Proc. Indian Natn. Sci. Acad.
Vol. **43**, Part B, No. 4 pp. 133-143 (1977)
Plant Embyology

SEED DEVELOPMENT IN DIPLOID AND AUTOTETRAPLOID OF *VINCA ROSEA* SYN. *CATHARANTHUS ROSEUS* (*LOCHNERA ROSEA*)*

by V. R. DNYANSAGAR, *Botany Department, Nagpur University, Nagpur* 440 010
and I. V. SUDHAKARAN, 659 *Seth Niwas, 16th Khar, Bombay* 400 052

(*Received* 30 *July*, 1977)

A day-to-day development study of seed in the diploid and tetraploid plants was carried out. Pollen tubes reached the megagametophyte within 12 to 24 hr after pollination in both diploid and teraploid plants. A high percentage of pollen tubes did not enter the megagametophyte in the induced tetraploids, and in many cases megagametophytes degenerated even before fertilization. The endosperm development is of the Nuclear type and ultimately, the endosperm becomes cellular in both diploid and tetraploid plants. The embryo development in both types of plants follows Caryophyllad type of Johansen, or Megarchetype VI, Series A of the Second Period of Souèges. The embryo is larger in size in the tetraploid seed than in the diploid. The diploid seed matures within 24 days after pollination, while in tetraploids the period is 28 to 32 days. The breakdown of seed in the autotetraploid plants can be attributed to pre-fertilization abnormalities called 'haplontic' sterility.

INTRODUCTION

Anderson (1931) made a comparative comprehensive study of embryology of the Apocynaceae. The embryo development was not, however, studied by him in any detail and hence no complete picture of the seed could be obtained. The embryo development was reported to be uniform in almost all members studied by him. Rao (1940) worked out the embryology of *Cerbera odollam, Vallaris heynei, Ichnocarpus frutescens. Wrightia tinctoria, Carissa carranda* and *Funtrimia elastica.* Neither Anderson nor Rao classified the development of embryo. There is no work on day-to-day development of seed in the family.

Lochnera rosea Reichb. syn. *Vinca rosea* L. syn. *Catharanthus roseus* (L) G. Don, a member of the Apocynaceae, is popularly known as 'Madasgascar Periwinkle' or Cape Periwinkle. It is cultivated in the gardens as an ornamental plant, and grows as a weed all over tropics. It occurs in abundance on sandy beaches and deserted placet. It finds its use in medicine — as a remedy in diabetes carbaniles. More than 20 alkaloids have been reported from different parts of the plant and their biological uses have been widely explored. Among the 15 bases which have already been isolated from the plant, ajamalicine and serpentina are found to be in the largest proportion and may account for most of the activity of total vinca alkaloids. The plant has assumed more importance particularly after the isolation of vinca leucoblastine (VLB) and some other alka-

*The paper was presented at the Indo-Soviet Symposium on Embryology of 'Crop Plants', held at Delhi, during 2-6 March, 1976 (Convener : Prof. B. M. Johri)

loids such as vincarodine and vindolidine. VLB has proved to be very effective in the treatment of cancer.

As no detailed cytogenetic study of this important medicinal plant wmas ade before, we selected this plant for such a study. We also thought it desirable to work out seed development in diploid and induced tetraploid plants noting day-to-day development.

Materials and Methods

The flowers or carpels of diploid and tetraploid plants were collected daily for 30 days after artificial self-pollination. The material was fixed in Cornoy's fluid (3:1) and formalin-acetic-alcohol. It was embedded in paraffin and sectioned at 16-20 μ and stained in iron-alum haematoxylin and destained in concentrated picric acid.

The seeds of the diploid plants were soaked in water for 24 hour and transfered to vials containing various concentrations of colchicine from 0.01% to 0.5% and were immersed for the period ranging from 6-48 hr. The seeds after treatment were washed in running water and sown in the garden soil. The seedlings which survived the treatment were examined for chromosome number. Those showing 32 chromosomes were scored as autotetraploids. The most successful treatment was with 0.3% and 0.4% colchicine for 12 and 24 hr. An alternative method of treatment was also used to save the radicles from coming in contact with the colchicine solution. Germinating seeds were suspended between two glass slides in petri dishes so that the seeds were below and radicles were above the slides; and concentrations of colchicine which induced tetraploidy were 0.2%, 0.3% and 0.4% for duration of 12 to 18 hr. Seedling immersion was also employed but it was found unsatisfactory since most of the seedlings died.

Pollen sterility was studied by staining fresh pollen grains in acetocarmine. Pollen grain viability was studied by germinating pollen in various concentrations of sugar (5% to 90% in 0.5% agar) on a slide for different periods of time varying from 1 to 24 hr. The pollen viability was determined using the standard formula, $W = \frac{s \times d}{100}$. The morphology of pollen grains was studied employing the methods of acetolysis.

Diploid Plant

Observations

Megagametophyte

The mature megagametophyte is elongated in shape, broader at the chalazal end and tapering to the micropylar end. It is of the Normal or Polygonum type (Fig. 1) consisting of 7 cells namely two synergids and one egg constituting the egg apparatus, two polar nuclei and three antipodal cells which are ephemeral and disintegrate soon after fertilization. The polar nuclei migrate to the micropylar region, lying very near to the egg.

Pollination and fertilization

Each microsporangium of the anther contains plenty of pollen, their stainability being 92-96%. The pollen grains germinate on the stigma at different intervals. The

pollen tube passes through the style and it enters through the micropyle. It reaches the female gamete passing between the egg and synergids (Fig. 2). The double fertilization takes place usually 48-72 hr after the day of pollination (Fig. 3). Sometimes the secondary pollen tube seems to be present even after the primary endosperm nuclei begin to divide.

Endosperm

The endosperm follows the Nuclear type of development. The primary endosperm nucleus divides before the division of the zygote. The free nuclei are formed as a result of repeated divisions, commencing from the division of primary endosperm nucleus. They are at first distributed at the periphery of the embryo-sac and the central portion is occupied by a large vacuole (Figs. 4-6). The process of wall formation begins within 5-6 days after pollination even when the embryo is four-celled (Fig. 7) and is completed before the embryo assumes the club shape after 9-10 days of pollination. The endosperm cells are filled with starch. The endosperm persists in the mature seed and it lies extensively all around the embryo.

Embryogeny

The zygote undergoes a period of rest. It divides transversely 4-5 days after pollination. The division results in the terminal cell, *ca* and the basal cell *cb* (Fig. 6). The *ca* divides transversely forming two cells, *cc* and *cd*. The former divides vertically to form two cells. In the meanwhile, *cd* is also segmented transversely into *m* and *ci* (Fig. 8). The basal cell, *cb* remains undivided throughout. It does not take part in the formation of embryo-proper at all. It enlarges and becomes vesicular and forms a part of the suspensor (Fig. 11).

The two juxtaposed cells of derivatives of *cc* divide by a wall at right angle to that by which they are formed, to give rise to quadrants (Figs. 8-10). The embryo assumes this stage within 6 or 7 days after pollination. A transverse division of each of the quadrant cells results in the formation of octants which are arranged in two tiers of cells designated as *l* and *l'* (Figs. 12, 13). The *l* is destined to give rise to the stem apex, *pvt* and the cotyledonary initials, *pco*, while the hypocotyl, *phy* is derived from *l'*. The cell, *m* gives rise to initials of root, *icc*, cortex and root cap, *co*. The cells, *n* and n' divide transversely and their derivatives together with *f* constitute the suspensor. The suspensor attains the maximum development 10 or 11 days after pollination.

The outer octant cells divide periclinally to form the initials of the epidermis and the embryo assumes club shape 9 or 10 days after pollination (Fig. 14). It consists of a long filamentous suspensor and a globular embryo proper. It appears that vesicular cell, *cb* gets detached from the rest of the suspensor when the embryo becomes 12-celled. The cell, *m* divides and forms *d* and *f*. The cell, *d* gives rise to *icc* and *co*, while *f* constitutes the suspensor (Fig. 15).

The following recapitulation table summarises the development of the embryo.

First cell generation

2 cells arranged in two tiers $\begin{cases} ca = pvt+pco+phy+icc+co \\ cb = s \text{ in part} \end{cases}$

Second cell generation

4 cells disposed in three tiers $\begin{cases} cc = pvt+pco+phy \\ m = icc+co+s \text{ in part} \\ ci = s \text{ in part} \\ cb = s \text{ in part} \end{cases}$

Third cell generation

As in the second cell generation.

Fourth cell generation

12 cells arranged in six tiers $\begin{cases} l = pvt+pco \\ l' = phy \\ d = icc+co \\ f = s \text{ in part} \\ n = s \text{ in part} \\ n' = s \text{ in part} \end{cases}$

There appears a depression in the apex of the club-shaped embryo and thus the embryo passes from the radial symmetry to the bilateral symmetry. The depression deepens and the embryo assumes heart shape within 15-16 days after pollination.

The periblem and pleurome are differentiated as well as the initials of root cortex and root cap. The cotyledons elongate and embryo becomes torpedo-shaped (Figs. 16, 17). The embryo takes 3-4 days to assume this shape after assuming heart-shape. The suspensor seems to disintegrate in most of the cases, within 15-16 days after pollination. The embryo enlarges in size by repeated divisions. A mature embryo is formed 25-26 days after pollination (Fig. 18). It consists of two thick flat and short cotyledons and a thick and long radicle. The cells of the embryo contain starch grains in abundance (Fig. 22).

The data regarding the length of the embryo and seed for various stages of growth in relation to the period of growth are given in Table I and are plotted as curves (Fig. 23). The latter indicate that the grand period of growth of the embryo is within 10-16 and 16-25 days after pollination. During the early phase of development, the length increases slowly. The grand period of growth of the seed is within 1-4, 6-9 and 16-25 days after pollination. During the period of 10-14 days, the length of the seed is rather constant while that of the embryo increases slowly.

Development of testa

The integument of the mature ovule at the time of fertilization consists of 6-8 layers of cells (Fig. 19). The cells of the outermost layer are larger in size with a large nucleus and granular cytoplasm. During the course of development of the ovule, the outermost cells enlarge and they become elongated radially (Fig. 20). A centrally situated prominent vacuole is formed in each cell and the nucleus shrinks to a considerable extent. The granular nature of the cytoplasm becomes more and more conspicuous. The cell wall becomes thickened. At different places, a few cells of the outermost layer elongate radially more than the others. Thus the mature testa is composed of epidermis and one or two layers of inner cells (Fig. 21).

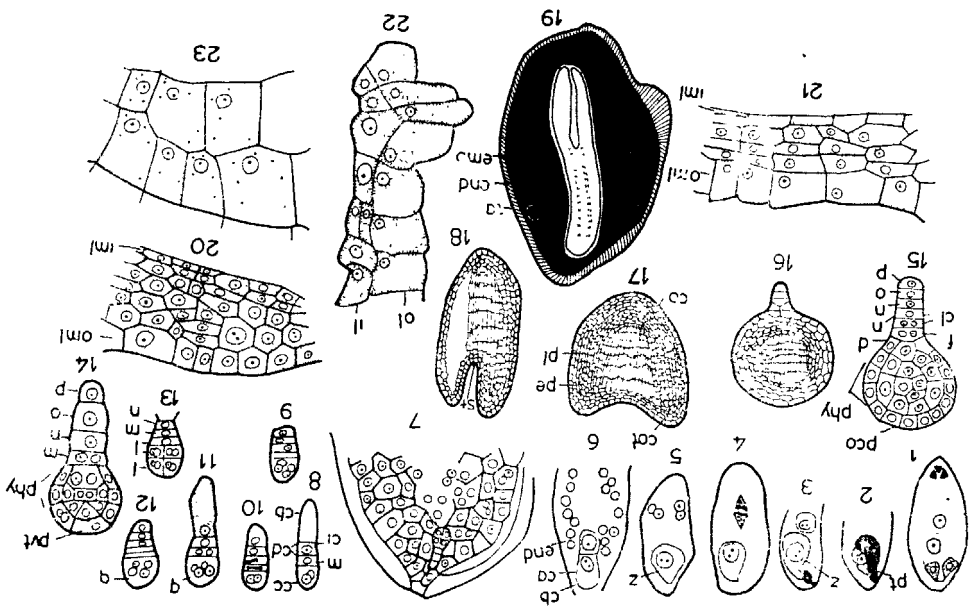


Fig. 1. Mature megagametophyte possessing egg apparatus, polar nuclei (one only seen) and degenerating antipodals.

Fig. 2. Micropylar part of megagametophyte showing pollen tube and egg.

Fig. 3. Mature megagametophyte showing double fertilization and remnants of the pollen tube.

Figs. 4-7. Development of endosperm. 4, Embryo-sac showing zygote and division of primary endosperm nucleus; 5, Embryo-sac showing zygote and four nuclei of endosperm (*Z*, zygote) 6, Part of embryo-sac with two-celled pro-embryo and many peripherally arranged endosperm nuclei (*ca*, terminal cell; *cb*, basal cell; *end*, endosperm nucleus); 7, Micropylar part of the embryo-sac showing club-shaped embryo surrounded by several endosperm nuclei in which wall formation has already commenced.

Figs. 8-18. Stages in development of embryo. For explanation see text.

Fig. 19. L. S. of mature seed (*end*, endosperm; *emb*, embryo; *ta*, testa).

Figs. 20-22. Stages in the development of testa, 20, at the mature megagametophyte stage; 21, at the club-shape stage of embryo; 22, at the mature seed stage.

Fig. 23. Part of endosperm in the mature seed. Cells are rich in starch.

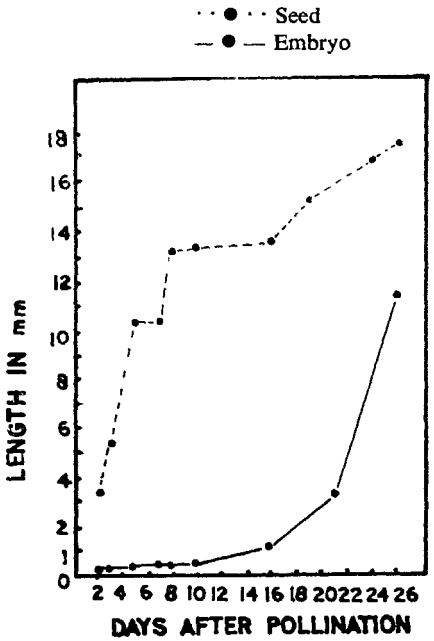


Fig. 24A. Linear development of embryo and seed for various stages of growth in relation to the period of growth.

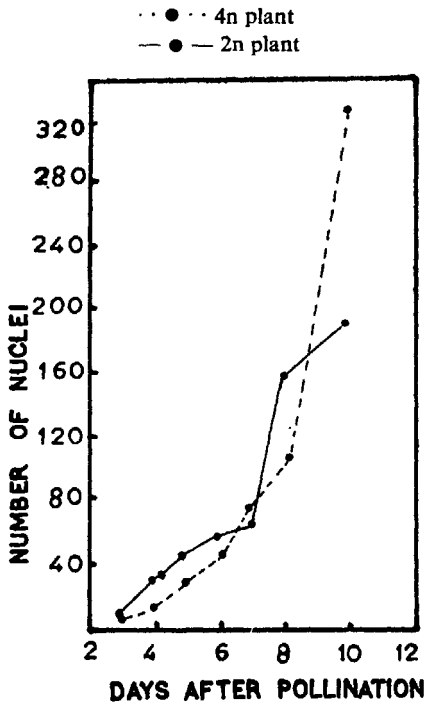


Fig. 24B. Rate of development of endosperm in both diploid and tetraploid.

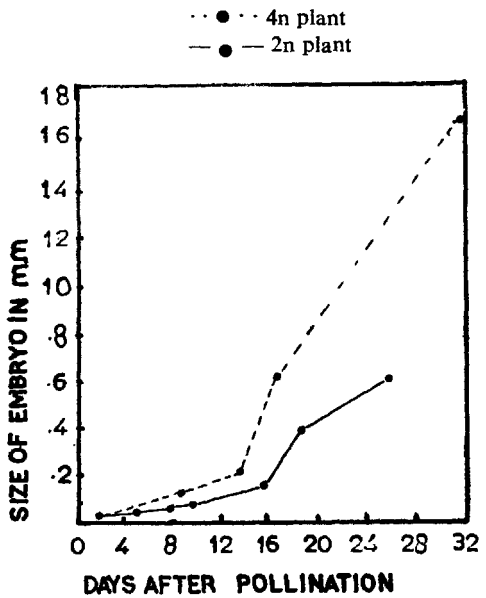


Fig. 25. Comparative growth rate of both diploid and tetraploid plants.

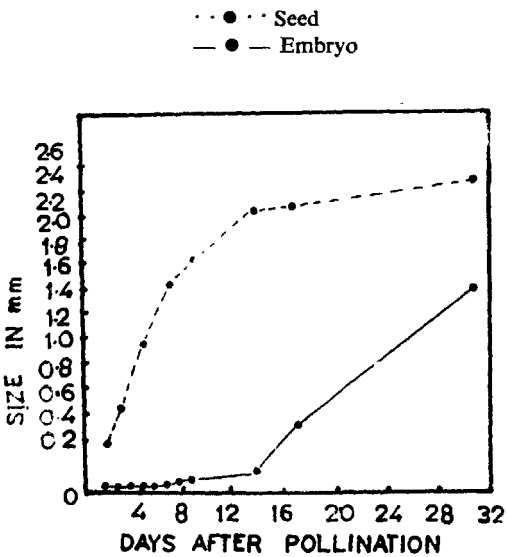


Fig. 26. Linear development of seed and embryo in tetraploid plant.

TABLE I

Linear development of seed and embryo

Period of pollination	Stage of embryo	Embryo length (in mm)	Length of seed or ovule
24-48 hr	Mature egg	0.0155	0.346
48-72 hr	Zygote	0.020	0.564
5th day	2-celled	0.0274	1.051
6-7th day	Tetrad	0.0504	1.06
8th day	Octant	0.0520	1.344
9-10 day	Club-shaped	0.0545	1.355
15-16 day	Heart-shaped	0.146	1.377
18-19 day	Torpedo	0.373	1.534
24-26 day		1.166	1.755

Discussion

Megagametophyte

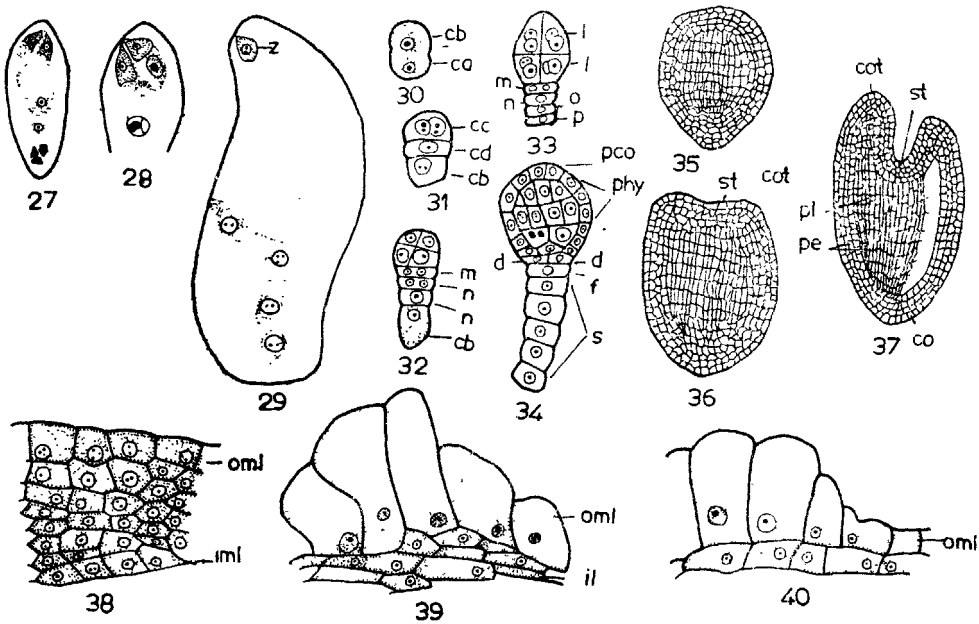
The mature embryo-sac in *Vinca rosea* is of the Normal or Polygonum type as in the other members of the Apocynaceae. The synergids are long. Anderson (1931) and Rao (1940) have also reported longer synergids in other members of the Apocynaceae and very long ones in *Vallaris Heynii*, *Carissa carandas*, and in *Echocanthera spectabilis*. In the last two plants, they are very long, almost reaching the middle of the embryo-sac. The polar nuclei in *Vinca rosea* come very near the egg. Rao (1940) has also pointed out the migration of polar nuclei towards the egg in a few members of the family. The ephimeral type of antipodal cells which are present in *Vinca rosea* seems to be a characteristic feature of the Apocynaceae. Anderson (1931) has observed early disintegration of the antipodal cells in plants of the Apocynaceae studied by him except in *Apocynum connabenum* and *Rhazya orientalis* where they are present even at the time of fertilization. Rao's (1950) similar observations also lead to the same conclusion.

Fertilization

The fertilization in the present plant is porogamous as in other members of the Apocynaceae so far studied. In *Vinca rosea*, the pollen tube has been observed to be persisting in the embryo-sac in a few cases even after the division of the endosperm nucleus. The persisting pollen tube in the embryo-sac even after the division of the endosperm nucleus as well as the formation of the pro-embryo has been reported by Anderson (1931) in the Apocynaceae.

Endosperm

The endosperm formation is of Nuclear type and similar is the case in all other members of the Apocynaceae investigated so far (Anderson, 1931 and Rao, 1940). The wall formation which starts from the periphery towards the centre in the present plant, appears to be the characteristic feature of this family. The endosperm



Figs. 27-40. tetraploid Mature megagametophyte possessing egg apparatus, polar nuclei (one) and degenerating antipodals, 28, Micropylar part of mature megagametophyte with double fertilization; 29, Zygote with four nuclei of endosperm, 30, Two celled pro-embryo (*ca*, apical cell; *cb*, basal cell); 31, 4-celled T-shaped pro-embryo; 32, Embryo showing the formation of quadrants; 33, Octant stage of an embryo; 34, Club-shaped embryo; 35, Embryo pior to heart-shaped stage; 36, beginning of heart-shaped stage of embryo; 37, Beginning of torpedo stage. (*st*, stem apex; *cot*, cotyledon; *co*, root cap; *pe*, periblem; and *pl*. plerome), 38-40. Developmental stages of testa. 38, At mature megagametophyte stage (*oml*, outermost layer; *inl*, innermost layer); 39, At the club-shaped stage of embryo; 40, At the mature seed stage (*oml*, outermost layer *il*, inner layer).

persists in the mature seed and the cells are filled with starch. Anderson (1931) has reported similar case in other members of the Apocynaceae.

Embryogeny

The present study indicates that the embryo development follows the Caryophyllad type of Johansen or Second Period, Series A, Megarchtype VI of Souèges. This study of the embryo development in *Vinca rosea* is based on day-to-day observations. While in all the other previous investigations in the embryogeny of the Apocynaceae, no such attempt was made. None of the previous workers have classified the type of embryo development studied by them. Johansen (1950) who has reviewed the related literature states that in the Apocynaceae, the embryo development in several species is very similar to that in the species of the Hypericaceae and it may, therefore, be assigned to the Sedum variation of the Caryophyllad type. It may be mentioned here that the Sedum variation is based on the presence of hypophysis. In the case of *Vinca rosea*, however, definite formation of the hypophysis has not been observed. A close study of the figures of the embryo development in *Lochnera rosea* and *Vinca minor* given by Anderson (1931) and those in *Garissa carandas*, *Cerbera odollam* and *Ichnocarpus frutescence* also (Rao, 1940) indicates no definite formation of hypophysis and none of these authors have also mentioned about the presence of this structure in their papers.

The suspensor in *Vinca rosea* is elongated and filamentous. Anderson (1931) has reported that it is made up of two layers of broader cells at the base in *Vinca minor*. While Rao (1940) states that the suspensor is uniformly broad and appears 3-4 seriate in most of the plants studied by him.

The seed coat is formed from the epidermis and compressed inner cells of integument, and it seems to be a characteristic feature of the Apocynaceae. Anderson (1931) has also observed similar type of seed-coat in this family.

Autotetraploid Plant

Observations

The percentage of fertile pollen in the induced tetraploid is 32-43%. The percentage of flowers fallen off after pollination is also considerably high, the range being 80-90%. It is, therefore, not surprising to note that the percentage of seed setting in relation to the total number of flowers pollinated is very less, the range being 17.5-22.5%. This has been supported by the data obtained from the study of the development of the seed. It is only in a few cases that the development of seed proceeds normally (Figs. 26-39). While in majority of cases, several abnormalities occur resulting in seed failure. These are described below :

(i) Many ovules (37-42%) shrink and collapse in the ovary.

(ii) The percentage of ovules without embryo-sacs, incompletely organised embryo-sacs and those which are delayed in organisation is 52-61%.

(iii) About 9-13% of the viable pollen grains do not germinate on stigma even 28 hours after pollination.

(iv) The pollen tube seems to fail to reach the ovary by the time the flower open and many of the pollen tubes seem to terminate their growth at different sections of the style.

(v) It seems that the pollen tube in many cases has not entered the ovary even 3 days after pollination.

(vi) In a few cases, the embryo degenerates during the first 5 to 7 days after pollination. But the endosperm development is found to be almost normal in these ovules.

Some of the abnormalities are given in the table below :

Plants	1	2	2
Number of flowers pollinated	40	30	40
Percentage of flowers fallen off	86 5	82.0	91.1
Number of seed sets	9	6	7
Percentage of seed set per 100 flowers pollinated	22.5	20.0	17.5
Percentage of undeveloped ovules	40.2	41.8	37.4
Percentage of abnormal embryo-sacs	52.6	61.2	37.5
Percentage of fertile pollen	43	32	40.5

Differences in the development of seed as compared with that of the diploid

(i) The egg cell of the egg apparatus is large. The polar nuclei in many of the embryo-sacs exhibit delay in fusion by the time flower opens and then lie distantly apart from each other.

(ii) The size of the nucleus in the egg as well as polars is large.

(iii) The rate of development of the endosperm during the first few days (3-8) is comparatively less and during the later period more endosperm nuclei are formed (Fig. 24 a).

(iv) The size and shape of nuclei of endosperm cells vary considerably. The nuclei of different sizes vary in numbers from 2-6 per nucleus.

(v) Embryo assumes club-shape in 8-9 days.

(vi) The transition from the radial to the bilateral symmetry by assuming torpedo shape, occurs in 16-17 days after pollination.

(vii) The seeds mature after 28-30 days.

(viii) A comparative study of the growth rate of both 2n and 4n seeds (Fig. 24b) indicates that during early stage, the tetraploid embryo grows faster and slows down during the later period. It is larger in size.

(ix) The cells of the testa are larger and they become suberized.

Comparative data concerning the linear development of the seed and embryo in the tetraploid (Fig. 25) indicates that the growth of embryo is slow and becomes fast during the second phase of growth (8-11 days) and the grand period of growth is 12-17 days. On the contrary, the grand period of growth of the seed is 5-14 days and the growth seems to be very low during the later period.

Discussion

It has been observed from the data on the development of seed of diploid and tetraploid plants that doubling of chromosomes in the tetraploid does not affect the normal pattern of development of embryo. However, polyploidization seems to alter the

rate of growth of embryo and endosperm therein. It has been noticed that the embryo grows faster during the early period while in later stages the growth seems to slow down. On the contrary, the rate of endosperm development during the first few days is comparatively slow and becomes more active towards the later period resulting in the formation of many more nuclei than in the developing seed of the diploid. It seems that doubling of chromosomes also results in an increase in the size of embryo. This can be attributed to the formation of a large number of cells which are also comparatively larger in size than in the diploid.

It has been noticed that seed fails to develop in majority of the cases in the tetraploid *Vinca rosea*. Several workers have observed reduced seed setting in autotetraploids. The sterility has been attributed to the pre-fertilization upsets called as "haplontic" sterility and post-fertilization abnormalities leading to the aberrant endosperm and embryo development. These abnormalities are referred to as "zygotic,' sterility. It can be visualised that the reduced seed setting in the present plant may be due to mostly pre-fertilization abnormalities. It has been observed that here only 32-43% of the pollen grains are fertile. Of the viable pollen grains, 9-10% seem to fail to germinate and of the germinated ones, the pollen tubes in many cases seem to develop incompletely within the style. They may even fail to enter the embryo-sac. This may result in a very poor percentage of fertilization and consequently flowers may shrivel away. It has been noticed that 82-90% of pollinated flowers fall off in the present plant. It has been observed that 37-42% of the ovules do not develop and they collapse. The ovules which develop normally may exhibit irregularities. About 50-60% of these may be without the embryo-sac and others with an un-organised embryo-sac. Thus there are a very few ovules left which develop normal embryo-sac in the case of the tetraploid *Vinca rosea*. Hence it seems that pre-fertilization abnormalities such as undeveloped ovules, lack of normal organisation of the embryo-sac, delayed organisation of the same and the delay in fertilization are all playing an important role in the break-down of seed in the autotetraploid.

Vinca rosea

The pre-fertilization abnormalities have been reported by many previous workers. Pandey (1951) in *Linum usitatissimum* and Parthasarathy in *Brassica campestris* (1953) have reported that the low seed setting in autotetraploids of these species is mainly due to pre-fertilization abnormalities leading to abnormal degeneration of the embryo-sac and also to non-fertilization of a great number of apparently normal ovules. Sensome *et al.* (1942) and Rajan and Ahuja (1956) have opined that the seed sterility in *Datura* and *Toria* autotetraploid plants is partly due to failure of fertilization and partly to "zygotic" sterility. Cooper and Brink (1945) have found that the seed collapse following mating between 2n and 3n races of *Lycopersicum pimpinelli-folium* may be largely due to restricted growth of pollen tube with consequent non-fertilization. Hakansson (1952) has reported the low seed setting in the cross between the diploid and tetraploid in *Galeopsis pubescens*. He suggests that "haplontic" sterility plays only a minor part in inducing sterility in high percentage of un-fertilized and apparently normal embryo-sacs. Datta and Banerjee (1960) remark that the failure of seed setting in 4n cultivated *Corchorus* and their crosses with 2n, may be the result of the failure of fertilization of the egg in time.

It has been observed that the post-fertilization abnormality such as degeneration of the embryo may also contribute partly to the seed break-down in the present plant. "Zygotic" sterility has been reported by previous investigators. Pandey (1954) has suggested that "zygotic" sterility like the abnormal behaviour of the endosperm can be attributed to the break-down of embryos in the autotetraploid *Trifolium pratense*. Hakansson and Ellerstorm (1950) have attributed the low seed setting in reciprocal crosses between 2n and 4n rye, to the post-fertilization abnormalities such as degeneration of the endosperm, and rarely embryos. Kihara and Nishiama (1932), Boyes and Thompson (1937), Brink and Cooper (1944), Thompson and Johnston (1945), and Pandey (1954) have also reported irregularities in the endosperm development leading to break-down of seed in the autotetraploid plant.

Thus it appears that probably the important source of seed sterility in the present study is "haplontic" sterility, an inherent inability in the formation of normal pollen grains and their subsequent growth and development during germination and also in the development and organisation of ovules. This inability may be attributed to the "disturbed" physiological and genetic factors induced in the cells of the autotetraploids.

References

Anderson, A. (1931). Studien uber die Embryologie der Familen Celastraceae, Oleaceae and Apocynaceae. *Act. Univ. Lund. Adv.* 2, 27. 1-112.

Boyes, J. W. & Thompson, W. P. (1937). The development of endosperm and embryo in reciprocal interspecific crosses in cereals. *J. Genetics*, **34**, 203-227.

Brink, R. D. & Cooper, D. C. (1944). The antipodals in relation to abnormal endosperm behaviour in *Hordeum jabatum* × *Secale cereale* seeds. *Genetics*, **29**, 391-406.

Cooper, D. C. & Brink R.D. (1945). Seed collapse following mating between 2n and 4n races of *Lycopersicum pimpinellifolium*. *Genetics* **30**, 370-390.

Datta & Banerjee (1960). On the cause of failure of seed setting in 2n cultivated *Corchorus capsularis* L × 2n cultivated. *capsularis* and 4n cultivated *C. olitorus* L. × 2n cultivated *C. olitorus* and their reciprocals through cytogenetical studies. *Genetics* **31**, 384-409.

Hakanson, A. & Ellerstrom, T.S. (1950). Seed development after reciprocal crosses between diploid and tetraploid rye. *Hereditas*, **36**, 256-296,

Hakanson, A. (1952). Seed development after 2n×4n crosses of *Galeopsis pubescens*. *Hereditas*, 38, 425-448.

Johanson, A.D. (1950). *Plant Embryology*. Chronica Botanica, Waltham, U.S.A.

Kihara, H. & Nishiayama, I. (1932). Different compatibility in reciprocal crosses of *Avena* with special reference to tetraploid hybrid between hexaploid and diploid species. *Jap. J. Bot.*, **6**, 245-305.

Pandey, K. K. (1951). Comparative study of diploid and autotetraploid of *Linum usitatissimum*. Thesis submitted for the Assoc., I.A.R.I., New Delhi.

———(1954). Diploid and tetraploid crosses of *Trifolium pratense* L. *Indian J. Genet. & Pl. Breeding*, **14**, 25-35.

Parthasarathy, N. (1953). The use of induced autotetraploids in plant breeding. *Indian J. Genet. Breeding*, **13**, 1-6.

Rajan, S. S. & Ahuia, Y. R. (1956). Seed development in colchicine-induced autotetraploids of *Toria*. *Indian. J. Genet. & Pl. Breeding*, **16**, 63-70.

Rao, A. M. (1940). Studies in the Apocynaceae. *J. Indian Bot. Soc*,, **19**, 33-44.

Sansome E. R., S. Satina A. F. Blakeslee, (1942). Disintegration of ovule in tetraploid-diploid in compatible species crosses in *Datura*. *Bull. Torr. Bot. Club*, **69**, 405-420.

Thompson, W. P. & D. Johnston. (1945). The cause of incompatibility between barley and rye *Canadian J. Res.*, **23**, 1-15.