Changes in Nucleic Acid Fractions of Seed Components of Red Pine (Pinus resinosa Ait.) During Germination

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Abstract. Changes in nucleic acid fractions of Pinus resinosa during seed germination were studied. At various stages of seed germination, embryos and megagametophytes were surgically separated and nucleic acids were extracted separately by a phenol-method. Total nucleic acids were fractionated on single-layer methylated albumin kieselguhr (MAK) columns. Total nucleic acids in embryos increased significantly 2 days after seeds were moistened, whereas, in megagametophytes, total nucleic acids stayed almost at a constant level until they degenerated at the time of shedding. In embryos, ribosomal RNAs (rRNA) increased 2 days after seeds were sown, whereas soluble RNA (sRNA) increased at 3 days. By comparison, nucleic acid fractions of megagametophytes did not show any quantitative changes during germination, except that rRNA fractions decreased shortly before shedding of seed coats. In dormant embryos the proportion of DNA was high and the proportions of sRNA and rRNA were low, whereas in megagametophytes at dormancy the proportions were completely reversed. As seed germination progressed, proportions of nucleic acid fractions in embryos changed significantly. In megagametophytes, although proportions of individual fractions remained almost constant throughout the experimental period, incorporation of 32P into sRNA and rRNA of megagametophytes indicated turnover of these fractions.

The dormant seed of red pine (Pinus resinosa Ait.) consists of a seed coat, a megagametophyte (or female gametophyte) and an embryo which is not completely differentiated. During seed germination under controlled environment, the embryo undergoes extensive differentiation including formation of vascular systems, stomata on the hypocotyl and cotyledons, and primary needles. In contrast, the megagametophyte, which consists of large storage cells with an abundance of reserve particles, does not show any significant changes. Only minor changes are observed in the megagametophyte during germination such as swelling of nuclei and disappearance of reserve particles (25). Such extensive differentiation in red pine embryos suggests changes in nucleic acid metabolism during seed germination and subsequent seedling development (1, 6, 9, 11, 16, 17, 18, 23, 27, 28, 29). Although in general, nucleic acids quantitatively increase in embryos during germination, patterns of nucleic acid metabolism and protein synthesis show differences between species, and even between seed groups of the same species (14). Patterns of nucleic acid metabolism in storage tissues such as endosperm and cotyledons vary greatly among different species (7, 12, 19, 22, 26). Although cotyledons of many angiosperms play a role as storage tissue, cotyledons in red pine differentiate and function as an important photosynthetic apparatus in early stages of seedling development (24, 25). In contrast, megagametophytes in gymnosperms have functions comparable to those of storage tissues in angiosperms (8). Therefore, the present experiments were designed to study changes in nucleic acid patterns with morphological development of the embryos during germination.

Materials and Methods

Seedlings. Red pine (Pinus resinosa Ait.) seeds, collected in Northeastern Minnesota were used. The seeds were washed with distilled water and placed on wet filter paper in Petri dishes. The Petri dishes were kept in a growth chamber at a constant temperature of 25°. Light intensity was maintained at 1000 ft-c on a 16-hr day. The radicle began to emerge from the seed coat at 5 days, and the cotyledons began to emerge from the seed coat at 10 days. At 14 days, the seed coat containing the megagametophyte was attached to tips of the cotyledons. Samples for nucleic acid determination were taken from dormant seeds, and seeds or seedlings at 1, 2, 3, 5, 7, 10, and 14 days after the seeds were sown. At each time of sampling, 200 plants were selected, and embryos and megagametophytes were surgically separated. Immediately after separation, embryos and megagametophytes were dropped separately into liquid nitrogen. At least 2 replications were made at each stage of seed germination.

Extraction. Total nucleic acids were extracted by a phenol-procedure similar to that used by Kirby (13). Modifications were as follows: frozen tis-
sues were ground in a glass mortar containing 6.0 ml of 0.1 M tris buffer, pH 7.6; 0.5 ml of 50 mg/ml bentonite suspension; 1 ml of 11% dupanol (sodium lauryl sulfate); and 10 ml of water-saturated phenol. Homogenates were transferred to a VirTis “45” homogenizer flask, and the mortar was washed with an equal volume of homogenizing solution which also was transferred to the homogenizer flask. The combined solutions were homogenized for 2 min at the highest speed, and combined with a rinse from the VirTis flask. The resulting homogenate solution was shaken for 2 hr at room temperature and subsequently refrigerated overnight at 2°C. Phenol and aqueous layers were separated by centrifugation at 23,000 g for 10 min. The aqueous layer was treated with 1 ml of the bentonite suspension and an equal volume of water-saturated phenol. The solution was well mixed and again centrifuged. Further phenol treatment of the aqueous layer was repeated until all interphase precipitate was removed. The final aqueous layer was mixed with an equal volume of 2.0 M potassium acetate buffer, pH 5.0, and 2.5 combined volumes of cold 95% ethanol and kept in a freezer at −30°C overnight. Nucleic acid precipitates were centrifuged at 40,000 g for 40 min. The pellet was suspended in 7 ml of 0.05 M phosphate buffer, pH 6.7, and dialyzed against 0.05 M phosphate buffer, pH 6.7, for 24 hr at 4°C. During dialysis the buffer was changed after 16 hr.

**Fractionation.** Nucleic acids were fractioned by single-layer columns of methylated albumin kieselguhr (MAK) similar to that used by Mandell and Hershey (15). A salt gradient from 0.1 to 1.5 M NaCl was adopted to elute nucleic acid fractions. Quantities of total nucleic acids loaded on columns varied between 12 and 50 OD units, depending on the level of nucleic acids in each sample. Eluates from the columns were continuously monitored by an ISCO U. V. monitor, and collected in 10 ml fractions. Nucleic acid concentrations in individual fractions were determined spectrophotometrically at 260 mμ. Quantities of nucleic acids in individual peaks were calculated by summation of OD readings and volumes within each peak. Peaks were classified as sRNA (tRNA + 5sRNA), DNA and rRNA (light and heavy ribosomal nucleic acids + a shoulder). Over 90% recovery was obtained from the columns.

**Incorporation of **$^{32}$P. Seeds were sown in Petri dishes as described above. One day after seeds were sown, 1 ml (50 μc) of $^{32}$P was pipetted to a Petri dish containing 50 seeds. Other groups of 150 seeds each were maintained without $^{32}$P. At 3 days, embryos and megagametophytes of the seeds with and without $^{32}$P were surgically separated and frozen in liquid nitrogen. Nucleic acids were extracted from embryos or megagametophytes (with and without $^{32}$P combined). After fractionation of the nucleic acids, the radioactivity of each fraction was determined by liquid scintillation counting.

**Results and Discussion**

Changes in total nucleic acids during seed germination were significantly different between embryos and megagametophytes (Fig. 1). In embryos, changes in total nucleic acid levels showed 4 different stages: Stage I, 0 to 1 day when no changes were detected; Stage II, 1 to 2 days when total nucleic acids increased; Stage III, 2 to 3 days when slight changes were observed; and Stage IV, over 3 days when total nucleic acid levels continuously increased. In contrast, total nucleic acid levels in megagametophytes remained almost constant until shortly before shedding of seed coats. Variations among replications were larger for megagametophytes than in embryos, possibly because of higher nuclease activities in megagametophytes.

Fractionations of nucleic acids on MAK columns were repeatable (Fig. 2). The DNA peak was the only fraction showing a significant reaction to the DNA-Ceriotti test (5). Individual fractions of nucleic acids showed marked changes during germination. In dormant embryos, nucleic acid fractions consisted of a high level of DNA, and relatively low levels of sRNA and rRNA (Fig. 2 and Fig. 3). The fractions did not show any quantitative changes for at least 24 hr after seeds were moistened (Fig. 3). Ribosomal RNA first began to increase at 2 days, whereas sRNA did not increase until 3 days after seeds were moistened, suggesting that rRNA synthesis preceded that of sRNA (Fig. 3).

During the first 3 day period, proportions of the fractions in embryos changed greatly, with a rapid

![Fig. 1. Changes in total nucleic acids (RNA + DNA) during red pine seed germination.](image-url)
increase in rRNA and a rapid decrease in DNA. Thereafter, a slight increase in the sRNA proportion and a decline in the DNA proportion were noted. These changes in individual nucleic acid fractions of pine embryos during the first 3 days after sowing might be explained as stepwise processes of seed germination before the onset of mitosis (25). Stepwise increases in total nucleic acids of pine embryos (Fig. 1) possibly reflect sequences of nucleic acid synthesis in cells. Changes in total nucleic acids during the synchronous early development of Jerusalem artichoke callus (20) showed 3 stepwise increases similar to those described above. In the slime mold, Physarum, RNA synthesis was low during mitosis and mid-interphase, while maximal synthesis occurred before and after mitosis (2, 3, 21). The observations reported herein suggest that mitosis in pine embryos may occur after rRNA and sRNA have reached specific levels.

Quantitative and proportional increases in sRNA of embryos after 5 days (Fig. 3) occurred concurrently with differentiation of stomata, parenchyma with chloroplasts, vascular systems, and primary needles (25). During this period, relative proportions of sRNA in red pine embryos increased significantly, while rRNA remained relatively constant, supporting the concept of tRNA regulation in tissue differentiation. Similarly, sRNA increases were observed in developing tissues such as wheat embryos (27, 28) and mimosa epicotyls (4). Furthermore, base compositions of total sRNA appeared to be different during chlorophyll formation in cotton cotyledons (12).

In contrast to nucleic acids in the embryos, the level of each nucleic acid fraction of the megagametophytes remained almost constant during seed germination. The relative proportions of rRNA and sRNA in megagametophytes were always higher than those of DNA, showing marked differences from similar comparisons in embryos. Only a slight decrease in rRNA was noted 14 days after seeds were moistened (Fig. 3). At that time, the megagametophytes, with their seed coats, approached the stage of shedding. In contrast, Douglas fir megagametophytes showed increases in total RNA during mid-germination stages (8). No significant changes were observed in relative proportions of rRNA, sRNA and DNA in red pine megagametophytes during seed germination. Similar trends in nucleic acid metabolism of storage tissues have been reported for peanut cotyledons (7), and potato tubers (10).

Table I and Fig. 4 report results of $^{32}P$ incorporation experiments for both embryos and megagametophytes. Megagametophyte fractions showed higher total $^{32}P$ incorporation than those of embryos. However, since embryos at 3 days were still imbedded within the megagametophytes, differences in $^{32}P$-uptake between these tissues probably reflect length of exposure to $^{32}P$. All 3 fractions (sRNA, DNA, and rRNA) in embryos showed $^{32}P$ incorporation. In contrast, $^{32}P$ incorporation into the megagametophyte DNA fraction was negligible. At 3 days, incorporation rates of $^{32}P$ into sRNAs were higher in both tissues than for RNAs. However, relative turnover rates of embryo rRNA were higher than those of megagametophyte rRNA.

Although no significant changes in proportions and quantities of nucleic acids of red pine megagametophytes were observed, incorporation of $^{32}P$...
Table I. $^{32}P$-incorporation Into Fractions of Nucleic Acids in Red Pine Seed Components 3 Days After the Onset of Seed Germination

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Specific activity</th>
<th>Relative turnover rates among fractions</th>
<th>Specific activity/total specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Embryos</td>
<td>Megagametophytes</td>
<td>Embryos</td>
</tr>
<tr>
<td>sRNA</td>
<td>2698</td>
<td>48238</td>
<td>2.13</td>
</tr>
<tr>
<td>DNA</td>
<td>964</td>
<td>...</td>
<td>0.76</td>
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<tr>
<td>rRNA</td>
<td>1289</td>
<td>20590</td>
<td>1.02</td>
</tr>
</tbody>
</table>

1 As OD 260 readings and radioactivity of the DNA fraction were negligible, specific activity and turnover rates were not calculated.

2 Values were obtained by dividing specific activity of each fraction by total specific activity for each tissue (Counts for total fractions in each tissue/total OD 260 for each tissue.) Therefore, values over 1 indicate above average turnover rates, whereas values below 1 indicate below average turnover rates, compared with the overall turnover rate for that tissue.

Based on data presented in this paper, differences in functional roles of red pine embryos and megagametophytes reflect differences in nucleic acid metabolism during germination.

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Literature Cited


