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Phosphorus Deprivation Effects on Productivity, Photosynthesis and Carbohydrates Accumulation in Hydroponically Grown *Brassica Alboglabra* Baileyat Different Growth Stages

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Abstract: *Brassica alboglabra* Bailey plants were first grown under full nutrient solution for 2, 3 and 4 weeks and then were transferred to phosphorus (P)-deprivation solution for 3 weeks (–P3), 2 weeks (–P2) and 1 week (–P1), respectively. The total growth duration was 5 weeks for all plants and the full P plants were grown under complete nutrient solution for 5 weeks. Full P and –P1 plants had similar productivity which was significantly higher than –P2 and –P3 plants. P-deprivation treatments caused a reduction of the P concentration in all treated plants compared to full P plants. The total P content per plant was significantly higher in full P than any other P-deprivation plants. P-deprivation did not cause significant changes in chlorophyll (Chl) fluorescence F_v/F_m ratio and Chl content. There were no differences in light saturated photosynthetic CO_2 assimilation (A_{sat}) and stomatal conductance (g_{ssat}) in young leaves (YL) among all plants. However, P-deprivation resulted in reduction of g_{ssat} for old leaves (OL).Full P plants had the lowest soluble sugarsaccumulated inboth YL and OL compared to P-deprivation plants. There were no significant differences in the concentration of insoluble sugar of YL among all plants. The concentration of insoluble sugar in OL of full P and –P3 plants were significantly lower than in –P1 and –P2 plants. This study concludes that *B. alboglabra*Baileyplants are able to accumulate adequate P in its early growth stages and reserves of P are sufficient one week before harvest for quality crop yield. Relationships among productivity, photosynthesis and carbohydrate levels under P deprivation at different growth stage were discussed.

Keywords: Carbohydrates accumulation, Phosphorus concentration, Phosphorus deprivation**,** Photosynthesis, Productivity.

INTRODUCTION

Phosphorus (P) is a macronutrient that is important for plant growth and development. It is probably the most important fertilizer for crop plants [1] as it is involved in energy generation, photosynthesis, carbohydrate metabolism, membrane biosynthesis and stability and enzyme activation or inactivation [2-4]. Under P deficiency, whereby P supply is restricted, the plant growth rate decreases before there is any significant effect on photosynthesis [3]. During P deficiency, the export of triose-Pi from the chloroplast to cytosol is reduced, resulting in its conversion to starch in the chloroplast [4].

Although it is a primary macronutrient, P is required in the lowest amount of the three primary macronutrients, usually 50% less than either N or K. For vegetable plants grown in hydroponics system, full nutrient solution is continuously supplied to the roots. Generally, P tends to be moderately immobile in soil-based media while soilless media of hydroponics culture gives the root immediate access to even the least mobile ions [5]. In the study with *Sesbania rostrata* seedlings, Aono *et al*., [6] pre-cultured the min hydroponic solutions with normal hydroponic levels of P and then transferred them to hydroponics with or without P. They found that the seedlings without P for a few days had adequate reserve P levels, allowing them to respond to the absence of P for several additional days before internal concentrations begin to inhibit growth [6].During the active vegetative growth stage, hydroponically grown vegetables may be able to accumulate excessive P and store it in their vegetative tissues and re-use it when plants are subjected to P deficiency [7]. However, to what extent which P deprivation affects the final yield and photosynthesis is poorly understood. On the other hand, over application of P for soilless culture could result in P pollution when excessively applied portions of P may runoff into and create ecological imbalances in nearby waters [8-10]. Of environmental hazards and the idea that P recycling is possible, this paper aims to investigate if reduced application of P in hydroponic culture, by providing plants with adequate P first and then depriving it at a later stage, can be done without compromising on crop yield. We hypothesize that it may depend on the growth stage when plants are subjected to P deprivation. The objectives of this project are to investigate the effects of P deprivation on not only productivity especially the partitioning of shoot and

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root dry matter and photosynthesis but also levels of both leaf starch and soluble carbohydrates of hydroponically grown *B. alboglabra* at different growth stages.

MATERIALS AND METHODS

Plant Material and Culture Methods

Brassica Alboglabra

Bailey(cultivar: #1816 New Veg-Gin) also known as Chinese broccoli was used for this study. Three days after germination, seedlings were inserted onto polyurethane cubes. The seedlings were then grown in the greenhouse for 4 days for acclimatization before transplanting onto the hydroponic trays. All plants were exposed to fluctuating ambient temperature (26-36^{o}C) under 100% of prevailing solar radiation with average maximal photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1}. All plants were supplied with full strength Netherlands Standard Composition [11]. Nutrient solution conductivity and pH were maintained at 2± 0.2mS and 6.5 ± 0.5, respectively. The composition of full strength nutrient solution in mg l^{-1} was: K_2HPO_4, 187; Ca $(NO_3)_2.4H_2O$, 1237; $MgSO_4.7H_2O$, 609; K_2SO_4, 252; KNO_3, 293; Fe EDTA, 20.52; $ZnSO_4.7H_2O$, 0.06; $CuSO_4.5H_2O$, 0.06; H_3BO_3, 0.59; $MnSO_4.H_2O$, 0.73; and $(NH_4)_6$ $Mo_7O_{24}.4H_2O$, 0.75.

P-deprivation Treatments

In this study, the experiments were carried out by first growing all plants under full nutrient solutions for 2, 3 and 4 weeks and then transferring them to P-deprivation solution for 3, 2 and 1 weeks respectively. Hence, the different periods of P deprived treatment were 3weeks (–P3), 2 weeks (–P2) and 1 week (–P1), respectively and the total plant growth period was 5 weeks for all plants. Those grown under full nutrient solution stated above were defined as full P plants. P-deprivation plants received an iso-osmotic P free solution in which P was replaced by chloride and the cat ions were unchanged. Before P-deprivation treatment, the youngest fully developed leaves were marked and defined as old leaves (OL) and those that emerged after the treatment were termed as young leaves (YL).

Measurement of Fresh Weight (FW) and Dry Weight (DW)

At harvest (5 weeks after transplanting), 8 plants were randomly selected from the trays between 4:00 and 5:00 in the afternoon. Photographs of the harvested plants were taken and the morphological appearances of the plants were observed and recorded. The harvested plants were separated into root, stem, petiole, YL and OL and the FW of these plant tissues were measured separately using a weighing balance (Sartorius, Fisher General Scientific Private Limited, Singapore). The roots were washed in tap water and dabbed dry with tissue paper immediately before weighing. After that, the separated plant parts were wrapped up in aluminum foil and dried in an oven at 80^{0}C. After about one week, these plant tissues were reweighed for DW. The shoot/root ratio was calculated for FW and DW, respectively.

Determination of P Concentration and Total P Content

The same plant materials used for measurement of shoot and root FW and DW were used for this analysis. A dry sample of 0.03 g was placed into a digestion tube with a Kjeldahl tablet with 3ml of concentrated H2SO4. After the digestion was completed, 2.5ml of digested liquid was transferred into a 100ml volumetric flask and the mixture was topped up to 100ml with deionised water. Then 5 ml of the diluted solution was transferred into a 25 ml volumetric flask and 2.5 ml of ammonium molybdate reagent and 2 ml of stannous reagent were added subsequently. The mixture was made up to 25 ml with deionised water and mixed thoroughly before it was allowed to react for 30 min. The absorbance of the sample was measured at 700 nm using a spectrophotometer (DU650, Beckman, USA). The amount of P present in the sample was determined from standard assay. Using the sample DW measured before this analysis, the total P content was calculated as [P concentration × DW].

Measurements of Light Saturated Photosynthetic CO_2 Assimilation, A_{sat} and Stomatal Conductance, g_{ssat} in the Greenhouse

These parameters were measured for both YL and OL using LI-COR Portable Photosynthesis System (LI-6400, LI-COR Biosciences-U.S., USA) between 10:00 and 11:00 in the morning in the greenhouse with an open infrared gas analysis system with a 6-cm^2 chamber (LI-6400, Biosciences-U.S.). Readings were taken with a LED light source which supplied 1200 μmol m^{-2} s^{-1}ofPPFD. The light source emitted in the wavelength ranged from 660 to 675 nm. Average [CO_2] and relative humidity in the chamber were 400 ± 5 μmol mol^{-1} and 70%, respectively. Leaf chamber temperature was set according to prevailing ambient conditions (32^{o}C).Preliminary experiments measured A_{sat} and g_{ssat} using natural sunlight at about 1200 μmol m^{-2} s^{-1}ofPPFD at 32-33^{o}C and obtained the similar levels of A_{sat} and g_{ssat}. The values were recorded when both A_{sat} and g_{ssat} were stable (usually within 3-5 min). For each treatment, five measurements were made from five different leaves of five different plants (n = 5).

Determination of Chl Fluorescence F_v/F_m Ratio

F_v/F_m ratios were taken with the Plant Efficiency Analyser (Hansatech Instruments Ltd, England) from the same leaves from which A_{sat} and g_{ssat} were recorded. The readings were carried out from 8:30 to 9:00 in the morning. Attached leaves were pre-darkened with clips for 15 min prior to measurements. Dark-adapted leaves were placed under the light pipe and irradiated with the pulsed lower intensity-measuring beam to measure F_0, initial Chl fluorescence. F_m, maximum Chl fluorescence was assessed by 0.8 s of saturated pulse (>6000 μmol m^{-2} s^{-1}). The variable fluorescence yield, F_v, was determined by F_m–F_0. The efficiency of excitation energy captured by open PSII reaction centres in dark-adapted plant samples was estimated by F_v/F_m ratio.

Determination of Chl Content

Samples of 0.05 g of fresh leaves were weighed and cut into small pieces. Chl was extracted from these samples us-

ing dimethylformamide and quantified spectrophotometrically at 647 and 664 nm [12].

Determination of Soluble and Insoluble Sugar

Soluble sugars were extracted using a method modified from Buy see and Merckx [13]. Samples of about 0.05 g of dried YL and OL were extracted three times in 10 ml of hot 80% ethanol (65^0C) without grinding. The supernatants were pooled and made to a convenient volume. To 1 ml of extraction solution, 1 ml of 28% phenol was added, followed by 5 ml of concentrated H_2SO_4. The reaction mixture was mixed thoroughly and then allowed to stand for 15 min. A small aliquot was poured into a 1 ml glass cuvette, and the absorbance was recorded at 490 nm using a spectrophotometer. Total sugar content of the sample was calculated based on a calibration curve from a glucose working standard. Insoluble sugars were extracted from the residual plant material from the soluble sugar extraction described above. This was done by incubating the dry pellet with 5 ml of 3% HCl in a boiling water bath for 3 h. The soluble products were assayed by the same phenol-sulphuric method as that of soluble sugars.

Statistical Analysis

Statistical analysis was conducted by ANOVA to discriminate means across all four treatments, followed by using Tukey's multiple comparison test. The difference between treatment means were considered significant at $p < 0.05$. All statistical analyses were carried out using MINITAB software (MINITAB, Inc., Release 15, 2007).

RESULTS

Plant Appearance

All plants shown in Plate (**1**) were the same age and they had been grown for 5 weeks after transplanting. There were no significant differences in plant size between full P and –P1 plants. However, –P2 and –P3 plants were smaller than full P and –P1 plants. There were no differences in the leaf colour and they all seemed to be healthy and they were similarly dark green regardless of P-deprivation treatments (Plate **1**).

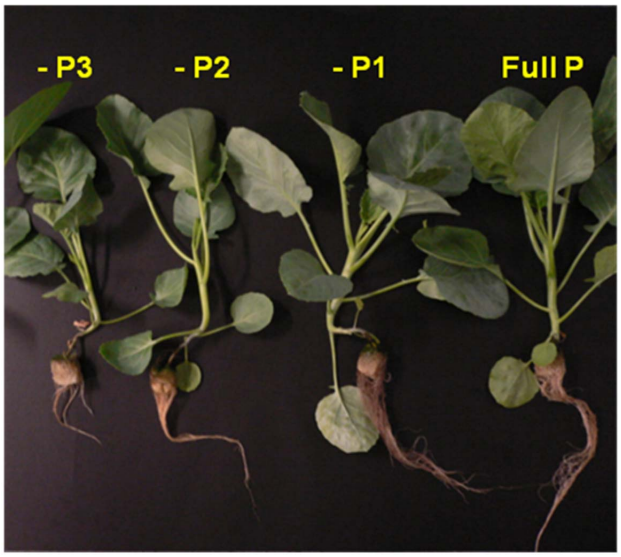

Plate (1). *B. alboglabra* plantsgrown under different P treatments for 5 weeks. Full P, 5 weeks full P; –P1, 4 weeks full P + 1 week P-deprivation; –P2, 3 weeks full P + 2 weeks P-deprivation; –P3, 2 weeks full P + 3 weeks P-deprivation.

Productivity

There were no significant differences in shoot FW (Fig. **1A**) and root FW (Fig. **1B**) between the full P and –P1 plants. However, shoot and root FW were significantly

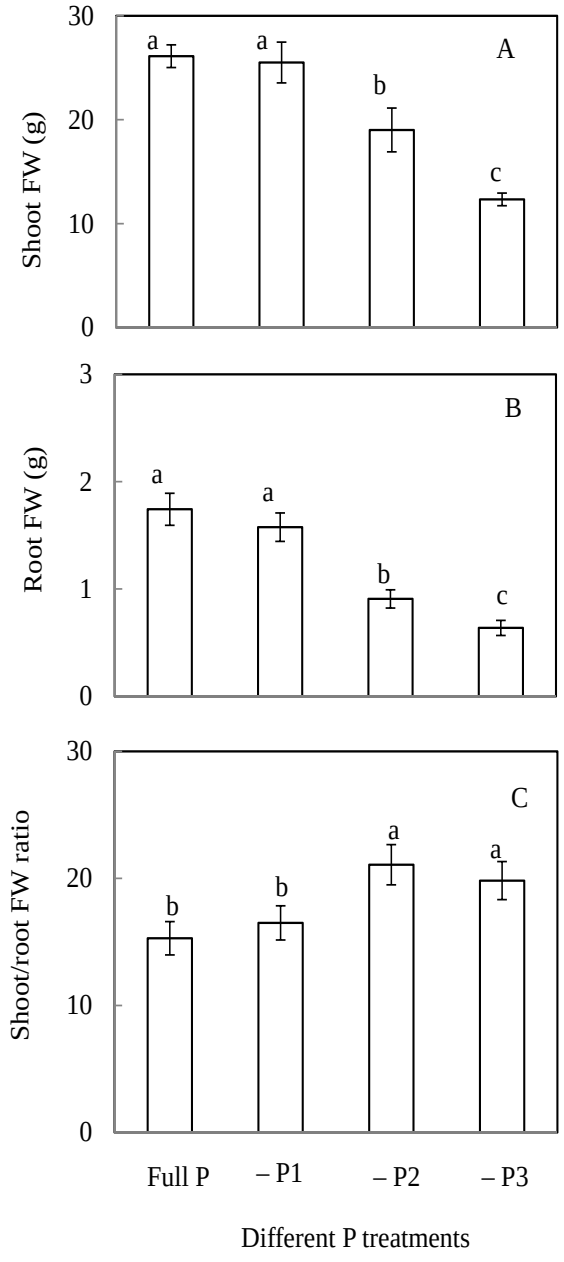

Fig. (1). FW of shoot (**A**), root (**B**), shoot/root FW ratio (**C**) of *B. alboglabra* plants after different P treatments for 5 weeks. Each point is the mean of 8 measurements of 8 different plants. Vertical bars represent the standard errors. Means with different letters above the columns are statistically different ($p<0.05$) as determined by Tukey's multiple comparison test.

higher in full P and –P1 plants than –P2 and –P3 plants (Figs. **1A** and **1B**). These data agreed well with the observations shown in Plate 1. The –P2 and –P3 plants had significantly higher shoot/root FW ratios than full P and –P1 plants (Fig. **1C**). Changes of shoot DW, root DW and shoot/root DW ratio after different P-deprivation treatments were similar to those of shoot FW, root FW and shoot/root FW ratio (data not shown).

P Concentration and Total P Content

For full P plants, the P concentration of YL was significantly higher than that of OL (Fig. **2A**). The P concentrations of YL and OL of –P1, –P2 and –P3 plants were all significantly lower than those of full P plants. However, there were no significant differences in P concentrations between YL and OL for any of P-deprivation treated plants (Fig. **2A**). It was interesting that roots had significantly higher P concentration than those of stems and petiole of any P treated plants (Fig. **2B**). P concentration of root was highest in full P plants. However, there were no differences in P concentrations between stem and petiole among all plants (Fig. **2B**). The total P content of both shoot and root per plant was significantly higher in full P plants than any other P-deprivation plants (Fig. **2C**). The longer the plants were subjected to P-deprivation, the lower the total P contents were. For total P shoot/root ratio, –P1, –P2 plants were significantly higher than those of full P and –P3 plants, indicating that when plants subjected to short periods of P-deprivation, more P was transported to the shoot (Fig. **2D**).

A_{sat} and g_{ssat}

For full P plants, there was no significant difference in A_{sat} (Fig. **3A**) between YL and OL. When comparing A_{sat} of the YL among the treatments, –P1 plants were significantly lower than other plants. However, OL of full P plants had A_{sat} significantly higher than those of any other P-deprivation plants. OL of –P3 plants had the lowest A_{sat}. Similar to A_{sat}, there were no differences in g_{ssat} between YL and OL of full P plants and no differences in this parameter for all YL among all plants (Fig. **3B**). But, OL of full P plants had significant higher g_{ssat} than any of the other plants which were subjected to different periods of P-deprivation.

Chl*a* Fluorescence F_v/F_m Ratio

Both YL and OL of all plants had F_v/F_m ratios ranged from 0.800 to 0.820 (Table **1**), indicating that none of them experienced photoinhibition including the P-deprivation treated plants.

Chl Content

Chl content was measured for all plants after they were grown under different P conditions for 5 weeks. For plant grown under the same P condition, YL had significantly higher Chl content than OL did (Table **1**). However, there were no significant differences in Chl content of either OL or YL among different P-deprivation treatments (Table **1**). These findings imply that P treatment did not result in changes of Chl content and these were well matched with the

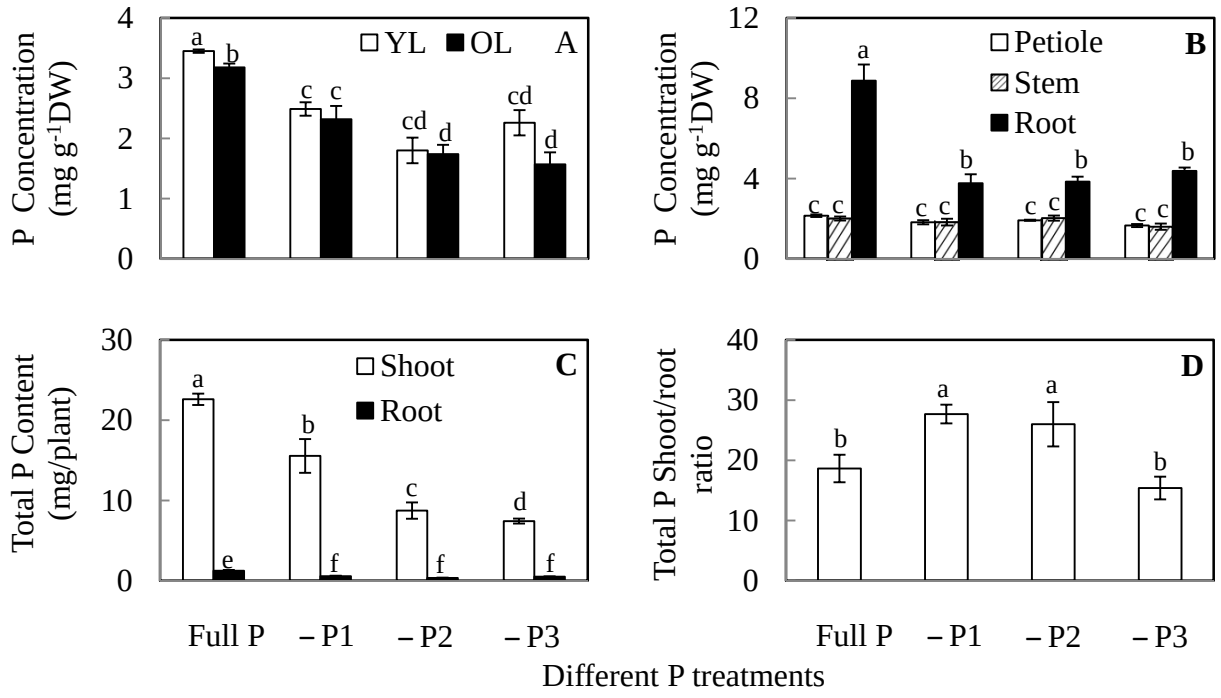

Fig. (2). P concentration of YL and OL (**A**), petiole, stem and root (**B**); total P content per plant (**C**) and total P shoot/ratio (**D**) of *B. alboglabra* plants after different P treatments for 5 weeks. Each point is the mean of 5 measurements of 5 different plants. Vertical bars represent the standard errors. Means with different letters above the columns are statistically different (p<0.05) as determined by Tukey's multiple comparison test.

Table 1. Chl Fluorescence F_v/F_m Ratio and Total Chl Content of YL and OL of *B. Alboglabra* Plants After Different P Treatments for 5 Weeks. Each Value is the Mean of 5measurements of 5different Plants. Means with Different Letters are Statistically Different (p<0.05) as Determined by Tukey's Multiple Comparison Test

P Treatments	F_v/F_m Ratio	Total Chl Content (μg g^{-1} FW)
Full P		
YL	0.809 ± 0.004^a	2028.32 ± 7.44^a
OL	0.809 ± 0.004^a	1697.69 ± 11.25^b
–P1		
YL	0.809 ± 0.004^a	2149.45 ± 6.52^a
OL	0.809 ± 0.004^a	1881.74 ± 9.11^b
–P2		
YL	0.812 ± 0.015^a	2114.13 ± 5.39^a
OL	0.820 ± 0.021^a	1802.72 ± 4.23^b
–P3		
YL	0.812 ± 0.015^a	2037.07 ± 9.45^a
OL	0.820 ± 0.021^a	1707.88 ± 10.02^b

observations shown in Plate 1. Our results also show that there were no significant differences in Chl a/b ratio among all plants (data not shown).

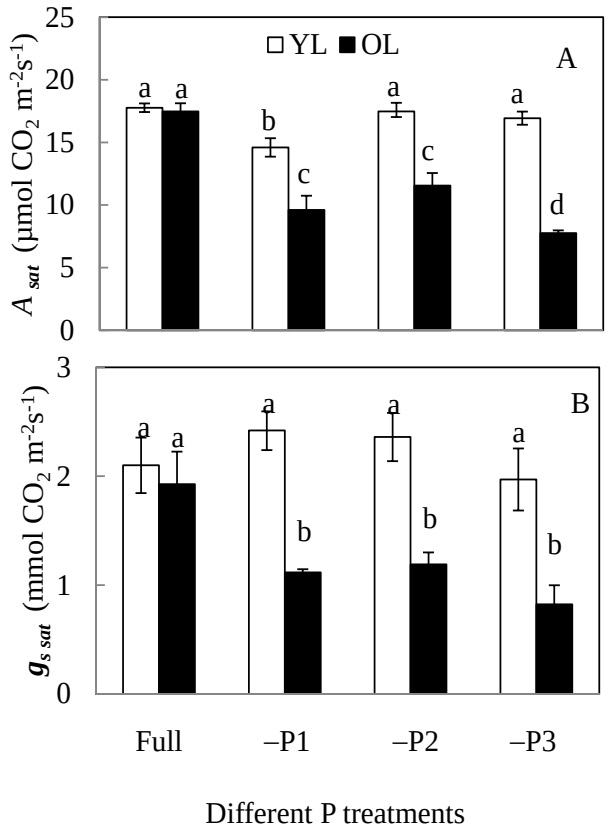

Fig. (3). A_{sat}(**A**) and g_{ssat}(**B**) of YL and OLof *B. alboglabra* plants after different P treatments for 5 weeks. Each point is the mean of 5 measurements of 5 different plants. Vertical bars represent the standard errors. Means with different letters above the columns are statistically different (p<0.05) as determined by Tukey's multiple comparison test.

Soluble and Insoluble Sugars

Full P plants had the lowest soluble sugars accumulated in both YL and OL compared to any other P-deprivation treated plants (Fig. **4A**), indicating that translocation of carbohydrate out of photosynthetic leaves during the photoperiod was most effective in plants supplied with full P nutrient. Among the different P-deprivation treated plants, –P1 plants had significantly higher concentration of total soluble sugar than –P2 and –P3 plants did (Fig. **4A**). There were no significant differences in the concentratin of insoluble sugar of YL among different P treatments (Fig. **4B**). However, the concentration of insoluble sugar in OL of full P and –P3 plants were significantly lower than in –P1 and –P2 plants (Fig. **4B**). Total insoluble/soluble sugar ratios of YL were lower in all P-deprivation plants than in full P plants (Fig. **4C**). However, there were no significant differences in this ratio in the OL among different P treated plants (Fig. **4C**).

DISCUSSION

In this studythe withdrawlof P from nutrient solution for one weekbefore harvest(–P1 plants) did not result in reduction of shoot and root FW compared to full P plants (Figs. **1A** and **1B**). These findings indicate that hydroponically grown *B. alboglabra* plants accumulated adequate P during its early growth stages and P could be totally withdrawn one week before harvest. Aono *et al*. [6] precultured *S. rostrata* seedlings with full P and then withdrew P for a few days. They found that *S. rostrata* seedlings had adequate reserve P levels for several additional days before internal concentrations begin to inhibit growth [6]. This knowledge could be put to practice as it would reduce environmentally hazardous waste [8-10]. However, when subjected to longer periods of P-deprivation for 2 or 3 weeks, *B. alboglabra* plants exhibited marked reductions in both shoot FW and root FW (Figs. **1A** and **1B**). These results agree with that when P supply is restricted for prolonged periods, the plant growth rate decreases [3]. Higher shoot/root

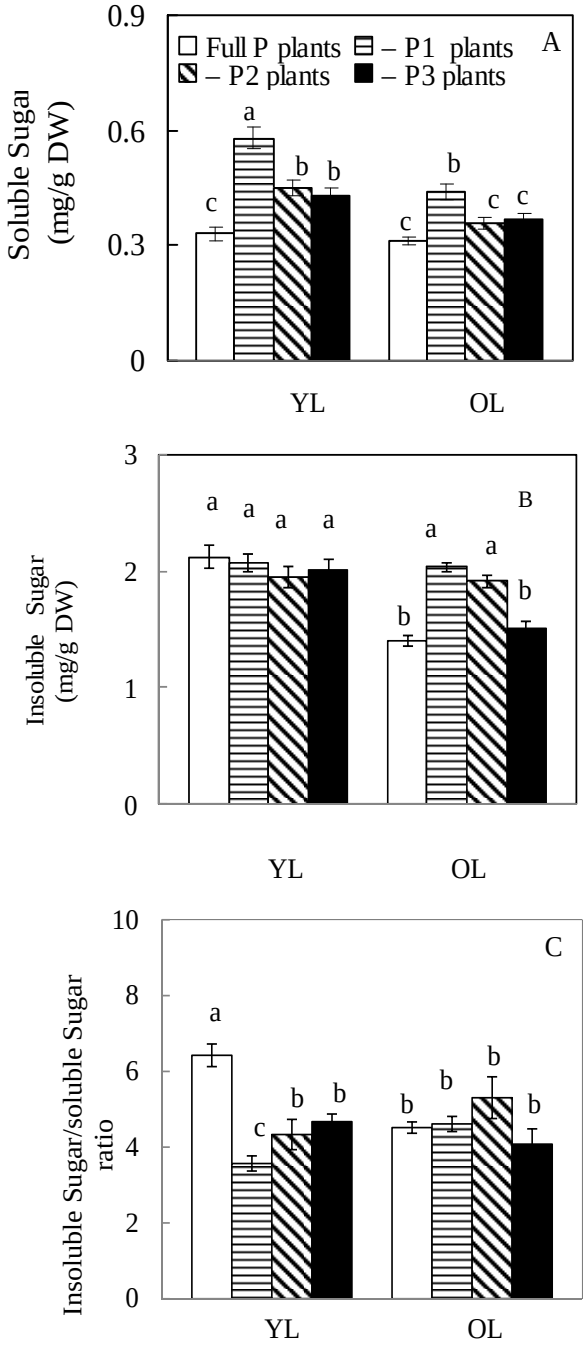

Fig. (4). Concentration of total soluble (**A**), insoluble sugar (**B**) and total insoluble sugar/soluble sugar ratio (**C**) of YL and OLof *B. alboglabra* plants after different P treatments for 5 weeks. Each point is the mean of 5 measurements of 5 different plants. Vertical bars represent the standard errors. Means with different letters above the columns are statistically different (p<0.05) as determined by Tukey's multiple comparison test.

FW ratios were observed for –P2 and –P3 plants (Fig. **1C**), indicating that these hydroponically grown plants have evolved different developmental adaptations to low P supply as compared to many plant species grown in soil. According to literature, the shoot/root ratio is often decreased by a soil factor such as low availability of P [4, 14-19]. Developmental responses often involve changes in root architecture, giving a typical increase in root/shoot growth ratio (or decrease in shoot/root growth ratio) under P deficiency [19].These adaptations generally serve to increase the efficiency of uptake and managing P through remobilization or recycling activities and to prioritize the use of P for prolonged usage [20]. However, in the present study, although P-deprivation caused a reductionin P concentration, the P concentration indifferent tissues of all plants fall in the range of2 mgg^{-1} DW or 0.2% dry mass (Figs. **2A** and **2B**) a recommended minimum average level that should be present in plant tissues (Epstein as cited in Taiz & Zeiger, 2006, p.74) [21]. Furthermore, for other organs such as petioles, stems and roots, they had high P concentration (Fig. **2B**) and contributed substantially to the total accumulation of P per plant (Fig. **2C**). According to Akhtar [22], variations in P concentrations of the various plant organs under P stress is due to the tendency of *Brassica* cultivars to ration resources to metabolically active sites such as the leaves and roots. The high concentration of P in roots could be transported to shoot when plants were subjected to short periods of P deprivation. This was supported by the higher total P shoot/root ratio in –P1 and –P2 plants (Fig. **2D**). Lalitha [23] reported that a hydroponics technique allows for increased P mobility into the plants. Thus, it is evident that initial periods of P-deprivation or having shorter periods of P deprivation, would result in a remarkable increase in P uptake and an activation of biochemical adaptations to conserve pools of acquired P [20].

Mikulska *et al*., [24] found that P starvation resulted in photosynthetic rate declined to 50% of the control bean plants. The observation that a low P level in the plant tissue decreases the rate of photosynthesis was also reported in other crops such as barley seedlings [25], sugar beet [26] and sunflower, maize and wheat plants [27-29]. However, in the present study, there were no differences in A_{sat} and g_{ssat} YL among full P, –P2 and –P3 plants (Fig. **3**), suggesting that P-deprivation has caused internal P recycling from other tissues to support the growth and normal functioning of YL photosynthesis. Although P-deprivation caused decreases in P concentration, both YL and OL had the minimum average level of P concentration of 2 mg g^{-1} DW or 0.2% dry mass (Fig. **2A**) [21]. Lower A_{sat} of YL in –P1 plants was due to the fact that they werenot yet fully matured when the measurement was carried out. However, P-deprivation resulted in reduction of A_{sat} and g_{ssat} for all OL. Clarkson *et al*., [30] reported that P-deficiencies result in major reductions of root hydraulic conductivity which may lead to lowered stomatal conductance. Reductions of A_{sat} and $g_{ssat}$of OL were partly responsible for the lower productivity of P-deprivation plants (Plate **1** and Fig. **1**). However, P-deprivation did not cause significant changes in Chl fluorescence F_v/F_m ratio and Chl content (Table **1**).

In the present study, full P plants had the lowest soluble sugars in both YL and OL compared to any other P-deprivation plants (Fig. **4A**), indicating that translocation of carbohydrate out of photosynthetic leaves during the photoperiod is most active in plants supplied with full P nutrient. Higher translocation of newly fixed carbon resulted in higher productivity (Plate **1** and Fig. **1**) [31]. However,–P1 plants had significant higher concentration of total soluble sugar than those of –P2 and –P3 plants.

This could be due to the observation that the YL of –P1 plants have not fully matured when they were harvested. For leaves which are not fully developed, they act as both source and sinks, and retained larger amount of carbohydrate for their own development [31]. High sink strength of other non-foliar tissues increases sucrose synthesis of matured source leaves, which in turn brings on the recycling of more cytosolic inorganic P into the chloroplast, resulting in the possibility of replenishing ATP and RuBP pool for photosynthesis and thus growth [3]. There were no significant differences in the concentration of insoluble sugar of YL among different P treatments (Fig. **4B**). The concentration of insoluble sugar in OL of full P and –P3 plants were significantly lower than in –P1 and –P2 plants (Fig. **4B**). When a plant is subjected to P starvation, its typical response is to accumulate insoluble starch in order to recycle substantial amounts of P from phosphorylated precursors and to protect the plant from photoinhibition [32]. All plants had Chl fluorescence F_v/F_m ratios greater than 0.8 (Table **1**), which explain that photoinhibition did not occur in any plants in the present study. It was also reported that an increase in the insoluble starch/soluble sucrose ratio with low P treatment plants [33-34]. However, in the presents study, we found that total insoluble/soluble sugar ratios of YL were higher in full P plants than all P-deprivation plants (Fig. **4C**). There were no significant differences in this ratio in the OL among different P treated plants (Fig. **4C**). Effects of P-deprivation on the allocation and partitioning of different carbohydrates merit our further studies. On the other hand, it is well known that P deficiency modifies not only primary metabolism but also secondary metabolism of plants [35]. Whether P-deprivation for one week would significantly affect the quality of *B. alboglabra* should also be addressed in our future study.

CONCLUSION

This study found that *B. alboglabra*Bailey plants are able to accumulate adequate P in its early growth stages and reserves of P are sufficient for one week before harvest for quality crop yield. One week of P withdrawal in agriculture not only reduces pollution to the environment but also reduces the costs of P fertilizers.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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