Taiwania, **46**(3): 204-216, 2001

A Comparative Account of the Gametophytes of Pollen Haploids and Parental Plants of *Nicotiana tabacum* L.

V. V. Anand

(Manuscript received 2 March, 2001; accepted 18 June, 2001)

ABSTRACT: Pollen haploids from *Nicotiana tabacum* L. cv FCV Special were raised by culturing their anthers on MS medium supplemented with .001mg BAP. The haploid plants were grown to adult stage. They flowered profusely but did not produce seeds. A comparison is made between the haploids and their normal parental diploids with reference to the ontogeny and organization of microsporangium, male gametophyte, megasporangium and female gametophyte. The development of microsporangium and male gametophyte in haploids revealed several interesting features. The events pertaining to the development of sporophytic features of microsporangia in haploids were more or less recapitulative of those in normal parental plants. Meiosis in microsporocytes is highly irregular and commences in a few microsporocytes, precociously, at the sporogenous tissue stage itself. Cytomixis, chromosomal disarray and occurrence of laggards are common. Very few 'tetrads' of irregular configurations are organized. Despite anomalous gametogenesis and production of a few microspores, consistently sterile, the anthers dehisced normally as observed in the parental plants. Megasporogenesis and female gametophyte development were highly irregular and unpatternized in haploids. The ovaries were distinctly smaller and possessed fewer ovules than in the diploids. Many ovules degenerated early in their ontogeny. A few that did not, exhibited merely a semblance of embryo sac development and organization, far from the normal patternized events seen in the normal diploids. A bisporic mode of embryo sac ontogeny is prevalent in the haploid ovules. The female gametophytes exhibited precocious organization but never conformed to a uniform pattern. Very few embryo sacs organized into an 8-nucleate Polygonum type of embryo sac. A few apomictic embryoids were noticed in the haploid ovules.

KEY WORDS: ***Nicotiana tabacum*** **L. FCV Spl., Haploids, Male and female gametophytes.**

INTRODUCTION

Although a great deal of information is available on the gametophytes of angiosperms, information on haploid gametophytes is scanty (Chase, 1969). There exists only two reports on haploid gametophytes of *Nicotiana tabacum*; de Fossard (1976) has given a comparative account of sporogenesis and gametogenesis in diploid and haploid N. *tabacum* cv Maryland Mammoth; Tsikova and Molhova (1978) describe the same in haploids produced from both fertile and cytoplasmic male sterile tobacco. These works, however, fail to give a comprehensive and sequential account of male and female gametophyte ontogeny and organization. In the present work, a detailed investigation of the ontogeny and organization of both male and female gametophytes in the haploids of *N. tabacum* cv FCV Spl. is attempted. Further, the causes for degeneration of female gametophytes and the structural changes in the ovule following degeneration of the embryo sac are discussed. A detailed investigation of the normal diploid parental plants of the same variety was also done so that a vis-à-vis comparison could be made between them and the pollen haploids obtained from them.

1. Department of Botany, Regional Institute of Education, Ajmer 305 004, India.

MATERIALS AND METHODS

Plants of *Nicotiana tabacum* cv FCV Spl. were raised in the botanical gardens, Manasagangotri, Mysore-6. Anthers of these plants were cultured on suitable media (Anand *et al.*, 1980). Haploid pollen embryoids and subsequently haploid pollen plantlets were obtained. Plantlets were transferred to soil and grown to the adult stage. The adult haploid pollen plants flowered profusely.

Flower buds and flowers at suitable developmental stages from both parental and pollen haploid plants were fixed for 24-48 hours in FAA and preserved in 70% ethanol. Dehydration was done by the customary alcohol-xylol series (Johansen, 1940). The materials were embedded in paraffin wax of 54-56℃ and serial sections were cut in a Lipshaw rotary microtome at 8-10 μm thickness. The staining schedule was either Heidenhain's iron-alum haematoxylin (Johansen, 1940) or phenolic haematoxylin (Shortt, 1923), with erythrosin in clove oil as the cytoplasmic stain. Dilute Picric acid was used to destain the sections. Drawings were made using an Abbe camera lucida and photomicrographs were taken with a Leica camera.

RESULTS

Microsoprogenesis and male gametophyte in Normal Diploid Plants

The flowers of diploid parental plants are strongly protandrous. The anthers are tetrasporangiate. A young anther in transection is lobed and position of future microsporangia could be recognized at the four corners.

A plate of hypodermal archesporial cells becomes apparent in each of the microsporangial sites. Individual cells of the archesporial plate are densely cytoplasmic and lodge a conspicuous nucleus. The archesporium soon undergoes a periclinal division resulting in an outer row of primary parietal cells and an inner row of primary sporogenous cells. Futher, the primary parietal layer by two more periclinal divisions gives rise to four layers of cells beneath the already existing outermost epidermal layer. Cell divisions in the epidermal layer occur only in anticlinal plane. The cells of the remaining wall layers also undergo anticlinal divisions to keep pace with the growing microsporangium. The wall of microsporangium consists of five layers (Fig. 1, A): the epidermis containing transversely stretched rectangular cells, the endothecium, two middle layers and the innermost tapetal layer. Cells on the connective side of microsporangium and lying adjacent to the sporogenous tissue also differentiate into tapetal cells. This layer aligns itself with the tapetal strip derived from primary parietal layer and forms a continuous sheath of glandular tapetum enclosing the sporogenous cells. Tapetal cells are radially elongated, and often become binucleate (Pl. 1, Fig. B).

As these changes are taking place in the parietal zone, cells of the sporogenous layer undergo a few divisions and organize the sporogenous tissue. Individual cells of sporogenous tissue separate from each other and become potential microspore mother cells (Fig. 1, B).

Meiotic division commences in each microspore mother cell. Two daughter nuclei result from meiosis I (Figs. 1, C, D) and after the second division (Fig. 1, E) a tetrahedral tetrad of microspores enclosed within a callose wall is organized (Figs. 1, F-H). At maturity, adjoining microsporangia coalesce owing to dissolution of separating layers of cells. Cells of endothecium and outer middle layer acquire bands of fibrillar thickenings. At a

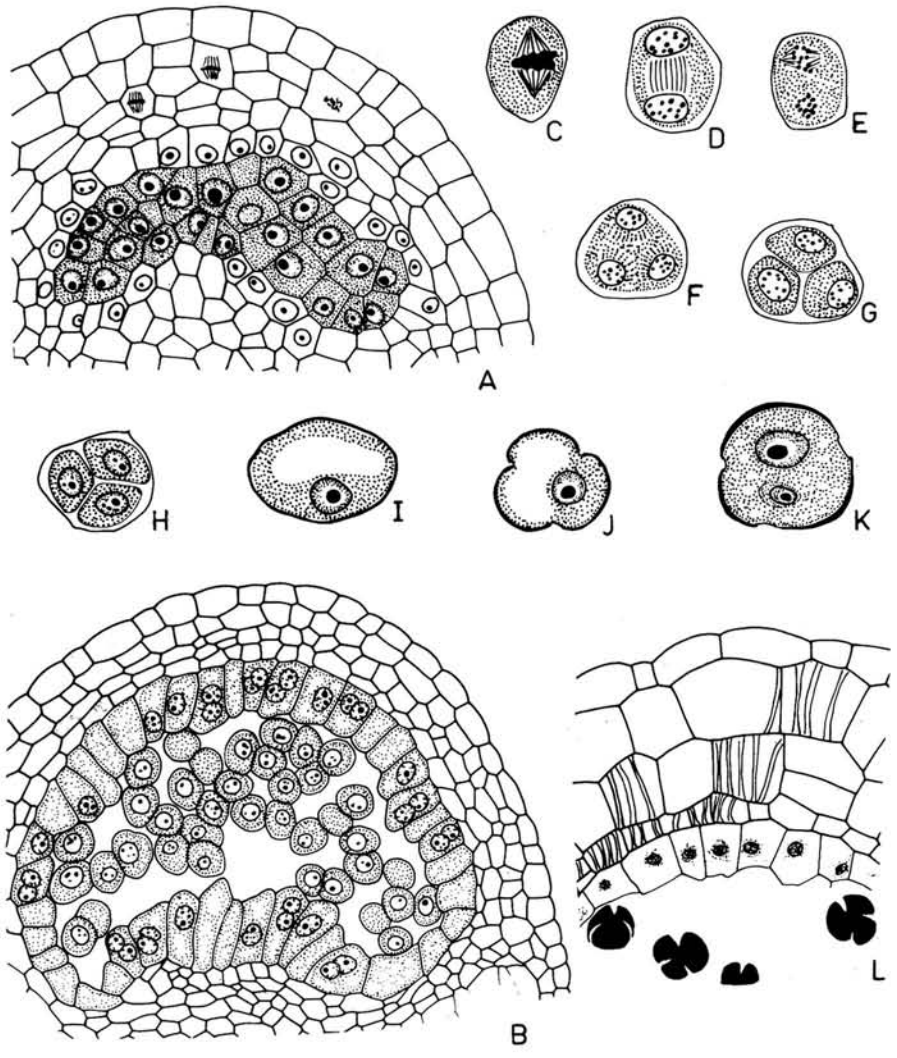

Fig. 1: Microsporogenesis and male gametophyte development in parental plants of *Nicotiana tabacum*. A: Portion of young microsporangium in transection showing sporogenous tissue, x 325. B: A young microsporangium in transection (note the bimodal origin of tapetum), x 325. C-H: Stages during microsporogenesis, x 780. I: Early uni-nucleate microspore, x 780. J: Late uni-nucleate microspore, x 780. K: Mature pollen showing vegetative (larger) and generative (smaller) cells, x 780. L: Transection, portion of mature anther to show fibrillar bands in endothecium and middle layers, x 300.

slightly later stage, such thickenings are laid down even in the cells of inner middle layer (Fig.1, L). Tapetum disintegrates by the time tetrads are formed. A group of subepidermal cells, which are large and hyaline, become conspicuous at the site of anther dehiscence at the microsporocyte stage itself.

Microspores of the tetrad separate and enlarge in size. The centrally located nucleus of the microspore shifts to a side because of the organization of a vacuole in the cytoplasm (Figs. 1, I, J). The nucleus divides, and organizes into a generative cell and a vegetative cell. The densely cytoplasmic generative cell is liberated into the cytoplasm of vegetative cell. Pollen is shed at the two-celled stage. It has a well-developed exine and is tricolpate (Fig. 1, L).

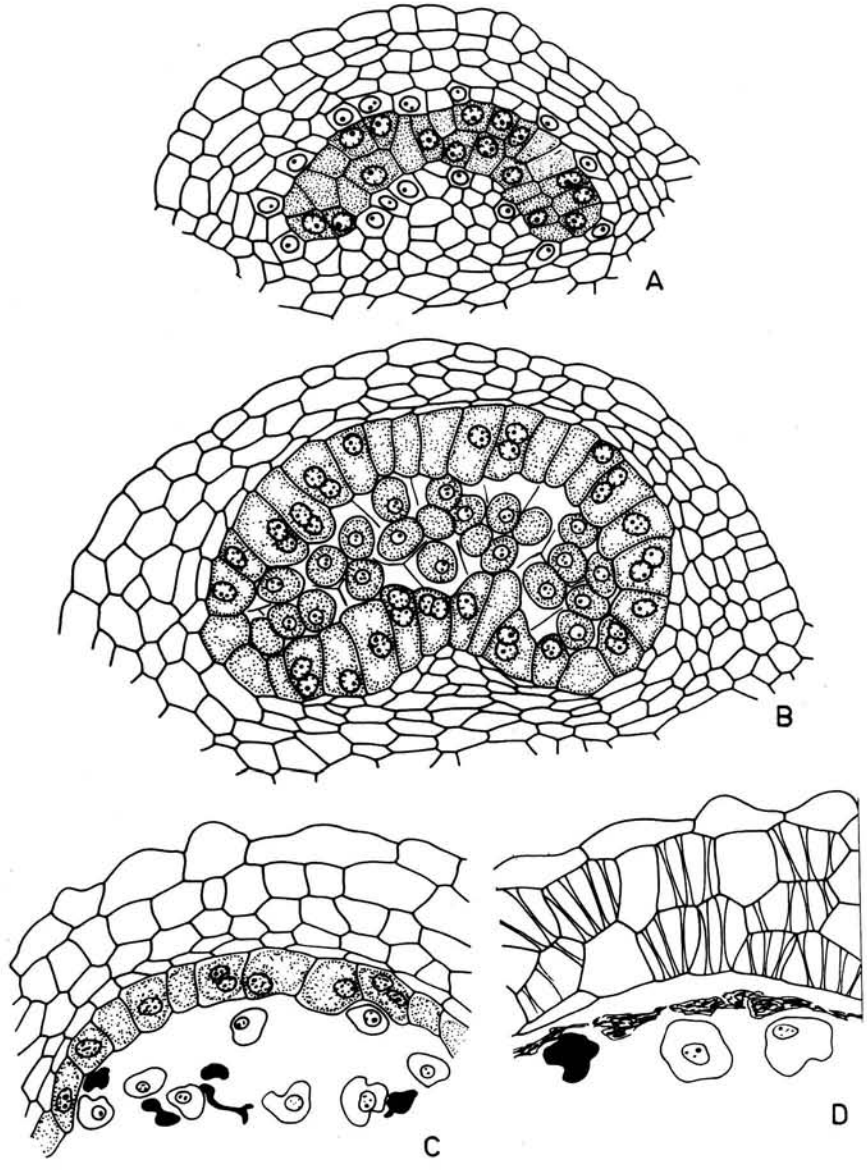

Fig. 2: Microsporogenesis in haploid plants of *Nicotiana tabacum*. A: Transection, portion of young microsporangium showing sporogenous tissue. B: Transection of microsporangium showing bimodal origin of tapetum. C: Transection, portion of mature anther (note, persistence of tapetum and abnormal microspores). D: Transection, portion of dehisced anther showing fibrillar bands in cells of endothecium and middle layers, (all Figs., x 325).

Microsporogenesis and Male gametophyte in Haploids

In haploid plants, events beginning with differentiation of a hypodermal archesporial plate upto differentiation of wall layers and sporogenous tissue are more or less recapitulative of events occurring in anthers of diploid plants (Fig. 2, A; Figs. 3, A-E). The wall consists of five layers, namely, epidermis, endothecium, two middle layers and tapetum (Fig. 2, B). Tapetal cells are of bimodal origin, generally binucleate and of glandular type.

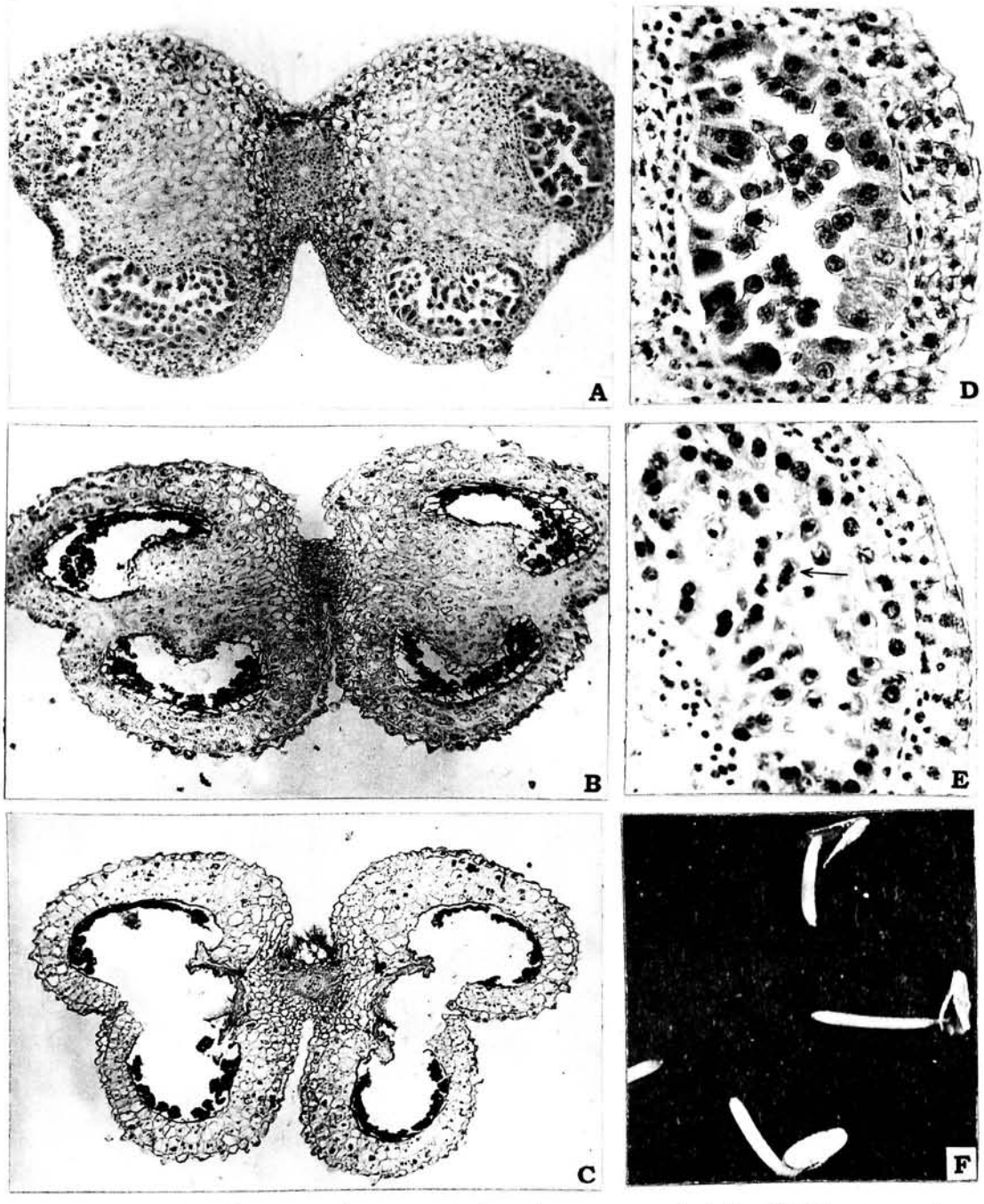

Fig. 3: Photomicrographs of representative stages during microsporogenesis in haploid *Nicotiana tabacum*. A: Transverse section of a young anther at the microspore mother cell stage (note, hyaline cells between two microsporangia of each anther lobe), x 126. B: Transverse section of anther showing degeneration of contents, x 126. C: Transverse section of a mature dehisced anther (note, transverse fibrillar bands in cells of endothecium and middle layers), x 108. D-E: Transverse sections of young microsporangia - enlarged view (note, cytomixis in Fig. 1, E, marked by an arrow), x 297. F: A group of dehisced anthers, x 5.4.

While the ontogeny of wall layers show remarkable similarity with anthers of diploid plants, meiosis of microsporocytes is highly irregular. In many anthers, contents of the pollen sac show complete and very early degeneration (Fig. 3, B). In some anthers, the microsporocytes showed traces of incipient separation from the group, but in a majority of

anthers the microsporocytes clump together. Deposition of callose was noticed around the cells of the sporogenous tissue. In a few microsporocytes, meiotic division seems to commence at the sporogenous tissue stage itself owing to the failure of separation of microsporocytes. Meiosis in microsporocytes is of cytological interest since it is occurring in a monoploid system. The events are highly irregular. Chromosomal disarray during division, presence of laggards and other kinds of anomalies are common. An instance of cytomixis (Fig. 3, E) was noticed in the pollen sac. Inspite of the haploid status and associated abnormalities observed in meiotic division, a few microsporocytes organize into sructures resembling 'tetrads' that are of irregular configurations. Occasionally, a rhomboidal tetrad is formed. A great deal of variation in size is noticed among the 'tetrads'. Most of the 'tetrads' degenerate rather early. The few 'tetrads' which exhibit delayed degeneration and the undivided cells of sporogenous tissue are often seen traped in the matrix of tapetal residue giving the appearance of a black, deeply staining residue (Fig. 3, C) at the periphery of the pollen sacs leaving a large central empty space. No normal pollen grains were noticed in any of the anthers.

By about the time anthers are mature, cells of the endothecium and outer middle layer enlarge radially and acquire band-like thickenings. This occasionally reaches the inner middle layer (Fig. 2, D). A few subepidermal cells at the junction of microsporangia become hyaline and represent sites of future dehiscence. This group of cells becomes differentiated at the sporogenous tissue stage itself (Fig. 3, A). Sometimes the contents of the microsporangium completely degenerate (Fig. 3, B). Despite exhibiting highly abnormal and defective microsporogenesis, anthers of haploids generally dehisce (Fig. 3, F) as in normal plants. In these anthers, the fibrillar bands of endothecium and middle layers are also distinct.

Megasporogenesis and Female Gametophyte in parental plants

In diploid parental plants, the gynoecium is superior, bicarpellary, syncarpous and the ovary bilocular. A large number of unitegmic anatropous tenuinucellate ovules are borne on an axile placenta.

A hypodermal archesporial cell appears in each ovular primordium and directly functions as the megaspore mother cell (Fig. 4, A). Meiosis of MMC results in a linear tetrad of megaspores (Fig. 4, B). The upper three megaspores degenerate while the lower one becomes functional (Fig. 4, C). Cytoplasm of functional megaspore becomes vacuolated; its nucleus divides and subsequently passes through the 2-, 4- and 8-nucleate stages before it organizes into a typical Polygonum type of embryosac (Figs. 4, D, F-H). During the embryo sac ontogeny, the surrounding nucellar cells get crushed and are absorbed by the enlarging embryo sac. Consequently the embryo sac establishes direct contact with inner epidermis of the integument (Fig. 4, E).

Megasporogenesis and female gemetophyte in haploids

The development and structure of ovary and ovules in haploids are in no way different from the parental plants. However, size of the ovary and the number of ovules are reduced in haploids.

Significant differences were however noticed in the mode of megasporo- and megagameto-genesis. The patternized monosporic Polygonum type of embryo sac seen in parental plants is substituted by events that are highly erratic in the haploids. A distinctive feature of haploid ovules is their total sterility.

A hypodermal archesporial cell of a young ovular primordium functions as the megaspore mother cell. It has dense cytoplasm and a prominent nucleus. Although meiosis-I occurs in

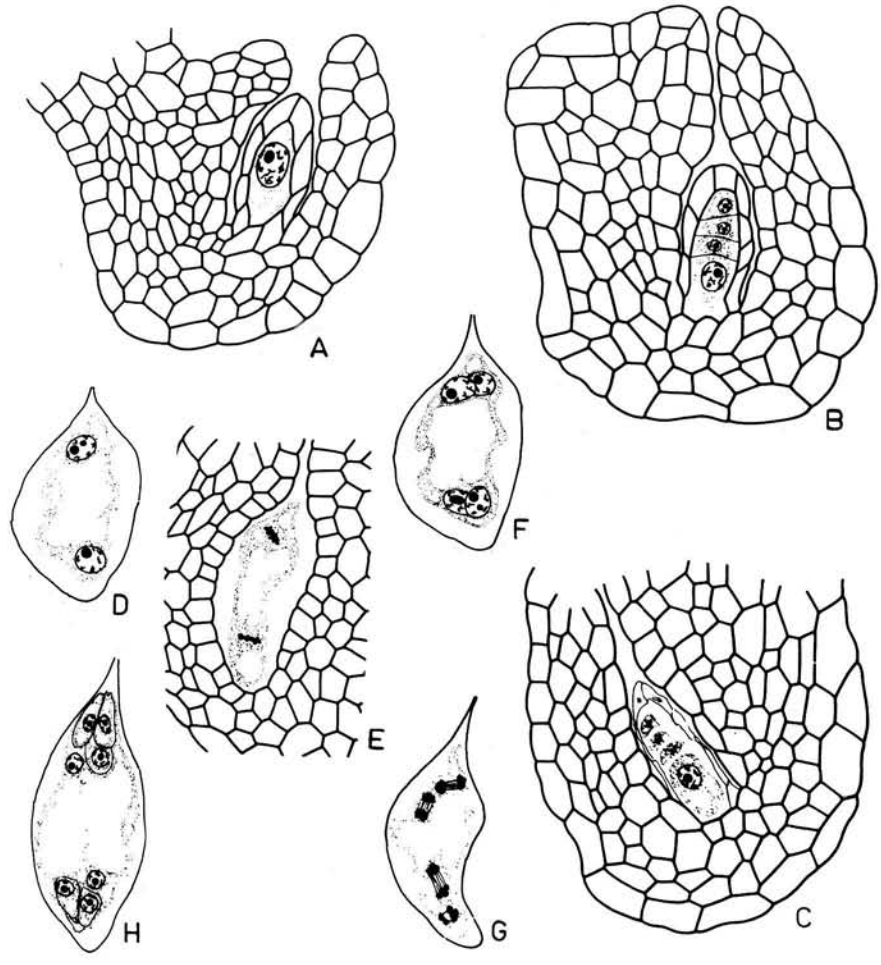

Fig. 4: Megasporogenesis and development of female gametophyte in parental plants of *Nicotiana tabacum*. A-C: Longisections of ovules showing: Fig. A: megaspore mother cell. B: linear tetrad of megaspores. C: Functional chalazal megaspore and degenerating micropylar megaspores. D: 2-nucleate embryo sac. E: Longisection, portion of ovule (note, dividing nuclei of 2-nucleate embryo sac). F: 4-nucleate embryo sac. G: Division of the 4-nucleate embryo sac. H: Mature, organized embryo sac. (all Figs., x 465).

most ovules engendering a dyad, uniformity is lacking in the process of meiosis II. It occasionally occurs in both dyads organizing a linear tetrad of megaspores (Fig. 5, A). It is interesting to note that contrary to the situation in ovules of parental plants, formation of a linear tetrad is very rare in haploids. A slight difference in the orientation of spindles would result in a tetrad of the type represented in Fig. 5, B. In a great majority of ovules, the micropylar dyad cell degenerates and only the chalazal one completes meiosis II (Figs. 5, C, D), suggestive of a probable bisporic ontogeny of embryo sac. This division may either be followed by a wall (Figs. 5, C, D) or not. In the latter case, the completion of meiosis II initiates a 2-nucleate embryo sac (Fig. 7, H). Occasionally, nuclei much smaller than the normal size are noticed (Figs. 7, D, E).

A statistical analysis to determine the type of megasporogenesis was made by counting the number of ovules exhibiting different configurations of megaspores in the tetrad. Only in about 22% of ovules a linear tetrad was organized, the rest exhibited dyad cells or some

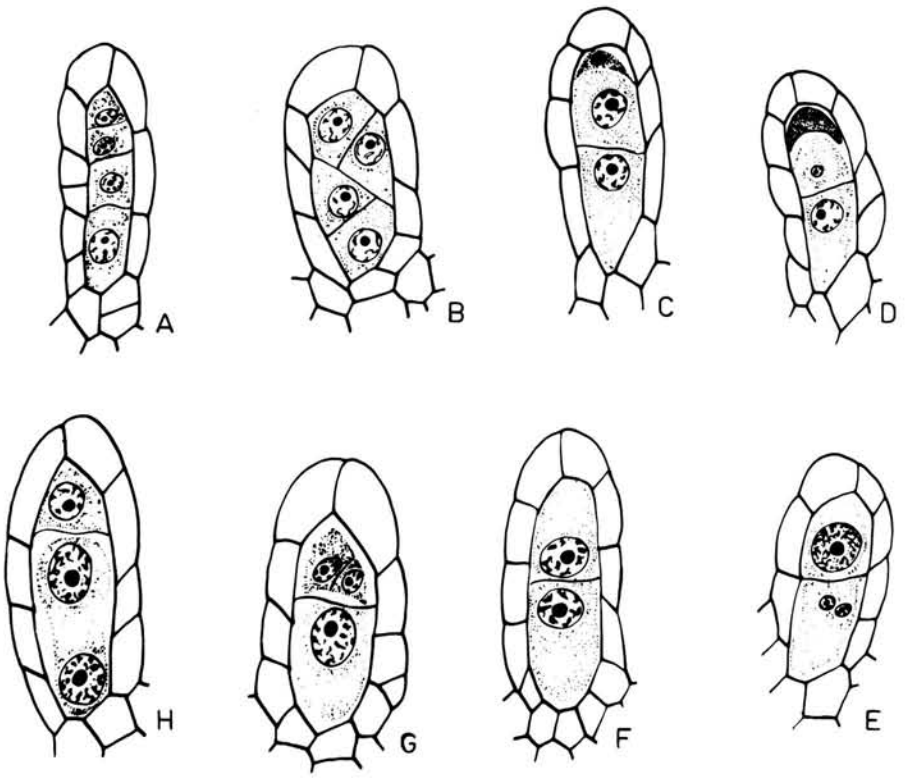

Fig. 5: Megasporogenesis in haploid plants. A: Nucellus and linear tetrad of megaspores. B: Nucellus and tetrad of megaspores with oblique walls. C & D: Triad of megaspores (note, degenerating micropylar dyad; small megaspore nucleus in Fig. 5, D). E-H: Stages during abnormal megasporogenesis (note, small megaspore nuclei in Fig. 5, E), (all Figs., x 940).

aberrant configuration (Figs. 5, D, E). In many ovules, the development is arrested at the megaspore stage itself. Quite frequently, persistent dyads are observed amidst ovules in which advanced stages of female gametophyte are seen (Figs. 6, H, I).

It is practically impossible to sequentially follow the further development of female gametophyte because of a high degree of variability that exists therein. Thus, an account of some frequently occurring types is given below. Pl. 6 presents the variations/ abnormalities noticed in the female gametophytes of haploids.

In all the illustrated cases except in Fig. 6, P, we notice a precocity in the organization of female gametophyte. This precocity is very clear in the embryo sacs represented in Figs.6, A-E, F, M-O. In Figs. 6, A and C, organization sets in at the 4-nucleate stage itself. The two nuclei at the micropylar end in Fig. 6, A do not organize into cells while at the chalazal end two cells are organized. In Fig. 6, C, there is an apparition of an egg apparatus of 2 cells. The single antipodal cell and the chalazal polar are probably derivatives of division of lower chalazal megaspore nucleus. In Fig. 6, D, a single cell is present in the position of egg apparatus. This embryo sac is 5-nucleate with 2 antipodals and 2 polars.

In Fig. 6, F, probably representing a developmental stage, 4 nuclei in a 1+2+1 arrangement are seen which perhaps organizes an embryo sac with a 1+4+1 arrangement of nuclei (Fig. 6, G) because of failure of division of nuclei situated at the two poles.

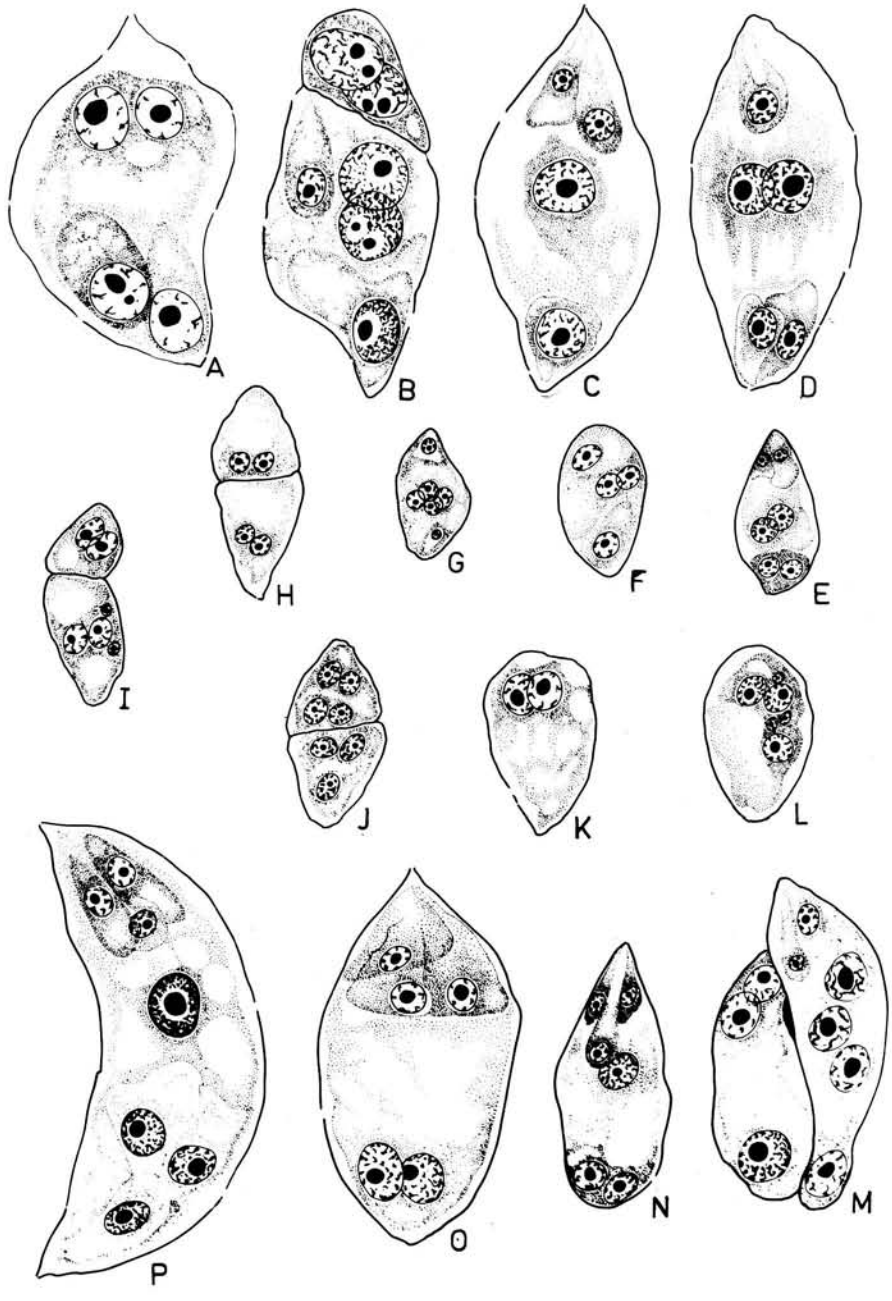

Fig. 6: A few representative embryo sacs of haploid plants of *Nicotiana tabacum*. A-C, F: 4-nucleate embryo sacs showing precocious organization (note, non-functional micropylar dyad, egg, 2 polars and 1 antipodal cell in Fig. B; one egg, one synergid, one polar and one antipodal cell in Fig. C). D: Organized 5-nucleate embryo sac (note, one egg, two polars and two antipodal cells). E: Organized 6-nucleate embryo sac (note, one egg, one synergid, two polars and two antipodal cells). F: 4-nucleate embryo sac. G: 6-nucleate embryo sac (note, one egg, one antipodal and four 'polars'). H-J: Abnormalities in dyads (note, micronuclei in the chalazal dyad cell in Fig. I). K: 2-nucleate embryo sac (note, absence of poleward movement of nuclei). L: 6-nucleate embryo sac (note, micronuclei). M: Twin embryo sacs. N-P: Organized embryo sacs showing variation in number of nuclei. N: 6-nucleate embryo sac (note, one egg, two synergids, two antipodals and a diploid secondary nucleus). O: 5-nucleate embryo sac (note, failure of organization of chalazal nuclei). P: Organized embryo sac (note, normal egg apparatus, one secondary nucleus and three antipodals). (Note, all Figs., x 504; micropylar end directed upwards in all Figs.).

In Fig. 6, H, the megaspore nuclei of the dyad have undergone meiosis - II forming 4 nuclei. Walls, however, fail to appear after meiosis II. In Fig. 6, I, the chalazal binucleate dyad has undergone another division resulting in 4 nuclei while the micropylar binucleate dyad fails to divide. In Fig. 6, J, another abnormality is seen wherein the upper binucleate dyad has divided and only one of the 2 nuclei of the chalazal dyad has divided resulting in 3 nuclei. In Fig. 6, K, the 2-megaspore nuclei are located side by side at the micropylar end. Fig. 6, L, shows 6 nuclei, clumped together without exhibiting any polarity in distribution. It is interesting to note that 3 nuclei are distinctly smaller than the rest.

Figure 6, M, represents a lone instance of twin embryo sacs within an ovule. The 2 embryo sacs are at different stages of growth. While the one towards left is 3- nucleate with a 2+1 arrangement the other has a 2+3+1 arrangement. It is interesting to note that one of the 2 nuclei in the micropylar end has organized into a cell, which is anteriorly vacuolate reminiscent of a typical egg cell.

Figures 6, N-P, though represent organized embryo sacs, there is no uniform pattern in oganization. Pl. 6, Fig. N, has a 3-celled egg apparatus, a large polar and 2 antipodal nuclei. Fig. 6, O, also has a 3-celled egg apparatus, but curiously, no polars and 2 antipodal nuclei. Fig. 6, P, was the lone case of a nearly normal mature embryo sac with a typical 3-celled egg apparatus, a large secondary nucleus and 3 distinctly large antipodal cells.

Although the above illustrated cases of female gametophytes are seen in haploids, not a single instance of fertilization and seed set was observed. All the embryo sacs and most of the ovules ultimately degenerated even before the abscission of flowers from the plant. But amidst these degenerating ovules, there were a few which presented peculiar morphological features. In ovules where degeneration set in early, the innermost layer of integumentary cells elongate and extends towards the centre of the ovule (Figs. 7, A, B). Subsequent breakdown and pectinization of the remaining cells of integument except the outermost epidermal layer followed this. As a result, a conspicuous cavity organizes within the ovule (Figs. 7, C-E). In some ovules, the enlarged integumentary cells coalesce and assume the appearance of 'apomictic embryoids'. (Fig. 7, E).

DISCUSSION

The development of the sporophytic zones of microsporangium in diploid and haploid plants presents a close parallel. In both, the microsporangial wall is constituted of 5 layers, an epidermis, an endothecium, 2 middle layers and a glandular tapetum. Significant differences were however noticed in the behavior of sporogenous cells and in the meiotic process. The early onsent of meiosis, even before the isolation of pollen mother cells is characteristic of haploids. The events of meiosis are highly irregular due to the absence of an entire complement of chromosomes. A few 'tetrads' of unusual configurations are organized contrary to the normal diploid plants, which predominantly organize tetrahedral tetrads. In haploids, tetrads can occasionally develop into pollen but the latter vary greatly in size. The tapetum persists upto anther dehiscence. Cytomixis, which was noticed in some cells of the sporogenous tissue, is probably an attempt at restoration of diploid status. It is likely that in reports of viable pollen production in haploids, cytomixis is one of the fertility restoring processes. de Fossard (1976) has reported successive development of fibrillar thickenings, first in the outer middle layer and subsequently, at the time of dehiscence, in the endothecium and inner middle layer. The development of fibrillar bands of thickenings in endothecium and middle layers is however simultaneous in the present investigation.

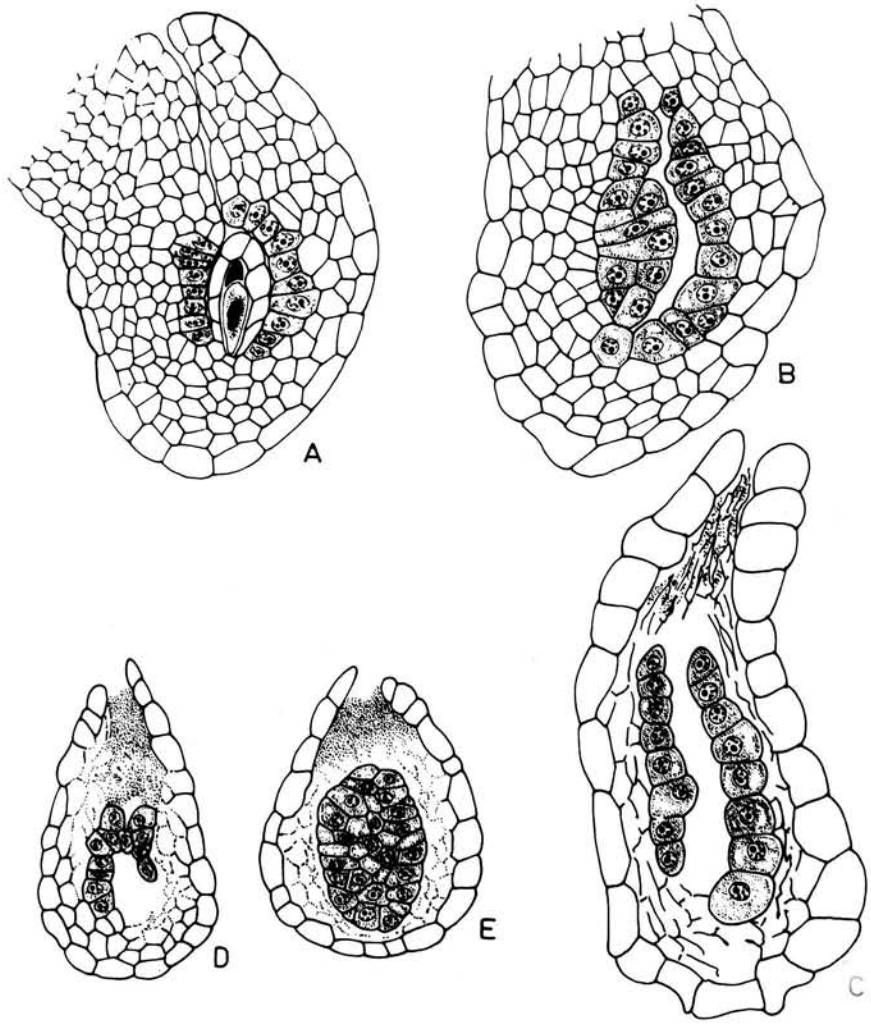

Fig. 7: Ovule anomalies in haploid plants of *Nicotiana tabacum*. A: Longisection of young ovule (note, degeneration of gametophytic cells and enlargement of inner epidermal cells of integument). B: Longisection of ovule showing migration of integumentary cells into the lumen of ovule. C-E: Represent longisections of ovules depicting sages during pectinization and formation of apomictic embryoids. (all Figs., x 300).

The ovules of haploids exhibit numerous abnormal events. The ovary is smaller in size with fewer ovules. Not a single instance of fruit set was recorded in the haploids. Associated with the monoploid status, early abscission of flowers from the plant reduced the chances of fertilization or fruit set. Degeneration of the female gametophyte appears to be the general rule but its onset may be either at the MMC stage or at any later stage of female gametophyte development. A tendency towards early degeneration is however prevalent in most ovules. In a few ovules, where advanced stages of female gametophyte development is noticed, we failed to notice any regular pattern in development, unlike in the parental plants where typical monosporic type of female gametophyte development and organization was noticed. A peculiar observation in haploids is the high incidence of bisporic ontogeny. In fact, a linear tetrad of megaspores is very rarely organized in the haploids. This shift from a normal

monosporic mode of female gametophyte development seen in parental plants to a more frequent bisporic mode in haploids is rather difficult to explain. Another interesting feature of the female gametophyte of haploids is their ability towards precocious organization. Although one may attribute this to either the smaller size of ovules or meiotic abnormalities because of a monoploid complement of chromosomes or a spatial adjustment, it is rather difficult to pin down the exact cause. It could well be a combination of all these factors. The specific polarizing forces that control the female gametophyte development in normal embryo sacs are lacking from the very early stages in haploids. This is probably another reason for the unpatternized female gametophyte ontogeny and organization seen in the haploids.

Contrary to the findings of de Fossard (1976) who reported total degeneration of ovules after the dyad stage, the present study has revealed embryo sacs at advanced stages of development. Very few typically organized, 8-nucleate embryo sacs were encountered, but it is hard to say if they are functional. de Fossard (1976) reported a lone instance of linear megaspore tetrad formation but not any stage beyond that. Though this suggests a monosporic ontogeny, his statement that, 'the nucleus of micropylar dyad either failed to divide or divided without cytokinesis' hints at a bisporic mode of ontogeny. This has been amply confirmed in the present work. A few apomictic tendencies recorded are probably attempts to circumvent the difficulties of abnormal sexual reproduction.

ACKNOWLEDGEMENTS

I express my sincere gratitude to Professor Govindappa D. Arekal and Professor B. G. L. Swamy, both deceased, for their encouragement and academic support in undertaking this study.

LITERATURE CITED

Anand, V. V., G. D. Arekal and B. G. L. Swamy. 1980. Androgenic chimeral plantlets in tobacco. Curr. Sci. **49**: 750.

Chase, S. S. 1969. Monoploid and monoploid derivatives of maize (*Zea mays* L.) Bot. Rev. **35**: 117-167.

De Fossard, R. A. 1976. Sporogenesis and gametogenesis in 2n and n tobacco (*Nicotiana tabacum* Linn.) New Bot. **3**: 24-32.

Johansen, D. A. 1940. Plant Microtechnique. McGraw Hill Book Co Inc., New York.

Shortt, H. E. 1923. Rapid method for Heidenhain stain. In Bolles Lee's Microtomists Vademecum, IX Ed., J and A Churchill, London.

Tsikova, E. and E. Molhova. 1978. Microgametogenesis, macrosporogenesis and macrogameto-genesis of *in vitro* produced haploids from fertile and CMS tobacco. Genet. Sel. **11**: 359-366.

菸草花粉單倍體和親代植株配子體之比較說明

V. V. Anand

(收稿日期：2001 年 3 月 2 日；接受日期：2001 年 6 月 18 日)

摘　　要

花粉單倍體是來自菸草栽培變種 FCV (*Nicotina tabacum* L. cv FCV) 的花藥，而此花藥培養於添加 0.001 mg BAP 的 MS 培養基中。單倍體植株可生長至成熟，其開花數眾多但不能產生種子。依據小孢子囊、雄配子體、大孢子囊和雌配子體的發生與結構，比較單倍體和其正常親代雙倍體的異同。單倍體植株之小孢子囊和雄配子體的發育有許多有趣的現象，而單倍體內小孢子囊之雄配子體特徵之發育大致和正常親代植株相同。小孢子母細胞之減數分裂呈高度不規則，有些提早在造孢組織發育階段就開始進行。胞質融合、染色體脫序和發育遲緩的現象經常發生，其後可產生少許不規則形狀的四分小孢子。雖然有不正常的小孢子發生過程和許多不孕性的小孢子產生，但如同親代植株般，其花藥是可正常開裂的。在單倍體中，大孢子發生和雌配子體發育呈高度不規則且沒有固定模式。比起雙倍體，單倍體的子房較小且內含較少的胚珠，而且很多胚珠在發育早期即已瓦解。一些未瓦解的胚珠僅呈現出類似雙倍體之胚囊發育與構造，而非如雙倍體般有正常的發育模式。在單倍體胚珠中，較普遍的胚囊發生是雙孢子型，而雌配子體呈現出早熟的構造但沒有一固定不變的模式，且少有胚囊能組織成一個八核的蓼型胚囊。在單倍體胚珠中可發現一些無性繁殖的胚狀體。

關鍵詞：菸草栽培變種 FCV、單倍體、雄和雌配子體。

1. Department of Botany, Regional Institute of Education, Ajmer 305 004, India.