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The Effect of Calcium and its Distribution in Cell Wall Components of Sweet Potato (*Ipomoea batatas* Lam) Tuberous Roots

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Abstract: To clarify Ca distribution in the cell wall of the tuberous roots of the sweet potato plant, two varieties, Beniotome and Benisatsuma, were cultured in river-sand with three levels of Ca. The fresh and cell wall weights of the tuberous roots of both varieties decreased with an increase of Ca level, with the cell wall weight of Beniotome being greater than that of Benisatsuma in each treatment. Both varieties contained more hemicellulose fraction than any other fraction in their tuberous roots. The lignin fraction increased in both varieties with increasing Ca level at 90 and 120 days, with that of Beniotome, this variety being more slender than Benisatsuma, higher at 90 days than that of Benisatsuma. In both varieties, the Ca content among all fractions was highest in the hemicellulose fraction, followed by the pectin fraction; and Ca contents increased with the increase of Ca level. In both fractions, however, in accord with the Ca supply, the increasing ratio of Ca in the fraction was higher in Beniotome than in Benisatsuma. These results suggest that the formation of slender tuberous roots by the increase of Ca supply may be due to the increase of the lignin ratio in spite of the depression of the cell wall, and that the difference of Ca requirement between the varieties is due to different amounts of Ca in the cell wall that combines with pectin and hemicellulose.

Key words: Calcium, cell wall, hemicellulose, lignin, sweet potato

INTRODUCTION

Calcium (Ca) is essential for the maintenance of cell wall structural integrity, and plays in important role in many physiological functions of higher plants. In an earlier paper[1], indicated that when sweet potato plants were cultured under a greater application of Ca in river-sand, the weight of vegetative tops, roots and tuberous roots all decreased and that slender tuberous roots were produced; but that the carbohydrate content of these increased as Ca levels increased. At the same time, under microscope observation, the cell numbers in the secondary xylem parenchyma decreased but the cell size expanded as the Ca level increased. Cell numbers were greater in Beniotome than in Benisatsuma (unpublished data). Furthermore, in Beniotome, in the early occurring physiological injury to the tuberous roots, only a little Ca was translocated to the vegetative tops, because compared with Benisatsuma more Ca remained in the tuberous roots[1]. These results suggest that there may be a difference between the varieties in Ca distribution in the cell walls of the tuberous root.

In this study, the cell wall composition was investigated using tuberous roots of Beniotome and Benisatsuma cultured in river-sand at different Ca levels.

MATERIALS AND METHODS

Plant culture: The effect of Ca and its distribution in cell wall components of sweet potato tuberous roots was carried out during cropping season (June to October 2001), in the experimental farm of the Faculty of Agriculture, Kagoshima University, Kagoshima City, Japan. Two sweet potato (*Ipomoea batatas* Lam.) varieties, Beniotome and Benisatsuma were used in this study. Vines 25 cm in length with 7 stem nodes were transplanted into plastic trays with bottoms (60 cm length x 50 cm width x 10 cm depth) filled with about 10 kg of wet river-sand containing 17 mg kg^{-1} of exchangeable Ca and 2 mg kg^{-1} of soluble Ca in water. The trays were place on a 15° tendency downward slope and covered with silver sheets as mulching. One plant was planted on one tray. The plants were supplied weekly with 500 ml of a nutrient solution containing the following elements; 1.43 g NH_4NO_3, 1.51 g KNO_3, 3.0 g $MgSO_4$, 0.95 g KH_2PO_4, 0.6 g Fe-EDTA, 0.07 g H_3BO_3, 0.006 g $ZnSO_4 \cdot 7H_2O$, 0.002 g $CuSO_4 \cdot 5H_2O$, 0.01 g $MnCl_2 \cdot 4H_2O$ and 0.0009 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ with 3 Ca levels, 0 mg L^{-1} Ca (L treatment), 4 mg L^{-1} Ca (M treatment) and 28 mg L^{-1} Ca (H treatment) as $CaCl_2$. The optimum range pH of the

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nutrient solution was 6.0-7.0. Ten trays were prepared for each treatment.

Cell wall preparation: Five plants each were harvested at 90 and 120 days after transplanting. The cut tuberous roots from each treatment were homogenized separately in cold water, and the homogenates filtered through four layers of cheesecloth and washed with cold water. The residues were then rehomogenized and washed. This was repeated four times. The final residues were washed with ethanol, acetone and diethyl ether, successively until no starch particles could be observed in the residues under microscopy. The dry weights of the cell walls were determined after 48 h of air-drying.

Fractionation of cell wall components: Determinations of cell wall components were made in walls prepared according to the modification method of by Ito and Fujiwara[2]. The cell wall was extracted with water at 80°C, giving fraction 1 (F1), 0.25% ammonium oxalate plus 0.25% oxalic acid at room temperature (F2), 0.25% ammonium oxalate plus 0.25% oxalic acid at 80-85°C (F3), water at 80-85°C containing 0.5 ml of glacial acetic acid and 0.5 g of sodium chlorite (F4), 5% aqueous potassium hydroxide at room temperature (F5) and 24% aqueous potassium hydroxide at room temperature (F6), successively. The extracted residue was F7. According to Anderson and King[3,4] the fractions extracted mainly contain the following materials: a) fraction 1; hot water soluble materials, b) fractions 2 and 3; pectic substances, c) fraction 4; oxidized lignin, d) fractions 5 and 6; hemicellulose materials, and e) fractions 7; cellulose.

Determination of Ca content: The Ca content of each fraction was determined after digestion using an atomic absorption spectrometer following the procedure of Trevelyan and Harrison[5].

Data analysis: Data were subjected to one-way analysis of variance (ANOVA). When ANOVA indicated $P<0.05$, the means were separated using Duncan's multiple range test in accordance with Sokal and Rohlf[6].

RESULTS

The fresh weight of sweet potato tuberous roots increased in both varieties in each treatment from 90 to 120 days (Table 1). However, this decreased with increasing levels of Ca at 90 and 120 days, and was generally lower in Beniotome for each treatment. While, the weight of the cell walls also increased in the tuberous roots of Beniotome and Benisatsuma from 90 to 120 days, with the increase being higher in Beniotome and tended to decrease with an increase of Ca level. Both varieties showed increases in Ca content in cell walls, which became higher with the increase of Ca levels (Table 2). Comparing the varieties, Beniotome was higher in H treatment at 90 and 120 days than that of Benisatsuma.

Table 1: Effect of different Ca levels on the tuberous root and cell wall weights of two sweet potato varieties

Varieties	Days	Treatments	Tuberous root FW (g plant^{-1})	Cell wall DW (g plant^{-1})
Beniotome	90	Low	90.36a	10.75a
		Medium	72.63b	6.25b
		High	63.48b	3.30b
	120	Low	133.80a	16.32a
		Medium	128.04a	10.01b
		High	89.80b	7.27b
Benisatsuma	90	Low	147.30a	8.83a
		Medium	122.13a	5.86a
		High	65.40b	2.20b
	120	Low	202.14a	13.54a
		Medium	174.94a	10.49a
		High	109.80b	5.16b

Low: (0 mg L^{-1} Ca), Medium: (4 mg L^{-1} Ca), High: (24 mg L^{-1} Ca), FW: fresh weight, DW: dry weight. At each harvesting time, means within a column followed by the same letters are not significantly different at 5% level by Duncan's multiple range test

Table 2: Effect of different Ca levels on the Ca content in the cell walls of tuberous root

Varieties	Days	Treatment	Ca content (mg g^{-1})
Beniotome	90	Low	1.751a
		Medium	1.943a
		High	2.514b
	120	Low	1.739a
		Medium	1.852a
		High	2.309b
Benisatsuma	90	Low	1.701a
		Medium	1.897a
		High	2.125b
	120	Low	1.511a
		Medium	1.777a
		High	1.891b

Low: (0 mg L^{-1} Ca), Medium: (4 mg L^{-1} Ca), High: (24 mg L^{-1} Ca). At each harvesting time, means within a column followed by the same letters are not significantly different at 5% level by Duncan's multiple range test

The weights of each fraction in the cell walls of the tuberous roots of the varieties at 90 and 120 days are shown in Fig. 1. In both varieties, total recovery of cell wall increased from 90 to 120 days, and H was the lowest among all treatments. Further, the hemicellulose fraction was higher than other fractions, but was lower in the H treatment at both time points than in the L and M treatments; while the cellulose fraction increased from 90 to 120 days, but was lower in the H treatment than in the L. Lignin formation also increased with the increase of Ca level.

In each treatment, the lignin fraction of Beniotome, which decreased from 90 to 120 days, was higher at both time points than that of Benisatsuma, which increased in L and M treatments. However, the hot water soluble and the pectin fractions in M and H treatments were lower in

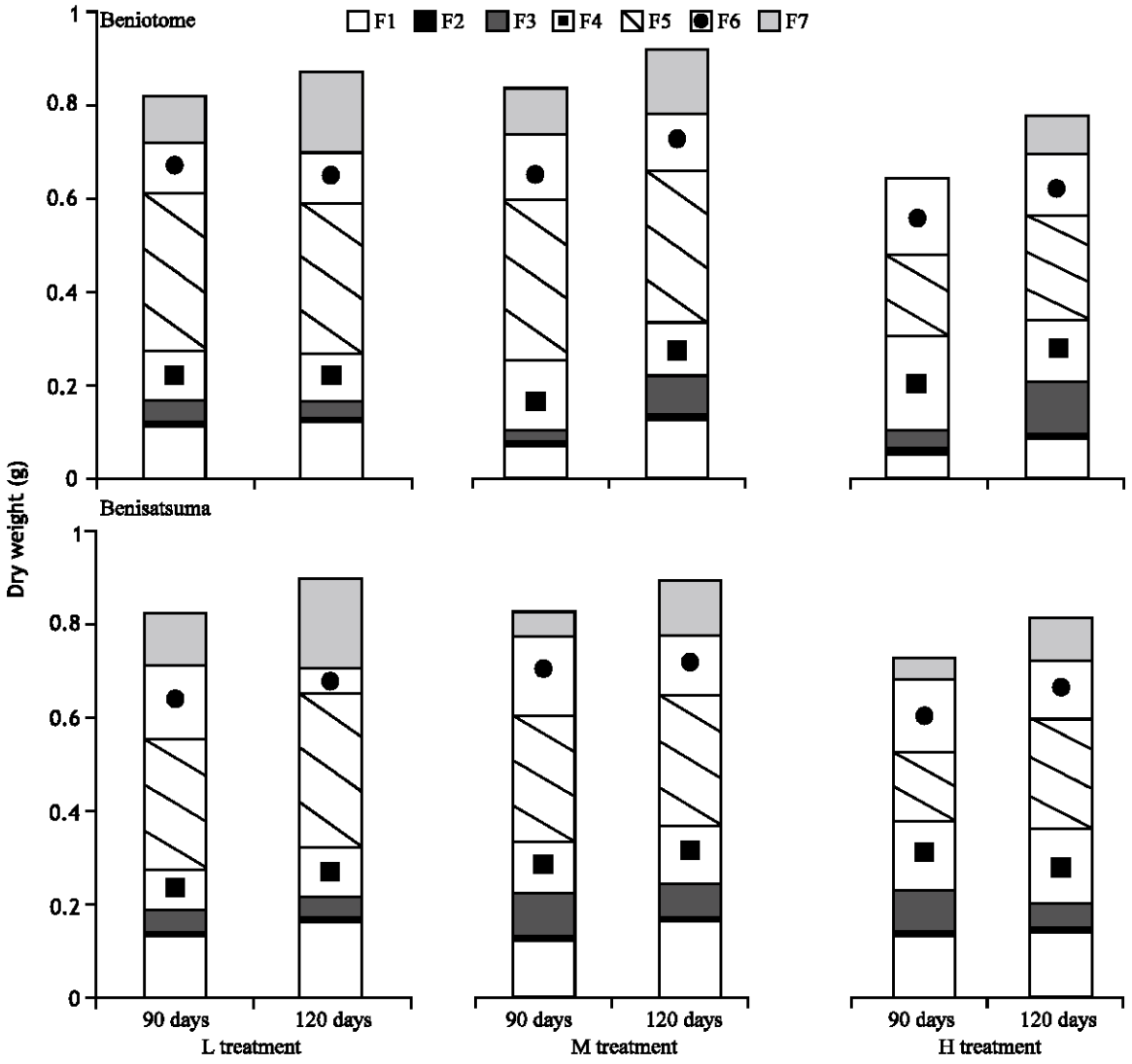

Fig. 1: Changes in the dry weight in each fraction of tuberous root cell walls. L treatment: 0 mg L^{-1} Ca, M treatment: 4 mg L^{-1} Ca, H treatment: 28 mg L^{-1} Ca, F1: hot water soluble materials, F2 and F3: pectic substances, F4: oxidized lignin, F5 and F6: hemicellulose materials, F7: cellulose

Beniotome at 90 days than in Benisatsuma, but no differences were observed at 120 days between the varieties. At 90 days, the pectin fraction was lower in Beniotome than in Benisatsuma.

The total Ca content of cell walls in both varieties increasing with Ca supply, decreased from 90 to 120 days (Fig. 2). In both varieties, the Ca content of the fractions was the highest in the hemicellulose fraction, following by the pectin fraction, and again increased with Ca supply. The increasing Ca ratios of both fractions were higher in Beniotome than in Benisatsuma. In both varieties, the lignin fraction was lower than those of hemicellulose and pectin, but increased with Ca supply. Further, the Ca content of the lignin fraction was higher in Beniotome at 90 and 120 days compared with Benisatsuma.

DISCUSSION

A slender tuberous root with higher starch and sugar contents, such as is preferred by consumers, can be produced by an increase in the Ca supply, but with a lower fresh weight[1]. Further, from results of microscopy observation in the secondary xylem parenchyma, the lower fresh weight from the increase in the Ca supply might be considered as a decrease of cell walls due to the restrained ability of cell multiplication (unpublished data).

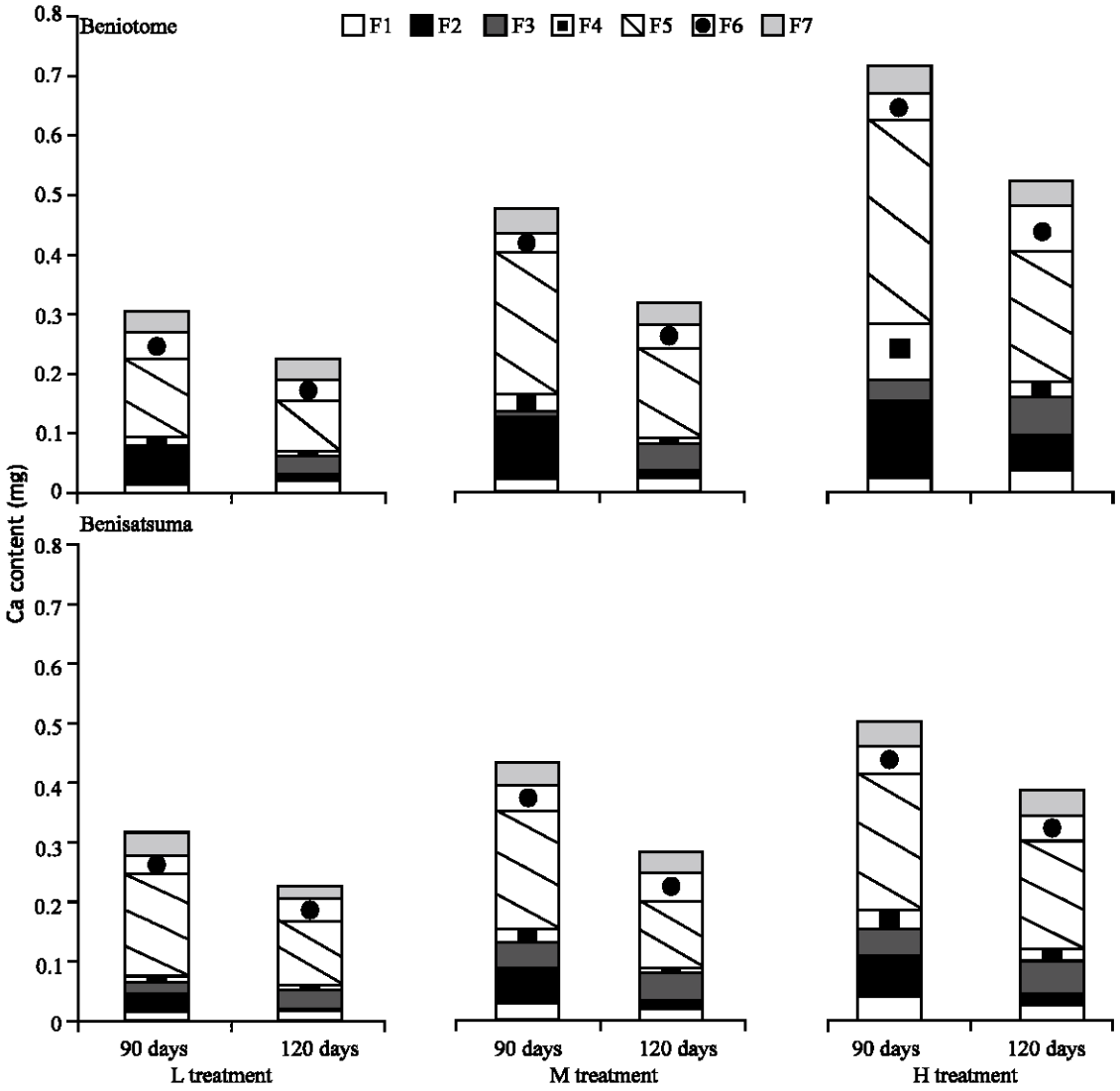

Fig. 2: Changes in the calcium content in each fraction of tuberous root cell walls. L treatment: 0 mg L^{-1} Ca, M treatment: 4 mg L^{-1} Ca, H treatment: 28 mg L^{-1} Ca, F1: hot water soluble materials, F2 and F3: pectic substances, F4: oxidized lignin, F5 and F6: hemicellulose materials, F7: cellulose

The fresh weights of both varieties decreased at 90 and 120 days with the increase of the Ca supply, but the cell wall weights increased from 90 to 120 days (Table 1). Furthermore, the cell wall weights of Beniotome, being lighter in fresh weight than Benisatsuma, were higher in every treatment at 90 and 120 days compared with those of Benisatsuma. These results support that the tuberous roots of sweet potato continue to form cell walls during their maturing stage (90 to 120 days), and that the lower fresh weight from an increase of the Ca supply depends upon a decrease of cell walls.

With an increase of the Ca supply, the Ca content in the cell wall of the tuberous roots of both varieties increased with the decrease of the fresh and cell wall weights, and the increasing ratio with the increased Ca supply was smaller in Benisatsuma than in Beniotome (Table 2); indicating that if cell wall weights showed cell numbers, Ca may play a role for depression of cell division near secondary xylem through cell wall formation in sweet potatoes. The fact that in spite of the increase of cell wall weights from 90 to 120 days the Ca content did not increase, suggests that Ca absorbed during this period did not remain in the cell wall.

The recovery ratio of total fraction from cell walls was about 80-90% in leaves of rice plants and shells of peanut plants[7,8]. However, at 90 days in the H treatment group,

it was about 65-75%, being due to the decrease of hemicellulose fraction with its greater Ca distribution (Fig. 1). During the process of extracting the cell wall with KOH, the hemicellulose fraction combined with Ca might be lost. However, the mechanism is unclear.

In rice leaves and peanut shells, the components of the cell wall with much more cellulose and less pectin fractions were not affected by the Ca level[7,8]. Those of an enlarging root, such as the radish that has much more pectin and less cellulose fractions than those of rice leaves, are affected by a greater Ca level[9]. However, at 90 and 120 days in the tuberous root of the sweet potato, the hemicellulose fractions were the greatest among the fractions; this being different from other crops.

As described previously, a slender tuberous root was formed with an increase of the Ca supply, Beniotome depending upon its longer length and narrower width, while Benisatsuma upon only narrower width[1]. The ratio of the lignin fraction increased with an increase of the Ca level at both time points in both varieties (Fig. 1) and that of Beniotome, which produced longer tuberous roots than Benisatsuma[1] was greater than that of Benisatsuma. While, the Ca content of the lignin fraction also increased with an increase of the Ca supply (Fig. 2) and at 90 days that of Beniotome in each treatment contained more than that of Benisatsuma. Ito and Fujiwara[7] suggested that Ca might play an important role in cell wall formation because more Ca was contained in the lignin fraction of growing rice leaves. Inanaga *et al.*[10] reported that Ca is present in binding with the lignin-carbohydrate complex. Lignin is also the material forming the vascular bundle[11]. The formation of long tuberous roots with an increase of the Ca supply might be due to the increase of the lignin fraction. The Ca content of the pectin fraction increased with an increasing Ca supply, and that of Beniotome with its narrower width than Benisatsuma[1] was more than that of Benisatsuma. In sweet potato, pectins with low methoxyl contents form gels by cross-linkages between free carboxyl groups and metallic ions, especially Ca[12]. When carboxyl-residue in pectin acid is methylated, a smaller amount of Ca combines with pectin acid[13,14]. As the pectin fraction was more methylated in Benisatsuma, its Ca content may be less than in Beniotome. It was observed that in the rice plant, most of the Ca in the cell wall was found in the pectic substances and lignin fractions during the growth of rice leaves[7]. Ca forms the cell wall with smaller plasticity through binding with pectin. From the above results, the occurrence of a broad tuberous root from reduced Ca supplies might depend on the relaxation of the cell wall from the weak binding of Ca with pectin.

In Beniotome, the Ca content of the tuberous root was higher than in Benisatsuma, but that of the vegetative tops was lower[2]. Among the fractions the Ca content in both varieties was the greatest in the hemicellulose fraction, followed by the pectin fraction, where the Ca content increased with the increase of the Ca supply (Fig. 2). However, the increasing ratio that accorded with the Ca supply was larger in Beniotome than in Benisatsuma. Furthermore, cell walls of the tuberous roots and the hemicellulose fraction were greater in Beniotome than in Benisatsuma. These results suggest that the difference between both varieties in the Ca requirements of the tuberous roots, and the Ca amount translocating to the vegetative tops, are due to the different Ca amounts combining with the pectin and hemicellulose fractions.

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