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EFFECT OF CYCLOHEXIMIDE ON METABOLISM OF PYRIMIDINES IN COTYLEDONS OF GERMINATING BLACK GRAM SEEDLINGS

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MARKED changes in the patterns of metabolism of pyrimidines have been observed in germinating seeds of leguminous plants[1–3], but detailed mechanisms of such changes remain to be resolved. The relative activities of the enzymes already present in dry seeds and those synthesized during germination seem to play a major role. Thus, we have compared the metabolism of pyrimidines in black gram cotyledons incubated with or without cycloheximide, an inhibitor of protein synthesis in eukaryotic cells.

Seeds of black gram (*Vigna mungo* L.) were sterilized and germinated on 0.55% agar-gel with or without cycloheximide (25 $\mu g.ml^{-1}$) in the dark at 27°C under aseptic conditions[3]. More than 90% of the incorporation of [^{3}H]leucine into the trichloroacetic acid-insoluble fraction was inhibited by this concentration of cycloheximide[4]. After 24 or 48 h, each pair of cotyledons was divided into 6 segments. Eighteen segments from three pairs of cotyledons were incubated in 2 ml of sodium phosphate buffer (10 mM, pH 6) that contained 0.3% glucose and labelled compounds (37 KBq) for 4 h at 37°C. The specific activities of [2-^{14}C]uracil, [2-^{14}C]uridine and [6-^{14}C]orotic acid were 2.00, 1.97 and 2.18 MBq.μmol^{-1}, respectively. Labelled compounds were analysed by the methods described earlier[3]. The activities of uracil phosphoribosyltransferase, uridine kinase, orotate phosphoribosyltransferase (OPRTase) plus orotidine-5'-monophosphate decarboxylase (ODCase) and phosphoribosylpyrophosphate (PRPP) synthetase in cell extracts were determined as described earlier[5].

Table 1 provides details of metabolism of [2-^{14}C]uracil, [2-^{14}C]uridine and [6-^{14}C]orotic acid in cotyledons incubated with and without cycloheximide. In day-old cotyledons, approximately 45% of exogenously supplied [2-^{14}C]uracil was incorporated into nucleotides and nucleic acids. More than half of the [2-^{14}C]uracil was catabolized reductively, most of the radioactivity being released as $^{14}CO_2$, with some found in β-ureidopropionate, an intermediate in the reductive degradation of pyrimidines[6]. The anabolic metabolism of uracil decreased in two-day-old cotyledons, while the catabolic metabolism of uracil increased. In both day-old and two-day-old

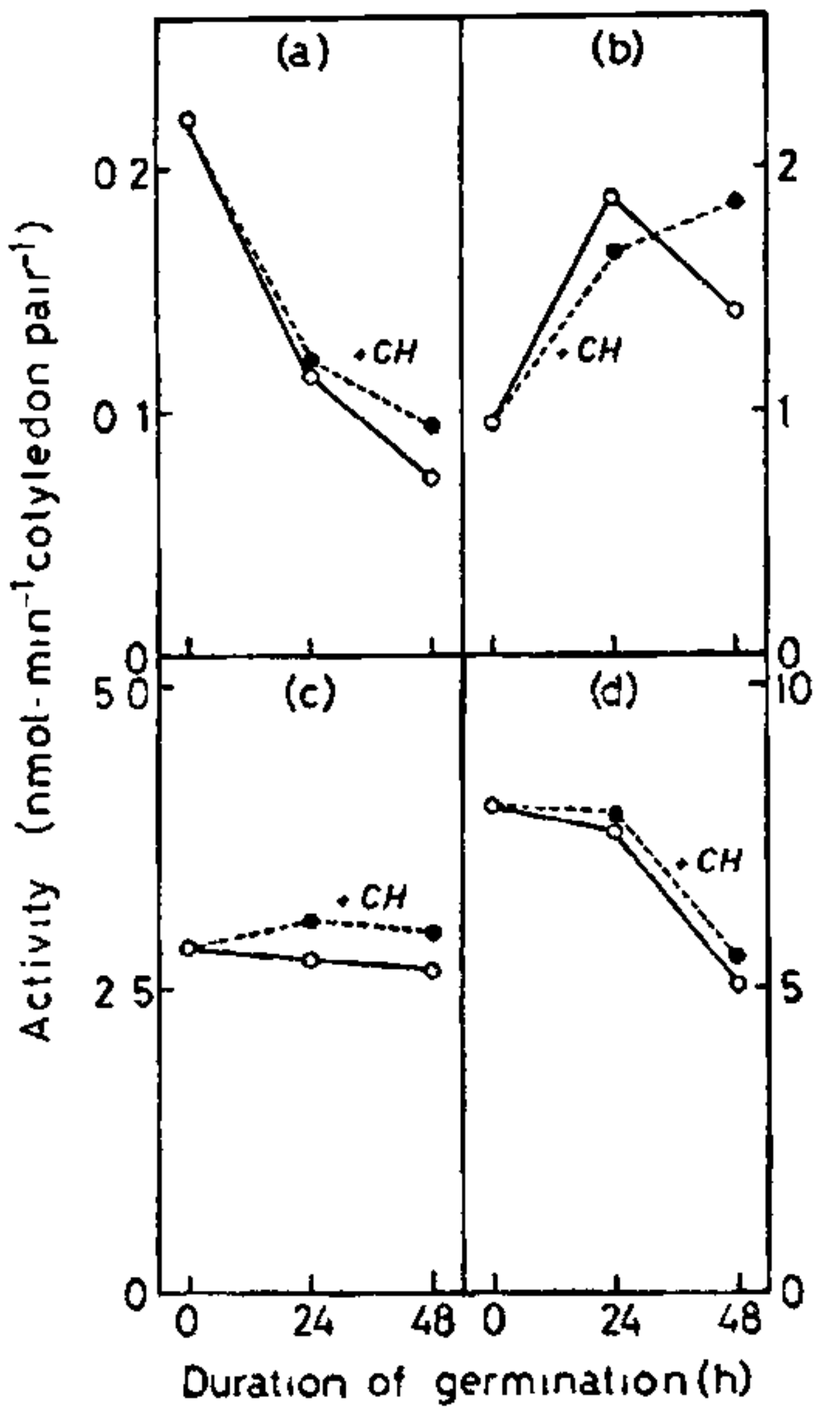

Figure 1a–d. a. Changes in activities of uracil phosphoribosyltransferase; b. Uridine kinase; c. Orotate phosphoribosyltransferase and orotidine-5'-monophosphate decarboxylase; d. Phosphoribosylpyrophosphate transferase in cotyledons of black gram seedlings incubated with (-●-) or without (-○-) cycloheximide (25 $\mu g.ml^{-1}$). The enzymatic activities are expressed as nmol per min per pair of cotyledons. The values are averages of results from duplicate samples from a typical experiment.

Table 1 *Metabolism of [2-^{14}C]uracil, [2-^{14}C]uridine and [6-^{14}C]orotic acid in cotyledons of Phaseolus mungo seedlings grown in the presence or absence of cycloheximide (25 μg.ml^{-1})*

	Duration of germination			
	24 h		48 h	
	Control	+CH	Control	+CH
[2-^{14}C]Uracil				
Total uptake	2.93*	3.47	3.12	3.06
Nucleotides	42.5**	56.9	34.4	46.8
Nucleic acids	3.3	4.2	1.3	2.4
Degradation products	50.1	33.0	60.4	45.1
Others	4.1	5.9	3.9	5.7
[2-^{14}C]Uridine				
Total uptake	1.60	1.53	2.51	1.67
Nucleotides	85.0	87.8	88.5	85.6
Nucleic acids	4.8	6.4	2.3	4.8
Degradation products	5.6	1.4	4.1	1.9
Others	4.6	4.4	5.1	7.7
[6-^{14}C]Orotic acid				
Total uptake	0.53	0.19	0.86	0.32
Nucleotides	83.4	55.6	84.5	63.3
Nucleic acids	5.3	4.0	2.8	4.9
Degradation products	1.3	3.5	1.5	2.6
Others	10.0	36.9	11.2	29.2

*Total uptake of labelled precursors is expressed as nmol per pair of cotyledons per 4 h; **Incorporation of radioactivity into metabolites is expressed as % of total uptake. Major components of 'degradation products' are CO_2 and β-ureidopropionate, and those of 'others' are unmetabolized precursors. The values are averages of results from duplicate samples from a typical experiment. CH, cycloheximide.

cycloheximide-treated cotyledons, the incorporation of radioactivity into anabolites (nucleotides and nucleic acids) was greater than in appropriate controls, but incorporation into catabolites (CO_2 and β-ureidopropionate) was significantly reduced. Anabolism of uracil by the 'salvage pathway' is catalysed by uracil phosphoribosyltransferase[5]. The activity of this enzyme was highest in dry seeds and decreased as germination progressed. Cycloheximide seemed slightly to inhibit degradation of the enzyme (figure 1a). The salvaging of uracil *in vivo* is also influenced by the activities of enzymes involved in catabolism of uracil. It has been reported that many hydrolytic enzymes that participate in degradation of storage materials are synthesized in cotyledons of legume-type dicots[2, 7]. The enzymes for degradation of uracil may also be synthesized during germination, but it is difficult to detect of these enzymes in cell-free extracts of higher plants[8].

Uridine was easily salvaged by cotyledons from both day-old and two-day-old seedlings independently of treatment with cycloheximide. The activity of uridine kinase increased during the first 24 h of germination, but decreased within 48 h (figure 1b). Apparently, cycloheximide retards both synthesis and degradation of uridine kinase. Catabolism of uridine was much less efficient than that of uracil, but cycloheximide also depressed the rate of the degradation of uridine. Synthesis of uridine hydrolase *de novo* may occur in germinating pea seeds[2].

[6-^{14}C]Orotic acid, an intermediate in the biosynthesis of pyrimidine nucleotides *de novo*, was incorporated into nucleotides and nucleic acids in black gram cotyledons. However, the rate of uptake was extremely low, and was further reduced by treatment with cycloheximide (table 1). Unmetabolized orotic acid, comprising more than 95% of radioactivity in the fraction designated 'others' in table 1, made up 10–40% of the total radioactivity taken up by cotyledons. The level of labelled orotate was 2.6–3.7 times higher in cycloheximide-treated tissues than in control ones. Orotate is converted to UMP in the presence of PRPP, in reactions catalysed by OPRTase and ODCase which seem to

be present as a complex in plant cells[9]. OPRTase and ODCase were found in dry seeds and in cotyledons. PRPP synthetase was also detected in dry seeds, but its level decreased over 48 h. Activities of these enzymes were slightly higher in cycloheximide-treated cotyledons than in controls (figure 1c, d). Ross *et al*[1] reported that biosynthesis of UMP from [6-^{14}C]orotate in pea cotyledons was limited during early stages of germination, although high levels of OPRTase, ODCase, and PRPP were present in the cotyledons. Similar results have been found in germinating black gram seeds[3]. The present results indicate that, at least in the case of black gram, the limitation in biosynthesis of UMP from externally supplied [6-^{14}C]orotic acid seems to be due to the poorer permeability of cells to orotate during early stages of germination. Significant amounts of [6-^{14}C]orotate, taken up by cotyledons, failed to reach the site of active synthesis of nucleotides.

From the present results, it appears that enzymes for *de novo* and salvage pathways of pyrimidine biosynthesis are present in dry seeds and are functional after imbibition, while degradation of pyrimidines is mostly catalysed by enzymes synthesized in cotyledons during germination.

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MORPHOGENETIC STUDIES IN ANDROGENIC CALLUS OF *SOLANUM MELONGENA* L.

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STUDIES on morphogenesis in callus cultures have not received due attention. However, some scattered reports are available[1-3]. It is important to study plant regeneration at morphological, physiological and biochemical levels for successful application of plant tissue culture techniques to crop improvement. In this communication, we describe the morphogenetic events leading to shoot and root formation in androgenic callus cultures of *Solanum melongena* L. cv. H4.

Androgenic callus was obtained by culturing anthers at the uninucleate stage of development on GD medium[4] supplemented with 2 mg/l IAA and 1 mg/l kinetin in the dark at 25±2°C. Calli were subcultured on differentiating medium containing GD basal medium, 0.1 mg/l NAA and 2 mg/l kinetin. Shoots and roots developed on the same medium. These cultures were incubated in a 16 h light, 8 h dark cycle. Calli at different stages of development were fixed and sectioned by conventional procedures and stained with safranin and fast green[5]. Details of callus development and differentiation have been discussed earlier[6].

The callus that grew out of anthers started growth by the activity of peripheral meristematic cells. The cell walls of intermittent group of cells became suberized, resulting in irregular growth of the callus (figures 1 and 2). The callus cells were large and loosely arranged. The cells destined to form meristemoids were rich in starch (figure 3). The first indication of vascularization was the appearance of tracheidal cells and vessel elements. These were randomly distributed at different positions over the callus but formed a continuous ring marking the initiation of procambium (figures 4 and 5). The procambium gave rise to xylem towards the outer side and phloem towards the inner side, and a multi-layered cambium in between. The protoxylem showed exarch condition at many places, which is a feature of roots. In shoot-forming callus, meristematic activity was located in peripheral regions. These cells were small, with prominent nuclei and dense cytoplasm. The meristematic tissue later