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Enzymatic and Anatomical Changes in Abscission Zone Cells of Apple Fruits Induced by Ethephon

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Abstract. The enhancement of fruit abscission zone formation with ethephon treatment caused an increase in soluble proteins, endo-cellulase, exo-polygalacturonase and peroxidase activities. Exo-cellulase and endo-polygalacturonase did not show any relationship with apple abscission. The separation of cells initiated in the cortex region and progressed towards vascular tissue. Cell separation in the cortex appeared to be due to dissolution of middle lamella but vascular tissues ruptured mechanically.

Cell wall degrading enzymes play an important role in abscission phenomenon. Both cellulases and polygalacturonases have been implicated with the abscission process (Rascio *et al.* 1985). In addition to these enzymes, the peroxidase isoenzymes profile also changes in ethylene-induced abscission zone cells (Poovaiah and Rasmussen 1973). Since most of the earlier studies have been related to leaf abscission phenomenon, in this paper we report some enzymatic and anatomical changes in ethephon-induced pedicel/spur fruit abscission zone cells of apple.

MATERIAL AND METHODS

The present studies were carried out at the experimental orchards of Department of Pomology and Fruit Technology, University of Horticulture and Forestry, Solan (India).

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Induction of Fruit Abscission

Fruit bearing branches of Starking Delicious apple/M.7 were sprayed with ethephon (2 mg ml^{-1}) at mean fruit diameter of 2.06 cm. The untreated trees were sprayed with water only. About 2–5 mm abscission zone segments were removed at various intervals till abscission reached the maximum.

Extraction

One hundred fruits per replication treated with ethephon were selected at random for each interval. For comparison an equal number of untreated fruits was also selected. The excised abscission zone segments thus collected were weighed and added to cold aqueous solution of 12% polyethyleneglycol 4000 and 10.5 mM Na-bisulphite. Enzyme extraction was done according to the method of Hinton and Pressey (1974). The crude enzyme preparations were assayed for soluble protein content (Lowry *et al.* 1951) and later frozen at – 10 °C until required for cell wall degrading enzyme estimations.

Cellulase Assay

Viscosimetric and reductometric assays were performed. The enzyme assay adopted was essentially the same as described by Hinton and Pressey (1974) with some modifications as suggested by Rascio *et al.* (1985). The endo-cellulase activity was determined by measuring per cent loss in viscosity of 0.5% carboxymethylcellulose (CMC) in Na-citrate phosphate buffer (PH 6.0).

The exo-cellulase activity was determined by measuring increase in free reducing sugars present after incubation of the enzyme mixture with 0.2 % solution of CMC over a period of 24 h according to Nelson (1944).

Polygalacturonase Assay

Exo- and endo-polygalacturonase activity was determined according to the method of Rascio *et al.* (1985). Substrate used for PG was 0.5% (m/v) solution of pectic acid.

Peroxidase Assay

Peroxidase activity was estimated by the method of Kar and Mishra (1976) with some modifications (Kumar and Khan 1982). Assay mixture for peroxidases contained 2 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O and 1 ml of 1 : 10 diluted enzyme source. Reaction was stopped by adding 1 ml of 2.5 N H_2SO_4 after 5 min of incubation at 25 °C. The amount of purpurogallin formed was determined by

measuring absorbance at 420 nm. Enzyme activity was expressed in absorbance units.

Anatomical Studies

Tissues were fixed in formaldehyde-acetic acid-alcohol (FAA), dehydrated through TBA and embedded in paraffin wax (Johanson 1940). The sections of 10–20 μm thickness were cut with an Erma rotary microtome. Light microscopy was performed on sections stained with safranin-fastgreen or iron-hematoxylin.

RESULTS AND DISCUSSION

Application of ethephon (2 mg ml^{-1}) to Starking Delicious apples resulted in fruit abscission (Fig. 1). Abscission of fruits started one day after ethephon treatment reaching maximum (78.4 %) after 11 d of its application. During the experimental period abscission in untreated trees remained very low (5.5%).

Soluble Proteins

Activation of the pedicel/spur abscission zone was related to an increase in soluble protein content (Fig. 2a). Ethephon-induced abscission zone cells contained higher soluble protein levels over that of untreated controls at each interval. A similar phenomenon has been observed by Abeles *et al.* (1979) and Lewis and Varner

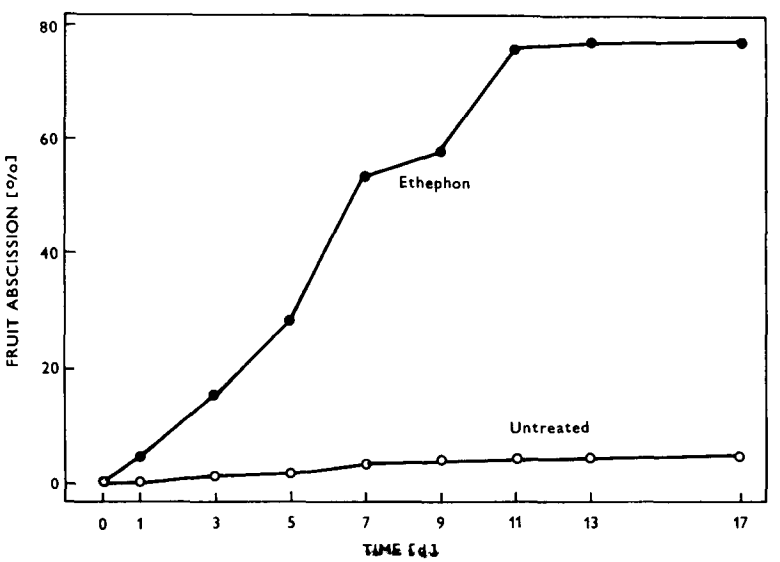

Fig. 1. Effect of ethephon on per cent fruit abscission.

(1970) in peas, who reported a concurrent increase in RNA and protein synthesis with ethylene treatment. The increase in soluble proteins in the present studies appears to be related to increase in hydrolytic enzymes responsible for cell wall degradation.

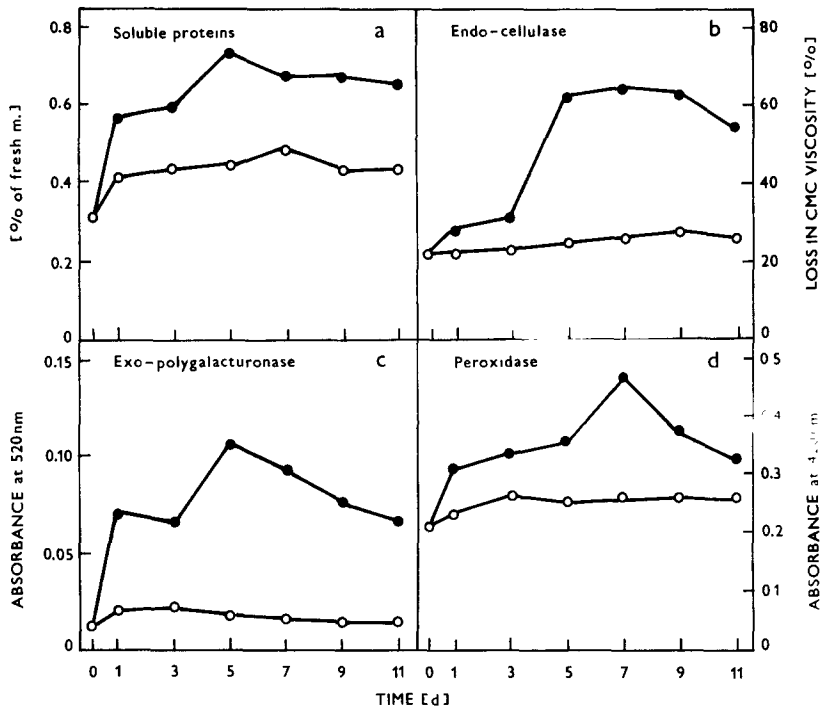

Fig. 2. Effect of ethephon(●)on soluble protein and enzyme activities in apple fruit abscission zone cells. ○–control.

Cellulases

Both endo-and exo-cellulases were present in fruit abscission zones. The endoform appears to be more related to abscission phenomenon as a parallelism was established between activation of the abscission zone and an increase in endo-cellulase activity (Fig. 2b). No difference was recorded in exo-cellulase activity between treated and untreated abscission zones throughout the experimental period (data not shown). Substantial increase in grapefruit was reported in cellulases, the enzyme responsible for cell wall degradation during abscission (Oliva *et al.* 1985). An increased endo-cellulase activity was associated with activation of abscission zone cells of peaches induced by ethephon and embryoctomy (Rascio *et al.* 1985).

Polygalacturonases

Pressey and Avants (1973) extracted both exo- and endo-polygalacturonases from higher plants. Polygalacturonases localized in apple abscission zone were found to be exo-hydrolases (Fig. 2c). An increased exo-polygalacturonase activity was found in ethylene induced fruit abscission zones. Endo-polygalacturonases were absent in fruit abscission zones as no loss in viscosity was recorded over a period of 24 h. Huberman and Goren (1979) also found higher polygalacturonase activity in ethylene-induced abscission zone cells of organges.

Peroxidases

The present data indicated that ethylene treatment increased peroxidase activity in abscission zone cells over that of untreated control (Fig. 2d). Wittemnack and Bukovac (1975) in cherry and Gaspar *et al.* (1978) in citrus noted greater activity of peroxidase enzyme in abscission zone. Peroxidases may be involved in abscission by reducing IAA levels in the abscission zone.

Anatomical Observations

A clearly defined abscission zone was present externally at the pedicel/spur junction. The abscission zone consisted of the distal region with compact and darkly stained cells (Fig. 3). The dissolution of cells started in the cortex (Fig. 4a) and spread to the vascular tissue (Fig. 4b). The final separation was achieved by mechanical rupturing of the vascular strands leading to complete separation of cells between the pedicel and spur (Fig. 5). Similar observations were obtained by McCown (1943) in apples and Wittenback (1970) in cherries.

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Figs. 3–5 at the end of the issue

Moore, T. C.: Biochemistry and Physiology of Plant Hormones, Second Edition. – Springer Verlag, New York – Heidelberg–Berlin–London–Paris–Tokyo–Hong–Kong 1989. 330 pp. Hardcover DM 98.–.

Second edition of Thomas C. Moore's well known textbook on phytohormones appears just 10 years after the first one. The textbook clearly reflexes author's experience in teaching: it is very well, didactically arranged and the text is supplemented with many (176) carefully chosen figures. The book consists of the following chapters: Introduction, Auxins, Gibberellins, Cytokinins, Abscisic acid and related compounds, Ethylene, Brassinosteroids, Phytochrome.

The Introduction is very general, reviewing basic terms and growth kinetics of cells, tissues, organs and whole plants. All chapters dealing with hormones have a similar arrangement covering the history of discovery, basic terminology, chemistry, metabolism, physiological effects, and mechanism of action. The textbook was updated in comparison with the first edition. Maybe I would expect more data on hormone receptors, practical use and in places more of the newest data on the mechanism of action. But the author himself explains in the Preface, why he preferred to ommit some of them.

The second edition of T. C. Moore's textbook is as good as the first one and the students in the field are highly recommended to use it as a basic source of information on phytohormones.

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