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Laboratory of Plant Production Physiology, Faculty of Agriculture, Kyushu University, Hakozaki, Higashi-ku, Fukuoka, Japan

Effects of Exogenous Injection of Different Sugars on Leaf Photosynthesis, Dry Matter Production and Adenosine 5′-Diphosphate Glucose Pyrophosphorylase (AGPase) Activity in Sweet Potato, *Ipomoea batatas* (Lam.)

M. Kadowaki, F. Kubota and K. Saitou

Authors' address: Mr M. Kadowaki, Drs F. Kubota (corresponding author) and K. Saitou, Laboratory of Plant Production Physiology, Division of Bioresource and Bioenvironmental Sciences, Graduate School, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812–8581, Japan

With 1 figure and 2 tables

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Abstract

Solutions of sucrose, glucose and fructose were artificially injected into the stems of sweet potato plants. The effects of solution injection on both dry matter production and the activity of adenosine 5′-diphosphate glucose pyrophosphorylase (AGPase) in tuberous roots were investigated and compared. The total weight of carbon (T_C) artificially and photosynthetically supplied to a plant during the treatment period of 40 days was 0.987–1.869 times the weight of photosynthetically assimilated carbon alone. At the final sampling time, the dry matter weight of tuberous roots in the plants injected with sugar solutions showed a 2.73–9.13-fold increase over that of the control plant. The root weight linearly increased with T_C. The activity of AGPase was also enhanced by solution injections, with 27–63 % increases compared to the control, but was not significantly related to T_C. The injection of sugar solutions is concluded to have a dual effect on root production in sweet potato. One effect is that the increased sugar concentration in the plant increases AGPase and sink activities, and the other effect is that the increased carbon supply quantitatively promotes starch synthesis and accumulation in roots.

Key words: adenosine 5′-diphosphate glucose pyrophosphorylase (AGPase) — fructose — glucose — sucrose — sweet potato — tuberous root production

Introduction

Many of the studies on sweet potato have reported that the activity level of sink function plays a dominant role in determining tuberous root production (Hojo and Park 1971, Hojo et al 1971, Hojo and Kato 1976, Nakatani and Komeichi 1988, Kubota et al. 1993, Yatomi et al. 1996), and enhancement of sink activity is indispensable for the improvement of sweet potato production. However, other studies have indicated that tuberous root production depends not only on sink activity but also on source potential (Nakatani et al. 1988, Tsubone et al. 1997, Ishida et al. 1999). We reported in a previous paper (Tsubone et al. 2000) that the activity of adenosine 5′-diphosphate glucose pyrophosphorylase (AGPase) and the dry matter weight of tuberous roots were increased by exogenous injection of sucrose into sweet potato plants. This result suggests the importance of source activity as a determinant of root production.

The finding of our previous paper that the injection of sucrose solution may increase tuberous root production is readily understandable because sucrose is one of the main substances produced by photosynthesis in leaves and then transported to sink organs. As the next step in this investigation, we are interested in determining the responses of sweet potato plants to injection of solutions of sugars other than sucrose. In this study, solutions of glucose, fructose and sucrose at different concentrations were injected into sweet potato plants, and their effects on leaf photosynthetic rate, tuberous root production and AGPase activity were investigated and discussed from the viewpoint of production improvement in sweet potato.

Materials and Methods

The sweet potato cultivar 'Koganesengan' was used as the experimental material. Plantlets with two leaves on the stem were treated and grown in pots during the summer season in 1999 in a greenhouse set up in an experimental field of

Kyushu University (33°35′ N, 130°23′ E) in Japan. The cultivation and treatment methods were similar to those described in the previous paper (Tsubone et al. 2000). The cut end of the stem of the sweet potato plantlet was connected to a glass tube, from which the solution of sucrose (4 or 6 % concentration), glucose (1 or 2 %) or fructose (1 or 2 %) was injected into the plant. As a control, distilled water was injected into plantlets. Each treatment was repeated five or six times.

To mitigate the high osmotic stress caused by sugar solution injection, shade and mist treatments were applied for 24 h after the initiation of injection to plantlets, each of which had three fully expanded leaves. On the second day, the top leaf was removed because it was apt to be most easily damaged by injection. Thereafter, plantlets with two leaves were supplied with sugar solutions for 40 days in a greenhouse under natural light conditions.

The level of solution in the glass tubes was monitored every day to determine the volume of absorbed solution and the weight of incorporated carbon. Glass tubes were filled up with each solution periodically. The leaf photosynthetic rate was measured with a portable photosynthesis system (SPB-H3, Analytical Development Co. Ltd., Herts, UK) every week under conditions of 1600–1800 μE light intensity, 32.0 $\pm$ 3.4 °C chamber temperature and 40.7 $\pm$ 1.5 % relative humidity. The plantlets were sampled on the 40th day after the start of the treatment to measure the growth parameters. Tuberous roots of > 5 mm diameter were sampled to determine the activity of AGPase. Enzyme extraction was carried out according to the method described by Yatomi et al. (1996). The activity of AGPase was measured using the procedure described by Nakamura et al. (1989). The measurement of enzymatic activity was repeated three or four times for each treatment.

The weight of carbon fixed photosynthetically in a plant over 40 days (P_C) was calculated by equation 1:

$$P_C = \text{photosynthetic rate} \times \text{leaf area} \times \text{sunshine duration} \times 0.273 \times 12, \quad (1)$$

where 0.273 is calculated from 12/44, the ratio of the molecular weight of carbon present in CO_2.

The weight of carbon supplied to a plant over 40 days by solution injection was termed S_C, and the total weight of carbon supplied to a plant (T_C) was calculated as $T_C = P_C + S_C$.

Results and Discussion

The osmotic potential of the sugar solutions, photosynthetic rate, leaf area, P_C, S_C and T_C are listed in Table 1. Leaf wilting and photosynthetic reduction were not detected in the plantlets injected with sugar solutions with osmotic potentials of −0.14 to −0.43 MPa. The water mist and shade treatments applied on the day injection was initiated were effective in preventing leaves from functional reductions caused by increased osmotic pressure in plants.

Table 1: Changes in leaf photosynthetic rate, leaf area and carbon gain of plantlets injected with a sugar solution

Injection treatment	Osmotic potential of solutions (MPa)	Average rate of photosynthesis (gmol m^{-2} s^{-1})	Leaf area (cm^2 $plant^{-1}$)	Total sunshine duration (h)	Carbon assimilated by photosynthesis P_C (g)	Volume of absorbed solution (cm^3)	Carbon supplied by sugar solutions S_C (g)	Total carbon T_C (g)	TC/PC (%)
Control	0	15.8	400.7	148.2	1.104	73.45	0	1.104 (100)	100
4 % Suc	−0.29	16.2	449.5	148.2	1.272	33.41	0.563	1.835 (166.2)	144
6 % Suc	−0.43	14.3	446.4	148.2	1.111	37.68	0.952	2.063 (186.9)	186
1 % Glu	−0.14	15.8	386.6	148.2	1.068	45.45	0.181	1.249 (113.1)	117
2 % Glu	−0.27	15.7	376.6	148.2	1.032	40.53	0.324	1.356 (122.8)	131
1 % Fru	−0.14	14.8	353.0	148.2	0.912	44.51	0.178	1.090 (98.7)	120
2 % Fru	−0.27	16.0	368.2	148.2	1.032	42.59	0.341	1.373 (124.4)	133

P_C = leaf photosynthetic rate × leaf area × sunshine duration × 0.273 × 12.
$T_C = P_C + S_C$.
Suc, sucrose; Glu, glucose; Fru, fructose.

The leaf area of the plantlets was slightly increased by injection of sucrose solutions, and hence a slightly higher value of P_C was calculated for plantlets injected with sucrose. However, overall, P_C did not show large variation amongst the various sugar treatments. T_C did differ amongst the treatments, ranging from 98.7 to 186.9 relative to the control (100). The value of P_C determined by equation 1 might be underestimated, because it was calculated based on sunshine duration, in which hours of a relatively low light intensity were not included. Of the treatments, the 6 % sucrose solution injection gave the highest values for S_C and T_C, 0.952 and 2.063 g, respectively, while the 1 % fructose solution gave the lowest values, 0.178 and 1.090 g for S_C and T_C, respectively, which were a little lower than the control value (1.104 g).

AGPase activity and dry matter weight in tuberous roots were changed by sugar solution injection (Table 2). Among the enzymes operating in the process of starch synthesis, AGPase is regarded as one of the key enzymes, and its activity in amyloplasts is considered to be one of the main regulators of starch synthesis and tuberous root production (Sowokino 1976, Nakatani and Komeichi 1992). Injection of sugar solutions enhanced AGPase activity by 27–63 % over the control. The point to note is that the activity of AGPase was increased by injecting higher concentration solutions, but did not show a significant relationship with T_C. In the case of the 1 % fructose solution injection, T_C was lower than that of the control (Table 1), but AGPase activity was enhanced by 27 % compared to the control, as shown in Table 2.

There are many genes whose expression is controlled by the sugar concentration in the plant (Koch 1996). But the sugar sensing system is likely different with genes. Chio et al. (1998) regarded sucrose as a signalling molecule for the expression of the sucrose transporter gene in sugar beet, *Beta vulagris* (L.). Mita et al. (1995) reported that expression of the gene for β-amylase was induced not only by sucrose but also by glucose or fructose in *Arabidopsis thaliana* (L.). AGPase activity in sweet potato was also enhanced by glucose, fructose and sucrose injection in our experiment. This suggests that the gene for AGPase, as well as the gene for β-amylase, might be subject to regulation by a carbohydrate metabolic signal.

In our experiment, the ratios of tuberous root weight to whole plant weight were increased from 6.6 % in the control to 15.8–38.5 % in plants with sugar solution, as shown in Table 2. As reported for fava bean plants, the decreased ratio of hexose to sucrose content promoted storage product synthesis in cotyledons (Weber et al. 1995). However, this is unlikely to be the explanation for our experimental result in sweet potato roots. The increase in tuberous root weight by sugar injection into sweet potato depends solely on T_C, but the ratio of sucrose to glucose is probably ineffective.

As shown in Fig. 1, the dry matter production of tuberous roots linearly increased with T_C. Such a relation was not obtained between AGPase activity and T_C whereas AGPase activity was enchanced by injecting sugar solution, as mentioned above (Table 2). This suggests supplying a large quantity of carbon is important to increase

Table 2: Effects of sugar solution injections on the activity of AGPase and dry matter weight in tuberous roots

Injection treatment	Activity of AGPase (unit gFW^{-1})	Dry matter weight of whole plant (A) (g)	Dry matter weight of tuberous roots (B) (g)	Percentage dry matter weight of tuberous roots (B/A) (%)
Control (H_2O)	0.433 (100)	8.48 (100) c	0.56 (100) c	6.6
4 % Suc	0.552 (128)	13.64 (161) a	4.24 (761) ab	31.1
6 % Suc	0.647 (149)	13.21 (156) ab	5.09 (913) a	38.5
1 % Glu	0.572 (132)	10.01 (118) bc	1.58 (283) c	15.8
2 % Glu	0.704 (163)	9.37 (110) c	2.30 (413) bc	24.6
1 % Fru	0.552 (127)	7.90 (93) c	1.52 (273) c	19.2
2 % Fru	0.637 (147)	10.65 (126) ac	2.41 (432) bc	22.6

Mean values followed by the same letters are not significantly different at the 5 % level according to Duncan's multiple range test. The figures in parentheses are values relative to the control.
Coefficients of correlation: between T_C and AGPase, $r = 0.396$ (ns); between T_C and dry matter weight of whole plant, $r = 0.949$ ($P < 0.01$); between T_C and dry matter weight of tuberous roots, $r = 0.980$ ($P < 0.001$).

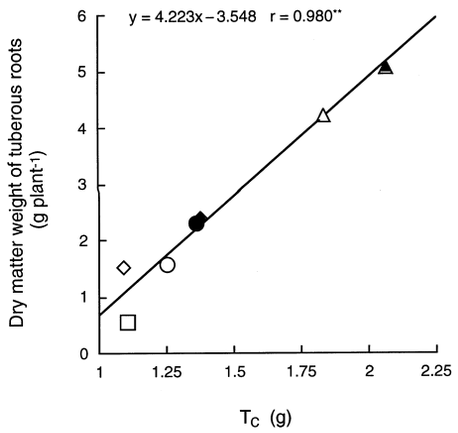

Fig. 1: Relation between T_C and dry matter weight of tuberous roots. □, control; △, 4 % sucrose; ▲, 6 % sucrose; ○, 1 % glucose; ●, 2 % glucose; ◇, 1 % fructose; ◆, 2 % fructose

tuberous root production in addition to enhancement of AGPase activity. For further improvement of root production in sweet potato, the primary requirement is increase in leaf photosynthetic ability. It is also necessary that AGPase is genetically improved to increase its activity at a lower sugar or carbon concentration level in roots.

Zusammenfassung

Einfluss einer exogenen Injektion mit verschiedenen Zuckersorten bei Süßkartoffelpflanzen, *Ipomea batatas* (Lam) auf die Blattfotosynthe, die Trockenmasseproduktion und die Aktivität von Adenosine 5′-Diphosphat-Pyrophosphorylase (AGPase)

Lösungen von Sukrose, Glukose und Fruktose wurden künstlich in den Stengel von Süßkartoffelpflanzen injiziert. Die Einflüsse der Lösungsinjektion auf die Trockenmasseproduktion und die Aktivität von Adenosin 5′-Diphosphat-Pyrophosphyrolase (AGPase) in Knollenwurzeln wurde untersucht und verglichen. Das Gesamtgewicht von Kohlenstoff (T_C), künstlich und fotosynthetisch in der Pflanze während der Behandlungsperiode von 40 Tagen zugeführt, betrug das 0987 bis 1,896-fache des Gewichtes des fotosynthetisch assimilierten Kohlenstoffs. Zur abschließenden Probenahmezeit zeigte das Trockengewicht der Knollenwurzeln in den Pflanzen, die mit Zuckerlösungen behandelt worden waren, eine 2,73–9,13-fache Zunahme über die Kontrollpflanze. Das Wurzelgewicht nahm linear mit T_C zu. Die Aktivität von AGPase war ebenfalls durch die Lösungsinjektion erhöht und zeigte 127–163 im Vergleich zur Kontrolle (100), hatte aber keine signifikante Beziehung zu T_C. Die Injektion von Zuckerlösungen könnte daher einen dualen Effekt auf die Wurzelproduktion bei Süßkartoffel ausüben. Die erste Einwirkung ist eine Erhöhung der Zuckerkonzentration in der Pflanze und regt die Erhöhung von AGPase-Aktivität an und resultiert in einer Verstärkung der sink-aktivität, der zweite Effekt zeigt sich darin, dass eine Erhöhung der Kohlenstoffzufuhr quantitativ die Stärkesynthese und -akkumulation in den Wurzeln fördert.

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