Control of deterioration in harvested asparagus

A report prepared for the
NZ Asparagus Council

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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. Key points of the report are:

- Invertase, rather than sucrose synthase, is the principal sucrose-metabolising enzyme in harvested asparagus.
- Feeding cut spears sugar solutions did not prolong shelf-life.
- Genetically transformed asparagus callus is now growing on antibiotic selection media to separate transformed from untransformed cells.
- Cell walls from asparagus tips can release more galactose than any other sugar, for up to three days after harvest.

Our research combines both underpinning knowledge of the physiological, biochemical and molecular changes that characterise asparagus spear senescence, and the biotechnology that may be used both to understand the impact of these changes on quality and as a future means of delaying postharvest deterioration of asparagus.

2 INTRODUCTION

Postharvest quality contributes significantly to the value and market image of fresh asparagus. Our research is aimed at understanding the interaction of physiological, biochemical and molecular processes occurring after harvest that contribute to deteriorating quality and eventual senescence of the asparagus spear. Control of these processes may extend the period of acceptable spear quality after harvest and thereby enhance market access and competitiveness of New Zealand asparagus.

Last year we reported on the influence of controlled atmospheres on sucrose-metabolising enzymes, the impact of cell wall and turgor changes on the texture of harvested spears, and the introduction of antisense gene technology into research strategy. This year we have built on these research themes by:

1) characterising the activity of sucrose-metabolising enzymes, invertase and sucrose synthase, and assessing the effectiveness of feeding spears sugar solutions;

2) using a gene gun to insert an antisense asparagine synthetase gene construct into asparagus callus; and

3) quantifying the capacity of asparagus cell walls to release galactose after harvest.

3 EXPERIMENTAL RESULTS

3.1 Sucrose Metabolism

We have found that invertase, rather than sucrose synthase, is the principal sucrose-metabolising enzyme in harvested asparagus, and activity data suggest that the increased invertase activity after harvest is a response to rapidly reduced sugar levels in the spear tip, rather than being the initial cause of this reduction.

Increasing the internal levels of sugar by feeding spears sucrose solutions did not prolong spear shelf-life noticeably, but did slightly increase the rate of accumulation of free amino acids after harvest (Fig. 1), while having no effect on the accumulation of asparagine (Fig. 2). Similar results were found after feeding spears mannitol solutions (as an osmotic control) which suggests that the increased accumulation of amino acids was simply a response to equalise the osmotic effects of the feeding solutions.

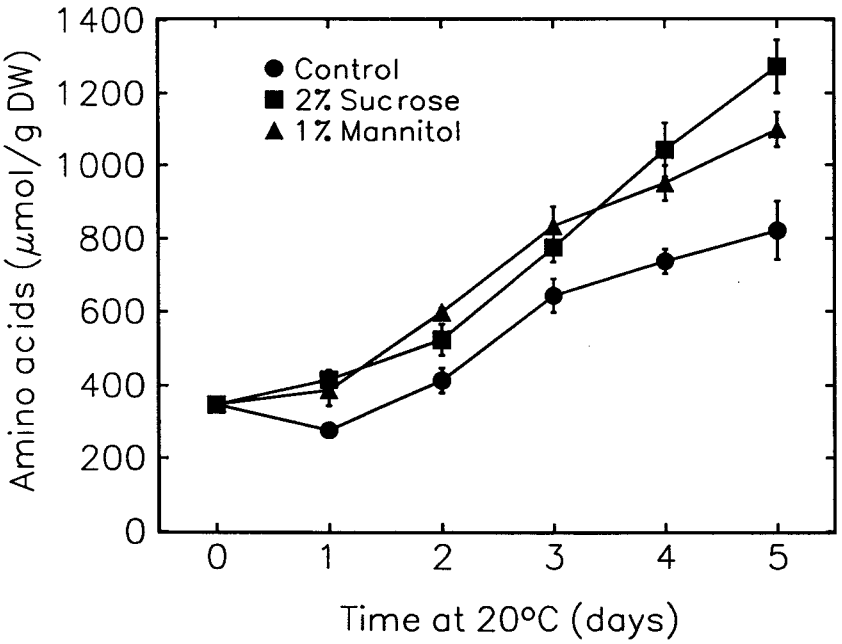

Figure 1 Effect of sucrose feeding on the total free amino acid content of spear tips.

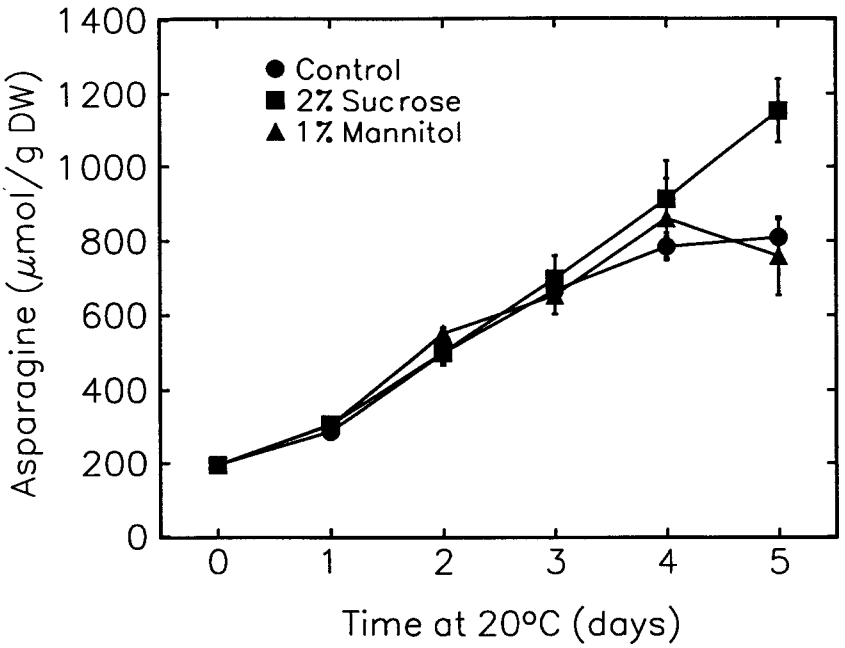

Figure 2 Effect of sucrose feeding on the asparagine content of spear tips.

3.2 Gene transformation

Asparagus has been genetically transformed by direct-shooting DNA into callus tissue using a particle gun. This year we have optimised transformation parameters such as shooting pressure and DNA quantity, and have assessed the effectiveness of selection marker genes and selection media. Clone X, one of the new clonal varieties developed through the Asparagus SuperClone Project has been used for these experiments.

The first of our asparagus genetic transformation experiments is aimed at obtaining stable transformed asparagus tissue with decreased asparagine synthetase. RNA transcripts coding for asparagine synthetase are up-regulated within 2 hours of spear harvest. The DNA that codes for this enzyme has been inserted into asparagus callus in the reverse (antisense) orientation, with the aim that spears generated from the callus will not be able to synthesize the asparagine synthetase enzyme after harvest. The transgenic callus is now growing on antibiotic selection media to separate transformed from untransformed cells. Transformation of asparagus is a long-term project because we can only assess the effectiveness of lowering asparagine synthetase activity (and consequently lowering asparagine production) on the postharvest quality of transgenic spears from mature established, asparagus plants.

3.3 Galactose metabolism

β-Galactosidase is an enzyme that removes single sugar units of galactose from cell wall polymers. Our previous work has shown that expression of the gene coding for this enzyme is up-regulated within 2 hours of spear harvest, and that β-galactosidase enzyme activity increases, especially in the bracts/buds and mid region of the spear, after harvest.

We have proposed that free galactose resulting from β-galactosidase activity may be used by the spear to supplement respiratory substrate. To test aspects of this hypothesis we have quantified and characterised the sugars that can be released from asparagus cell walls due to the activity of hydrolytic enzymes already present in the walls, and we have investigated whether the release of sugars changes after harvest.

The release of single sugars was greatest in the bracts, buds and tip regions of the spear, and tended to increase after harvest. Our results show that asparagus cell walls released much higher levels of galactose than any other neutral sugar present in the wall at harvest, but as the proportion of galactose in the wall declines after harvest, the quantity of galactose released also declines. We conclude that there are two factors that balance the release of galactose from cell walls - the level of galactosidase activity, and the quantity of galactose incorporated into wall polymers.