Phosphate deficiency suppresses expression of light-regulated psbO and psbP genes encoding extrinsic proteins of oxygen-evolving complex of PSI

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Phosphate (Pi) is one of the important mineral nutrients influencing growth and development of plants. Prolonged Pi starvation is known to influence the light-saturated rate of photosynthetic evolution of O₂. The genes psbO and psbP encode 33 and 23 kDa extrinsic proteins respectively, that play a critical role in the structural and functional integrity of oxygen-evolving complex (OEC) of the photosystem II (PSII). Although the role of Pi in photosynthesis is well documented, the effect of its deficiency on the expression of these genes has not been elucidated. In this study we analysed the expression of psbO and psbP genes in Arabidopsis thaliana supplemented with different concentrations of Pi. A concurrent increase in the transcript levels of these genes was observed with an increase in the concentration of Pi in the medium, suggesting a role for Pi in the regulation of these genes. These results were further substantiated by time-course studies where a complete suppression of psbO and psbP genes was observed in plants starved of Pi for 7 d. The suppressive effect of Pi deficiency on these genes could be alleviated by replenishment with Pi. Fe deficiency had only a moderate effect on the expression of these genes. The effects of Pi stress on the expression of these genes could have potential implications on the structural integrity of OEC and consequently its O₂ evolving efficacy.

Keywords: Arabidopsis thaliana, oxygen-evolving complex of PSI, phosphate deficiency, psbO, psbP.

INORGANIC phosphate (Pi) is one of the important mineral nutrients influencing metabolism, growth, development and consequently yield of plants¹. However, in terrestrial and freshwater ecosystems orthophosphate ions (H₂PO₄⁻), a preferentially assimilated form of P, are either limited or present in inorganic and organic complexes, which are not readily available to plants². To circumvent phosphate deficiency, plants have evolved several morphological, biochemical, physiological and molecular adaptations³ that result in increased availability, uptake and efficient utilization of Pi. Despite many of these adaptations, during prolonged Pi-deprivation cytoplasmic Pi concentration may reach sub-optimal levels to affect different metabolic processes in plants. ³³P-NMR studies indicated that cytoplasmic concentration


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of Pi in Pi-deficient leaves of soybean was in the range of 0.01 to 0.23 mM, which was significantly lower than the concentration (5–8 mM) in Pi-sufficient leaves. Such a drastic reduction of cytoplasmic Pi concentration during Pi stress affects various aspects of photosynthetic processes and consequently growth and vigour of plants. Studies on maize and sunflower had linked phosphate deficiency to a decline in the in vivo light-saturated rate of photosynthesis, which was largely attributed to a lower capacity for regeneration of RuBP and ATP and also due to the reduced enzymatic activities involved in photosynthetic CO$_2$ assimilation. Alterations in these photosynthetic processes during Pi deficiency had also been correlated with limited sucrose synthesis and subsequent carbon export from source leaves in favour of starch accumulation. The effects of Pi deprivation on the lower efficacy of light harvesting and electron transport components of photosystem II (PSII) in Chlamydomonas reinhardtii and Pinus pinaster have also been well documented. However, at the molecular level, relatively little is known about Pi-starvation response of PSII and even lesser about its regulation. Since evolution of molecular oxygen is one of the important determinants of PSII functional integrity, it is important to know if phosphate stress has any effect on the regulation of nuclear psbO and psbP genes encoding 33 and 23 kDa extrinsic proteins respectively. These extrinsic proteins apparently interact with intrinsic membrane proteins of PSII and possibly with each other to form physiologically functional PSII. In this communication we report the role of phosphate in the regulation of the expression of psbO and psbP genes in Arabidopsis thaliana. Since Fe deficiency has been correlated with decreased abundance of the photosynthetic machinery, its effect on the expression of psbO and psbP genes was also evaluated.

Seeds of A. thaliana (L.) ecotype Columbia were used in the present study to raise the plants in liquid culture following the protocol described earlier. To study the effects of different concentrations of Pi on the expression of genes, 7-day-old seedlings grown initially in half-strength MS medium were transferred to 20 ml of full-strength MS medium supplemented with different concentrations of Pi (0, 0.01, 0.1, 0.5 and 1.25 mM) and grown for another 7 d. For 0 mM Pi (P-), KH$_2$PO$_4$ in MS medium was replaced with K$_2$SO$_4$. For time course study, 7-day-old seedlings grown in half-strength MS medium were transferred to 20 ml full-strength MS medium with Pi (1.25 mM) or without Pi (0 mM). Plants grown for 3, 5 and 7 d were then harvested sequentially. In another study, plants were starved of Pi for 7 d and then subsequently replenished with 1.25 mM Pi for 0 h (control), 12 h, 1 and 3 d. Effect of Fe deficiency on the expression of genes was evaluated by transferring 7-day-old seedlings grown in half-strength MS medium to Fe deficient (Fe–) medium for 7 d. All the experiments in liquid culture were repeated at least twice with a minimum of 8–10 replicates for each set of experiment. Replicates were pooled, rinsed with distilled water, blotted-dried to remove excess water, frozen immediately in liquid nitrogen and stored at –80°C till further use for RNA extraction. Total RNA was extracted using Trizol reagent according to manufacturer’s instructions (Life Technologies/Gibco-BRL, Cleveland, OH, USA). Ten micrograms of total RNA was used for Northern analysis following standard protocol. DNA sequences of psbO2 (At3g50820) and psbP (At1g06680) genes were obtained from the GenBank database of NCBI (Bethesda, MD, USA) and their ESTs (psbO2, 180F24T7 and psbP, 78E1T7) were acquired from ABRC (OH, USA). ESTs were amplified using SP6 and T7 primers and amplification products were used as probes. The EST 180F24T7 showed 90 and 73% homology with psbO2 (At3g50820) and psbO1 (At5g66570) respectively. Furthermore, psbO1 and psbO2 share about 80% homology at the nucleotide level. Therefore, in the present study there is a distinct possibility that the probe used for psbO2 could also cross-hybridize with the transcripts of psbO1. For the determination of dry weights and quantifications of Pi the contents, Arabidopsis plants were raised in liquid culture for 7 d in half-strength MS medium and then transferred to full MS medium containing (+) or deficient (−) of Pi for 7 d. Arabidopsis plants were raised in liquid culture supplemented with (P+) or without (P−) Pi (Figure 1). After 7 d of Pi deprivation, plants exhibited a significant reduction in their dry weight (Figure 1a), lower Pi content (Figure 1b) and a concomitant accumulation of anthocyanin in the shoots (Figure 1c). An inadequate supply of Pi limits the availability of adenylate energy and various phosphorylated intermediates, which are critical for photosynthetic carbon reduction cycle. Furthermore, accumulation of anthocyanins in photosynthetic tissues, which is one of the most characteristic visible symptoms of phosphate deficiency, appears to serve a photoprotective role by optically masking chlorophyll and facilitating the conversion of excess absorbed light energy to heat. These modulations in photosynthetic tissues during Pi stress apparently result in channelling of lesser amount of light energy into PSII reaction centres, which is reflected in a decline in light-saturated rate of in vivo O$_2$ evolution. This view was substantiated by a study on C. reinhardtii, where nearly 75% reduction in functional PSII reaction centres was observed when the cells were starved of phosphate for 4 d. Earlier studies had shown that, in addition to light, various other components such as plant growth regulators, endogenous developmental processes, sugars and circadian rhythm also regulate the genes encoding different components of PSII. Since light-regulated psbO and psbP genes encode 33 and 23 kDa extrinsic proteins of OEC, which are important constituents of PSII, we were interested to know whether Pi deficiency has any affect on the regulation of these genes. To decipher this, 7-d-old seedlings of Arabidopsis, grown in half-strength MS liquid medium, were transferred to full-strength MS medium supplemented with different concentrations of phosphate ranging from 0 to 200 mM. The expression of psbO and psbP genes was evaluated by transferring 7-day-old seedlings grown in half-strength MS medium to Fe deficient (Fe–) medium for 7 d. All the experiments in liquid culture were repeated at least twice with a minimum of 8–10 replicates for each set of experiment. 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Furthermore, accumulation of anthocyanins in photosynthetic tissues, which is one of the most characteristic visible symptoms of phosphate deficiency, appears to serve a photoprotective role by optically masking chlorophyll and facilitating the conversion of excess absorbed light energy to heat. These modulations in photosynthetic tissues during Pi stress apparently result in channelling of lesser amount of light energy into PSII reaction centres, which is reflected in a decline in light-saturated rate of in vivo O$_2$ evolution. This view was substantiated by a study on C. reinhardtii, where nearly 75% reduction in functional PSII reaction centres was observed when the cells were starved of phosphate for 4 d. Earlier studies had shown that, in addition to light, various other components such as plant growth regulators, endogenous developmental processes, sugars and circadian rhythm also regulate the genes encoding different components of PSII. 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Figure 1. *Arabidopsis* seeds were grown in liquid culture in half-strength MS medium for 7 d and seedlings were transferred to full-strength MS supplemented with 1.25 mM Pi (P+) or without Pi (P–). Pi deprivation resulted in a significant reduction in the dry weight of seedling (a), Pi content (b) and accumulation of anthocyanin in the shoots (c). Values represent means ± SE for five replicates.

Figure 2. a, Low Pi concentrations suppress expression of *psbO* and *psbP* genes. Ten micrograms of total RNA isolated from the whole plant, was separated on agarose gel and transferred to nylon membrane. The membrane was hybridized with 32P-labelled cDNA fragments of *psbO* and *psbP* genes. As control, the membrane was hybridized with 32P-labelled cDNA fragments of *AtPT2* gene. Equivalence of RNA loading in all the lanes is shown by 32P-labelled tubulin hybridization and ethidium bromide-stained rRNA (bottom panel). b, Expression of *psbO* and *psbP* genes is affected by duration of Pi deficiency. c, Phosphate plays a role in the transcriptional regulation of *psbO* and *psbP* genes. d, Iron deficiency does not suppress expression of *psbO* and *psbP* genes. Conditions for Northern analysis and probes used for (b), (c) and (d) were same as described in (a).
on a C. reinhardtii mutant that lacked 33 kDa extrinsic protein of OEC, due to which it failed to assemble the functional PSII centres and as a consequence did not evolve oxygen\textsuperscript{23}. Furthermore, significant growth retardation was observed\textsuperscript{23} in A. thaliana mutant with a defect in psbO. Likewise, the C. reinhardtii mutant, which was unable to synthesize 23 kDa extrinsic protein of OEC, showed lower oxygen evolving activity compared to its wild type\textsuperscript{23}. In this context, suppression of psbO and psbP genes during phosphate starvation could possibly be an adaptive mechanism to lower PSII energy capture efficiency during higher irradiance. This reduction in PSII activity may work in concert with increased anthocyanin accumulation during Pi deficiency thereby negating the ill-effects of transfer of light energy to PSII. It is well known that during Pi deficiency photosynthesis is severely inhibited\textsuperscript{25} and this could thus be in part due to the down regulation of psbO and psbP genes. The concurrent suppression of psbO and psbP and induction of high-affinity Pi transporter AtPT2 (Phl; 4) in P- Arabidopsis thus points to an adaptive response of a plant to phosphate deficiency. This mode of differential gene regulation perhaps facilitates the plant to cope with Pi deficiency by increasing its uptake by the induction of genes encoding Pi transporters on the one hand, and economizing further its available pool in the cytoplasm by down-regulating some of genes involved in the energy requiring metabolic processes\textsuperscript{26}. In addition, the role of Fe in the regulation of psbO and psbP genes was also investigated (Figure 2d). Among the micronutrients, Fe deficiency has often been associated with changes in chloroplast ultrastructure and decreased concentrations of photosynthetic pigments in the leaf, which results in the development of chlorosis in young leaves\textsuperscript{1}. This is largely attributed to the fact that about 80% of the leaf iron is localized in the chloroplast, primarily in the molecular complexes involved in the photosynthetic electron transport chain which comprise almost 60% of total leaf iron\textsuperscript{25}. In the present study, Arabidopsis plants were grown for 7 d in half-strength MS liquid medium and then transferred to full-strength MS medium deprived of Fe for 7 d. As controls, plants were also grown in P+ and P- media for similar length of time. Though Fe-plants exhibited typical deficiency symptoms in form of interveinal chlorosis in young leaves, no significant changes in the expression of psbO and psbP genes could be observed (Figure 2d). This suggests that the effect of Fe deficiency could be more pronounced on the other processes of photosynthesis, but not directly on the regulation of psbO or psbP gene expression. However, the likelihood of other macro and micro-nutrients exerting their influence on the regulation of psbO and psbP genes or those involved in other processes of PSII could not be ruled out. In this context, it would be interesting to evaluate the role of other plant nutrients, particularly those that influence the functional activity of PSII. One such likely candidate could be sulphur, whose deficiency reduces the PSII activity to about 50% in C. reinhardtii\textsuperscript{18}.

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RESEARCH COMMUNICATIONS


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Citrus yellow mosaic virus is associated with mosaic disease in Rangpur lime rootstock of citrus

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A mosaic disease was observed in Rangpur lime rootstock in a citrus orchard, Tirupati. Electron microscopy showed bacilliform virus particles in the diseased leaf tissues. We have cloned and sequenced part of intergenic region and ORF III of the viral genome. Sequence analysis showed that it has high sequence identity in ORF III with other Indian isolate of Citrus yellow mosaic virus (CYMV) infecting sweet orange of citrus, but showed variability in intergenic region. Phylogenetic analysis of badnaviruses indicated that CYMV was most closely related to *Cacao swollen shoot virus*.

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The intergenic region contained putative transcriptional elements similar to other badnaviruses.

**Keywords:** Badnavirus, Citrus yellow mosaic virus, PCR, sequencing.

In India, mosaic diseases in citrus were reported in sathgudi sweet orange and khasi mandarins1,2 but etiology of these diseases was not established. A mosaic disease of citrus was also reported from Japan, where a spherical virus was reported to be associated with it3. Ahlawat et al.4 reported a yellow mosaic disease on pummelo from Karnataka and showed the association of a badnavirus with the disease. Subsequently, a badnavirus was reported infecting acid lime5. Since the infected citrus trees in India showed yellow mosaic symptoms, it was decided at the 13th International Organization of Citrus Virologists Conference held in China in 1995, that the name of disease may be changed to citrus yellow mosaic disease and the citrus mosaic badnavirus be called as Citrus yellow mosaic badnavirus (CYMBV). However, the causal virus has been now designated as Citrus yellow mosaic virus (CYMV)6. CYMV has been found to be serologically related to *Banana streak virus* (BSV), *Cacao swollen shoot virus* (CSSV), *Commelina yellow mosaic virus* (ComYMV), Kalanchoe top-spotting virus (KTSV), *Sugarcane bacilliform virus* (ScBV), and Taro bacilliform virus (TaBV) in ISEM tests and with BSV and ComYMV in PCR using degenerate primers7. Preliminary results showed that mealybug transmits the virus, but its role under natural conditions is not known5. During surveys, typical symptoms of citrus yellow mosaic disease were observed on Rangpur lime trees in Tirupati (Figure 1). Since Rangpur lime is used as rootstock in citrus propagation, studies were undertaken to establish the relationship of the causal virus by sequencing its genome.

The symptomatic leaf tissue of Rangpur lime plants was used for leaf dip electron microscopy using 2% uranyl acetate8. Total DNA was isolated from 100 mg of symptomatic leaves of Rangpur lime or sweet orange grafted with diseased Rangpur lime budwood, as well as from healthy seedling of sweet orange or Rangpur lime9. Specific primer pair designed and synthesized previously from RNase H and Reverse Transcriptase of ORF 3 (5567F 5′GTTGCTTTTCATAGGTAGC and 6204R 5′CATG CATCCATCGTTCG)9 and a primer pair 7011F 5′ GAGCTTTAGAAGAATCCT and 20R 5′ATAA CCAAGCTCTGATACCA designed and synthesized (Qiagen Operon, GMBH, Germany) for amplification of intergenic region were used to amplify the viral genome (Figure 2). PCR amplification was performed in 50 μl reaction mixtures using 1 μM of primer, 200 μM each of dNTPs, 0.05 unit/μl Taq DNA polymerase, 1X reaction buffer, 1.5 mM MgCl2, and 5 μl DNA template either from symptomatic or non symptomatic (healthy) plants. Samples were amplified for 30 cycles using a thermocycler (Biometra, Germany).

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