at about 150 m during the normal and the El Nino years. Figure 4 is a schematic diagram of the PTGs during normal conditions and during El Nino.

The results bring out the presence of PTGs in the El Nino region, especially in the 3.4 domain. The gyres are a result of the interactions between the Kelvin and Rossby waves. They are also responsible for increased SSH and SST and for the westward propagation of the warm temperatures along the equatorial Pacific during El Nino. During non-El Nino years, the PTGs once again form a mode by which upwelling takes place prominently on the southern side through the cyclonic circulations of the southern gyre of the PTG, while the northern gyre with anticylonic circulation accounts for the warmer surfaces north of the equator. The PTGs also call for more dynamical search to be carried in order to bring out the role of such meso-scale features during El Nino and also their role and impact in the deepening of the thermocline. Majority of the studies carried out are with seasonal or monthly forcings; these often did filter the meso-scale moving/oscillating twin gyres in the Indian Ocean or in the Pacific Ocean. Hence, the role of the reduced timescale forcings also seems to be important in order to disseminate complex phenomena like the El Nino.

1. Rahul Chand Reddy, P., Salvekar, P. S., Deo, A. A. and Ganer, D. W., Westward propagating twin gyres in the tropical Indian Ocean. *Geophys. Res. Lett.*, 2004, **31**, L01304.
2. Wallace, J. M., Rasmusson, E. M., Mitchell, T. P., Kousky, V. E., Sarachik, E. S. and von Storch, H., On the structure and evolution of ENSO-related climate variability in the tropical Pacific: Lessons from TOGA. *J. Geophys. Res.*, 1998, **109**, 14241–14259.
3. Neelin, J. D, Battisti, D. S., Hirst, A., Jin, F. F., Wakata, Y., Yamagata, T. and Zebiak, S., ENSO theory. *J. Geophys. Res.*, 1998, **109**, 14261–14290.
4. Battisti, D. S., Dynamics and thermodynamics of a warming event in a atmosphere–ocean model. *J. Atmos. Sci.*, 1988, **46**, 2889–2919.
5. Suarez, M. J. and Schopf, P. S., A delayed action oscillator for ENSO. *J. Atmos. Sci.*, 1988, **45**, 3283–3287.
6. Battisti, D. S. and Hirst, A. C., Interannual variability in the tropical atmosphere/ocean system: Influence of the basic state, ocean geometry and nonlinearity. *J. Atmos. Sci.*, 1989, **46**, 1687–1712.
7. Philander, S. G. H., Pacanowski, R., Lau, N. A. and Nath, M. J., Simulation of ENSO with a global atmospheric *GaM* coupled to a high-resolution, tropical Pacific Ocean *GaM*. *J. Climate*, 1992, **5**, 308–329.
8. Lu, P., McCreary Jr., J. P. and Klinger, B. A., Meridional circulation cells and the source waters of the Pacific equatorial undercurrent. *J. Phys. Oceanogr.*, 1998, **28**, 62–84.
9. Jin, F.-F., An equatorial recharge paradigm for ENSO, I. Conceptual model. *J. Atmos. Sci.*, 1997, **54**, 811–829.
10. Jin, F.-F., An equatorial recharge paradigm for ENSO, II. A stripped-down coupled model, *J. Atmos. Sci.*, 1997, **54**, 830–845.

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

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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 O_2. 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 O_2 evolving efficacy.

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

INORGANIC phosphate (Pi) is one of the important mineral nutrients influencing metabolism, growth, development and consequently yield of plants[1]. However, in terrestrial and freshwater ecosystems orthophosphate ions (H_2PO_4), a preferentially assimilated form of P, are either limited or present in inorganic and organic complexes, which are not readily available to plants[2]. To circumvent phosphate deficiency, plants have evolved several morphological, biochemical, physiological and molecular adaptations[3] 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. ^{31}P-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[4]. Such a drastic reduction of cytoplasmic Pi concentration during Pi stress affects various aspects of photosynthetic processes and consequently growth and vigour of plants[5]. 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[6]. 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[7]. 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[8]. 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[9] 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[10], 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[11]. 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_2PO_4 in MS medium was replaced with K_2SO_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, blot-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[12]. DNA sequences of *psbO2* (At3g50820) and *psbP* (At1g06680) genes were obtained from the GenBank database of NCBI (Bethesda, MD, USA http://www.ncbi.nlm.nih.gov) 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[13]. 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[14] and anthocyanin[15] 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 1 *a*), lower Pi content (Figure 1 *b*) and a concomitant accumulation of anthocyanin in the shoots (Figure 1 *c*). An inadequate supply of Pi limits the availability of adenylate energy and various phosphorylated intermediates, which are critical for photosynthetic carbon reduction cycle[5]. 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[16]. 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[17]. This view was substantiated by a study on *C. reinhardtii*, where nearly 75% reduction in functional PSII reaction centres was observed[18] when the cells were starved of phosphate for 4 d.

Earlier studies[19] 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[20,21] encode 33 and 23 kDa extrinsic proteins of OEC, which are important constituents[9] 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

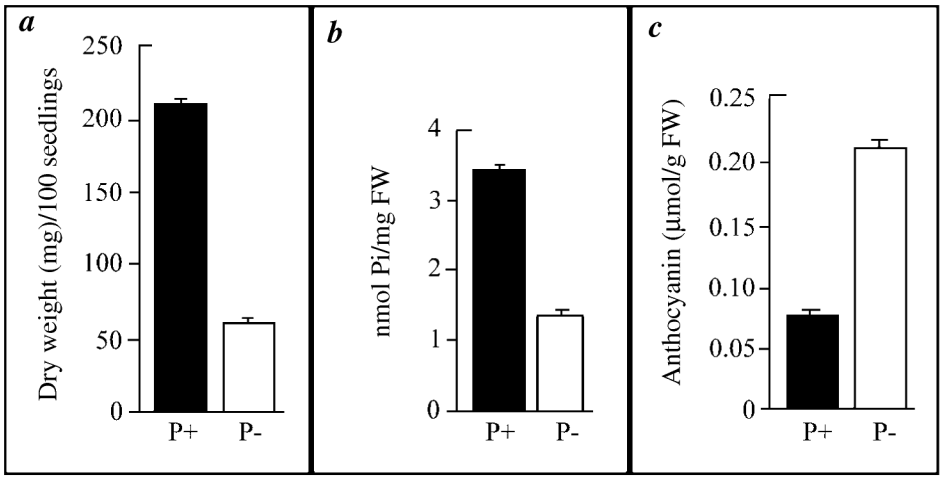

Figure 1. *Arabidopsis* seeds were grown in liquid culture in half-strength MS medium for 7 d and seedlings were transferred to full-strength MS supplemented with 1.25 mM Pi (P+) or without Pi (P–). Pi deprivation resulted in a significant reduction in the dry weight of seedling (***a***), Pi content (***b***) and accumulation of anthocyanin in the shoots (***c***). Values represent means ± SE for five replicates.

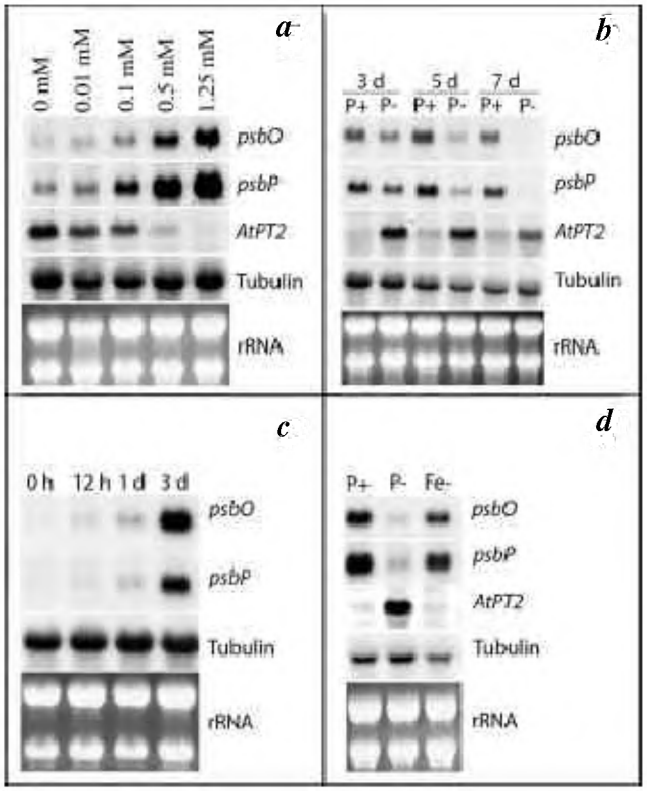

Figure 2. ***a***, Low Pi concentrations suppress expression of *psbO* and *psbP* genes. Ten micrograms of total RNA isolated from the whole plant, was separated on agarose gel and transferred to nylon membrane. The membrane was hybridized with ^{32}P-labelled cDNA fragments of *psbO* and *psbP* genes. As control, the membrane was hybridized with ^{32}P-labelled cDNA fragments of *AtPT2* gene. Equivalence of RNA loading in all the lanes is shown by ^{32}P-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***).

(P–) to 1.25 mM (P+). Plants were harvested after 7 d of treatment and used for Northern analysis of *psbO* and *psbP* genes (Figure 2 *a*). Low abundance of *psbO* and *psbP* transcripts was observed under Pi starvation (0 mM) and low Pi concentration (0.01 mM). There was a concurrent increase in their levels with an increase in the concentration of phosphate. Northern analysis indicated a positive correlation between Pi concentration in the medium and the level of expression of these genes. To ensure the fidelity of phosphate-dependent gene expression in liquid culture medium, a high affinity phosphate transporter *AtPT2* (Pht1; 4) was used as a control, as it is known to be induced preferentially in Pi-deficient roots[11]. To further investigate as how rapidly the expression of *psbO and psbP* genes is suppressed under phosphate deficiency, a time course study was conducted (Figure 2 *b*). No reductions were observed in the expression of *psbO* and *psbP* genes up to 7 d of growth in P+ medium. On the contrary, when the plants were grown in P– medium, there was gradual reduction in the abundance of transcripts and the expression could barely be detected for either of the two genes after 7 d of starvation. Furthermore, when *Arabidopsis* starved of Pi for 7 d was replenished with Pi (1.25 mM) for different time intervals, an appreciable up-regulation of these genes was observed after 3 d of replenishment, which suggests their transcriptional regulation by phosphate (Figure 2 *c*). The reversibility of long-term Pi deficiency stress response has also been shown in spinach and barley plants[22], where the P-deficient plants recovered photosynthetic quantum yield after a day of replenishment with Pi.

Since *psbO* and *psbP* genes encode 33 and 23 kDa extrinsic proteins of OEC, which are important constituents[9] of PSII, their low abundance during phosphate stress could potentially affect the oxygen evolving capacity of PSII. This view could be substantiated by the earlier report

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[23]. Furthermore, significant growth retardation was observed[24] 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[25]. 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[5] 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* (*Pht1*; *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[26].

In addition, the role of Fe in the regulation of *psbO* and *psbP* genes was also investigated (Figure 2 *d*). 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[27]. 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[28]. 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 2 *d*). 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 micronutrients 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*[18].

1. Marschner, H., In *Mineral Nutrition of Higher Plants*, Academic Press, London, 1995, 2nd edn.
2. Raghothama, K. G., Phosphate acquisition. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1999, **50**, 665–693.
3. Raghothama, K. G., Phosphate transport and signaling. *Curr. Opin. Plant Biol.*, 2000, **3**, 182–187.
4. Lauer, M. J., Blevins, D. G. and Sierzputowska-Gracz, H., ^{31}P-Nuclear magnetic resonance determination of phosphate compartmentation in leaves of reproductive soybeans as affected by phosphate nutrition. *Plant Physiol.*, 1989, **89**, 1331–1336.
5. Rao, M., The role of phosphorus in photosynthesis. In *Handbook of Photosynthesis* (ed. Pessarakli, M.), Marcel Dekker, New York, 1997, pp. 173–194.
6. Jacob, J. and Lawlor, D. W., *In vivo* photosynthetic electron transport does not limit photosynthetic capacity in phosphate-deficient sunflower and maize leaves. *Plant Cell Environ.*, 1993, **16**, 785–795.
7. Pieters, A. J., Paul, M. J. and Lawlor, D. W., Low sink demand limits photosynthesis under Pi deficiency. *J. Exp. Bot.*, 2001, **52**, 1083–1091.
8. Loustau, D., Ben, B. M., Gaudillere, J. P. and Dreyer, E., Photosynthetic response to phosphorus nutrition in two-year-old maritime pine seedlings. *Tree Physiol.*, 1999, **19**, 707–715.
9. Bricker, T. M. and Frankel, L. K., The structure and function of the 33 kDa extrinsic protein of Photosystem II: A critical assessment. *Photosynthesis Res.*, 1998, **56**, 157–173.
10. Terry, N., Limiting factors in photosynthesis. I. Use of iron stress to control photochemical capacity *in vivo*. *Plant Physiol.*, 1980, **65**, 114–120.
11. Karthikeyan, A. S., Varadarajan, D. K., Mukatira, U. T., D'Urzo M. P., Damsz, B. and Raghothama, K. G., Regulated expression of *Arabidopsis* phosphate transporters. *Plant Physiol.*, 2002, **130**, 221–233.
12. Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989, 2nd edn.
13. Dasgupta, U., Jain, M., Tyagi, A. K. and Khurana, J. P., Regulatory elements for light-dependent and organ-specific expression of *Arabidopsis thaliana PSBO1* gene encoding 33 kDa polypeptide of the oxygen-evolving complex. *Plant Sci.*, 2005, **168**, 1633–1642.
14. Ames, B. N., Assay of inorganic phosphate, total phosphate and phosphatases. *Methods Enzymol.*, 1966, **8**, 115–118.
15. Lange, H., Shropshire Jr., W. and Mohr, H., An analysis of phytochrome-mediated anthocyanin synthesis. *Plant Physiol.*, 1971, **47**, 649–655.
16. Field, T. S., Lee, D. W. and Holbrook, N. M., Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. *Plant Physiol.*, 2001, **127**, 566–574.
17. Allen, J. F., Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta*, 1992, **1098**, 275–335.
18. Wykoff, D. D., Davies, J. P., Melis, A. and Grossman, A. R., The regulation of photosynthetic electron transport during nutrient deprivation in *Chlamydomonas reinhardtii*. *Plant Physiol.*, 1998, **117**, 129–139.
19. Gray J. C., Regulation of expression of nuclear genes encoding polypeptides required for the light reactions of photosynthesis. In *Oxygenic Photosynthesis: The Light Reactions* (eds Ort, D. R. and Yocum, C. F.), Kluwer, The Netherlands, 1996, pp. 621–641.
20. Jain, P. K., Kochhar, A., Khurana, J. P. and Tyagi, A. K., The *psbO* gene for 33 kDa precursor polypeptide of the oxygen evolving complex in *Arabidopsis thaliana*: nucleotide sequence and control of its expression. *DNA Res.*, 1998, **5**, 221–228.
21. Kochhar, A., Khurana, J. P. and Tyagi, A. K., Nucleotide sequence of the *psbP* gene encoding precursor of 23 kDa polypeptide of oxygen-evolving complex in *Arabidopsis thaliana* and its expression in the wild-type and a constitutively photomorphogenic mutant. *DNA Res.*, 1996, **3**, 277–285.

22. Dietz, K. J. and Foyer, C., The relationship between phosphate status and photosynthesis in leaves. Reversibility of the effects of phosphate deficiency on photosynthesis. *Planta*, 1986, **167**, 376–381.
23. Mayfield, S. P., Bennoun, P. and Rochaix, J. D., Expression of the nuclear encoded OEE1 protein is required for oxygen evolution and stability of photosystem II particles in *Chlamydomonas reinhardtii*. *EMBO J.*, 1987, **6**, 313–318.
24. Murakami, R., Ifuku, K., Takabayashi, A., Shikanai, T., Endo, T. and Sato, F., Characterization of an *Arabidopsis thaliana* mutant with impaired *psbO*, one of the two genes encoding extrinsic 33-kDa proteins in photosystem II. *FEBS Lett.*, 2002, **523**, 138–142.
25. De Vitry, C., Olive, J., Drapier, D., Recouvreur, M. and Wollman, F. A., Post-translational events leading to the assembly of photosystem II protein complex: A study using photosynthesis mutants from *Chlamydomonas reinhardtii*. *J. Cell Biol.*, 1989, **109**, 991–1006.
26. Plaxton, W. C. and Carswell, M. C., Metabolic aspects of the phosphate starvation response in plants. In *Plant Responses to Environmental Stresses: From Phytohormones to Genome Organization* (ed. Lerner, H. R.), Marcel Dekker, New York, 1999, pp. 349–372.
27. Abadia, J., Morales, F. and Abadia, A., Photosystem II efficiency in low chlorophyll, iron-deficient leaves. *Plant Soil*, 1999, **215**, 183–192.
28. Abadia J., Leaf responses to Fe deficiency: A review. *J. Plant Nutr.*, 1992, **15**, 1669–1713.

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

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

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IN India, mosaic diseases in citrus were reported in sathgudi sweet orange and khasi mandarins[1,2] 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 it[3]. 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 lime[5]. 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 primers[4]. Preliminary results showed that mealybug transmits the virus, but its role under natural conditions is not known[7]. 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 acetate[8]. 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 lime[9]. Specific primer pair designed and synthesized previously from RNase H and Reverse Transcriptase of ORF 3 (5567F 5′GTGGCTTTCATCAGGTAGC and 6204R 5′ CATG CATCCATCCGTTTCG)[9] and a primer pair 7011F 5′ GAGCTATTAGAAGGAATCTC 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 $MgCl_2$ and 5 µl DNA template either from symptomatic or non symptomatic (healthy) plants. Samples were amplified for 30 cycles using a thermocycler (Biometra, Germany).