Phytochemistry, 1981, Vol. 20, pp. 31–33. © Pergamon Press Ltd. Printed in England. 0031-9422/81/0101-0031 $02.00/0

PEROXIDASE LEVELS IN THE COTYLEDONS OF HAZEL SEED (*CORYLUS AVELLANA*)

PETER G. GOSLING* and JAMES D. ROSS

Department of Botany, University of Reading, Reading, Berkshire, U.K.

(*Received* 3 *April* 1980)

Key Word Index—*Corylus avellana*; Corylaceae; peroxidase; dormancy breakage; accelerated ageing.

Abstract—Peroxidase activity was demonstrated in extracts from hazel cotyledons and the cytoplasmic enzyme was partially characterized. Levels of cytoplasmic and organellar peroxidase were monitored in seeds stratified at 5° and incubated under moist conditions at 20°. Specific activities of the enzymes in both subcellular localizations remain constant throughout nine weeks stratification but show a steady rise in activity from seeds maintained at 20°.

INTRODUCTION

Peroxidase is known to be present in many seeds. Amongst numerous suggested roles [1] it has been postulated to be involved in the regulation of plant growth and development [2, 3], and more specifically in metabolic activities concerned with germination [4, 5].

A previous demonstration of increased pentose phosphate pathway (PPP) enzyme activity during stratification of hazel seed (*Corylus avellana*) [6], has caused us to re-examine whether these increases are an isolated response to chilling and play an important role in the after-ripening of this seed, or are simply part of a general increase in enzyme activities prior to germination. For these reasons an enzyme characterization of hazel peroxidase followed by an investigation of total levels of the enzyme during moist incubation of the fruits at 5° and 20 has been carried out.

RESULTS AND DISCUSSION

Michaelis constants were determined for cotyledonary peroxidase from dormant seeds. The double-reciprocal plot for H_2O_2 revealed a non-Michaelean behaviour of the enzyme with respect to substrate. The K_m at less than 0.2 mM H_2O_2 was 1.5×10^{-3} M, at more than 0.2 mM was 0.1×10^{-3} M. The mechanism responsible for this was not elucidated. The Lineweaver–Burk plot of $1/v$ vs.

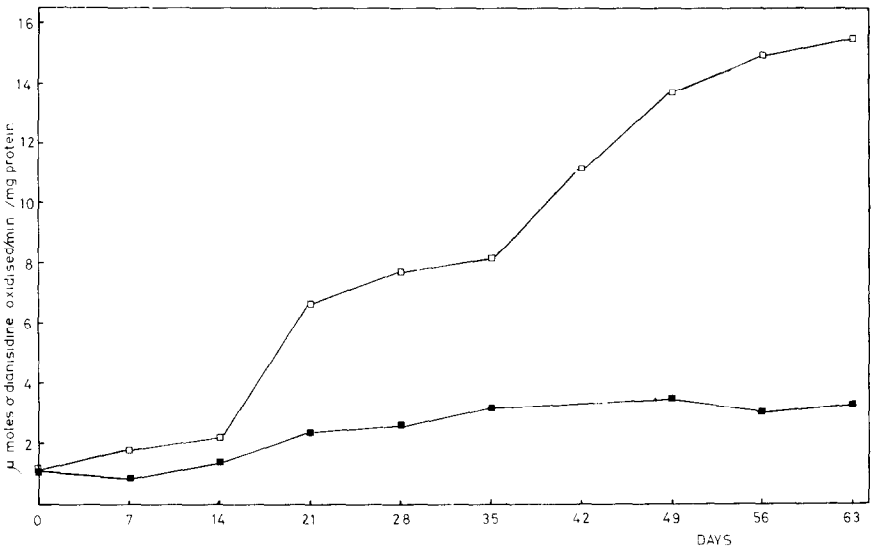

Fig. 1. Levels of cotyledonary cytoplasmic peroxidase during moist incubation of fruits at 20 (□——□) and 5 (■——■) for varying lengths of time.

* Present address: Department of Biology, Queen Elizabeth College, Campden Hill Road, London.

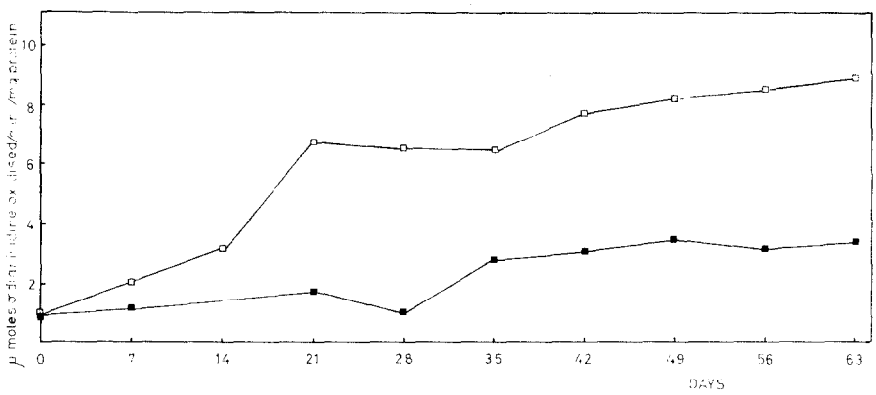

Fig. 2. Levels of cotyledonary organellar peroxidase during moist incubation of fruits at 20 (□——□) and 5 (■——■) for varying lengths of time.

1/[σ-dianisidine] extrapolated to a K_m value of 1.3×10^{-3} M.

Total levels of peroxidase extracted from the cotyledons of hazel nuts during moist incubation at 5° and 20° are shown in Figs. 1 and 2. It is apparent that the levels of both cytoplasmic and organellar enzymes remain constant throughout stratification. However, in the case of nuts incubated under moist conditions at 20° the cotyledonary cytoplasmic peroxidase activity increased 14-fold over the course of the investigation. Changes in the organellar peroxidase of seeds incubated at 20° were less pronounced but still show an eight-fold increase in specific activity after nine weeks.

The induction of several enzymes in plant tissues has been reported when they are subjected to wounding [7], infection by pathogens [8, 9], ageing [7] or other conditions of stress. Peroxidase is amongst one of the most studied of these enzymes and has been shown to increase dramatically under all the above conditions [10]. The increase in peroxidase activity during incubation of intact hazel fruits at 20° is clearly not due to wounding. The possibility of pathogenic infection is also unlikely since every precaution was taken to ensure that the fruits and seeds were kept axenic. Furthermore, the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase, which increase in plants infected by pathogens [11, 12], were shown to rise during stratification but not during moist incubation at 20° [6]. The combined evidence that the PPP dehydrogenase enzymes only increased at 5°, while the peroxidase enzyme only increased at 20°, tends to indicate that neither increase in hazel was due to pathogenic causes. By a process of elimination and also the fact that warm, moist incubation of many seeds is known to accelerate ageing [13, 14], this evidence suggests that peroxidase increases in seeds of hazel maintained at 20° are an ageing response.

The results clearly demonstrate that increases in peroxidase activity do not accompany dormancy breakage in hazel seed. This verifies that the PPP enzyme increases shown to occur during stratification [6] are not part of an overall enzyme multiplication during afterripening. Such evidence lends further credence to the suggestion of Gosling and Ross [6] that increased PPP enzyme activity may play an important role in the afterripening of this seed.

EXPERIMENTAL

Hazel fruits (*Corylus avellana* L.) were commercially obtained at the end of September, 1978, and subsequently stored and incubated at 5° or 20° as detailed by Gosling and Ross [6]. At intervals of 1 week approximately 5 g of cotyledons from each incubation treatment were ground at 5° with a pestle and mortar containing 30 ml of extraction medium (0.02 M acetate buffer pH 5.2 plus 0.48 M mannitol). The macerate was then treated as in the preparation of cotyledonary cytoplasmic dehydrogenase enzymes [15]. In the case of the peroxidase extract, further purification of this fraction was not necessary.

Enzyme assay. The activity of the peroxidase enzyme was measured spectrophotometrically by following the maximum rates of change in absorbance of oxidized σ-dianisidine at 460 nm. Unless otherwise shown, reaction mixtures contained 0.5 mM H_2O_2, 0.1 mM σ-dianisidine and between 10 and 50 μl extract in a final volume of 3 ml adjusted with 0.02 M acetate buffer pH 5.2. All enzyme assays were repeated until three consecutive reactions achieved an accuracy within 5%. The temperature of the reaction mixture was approximately 30° and reactions were initiated by the addition of H_2O_2.

Protein assay. Three dilutions of the original extract were prepared and the protein concentration of each dilution measured in triplicate by the method of Sedmak and Grossberg [16]. The results were used to calculate the mean value for the protein concentration within the original extract.

Bovine serum albumin was used in the construction of a standard curve.

REFERENCES

1. Saunders, B. C., Holmes-Stedle, A. G. and Stark, B. P. (1964) in *The Properties and Uses of a Versatile Enzyme and of some Related Catalysts.* Butterworths, London.
2. Gaspar, T., Khan, A. A. and Fries, D. (1973) *Plant Physiol.* **51,** 146.
3. Rychter, A. and Lewak, S. (1971) *Phytochemistry* **10,** 2609.
4. Hendricks, S. B. and Taylorson, R. B. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72,** 306.
5. Lewak, H., Asahi, T. and Uritani, I. (1968) in *Biochemical Regulation in Diseased Plants and Injury* (Hivai, T., Hidaki, J. and Uritani, I., eds.) Vol. 189. Phytopathological Society of Japan.
6. Gosling, P. G. and Ross, J. D. (1980). *Planta* **148,** 362.
7. Lavee, S. and Galston, A. W. (1968) *Am. J. Bot.* **55,** 890.

8. Shannon, L. M., Uritani, I. and Imaseki, H. (1971) *Plant Physiol.* **47,** 493.
9. Stahmann, M. A., Clare, B. G. and Woodbury, W. (1966) *Plant Physiol.* **41,** 1505.
10. Gayler, K. R. and Glasziou, K. T. (1968) *Phytochemistry* **7,** 1247.
11. Scott, K. J., Craigie, J. S. and Smillie, R. M. (1964) *Plant Physiol.* **39,** 323.
12. Farkas, G. L. and Lovrekovich Jr., F. P. (1965) *Phytopathology* **55,** 519.
13. Heydecker, W. (1972) in *Viability in Seeds* (Roberts, E. H., ed.). Chapman and Hall, London.
14. Villiers, T. A. (1974) *Plant Physiol.* **53,** 875.
15. Gosling, P. G. and Ross, J. D. (1979) *Phytochemistry* **18,** 1447.
16. Sedmak, J. J. and Grossberg, S. E. (1977). *Analyt. Biochem.* **79,** 544.