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SHORT REPORTS

ATP SYNTHESIS IN LETTUCE SEEDS DURING RIPENING

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Key Word Index—*Lactuca sativa*; Compositae; lettuce; seed ripening; ATP; malate dehydrogenase; PEP-carboxylase; pyruvate kinase; ATP synthesis.

Abstract—During the ripening of lettuce seeds, ATP, AMP + ADP, and moisture decrease to very low levels, and the ability to produce ATP from AMP + PEP (phosphoenolpyruvate) and the PEP-carboxylase (EC 4.1.1.38) activity is diminished. Malate dehydrogenase (EC 1.1.1.37) and pyruvate kinase (PK) (EC 2.7.1.40) decreased up to 10 days after anthesis, after which a sharp increase occurred.

During the early stage of germination, increasing activities of malate dehydrogenase (EC 1.1.1.37) and phosphoenolpyruvate (PEP)-carboxy-phosphotransferase (EC 4.1.1.38) have been demonstrated [1–3]. Recently, it was shown that ATP may be synthesized in imbibed seeds from AMP and PEP [4, 5]. The ATP seems to be produced by pyruvate kinase (PK) (EC 2.7.1.40) and by adenylate kinase (AK) (EC 2.7.4.3). Both enzymes are very active in all seeds examined so far [6]. Since AMP and malate accumulate in seeds [7, 8], it is suggested that the above-mentioned enzymes serve as an ATP-providing system in seeds at their early stage of germination. In this work an attempt was made to examine the development of these enzymes as well as the amounts of ATP and ATP-precursors in seeds during the ripening process.

Seeds produced from manually pollinated flowers were collected at various time intervals after pollination. The seeds were examined for enzyme activities and for ATP and ATP-precursors (AMP + ADP). Fig. 1 shows that all enzymes examined exhibit high activity at the early stage of seed development. During the ripening process there is a sharp decrease in the enzyme specific activities. About 10 days after anthesis the PK and the malate dehydrogenase tend to increase until the ripe stage. The ability to synthesize ATP from AMP as well as the PEP-carboxylase activity reach a relatively low, steady-state, level in ripe seeds. A similar increase in malate dehydrogenase and PEP-carboxylase during the late stage of seed ripening has been shown in onion seeds [9]. Since the examinations were made with crude extracts, it is not clear whether the changes in the activities are a result of changes in enzyme concentrations or in promoter–inhibitor-like substances in the cell.

The amount of ATP decreases almost to zero. The precursors for ATP decrease sharply, but still maintain measurable amounts in the ripe seeds. The amount of PEP seems to be unchanged during seed ripening.

The biochemical changes during seed ripening may be a result of the decrease in the moisture content. Alterations in membrane structure caused by loss of water may lead to a loss in ATP, followed by a decrease in enzyme synthesis. The late increase in PK and malate dehydrogenase could be explained by a de-repressed protein-synthesizing system with a high affinity toward ATP. A role in this phenomenon of hormones, which appear in seeds at the late stage of ripening [8], is not excluded. Moreover, the seeds are able to germinate from the 10th day after anthesis, independently of light. About 4 days later, light seems to be more and more necessary for seed germination at 25° (germination data are not shown); this time period coincides with the stage of minimal activity of all examined enzymes.

EXPERIMENTAL

Lettuce (*Lactuca sativa* cv cos type) seeds were grown in a greenhouse. At anthesis the flowers were pollinated manually and marked. From the 8th day after anthesis about 50 fruits were collected at random every 2 days and the seeds were examined for the following components and activities: (a) ATP content with the luciferin–luciferase system [4]; (b) PEP content, by the addition of AMP to cut seeds [4]; (c) AMP + ADP content, by the addition of PEP to cut seeds [4]; (d) H_2O content, by measuring the differences in wt between fresh seeds and after drying them at 96° for 24 hr; (e) PK activity in crude extracts, as described in ref. [5]; (f) Malate dehydrogenase, as described in ref. [1]; (g) PEP-carboxylase [2]; and (h) per cent germination (radical protrusion in light and dark at 25°). All examinations were done in three replicates in at least two different experiments.

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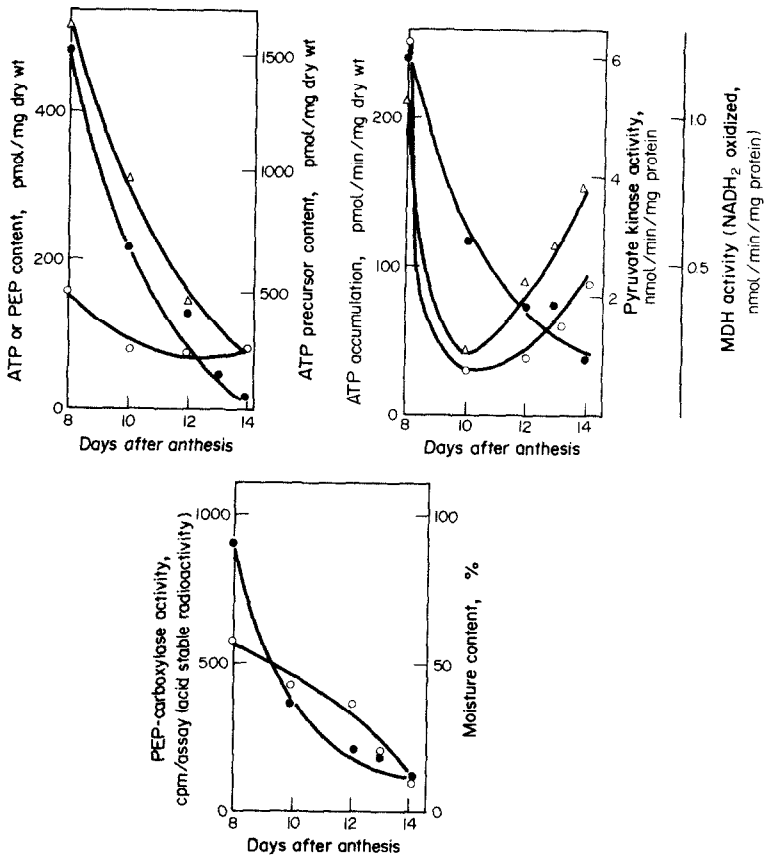

Fig. 1. Biochemical changes in lettuce seeds during the ripening process. At various times after anthesis, the following materials and activities were examined: ATP (●), PEP (○) and AMP + ADP (△) (upper left figure). The rate of ATP accumulation in the presence of AMP and PEP in the incubation mixture with cut seeds (●), PK activity (○), and malic dehydrogenase activity (△) (upper right figure). PEP-carboxylase activity (●) and water content (○) (lower figure). For explanation of methods used, see Experimental. All results are means of 3 replicates.