The phosphatase/kinase balance affects phytochrome A and its native pools, phyA' and phyA'', in etiolated maize roots: evidence from the induction of phyA' destruction by a protein phosphatase inhibitor sodium fluoride

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Abstract

Phytochrome A (phyA) comprises two native types, phyA' and phyA'', with distinct spectroscopic, photochemical, and functional properties, differing at the *N*-terminal extension, probably, by the state of phosphorylation. To find out if and how protein phosphatases (PP) affect the state of the phyA species *in planta*, we studied the effect of the non-specific phosphatase inhibitor NaF on etiolated maize seedlings with the use of low-temperature fluorescence spectroscopy and photochemistry. In roots, phosphatase inhibition facilitated photoreceptor destruction in its labile phyA' form and shifted the phyA'/phyA'' ratio towards the more stable phyA''. The effect of NaF was not observed in stems. It was similar, though less pronounced, in comparison to the effects of the serine/threonine PP inhibitors, okadaic and cantharidic acids (OA and CA), which likewise facilitate the destruction of phyA' in etiolated maize stems, not, however, in roots (Sineshchekov et al., Photochem. Photobiol 89:83–96, 2013). The phyA'/phyA'' balance thus depends on the kinase/phosphatase equilibrium in the root cells. The relatively low effect of NaF on phyA in roots, together with the lack of the effect of OA and CA in them, may imply that the mechanism controlling the phyA'/phyA'' balance in roots can be different from that in shoots.

Graphic abstract

Keywords Phytochrome A (phyA) · phyA pools · Fluorescence spectroscopy · Photochemistry · Phosphorylation · Protein phosphatases (PP) · PP inhibitors · NaF · Maize · Roots

Abbreviations

CA	Cantharidic acid
FR	Far-red light
γ_1	The extent of the Pr $\rightarrow$ lumi-R phototransformation
HIR	High-irradiance response
JA	Jasmonic acid
λ_a , λ_e , λ_{max}	Wavelengths of the actinic and excitation light and of the maximum of phytochrome fluorescence, respectively
LFR	Low-fluence response
lumi-R	The first photoproduct of the Pr phototransformation at 77–85 K
NaF	Sodium fluoride
NTE	N-Terminal extension
phy	Phytochrome
P_{tot}	Total phy content
Pr	The red-light-absorbing phy form
Pfr	The far-red-light-absorbing phy form
R	Red light
PP1 and PP2A	Protein phosphatases 1 and 2A
phyA and phyB	Phytochromes A and B
phyA' and phyA''	Subpopulations of phyA
T	Temperature
VLFR	Very low-fluence response

1 Introduction

Phytochrome (phy)-mediated light regulation in plants is primarily associated with the action of the two major phy species, phyA, and phyB [1]. phyB is a light-stable and major species in de-etiolated tissues participating in the classical red-light (R) induced/far-red-light (FR)-reversed low-fluence responses (LFR). phyA, on the contrary, is light-labile. In etiolated seedlings, it dominates by its content and functional activity mediating the irreversible very low-fluence responses (VLFR) and FR-induced high-irradiance responses (HIR) in the process of de-etiolation [2]. It may perform the low-fluence response (LFR) functions characteristic for phyB [3–5].

This functional uniqueness of phyA may be explained, at least partially, by the fact that it comprises two native pools—post-translationally modified products of the PHYA gene found in monocots and dicots (see review [6] and the literature by the same author cited therein). They differ spectroscopically, photochemically and by their phenomenological properties in plant tissues. phyA' is a longer wavelength species capable of low-temperature (T) photoconversion from the Pr form into the first photoproduct lumi-R, whereas the shorter wavelength phyA'' is photochemically inactive at low T. The fluorescence spectroscopy of phyA and these photochemical features of its two types were used

to monitor their proportion and content in etiolated plants. phyA' dominates in growing etiolated tissues, its concentration increases almost linearly with the rise of the total phyA content, whereas phyA'' is a minor and saturated by its content species. phyA'' is membrane- (protein-) associated, and the number of the association sites can be limited, what may explain the relatively low phyA'' content compared to that of phyA'. phyA', on the contrary, is water-soluble. They differ also by their stability—phyA' is light-labile, whereas phyA'' is relatively light-stable.

phyA' is likely to be responsible for VLFR, whereas phyA'', for HIR, and possibly, for LFR [6]. This is implied by the following facts. The $\Delta 6-12$ truncated (oat) phyA (expressed in phyA-deficient *Arabidopsis thaliana*) forms primarily, if not exclusively, phyA' [7], what positively correlates with the promotion of VLFR and suppression of HIR in this *Arabidopsis* species [8] supporting the notion that phyA' is responsible for VLFR. On the contrary, rice phyA with the S/A substitution of 10 serines at the N-terminal extension (NTE) (expressed in transgenic *Arabidopsis*) is represented only by phyA'' [9], and the suppression of VLFR and promotion of HIR were observed in this transgenic plant by Kneissl et al. [3]. Interestingly, the regulation of the phyA state and functions under R and FR light is accomplished in cooperation with the phytohormone jasmonic acid (JA) responsible for plant's reactions to wounding and infection [10–12].

It is well established that phyA is a phosphoprotein and a light-regulated kinase [13–17]. It was shown that in darkness oat phyA autophosphorylates at serines 8 and 18 (Ser8 and Ser18) in the N-terminus, whereas S599 in the hinge region is phosphorylated upon illumination. Recent observations [18] indicate that in *Arabidopsis* one residue in the hinge region, threonine 593 (T593), is also likely to be a phosphorylation site. The phyA phosphorylation at the N-terminus (Ser8 and Ser18) lowers its functional activity by increasing the light-lability of phyA since phosphorylated phyA is a preferred substrate for the COP1/SPA complex-mediated degradation [19]. Phosphorylation in the hinge region reduces pigment activity by preventing the interaction of phyA with its signal transducers [20]. On the other hand, of interest is the fact, that far-red light increases the content of phosphorylated phyA (in the nucleus, but not in the cytosol), and this increase is associated with the rise of its functional activity [21]. A positive relationship between the kinase activity of phytochrome A and its photoresponses in plants was also recently demonstrated with the use of the mutant (*phyA-201*) *Arabidopsis* expressing point-mutated *Avena* phyA with decreased [22] or increased [23] kinase activity.

Our experiments with truncated and serine/alanine substituted phyA mutants indicate that phosphorylation in the dark can be also responsible for the phyA differentiation into the

phyA' and phyA'' species, and the site(s) participating in this process is (are) at the serine cluster (10 serine residues) in the NTE [6.] It should be noted, however, that the sites of phyA autophosphorylation at Ser8 and Ser18 are not involved in the hypothetical phyA'' phosphorylation causing its conversion into phyA' [24]. To verify this hypothesis and to try to find out if the phosphatase/kinase balance in the cell may affect the phyA'/phyA'' equilibrium, we have undertaken experiments with inhibitors of the Ser/Thr protein phosphatases (PP) of the PP1 and PP2A types active against phytochrome—okadaic and cantharidic acids (OA and CA). It was found that the phyA'/phyA'' proportion is indeed connected with the kinase/phosphatase equilibrium in the tissues of etiolated maize seedlings [25]. The PP inhibition shifted the phyA'/phyA'' ratio towards the latter in etiolated maize stems at the expense of stimulated phyA' destruction. It is worth mentioning that it failed to produce this effect in roots although OA and CA enter the root cells [26]. The direction of the phyA pools' balance change contradicted, however, the hypothesis, that phyA' is produced upon phyA'' phosphorylation. This was, probably, because of the strong stimulation of the phyA' destruction process and its domination over the others. The specific state of phyA'' in the cell (membrane-/protein-associated) may also prevent its phosphorylation.

These observations, suggesting the intimate participation of the phosphatase/kinase equilibrium in the regulation of the phyA'/phyA'' state in the cell, and the fact that there was no effect of OA and CA in roots, prompted us to proceed with the experiments in this direction using a PP inhibitor with a wider spectrum of possible targets of its activity—sodium fluoride (NaF). It is a well-known inhibitor of the serine/threonine phosphatases and a strong inhibitor of tyrosine phosphatases, which are active in maize roots [27]. It was found that, in etiolated root tissues, NaF facilitated phyA' destruction shifting the phyA'/phyA'' ratio towards phyA'' similarly to the effect of the specific inhibitors OA and CA in shoots, but with a lower extent, whereas in coleoptiles NaF was not active. This supports the notion [25] that the phosphorylation status of the cell regulates the phyA turnover in maize roots as well. However, the facts that NaF is active in roots, where the specific PP inhibitors, OA and CA, are inactive, whereas in stems the situation is reverse, suggest a different state of phyA and/or a different mechanism of action of the phyA destruction system in these organs.

2 Materials and methods

2.1 Plant material

Zea mays L. plants of the Kubanskaya variety were used in this work. The seedlings were grown at 23 °C on filter paper

soaked with tap water in total darkness. The material for measurements was roots (7–10 mm long) and coleoptiles (22–30 mm) of 2- to 3-day-old and 4-day-old, etiolated seedlings, respectively. Tips of roots and coleoptiles (usually, two tips 3 ± 0.3 mm long) were taken for sample preparation and glued to a Plexiglas sample holder with a water–glycerol mixture (50:50) and frozen at 85 K in a cryostat. All the manipulations with the samples were carried out under photochemically green safelight (incandescent lamps in combination with green band-pass filters or green light diodes LG 3330/HV12).

2.2 Treatment with inhibitors

Sodium fluoride (NaF) was used as a PP inhibitor with a wider range of the targets of its activity than the specific PP inhibitors (okadaic and cantharidic acids) previously employed in our experiments [25]. NaF is the source of the fluoride ions (F[−]) which irreversibly inhibit acidic serine-threonine phosphatases [28–31]. Concentrations of NaF, sufficient to produce an effect but at the same time not toxic for the plant, were determined to be 0.5–1 mM and minimal incubation time of 10 min [32, 33]. Higher concentrations of NaF cause a phytotoxic effect, which is seen in the inhibition of root and stem growth [33, 34]. In our preliminary experiments, optimal NaF concentrations were also obtained within this range. The concentration of 1 mM proved to be the minimal concentration sufficient to induce an effect on phyA in roots within 10 min of their incubation with NaF, which was significantly different from that caused by 10 min incubation in water (as a control). In addition, it is known that for NaF, there is a delay of its phytotoxic action for approximately 1 day after its penetration into the cell [35]. Therefore, in our experiments, its action was followed within the first hours of exposition—maximal time of incubation with the inhibitors did not exceed 4 h (+ 12 h in water). Thus, we may conclude that the native state of the plant tissues remained unaffected under these experimental conditions.

Treatments with the inhibitor solution were carried out in the dark at 23 °C according to the following schemes: (1) 10 min, (2) 4 h, and (3) 4 h + 12 h in tap water to detect possible delayed effects. The time regimes were taken like those used in our earlier experiments [25] with the specific PP inhibitors, okadaic and cantharidic acids, to comparatively follow their possible effects. The seedlings were put in solution either as a whole intact seedling with roots down into solution (in the case of the root samples) or as shoots cut at the level of coleoptile or coleoptile plus mesocotyl with the lower end put down into the solution and the upper part above the solution. Samples without treatment or after incubation in tap water for the same period as in the experiment with the inhibitor were used as a control.

2.3 Fluorescence spectroscopy of phytochrome

We used the same low-temperature (85 K) in situ phytochrome fluorescence and photochemical approach as earlier [36]. Fluorescence emission spectra of phytochrome in etiolated tissues were taken at 85 K ($\lambda_e = 633$ nm) in its initial red-absorbing Pr form (F_0) and after transformation into the first, stable at low temperatures product lumi-R upon saturating red illumination ($\lambda_a = 633$ nm) at 85 K (F_1 —state of photoequilibrium between Pr and lumi-R at 85 K). The spectrofluorimeter used was a lab-designed instrument based on two double-grating monochromators of the DFS-12 and DFS-24 types (LOMO, Leningrad, USSR) conventionally used for Raman spectroscopy. The source of the exciting and actinic light was a He–Ne laser of the LG type (5 mW) ("Plasma" factory, Ryazan, Russia) in a combination with a grating monochromator MDR-2 (LOMO, Leningrad, USSR) to cut off pumping light. In the case of the exciting light, its intensity was reduced by approximately two orders of magnitude to practically exclude its photochemical effect during spectra measurements.

Fluorescence intensity of phytochrome in the initial Pr form (F_0) was a measure of the total pigment content ($[P_{tot}]$ in relative units, r. u.), whereas its relative changes under saturating red illumination at low temperature (T) were taken as the extent of the Pr $\rightarrow$ lumi-R conversion to reach a photoequilibrium (γ_1). The latter value allows evaluation of the relative content of the two native phyA species, phyA' and phyA'', which are active and inactive, respectively, in the Pr $\rightarrow$ lumi-R photoconversion at 85 K with the individual $\gamma_1' = 0.49 \pm 0.03$ and $\gamma_1'' = 0$ (see the procedure in [34]). To get $[P_{tot}]$ and γ_1 values, two raw spectra of a sample under investigation (in the F_0 and F_1 states) were corrected for the background fluorescence by subtraction of the spectrum (not shown) of the reference sample—mature roots at their base after saturating R illumination converting Pr into Pfr, which did not practically contain phytochrome in its Pr form. After normalization of the raw spectrum and the reference spectrum to unity at 660–662 nm, where the input of the phytochrome emission intensity is less than 3–5%, the reference spectrum was subtracted from the raw spectrum to give the spectrum of phytochrome in the sample (Fig. 1). From them, we obtained the position of the emission spectrum (λ_{max}), $[P_{tot}]$, and γ_1 . The latter parameter was calculated as the difference in the intensity of the two spectra in the maximum ($\Delta F = F_0 - F_1$) related to F_0 , i.e., $\gamma_1 = \Delta F / F_0$ (Fig. 1). Knowing $[P_{tot}]$ and γ_1 , it was easy to evaluate [phyA'] and [phyA'' (+ phyB)] in the sample in % and r. u. To get these phyA parameters, from 5 to 20 independent samples were measured and the data are presented as means $\pm$ SE. The Student's *t* test (*t*-criterion) was used (with the level of confidence $P < 0.05$) for independent distributions.

Fig. 1 Low-temperature (85 K) fluorescence emission spectra ($\lambda_e = 633$ nm) of phytochrome in etiolated maize root tips and its red-light-induced changes. 1—Initial Pr state (F_0); 2—state of photoequilibrium, Pr + lumi-R, after saturating actinic illumination ($\lambda_a = 633$ nm) at 85 K (F_1). The spectra here and below (obtained from raw experimental spectra by subtraction the spectrum of background emission of the sample, not shown) are not corrected for the spectral sensitivity of the spectrofluorimeter. The spectra here and below are the average of 5–7 independent samples, and bars ($\pm$ SE) reflect variations of the phytochrome content in the samples and noise of registration

3 Results

Experiments with maize coleoptiles treated with NaF at 1 or 5 mM for up to 4 h did not reveal any statistically reliable effect as compared to control samples (data not shown). The absence of an NaF effect in shoots can be explained by their ineffective NaF uptake [37]. Roots, which display an effective NaF uptake, were thus much more suitable for investigating inhibitor effects on phytochrome and the obtained results are presented below. Since the content of the phyA native species depends on the developmental state of organ/tissue (for the situation in maize see [23]), the roots were taken primarily at the age of two–three days (seedlings without shoots, root length of 7–10 mm), when maximum concentration of phyA and comparable relative amounts of its two pools were achieved.

Control root tips (without treatment or after 10 min in water) had the following phyA spectroscopic and photochemical characteristics and content: $\lambda_{max} = 684$ nm; half-band width of the spectrum, $\Delta\lambda$, of 28 nm; $[P_{tot}] = 3.1$ r. u.; $\gamma_1 = 0.34$ (Figs. 1 and 2). For further control, fluorescence spectra of root tips were taken after 4 h and 16 h exposition in water (Fig. 3). There was no difference in the position and shape of the spectra under these experimental conditions (Fig. 2), however, a decline of total phytochrome concentration ($[P_{tot}]$) by approx. 25% of the initial value (Fig. 3a) and a lowering of the γ_1 by approx. 10% were observed (Fig. 3b).

Fig. 2 Low-temperature (85 K) fluorescence emission spectra of phytochrome A in the initial Pr state (F_0) ($\lambda_e=633$ nm) in etiolated maize root tips after treatment for 4 h with a non-specific protein phosphatase inhibitor NaF (curve 1) as compared with water control (curve 2). The spectra are normalized at the maximum after subtraction of the background spectrum (not shown)

In our earlier works (see review [6]), we have shown that variations of the phyA parameters in plant tissues are explained by the existence of the two phyA native species—the longer wavelength (with the low-T absorption/emission maxima at 673/687 nm) labile phyA' form photochemically active at low T and the shorter wavelength (668/683 nm) relatively stable phyA'' inactive at low T. phyA' dominates in the young actively growing tissues, whereas phyA'', in mature tissues (see “Introduction”). The obtained experimental data are considered here and below in this context. Evaluations of the phyA' and phyA'' content based on the variations of the experimental $[P_{tot}]$ and γ_1 values have shown changes in their equilibrium towards phyA'' already in the control root samples upon their prolonged incubation in water (from 68/32 to 60/40% after 4 h) (the 10 min treatment was not effective with all the parameters remaining virtually the same as in intact roots) (Figs. 3, 4). The decline in $[P_{tot}]$ by $25 \pm 3\%$ was achieved primarily at the expense of the labile phyA' whereas the content of phyA'' remained relatively unchanged; [phyA'] reduced from 2.0 r. u. to 1.44 and 1.38 r. u. and [phyA''] from 1.0 r. u. to 0.96 and 0.85 r. u. after 4 h and 16 h in water, respectively (Fig. 4a, b).

Under the action of NaF, the shape and position of the phytochrome spectra do not undergo changes after all the regimes of treatment (Fig. 2) as in the case of the specific inhibitors—okadaic and cantharidic acids [25]. At the same time, the other two key parameters, fluorescence intensity F_0 (proportional to $[P_{tot}]$), and parameter γ_1 , varied depending on the incubation time (Fig. 3). Fluorescence intensity F_0 drops down by approximately 30% even after 10 min incubation of etiolated maize root with NaF (from 3.1 to 2.18

Fig. 3 Phytochrome A content ($[P_{tot}]$) proportional to the fluorescence intensity F_0 in the dark-adapted state (a) and extent of the Pr→lumi-R photoconversion ($\gamma_1=(F_0-F_1)/F_0$, where F_1 is the fluorescence intensity at the photoequilibrium between Pr and lumi-R) at 85 K (b) in root tips incubated at 28 °C in water and NaF. (Here and in Fig. 4, the columns differing significantly are indicated by lines and asterisks.)

r. u.) and the γ_1 parameter reduces at the same exposition by approximately 10% (from 0.34 to 0.31). As a result, the phyA'/phyA'' proportion decreased to 62/38% and [phyA']/[phyA''] to 1.35/0.83 r. u. compared to those in the water control—68/32% and 2.0/1.0 r. u. (Fig. 3). Longer treatment (4 h NaF and 4 h NaF + 12 H₂O) results in a more significant lowering of $[P_{tot}]$ (to 1.9 and 1.58 r. u., respectively) and γ_1 (to 0.25 and 0.23) (Fig. 3a, b). This signifies the further decline of the phyA' proportion and content at a relatively constant phyA'' level, and because of that, the phyA'/phyA'' pools' ratio shifts towards phyA'' (to 50/50% and 0.95/0.95

Fig. 4 phyA pools in etiolated maize root tips after treatment with NaF at 28 °C as compared with untreated root tips in water (phyA'—light grey, phyA''—grey): proportion (a) and content in rel. units (b)

r. u. after 4 h NaF treatment and to 46/54% and 0.73/0.85 r. u. after the following 12 h exposition in water) (Fig. 4a, b).

4 Discussion

The data obtained show that the unspecific PP inhibitor NaF affects the state of phyA in etiolated maize roots. The dominating effect is the destruction of the pigment in its labile phyA' form; it leaves the phyA'' content relatively unchanged and leads to the shift in the phyA'/phyA'' equilibrium towards the latter. These changes may result in the modulation of the regulatory phyA functions since its two

Fig. 5 Hypothetical scheme of the action of the two native phytochrome A types, phyA' and phyA'', and of the effect on them of the non-specific phosphatase inhibitor NaF and specific Ser/Thr phosphatase inhibitors okadaic and cantharidic acids (OA and CA) in etiolated maize seedlings. phyA is sensitized in the phyA'' form, which converts in darkness into phyA' upon a post-translational modification at the NTE, possibly, via serine phosphorylation. Upon illumination, phyA'' mediates the high-irradiance reactions (HIR) and the low-fluence responses (LFR), whereas phyA', the very low-fluence responses (VLFR). NaF (in roots) and OA and CA (in coleoptiles) inhibit the action of phosphatases and thus activate the phosphorylation of the labile phyA' form, what facilitates its recognition and destruction by the proteolytic system. This results in the suppression of the VLFR. The stable phyA'' and its photoresponses (HIR and LFR) remain unaffected

species mediate different types of photoresponses (Fig. 5). This phenomenological picture is well in line with that found earlier in etiolated coleoptiles under the action of the specific PP inhibitors, okadaic and cantharidic acids [25]—the decline in the total phyA content, which is achieved through the phyA' destruction (Fig. 5). In the case of the specific PP inhibitors, the $[P_{tot}]$ decline could reach approximately twofold; however, because $[phyA'']$ remained essentially unaffected, $[phyA']$ dropped down considerably deeper, by fivefold. Under the action of the non-specific PP inhibitor in roots, the effect was less pronounced: total $[phyA]$ decline was approximately 1/3 of the initial control level, and $[phyA']$ dropped maximally by approximately twofold. In general, however, the qualitative similarity of the phenomenological effects of the non-specific PP inhibitors in shoots supports the notion [25], that the state of the pigment depends on the phosphatase/kinase equilibrium in the cell. The facts that the phyA' content drops down, although to a lesser extent, in the control samples without any treatment by NaF, and that NaF acts also as an inhibitor of respiration [37] suggest that the phyA' destruction may be connected, at least, partially, with the block of the energy metabolism.

To understand the nature of the observed effects, it could be instrumental to compare them with $[phyA']/[phyA'']$ variations under normal physiological conditions. In particular, the constant red light (Rc) induces a fast (tens of minutes)

and deep $[P_{tot}]$ decline at the expense of the destruction of the phyA' form not affecting phyA'', and hence with a phyA'/phyA'' ratio shift towards the latter [38]. The decline of [phyA'] upon prolonged (4 h) action of NaF in this work and of OA and CA [25] looks very similar to it. From this, we may assume that the shift in the phosphatase/kinase equilibrium upon the PP inhibitors action could promote phosphorylation of phyA in its phyA' form. This can make it susceptible to recognition and destruction by the proteolytic system as it happens during the red-light-induced phyA degradation upon Pr conversion into Pfr [17, 19, 39] and into the light-labile Pr*, i.e., Pr cycled through Pfr [40].

The fact that NaF affects phyA in roots, whereas OA and CA do not, may relate to a specific state of the pigment in this organ which differs from that in shoots or with lower activity of the phyA destruction system. It is known that phytochrome in maize roots is distinguished from that in shoots by its immunological properties and by the kinetics of its light-induced destruction [41]. In *Arabidopsis* roots, phyA also proved to be more stable than in shoots [42]. Of interest in this connection is the fact that the "de-etiolating" *lip* mutation [43] did not affect the content of phyA in pea roots, whereas it dropped down by approximately threefold without changes in the phyA'/phyA'' proportion in epicotyls [44]. One may hypothesize in this connection that, for the facilitation of the destruction processes in roots, more profound changes in the phosphatase/kinase system are to be brought about, which can be achieved using non-specific PP inhibitors with a wider spectrum of possible targets of their action. This and the fact that FyPP, the only PP of the PP2A type known to interact with phytochrome, is absent in roots [45], whereas those present in roots, PAPP5 [46] and PAPP2C [47], are not of the PP1 or PP2A types, could be the reason for the differences in the effects of the specific and non-specific PP inhibitors in these organs.

The specificity of the phyA state and turnover in roots may have a relation to its functions in this organ. It is well established that the underground roots directly sense stem-piped light, which induces roots' photomorphogenesis comprising such complex processes as root gravitropism and elongation, the development of root hairs, the orientation of lateral roots, and jasmonic acid responses (see [48, 49] and the literature cited therein). They are controlled primarily by phyA because light, which travels from stems to roots through the vascular tissue, is highly enriched with the far-red component sensed exclusively by phyA. Our experiments with rice seedlings grown in darkness and under constant and pulsed far-red light (light conditions favorable for the HIR and VLFR response types, respectively) have shown that the downregulation of roots' growth is observed only under constant FR [12]. Taking in consideration that phyA'' and phyA' mediate HIR and VLFR, respectively [9], we may conclude that this root growth rate reduction is controlled

by phyA'', whereas phyA' is inactive. The content of the two phyA species, which is hypothetically regulated by the phosphatase/kinase equilibrium in the cell, may thus affect the character and extent of the roots' photoresponses.

Experiments with rice mutants deficient in the hormone jasmonic acid (JA) (*hebiba* and *cpm2*) have shown [12] that JA is intimately involved in the phyA activity in roots. In the mutants, both constant FR and pulsed FR were active in rice roots' growth reduction, in contrast to the wild type where pulsed FR was inactive. This phyA activity in the mutants correlated with the higher phyA' content and phyA'/phyA'' proportion in them compared with the wild type [12]. This suggests that phyA' is active in the mutants, but in the wild-type JA suppresses its activity. This modification of the phyA pools' activity is achieved through the regulation of the phyA turnover because JA was shown to participate in light-induced phyA destruction primarily in the phyA' form [10, 11]. We may hypothesize, that this hormone can participate in the regulation of phyA abundance and phyA' destruction in darkness as well.

The data obtained can be also considered in the context of the problem of the fluoride stress [29]. Inorganic fluoride (F) exposure generates diverse toxic effects in plants including the suppression of respiration and photosynthesis through disruption of enzyme activity. In general, F increases oxidative stress and affects energy metabolism by inhibiting the ATP synthase enzymes. As far as we know, our data for the first time show that F also affects the state of phyA and its native pools via stimulation of the destruction of the labile phyA' species in roots even at non-toxic concentrations of F. Since the two phyA species differ by the type of photoreponses they mediate (see "Introduction"), it is likely that the fluoride stress may affect the plant also through modification of the phyA photoregulation activity (Fig. 5).

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Declarations

Conflict of interest There are not conflicts to declare.

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