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*Letters*

# Shoot thermosensors do not fulfil the same function in the root

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Received, 17 February 2022; accepted, 11 June 2022.

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Key words: thermomorphogenesis, root growth, phytochrome (phyB), EARLY FLOWERING 3 (ELF3), PHYTOCHROME INTERACTING FACTOR 7 (PIF7).

## Introduction

Increasing ambient temperature within the physiological, non-stressful, range promotes the growth of the hypocotyl (Gray *et al.*, 1998) and of the primary root (Hanzawa *et al.*, 2013) whilst reducing the expansion of the cotyledons (Hahm *et al.*, 2020) of *Arabidopsis thaliana* seedlings.

We currently know three thermosensors, phytochrome B (phyB) (Jung *et al.*, 2016; Legris *et al.*, 2016), EARLY FLOWERING 3 (ELF3) (Jung *et al.*, 2020) and PHYTOCHROME INTERACTING FACTOR 7 (PIF7) (Chung *et al.*, 2020), which were uncovered by their role in the control of hypocotyl growth. phyB is a photo-sensory receptor, activated by red light and inactivated by far-red light, and a thermosensor by virtue of the instability of its active form, undergoing thermal reversion to its inactive conformer accelerated by warmth (Jung *et al.*, 2016; Legris *et al.*, 2016; Burgie *et al.*, 2021). ELF3 is a transcriptional regulator that integrates the evening complex, and warm temperatures reduce the association of this complex to the target gene promoters (Box *et al.*, 2015; Ezer *et al.*, 2017; Silva *et al.*, 2020) by eliciting ELF3 phase transition from active to the inactive state (Jung *et al.*, 2020). PIF7 is a transcription factor and warm temperatures modify the structure of the RNA hairpin present at the 5′-untranslated region of the *PIF7* transcript, increasing its rate of translation and hence increasing PIF7 protein abundance (Fiorucci *et al.*, 2020; Chung *et al.*, 2020). In summary, warm temperatures reduce the activities of phyB and ELF3, while increasing that of PIF7. Both phyB and ELF3 repress hypocotyl growth and are active at control temperatures; therefore, the *phyB* and *elf3* mutants have elongated hypocotyls at control temperatures. Conversely, PIF7 promotes hypocotyl growth and its activity increases at warm temperatures; therefore, the *pif7* mutant has a short hypocotyl at warm temperatures.

The root captures water and nutrients and provides of anchorage to the soil. Given these crucial functions, there is a growing interest in understanding the mechanisms involved in the control of primary root elongation by temperature (Hanzawa *et al.*, 2013; Wang *et al.*, 2016; Ibañez *et al.*, 2017; Martins *et al.*, 2017; Yang *et al.*, 2017; Zhu *et al.*, 2018; Fei *et al.*, 2019; Feraru *et al.*, 2019; Gaillochet *et al.*, 2020; Fonseca de Lima *et al.*, 2021; Lee *et al.*, 2021). The aim of this work is to investigate whether the shoot thermosensors phyB, EFL3 and PIF7 fulfil the same function in the root.

## Primary root growth

In plants with the shoot exposed to light (photoperiod: 12 h : 12 h) and the root in darkness (Notes S1), 28°C enhanced primary root growth (Fig. 1a-b, S1a) and cell elongation (Fig. 1c) compared to 20°C. Warmth reduced meristem size (Fig. 1d, see also Yang et al., 2017) and enhanced the staining driven by *pCYCB1;1:GUS* (Fig. 1e); a combination also observed in the *bri1-116* mutant, and interpreted in terms of delayed cell cycle progression (González-García *et al.*, 2011). The promotion of primary root growth by warm temperature was rapid (detectable at 4h, Fig. 1f) and continued steadily during day and night (Fig. 1g). Growth increased between 15°C and 25°C but decreased at 30°C (Fig. 1f, see also Ibañez *et al.*, 2017). Direct exposure to light reduced primary root elongation (Zhang *et al.*, 2019; Cabrera *et al.*, 2021) but did not affect the absolute root response to temperature (no significant interaction, Fig. 1a, S1a).

## The *phyB*, *elf3* and *pif7* primary root growth phenotypes

Compared to the wild type, the *phyB* (Fig. 1h, see also Mayfield *et al.*, 2012; van Gelderen *et al.*, 2018), *elf3* (Fig. 1i) and *pif7* mutants (Fig. 1j) showed reduced primary root growth, particularly at 28°C. These mutants also showed reduced cell length, particularly at 28°C (Fig. 1k). Thus, *phyB* and *elf3* phenotypes are not compatible with phyB or ELF3 functions as thermosensors in the control of primary root growth (they should exhibit enhanced elongation at 20°C as observed for hypocotyl growth). The *pif7* phenotype does not exclude a thermosensor function of PIF7.

## The function of phyB in primary root growth does not require normal thermal reversion

Since thermal reversion is crucial for the thermosensing function of phyB (Jung *et al.*, 2016; Legris *et al.*, 2016; Burgie *et al.*, 2021; Murcia *et al.*, 2021), we compared the *phyB* mutant lines complemented with either wild-type phyB or the mutant versions phyB$^{Y361F}$ and phyB$^{R582A}$, which show severely reduced thermal reversion (Zhang *et al.*, 2013). The wild-type phyB, phyB$^{Y361F}$ and phyB$^{R582A}$ variants similarly rescued the root growth defect of the *phyB* mutant at 20°C and the response to 28°C (note significant effects of phyB and no effects of phyB variants, Fig. 1l, S1b).

## Expression of growth-related genes

To analyse the phenotypes at the molecular level we selected the *XYLOGLUCAN ENDOTRANSGLUCOSYLASE /HYDROLASE 24 (XTH24)* and *EXPANSIN-LIKE A 1 (EXLA1)* genes because they belong to the GO terms cell growth / cell wall organisation or biogenesis, and warm temperatures consistently enhanced their expression in the primary root (Martins *et al.*, 2017; Bellstaedt *et al.*, 2019; Gaillochet *et al.*, 2020; Lee *et al.*, 2021; Fig. 1m). The *phyB* and *elf3* mutants showed reduced expression of both genes and the *pif7* mutant showed reduced expression of the *XTH24* gene, in all cases particularly at warm temperature (Fig. 1m). The expression patterns observed in these mutants is consistent with their growth phenotype and actually, *EXLA1* showed a tight correlation with primary root growth (Fig. 1m).

## The root itself senses temperature

Selectively exposing the root to 28°C while keeping the shoot at 20°C was enough to promote its elongation in intact seedlings (Fig. 1n, S1c). Detached roots responded to temperature treatments applied once isolated from the shoot (Fig. 1o-p, S1d, see also Bellstaedt *et al.*, 2019). In detached roots, the phenotypes of the *phyB*, *elf3* and *pif7* mutants were similar to those observed in entire seedlings, despite the differences in growth capacity (Fig. 1o-p). Etiolated seedlings use phyB and other photoreceptors to achieve the photosynthetic competence of the shoot and generate sugars that travel to the root and promote its elongation (Kircher & Schopfer, 2012); added sucrose bypasses the need of these photoreceptors. Here, the *phyB* root phenotype persisted despite the addition of sucrose to the substrate in the experiments with severed shoots (Notes S1), suggesting different pathways of phyB action on root growth in etiolated and light-grown seedlings.

In other experiments, we severed the shoot and transferred the roots to darkness (20°C or 28°C) either directly or after a pulse of red and/or far-red light to establish different proportions of active phyB (Pfr%). If phyB were acting as a thermosensor, at 20°C there should be more Pfr (less thermal reversion) than at 28°C and lowering Pfr by light should enhance root elongation as temperature does. Warmth increased root elongation but modifying Pfr across the whole range (from long-wavelength far red to pure red light) did not significantly affect root growth at 20°C (Fig. 1q). Increasing Pfr by red light reduced the response to warmth in detached roots (Fig. 1q) an effect of light not observed in entire seedlings (Fig. 1a).

## Lateral root development

Light perceived by phys in the shoot controls the development of lateral roots via a mobile signal (van Gelderen *et al.*, 2018). Warm temperatures enhance lateral root development in light-exposed roots (Wang *et al.*, 2016) but in our experiments with dark grown roots, 28°C reduced the generation of lateral roots compared to 20°C (Fig. S2). The *phyB* mutants showed reduced number of lateral roots at 20°C (see also van Gelderen *et al.*, 2018) and no further response to 28°C (Fig. S2). This constitutive phenotype typical of warm conditions is compatible with a role of phyB as thermosensor in this process. The *elf3* and *pif7* mutations had no significant effects on the rate of appearance of secondary roots (Fig. S2).

## Warmth did not reduce the size of phyB nuclear bodies in the root

Stem piping of the light induces the accumulation of phyB in the nucleus of root cells and its condensation in nuclear bodies (NBs) that do not increase in size upon direct exposure of the root to light (Fig. 2a and S3, see also Lee *et al.*, 2016; van Gelderen *et al.*, 2018). The NBs were larger in elongating than root tip cells, but more nuclei had detectable phyB in the tip than in elongating cells (Fig. 2a-b). In the hypocotyl, the NBs relate to phyB activity (Van Buskirk *et al.*, 2014) and warm temperatures, by lowering Pfr levels reduce NB size (Legris *et al.*, 2016; Hahm *et al.*, 2020; Murcia *et al.*, 2021). In contrast, 28°C did not reduce the size of the NBs in root cells of the elongation zone and actually caused a small increase in tip cells (Fig. 2a and S4a-b). These observations suggest that in the root, phyB Pfr might be relatively stable against thermal reversion. This apparent Pfr stability, in combination with higher abundance in the elongation zone (Fig. 2b) would yield more active phyB at 28°C than at 20°C. Since phyB promotes root growth (Fig. 1h), we reasoned that such higher phyB activity could per se mediate the growth promotion. However, arguing against this possibility, overexpression of phyB did not affect primary root growth (Fig. S1e).

## Warmth reduced the number of ELF3 nuclear bodies in the root

Phase changes induced by warm temperatures favour ELF3 NB formation (Jung *et al.*, 2020) but the link between the formation of NBs and ELF3 activity is not univocal (Ronald *et al.*, 2021). In hypocotyl cells, the number of ELF3 NBs either increases or decreases in response to warmth, depending on the time of the day (Murcia *et al.*, 2022). We observed a reduction by warm temperature of the number of ELF3 NBs and the partitioning of fluorescence towards NBs from the nucleoplasm in cells of the primary root elongation zone

(Fig. 2c-d, Ronald *et al.*, 2021 also observed a reduction in the number of NBs). Warmth did not affect the average size of each ELF3 NB (Fig. S4) but increased total ELF3 fluorescence in cells of the elongation zone (Fig. 2e).

## Warmth did not increase PIF7 protein abundance in the root

Warm temperatures increase the translatability of the *PIF7* transcript and the abundance of PIF7 in the shoot (Fiorucci *et al.*, 2020; Chung *et al.*, 2020) (Fig. 2 g). However, PIF7 was less abundant in the root (Fig. 2 g) in seedlings treated with 28°C than in the controls at 20°C. Consistently with previous reports, we did not observe changes in *PIF7* expression in the roots (Fig. S5, see also Martins *et al.*, 2017; Bellstaedt *et al.*, 2019; Gaillochet *et al.*, 2020; Lee *et al.*, 2021), indicating the effect is post-transcriptional.

## Conclusions

phyB, ELF3 and PIF7 did not act as thermosensors in the primary root growth response to temperature. Since 28°C compared to 20°C promotes primary root growth and warm temperatures reduce phyB and ELF3 activities when they act as thermosensors, had phyB and ELF3 acted as thermosensors, the primary roots of their loss-of-function mutants should have been long at 20°C. In contrast, they were short. Furthermore, whilst thermal reversion is essential for phyB function as thermosensor (Jung *et al.*, 2016; Legris *et al.*, 2016; Burgie *et al.*, 2021; Murcia *et al.*, 2021), phyB variants with severely compromised thermal reversion complemented the *phyB* mutant similarly to the wild-type phyB. Thermal reversion typically reduces the size of phyB NBs between 20°C and 28°C but such reduction was not observed in root cells. Whilst increased PIF7 abundance is a direct consequence of *PIF7* sensation of warmth (Chung *et al.*, 2020), in the root 28°C actually reduced PIF7 abundance compared to 20°C (via post-transcriptional mechanisms), indicating a dynamic that is not compatible with the mechanism of action of PIF7 as thermosensor.

Although phyB, ELF3 and PIF7 do not act as root thermosensors, the three are important for root thermomorphogenesis. In the hypocotyl, phyB and ELF3 inhibit whilst PIF7 promotes cell elongation. Conversely, the three conditioned the ability of the primary root cells to elongate in response to warm temperature. In addition, phyB could convey shoot temperature information for the control of root branching. In fact, the *phyB* mutant had

a phenotype compatible with its role as thermosensor for lateral root development; a response controlled by shoot-derived signals (van Gelderen *et al.*, 2018).

In natural conditions, the root and shoot environments, including their temperature patterns, are substantially different (Walter *et al.*, 2009). Therefore, root and shoot thermomorphogenesis likely evolved independently to fulfil different functions (De Smet *et al.*, 2021; Ludwig *et al.*, 2021). The use of at least partially different sets of thermosensors in the root and the shoot could serve to the purpose of organ specific functions.

## Acknowledgements

We thank Malcolm Bennett (University of Nottingham) for his encouragement to study temperature sensing in the roots. This work was supported by grants from the University of Buenos Aires (grant no. 20020170100505BA to JJC) and *Agencia Nacional de Promoción Científica y Tecnologica* (grant no. PICT-2018-1695 to JJC and PICT-2019-2252 to CC-R).

## Author contributions

M.B.B. and J.J.C. planned and designed the research, M.B.B. and C.C.-R. performed experiments, M.B.B. and J.J.C. analysed data and J.J.C. wrote the manuscript with input from the other authors.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Supporting Information**

The following Supporting Information is available for this article:

**Fig. S1.** Light and temperature effects on primary root growth.

**Fig. S2.** Temperature effects on the rate of lateral root appearance.

**Fig. S3.** Direct exposure to light has no significant effects on the size of phyB nuclear bodies in primary root cells.

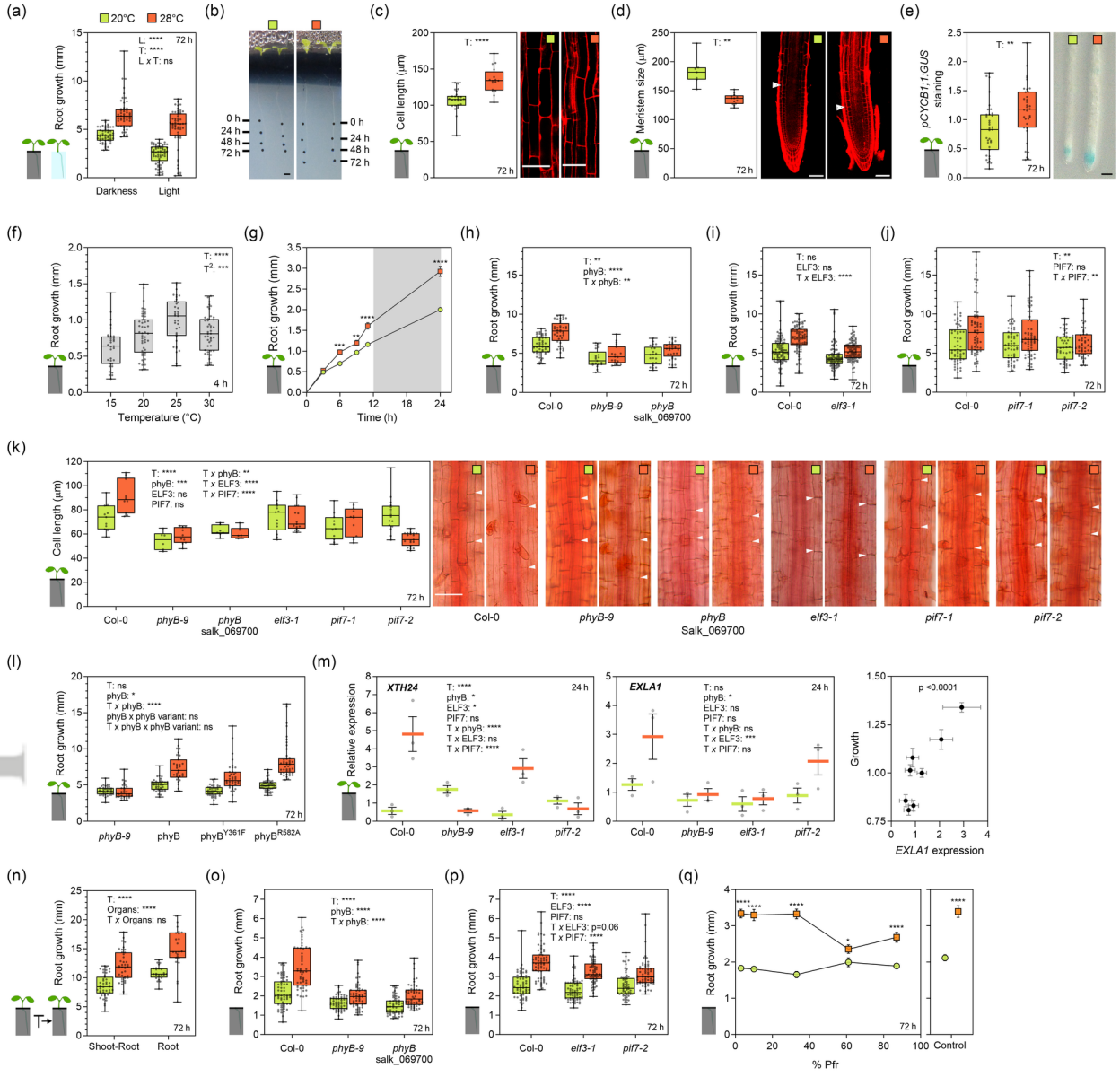
**Fig. S4.** Nuclear bodies of phyB and ELF3 at two different temperatures.

**Fig. S5.** Warm temperature did not affect PIF7 expression in the root.

**Notes S1.** Details of methodology

**Fig. 1** phyB, ELF3 and PIF7 affect primary root growth responses to temperature. (a) Light reduces primary root growth without affecting its absolute response to temperature in *Arabidopsis thaliana* Col-0. (b) Representative seedlings grown at different temperatures after removal of the black plastic cover to visualise the roots (Supporting Information Notes S1). (c) Warm temperature increases the length of primary root epidermal cells within the elongation zone (confocal images of seedlings in propidium iodide, see Notes S1). (d-e) Warm temperature reduces meristem size (d) and enhances the activity of the *CYCB1;1* promoter in the meristem (e). (f) Response of primary root growth to a range of different temperatures. (g) Time course of the growth response (shaded area represents the darkness of the night period). (h-j) Phenotype of *phyB* (h), *elf3* (i), and *pif7* mutants (j). (k) Cell length in the *phyB*, *elf3* and *pif7* mutants. (l) Complementation of the *phyB* mutant with wild type (*phyB-9 pUBQ10:PHYB)* or stable (*phyB-9 pUBQ10:PHYB*$^{Y361F}$*, phyB-9 pUBQ10:PHYB*$^{R582A}$) versions of phyB. (m) Expression of *XTH24* and *EXLA1* in the roots (note the correlation between EXLA1 expression across genotypes and temperatures). (n) Response to selective root warming (differences in basal growth rates with respect to other experiments caused by the use of a different growth chamber with stronger ventilation to maintain shoot temperature unaffected by root treatments). (o-p) Temperature responses of the roots of Col-0, *phyB* (o), *elf3* and *pif7* (p) after severing the shoot (see Notes S1). (q) Temperature responses of isolated roots exposed to a 20-min pulse of red plus far-red mixtures that establish different proportions of Pfr (Yanovsky *et al.*, 2002) given immediately before the temperature treatments and followed by darkness, compared to dark controls. Scale bar: 1 mm (b), 50 µm (c-d, k), 100 µm (e). Box-plots show median, 1–3 interquartile range, maximum-minimum interval and individual values (a, c-f, h-l, n-p). Bars indicate ±SE (g, m, q). Significance of the effects of temperature (T) in *t* test (a, c, d, e, g, q) or the effects of T, genotype or light (L) and their interactions in multiple regression analyses (f, h-p): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant.

**Fig. 2** phyB, ELF3 and PIF7 responses to warmth in primary root cells of *Arabidopsis thaliana*. (a-b) Size of phyB nuclear bodies (NB, a) and total fluorescence (b) driven by *pUBQ10:PHYB-YFP* in cells of the elongation zone and tip of the primary root (see confocal microscopy in Supporting Information Notes S1). (c-e) Number of ELF3 NBs (c), fluorescence ratio between NB and nucleoplasm (NP, d) and total fluorescence (e) driven by *p35S:YFP-ELF3* in cells of the elongation zone of the root. (f) Abundance of PIF7 in the shoot, the root or the root without tip in *pif7-2 pPIF7:PIF7-3HA-tPIF7* (see protein blots in Notes S1). Grey and black arrowheads indicate different PIF7 isoforms. Scale bar is 1 µm (a, c) or 50 µm (b, e). Box-plots show median, 1–3 interquartile range, maximum-minimum interval and individual values. Fluorescence data and protein abundances relative to the abundance of loading control were normalised to the average of each experiment or to the average of each pair of biological replicates in the case of roots without tip because their harvest was slower. Significance of the effects of temperature and position (Pos.) of the cell or time and their interaction in multiple regression analysis (a-c) and of the effects of temperature (T) in *t* tests (d-f): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant.

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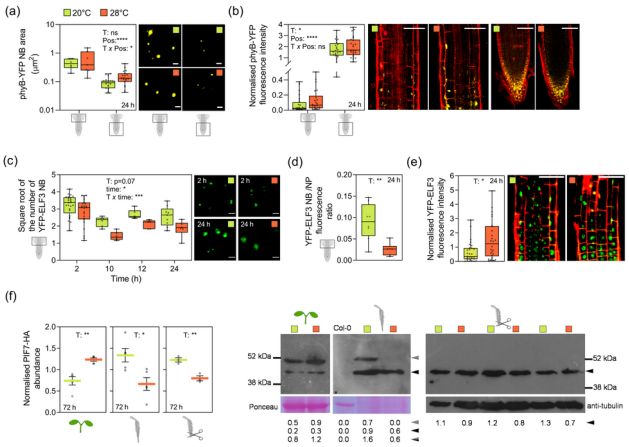


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