Root Photomorphogenesis in Laboratory-Maintained Arabidopsis Seedlings

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Abstract

In nature, root systems of most terrestrial plants are underground in darkness. Yet several photoreceptors have been found recently in roots and light-responsive mechanisms of plant roots have been discovered, allowing roots to escape from unfavorable light conditions. Regular light exposure affects root morphology and behavior in transparent Petri dishes. We advocate the use of darkened Petri dishes which would allow the roots to be kept in darkness, mimicking more closely the conditions in nature.

Roots and light

Light is one of the most important physical factors for plant life, yet it can also induce serious damage to plant cells at strong light intensities. Especially UV is a harmful factor to living organisms. During evolution of land plants, their roots evolved in darkness whereas shoots were exposed regularly to light during daytime. As a result of this different evolution, roots are better adapted to grow in darkness whereas shoots show positive

phototropism, thereby growing towards the light. Plants perceive the external light sources and perform positive or negative tropisms of their corresponding organs. In order to be able to perform phototropism, plants use several plasma membrane-associated photoreceptors which are activated by absorbing a specific wavelength of light and converting the perception of light into the cellular and hormonal responses [1].

In the earlier studies using laboratory grown Arabidopsis seedlings, attention to roots has not been sufficient as Arabidopsis roots grow well even when illuminated. Moreover, some 50 years ago, when this Petri dish methodology for growing Arabidopsis in the laboratory was developed, only one photoreceptor was known in Arabidopsis [2]. Currently, we know 14 photoreceptors [1,2] and most of them are also expressed and active also in plant roots [3-5]. In addition to pigments absorbing visible wavelengths, the presence of UV-B responsive proteins, ROOT UV-B SENSITIVE1, 2 (RUS1, 2) and UV RESISTANCE LOCUS 8 (UVR8) are found in root cells [3-5]. Intriguingly, RUS1 and RUS are root-specific UV-B receptors not expressed in shoots, suggesting that the complexity of UV-B sensing and signaling is even greater in roots than in shoots.

Expression of photoreceptors in roots is relevant even for soil-grown plants as roots receive some light via cracks within the soil [6,7].

Here, we discuss recent reports showing that exposure of roots of Arabidopsis to light induces stress-responses affecting the whole seedlings. We are suggesting that the common scientific practice of growing Arabidopsis seedlings in transparent Petri dishes should be modified to keep roots in darkness. This can be achieved relatively simply by partial darkening of the conventional transparent Petri dishes (Figure 1). Importantly, shading of just the roots is enough to inhibit the light-induced root growth [8,9].

Increased growth of light-exposed roots: an 'escape tropism' of stressed roots?

Recent advances in sensory root biology revealed that root growth is stimulated by light in Arabidopsis [8-10]. This light-induced root growth is driven by photoreceptors active in roots [10-13] and emerges as a stress response, complementing the well-known negative root phototropism [9,14]. We have previously reported that an immediate burst of reactive oxygen species (ROS) was observed in the root apex after just 10 seconds of blue light illumination of the root apex region [9]. ROS seems to be a primary emergency alert, as

it can function as a rapid and systemic signaling molecule enhancing further signaling cascades leading to physiological responses and organ movements [15].

As the figure 2 shows, while the soil grown seedlings (having their roots within darkness) have approximately 1:1 root-shoot ratio, the light-exposed seedlings (16 h/8 h, L/D cycle) increase their root growth; resulting in a major increase in this root-shoot ratio to 10:1 (Figure 2). This light-induced root growth can be regarded physiologically relevant since it would, outside in the nature, allow illuminated roots to get back into darkness (soil). This negative root phototropism, when combined with increased root growth, can be considered to represent a stress-induced root 'escape tropism'. This suggests that roots have powerful mechanisms to increase their growth rate or movement direction in response to light. Importantly, using a newly-developed cell-permeable [16] ROS-detecting fluorescence dye PeroxyYellow-1 (Figure 2C), we have observed an almost instantaneous generation of ROS in the root apex after a brief illumination. After just 1 min of illumination, the fluorescence signal reflecting ROS generation in the root apex region lasted up to 10 min [9].

The light-induced ‘root escape’ pathway is based on the actin cytoskeleton [8], as well as on PIN1 and PIN2 proteins, which are more active in illuminated roots, supporting more active polar auxin transport [10,13,17,18]. Importantly, this process is also under negative control. For example, the basic/helix-loop-helix (bHLH) transcription factor UPBEAT1 emerges as a negative regulator of this light-induced root escape pathway [19]. This protein acts as some kind of molecular ‘brake’, preventing roots from growing even faster when illuminated. Similarly the master photomorphogenesis repressor COP1 is relevant as illuminated *cop1* roots are shorter than wild-type roots, whereas the *cop1* roots are longer than wild-type roots when grown in darkness [17]. UPBEAT1 emerges as part of a putative negative feedback loop, to safeguard stressed seedlings, cultivated in the conventional transparent Petri dishes, against exaggerated photomorphogenic root responses [9].

Root photomorphogenesis in laboratory-maintained Arabidopsis seedlings?

Arabidopsis thaliana has been used as a model plant for several decades in laboratories around the globe. On a routine basis, roots have been exposed to the light by using the

transparent Petri dishes. As plants are capable of very rapid adaptive evolution, it might be that numerous generations of laboratory cultivated Arabidopsis, with permanent exposure of roots to light, resulted in adaptation of these laboratory Arabidopsis lines to this environmental (light) stress. Therefore, it is urgently needed to compare root growth rates and their physiological-, genetical aspect including root-shoot communication between laboratory maintained lines and wild-grown lines of Arabidopsis. In addition, it was observed that expression patterns of certain proteins, such as for example AnnAt4, are fundamentally altered when soil- or Petri dish-grown (illuminated) Arabidopsis roots were compared [see Figure 1 in 20]. It is essential to perform specifically designed experiments, which will allow us to study the phototropism of roots, as well as root-to-shoot communication in order to understand plant development in varying light environments. One can solve the problem of illuminated roots quite simply using covered Petri dishes (Figure 1). The root-shoot junction can be split via a dark barrier and the surfaces of Petri dishes can be covered with a light-shielding material to keep roots in the darkness. In such darkened Petri dishes, the light-induced root growth rate is lowered when compared to illuminated seedlings (Figure 1, Figure 1E in 9). Similarly,

Dyachok et al. [9] reported that shading of just the roots is enough to inhibit the light-induced root growth. In conclusion, light is a critical factor affecting to the plant life cycle and can easily be controlled in laboratories. In order to interpret the light effects on roots and plants properly, the experimental design should include, besides the darkened Petri dishes proposed here, also soil-grown samples. Furthermore, it would be invaluable to compare wild-grown Arabidopsis under the same array of conditions to see if the laboratory-reared plants have, indeed, adapted (evolved) to these unnatural conditions.

References

1. Chen, M. *et al.* (2004) Light signal transduction in higher plants. *Annu Rev Genet.* 38, 87-117

2. Briggs, W. and Lin, C.-T. (2012) Photomorphogenesis - from one photoreceptor to 14: 40 years of progress. *Mol. Plant* 3, 531-532

3. Tong, H. *et al.* (2008) Role of root UV-B sensing in Arabidopsis early seedling development. *Proc. Natl. Acad. Sci. USA* 105, 21039-21044

4. Leasure, C.D. *et al.* (2009) ROOT UV-B SENSITIVE2 acts with ROOT UV-B SENSITIVE1 in a root ultraviolet B-sensing pathway. *Plant Physiol.* 150, 1902-1915

5. Rizzini, L. *et al.* (2011) Perception of UV-B by the Arabidopsis UVR8 protein. *Science* 332, 103-106

6. Mandoli, D.F. *et al.* (1990) Some spectral properties of several soil types: implications for photomorphogenesis. *Plant Cell Environ.* 13, 287-294

7. Galen C *et al.* (2007) Functional ecology,of a blue light photoreceptor: effects of phototropin-1 on root growth enhance drought tolerance in *Arabidopsis thaliana*. *New Phytol.* 173, 91-99

8. Dyachok, J. *et al.* (2011) SCAR mediates light-induced root elongation in Arabidopsis through photoreceptors and proteasomes. *Plant Cell* 23, 3610-3626

9. Yokawa, K. *et al.* (2011) Illumination of Arabidopsis roots induces immediate burst of ROS production. *Plant Signal. Behav.* 6, 1457-1461

10. Laxmi, A. *et al.* (2008) Light plays an essential role in intracellular distribution of auxin efflux carrier PIN2 in *Arabidopsis thaliana*. *PLoS ONE* 3, e1510

11. Costigan, S.E. *et al.* (2011) Root-localized phytochrome chromophore synthesis is required for photoregulation of root elongation and impacts root sensitivity to jasmonic acid in Arabidopsis. *Plant Physiol.* 157, 1138-1150

12. Warnasooriya, S.N. and Montgomery, B.L. (2011) Spatial-specific regulation of root development by phytochromes in *Arabidopsis thaliana*. *Plant Signal. Behav.* 6, 2047-2050

13. Wan, Y.-L. *et al.* (2012) The signal transducer NPH3 integrates the Phototropin1 photosensor with PIN2-based polar auxin transport in Arabidopsis root phototropism. *Plant Cell* 24, 551-565

14. Burbach, C. *et al.* (2012) Photophobic behavior of maize roots. *Plant Signal. Behav.* 7, 876-880

15. Miller, G. *et al.* (2009) The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Sci. Signal.* 2, ra45

16. Miller, E.W. *et al.* (2010) Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. *Proc. Natl. Acad. Sci. USA* 107, 15681-15686

17. Sassi, M. *et al.* (2012) COP1 mediates the coordination of root and shoot growth by light through modulation of PIN1- and PIN2-dependent auxin transport in Arabidopsis. *Development* 139, 3402-3412

18. Kleine-Vehn, J. *et al.* (2008) Differential degradation of PIN2 auxin efflux carrier by retromer-dependent vacuolar targeting. *Proc. Natl. Acad. Sci. USA* 105, 17812-17817

19. Tsukagoshi, H. *et al.* (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell* 143, 606-616

20. Huh, S.M. *et al.* (2010) Arabidopsis annexins AnnAt1 and AnnAt4 interact with each other and regulate drought and salt stress responses. *Plant Cell Physiol.* 51, 1499-1514

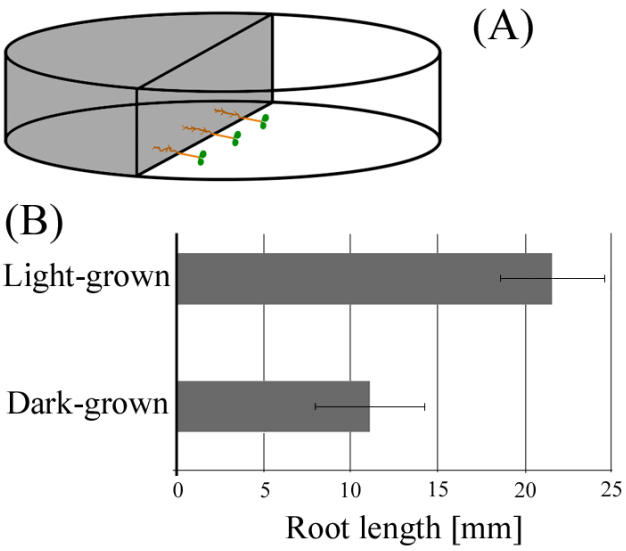

Figure 1

Root lengths of Arabidopsis seedlings grown in darkenned Petri dishes.

(A) Schematic view of partially darkenned Petri dish, adapted from transparent Petri dish using light-shielded material. (**B**) Comparison of root lengths in the completely darkenned and in transparent Petri dishes. Error bars indicate S.E. (darkenned; n=8, transparent; n=6). [adapted from Ref. 9].

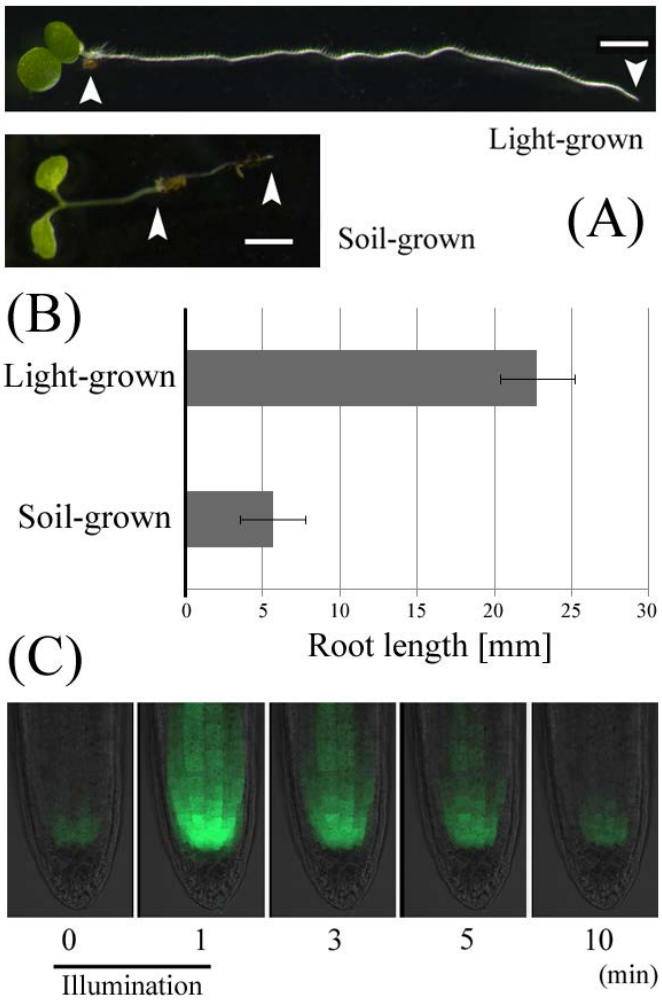

Figure 2

Arabidopsis seedlings grown in different light conditions.

(**A**) Seedlings grown in conventional transparent whole-illuminated Petri dishes (upper) and in soil (lower). Pictures were taken 6 days after germination. White arrowheads indicate root/shoot junction and root tip. (**B**) Comparison of shoot and root length. Error bars indicate S.E. (light grown; n=6, Soil grown; n=274). (**C**) The generation of ROS after 1 min blue light illumination (80 μmol/m^2/s) to root apex. ROS signals were visualized by the fluorescence indicator, PeroxyYellow-1 [16]. After the illumination, ROS levels decrease to the basal level within 10 min. Bar = 2 mm. [adapted from Ref. 9].