FoodInfo Confidential Report No. 182

Regulation of deterioration in harvested asparagus

A report prepared for
New Zealand Asparagus Council

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June 1996

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CROP & FOOD RESEARCH
Mana Kai Rangahau

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1 EXECUTIVE SUMMARY

This report describes progress on the research project supported by both the New Zealand Asparagus Council and the Foundation for Research, Science and Technology.

- cDNA clones that code for essential sucrose-metabolising enzymes that are active in harvested asparagus have been isolated, and the impact of controlled atmosphere storage on the activity of these enzymes has been studied.

- While the texture of asparagus spears deteriorates dramatically within 24 hours of harvest, cell walls continue to be synthesized over this time and spear water content does not change significantly. This suggests that the initial loss of spear firmness is linked to a repartitioning of water.

- Genetic transformation of asparagus has begun by inserting the asparagine synthetase promoter and a 'reporter' gene into cells from asparagus callus tissue. We have collaborated with Canadian researchers on this project.

This year has seen the beginning of a second phase of our asparagus senescence research. Now that we have detailed knowledge of spear physiology after harvest, and have isolated and characterized some of the genes with altered expression after harvest, we have been able to begin antisense gene transformation of asparagus. This is long-term and difficult research, but provides us with a mechanism to test whether altered expression of particular genes after harvest can successfully delay postharvest deterioration.

2 INTRODUCTION

The resale value and market image of fresh asparagus depends on postharvest quality. The long-term goal of our asparagus research programme is to enhance the quality of harvested asparagus by delaying the onset of spear senescence after harvest. To achieve this goal we have aimed firstly to understand the spear physiology to an extent that key processes in the harvest response are identified, and then to use both current and new technological approaches to modify these responses.

Last year we reported on the effects of a postharvest storage treatment that changed the sucrose metabolism of harvested spears, the localization of harvest responses in the very youngest regions of the spear tip, and the isolation of a gene promoter that turns on asparagine synthetase production in harvested spears. We have continued to make progress in our research into sucrose metabolising enzymes as influenced by controlled atmosphere technology, and we also report on the impact of cell wall and turgor changes on the texture of harvested spears and the introduction of antisense gene technology.

3 RESULTS AND DISCUSSION

3.1 Sucrose Metabolism

The sucrose content of asparagus spears is rapidly depleted after harvest. Controlled atmosphere (10% CO_2, 2% O_2) storage at room temperature has been shown to extend the shelf-life of harvested asparagus by up to three days, while also maintaining pre-harvest sucrose levels and repressing the harvest-induced increase in acid invertase (a sucrose metabolising enzyme) activity. This year we tested the effect of varying the O_2 content of controlled atmospheres on the levels of glucose, fructose and sucrose and on invertase and sucrose synthase activity. Our results show that controlled atmospheres delay the normal decline in sugars as well as suppressing the harvest-induced increase in acid invertase activity. When asparagus is transferred from controlled atmospheres into air, there is no residual effect and sugar metabolism again proceeds at its normal rate. Additionally, we have isolated and characterized cDNA clones for acid invertase and sucrose synthase in asparagus. We have found that harvest induces rapid up-regulation of transcripts coding for acid invertase, but not for sucrose synthase.

3.2 Gene Expression

This year we have attempted to genetically transform asparagus cells in culture to test the responsiveness of the asparagine synthetase (AS) gene promoter to sugar levels. The AS gene promoter 'turns on' expression of the AS gene. By inserting this promoter attached to a 'reporter gene' the biochemical factors that influence the promoter can be assessed through the presence of coloured products of the reaction catalyzed by the enzyme encoded by the reporter gene. The electroporation technique to insert the gene constructs into asparagus cells was developed at the University of Laval, Quebec, and requires the application of an electrical current to temporarily disrupt cell membranes. We have had difficulties with the viability of asparagus protoplasts from callus cultures prior to electroporation which has delayed progress with the research. This work is continuing, and we are also continuing our collaboration with the University of Laval group - Mr Richard Moyle, a PhD student supported by our research programme visited the Laval lab, and we have hosted Dr Yves Desjardins in New Zealand for an in-house workshop on asparagus gene transformation. Dr Desjardins met with Mrs Lesley McKeown, Chair, NZ Asparagus Council during his visit here in April.

3.3 *Walls and Membranes*

Cell walls generally provide rigidity and strength in plant tissue, and various membranes can control the water pressure (turgor) within each cell. We have studied cell walls and cell membranes in harvested asparagus spears because softening of the spear tip in asparagus and general flaccidity of the spear influences the visual perception of texture and quality.

We have found that harvested asparagus stored dry, as normal, becomes much more flexible during the first 24 hours after harvest than spears stored in perforated plastic bags or stored with bases immersed in water. The turgor of spears is not necessarily related to total water content, but to the partitioning of water within the spear after harvest. The turgor of bracts of spear tips declines much more rapidly than that of the underlying tissue of the main axis of the spear. In harvested spears, components of the cell wall continue to accumulate over the first 24 hours after harvest, and increased flexibility of the spear does not appear to be related to any major changes in the length of cell wall polymers.