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Full Length Research Paper

Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on photosynthesis and chlorophyll content in *Beta vulgaris subsp. Cycla* 'fordhook giant' grown in hydroponic cultures

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The assessments of photosynthetic rate, stomatal conductance, evapotranspiration, intercellular CO_2 concentration and chlorophyll content in *Beta vulgaris subsp. cycla* 'Fordhook Giant' grown in hydroponic cultures containing different compositions of hydroponic solutions were evaluated in this study. The aim of the study was to quantify the effects of different combinations of Hoagland's solution and *Azolla filiculoides* on photosynthesis processes and chlorophyll content in *B. vulgaris* grown in hydroponic cultures. The following treatments were evaluated in four replications: (1) Control (Hoagland's solution minus N solution excluding *Azolla*; (2) Hoagland's minus N solution including *Azolla*; (3) full Hoagland's solution plus *Azolla*; and (4) full Hoagland's solution excluding *Azolla*. Results show that photosynthetic rate, evapotranspiration, intercellular CO_2 concentration and chlorophyll were generally higher in full Hoagland's solution. This was closely followed by full Hoagland's solution plus *Azolla*, and Hoagland's minus N solution plus *Azolla* treatments. The lowest photosynthetic rates and chlorophyll contents were found in the control (Hoagland's minus N solution) treatment.

Key words: Photosynthetic rate, stomatal conductance, evapotranspiration, intercellular CO_2 concentration, chlorophyll a, chlorophyll b.

INTRODUCTION

Photosynthesis and chlorophyll concentration are directly related to N inputs in plants (Schepers et al., 1992; Blackmer and Schepers, 1995; Guo, 2001). In the photosynthesis process, nitrogen plays an important function in electron transport and photophosphorylation (Terashima and Evans, 1988). Chlorophyll is also constituted by nitrogen as it forms an important part in the formation of chloroplasts resulting in N being stored in the chloroplasts (Evans, 1989). It has been noted that the rate of photosynthesis and chlorophyll concentration are directly affected by nitrogen. Hence photosynthesis will increase if N is adequate during the process (Paul and Driscoll, 2008). The green pigmentation in the plant (chlorophyll) is directly related to N content, as N is stored in the chloroplasts (Evans, 1989; Schepers et al., 1992; Blackmer and Schepers, 1995). N richer plants are greener, whereas the deficient ones have yellow discolouration.

Nitrogen can be applied through biological and chemical nitrogen fertilizers (Roger and Reynaud, 1982;

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Abbreviations: DMSO, Dimethyl sulphoxide; **DWC,** deep water channel; **PVC,** polyvinylchloride.

Kumarasinghe and Eskew, 1995). Chemical nitrogen fertili-zers can be applied in different forms such as nitrate and ammonium nitrogen (Cox and Reisenauer, 1973; Reddy et al., 1989; Pahlsson, 1992; Lu et al., 2005). These substances are a main component in agrarian applications as they are able to ensure sufficient crop harvests (Watanabe and Liu, 1992; Kumarasinghe and Eskew, 1995). Biological nitrogen systems such as those involving *Azolla filiculoides* are able to fix nitrogen from the atmosphere into the surrounding aqueous environment (Roger and Ladha, 1992). *A. filiculoides* achieves this by having a symbiotic relationship with cyanobacteria *Anabaena azollae* (Shi and Hall, 1988). *Anabaena* occurs extracellularly and fixes nitrogen in the leaf fronds of *A. filiculoides* (Peters, 1977, 1978). *Azolla* provides suitable surroundings for the *Anabaena* to survive in. *A. filiculoides* and *A. azollae* affiliation has been used as a biological source of N in rice cultivation (Fogg et al., 1973; Tran and Dao, 1973; Becking, 1976; Talley and Rains, 1980; Roger and Reynaud, 1982; Watanabe, 1984; Watanabe and Liu, 1992; Wagner, 1997).

The production of N from biological sources namely that of the *Azolla-Anabaena* symbiosis has shown promising potential to be used as a source N in crop cultivation. In this study, an experiment was conducted to evaluate the effect of this symbiosis on other food crops such as *B. vulgaris* in hydroponic cultures. This study was conducted with the objective of quantifying the effects of different combinations of Hoagland's solution and *A. filiculoides* on photosynthesis processes and chlorophyll content in *B. vulgaris* grown in hydroponic cultures.

MATERIALS AND METHODS

The physiological responses photosynthesis and chlorophyll concentration of *B. vulgaris* was measured throughout an 8 week period. An actively ventilated greenhouse sited at the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa was used to facilitate the experiment. A polyvinyl chloride (PVC) pipe deep water channel (DWC) hydroponic system on a four block experiential design was employed (Roberto, 2000). Each treatment comprised of four PVC pipes containing 20 plants resulting in 80 plants for the experiment. A 3500 L/h submersible pump circulated the nutrient solutions contained within a 70 L reservoir.

The PVC systems discussed by Roberto (2000) were suitable to cultivate *B. vulgaris* and *A. filiculoides. B. vulgaris* was positioned in the channels and allowed to establish while being supplied with flowing nutrient solution within the gully. *A. filiculoides*, a floating water fern located in nutrient reservoir, was allowed to drift on the nutrient solution. Both plants species were exposed to the nutrient solution within each treatment. The plant identification section at CPUT rendered the botanical material of *A. filiculoides* and a garden centre located in Cape Town provided *B. vulgaris* seedlings. A total of 70 g of *A. filiculoides* was introduced to two of the systems one week earlier to that of the *B. vulgaris*. A one week period allowed for the fixation of N into the nutrient solution while *A. filiculoides* stabilised. *B. vulgaris* was placed at a plant spacing of 40 cm. Two compositions were utilized: a full Hoagland's solution and a Hoagland's minus nitrogen nutrient solution (Hershey, 1994, 1995). Macro elements were characterized by 2 M KNO_3; 2 M $Ca(NO_3)_2 \times 4H_2O$; 1 M NH_4NO_3; 1000 mg/L Fe-EDTA; 2 M $MgSO_4 \times 7H_2O$; 1 M KH_2PO_4 and minor elements constituted 0.86 g H_3BO_3; 1.81 g $MnCl_2 \times 4H_2O$; O.22 g $ZnSO_4 \times 4H_2O$; 0.051 g $CuSO_4$; 0.09 g $H_3MoO_4 \times H_2O$ portrayed the full Hoagland's solution. Combination of 0.05 M CaH_2PO_4; 0.01 M $CaS0_4 \times 2H_2O$; 0.5 M K_2SO_4; 1 M $MgSO_4$; 1000 mg/L Fe-EDTA; 1 M KH_2PO_4 as macro elements and 0.86 g H_3BO_3; 1.81 g $MnCl_2 \times 4H_2O$; O.22 g $ZnSO_4 \times 4H_2O$; 0.051 g $CuSO_4$; 0.09 g $H_3MoO_4 \times H_2O$ were used as minor elements for the minus N solution. The research treatments were represented by the control: Hoagland's minus N solution; treatment 1: Hoagland's minus N solution plus *Azolla*; treatment 2: full Hoagland's solution plus *Azolla* and treatment 3: full Hoagland's solution.

Measurement of photosynthesis

A portable photosynthesis system LCpro + 1.0 ADC, (Bioscientific Limited, 12 Spurling Works, Pinder Road, Hoddesdon, Hertfordshire, EN11 ODB, UK) was used to take photosynthesis readings. Measurements were taken on a healthy fully functional third leaf on the apical growth point of each plant. Readings taken between 09.00 to 11.00 a.m from the apparatus included: photosynthetic rate, stomatal conductance, intercellular carbon dioxide concentration and where water in the nutrient solution is absorbed and expelled as the water vapour or evapotranspiration rate.

Determination of chlorophyll concentrations in leaves

A method determined by Hiscox and Israelstam (1979) was used to compare chlorophyll concentrations. Dimethylsulphoxide (DMSO) was added to the plant matter to extract the chlorophyll pigment. Plant material collected from *B. vulgaris* comprised of a small section removed from the tip of the leaf. From this, a 100 mg sample was removed and placed into 50 ml container. 7 ml of DMSO was added and incubated for 72 h at 4°C. 3 ml of DMSO was added to dilute the extraction to 10 ml. Absorbance values were then determined by adding 3 ml of the extracted samples to curvets and measuring them with a spectrophotometer (UV/Visible Spectrophotometer, Pharmacia LKB. Ultrospec II E). Values were determined at 645 and 663 nanometres (nm) by comparing the extracted samples to pure DMSO. Total leaf chlorophyll, chlorophyll a and chlorophyll b were calculated by using the formulae developed by Arnon (1949).

$$Chl_t = 20.2D_{645} + 8.02D_{663}$$

$$Chl\ a = 12.7D_{663} - 2.69D_{645}$$

$$Chl\ b = 22.9D_{645} - 4.68D_{663}$$

Statistical analysis

The collected data was analysed using a One-way analysis of variance (ANOVA). The analysis was performed using STATISTICA Software Programme 2010 (StatSoft Inc., Tulsa OK, USA). Where F-value was found to be significant, Fisher's least significant difference (LSD) was used to compare the means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

Table 1. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) of *B. vulgaris.*

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control (Hoagland's solution minus N)	0.6±0.1^{c}	0.2±0.0^{a}	0.0±0.3^{b}	0.3±0.1^{b}	0.3±0.0^{b}	0.3±0.0^{c}	0.3±0.0^{c}	0.2±0.0^{c}
Azolla plus Hoagland's minus N solution	0.4±0.0^{c}	0.2±0.1^{a}	0.0±0.3^{b}	0.4±0.0^{b}	0.5±0.0^{b}	0.5±0.1^{b}	0.5±0.1^{c}	0.6±0.0^{b}
Azolla plus full Hoagland's solution	0.9±0.1^{b}	0.5±0.1^{a}	0.1±0.6^{a}	1.0±0.1^{a}	1.0±0.1^{a}	1.1±0.0^{a}	1.1±0.1^{b}	0.8±0.0^{b}
Full Hoagland's solution	1.3±0.0^{b}	0.6±0.2^{a}	0.1±0.7^{a}	1.1±0.0^{a}	0.9±0.0^{a}	1.2±0.0^{a}	1.6±0.1^{a}	1.2±0.2^{a}
One-way ANOVA (F-statistic) Rep	45.4***	3.1^{NS}	13.9***	64.9***	25.5***	91.9***	44.0***	16.1***

Values presented are means ± SE. *** = significant at $P \leq 0.001$; NS = not significant; SE = standard error. Means followed by dissimilar letter in a column are significantly different from each other at $P = 0.05$ according to Fischer least significance difference.

RESULTS

Effects of different combinations of Hoagland's solution and *A. filiculoides* on photosynthesis of *B. vulgaris*

Table 1 portrays weekly measurements of the photosynthesis of *B. vulgaris*. Results show that the treatment with *Azolla* plus full Hoagland's solution produced significant ($P \leq 0.001$) results when compared with the control, where the photosynthetic rate was higher in weeks 4, 6 and 8. Divergences were noted between the control, and the treatments comprised of full Hoagland's solution and *Azolla* plus full Hoagland's, where the treatments were significantly ($P \leq 0.001$) higher in weeks 1, 4 and 8. In week 2, no significant differences were noted between all other treatments relative to the control. In weeks 3, 5 and 7, significant differences ($P \leq 0.001$) were noted between the two treatments containing nitrogen and *Azolla* plus Hoagland's minus N solution and the control (Hoagland's minus N solution), where the full Hoagland's solution and *Azolla* plus full Hoagland's solution treatments had a higher photosynthetic rate relative to the *Azolla* plus Hoagland's minus N solution and the control (Hoagland's minus N solution). Whilst no significant differences were observed between the full Hoagland's solution and *Azolla* plus full Hoagland's solution in weeks 1 and 4, a significant ($P \leq 0.001$) difference was acquired in week 8, where full Hoagland's solution had the highest photosynthetic rate. In conclusion, the treatment with full Hoagland's solution had the highest photosynthetic rate succeeded by *Azolla* plus full Hoagland's, then *Azolla* plus Hoagland's minus N solution, and the lowest rate was found in the control (Hoagland's minus N solution). Even though week to week differences were noted in the photosynthetic rates of all the treatments, this is most probably due to varying light intensities during the data collection. Possibilities to improve the measurement of plants using the LCpro+ 1.0 ADC would either be to provide a constant light intensity such as using horticultural lighting according to the crop/plant type in an artificial growth chamber, or by using numerous LCpro+ 1.0 ADC instruments to measure all treatments simultaneously.

Effects of different combinations of Hoagland's solution and *A. filiculoides* on stomatal conductance of *B. vulgaris*

Table 2 presents results on stomatal conductance of *B. vulgaris*. No significant results were observed in weeks 2, 3, 5 and 8. Significant differences were noted in weeks 1 ($P \leq 0.001$), 4 ($P \leq 0.01$), 6 ($P \leq 0.001$) and 7 ($P \leq 0.01$). In week 1, the *Azolla* plus Hoagland's minus N solution treatment was not statistically different from the control (Hoagland's minus N solution) although the *Azolla* plus Hoagland's minus N solution treatment had slightly higher value of stomatal conductance than the control. The highest stomatal conductance rate in week 1 was recorded in the full Hoagland's solution treatment proceeded by *Azolla* plus full Hoagland's solution then *Azolla* plus Hoagland's minus N solution and the lowest in the control. Outcomes in week 4 showed that the highest significant results were recorded in treatment containing *Azolla* plus full Hoagland's solution followed by the full Hoagland's solution treatment. The control treatment had the lowest stomatal conductance which was similar to the *Azolla* plus Hoagland's minus N solution treatment. In weeks 6 and 7, the *Azolla* plus Hoagland's minus N solution treatment had a significantly ($P \leq 0.001$) higher stomatal conductance than the control (Hoagland's minus N solution), but was very similar to the *Azolla* plus full Hoagland's solution treatment. The full Hoagland's solution treatment had the highest results in weeks 6 and 7 but was very similar to *Azolla* plus full Hoagland's solution in week 7.

Effects of different combinations of Hoagland's solution and *A. filiculoides* on evapotranspiration of *B. vulgaris*

The evapotranspiration rate of *B. vulgaris* is documented

Table 2. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the stomatal conductance (mmol H_2O m^{-2} s^{-1}) of *B. vulgaris.*

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control (Hoagland's solution minus N)	0.015±0.003^{c}	0.000±0.000^{a}	0.003±0.003^{a}	0.000±0.000^{c}	0.003±0.003^{a}	0.003±0.003^{c}	0.003±0.003^{c}	0.003±0.003^{a}
Azolla plus Hoagland's minus N solution	0.010±0.000^{bc}	0.003±0.003^{a}	0.003±0.003^{a}	0.005±0.003^{bc}	0.010±0.000^{a}	0.010±0.000^{b}	0.010±0.000^{b}	0.010±0.000^{a}
Azolla plus full Hoagland's solution	0.025±0.006^{b}	0.005±0.003^{a}	0.005±0.003^{a}	0.013±0.003^{a}	0.013±0.006^{a}	0.010±0.000^{b}	0.013±0.003^{ab}	0.010±0.004^{a}
Full Hoagland's solution	0.055±0.003^{a}	0.005±0.005^{a}	0.008±0.003^{a}	0.010±0.000^{ab}	0.013±0.003^{a}	0.180±0.003^{a}	0.018±0.003^{a}	0.013±0.003^{a}
One-way ANOVA (F-statistic) Rep	27.9***	0.58^{NS}	0.85^{NS}	8.4**	1.7^{NS}	12.0***	8.3**	2.6^{NS}

Values presented are means ± SE. **; *** = significant at P≤0.01, P≤0.001, respectively. NS = not significant. Means followed by dissimilar letter(s) in a column are significantly different from each other at P=0.05 according to Fischer least significance difference.

Table 3. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the evapotranspiration (mmol m^{-2} s^{-1}) of *B. vulgaris.*

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control (Hoagland's solution minus N)	0.140±0.025^{c}	0.050±0.006^{a}	0.128±0.023^{b}	0.093±0.006^{c}	0.090±0.023^{c}	0.090±0.013^{c}	0.110±0.019^{c}	0.108±0.020^{b}
Azolla plus Hoagland's minus N solution	0.098±0.010^{bc}	0.060±0.018^{a}	0.105±0.024^{b}	0.140±0.015^{b}	0.173±0.010^{ab}	0.158±0.017^{b}	0.200±0.013^{b}	0.228±0.009^{a}
Azolla plus full Hoagland's solution	0.178±0.015^{b}	0.113±0.015^{a}	0.110±0.011^{b}	0.150±0.020^{b}	0.145±0.033^{bc}	0.170±0.007^{b}	0.183±0.009^{b}	0.155±0.025^{b}
Full Hoagland's solution	0.23±0.000^{a}	0.095±0.028^{a}	0.293±0.044^{a}	0.270±0.004^{a}	0.220±0.014^{a}	0.255±0.009^{a}	0.268±0.005^{a}	0.213±0.008^{a}
One-way ANOVA (F-statistic) Rep	13.4***	2.5^{NS}	10.3***	33.9***	6.2**	32.5***	27.3***	10.3***

Values presented are means ± SE. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$, respectively; NS = not significant. Means followed by dissimilar letter(s) in a column are significantly different from each other at $P = 0.05$ according to Fischer least significance difference.

in Table 3. The results show that there were significant ($P \leq 0.001$) differences between the treatments. The *Azolla* plus Hoagland's minus N solution treatment exhibited similar results to the control (Hoagland's minus N solution) in week 1, but *Azolla* plus full Hoagland's solution and full Hoagland's solution treatment were significantly ($P \leq 0.001$) superior to the rest. From weeks 3 to 8, the evapotranspiration rate in *B. vulgaris* were significantly superior in full Hoagland's solution treatment relative to all other treatments. This was followed by *Azolla* plus full Hoagland's solution and *Azolla* plus Hoagland's minus N solution when compared with the control. However, in week 8, *Azolla* plus Hoagland's minus N solution and *Azolla* plus full Hoagland's solution had better evapotranspiration rate relative to *Azolla* plus full Hoagland's solution and control (Hoagland's minus N solution).

Effects of different combinations of Hoagland's solution and *A. filiculoides* on intercellular CO_2 concentration of *B. vulgaris*

The intercellular CO_2 concentration of *B. vulgaris* in Table 4 shows no significant results in weeks 1, 3, 4, 5, 7 and 8. Significant differences are noted in weeks 2 and 6 ($P \leq 0.01$). In week 2, the *Azolla* plus Hoagland's minus N solution treatment and the control had significantly higher intercellular CO_2 concentration compared with the *Azolla* plus full Hoagland's solution and the treatment containing full Hoagland's solution. In week 6, the highest intercellular CO_2 concentrations were recorded in the *Azolla* plus Hoagland's minus N solution and full Hoagland's solution treatments.

Effects of different combinations of Hoagland's solution and *A. filiculoides* on photosynthetic rate of *B. vulgaris* chlorophyll content of *B. vulgaris*

Chlorophyll content of *B. vulgaris* represented by

Table 4. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the intercellular CO_2 concentration of *B. vulgaris*

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control(Hoagland's solution minus N)	275.8±5.0^{a}	243.0±12.0^{ab}	206.0±33.6^{a}	217.3±44.7^{a}	140.3±57.0^{a}	188.0±39.8^{bc}	221.5±51.4^{a}	207.3±41.2^{a}
Azolla plus Hoagland's minus N solution	262.0±12.0^{a}	281.0±8.9^{a}	207.8±23.2^{a}	218.0±6.6^{a}	244.5±9.1^{a}	261.5±5.1^{a}	249.3±6.4^{a}	248.5±4.1^{a}
Azolla plus full Hoagland's solution	257.3±7.5^{a}	194.0±31.1^{b}	167.3±27.6^{a}	183.5±23.3^{a}	150.5±33.7^{a}	137.0±20.3^{c}	143.8±20.3^{a}	214.8±17.1^{a}
Full Hoagland's solution	276.0±3.9^{a}	215.3±19.9^{b}	218.8±21.7^{a}	217.0±4.9^{a}	223.5±9.7^{a}	208.8±9.0^{ab}	219.5±9.0^{a}	245.3±22.0^{a}
One - Way ANOVA (F-Statistic) Rep	1.5^{NS}	3.6**	0.7^{NS}	0.4^{NS}	2.4^{NS}	5.1**	2.6^{NS}	0.7^{NS}

Values presented are means ± SE. ** = significant at $P \leq 0.01$. NS = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 5 shows significant outcomes ($P \leq 0.001$). These results were noted in total chlorophyll ($P \leq 0.001$), chlorophyll a ($P \leq 0.001$) and chlorophyll b ($P \leq 0.01$) concentrations. The full Hoagland's solution treatment achieved the highest concentrations succeeded by the *Azolla* plus full Hoagland's, *Azolla* plus Hoagland's minus N solution and control (Hoagland's minus N solution) treatments. Similarities were noted between the full Hoagland's solution and *Azolla* plus full Hoagland's solution treatments in the chlorophyll a concentrations. The *Azolla* plus full Hoagland's, and *Azolla* plus Hoagland's minus N solution treatments also produced results that were similar in the chlorophyll b concentration.

DISCUSSION

Results in Tables 1 to 5 show that photosynthetic rate, evapotranspiration, intercellular CO_2 concentration and chlorophyll were generally higher in treatments with nitrogen which was readily available to plants. This was specifically evident in the treatment composed of the full Hoagland's solution. This was followed by *Azolla* sp. plus full Hoagland's solution and *Azolla* sp. plus Hoagland's minus N solution treatment. The control (Hoagland's minus N solution) had lowest values for these para-meters. It is widely reported that nitrogen plays a significant role in the function of physiological responses of plants namely that of photosynthesis (Bottrill et al., 1970; Evans and Terashima, 1987; Terashima and Evans, 1988; Evans, 1989; Paul and Driscoll, 2008), chlorophyll formation (Evans, 1989; Schepers et al., 1992; Blackmer and Schepers, 1995) and proteins such as RubisCO represent 30% of the part of soluble proteins and in relation to nitrogen utilised in the synthesis of proteins in plant organs such as seeds (Dalling et al., 1976; Ellis, 1979).

In photosynthesis, nitrogen has crucial function in electron transport and photophosphorylation (Terashima and Evans, 1988). Several other studies have shown that chlorophyll is directly linked to nitrogen, and the amount available will determine the chlorophyll concentration in leaf tissues (Evans, 1989; Schepers et al., 1992; Blackmer and Schepers, 1995; Tadahiko, 1997; Zhouping et al., 2000). An adequate supply of nitrogen will promote these functions, and an insufficiency will result in a disruption of the processes. Therefore, nitrogen in sufficient amounts will encourage a higher rate of photosynthesis, evaporative transpiration, inter-cellular CO_2 concentration and chlorophyll formation, a phenomenon which was also observed in our study.

The *Azolla* sp. plus Hoagland's solution minus N treat-ment alone in this study displayed improved photo-synthesis, evaporative transpiration, intercellular CO_2 concentration and chlorophyll content. *Azolla* sp. has the capability of fixing atmospheric nitrogen into usable forms (Lumpkin and Plucknett, 1982; Peters et al., 1982; Lambers and Poorter, 1992). Based on the settings of the hydroponic system in this study, it is possible that N was released from the *Azolla* into the hydroponic solution and then absorbed by *B. vulgaris* hence contributing to improving the photosynthesis, evaporative transpiration, intercellular CO_2 concentration and chlorophyll content at different measurements dates.

The growth and development of *A. filiculoides* in the experiment showed various differences. The *Azolla* sp. was exposed to light in its nutrient reservoir on a continual basis, so that the *Anabaena* sp. was able to use this light for N-fixation. From visual observation, it was noted that the surface area covered by *A. filiculoides* was noticeably larger in the Hoagland's minus N solution, even though the surface areas had increased in both solutions after inoculation.

Another visible factor was that the leaf fronds

Table 5. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the chlorophyll concentration (mg L^{-1}) of *B. vulgaris.*

Treatment	Total chlorophyll content	Total chlorophyll a content	Total chlorophyll b content
Control (Hoagland's solution minus N)	0.3±0.1[d]	0.3±0.1[c]	-0.1±0.0[c]
Azolla plus Hoagland's minus N solution	1.2±0.0[c]	0.9±0.0[b]	0.3±0.1[b]
Azolla plus full Hoagland's solution	2.5±0.1[b]	2.2±0.1[a]	0.3±0.0[b]
Full Hoagland's solution	2.8±0.0[a]	2.2±0.1[a]	0.5±0.1[a]
One-way ANOVA (F-statistic) Rep	531.0***	161.7***	25.0**

Values presented are means ± SE. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively. Means followed by dissimilar letter in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

were larger and darker green in the Hoagland's minus N solution as opposed to the full Hoagland's solution. These observations suggest that *A. filiculoides* in the Hoagland's minus N solution thrived and excreted N-rich substances into the solution which aided *B. vulgaris* to thrive. In comparison to the full Hoagland's solution, *A. filiculoides* competed with *B. vulgaris* for the nitrogen that was readily available in the solution, and thus resulted in poorer growth of both *A. filiculoides* and *B. vulgaris.* These results are of valuable agricultural importance in using *A. filiculoides* successfully as a possible nitrogen fertilizer in the hydroponic cultivation of *B. vulgaris* and other food crops. To the best of our knowledge, this is the only study in literature to evaluate the effect of *Azolla* on the physiological responses of *B. vulgaris* in hydroponic cultures. Further insight must be gathered on the execution of more efficient hydroponic cultivation method, where a more prominent amount, growth and nitrogen fixation of *Azolla* can be achieved for effective crop production in hydroponic cultures.

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