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Hormone-Like Effects of Sucrose in Plant in vitro Cultures

By

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Summary

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Putative hormone-like effects were investigated in a number of plant species cultured in vitro among which most interesting findings were observed in *Dracaena fragrans* Ker-Gawl. and carob (*Ceratonia siliqua* L.). In carob, increased sucrose nutrition prevented appearance of lenticel hypertrophy, increased leaf size and in dark grown cultures enhanced etiolated growth. In *Dracaena fragrans,* increased sucrose nutrition induced two distinct morphogenetic responses, branching of adventitious roots and formation of axillary buds.

Introduction

In recent years the nutritional and osmotic role which in plant physiology is usually attributed to sucrose is gradually changing due to many new findings which indicate that sucrose in plants plays an important regulatory role. KOCH 1996 reviewed a number of these reports concluding that plants have sensor systems by which they can detect feast-famine conditions ie. changes in levels of available carbohydrates. KRUGER 1997 states that sucrose is the most important plant carbohydrate since it is the principle product of photosynthesis and major translocation and storage form of carbohydrates.

Sucrose at 2-3% concentration is the carbohydrate of choice for establishment and maintenance of in vitro cultures of most plant species. Shoot cultures although functionally equipped to perform photosynthesis are usually

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mixotrophic (KOZAI 1991) and obtain most of the required energy from dissimilation due to the low concentration of carbon dioxide which is usually available to in vitro cultures.

We investigated the effect of increased sucrose nutrition in shoot cultures of many species looking for morphogenetic responses which could be used as proof for the hormone-like effects of sucrose. Although some interesting sucrose effects were registered in shoot cultures of several species including potato (VINTERHALTER & al. 1996) true morphogenetic responses were registered only in cultures of *Dracaena fragrans* and carob *(Ceratonia siliqua* L.).

Material and Methods

All cultures were maintained on MS (1962) type medium in a growth room adjusted to 25 ± 2 °C, photoperiod 16/8 hours light to darkness with cool-white fluorescent lamps providing 33-46.5 $\mu mol.m^{-2}s^{-1}$. Details for the establishment of shoot cultures were published as follows: *D. fragrans* (VINTERHALTER & VINTERHALTER 1997) and carob (VINTERHALTER & al. 1992). Experiments with carob were performed in 100 ml wide neck Erlenmeyer flasks with sealed cotton-wool plugs and basal medium comprising 2.22 µM BA and 0.49 µM IBA, with 5-6 explants per flask. Dracaena cultures were maintained on medium with 0.44 µM BA and 5.37 µM NAA. Treatments were performed in Φ 18 x 180 mm test tubes with stationary liquid medium and stainless steel supports for explants. All experiments were scored after 5 weeks and repeated at least three times with 24-32 replicates per treatment.

Results and Discussion

In carob sucrose manifested a number of interesting growth effects. One of the outstanding finding was that increased sucrose nutrition can prevent appearance of in vitro lenticel hypertrophy, physiological disorder characteristic for carob and certain *Populus* species (LLEDO & al. 1995). Thus on media supplemented with more than 3% sucrose the number of hypertrophied lenticels per internode is significantly reduced. Sucrose affects development of leaf and there was a distinct maximum at 5% sucrose. Recently we demonstrated that sucrose is also indispensable for the induction of etiolated growth in darkness. At low sucrose concentrations (2-3%) growth of cultures transferred to continuous darkness is temporarily arrested but it later continues by activation of axillary buds which then develop as etiolated shoots. Etiolated cultures have much higher multiplication rates than cultures maintained in light (Fig 1.). The terminal bud of the explant can also grow in darkness as a shoot with etiolated structure but only on media containing 5% or more sucrose. Under this condition it directly converts structure from green photosynthetic into etiolated shoot, often within single internode. It is interesting to note that fructose and glucose could not replace sucrose as carbohydrate source for etiolated dark growth.

In *Dracaena*, sucrose triggers two distinct morphogenetic effects: (i) formation of axillary buds and, (ii) first order branching of adventitious roots. These two processes appeared concomitantly during rooting (VINTERHALTER &

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VINTERHALTER 1997) and we decided to investigate how they are affected by IBA and inorganic nutrition.

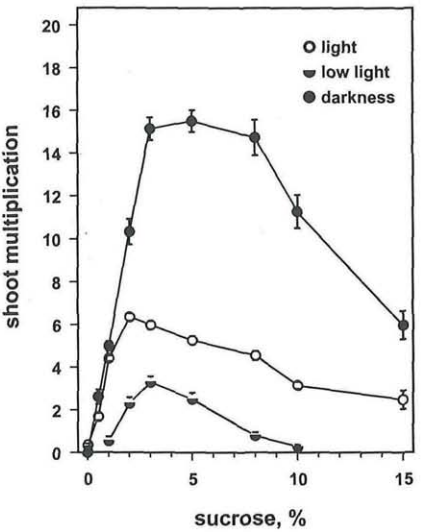

Fig. 1. Effect of sucrose on shoot multiplication (total axillary buds) in light (33-46.5 $\mu mol.m^{-2}s^{-1}$), low light (2 $\mu mol.m^{-2}s^{-1}$) and continuous darkness.

A simplified DE FOSSARD 1976 broad spectrum experiment showed that root and shoot branching were independent processes each of them with specific requirements (Table 1). In single excised shoot explants, adventitious roots appeared even on "empty" medium consisting only of deionized water. It enabled highest number of lateral roots to form but root elongation was better if 1/10 strength MS salts were included. IBA clearly inhibited root branching and root elongation. In absence of sucrose media with full strength MS salts inhibited lateral root formation. This inhibitory effect was cancelled by addition of 5% sucrose.

Axillary bud formation apart from high (5%) sucrose nutrition required also presence of inorganic salts. Furthermore, axillary bud formation increased with increased concentration of inorganic salts indicating a synergistic effect of sucrose and inorganic nutrition over axillary bud formation. IBA had no apparent effect on axillary shoot formation as in lateral root formation where it was clearly inhibitory.

D. fragrans is thus a model system of a monocotyledonous plant in which shoot multiplication can occur on hormone-free medium through a synergistic action of sucrose and inorganic salts.

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Table 1. Effect of inorganic nutrition, IBA and sucrose on shoot and root branching.

Sucrose %	MS salts	IBA µM	Root length mm ± SE	Roots with laterals,%	No.of lateral roots ± SE	No.of axillary buds ± SE
0	0	0	50.6 ± 1.5	90.6	12.3 ± 0.5	0
0	0	2.46	32.5 ± 1.3	22.1	1.5 ± 0.3	0
0	1/10	0	80.3 ± 1.3	56.2	5.3 ± 0.4	0
0	1/10	2.46	57.4 ± 1.5	7.4	0.6 ± 0.1	0
0	full	0	27.4 ± 2.5	0	0	0
0	full	2.46	22.2 ± 1.5	0	0	0
5	0	0	50.0 ± 2.1	57.7	7.8 ± 1.0	0*
5	0	2.46	17.1 ± 1.1	27.0	2.7 ± 0.5	0*
5	1/10	0	71.6 ± 1.4	61.2	8.7 ± 0.6	0.9 ± 0.08
5	1/10	2.46	47.6 ± 1.3	31.1	3.5 ± 0.4	1.1 ± 0.12
5	full	0	86.8 ± 1.9	31.9	7.5 ± 1.0	2.2 ± 0.17
5	full	2.46	59.8 ± 1.1	36.8	6.7 ± 0.6	2.1 ± 0.16

* - less than 0.05

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