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Carbohydrate Status and Sucrose Metabolism in Asparagus Roots over an Extended Harvest Season

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Abstract: Changes in soluble sugar content and the activity of acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) in the roots of two cultivars of asparagus (*Asparagus officinalis* L. cvs. Welcome and E414) grown using the mother stalk culture were studied. The primary objective of this study was to understand the effect of long-term harvesting (March-October) on the carbohydrate status of asparagus roots. All the three soluble sugars sucrose, glucose and fructose declined from March to a minimum in May as the new shoots developed. After that root sugar level remained almost unchanged until August and thereafter started to decline again. The activities of both the soluble and cell wall-bound acid invertase increased until May and after that they started to decline. On the other hand, SS and SPS activity decreased to a maximum in May and changed little after that. The activity of acid invertase was higher in 'E414' than 'Welcome' (WC), whereas those of SS and SPS were higher in 'WC' than in 'E414', although there were no significant differences in soluble sugar content in the two cultivars.

Key words: *Asparagus officinalis*, invertase, sucrose synthase, sucrose phosphate synthase, soluble sugars, roots

Introduction

In asparagus the commercial crop (spears) is produced early in the spring and production is largely dependent upon food reserves produced during the previous season and stored in the roots (Pressman *et al.*, 1993). Unlike most vegetable crops, asparagus is a perennial plant and current photosynthesis does not directly contribute to spear quality. Seasonally, newly formed assimilates are first translocated into underground storage roots and then utilized for spear growth. This has led to studies on the changes in root carbohydrate levels throughout the annual growth cycle (Scott *et al.*, 1939; Haynes, 1987 and Shelton and Lacy, 1980). However, these studies were carried out in temperate climates with a limited harvest season (6 to 8 weeks each spring). Neither of these studies monitored the changes that occur over an extended harvest season. In the south western part (warm regions) of Japan, now a days long term harvesting of asparagus has become possible from March to October using mother stalk cultivation method. The basic concept of mother stalk culture is to allow three to five stalks per plant to develop into fern that produces photosynthate, while harvesting of adjacent spears continues for an extended period. Although the mother stalk method allows extending the harvest season, spear quality varies

with harvest month. Our previous experiments indicated that carbohydrate content at harvest in asparagus spears from mother stalk culture were affected by fluctuation of temperature and by the loss of crown storage carbohydrates over the harvest season (Bhowmik *et al.*, 2001). In general, spear quality has changed due to the changes of crown storage carbohydrates necessary for the following-season's spear production and sucrose metabolizing enzymes such as AI, SS and SPS played a major role in cleaving imported sucrose. We also examined whether there is a seasonal effect on spear quality and shelf life linked to mother stalk culture and found both the storage quality and shelf life to decline over the harvest season (Bhowmik *et al.*, 2002).

In the present work we analyzed the carbohydrate composition and the activity of carbohydrate metabolizing enzymes in asparagus storage roots to interpret the effects of long term harvesting on spear quality and crown storage carbohydrate level.

Materials and Methods

Three year old asparagus plants grown in the Agricultural Experiment Station, Miki branch, Kagawa, Japan were used during the 2000 growing season (Bhowmik *et al.*, 2002). The usual cultural practices were employed and

harvest took place at the middle of each month starting from March to October. Roots of six representative plants of each cultivar were sampled, washed and stored at -30°C until sugar and enzyme analysis.

Enzyme extraction and assay was performed as in Bhowmik *et al.* (2001). A 0.2 M citrate-phosphate (C-P) buffer at pH 5.0 for soluble acid invertase and 0.2 M NaCl C-P buffer at the same pH for cell wall-bound acid invertase were used. On the other hand, 0.2 M potassium-phosphate (K-P) buffer at pH 7.8 containing 10 mM ascorbate, 15 mM MgCl₂, 1 mM EDTA and 1mM dithiothreitol (DTT) was used for the extraction of sucrose synthase (SS) and sucrose phosphate synthase (SPS). The amount of reducing sugars was estimated by the method of Somogyi (1952). Soluble protein content was determined by the method of Lowry (Lowry *et al.*, 1951) using bovine serum albumin as the standard. The enzyme activity was expressed as the amount of glucose produced per min per mg of protein. Soluble sugars, sucrose, glucose and fructose were determined by HPLC, as previously reported (Bhowmik *et al.*, 2001).

A randomized complete block design was adopted with three replications. The level of significance was calculated from the *F* value of ANOVA. The relationship between sugars and enzyme activities were described with linear correlation analysis using mean values.

Results and Discussion

The present study shows that sucrose was the major soluble carbohydrate in asparagus root and marked changes occurred in sucrose metabolism over the extended harvest season. From March to May, there was a steady decline in soluble sugar contents in both cultivars (Fig. 2). After that sugar contents remained almost unchanged until August before declining from September to October. In late winter and early spring, the emergence and growth of new shoots utilize stored carbohydrates which causes the drop in sugar levels especially during April and May. Wilson *et al.* (1997) reported that carbohydrate content declines steadily during harvest, but especially during periods when fern are produced. Once ferns had developed and carbohydrates began to be translocated downward to the rhizome, the increase in soluble sugar contents proceeds. It happens for the natural growth pattern of asparagus plants in a temperate environment where new shoots emerge in early spring and quickly develop into ferns. But in case of long term harvesting using mother stalk culture sugar levels remain unchanged during harvesting as only three to five stalks per plant were allowed to develop fern. As the cutting period extended carbohydrate levels started to decline again from September.

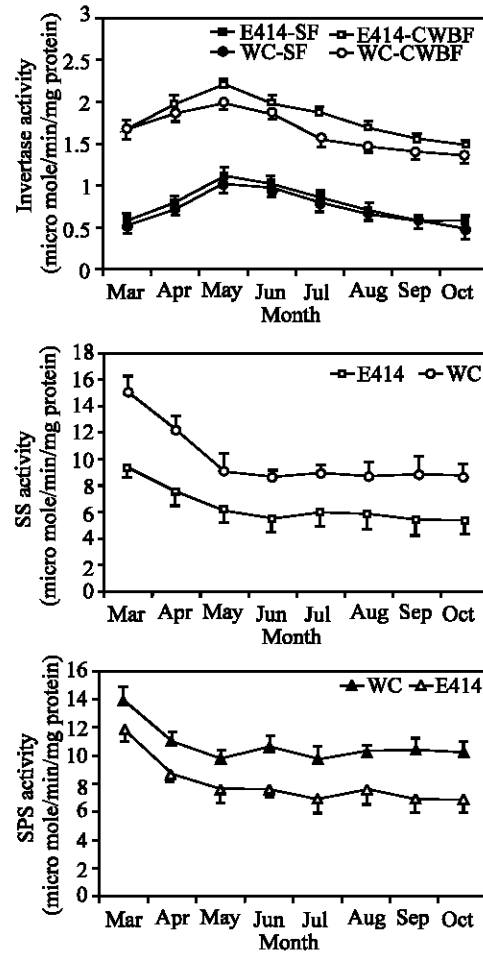


Fig. 1: Changes in enzyme activities in the roots of two asparagus cultivars over an extended harvest season. Each point represents the mean of three replications. Vertical bars indicate S.E.

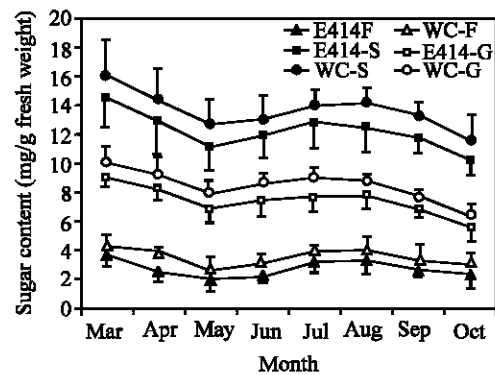


Fig. 2: Changes in soluble sugar content in the roots of two asparagus cultivars over an extended harvest season. Each point represents the mean of three replications. Vertical bars indicate S.E.

Table 1: Correlation coefficients (r) among enzyme activities and sugar contents in the roots of two asparagus cultivars over an extended harvest season

Sugar Contents	Cultivars	Enzyme activity			
		SF	CWBF	SS	SPS
Sucrose	E414	-0.482	-0.731*	0.210	0.123
	Welcome	-0.412	-0.823**	0.321	0.129
Glucose	E414	0.056	0.302	-0.098	-0.087
	Welcome	0.453	0.076	-0.543	-0.345
Fructose	E414	0.797*	0.687*	-0.698*	-0.231
	Welcome	0.143	0.432	-0.987**	-0.788*

SF= Soluble fraction, CWBF= Cell wall-bound fraction, SS= Sucrose synthase, SPS= Sucrose phosphate synthase, * and ** denote significant at $p < 0.05$ and $p > 0.01$ respectively, n=12

Dufault (1999) reported almost the same pattern of changes in carbohydrate levels for summer-forced asparagus but did not interpret in the light of changes in activity of sucrose metabolizing enzymes. The fall in sucrose concentration was correlated well with a increase in invertase activity, seen here and previously (Bhowmik *et al.*, 2002b). In our experiment the activities of both soluble and cell wall-bound acid invertases increased (Fig.1) until May and after that they started to decline. On the other hand, SS and SPS activity decreased to a maximum in May and changed little after that. The highly significant negative correlation (Table 1) between invertase activity and sucrose content indicated that in asparagus root invertase only acts in breakdown of sucrose. The same pattern of invertase activity has also been reported in some other plant tissues such as that of carrots (Ap Rees, 1984).

The results indicated that in case of long term harvesting of asparagus the growth pattern is altered. The more extended the cutting period, the greater will be the depletion of storage carbohydrates and the longer will fern growth be delayed.

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