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DORMANCY AND ENZYME LEVELS IN SEEDS OF WILD OATS

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Key Word Index—*Avena fatua*; Gramineae; wild oats; dormancy; enzyme levels.

Abstract—Nine enzymes were compared in dry and steeped mature dormant and non-dormant seeds of wild oats. In dry seeds only glutamate–pyruvate transaminase and phosphoglycerate kinase were greater in non-dormant seeds. In steeped non-dormant seeds glucose-6-phosphate dehydrogenase activity doubled while the enzyme declined sharply in dormant seeds. Increases in isocitrate dehydrogenase, glutamate-oxaloacetate transaminase and acid phosphatase in non-dormant seeds, during steeping, are consistent with the hypothesis that the pentose phosphate and glycolysis–tricarboxylic acid pathways are involved in the control of dormancy of wild oat seed.

INTRODUCTION

Failure of seeds to germinate under favorable conditions is a major factor in the persistence of weeds. Seed dormancy of wild oats (*Avena fatua*) has been investigated since the turn of the century from both a physiological and ecological view point [1]. During the initial stages of seed germination increases in respiration [2–4] and activity of the oxidative pentose phosphate pathway relative to the tricarboxylic acid cycle [5,6] have been reported. The shift between the above two pathways of oxidation of glucose has also been demonstrated during and after ripening and also following GA treatment of dormant wild oats [6].

The importance of the redox system in maintaining a high level of nicotinamide adenine dinucleotide phosphate (NADP) for germination has been demonstrated [2,4,7–9]. In sweet potato reduced NADP competitively inhibits glucose-6-P dehydrogenase, a key enzyme of the pentose phosphate pathway [10]. Moreover, the steady state concentration of the pentose phosphate pathway intermediates suggested that the oxidation of glucose-6-P by glucose-6-P dehydrogenase may be a key step in the control of the pentose phosphate pathway [11]. Catalase can also be involved by preventing the oxidation of reduced NADP which is required for the pentose phosphate pathway [9]. Seed dormancy of lettuce and pigweed can be overcome by the inhibition of catalase [12].

The objective of this investigation was to study the relationship between levels of certain key enzymes of glucose metabolism and dormancy of mature wild oat seeds. Enzymes investigated were (a) *Oxidative pentose phosphate pathway*; glucose-6-P dehydrogenase (EC 1.1.1.49), 6-P-gluconate dehydrogenase (EC 1.1.1.44), (b) *Glycolysis*; fructosediphosphate aldolase (EC 4.1.2.13), NAD-dependent glyceraldehyde-3-P dehydrogenase (EC 1.2.1.12), phosphoglycerate kinase (EC 2.7.2.3), (c) *Tricarboxylic acid cycle*; isocitrate dehydrogenase (EC 1.1.1.42), (d) *Amino acid and protein synthesis*; glutamate-pyruvate transaminase (EC 2.6.1.2), glutamate–oxaloacetate transaminase (EC 2.6.1.1), (e) *Phosphate metabolism*; acid phosphatase (EC 3.1.3.2).

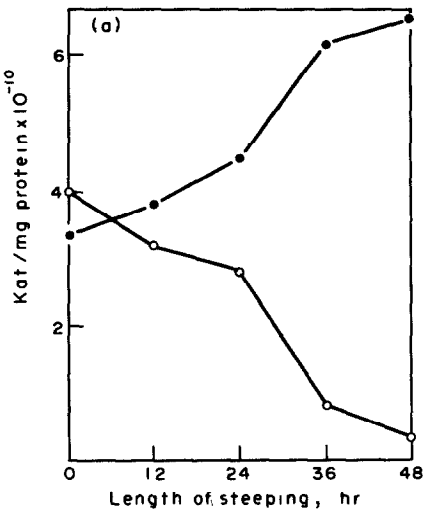
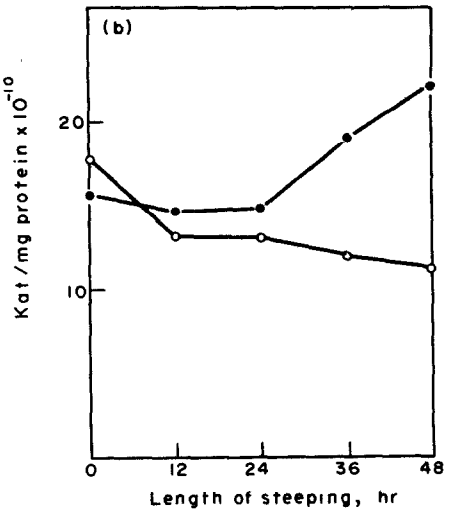


Fig. 1(a).



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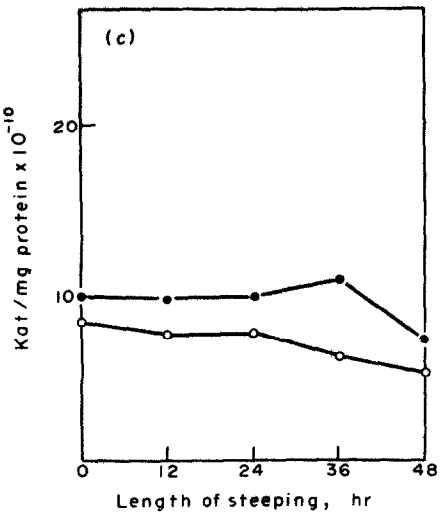

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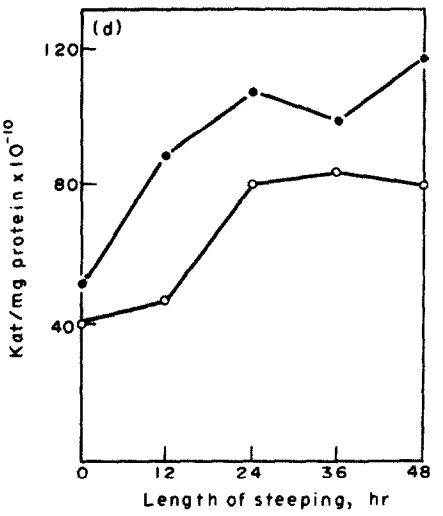

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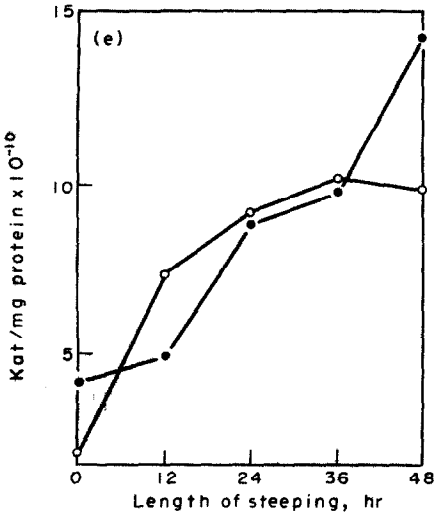

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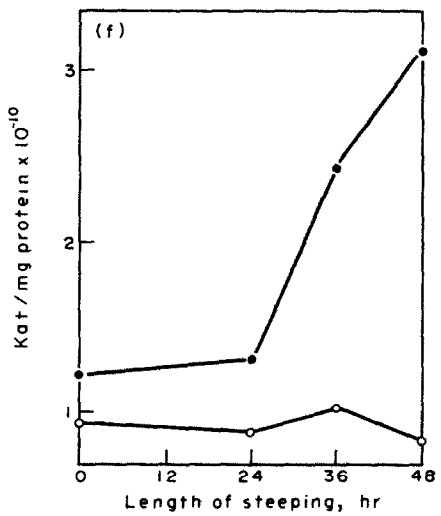

Fig. 1(f).

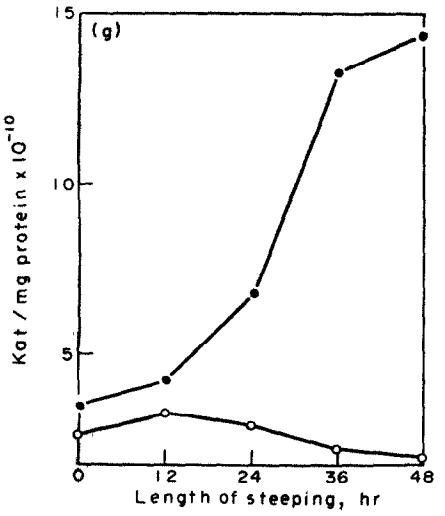

Fig. 1(g).

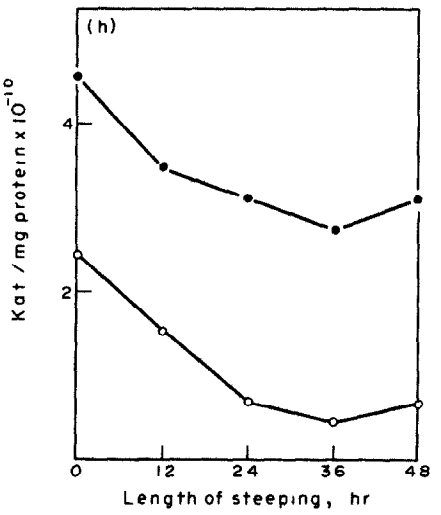

Fig. 1(h).

Fig. 1. Enzyme activities during steeping of dormant (○——○) and non-dormant (●——●) wild oat seeds. Activities are expressed in Katals (activity effecting the conversion of one mol of substrate per sec). (a) glucose-6-phosphate dehydrogenase; (b) 6-phosphogluconate dehydrogenase; (c) fructose diphosphate aldolase; (d) glyceraldehyde-3-phosphate dehydrogenase; (e) phosphoglycerate kinase; (f) isocitrate dehydrogenase; (g) glutamate-oxalacetate transaminase; (h) glutamate–pyruvate transaminase.

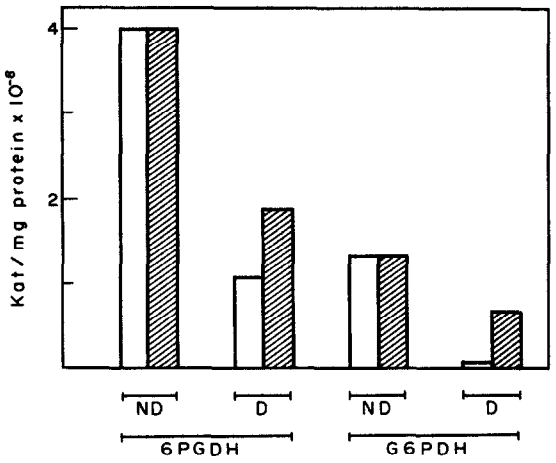

Fig. 2. Effect of gibberellic acid (0.2 mM) ■ on the levels of 6-phosphogluconate dehydrogenase (6PGDH) and glucose-6-phosphate dehydrogenase (G6PDH) in dormant (D) and non-dormant (ND) seeds of wild oats after five days of steeping.

RESULTS

In both dormant and non-dormant mature dry seeds all the enzymes studied except glutamate–pyruvate transaminase and phosphoglycerate kinase were approximately equal in activity (Fig. 1a–h). The activities of glutamate–pyruvate transaminase (Fig. 1g) and phosphoglycerate kinase (Fig. 1e) were four- and two-fold higher, respectively, in non-dormant vs dormant seeds.

During the first 48 hr of germination, following steeping, the activity of glucose-6-P dehydrogenase increased two-fold in non-dormant seeds whereas in dormant seeds virtually no activity could be found at the end of this period (Fig. 1a). A similar pattern, but to a less marked degree, was seen for 6-P-gluconate dehydrogenase (Fig. 1b). In both dormant and non-dormant seeds the activity of 6-P-gluconate dehydrogenase was three- to five-fold higher than the activity of glucose-6-P dehydrogenase.

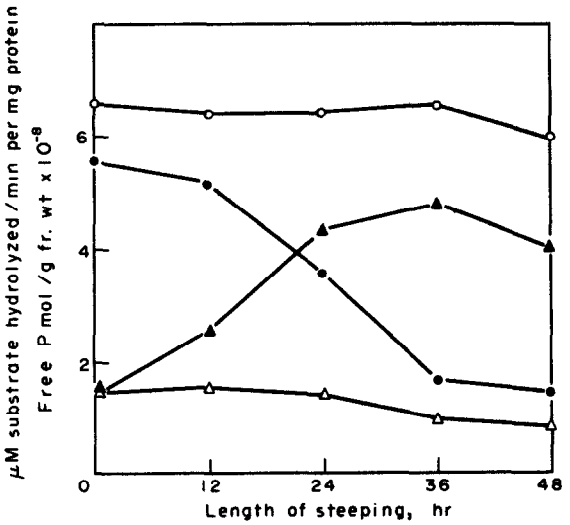

Fig. 3. Levels of (a) acid phosphatase in dormant (—△—) and non-dormant (—▲—) seeds (b) free phosphate in dormant (—○—) and non-dormant (—●—) seeds of wild oats.

There was no significant change in aldolase activity during 48 hr in either dormant or non-dormant steeped seed (Fig. 1c). Glyceraldehyde-3-P dehydrogenase and phosphoglycerate kinase each increased significantly in both dormant and non-dormant steeped seed (Fig. 1d,e).

Isocitrate dehydrogenase increased only in the non-dormant steeped seeds by approximately three-fold between 24 and 48 hr (Fig. 1f). A similar pattern of increase in activity of glutamate oxaloacetate transaminase was observed in non-dormant seed, with little change in activity in the dormant seed (Fig. 1g). While the levels of glutamate–pyruvate transaminase activity were different in dormant and non-dormant seeds the pattern of decrease following steeping was similar in both types.

Five days of steeping dormant seeds in GA_3 (0.2 mM) increased the activities of glucose-6-P dehydrogenase and 6-P-gluconate dehydrogenase six- and two-fold, respectively (Fig. 2). The same treatment on non-dormant seeds produced no detectable change in these dehydrogenases.

Free phosphate levels (fr. wt basis) declined in non-dormant seeds whereas the level remained constant in dormant seeds (Fig. 3). At the same time acid phosphatase increased up to 24 hr in non-dormant seed while it remained constant in dormant seed (Fig. 3).

DISCUSSION

Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase catalyze the initial steps of the pentose-phosphate pathway. Comparison of the relative activities of these enzymes in dormant and non-dormant wild oats showed that initially (mature dry seed) the enzyme levels are similar but there is a rapid change whereby activities of both enzymes increase in non-dormant seeds and both enzymes decrease in dormant seeds. This suggests that the initial level is critical for germination and that the ability to maintain or increase this level following steeping is obligatory for germination.

The activity of glucose-6-P dehydrogenase, the first step in the pentose phosphate pathway, is lower than 6-P-gluconate dehydrogenase the second enzyme in the pathway. This observation is consistent with the idea [11] that glucose-6-P dehydrogenase participates in control of the pentose pathway by limiting the breakdown of glucose in the early stages of germination.

The observations that the two enzymes of the pentose phosphate pathway (glucose-6-P dehydrogenase, 6-P-gluconate dehydrogenase) increased considerably, that glycolysis (fructose diphosphate aldolase) remained essentially unchanged and that the tricarboxylic acid cycle (isocitrate dehydrogenase) increased are all consistent with the view, put forward by others [3,6,13] that release of dormancy requires an increase in glucose oxidation via the pentose shunt for the purpose of forming those metabolites particularly required for synthetic reactions such as protein synthesis which use NADPH (14–16). The fact that glutamate–oxaloacetate transaminase increased markedly when dormancy was lost indicates enhanced metabolism of amino-acids with the possibility of further control steps. There is no obvious significance in the decline of glutamate–pyruvate transaminase in the first 48 hr in both dormant and non-dormant seeds. It is possible that this enzyme has significance at a later period in germination.

The increase in the C_6/C_1 ratio in the same dormant wild oat seeds treated with GA_3 [6] implies three possibilities in the relative activities of the pentose–phosphate and tricarboxylic acid pathways. The observations reported here that steeping dormant seeds in GA increased the activities of the two first key enzymes of the pentose phosphate pathway exclude that possibility where there would be no change in activity of that pathway. Furthermore, the considerably larger response to GA_3, in dormant seeds, in activity of glucose-6-P dehydrogenase compared with 6-P gluconate dehydrogenase suggests a more critical role for the former enzyme in the control of dormancy. The explanation for lack of response to GA_3 in the activities of the same enzymes in non-dormant seeds indicates there is no deficiency of the hormone in these seeds, as previously suggested [17].

The lack of change in phosphatase and free phosphate in dormant seeds compared to the marked changes in non-dormant seeds corroborates earlier evidence [18,19] that germination is correlated with a high rate of phosphorus metabolism.

EXPERIMENTAL

Plant material. Dormant (freshly harvested) and non-dormant (after ripened 4 yr under laboratory conditions) seeds were obtained from the same uniformly inbred line of wild oats (*Avena fatua* L.) grown in the field [20].

Preparation of extracts. The following procedure was used for all the enzyme assays except acid phosphatase. Samples (100 seeds) of both dormant and non-dormant seeds were steeped for either 0, 12, 24, 36 and 48 hr at 20°; the samples were homogenized in 10 ml of medium at half speed, first for 30 sec and then for another 30 sec with cooling by an ice bath. The extraction medium contained 10^{-2} M KPi buffer (pH 6), 10^{-3} M EDTA and 0.1 M β-mercaptoethanol. The homogenate was centrifuged at 15 000 *g* for 5 min. The resulting supernatant was further centrifuged for 30 min at 48 000 *g*. The final supernatant was used as the enzyme source. All the extractions were carried out between 0–4°. For the preparation of acid phosphatase 10^{-2} phthalate buffer (pH 6) was used.

Enzyme assay. Activities of 6-P-gluconate dehydrogenase and glucose-6-P dehydrogenase were determined by the initial rate of reduction of pyridine nucleotide phosphate measured by $A_{340\ nm}$ at 37°. Two assays were carried out, one containing 5×10^{-2} M Tris–HCL (pH 7.8), 2×10^{-3} M glucose-6-P, 2×10^{-3} $NADP^+$ and 50 to 100 μl enzyme extract in 1 ml. The second contained all the above except glucose-6-P. The difference in the reduction rate of $NADP^+$ between these two assays was used to calculate glucose-6-P dehydrogenase activity. The activities of aldolase, glyceraldehyde phosphate dehydrogenase (NAD dependent), glycerate phospho·kinase, isocitrate dehydrogenase, glutamate pyruvate transaminase and oxaloacetate transaminase were assayed spectrophotometrically [21].

Activity of acid phosphatase was determined as follows. Enzyme extract (50 μl) was incubated at 37° for 30 min in a reaction mixture containing 5×10^{-3} M Tris–maleate buffer (pH 6), 10^{-2} M $MgSO_4$ and 2.5×10^{-2} M *p*-nitrophenyl phosphate. (Total vol 1 ml). The reaction was stopped by adding 2 ml of 30% TCA and a suitable aliquot of supernatant was then diluted. Release of *p*-nitrophenol was determined by addition of 2 ml of saturated Na_2CO_3 to 2 ml of the diluted sample and measurement of $A_{430\ nm}$. Free Pi [22] was determined following $HClO_4$ treatment of the extract [23]. Protein was determined by a modified Biuret method [24].

REFERENCES

1. Atwood, W. M. (1914) *Bot. Gaz.* **57,** 386.
2. Koller, D., Mayer, A. M., Poljakoff-Mayber, A. and Klein, S. (1962) *Ann. Rev. Plant Physiol.* **13,** 437.
3. Roberts, E. H. (1969) *Symp. Soc. Exp. Biol.* **23,** 161.
4. Roberts, E. H. (1973) In *Seed Ecology* (Heydecker, W., ed.), pp. 189–218. Penn. State Univ. Press, University Park.
5. Major, W. and Roberts, E. H. (1968) *J. Exp. Botany* **19,** 90.
6. Simmonds, J. A. and Simpson, G. M. (1971) *Can. J. Botany* **49,** 1833.
7. Ballard, L. A. T. and Grant-Lipp, A. E. (1967) *Science* **156,** 398.
8. Hendricks, S. B. and Taylorson, R. B. (1972) *Nature* **237,** 169.
9. Hendricks, S. B. and Taylorson, R. B. (1974) *Plant Physiol.* **54,** 304.
10. Muto, S. and Uritani, I. (1972) *Plant Cell Physiol.* **13,** 377.
11. Ashihara, H. and Komamine, A. (1974) *Plant Sci. Letters* **2,** 331.
12. Hendricks, S. B. and Taylorson, R. B. (1975) *Proc. Natl. Acad. Sci. U.S.* **72,** 306.
13. Lacroix, L. J. and Jaswal, A. S. (1967) *Plant Physiol.* **42,** 479.
14. Gibbs, M. and Beevers, H. (1955) *Plant Physiol.* **30,** 343.
15. Neish, A. C. (1960) *Ann. Rev. Plant Physiol.* **11,** 55.
16. Karlson, P. (1969) *Introduction to Modern Biochemistry,* Academic Press, New York.
17. Simpson, G. M. (1965) *Can. J. Botany* **43,** 793.
18. Prieley, H. J. and Fowden, L. (1965) *Phytochemistry* **4,** 169.
19. Hall, R. and Hodges, T. G. (1966) *Plant Physiol.* **41,** 1459.
20. Black, M. (1959) *Can. J. Botany* **37,** 393.
21. Bucher, T., Luh, W. and Petter, D. (1964) In *Handbuch der Physiologisch und Patologisch-chemischen Analyse* (F. Hoppe-Seyler and Thierfelder H. eds.) Vol. 6, pp. 292–339. Springer, Heidelberg.
22. Lowry, O. and Lopez, J. A. (1946) *J. Biol. Chem.* **162,** 421.
23. Hohorst, H. J., Kreutz, F. H. and Bucher, T. (1959) *Biochem. Z.* **332,** 15.
24. Holl, J. S., Simmonds, D. H. and Bushuk, W. (1974) *Cereal Chem.* **38,** 467.