BIOLOGIA PLANTARUM 54 (4): 671-676, 2010

Alteration in growth and thylakoid membrane lipid composition of *Azolla caroliniana* under phosphate deficiency

G.S.M. ISMAIL* and H.E. MOHAMED

Botany Department, Faculty of Science, Alexandria University, 21511, Alexandria, Egypt

Abstract

The changes in the fresh biomass accumulation, photosynthetic and anthocyanin pigments, photosystem 2 (PS 2) activity, ultrastructure of chloroplast, total lipids and fatty acid composition of thylakoid membrane were followed in the aquatic fern *Azolla caroliniana* grown on medium either deficient or supplied with various phosphorus concentrations. The content of photosynthetic pigments and the anthocyanin/chlorophyll ratio increased significantly with increasing PO_4^{3-} concentration. Phosphate deficiency inhibited growth and PS 2 activity and decreased content of total lipids and phospholipids in isolated thylakoids. This was accompanied with a significant increase in the percentage of galalactolipids.

Additional key words: anthocyanin, aquatic fern, chlorophyll, chloroplast ultrastructure, fatty acids, galactolipids, phospholipids.

Introduction

Phosphorus is an essential constituent of many biomolecules and plays a pivotal role in energy conservation and metabolic regulation. It is a plant macronutrient, and the effects of a limited supply have been extensively studied (Raghothama 1999, Zaheer *et al*. 2001, Vance *et al*. 2003; Raghothama and Karthikeyan 2005). Phosphorus deficiency decreased fresh and dry mass in *Glycine max* (Tsvetkova and Georgiev 2003) and *Arabidopsis thaliana* (Li *et al*. 2006), leaf photosynthetic rate in barley (Foyer and Spencer 1986), sugar beet (Rao and Terry 1989), and soybean (Fredeen *et al*. 1989). Plesnicar *et al*. (1994) showed that low P in the medium inhibited growth, decreased photosynthetic oxygen evolution and efficiency of photosystem 2 (PS 2) in *Helianthus annuus.* Thomson *et al*. (1964) working with *Phaseolus vulgaris* and Hall *et al*. (1972) working with *Zea mays* found that P-deficiency caused marked alterations in the ultrastructure of the chloroplasts.

It is known that the thylakoid membranes in chloroplasts are mainly composed of glycerolipids forming lipid bilayers and of protein complexes involved in photosynthetic electron transport and energy conversion (Siegenthaler 1998, Hagio *et al.* 2000). Dörmann and Benning (2002) showed drastic alterations in the thylakoid lipid composition under phosphate starvation. Phospholipid content fell concomitantly with an increase in galactolipid content (Essigmann *et al*. 1998). In addition, an enhanced content of unsaturated fatty acids, which could cause greater membrane fluidity, has also been found in phosphorus-starved cells of *Chlorella kessleri* (El-Sheek and Rady 1995).

The aim of present study was to follow the effect of P-deficiency on the biomass accumulation, PS 2 activity, ultrastructure of chloroplasts, total lipids and fatty acid composition of the thylakoid membranes in the aquatic fern *Azolla caroliniana*.

Materials and methods

Azolla caroliniana Wild. (known as water velvet) was provided by Prof. C. Van Hove, Catholic University of Louvain, Belgium. The plants were acclimated in the greenhouse of the Faculty of Science, Alexandria

Received 1 May 2009, *accepted* 15 December 2009.
Abbreviations: Anth - anthocyanins; Car - carotenoids; Chl - chlorophyll; PS - photosystem.
* Author for correspondence; fax: (+203) 3911794, e-mail: ghada5f@yahoo.com

University, Egypt, in 2500 cm^3 polyethylene vessels filled with 2/5 strength modified Hoagland nutrient solution. About 3 g fresh mass (FM) of *Azolla* plants from the stock material were sub-cultured every two weeks.

Before being used, the plants were freed from epiphytic microorganisms by thorough washing with distilled water, surface-sterilized with 0.2 % *Clorox*, and then thoroughly washed again with distilled water. The sterilized plants were sub-cultured into 250 cm^3 vessels containing the 2/5 Hoagland solution and maintained in the growth chamber under a 16-h photoperiod at an irradiance of 1200 µmol m^{-2} s^{-1} (cool white fluorescent tubes) and day/night tempearture of 29/24 ± 2 °C for 7 d. The 7-d-old plants (initial mass 3 g) were transferred to 250 cm^3 vessels containing the modified Hoagland solution either deficient in PO_4^{3-} or supplemented with 0.4, 1.5 or 4.5 mM of PO_4^{3-} as KH_2PO_4. In the phosphorus-deficient nutrient solution, KH_2PO_4 was replaced by KCl. The experiment was extended for 20 d. At an interval of 5 d, the biomass was collected, weighed and used for chemical analyses.

The photosynthetic pigments were determined after grinding of *Azolla* samples with acetone following the method of Metzner *et al*. (1965). Absorbance was measured with a spectrophotometer (*Jenway 6305* UV/Vis, Essex, UK). The chloroplasts were isolated as described by Osman and El-Shintinawy (1988). The PS 2 activity was measured according to Biswall and Mohanty (1976) using the isolated thylakoids.

Total lipids of freshly isolated thylakoids of P-deficient (0.0 mM PO_4^{3-}) and P-sufficient (1.5 mM PO_4) *Azolla* plants were estimated as described by Melton *et al*. (1979). The phospholipid content was determined colorimetrically after the method of Yan *et al*. (1998). Lipids were digested with perchloric acid and the released phosphate was quantified with the method of Stewart and Grimshaw (1974). The preparation of fatty acid methyl esters of lipids extract was carried out according to Kock *et al*. (1985). The methyl esters were separated and quantified by gas chromatography (*Shimadzu 12A*, Kyoto, Japan) after the method of Augustin (1989) and identified through comparison with standard fatty acid methyl esters. The unsaturation degree was calculated by the equation mentioned by De Santis *et al*. (1999).

Transverse sections of *Azolla* plants were examined by both light microscopy (LM) and transmission electron microscopy (TEM). Sectioning and staining were done as described by Doncheva *et al.* (2001), and examined by a *Jeol 100 CX* electron microscope (Tokyo, Japan). Two digital TEM micrographs were taken from random cells of *Azolla* plants grown on 0.0 and 1.5 mM PO_4^{3-} to discern clearly the structure of the chloroplasts.

Results

Supplementing the nutrient solution with 0.4, 1.5 or 4.5 mM PO_4^{3-} resulted in a significant increase in the fresh biomass (Table 1). At the end of experiment the fresh biomass of *Azolla* plants grown at 4.5 mM PO_4^{3-} increased by 27.15 g per culture, the corresponding value of P-deficient plants was only 5.22 g per culture.

The pigment contents significantly increased with increasing PO_4^{3-} concentration. This increase was mainly related to chlorophyll (Chl) *a*. At the end of the experiment, the ratio of Chl *a*/Chl *b* increased from 1.82 to 3.23 by the increase of PO_4^{3-} concentration from 0.0 to 4.5 mM. In addition, there was a slight change in the carotenoid (Car) content during the experiment but the ratio of Car/Chl *a+b* was always highest in P-deficient plants (Table 1).

The anthocyanin (Anth) content of P-deficient *A. caroliniana* increased steadily during the experimental period while that of P-sufficient plants decreased. Moreover, at the end of the experiment, the ratio of Anth/Chl *a+b* in P-deficient plants was 0.23 compared to 0.02 in the plants grown on 4.5 mM PO_4^{3-} (Table 1).

Phosphorus deficiency significantly inhibited PS 2 activity. Supplementing the nutrient solution with various concentrations of PO_4^{3-} significantly increased PS 2 activity, particularly at 1.5 mM concentration.

There was a notable alteration in the ultrastructure of chloroplast under phosphorus deficiency (Fig. 1). They become more or less rounded and exhibited a dense lamellar system, the grana stacks that were difficult to distinguish and large starch grain was present.

Deficiency of phosphorus resulted in a significant decrease in both total lipids and phospholipids in isolated thylakoids. This was accompanied with a significant increase in the percentage of galalactolipids relative to the total lipids (Table 2). In addition, a change in the fatty acid composition was recorded (Table 2). Content of unsaturated fatty acids of thylakoid membrane of P-deficient plants was higher than the content of saturated fatty acids. Moreover, the unsaturation degree of the P-deficient plants was greater than that of plants sufficiently supplied with P, denoting the shift from saturated to unsaturated fatty acids and increasing membrane fluidity in P-deficient plants (data not shown).

Table 1. Changes in fresh biomass, PS 2 activity and contents of anthocyanins, chlorophylls, carotenoids and their ratios in *Azolla caroliniana* plants in response to PO_4^{3-} concentrations and time of cultivation [d]. Values are the means of 3 independent replicates ± SE; means followed by different letters are significantly different at $P \leq 0.05$ according to the least significant difference (LSD) test.

Parameter	PO_4^{3-} conc. [mM]	5 d	10 d	15 d	20 d
Fresh biomass [g culture^{-1}]	0.0	7.43 ± 0.42^a	10.75 ± 0.09^c	11.55 ± 0.20^b	8.22 ± 0.04^c
	0.4	7.37 ± 0.50^a	14.34 ± 0.34^b	14.11 ± 0.06^b	12.92 ± 0.30^c
	1.5	8.48 ± 0.20^a	21.33 ± 0.21^a	22.88 ± 0.08^a	23.91 ± 0.51^b
	4.5	7.18 ± 0.48^a	20.91 ± 0.02^a	25.49 ± 0.07^a	30.15 ± 0.12^a
PS 2 activity	0.0	0.02 ± 0.01^b	0.04 ± 0.02^c	0.03 ± 0.01^c	0.03 ± 0.00^d
[μmol(reduced DCPI) mg^{-1}(f.m.) s^{-1}]	0.4	0.05 ± 0.01^b	0.15 ± 0.02^b	0.72 ± 0.02^b	0.81 ± 0.10^c
	1.5	0.22 ± 0.02^a	0.33 ± 0.03^a	1.31 ± 0.10^a	1.62 ± 0.20^a
	4.5	0.14 ± 0.04^a	0.32 ± 0.05^a	1.14 ± 0.12^a	1.36 ± 0.10^b
Anthocyanins	0.0	4.22 ± 0.28^a	4.27 ± 0.05^a	8.35 ± 0.32^a	7.02 ± 0.02^a
[mg g^{-1}(d.m.)]	0.4	3.00 ± 0.20^b	3.69 ± 0.09^b	6.84 ± 0.81^a	7.17 ± 0.17^a
	1.5	3.53 ± 0.11^b	3.70 ± 0.20^b	3.45 ± 0.45^b	2.93 ± 0.03^b
	4.5	3.00 ± 0.50^b	3.30 ± 0.20^b	3.28 ± 0.28^b	2.90 ± 0.19^b
Chlorophyll *a*	0.0	29.19 ± 0.06^b	29.05 ± 0.12^c	24.07± 0.16^d	19.34± 0.20^c
[mg g^{-1}(d.m.)]	0.4	36.93± 0.03^a	41.09 ± 0.09^b	38.50± 0.20^c	32.37 ± 0.30^b
	1.5	34.64 ±0.04^a	49.86 ± 0.14^a	47.87 ± 0.90^b	48.47 ± 0.20^a
	4.5	39.86 ± 0.14^a	52.34 ± 0.16^a	55.43 ± 0.31^a	55.16 ± 0.41^a
Chlorophyll *b*	0.0	17.28 ± 0.06^b	17.05 ± 0.12^b	12.04 ± 0.20^c	10.61 ± 0.50^c
[mg g^{-1}(d.m.)]	0.4	24.89 ± 0.10^a	21.34 ± 0.40^b	18.56 ± 0.18^b	15.80 ± 0.37^b
	1.5	24.38 ± 0.24^a	28.67 ± 0.16^a	28.25 ± 0.45^a	20.33 ± 0.18^a
	4.5	19.83 ± 0.90^b	29.66 ± 0.27^a	26.51 ± 0.12^a	17.04 ± 0.60^a
Carotenoids	0.0	11.01 ± 0.50^a	11.41 ± 0.30^b	9.44 ± 0.16^b	9.75 ± 0.40^b
[mg g^{-1}(d.m.)]	0.4	11.16 ± 0.16^a	11.00 ± 0.20^b	10.26 ± 0.40^b	7.90 ± 0.51^b
	1.5	11.89 ± 0.80^a	16.46 ± 0.70^a	19.38 ± 0.21^a	19.51 ± 0.15^a
	4.5	12.41 ± 0.40^a	19.44 ± 0.20^a	19.26 ± 0.18^a	17.52 ± 0.19^a
Chl *a*/*b*	0.0	1.68	1.70	1.99	1.82
	0.4	1.48	1.92	2.07	2.04
	1.5	1.42	1.73	1.69	2.38
	4.5	2.01	1.76	2.09	3.23
Car/Chl *a*+*b*	0.0	0.23	0.24	0.26	0.32
	0.4	0.18	0.17	0.17	0.16
	1.5	0.20	0.20	0.25	0.28
	4.5	0.20	0.23	0.23	0.24
Anth/Chl *a*+*b*	0.0	0.09	0.09	0.23	0.23
	0.4	0.04	0.05	0.06	0.08
	1.5	0.05	0.04	0.04	0.04
	4.5	0.05	0.04	0.04	0.02

Discussion

Phosphorus deficiency had a significant inhibitory effect on the growth of *Azolla*. This was accompanied with a significant decrease in contents of photosynthetic pigments and PS 2 activity (Table 1). Inhibition of growth due to phosphorus deficiency has previously been reported for many plants [*Glycine max* (Tsvetkova and Georgiev 2003), *Arabidopsis thaliana* (Ajay-Jain *et al*. 2005, Li *et al*. 2006), *Brassica* (Akhtar *et al*. 2007)] including *Azolla caroliniana* (Adalberto *et al*. 2004). Furthermore, Adalberto *et al*. (2004) and Khozin-Goldberg and Cohen (2006) reported that under P-deficiency, chlorophyll synthesis was severely retarded in *Azolla caroliniana* and *Monodus subterraneus*, respectively. However, Ticconi *et al*. (2001) reported that total chlorophyll content did not significantly differ between *Arabidopsis* seedlings grown on P-deficient or P-sufficient medium. In present experiments, the decrease in Ch *a*+*b* in P-deficient plants was mostly related to the decrease in Chl *a*.

It has been reported by many authors that P deficiency significantly inhibited leaf photosynthetic rate and carbon metabolism in plants (Rao 1997, Ripley *et al*. 2004**,** Li

Table. 2. Changes in the contents of phospholipids and galactolipids [mg g^{-1}(d.m.)] and fatty acid composition of thylakoid membranes [% of total] of *Azolla caroliniana* plants in response to PO_4^{3-} concentrations [mM] and time of cultivation [d]. Values are the means of 3 independent replicates ± SE; means followed by different letters are significantly different at $P \leq 0.05$ according to the least significant difference (LSD) test. Saturated fatty acids: C10- decanoic, C12 - lauric, C14 - myristic, C16 - palmitic, C18 - stearic; unsaturated fatty acids: C16:1 - palmitoleic, C18:1 - oleic, C18:2 - linoleic, C18:3 - linolenic.

Time	PO_4^{3-}	Phospholipids	Galactolipids	C10	C12	C14	C16	C18	C16:1	C18:1	C18:2	C18:3
5	0.0	0.30 ± 0.10^{b}	11.10 ± 0.10^{a}	0.931	3.722	5.584	31.002	6.381	5.318	26.588	12.284	8.190
	1.5	0.96 ± 0.07^{a}	12.36 ± 0.16^{a}	6.219	8.291	5.389	34.528	5.774	3.553	11.845	9.950	14.451
10	0.0	0.27 ± 0.10^{b}	7.65 ± 0.09^{b}	2.219	5.270	4.583	22.32	6.874	3.600	26.187	19.247	9.624
	1.5	4.90 ± 1.00^{a}	13.50 ± 0.09^{a}	2.510	29.844	4.500	27.961	2.411	1.607	2.411	23.23	5.510
15	0.0	0.23 ± 0.03^{b}	8.61 ± 0.03^{b}	2.009	5.023	6.530	18.369	5.382	2.842	20.666	29.535	9.644
	1.5	7.19 ± 1.00^{a}	17.29 ± 1.00^{a}	4.767	24.117	7.066	24.145	4.319	0.982	3.337	26.219	5.048
20	0.0	0.24 ± 0.10^{b}	7.35 ± 0.10^{ba}	1.029	2.917	2.574	18.873	4.044	2.451	34.314	28.308	5.490
	1.5	7.52 ± 1.20^{a}	16.48 ± 1.20	5.318	12.284	8.192	26.588	3.722	0.931	6.381	31.002	5.584

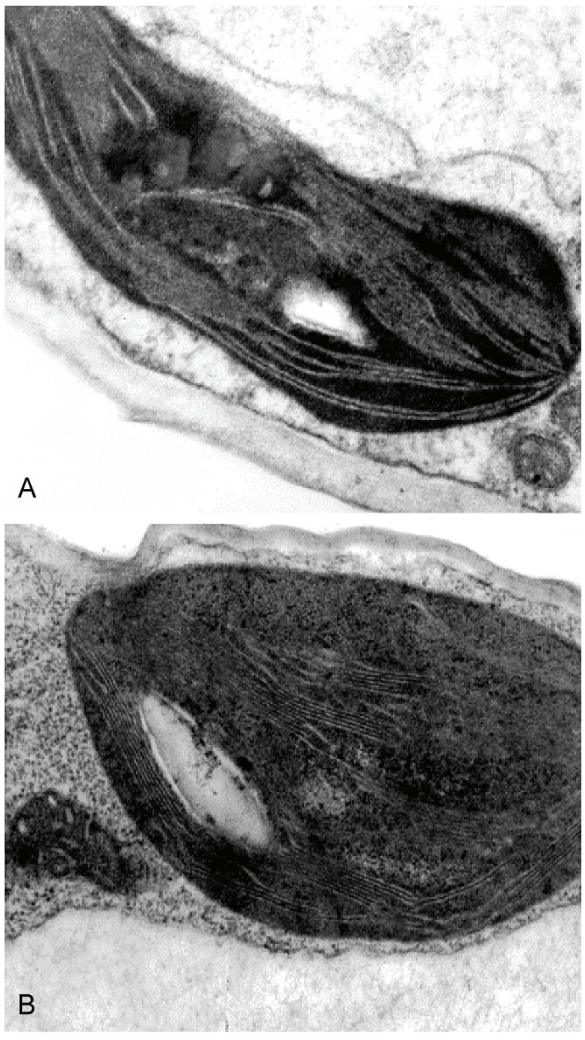

Fig. 1. Chloroplast from P-sufficient (*A*) and P-deficient (*B*) *Azolla caroliniana* plants. The chloroplast in P-deficient plants became rounded instead of lobate and exhibiting a dense lamellar system.

et al. 2006, Zhou *et al.* 2009). In agreement with these observations, PS 2 activity of *A. caroliniana* plants was significantly retarded at P-deficiency and low P concentration (0.4 mM). On the other hand, Yang *et al*. (2004) reported that phosphate deficiency had no clear effect on PS 2 oxygen evolving activity. An inadequate supply of Pi can limit ADP or ATP formation, which is critical for the photosynthetic carbon reduction cycle (Rao 1997). P-deficiency in *A. caroliniana* obviously changed the ultrastructure of the chloroplast which may explain the decrease in chlorophyll content and PS 2 activity; and accordingly the chloroplasts were affected both morphologically and functionally.

In the present investigation, the increase in PO_4^{3-} concentration resulted in a decrease in the Anth/Chl ratio (Table 1), which agrees with the results of Adalberto *et al*. (2004). The accumulation of anthocyanins in the photosynthetic tissues of P-deficient *Azolla* appears to serve a photoprotective role by optically masking chlorophyll and facilitating the conversion of excess absorbed light energy to heat (Feild *et al*. 2001).

Phosphorus deficiency has been shown to cause significant changes in membrane lipids in several plants suggesting that this adaptation mechanism for the conservation of phosphorus is widespread in plants (Andersson *et al*. 2003, Jouhet *et al*. 2003, 2004). In our experiments, total lipid content of the thylakoid membrane of P-deficient plants was significantly reduced (Table 2) and the percentage of phospholipids fell concomitantly with increase in percentage of galactolipids. In this context, phospholipid hydrolysis may be considered as a key step that provides valuable Pi source and the primary substrate for galactolipid biosynthesis under Pi starvation. Similar alteration in the thylakoid and/or plasma membrane lipid composition under phosphate deprivation was also reported for many algal species such as *Monodus subterraneus* (Khozin-Goldberg and Cohen 2006) and *Dunaliella tertiolecta*

(Siron *et al*. 1989), and higher plants such as *Avena sativa* (Andersson *et al*. 2005) and *Arabidopsis* (Hartel *et al*. 2000, Kobayashi *et al*. 2006).The decrease in lipid content in P-deficient *Azolla* plants may reveal the reduction in lipid biosynthesis and/or acceleration of lipid degradation and therefore the structure of the cell membrane systems was greatly disrupted, leading to decreased water and nutrient uptake and hence suppression of growth.

Siron *et al*. (1989) reported that total lipid unsaturation of the alga *Dunaliella tertiolecta* was slightly affected by phosphorus limitation. On the other hand, El-Sheek and Rady (1995) recorded an enhanced content of unsaturated fatty acids in phosphorus-starved cells of *Chlorella kessleri*. In our experiments, the increased unsaturation degree of the fatty acids of the thylakoid membrane of P-deficient *Azolla* plants could cause greater membrane fluidity, disruption of thylakoid membrane integrity and hence alteration in its function.

In conclusion, this study showed that P-starvation induced several inhibitory changes in the growth of *A. caroliniana*, the ultrastructure of the chloroplast and the fatty acid and lipid composition of the thylakoid membrane. The presumed function of the observed changes in lipid composition would be the conservation of phosphorus by reducing the amount of the phosphorus bound in membrane lipids without compromising the function of photosynthetic membranes.

References

Adalberto, P.R., Massabni, A.C., Goulart, A.J., Monti, R., Lacava, P.M.: [Effect of phosphorus on the mineral uptake and pigmentation of *Azolla caroliniana* Willd. (Azollaceae).] - Rev. bras. bot. **27**: 581-585, 2004. [In Port.]

Ajay-Jain, A., Cao, A., Karthikeyan, A.S., Baldwin, J.C., Raghothama, K.G.: Phosphate deficiency suppresses expression of light-regulated *psbO* and *psbP* genes encoding extrinsic proteins of oxygen-evolving complex of PSII. - Curr. Sci. **89**: 1592-1596, 2005.

Akhtar, M.S., Oki, Y., Adachi, T.: Genetic diversity in *Brassica* cultivars under deficiently buffered P-stress environment: I. Biomass accumulation, P-concentration, P-uptake, and related growth parameters. - J. amer. Sci. **3**: 55-63, 2007.

Andersson, M.X.., Larsson, K.E., Tjellstrom, H., Liljenberg, C., Sandelius, A.S.: Phosphate-limited oat**.** - J. biol. Chem. **280**: 27578-27586, 2005.

Andersson, M.X., Stridh, M.H., Larsson, K. E., Liljenberg, C., Sandelius, A.S.: Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol. - FEBS Lett. **537**: 128-132, 2003.

Augustin, O.P.H.: Differentiation between yeast species, and strains within a species by cellular fatty acid analysis. 2 *Saccharomycetes cerevisiae*. - South Afr. J. Enol. Viticult. **10**: 8-15, 1989.

Biswall, U.C., Mohanty, P.: Aging induced changes in photosynthetic electron transport of detached barley leaves. - Plant Cell Physiol. **17**: 323-332, 1976.

De Santis, A., Landi, P., Genchi, G.: Changes in mitochondrial properties in maize seedlings associated with selection for germination at low temperature. Fatty acid composition, cytochrome oxidase and nucleotide translocase activities. - Plant Physiol. **119**: 743-754, 1999.

Doncheva, S., Vassileva, V., Ignatov, G., Pandev, S.: Influence of nitrogen deficiency on photosynthesis and chloroplast ultrastructure of pepper plants. - Agr. Food Sci. Finland **10**: 59-64, 2001.

Dörmann, P., Benning, C.: Galactolipids rule in seed plants. - Trends Plant Sci. **7**: 112–118, 2002.

El-Sheek, M.M., Rady, A.A.: Effect of phosphorus starvation on growth, photosynthesis and some metabolic processes in the unicellular alga *Chlorella kessleri*. - Phyton **35**: 139-151, 1995.

Essigmann, B., Güler, S., Narang, R.A., Linke, D., Benning, C.: Phosphate availability affects the thylakoid lipid composition and the expression of SQD1, a gene required for sulfolipid biosynthesis in *Arabidopsis thaliana*. - Proc. nat. Acad. Sci. USA **95**: 1950-1955, 1998.

Feild, T.S., Lee, D.W., Holbrook, N.M.: Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. - Plant Physiol. **127**: 566-574, 2001.

Foyer, C., Spencer, C.: The relationship between phosphate status and photosynthesis in leaves: Effects on intracellular orthophosphate distribution, photosynthesis and assimilate partitioning. - Planta **167**: 369-375, 1986.

Fredeen, A. L., Rao, I.M., Terry, N.: Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max*. - Plant Physiol. **89**: 225-230, 1989.

Hagio, M., Gombos, Z., Varkonyi, Z., Masamoto, K., Sato, N., Tsuzuki, M., Wada, H.: Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis. - Plant Physiol. **124**: 795-804, 2000.

Hall, J.D., Barr, R., Al-Abbas, A.H., Crane, F.L.: The ultrastructure of chloroplasts in mineral-deficient maize leaves. - Plant Physiol. **50**: 404-409, 1972.

Hartel, H., Dormann, P., Benning, C.: DGD1-independent biosynthesis of extraplastidic galactolipids after phosphate deprivation in *Arabidopsis*. - Proc. nat. Acad. Sci. USA **97**: 10649-10654, 2000.

Jouhet, J., Maréchal, E., Baldan, B., Bligny, R., Joyard, J., Block, M. A.: Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria. - J. cell. Biol. **167**: 863-874, 2004.

Jouhet, J., Maréchal, E., Bligny, R., Joyard, J., Block, M.A.: Transient increase of phosphatidylcholine in plant cells in response to phosphate deprivation. - FEBS Lett. **544**: 63-68, 2003.

Khozin-Goldberg, K., Cohen, Z.: The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneus*. - Phytochemistry **67**: 696-701, 2006.

Kobayashi, K., Masuda, T., Takamiya, K., Ohta, H.: Membrane lipid alteration during phosphate starvation is regulated by phosphate signaling and auxin/cytokin cross-talk. - Plant J. **47**: 238-248, 2006.

Kock, J.L.F., Botes, P.J., Erasmus, S.C., Lategan, P.M.: A rapid

method to differentiate between four species of Endomycetaceae. - J. gen. Microbiol. **131**: 3393-3396, 1985.

Li, M., Welti, R., Wang, X.: Quantitative profiling of *Arabidopsis* polar glycerolipids in response to phosphorus starvation. Roles of phospholipases Dz1 and Dz2 in phosphatidylcholine hydrolysis and digalactosyldiacylglycerol accumulation in phosphorus-starved plants. - Plant Physiol. **142**: 750-761, 2006.

Metzner, H., Rauh, H.J., Senger H.: The synchronization of some pigment-deficient mutants of *Chlorella*. - Planta **65**: 186-190, 1965.

Melton, S., Moyers, R., Play-Ford, C.: Lipid extracted from soy bean products by different procedures. - J. amer. oil chem. Soc. **56**: 489-493, 1979.

Osman, M.E.H., El-Shintinawy, F.: Photosynthetic electron transport under phosphorylating conditions of various salts. - J. exp. Bot. **39**: 859-863, 1988.

Plesnicar, M., Kastori, R., Petrovic, N., Pankovic, D.: Photosynthesis and chlorophyll fluorescence in sunflower (*Helianthus annuus* L.) leaves as affected by phosphorus nutrition. - J. exp. Bot. **45**: 919-924, 1994.

Raghothama, K.G.: Phosphate acquisition. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 665-693, 1999.

Raghothama, K.G., Karthikeyan, A.S.: Phosphate acquisition. - Plant Soil **274**: 37-49, 2005.

Rao, I.M.: The role of phosphorus in photosynthesis. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 173-194. Marcel Dekker, New York 1997.

Rao, I.M., Terry, N.: Leaf phosphate status, photosynthesis, and carbon partitioning in sugar beet: I. Changes in growth, gas exchange, and Calvin cycle enzymes. - Plant Physiol. **90**: 814-819, 1989.

Ripley, B.S., Redfern, S.P., Dames, J.: Quantification of the photosynthetic performance of phosphorus-deficient *Sorghum* by means of chlorophyll-*a* fluorescence kinetics. - South Afr. J. Sci. **100**: 615-618, 2004.

Siegenthaler, P.A.: Molecular organization of acyllipids in photosynthetic membranes of higher plants. - In: Siegenthaler, P.A., Murata, N. (ed.): Lipids in Photosynthesis. Pp. 119-144. Kluwer Academic Publishers, Dordrecht 1998.

Siron, R., Giusti, G., Berland, B.: Changes in the fatty acid composition of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* during growth and under phosphorus deficiency. - Mar. Ecol. **55**: 95-100, 1989.

Stewart, E.A., Grimshaw, H.M.: Chemical analysis of ecological materials. - In: Stewart, E.A. (ed.). Phosphorus and Compounds. Pp. 206-209. Blackwell, Madison 1974.

Thomson, W.W., Weier, T.E., Drever, H.: The fine structure of chloroplasts from mineral deficient leaves of *Phaseolus vulgaris*. - Amer. J. Bot. **49**: 1047-1055, 1964.

Ticconi, C.A., Delatorre, C.A., Abel, S.: Attenuation of phosphate starvation responses by phosphite in *Arabidopsis.* - Plant Physiol. **127**: 963-972, 2001.

Tsvetkova, G.E., Georgiev, G.I.: Effect of phosphorus nutrition on the nodulation, nitrogen fixation and nutrient-use efficiency of *Bradyrhizobium japonicum*-soybean (*Glycine max* L. Merr) symbiosis. - Bulg. J. Plant Physiol. **2003** (Special issue): 331-335, 2003.

Vance, C.P., Uhde-Stone, C., Allan, D.H.: Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. - New Phytol. **157**: 423-47, 2003.

Yan, F.R., Schaffer, S., Fortmeier, H., Schubert, S.: Adaptation of active proton pumping and plasmalemma ATP-ase activity of corn roots to low root medium pH. - Plant Physiol. **117**: 311-319, 1998.

Yang, W., Liu, S., Feng, F., Hou, H., Jiang, G., Xu, Y., Kuang, T.: Effects of phosphate deficiency on the lipid composition in cucumber thylakoid membranes and PSII particles. - Plant Sci. **166**: 1575-1579, 2004.

Zaheer, A., Gill, M.A., Qureshi, R.H.: Genotypic variations of phosphorus utilization efficiency of crops. - J. Plant Nutr. **24**: 1149-1171, 2001.

Zhou, Y.H., Wu, J.X., Zhu, L.J., Shi, K., Yu, J.Q.: Effects of phosphorus and chilling under low irradiance on photosynthesis and growth of tomato plants. - Biol. Plant. **53**: 378-382, 2009.