless fruit the ovules may have been fertilized and later aborted, as in some strains of *Vitis vinifera* var. *concord* (Nitsch et al. 1960). Also, there may be parthenocarpic fruits with seeds in them. Auxin treatments are known to produce seeded parthenocarpic fruits in *Citrus* sp., grapes, watermelons and pineapples.

The seeds in such fruits are really pseudo-seeds, lacking a sexual embryo: parthenogenetic or adventive embryo may, however, be present. Nitsch (1963) had recognized three types of parthenocarpy, namely: (a) genetic, (b) environmental, and (c) chemically induced. Recently, parthenogenetic or adventive embryo may, however, be present. Nitsch (1963) had recognized the types of parthenocarpy, namely (a) genetic, (b) environmental, and (c) chemically induced. Recently, parthenocarpic plants have been developed by genetic engineering (Spencer and Rotina, 2001).

Genetic Parthenocarpy

Many of the plants cultivated for their fruits show seeded as well parthenocarpic varieties. This type of parthenocarpy is known to arise due to mutations or hybridization. The famous navel oranges arose from a hormonal seeded Citrus variety through mutation in an axillary bud which grew out into a branch bearing seedless oranges. Besides navel oranges, parthenocarpy occurs in Citrus, Cucurbita, Eugenia, Musa, Punica and Vitis.

Wellington and Hawthorn (1929) obtained a parthenocarpic hybrid by crossing an English forcing variety with "Arlington White spine". Kihara (1951) developed a method of crossing tetraploids and diploids to produce triploid, parthenocarpic seedless watermelon. The cultivated plantain and banana (*Musa* sp.) are highly sterile polyploid (mainly triploid) plants which develop parthenocarpic fruits. At least three complementary dominant genes control parthenocarpy.

Environmental Parthenocarpy

Variations in environmental conditions such as frost, fog, and low temperatures, interfere with the normal functioning of sexual organs and bring about parthenocarpy. Campbell (1912) observed that a heavy fog in the month of June caused the formation of seedless olives. Lewis (1942) obtained parthenocarpic pears by exposing the flowers to freezing temperatures for 3-19 hours. Cochran (1936) increased fruit setting and obtained parthenocarpic fruits in *Capsicum* by shifting the plants from temperatures of 32-38°C to 10-16°C at the time of anthesis. Osbrone and Went (1953) were able to induce parthenocarpy in tomatoes with temperatures and high light intensity. Under these conditions, pollination is poor.

Chemically Induced Parthenocarpy

Auxins and gibberellins at low concentrations (about 10^{-7} - 10^{-6} M) have been successfully used to induce parthenocarpy in a number of plants which normally bear seeded fruits. These substances are applied to flowers in the form of a lanolin paste or as sprays. The latter is more convenient for commercial purposes.

Balasubramanyam and Rangaswamy (1959) noted that as a result of artificial pollination, most of the varieties of *Psidium guajava* developed into seeded fruits, but the variety "Allahabad Round" yielded seedless parthenocarpic fruits. In this variety, parthenocarpy could also be induced by treating the emasculated flowers with an aqueous extract of

pollen. This "pollen hormone" effect could be duplicated by the application of Indoleacetic acid and indolebutyric acid. Other plants where auxins (IAA, IBA, NAA, NOAA 2 4-D, 2, 4, 5-T) could bring about parthenocarpic development of fruits include tomato, blackberry, strawberry, figs, cucurbits, Citrus and Rosa. Gibberellic acid has been reported to induce parthenocarpy

in a number of rosaceous fruit trees, grapes, figs and tomato It has been suggested that for the induction of parthenocarpy through auxin treatment, the growth substance should be applied sometime after anthesis because an early application may damage the flowers and cause seed abortion and consequently, fruit drop. On the contrary, where gibberellic acid is used for this purpose it should be applied as early as possible, preferably at anthesis (Nitsch, 1963).

Recently, Ding et al. (2013) reported the production of parthenocarpic tomato fruits by the application of a cytokinin, 1-2-chloro-4-pyridyl-3 phenylurea (CPPU). The fruit size was comparable with those induced by GA₃

Genetically Engineered Parthenocarpy

Genetically engineered parthenocarpy is achieved through two mechanisms. Either normal development of embryo and/or seed is blocked without curtailing fruit development or desired hormone activity is induced in the desired organ (Fig. 17.15).

By inserting a chimeric gene Def H9-iaam, Rotino et al. (1997) and Ficcadenti et al, (1999) were able to induce parthenocarpy in tobacco and several lines of eggplant and tomato. The iaam gene from *Pseudomonas syringae* pv saustanoid increases auxin synthesis in the tissues and organs of the transgenic plants and the ovule-specific promoter and regulatory gene DefH9, from *Antirrhinum majus*, regulates the expression of iaam only in the ovules without affecting vegetative growth.

The transgenic plants of eggplant carrying the gene Defu9-aam produced fruits with viable seeds when pollinated and parthenocarpic fruits in the absence of pollination. Thus, in glasshouses where non-transformed plants produce normal fruits only during summer, the transgenic plants continue to produce fruits with normal appearance throughout the year which is commercially very attractive. The parthenocarpic character was transmitted to the progeny in a Mendelian fashion as a dominant trait.

Parthenocarpy offers the possibility of improving fruit quality and productivity in many crop plants grown for their fruits (Spena and Rotino, 2001). Parthenocarpy is regarded as the most efficient way to produce fruits under environmental conditions not favourable for pollen production, germination and fertilization. Whereas in plants such as eggplant parthenocarpy can improve fruit quality, in others, such as kiwifruit, it might also improve the productivity because pollinator plants are not required. For many horticultural crops seedlessness of parthenocarpic fruits is a highly desirable trait for their consumption as fresh fruit or for juice and jam industries. One can imagine the annoyance caused by seeds while eating watermelon. Similarly, seedlessness in table grapes is an important trait meeting the preference of consumers.

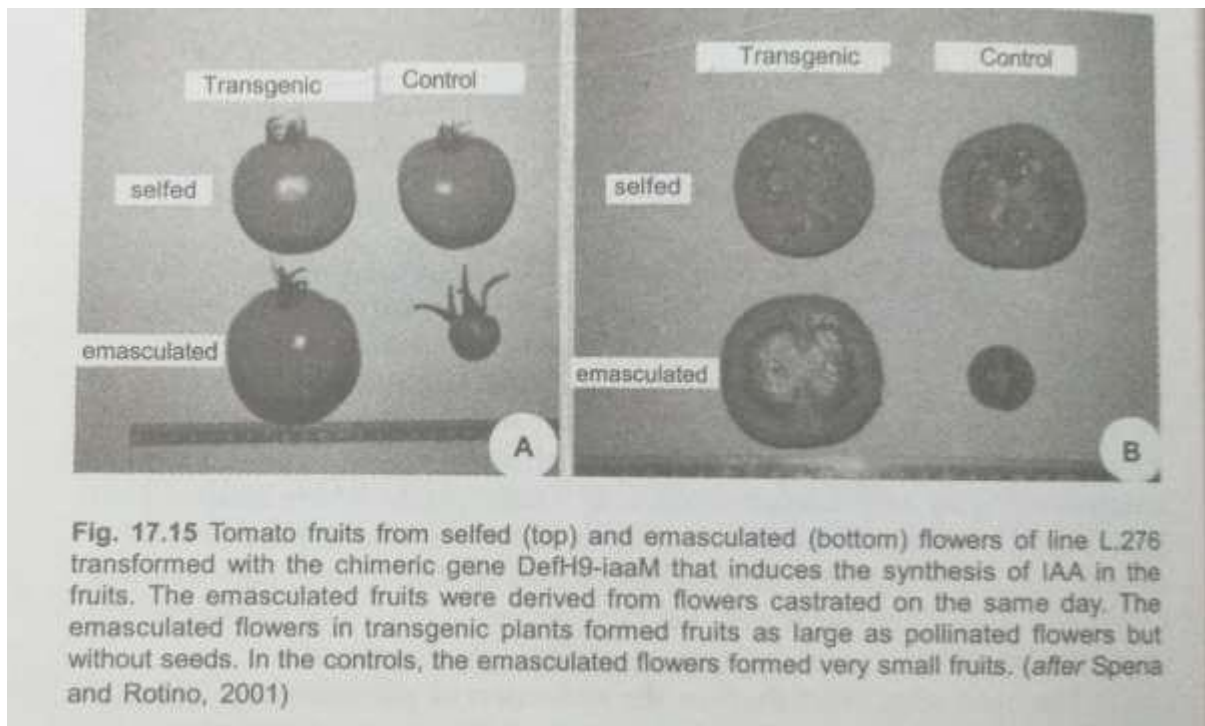


Fig. 17.15 Tomato fruits from selfed (top) and emasculated (bottom) flowers of line L.276 transformed with the chimeric gene *DefH9-iaaM* that induces the synthesis of IAA in the fruits. The emasculated fruits were derived from flowers castrated on the same day. The emasculated flowers in transgenic plants formed fruits as large as pollinated flowers but without seeds. In the controls, the emasculated flowers formed very small fruits. (after Spena and Rotino, 2001)