REVIEW ARTICLE

Seedling development in buckwheat and the discovery of the photomorphogenic shade-avoidance response

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ABSTRACT

Numerous botanists of the early 19th century investigated the effect of sunlight on plant development, but no clear picture developed. One hundred and fifty years ago, Julius Sachs (1863) systematically analysed the light–plant relationships, using developing garden nasturtium (*Tropaeolum majus*) and seedlings of buckwheat (*Fagopyron esculentum*) as experimental material. From these studies, Sachs elucidated the phenomenon of photomorphogenesis (plant development under the influence of daylight) and the associated 'shade-avoidance response'. We have reproduced the classical buckwheat experiments of Sachs (1863) and document the original shade-avoidance syndrome with reference to hypocotyl elongation and cotyledon development in darkness (skotomorphogenesis), white light and shade induced by a canopy of green leaves. In subsequent publications, Sachs elaborated his concepts of 1863 and postulated the occurrence of 'flower-inducing substances'. In addition, he argued that the shade-avoidance response in cereals, such as wheat and maize, is responsible for lodging in crowded plant communities. We discuss these processes with respect to the red- to far-red light/phytochrome B relationships. Finally, we summarise the phytochrome B–phytohormone (auxin, brassinosteroids) connection within the cells of shaded *Arabidopsis* plants, and present a simple model to illustrate the shade-avoidance syndrome. In addition, we address the relationship between plant density and health of the corresponding population, a topic that was raised for the first time by Sachs (1863) in his seminal paper and elaborated in his textbooks.

INTRODUCTION

Farmers and biologists have known for a long time that sunlight is an essential environmental factor for plants to grow and thrive. However, until the early 19th century, these people had no notion that different colours of light might have selective effects on plant growth and development. Poggioli (1817) first demonstrated that it was 'violet light' that caused the leaves of *Mimosa pudica* (sensitive plant) to turn to face the light source, and that plants did not do at all well when grown under red light alone. Two decades later, Daubeny (1836) published a lengthy treatise in which he examined the effects of different coloured light on plant growth. He reported, first, that red was the colour of light that caused the 'secretion of green matter' into bean leaves, and, second, that blue light induced much more water loss from bean plants than red light; the latter much later confirmed in studies on the effect of different wavelengths of light on the aperture of stomata (see Briggs 2006). In a subsequent report, Payer (1842) demonstrated unambiguously that plants curved only in the direction of a blue light source (for a detailed discussion of this early history, see Briggs 2006).

We now know that light, through a multitude of photoreceptors excited by several different wavelengths, plays a crucial role in a plant's development throughout its life cycle. The seeds of many non-domesticated flowering plants have low levels of stored reserves that become rapidly exhausted during the period of growth below the surface of the soil. For buried, imbibed seeds of such species, light is the only signal indicating the proximity of the seedling to the soil surface, so that it acts as a reliable cue for the initiation of germination (see Mandoli *et al.* 1990 for a discussion of light penetration through soil). During subsequent seedling establishment and greening of the aboveground organs (stems, cotyledons, primary leaves), the juvenile plant develops from a heterotrophic into a fully photoautotrophic organism (*i.e.* from skotomorphogenesis to photomorphogenesis). These key events, which are associated with light-induced directional growth of the stems or leaves, are also regulated by light (Mohr 1972).

In mature angiosperms, light cues also provide information that can regulate the position of the leaves, and are used to sense circadian and seasonal rhythms. As a result, the induction and breaking of bud dormancy, the orientation of leaves, the initiation of flowering and the movement of stomata are among the light-mediated processes that are imperative for optimising growth, development and reproduction of the green, sessile, photosynthetic organisms (Srivastava 2002; Briggs & Spudich 2005; Briggs 2006; Schäfer & Nagy 2006; Koller 2011).

LIGHT AND PLANT GROWTH: THE EMERGING CONCEPTS OF JULIUS SACHS

Although the writings of 19th century naturalists noted and summarised most of the light-mediated processes mentioned

above, they did not present a clear picture as to the effects of light on plant development. One hundred and fifty years ago, the German botanist Julius Sachs (1832–1897), who was the founding father of experimental plant physiology (Morton 1981; Kutschera & Briggs 2009, 2012), published an article entitled *Ueber den Einfluss des Tageslichts auf Neubildung und Entfaltung verschiedener Pflanzenorgane* (On the influence of daylight on the formation and growth of different plant organs; Sachs 1863). This paper appeared as a *Beilage* (Supplement) to Vol. 21 of the *Botanische Zeitung* (Botanical Magazine), a leading journal devoted to all branches of the plant sciences at that time. Sachs (1863) pointed out, with reference to DuHamel (1758) and other earlier investigators, that the literature on light and plant development was not very revealing. To fill this void in botanical knowledge, he carried out numerous experiments with a variety of crop species, and described his results in the 1863 publication. Among many other interesting data provided by the author, Sachs (1863) summarised his studies on the effect of different wavelengths of daylight on plant development. Within this context, the German botanist described original experiments carried out with buckwheat seedlings that led him to the following conclusion (Sachs 1863): '*Wird der Gipfel der Keimpflanze durch andere, benachbarte Pflanzen beschattet, so verlängert sich das hypocotyle Glied* (If a seedling is shaded by other, neighbouring plants, the hypocotyl elongates)'.

In the present contribution, which marks the 150th anniversary of the discovery of photomorphogenesis and the related shade-avoidance response (Sachs 1863), we first summarise some classical key experiments on the effect of light on plant development. In the second part, we have reproduced Sachs's original buckwheat experiments of 1863 and describe our results, in a modern version, to illustrate the shade-avoidance behaviour of this crop species (Franklin 2008; Casal 2013). Finally, we provide a simple model of the shade-avoidance response that is based, in part, on our recent work in the field of phytohormone research (Kutschera *et al.* 2010a; Deng *et al.* 2012; Kutschera & Wang 2012; Wang *et al.* 2012).

DISCOVERY OF PHOTOMORPHOGENESIS AND FLOWER-INDUCING SUBSTANCES

In his seminal paper, Sachs (1863) referred to the work of some famous 'older physiologists' such as Charles Bonnet (1720–1793), Henri-Louis DuHamel (1700–1782), and Jean Senebier (1742–1809). All three botanists studied and described the phenomenon of 'etiolement', *i.e.* growth and development of seedlings in complete darkness. However, according to Sachs (1863), these scientists focused on the lack of chlorophyll in etiolated plant organs, but failed to provide a general description or definition for this phenomenon. Based on his own experiments on seedlings of sunflower (*Helianthus annuus*), buckwheat (*Fagopyron esculentum*) and garden nasturtium (*Tropaeolum majus*), Sachs (1863) concluded that, in darkness, plant development proceeds in an 'abnormal' way, whereas daylight 'regulates the normal growth', and causes the healthy green colour of the aboveground plant organs.

Under the heading *Tropaeolum majus*, Sachs (1863) described experiments he had carried out in June 1862 (Fig. 1). The botanist used a *T. majus* plant raised in a natural daylight/night cycle that had already developed numerous green leaves.
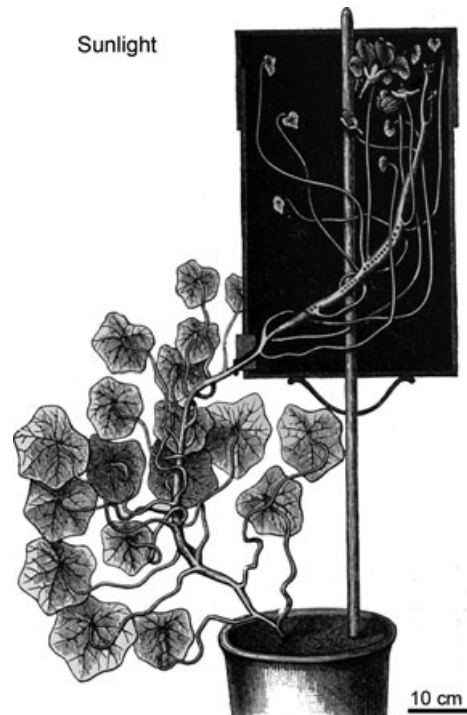

Fig. 1. Experimental demonstration of the effect of daylight on plant development by Julius Sachs (1863). The tip of the stem of a garden nasturtium (*Tropaeolum majus*) was placed in a dark box and subsequent development of the organs in the absence of light was recorded over the following 3 weeks. The author concluded that light regulates 'normal' organ growth, and a lack of solar irradiation causes 'abnormal' plant development. In addition, he suggested that the green leaves outside the box produce and send substances that cause the formation of flowers (adapted from Sachs 1887). This experiment provided a dramatic example of re-etiolation.

He then placed the tip of the stem into a dark box. In complete darkness, the developing stems were thinner and longer than in the light, and the smaller pale leaves developed long petioles. Most interestingly, after several weeks of growth in darkness, the pale stem within the black box developed colourless flowers (see Fig. 1, top). Accordingly, Sachs (1863) concluded that the green assimilating leaves of the *Tropaeolum* plant produce nutrients that are transferred *via* the stem into the developing organs of the etiolated upper half of the plant. In addition, he hypothesised that these leaves also produce substances that cause the formation of flowers (see Danielson & Frommer 2013 for a recent update on this topic). In his 1863 paper, Sachs for some reason failed to illustrate this seminal experiment. Two years later, the author published a second series of *Tropaeolum* experiments, again without illustration. These studies yielded very similar results (Sachs 1865a). Twenty-five years after the first description of the effect of light on plant development, and the proposal that the floral stimulus is produced in the green leaves under the influence of irradiation, Sachs (1887) extended his earlier studies for a third time. Moreover, he finally illustrated his experiment of 1863 (Fig. 1). In his textbook, *Handbuch der Experimental-Physiologie der Pflanzen* (Experimental Physiology of Plants), Sachs (1865b) ultimately published a figure showing an experimental set up similar to that reproduced in Fig. 1. Finally, his famous *Lectures*

on the Physiology of Plants (1882), included a splendid figure showing a large squash (Cucurbita pepo) plant growing in a botanical garden, with the tip first placed into a large dark box and later, after the development of stems and leaves, returned into sunlight (Fig. 2). This experiment documented for the first time that the symptoms caused by a lack of light are reversible. Sachs (1882) also described the effect of light on organ development, with reference to flowering and the production of fruits in darkness.

It should be noted that Sachs (1863), who must be credited with the discovery of photo- and skotomorphogenesis, i.e. plant development in the light or darkness, respectively, did not use our modern terminology. He referred to 'die Krankheit des Etiolements (the disease of etiolement)', or 'plant sickness in the absence of light', and only considered organ development in a daylight/dark cycle as the 'normal' mode of growth (Sachs 1882). It was Wilhelm Pfeffer (1845–1920), the co-founder of modern plant physiology, who coined the term 'photomorphogenesis' (Photomorphose, later transformed into Photomorphogenese) in his famous textbook (Pfeffer 1897/1904). His well-known 'potato' picture, showing two adult Solanum tuberosum plants, one grown in complete darkness, and the second in a light/dark-regime, is shown in Fig. 3.

It is obvious from all of these experiments that sunlight exerts a strong effect on the development of aboveground organs (stem, petioles and leaf blades), which is of major significance for survival of the organism. In darkness, stem elongation is the key process – nothing else matters, until the tip of the etiolated plant axis reaches a light environment.

REPRODUCTION OF THE BUCKWHEAT EXPERIMENTS OF JULIUS SACHS

In the 19th century, the crop plant, common buckwheat (F. esculentum), was cultivated in many agricultural areas of Europe and Russia (Quinet et al. 2004). As a result, botanists used such 'pseudo-cereals' of the dicot family Polygonaceae in their experimental studies. Accordingly, buckwheat seeds, which, like sunflower kernels, are achenes containing an embryo surrounded by a hard outer hull, are among the several materials that Sachs (1863) used. The botanist briefly described the germination of buckwheat achenes and their growth in darkness. According to Sachs (1863), etiolated buckwheat seedlings can reach a stem length of up to 40 cm before they die in darkness, whereas plants of the same age raised in sunlight are only ca. 3-cm tall. Sachs (1863) also pointed out that when the seedlings were grown in the shade of neighbouring vegetation, they elongated more rapidly than in full sunlight, and could reach a stem length of up to 15 cm. In addition, Sachs (1863) mentioned a weakened greening response of the cotyledons for plants under a canopy in sunlight.

Fig. 2. Demonstration of the effect of sunlight on plant development. Sachs's image of a mature squash plant (Cucurbita pepo), showing both re-etiolation and de-etiolation. The experiment documents that light action on plant morphology is completely reversible (adapted from Sachs 1882).

Fig. 3. The effect of daylight on plant development in potato (Solanum tuberosum). Tubers obtained from the same mother plant were either grown in a dark box (A) or in a greenhouse under a natural dark/light cycle (B). The numbers 1 to 8 denote the internodes, counted from below. Within the context of these experiments, Wilhelm Pfeffer introduced the term photomorphose (adapted from Pfeffer 1897/1904).

We have reproduced and extended these classic buckwheat experiments under standard laboratory conditions (25 °C) with the following results. Within 12 h after sowing of achenes in moist soil, the radicle emerged. Thereafter, the primary root elongated and reached a length of *ca*. 2 cm 1 day after planting (Fig. 4). After 1.5 days, lateral roots started to develop, and by 2 days the root had elongated extensively and developed many lateral branches. The stem emerged *ca*. 12 h later than the radicle. One day after sowing, the angle between the axis of the hypocotyl and that of the cotyledons (with attached seed coat) was about 90°, rather than the usual 180° inverted U-shaped hook. Hence, in buckwheat seedlings, the curved hook did not develop until between days 1 and 2 after sowing; it was maintained over the subsequent 4 days. By day 7 after sowing, the apical hook was actually partially open in the majority of etiolated seedlings (Fig. 5A). This mode of organ development in darkness differs from that in seedlings of sunflower and garden cress; species in which the apical hook develops concomitant with the onset of germination (Kutschera & Niklas 2009, 2011, 2012, 2013; Kutschera & Briggs 2012).

In the next set of experiments, we reproduced the shade avoidance experiment as described by Sachs (1863). Since by day 7 after sowing, *ca*. 20% of the dark-grown control seedlings had reached their final size, we compared batches of 1-week-old buckwheat seedlings that were raised in darkness, in a white light/dark cycle (WL) or, under the latter conditions but in shade provided by the green leaves of mature plants. Representative 7-day-old seedlings of average size are depicted in Fig. 5. It is obvious that, under the conditions used by Sachs (1863), skoto- and photomorphogenesis of the buckwheat seedlings are clearly distinguishable developmental strategies. In dark-grown seedlings, a long, thin pale hypocotyl and small yellowish cotyledons develop (Fig. 5A), whereas in light-grown plants the stem is much shorter and sturdier, the cotyledons are fully expanded (Fig. 5B) and the primary leaves are emerging (see Fig. 6B). In 7-day-old seedlings that were raised in the shade of green leaves of larger plants, a photomorphogenic elongation

response is visible (Fig. 5C). Moreover, the unfolded cotyledons are much smaller than in the white light control, and are not fully green.

Taken together, our reproduction of the original buckwheat experiments of Sachs (1863) again demonstrated the phenomenon of scoto- *versus* photomorphogenesis (Figs 1–3), as well as the shade-avoidance response, in this crop species.

SHADE AVOIDANCE AND COTYLEDON DEVELOPMENT

Sachs (1863) mentioned that, during growth in daylight, the greening of cotyledons proceeds 'normally', whereas in dark-grown or shaded plants, only pale 'abnormal' (*i.e.* unhealthy) organs are visible. We have analysed this phenomenon quantitatively, using buckwheat seedlings as experimental material. Representative pairs of cotyledons from 7-day-old seedlings raised in darkness, white light or shade are depicted in Fig. 6. In darkness, the hook displayed an angle of 90° (Fig. 6A), which is similar to that in 1-day-old seedlings (see Fig. 4), and the cotyledons were small, still folded and yellow. In light-grown, fully de-etiolated seedlings, the cotyledons were dark-green, and the primary leaves had emerged (Fig. 6B). This pattern is in sharp contrast to the morphology of shaded seedlings. Although hook opening proceeded as in white light (WL) controls, the unfolded cotyledons were much smaller and pale-green, and no primary leaves were visible (Fig. 6C). Our quantitative data are summarised in Table 1. In this study, the average hypocotyl lengths of 7-day-old buckwheat seedlings, raised in darkness, WL or shade, were *ca*. 21, 8 and 13 cm, respectively. In etiolated seedlings, variability was very large,
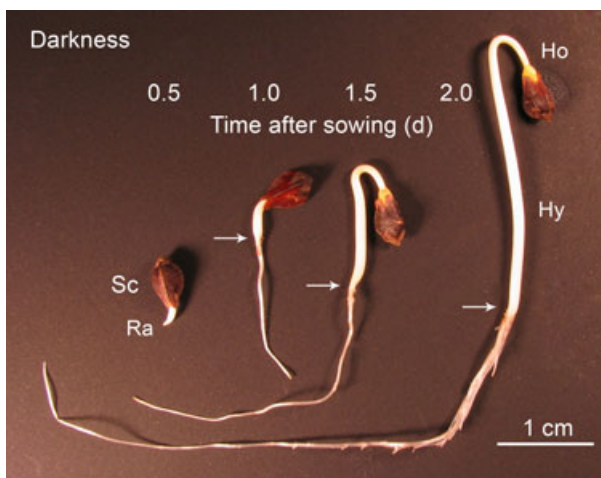
Fig. 4. Development of buckwheat (*Fagopyron esculentum*) achenes in darkness. Between 0.5 and 2.0 days after sowing, representative seeds taken from populations of individuals (25 per plastic box) raised in moist soil at 95% RH (25 °C) were selected and photographed. Ho = apical hook, Hy = hypocotyl, Ra = radicle, Sc = seed coat. The transition zone between the hypocotyl and the primary root is indicated with arrows.
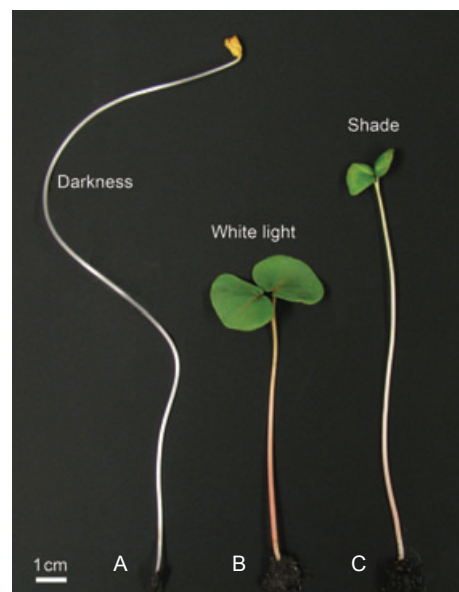
Fig. 5. Documentation of skoto- and photomorphogenesis, as well as the shade-avoidance response, in seedlings of buckwheat (*Fagopyron esculentum*). Representative plants, photographed 7 days after sowing, are depicted. The seedlings were grown in darkness (A), in a 16-h white light/dark cycle (bright WL, 120 µmol photons m^{-2}·s^{-1}) (B), or under this light regime but in shade from green leaves (dim WL, 12 µmol photons m^{-2}·s^{-1}) (C) at 25 °C. Full-length hypocotyls from the root–shoot transition zone to the cotyledons are shown.

Fig. 6. Cotyledon development in buckwheat (*Fagopyron esculentum*) seedlings raised in darkness (A), white light (B) or shade (C), as described in the legend to Fig. 5. Photographs of 7-day-old plants of representative size and morphology are shown. Co = cotyledons, Ho = apical hook, Hy = hypocotyl, Pl = plumule (primary leaves).
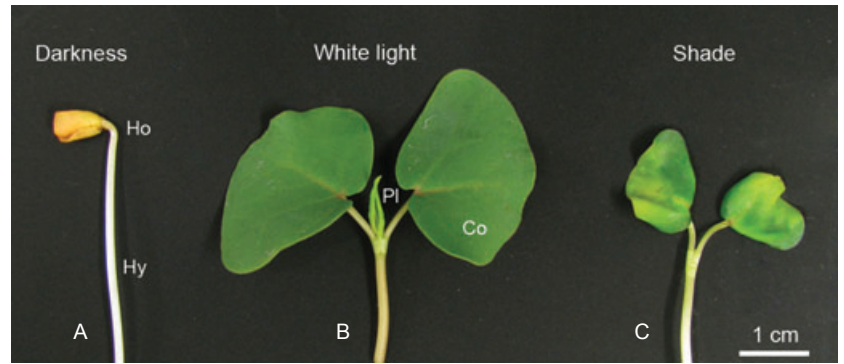
Table 1. Effects of white light (120 µmol photons $m^{-2} \cdot s^{-1}$) and shade (12 µmol photons $m^{-2} \cdot s^{-1}$) on seedling development in buckwheat (*Fagopyron esculentum*).

parameter	treatment	length or surface area
A. Length of hypocotyl (cm)	D	21.2 ± 0.3
	WL	7.6 ± 0.2
	S	12.8 ± 0.2
B. Surface area of cotyledons (mm²)	D	42 ± 1
	WL	250 ± 4
	S	130 ± 2

Batches of 12 plants were grown in garden soil for 7 days in darkness (D), in a 16-h white light (WL)/dark cycle, or under this regime in shade (S), as shown in Figs 5 and 6 (data represent means ± SE of six independent experiments each).

Table 2. Effects of white light (120 µmol photons $m^{-2} \cdot s^{-1}$) and shade (12 µmol photons $m^{-2} \cdot s^{-1}$) on pigment accumulation in cotyledons of buckwheat seedlings (*Fagopyron esculentum*) (chlorophyll *a/b*, carotenoids).

treatment	Chl *a*	Chl *b*	carotenoids (mg·g⁻¹ FM)
D	0.00	0.00	0.13 ± 0.01
WL	1.50 ± 0.1	0.48 ± 0.02	0.38 ± 0.02
S	0.74 ± 0.1	0.23 ± 0.01	0.19 ± 0.01

Batches of 12 plants were grown for 7 days in darkness (D), white light (WL) or shade (S), as detailed in the legend to Table 1, and pigments quantified as described in Kutschera *et al.* (2010b) (data represent means ± SE of 9 independent experiments each).

with stem lengths ranging from 16 to 34 cm. These data are in accordance with the measurements of Sachs (1863), who found that some hypocotyls of etiolated buckwheat seedlings were 30 to 40 cm in length.

White light (WL) had a large effect on the expansion of the cotyledons (surface areas: 250 *versus* 42 mm² in WL-grown *versus* etiolated seedlings), and in shaded plants the surface area had an intermediate value of *ca*. 130 mm² (Table 1). The concentrations of photosynthetic pigments (chlorophylls *a*, *b*, carotenoids) were determined using the spectrophotometric assay as described in Kutschera *et al.* (2010b). In etiolated cotyledons, no chlorophyll was detected, and the level of carotenoids was low (Table 2). Dark-green cotyledons of fully de-etiolated buckwheat seedlings contained high concentrations of chlorophyll *a* and *b*, and a *ca*. three-fold higher level of carotenoids than was measured in the dark control. In shaded plants, pigment levels were intermediate between those measured in the WL- and dark-grown seedlings, indicating that the shade avoidance-response, as described by Sachs (1863), is associated with a reduction in the biosynthesis of photosynthetic pigments.

THE EFFECT OF RED LIGHT ON LEAF DEVELOPMENT

In his classic paper, Sachs (1863) discussed the shade-avoidance response with respect to skoto- and photomorphogenesis, and interpreted his buckwheat experiments (Figs 5 and 6) as a partial etiolation reaction, which is attributable to the lack of sunlight. One year later, the author published a comprehensive

article entitled *Wirkung farbigen Lichts auf Pflanzen* (Effect of coloured light on plants), wherein he studied the influence of different wavelengths of sunlight on greening and photosynthetic activity in different crop species, and in water plants such as the dicot *Ceratophyllum* sp. (Sachs 1864). In this paper, he pointed out that light-mediated greening of etiolated stems and cotyledons occurs in all monocotyledonous and dicotyledonous plants, but not in the seedlings of gymnosperms. In *Pinus sylvestris* and related taxa, the organs become green even in the absence of any sunlight.

Sachs (1864) compared the effects of orange *versus* blue light (Fig. 7) on accumulation of chlorophyll (*i.e.* the greening response) in etiolated seedlings that were transferred into the light. Based on numerous experiments, he concluded that the light-induced greening in cotyledons and leaves of crop species, such as white mustard (*Sinapis alba*) and maize (*Zea mays*), is dependent on the colour of the irradiation, *i.e.* light quality. Sachs (1864) noted that light in the orange and blue regions of the spectrum caused a rapid greening response, but phototropic bending of axial organs only occurred when unilateral blue light was applied (see Briggs 2006 and Kutschera & Briggs 2012 for historic accounts of this phenomenon). In addition, he observed that plant growth occurs more rapidly when seedlings are raised in orange light, compared to blue light-treated controls. These qualitative observations strongly confirmed the earlier experiments showing that plants can sense light quality *via* separate pigment systems (Briggs 2006).

It should be noted that Sachs (1864) did not draw a general conclusion from his experiments. However, he mentioned that, with respect to cotyledon expansion, orange light acts like shade. This was the first indication that light in the red region of the spectrum (Fig. 7) may be causally involved in the
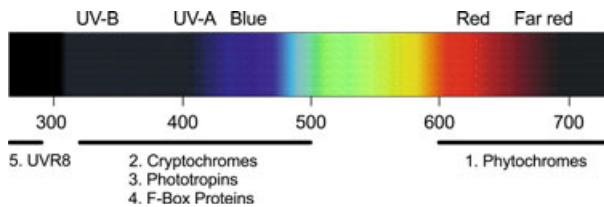
Fig. 7. The five classes of plant photoreceptors. Phytochromes (1) absorb in the red/far-red region; cryptochromes, phototropins and F-box proteins (2, 3 and 4) are UV-A/blue receptors, and the UVR8 photoreceptor (5) absorbs light in the UV-B part of the sunlight spectrum.

perception of shade by plants that grow below dense populations. We now know that, as chlorophyll and the various carotenoid pigments absorb light strongly in the blue region of the spectrum, it is partially a lack of activation of blue light-sensitive photoreceptors in shade under green leaves that accounts for the partial etiolation that Sachs (1864) observed.

LODGING IN CROWDED PLANT COMMUNITIES

In all of his research papers, Sachs (1863, 1864, 1865a, 1887) discussed his findings with respect to those of other earlier investigators, but without reference to agricultural practises. The significance of the shade-avoidance response of plants raised in dense populations (Sachs 1863) was fully recognised by the discoverer of this phenomenon, but in an entirely different context. In chapter V of his *Handbuch der Experimental-Physiologie der Pflanzen* (Handbook of Experimental Physiology of Plants), Sachs (1865b) summarised the nutrients that crop plants need in order to grow, flower and set seed (within the limits of the chemistry of the time). Under the headline *Die Kieselsäure* (Silicic acid), Sachs (1865b) pointed out that according to his own extensive studies on juvenile crop plants raised in aquaculture, silicon is a major component of the ash of dried and burned vegetation. However, in the absence of added silicic acid $(Si(OH)_4)$, crops such as maize (*Z. mays*; Fig. 8, Inset) develop normally, and therefore this element does not appear to be an essential nutrient for plants. Despite these insights, leading agriculturalists, such as Wilhelm Knop (1817–1891), argued that silicon is necessary for plants, notably cereals, in order for them to develop sturdy resistant internodes and leaves.

Based on this unfounded assumption, European agriculturalists believed that the '*Lagern des Getreides* (the lodging of cereal plants)' is caused by a lack of silicic acid in the soil. Sachs (1865b) refuted this hypothesis, an idea that had developed around 1860 into a dogma among agriculturalists, and attributed this major problem to the shade-avoidance response. In crowded populations of cereal plants such as wheat (*Triticum aestivum*) or maize (Fig. 8) the lower internodes are shaded from the green leaves of neighbouring conspecifics. As a result, the basal regions of the stems become etiolated and develop thin weak internodes that are prone to lodging, *i.e.* become permanently displaced from the upright position due to external forces exerted by wind, rain or hail.

Sachs (1865b) provided the following empirical evidence for his shade-avoidance hypothesis in dense stands of cereals. (i) When seeds are planted at lower densities, the resulting organisms do not lodge. (ii) Single crop plants that 'escaped' from
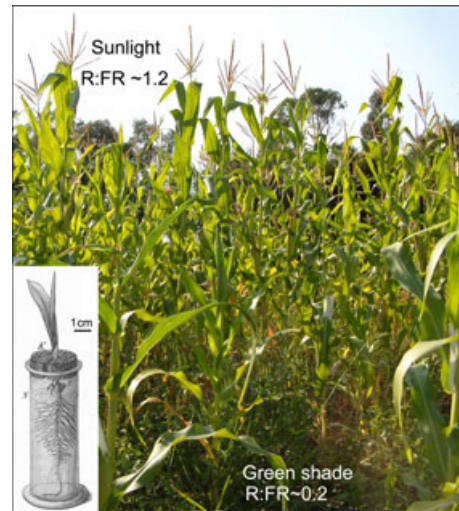

Fig. 8. Maize (*Zea mays*), one of the most important crops in the USA, is grown in dense populations so that shade-avoidance responses are induced. R = red, FR = far-red light; the R:FR data from Smith (1982) (Original photograph, Carnegie Institution for Science, Stanford, CA, USA, September 2012). Julius Sachs was one of the first to use maize seedlings for experimental analyses (hydroculture experiments, inset) (adapted from Sachs 1865b).

dense populations and grow without competitors are sturdy and never succumb to wind or rain. (iii) Crowded populations that are supplied with mineral nutrients, and hence grow more rapidly than 'normal' stands, show the highest tendency to lodge, even without strong wind or heavy rainfall. In addition, Sachs argued against the 'silicon hypothesis' as follows: only the soft nodes, but not the harder silicon-rich leaves respond to gravity and cause upward bending of the cereal plants. Hence, the addition of silicic acid to the soil to prevent the lodging response is useless (Sachs 1865b).

These facts show that Sachs (1863) not only discovered the shade-avoidance response, but, 2 years later, used this knowledge to explain a phenomenon of tremendous practical importance, *i.e.* the reduction of crop yield caused by lodging in dense populations of cereals. Sachs (1865b) recommended that farmers plant the seeds at larger distances, and refuted the silicic acid dogma by showing that this chemical element did not play any role in plant metabolism (again within the limitations of the chemistry of the time). However, today we know that silicon is always present at low levels in glass jars used in experimental systems, so that a Si-free control is difficult to achieve. We also know that silicon is found in the epidermal cells of grasses, including crop species. Indeed, Sachs (1865b) had already suggested that his glass container for aquaculture experiments (see Fig. 8, Inset) might release some silicon into the culture medium, and this assumption was much later verified (Rafi *et al.* 1997).

Was Sachs (1865b) correct when he concluded that lodging in wheat, barley and oats is attributable to the shade-avoidance response? Lodging may occur between ear emergence and maturity of the plants and can cause significant reductions in grain yield and quality (Pinthus 1974). Quantitative studies have shown that the percentage of the crop lodged at harvest is a function of plant density. When 50 plants m^{-2} are raised in

the field, the percentage lodging was zero. However, in crowded populations of crops (500 individuals m^{-2}) at the time of harvest, 80–90% of the area consisted of lodged individuals (Berry *et al.* 2004). Although increased plant density reduced the diameter and wall thickness of the basal grass internodes, it is not yet clear whether the shade-avoidance response is the only factor that accounts for lodging. According to laboratory studies of Sparkes & King (2008), it is likely that a reduction in photosynthetically active radiation, and hence light quantity, is a second factor that also contributes in the partial etiolation response to lodging in wheat and other cereals.

PHOTORECEPTORS AND THE SHADE-AVOIDANCE SYNDROME

In the experiments of Sachs (1863, 1864, 1865a,b, 1887), polychromatic white light (WL, wavelength *ca.* 380–800 nm) and light in the orange or blue region of the spectrum was used. Although speculations as to the presence of photoreceptors in plant cells had been published by Pfeffer (1897/1904), it took decades of research effort until, in 1959, the first signal-transducing photoreceptor pigment, the red/far-red light-absorbing phytochrome, was discovered (Butler *et al.* 1959; Mohr 1972; Batschauer 2003).

Today, five classes of photoreceptors in plant cells are known: (i) phytochromes; (ii) cryptochromes; (iii) phototropins; (iv) F-box proteins; and (v) the UVR8 photoreceptor (Franklin & Quail 2010; Rizzini *et al.* 2011; Briggs & Lin 2012; Kutschera & Briggs 2012). Photoreceptors (i) (the phytochromes, *i.e.* phytochromes A through E) absorb in the red/far-red region of the sunlight spectrum, whereas the other four classes [(ii)–(v)] are blue/UV-A light-absorbing sensor pigments (cryptochromes and phototropins) or a UV-B-absorbing photoreceptor (UVR8; Fig. 7).

Three decades ago, Smith (1982) summarised the effect of different light qualities on plant development in dense communities, with special reference to the ratio of red (660 nm; R) to far-red (730 nm; FR) light. In daylight, R:FR was found to be relatively constant (*ca.* 1.2) irrespective of weather conditions and time of year (Fig. 8). However, on clear days, dawn and the onset of dusk were associated with a significant drop in R:FR to values of *ca.* 0.8–0.7. Light quality under vegetation canopies (*i.e.* in the shade of green leaves) was more drastically changed: under the canopies of maize, wheat and sugar beet, R:FR of 0.2, 0.5–0.6 and 0.1–0.4, respectively, were measured (Smith 1982, 1995). These data document that, dependent on the presence of other plants, light quality in the red region of the sunlight spectrum (Fig. 7) may show drastic changes and can cause the so-called 'green shade' (*i.e.* dim light enriched in FR; Fig. 8). These changes will dramatically alter the ratio of Pr (inactive phytochrome) to Pfr (active form of the sensor pigment). Indeed, Morgan & Smith (1979) demonstrated dramatically that the lower the ratio of R:FR, the taller and weaker plants of *Sinapis alba* became – precisely the consequence of growing in the shade of other leaves. At the time of their study, nothing was known about the blue light receptor, but it is now clear that the absence of blue light in green shade from leaves is also of importance in the partial de-etiolation response seen under a vegetation canopy (Mathews 2006; Casal 2013). Moreover, the blue light receptor has morphed into cryptochromes,

phototropins and F-box proteins – forming an array of blue light receptors whose activation is required for normal plant development in full sunlight (Bae & Choi 2008; Tong *et al.* 2008).

Based on these insights, it was suggested that phytochromes might be primary photoreceptors in the perception of 'green shade' caused by leaves. In dark-grown plant organs, phytochrome, which is a cytoplasmic homodimer of two independently reversible subunits, is synthesised in the red light (R-absorbing) and inactive form (Pr). Upon photo-conversion *via* red irradiation, the far-red (FR)-absorbing version of the sensor pigment, which represents the active phytochrome (Pfr), is generated. Under natural conditions, the relative amounts of R and FR will determine the relative concentration of Pfr, which is translocated from the cytoplasm into the nucleus. Thereafter, a Pfr-mediated signal cascade is initiated, resulting in differential gene activity (Smith 2000; Briggs & Spudich 2005; Mathews 2006). However, with respect to photomorphogenesis and shade avoidance, it remained unclear how Pfr exerts its multiple effects. Four decades ago, Mohr (1972) suggested that phytohormones (auxins, gibberellins) might act as second messengers of the active phytochrome (Pfr). We now know that this is likely the case, and will briefly discuss this topic in the next section.

THE PHYTOCHROME–GROWTH HORMONE CONNECTION

Numerous studies have shown that the phytochromes A and B (phy A/B) have different functions during plant development (Franklin & Quail 2010). All available evidence indicates that phy B is involved in perception of the light stimulus under dense plant canopies (Franklin 2008; Casal 2013). However, in earlier studies, phytochrome action was studied almost entirely with a focus on skoto- *versus* photomorphogenesis (Mohr 1972; Smith 1982).

The shade-avoidance syndrome (Sachs 1863) documented here (Fig. 5C) was re-discovered during the 1980s (Ballaré *et al.* 1990), and numerous papers summarising this physiological response have been published over the past 20 years (Casal 2013). It is well established that in dense populations, the low R:FR (*i.e.* light enriched with FR, or 'green shade'; Fig. 8) is perceived *via* phy B, resulting in an enhancement of stem and petiole extension. By this means, an intra-specific 'struggle for a top place in the sun' is elicited, leading to competition for the resource of light through overtopping of neighbours (Smith 1982, 2000; Briggs & Spudich 2005; Franklin 2008; Ballaré 2009; Kutschera & Briggs 2009; de Wit *et al.* 2012). However, despite these insights, the key question as to how a shade-induced, phytochrome-mediated perception of light enriched in FR (*i.e.* 'green shade') is translated into an enhancement of growth in crop species such as wheat and maize remained a mystery (Ballaré *et al.* 1990; Srivastava 2002; Mathews 2006; Sparkes & King 2008).

Experiments with the model plant *Arabidopsis thaliana*, which displays a strong shade-avoidance response under laboratory conditions, yielded novel insights that are summarised as follows (Bai *et al.* 2012a,b; Hornitschek *et al.* 2012; Li *et al.* 2012; Oh *et al.* 2012; Wang *et al.* 2012). First, 'green shade'-induced low R:FR within the cells of plants overgrown by competitors 'inactivate' phy B (Fig. 9). This initial response occurs

by transforming the active form of the cytoplasmic photoreceptor (Pfr), which is normally transferred into the nucleus, into the inactive version of the sensor pigment (Pr). As a result of Pfr inactivation (*i.e.* Pr formation), basic helix-loop-helix transcription factors (PIFs, or phytochrome-interacting factors), which are negative regulators of photomorphogenesis (Leivar & Quail 2011), accumulate in the nucleus and suppress the gene expression required for normal photomorphogenesis. Moreover, at the same time, positive regulators of photomorphogenesis are suppressed (see Bae & Choi 2008 for a more detailed scheme). This Pr–PIF-mediated transcriptional re-programming leads to increased biosynthesis and/or activity of phytohormones, such as auxin (indole-3-acetic acid, IAA), brassinosteroids (BRs) and gibberellins (GAs). These growth-promoting substances enhance the rate of cell elongation and, as a result, stimulate elongation of stems and petioles in the shaded plants (Sánchez-Bravo *et al.* 2008; Kutschera & Wang 2012; Niklas & Kutschera 2012; Zhao 2012).

It should be stressed that this simple model of Pr–phytohormone-mediated growth promotion (Fig. 9) does not take into account that in *Arabidopsis*, IAA increases the biosynthesis of BRs (Wang *et al.* 2012), nor does it consider the roles of the various blue light receptors, especially the cryptochromes. However, the relationships between these major groups of phytohormones are complex and not yet fully elucidated. Nevertheless, the results summarised here clearly document that the photomorphogenic shade-avoidance response in *Arabidopsis* is a phytohormone-mediated reaction, and auxin and BRs are the central players in this complex phenomenon (Kurepin *et al.* 2012; Wang *et al.* 2012; Casal 2013).

We want to point out that the shade-avoidance syndrome discovered by Sachs (1863; Fig. 5C) and analysed here with reference to recent work on populations of *A. thaliana* is clearly
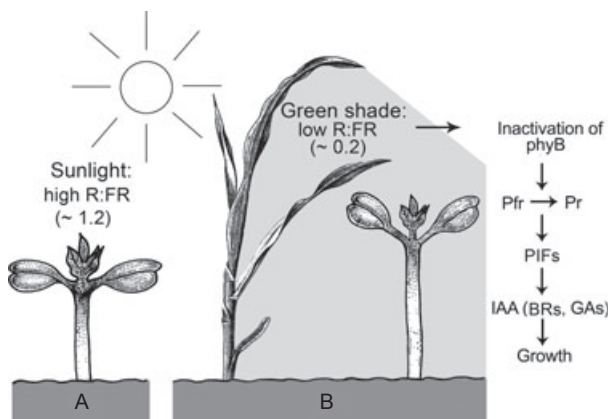
an undesirable feature of most major crop species. In monocultures, these green, sessile organisms compete for light (Fig. 8) and therefore display a more-or-less pronounced shade-avoidance response (Franklin 2008; Casal 2013). It is obvious that shade-*tolerant* plant species that grow in forest understories are not capable of outgrowing surrounding trees. These plants, herbs and small bushes that inhabit the forest soil, have evolved shade-tolerance behaviour to optimise photosynthetic carbon gain (increase in specific leaf area, *etc.*). As pointed out in Gommers *et al.* (2013), the mechanisms of shade tolerance (as opposed to shade avoidance) are, at present, only poorly understood.

CONCLUSIONS: SHADE AND PLANT HEALTH

In his original paper published 150 years ago, Sachs (1863) mentioned that, in darkness (or deep shade), plants display 'abnormal' or 'unhealthy' modes of development. In subsequent articles, and in his textbooks, he elaborated on this concept of the 'disease of etiolation' (Sachs 1882). Despite the fact that later investigators discovered that photo- and skotomorphogenesis are alternative developmental strategies of healthy land plants (Mohr 1972; Smith 1982, 1995, 2000; Briggs & Spudich 2005), recent studies appear to indicate that Sachs (1863, 1882) was, at least in part, correct.

The innate immune system of plants is, like the shade-avoidance response, modulated by phytohormones, notably by brassinosteroids (Wang 2012). Recent studies have shown that the occurrence of pests and diseases, which may be responsible for up to 25% of pre-harvest crop losses (Kutschera & Hossfeld 2012), is dependent on the environmental factor, light. For instance, the severity of disease caused by pathogenic fungi and bacteria is positively correlated with plant density (Ballaré 2009). Moreover, in many leaf-chewing insects, herbivory is largely suppressed in foliage and stems exposed to full sunlight, and induced when these green organs grow in the shade. Hence, light may in some way be important for the development of plant resistance *via* innate immunity (Roberts & Paul 2006).

Recent studies have shown that as a result of shading, the resistance of crop plants to insect herbivory is reduced because of inactivation of phy B. In addition, UV-B irradiation at very low irradiances was shown to have positive effects on plant health (Ballaré *et al.* 2012). Taken together, these novel findings document that the shade-avoidance response, which occurs when plants grow at high densities, and may be interpreted as a 'crowding effect', is clearly associated with health of the individual within the variable population (Ballaré *et al.* 2012). Hence, the suggestion of Sachs (1863, 1882) that dark-grown (or shaded) plants suffer from the 'sickness of etiolation' (Figs 1 and 2) may be interpreted in the light of these new findings, 150 years after the discovery of the phytohormone-mediated, photomorphogenic shade-avoidance response.

Fig. 9. Schematic representation of the shade-avoidance response in seedlings of dicotyledonous plants, such as thale cress (*Arabidopsis thaliana*). In sunlight (A), at a high R:FR, photomorphogenesis is mediated by phytochromes and blue light photoreceptors (see Fig. 7), whereas in shade (B), at low R:FR, phyB is largely converted to Pr. Lacking Pfr in the nucleus, negative regulators of photomorphogenesis are not inactivated and positive regulators are, on the contrary, suppressed, leading to partial etiolation. A reduction in the level of active phytochrome (Pfr) ultimately leads, *via* phytochrome-interacting factors (PIFs), to increased phytohormones (auxin, IAA; brassinosteroids, BRs; gibberellins, GAs), and promotion of growth of the stem and petioles is induced.

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