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Enhancement of Tuberous Root Production and Adenosine 5′-Diphosphate Pyrophosphorylase (AGPase) Activity in Sweet Potato (*Ipomoea batatas* Lam.) by Exogenous Injection of Sucrose Solution

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With 2 figures and 4 tables

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Abstract

Adenosine 5′-diphosphate pyrophosphorylase (AGPase) is a key enzyme governing starch synthesis and is regarded as an important determinant of the sink activity of the sweet potato root. In this study, assuming that the expression of AGPase is under the direct or indirect control of sucrose, we investigated the effect of exogenous injection of sucrose solution into a plant on the activity of AGPase and tuberous root production. Sucrose solutions of 6 and 12 %, and distilled water as the control, were injected into the top of the shoot. The application of sucrose solution was effective in increasing tuberous root production and increasing the ratio of tuberous root weight to total root weight in a plant. AGPase activity in roots was enhanced by about 25 % by injecting sucrose solution. These results suggest that AGPase and sink activities are controlled by sucrose transported from the leaves. To increase sweet potato production effectively, AGPase activity and sink function must be enhanced, and so a genetic and physiological improvement in photosynthetic function or sucrose productivity in the leaves is necessary to increase AGPase activity in sink organs.

Key words: Adenosine 5′-diphosphate pyrophosphorylase (AGPase) — photosynthesis — sink — source — sucrose — sweet potato — tuberous root production

Introduction

Many studies have shown that the activity of the sink organ in sweet potato, *Ipomoea batatas* Lam., is one of the most important determinants of tuberous root production. Hozyo et al. (1971) and Hozyo and Kato (1976), for example, performed sophisticated experiments to clarify the role of sink and source functions in sweet potato production, using experimental plants drafted with various cultivars with different sink and source activities. Recently, several biochemical and physiological studies have been carried out to identify the enzymes that control and adjust the process of starch synthesis, and adenosine 5′-diphosphate pyrophosphorylase (AGPase) (EC 2.7.7.27) has been determined to be a major enzyme governing starch synthesis and sink activity in sweet potato (Nakatani et al. 1992). It has also been reported that AGPase may play an important role in the starch production of several crops. and plants (Sowokinos 1976, Lin et al. 1988, Tyynela et al. 1995), and one notable finding is that a significantly higher yield was obtained in transgenic potato plants into which genes of highly active AGPase were introduced by genetic engineering techniques (Stark et al. 1992). Tsubone et al. (1997) also pointed out the importance of AGPase as a determinant of starch production and sink activity in sweet potato roots.

In sweet potato, the gene encoding the small subunit of AGPase is induced in leaves and petioles when leaf petiole cuttings are supplied with high concentrations of sucrose (Takeda et al. 1994). Plant AGPases, however, are regulated by allosteric effectors (Preiss 1998), and so it has not yet been clarified whether an increase in AGPase activity caused by sucrose may lead to high starch production in sweet potato.

Sink function has been believed to restrict source activity or leaf photosynthesis, but the recent evidence described above may indicate that sink activity in sweet potato roots is restricted by source function. With the assumption that sucrose is a major factor inducing and promoting gene expression of AGPase, we exogenously injected sucrose solution into a plant, and investigated its

effect on AGPase activity, tuberous root production and leaf photosynthesis with a view to the possibility of increasing sweet potato production.

Materials and Methods

The sweet potato cultivar 'Kokei14', which has a high yield capacity and early maturity of tuberous root growth, was used as the experimental material, and was grown during the summer of 1997 in a greenhouse set up in an experimental field of Kyushu University (33° 35′ N, 130° 23′ E). Young shoots with eight unfolding leaves were transplanted on 21 July in pots (8.0 l in capacity) containing sandy soil. To achieve complete rooting, the basal three nodes of each shoot were placed into the soil. At the time of transplanting, compound fertilizer (N:P:K = 16:16:16 in percentage) was applied at 5.0 g pot^{-1}. On the 10th day after transplanting, sucrose solutions were introduced into the plants using the injection tool shown in Fig. 1, and thereafter the supply of solution was continued for 28 days. Just before the injection treatment started, the top of the shoot stem was cut off, and a glass tube (diameter 8.0 mm × height 50 cm) was connected to the cut end of the stem by a silicone rubber tube. Immediately afterwards, the glass tube was filled with a 6 or 12 % solution of sucrose. As a control, distilled water, instead of sucrose solution, was injected into some of the plants. The top of the glass tube was covered with aluminium foil to prevent water loss by evaporation. The volume of solution or water absorbed by the plant was compensated every day. Water was supplied to the soil every day before application of the solution, but during the period of application of solution the watering frequency was reduced to every other day to dry the soil and thus promote solution absorption from the stem of the shoot.

The CO_2 exchange rate (CER), stomatal conductance (Gs) and mesophyll conductance (Gm) of leaves were measured at a photosynthetically saturating light intensity (PAR = 1600–1800 $\mu mol\,m^{-2}\,s^{-1}$) with a portable photosynthesis system (SPB-H3, Analytical Development Co. Ltd., Herts,UK) on the 14th and 28th days after the start of the sucrose treatment.

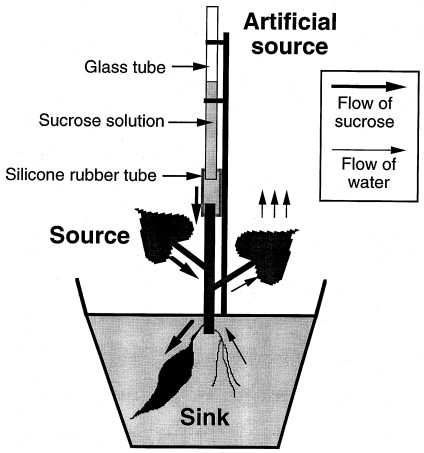

Fig. 1: Method of exogenous application of sucrose into a plant

After measurement of these parameters, three or four plants were sampled from each treatment plot to determine leaf area (LA), dry matter weight and the activity of AGPase in tuberous roots. Roots were classified into three types: tuberous root of more than 5 mm diameter, thick root of 2–5 mm and fibrous root of less than 2 mm. Immediately after these measurements, soil was carefully washed off the roots, and tissue segments were cut from the tuberous roots, frozen in liquid nitrogen and stored at –80 °C until used for enzyme extraction.

Enzyme extraction was carried out according to the method described by Yatomi et al. (1996). All the procedures were carried out at 0–4 °C. For the assay of AGPase activity, a tissue segment (about 0.5 g fresh weight) was quickly homogenized with a pestle in an ice-cold mortar that contained 250 mM Tricine-NaOH (pH 8.0), 20 mM $MgCl_2$, 2 mM Na_2-EDTA, 12.5 % (v/v) glycerol, 10 mM dithiothreitol and 10 % (w/v) polyvinylpyrrolidone at a ratio of segment to buffer of 1 to 10 (g ml^{-1}). The homogenate obtained was centrifuged at 10 000 × ***g*** for 5 min, and the resulting supernatant was used as the preparation for enzyme assay. The activity of AGPase was measured using the procedure described by Nakamura et al. (1989).

Results

Figure 2 shows the changes in the dry matter weights of (a) the whole plant and (b) tuberous roots produced by the sucrose treatments. The injection of a 6 % sucrose solution was not effective in increasing the total dry matter weight of a plant, but when a 12 % sucrose solution was injected plant weight increased by 25 and 8 % in samples taken on the 14 and 28th days, respectively, compared to the control, although these increases were not statistically significant. There was, however, a significant inter-treatment difference in the weight of tuberous roots. The highest weight, 3.19 g $plant^{-1}$, was found for the plant injected with a 12 % sucrose solution; this value is about 3 times that of the control (1.03 g $plant^{-1}$) and 1.37 times that of the plant supplied a 6 % sucrose solution (2.33 g $plant^{-1}$).

The ratio of biomass partitioning to each individual organ in a plant is an important indicator of the sink–source balance and the harvest index. The effect of an exogenous supply of sucrose on the partitioning ratio is shown in Table 1 as the dry matter weight of each organ as a percentage of that of the whole plant. The ratios for above-ground organs such as leaf, petiole and stem in the control

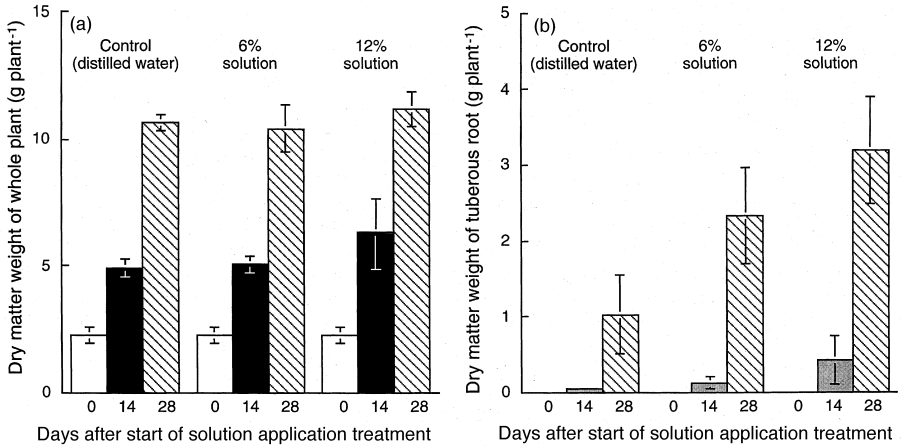

Fig. 2: Changes in dry matter weight of (a) the whole plant and (b) tuberous roots after treatment with different concentrations of sucrose. Vertical bars indicate the standard errors of means

Table 1: Dry matter weight of each organ as a percentage of that of the whole plant on the 28th day after commencement of solution application

	Leaf blade	Petiole	Stem	Tuberous root	Thick root	Fibrous root	Tuberous root/ total root
Control	21.03 ± 1.89a	5.62 ± 0.54a	40.32 ± 4.70a	9.45 ± 4.55a	4.51 ± 0.30a	19.08 ± 2.35ab	24.44 ± 6.98a
6 %	16.49 ± 0.44ab	4.37 ± 0.18a	32.20 ± 3.25a	21.95 ± 4.89ab	3.73 ± 0.58ab	21.27 ± 1.99a	45.19 ± 7.61b
12 %	16.54 ± 1.38b	4.81 ± 0.55a	33.72 ± 4.17a	27.74 ± 4.19b	2.61 ± 0.64b	14.58 ± 1.02b	45.19 ± 7.61b

Values are means of four replications ± SE. Mean values followed by the same letter are not significantly different at the 5 % level according to Duncan's multiple range test.

plant were higher than those in the plants injected with sucrose solutions, but the inter-treatment difference was not significant except for the leaf ratio. However, a large difference was found for the weight ratio of tuberous root/total root between the treated plants and the control; the ratios in plants supplied with 6 and 12 % sucrose solutions were 45.19 and 61.29 %, respectively, both of which were significantly higher than the value obtained for the control (24.44 %). The exogenous application of sucrose was effective in stimulating sink activity, which resulted in accumulation of more photosynthate in the tuberous roots.

To investigate the effect of sucrose application on source activity, four parameters, CER, Gs, Gm and leaf area (LA) per plant, were measured on the 14th and 28th days after the treatments, and values obtained are given in Tables 2 and 3, respectively. CER on the 14th day decreased as the solution concentration increased, with values of 22.98 μmol m^{-2} s^{-1} in the control plant, 20.68 μmol m^{-2} s^{-1} (10 % less than the control) in the plant injected with a 6 % solution and 18.0 μmol m^{-2} s^{-1} (22 % less) in the plant supplied with a 12 % solution. Gm also decreased as the sucrose concentration increased, suggesting that sucrose application restricted physiological activity in the mesophyll and leaf photosynthesis.

The average control values of CER, Gs and Gm measured on the 28th day were lower than those measured on the 14th day, decreasing to 55, 52 and 60 %, respectively (Tables 2 and 3). In the treatment in which a 12 % sucrose solution was injected, these three parameters were significantly lower than the control values, while for the 6 % solution treatment they were little different. LA increased with growth but an inter-treatment difference was not detected.

AGPase activity g^{-1} fresh weight of tuberous roots on the 28th day is shown in Table 4 in a comparison of the effects of sucrose application on enzymatic activity. The activity in the control plant was 0.68 units g^{-1} FW, but when a 6 or 12 % sucrose solution was injected, the activity increased by about 25 % up to 0.86 and 0.85 units g^{-1} FW, respectively. The activity of AGPase in tuberous roots on the 14th day was very similar to that on the 28th day (data not shown).

Table 2: CO_2 exchange rate (CER), stomatal conductance (Gs), mesophyll conductance (Gm) and leaf area (LA) on the 14th day after commencement of solution application

	CER (μmol m^{-2} s^{-1})	Gs (mol m^{-2} s^{-1})	Gm (mol m^{-2} s^{-1})	LA (m^2 $plant^{-1}$ m)
Control	22.98 ± 0.46a	0.53 ± 0.01a	0.093 ± 0.003a	0.024 ± 0.001a
6 %	20.68 ± 0.52b	0.50 ± 0.01a	0.081 ± 0.001b	0.023 ± 0.002a
12 %	18.00 ± 0.91c	0.46 ± 0.02b	0.069 ± 0.003c	0.023 + 0.003a
Mean	20.55	0.50	0.081	0.023

Values are means ± S.E. of six replications for CER, Gs and Gm and means ± S.E. of three replications for LA. Mean values followed by the same letter are not significantly different at the 5 % level according to Duncan's multiple range test.

Table 3: CO_2 exchange rate (CER), stomatal conductance (Gs), mesophyll conductance (Gm) and leaf area (LA) on the 28th day after commencement of solution application

	CER (μmol m^{-2} s^{-1})	Gs (mol m^{-2} s^{-1})	GM (mol m^{-2} s^{-1})	LA (m^2 $plant^{-1}$0
Control	12.80 ± 0.57a	0.28 ± 0.01a	0.055 ± 0.003a	0.038 ± 0.002a
6 %	11.63 ± 0.52a	0.29 ± 0.01a	0.049 ± 0.002ab	0.030 ± 0.002a
12 %	9.65 ± 0.54b	0.21 ± 0.01b	0.042 ± 0.003b	0.030 ± 0.003a
Mean	11.36 (55 %)	0.26 (52 %)	0.049 (60 %)	0.033 (143 %)

Values are means ± S.E. of 4–6 replications for CER, Gs and Gm and means ± S.E. of four replications for LA. Mean values followed by the same letter are not significantly different at the 5 % level according to Duncan's multiple range test. The figures in parentheses are percentages relative to values obtained on the 14th day.

Table 4: AGPase activity in tuberous roots on the 28th day after commencement of solution application

	AGPase activity (units g^{-1} FW)
Control	0.68 ± 0.05a
6 %	0.86 ± 0.01b
12 %	0.85 ± 0.06b

Values are means ± S.E. of three replications. Mean values followed by the same letter are not significantly different at the 5 % level according to Duncan's multiple range tast. The term 'units g^{-1} FW' represents nmol min^{-1} g^{-1} FW.

Discussion

The application of sucrose solution was effective in increasing sink organ production and AGPase activity, but had a negative effect on photosynthetic activity. As shown in Table 2, plants supplied with a 12 % sucrose solution had 22–25 % lower CER than the control plant. Azcon-Bieto (1983) and Morcuende et al. (1997) reported that increased sucrose concentration in wheat leaves restricted photosynthesis, and Martınez Carrasco et al. (1993) also described a negative relationship between photosynthetic rate and hexose concentration in the leaf. Hexoses are released through hydrolysis of sucrose accumulated in the mesophyll. Huber (1989) postulated that hexoses are phosphorylated and consume orthophosphate in the cytosol, reducing the amount of orthophosphate re-transferred into chloroplasts and restricting the production of adenosine 5′-triphosphate (ATP). A reduction of ATP synthesis is likely to be a limiting factor for photosynthesis. Exogenous application of sucrose may have promoted photosynthate accumulation in the leaves, causing a reduction of photosynthesis in the leaves of the sweet potato.

In the penultimate step in the process of starch synthesis, AGPase catalyses the reaction producing adenosine 5′-diphosphate glucose from glucose-1-phosphate in amyloplasts. It has been reported that there is a significant difference in AGPase activity amongst sweet potato cultivars, and also that the

activity shows a positive relationship with starch content or dry matter weight in the tuberous root (Nakatani and Komeichi 1992, Tsubone et al. 1997). In our experiment also, AGPase activity showed a close positive relationship with sink organ weight (Fig. 2 and Table 4). Stark et al. (1992) successfully increased root production in the white potato, so it may be predicted that an enhancement of AGPase activity in sweet potato root by genetic treatment may also produce an increase in root production.

It has frequently been reported that decreased sink function or capacity in root crops may cause photosynthate accumulation in leaves, with a resultant reduction in photosynthesis. Sink activity is therefore generally regarded as a primary determinant of starch production, and an improvement in sink activity is thought to increase root production directly. However, Tsubone et al. (1997) reported that production was regulated by source activity, not by sink activity, in sweet potato, and suggested that the increase in photosynthesis or source activity stimulated and enhanced AGPase activity. This suggestion is supported by evidence that one of the genes of AGPase in white potato is strongly inducible by sucrose at the mRNA level (Muller-Rober et al. 1990). Furthermore, Saitou et al. (1997) reported that sucrose synthase activity in the petiole of an excised leaf of sweet potato was greatly enhanced by exogenous application of a 6 % sucrose solution.

These findings and our results provide new information on the source–sink relationship. Exogenous application of a sucrose solution enhances the sucrose concentration in roots, and this triggers an increase in AGPase activity in roots. The functional activation in sink then allows roots to import sucrose more quickly from leaves and to accumulate it more efficiently as starch in amyloplasts. We conclude that improvement of leaf photosynthetic activity is a prerequisite for enhancing AGPase activity and sink production of sweet potato.

In our experiment, the effect of sucrose application on sink and source was clear, and the exogenous application of sucrose is thus regarded as a useful technique for clarifying the roles of sink and source functions in tuberous root production *in vivo*. However, the actual concentration of sucrose in plant tissues was not determined here. We are planning to carry out a further study which will include determination of this parameter, in a wide-ranging investigation using different genotypes of *Ipomoea batatas*, in order to elucidate more precisely the biochemical and physiological roles of sucrose in sweet potato.

Zusammenfassung

Erhöhung der Knollenproduktion und der Adenosin 5′-diphosphat-pyrophosphorylase (AGPase) Aktivität in Süßkartoffeln, *Ipomea batatas* Lam., durch exogene Injektion von Sukroselösung

Adenosin 5′-diphosphat-pyrophosphorylase (AGPase) ist ein Schlüsselenzym in der Stärkesynthese und wird als eine der bedeutendsten Faktoren für die Sinkaktivität bei Süßkartoffeln betrachtet. In dieser Untersuchung kann man von der Annahme ausgehen, daß die Expression von AGPase unter einer direkten oder indirekten Kontrolle von Sukrose steht; wir untersuchten daher den Einfluss einer Injektion von Sukroselösung in die Pflanze im Hinblick auf die Aktivität von AGPase und Knollenproduktion. Sukroselösungen von 6 und 12 % sowie destilliertes Wasser als Kontrolle, wurden in die Spitzen der Sproßachsen einer Pflanze injiziert. Die Anwendung von Sukroselösung war wirksam im Hinblick auf die Knollenproduktion und Erhöhung der Gewichtsrelation von Knollen zum Gesamtwurzelgewicht einer Pflanze. Die AGPase-Aktivität in den Wurzeln wurde erhöht durch eine etwa 25 %ige injizierte Sukroselösung. Aus diesen Ergebnissen kann gefolgert werden, daß AGPase und Sinkaktivitäten kontrolliert werden durch den Sukrosetransport aus den Blättern. Für eine effiziente Erhöhung der Süßkartoffelproduktion ist es unverzichtbar, die AGPase-Aktivat und Sinkfunktion zu vergrößern, und um eine höhere Enzymaktivität in den Sinkorganen zu erhalten, bedarf es einer genetischen und physiologischen Erhöhung der photosynthetischen Funktion oder Sukroseproduktivität in den Blättern als Voraussetzung.

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