Phytochemistry, 1976, Vol 15, pp. 79–81. Pergamon Press. Printed in England.

ISOCITRATE LYASE IN GERMINATING SEEDS OF *PRUNUS DULCIS*

JOHN S. HAWKER and DIANE M. BUNGEY

C.S.I.R.O. Division of Horticultural Research, G.P.O. Box 350, Adelaide, South Australia, 5001

(*Received* 18 *June* 1975)

Key Word Index—*Prunus dulcis*; Rosaceae; almond kernel; stratification; germination; gibberellic acid; isocitrate lyase.

Abstract—Isocitrate lyase activity increased in cotyledons of germinating seeds of *Prunus dulcis* (almond) which had been induced to germinate by either stratification or treatment with gibberellic acid (GA). Germination of embryos, growth of the embryonic axis and activity of isocitrate lyase increased with increasing concentrations of GA from 10^{-7} to 10^{-3} M.

INTRODUCTION

The control of seed germination has been reviewed recently [1]. Before many seeds can germinate they need to be stratified (chilled in a moist condition) for a suitable period or treated with a chemical, e.g. GA. Abscisins also seem to play a role in dormancy [1]. In fatty seeds work is continuing on the hydrolysis of lipid and its conversion to sucrose via the glyoxylate cycle [2–4]. It has been reported that GA promotes the activity of one of the enzymes of the glyoxylate cycle (isocitrate lyase, E.C. 4.1.3.1) in hazel cotyledons [5] whereas in ponderosa pine seeds GA inhibited the development of isocitrate lyase activity [4].

The present paper describes the effect of stratification and gibberellin on the germination and development of isocitrate lyase activity of the fatty seed of almonds (*Prunus dulcis* (Mill.) D. A. Webb) [6].

RESULTS

Effect of stratification and gibberellin on germination

Almond seeds did not germinate within 35 days when soaked in distilled water overnight and plated on moist filter paper at 25°. Removal of testas (to give embryos) resulted in a low percentage germination within 7 to 13 days (Figs. 1, 2). After seeds were stratified with testas intact at 4° for from 4 to 8 weeks, germination occurred rapidly upon placing the seeds at 25° (Fig. 3). Treatment of unstratified embryos with GA caused a markedly increased rate of germination at all concentrations from 3×10^{-7} to $1{\cdot}5 \times 10^{-3}$ M, the effect being particularly pronounced at 3×10^{-5} to $1{\cdot}5 \times 10^{-3}$ M GA (Fig. 1). In another experiment using 2 concentrations of GA the initial growth rates of roots and shoots at 3×10^{-4} M GA were faster than at 3×10^{-6} M GA (Fig. 2).

Enzymes of glyoxylate cycle. Malate synthase (E.C. 4.1.3.2, 18·5 nkat/g fr. wt) and isocitrate lyase (13·0 nkat/g fr. wt) were present in cotyledons of almonds after 55 days of stratification. In the cotyledons of unstratified almonds no isocitrate lyase could be detected, but after 28 days of stratification the activity present had increased to 3 nkat/g fr. wt. In another experiment the activities of isocitrate lyase at pH 6, 6·9, 7·5 and 8 were 1, 3·4, 4·8 and 4 nkat/g fr. wt, respectively.

During germination isocitrate lyase activity increased in the cotyledons in both stratified seeds and in embryos treated with GA reaching maxima in about 10 days (Figs. 3, 4). Thereafter activity decreased and by about day 17 had fallen to less than half the maximum values. Embryos on distilled water had relatively low enzyme activity until about day 15 (Fig. 4).

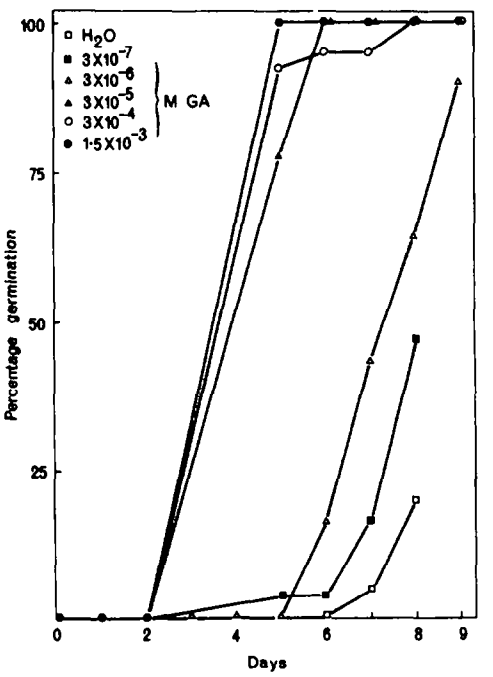

Fig. 1. The effect of GA on the germination of almond embryos.

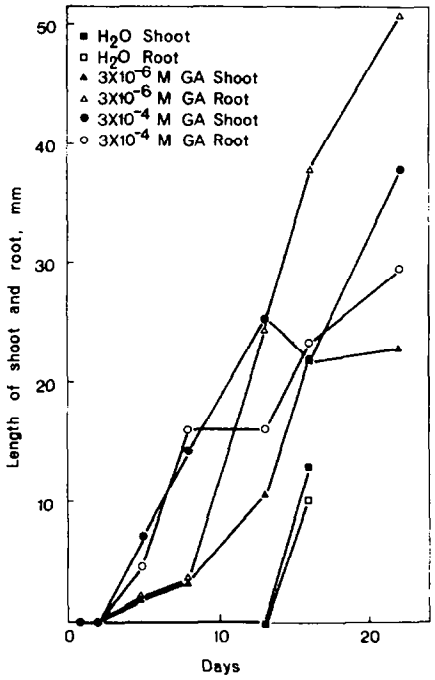

Fig. 2. The effect of GA on the growth of the embryonic axis of almond embryos during germination.

Seeds in the dry state contained 50% lipid and 20% protein. After 22 days germination on GA, about 60% of the lipid and 50% of the protein of the cotyledons remained even though at this stage isocitrate lyase activity had fallen to 20% of its maximum value (Fig. 4). Cotyledons of stratified seeds also had much of their lipid and protein remaining after 19 days germination.

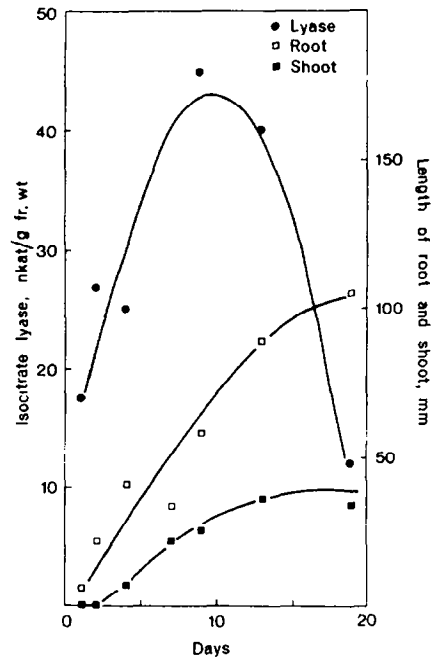

Fig. 3. The growth and isocitrate lyase activity of stratified almonds during germination. Stratification was for 8 weeks.

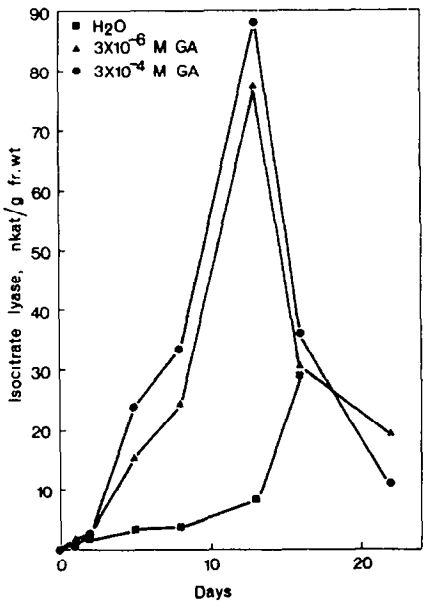

Fig. 4. Isocitrate lyase activity in cotyledons of germinating almond embryos with or without GA. Cotyledon fr wt/seed was 2·4, 3·7, and 4·1 g for day 1, 13, and 22 respectively at 3×10^{-4} M GA.

In another experiment the length of roots and shoots and the activity of isocitrate lyase was increased with increasing concentrations of GA (Fig. 5).

DISCUSSION

The dormant seeds of almond could be induced to germinate either by stratification or by removal of testas and treatment with GA. Isocitrate lyase activity appeared during stratification and further increases occurred dur-

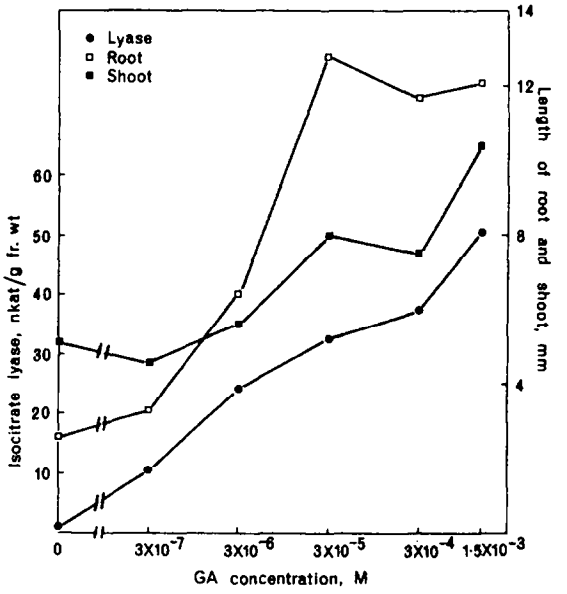

Fig. 5. The effect of GA concentration on the growth of the embryonic axis and on the activity of isocitrate lyase in the cotyledons of almond embryos at day 8 of germination.

ing germination of stratified seeds. Almond embryos induced to germinate by GA treatment also showed increases in isocitrate lyase activity and the activity of the enzyme seemed to be more dependent on GA concentration than was the percentage germination or growth of root and shoot (Fig. 5). Pinfield [5] has shown that exogenously supplied GA stimulates isocitrate lyase activity in germinating hazel seeds. Although in the present work it is not possible to completely separate the effect of GA on growth and enzyme activity it is clear that GA does not inhibit the development of activity of isocitrate lyase as has been shown in pine seeds [4]. GAs control the *de novo* synthesis of α-amylase in barley endosperm and it seems very likely that the *de novo* synthesis of isocitrate lyase occurs in many fatty seeds [1]. The results with almond support the suggestion that GA is involved in the stimulation of isocitrate lyase activity in fatty seeds but more detailed work is required on almonds or other seeds.

Compared to other plant seeds isocitrate lyase activity increased relatively slowly in the cotyledons of germinating almonds to reach a maximum at about 10 days or later. Seed storage tissues of castor bean [7], cucumber [8], cotton [9] and pine [4] have a relatively rapid increase of isocitrate lyase activity which then virtually disappears by day 10 of germination, by which time most of the storage compounds have been depleted. The germination of almonds is hypogeal (the cotyledons remain at or below ground level) and the large cotyledons of the commercial variety used here still retained about half their protein and lipid when the activity of isocitrate lyase had dropped markedly. Almonds have been selected for their food value, not their germination characteristics and this fact may explain both the relatively slow build up of isocitrate lyase activity and the incomplete early use of reserve materials.

Enzyme changes occurring during almond germination have not been studied previously. The occurrence of malate synthase and the changes in isocitrate lyase activity suggest that almonds belong to the group of fatty seeds which exhibit the glyoxylate cycle. Presumably they contain glyoxysomes and it would be interesting to determine their fate as the isocitrate lyase activity decreases. Although almond cotyledons do not become leaflike (as in cucumber [8]) they nevertheless remain healthy with some chlorophyll present for several weeks after isocitrate lyase activity drops.

EXPERIMENTAL

Germination of almonds (Prunus dulcis *cv Chellaston*). Seeds were stratified by placing in moist vermiculite at 4° for from 4 to 8 weeks and then germinated on filter paper moistened with 20 ml H_2O in covered Petri dishes (10 seeds/dish) in the dark at 25°. Unstratified seeds were soaked in H_2O for 30 min, the testas were removed and the embryos (cotyledons plus root-shoot axis) were shaken in a filtered 7% solution of $CaOCl_2$ for 30 min. After 3 washes in sterile H_2O 10 embryos were plated per 15 cm Petri dish in 20 ml of H_2O or GA_3 soln (Figs. 3 and 5) or soln containing 100 units/ml mycostatin, 250 units/ml penicillin, 100 ppm streptomycin and GA_3 as required (Figs. 1, 2 and 4). Embryos sterilised with $CaOCl_2$ prior to germination in H_2O frequently developed fungal or bacterial infections. The mycostatin, penicillin and streptomycin largely controlled both the fungi and bacteria.

Assays. Enzymes were extracted at 0–4° by grinding 3 g of cotyledons with 5 ml of 0·5 M Tris–HCl buffer, pH 8·5 containing 20 mM EDTA, 11 mM Na diethyldithiocarbamate and 15 mM cysteine–HCl. The brei was centrifuged at 12000 *g* for 15 min and 3 ml of the clear supernatant (between ppt. and fat layer) was withdrawn and desalted on a 15 ml column of Sephadex G-25 which had been washed with 5 mM Tris–HCl pH 7. Isocitrate lyase and malate synthase were assayed at 30° spectrophotometrically [10]. Total lipids were determined gravimetrically [11]. Protein was determined in extracts of TCA washed cotyledon ppts in 3% Na_2CO_3 in 0·1 N NaOH [12]. Germination percentages were calculated as means of 30 seeds, growth studies as means of 5 or more seeds and enzyme assays as means of at least duplicate assays on duplicate bulked samples of 10 cotyledons.

Acknowledgement—Gifts of almonds by H. C. T. Stace and the Almond Producers Co-op. Ltd., Adelaide, are gratefully acknowledged.

REFERENCES

1. Mayer, A. M. and Shain, Y. (1974) *Ann. Rev. Plant Physiol.* **25**, 167.
2. Muto, S. and Beevers, H. (1974) *Plant Physiol.* **54**, 23.
3. Lopez-Perez, M. J., Gimenez-Solves, A., Calonge, F. D. and Santos-Ruiz (1974) *Plant Sci. Letters* **2**, 377.
4. Bilderback, D. E. (1974) *Physiol. Plant.* **31**, 200.
5. Pinfield, N. J. (1968) *Planta* **82**, 337.
6. Terrell, E. E. and Winters, H. F. (1974) *Hort. Sci.* **9**, 324.
7. Carpenter, W. D. and Beevers, H. (1959) *Plant Physiol.* **34**, 403.
8. Trelease, R. N., Becker, W. M., Gruber, P. J. and Newcomb, E. H. (1971) *Plant Physiol.* **48**, 461.
9. Smith, R. H., Schubert, A. M. and Benedict, C. R. (1974) *Plant Physiol.* **54**, 197.
10. Cooper, T. G. and Beevers, H. (1969) *J. Biol. Chem.* **244**, 3507.
11. Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.