Proc. Indian natn. Sci. Acad. **B52** No. 5 pp 679–684 (1986)

Cytochemical Studies of Suspensor Cell during Embryo Development in *Najas marina* L*

B K JAIN†
Botany Department, Gujarat University, Ahmedabad 380 009

(Received 17 October 1986)

Changes in the extinction values and contents of RNA, DNA, −SH and total proteins were studied in the suspensor cell during different stages of embryo development in *Najas marina* L. Attempts were also made to study the changes in the intensity of insoluble polysaccharides qualitatively. The suspensor cell shows five to eight fold increase in these macromolecular substances during early embryogenesis and a gradual decrease in their levels during cotyledon and plumule initiation. At cotyledon initiation stage, the DNA content shows 242 C level as compared to that of the haploid nucleus (1C) of microspore of the same plant. This higher amount of the DNA points to endopolyploidy.

Key Words: *Najas marina*, **Suspensor cell, Embryo, Nucleic acids, Proteins, Endopolyploidy**

Introduction

Suspensor is an enigmatic structure with respect to its homology with other embryonal parts as well as its function. Recent structural (Nagl 1976, Yeung & Sussex 1979), physiological (Alpi et al. 1975, Lorenzi et al. 1978), biochemical (Walbot et al. 1972, Sussex et al. 1973, Clutter et al. 1974), histochemical (Prabhakar & Vijayaraghavan 1983) and embryo culture studies (Cionini et al. 1976, Yeung & Sussex 1979) indicate that suspensor plays an active role during early embryo development.

With a view to extend our knowledge of embryo-suspensor relations at different stages of seed development the present work was carried out, which outlines the relative changes of some physiologically active macromolecular substances, viz. RNA, DNA, total and −SH proteins and insoluble polysaccharides in the suspensor cell and their role in embryo development in *Najas marina* L.

Material and Methods

The fertilized ovules of *Najas marina* L. were fixed in Carnoy's fluid and processed for microtomy. For histochemical localization, the standard procedures and their control methods were followed, as listed below:

Sub-stances	Procedures	Controls
RNA	Pyronin-G method (Tepper & Gifford 1962)	Enzymatic digestion with RNase
DNA	Feulgen reaction (Jensen 1962)	Hydrolysis process was omitted
Total proteins	Mercuric bromophenol blue (Mazia et al. 1953)	Sections were treated with trichloroacetic acid
−SH proteins	DDD method (Barrnett & Seligman 1952)	— do —
Insoluble polysacc-harides	Periodic acid Schiff's (PAS) method (Jensen 1862)	Sections were brominated before oxidation

For the quantitative determination of the cell constituents, the staining intensities were measured with

*This forms a part of the Ph.D. Thesis submitted to the Gujarat University, Ahmedabad
†Present address: Botany Department, Arts, Science & Commerce College, Shahada 425 409 Dist: Dhule (Maharashtra)

the help of cytophotometer (Bhatt et al. 1983). Two parameters, viz. extinction value (*e* value) and content per cell or nucleus were calculated from absorption values as suggested by Pollister et al. (1969) and expressed in Arbitrary units (Au).

Results

The embryogeny of *Najas marina*. L. conforms to the Aponogetonad type (Shah 1970). The basal cell(cb) does not undergo further divisions but enlarges enormously and becomes a large vesicular cell, while the apical cell(ca) is destined to form embryo proper.

Micrometric Changes

Area and nuclear volume of the suspensor cell reveal five and eight time increase respectively from cb+2ca to plumule initiation stage of the embryo development but decrease during further development (figure 3A).

Histochemical Changes

RNA: After the first division of zygote, the suspensor cell manifests a continuous rise in the *e* value (figure 2A) and content (figure 2B) up to globular stage of embryo. However, *e* value falls down later on but content reveals a peak at cotyledon initiation stage. The suspensor nucleolus and cytoplasm are highly pyroninophilic during all the stages of embryogeny (figure 1A, C) but maximum staining is observed at globular stage. After cotyledon initiation, the intensity of cytoplasmic RNA increases in the radicular region of the embryo with simultaneous decrease in suspensor cell (figure 1G).

Total proteins: Like RNA, total proteins also show high level of their *e* value (figure 2A) at globular stage. Content manifests a five- to seven-fold increase from cb+6 ca to plumule initiation stage (figure 2B). Minimum *e* value and content are observed at the mature embryo stage. The cytoplasm and nucleolus of the suspensor cell are proteins rich throughout embryogeny (figure 1D, E). The radicle cells of embryo proper show an increasing staining intensity of proteins with a simultaneous decrease in the suspensor cell.

–SH proteins: The suspensor cell reveals a gradual rise in the *e* value from cb+6 ca to globular stage of embryo development (figure 2A). The *e* value is double at globular stage as compared to that of the cb + 6 ca stage. Content also shows a seven- to eight-fold increase from cb+6 ca to elongated stage of embryo (figure 2B). The trend of relative changes of –SH proteins very closely resembles with total proteins and RNA.

DNA: *E* value of DNA manifests a peak at globular embryo stage, where it is double than that of the cb+6 ca stage (figure 3B). Content shows its peak value at cotyledon initiation stage which is five times more than the value at cb+6 ca stage (figure 3B). The nucleus of suspensor is deeply stained and does not show any mitosis-like structural changes during entire period of embryo development (figure 1B, F). The amount of DNA increases within nuclear envelope without spindle formation.

A quartet nucleus of developing pollen (microspore) of the same plant is selected as a standard to estimate DNA content. Each nucleus of quartet has 1C (8.5 Au ± 0.062) DNA of haploid cell. The amount of DNA in developing suspensor cell was compared with it and the maximum 242C (2063.2 Au ± 0.09) level of DNA is reported at the cotyledon initiation stage.

Insoluble polysaccharides: In early proembryo stages (i.e. up to cb+16 ca) the PAS reaction is mostly associated with the wall of the suspensor cell. Cytoplasm shows the presence of diffused polysaccharides but no starch. The cell walls, at the points of attachment of suspensor with the embryo sac on one side and embryo on the other side are thick and stained deeply. No accumulation of starch grains occurs in the suspensor cell up to plumule initiation stage. But later, when embryo is fully mature and cuts off from the suspensor cell, the suspensor cell stores starch grains in the peripheral region (figure 1H).

The regression graphs of RNA/DNA contents (figure 2C) and RNA/total proteins contents (figure 2D) show positive correlation in the suspensor cell during all the stages of development. However, the points of experimental and calculated values lie near the central trend line reveal sharp positive correlation while those which are slightly away manifest weak correlation with respect to their stages. Correlation between RNA/total proteins is much stronger than between DNA/RNA.

Discussion

Five- to seven-fold increase of RNA, total proteins, –SH proteins and DNA contents in the suspensor cell (cb) of *N. marina* during early embryo development and a continuous decrease in their levels during later stages of embryogenesis, i.e. cotyledon and plumule initiation, indicate an important functional role of the suspensor, and it appears that the most physiologically active period for the suspensor cell lies between the mid globular to the elongated stage. Walbot et al. (1972), Sussex et al. (1973) and Clutter et al. (1974) reported highest rate of RNA and proteins syntheses in the suspensor cells of *Phaseolus coccineus* during early embryogeny. Similar observations were also made by Prabhakar and Vijayaraghavan (1983) in *Alyssum maritimum*. Latter, however, this activity declines in the suspensor and increases instead in the embryo proper.

A continuous increase in the intensity of RNA, total proteins, –SH proteins and insoluble polysaccharides occurs in the radicular region of the embryo with corresponding decrease in the suspensor cell during

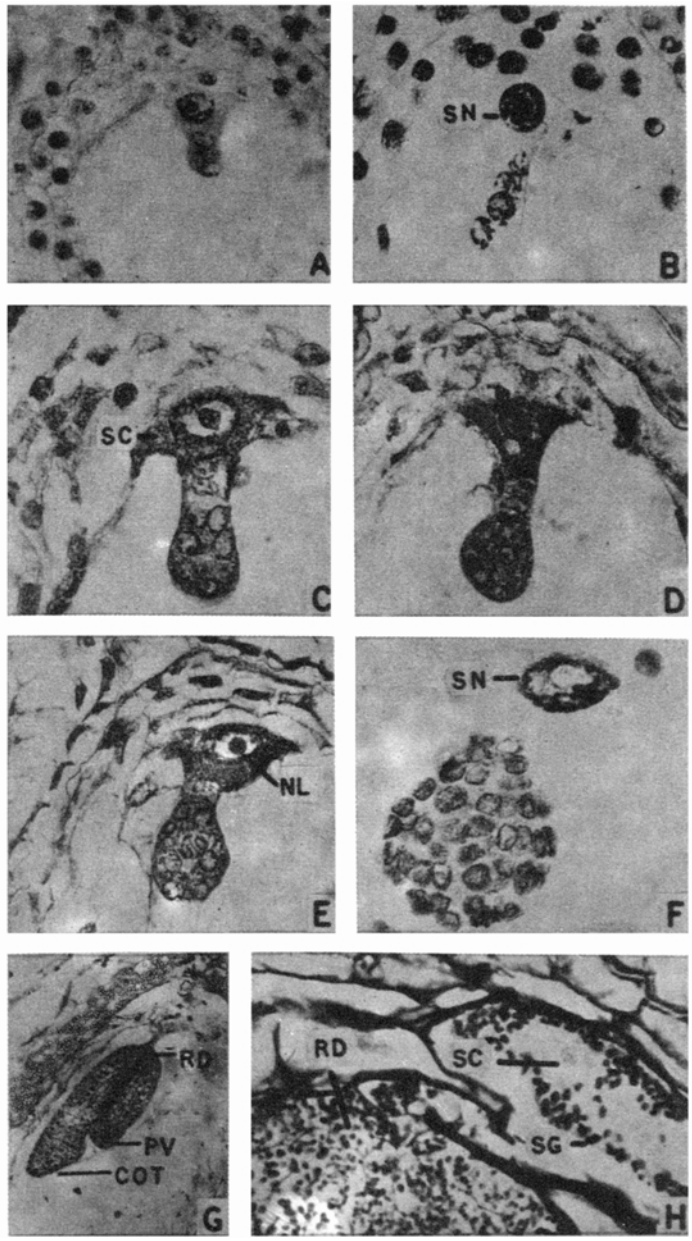

Figure 1. **A–H** LS of suspensor cells at different stages of embryo development. **A**, *cb* + 3 *ca* celled proembryo stained for RNA (×450); **B**, *cb* + 6 *ca* celled proembryo. Note big and deeply stained suspensor nucleus for DNA (×450); **C**, Proembryo stained for RNA. Note deeply stained cytoplasm and nucleolus (×450); **D**, cb + 14 *ca* celled proembryo stained for total proteins (×450); **E**, Mid globular embryo stained for total proteins (×450); **F**, Globular embryo showing a big deeply stained suspensor nucleus for DNA (×450); **G**, Early mature embryo showing plumule and radicle. Note higher accumulation of RNA in the radicular region (×100); **H**, Suspensor cell with radicular portion of the mature embryo stained for insoluble polysaccharides. Note the stored starch grains in suspensor cell (×270). (COT, cotyledon; NL, nucleolus; PV, plumule; RD, radicle; SC, suspensor cell; SG, starch grains; SN, suspensor nucleus)

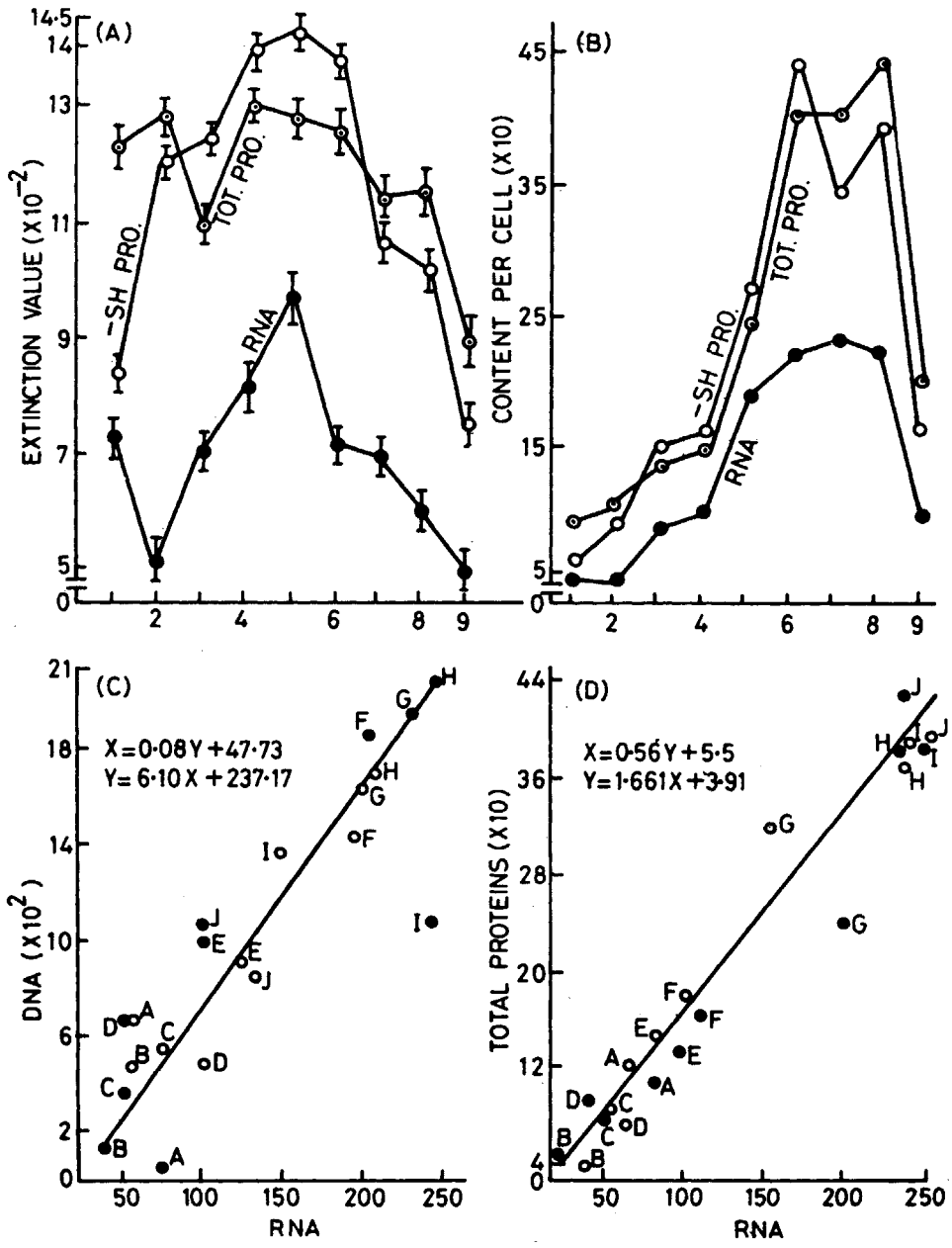

Figure 2 A–D. A & B, Changes in the *e* values and contents of RNA, total and −SH proteins in the suspensor cell during different stages of embryo development (on axis OX 1, *cb*+6 *ca*; 2, *cb*+8 *ca*; 3, *cb*+14 *ca*; 4, mid globular embryo; 5, globular embryo; 6, elongated embryo; 7, cotyledon initiation; 8, plumule initiation; 9, mature embryo. *ca*, apical cell, *cb*, basal cell); **C & D:** Regression graphs showing correlation between contents of RNA/DNA and RNA/total proteins in the suspensor cell during different stages of embryo development (on axis OX A, Zygote; B, *cb*+4 *ca*; C, *cb*+6 *ca*; D, *cb*+8 *ca*; E, mid globular embryo; F, globular embryo; G, elongated embryo; H, cotyledon initiation; I, plumule initiation; J, mature embryo) and represent experimental and calculated values respectively

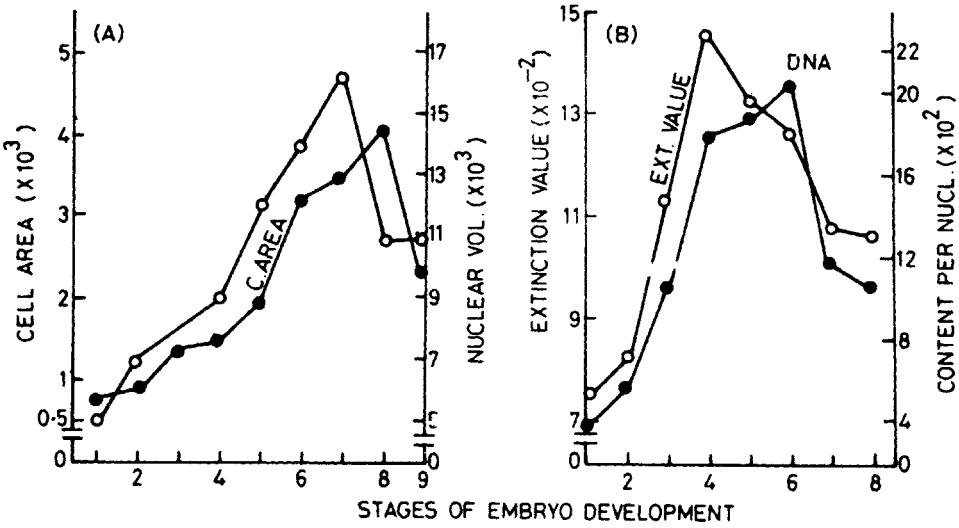

Figure 3 A–B A, Changes in cell area and nuclear volume of the suspensor cell during various stages of embryo development (on axis OX 1, *cb* + 6 *ca*; 2, *cb* + 8 *ca*; 3, *cb* + 14 *ca*; 4, mid globular embryo; 5, globular embryo; 6, elongated embryo; 7, cotyledon initiation; 8, plumule initiation; 9, mature embryo); **B,** Changes in e value and content per nucleus of DNA in suspensor cell during different stages of embryo development (on axis OX 1, *cb* + 5 *ca*; 2, *cb* + 6 *ca*; 3, mid globular embryo; 4, globular embryo; 5, elongated stage; 6, cotyledon initiation; 7, plumule initiation; 8, mature embryo)

later stages of embryogenesis, i.e. after cotyledon initiation. This gradient suggests a transport of histochemical substances from suspensor cell to the embryo proper.

N. marina suspensor shows highest DNA content at cotyledon initiation stage. This value is 242 times more than DNA contents of a haploid microspore nucleus (1C) of the same plant. The suspensor often enlarges much quickly than the organ forming part, i.e. embryo proper, and an increase in the size of its nucleus is brought about by the endopolyploidy of its DNA content which does not show any mitotic division and chromosome condensation throughout its development. Nagl (1974) has suggested a possible significance of endopolyploidy for increased levels of RNA and protein syntheses in highly specialized suspensor cell. The increase in the nuclear DNA contents, several folds to that of diploid amount by endopolyploidy cycles supplies the suspensor cell with multiple amount of DNA templates. This increases the general synthesizing capacity of the cell. Intense metabolic status of the suspensor cells due to high levels of endoploidy and polyteny is further suggested by Avanzi et al. (1971), Clutter et al. (1974) and Brady (1973) in *Phaseolus coccineus*, Corsi (1972) in *Eruca sativa* and Nagl (1976) in *Tropaeolum majus*.

At cotyledon initiation stage, the degenerating suspensor cell is accompanied by rapid decline in proteins and total nucleic acids contents. In *Tropaeolum majus*, the progressive autolysis of the suspensor cell occurred by the release of hydrolytic enzymes from tonoplast (Singh et al. 1979). Nagl (1976) also reported that the activity of hydrolysing enzymes is correlated with the autolysis of the suspensor cells of *Phaseolus* embryo. These findings suggest that the death of suspensor cell is due to hydrolysing enzymes but not due to mechanical pressure imposed by the developing embryo.

Acknowledgements

Author gratefully acknowledges the constant encouragement and guidance of Prof. C K Shah. He thanks Hon'able Anna Saheb P K Patil and Dr D K Sonar for encouragement and their keen interest in the research activity. The financial assistance by the University Grants Commission, is highly acknowledged.

References

Alpi A, Tognoni F and D'Amato F 1975 Growth regulator levels in embryo and suspensor of *Phaseolus coccineus* at two stages of development; *Planta* **127** 153–162

Avanzi S, Buongiorno-Nardelli M, Cionini P G and D'Amato F 1971 Cytological localization of molecular hybrids between rRNA and DNA in the embryo-suspensor cells of *Phaseolus coccineus*. A preliminary note; *Rend. Classe. Sci. Fisiche. Matemat. Nat. Ser.* **50** 357–361

Bárrnett R J and Seligman A M 1952 A new histochemical demonstration of protein-bound sulphydryl groups; *Science* **116** 323–327

Bhatt P N, Srivastav D and Shah C K 1983 A design of an inexpensive cytophotometer; *Indian J. exp. Biol.* **21** 472–474

Brady T 1973 Cytological studies on the suspensor polytene chromosomes of *Phaseolus*; DNA content and synthesis and the ribosomal cistrons; *Caryologia* **25** (suppl.) 233–259

Cionini P G, Bennichi H, Alpi A and D'Amato F 1976 Suspensor, gibberellin and *in vitro* development of *Phaseolus coccineus* embryo; *Planta* **131** 115–117

Clutter M, Brady T, Walbot V and Sussex I 1974 Macromolecular synthesis during plant embryogeny. Cellular rates of RNA synthesis in diploid and polytene cells in bean embryo; *J. cell Biol.* **63** 1907–2102

Corsi G 1972 The suspensor of *Eruca sativa* Miller (Cruciferae) during embryogenesis *in vitro; G. Bot. Ital.* **106** 41–54

Jensen W A 1962 *Botanical Histochemistry. Principles and Practice* (San Francisco and London: Freeman and Co.)

Lorenzi R, Bennici A, Cionini P G, Alpi A and D'Amato F 1978 Embryo-suspensor relationship in *Phaseolus coccineus*: Cytokinins during seed development; *Planta* **143** 59–62

Mazia D, Brewer P A and Alfert M 1953 The cytochemical staining and measurement of protein with mercuric bromophenol blue; *Biol. Bull.* **104** 57–67

Nagl W 1974 The *Phaseolus* suspensor and its polytene chromosomes; *Z. Pflanzenphysiol.* **73** 1–44

—— 1976 Early embryogenesis in *Tropaeolum majus* L. Ultrastructure of the embryo suspensor; *Biochem. Physiol. Pflanzen.* **170** 255–260

Pollister A W, Swift H and Rasch E 1969 Microphotometry with visible light; in *Physical Techniques in Biological Research* Vol. 3 ed. A W Pollister (New York: Academic Press Inc.)

Prabhakar Kumkum and Vijayaraghavan M R 1983 Histochemistry and Ultrastructure of suspensor cells in *Alyssum maritimum*; *Cytologia* **48** 389–402

Shah C K 1970 Embryogenesis in monocotyledons. III. *Aponogeton natans* (L.) Engl, et. Krause; *Proc. natn. Acad. Sci. India* **B40** 1–5

Singh M B, Bhalla P L and Malik C P 1979 Peroxidase localization during early embryogenesis in *Tropaeolum majus* L.; *Phytomorphology* **29** 306–309

Sussex L, Clutter M, Walbot V and Brady T 1973 Biosynthetic activity of the suspensor of *Phaseolus coccineus*; *Caryologia* **25** (suppl.) 261–272

Tepper H B and Gifford Jr. E M 1962 Detection of ribonucleic acid with pyronin; *Stain Technol.* **37** 52–53

Walbot V, Brady T, Clutter M and Sussex I 1972 Macromolecular synthesis during plant embryogeny. Rates of RNA synthesis in *Phaseolus coccineus* embryo and suspensor; *Develop. Biol.* **29** 104–111

Yeung E C and Sussex I M 1979 Embryology of *Phaseolus coccineus*: The suspensor and the growth of the embryo proper *in vitro*; *Z. Pflanzenphysiol.* **91** 423–433