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# phyA-GFP is Spectroscopically and Photochemically Close to phyA and Comprises Both its Native Types, phyA' and phyA''

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Abstract

Low-temperature fluorescence investigations of phyA-GFP used in experiments on its nuclear-cytoplasmic partitioning were carried out. In etiolated hypocotyls of phyA-deficient *Arabidopsis thaliana* expressing phyA-GFP, it was found that it is close to phyA by spectroscopic parameters with both its native types, phyA' and phyA'', present and their ratio shifted towards phyA'. In transgenic tobacco hypocotyls, native phyA and rice phyA-GFP were also identical to phyA in the wild type whereas phyA-GFP belonged primarily to the phyA' type. Finally, truncated oat Δ6-12 phyA-GFP expressed in phyA-deficient *Arabidopsis* was represented by the phyA' type in contrast to full-length oat phyA-GFP with approximately equal proportion of the two phyA types. This correlates with a previous observation that Δ6-12 phyA-GFP can form only numerous tiny subnuclear speckles while its wild-type counterpart can also localize into bigger and fewer subnuclear protein complexes. Thus, phyA-GFP is spectroscopically and photochemically similar or identical to the native phyA suggesting that the GFP tag does not affect the chromophore. phyA-GFP comprises phyA'-GFP and phyA''-GFP suggesting that both of them are potential participants in the nuclear-cytoplasmic partitioning what may contribute to its complexity.

## INTRODUCTION

Photoregulation in plants is achieved through cooperation of a number of the complex photoreceptor systems in which phytochromes are the main ones and the most profoundly investigated[1,2]. In the recent two decades, we are witnessing considerable progress in phytochrome (phy) research both with regard to the understanding of the nature of their native forms and the mechanism of downstream signal transduction from them. The observed phytochrome heterogeneity[3-6] turned out to be the manifestation of the existence of a small family of its gene products, which has five members in *Arabidopsis*[7], among them phyA and phyB being the major ones. phyA massively dominates the phytochrome pool in the etiolated plants; it is the most versatile pigment, mediating the so-called very low fluence responses (VLFR) and high irradience reactions (HIR)[1] and also the classical photoreversible low fluence responses (LFR)[8-10]. phyA is the light-labile species and is characterized by light-induced down-regulation of its own synthesis. Because of that, light-stable phyB becomes the major phy in de-etiolated tissues; it is responsible only for LFR[1].

The mechanism of the light signal transduction from phytochromes unravels along two major lines: first is the elucidation of their localization in the cell and intracellular trafficking, and second, determination of their signal transduction partners. Fusing phyA and phyB to the green fluorescent protein (GFP) (phyA-GFP and phyB-GFP), it was discovered that both phyA and phyB imported to the nucleus in a light-dependent manner but with markedly different dynamics[11-14]. The phyA transfer occurs as VLFR in the minutes time domain whereas the phyB transfer is LFR and is completed within 1-2 hours[15]. To achieve nuclear import, phyA in its Pfr form needs association with plant-specific proteins FHY1 (Far-red elongated Hypocotyl 1) and FHL (FHY1-like)[16-19] . The phyA amino-terminal extension (NTE) domain mediates the formation of this complex[20], which rapidly dissociates when phyA is converted to Pr[21,22]. This shuttle-like process of the phyA transport in the nucleus may explain the HIR action spectrum with the maximum beyond 700 nm[23] .

There are two principal modes and sites of phytochrome action. On one hand, cytoplasmic phyA is likely to be engaged in several biophysical and biochemical events, mediation of root phototropism and modification of gravi- and phototropism (see Refs 24,25 for a review). On the other hand, most of the examined phyA actions require nuclear import of the protein[19]. In the nucleus, phyA Pfr interacts with different factors, it regulates

transcription and light-induced proteolysis of partner proteins and itself[26-31]. Vast majority of the nuclear phytochrome is localized to protein complexes, termed photobodies or speckles or spots[32,33]. While their presence is required for proper signaling our knowledge about their functionality is limited. The appearance of these speckles, however, is well known: phyA can aggregate to many small or a few large nuclear complexes[11,15,32,34]. Interestingly, phyA lacking the 6-12 amino acids from its N-terminus (Δ6–12 phyA-GFP) can form only many tiny spots[35]. Recently, it was shown that modification of transcriptional processes may also take place in the cytoplasm[36].

The complex phenomenology of the phyA photoresponses can be explained, at least partially, by its polymorphism. Circumstantial evidence pointed to phyA heterogeneity *in vivo*. In particular, phyA populations differing by light-induced destruction[37,38,39] and association of phyA with membrane / proteins have been observed[40,41]. Our direct investigations of phyA in phytochrome mutants and overexpressors with the use of *in planta* fluorescence spectroscopy and photochemistry have revealed two populations (phyA' and phyA'') of the pigment in monocots and dicots which differ by a number of phenomenological properties (see reviews[42-44] and original papers by the same author cited therein). phyA' is the longer-wavelength species capable of undergoing photochemical transformation from the initial Pr form into the first stable at low temperatures (T < 85 K) photoproduct lumi-R upon saturating R with the extent of Pr photoconversion to reach the Pr↔lumi-R photoequilibirum ($\gamma_1$) of around 0.5. It is the major and light-labile species in the etiolated plant tissues and is water-soluble. phyA'', on the contrary, is the shorter-wavelength species incapable of photoconversion at low T ($\gamma_1$=0). It is the minor, saturable, relatively light-stable and, possibly, membrane (protein)-bound species. Their photochemical distinction at low T is interpreted in terms of the existence of the activation barrier $E_a$ for the Pr→lumi-R photoreaction in the Pr excited state, which is low for phyA' and high for phyA''. At ambient temperature, this barrier is easily overcome and there is practically no difference in the extent of the Pr→Pfr conversion. Experiments with phyA expressed in transgenic yeast have shown that both its species are the products of one and the same phyA gene plant-specifically post-translationally modified at the N-terminal extention (NTE). phyA' is responsible for de-etiolation under FR (HIR and VLFR) whereas the relatively light-stable phyA'' is likely to be active under R and could be functional together with phyB throughout the plant's life cycle. phyA'' suppresses the action

of phyA' and the regulation of the ratio between phyA' and phyA'' could be the instrument of fine-tuning of their activity.

The data on phyA-GFP nuclear/cytoplasmic partitioning revealing different types of speckle formation and retention of a part of the phyA pool in the cytoplasm after illumination[2,35] are as yet another manifestation of the possible heterogeneity of phyA. Taking this into consideration and also that exact functional distinctions between the two phyA isoforms, as well as the mechanisms of their action, are far from being fully understood, we have undertaken the present investigation in order to establish a connection between the two native phyA pools and the GFP – tagged phyA used in the intracellular trafficking experiments. In particular, the question arises which of the two phyA pools, phyA' or phyA'' (or both), undergoes light-induced transport from the cytoplasm to the nucleus. This question could be answered by clarifying to which of the two phyA species phyA-GFP belongs. It is also interesting to characterize phyA-GFP and compare it with the native phyA spectroscopically and photochemically.

## MATERIALS AND METHODS

### Plant Materials and Growth Conditions

*Arabidopsis thaliana* L. expressing Arabidopsis *35S:AtPHYA-GFP* in the phyA-deficient (*phyA-201*) *Arabidopsis* and tobacco (*Nicotiana tabacum,* SR1) expressing rice (*Oryza sativa* L.) *35S:OsPHYA-GFP* was described by Kim *et al*.[15]. The *Arabidopsis* phyA-deficient mutant overexpressing full-length and Δ6-12 truncated *Avena sativa* (oat) phyA-GFP (*35S: As*(6-12 PHYA-GFP) was obtained by Casal et al.[35]; *phyA-201* was described by Nagatani et al.[45]; and *phyAphyB* (*phyA-2phyB-1*) was generated by the G. C. Whitelam laboratory[46]. Experiments were carried out on etiolated seedlings (without cotyledons which contain Pchl(-ide) interfering with determination of phyA fluorescence phyA determination). The 3-day-old *Arabidopsis* and 8-day-old tobacco seedlings were grown on filter paper moistened with tap water and frozen at 85 K in the dark. Sample manipulations were carried out under photochemically inactive green light (light sources with green filters SZS-21, Krasnogorsk, USSR or light diodes LG 3330/HV12).

### Spectrofluorimetry

The measurements were carried out with the use of custom-built spectrofluorimeter[47] based on two monochromators of the DFS-12 and DFS-24 (LOMO, Leningrad, USSR) conventionally used for the Raman spectroscopy. The source of exciting and actinic light was a He-Ne laser (LGN-207B, 1 mW, Ryazan, Russia) in combination with a monochromator of the MDR-2 type (LOMO, Leningrad, USSR) to cut-off the pumping light. However in the case of the former its intensity was reduced approx. 50-fold with neutral filters to exclude any photochemical changes in the sample during spectra measurements at low temperatures. Red cut-off filter (transmitting at λ> 650 nm, KS-10, Krasnogorsk, USSR) was placed at the entrance slit of the analyzing monochromator. The sample was frozen in a cryostat at 85 K in darkness, when all the pigment is in its red light absorbing Pr form, and the low-temperature fluorescence emission spectrum of phytochrome was first registered upon red excitation ($\lambda_e$ = 633 nm) with He-Ne laser ($F_0$ state). After that, the sample was illuminated at 85 K with full power laser light to induce saturating photoconversion of the Pr form into its first photoproduct (lumi-R) stable at low temperatures and the emission spectrum of the sample with phytochrome in the Pr-lumi-R

photoequilibrium was recorded ($F_1$ state). These raw (experimental) spectra of the sample were corrected for the background emission of the tissues to get the spectrum of phytochrome. For that, a low-temperature (85 K) emission spectrum of *phyA* or *phyAphyB* mutant of *Arabidopsis* was taken for the correction as a spectrum of the background fluorescence. In the case of tobacco samples, the background spectrum was taken from the root tissues at its base after Pr conversion into Pfr upon red illumination, which practically coincides with the spectrum of the *phyA* mutants of *Arabidopsis*[48]. After its subtraction from the experimental spectra, we obtained the spectra of phytochrome and from them, a number of parameters describing the pigment in its native state in the cell. These are (1) spectroscopic characteristics (position, $\lambda_{max}$, shape and half-band width, $\Delta\lambda$, of the spectrum); (2) total phytochrome content ($P_{tot}$ in relative units, RU) proportional to the fluorescence intensity related to the intensity of the background fluorescence at 660 nm reflecting the mass of the plant tissue in the sample under the exciting light beam, $P_{tot} = F_0/F_b$; and (3) extent of the Pr→lumi-R conversion at 85 K to reach a photoequilibrium, $\gamma_1=(F_0-F_1)/F_0$. The experimental $\gamma_1$ parameter is very important in the context of the investigation of the native phyA pools because it allows determination of the phyA'/phyA'' ratio based on the fact that phyA' is photoactive at low temperatures (with individual $\gamma_1$' value of 0.49±0.03) and phyA'' is inactive (with $\gamma_1$'' = 0): (proportion of phyA' is determined as $\gamma_1/\gamma_1$' (i. e. = $\gamma_1/\approx0.5$) [47]. From this ratio and total phytochrome content $P_{tot}$, the concentration of the two phyA pools can also be calculated (in RU). The error of raw spectra registration (noise to signal ratio) is around 5 %. We performed 5 to 10 measurements of independent samples (biological replicates) for each parameter determination. The calculated standard error values are also indicated in the figures.

## RESULTS

The phyA-deficient (*phyA-201*) *Arabidopsis* expressing *Arabidopsis* PHYA-GFP fusion protein has an experimental (raw) low-T fluorescence emission spectrum with a pronounced band at 683 nm (Fig. 1a , curves 1, 2) characteristic in general for phytochrome in the wild-type *Arabidopsis*. The respective *phyA* mutant of *Arabidopsis* used as a control has the experimental spectrum which practically does not show the Pr phytochrome band (Fig. 1a, curve 3) similarly to what was observed earlier on *phyA* mutants of *Arabidopsis*[48]. In order to obtain real spectra of AtphyA-GFP (Fig. 1b) we have subtracted the spectrum of the *phyA* mutant taken as a spectrum of the background emission (Fig. 1a, curve 3) from the experimental spectra of the AtphyA-GFP (Fig. 1a, curves 1, 2). The position, half-band width and the shape of the AtphyA-GFP spectra are very close to those of the native AtphyA (Fig. 1c). However, the content of phyA-GFP is higher than that of the native phyA, 2.20±0.40 RU vs. 0.82±0.12 RU. (Fig. 2a). The extent of the Pr→lumi-R conversion, $\gamma_1$, characterizing the photochemical activity of phytochrome at cryogenic temperatures and indicative of the phyA' and phyA'' abundance[47,48] is also higher (0.33±0.03 vs. 0.24±0.05). Finally, evaluations of the phyA' and phyA'' content based on $P_{tot}$ and $\gamma_1$ give their proportion as 66/34 and concentration of 1.45 and 0.75 RU in the phyA-GFP overexpressor; while for WT *Arabidopsis* they were 49/51 and 0.40 and 0. 42 RU (Fig. 2b,c). The phyA' and phyA'' content is thus ≈ 3.5 – fold and ≈ 2 - fold higher, respectively, in the transgenic *Arabidopsis* than in the WT suggesting that the phyA'/phyA'' equilibrium is shifted towards phyA' in phyA-GFP. These parameters of phyA-GFP are comparable with those of phyA in *Arabidopsis* overexpressing oat phyA, (AsphyA-OX, lines 21k15 and 13k7[49], used in our work earlier[48]. For istance, in the case of AsphyA-OX 21k15 they are: $P_{tot}$=1.94±0.23 RU, $\gamma_1$=0.29±0.02; phyA' and phyA'' make up 59 and 41 % what amounts to 1.15 and 0.79 RU respectively (Fig. 2 b,c). Thus, we may conclude that the AtphyA-GFP transgenic has both phyA' and phyA'' in quantities more than 2-fold higher than in WT, with the equilibrium between the two species shifted towards the former.

In the case of tobacco overexpressing rice OsphyA-GFP the situation is more complex since the overexpressor contains both endogenous NtphyA and heterologous OsphyA-GFP. Also, the level of phyA overexpression turned out to be low. Total phytochrome content in etiolated WT tobacco seedlings is 0.96±0.08 RU and is only slightly higher in the

OsphyA-GFP overexpressor (1.17±0.14 RU, ≈ 20 % excess). A higher value for $\gamma_1$ is found in the overexpressor than in the WT, 0.37±0.04 and 0.34±0.02, respectively. Spectroscopical parameters of the native NtphyA in WT and endogenous NtphyA plus OsphyA-GFP in the overexpressor line are practically identical (Figs. 3a-c) suggesting that fluorescence parameters of OsphyA-GFP are highly similar to the WT NtphyA. However, evaluations of the phyA' and phyA'' content of NtphyA-GFP suggest, that NtphyA-GFP is primarily represented by phyA'. In the overexpressor, the content of phyA' and phyA'' is 74 and 26 % what amounts to 0.87 and 0.30 RU respectively, whereas in WT, the values are 68 and 32 % (or 0.66 and 0.30 RU). Consequently, the excess of the phyA content in the phyA-GFP overexpressor is achieved primarily through the input of the phyA' species (surplus of 0.21 RU). This finding suggests that the effect of the phyA-GFP translocation in transgenic tobacco via VLFR[11,15] is likely to be attributed to the phyA'-GFP species. On the other hand, there is a correlation between the types of phyA responses and the phyA types present in the cell: in the transgenic *Arabidopsis*, which contains phyA'-GFP and phyA''-GFP (see above), both LFR and HIR controlled nuclear accumulation were observed[15] . This agrees with our previous findings that phyA' mediates VLFR and HIR, whereas phyA'', LFR[42,43] .

In order to find out the nuclear/cytoplasmic partitioning features of phyA'-GFP and phyA''-GFP, we examined phyA-GFP molecules truncated at their N-terminal NTE region. It was already demonstrated that the extreme N-terminal region of phyA (NTE) is responsible for the phyA'/phyA'' differentiation[42,43]. Our fluorescence emission measurements of full-length (FL) and Δ6-12 truncated oat phyA-GFP (AsΔ6-12 phyA-GFP) expressed in phyA-deficient *Arabidopsis*[35] revealed that FL phyA-GFP contained both phyA species in comparable amounts, what is characteristic of WT *Arabidopsis*, whereas AsΔ6-12 phyA-GFP was represented primarily, if not exclusively, by phyA'. These evaluations were done based on the experimental fluorescence emission spectra of transgenic *Arabidopsis* overexpressing both AsphyA-GFP species (Fig. 4a) and AsphyA-GFP spectra obtained after correction for the background emission (Fig. 4b). As is seen from Fig. 4b the spectra of both phytochrome species in their initial Pr forms ($F_0$ state) have similar intensities and the same profile with their maximum at 683 nm, whereas there is a difference in the longer region beyond 690-700 nm where lumi-R emits in the $F_1$ state (for details see Ref. 42). This difference is explained by the fact that although the content of the

pigment is similar in both lines (Fig. 5a), the extent of the Pr→lumi-R conversion ($\gamma_1$) is 1.5-fold higher in the case of the truncated phyA species (0.40±0.01 and 0.26±0.02, respectively) (Fig. 5b). This gives the ratio between the phyA'-GFP and phyA''-GFP of 80/20 and 52/48 for AsΔ6-12 phyA-GFP and AsphyA-GFP, respectively (Fig. 5c). Given that phyB makes up to 10-20% in $P_{tot}$[48], this points to almost full domination of the truncated species in the etiolated tissues (Fig. 5c,d).

## DISCUSSION

The fusion phyA-GFP phytochrome is extensively and effectively used for investigation of localization of the pigment in the cell and its changes during the process of light signal transduction. The major concrete aim of this work was to find out whether it is spectroscopically and photochemically identical to the native pigment. More specifically, since phyA in the cells of monocots and dicots is represented by its two phenomenological types (phyA' and phyA'')[43,44], it was important to find out whether there are also two types of phyA within the phyA-GFP population with the known photochemical and spectroscopic parameters. With this in mind, we have investigated properties of the fusion phyA-GFP in transgenic *Arabidopsis* and tobacco plants with the use of low temperature fluorescence spectroscopy and photochemistry[42]. In our previous works, this approach proved to be efficient in differentiation of the phyA pool into its two native subpopulations. It was found that the phyA-GFP possesses the same spectroscopic and photochemical properties as the native phyA and that it is represented in the cell as the two phyAs – phyA'-GFP and phyA''-GFP. This fact indicates, in particular, that the GFP fused in the phyA molecule does not affect the chromophore in its protein pocket. This complements well the literature data on full functional competence of the fusion phyA-GFP[11,15,20,35].

Quantitative determinations of total phyA-GFP and of the phyA'-GFP/phyA''-GFP proportion have shown that they follow well the earlier observations of the general dependency of the phyA'/phyA'' ratio on $P_{tot}$[42-44]. For a number of plant species, it was found that [phyA'] rises almost linearly with $P_{tot}$ whereas [phyA''] suffers early saturation. In this work, the content of the overexpressed phyA-GFP in phyA-deficient *Arabidopsis* was higher than that of native phyA in the wild type and the ratio phyA'-GFP/phyA''-GFP was

shifted towards the former. Also, our earlier experiments with *Arabidopsis* overexpressors of oat phyA revealed similar increase of $P_{tot}$ and concomitant shift of the ratio phyA'/phyA'' towards the former[48]. From this, we may speculate that the changes in the phyA'-GFP/phyA''-GFP content and proportion are likely to be connected with the increase in $P_{tot}$ in the overexpressor rather than with the modification of the phyA molecule upon introduction of the GFP fragment into it. This assumption needs, however, further direct verification by comparing the content of *Arabidopsis* phyA-GFP and phyA and distribution of their subpopulations using *Arabidopsis* overexpressors with *phyA* background.

The fact that phyA-GFP is represented in the cell as phyA'-GFP and phyA''-GFP implies that both of them are potential participants of the light-induced nuclear-cytoplasmic partioning. More to that, this finding and the observation that there appear two types of light-induced phyA nuclear speckle formation prompt us to suggest that the latter may be connected with the existing of the two phyA species. Our experiments with the truncated AsΔ6-12 phyA-GFP, which forms primarily or exclusively the phyA'-GFP type, support this hypothesis. It was observed that AsΔ6-12 phyA-GFP forms only numerous tiny subnuclear speckles compared to the WT AsphyA-GFP which forms large ones as well[35]. This proves that phyA' participates in the nuclear translocation with this type of speckle formation. In the case of full-length AsphyA-GFP consisting of phyA' and phyA'', both nuclei with many tiny speckles and nuclei with few large speckles were found[35]. Thus, the large speckles may be associated with phyA''. Interestingly, the photoresponses in transgenic *Arabidopsis* induced by the full-length oat phyA comprising both the phyA species and by the truncated AsΔ6-12 phyA forming primarily phyA' are different[35]. AsΔ6-12 phyA was as active as full-length AsphyA for the VLFR of hypocotyl growth and cotyledon unfolding and for the VLFR blocking subsequent greening under white light. It showed also a dominant-negative suppression of HIR. This suggests that phyA' is responsible for the VLFR. These observations, on the other hand, imply alternative schemes for differences in the intracellular localization of phyA' and phyA'' species: (i) both of them are present in each cell in a ratio close to the experimental average ones and (ii) there are two different groups of cells containing primarily one or the other phyA type. The latter type may be connected with the stage of cell development or their specialization. The data obtained with the Δ6-12 truncated phyA seem to be in favor of the second possibility.

Finally, it is of interest to mention the fact that fern *Adiantum capillus veneris* phy1 comprises two isoforms (in transgenic *Arabidopsis*) similar to those of phyA[50]. Certain similarities between them were found also with regard to their light-induced localization in the nucleus and function in gene expression[51,52] . These data indicate that the observed complex phenomenon of participation of this type of phytochrome in the light-induced nuclear / cytoplasmic partitioning may be of a universal character which appears at the early stages of its molecular evolution.

Thus, the following major conclusions can be drawn from the obtained results: Firstly, the spectroscopic and photochemical properties of the phyA-GFP chimerical protein are very similar to the native phyA. This suggests that the GFP tag does not affect the chromophore and its immediate protein surrounding and is in good agreement with the previous studies showing that phyA-GFP is a functional photoreceptor[11,15,20,35]. Secondly, similarly to the endogenous phyA, phyA-GFP is also represented by the two native phyA subpopulations, phyA’ and phyA’’ *in planta*. Thirdly, the phyA’-GFP/phyA’’-GFP ratio is higher than that of phyA’/phyA’’. This shift is characteristic for the other investigated *Arabidopsis* phyA overexpressors[48]. The fact that phyA-GFP has two phyA forms similar to those of the native phyA suggests that the phyA-GFP fusion protein also undergoes the post-translational modification proposed for the native phyA[53]. This observation shows that either of the two phyA pools can participate in the nuclear-cytoplasmic partitioning of the receptor and thus of the phyA signal transduction. As a working hypothesis, we suggest that the phyA’-GFP translocation is likely to be mediated by both VLFR and HIR, whereas that of phyA’’-GFP, by VLR. And finally, we may speculate that the fraction of phyA retaining in the cytoplasm after light-induced nuclear translocation and relatively light stable could be possibly associated with the less light-labile and membrane (protein)-associated phyA’’.

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## FIGURE LEGENDS

Figure 1. Fluorescence emission spectra of *Arabidopsis* phyA fused to GFP (*35S:PHYA-GFP*) (AtphyA-GFP). (a) Raw fluorescence spectra ($\lambda_e$=633 nm, 85 K) of etiolated seedlings without cotyledons of *phyA-201* mutant *Arabidopsis* expressing AtphyA-GFP (curves 1 and 2) and of the *phyA-201* mutant (curve 3). Curves 1 and 3 show data obtained using dark-grown seedlings (state $F_0$); curve 2 -- the same samples as in the case of curve 1 but after saturating illumination with $\lambda_a$=633 nm at 85 K to convert Pr into lumi-R (state $F_1$). (b) Fluorescence spectra of AtphyA-GFP obtained by subtraction of the spectrum 3 from the spectra 1 and 2 in (a). (c) Comparisson of the spectra of AtphyA-GFP (from (b)) and of native *Arabidopsis* phyA (AtphyA) (initial raw spectra not shown). The spectra in the state $F_0$ were normalized to the maximum value of each curve. Here and below, the spectra were not corrected for the spectral sensitivity of the instrument. Error bars -- standard error of the mean of 5-10 measurements.

Figure 2. Total phytochrome content (a), percentage (b) and abundance of phyA' (black bars) and phyA'' (grey bars) (c) in etiolated seedlings (without cotyledons) of transgenic *Arabidopsis* line expressing fusion AtphyA-GFP in the *phyA-201* background (AtphyA-GFP) as compared with the wild type (WT) and the transgenic line overexpressing oat phyA (AsphyA-OX, line *21k15*[48,49]).

Figure 3. Fluorescence spectra of native tobacco phyA + rice phyA-GFP in the tobacco overexpressor and of phyA in wild type tobacco. (a) Raw fluorescence emission spectra ($\lambda_e$=633 nm, 85 K) of etiolated hypocotyls of tobacco seedlings overexpressing OsphyA-GFP (curves 1 and 3) and of the wild-type control (curves 2 and 4) and also of the wild-type tobacco roots at their base after saturating red ($\lambda_a$=633 nm) illuminatiation at room temperature to convert traces of phytochrome into its non-fluorescencent Pfr form (curve 5). Curves 1 and 2 show spectra of dark-grown seedlings; curves 3 and 4 indicate the same as curves 1 and 2 (state $F_0$) after saturating illumination with $\lambda_a$=633 nm at 85 K to convert Pr into lumi-R (state $F_1$). (b) Fluorescence spectra of tobacco phyA + OsphyA-GFP (1, 3) and of wild type tobacco phyA (2, 4) obtained from the spectra 1, 3 and 2, 4 in (a) by subtraction of the spectrum of background emission (curve 5). (c) Fluorescence spectra of

phyA+phyA-GFP in the initial $F_0$ state in the tobacco overexpressor (1) and of phyA in the wild type (2) normalized for comparisson in the maximum.

Figure 4. Fluorescence spectra of fusion oat phyA truncated at a.a. 6-12 (AsΔ6-12 phyA-GFP) and of full-length AsphyA-GFP. (a) Raw fluorescence emission spectra ($\lambda_e$=633 nm, 85 K) of etiolated seedlings (without cotyledons) of the phyA-deficient *Arabidopsis* expressing AsΔ6-12 phyA-GFP (curves 1, 3) and of the full-length AsphyA-GFP (curves 2, 4). phyAphyB-deficient *Arabidopsis* (*phyAphyB*) etiolated seedlings are taken as a background control (5).1, 2 – dark-grown seedlings (state $F_0$); 3, 4 – same samples as in the case of curves 1, 2 after saturating illumination with $\lambda_a$=633 nm at 85 K to convert Pr into lumi-R (state $F_1$). (b) Fluorescence spectra of AsΔ6-12 phyA-GFP (curves 1, 3) and of AsphyA (2, 4) obtained from (b) by subtraction of spectrum 5 from spectra 1-4.

Figure 5. Total phytochrome content (a), extent of the Pr→lumi-R photoconversion upon saturating red illumination ($\lambda_a$=633 nm, 85 K), $\gamma_1$, (b), and proportion (c) and concentration in relative units (d) of the two distinct photochemical species of truncated oat phyA fused to GFP (AsΔ6-12 phyA-GFP) and of the full-length AsphyA-GFP both expressed in the phyA-deficient *Arabidopsis*.

**FIGURES**:

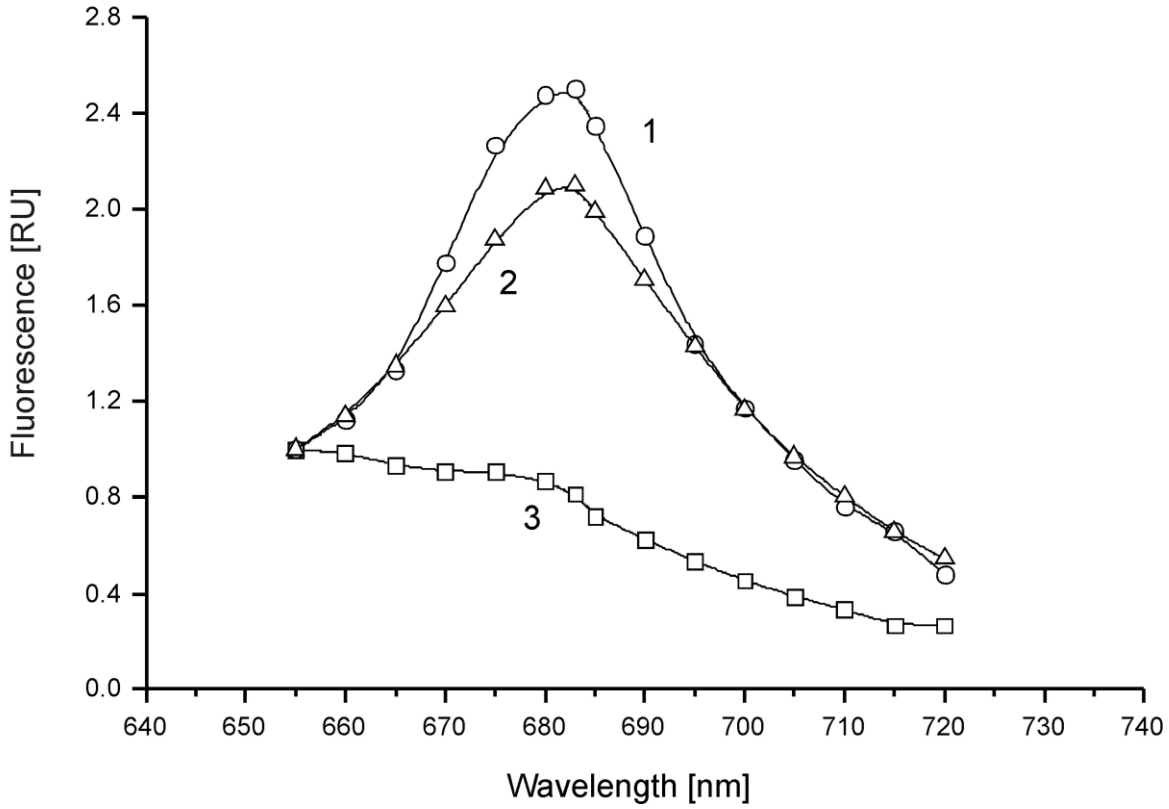


Fig.1a

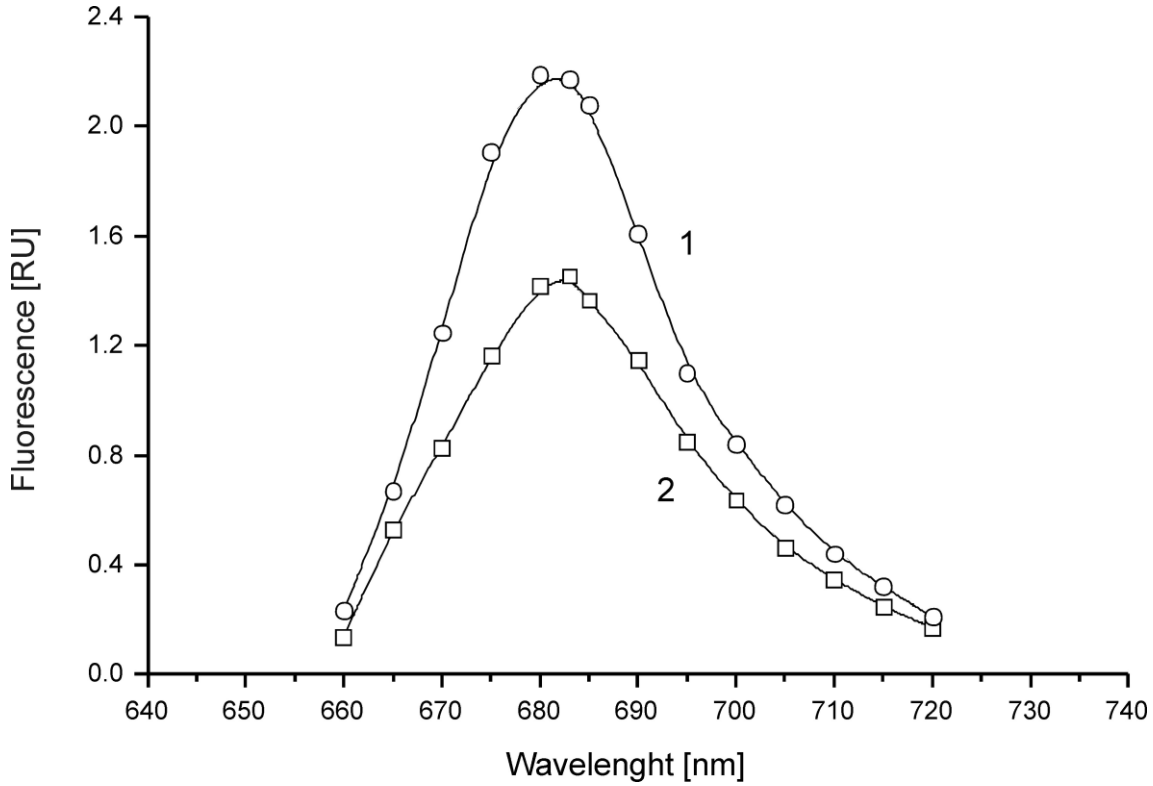


Fig. 1b

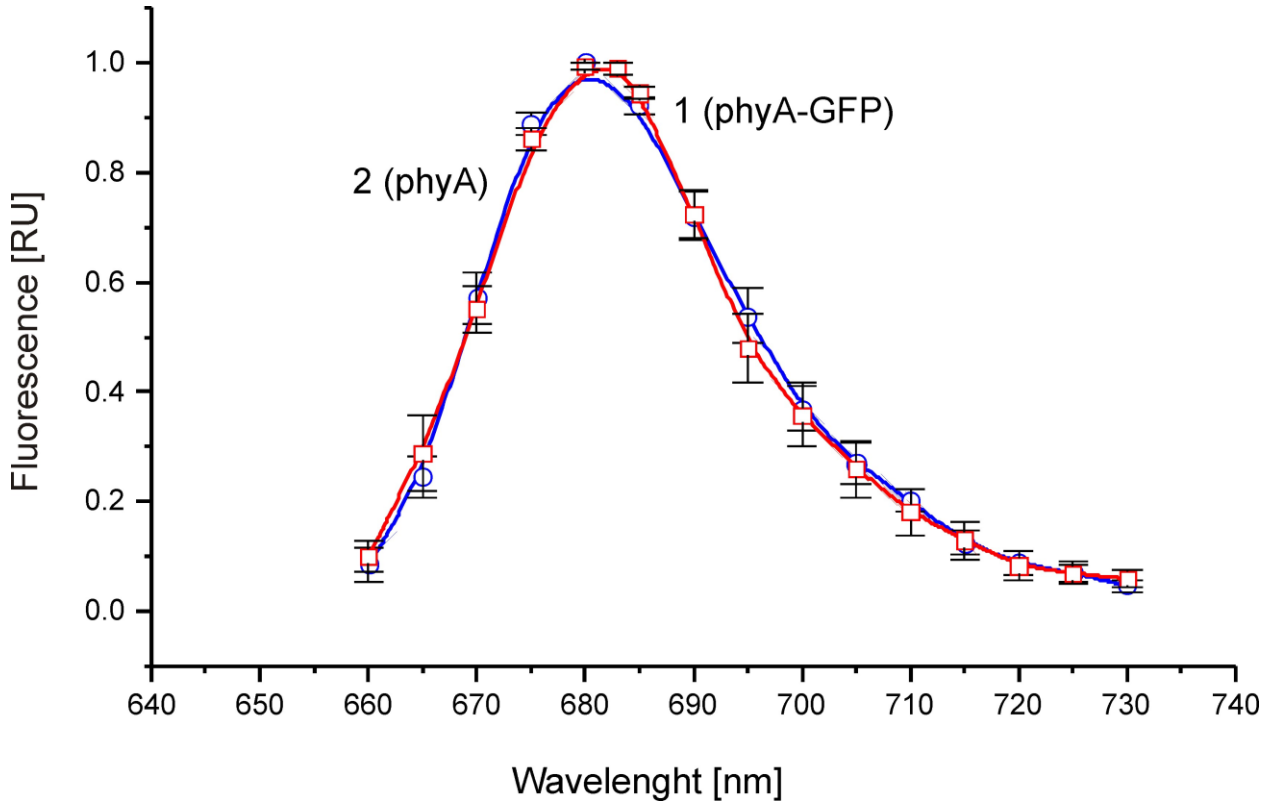


Fig. 1c

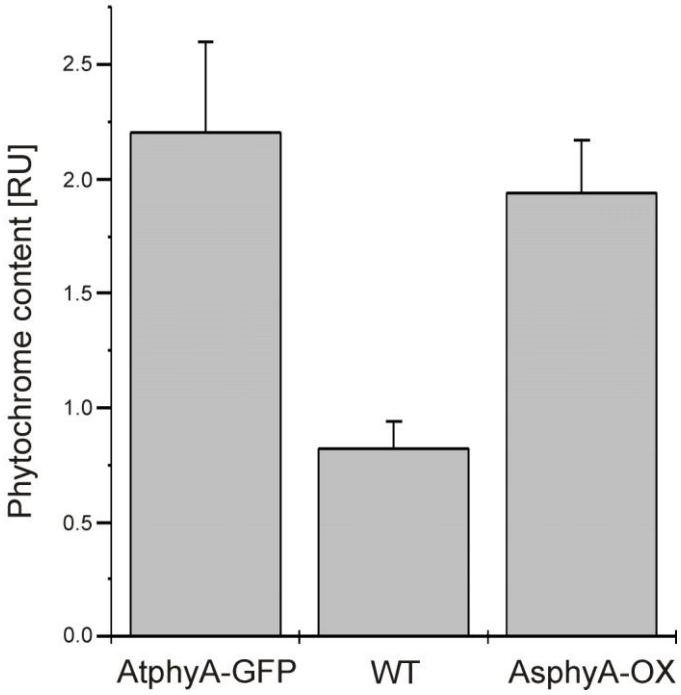


Fig. 2a

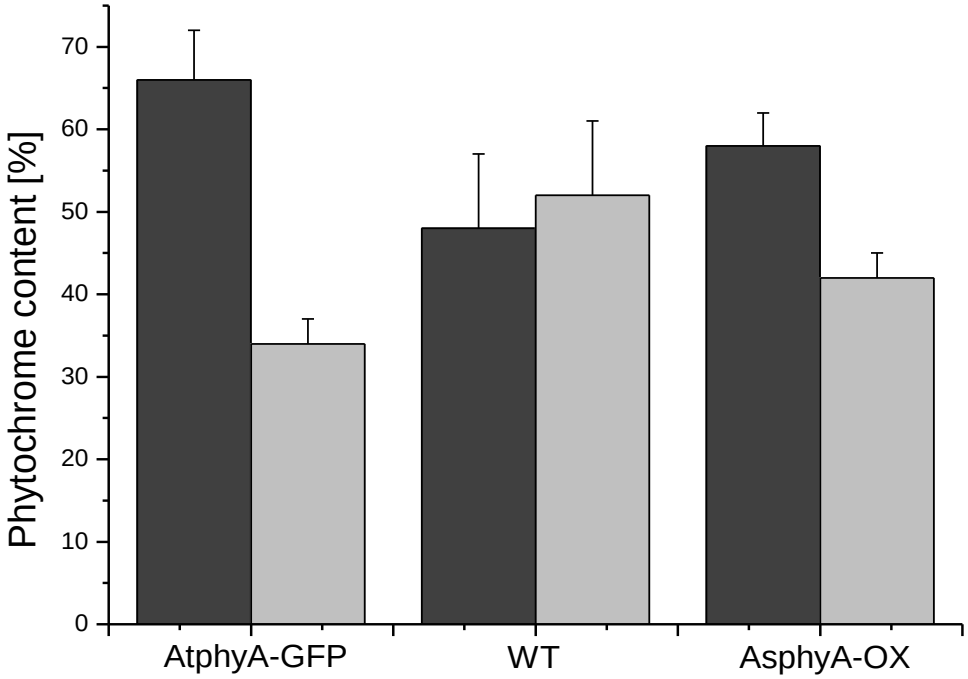


Fig. 2b

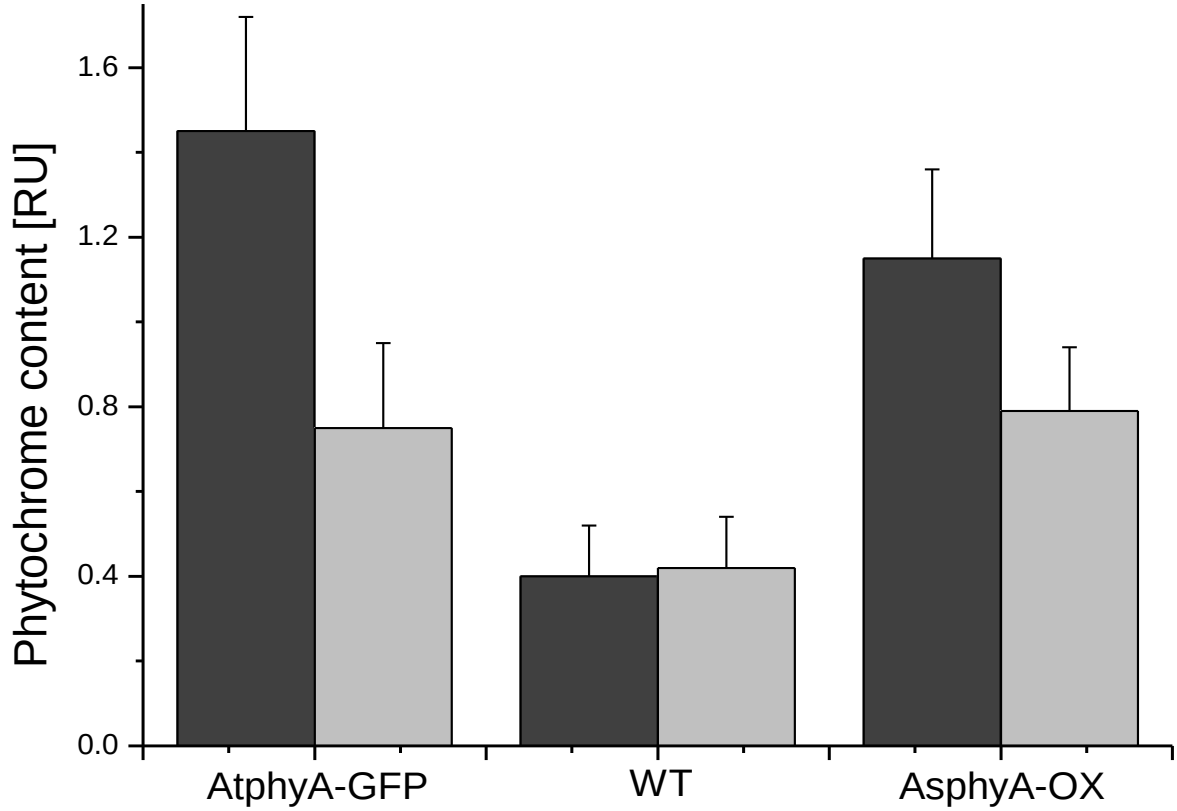


Fig. 2c

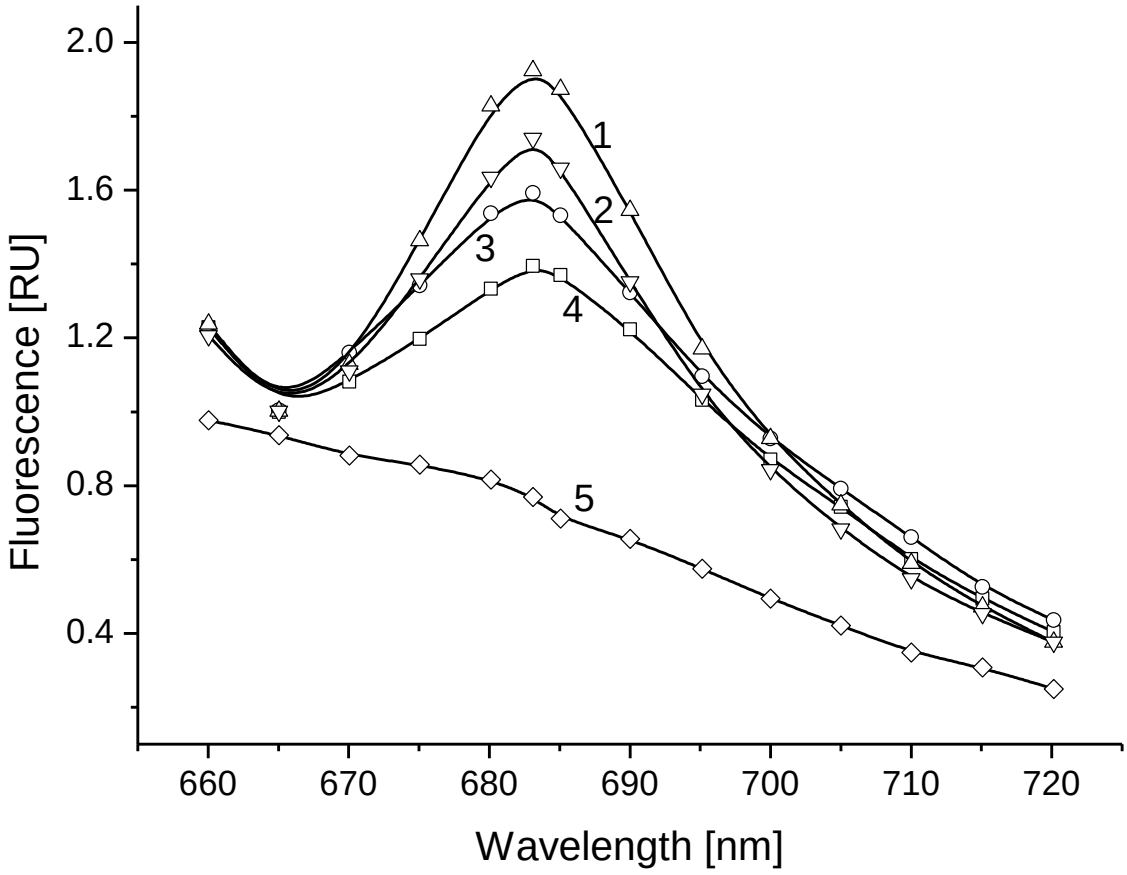


Fig. 3a

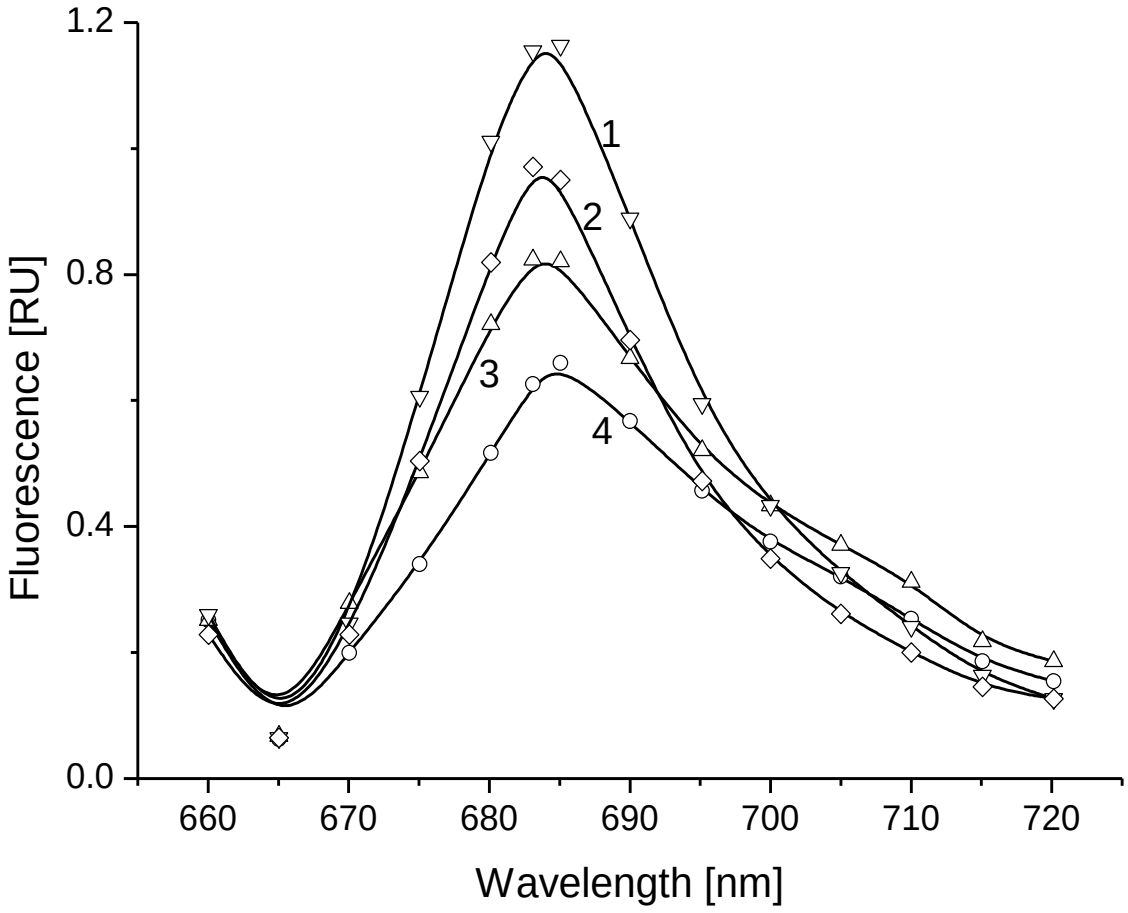


Fig. 3b

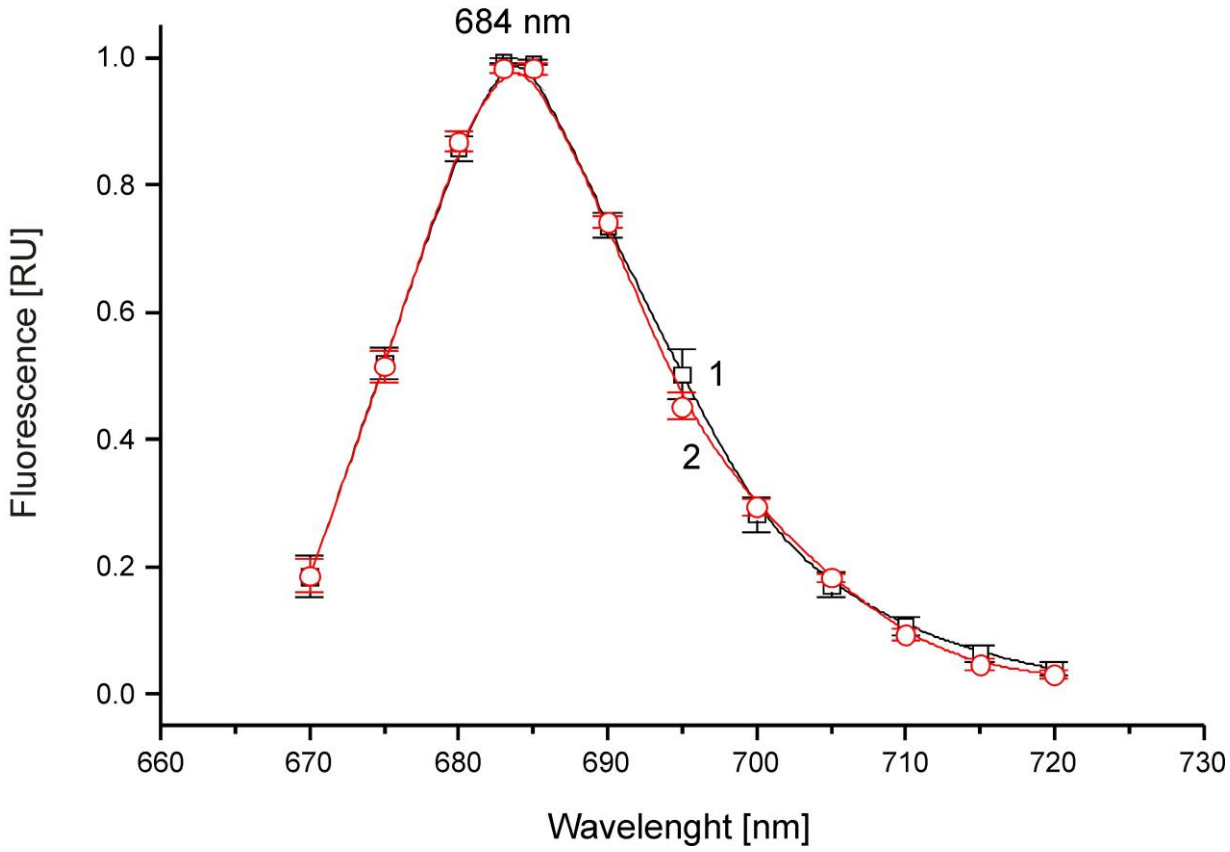


Fig. 3c

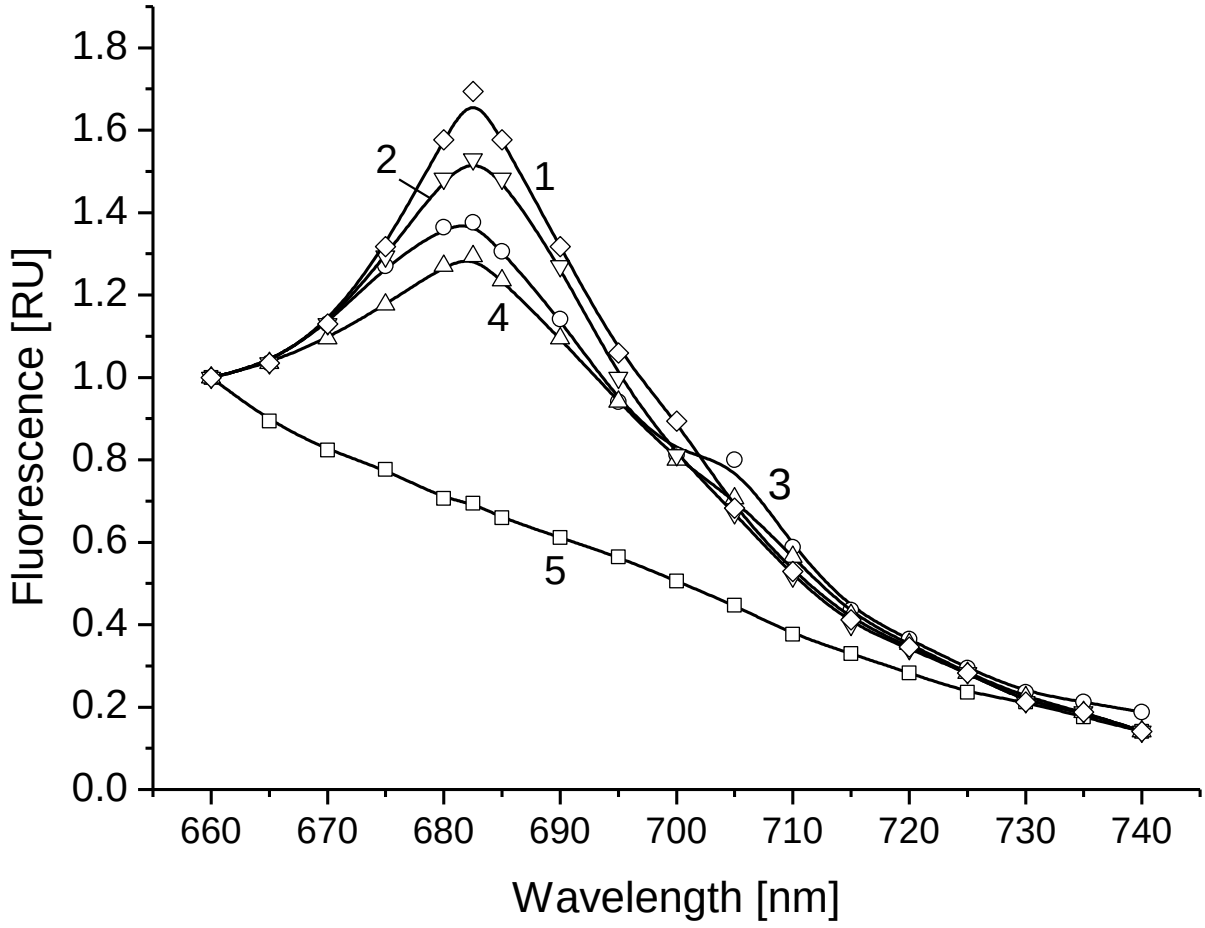


Fig. 4a

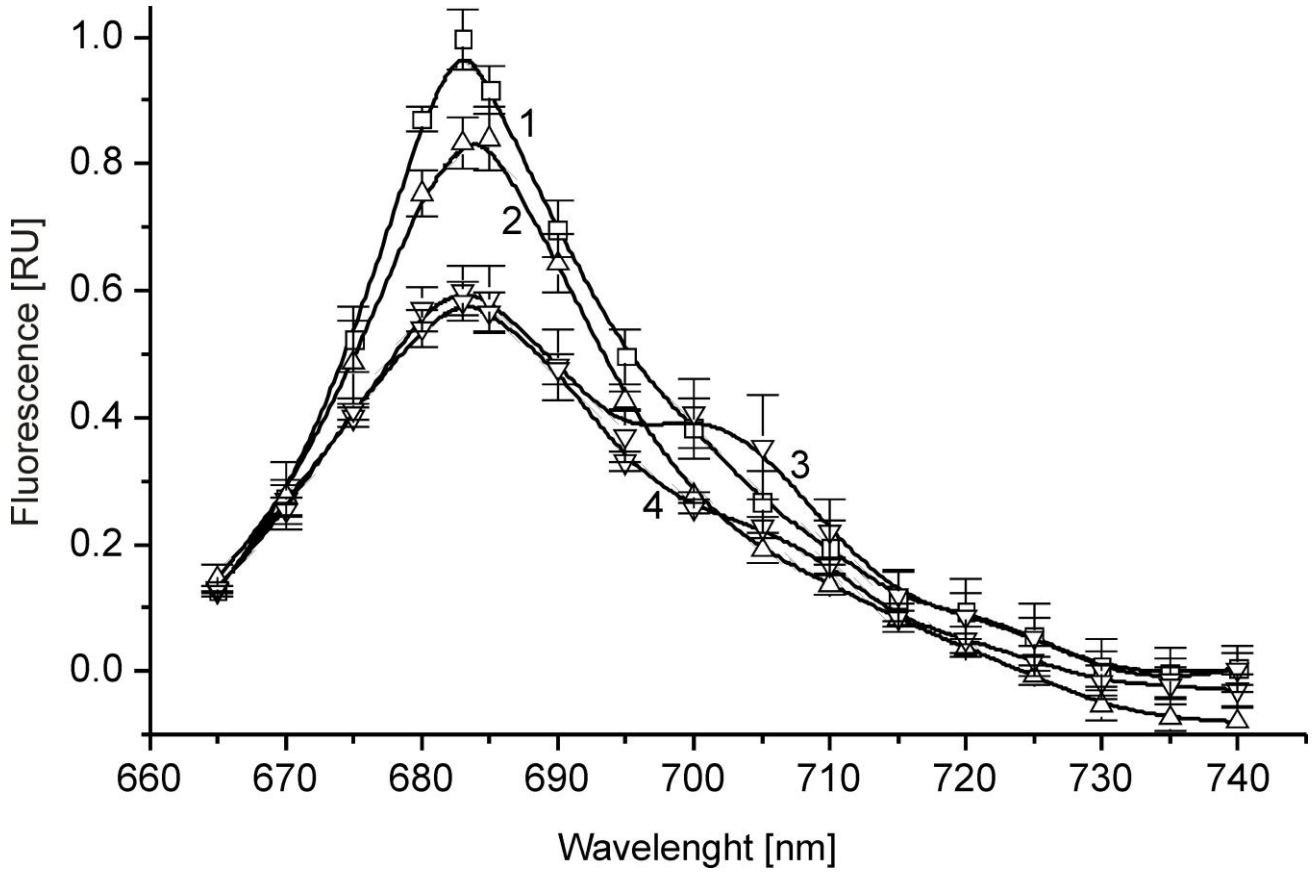


Fig. 4b

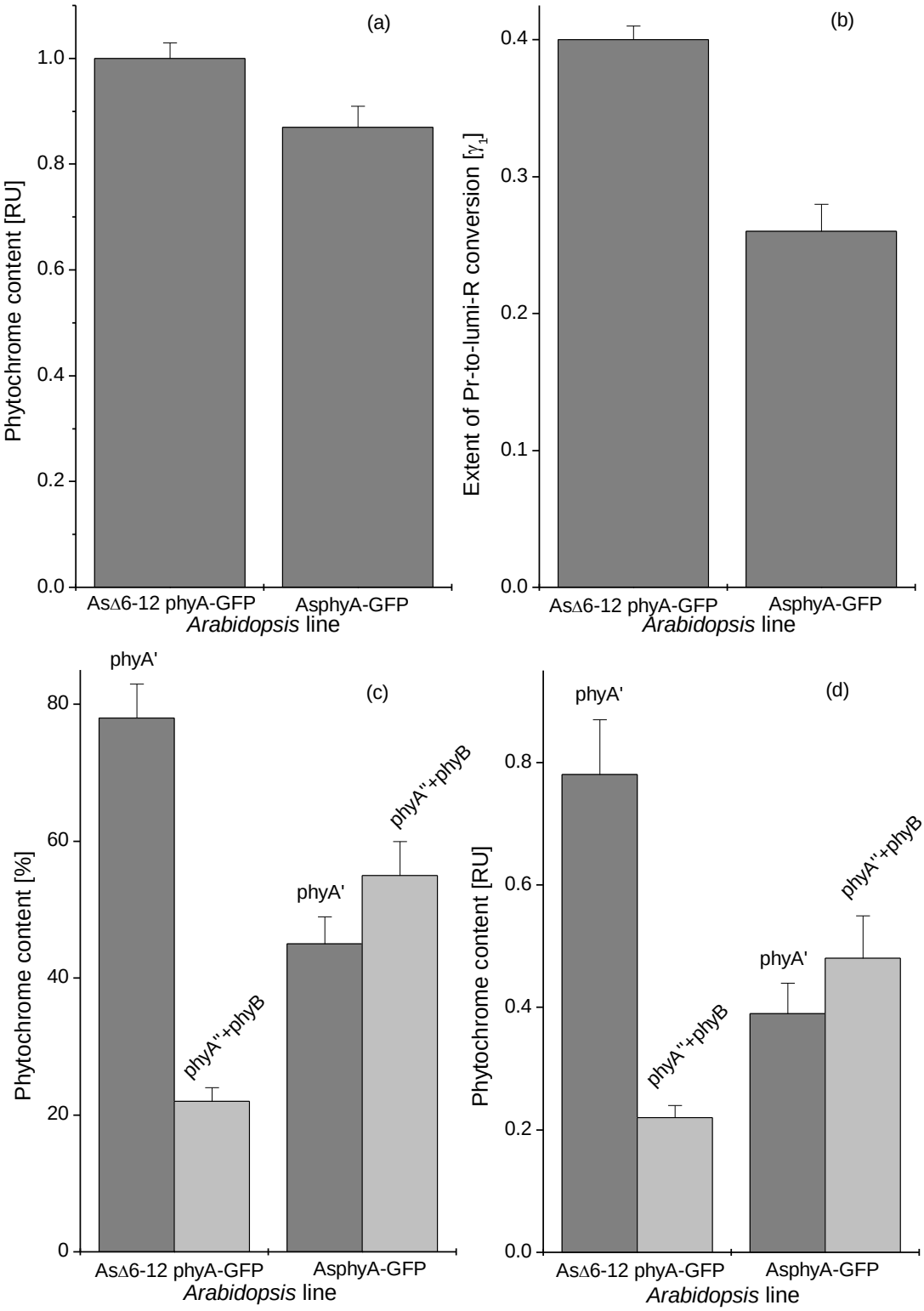


Fig. 5