Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences 6 (3): 470-476, 2007
ISSN 1682-3974

Effects of Mulching on the Activity of Acid Invertase and Sugar Contents in Japanese Radish

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Abstract: The effects of mulching on soil moisture and temperature and sugar metabolism in Japanese radish (*Raphanus sativus* L.) were studied. Soil moisture content and temperature were higher in mulched plots than the none-mulched plots throughout the experimental period. Radish roots grown in plots with mulch were heavier than those without one. Acid invertase activities in soluble fraction (SF) and cell wall-bound fraction (CWBF) of the roots grown in plots with and without mulch showed the same pattern. However, the enzyme activity was higher in roots grown in mulched plots. The acid invertase activity in SF gradually increased during growth and development while no specific inclining or declining pattern was found in CWBF. Mulching did not significantly affect the amount of fructose (Fru) and glucose (Glc) contents during growth and development. However, sucrose (Suc) content in roots grown in plots with mulch was higher than those grown without mulch throughout the experimental period. Fru and Glc contents in the bottom portion of the root were also found to have same pattern as the top portion. Present results suggest that mulching could increase crop yield by producing heavier root weight and improve product quality such as sweetness due to higher Suc content of the roots.

Key words: Daikon, soil moisture, soil temperature, sucrose

INTRODUCTION

Japanese radish (*Raphanus sativus* L.), also known as daikon, oriental radish and winter radish (Stephens, 1994) is a major commercial vegetable grown in Japan. The most important quality components of this root vegetable are sweetness and fresh flavor. Sweetness of horticultural commodities is attributed by tissue composition particularly sugar contents. It has been reported that chemical composition of agricultural commodities is influenced by environmental factors to which the crop is exposed during growth and development. For instance, total available heat and the extent of low and high temperatures are singled out to be important determinant of growth rate and chemical composition of horticultural crops (Lee and Kader, 2000). Temperature, therefore, influences various metabolic processes in the plant system which eventually affects yield and product quality. In field production, however, environmental conditions such as temperature are often unmanageable but have strong implication to crop quality (Weston and Barth, 1997). To modify the underground growing environment, the use of polyethylene film mulch could be employed considering its effects on soil conditions by increasing the temperature and conserving moisture and consequently increasing growth and yield of plants (Teasdale and Abdul-Baki, 1995).

Although there are a lot of metabolic processes affected by temperature, sugar metabolism would be an interesting aspect to elucidate since sweetness which is attributed by sugars is an important product quality. Sugar metabolism involves complex biochemical reactions. In plant tissue, sucrose is synthesized by sucrose phosphate synthase (SPS; EC 2.4.1.14) and degraded by invertase (EC 3.2.1.26) (Coupe *et al*., 2002) and sucrose synthase (SS; EC 2.4.1.13) (Hurst *et al*., 1993). In soybean leaves, lower SPS activity was observed under cool conditions (18/14°C) than under warm conditions (26/22°C) (Rufty *et al*., 1985). In addition, SPS and acid invertase activities in mature leaves of roses were greater in plants grown under higher night temperature than under lower temperature (Khayat and Zieslin, 1987).

To our knowledge, there is no research conducted relating to mulch as a way of altering soil temperature on sugar metabolism in plants. Hence, this study was conducted to investigate the effect of soil temperature as

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influenced by mulch application on acid invertase activity in relation to sugar contents during growth and development of Japanese radish.

MATERIALS AND METHODS

Plant material: This experiment was conducted at the experimental field of the Faculty of Agriculture, Kagawa University from April to June, 2006. Polyethylene film (0.02 mm thick) was used as mulching material. Japanese radish was planted with a plant spacing of 25×50 cm in plots with and without mulch. Japanese radish was randomly harvested from each plot at 40, 50, 60, 70 and 80 days after sowing. The harvested root was divided into two equal halves (top and bottom) after harvest and kept at -30°C until sugar analysis and acid invertase extraction and assay.

Soil temperature and soil moisture determination: Soil temperature was measured by thermocouples (t-type) at 10 cm depth, 10 points per plot at the edge of planting hole. It was measured at 6:00 and 15:00. Soil moisture was determined gravimetrically at 10 cm depth every 10 days after sowing.

Extraction and assay of acid invertase: Enzyme extraction and assay procedure were performed as described by Kassinee *et al*. (2004). Briefly, approximately 5 g of fresh-weight root sample were added with 1% of polyvinylpolypyrrolidone (PVPP) and 1 g sea sand. The mixture was homogenized using a cooled mortar and pestle with 5 mL of 0.2 M citrate-phosphate (C-P) buffer at pH 5.0. The homogenate was then filtered through four layers of cotton cloth and the filtrate was centrifuged at 12,000 x g, at 2°C for 10 min. The total supernatant was dialyzed with 0.2 M C-P buffer (pH 5.0), diluted 40 times for 12 h and the inner solution was designated as Soluble Fraction (SF). The residual tissues were re-extracted with 5 mL of 0.2 M NaCl C-P buffer (pH 5.0) for about 24 h with constant stirring. The supernatant was dialyzed and the solution was designated as cell wall-bound fraction (CWBF). The extraction procedure was carried out at 0-4°C.

The standard assay medium for acid invertase consisted of 0.2 mL of 0.2 M C-P buffer (pH 5.0), 0.1 mL of 0.5 M sucrose, 0.1 mL of distilled water and 0.1 mL of crude enzyme solution. The blank experiment contained distilled water instead of sucrose. The assay mixture was incubated at 45°C for 15 min. After the reaction, the assay mixture was neutralized with 0.1 N NaOH and added with a coloring Somogyi's copper reagent. The mixture was heated for 10 min in boiling water. The amount of reducing sugars was estimated by the method of Somogyi (1952). Soluble protein content was determined by Lowry method (Lowry *et al*., 1951) using bovine serum albumin as the standard. The enzyme activity was expressed as the amount of glucose produced per minute per milligram of protein.

Carbohydrate determination: Approximately 2 g of fresh-weight root sample was mixed with 1 g sea sand and homogenized in a cooled mortar and pestle. Ten milliliter of distilled water was added to the homogenate and centrifuged at 12,000 x g, at 2°C for 10 min. The mixture was filtered through a cellulose nitrate membrane filter (0.2 μm pore size). Soluble sugars were analyzed by HPLC having a stainless steel column of 10.7 mm ID × 30.0 cm packed with silica gel (gel pack C610). The mobile phase (filtered water) was pumped through the column at a flow rate of 1.0 mL min^{-1}. The pressure was adjusted to 14-15 kg cm^{-2} and the temperature to 60°C. Sucrose, glucose and fructose were identified by their retention times and were quantified according to standards.

Data analysis: A completely randomized design with three replications was adopted. The level of significance was calculated from the F-value of ANOVA. The relationships between sugars and enzyme activity were described with linear correlation analysis.

RESULTS

Soil moisture content and temperature: Soil moisture content in plots with mulch was higher than those without mulch (Fig. 1A). Soil temperatures in both mulched and none-mulched plots increased throughout the experimental period. However, soil temperature in mulched plots was higher than the plots without mulch (Fig. 1B).

Root weight: The root weight increased gradually throughout the experimental period (Fig. 2). This pattern was found in both plots with and without mulch. However, root weight of plants grown in the plots with mulch was higher than those grown in none-mulched plots.

Acid invertase activity: The acid invertase activity in soluble fraction (SF) gradually increased during growth and development until the end of the experimental period while no specific inclining or declining pattern was found in cell wall-bound fraction (CWBF). The same patterns of enzyme activity in SF and CWBF were found in roots grown in both none-mulched and mulched plots (Fig. 3A).

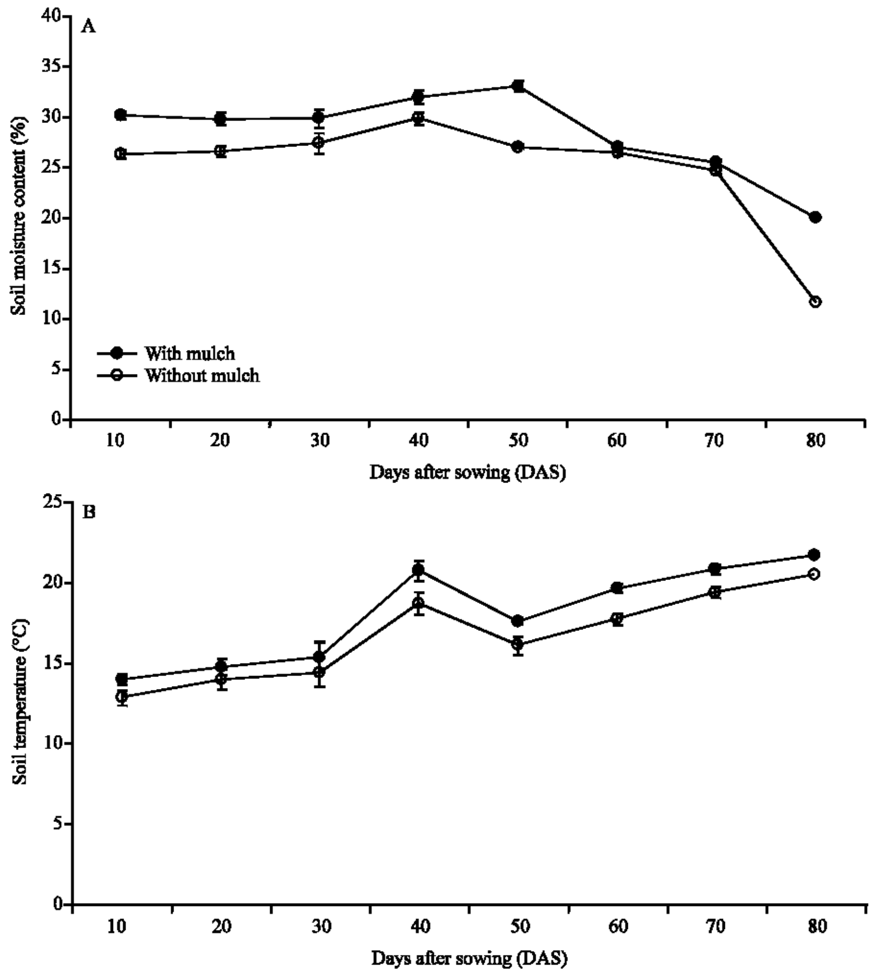

Fig. 1: Average soil moisture content and temperature taken every 10 days after sowing in mulched and none-mulched plots from April to June 2006. Vertical bars indicate SE. SE bars were not shown under masked by the graph symbols. A: Moisture content, B: Temperature

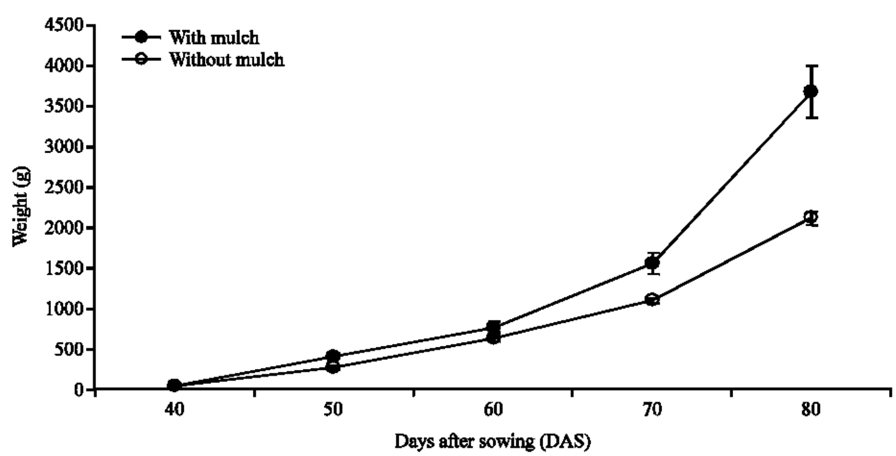

Fig. 2: Root weight of Japanese radish during growth and development. Each point represents the mean of 3 replications. Vertical bars indicate SE. SE bars were not shown under masked by the graph symbols

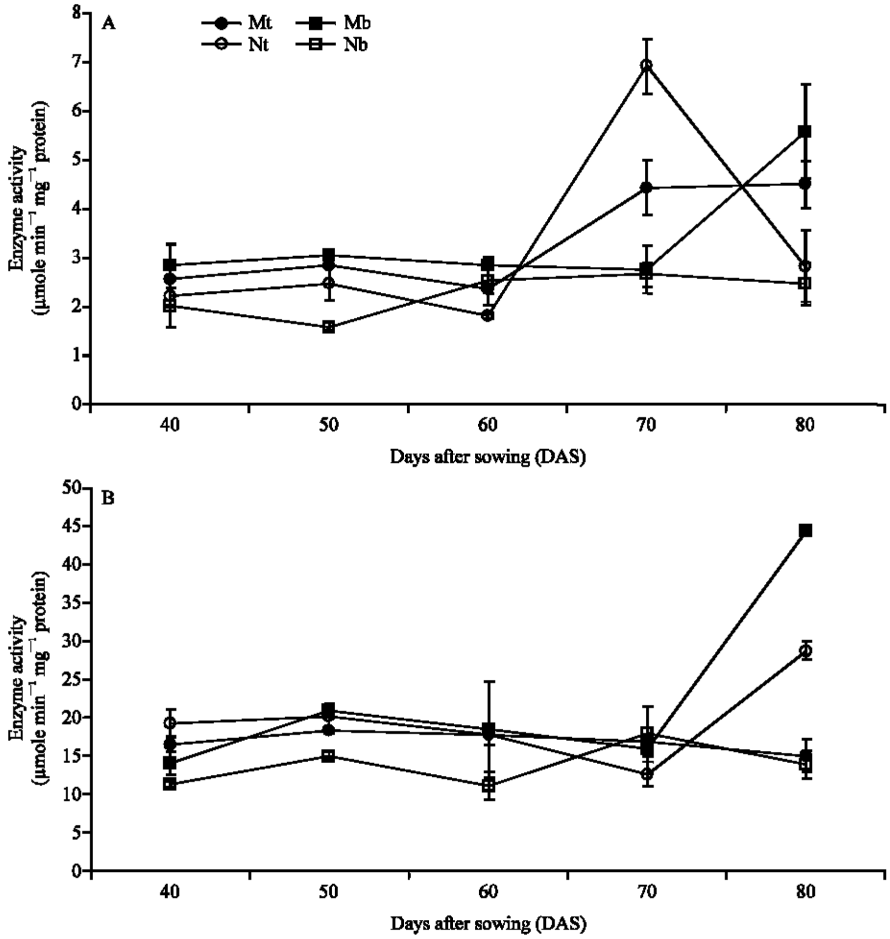

Fig. 3: Changes in acid invertase activity in the soluble fraction (SF, A) and cell wall-bound fraction (CWBF, B) of both portions of Japanese radish roots during growth and development. Each point represents the mean of 3 replications. Vertical bars indicate SE. Legend: Mt-top portion with mulch, Mb-bottom portion with mulch, Nt-top portion without mulch and Nb-bottom portion without mulch

Comparing the two portions, higher enzyme activity was found in the bottom portion of roots grown with mulch than that in the top portion. On the other hand, in the none-mulched plot, the enzyme activity in the bottom portion was lower than that in the top portion. Acid invertase activity in CWBF was highest at 80 DAS in the bottom portion of roots grown in plot with mulch. Acid invertase activity in CWBF increased in Mb (Bottom with mulch) and Nt (Top without mulch) throughout the growing period while there was no specific inclining or declining trends observe in Mt (Top with mulch) and Nb (Bottom without mulch) (Fig. 3B). Acid invertase activity in roots of each treatment was not significantly different at 40 DAS. However, the enzyme activity was high at 50, 60 and 80 DAS in SF of Mb. The enzyme activity in CWBF was higher than SF (Table 1).

Table 1: Effects of mulching on invertase activity during growth and development of Japanese radish

Acid invertase	Plot	Days after sowing (DAS) 40	50	60	70	80
SF	Mt	2.55ns	2.85ab	2.36ab	4.42b	4.49b
	Mb	2.83ns	3.05a	2.84a	2.76cd	5.57a
	Nt	2.20ns	2.48b	1.80b	6.90a	2.82b
	Nb	2.01ns	1.58c	2.52ab	2.66d	2.47b
CWBF	Mt	16.45ab	18.23a	17.77ns	16.76ns	15.02cd
	Mb	13.94b	20.93a	18.41ns	15.94ns	44.35a
	Nt	19.22a	20.06a	17.80ns	12.60ns	28.66b
	Nb	11.24b	14.97b	11.05ns	17.87ns	13.92d

SF: Soluble fraction; CWBF: Cell wall bound fraction; Mt: Top portion with mulch; Mb: Bottom portion with mulch; Nt: Top portion without mulch; Nb: Bottom portion without mulch; ns: Not significant at 5% level; Means followed by the same letter(s) are not significantly different

Soluble sugar contents: Fructose (Fru) and glucose (Glc) contents in roots grown in mulched plots were lower than those grown without mulch throughout the growing period except at 40 DAS. On the other hand, sucrose (Suc) content in the roots grown in plots with mulch was higher than those grown in the plots without mulch throughout

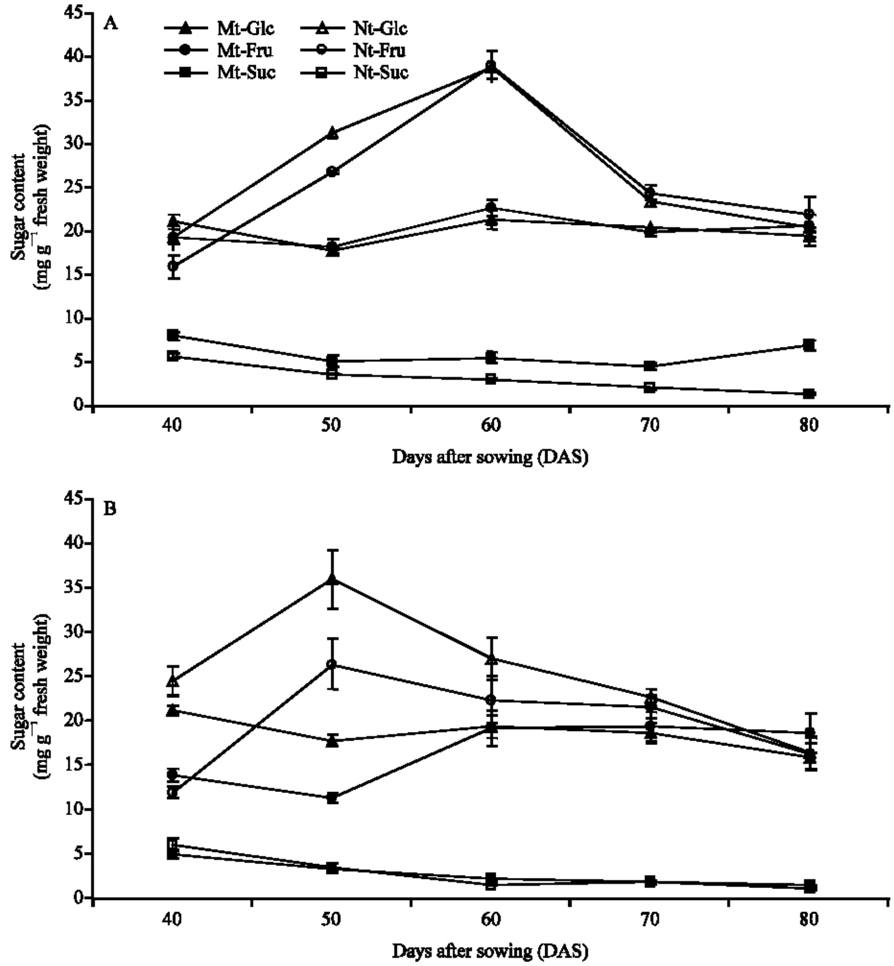

Fig. 4: Changes in soluble sugar contents in both portions of Japanese radish roots during growth and development. Each point represents the mean of 3 replications. Vertical bars indicate SE. Legend: Mt-top portion with mulch, Mb-bottom portion with mulch, Nt-top portion without mulch and Nb-bottom portion without mulch, Glc-glucose, Fru-fructose, Suc-sucrose

the experimental period (Fig. 4A). In general, the amount of Fru and Glc was increasing from 40 to 60 DAS. Thereafter, the amount of Fru and Glc decreased until 80 DAS particularly in none-mulched plots. In bottom portion, Fru and Glc contents in roots grown in none-mulched plots gradually decreased from 50 DAS until the end of the experimental period while no specific inclining or declining pattern was found in those grown in mulched plot (Fig. 4B). There was no significant difference observed in the amount of Fru and Glc in the top and bottom portions of the roots. However, the amount of Suc content was significantly higher in the top than bottom portions in mulched plot at 70 and 80 DAS, while there was no significant difference in none-mulched plot (Table 2).

Table 2: Effects of mulching on sugar contents during growth and development of Japanese radish

Sugar content	Plot	Days after sowing (DAS) 40	50	60	70	80
Glc	Mt	21.15ns	17.70ns	21.32ns	20.35ns	19.39ns
	Mb	21.18ns	17.73ns	19.32ns	18.56ns	15.88ns
	Nt	19.28ns	21.25ns	38.65ns	23.30ns	20.27ns
	Nb	24.40ns	35.88ns	26.97ns	22.56ns	19.38ns
Fru	Mt	19.29ns	18.18ns	22.59ns	19.85ns	20.62ns
	Mb	13.85ns	11.27ns	19.14ns	19.24ns	18.56ns
	Nt	15.86ns	26.67ns	38.90ns	24.34ns	21.88ns
	Nb	11.89ns	26.32ns	22.28ns	21.50ns	16.26ns
Suc	Mt	7.94ns	5.05ns	5.48ns	4.55a	6.85a
	Mb	5.00ns	3.02ns	2.13ns	1.89b	1.05b
	Nt	5.65ns	3.62ns	3.05ns	1.96b	1.38b
	Nb	5.98ns	3.52ns	1.52ns	1.87b	1.37b

Mt: Top portion with mulch; Mb: Bottom portion with mulch; Nt: Top portion without mulch; Nb: Bottom portion without mulch; ns: Not significant at 5% level; Means followed by the same letter(s) are not significantly different

Table 3: Correlation coefficient (r) values between acid invertase activity and sugar contents of two portions of Japanese radish roots during growth and development

		Correlation coefficient value			
		Acid invertase			
Sugar content	Plot	SF		CWBF	
Glc	Mt	-0.226	ns	-0.179	ns
	Mb	-0.785	**	-0.855	**
	Nt	-0.226	ns	-0.295	ns
	Nb	-0.792	**	-0.025	ns
Fru	Mt	-0.078	ns	-0.117	ns
	Mb	0.260	ns	0.262	ns
	Nt	-0.182	ns	-0.226	ns
	Nb	-0.188	ns	-0.433	ns
Suc	Mt	-0.198	ns	-0.572	*
	Mb	-0.570	*	-0.651	**
	Nt	-0.465	ns	-0.214	ns
	Nb	-0.658	**	-0.351	ns

Mt: Top portion with mulch; Mb: Bottom portion with mulch; Nt: Top portion without mulch; Nb: Bottom portion without mulch; *, ** Denote significant correlation at $p<0.05$ and $p<0.01$, respectively, ns: No significantly difference, n = 18

Correlation coefficient (r) between acid invertase activity and sugar contents: There was significant negative correlation observed between the acid invertase activity in both SF and CWBF and sugar contents (Suc and Glc) in the bottom portion of the roots grown in the plots with and without mulch except in CWBF under none-mulched plots (Table 3). On the other hand, there was no correlation observed between Fru and acid invertase activity in both top and bottom portions. In CWBF, Suc and Glc were negatively correlated with acid invertase in the roots grown in mulched plots, while this correlation was not found in roots grown in none-mulched plots. No correlation was observed between Fru and acid invertase activity in roots grown in both mulched and none-mulched plots.

DISCUSSION

Mulching with black polyethylene plastic conserved soil moisture content and increased soil temperature. This could be due to high short wave absorbance (black plastics) or transmittance coupled with low long wave transmittance (Ham *et al.*, 1993; Teasdale and Abdul-Baki, 1995) of the mulching material used. In addition, the black polyethylene plastic prevented the flow of evaporation from coming out of the plot (Begum *et al.*, 2001). This soil condition favored the growth of roots as indicated by higher root weight in plants grown in plots with mulch than those without mulch throughout the growing period (Fig. 2). These results agree with the findings of Wien *et al.* (1993) and Teasdale and Abdul-Baki (1995) that mulching could increase an early root and shoot growths due to favorable growing environment. In addition, the reduction of nutrient leaching (Clarkson, 1960) could have contributory role in the improvement of crop yield.

Mulching influenced the activities of sugar metabolizing enzyme by affecting soil moisture content and temperature. Acid invertase activity in soluble fraction (SF) of both top and bottom portions in roots grown in plots with mulched was higher than those grown in plots without mulched throughout the experimental period (Fig. 3A). This could be due to higher soil moisture content (Fig. 1A) and temperature (Fig. 1B) in plots with mulched than those without one. In sugar cane, acid invertase was found to have higher activity when the plants grown under high water regime (Hatch and Glasziou, 1962) and with increasing soil temperature (Khayat and Zieslin, 1987). It was further observed in mature leaves of roses that acid invertase activities were higher in plants grown under higher temperature than under lower temperature (Khayet and Zieslin, 1987). Suc content in roots grown in mulched plots was higher than those grown in none-mulched plots. This could be due to higher activities of other enzymes related to sucrose metabolism such as SS or SPS. In rose (Khayat and Zieslin, 1987) and potato (Lafta and Lorenzen, 1995), SPS activity was higher in plants grown under higher night temperature than under lower temperature. In this experiment we found that acid invertase activity was negatively correlated with sugar contents. This could be due to the activity of acid invertase which mainly broke down Suc into Glc and Fru (Coupe *et al.*, 2002). These results were in agreement with the findings of Pramanik *et al.* (2004) in broccoli and Kassinee *et al.* (2004) and Sitthiwong *et al.* (2005) in vegetable soybean that acid invertase activity was negatively correlated with sugar contents. Thus, it is likely that acid invertase activity functions mainly in the breaking down of Suc. However, no correlation was observed between Fru and acid invertase activity, both in top and bottom portions of roots grown in plots with and without mulch. Ross *et al.* (1994) reported that during potato tuber development, the Glc content increased while the fructose content remained relatively constant. In general, acid invertase activity in the bottom portion of the roots grown in mulched plots was higher than that of the top portion at 40, 50, 60 and 80 DAS in SF and 50, 60 and 80 DAS in CWBF (Table 1). The higher acid invertase activity in the bottom portion could be due to the effect of higher soil temperature and moisture content in the plots with mulched as described above. The results of this study suggest that mulching could increase crop yield by producing heavier root weight and improve product quality such as sweetness due to higher Suc content of the roots. Studies on the activities of other sugar-metabolizing enzymes will further improve understanding on the benefits of mulch in improving product quality.

ACKNOWLEDGMENT

The authors would like to thank the Government of Japan through its Ministry of Education, Culture, Science and Technology for the financial support.

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