Histones and Nucleic Acids during Seed Development and Germination in *Linum usitatissimum*

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In the nuclei of higher organisms, histones are the basic proteins that are associated with DNA. Histone synthesis is partially coordinated with DNA replication. The present paper deals with histones and nucleic acids distribution in various parts of seeds of *Linum usitatissimum*. The changes that occur in these macromolecules during seed germination are also described. These changes perhaps involve the elimination of the quiescent state which is replaced by another phase that ensures activation of various developmental potencies.

Material and methods

The seeds of *Linum usitatissimum*, at various developmental stages were fixed either in AA (25 ml glacial acetic acid: 75 ml absolute alcohol) or in 10% neutral formalin. The fixed material was dehydrated in tertiary butyl alcohol series and embedded in paraffin wax. Sections were cut at 12 μm.

DNA: AA fixed material is used for the localisation of DNA. The xylene deparaffinised slides are brought through graded alcohol series to water; later immersed in 5N HCl for 10 min at room temperature and washed in running water. The slides are stained for one hour in dark with Schiff’s reagent (Kallarackal 1974), rinsed in water and placed in 2% sodium bisulphite for 2 min. After staining the slides are dehydrated through graded alcohol series, cleared in xylene two changes and mounted in Canada balsam. The nuclei are stained magenta.

RNA: AA fixed material is used for the localisation of ribose nucleic acid. The deparaffinised slides are brought through descending alcohol series to water; later immersed in 2% solution of Pyronin Y for 2 min, washed in distilled water (Tepper and Gifford 1962), dehydrated through ascending graded alcohol series, cleared in xylene and finally mounted in Canada balsam. RNA containing regions stain deep pink.

Histones: Neutral formalin fixed material is used for the localisation of histones. The deparaffinised slides are brought through graded alcohol series to 90% ethanol. Thereafter, the slides are dipped in 0.5% solution of Celloidin (0.5 g celloidin, 50 ml absolute alcohol and 50 ml solvent ether) and then through graded alcohol series brought to water; placed in 5% trichloroacetic acid at 90°C for 15 min, washed in running water and rinsed in distilled water at pH 8.0. These slides are stained in 0.1% aqueous solution of Fast Green at pH 8.1 for 3 hours (Alfert and Geschwind 1953). The stained slides are dehydrated through ascending alcohol series, cleared in xylene and mounted in Canada balsam. Histones and cytoplasmic proteins stain green.

Observations

Results with Feulgen reaction for DNA

Testa: After fertilization, the nuclei in the cells of outer integument and endothelium...
stain intensely than that in the inner integument. At the globular proembryo stage, the staining intensity in the cells of both the integuments, except the endothelium markedly decreases.

Endosperm: At the zygote stage, the endosperm nuclei stain feebly but during progressive embryogenesis there is a rise in the staining intensity of the endosperm nuclei which, however, decreases sharply at the dicotyledonous embryo stage.

Embryo: The zygote nucleus stains feebly (Fig. 1A), whereas in the 4-celled and octant proembryos all the nuclei are equally well stained. In globular proembryo, however, the embryonal nuclei are more intensely stained than that of the suspensor nuclei (Fig. 1B). In a young dicotyledonous embryo, all the cells are well stained (Fig. 1C) and at later stages, the nuclei in the cells of protoderm, procambium and root and shoot apices are deeply stained.

Fig. 1. Distribution of DNA. A, zygote with poorly stained nucleus. ×482. B, C, nuclei of embryonal cells are deeply stained. ×482, ×145. D, dicotyledonous embryo. Nuclei of the cells of protoderm, procambium, root and shoot apices are intensely stained. ×115.
Three days after sowing the root tip shows deep staining followed by the shoot apex and leaf primordia. The cotyledonary cells, however, reveal poor staining and remain so until six days.

Results with Pyronin Y for RNA

Testa: At the zygote stage the outer integument is deeply stained. In the inner integument, the endothelium and two or three layers adjacent to it are intensely stained (Fig. 2A). The cytoplasm in the rest of the cells shows feeble hue while the nucleoli are deeply stained. During early proembryonal stages the staining intensity is decreased in both the integuments.
Endosperm: The nucleoli of the endosperm cells are deeply stained during early stages.

Fig. 3. Distribution of RNA. A, shoot apex after 10 hr of sowing. Note nucleolar RNA. ×482. B, shoot tip two days after sowing showing deeply stained shoot apex, procambium and leaf primordium (lp). ×115. C, shoot tip after six days of sowing. Leaf buttress (lb) and second leaf primordium (lp) are more intensely stained than the first leaf primordium. ×482.
of embryogenesis (Fig. 2C). At dicotyledonous proembryo stage endosperm cells adjacent to the endothelium stain deeply. In the dry seed the endosperm cells are stained feebly.

Embryo: The zygote cytoplasm and nucleolus are well stained. In the two-celled pro-

embryo the terminal cell stains well than the basal cell, albeit the nucleoli of both the cells are equally well stained. In the 4-celled proembryo, the terminal cell derivatives stain better than that of the basal cell (Fig. 2B). At the octant and the globular proembryo stages, the embryon-
al cells stain more intensely than the suspensor cells (Fig. 2C). The cells of the cotyledonalery
primordia are intensely stained in the heart-shaped embryo and in young dicotyledonous
embryo the cells of protoderm and the procambium stain more intensely than the ground
meristem. The organogenetic parts of the embryo are more pyroninophilic than the suspensor
cells (Fig. 2D). At later stages, the cells of root cap and root apex show maximum staining
intensity. In a dry seed all the cells of the embryo stain feebly except the shoot apex and leaf
primordia.

Ten days after imbibition, the staining intensity in the embryo increases gradually in the
cotyledonary cells (Fig. 3A) mainly due to the formation of protein bodies. Two days later
the cells of shoot apex and leaf primordia stain deeply (Fig. 3B) and after three days, the stain-
ing intensity in the shoot tip increases. Four days after sowing, the second pair of leaf prim-
ordia stains more deeply than the first pair of leaf primordia. After six days, leaf buttresses
are formed, and are deeply stained (Fig. 3C).

Results with alkaline Fast Green for histones

Testa: At zygote stage, the outer integument and the endothelium stain deeply but sub-
sequently the staining intensity decreases in all the cells except the endothelium.

Endosperm: At zygote stage, the endosperm nuclei stain feebly. There is a sharp in-
crease in its staining at globular proembryo stage and thereafter there is a steep fall in staining
reaction.

Embryo: The zygote nucleus stains feebly. During early stages of embryogenesis, the
embryonal cells are well stained when compared to that of the suspensor cells (Fig. 4A, B).
In the dicotyledonous embryo, the procambium, the cotyledons and the cotyledonary protein
bodies stain well.

Two days after sowing, deeply stained nuclei and faintly stained protein bodies are ob-
served in the embryo. The cells of shoot tip are well stained. After four days, the cells of
the shoot apex, leaf primordia and procambium show prominent staining (Fig. 4C). Six days
later, the cells of the hypocotyl and cotyledons stain feebly while the shoot apex and the leaf
primordia are deeply stained (Fig. 4D).

Discussion

The zygote is feebly stained for DNA and histones but is deeply stained for RNA. The
faint staining for DNA may be due to the large volume of the nucleus (Pritchard 1964a) or the
state of the chromatin (Vassileva-Dryanovska 1964) or due to the stretching of DNA mole-
cule due to which the reacting aldehyde groups, released on hydrolysis, are linearly separated
(Sterba 1963).

The cells of the two-celled proembryo are equally stained for histones, DNA and nucleolar
RNA. The cytoplasm of the terminal cell is, however, deeply stained (present work) as seen
in Vanda (Alvarez and Sagawa 1965). In Stellaria media (Pritchard 1964b), Capsella bursa-
pastoris (Schulz and Jensen 1968) and Limnophyton obtusifolium (Shah and Pandey 1978), the
basal cell of the two-celled proembryo is rich in DNA and RNA. In heart-shaped and dicot-
yledonous embryos, the cells of protoderm, ground meristem and procambium can be histologi-
cally and histochemically demarcated.

The dry seed of Linum usitatissimum (present work) stains feebly for nucleic acids and
histones. Brunori (1967) suggested that in a dehydrated embryo, the available water supply
might limit DNA synthesis. The procambium, root and shoot apices and leaf primordia
stain deeply for these metabolites during seed germination. Similar increase in DNA and
RNA in the embryonic axis was seen in peanut (Marcus and Feeley 1962) and Quercus nigra
(Vozzo and Young 1975) and in shoot apex of Paulownia tomentosa (Rickson 1968) and
Pinus radiata (Riding and Gifford 1973). An increase in RNA content has been noted in
Arachis hypogaea (Cherry 1963a) and Yucca spp. (Horner and Arnott 1966) during initial stages of germination.

Cherry and Hageman (1961) reported a decrease in RNA concentration at the beginning of germination, indicating that RNA is primarily a reserve metabolite. Decrease in RNA during germination has also been reported in Pisum arvense (Smith and Flinn 1967). Oota and Osawa (1954) found that the decrease of RNA in cotyledons of Vigna sesquipedalis was accompanied by an increase of this metabolite in the hypocotyl, and they stated that RNA present in the cotyledons was “storage RNA”. On the contrary, increase in the staining intensity of Pyronin Y during germination, in Linum usitatissimum (present study) can be correlated with enzymic changes which suggest its metabolic role (see also Cherry 1963b, Walbot 1971).

Summary

In Linum usitatissimum, the endothelium stains well for nucleic acids and histones. In endosperm, the staining intensity for histones, DNA and RNA increases during embryogeny. The basal and terminal cells of the two-celled proembryo are equally stained for DNA, nucleolar RNA and histones. The terminal cell is stained deeply with Pyronin Y. The embryonal cells stain intensely for these macromolecules than the suspensor cells. In a dry seed the staining intensity of histones and nucleic acids is low but after germination it increases in the shoot apex and leaf primordia.

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References


* Not seen in original.