Sugar Use in Relation to Shoot Induction by Sorbitol and Cytokinin in Apple

Saila T. Karhu

Agricultural Research Centre, Institute of Horticulture, Toivonlinnantie 518, FIN-21500 Piikkiö, Finland

ADDITIONAL INDEX WORDS. apical dominance, benzyladenine, carbohydrate, invertase, in vitro culture, *Malus domestica*, micropropagation

ABSTRACT. Microshoots of 'McIntosh' apple (*Malus domestica* Borkh.) were grown on Murashige-Skoog (MS) nutrient medium supplemented with either sucrose or sorbitol or with sucrose and an elevated level of cytokinin. Shoot growth was recorded and concentrations of fructose, glucose, sorbitol, and sucrose were analyzed in nutrient media and shoots during a 6-week subculture period. Axillary branching was stimulated by high cytokinin and sorbitol media, with increased biomass production and carbohydrate use on the high-BA medium only. The sucrose in the nutrient medium was hydrolyzed to fructose and glucose, which were equally taken up by shoots. Sorbitol was taken up somewhat less effectively. The elevated level of BA decreased sucrose hydrolysis in the nutrient medium. There were high concentrations of sorbitol in shoots grown on the sorbitol medium, and sorbitol also accumulated at the end of the culture period in shoots grown on sucrose. The amount of sucrose was low, and glucose was more abundant than fructose in microshoots. The starch content of leaves was not affected by treatments or sampling time. Chemical names used: N⁶-benzyladenine (BA).

Carbohydrate supplements are essential in the tissue culture of plants when heterotrophic or mixotrophic systems are used. A number of carbohydrates support growth, but their requirement and suitability can vary according to the plant species. The exogenous carbohydrates of the nutrient medium serve as energy and carbon sources, and these carbohydrates affect the physiology and differentiation of tissues (George, 1993). They also influence tissue growth, organ induction, and differentiation (Aloni, 1980; Steffen et al., 1988). Interactions of carbohydrates with other constituents of nutrient media are reported in several studies (Cabasson et al., 1995; Lee and Titus, 1993; Simko, 1994).

The carbon metabolism of apple, like that of several other woody *Rosaceae* species, is special in the sense that sorbitol (D-glucitol) is the main product of photosynthesis and the form of transportation of carbohydrates (Bielecki, 1982). There are reports on successful shoot cultures of *Malus* species in the presence of either sorbitol, sucrose, fructose, or glucose, but the type of carbohydrates affects the growth and the frequency of shoots developed (Chong and Pua, 1985; Karhu, 1995; Pua and Chong, 1984; Welander et al., 1989). The carbohydrate supply seems to affect the apical dominance of shoots, resembling the effect of cytokinins that are generally used to induce axillary shoot formation in shoot culture systems in vitro.

To my knowledge, there is no information on the carbohydrate use of apple shoots cultured on medium containing sorbitol or sucrose. The aim of my study was to investigate the efficiency of these carbohydrates in the shoot regeneration process. Changes in the content of soluble carbohydrates and starch in shoots also were compared using two levels of cytokinin to discover whether changes in carbohydrate metabolism caused by sorbitol supply are consistent with those related to cytokinin.

Materials and Methods

GROWING AND SAMPLING OF MATERIAL. Shoot cultures of 'McIntosh' apple were initiated from nodal shoot sections from half-year-old, own-rooted plantlets micropropagated and grown in a greenhouse. Shoot cultures grown in vitro with 6-week subculture periods were used in the experiment. The basal multiplication medium consisted of Murashige-Skoog (MS) nutrients (Murashige and Skoog, 1962) without glycine but with 3.0 μM thiamine-HCl, 4.4 μM BA, 0.5 μM indole-3-butyric acid, and Difco Bacto-agar at 6 g·L⁻¹, with pH adjusted to 5.5 before autoclaving. The stock cultures had been initiated and maintained for 2 years on 165 mM (3%) sorbitol (Sigma, St. Louis). Because adaptation of apple shoots to the carbon source in the culture medium has been suggested (Pua and Chong, 1985), shoots were transferred onto a medium containing 88 mM (3%) sucrose (BDH, Dorset, England) to check their ability to grow on this carbohydrate. The experiment was started with shoots grown on the sucrose medium for one passage. Culture jars were kept in a growth room at 25 $\pm$ 2 °C with a 16-h photoperiod provided by cool-white fluorescent lamps at a photosynthetic photon flux density of 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Shoots were cultured on the basal multiplication medium with 88 mM sucrose (control sucrose medium) or on the same medium but either with 165 mM sorbitol substituted for sucrose (sorbitol medium) or with the sucrose concentration kept unchanged but the concentration of BA increased from 4.4 to 13.3 $\mu\text{mol}\cdot\text{L}^{-1}$ (high-BA medium). Sorbitol and sucrose were filter sterilized and added to the autoclaved nutrient medium. Four shoot-tips (1 to 2.5 cm long) were cultured on 16 mL nutrient medium in a 130-mL glass jar covered with a polypropylene lid.

The number of visible axillary shoots of each shoot explant was recorded in three jars per treatment after 2, 4, and 6 weeks in culture. Traces of nutrient medium adhering to shoots were removed with tissue paper, and leaves, stems, and callus tissues were separated and their fresh masses measured. Leaves and stems from each culture jar were frozen separately in liquid N and were ground to a powder in a cooled mortar. Samples were stored at -70 °C before carbohydrate analysis. Part of the material was dried at 80 °C for 24 h to obtain dry mass of leaf and stem tissues.

Nutrient media in culture jars also were frozen for carbohydrate analyses at each sampling period. In addition to the jars containing

Received for publication 3 June 1996. Accepted for publication 8 Jan. 1997. I thank Anton F. Croes for valuable comments and Katriina Ahanen and Päivi Tuomola for skillful technical assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

shoot explants, reference jars with nutrient medium but without plant material were incubated in the growth room to test for stability of the carbohydrate composition in media. Media were sampled at 2-week intervals.

CARBOHYDRATE ANALYSIS. To prepare samples of nutrient media for carbohydrate analysis, the agar medium in each jar was mixed, and then, 1 g was homogenized with 100 mL of water. To prepare plant samples, 0.1 g of leaf or stem powder was treated in the extraction medium by sonication at 60 °C (model 3200; Branson, Soest, The Netherlands). Soluble carbohydrates were extracted from plant material in 5 mL water during 3 h. Starch was extracted over 40 min in 1 mL DMSO plus 0.25 mL 8 M HCl. To the starch samples, 0.25 mL 8 M NaOH and 0.112 M Na-citrate buffer (pH 4.0) was added for a final volume of 5 mL (Lukaszewska and Gorin, 1988). Samples were filtered through a membrane filter (model Me 27; Schleicher & Schuell, Dassel, Germany).

Fructose, glucose, sorbitol, sucrose, raffinose (plus other α -galactosides), and starch were determined by enzymatic assays (Bergmeyer and Bernt, 1974; Boehringer Mannheim, 1989) using assay kits of Boehringer Mannheim (Boehringer, Mannheim, Germany). The absorbance was determined using a UV-VIS spectrophotometer (Ultrospec III, Pharmacia LKB Biochrom, Cambridge, England) at 340 nm. All analyses were made in triplicate. Each sample represented the material of one culture jar, except at week 2 when shoots from two culture jars were pooled to obtain enough material for one sample.

The total concentration of soluble carbohydrates refers to the sum of concentrations of all measured sugars and sorbitol. Carbohydrate uptake from the medium was calculated by subtracting the measured carbohydrate concentration of the medium at each analysis time from the initial carbohydrate content of the medium. The total amount of carbohydrates accumulated in the shoot mass in each culture jar was calculated by multiplying the carbohydrate concentration in leaves and stems with the total leaf and stem biomasses, respectively, and summing up these values.

STATISTICAL ANALYSIS. The effects of treatments, with three

Table 1. Axillary shoot count and their biomass produced by a shoot explant during a 6-week subculture period (means $\pm$ SE, $n = 12$).

Medium	Axillary shoots (no.)	Fresh mass at week 6 (mg)			
		Leaves	Stems	Calli	Total
Sorbitol (165 mM sorbitol 4.4 μ M BA)	3.9 $\pm$ 0.2	169 $\pm$ 11	161 $\pm$ 5	31 $\pm$ 2	361 $\pm$ 17
Control sucrose (88 mM sucrose 4.4 μ M BA)	3.2 $\pm$ 0.2	180 $\pm$ 15	162 $\pm$ 11	35 $\pm$ 10	377 $\pm$ 13
High BA (88 mM sucrose 13.3 μ M BA)	4.1 $\pm$ 0.3	209 $\pm$ 21	251 $\pm$ 8	45 $\pm$ 7	505 $\pm$ 28
ANOVA ²	*	NS	***	NS	***
Contrasts ³					
Sorbitol vs. control sucrose	*	NS	NS	NS	NS
High BA vs. control sucrose	**	NS	***	NS	**

²ANOVA = analysis of variance.

³Single degree of freedom contrasts.

NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

replications at each sampling time, were tested over all sampling times and separately at each time with analysis of variance using the SAS GLM procedure (SAS Institute, Cary, N.C.). No transformation of data was necessary. To detect differences between the control sucrose and sorbitol treatments and between the control sucrose and high-BA treatments, these were contrasted pairwise at each sampling time by the SAS GLM contrast statement.

Results

SHOOT PRODUCTION. High-BA and sorbitol medium stimulated axillary shoot production more than the control sucrose medium (Table 1). At a higher BA concentration, biomass production was highest because of stimulated axillary shoot growth. At the end of the culture period, the biomass of shoots grown in the presence of sorbitol did not, however, differ from that of the shoots grown on the sucrose medium.

CARBOHYDRATES IN NUTRIENT MEDIA. The carbohydrates in the nutrient media were used only in part: at the end of the culture period, half of the initial amount was still present in the control sucrose medium (Table 2). The carbohydrate uptake was higher in the high-BA medium (57%) than in the sorbitol medium (40%). Sucrose in the nutrient media was hydrolyzed to fructose and glucose during the subculture period in the presence of shoot explants. There were no differences between the concentrations of monosaccharides, indicating an equal uptake of glucose and fructose. The hydrolysis of sucrose was somewhat slowed by the high BA concentration (Table 2). Only traces (<0.5 g·L⁻¹) of sorbitol were detected in the two media with sucrose. Similarly, there were only traces of fructose, glucose, and sucrose in the sorbitol medium. Sucrose and sorbitol proved stable in the jars without shoot explants (data not presented).

CARBOHYDRATES IN SHOOTS. The highest total concentration of soluble carbohydrates was in the shoots grown on the sorbitol medium, and the lowest total concentration was in the shoots on the high-BA medium (Table 3). The differences were decreased toward the end of the culture period, mainly because of the increased carbohydrate concentrations in the shoots on the two media with sucrose. The total amount of soluble carbohydrates at week 6 accumulated in the axillary shoot mass in each culture jar, calculated based on the total biomasses and carbohydrate concentrations of leaves and stems, was lower ($P = 0.0254$) on the high-BA medium than on the control sucrose medium (19.3 and 23.3 mg, respectively), with intermediate values in the shoots on sorbitol medium. The lower concentrations of carbohydrates on the high-BA medium, thus, were not caused by dilution related to a higher biomass but because of their higher use.

Sorbitol was the main soluble carbohydrate in the shoots grown on sorbitol medium (Table 3). Its concentration was lowest on the high-BA medium, but, toward the end of the culture period, sorbitol also accumulated in the shoots on the two media with sucrose. The amount of sucrose in shoots was low for all treatments (Table 3).

Concentrations of glucose and fructose in stems grown on the control sucrose medium or on the high-BA medium were higher than on the sorbitol medium (Table 3), resulting from the transport of these monosaccharides after hydrolysis of sucrose. There were high amounts of glucose in shoots. The difference between the concentrations of glucose and fructose was particularly high in the leaves. The starch concentration of leaves was low and stable, varying between 5.3 and 6.8 mg·g⁻¹ (on a dry mass basis), and it was not affected by the treatments and sampling time. No raffinose or other α -galactosides were detected in shoots.

Discussion

The cytokinin concentration and the carbohydrate type of the nutrient medium affected axillary shoot production as well as the concentrations of different carbohydrates in apple shoot tissues. BA stimulated shoot formation and biomass production. Higher uptake of carbohydrates from the nutrient medium but lower concentrations in leaves and stems also showed that more carbohydrates were used in the presence of high-BA concentration.

Sorbitol also induced shoot formation, although to a lesser extent than BA. Unlike on the high-BA medium, the use of carbohydrates by microshoots, however, was not stimulated on the sorbitol medium, but the carbohydrate accumulation was highest in shoots. Thus, the sugar metabolism is different in these two treatments, although the apical dominance of shoot explants was affected by both. Sorbitol did not cause higher biomass production, but resulted in production of shorter shoots. Thus, sorbitol affected induction rather than subsequent growth of axillary shoots. Analogously, sorbitol has been preferred to sucrose, glucose, and fructose in bud initiation of apple shoot-tip explants as well as in adventitious shoot regeneration of some apple genotypes, although all these sugars can support shoot growth (Pawlicki and Welander, 1994; Pua and Chong, 1984).

The mechanisms by which sorbitol can affect axillary branching is not clear. In potato tuber production, high levels of soluble carbohydrates accumulated in plants led to decreased apical dominance and allowance of resting buds to grow (Kobmann et al., 1995). The results of that study and my experiment suggest that control mechanisms other than carbohydrate requirement and use are involved. Different uptake and use of sucrose and sorbitol led to different carbohydrate concentrations and, consequently, to different osmotic potentials in the shoots and media. The increased carbohydrate concentration of plant tissues, as was in shoots grown on sorbitol, has been proposed to act as a message-like factor in morphogenetic events (Kinet, 1995; Perl et al., 1991), and, also, osmoregulation has been associated with plant organ initiation (Brown et al., 1979; Galiba and Erdei, 1986). The interaction of carbohydrates with growth regulator effects also has influenced organ development (Simko, 1994). The influences on nutrient use, for example, through controlling enzymatic activity (Lee and Titus, 1993) and through direct reactions with mineral nutrients (George, 1993), are also possible in the regulation of morphogenesis by carbohydrates. Effects of carbohydrates on the mineral nutrient content of apple shoot tissues are currently under investigation.

The complete hydrolysis of sucrose in the nutrient medium with shoot explants, as shown in this study, can be attributed to the action of acid invertase that has been transferred into the culture

Table 2. Content of soluble carbohydrates of nutrient media during a 6-week subculture period. Values are means $\pm$ SE of three replications.

Weeks in culture	Nutrient medium			ANOVA ^y	Contrasts ^z	
	Sorbitol	Control sucrose	High BA		Sorbitol vs. cont.	High BA vs. cont.
Soluble carbohydrates, total ^x (g·L ⁻¹)						
0 ^w	30.8 ± 0.2	30.1 ± 0.3	30.4 ± 0.4	NS	NS	NS
2	28.4 ± 0.3	27.0 ± 0.1	27.5 ± 0.6	NS	NS	NS
4	21.9 ± 0.4	19.9 ± 0.3	18.3 ± 1.0	*	NS	NS
6	18.5 ± 0.1	15.6 ± 0.8	12.9 ± 1.4	*	NS	NS
Sucrose (g·L ⁻¹)						
0	0.2 ± 0.04	29.7 ± 0.2	29.9 ± 0.2	***	***	NS
2	0.2 ± 0.1	18.2 ± 0.3	20.7 ± 1.2	***	***	*
4	0	1.5 ± 0.6	3.9 ± 1.0	*	NS	*
6	0.4 ± 0.3	0.3 ± 0.2	0.4 ± 0.3	NS	NS	NS
Glucose (g·L ⁻¹)						
0	0	0.2 ± 0.1	0.04 ± 0.04	NS	NS	NS
2	0.1 ± 0.1	4.6 ± 0.2	3.3 ± 0.4	***	***	**
4	0.4 ± 0.1	9.5 ± 0.5	7.4 ± 0.4	***	***	**
6	0	7.5 ± 0.3	6.2 ± 0.7	***	***	NS
Fructose (g·L ⁻¹)						
0	0.1 ± 0.05	0	0	*	*	NS
2	0.02 ± 0.02	4.2 ± 0.1	3.5 ± 0.7	***	***	NS
4	0.02 ± 0.02	8.9 ± 0.5	7.0 ± 0.4	***	***	**
6	0.04 ± 0.04	7.8 ± 0.3	6.3 ± 0.6	***	***	*

^zSingle degree of freedom contrasts, cont. = control sucrose medium.

^yANOVA = analysis of variance.

^xTotal = sorbitol + sucrose + glucose + fructose.

^wWeek 0 = Beginning of experiment, no shoots grown on medium.

NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

medium by plant tissues (Masuda et al., 1989). Slower inversion to glucose and fructose in the presence of higher BA suggests interaction between the enzyme activity and cytokinin. Equal uptake of glucose and fructose found in this study also has been reported in some, but not all, apple genotypes (Ribaux et al., 1995; Singha et al., 1987).

It was noteworthy that glucose and fructose were suitable carbon sources for shoot proliferation, although the sucrose concentration of shoot tissues was low. At the end of the culture period, there was a tendency for sugars to be converted to sorbitol, even in the shoots cultured on sucrose. This result is similar to in vivo apple leaves in which sorbitol is the principal form of transport and storage of carbohydrates, followed by sucrose, and in equal concentrations, glucose and fructose (Chong and Taper, 1971; Lee and Titus, 1993; Loescher et al., 1982).

Glucose occurred remarkably more abundantly than fructose in the microshoot leaves. To my knowledge, this result has not been reported in apple leaves in vivo but might be related to the effects of cytokinin on carbohydrate metabolism (Simpkins and Street, 1970). The starch content of leaves was rather low and did not vary during the culture period. Similarly, an increase in sucrose concentration of nutrient medium from 3% to 5% has been a prerequisite for starch accumulation in leaves of rose shoot cultures (Capellades et al., 1991).

In conclusion, although sorbitol stimulated shoot initiation, neither higher biomass production nor carbohydrate use was involved, as was the case in the presence of higher cytokinin content in the nutrient medium. Thus, the higher shoot production with sorbitol as opposed to with sucrose was not caused by more efficient carbohydrate metabolism, but by other control factors

Table 3. Content of soluble carbohydrates in leaves and stems of shoots grown on different nutrient media. Values are means of three replications.

Weeks in culture	Nutrient medium						Contrasts ²					
	Sorbitol		Control sucrose		High BA		ANOVA ³		Sorbitol vs. cont.		High BA vs. cont.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Soluble carbohydrates, total⁴ [mg·g⁻¹ (on a dry mass basis)]												
0 ^w			149.0	49.0								
2	155.5	68.9	115.6	46.4	101.3	33.8	*	***	*	**	NS	*
4	145.7	72.2	113.6	62.8	93.5	50.3	***	***	**	**	*	***
6	143.7	68.1	140.6	62.2	106.5	58.8	***	*	NS	NS	***	NS
Sorbitol [mg·g⁻¹ (on a dry mass basis)]												
0			56.6	19.5								
2	76.6	54.1	22.5	8.6	14.8	2.3	***	***	***	***	NS	NS
4	70.8	49.8	23.6	12.9	13.3	5.6	***	***	***	***	**	*
6	75.4	49.9	42.3	23.0	22.2	17.8	***	***	***	***	***	NS
Sucrose [mg·g⁻¹ (on a dry mass basis)]												
0			0.9	1.6								
2	2.3	2.7	5.5	5.9	3.6	6.0	NS	NS	*	NS	NS	NS
4	1.0	1.8	0.9	4.4	9.4	7.2	***	**	NS	*	***	*
6	1.0	1.6	6.0	3.8	3.0	3.2	NS	*	*	**	NS	NS
Glucose [mg·g⁻¹ (on a dry mass basis)]												
0			72.9	18.0								
2	61.7	7.5	61.9	18.0	59.2	14.5	NS	***	NS	***	NS	**
4	59.7	15.4	64.6	25.8	47.7	20.2	*	***	NS	***	*	***
6	56.9	11.5	69.9	20.0	56.1	21.1	**	***	**	***	**	NS
Fructose [mg·g⁻¹ (on a dry mass basis)]												
0			18.6	10.0								
2	14.9	4.6	25.6	13.9	23.7	11.0	***	***	***	***	NS	***
4	14.3	5.2	24.4	19.7	23.1	17.3	**	***	***	***	NS	***
6	10.4	5.2	22.3	15.5	25.1	16.7	***	***	***	***	NS	NS

²Single degree of freedom contrasts, cont. = control sucrose medium.³ANOVA = analysis of variance.⁴Total = sorbitol + sucrose + glucose + fructose.^wWeek 0 = Beginning of experiment, shoots grown for one passage on control sucrose medium.NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

interacting with the carbohydrate supply. The carbohydrate content of leaves differed from that reported on apple leaves in vivo, showing modification of carbohydrate metabolism by the in vitro growth conditions.

Literature Cited

- Aloni, R. 1980. Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. *Planta* 150:255–263.
- Bergmeyer, H.U. and E. Bernt. 1974. Sucrose, p. 1176–1179. In: H.U. Bergmeyer (ed.), *Methods in enzymatic analysis*, 2nd ed. vol. 3. Academic Press, New York.
- Bielecki, R.L. 1982. Sugar alcohols, p. 158–192. In: F.A. Loewus and W. Tanner (eds.), *Encyclopedia of plant physiology*, New series. vol. 13A, Springer Verlag, Berlin.
- Boehringer Mannheim. 1989. *Methods of biochemical analysis and food analysis using test-combinations*. Boehringer Mannheim GmbH, Mannheim, Germany.
- Brown, D.C.W., D.W.M. Leung, and T.A. Thorpe. 1979. Osmotic requirement for shoot formation in tobacco callus. *Physiol. Plant* 46:36–41.
- Cabasson, C., P. Ollitrault, F.-X. Côte, N. Michaux-Ferrière, D. Dambier, R. Dalnic, and C. Teisson. 1995. Characteristics of *Citrus* cell cultures during undifferentiated growth on sucrose and somatic embryogenesis on galactose. *Physiol. Plant* 93:464–470.
- Capellades, M., R. Lemeur, and P. Debergh. 1991. Effects of sucrose on starch accumulation and rate of photosynthesis in *Rosa* cultured in vitro. *Plant Cell Tiss. Org. Cult.* 25:21–26.
- Chong, C. and E.-C. Pua. 1985. Carbon nutrition of Ottawa 3 apple rootstock during stages of in vitro propagation. *J. Hort. Sci.* 60:285–290.
- Chong, C. and C.D. Taper. 1971. Daily variation of sorbitol and related carbohydrates in *Malus* leaves. *Can. J. Bot.* 49:173–177.
- Galiba, G. and L. Erdei. 1986. Dependence of wheat callus growth, differentiation and mineral content on carbohydrate supply. *Plant Sci.* 45:65–70.
- George, E.F. 1993. *Plant propagation by tissue culture*, Part 1: The technology. Exegetics Ltd., Edington, England.
- Karhu, S. 1995. The quality of applied carbohydrates affects the axillary branching of apple microshoots. *Bul. Recherches Agron. Gembloux* 30:21–27.
- Kinet, J.M. 1995. Recent developments in understanding flowering control. *Mededelingen Faculteit Landbouww. Univ. Gent* 60:1589–1596.
- Koßmann, J., B.-M. Röber, J. Riesmeier, U. Sonnewald, and L. Willmitzer. 1995. The influence of source-sink relations on tuber development in potato. *Mededelingen Faculteit Landbouww. Univ. Gent* 60:1653–1658.
- Lee, H.J. and J.S. Titus. 1993. Relationship between nitrate reductase activity and level of soluble carbohydrates under prolonged darkness in MM.106 apple leaves. *J. Hort. Sci.* 68:589–596.
- Loescher, W.H., G.C. Marlow, and R.A. Kennedy. 1982. Sorbitol metabolism and sink-source interconversions in developing apple leaves. *Plant Physiol.* 70:335–339.
- Lukaszewska, A.J. and N. Gorin. 1988. Simplification of the Boehringer Mannheim enzymic analysis of starch in the methanol-insoluble residue of freeze-dried corollas from cut 'Sonia' roses. *Gartenbauwissenschaft* 53:49–51.
- Masuda, H., T. Takahashi, and S. Sugawara. 1989. Acid and alkaline

- invertases in suspension cultures of sugar beet cells. *Plant Physiol.* 86:312–317.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473–497.
- Pawlicki, N. and M. Welander. 1994. Adventitious shoot regeneration from leaf segments of in vitro cultured shoots of the apple rootstock Jork 9. *J. Hort. Sci.* 69:687–696.
- Perl, A., D. Aviv, L. Willmitzer, and E. Galun. 1991. In vitro tuberization in transgenic potatoes harboring β -glucuronidase linked to a patatin promoter: effects of sucrose levels and photoperiods. *Plant Sci.* 73:87–95.
- Pua, E.-C. and C. Chong. 1984. Requirement for sorbitol (D-glucitol) as carbon source for in vitro propagation of *Malus robusta* no. 5. *Can. J. Bot.* 62:1545–1549.
- Pua, E.-C. and C. Chong. 1985. Regulation of in vitro shoot and root regeneration in 'Macspur' apple by sorbitol (D-glucitol) and related carbon sources. *J. Amer. Soc. Hort. Sci.* 110:705–709.
- Ribaux, M., J. O'Rourke, S. Gavillet, and C. Moncousin. 1995. Carbohydrate absorption during the in vitro proliferation of *Malus* EM IX. *Bul. Recherches Agron. Gembloux* 30:103–111.
- Simko, I. 1994. Sucrose application causes hormonal changes associated with potato tuber induction. *J. Plant Growth Regulat.* 13:73–77.
- Simpkins, I. and H.E. Street. 1970. Studies on the growth in culture of plant cells. VII. Effects of kinetin on the carbohydrate and nitrogen metabolism of *Acer pseudoplatanus*, L cells grown in suspension culture. *J. Expt. Bot.* 21:170–185.
- Singha, S., G.H. Oberly, and E.C. Townsend. 1987. Changes in nutrient composition and pH of the culture medium during in vitro shoot proliferation of crabapple and pear. *Plant Cell Tiss. Org. Cult.* 11:209–220.
- Steffen, J.D., R.M. Sachs, and W.P. Hackett. 1988. Growth and development of reproductive and vegetative tissues of *Bougainvillea* cultured in vitro as a function of carbohydrate. *Amer. J. Bot.* 75:1219–1224.
- Welander, M., N.T. Welander, and A.-S. Brackman. 1989. Regulation of in vitro shoot multiplication in *Syringa*, *Alnus* and *Malus* by different carbon sources. *J. Hort. Sci.* 64:361–366.