INCREASE IN NUCLEIC ACID SYNTHESIZING CAPACITY DURING COLD TREATMENT OF DORMANT PEAR EMBRYOS*

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Seeds of many rosaceous genera, such as Pyrus, Malus, Sorbus and Chaenomeles require an extended period of moist prechilling treatment ("stratification") at 1 to 10° to break the dormancy of the embryo. Embryos excised from such seeds develop into normal seedlings. Embryos from unchilled seeds, on the other hand, grow very slowly and eventually develop into abnormal seedlings ("physiological dwarfs"). The physiological and biochemical changes in the embryo accompanying cold treatment remains obscure. An increase in growth promoting hormones or a decrease in growth inhibiting hormones or their interaction may be a consequence of prechilling (Wareing, 1965). It was recently shown (Sondheimer et al, 1968) that the level of abscisic acid (dormin), a germination inhibitor (Khan, 1967a), widely distributed in plant tissues (Milborrow, 1967) decreases by 70% during cold temperature treatment of dormant Fraxinus embryos. Work in this laboratory has shown that cytokinins (a class of growth regulators) overcome the inhibitory action of several natural inhibitors on seed germination (Khan and Tolbert, 1965; Khan, 1967a, 1968, 1969) and dormancy (Khan, 1966, 1967b). Preliminary studies with rosaceous embryos indicated that both kinetin (a cytokinin) and gibberellic acid (GA_3) caused the dormant embryos to grow somewhat. A combination of kinetin and GA_3 proved more effective than either of them alone in releasing dormancy of these embryos.

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A
B
C
D
OD 260 mμ
CPM/ml.
FRACTION NUMBER
SRNA
DNA-RNA
hr RNA
m RNA

Fig. 1. MAK (methylated-albumin kieselguhr) column fractionation. 80 embryos were preincubated for 2 hours in the heat sterilized medium containing 0.03 M sucrose, 0.01 M tris-HCl pH 6.5, 0.01 M $MgCl_2$ and 0.06 M KCl in 5 ml solution prior to 4 hour period with the addition to the medium of 25 μc of carrier-free $H_3{}^{32}PO_4$. Incubation was carried out at 25° on a metabolic shaker. Extraction and purification of total nucleic acid from embryos were as described by Cherry and Chroboczek, (1966). The embryos were homogenized on ice in a mixture containing 15 ml 0.01 M tris HCl, pH 7.6, 0.06 M KCl, 0.01 M $MgCl_2$, 1 ml (40 mg) bentonite, 3 ml 11% sodium lauryl sulfate and 15 ml phenol (washed with tris buffer). The aqueous layer was drawn off after centrifugation and reextracted twice with equal volume of cold phenol in presence of bentonite. Nucleic acids were precipitated with 2 volumes of cold ethanol in presence of 0.4 g of potassium acetate, dissolved in 0.05 M sodium phosphate, pH 6.7 and dialyzed 2 days against the same buffer at 12°. Purified total nucleic acid was fractionated on MAK column with a linear gradient of NaCl from 0.35 to 1.1 M in 0.05 M phosphate buffer pH 6.7. Fractions containing 5 ml were collected and assayed for absorbency at 260 mμ and for radioactivity. Embryos from 1 day (A), 7 day (B), 21 day (C) and 38 day (D) prechilled (5°) Pyrus communis seeds.

This communication describes the effect of different lengths of cold treatment of pear seeds on subsequent growth and nucleic acid synthesis by the excised embryos as studied by MAK column fractionation. It will be shown that with an increasing length of cold treatment the embryos show a progressively increased capacity for synthesis of nucleic acids, notably a fraction eluting just before the DNA peak. A definite peak corresponding to the region of mRNA is reported for the first time in two dicotyledonous species of Pyrus.

Materials and Methods.

Pear seeds, Pyrus communis, var. Bartlett (common pear) and Pyrus calleryana (a wild pear) were prechilled at 5° on moistened blotters for various lengths of time. Seeds were soaked for 20 minutes in 1% sodium hypochlorite solution and then thoroughly washed with distilled water. Embryos were then dissected out and used immediately for various experiments.

In growth experiments, embryos were grown for 4 days on 0.75% agar (Bactoagar) at 25° in continuous light (cool fluorescent, 10 ft.-c). In metabolic studies the method of Cherry and Chroboczek (1966) was used with minor modifications for preparation and fractionation of nucleic acids.

Results and Discussion.

The MAK chromatography profile of nucleic acids of pear embryos followed the pattern for other plant tissues (Fig. 1, D). Designation of various

nucleic acids as sRNA (soluble), DNA-RNA, lrRNA (light ribosomal), hrRNA (heavy ribosomal) and mRNA is based upon characterization of these fractions in peanut cotyledons (Cherry and Chroboczek, 1966; Chroboczek and Cherry, 1966) and Soybean hypocotyls (Ingle et al, 1965). A distinct peak corresponding to the region of mRNA was present in all preparations in both Pyrus communis (Fig. 1) and in Pyrus calleryana (Fig. 2). It is the first report of a distinct peak in this region among dicotyledonous species studied and corresponds to X- RNA, believed to be a long-lived mRNA, shown in the monocotyledonous species (Cherry and Lessman, 1967). This peak was present in pear embryos with (Fig. 1, A-D; Fig. 2) or without cold treatment. Whether this peak is characteristic of pear embryos or is present in all embryos requiring cold treatment is not yet known. The characterization of this peak is in progress.

The capacity of the excised pear embryos to synthesize nucleic acids after different lengths of cold treatment of the seeds is shown in Fig. 1 (A-D). There was a progressive increase in the synthesis (CPM/OD) of all nucleic acids with the increase in length of cold treatment. The synthesis of sRNA and DNA-RNA was higher than any other nucleic acids up to 21 days of cold treatment

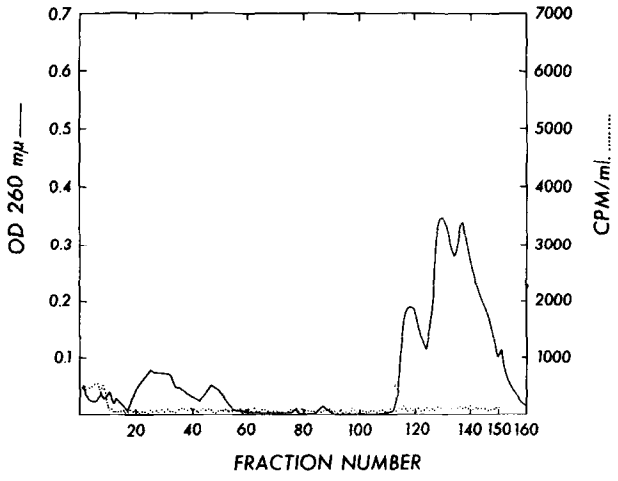

Fig. 2. MAK column fractionation of nucleic acids of embryos of Pyrus calleryana seeds prechilled 7 days. Other details same as in Figure 1.

Table 1. Effect of different lengths of cold treatment (5°) of Pyrus communis seed on the synthesis of various nucleic acids by the excised embryos. See Fig. 1 for details.

	Specific Activity, CPM/OD				
Prechilled	sRNA	DNA-RNA	lrRNA	hrRNA	mRNA
1 day	8,488	7,620	5,345	6,096	4,220
7 days	11,835	13,238	11,537	10,961	5,872
21 days	22,718	20,426	18,721	18,490	16,506
38 days	50,517	102,835	72,174	45,635	56,855

Table 2. Effect of different lengths of cold treatment of Pyrus communis seeds on subsequent development of excised embryos. 80 4-day old embryos were weighed in each case.

Seeds prechilled (days)	Wt. of embryos (gm)
0*	2.92
1	3.10
7	3.75
21	4.13
38	8.45

*Seeds were soaked for a few hours before dissecting the embryos.

(Fig. 1, A-C; Table 1) when the dormancy of the embryo is only partially released (Table 2). After 38 days the embryo development was highest and DNA-RNA was nearly twice that of any other nucleic acids (Fig. 1, D; Tables 1, 2). Thus, it would appear that the fraction represented as DNA-RNA may have an important role in dormancy release and the development of the embryo. The proportion of label in DNA-RNA peak varies with tissues studied and according

to Cherry (1964) half of the radioactivity is RNA bound as RNA-DNA hybrid in peanut cotyledons. It has been suggested by some workers including Sabota et al (1968) that an increased labelling in this fraction may be due to bacterial contamination. However, the data presented here clearly indicate that it is not so. The rate of synthesis of DNA-RNA (as well as other nucleic acids) in embryos (all incubated under identical conditions) parallels the metabolic state or growth potential of the embryos as determined by the length of cold treatment. Incorporation of radioactivity due to bacterial contamination, if any, must be very low as there was practically no DNA-RNA synthesis in embryos after 1 day of cold treatment (Fig. 1 A).

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