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RESEARCH PAPER

Photomorphogenesis of the root system in developing sunflower seedlings: a role for sucrose

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Keywords

Auxin action; brassinosteroids; photomorphogenesis; phytohormones; sucrose.

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Dedicated to the memory of Hans Mohr (1930–2016), a pioneer in photomorphogenesis research.

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ABSTRACT

- The domestic sunflower (*Helianthus annuus* L. cv. 'Giganteus') has been used since the 19th century as a model plant for the study of seedling development in darkness and white light (WL) (scoto- *versus* photomorphogenesis). However, most pertinent studies have focused on the developmental patterns of the hypocotyl and cotyledons, whereas the root system has been largely ignored.
- In this study, we analysed entire sunflower seedlings (root and shoot) and quantified organ development in the above- and belowground parts of the organism under natural (non-sterile) conditions.
- We document that seedlings, raised in moist vermiculite, are covered with methylobacteria, microbes that are known to promote root development in *Arabidopsis*. Quantitative data revealed that during photomorphogenesis in WL, the root system expands by 90%, whereas stem elongation is inhibited, and hook opening/cotyledon expansion occurs. Root morphogenesis may be mediated *via* imported sucrose provided by the green, photosynthetically active cotyledons. This hypothesis is supported by the documented effect of sucrose on the induction of lateral root initials in sunflower cuttings. Under these experimental conditions, phytohormones (auxin, cytokinin, brassinolide) exerted little effect on root and cotyledon expansion, and no hormone-induced initiation of lateral roots was observed.
- It is concluded that sucrose not only acts as an energy source to fuel cell metabolism but is also a shoot-derived signalling molecule that triggers root morphogenesis.

INTRODUCTION

Five decades ago, the German plant physiologist Hans Mohr (1930–2016) published a monograph entitled *Lectures on Photomorphogenesis*, wherein he summarised the state of the art of plant developmental physiology at that time. In his Preface, Mohr (1972) briefly summarised the discovery of the 'reversible red/far-red control of plant growth' *via* the 'photoreceptor pigment phytochrome' and he dedicated his monograph to the discoverers of these phenomena, the botanist Henry A. Borthwick (1898–1974) and the chemist Sterling B. Hendricks (1902–1981) (see Briggs 1976). In addition, Mohr (1972) mentioned that he 'began to work with the mustard seedling 15 years ago' (*i.e.* in 1957). Accordingly, his first research paper dealing with the development of seedlings of white mustard (*Sinapis alba* L.) was published in the same year (Mohr 1957).

With reference to the classical 'Wilhelm Pfeffer – light vs. dark-grown potato – experiment' (see Kutschera & Briggs 2013), he defined photomorphogenesis as the light control of 'growth and differentiation (and therewith development) of a plant independently of photosynthesis' (Mohr 1972, p. 1). In this context, he introduced seedlings of *S. alba* as a suitable model system for photomorphogenesis research. According to Mohr (1972), the 'photomorphogenic' effect of light on seedling development can be experimentally de-coupled from photosynthesis: 3-day-old seedlings, raised in continuous white light *versus* far-red light (which does not cause greening), display the same photomorphogenic patterns (short hypocotyl, expanded cotyledons, etc.) with reference to dark controls. Therefore, in land plants (embryophytes), light acts as a developmental signal, as well as an energy source to drive photosynthesis.

During the early 1980s, Mohr's 'white-seeded mustard (*S. alba*) system' was gradually replaced by the use of a relative of this crop species, the mouse-ear cress, *Arabidopsis thaliana* (L.) Heynh. Over the past decades, in both the *Sinapis* and *Arabidopsis* research programmes, it was common to use sterilised seeds. These aseptic seeds were raised on a germ-free medium that either consisted of distilled water (*S. alba*) or a mixture of mineral salts (*A. thaliana*; Mohr 1972; Kircher & Schopfer 2012; Deng *et al.* 2014; Moni *et al.* 2015). Under these artificial conditions (*i.e.* seedlings with light-exposed roots growing in the absence of soil microbes), organ development in the lower part of the juvenile plant is severely retarded, so that only the shoot appears to grow normally (Kutschera 2007; Klikno & Kutschera 2017). Accordingly, using this experimental system in plant research, a comparative analysis of photomorphogenesis of the shoot *versus* root system may yield experimental artefacts.

Although it has been shown that light not only controls the development of the shoot, but also exerts an effect on the subterranean root system (Mandoli *et al.* 1990; van Gelderen *et al.* 2018), quantitative data on photomorphogenesis of entire seedlings, grown under real-world conditions, are lacking. The

experiments described here were designed to elucidate the effect of WL on the developmental patterns of entire sunflower seedlings raised in moist vermiculite under non-sterile conditions. In addition, we investigated the role of sucrose as a cotyledon-derived signal with respect to the initiation of lateral roots, and explored the effect of phytohormones on this physiological process in our test system.

MATERIAL AND METHODS

Sunflower seedlings (*Helianthus annuus* L. cv 'Giganteus') were raised under non-sterile standard conditions (moist vermiculite), as described by Kutschera *et al.* (2010). Batches of 20 achenes were soaked in H_2O for 2 h and planted into vermiculite saturated with tap water in closed, transparent plastic boxes (20 × 30 × 12 cm). After 3 days of growth in darkness (25 °C, relative humidity ca. 100%), some boxes were transferred into constant white light (ca. 100 μmol photons m^{-2} s^{-1}), others were left in darkness as controls. After 2–6 days of growth in the dark or white light (WL; Osram L Daylight, Lumilux.de/15 W, photon fluence: 100 μmol photons m^{-2} s^{-1}), representative seedlings were removed from the vermiculite and washed several times in tap water in order to remove the attached particles from the roots, without damaging the root system. The harvested seedlings were blotted dry on filter paper and separated into cotyledons, hypocotyl and root system. All three parts of the developing seedlings were weighed separately, and fresh mass (in g) of these three organs of the developing plant recorded. Thereafter, the separated parts were placed into a chamber at 70 °C in order to desiccate the tissues. After 2 h, the dry mass of the root system and the cotyledon/hypocotyl was measured (in g per seedling).

To quantify the relative surface area of the developing root system, a dye absorption method was used (Beneš 1968). One to five juvenile root systems, cut from 2-day-old etiolated sunflower seedlings, were incubated for 2 h in Alcian blue (0.05%, w/v) solution, until the surface of the root (*i.e.* cell wall polysaccharides) was stained. After removal of the dye with 50% acetic acid, the absorbance of the solution was measured at 620 nm. This A620 value reflects the relative amount of dye attached to the wall polysaccharides of the root surface. Based on a linear calibration curve (number of roots *versus* A620, *i.e.* the absorbance of dye by cell wall surfaces), the relative size (*i.e.* surface area) of roots from 2- to 6-day-old etiolated *versus* irradiated seedlings was calculated (relative units). The vermiculite did not significantly absorb the dye.

To determine whether or not the observed light effect on root development is attributable to a phytochrome-mediated response, single 3-day-old etiolated seedlings were raised in moist vermiculite (plastic boxes, 6 × 6 × 8 cm). Individual seedlings were either kept in darkness or irradiated for 10 min with red light (680 nm), or with red followed by far-red light (730 nm), as described by Iino & Briggs (1984). After 3 days of growth in darkness, the cotyledons and root systems were harvested and their fresh mass determined (g per organ).

The concentration of sucrose and glucose in extracts from entire (thoroughly washed) root systems and the specific catalytic activities of the enzymes acid invertase (INV) and sucrose synthase (SS) from corresponding protein extracts were determined as previously described (Pfeiffer & Kutschera 1995; Kutschera & Heiderich 2002).

The effect of sucrose (10 mM) and phytohormones (auxin, IAA, 10 μM; the cytokinin 6-benzyl aminopurine, 10 μM; brassinoide, BL, 1 μM) on the cotyledons and the root system were investigated as follows. Pairs of cotyledons and roots of average size were cut from 3-day-old etiolated seedlings (under dim green safe light). The samples were incubated in water (control) or corresponding solutions for 3 days in darkness (Petri dishes, five roots per 10 ml solution/shaker, 50 rpm, 25 °C). Thereafter, the fresh masses of the cotyledons were measured, and photographs of the roots were taken to determine the length increase of the samples. To test for the presence of methylobacteria on the shoot and root system of sunflower seedlings, the agar impression method was employed. Scanning electron micrographs of batches of isolated methylobacteria were prepared as described by Kutschera (2002).

All experiments were repeated six to nine times with independent batches of seedlings. Arithmetic means and standard error of the mean (SEM) were calculated for each data point. These values were usually < 3% and, in most cases, within the size of the symbols. The SEM values are depicted if they are larger than the size of the corresponding symbols. Figures 5, 6, and 8 show the means (± SEM) of six to nine separate experiments. Each sample consisted of five to 12 seedlings (or organ cuttings). To further analyse the data, we performed one-way ANOVA, followed by significance tests. The corresponding *F*-values are given in the text where appropriate.

RESULTS

Scoto- *versus* photomorphogenesis of shoot and root system

The morphology of representative 6-day-old sunflower seedlings that were either raised in darkness or for 3 days in the dark followed by 3 days in continuous white light (WL) is shown in Fig. 1. In etiolated seedlings, the thin, curved hypocotyl is long, and the yellow cotyledons are closed (scotomorphogenesis). Irradiated seedlings are characterised by a short, sturdy hypocotyl and fully expanded green cotyledons (photomorphogenesis), as described for numerous other dicot species (Mohr 1972). Although the root system was only briefly washed and therefore still covered with a few particles of vermiculite, it is apparent that this organ is larger in irradiated seedlings than in the dark controls. Figure 2 shows that these sunflower seedlings, raised in non-sterile vermiculite, were covered with bacteria, which originated from the outer surface of the seed coat and/or the substrate (Kutschera 2002). The result documents that under our experimental conditions, epiphytic root- (and shoot)-associated bacteria are attached to the peripheral cells of all organs of the juvenile sunflower plant.

Kinetics of organ growth and description of photomorphogenesis

Quantitative data on the increase in fresh mass of the cotyledons, hypocotyl and the completely washed root system during seedling development in darkness and WL are shown in Fig. 3. Cotyledon expansion, as determined by fresh mass change, is rapidly enhanced, whereas hypocotyl growth is inhibited (Fig. 3A and B). Concomitantly, the root system responds with an enhancement of fresh mass upon irradiation of the seedling with WL. As a result, by day 6, the fresh mass of the

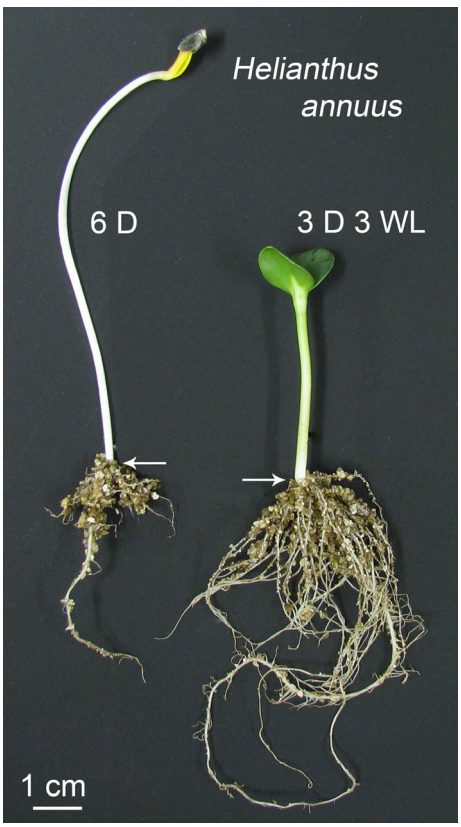

Fig. 1. Photograph of 6-day-old sunflower seedlings grown either in darkness (6 D; scotomorphogenesis) or for 3 days in the dark and 3 days in continuous white light (WL; 3 D 3 WL). Substrate: moist vermiculite. Note that the root system is, in part, covered by particles of vermiculite. Arrows: hypocotyl–root transition zone.

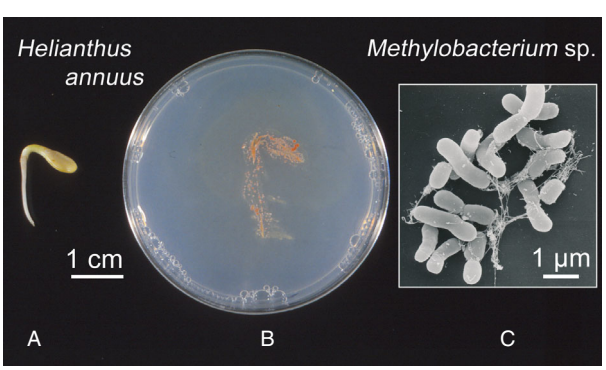

Fig. 2. Documentation of the presence of epiphytic methylobacteria on the shoot and root of a 2-day-old sunflower seedling. The juvenile plant (A) was raised in non-sterile moist vermiculite and, after removal of the seed coat, placed on a sterile agar plate containing methanol as sole carbon source. After 24 h, the seedling was removed and the plate incubated for 7 days at 27 °C (darkness). As a result (B), numerous colonies of pink-pigmented methylobacteria developed (see the SEM image in C).

subterranean half of the plant had almost doubled, compared to the etiolated control.

The corresponding values for dry mass of the three parts of the seedlings are shown in Fig. 4. Cotyledon dry mass declined by 45% between days 2 and 6 after sowing; this decrease in cotyledon material is in part attributable to the conversion of storage fats to

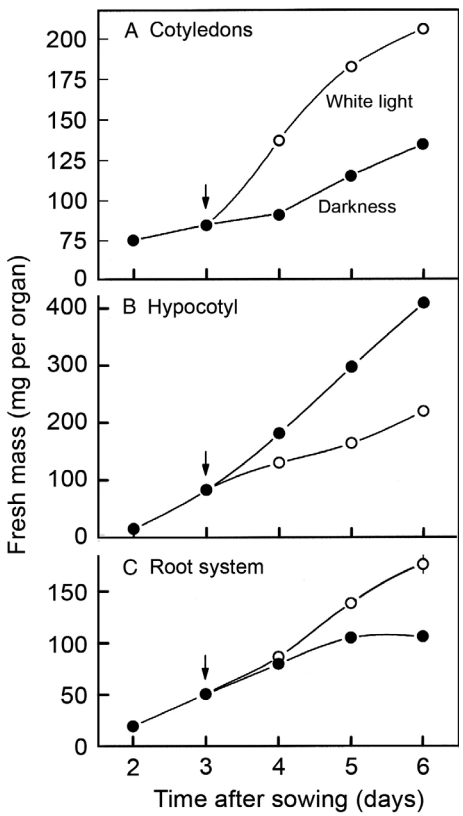

Fig. 3. Time course changes in fresh mass of the cotyledons (A), hypocotyl (B) and root system (C) in sunflower seedlings raised in darkness or irradiated with continuous white light (WL). Arrows indicated the onset of WL treatment.

sucrose, which is exported towards the developing stem and root system of the growing organism (Kutschera & Niklas 2013). WL did not exert a significant effect on this process (*F*-value light/darkness: 0.743). Dry mass accumulation in the stem of irradiated seedlings was lower than in the dark control, which reflects light-mediated inhibition of hypocotyl elongation during photomorphogenesis. The dry mass of the root system reached a constant value between days 5 and 6 after sowing (darkness). In irradiated seedlings, a steady increase in root mass was recorded so that, by day 6, the dry mass of the root system in WL-treated plants was ca. 90% higher than that of the etiolated control (Fig. 4C).

In order to investigate this apparent light-mediated promotion of root development in *H. annuus*, a dye attachment method was employed. The calibration curve (Fig. 5A) documents that the number of roots of similar size is proportional to absorbance of the dye that attaches to the surface of the organs. Quantitative data revealed that the relative root surface area of 6-day-old etiolated seedlings is about 50% of that in WL-treated (green) plants (Fig. 5). These data are in accordance with the fresh and dry mass measurements summarised in Figs 3 and 4, i.*e.* WL causes expansion of the root system at the expense of stem elongation. Based on these results, the sequence of developmental patterns of entire sunflower seedlings during growth in darkness and WL (skoto- *versus* photomorphogenesis) was reconstructed (results not shown). The images document that lateral root development is drastically promoted by WL, and therefore represents an important component of the photomorphogenic response of sunflower seedlings.

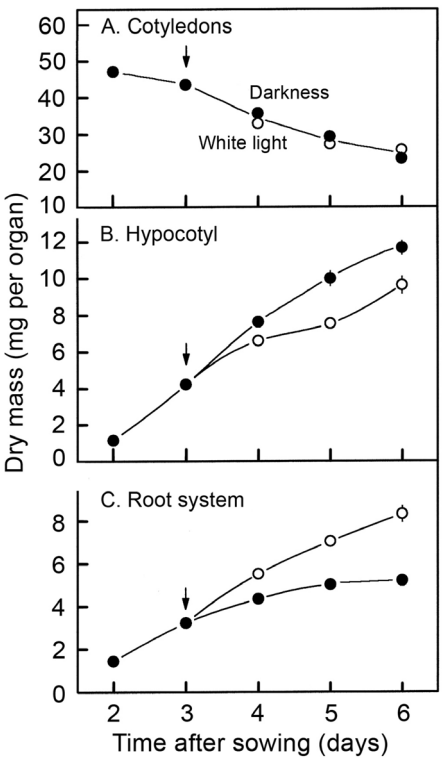

Fig. 4. Time course changes in dry mass of the cotyledons (A), hypocotyl (B) and root system (C) in sunflower seedlings raised in darkness or irradiated with continuous white light (WL). Arrows indicate the onset of WL treatment.

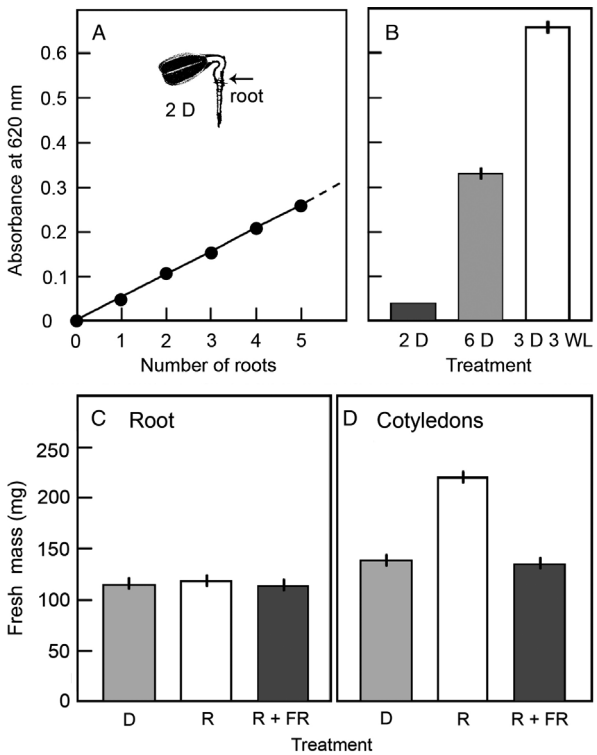

Fig. 5. Number of 2-day-old roots *versus* absorbance at 620 nm (A) and quantification of the relative surface area of the root system in juvenile and 6-day-old dark-grown *versus* WL-treated seedlings (B). Inset in (A) shows morphology of a 2-day-old etiolated seedling, with seed coat attached. Arrow: hypocotyl–root transition zone. Effect of red and far-red light on the changes in fresh mass of the root system (C) and cotyledons (D) in etiolated sunflower seedlings. Three-day-old dark-grown seedlings were irradiated for 10 min with red or 10 min with red and another 10 min with far-red light. Thereafter, seedlings were placed in darkness and analysed on day 6 after sowing. D = dark, R = red, FR = far-red.

Phytochrome-mediated effects and sucrose metabolism

In order to examine whether the WL-induced promotion of root development is due to a phytochrome-induced photomorphogenic response, 3-day-old etiolated seedlings were treated with saturating red and far-red light pulses (10 min each) and analysed after another 3 days of growth in darkness. The results (Fig. 5C) show that root fresh mass of 6-day-old etiolated seedlings is about 110 mg; red/far-red treatments did not have a significant effect on root development. In contrast, the cotyledons responded with an enhancement of growth upon red light treatment (Fig. 5D); this effect was reversed by a subsequent far-red pulse of the same duration, indicating that phytochrome was involved in the regulation of WL-induced cotyledon expansion.

The concentrations of sucrose and glucose in the expressed tissue sap of juvenile (2-day-old) and fully developed (6-day-old) roots are shown in Fig. 6A and B. In 2-day-old roots, the concentration of sucrose was ca. 4 mM, whereas that of glucose was 100 mM. Both sugars were quantified in the same tissue extract. The 25-fold higher concentration of glucose is in accordance with the fact that this monosaccharide is a mass component of the vacuolar fluid, whereas sucrose is transported, at a high concentration, in the sieve elements of the phloem (Hedrich *et al.* 2016). During root development in darkness, the concentrations of sucrose and glucose both declined by ca. 70%. White light had a significant positive effect on this apparent dilution (or consumption) of sucrose (and glucose; *F*-values < 0.001).

The activities of two key enzymes of sucrose metabolism, acid invertase and sucrose synthase, were quantified in roots harvested from the same batches of seedlings. The changes in acid invertase activity were similar to those in sucrose concentration (Fig. 6C). The specific catalytic activity of sucrose synthase was lower than that of invertase and did not show a positive correlation with WL-induced root development (Fig. 6C).

Effects of sucrose on lateral root development

The positive light effects on lateral root growth (Figure S1) and sucrose metabolism (Fig. 6A and C) prompted the question as to whether or not this disaccharide exerts a morphogenic effect on isolated roots cultivated *in vitro*. The results (Fig. 7A–C) show that roots, cut from 3-day-old etiolated sunflower seedlings, can be maintained in distilled water over several days in darkness, without morphological deterioration. In the presence of sucrose (10 mM), numerous lateral root initials developed within 3 days of incubation (Fig. 7D). As a control, pairs of cotyledons were incubated under identical conditions. Despite the fact that cotyledon growth was apparent in excised organ pairs floating on water, sucrose had little effect on this process (Fig. 7A–C).

Effects of phytohormones on cotyledon expansion and root development

It has been documented that in seedlings of *A. thaliana*, lateral root development is regulated by the phytohormone auxin

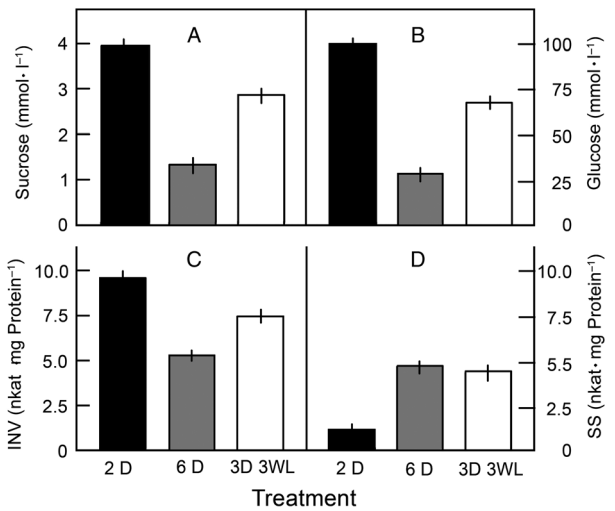

Fig. 6. (A, B) Concentrations of sucrose and glucose in cell sap extracted from the root system of sunflower seedlings raised for 2 or 6 days in darkness (2 D, 6 D) or irradiated for 3 days with continuous white light (3 D 3 WL). Specific catalytic activity of the enzymes acid invertase (INV) (C) and sucrose synthase (SS) (D) determined from protein extracts of seedlings harvested from the same batches of plants.

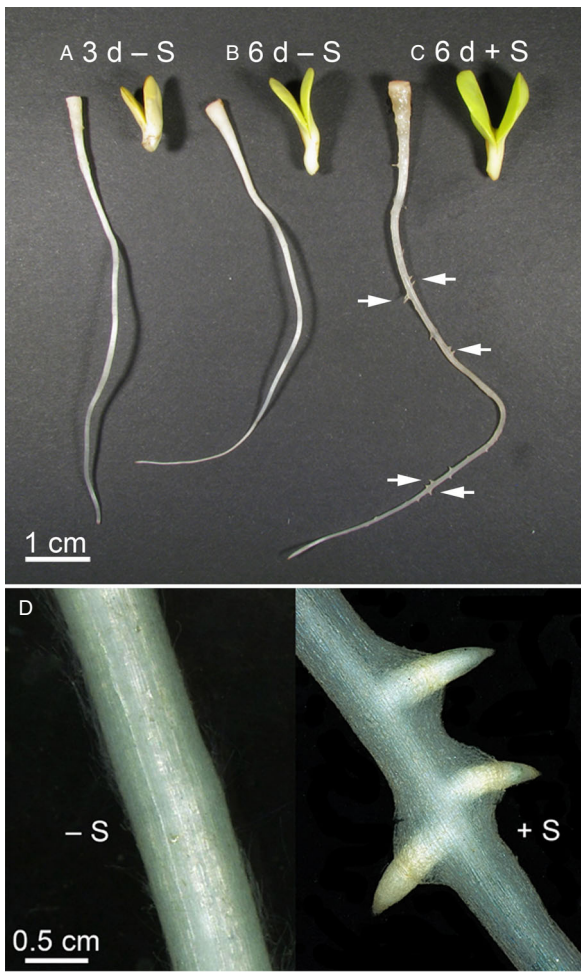

Fig. 7. Effect of sucrose (S) on root development in etiolated sunflower seedling cuttings. Roots were cut from 3-day-old etiolated seedlings and incubated for another 3 days on water (– S; A). After 6 days, roots were photographed and analysed again (B). In samples incubated for 3 days in sucrose (10 mM), numerous lateral root initials were observed (arrows; C). Cotyledons from the same seedlings are also depicted. Sucrose-induced initiation of lateral roots in etiolated sunflower seedlings (D). Note that the three lateral root initials emerged from the central cylinder and have pierced the outer layer of the primary root.

(IAA), originating in the root tip, whereas emergence is dependent on IAA supplied by the whole root system (Moni *et al.* 2015). To test for possible involvement of IAA (and two other phytohormones) in root development, the experiments summarised in Fig. 8 were carried out. Root cuttings and excised pairs of cotyledons (see Fig. 7A–C) were incubated for 3 day in darkness and thereafter analysed. In the control (water), the cotyledons expanded by ca. 30%. Sucrose, IAA and cytokinin had little effect on this growth process. However, brassinolide (BL) significantly promoted cotyledon expansion in this test system (Fig. 8A; F-value < 0.001). Similar to the cotyledons, the excised roots elongated in the water control (+25%). Sucrose had no measurable effect on this process; however, IAA and cytokinin completely inhibited this 'endogenous' root elongation response in water (Fig. 8B; F-value H_2O/IAA: < 0.001). In contrast to IAA and cytokinin, BL had no influence on the length increase of the root cuttings. The hormone concentrations applied in these tests had strong physiological effects on rice and maize seedlings (lamina bending assay/coleoptile elongation, respectively; Kutschera & Wang 2012, 2016). Hence, the relatively weak responses reported here are not attributable to a suboptimal concentration of the corresponding phytohormone.

Morphological examinations of the root cuttings (see Fig. 7) revealed that only in the presence of sucrose, but not when IAA, cytokinin or BL were applied, were lateral root initials induced. Hence, the three phytohormones investigated here are not capable of inducing the sucrose-mediated morphogenic effects shown in Fig. 7D, although they modulate primary root elongation (Fig. 8).

DISCUSSION

The domesticated sunflower is an important oil crop that is characterised by drought resistance and a large, highly repetitive genome (Badoin *et al.* 2017). Since *Helianthus* populations can maintain approximately constant yields under different environmental conditions (*e.g.* in dry soil), this iconic plant may become a model crop for the study of adaptation of vegetation to climate change (Martinez-Force *et al.* 2015).

In a previous study, we analysed organ development and phototropic solar tracking in shoots of 12-week-old, fully de-etiolated (green) sunflower plants grown in northern California (Kutschera & Briggs 2016). For technical reasons, it was not possible to analyse the behaviour of the root system under these conditions. Here, we attempted to obtain a more 'holistic' view of organ development in 2- to 6-day-old sunflower seedlings raised in non-sterile vermiculite (± WL). Our analyses clearly revealed that as the cotyledons become green and expand in WL, hypocotyl elongation is inhibited and lateral root expansion is drastically enhanced. Therefore, during photomorphogenesis of the juvenile plant, resources (notably sucrose derived from fats stored in the cotyledons) are re-distributed. Whereas in etiolated

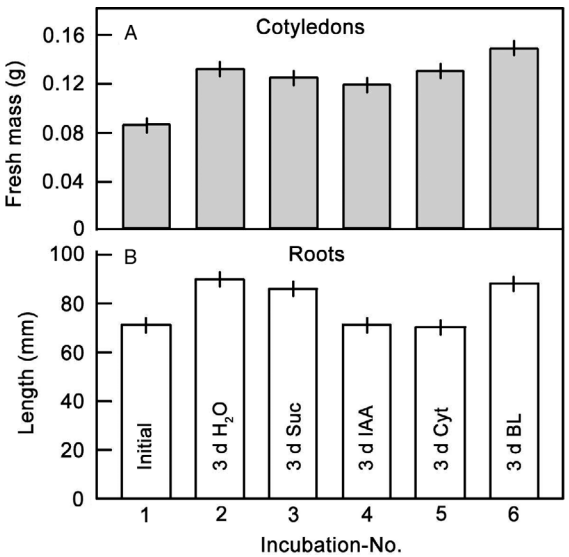

Fig. 8. Effects of sucrose (Suc, 10 mM), auxin (IAA, 10 μM), the cytokinin benzylaminopurine (Cyt, 10 μM) and brassinolide (BL, 1 μM) on cotyledon expansion (A) and root elongation (B) in sunflower cuttings. Pairs of cotyledons and roots were excised from 3-day-old etiolated seedlings, as shown in Fig. 9. Samples were incubated for 3 days on water, Suc, IAA, Cyt or BL, then cotyledon fresh mass and root length were measured.

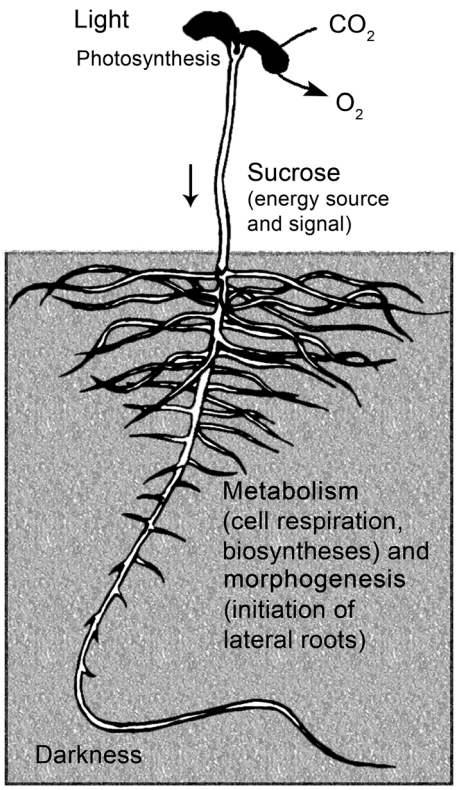

Fig. 9. Summary of the effect of white light (WL) on root development in sunflower seedlings. In this model, sucrose is transferred from the photosynthetically active cotyledons towards the root system. In the subterranean part of the seedling that grows in non-sterile vermiculite (or soil), sucrose maintains cell metabolism and, in addition, acts as a signal for the initiation of lateral roots (photomorphogenesis belowground in the phytosphere).

seedlings, axial elongation of the hypocotyl and primary root is promoted, upon irradiation with WL, an alternative developmental strategy prevails. During de-etiolation, not only the cotyledons grow and become green, in the soil (darkness) the root system expands laterally to anchor the developing system in the moist substrate. As a consequence, the surface area of the root system doubles, resulting in enhanced water (plus ions) uptake.

Which signals trigger this subterranean photomorphogenic response? Based on our results, a light effect *via* phytochrome action can be ruled out. We suggest that sucrose, provided *via* the photosynthetically active cotyledons, is not only the energy source to maintain cell metabolism and fuel ATP-dependent ion uptake of the root; but may also act as a signal to initiate lateral root primordia in the subterranean part of the seedling (Fig. 9). However, the mode of sucrose transport and unloading at the cellular level is still not yet clear (Eom *et al.* 2015; Ross-Elliott *et al.* 2017; van Gelderen *et al.* 2018). Likewise, the exact molecular mode of action of this disaccharide as a 'morphogene' is still a matter of debate (Li & Sheen 2016; Petrillo *et al.* 2018).

The morphogenic action of sucrose in cuttings from sunflower seedlings is depicted in Fig. 7D. It is apparent that lateral root initials, induced by sucrose, emerge from the inner cylinder of the organ. Kircher & Schopfer (2012) used aseptically grown seedlings of *A. thaliana* and obtained similar results. However, since these authors raised their seedlings in the absence of soil microbes, their seedlings almost entirely lacked lateral roots. Hence, these elegant experiments should be reproduced under non-sterile (real world) conditions in order to explore the full developmental potential of this model plant. Lateral root development consists of a 're-embryonalisation' of the totipotent cells of the pericambium, a process that has been described in a number of plant systems in some detail (Josten & Kutschera 1999).

Are the classical phytohormones involved in the initiation of lateral roots, as proposed by Kazan (2013), Moni *et al.* (2015) and others? Based on our results, we have to conclude that in none of these phytohormone experiments carried out with sunflower cuttings, were lateral root initials induced. This finding indicates that other factors are necessary for root development to occur in this system. Butcher (1964) has shown that a mixture of substances (root hormones) are responsible for the continued growth of isolated roots in liquid culture. Since sucrose is usually one component of this cocktail of ions (plus organic molecules), we suggest that the failure to initiate lateral root elongation is due to a lack of one or several substances in our system. More work is required to elucidate the factors responsible for the continuation of lateral root development in the model plant *H. annuus*. Since the crop species *H. annuus* is not only of economic value, but also important for climate change research (Badoin *et al.* 2017), it is necessary to further explore these processes using more sophisticated techniques.

About six decades ago, Hans Mohr introduced the mustard seedling (*S. alba*) as a suitable experimental system for the study of plant development (Mohr 1957). Over subsequent decades he published numerous monographs and textbooks on this topic (Mohr 1968, 1972; Mohr & Schopfer 1978). Despite the fact that the *Arabidopsis* agenda has now overshadowed the era of such classical photomorphogenesis research (Schäfer & Nagy 2006; Tong *et al.* 2008; Wang *et al.* 2012; Chen *et al.*

2016; Pham *et al.* 2018), and sunflower is a model organism used to study global warming (Badoin *et al.* 2017), the pioneering *S. alba* work of Mohr and collaborators laid the foundations for these more recent developments in plant sciences.

Finally, we want to stress that in addition to sucrose, other mobile signalling chemicals may be of importance during photomorphogenesis of the root system in developing seedlings. Chen *et al.* (2016) have shown that the transcriptions factor HY5 acts as a key integrator during light-induced, hormone-mediated organ development. Upon light activation in the shoot, HY5 is transported into the root system, where it exerts a positive effect on lateral root development (Chen *et al.* 2016; van Gelderen *et al.* 2018). HY5 is involved in light–hormone signalling, pathways that interact in the cells of the juvenile plant (Luo & Shi 2019). Therefore, we conclude that our simple 'sucrose model' (Fig. 9) represents only a crude scheme of those much more complex (auxin-dependent) processes that lead to the initiation and development of lateral roots in developing *Helianthus* seedlings.

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