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# Effect of sucrose application, minerals, and irradiance on the *in vitro* growth of *Cistus incanus* seedlings and plantlets

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## Abstract

To study the effect of sucrose on the sink-source relationship in *in vitro*-grown plants, *Cistus incanus* seedlings and plantlets were grown horizontally in a two-compartment Petri dish (split dish), with the root system in one compartment and the shoot in the other. Shoots and roots were exposed to different sucrose concentrations (0 - 30 g $dm^{-3}$), two irradiance levels (25 and 160 μmol $m^{-2}s^{-1}$) and the presence or absence of a minimum medium containing minerals and vitamins (M medium). Root and shoot biomass of the seedlings was enhanced by an increase in irradiance when the growth medium was not supplemented with sucrose indicating the role of photosynthesis in biomass production. When sucrose was added to either organ growth was enhanced as well. In the presence of sucrose in the root compartment, sucrose applied to the shoot compartment enhanced growth of both organs under low irradiance, while under high irradiance, sucrose had no further additive effect. In the absence of sucrose in the root compartment, the enhancement of root biomass by sucrose added to the shoot compartment was lower under high irradiance than under low irradiance. The response of *Cistus* plantlets to sucrose and irradiance differed from that of seedlings, probably reflecting a greater susceptibility of the plantlets to sucrose feedback inhibition on photosynthesis and biomass accumulation. The decrease in root and shoot growth when M medium was added to the shoot compartment and the relatively better growth of these organs when the roots were supplied with minerals and the shoot with sucrose, indicate that growth of the two organs in our experimental set-up was regulated by opposing fluxes of C and nutrients.

*Additional key words*: sink-source relationship, two-compartment Petri dish.

## Introduction

Sugars have essential functions in plant metabolism, serving as carbon skeletons, energy sources (Thorpe 1974), osmotic agents (Brown *et al*. 1979), and signals (Smeekens 2000). *In vitro*, it is essential to ensure the supply of sugars to the cultured plants, since photosynthetic activity is often low, mainly due to low irradiance and limited gas exchange (Kozai 1991). Different studies have shown opposite effects of exogenously applied sucrose to both plantlets and seedlings grown *in vitro* – in some promotion and in others inhibition of growth (see references in Le *et al*. 2001). It has been argued that these contradictory results are due to differences in irradiance and $CO_2$ concentrations, which determine the plant sink-source equilibrium: under source-limiting conditions, exogenously applied sucrose contributes to biomass production and development of the photosynthetic apparatus, while under sink limitation the sucrose increases plant susceptibility to feedback inhibition (Le *et al*. 2001). The above experiments on the effect of exogenous sucrose on *in vitro* growth and photosynthesis were conducted in the traditional way of seeding or plantlets growing in solid agar containing the nutrients plus sucrose. However, to our best knowledge, notions of a non-storing root serving as the source of sugars (especially, sucrose, glucose or fructose) *in vitro* and of the transport of sugars from roots to shoots have never been addressed in *in vitro* experiments. Since water potential gradient, a driving force for both phloem and xylem transport, is limited under *in vitro* conditions (Ikeda *et al*. 1999), it may be assumed that a sugar concentration gradient is the main driving force for the diffusion of the sucrose and that sugar transport is, therefore, slow. In plants grown under photomixotrophic conditions, this slow transport of

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sugars from root to shoot together with the existence of two sources of sugars, one from the root (fed with sugars) and the other from the shoot (especially under growth conditions of adequate irradiation and supply of $CO_2$) may result in a discordance in the signalling of sink-source equilibrium.

In an *ex vitro* study of the effect of sucrose on the growth of soybean plants, it was shown that long-term injection of sucrose into the plant stems resulted in increases in leaf area, pod number and nodule dry mass (Abdin *et al*. 1998). Since it is difficult to implement such a system *in vitro*, in our experimental set-up, sucrose was applied to the shoots of intact *Cistus incanus* seedlings and plantlets grown in two-compartment Petri dishes (split dishes), with the root system in one compartment and the shoot in the other.

## Materials and methods

**Plants and culture media:** Seeds of *Cistus incanus* L. were collected from wild stands on Mount Carmel (north of Israel). Seeds were surface disinfected with sodium hypochlorite (1 or 10 %) for up to 40 min and then germinated on solidified 6 g $dm^{-3}$ agar-$H_2O$. When the first leaves started to develop, 18 - 21 d after sowing, the seedlings were pulled out of the agar and grown as described below. To obtain plantlets *in vitro*, cultures of clone DC2 were propagated as described before (Mills *et al*. 2002): shoot propagules of four leaves were prepared from two-month-old plantlets and cultured on MS medium (Murashige and Skoog 1962) modified such that the nitrogen content was only 20 %. After 10 d, rooted plantlets were pulled out of the agar and grown as described below. Seedlings and plantlet cultures were grown in 25 × 100 mm glass tubes in a growth room maintained at 25 ± 1 °C, under 25 µmol $m^{-2}s^{-1}$ photon flux density at the plant level and a 16-h photoperiod.

**Experimental set-up:** Experiments were conducted in two types of vessels, 25 × 100 mm glass tubes (vertical growth) or two-compartment 90-mm Petri dishes (horizontal growth). For both, the root M-medium comprised *Phytagel*™ (*Sigma*, Rehovot, Israel), the minimal medium of Balaji *et al*. (1995), supplemented with 0 - 30 g $dm^{-3}$ sucrose. In the two-compartment dishes, the shoot medium comprised *Phytagel*™ with or without M-medium or 0 - 30 g $dm^{-3}$ sucrose. In all the experiments, 15 $cm^3$ of medium were used unless otherwise indicated. For the Petri-dish experiments, a notch was made in the middle of the divider with a hot scalpel to a depth of about 2 mm, *i.e*., above but very close to the level of the solidified medium. Seedlings or rooted plantlets were placed horizontally on the top of the medium such that the shoot lay in one compartment and the root in the other. Care was taken to assure good contact of both the shoot and the root with the medium. The root compartment was covered with aluminum foil. The dishes were placed horizontally (upside up) on shelves in a growth room under 1 or 6 cool white fluorescence tubes , irradiance at the plant level of 25 or 160 µmol $m^{-2}$ $s^{-1}$, respectively. For the vertical cultures, the seedlings were placed in the tubes with their roots in the medium, and the tubes were covered with foil up to the level of the medium and placed vertically on the same shelves as the Petri dishes. Once a week, root and shoot tips were marked on the dishes. In experiments with tubes, only biomass was recorded. Growth was expressed in terms of a growth factor (GF) as follows: $GF = (GF_f - GF_s)/GF_s$, where $GF_s$ and $GF_f$ are the values at start and at end of the experiment, respectively. After 28 d, roots were separated from the shoots, washed and blotted dry, and root fresh mass was determined. Dry masses of roots and shoots were determined after 48 h at 70 °C.

**Statistical analysis:** Data were analyzed statistically by one-way analysis of variance (*ANOVA*) using *JMPIN* software, version *4.04*. Means were separated by Student's t-test at the $P \leq 0.05$ level.

## Results

**Effect of sucrose supplied to the root:** An increase in the concentration of sucrose applied to the root of a *C. incanus* seedling grown in the traditional vertical manner under low irradiance resulted in slight increase in root and shoot fresh and dry biomass (Fig. 1). Such an increase was also observed when seedlings were placed horizontally in the two-compartment dishes with the root lying in 5 $cm^3$ of M-medium supplemented with increasing concentrations of sucrose and with the shoot in contact with *Phytagel*-$H_2O$ (Fig. 1). Root and shoot biomass in the dishes was higher than that in the tubes.

**Effect of sucrose supplied to the shoot:** *Cistus* seedlings were grown in M-medium plus sucrose in tubes or in two-compartment dishes in which the root was placed in M-medium with sucrose in one compartment, while the shoot lying in the other compartment was subjected to one of four different treatments: empty compartment (air alone); three drops of sucrose on the bottom of an otherwise empty compartment; *Phytagel*-$H_2O$ alone; or

*Phytagel* with sucrose. Irradiance was 25 µmol $m^{-2}$ $s^{-1}$. Root and shoot dry masses were significantly higher in horizontally grown seedlings with the shoots in contact with *Phytagel* + sucrose than in the seedlings grown vertically (Table 1). There were no significant differences in shoot and root growth between seedlings grown vertically and those grown horizontally in the other three treatments (Table 1). These results indicate that sucrose is absorbed by the shoot to support its growth and that the sucrose is transported to the root. The results also indicate that neither the moisture of the *Phytagel* nor any possible impurities in the *Phytagel* influenced the growth of the seedlings in the two-compartment dishes.

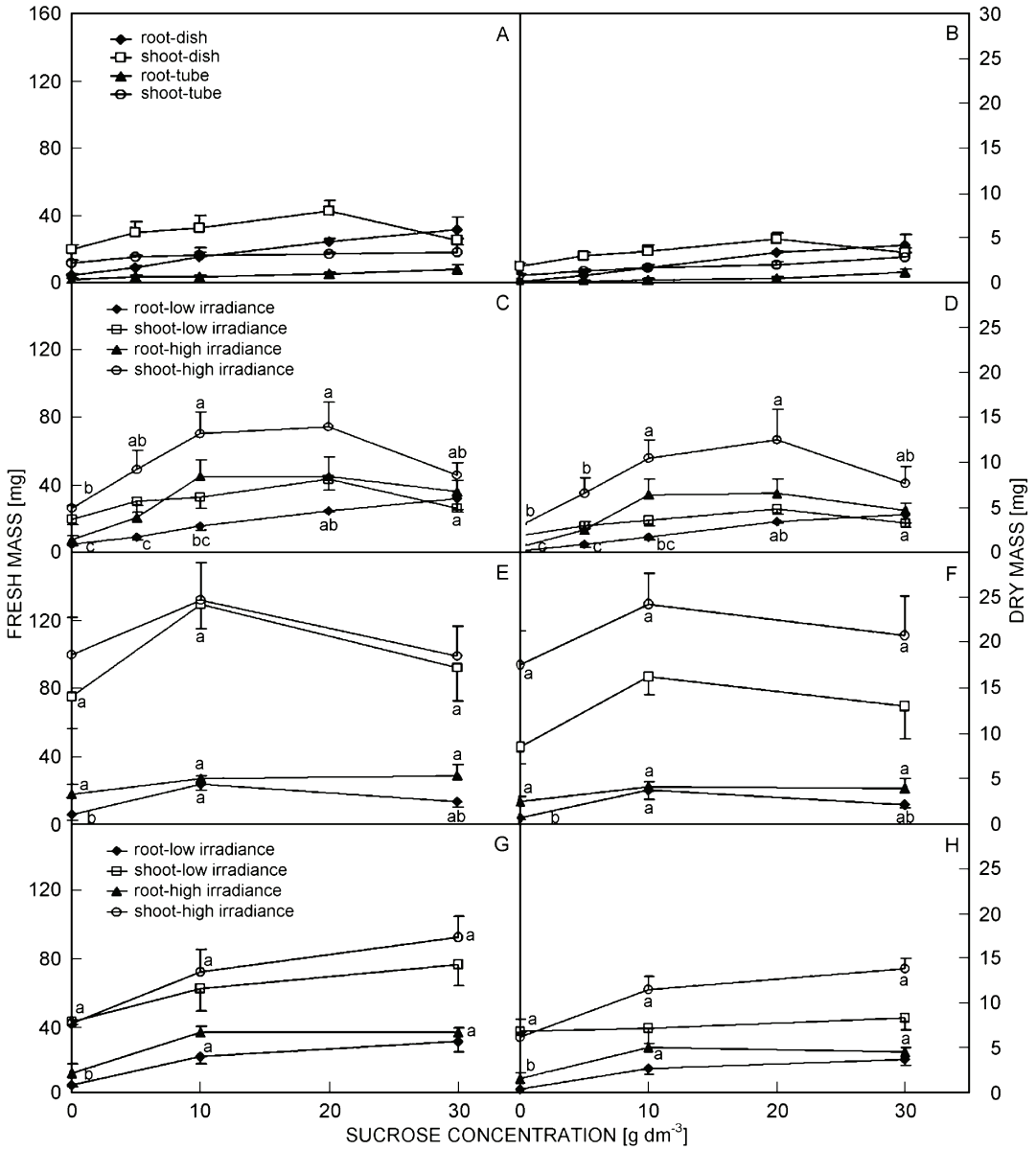


Fig. 1. *A*, *B* - Effect of sucrose concentration in M-medium on root and shoot fresh and dry biomass of seedlings grown either vertically in tubes or horizontally in dishes under irradiance of 25 µmol $m^{-2}s^{-1}$ for 4 weeks. *C*, *D* - Effect of sucrose applied to the root compartment on root and shoot fresh and dry biomass of seedlings grown horizontally in two-compartment dishes for 4 weeks under low (25 µmol $m^{-2}s^{-1}$) or high (160 µmol $m^{-2}$ $s^{-1}$) irradiance. M-medium was supplied to root compartment and *Phytagel*-$H_2O$ to the shoot compartment. *E*, *F* - Effect of sucrose applied to the shoot compartment on root and shoot fresh and dry biomass of plantlets grown for 4 weeks under low or high irradiance. M-medium was supplied to the root compartment. *G*, *H* - Effect of sucrose applied to the shoot compartment on root and shoot fresh and dry biomass of seedlings grown for 4 weeks under a low or high irradiance. M-medium was supplied to the root compartment. Values are means ± SE, $n$ = 3 (*A*,*B*), 4 (*C*,*D*,*E*,*F*) and 8 (*G*,*H*). Values followed by different letters are statistically different ($P < 0.05$).

Table 1. Biomass of seedlings grown with or without contact of the shoot with *Phytagel* or sucrose. The root medium in tubes and dishes consisted of *Phytagel* with M-medium (+ 10 g $dm^{-3}$ sucrose). Cultures were maintained under irradiance of 25 μmol $m^{-2}s^{-1}$. Values are means of 5 replicates. Values followed by different letters are statistically different ($P < 0.05$).

| Vessel | Shoot compartment | Root dry mass [mg] | Shoot dry mass [mg] |
|---|---|---|---|
| Tube | | 0.5b | 2.1b |
| Dish | empty | 0.6b | 2.7b |
| | 3 drops of sucrose | 0.6b | 2.8b |
| | *Phytagel*-$H_2O$ | 0.1b | 1.9b |
| | *Phytagel* + sucrose | 2.4a | 8.3a |

**Effect of sucrose supplied to the root or shoot under low irradiance:** Another set of experiments was conducted with seedlings and plantlets in the two-compartment set-up to study the differential effect of sucrose and M-medium applied either directly to the shoot or to the root system (Table 2). For the seedlings, addition of sucrose to the root compartment (experimental conditions designated F, Table 2) enhanced root elongation and root biomass production but not shoot biomass. Growth factors and biomass were higher when both compartments were filled with M-medium supplemented with sucrose (A). In the presence of M-medium in both compartments (B and C), addition of sucrose to the shoot compartment (B) had no effect on growth factor, but resulted in increased shoot biomass in comparison with treatment C. Root growth and biomass accumulation responded positively to the addition of sucrose to the shoot compartment (E *vs*. D). Applying sucrose directly to the roots did not promote root growth (F *vs*. E), but shoot growth was enhanced by addition of the sugar to the shoot compartment (E *vs*. F). The addition of minerals to the shoot compartment resulted in lower root growth and lower shoot biomass accumulation (E *vs*. B).

The growth patterns of the plantlets were, in general, different from those of the seedlings. Although the shoot elongation factor was similar in plantlets and seedlings, leaf number and leaf length factors were lower in plantlets than in seedlings. Plantlets gained more root and shoot biomass than seedlings, probably due to their initially higher biomass and better adaptation to the *in vitro* conditions. Nevertheless their response to the manipulation of medium was similar to that of seedlings. The best root growth of the plantlets was obtained when the shoot was exposed solely to sucrose and the roots solely to minerals (E). In plantlets, as in seedlings, addition of minerals to the shoot compartment resulted in lower root and shoot biomass accumulation (E *vs*. B).

**Effect of sucrose supplied to the root or shoot under high irradiance:** The performance of seedlings in two-compartment dishes was also tested under irradiance of 160 μmol $m^{-2}$ $s^{-1}$ (Table 3). With M-medium in the root compartments and water in the shoot compartment, addition of sucrose to the root compartment enhanced root elongation and root biomass production (F *vs*. D, Table 3), and in contrast to low irradiance it enhanced also shoot elongation and shoot biomass. There were no statistical differences in shoot growth factors and biomass between treatments containing both sucrose and M-medium in either of the compartments (A, B, E and F). In general, biomass production and the different growth factors were higher (1.5- to 2.5-fold) under high irradiance (Table 3) than under low irradiance (Table 2). In the treatment of M-medium plus 10 g $dm^{-3}$ sucrose in the root compartment and water alone in the shoot compartment (F), shoot and root biomass were three- and fourfold, respectively, higher under the high irradiance

Table 2. Effect of M-medium and 10 g $dm^{-3}$ sucrose on root and shoot dry mass [mg], growth factors (GF) for root and shoot elongation, leaf number and length of the longest leaf [cm] of *Cistus* seedlings and plantlets grown for 4 weeks in two-compartment dishes under irradiance of 25 μmol $m^{-2}$ $s^{-1}$. Values are means of 6 - 15 replicates. Values followed by different letters are statistically different ($P < 0.05$).

| Treatment | | Root comp. | Shoot comp. | Root d.m. | Shoot d.m. | Root GF | Shoot GF | Number | Length |
|---|---|---|---|---|---|---|---|---|---|
| Seedlings | D | M | $H_2O$ | 0.1c | 1.9c | 2.6b | 0.9b | 1.3b | 4.0ab |
| | C | M | M | 0.2c | 2.5c | 1.4b | 1.0b | 1.7ab | 7.8a |
| | B | M | M+sucrose | 1.2bc | 4.9ab | 5.7b | 1.4ab | 2.0a | 8.4ab |
| | E | M | sucrose | 2.2a | 7.2a | 15.6a | 1.5ab | 2.1a | 11.9a |
| | F | M+sucrose | $H_2O$ | 1.5ab | 3.8bc | 14.9a | 1.0a | 1.9a | 10.8a |
| | A | M+sucrose | M+sucrose | 2.6a | 5.9ab | 22.5a | 1.9a | 2.0a | 10.3ab |
| Plantlets | D | M | $H_2O$ | 1.9b | 15.1ab | 35.8a | 1.6a | 0.9a | 0.6a |
| | C | M | M | 2.1b | 15.1ab | 27.1a | 0.9b | 0.7a | 0.3a |
| | B | M | M+sucrose | 1.0b | 11.7b | 30.6a | 1.1ab | 0.8a | 0.3a |
| | E | M | sucrose | 3.5a | 16.9a | 34.7a | 1.6a | 0.8a | 0.5a |
| | F | M+sucrose | $H_2O$ | - | - | - | - | - | - |
| | A | M+sucrose | M+sucrose | 1.9b | 13.0ab | 20.6a | 1.3ab | 0.9a | 0.4a |

Table 3. Effect of M medium and 10 g $dm^{-3}$ sucrose on root and shoot dry mass [mg], growth factors (GF) for root and shoot elengetion, leaf number and length of the longest leaf [cm] of *Cistus* seedlings grown for 4 weeks in two-compartment dishes under irradiance of 160 µmol $m^{-2}$ $s^{-1}$. Values are means of 6 - 15 replicates. Values followed by different letters are statistically different ($P < 0.05$).

| Treatment | Root comp. | Shoot comp. | Root d.m. | Shoot d.m. | Root GF | Shoot GF | Number | Length |
|---|---|---|---|---|---|---|---|---|
| D | M | $H_2O$ | 1.3c | 4.7c | 12.6b | 1.0b | 2.8b | 8.2b |
| B | M | M+sucrose | 2.5bc | 7.1bc | 21.2b | 1.0ab | 3.0ab | 21.7a |
| E | M | sucrose | 4.6ab | 14.2a | 22.6b | 1.5ab | 3.4a | 17.4a |
| F | M+sucrose | $H_2O$ | 6.2a | 12.6a | 41.1a | 1.6a | 3.0ab | 16.8a |
| A | M+sucrose | M+sucrose | 6.4a | 10.7ab | 29.9ab | 1.2ab | 3.0ab | 7.8ab |

than under the low irradiance (Tables 2 and 3). Similarly as under low irradiance, root growth and biomass accumulation under high irradiance responded to addition of sucrose to the shoot compartment (E *vs*. D), but to a lesser extent (Table 3). The differences in shoot to root ratio between treatments E and F were higher under high than under low irradiance, indicating that the shoot becomes a better sink at the expense of the root.

Since shoot growth was enhanced in response to M-medium in the root compartment, we tested the effect of the omission from the medium of the macroelements N and P. Absence of N resulted in lowered shoot biomass accumulation (Table 4), while omission of P had no effect. A reduction in root growth under N starvation was observed in the plantlets but not in the seedlings.

Table 4. Effect of P and N starvation on root and shoot dry mass [mg] of *Cistus* seedlings and plantlets in two-compartment dishes under irradiance of 25 µmol $m^{-2}$ $s^{-1}$. Shoot compartment contained 10 g $dm^{-3}$ sucrose. Values are means of five replicates. Values followed by different letters are statistically different ($P < 0.05$).

| | | Root comp. | Root d.m. | Shoot d.m. |
|---|---|---|---|---|
| Seedlings | E | M | 2.4a | 8.3a |
| | L | M-N | 1.9a | 3.1c |
| | M | M-P | 1.9a | 5.7b |
| | N | M-N,P | 2.6a | 3.1c |
| Plantlets | E | M | 3.5a | 17.0a |
| | L | M-N | 2.2b | 11.3b |
| | M | M-P | 3.6a | 17.3a |
| | N | M-N,P | 3.0ab | 12.2b |

**Effect of sucrose at different concentrations supplied to the root or shoot under two irradiances:** The effects of low *vs*. high irradiance and of sucrose supplementation (0, 5, 10, 30 g $dm^{-3}$ to M-medium in the shoot compartment was also investigated in *Cistus* seedlings. The higher irradiance enhanced the growth of both shoots and roots (Fig. 1). The optimum sucrose concentration for enhancing root and shoot biomass accumulation was 30 g $dm^{-3}$ under low irradiance, while 10 - 20 g $dm^{-3}$ at high irradiance (Fig. 1).

Addition of sucrose in the shoot compartment of *Cistus* plantlets did not affect shoot growth but did increase root growth at the low irradiance (Fig. 1). No effect was observed when sucrose was increased from 1 to 30 g $dm^{-3}$. When the same experiment was repeated with seedlings, it was found that sucrose in the shoot compartment stimulated shoot and root growth under high irradiance and root growth under low irradiance (Fig. 1). The effect of irradiance was significant for root growth at 10 g $dm^{-3}$ sucrose and for shoot growth at 30 g $dm^{-3}$ sucrose. In contrast to the inhibition of shoot growth by 30 g $dm^{-3}$ sucrose in the root compartment (Fig. 1), addition of the sucrose to the shoot compartment did not have an inhibitory effect (Fig. 1).

## Discussion

This work was aimed at studying the interaction between sucrose and irradiance on the growth of *C. incanus* seedlings and plantlets in aseptic cultures by applying sucrose directly to the shoot. In seedlings, photosynthesis contributed to growth, as indicated by the increase in root and shoot biomass in response to higher irradiance without sucrose supplementation of the medium (D, Table 2 *vs.* 3). However, the addition of sucrose to the root medium further enhanced root and shoot growth. These observations support previous studies that showed enhancement of growth by sucrose under photomixotrophic conditions (Cournac *et al*. 1991, Paul and Stitt 1993, Furbank *et al*. 1997, Tichá *et al*. 1998, Fuentes *et al*. 2005). In those studies, the increase in biomass in response to exogenous sucrose occurred mainly under low irradiance and low $CO_2$ (Cournac *et al*. 1991, Tichá *et al*. 1998, Le *et al*. 2001). In other studies, it was found that sucrose inhibited shoot growth under photomixotrophic conditions by down regulation of photosynthesis (Capellades *et al*. 1991, Desjardins *et al*. 1995, Le *et al*.

2001). Although it had previously been argued that a lack of down regulation in photomixo-trophically grown plantlets might be due to the low irradiance which results in low photosynthetic rates and a source limitation of growth (insufficient C production in source to support sink growth), Tichá *et al*. (1998), working with tobacco plantlets, concluded that since under high irradiance sugars enhanced leaf number, leaf area, photosynthetic potential and resistance to photoinhibition, the problem is not source limitation but rather a sink limitation (insufficient C consumption). In *C. incanus*, sucrose at a concentration up to 10 g $dm^{-3}$ applied to the shoot enhanced growth of shoots and roots. Under high irradiance sucrose had stimulated more biomass accumulation than under low irradiance. This support the hypothesis of Tichá *et al*. (1998) that sugar affected positively the sink capacity of roots and shoots to consume the sugars, a situation of sink limitation. Increase of sucrose to 3 % had no additional effect. This might reflect a change in the equilibrium between a negative feedback of sugar on the photosynthetic processes on one hand and biomass build up by sugar on the other hand.

It is generally accepted that growth and development of roots and shoots are interdependent. Lack of nutrients in the root environment may lead to a reduction in shoot growth, an increase in sugar allocation to the root system, and eventually the production of more lateral roots to increase the supply of more nutrients to the plant. Under non-limiting nutrient supply, sugars are allocated to the shoot to increase photosynthetic leaf area and productivity of the plant. Different suggestions have been put forward for the mechanism(s) controlling regulation of the shoot/root ratio of sugars *in vivo*: these include cytokinins as root signals (Beck 1996), $NO_3^-$ as a root signal, and amino acids, sugars, proteins or plant hormones as shoot signals (see review by Forde 2002). We now ask how the shoot/root ratio is regulated *in vitro*. The results presented here demonstrate enhancement of shoot and root growth of *Cistus* plants when the roots are supplied with minerals, and the shoot, with a carbon source; these findings indicate that growth of the two organs is regulated by opposing nutrient fluxes and/or signals. Nitrogen is an important constituent of M-medium that supports growth of both shoots and roots (Table 4) and thus could serve as the root signal in the regulation of shoot and root growth *in vitro*. The presence of minerals and vitamins in the shoot compartment inhibited both shoot and root growth (Table 2), probably by disturbing the regulation of opposing fluxes between roots and the shoot. Since this finding was not statistically significant under high irradiance (Table 3), the inhibition of root and shoot growth may have been due to high assimilation or negation of a specific feed-back inhibitor in the shoot by the high irradiance. Sink-source regulation is complex and subjected to modulation and integration between different signalling pathways that respond to phytohormones, P, light, sugars and other stimuli (Roitsch 1999). Reduction in shoot and root growth was observed when 30 g $dm^{-3}$ sucrose was added to the roots in comparison to lack of inhibition when added to the shoot. If sucrose inhibits shoot growth under photomixotrophic conditions by down regulation of photosynthesis (Capellades *et al*. 1991, Desjardins *et al*. 1995, Le *et al*. 2001), it could have been expected also when the sugar was applied directly to the shoot. This can be explained by down regulation through a specific root signal such as a phytohormone.

The usual environmental conditions used for growing plants *in vitro* under tight closure are low irradiance, high humidity and limited gas exchange. These conditions result in low photosynthetic rates and low productivity (Kozai 1991). The high contents of $CO_2$, ethylene, and particularly water vapour are considered to be the major causes of abnormalities observed in organs and plantlets grown *in vitro* with low ventilation (Ziv 1991, Debergh *et al*. 1992). These abnormalities may include the lack of vascular bundles, decreased synthesis of lignin, poly-phenols and waxes, thin cell walls, stomatal malfunction, and low differentiation of mesophyll cells, all of which may affect plant-water and plant-ion relationships. In contrast to a seedling that has been exposed to *in vitro* conditions only once, plantlets that have been subcultured numerous times accumulate far more abnormalities. In our experiments, exposure of plantlets to high irradiance stimulated growth only in the absence of sucrose, and addition of sucrose to the shoot under high irradiance had no significant effect on shoot and root growth (Fig. 1). In contrast, exposure of seedlings to high irradiance promoted biomass production of shoots and roots, and addition of sucrose to the shoot enhanced shoot and root growth under both low and high irradiance (Fig. 1). It thus seems that plantlets are more susceptible to sucrose inhibition than seedlings.

The finding that shoot growth was enhanced when sucrose was added to the shoot compartment rather than to the root compartment indicates that sugar consumption and/or transport from root to shoot may be limiting. There is very little information in the literature on sugar transport in plants grown *in vitro* under conditions that are quite different from *ex vitro* conditions. Among the few studies that have been conducted, Ziv (1991) reported a reduction in lignification and in vascular tissue in hyperhydric plants (Ziv 1991). Dantas *et al*. (2001) found that ventilation improves water and mineral conductance from root to shoot and may therefore also be expected to improve sucrose transport from root to shoot. Shim *et al*. (2003), however, showed that ventilation improved shoot dry mass to a greater extent in the absence than in the presence of sucrose in the medium. The roles of the xylem and the phloem in transport and functionality *in vitro* are not clear, and research should be devoted to this aspect of *in vitro* plant culture.

**Conclusion**: The results of these experiments indicate that under low irradiance the plant is under source limitation and a transition towards less source limitation or sink limitation occurs when the irradiance is increased. An inhibition of the photosynthesis by sucrose under high irradiance cannot be ruled out, and it is possible that there is a balance between the inhibitory and enhancement effects of sucrose. The different responses of *Cistus* plantlets and seedlings to sucrose and irradiance probably reflect a greater susceptibility of the plantlets to sucrose inhibition.

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