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BARD

FINAL REPORT

PROJECT NO. IS-1111-86

Hormonal Regulation of Cell Wall Metabolism During Fruit Ripening

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Date: Dec 1991

BARD Project No. I-IIII-86

Title

HORMONAL REGULATION OF CELL WALL METABOLISM DURING

FRUIT RIPENING.

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Project's starting date: August 1987

Type of Report: 1st Annual _____ 2nd Annual _____ Final x

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630.72
BAR/BEN : 635.64 : 634.13 : 634.45 : 581.134.1 :
581.192.7 : 581.875 : 581.13
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TABLE OF CONTENTS

Abstract	2
Objectives of the original research proposal	4
Body of the report	5
A. Cell wall synthesis in ripening fruit.	
1. Analysis of cell wall synthesis in tomato pericarp tissue.	6
2. The effect of gibberellic acid.	12
B. Interaction of gibberellin and ethylene.	25
1. Pear cell cultures.	25
2. Persimmon fruits.	45
C. Cell wall degradation.	52
1. Tomato pericarp tissue.	
2. Persimmon fruits.	89
D. Gibberellin application in persimmon cultivation.	111
Description of Cooperation	135
Evaluation of Research Achievements	135
List of Publications	136

ABSTRACT

Hormonal regulation of cell wall metabolism during fruit ripening was studied from two aspects : polysaccharide synthesis and polysaccharide degradation. The systems used were at three levels: cell cultures derived from pear fruits, tomato pericarp tissue and intact persimmon fruits. The hormones studied were gibberellin (GA_3) and ethylene. Ethylene enhanced softening, gibberellin inhibited and delayed softening. The ethylene effect was studied only as it related to gibberellin. In growing and quiescent pear cell suspensions, both basal and ACC-induced ethylene production were inhibited by GA_3 , which also inhibited growth and accelerated their loss of viability. In tomato pericarp disks, treated at the green-mature stage, GA_3 inhibited certain aspects of ripening, including softening, but the effect on ethylene production was inconclusive, and still needs to be clarified. In persimmon fruits, GA_3 delayed fruit softening and the onset of the climacteric rise in respiration and ethylene evolution. Moreover, GA_3 -treated fruits were less sensitive to ethylene-induced softening.

The ripening of tomato pericarp disks was found to be accompanied by continuous polysaccharide biosynthesis, but the nature of the synthesis changed. Specific changes in wall biosynthesis noted include increased incorporation of xylosyl and mannosyl residues into hemicellulosic cell wall fractions as the disks mature and decreased incorporation of galactosyl into pectins. The response to GA_3 is rapid and most pronounced 24 h after application, showing a decrease in xylosyl incorporation, but increased incorporation into cellulose, with a transient rise

in the content of this fraction. These effects became less prominent at later stages of ripening.

An increased cellulose content was observed in GA₃-treated persimmon fruits at harvest, even though there was no significant difference in firmness at this time. Fruit softening was accompanied by increased activity of PG and of CMCase. The results of PG activity were observed in the increased soluble pectin content and decreased insoluble pectin of the cell wall material. The substrate for CMCase activity is not yet obvious. In GA₃-treated fruits, both hydrolytic activities were inhibited but in a different manner. The increase in PG activity was only delayed, whereas CMCase activity was inhibited even in soft fruit.

Attempts have been made to improve the efficacy of GA₃ treatment in persimmon orchards, by studying application techniques, with some success.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

The principal objective of the proposal was to examine the possible control of cell wall metabolism and tissue firmness in ripening fruits by gibberellin and ethylene. The term "cell wall metabolism" included synthesis and degradation of cell wall polysaccharides. The results of the research were expected to lead to a clearer understanding of the control of cell wall metabolism and tissue firmness in ripening fruits and to contribute to a wider and more educated application of plant hormones in the field of postharvest fruit conservation.

BODY OF THE REPORT

1. Plant Material

The research plan was to screen a number of fruits and to choose those which showed the clearest influences of gibberellins and ethylene on ripening. The results of preliminary trials with pears and apricots were inconclusive, and therefore were not continued and are not reported in detail. The body of the work was conducted with tomatoes and persimmons. However, cell suspension cultures prepared from pear fruit tissues were very responsive to gibberellin, and this response was studied and is reported.

2. Calcium compartmentation

One of the hypotheses proposed to explain the mode of action of the ripening regulating hormones was that they are involved in the binding and release of calcium from the cell wall. It was proposed that this hypothesis be examined by studying changes in compartmentation of calcium under the effect of the two hormones to be studied. Due to the cut in the budget allotted to the proposal, this section of the research plan was deleted, but an assessment was made of the connection between gibberellin and calcium, by comparing the effects of each on ripening and cell wall degradation in tomato pericarp disks.

DESCRIPTION OF COOPERATION

During the first year of the project, Dr. Carl Greve from JML's laboratory visited Israel and aided in setting up the GC for neutral sugar assays. RBA spent 6 months on sabbatical during the second year of the project in JML's laboratory at Davis, working on the tomato pericarp disk system and paid another 2-week visit in the third year to complete the assay of the experiments which had been frozen.

Throughout, there has been an exchange of information and ideas, and close contact has been maintained, chiefly by electronic mail.

EVALUATION OF RESEARCH ACHIEVEMENTS

The research conducted in the framework of this research project has contributed to the understanding of the involvement of cell wall metabolism in ripening and softening fruits. For the first time, biosynthesis of specific carbohydrates during maturation has been demonstrated by use of ^{14}C -labelled glucose. The importance of synthesis of the hemicellulosic fraction in the ripening process is suggested by its inhibition, following treatment with the ripening inhibitor - GA_3 . There are also indications that cellulose is of importance in the ripening process, on the basis of both increased substrate levels and reduced CMCase activity as a result of GA_3 application. These findings are of special interest in light of the recent recognition that pectic solubilisation by PG cannot provide an overall explanation for the softening of ripening fruits.

LIST OF PUBLICATIONS

- Ben-Arie, R., Roisman, Y., Zuthi, Y. and Blumenfeld, A. (1989) Gibberellic acid reduces sensitivity of persimmon fruits to ethylene. In H. Clijsters et al. (eds.) Biochemical and Physiological Aspects of Ethylene Production in Lower and Higher Plants, Kluwer Academic Publishers, pp. 165-171.
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- Campbell, A.D., Huysamer, M., Stotz, H.V., Greve, L.C. and Labavitch, J.M. (1990) Comparison of ripening processes in intact tomato fruit and excised pericarp discs. Plant Physiol. 94:1482-1589.

Greve, L.C. and Labavitch, J.M. (1991) Cell wall metabolism in ripening fruit. V. Analysis of cell wall synthesis in ripening tomato pericarp tissue using a $\text{D}[U-^{13}\text{C}]$ glucose tracer and gas chromatography-mass spectrometry. Plant Physiol. 97 (in press).