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Research Article

Phytochrome A and its Functional Manifestations in Etiolated and Far-red Light-grown Seedlings of the Wild-type Rice and its *Hebiba* and *Cpm2* Mutants Deficient in the Defense-related Phytohormone Jasmonic Acid

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ABSTRACT

Interaction between phytochromes and hormones is becoming one of the major issues in plant photophysiology. In this work, effects of defense-related jasmonic acid (JA) on phytochrome A (phyA) were investigated by fluorescence spectroscopy making use of two JA biosynthesis mutants of rice: *cpm2* with the inactivated gene allene oxide cyclase and *hebiba* with additional genes deleted. Constant far-red light (FRc) mediated by phyA reduced its content in the wild type (WT) and mutants, and brought about domination of its light-stable pool (phyA″) in WT and light-labile pool (phyA′) in the mutants. Pulsed FRp was much less effective. This FR effect classifies as primarily HIR with a low fluence threshold; it comprises inhibition of phyA biosynthesis, stimulation of phyA″→phyA′ transformation and phyA′ destruction. In the mutants, phyA suppresses [Pchlide] under FRp (VLFR) and stimulates it under FRc (HIR); these effects are lacking in WT. Similarly, phyA suppresses roots'growth under FRp in the mutants but not in WT. These JA mutant features suggest that JA reduces the phyA functional activity primarily in its phyA″ form mediating HIR. This modulating JA action on phyA functions under FR limiting their extreme manifestations may have contributed to the evolutionary advances of the land plants.

Abbreviations: AOC, enzyme allene oxide cyclase; *cpm2*, *hebiba*, JA-deficient rice mutants; FHY1 and FHL, proteins participating in the phyA transport into the nucleus; FHY3, protein activating FHY1 and FHL; FR, far-red light; FRc, constant FR; FRc-high, FRc of high fluence rate; FRc-low, FRc of low fluence rate; FRp, pulsed FR; HIR, high-irradiance responses; JA, hormone jasmonic acid; JAZs, proteins suppressing FHY3; LFR, low fluence responses; phy, phytochrome; phyA, phyB and phyC, phytochromes A, B and C; *PHYA*, phyA gene; phyA′ and phyA″, phyA types; Pchlide, protochlorophyllide; $Pchlide^{655}$ and $Pchlide^{633}$, active and inactive protochlorophyllides; P_{tot}, total phytochrome content; VLFR, very-low fluence responses; WT, wild type.

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INTRODUCTION

The phytochrome system, the most profoundly investigated photoreceptor apparatus in plants, comprises a small number of the phytochrome (phy) gene products (for instance phyA, phyB and phyC in rice (1)). The two major phys are phyA and phyB; phyA is light-labile, and it dominates quantitatively and functionally in etiolated seedlings, whereas the light-stable phyB, in light-grown plants. Both of them are active in the classical red light-induced/ far-red light-reversed photoresponses (the so-called low fluence responses, LFR). phyA is, however, more versatile —it mediates photoresponses initiated by far-red light (the photoirreversible inductive very-low fluence responses, VLFR, and the high-irradiance responses, HIR, which require constant illumination). This virtue makes phyA the only photoreceptor in the spectral region of the photosynthetically inactive far-red light, which governs de-etiolation processes in plants growing under the conditions of dense canopy shade.

This versatility of the phyA action may be explained, at least partially, by the existence of its two divergent species, phyA′ and phyA″ (see reviews (2,3) and the literature by the same author cited therein). They were detected *in situ* in etiolated seedlings of monocots and dicots with the use of low-temperature fluorescence spectroscopy and photochemistry. The heterogeneity of phytochrome (phy) in plant tissues was indicated by the variability of its physicochemical parameters and their dependence on plant species and organ/tissues, developmental state and physiological conditions. The fact that there was practically no phy emission in double *phyAphyB* mutants and that the heterogeneous phy population similar to that in the wild type was observed in the *phyB* mutants has shown that the heterogeneity arises from phyA. These data and also the fact that phyA accumulates in heterologous expression systems (*E. coli* and *P. pastoris*) in the phyA″ form support the notion that the minor phyA″ pool originates from the same *PHYA* gene, such that phyA exists in two forms, probably representing differing post-translational modification. These forms can be phenomenologically discriminated by their ability or inability to undergo the low-temperature photoconversion from the initial Pr form into the photoproduct lumi-R—phyA′ (here, the extent of the photoconversion is $\gamma_1 = 0.5$) while for phyA″ $\gamma_1 = 0$. Using this criterion, the relative contents of the two pools can be easily determined in plant tissues. The major pool, phyA′, is light-

labile, while the minor phyA″ pool is relatively light-stable. *In vitro* experiments with extracted phyA indicate that phyA′ is water-soluble, while phyA″ seems to be membrane-(protein-) bound. The two phyA species may differ by serine phosphorylation in the small serine cluster of 10 aa. at the very end of the *N*-terminal extension of the molecule as revealed by the experiments with truncated and point-mutated phyA. They were also shown to differ by the mode of the nuclear/cytoplasmic partitioning with phyA′ forming small speckles in the nucleus whereas phyA″ clusters into large speckles. These physicochemical distinctions between the two phyA pools are likely to determine their functional diversity. Making use of Ser/Ala substituted phyA mutants of rice expressed in transgenic *Arabidopsis* it could be shown that phyA′ mediates VLFR, whereas phyA″, HIR and, possibly, LFR. The second source of the complexity of the phyA action arises from the functional interaction between the phytochrome system and hormones. Recent investigations have shown a very close connection between phyA functions and the hormonal status of the plant (see reviews (4–9)).

The subject of our current study is the functional interconnection between phyA and the hormone jasmonic acid (JA), which is a key regulator for the activation of defense against biotic and abiotic stress (10,11). JA is formed from α-linolenic acid via the octadecanoid pathway with the participation of the enzyme allene oxide cyclase, AOC (12), and controls different aspects of plant growth and development, such as inhibition of seed germination and root growth, or the stimulation of leaf senescence including degradation of chloroplast proteins.

Mutants deficient in JA, such as *hebiba* (13) or *cpm2* (see below), allowed to dissect the role of this hormone not only in stress adaptation, but also in normal development. These mutants lack the inhibition of coleoptile growth in response to light, suggesting that jasmonates participate in the processing of light signals. Later on, it was found that JA is involved in the regulation of light-induced phyA turnover (14,15). Likewise, a role of JA was also manifest in the phyA-dependent regulation of protochlorophyllide (Pchlide) biosynthesis (14). In the WT, pulsed FR (a VLFR condition) stimulated biosynthesis of the active Pchlide^{655}/$_{650}$, whereas continuous FR (a HIR condition) was inhibiting. In contrast, both illuminations stimulated biosynthesis of the pigment in the mutant, that is the sign of the VLFR effect changed from negative in the WT to positive in *hebiba*. These observations agree with the data showing that JA contributes to the block of the greening response by FR (10). In general, JA and phytochrome signals are mutually antagonistic (for review, see (5)). It was shown recently (16) that JA initiates a complex molecular cascade modulating phyA signaling through repressing (by JAZs proteins) the activity of FHY3, a transcription factor activating the genes coding for the two proteins, FHY1 and FHL, which participate in the light-induced phyA nuclear accumulation of light-activated phyA and in the FR light responses.

In this work, we went on investigating the functional interaction between phyA and JA using low-temperature (85 K) fluorescence spectroscopy and photochemistry in wild-type rice and its mutants *hebiba* and *cpm2*, both defective in allene oxide cyclase (AOC) that is essential for JA synthesis. While *hebiba* harbors a deletion of approximately 170 kbp comprising the entire *AOC* locus, but also numerous additional genes, the second mutant, *cpm2* (*coleoptile photomorphogenesis 2*), just lacks 11 base pairs in an AOC exon. Nevertheless, the phenotype is close to that of *hebiba*. Therefore, *cpm2* can be considered as an *AOC*-specific mutant, while for *hebiba* additional genes are affected (17,18). It was found that, under FR, phyA considerably reduced its own content in WT and in the mutants. However, while in the WT, total domination of the phyA″ resulted, in the mutants, the phyA′ became predominant. JA seemed to participate primarily in phyA destruction and also in the suppression of the phyA action on Pchlide biosynthesis. The effect of JA on the growth responses to phyA was complex: in roots, JA abolished the phyA-induced suppression under pulsed FR (VLFR) and was dispensable for the action of constant FR (HIR). In contrast, coleoptile growth was not affected. In general, by all the parameters tested, the most distinctive phenotype was seen in the case of *cpm2* what may be explained by the fact that this line was defective only in the JA biosynthesis. This suggests participation of JA in the phyA turnover and modification of its signaling activity.

MATERIALS AND METHODS

Wild-type (WT) rice (Oryza sativa L. ssp. *Japonica* cv. Nihonmasari) and its mutants *hebiba* and *cpm2*, deficient in jasmonic acid, generated in the same cultivar (17) were used in the experiments. Both the mutants are similarly incapable of synthesizing JA. The seeds were sown on synthetic floating rafts and grew on tap water during 5 days at 23°C either in the dark or under constant of pulsed far-red illumination. The phenotypical characterization of the seedlings (by the length of coleoptiles, mesocotyls and roots) and preparation of samples for spectroscopic measurements were carried out under dim green safe light (tungsten bulb 20 Wt, blue-green and neutral filters SZS22 and NS10, respectively, fluence rate 10^{-4} μmol m^{-2} s^1; Optical Glass Plant, Krasnogorsk, Russia). The mutant *cpm2* and *hebiba* plants were identified by their unique long mesocotyls.

The source of FR light was light-emitting diode with cutoff filters [KS-17 + FS-9, Krasnogorsk Optical Plant, Russia] that provided illumination with the wavelength at 740 nm (λ_a = 740 nm) and a half-band of 15 nm. Two regimes of the illumination—constant (designated FRc-high) and pulsed (FRp, 6 min light, 54 min darkness) of the same fluence rate (0.2 μmol m^{-2} s^{-1})—were employed, which are favorable, respectively, for the manifestation of the high-irradiance responses (HIR) and very-low fluence responses (VLFR) (19). Constant illumination with low irradiance (FRc-low, 0.004 μmol m^{-2} s^{-1}) was also used (reduction by an additional neutral filter NS-9). The light intensity was measured with the PAR special sensor (Skye Instruments Ltd., UK).

The content of phytochrome A in etiolated seedlings and also the proportion of their native types phyA′/phyA″ were determined with the use of the in situ low-temperature (85 K) fluorescence spectroscopy and photochemistry. The spectra were taken with a FluoroMax-4P (Jobin-Yvon Horiba, France) spectrofluorimeter, λ_{ex} = 633 nm, T = 85 K. Briefly, the phyA content was evaluated in relative units (r. u.) by relating the fluorescence intensity in the phyA maximum at 683–685 nm to the background emission at 660 nm, where phyA does not emit, reflecting the mass of the sample under the exciting beam. And the proportion of the phyA′/phyA″ species was determined by the extent of the Pr → lumi-R conversion at 85 K under red (633 nm) illumination taking into account the fact that phyA′ is active in this low-temperature reaction (the individual extent (γ_1) of the photoconversion is 0.5 and phyA″, inactive (γ_1 = 0) (20) (Fig. 1).The samples for measurements were two 5 mm coleoptile tips kept for 2–5 min during the preparation procedure in 50:50 water: glycerol mixture and dried before measurements with filter paper. The Pchlide content in coleoptiles and leaves in the active Pchlide^{655} and inactive Pchlide^{633} was similarly determined (in r.u.) from their low-temperature (77 K) emission spectra (λ_{ex} = 435 nm) by relating the intensity in the respective emission maxima (633 and 655 nm) to the intensity of the background emission at 618 nm as described in (21) (Fig. 2). The statistical analysis of the obtained data (±SE from 3 to 15 independent measurements) was performed using the *t*-criterion (Student's *t*-test).

RESULTS

The work has been carried out along the following three experimental lines determining (1) seedlings' morphology, (2) phyA and its pools' content in coleoptiles and (3) inactive Pchlide^{633}

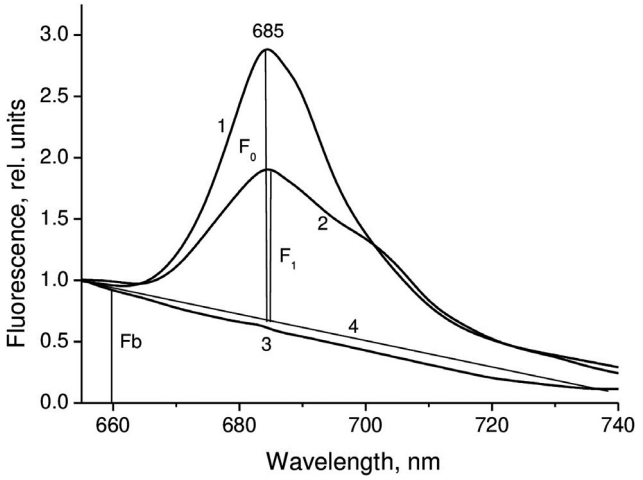

Figure 1. Low-temperature (85 K) fluorescence emission spectra (λ_{ex} = 633 nm) of the coleoptile tips of wild-type rice (*Oryza sativa* L. ssp. Japonica cv. Nihonmasari) (1,2) and of the phyA deficient rice, and their analysis leading to the evaluations of the total phytochrome A (phyA) content and the proportion of the two phyA native types (phyA′ and phyA″). Curve 1 was obtained from the dark-grown seedlings (state F_0), curve 2 was obtained from the same sample after its saturating red light (λ_a = 633 nm) illumination at 85 K (state F_1) and curve 3 was obtained from (23) (the spectrum of the coleoptiles of the *phyA* rice mutant taken as a spectrum of the background emission and linearly approximated by line 4). The phyA content was evaluated as the ratio of the phyA amplitude in the maximum at 685 nm to the amplitude of the background emission at 660 nm F_0/F_b (rel. units), and the phyA′/phyA″ (%) was taken from the extent of the Pr → lumi-R conversion $\gamma_1=(F_0-F_1)/F_0$ as described in (20). The spectra were not corrected for the spectral sensitivity of the spectrofluorimeter

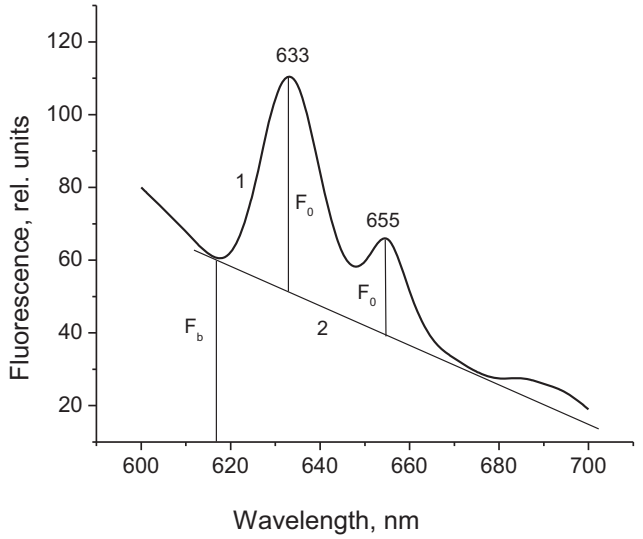

Figure 2. Low-temperature (77 K) fluorescence emission spectrum (λ_{ex} = 435 nm) of the coleoptile tips of wild-type rice (*Oryza sativa* L. ssp. Japonica cv. Nihonmasari) (1) and linear approximation of the background emission (2) for the determination of the content of the active (Pchlide^{655}) and inactive (Pchlide^{633}) protochlorophyllides. The Pchlide^{655} and Pchlide^{633} concentrations (in rel. units) were evaluated as the F_0/F_b ratio (as described in (21))

and active Pchlide^{655} content in coleoptiles and leaves of the dark and FRc- and FRp-grown seedlings of the wild-type, *hebiba* and *cpm2* rice lines.

Growth responses

Etiolated seedlings of the wild-type line were characterized by the length of coleoptiles, mesocotyls, and roots of 12.0 ± 0.4 mm, ≈2 mm and 34.0 ± 2.5 mm, respectively. Both the mutants, *hebiba* and *cpm2*, revealed in the dark a specific phenotype with the elongated mesocotyls of 10–12 mm, that was five- six-fold longer than those of the WT seedlings, and comparable lengths of the coleoptiles and roots—around 12 mm and 35 mm, see Fig. 3.

Upon illumination of the growing wild-type seedlings with FRc-low, there was practically no difference observed in the length of the coleoptiles and roots, although the mesocotyl virtually disappeared (Fig. 3). In the case of *hebiba*, the illumination brought about a considerable reduction in the length of the mesocotyl, in line with the effect in WT; however, the coleoptiles and roots under light were approx. 1.5 longer than in darkness and also in WT in darkness and under FRc-low. The *cpm2* plants reveal a somewhat different picture compared with the WT and *hebiba*: There is barely any effect of illumination on the coleoptile length, lower effect of mesocotyl reduction and strong effect (a two-fold reduction) of the roots.

FRc-high produced essentially similar effect as FRc-low in the WT seedlings—no change in the length of the coleoptiles and disappearance of the mesocotyls (Fig. 3). However, almost a two-fold reduction of the roots was obvious in contrast to the lack of the effect in darkness and under FRc-low. In *hebiba*, the

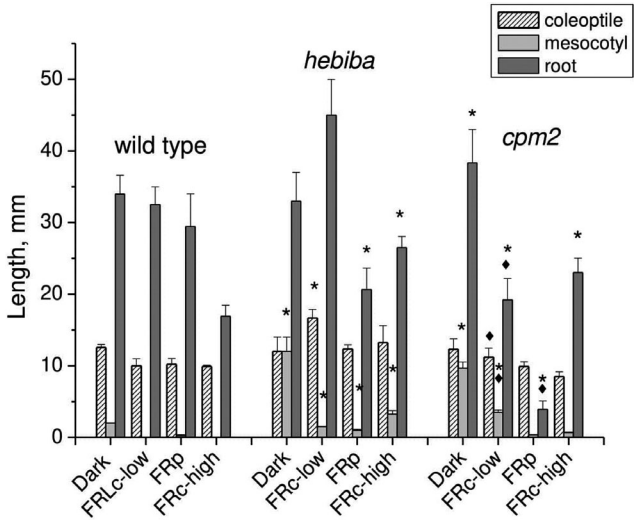

Figure 3. The length of the coleoptiles, mesocotyls and roots of dark-grown 5-day-old rice (*Oryza sativa* L. ssp. Japonica cv. Nihonmasari) seedlings and of its *hebiba* and *cpm2* mutants deficient in the hormone jasmonic acid (JA) and of the seedlings of the same lines grown under far-red constant light (FRc, λ_a = 740 nm) of two fluence rates (FRc-high, 0.2 μmol m^{-2} s^{-1}; FRc-low, 0.004 μmol m^{-2} s^{-1}) and pulsed FR (FRp, 6 min light/54 min dark, 0.2 μmol m^{-2} s^{-1}). In the wild type, coleoptile lengths under different illumination conditions are not significantly different; statistically significant light effects are seen in the case of mesocotyls; roots undergo significant growth inhibition only under FRc-high (Student's *t*-test; $P < 0.05$). Statistically significant light effects are seen: in *hebiba*—for coleoptiles under FRc-low, for mesocotyls under all the light conditions and for the roots under FRp; in *cpm2*—for coleoptiles under FRp and FRc-high, and for mesocotyls and roots under all the light conditions. Here and in Figs 4 and 5, the asterisks indicate the values of the mutants, which are significantly different from those of the wild type, and the diamonds point similarly to the values of *cpm2* significantly different from those of *hebiba*

FRc-high illumination is not effective with regard to the coleoptiles length and approx. similarly effective as the FRc-low in the reduction of the mesocotyl. However, it reveals an opposite effect to that under FRc-low for the root length—similarly to the situation in the WT, it is reduced by approx. 1.3-fold. For the *cpm2* line, FRc-high was more active in the coleoptile and mesocotyl reduction than FRc-low.

The pulsed FRp light was practically not effective in the WT with regard to the coleoptiles and root shortening and similarly effective in the mesocotyl reduction as in the case of FRc of both fluence rates (0.25 mm *vs* 2 mm in darkness) (Fig. 3). In *hebiba*, FRp did not affect coleoptiles, steeply reduced mesocotyls and brought about a two-fold shortening of the roots. The *cpm2* mutant revealed the same pattern of changes in the seedlings under FRp as did *hebiba,* but their extent in the case of the root reduction was much deeper: The coleoptile is not affected, the mesocotyl is not visible and the roots are reduced by two-fold.

Pchlide^{655} and Pchlide^{633}

In darkness, the total content of the active and inactive protochlorophyllides in the coleoptiles, [Pchlide^{655}] + [Pchlide^{633}], was 1.5- to 1.7-fold higher in *hebiba* and *cpm2* than in the WT (Fig. 4a). FRc-low was stimulating for their biosynthesis in the WT seedlings and FRp was neutral, whereas these illumination modes were inhibiting for the mutants. Interestingly, FRc-low was much more effective than FRp in this inhibiting effect, although the fluence for FRp was five-fold higher suggesting that the mode of the illumination (constant *vs* pulsed) proves to be imperative for the effect. FRc-high was either neutral in the case of the WT or stimulating in the mutants. The ratio between the two pigment forms, [Pchlide^{655}]/[Pchlide^{633}] %, remained, however, essentially similar in all the lines and under all the illumination conditions (Fig. 4b).

The content of the two protochlorophyllides was also followed in leaves of the same 5-day-old seedlings (data not shown). For Pchlide^{655}, it was, in general, four- to eight-fold higher in darkness in all the three lines than in the coleoptiles, and for Pchlide^{633}, this difference was lower, ≤ two- to three-fold. However, the pattern of the FR effects was essentially the same as in the coleoptiles. Of interest is the fact that under FRp the content of Pchlide^{633} and Pchlide^{655} and also their concentration ratio in leaves of the WT, *hebiba* and *cpm2* plants are very similar (in line with the observation on coleoptiles, see above) suggesting that JA is not involved in the phyA regulation of the protochlorophyllide state under FRp independently of plant's organ/tissue.

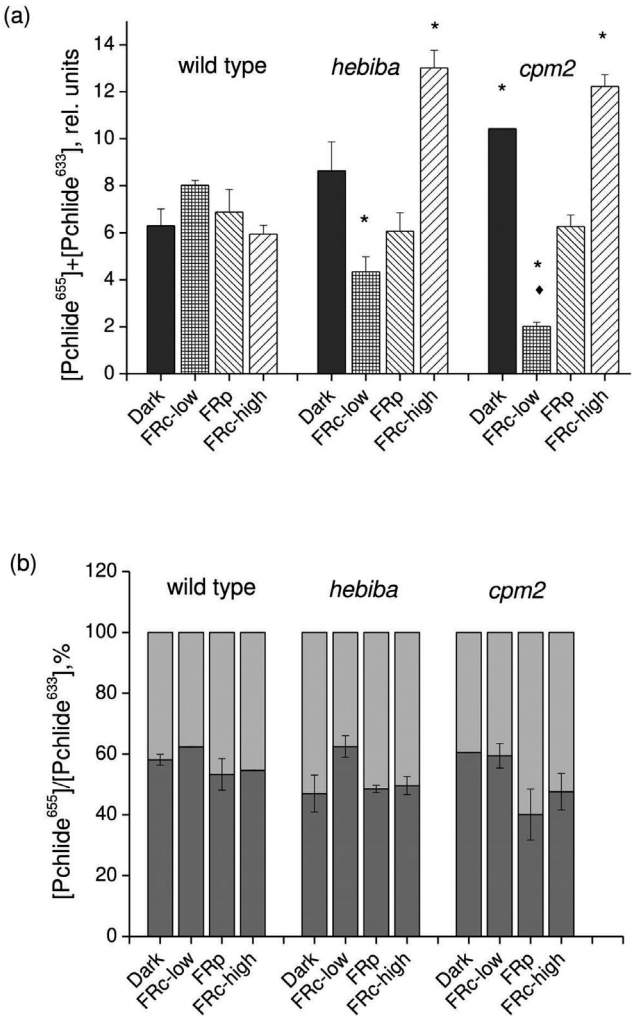

Figure 4. The content of the two protochlorophyllides ([Pchlide^{655}]+[Pchlide^{633}]) in the coleoptiles of the wild-type seedlings of rice (*Oryza sativa* L. ssp. Japonica cv. Nihonmasari) and of its *hebiba* and *cpm2* mutants (a) and the proportion of the active (light gray) and inactive (gray) protochlorophyllides (b). The pigments were determined by fluorescence emission spectra (as described in the Materials and Methods section and illustrated by Fig. 2). The effects of the illumination of the different regimes on the protochlorophyllides' content are not statistically significant in the wild type but they are in the mutants (indicated by the symbol (+) here and in Fig. 5). For the Pchlide^{655}/Pchlide^{633} proportion under all the illumination variants, the difference of the population means is not significantly different than Student's *t*-test difference at the 0.05 level. For the illumination conditions, see the legend to Fig. 3.

phyA and its two types phyA′ and phyA″

In the dark-grown plants, the content ([P_{tot}]) of phyA in WT coleoptiles was 1.71 ± 0.12 r.u. and the proportion is 61.5/38.5 % (Fig. 5). The *hebiba* mutant plants revealed a slightly lower content and a higher phyA′/phyA″ ratio—1.5 ± 0.17 r. u. and 73/28 %. The *cpm2* mutant showed even further reduction in the phyA content (1.5-fold lower) and a lower phyA′/phyA″ proportion (54/46%). Growing plants under FRc light brought about a decline of total phyA in the WT—by eight-fold under FRc-low and even further by 12-fold under FRc-high. The decline under the FRc-low was followed by an insignificant rise of the phyA′/phyA″ proportion, whereas the FRc-high brought about, on the contrary, a redistribution of phyA′/phyA″ toward phyA″ so that it almost completely dominated (more than 95 %). In *hebiba*, application of both low- and high-FRc treatment resulted in an eight- to 10-fold [P_{tot}] decline; however, there was no such a drop in the phyA′ content under high FRc, as in the case of the WT. More to that, in *hebiba,* practically all phyA was in the phyA′ form under FRc-high. The *cpm2* mutant essentially resembled *hebiba* for the low- and high-FRc effects, although the decline of [P_{tot}] was lower, by three- to four-fold (Fig. 5). Under pulsed FRp, the effects of the light treatment were much less pronounced in all the lines than those of the FRc-low and FRc-high and in WT, much less than in the mutants—approx. a two-

fold [P_{tot}] decline in WT and a three- to four-fold, in the mutants. This decline was followed by the increase of the phyA′ proportion in WT and its relative reduction in the mutants.

DISCUSSION

We have experimentally characterized etiolated seedlings of the investigated rice lines—the wild-type cv. Nihonmasari and its JA biosynthesis mutants *hebiba* and *cpm2*—with respect to the three major phenomenological features—phenotype, accumulation of the active and inactive protochlorophyllides, and the content of phytochrome A and the balance of its two native types, phyA′ and phyA″. These plants were also tested with regard to their phyA-mediated photoresponses—development and growth under illumination with constant FRc-high (favorable for HIR) and pulsed FRp (favorable for VLFR). We have also used FR constant of very-low irradiance (FRc-low), 50-fold lower than FRc-high, which may have the properties of the two response modes (HIR and VLFR).

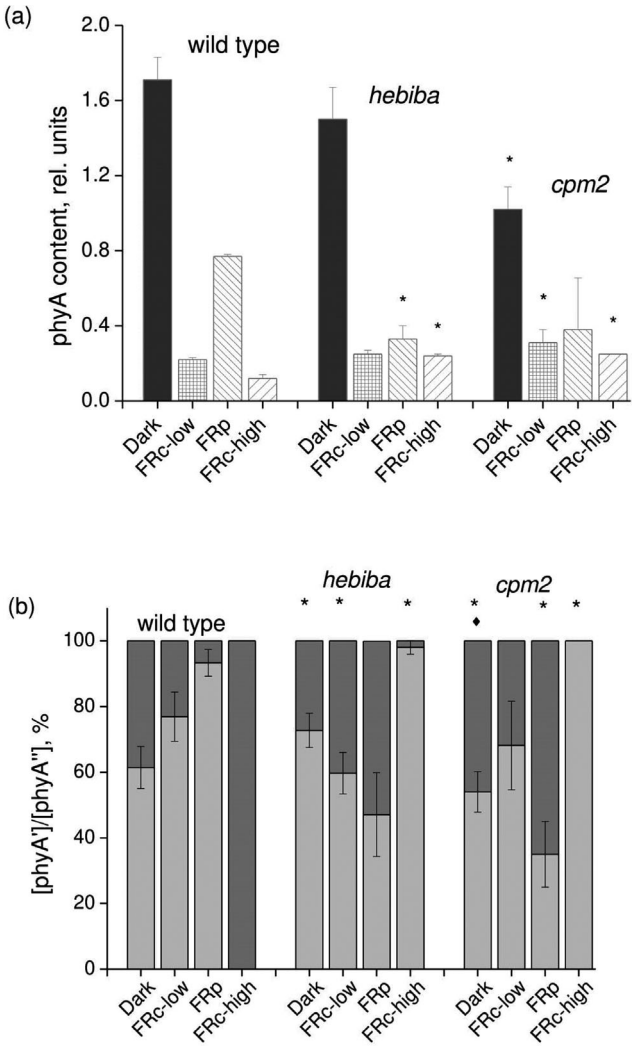

Figure 5. The total content of phytochrome A in the coleoptiles of the wild-type seedlings of rice (*Oryza sativa* L. ssp. Japonica cv. Nihonmasari) and of its *hebiba* and *cpm2* mutants (a) and the proportion of its two native types, phyA′ (light gray)/phyA″ (gray) (b). (a) The effects of the FR light under all the illumination regimes on the phyA content are statistically significant (Student's *t*-test; $P < 0.05$) for all the lines (indicated by the symbol (+)). (b) The statistically significant light effects on the phyA pools' ratio in all the lines are the variants "dark *vs* FRp and FRc-high" (symbol (+)). The statistically significant differences between the mutants and the WT for [P_{tot}] and the phyA pools' proportion are indicated by asterisks, and between the mutants, by the diamond symbol. The illumination conditions are the same as in Figs 3 and 4.

In darkness, the mutants revealed a specific phenotype with the elongated mesocotyls, whereas the coleoptiles and roots were of comparable lengths (Fig. 3). The dark level of the active Pchlide^{655} and inactive Pchlide^{633} was mildly affected (Fig. 4), and the content of phyA and the phyA′/phyA″ ratio varied also within a small range (Fig. 5). In light-grown seedlings, the JA mutant phenotype revealed itself, however, much more profoundly. In the wild type, mesocotyls drastically reduced their length and the seminal roots showed sensitivity to the light treatment with FRc-high; however, the coleoptile growth was not affected (Fig. 3). The latter is in contrast to the well-known inhibition effect of red and far-red light (see (22) and the literature cited therein) and to our earlier observations on the same rice line (14). This contradiction may be explained in the light of the observation (1,22) that FR treatment was not effective on coleoptile growth in relatively young rice seedlings. In our present experiments, we were likely dealing with seedlings of a younger physiological age, because they were grown under lower temperature, at 23 *vs* 27°C. This may relate to other variations in the character of the light effects observed on Pchlide and phytochrome (see below). Pchlide synthesis in WT coleoptiles was relatively unresponsive to the FR treatment under all the illumination regimes (Fig. 4a), what is in agreement with the data on Pchlide^{633} of the same rice line *Oryza sativa* L. Japonica cv. Nihonmassari (14) but in contrast to the strong inhibiting effect of FRc observed on the rice line *Oryza sativa* L. *cv*. Nipponbare (23). The Pchlide^{655}/Pchlide^{633} ratio was not practically affected by the light treatment, and in general, this parameter turned out to be rather conservative (Fig. 4b). This is in agreement with the data on FRp but in contrast to the situation under FRc, when strong inhibiting effect was seen only on Pchlide^{655} but not on Pchlide^{633} (14). This variability of the FR effects under different illumination regimes (FRp and FRc) observed even on the same plant species (rice) suggests dependence of their sensitivity on the physiological status of the plant, possibly, on their physiological age. The most pronounced and complex effects of the far-red treatment were observed, however, on the phyA content and the balance between its two pools. All the illumination regimes proved to be inhibiting for [phyA], and FRc was much more effective than FRp. This correlates well with our earlier observation (14,23) and suggests that this effect is of the HIR type. The phyA decline under FRp was followed by the domination of phyA′, whereas under FRc-high, of phyA″. Given that phyA′ mediates VLFR, whereas phyA″, HIR (24), this drastic difference in the phyA′/phyA″ proportion upon different modes of illumination (FRc-high, FRp), at the relatively even total phyA content, may be one of the sources of the observed differences in the character of the responses they cause.

The *hebiba* mutant is essentially similar to the WT with respect to the FRc-high growth effects. However, under FRp it shows an inhibitory effect on roots when in the WT it is ineffective. There is a reversion of the sign of the light effect under FRc-low with regard to the coleoptiles and roots—stimulation of their growth, whereas in the WT this treatment is ineffective. This agrees with the earlier observations on the *hebiba* mutant

(13,14). Of interest is the fact that the Pchlide content was considerably suppressed under FRc-low and FRp, whereas under FRc-high it was promoted suggesting that the sign of the phyA action on Pchlide under FR depends on the mode of illumination (FRc *vs* FRp) and, possible, on its fluence (FRc-high *vs* FRc-low) (Fig. 4). Our earlier observations have shown that, along with the well-known inhibition of the protochlorophyllide biosynthesis by FR (see review (25)), there was a stimulating affect of this light, and the sign of the effect depended on plant species/tissues, its genotype, and light conditions used (14,21). In particular, a similar stimulation of the $Pchlide^{655}$ and $Pchlide^{633}$ biosynthesis under FRp was observed in wild-type rice; however, FRc was strongly inhibiting for $Pchlide^{655}$, what is at variance with the current results when FRc-high of a comparable fluence rate was ineffective. This may be due to the different physiological age of the plants under investigation (see above) and underscores the importance of their physiological state for their responsiveness to the light treatment. The phyA content in *hebiba* coleoptiles was suppressed at all the illumination regimes as in the case of WT. However, there was a specificity of the FR action with regard to phyA′/phyA″ ratio: (1) Under FRc-high, the sign of the effect in *hebiba* was positive (domination of phyA′) (in contrast to WT with the domination of phyA″); (2) under FRc-low, the situation is reversed—the proportion of phyA′ is lower in *hebiba* than in WT; and (3) under FRp, both the lines (*hebiba* and WT) revealed a positive effect on the phyA′/phyA″ ratio. This suggests deep implication of JA into the determination of the phyA state and its dependence on the light conditions of the FR-induced de-etiolation.

The *cpm2* seedlings in general proved to be similar by their reactions to the FR treatment to those of *hebiba*, although their magnitude, and for some responses, their sign differed depending on the process under investigation. In roots, for instance, the inhibiting FR effects were higher in *cpm2* than in *hebiba,* and at the same time, under FRc-low, a reversion of the sign of the effect was observed—stimulation in *hebiba* and inhibition in *cpm2* (Fig. 3). The FR effects on [Pchlide] and [phyA] were similar in *cpm2* and *hebiba* (Figs 4 and 5), as well as on the phyA′/phyA″ ratio—increase of the relative phyA″ content, in contrast to WT where phyA′ is the major component (Fig. 5b). This specificity of the FR effects in *cpm2* suggests that mutations in *hebiba* other than the JA deficiency may contribute to the phenotype of the latter.

Thus, we observed a highly complex picture of the light responses to the three different modes of illumination. There are, however, two or three unifying features for all the light conditions and all plant lines used. First, there is a lack of the light effect on the coleoptile growth, whereas this effect was firmly established in the literature (13,14,22,23). The inhibitory effect was, however, observed in this work in mesocotyls in all the lines and under all the light illumination conditions suggesting a much lower sensitivity threshold for the effect. The roots were also sensitive to the light treatments—the effects were primarily inhibiting, although a positive effect (*hebiba*, under FRc-low) and lack of it (WT, under FRc-low and FRp) were documented. The lack of the FR light effect in coleoptiles in our current experiments may be explained by the relatively young physiological age of the seedlings in this work as compared to (14) (see above). Another possible cause is, possibly, the differences in the illumination conditions (the actinic light was a far-red emitting diode with λ_a = 740 nm *vs* a tungsten 100 W lamp with $\lambda_a \geq 720$ nm in our earlier experiments). According to (22), the wavelength of actinic light in the FR region of the spectrum is critical for its effects, as revealed by the experiments on the action spectrum of the light treatment. The two following facts established in this work speak well for the notion that the lack of the inhibitory effect on coleoptiles is not due to the photoreceptor itself and that the bottleneck is at the stage of the light signal transduction and/or its realization. The first fact is that the coleoptiles of the mutant lines contain spectroscopically and photochemically normal phyA in comparable concentrations to those of the WT and the second is that phyA is functional with regard to the other responses—modification of its state (FR brings about phyA decline and redistribution of its pools), protochlorophyllide synthesis and growth effects on mesocotyl and roots. Indeed, Takano *et al*. (1) and Xie *et al*. (22) have shown that the light tells on the length of the inner epidermal cells of the coleoptile and that the sensitivity to such a light treatment increases 2–3 orders of magnitude on the later stage of seedlings' growth (after 4^{th} day). Given that the seedlings used in this work were approx. of this age, this may account for the observed lack of the coleoptile inhibition.

The second unifying feature for all the plant lines used was the drastic reduction of the phyA content with a quite similar pattern of its dependence on the mode and fluence of the illumination (Fig. 5a). Proceeding from our earlier observations on pea and its phyA mutants (26), we may speculate that this FR-induced phyA decline and alteration of the phyA′/phyA″ equilibrium is the result of two processes—the inhibition of the total phyA biosynthesis (without changes in the phyA′/phyA″ balance) and regulation of its post-translational differentiation into the two native types. For instance, to explain the observed increase of the phyA′/phyA″ proportion (in *cpm2* under FRc-high and in the WT under FRp), we have to postulate the stimulation of the phyA″ into phyA′ conversion, the effect observed earlier (3,24). The decline in the phyA′/phyA″ ratio, on the contrary, is due to the preferential destruction under certain conditions of the light-labile phyA′ (26). These three processes—inhibition of the phyA synthesis, stimulation of the phyA″ conversion into phyA″ and phyA′ degradation—may account for the complex phenomenon of the phyA turnover under FR illumination (Fig. 6).The differences in these effects observed between the WT and the JA mutants point to theimplication of this hormone in them (Fig. 6). Taking into account that JA modifies the action of phyA through suppression of its FR-induced transport into the nucleus (16), we may speculate that the specific effects of JA on HIR (mediated by phyA″) and VLFR (mediated by phyA′) may be connected with the differences in the phyA pools' transport into the nucleus and speckle formation in them (27). As another possible cause of the JA effects on HIR and VLFR, one can consider the fact that JA participates in phyA destruction (14,15) and that it may primarily tell on the light-labile phyA′ than on the relatively light-stable phyA″. Certain variations of these effects seen in *hebiba* and *cpm2* suggest that the differences between these mutant lines may not be restricted to the pure lack of this hormone but most likely could be attributed to additional mutation in *hebiba*. Considering functional significance of the JA effects on phyA, we may speculate, taking into account the specific functions of phyA in seedlings' de-etiolation and growth under FR-enriched light conditions (deep canopy shade) (28), that JA, together with the light-induced lability of the photoreceptor and the FR-induced down-regulation of its synthesis, could limit extreme

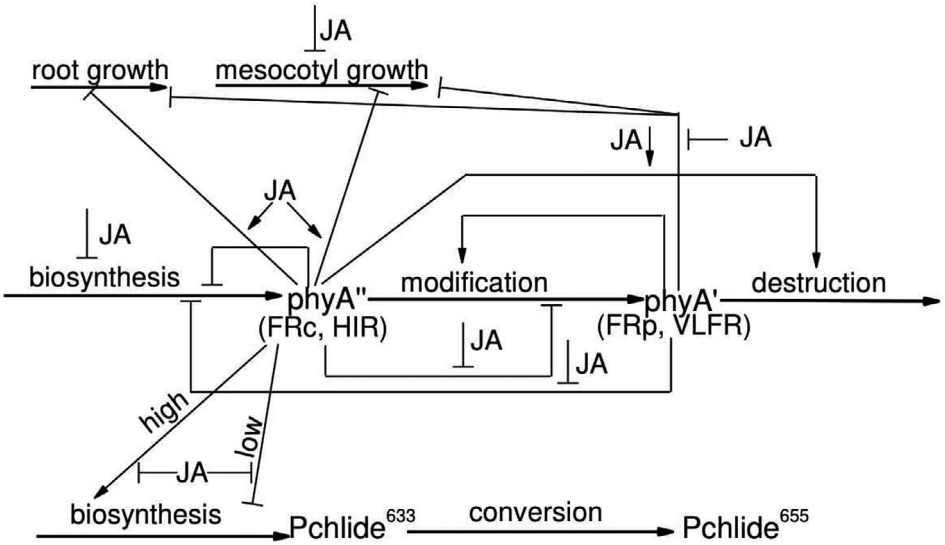

Figure 6. Hypothetical model of relationship between phytochrome A pools (phyA′ and phyA″) and phytohormone jasmonic acid signaling in etiolated 5-day-old seedlings of rice grown under far-red illumination (FR) of different regimes—FR constant (FRc, HIR) and FR pulsed (FRp, VLFR). Thin arrows indicate signaling and thicker arrows, metabolic processes. "High" and "low" stand for the light signaling under FRc of different (by approx. 50-fold) fluences. Lines with arrowheads correspond to positive regulation, lines with blunt ends, to negative regulation. Note that coleoptiles are not presented because their growth was not practically affected by the light treatment probably due to the young age of the seedlings (see Discussion), and mesocotyls are too short after light treatment to qualitatively evaluate its effect in all the plant lines. As one can see from the scheme, almost all the effects of JA on the light signals from phyA′ (FRp, VLFR) and from phyA″ (FRc, HIR) are inhibiting, except the three effects, when JA promotes phyA signaling—the phyA″ inhibition of phyA biosynthesis and root growth, and the stimulation of phyA′ destruction.

manifestations of its activity and thus may have contributed to the evolutionary advances of the land plants (29).

CONCLUSIONS

A comparative investigation of the etiolated and far-red (FR) light-grown seedlings of the wild-type rice and its mutants deficient in the defense-related phytohormone jasmonic acid—*hebiba* and *cpm2*—has been carried out to follow its functional interaction with phytochrome A. Both *hebiba* and *cpm2* have a block of the enzyme allene oxide cyclase, AOC, participating in the JA biosynthesis, but *hebiba* is even more profoundly genetically modified than *cpm2*. In darkness, all the three plant lines had essentially similar parameters—the length of their coleoptiles and roots, content of the active and inactive protochlorophyllides ($Pchlide^{655}$ and $Pchlide^{633}$) and phytochrome A pools (phyA′ and phyA″)—suggesting that JA is not critical for their regulation in darkness. The only exception was the mesocotyl which was much longer in the mutants. The seedlings grown under constant FR (of two fluence rates differing by 50-fold, FRc-low and FRc-high) and pulsed FR demonstrated, however, wide variations of their parameters depending on the illumination mode and, in particular, on the plant line. The coleoptile length was not considerably affected by all the light regimes in all the lines, in agreement with the observation (1) that the FR effect on coleoptiles is not seen at the early stage of development. However, strong mesocotyl reduction was seen in all the lines under all the light conditions and the seminal roots revealed a complex growth inhibition effect most pronounced in the mutants. In contrast to the WT roots affected only by FRc-high (HIR), the roots in the mutants underwent reduction both under FRc-high and FRp (VLFR). Of interest is the fact that the effects were more pronounced in *cpm2*, whereas there was even mild root growth stimulation (under FRc-low) in *hebiba*. This clearly demonstrates that JA negatively affects the phyA regulation of rice seedling growth differently modulating their extent and even their sign under the different light-growth conditions (Fig. 6). The regulation of Pchlide by phyA was observed only in the mutants—suppression under FRc-low and FRp (VLFR) and promotion under FRc-high (HIR), and there was practically no effect on the proportion of the Pchlide's active and inactive forms in all the lines. This suggests that phyA can differentially affect the biosynthesis of Pchlide under VLFR and HIR conditions and that JA counteract this action in WT (Fig. 6). Finally, phyA exerts strong down-regulation of its own synthesis in all the lines suggesting that JA has a relatively small effect on it. However, JA reverses the sign of the phyA′/phyA″ proportion changes under FRc-high (HIR) from the phyA″ domination in WT to phyA′ domination in the mutants. In general, the two mutant lines, *hebiba* and *cpm2*, reveal essentially similar phenotype, although some specificity in *hebiba* is seen suggesting that its phenotype may not be entirely due to the lack of JA. Of interest for further investigations is also the fact that JA differentially affects the two response types—HIR and VLFR. Since they are likely to be mediated by phyA″ and phyA′, respectively, we may expect different mechanisms of a possible JA interaction with them, possibly, via differential modification of their nuclear/cytoplasmic partitioning. It is also tempting to try to understand why the sign of the photoresponses from phyA depends on the illumination mode (for instance HIR *vs* VLFR in the regulation of the Pchlide biosynthesis) and clarify the mechanism of the involvement of the different phyA pools in this effect. Finally, the FR effects, their extent and even the sign, and also the character of the JA participation in them depend on the developmental state (physiological age) of the plant what needs further systematic investigation.

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