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Full Length Research Paper

Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on growth and development of *Beta vulgaris* subsp*. cycla* 'Fordhook Giant' grown in hydroponic cultures

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The comparisons of plant height, leaf number, foliage colour, fresh and dry weight of *Beta vulgaris* subsp. cycla 'Fordhook Giant' were evaluated in the glasshouse over a period of 8 week. The *B. vulgaris* plants were exposed to different nutrient solutions in which some contained *Azolla filiculoides.* Each treatment was replicated four times. The aim of this study was to assess the effectiveness of *A. filiculoides* as an alternative nitrogen fertilizer on growth and development of *B. vulgaris*. The treatments evaluated were: 1) Hoagland's solution minus N (control); 2) *A. filiculoides* plus Hoagland's minus N solution; 3) *A. filiculoides* plus a full Hoagland's solution and 4) a full Hoagland's solution. Results showed that fully supplemented Hoagland's solutions induced the best growth in terms of plant height, leaf number, foliage colour, fresh and mass of *B. vulgaris*. This was followed by *Azolla* plus full Hoagland's solution and then by *Azolla* plus Hoagland's minus N solution. The control gave the poorest results in all aspects. Although not as effective as the fully supplemented solutions, the *Azolla* plus Hoagland's minus N solution can be modified in future to produce more nitrogen and in turn increase the growth rate of *B. vulgaris*.

Key words: Height, leaf number, foliage colour, fresh and dry weight, full Hoagland's solution, Hoagland's minus N.

INTRODUCTION

Physiological processes within plants greatly affect mineral nutrients including nitrogen. This element enhances the metabolic processes, which in turn influences growth patterns such as protein formation (Fernandes and Pereyra, 1995), fruiting (Golomb and Goldschmidt, 1987; Conradie, 1992; Brown et al., 1995) and root growth (Caldwell et al., 1981). Nitrogen can be supplied to plants through chemical fertilizers and biological nitrogen fixation (BNF) (Roger and Reynaud, 1982; Watanabe and Liu, 1992; Kumarasinghe and Eskew, 1995). In agriculture chemical fertilization is the chosen method of supplying nitrogen to ensure sustained plant yields (Anjana et al., 2009). But this method is expensive and may be harmful to the environment (Lee and Nielsen, 1987; Pasternak et al., 1988; Vroomen, 1989, Byrnes, 1990; Madhusoodanan and Sevichan, 1992; Zhu and Chen, 2002; Ladha and Reddy, 2003; Crew and Peoples, 2004). Alternative biological methods such as those involving symbionts could possibly be used

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Abbreviations: BNF, Biological nitrogen fixation; **$kg.ha^{-1}$,** kilograms per hectare; **L,** litres; **L/hr,** litres per hour; **LECA,** light expanded clay aggregate; **m^2,** metres square; **PVC,** polyvinylchloride; **$t.ha^{-1}$,** tonne per hectare.

to replace or supplement the chemical fertilizers.

Azolla filiculoides is able to fix atmospheric N into water by having a symbiotic relationship with a cyanobacterium: *Anabaena azollae* (Shi and Hall, 1988). *Anabaena* occurs extracellularly in the leaf cavities of *Azolla* (Hill, 1976; Obreht et al., 1997). The role of *A. azollae* (the cyanobacteria) in the symbiosis is to fix N in leaf cavities of *A. filiculoides* (Peters, 1977, 1978) and in turn *A. filiculoides* provides carbon for the cyanobacteria (Peters, 1976; Van Hove, 1989).

Researchers such as Mian and Stewart (1985) and Rains and Talley (1979) have indicated that *A. filiculoides* has the ability to supply biological N to plants either by leaching N directly to the medium or through biodegradation processes. For example, studies have shown that *Azolla* contributed 25 to 162 $kg.ha^{-1}$ of nitrogen when applied to rice cultivation (Tran and Dao, 1973; Becking, 1976; Wagner, 1997). A study conducted by Wagner (1996) involving the incorporation of *Azolla nilotica* in rice fields showed a 19 to 103% increase in rice yield due to a substantial increase in plant height and number of tillers per rice plant. This positive increase is what is required by a biological N fertilizer, somewhat similar to chemical N fertilizer which has been used in modern agriculture to supply sufficient amounts of N to sustain growth and crop yields (Watanabe and Liu, 1992; Kumarasinghe and Eskew, 1995).

Therefore, well-designed hydroponic system may provide a suitable avenue of incorporating *A. filiculoides* into the system and hence allowing the *Azolla* and *Anabaena* to release the N fixed form the atmosphere into the hydroponic system and make it available to plants such as *B. vulgaris subsp. Cycla* 'Fordhook Giant' grown in hydroponics. *B Vulgaris* is an annual vegetable plant grown during winter period. It has deep tap root and shallow secondary root system. The objective of this experiment was to ascertain whether the N fixed from *Azolla* and *Anabaena* symbiosis would influence the growth and development of *B. vulgaris*.

MATERIALS AND METHODS

Experimental

The experiment was conducted in an actively vented greenhouse sited at the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa. The growth and development of *B. vulgaris* was evaluated over an 8 week period on a four block experimental design. The apparatus constituted by a hydroponic system in each block. Each arrangement comprised of a channel hydroponic system containing 4 PVC pipe channels. Individual channels contained 5 *B. vulgaris* plants a piece and therefore, 20 plants per treatment resulting in 80 plants for the entire experiment. Nutrient solution was invariably supplied by a 70 L reservoir powered by a 3500 L/h submersible pump.

The application of PVC pipe systems mentioned by Roberto (2000) was adequate to accommodate *B. vulgaris* and *A. filiculoides.* This was achieved by planting the chard into hydroponic net baskets supported by 4 to 8 mm light expanded clay aggregate (LECA) pebbles. These were positioned in the channels and allowed to root into the flowing nutrient solution within the channel.

A. filiculoides was placed in the nutrient reservoir and allowed to float on the nutrient solution. This permitted that both plants species were exposed to the nutrient solution within each system. Botanical material of *A. filiculoides* was derived from the plant identification section at CPUT and *B. vulgaris* was obtained from a garden centre situated in Cape Town.

Upon the commencement of the study *A. filiculoides* was introduced to 2 of the systems 1 week prior to that of the chard. This is to allow the *Azolla* to establish and to permit the fixation of N into the nutrient solution. *B. vulgaris* was placed into the systems at plant spacing of 40 cm between plants to allow sufficient growth space and data collection to be unobstructed. Nutrient solutions were comprised of a full Hoagland's solution and a Hoagland's minus N solution suggested by Hershey (1994, 1995). Macro elements composed of 2 Mole KNO_3; 2 Mole $Ca(NO_3)$ 2 X $4H_2O$; 1 Mole NH_4NO_3; 1000 mg/L Fe-EDTA; 2 Mole $MgSO_4$ X $7H_2O$; 1 Mole KH_2PO_4 and minor elements made up of 0.86 g H_3BO_3; 1.81 g $MnCl_2$ X $4H_2O$; O.22 g $ZnSO_4$ X $4H_2O$; 0.051g $CuSO_4$; 0.09 g H_3MoO_4 X H_2O represented the full Hoagland's solution. Whereas the minus Hoagland's solution was prepared from 0.05 Mole CaH_2PO_4; 0.01 Mole $CaS0_4$ X $2H_2O$; 0.5 Mole K_2SO_4; 1 Mole Mg_SO4; 1000 mg/L Fe-EDTA; 1 Mole KH_2PO_4 as macro elements and 0.86 g H_3BO_3; 1.81 g $MnCl_2$ X $4H_2O$; O.22 g $ZnSO_4$ X $4H_2O$; 0.051 g $CuSO_4$; 0.09 g H_3MoO_4 X H_2O as minor elements depicted the minus N Hoagland's 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 growth and development

Growth and development of *B. vulgaris* was determined by measuring the following parameters. These are plant height, total leaf number, leaf colour, fresh and dry weight. Plant height was evaluated in millimetres (mm) where the distance between the plant base and apex was taken into consideration. Total leaf number was determined by counting the developing and developed leafs Plant colour was visually assessed on a scale of 1 to 5 where 1 represented a pale yellow colour and 5 typified a dark green colour, these hebdomadally assessments were then compared at the end of the experiment. Plant harvest was conducted by removing 10 per treatment and therefore 40 *B. vulgaris* plants were harvested at week 4 and 8 respectively. Harvested plants were separated into roots and shoots and weighed to determine the fresh mass. Post weighed fresh *B. vulgaris* were allowed to dry for 48 h at 60°C to ascertain the dry mass.

Data collected were analysed using a One-Way analysis of variance (ANOVA). The analysis was performed using STASTICA 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).

RESULTS

Effect of different combinations of Hoagland's solution and *A. filiculoides* on plant height of *B. vulgaris*

Table 1 depicts the effect of *Azolla* on 4 different treatments on plant height of *B. vulgaris*. The outcomes

Table 1. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the height (mm) of *B. vulgaris*.

Treatment	Week 2	Week 4	Week 6	Week 8
Control(Hoagland's minus N solution)	75.3±7.1[c]	75.3±6.8[d]	52.8±12.7[d]	57.8±8.3[d]
Azolla plus Hoagland's minus N solution	160.0±12.2[b]	167.0±12.3[c]	166.3±15.0[c]	168.0±16.4[c]
Azolla plus full Hoagland's solution	203.3±23.6[ab]	263.0±31.0[b]	277.5±26.3[b]	340.5±48.2[b]
Full Hoagland's solution	232.3±22.4[a]	391.8±25.7[a]	444.0±12.0[a]	481.8±21.9[a]
One - Way ANOVA (F-Statistic) Rep	14.9***	40.3***	91.1***	44.7***

Values presented are means ± SE. *** = significant at *P*≤0.001. 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 2. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the total leaf number of *B. vulgaris*.

Treatment	Week 2	Week 4	Week 6	Week 8
Control(Hoagland's minus N solution)	4.8±0.3[b]	5.0±0.0[b]	2.8±0.9[c]	2.8±0.6[b]
Azolla plus Hoagland's minus N solution	5.5±0.5[ab]	5.8±0.6[b]	4.5±1.0[bc]	3.8±0.9[b]
Azolla plus full Hoagland's solution	5.5±0.3[ab]	5.5±0.5[b]	6.8±0.9[ab]	8.3±0.6[a]
Full Hoagland's solution	6.3±0.5[a]	8.0±0.4[a]	8.5±0.5[a]	10.3±0.9[a]
One - Way ANOVA (F-Statistic) Rep	2.4[NS]	8.7**	9.1**	22.8***

Values presented are means ± SE. **; *** = significant at *P*≤0.01, *P*≤0.001 respectively. 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.

showed that by applying *Azolla* plus Hoagland's minus N solution resulted in significant (*P*≤ 0.001) increase in height as opposed to the control (Hoagland's minus N solution) treatment in week 2, 4, 6 and 8. Significant (*P*≤ 0.001) differences in height were also noted in the treatments with *Azolla* plus full Hoagland's, and full Hoagland's solution as compared with the control (Hoagland's minus N solution). The treatment with full Hoagland's solution achieved the highest height, followed by *Azolla* plus full Hoagland's solution. Overall, all the treatments depicted a significantly (*P*≤ 0.001) higher height than the control (Hoagland's minus N solution) where full Hoagland's solution was the highest, followed by *Azolla* plus full Hoagland's solution and then by *Azolla* plus Hoagland's minus N solution.

Effect of different combinations of Hoagland's solution and *A. filiculoides* on total leaf number of *B. vulgaris*

The effects of *Azolla* on total leaf number of *B. vulgaris* are represented in Table 2. The total leaf number of the treatment containing *Azolla* plus Hoagland's minus N solution was not significantly higher in week 2 and 4 when compared with the control (Hoagland's minus N solution) where full Hoagland's solution achieved a significantly (*P*≤ 0.01) higher total leaf number. In week 6 and 8, differences were noted between the control (Hoagland's minus N solution) and the *Azolla* plus Hoagland's minus N solution and the treatments containing *Azolla* plus full Hoagland's solution and full Hoagland's solution. The *Azolla* plus full Hoagland's solution and full Hoagland's solution developed a higher total leaf number than the control (Hoagland's minus N solution) and *Azolla* plus Hoagland's minus N solution. Although there was no significant difference between the control (Hoagland's minus N solution) and *Azolla* plus Hoagland's minus N solution treatment, a difference in the mean was noted where *Azolla* plus Hoagland's minus N solution treatment had an average of 3.8 and 2.8 for the control (Hoagland's minus N solution).

Effect of different combinations of Hoagland's solution and *A. filiculoides* on plant colour of *B. vulgaris*

Plant colour of *B. vulgaris* presented in Table 3 showed significant (*P*≤ 0.001) differences between the control (Hoagland's minus N solution) and the other treatments. The treatments containing full Hoagland's solution and *Azolla* plus full Hoagland's solution produced the darkest colour when compared with the control (Hoagland's minus N solution). This was followed by *Azolla* plus Hoagland's minus N solution and lastly the control (Hoagland's minus N solution).

Effect of different combinations of Hoagland's solution and *A. filiculoides* on fresh weight of *B. vulgaris*

The effects of *A. filiculoides* on fresh weight of *B. vulgaris*

Table 3. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the plant colour of *B. vulgaris*.

Treatment	Week 2	Week 4	Week 6	Week 8
Control (Hoagland's minus N solution)	2.3±0.1[d]	2.5±0.0[b]	2.5±0.0[d]	2.5±0.0[c]
Azolla plus Hoagland's minus N solution	3.0±0.0[c]	2.9±0.1[b]	3.1±0.1[c]	3.3±0.1[b]
Azolla plus full Hoagland's solution	3.8±0.1[a]	3.8±0.1[a]	5.0±0.0[b]	5.5±0.0[a]
Full Hoagland's solution	3.4±0.1[b]	4.0±0.2[a]	5.4±0.1[a]	5.5±0.0[a]
One - Way ANOVA (F-Statistic) Rep	28.6***	25.8***	252.0***	459.0***

Values presented are means ± SE. *** = significant at $P \leq 0.001$. 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.

are shown in Table 4. Significant results were observed between the treatments for shoot, root and total fresh weight. During harvest 1 and 2, using of full Hoagland's solution solution resulted into getting significantly higher fresh mass of shoot and root relative to all other treatments tested. The second best results collected during harvest 1 and 2 were recorded in the *Azolla* plus full Hoagland's solution treatment. Except for roots collected in harvest 1, these organs were significantly weighing less than those produced by the full Hoagland's treatment solution. The treatment composed of *Azolla* plus Hoagland's minus N solution produced the 3rd best results which in most cases during harvest 1 were significantly superior to the control treatment (Hoagland's minus N solution). However, there was no significant fresh weight yield difference between the control and the *Azolla* plus Hoagland's solution minus N in harvest 2 although the *Azolla* plus Hoagland's minus N solution had higher shoot and root masses.

Effect of different combinations of Hoagland's solution and *A. filiculoides* on dry weight of *B. vulgaris*

As shown in Table 5, the treatments tested significantly affected the dry weight of shoots, roots and whole plant of *B. vulgaris* collected during harvest 1 and 2. With the exception of shoots collected during harvest 1, the control and the *Azolla* plus Hoagland's minus N solution treatment produced dry shoots, roots and whole plant that were significantly lower to *Azolla* plus full Hoagland's solution and the full Hoagland's solution. Generally, higher dry shoots, roots and whole plant were produced in the full Hoagland's solution. This was closely followed by the *Azolla* plus full Hoagland's solution.

DISCUSSION

Results depicted in Tables 1, 2, 3, 4 and 5 have shown that when *B. vulgaris* was exposed to different nutrient solutions, a difference in growth and development was noted (Figures 1 to 3). The nutrient solution containing *Azolla* plus Hoagland's minus N solution showed improved growth relative to the control (Hoagland's minus N solution) which had no nitrogen and no *Azolla* (Figure 1). Although there were two plant species (*Azolla* and *B. vulgaris*) in the treatment involving *Azolla* plus Hoagland's minus N solution and only one plant specie in the control treatment (*B. vulgaris*) the competition between the two species did not influence negatively the growth of *B. vulgaris*. This clearly suggests that N fixed from *Azolla* (data not shown) was responsible for the observed improved growth.

The most favourable results were achieved by the solutions containing full Hoagland's solution (Figure 3) and *Azolla* plus full Hoagland's solution (Figure 2) as all the characteristics of growth and development in these treatments were the highest relative to all other treatments. Release of nitrogen from *Azolla* in the solution containing *Azolla* plus Hoagland's minus N solution could possibly have influenced *B. vulgaris* to some degree and resulted in higher growth and development as compared with the control (Hoagland's minus N solution) which lacked nitrogen. Past studies have confirmed that *A. filiculoides* floating into water is able to fix nitrogen (Holst and Yopp, 1979; Kitoh and Shiomi, 1984; Liu and Zheng, 1992; Bharati et al., 2000). This therefore suggests that *B. vulgaris* was exposed to nitrogen supplied by the *Azolla* which was growing in the hydroponic system. Several studies have reported positive effects of nitrogen fixed from *Azolla* on plant growth (Kolhe and Mittra, 1990; Mahapatra and Sharma, 1989; Marwaha et al., 1992; Mohamed, 2005; Onwueme, 1999; Teckle-Haimanot, 1995). However, the *Azolla* plus Hoagland's minus N solution treatment had a minimal effect, as the amount of *Azolla* was not enough to sufficiently supply enough nitrogen to completely surpass the growth produced by the full Hoagland's solution and *Azolla* plus full Hoagland's solution.

The solutions comprised of full Hoagland's solution and *Azolla* plus full Hoagland's solution contained the highest amount of nitrogen as compared with the control (Hoagland's minus N solution) and the solution containing *Azolla* plus Hoagland's minus N solution. This is because both solutions were supplied with calcium nitrate ($CaNO_3$), potassium nitrate (KNO_3) and ammonium

Table 4. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the fresh weight (g) of *B. vulgaris*.

Treatment	Shoots harvest 1	Roots harvest 1	Total harvest 1	Shoots harvest 2	Roots harvest 2	Total harvest 2
Control (Hoagland's minus N solution)	1.5±0.4[c]	2.4±0.6[b]	3.9±1.0[d]	2.1±0.2[c]	4.9±3.2[c]	7.0±0.7[c]
Azolla plus Hoagland's minus N solution	4.2±0.3[c]	8.5±1.2[a]	12.8±1.4[c]	4.9±0.3[c]	8.5±6.1[c]	13.4±1.0[c]
Azolla plus full Hoagland's solution	25.9±1.4[b]	11.6±1.9[a]	37.5±2.4[b]	123.1±16.6[b]	52.5±39.2[b]	175.5±19.8[b]
Full Hoagland's solution	48.3±2.6[a]	8.7±0.9[a]	57.0±3.0[a]	268.5±33.2[a]	77.5±50.0[a]	346.0±38.5[a]
One - Way ANOVA (F-Statistic) Rep	216***	10.0**	133.2***	46.0***	53.4***	55.0***

Values presented are means ± SE. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively. 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.

Table 5. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the dry weight (g) of *B. vulgaris*.

Treatment	Shoots harvest 1	Root harvest 1	Total harvest 1	Shoots harvest 2	Root harvest 2	Total harvest 2
Control (Hoagland's minus N solution)	0.2±0.1[c]	0.2±0.1[c]	0.4±0.1[c]	0.5±0.1[c]	0.4±0.0[b]	0.9±0.1[b]
Azolla plus Hoagland's minus N solution	0.9±0.1[b]	0.7±0.1[c]	1.6±0.2[c]	0.8±0.0[c]	0.6±0.1[b]	1.5±0.1[b]
Azolla plus full Hoagland's solution	1.5±0.2[a]	3.8±0.2[b]	5.3±0.3[b]	16.7±2.1[b]	4.7±0.7[a]	21.4±2.6[a]
Full Hoagland's solution	1.2±0.2[ab]	5.3±0.6[a]	6.6±0.7[a]	29.0±3.2[a]	5.5±0.4[a]	34.5±3.5[a]
One - way ANOVA (F-Statistic) Rep	68.2***	15.6**	55.2***	50.9***	41.8***	55.8***

Values presented are means ± SE. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively. 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.

nitrate (NH_4NO_3) which are readily available for absorption (Hershey, 1994; 1995). Generally, commercial nitrogen fertilizers have proven to increase growth rate and yields of crops (Kirk, 2001). This is also confirmed by Bouldin (1986) who reported that nitrogen applied to rice attained considerable yields. Wagner (1996) found that *Azolla nilotica* integrated with rice achieved 19 to 103% in yield and a significant gain in number of tillers per plant and plant height. Positive effects on growth in other crops have been documented where increases in yields were attributed to the presence of *Azolla*. In a study by Mohamed (2005) banana growth was significantly increased by *Azolla*. A 56 to 69% increase in wheat yield due to *Azolla* fertilization was also reported by Mahapatra and Sharma (1989). Other experiments on Taro (*Colocasia esculenta*) resulted in significant increase in yield respectively, as compared with the control when combining *Azolla* with the growth medium (Onwueme, 1999; Teckle-Haimanot, 1995).

In conclusion, these results give positive insight into *A. filiculoides* as a potential nitrogen fertilizer in the hydroponic culture of *B. vulgaris* and other related vegetables. To the best of our knowledge, this is the only study in literature to evaluate the effects of *Azolla* on vegetable production in hydroponic systems. Further research must be conducted on the implementation of a more effective hydroponic system, which is able to contain a larger amount and effectively promote the growth and nitrogen fixation from *Azolla–Anabaena* symbiosis for effective crop production in hydroponic cultures. Furthermore, studies should also focus on the competition between the fixing and none fixing partner in the hydroponic cultures.

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Figure 1. Comparison between the control (left side) and *Azolla* plus Hoagland's solution minus N (right side).

Figure 2. Comparison between control (left side) and *Azolla* plus full Hoagland's solution (right side).

Figure 3. Comparison between the control (left side) and full Hoagland's solution (right side).

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