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Sucrose Application Causes Hormonal Changes Associated with Potato Tuber Induction

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Abstract. Stems of potato plants (*Solanum tuberosum* L. cv. Dianella) were immersed in solutions containing water (control), sucrose, glucose, paclobutrazol, and gibberellic acid. The effects of these treatments on the ethylene release, levels of endogenous gibberellins, glucose, sucrose, and starch were correlated with tuberization of nodal cuttings, excised from potato stems. Paclobutrazol and sucrose improved the percent of tuberization and/or increased tuber weight. In contrast, GA_3 inhibited tuber formation compared with the control. The level of endogenous free GAs was negatively correlated with percent tuberization. However, the level of conjugated GAs was positively correlated with both percent tuberization and tuber weight. The effect of sucrose on potato tuber induction in relation to the possible role of sucrose in GA-conjugate formation is discussed.

It has been suggested that tuber formation/induction is not determined by the concentration of a single compound but rather by balance between inhibitory (e.g., gibberellins) and promoting substance(s) (Okazawa and Chapman 1962). Sucrose may enhance tuber formation, although it is unlikely that sucrose itself is the tuber-inducing stimulus (Ewing 1990). In intact potato plants, assimilates may provide part of the stimuli for tuber formation (for review see Ewing and Struik 1992), probably in interaction with hormones (Vreugdenhil and Helder 1992). In vitro it is necessary to add sucrose to the agar medium in order to obtain tuberization (Gregory 1956). Recently it has been shown that the requirement for high sucrose levels does not represent an osmotic effect or an energy demand but rather a signal for tuber formation (Perl et al. 1991). It is not known, however, how the level of sucrose promotes tuber induction.

The objective of this study was to determine tuber formation in relation to levels of endogenous carbohydrates, ethylene, and gibberellins, especially, the role of sucrose in potato tuber induction. Therefore, the effect of sucrose was compared to that of another source of carbohydrate (glucose) and to the effect of compounds that either inhibit (gibberellic acid) or stimulate (paclobutrazol) tuber formation.

Materials and Methods

Plant Material and Condition of Cultivation

Potato plants (*Solanum tuberosum* L. cv. Dianella) were grown under field conditions. At 6 weeks after planting, the upper part of the main stem (30 cm) with leaves was removed and transferred to 1000-ml glass flasks. Each flask contained 100 ml of solution. There were five treatments: W [distilled water (control)], S (100 g L^{-1} sucrose), G (100 g L^{-1} glucose), P (1 mg L^{-1} paclobutrazol), GA (1 mg L^{-1} GA_3). Samples were incubated for 30 h in the light (100 μE m^{-2} s^{-1}) at 22°C (last 2 h the flasks were air-tight). Then the amount of released ethylene was determined. The upper part of the stem with the leaves was analyzed for glucose, sucrose, and starch content, and used for the assay of gibberellin-like substances. The bottom part of each stem was cut into nodal segments (four from each stem) with one bud and sterilized with 0.1% $HgCl_2$ (washed three times with sterile distilled water). The nodal segments were cultured in test tubes containing solidified (0.8% agar) MS medium (Murashige and Skoog 1962) with addition of sucrose (60 g L^{-1}). The cultures were maintained in the growth chamber for 2 weeks (22°C, 8-h photoperiod, 100 μE m^{-2} s^{-1} light intensity) and then either the number and the weight of microtubers were determined or the stem growth was measured.

There were two replicates, both comprised 15 stems per treatment. The four nodal cuttings were taken from each stem and

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they were randomly assigned to six replicates (20 cuttings per replicate). However, only noncontaminated cuttings were recorded for statistical evaluation. One-way ANOVA was used to calculate the levels of significant differences between treatments. The percentage data were transformed by arcsin prior to calculation. Data are presented in nontransformed form. Simple linear correlation was calculated between tuberization of cuttings (percent, tuber weight) and earlier measured characteristics.

Determination of Ethylene Release

A 1-ml gas sample was withdrawn by syringe from the air-tight flasks, and the ethylene content of the sample was determined using a CHROM 5 gas chromatograph.

Determination of Glucose, Sucrose, and Starch Content

Next, 10 g fresh weight of material was sequentially extracted with both 80% and 50% (v/v) ethanol for 1 h at 60°C, and the extracts were combined. The sucrose (after hydrolysis with invertase) and glucose contents were determined using a specific glucose oxidase assay Oxochrom glucosa (LACHEMA Brno, CZ).

For measurement of starch, remaining plant extracts were homogenized with (0.2 *N*) NaOH. After the mild alkali extraction, the starch was hydrolyzed with (1 *N*) HCl (100°C for 2.5 h), and the liberated amount of glucose was also measured by Oxochrom glucosa assay.

Determination of Free and Conjugated GA-Like Substances Activity

GA-like substances were extracted according to the modified method of Jureková and Repka (1973). We homogenized 20 g fresh weight of haulm with 80% aqueous methanol and filtered it on Whatman No. 1. The filtrate was evaporated in vacuum at 37°C until an aqueous solution remained. The aqueous filtrate was then adjusted to pH 8.5 with (0.5 *N*) $NaHCO_3$ and partitioned with petroleum ether. The petroleum ether phase containing pigments was discarded, and the aqueous phase was partitioned two times with ethyl acetate. Then the aqueous phase was acidified to pH 2.5 with (0.5 *N*) HCl and partitioned three times with ethyl acetate, obtaining an acidic fraction of free GAs. The remaining aqueous phase was partitioned two times with *n*-butanol in order to obtain the conjugated GAs. The butanol fraction was subjected to hydrolysis with (1 *N*) HCl for 1 h at 60°C. Both fractions, free and conjugated GAs, were dried and kept in a freezer. A bioassay to determine GA activity was performed with both fractions, using the lettuce (*Lactuca sativa* L., cv. Kamek) hypocotyl assay (Frankland and Wareing 1960). The biological activity is expressed as nanogram GA_3 equivalents per gram fresh weight of haulm.

Results

Sucrose, paclobutrazol, and GA_3 treatments significantly affected tuberization compared to the control (Fig. 1). The highest percentage of segments

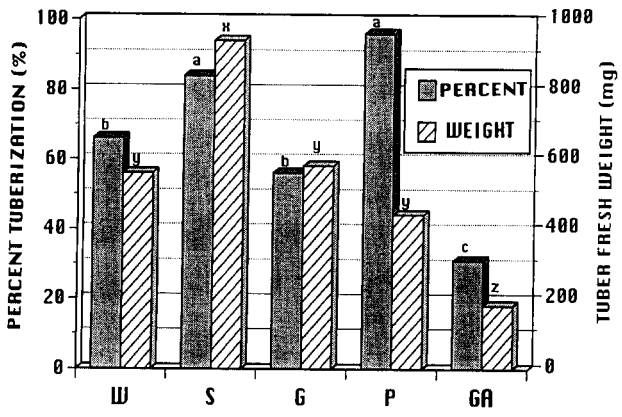

Fig 1. Effect of treatments on potato tuberization. Potato stem segments were immersed in 100 ml of the treatment solution for 30 h (100 $\mu E\ m^{-2}\ s^{-1}$ light intensity and 22°C temperature). Then single-nodal cuttings from the stems of all treatments were cultivated for 2 weeks on MS medium containing 60 g L^{-1} sucrose (22°C, 8 h photoperiod, 100 $\mu E\ m^{-2}\ s^{-1}$ light intensity). The percent tuberization means percentage of nodes that tuberize. The tuber fresh weight is the mean weight of buds that did tuberize. Treatments: W, water (control); S, sucrose (100 g L^{-1}); G, glucose (100 g L^{-1}); P, paclobutrazol (1 mg L^{-1}); GA, gibberellic acid (1 mg L^{-1}). Treatment means marked by different letters are significantly different at $p(0.05)$.

with tubers was observed after paclobutrazol treatment. It was, however, not significantly different from the sucrose treatment, which yielded the highest weight of microtubers. Significantly fewer and smaller tubers were obtained with GA_3 than in the control segments.

Other characteristics also changed when the potato stems were immersed in various solutions for 30 h (Table 1). The addition of sucrose increased the release of ethylene and the content of glucose, sucrose, and conjugated GA. Conversely, a lower free GA level and less stem growth were observed in this treatment.

Glucose treatment only increased ethylene release and glucose content, while the other characteristics were not significantly affected. Paclobutrazol inhibited in vitro stem growth and decreased the content of sucrose and free GAs, but increased the amount of conjugated GAs. Gibberellic acid treatment stimulated stem growth and showed a high level of free GAs and a low level of conjugated GAs. The glucose content in these plant stems and leaves was lower than that in control plants.

The percent tuberization showed a significant and positive correlation with the starch and conjugated gibberellin content in the plant haulm (Table 2). A negative correlation was observed with stem growth and the free gibberellin content. The fresh weight of microtubers showed positive correlations with ethylene release, glucose, sucrose, and conju-

Table 1. Effect of treatments on ethylene release, stem growth, glucose, sucrose, starch, and free and conjugated gibberellins

Characteristics	Treatment					$LSD_{(0.05)}$
	Water	Sucrose	Glucose	PBZ	GA_3	
Ethylene (nl g^{-1} (FW) hr^{-1})	0.34	1.26	1.19	0.48	0.58	0.41
Stem growth (mm)	43.5	6.3	36.5	4.8	56.4	12.3
Glucose [mg g^{-1} (FW)]	1.26	2.55	1.70	0.92	0.63	0.44
Sucrose [mg g^{-1} (FW)]	0.68	1.89	0.95	0.27	0.54	0.39
Starch [mg g^{-1} (FW)]	2.49	2.10	1.81	2.55	2.28	0.72
Conjugated GAs [ng (GA_3) g^{-1} (FW)][a]	1.38	1.99	1.50	2.53	0.16	0.46
Free GAs [ng (GA_3) g^{-1} (FW)][a]	1.67	1.13	1.98	0.29	4.07	0.39

Note. Potato stem segments were immersed in 100-ml solution for 30 h (100 μE m^{-2} s^{-1} light intensity and 22°C temperature). Measurements were made at the end of treatment, except stem growth which was observed after 2 weeks cultivation on MS medium containing 60 g L^{-1} sucrose (22°C, 8-h photoperiod, 100 μE m^{-2} s^{-1} light intensity). Treatments: water (control), sucrose (100 g L^{-1}), glucose (100 g L^{-1}), paclobutrazol (1 mg L^{-1}), gibberellic acid (1 mg L^{-1}).

[a] Lettuce hypocotyl bioassay.

Table 2. Simple linear correlation coefficients between tuberization and investigated characteristics

	Ethylene release	Stem growth	Glucose content	Sucrose content	Starch content	Conj.GA activity	Free GA activity
Percent tuberization	−	− − −	+	+	+ + +	+ + +	− −
Tuber fresh weight	+ + +	−	+ + +	+ + +	−	+ + +	−

Note: Potato stem segments were immersed in 100-ml solution for 30 h (100 μE m^{-2} s^{-1} light intensity and 22°C temperature). Then the single-nodal cuttings from treated stems were cultivated for 2 weeks on MS medium containing 60 g L^{-1} sucrose (22°C, 8 h photoperiod, 100 μE m^{-2} s^{-1} light intensity). The percent tuberization means percentage of nodes that tuberize. The tuber fresh weight is the mean weight of buds that did tuberize.

+, − Correlation coefficient is positive or negative respectively, but not significant.

− −, − − − Correlation coefficient is significantly negative at $p(0.05)$ or $p(0.01)$, respectively.

+ +, + + + Correlation coefficient is significantly positive at $p(0.05)$ or $p(0.01)$, respectively.

gated GAs levels. However, there was a time lapse of 2 weeks between evaluation of characteristics and the measurement of tuberization.

Discussion

The effect of paclobutrazol on endogenous GA level and tuberization were similar to the effect of CCC as described by Guiñazú et al. (1988). These authors observed that CCC not only lowered free GA levels through inhibition of their synthesis, but also stimulated the formation of conjugated GAs. GA conjugates *per se* are presumably biologically inactive hormones, mostly accumulated by ripening seeds (for review see: Sembdner et al. 1991). However, they were also found in potato shoots (Van den Berg et al. 1993), roots (Guiñazú et al. 1988) and tubers (Hayashi et al. 1962).

This investigation confirmed previous reports that paclobutrazol-treated plants show a decrease in the levels of most sugars (Balamani and Poovaiah 1985). On the other hand, GA_3 application in our experiments decreased the glucose content in haulms. This is in contrast to a report by Mares et al. (1981), which showed that GA_3 treatment increased reducing sugar content and lowered sucrose content in tuber tissue. This discrepancy may be explained by the ability of gibberellin to promote "sink strength" at the point of application (Mulligan and Patrick 1979). Because only the bottom 3 cm of the stem were immersed into the GA_3 solution, this part of the stem could increase the level of endogenous GAs more rapidly than the upper part (the part analyzed), and the reducing sugars shifted from the apical toward the basal part of the stem.

The high release of ethylene observed in sucrose and glucose treatment was similar to that described for the start of tuber formation in potato (Bečka et al. 1985), where a high content of glucose and fructose was associated with a high production of ethylene. The authors explained this association by the degradation of glucose via pyroacemic acid, acetic acid, and fumaric acid, which is finally decarboxylated. Consequently, the high production of ethyl-

ene at the beginning of tuberization later decreased along with the lower endogenous glucose level. Thus it seems that ethylene release is not in direct association with potato tuberization even though in our experiments a significant correlation with average tuber weight was found.

It was reported that tuberization is negatively correlated with the level of reducing sugars (mostly glucose) (Darpas et al. 1986; Hawker et al. 1979). In our experiments no significant correlation between glucose level and percentage of tuberized segments was found. Furthermore, a positive correlation existed between the level of glucose and the weight of microtubers; after sucrose treatment both glucose content and average microtuber weight increased substantially. These observations are in line with earlier results in which a high concentration of soluble sugars was found in stolon tips during the tuberization stage (Bečka et al. 1985; Burt 1964).

Unlike sucrose treatment, glucose treatment did not improve potato tuberization. Similar results were described for both tuber formation (Ewing 1985), and for the expression of patatin class I promoter (Wenzler et al. 1989).

Hypothesis of Sucrose Action

If the tuber induction process is primarily regulated by a balance between gibberellin(s) and promoting substance(s) (Okazawa and Chapman 1962), then there are two ways to change the GA/promoter ratio and cause potato tuber formation. One is increasing the level of naturally occurring substance(s) which is involved in tuberization—probably tuberonic acid, tuberonic acid glucoside, and/or tuberonic acid glucoside methyl ester (Koda 1992). The second is decreasing the level of biologically active GA(s), as by the use of "antigibberellins" that inhibit GA formation (e.g., paclobutrazol). There is, however, also a possibility of forming biologically inactive free and/or conjugated GAs. GA conjugates are storage forms preferentially involved in rapid changes in the levels of physiologically active GA (Sembdner et al. 1991). Conjugated GAs are built with glucosyl moieties, for which the exclusive donor is UDPglucose (Sembdner et al. 1985). The UDPglucose is formed mainly from sucrose by sucrose synthase (ap Rees and Morrell 1990). The present hypothesis is that a high exogenous sucrose supply causes the formation of excess UDPglucose, which in turn increases the conjugation of free GAs. In this way the sucrose flux would directly change the level of active hormones participating in potato tuber induction (Fig. 2). According to this hypothesis, glucose does not significantly affect potato tuberization, because only a small amount of endogenous glucose is converted to sucrose. Cells that contain a high glucose, but low sucrose level show weak sucrose synthase activity (Sowokinos and Varns 1992); and, therefore, less UDPglucose is formed (Geigenberger and Stitt 1993).

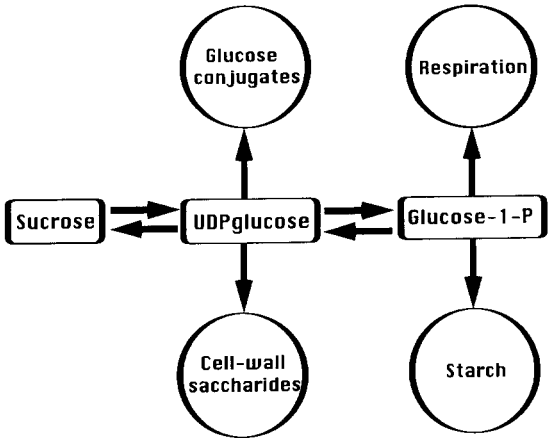

Fig. 2. A simplified scheme to show a sucrose role in potato tuberization via the formation of glucose conjugates.

The suggested hypothesis is consistent with several observations: (1) Culture on media containing gibberellin acid increased the amount of sucrose required to give maximal levels of patatin induction (Park 1990). (2) A higher level of sucrose was present in a transgenic potato plant and then significantly more tubers were formed (Müller-Röber et al. 1992). (3) As sucrose content in the apoplast of potato plants declined, the transformed plants had fewer tubers (Heineke et al. 1992). (4) A transgenic plant contained an increased UDPglucose/hexose–phosphate ratio, and the plants produced a large number of tubers (Jelitto et al. 1992; Sonnewald 1992).

Tuber induction in potato does not absolutely require a high level of endogenous sucrose, for example, after paclobutrazol treatment. However, the high sucrose apparently provides a favorable environment in which a tuberization stimulus can act.

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