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Sugar metabolism in 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 (NZAC) and the Foundation for Research, Science and Technology. The long-term aim of our research is to identify physiological, biochemical and molecular processes that cause deterioration in harvested asparagus. This year we have focused on sugar metabolism. Key points of the report are:

- β-galactosidase activity in zones of growing spears and in senescing asparagus fern indicates that galactose mobilisation accompanies growth and natural senescence;
- Galactose-metabolising enzymes are present in asparagus spears after harvest, suggesting that the galactose released from the cell walls of the spear by galactosidase could be used as an energy source for the spear after harvest;
- Controlled atmosphere storage of asparagus causes a transient increase in sucrose synthase gene expression.

Our research programme, which receives supplementary funding from the NZAC, combines experience in postharvest storage and handling with biochemical and molecular research. This interdisciplinary approach allows us to link experimental results to practical applications.

2 INTRODUCTION

The role of soluble sugars in asparagus after harvest is an exciting avenue for postharvest research, and our work reflects a strong international interest in this area. Soluble sugars are important as nutrient/energy sources and also appear to be used by plant cells as signals monitoring energy status during normal growth and development. Loss of sugar (glucose, fructose and sucrose), as happens in asparagus tips after harvest, results in a change in the signalling system thereby instigating effects well beyond that of loss of nutrient.

Last year we reported on the activity of sucrose metabolising enzymes and the effectiveness of feeding sugar directly to asparagus spears. This year we report on further developments in sugar metabolism research. While glucose, fructose and sucrose levels decline in asparagus spears after harvest, soluble galactose is retrieved from the cell wall. We have investigated if this also occurs during growth and natural senescence, and whether the retrieved galactose can be metabolised as an alternative energy source. Controlled atmosphere storage changes the activity of sugar metabolising enzymes (e.g. acid invertase) and we have investigated whether this effect is due to changed gene expression for these enzymes.

3 EXPERIMENTAL RESULTS

3.1 β-galactosidase activity during normal growth and natural senescence

The activity of β-galactosidase, which removes galactose residues from cell wall polymers, increases in asparagus after harvest. Increases in galactosidase activity are also found in the cell walls of ripening fruit, senescing flower petals, and parts of plants that are undergoing rapid growth. We were interested to find out whether galactosidase activity accompanied the rapid pre-harvest growth of asparagus and whether its postharvest increase reflected a role in senescence.

3.1.1 Normal growth

We measured the galactosidase activity in zones of spears at 6, 10 and 16 cm in height above the soil level. Each zone was 3 cm and was taken from regions at the spear tip, tissue immediately below the tip, and the base of each spear (see Fig. 1).

β-galactosidase activity was present in all zones in growing spears (see Fig. 2.). Activity is higher in the young tissue in the upper part of the spear than in the older tissue at the base, being probably associated with growth-related cell wall expansion in that region. The activity within the A zone remains fairly constant as the spear grows, but the activity in the B zone (where extension is occurring) increases slightly. From our previous results from spears after harvest, β-galactosidase activity associated with growing is lower than the postharvest activity.

3.1.2 Fern senescence

We sampled the senescing cladophylls from asparagus fern as it senesced. Fern senescence stages were based on cladophyll colour, with the colour ranging from dark green, half yellow, fully yellow, deep gold, and finally to brown. These samples were taken from labelled plants, over a 4 week period in March/April 1997. Total β-galactosidase activity (assayed with buffer and salt) increased in the dried samples as yellow colour appeared on the cladophylls (see Fig. 3). Activity reached a peak as the cladophylls turned a deep gold and then declined as the cladophylls became brown and shrivelled. Activity was reduced when the assay was run without salt in the buffer, but a pattern of increasing activity was still seen. The increased activity with salt indicates that an increased proportion of the enzyme was bound to the cell wall. We have also seen similar salt-related changes in β-galactosidase activity in harvested spears.

Conclusions

From these results we can conclude that galactosidase activity accompanies growth as well as senesence during the life-span of asparagus. The harvest-induced increase in activity is not simply a carry-over from growth because growth ceases at harvest, and is more in line with a senescence response since activity also increases in senescing fern.

3.2 Galactose metabolism

We have little information regarding the fate of galactose released from cell walls by β-galactosidase after spear harvest. One hypothesis is that the galactose is used as an energy source in place of the depleted glucose, fructose and sucrose supplies. If this is to occur, there must be enzymes present that convert galactose into a useable form.

We assayed the harvest-related activity of three enzymes (galactokinase, UDP-galactose pyrophosphorylase and UDP-galactose-4′-epimerase) that act sequentially to convert galactose to a form that can be used for energy. UDP-galactose-4′-epimerase activity increased for up to four days after harvest (Fig. 4). UDP-galactose pyrophosphorylase activity declined over this period although it was still present in physiologically relevant quantities. We were unable to find conclusive evidence of galactokinase activity; however, given that activities of other essential members of the kinase family are low but highly effective, it is likely that galactokinase is similarly present but hard to detect.

Conclusions

From these results we can conclude that asparagus cells do have the mechanisms in place to utilise galactose as a respiratory substrate. However, we do not have evidence yet to claim that this actually happens in harvested asparagus.

3.3 Effects of controlled atmosphere on gene expression

Controlled atmosphere storage slows down the rapid postharvest loss of sucrose from spear tips and also eliminates the transient rise of acid invertase seen in air-stored tips. However, we don't know whether this effect is due to a direct suppression of enzyme activity or whether it represents a more fundamental effect on gene expression. We intended to test this by storing asparagus continuously in an atmosphere of 5% O_2 and 10% CO_2 and measuring the gene expression patterns for acid invertase using northern analysis. Unfortunately, this has proved more difficult than first thought and we are unable to report on the results for the acid invertase expression at this time. We have, however, completed the same experiment for sucrose synthase. This enzyme can either synthesize or hydrolyse sucrose.

We previously reported ('Regulation of deterioration in harvested asparagus', 1996 FoodInfo Confidential Report No. 182) that sucrose synthase is active at low levels in asparagus stored in air, and that harvest does not affect the expression of sucrose synthase genes. We have now found that there is a strong but transient upregulation of transcripts coding for sucrose synthase in asparagus stored continuously in controlled atmospheres. This upregulation occurs between 3 and 12 hours after storage begins.

Conclusions

We conclude that the controlled atmosphere is provoking a change in gene expression that we have not seen before in air-stored asparagus. Based on this, we predict that there will also be differences in the expression patterns for the acid invertase gene. We will make a supplementary report to the Committee as the information becomes available.

4 DISCUSSION

Harvested spears rapidly become depleted of sucrose. This is a critical loss, since sucrose is important not only as a source of nutrient for energy but also as a component of a complex signalling mechanism that links biochemical processes to gene expression changes. We have found that it is possible for an asparagus spear to use another sugar - galactose - as a postharvest energy source, but whether this actually happens is another matter. Galactose mobilisation is a response that is not limited to postharvest senescence only - it also occurs during asparagus spear growth phases and during natural senescence of the asparagus fern. Therefore there are likely to be factors other than the depleted sugar and energy status of the harvested asparagus motivating galactose liberation.

We are also beginning to dissect the reasons behind the success of controlled atmosphere storage to maintain asparagus quality. We know that this treatment can delay sucrose breakdown in the harvested spears, but now we have evidence that suggests it is having this effect via fundamental changes in gene expression. This is an exciting result and we have plans to pursue it further in our long-term research programme funded by the Foundation for Research, Science and Technology.

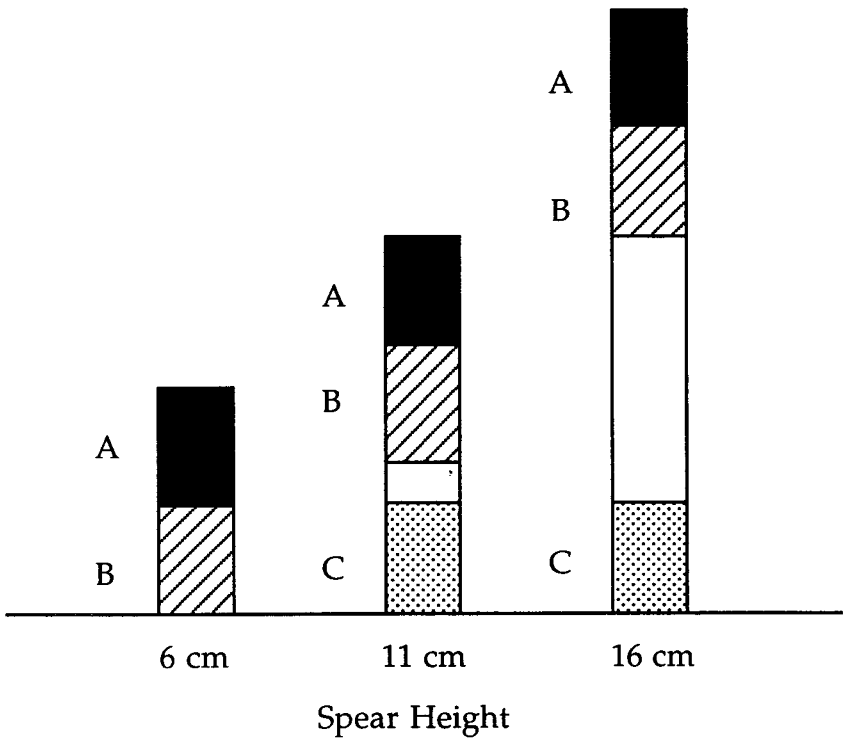

Fig. 1. Selected growing spear zones.

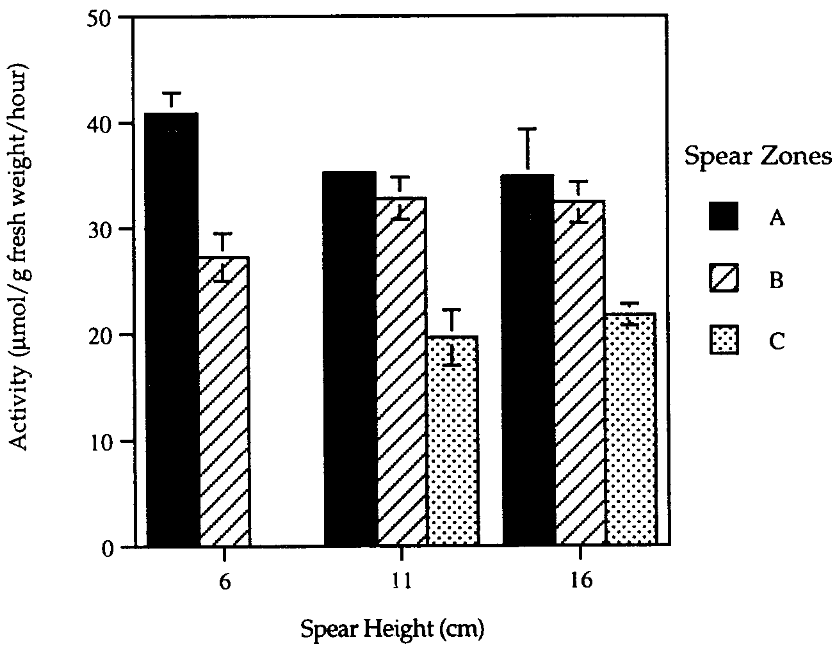

Fig. 2. β-Galactosidase activity in growing spears.

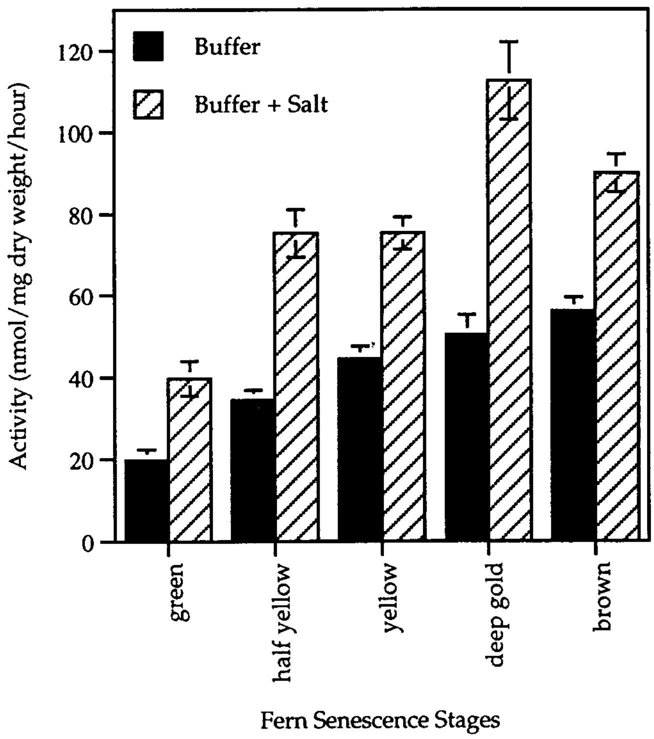

Fig. 3. Activity of β-galactosidase in senescing asparagus fern.

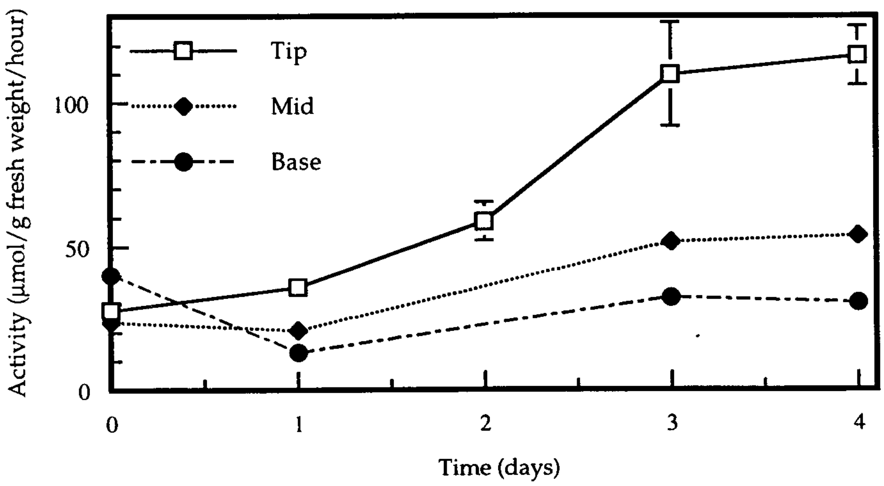

Fig. 4. UDP-galactose-4'-epimerase activity in harvested asparagus.