NOTES

Phytochrome controlled, long-day photoperiod-inducible protein in rice leaves

KANG Bin, TONG Zhe & KUANG Tingyun

Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
Correspondence should be addressed to Tong Zhe

Abstract A new protein in the leaves of NK58S and NK58 (*Oryza sativa* L. subsp. *japonica*), which can be induced by 10 d-long-day photoperiod (14 h light/d) and cannot be induced by 10 d-short-day photoperiod (10 h light/d), has been found by two-dimensional gel electrophoresis. The protein,whose molecular weight and isoelectric point are 36 ku and pH 5.2 respectively, is found to be controlled by phytochrome as shown by the experiment of red light induction-far red light reversion.The existence of this protein in both NK58S and NK58 reflects that some of the responses of NK58S and NK58 might be similar in response to long-day photoperiod, a mild stress.

Keywords: **rice, protein, photoperiod, phytochrome.**

Photoperiod-sensitive genic male-sterile rice Nongken58S (NK58S) is a spontaneous mutant originally discovered in a field of the cultivar Nongken58 (NK58). Under the proper temperature, this mutant shows male sterility under a long-day photoperiod and normal fertility under a short-day photoperiod[1]. This trait of NK58S laid the basis of two-line hybrid rice research[2]. Some problems such as the recovery of sterility under a long-day photoperiod encountered in the two-line hybrid rice production call for more research on the elucidation of the mechanisms underlying the fertility alteration. In our previous work, we demonstrated that the phytochrome in leaves is the photoreceptor which receives the signal of photoperiod and regulates the fertility alteration[3]. We also showed that the expression of phytochrome A in NK58S was more active than that in NK58, which maybe resulted in more sensitivity to photoperiod in NK58S[4]. We also found that the long-day photoperiod could decrease the GAs and IAA contents of leaves much more in NK58S than in NK58 during the stage of

fertility alteration[15]. A further question was raised naturally: are there any photoperiod-regulated proteins in the leaves of NK58S and NK58 which might have close relationship with fertility alteration? So, in this note the effect of photoperiod on the leave protein patterns of NK58S and NK58 was investigated.

1 Materials and methods

(i) The culture of rice and the treatment of diverse photoperiods. Seeds of NK58S and NK58 (*Oryza sativa* L. subsp. *japonica*) were planted in soil saturated with water at 28℃. When the sixth leaves of seedlings began to expand, they were treated by diverse photoperiods: part of the seedlings were subjected to long-day photoperiod (LD, 10 h natural irradiation + 4 h white fluorescent light illumination (2.0 W/m^2)) per day for 10 d; another part of seedlings were subjected to the short-day photoperiod (SD, 10 h natural irradiation) or to SD-nightbreaks for 10 d: illumination in the middle of the long dark phase using red light (R) alone or far red light (FR) alone for 20 min or illumination using FR for another 20 min after 20 min illumination of R. The light intensities of R and FR used were both 2.5 W/m^2. The λ_{max} and $\lambda_{1/2}$ of R are 660 and 25 nm respectively. The λ_{max} and $\lambda_{1/2}$ of FR are 730 and 120 nm respectively. At the end of treatment, the sixth leaves were harvested, weighed and stored at –70℃.

(ii) Analysis of leaf proteins by two-dimensional gel electrophoresis. Extraction of leaf proteins and separation by two-dimensional gel electrophoresis were carried out according to the method described in ref. [6] and the same amount of proteins of each sample measured according to the method in ref. [7] was applied to the analysis by two-dimensional gel electrophoresis.

2 Results

(i) Protein patterns of rice leaves subjected to different photoperiods. It can be seen from fig. 1 that a protein existed in the sixth leaves of NK58S subjected to 10 d-long-day photoperiod (14 h light/d)

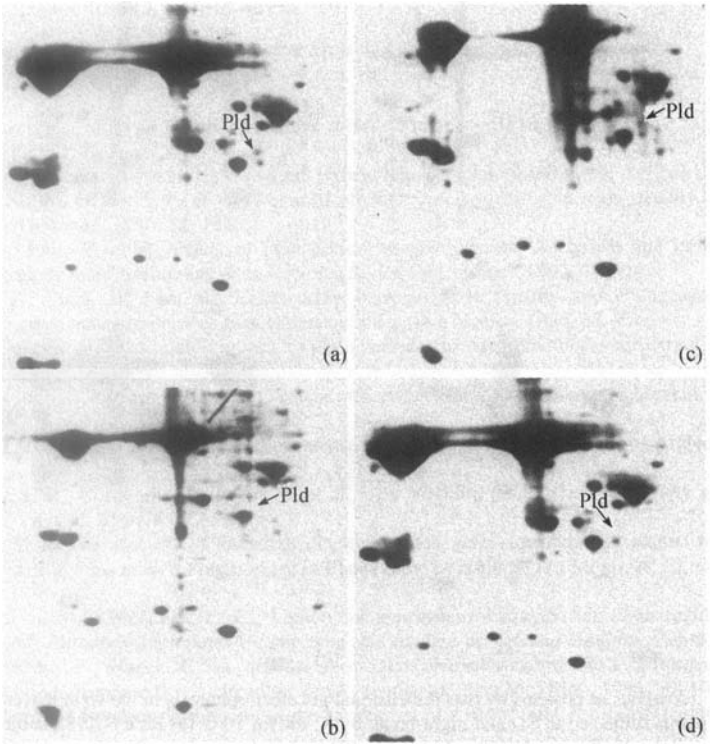

Fig. 1. Analysis of proteins by two-dimensional gel electrophoresis in the sixth leaves of NK58S and NK58 plants subjected to diverse photoperiods for 10 d. (a) Rice NK58S subjected to LD; (b) rice NK58S subjected to SD; (c) rice NK58 subjected to LD; (d) rice NK58 subjected to SD. Arrows show the presence of Pld in (a), (c) and the absence of Pld in (b), (d).

(fig. 1(a)), but it did not exist in the sixth leaves of NK58S subjected to the short-day photoperiod (10 h light/d) (fig. 1(b)). The molecular weight and isoelectric point of this protein (called Pld) are 36 ku and pH 5.2 respectively. It should be noted that Pld existed not only in the leaves of NK58S subjected to long-day treatment (14 h light/d) but also in the leaves of NK58 subjected to 10 d-long-day photoperiod (14 h light/d) (fig. 1(c)). It did not exist in the leaves of NK58 subjected to 10 d-short-day photoperiod (10 h light/d), either (fig. 1(d)). So, Pld can be induced by the long-day photoperiod (14 h light/d) in the sixth leaves of both NK58S and NK58 plants, and no such protein which existed in the leaves subjected to the short-day photoperiod (10 h light/d) and disappeared when the leaves were subjected to the long-day photoperiod (14 h light/d) was found in our experimental system.

(ii) Effect of short-day photoperiod-night breaks on the existence of Pld. It was found that Pld could be induced when R pulse was used to night-break short-day photoperiod (fig. 2(a), (b)) and

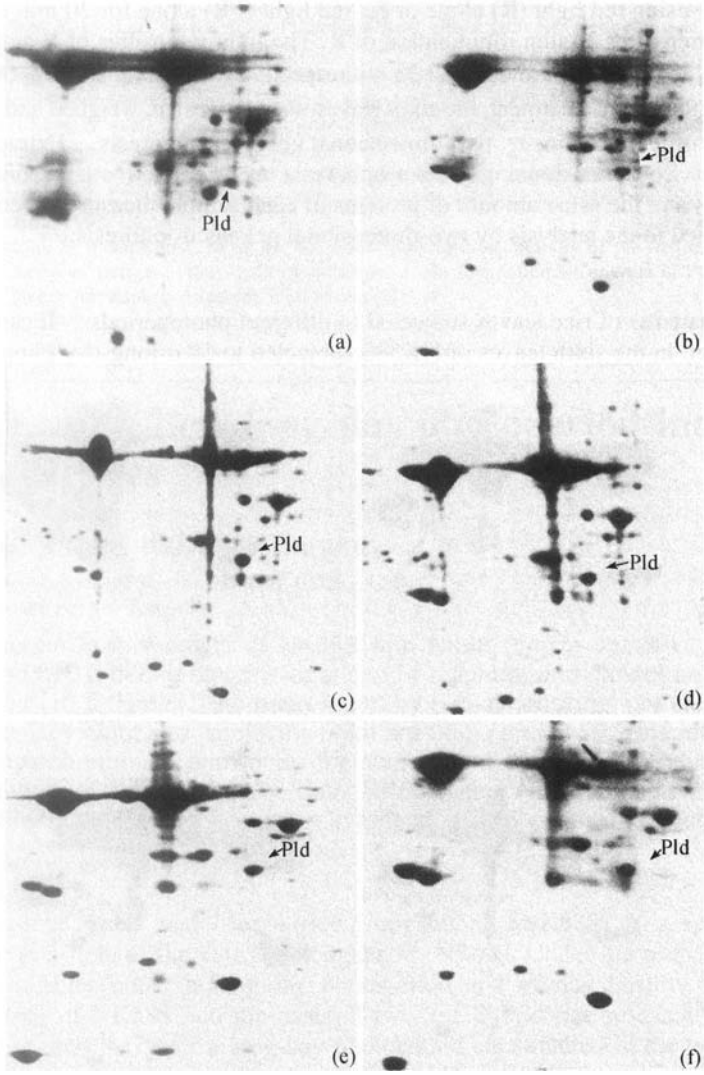

Fig. 2. Analysis of proteins by two-dimensional gel electrophoresis in the sixth leaves of NK58S and NK58 plants subjected to SD and night-break by R, FR for 10 d. (a) Rice NK58S subjected to SD and night-break by R; (b) rice NK58 subjected to SD and night-break by R; (c) rice NK58S subjected to SD and night-break by R + FR; (d) rice NK58 subjected to SD and night-break by R+FR; (e) rice NK58S subjected to SD and night-break by FR; F, rice NK58 subjected to SD and night-break by FR. Arrows show the presence of Pld in (a), (b) and the absence of Pld in (c), (d), (e) and (f).

disappeared when FR pulse was used just after R pulse (fig. 2(c), (d)).When FR pulse alone was used to night-break short-day photoperiod, Pld did not exist (fig. 2(e), (f)).

3 Discussion

It was demonstrated by recent research that the floral induction by proper photoperiod involved the signal receiving of nightlength and the regulation of inner circadian rhythm (biological clock). Some evidence indicated that both of phytochrome A and phytochrome B take part in the signal receiving of photoperiod[8,9] and many genes are controlled by circadian rhythm, most of which are related to photosynthesis, for instance, *Cab*, *RbcS*, *Rca* and the gene encoding Rubisco kinase, etc.[10]. In addition, genes encoding 1-aminocyclopropane-1-carboxylic acid oxidase involved in ethylene synthesis[11] and S-adenosylmethionine decarboxylase (a rate-limiting enzyme involved in polyamine synthesis)[12] are both circadian-clock-regulated. Some genes encoding proteins of unknown functions are also shown to be circadian-clock-regulated, for example, *Lir1*[13] in rice. So, a lot of evidence indicates that many genes in plants are circadian-clock-regulated. Here, we report another rice protein Pld in the sixth leaves of NK58S and NK58 (which began to be able to respond to photoperiod)[14]. It can be concluded from the results that Pld is a long-day photoperiod-inducible protein. Is it a phytochrome controlled protein? The criterion for the phytochrome as the photoreceptor in a light regulated response is that if the response can be induced by R pulse and reverted by immediate FR pulse after R pulse to the extent of FR action alone, the photoreceptor in this light regulated response is indeed phytochrome[3]. In order to verify whether Pld is controlled by phytochrome, we broke the long dark phase using R pulse or FR pulse according to the method of Tong et al.[3] and found that Pld could be induced by R pulse, but not by FR pulse alone and by FR pulse after R pulse. All the results indicate that Pld is indeed controlled by phytochrome.

Long-day photoperiod can be considered as a mild stress to rice as a short-day plant[15]. The existence of this protein in both NK58S and NK58 reflects that some of the responses of NK58S and NK58 are similar in response to LD, a mild stress.

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