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METABOLISM OF NUCLEIC ACIDS DURING EARLY STAGES OF THE GERMINATION PROCESS IN RYE (*SECALE CEREALE*)

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Abstract—A study of nucleic acid metabolism during the early stages of germination of rye (*Secale cereale*) has been made. A modified Schmidt and Thannhauser procedure was employed for the quantitative estimation of RNA and DNA in endosperm, plumule, radicle and scutellum. Changes in RNA and DNA levels during the early stages of germination have been measured. The increase in RNA levels found during the first few hours of imbibition in the plumule and radicle have been discussed in relation to initiation of cell division. A net increase in RNA content of the endosperm observed after an initial loss is discussed in relation to enzyme synthesis. The early increase in DNA levels noted in the plumule and radicle occurs prior to cell division; the later increases in DNA are a reflection of cell division.

INTRODUCTION

SEVERAL investigations on nucleic acid changes in germinating seeds and developing seedlings have been reported.[1–4] However, some workers used the Ogur and Rosen procedure which is now known to be unsuitable for quantitative analysis of RNA and DNA in plants.[5] In spite of this, the overall picture which emerges from these studies does tend to show that increases in RNA and DNA in the radicle and plumule are achieved at the expense of the nucleic acids of the endosperm and scutellum; this picture is based on analyses made primarily at daily intervals.[6] Because of the obviously rapid changes which must occur immediately after imbibition starts, a study of nucleic acid metabolism during the early stages of germination at shorter time intervals was considered desirable. The results of such a study, which employed a modified Schmidt and Thannhauser procedure[7,8] are presented here.

RESULTS

1. *The RNA Fraction*

Although a two stage purification procedure was employed, the RNA fraction from the endosperm was contaminated by extraneous phosphorus and carbohydrate (Table 1). Even so the pattern of changes in RNA content were similar irrespective of the assay procedure employed (carbohydrate, phosphorus, u.v. absorption). Since the u.v. spectra of the extracts

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TABLE 1. THE VARIATION IN RNA VALUES OF VARIOUS RYE TISSUES BASED ON PHOSPHATE, ORCINOL AND U.V. METHODS OF ASSAY

Tissue	Age (hr)	Estimates (μg RNA/plant part) based on phosphate	orcinol	u.v. absorption
Endosperm	0	25·5	40·9	10·8
Endosperm	32	16·5	22·9	12·6
Root	0	10·5	7·5	6·6
Root	32	9·6	8·3	10·7
plumule	0	11·5	7·0	5·9
Scutellum	32	7·2	6·8	6·9

appeared reasonably free from contamination by other absorbing substances, the values obtained by computing the RNA content from the difference in absorption at 265 mμ and 290 mμ were considered to be most accurate. A more reasonable agreement was obtained between all three methods of assay for RNA in the other tissues studied (Table 1) but the results based on u.v. absorption have been used to assess the changes which occur during the early stages of germination in the various parts of the seedling (Fig. 1).

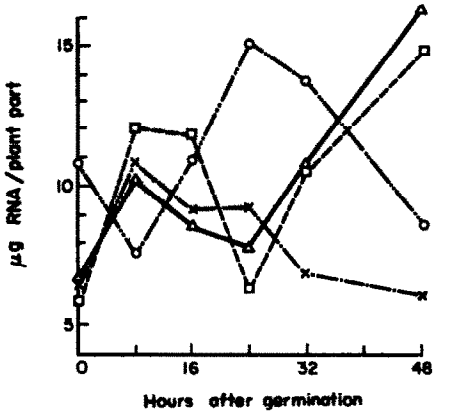

FIG. 1. CHANGES IN RNA CONTENT (μg/PLANT PART) DURING GERMINATION OF RYE.
O–·–·–O, endosperm; □– – –□, plumule; ×–·–·–×, scutellum; △——△, radicle.

2. *The DNA Fraction*

In general, u.v. absorption of the DNA fraction, could not be employed for assay purposes because of contaminating material. The more specific diphenylamine reaction was therefore used in all cases. The pattern of changes observed during germination are presented in Fig. 2.

3. *Replication and Reproducibility*

The RNA and DNA values presented are the average of at least four individual estimations and some endosperm values represent the average of at least ten estimations. In general the reproducibility of the results was within ∓4 per cent, and rarely exceeded ∓5 per cent.

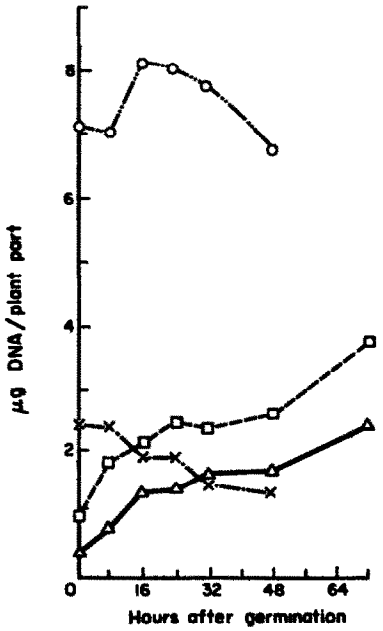

FIG. 2. CHANGES IN DNA CONTENT (µg/PLANT PART) DURING THE GERMINATION OF RYE.
○–·–·–·–○, endosperm; □——□, plumule; ×–·–·–·–×, scutellum; △——△, radicle.

DISCUSSION

DNA Metabolism

The DNA metabolism in the various tissues of the rye caryopsis shows little variation from what might be expected. The gradual increase in the DNA content in both the plumule and the radicle (Fig. 2) is clearly a synthesis of DNA prior to cell division, which in rye does not take place until after a period of expansion which may continue for 24 hr after imbibition starts. The further increase in DNA during the period studied is an expression of increased cell number.

The fall in the DNA content of the scutellum cannot be accounted for by known cellular differentiation. The significance of this phenomenon cannot be assessed from the data available. The increase in DNA content of the endosperm during the first 16 hr of the germination process is also rather unexpected. It could be accounted for by an increase in cell number, but no cytological evidence of cell division in the aleurone layers is known. Cherry[3] has recorded an increase in the DNA content of *Arachis hypogea* (peanut) cotyledons during the first 8 days of germination. In peanut the increase is gradual and sustained over a relatively long period with the result that the DNA content is approximately doubled. Again in peanut cotyledons cell division is absent. In rye the increase in DNA is probably confined to the aleurone layers and the periphery of the endosperm, the only regions hydrated at this stage of germination. In peanut the increase is probably more general. The significance of these increases in rye and peanut is difficult to assess, but it is tempting to suggest that they may be important in controlling enzyme synthesis in storage tissues.

RNA Metabolism

The initial fall in the RNA content of the endosperm, which then rises to a value above the original level (Fig. 1) could suggest the development, in part at least, of a new enzyme-synthesizing apparatus. It is of interest that the fall occurs so early in the germination process when only the outer layers of the endosperm are at all hydrated. There are two possible explanations for this loss; first, some RNA may be degraded by phosphodiesterases, and secondly the RNA could be transported as a macromolecule to the embryonic axis. The probability of macromolecular RNA translocation was suggested by the work of Oota and his colleagues,[9,10] which showed that ribosomal RNA may be transported from the cotyledons to the hypocotyl of bean (*Vicia sesquipedalis*) and by that of Ledoux and Huart[11] which demonstrated that in barley, exogenously labelled RNA is transported via the endosperm to the embryonic axis. In the latter case the amount of RNA translocated during a 5 hr period increased progressively with time and corresponded to the normal RNA increase in the embryonic axis during this period. It would therefore be logical to conclude that the initial RNA loss from rye endosperms occurs by translocation rather than by phosphodiesterase activity, which in any case has not been demonstrated at this early stage of germination.

The subsequent increase in RNA indicates a net synthesis; presumably any translocation of RNA would be continuing and at an increasing rate as more tissue becomes hydrated with time. The RNA synthesis must take place in the hydrated regions, that is, in the aleurone layers and peripheral cells of the starchy endosperm.

It has been demonstrated that enzymes involved in the degradation of endospermic food reserves tend to originate from the aleurone layers. It is therefore attractive to assume that the RNA synthesis, recorded in the endosperm, is intimately associated with *de novo* enzyme synthesis, but much further work is required to prove this.

The subsequent loss of RNA from the endosperm must be associated with complete hydration and the gradual increase in the activity of phosphodiesterases, which must result in the depolymerization of physiologically inactive RNA; in addition some possible RNA translocation is also probably occurring.

The comparatively rapid increase in the RNA content of the scutellum during the first few hours of imbibition appears to correspond to the loss of RNA from the endosperm, and thus lends support to the translocation concept.

A *de novo* synthesis of RNA has been shown to occur in the scutellum.[12] Cherry and Hageman[12] found that the increase in scutellum mitochondrial RNA was not only the result of a larger mitochondrial pellet but also a result of a net increase in RNA content. The relationship between oxidative phosphorylation and RNA is not clear but Hanson[13] showed that mitochondria treated with RNase lost their activity and he also observed the formation of holes in the mitochondrial membrane. Edelman *et al.*[14] have shown that substantial enzymic activity develops in the scutellum and demonstrated the absorption of glucose from the endosperm and its conversion into sucrose in the scutellum. The production of α-amylase in the scutellum is also well known. It therefore appears that the metabolic activity of the scutellum is complex, and in the results presented here the role of RNA is not clear, although

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part of the initial increase in the RNA content of the scutellum could be a result of *de novo* synthesis, and part could be derived from the endosperm as its RNA is being transferred to the embryonic axis. The loss of RNA from the scutellum after the first few hours of imbibition is probably a combination of degradation and translocation of physiologically inactive material.

The substantial increase in RNA content of the plumule and radicle during the first 8 hr of imbibition must be a result at least in part of a *de novo* synthesis; it is evident that the increase is not a result of translocation from the endosperm and scutellum. The *de novo* synthesis could be associated with DNA replication and the substantial increase in cell size which occurs during the first few hours of imbibition.

Synthesis of enzymes associated with DNA replication is almost certainly required, although no proof of such enzyme production has yet been presented for higher plant tissues. When the tuber cells of Jerusalem artichoke are stimulated to divide, a rapid synthesis of RNA and protein occurs prior to division.[15] After division the RNA content per nucleus falls and does not rise again, although cell division continues. The metabolism of RNA in the embryonic axis reported here is difficult to explain unless a situation exists which is similar to that in the artichoke. The fall in RNA content in both plumule and radicle after DNA replication strongly suggests an active role for RNA in the process of DNA production and initiation of cell division at the onset of germination. The subsequent sustained increase in RNA content of the embryonic axis is a result of the increased cell number.

Materials and Methods

Secale cereale (Var. King II) seeds, kindly supplied by R. Gunson (Seeds) Ltd., London, were soaked in deionized water for 24 hr, unless analysed before this time, and then sown in moistened vermiculite. Germination was allowed to take place at 28° in darkness. At harvest seedlings were killed by plunging into hot methanol. Material was stored in methanol at 0–2°. Seedlings were carefully dissected into endosperm, scutellum, plumule and radicle.

Extraction

Tissue samples, equivalent to between 180 and 250 mg dry weight, were homogenized in 85% methanol with a mortar and pestle. The homogenate was extracted by the modified Schmidt and Thannhauser procedure described elsewhere.[8] Each homogenate was extracted at 2° with 5·7 ml portions of trichloroacetic acid, 90% ethanol saturated with sodium acetate, and ethanol. The residue was made lipid-free by extraction with the following solvents at room temperature: ethanol: chloroform (3:1) twice, ethanol: ether (1:1) once, and ether once. The resulting air-dry, cold acid-soluble, lipid-free powder was extracted with 0·5 N KOH at 28° for 16 hr. This treatment converts the RNA almost completely into mononucleotides but leaves the DNA sufficiently unchanged that it can be precipitated by acidifying the cooled extract with 70% $HClO_4$ to pH 1 and mixing with 2 volumes of ethanol at 0°. The precipitate, collected after 90 min, was washed with cold 0·5 N $HClO_4$, and extracted with 0·5 N $HClO_4$ at 70° (three times) to extract DNA. The RNA fraction was purified by adsorption on and elution from charcoal followed by a second purification step on Dowex 1 × 4 Cl.[8,16]

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Estimation

DNA was estimated by the Burton[17] modification of the diphenylamine reaction, and by u.v. spectrophotometry. RNA was estimated by difference in absorption at 265 mμ and 290 mμ.

All chemicals used were of A.R. grade or of the highest purity available. Highly polymerized yeast RNA (British Drug Houses Ltd., Poole, Dorset, England) and highly polymerized DNA (Worthington Chemical Co., U.S.A.) treated in the same manner as the extracts were used as standards.

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