



Cape Peninsula  
University of Technology

**THE EFFECTIVENESS OF DIFFERENT COMBINATIONS OF HOAGLAND'S  
SOLUTION AND *AZOLLA FILICULOIDES* ON HYDROPONICALLY CULTIVATED  
*BETA VULGARIS SUBSP. CYCLA* 'FORDHOOK GIANT'**

by

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**Thesis submitted in fulfilment of the requirements for the degree**

**Master of Technology: Horticultural Sciences in the Faculty of Applied  
Sciences at the Cape Peninsula University of Technology**

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## **DECLARATION**

I, Alan de Bever, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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**Signed**

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**Date**

## ABSTRACT

This study evaluated the effects of different combinations of Hoagland's solution and *Azolla filiculoides* on *Beta vulgaris* subsp. *cycla* 'FORDHOOK GIANT' grown in different hydroponic nutrient solutions. These solutions were comprised of a full Hoagland's solution and a Hoagland's solution minus nitrogen solution and amalgamations of these with *Azolla* respectively. The objectives were to assess the effects of different combinations of Hoagland's solution and *A. filiculoides* on uptake of nitrogen and other nutrients, photosynthesis, chlorophyll content, growth and development in *B. vulgaris* grown in hydroponic cultures. The treatments were made up of 1) Hoagland's minus N solution (as the control), 2) *A. filiculoides* plus Hoagland's minus N solution, 3) *A. filiculoides* plus a full Hoagland's solution and 4) full Hoagland's solution. Each treatment was replicated 4 times. Nutrient uptake was measured at 4 and 8 weeks into the experiment. Photosynthesis was measured by analysing the photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration and the evapotranspiration rate of *B. vulgaris* on a weekly basis. Chlorophyll content was determined by analysing the samples at 4 and 8 weeks. Growth and development was determined by measuring plant height, leaf number, leaf colour, fresh weight and dry weight. Plant height, leaf number and leaf colour on a biweekly intervals, while fresh and dry weight were analysed at 4 and 8 weeks into the experiment.

In this study, the most favourable results were attained by the full Hoagland's solution. This treatment produced plants with the highest nutrient uptake, photosynthesis, chlorophyll content and best growth and development. Preceding this was the *Azolla* plus full Hoagland's solution, followed by the *Azolla* plus Hoagland's minus nitrogen solution. The poorest results were noted in the control (Hoagland's minus nitrogen solution) as all the tested parameters in this treatment were the lowest.

In this study, *Azolla* plus Hoagland's minus N solution treatment produced significant growth in *B. vulgaris*. Although nitrogen was not applied in this treatment, there was improved nitrogen content in *B. vulgaris* organs. It is postulated that, probably, *Azolla* released the fixed nitrogen in its surrounding environment making it available to *B. vulgaris* hence improving N uptake and growth. This implies that there was a

synergistic effect from *Azolla–Anabaena* symbiosis. More studies to understand the mechanisms involved in improving the plant growth are recommended.

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## **CHAPTER ONE**

### **INTRODUCTION AND LITERATURE REVIEW**

Review

## ***Azolla filiculoides* as an alternative N fertilizer in hydroponic cultures**

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### **1.1 ABSTRACT**

*Azolla filiculoides* and *Anabaena* symbiosis may produce an alternative Nitrogen (N) fertilizer due to its ability to rapidly and biologically fix N from the atmosphere and release it into the aqueous systems. Nitrogen fixation occurs in *Azolla* due to a symbiotic relationship with blue green algae called *Anabaena azollae*. Studies in rice cultivation have shown that *Azolla–Anabaena* symbiosis can contribute between 25 - 162 kgN.ha<sup>-1</sup>. Nitrogen plays an important role in the growth and development of crops. Nitrogen from whatever sources will influence functions such as growth, photosynthesis, chlorophyll content and nutrient uptake. Limited studies have been conducted on the use of the *Azolla–Anabaena* symbiosis on hydroponics. The possible advantages of using *Azolla filiculoides* and *Anabaena* symbiosis in agriculture and the hydroponic cultures are discussed in this review.

**Key words:** *Azolla–Anabaena* symbiosis, biological N fixation (BNF), hydroponics, relative growth rate (RGR), terrestrial crops

**Abbreviations:** BNF = Biological Nitrogen Fixation; kg.ha<sup>-1</sup> = kilograms per hectare; mg/L = milligram per litre; RGR = Relative Growth Rate; t.ha<sup>-1</sup> = tonne per hectare

## 1.2 INTRODUCTION

*Azolla–Anabaena* symbiosis has been used as a natural source of nitrogen (N) fertilizer in rice cultivation for centuries namely in China and Vietnam (Fogg et al., 1973; Talley and Rains, 1980; Watanabe, 1984; Watanabe and Liu, 1992). This is mainly due to its unique ability to fix atmospheric N into a useable form of ammonia N in water (Holst and Yopp, 1979; Wagner, 1997; Arora and Singh, 2003). The use of this natural source of N has recently been implemented in Africa and other countries in Asia (Lumpkin and Plucknett, 1982; Van Hove, 1989; Watanabe, 1984). In modern agriculture, this form of N has been phased out as large amounts of N are required to ensure sufficient yields to sustain increasing food requirements, this therefore has led to the development of chemically derived N fertilizers which are able to provide sufficient amounts of N (Watanabe and Liu, 1992; Kumarasinghe and Eskew, 1995). Chemical N fertilizers have numerous constraints viz: they are expensive (Madhusoodanan and Sevichan, 1992; Ladha and Reddy, 2003; Awodun, 2008), harmful to the environment (Lee and Nielsen, 1987; Pasternak et al., 1988; Vroomen, 1989; Byrnes, 1990; Cary and Weerts 1992; Zhu and Chen, 2002; Crew and Peoples, 2004), and their over application can lead to toxic N build up in the cultivated crops, which can affect human health negatively (WHO, 1995; Robert, 1997). Lastly, chemical N fertilizer has limited availability to subsistence farmers as they are unable to afford them (Wagner, 1997). *Azolla* in association with *Anabaena* can provide a suitable solution to these farmers as it fixes nitrogen, which is sustainable and inexpensive (Wagner, 1997). Therefore, more insight must be acquired on the effectiveness of *Azolla filiculoides* as N fertilizer on other food crops than rice.

Numerous studies and research have been conducted on this unique organism in the past. Literature published by Shi and Hall (1988) reviewed the history and the

symbiotic relationship between *Azolla filiculoides* and *Anabaena azollae*. However, limited research and reviewing has been conducted on the development of these numerous uses of *Azolla* (Wagner, 1997). An example is the use of *Azolla* as a bio fertilizer on other agricultural crops besides rice. This is mainly due to the fact that rice and *Azolla* can co-exist in similar conditions during the growth period in shallow aquatic environment (Wagner, 1997). Contrarily, most other agricultural crops require different conditions such as a soil based environment to thrive in. New research ought to be directed at developing techniques whereby *Azolla* and other crops can be dual cropped in other systems such as those involving hydroponics cultures.

*Anabaena azollae* occurs within the leaf cavities of *Azolla filiculoides* and it is the active principal in the nitrogen fixing process as it sustains the N needs of itself and *Azolla* (Peters, 1977; 1978). The role of *Azolla filiculoides* in this relationship is that of providing a suitable environment for the blue-green algae to thrive in and a source of carbon (Peters, 1976; Van Hove, 1989). Studies conducted by Ashton and Walmsley, (1976), Becking (1976) and Shi and Hall (1988) on *Anabaena azollae* showed that 50% of the nitrogen excreted was in the form of ammonium. This is then broken down by an enzyme called glutamine synthetase into amino acids, which is then absorbed by *Azolla* in its leaf cavities (Watanabe, 1982; Van Hove, 1989). The rate at which N fixation occurs in *Azolla* differentiates due to leaf maturity (Watanabe, 1982) as the number of leaf cavities in *Azolla* determine to what extent the algae multiply as a certain amount can only occupy the leaf cavities (Hill, 1976). The separated version of *Anabaena* showed that N fixation was lower as opposed to *Anabaena* combined with *Azolla* (Peters and Mayne, 1974; Peters, 1975). This is mainly due to the dependency of *Anabaena* on *Azolla* as it supplies some of the carbon required by the algae (Peters, 1976; Van Hove, 1989; Wagner, 1997). Therefore, more insight has to be gained to incorporate this symbiosis with the nitrogen needs of terrestrial crops that do not occur in water-saturated environments.

Hydroponics may provide a suitable solution as the conditions are emulated to that of the *Azolla* and the cultivated crop. This may be accomplished by manipulating the hydroponic system to provide an aqueous environment for *Azolla* to thrive in. An

example of this is to modify the nutrient reservoir so that it has maximum surface area to accommodate the greatest number of *Azolla* ferns. A flat rectangular container would be suitable with an appropriate volume to provide enough nutrient solution to sustain the development of the desired crop, growth and nitrogen fixation of *Azolla*. The cultivated crop can be incorporated by designing a specific component of the system to contain it. This is determined by growth characteristics of the crop such as the root structure, plant height and environmental conditions. The nutrient concentrations may be formulated to exactly meet the requirements of *Azolla* to provide maximum N fixation, which in turn will be transferred to the associated crops.

As mentioned before, hydroponics could be the link between N supplied by *Azolla* and non-aqueous growing crops, but research is limited on this topic. If more insight is gathered, this could lead to the cultivation of crops where N from the *Azolla*–*Anabaena* symbiosis is used instead of chemical N fertilizer and therefore leading to more sustainable agriculture.

### **1.3 Advantages of *Azolla filiculoides* as a nitrogen fertilizer**

*Azolla*–*Anabaena* symbiosis has been used as a N fertilizer in paddy rice for many centuries (Fogg et al., 1973; Talley and Rains, 1980; Watanabe, 1984; Watanabe and Liu, 1992). This is mainly due to certain characteristics of this symbiosis namely rapid growth, ability to convert atmospheric N to a utilizable form of N (Holst and Yopp, 1979; Lumpkin and Plucknett, 1982; Peters et al., 1982; Lambers and Poorter, 1992), to produce N and grow in N deficient conditions (Maejima et al., 2002). This symbiosis affects the crops that have been cultivated with it positively (Fogg et al., 1973; Watanabe and Liu, 1992; Ventura and Watanabe, 1993; Wagner, 1996; Ladha et al., 2000; Cissé and Vlek, 2003), recondition soil by increasing N, and organic carbon levels (Van Hove, 1989; Singh and Singh, 1990; Satapathy, 1993; Thangaraju and Kannaiyan 1993), to be dual cropped (Watanabe et al., 1977; Venkataraman, 1981; Roger and Ladha, 1992; Roger, 1996; Prasanna et al., 2003) and to release N through decomposition and leaching (Mian and Stewart, 1985; Rains and Talley, 1979). Possibilities are there for using the *Azolla*–*Anabaena* symbiosis to benefit other crops such as those grown in closed hydroponic systems.

#### **1.4 *Azolla filiculoides* as an alternative nitrogen fertilizer**

As mentioned by Wagner (1997) the most favourable crop to cultivate in conjunction with *Azolla* is lowland rice. Dual cropping rice and *Azolla* has been a long established process mainly in China and Vietnam (Moore, 1969; Fogg et al., 1973; Talley and Rains, 1980; Watanabe, 1984; Watanabe and Liu, 1992). This method of combining *Azolla* and rice has been used for centuries in the mentioned areas (Fogg et al., 1973; Talley and Rains, 1980; Watanabe, 1984; Watanabe and Liu, 1992). The discontinued use of the aquatic water fern in rice culture is mainly due to the development of chemical N fertilizer to sustain larger yields (Watanabe and Liu, 1992; Kumarasinghe and Eskew, 1995). Elevated production costs are mainly a result of the use of chemical N fertilizer (Madhusoodanan and Sevichan, 1992; Ladha and Reddy, 2003; Awodun, 2008) which is used to promote growth of crops and in turn their yield (Cox and Reisenauer, 1973). Reducing this expense would require an alternative supply of nitrogen, which is renewable, inexpensive and readily available. One source that suits these characteristics is that of *Azolla–Anabaena* symbiosis.

##### **1.4.1 The use of *Azolla filiculoides* in rice production**

Application of *Azolla* is accomplished by intercropping and mono-cropping. Intercropping is comprised of inoculating *Azolla* after the rice has been planted, it is then allowed to grow and is mixed into the soil where decomposition releases the available nutrients (Lumpkin, 1987a). Maximum benefits of *Azolla* in rice can be achieved by mono-cropping practices. This includes inoculating the *Azolla* before planting the rice and allowing it to grow. When rice is transplanted to the inoculated plots, they benefit from fixed nitrogen, which in turn promotes their growth (Fogg et al., 1973; Watanabe and Liu, 1992). Studies on nitrogen production from *Azolla* in rice cultivation show that *Azolla* is able to produce 25 - 162 kg.ha<sup>-1</sup> of nitrogen (Tran and Dao, 1973; Becking, 1976; Wagner, 1997).

Numerous studies have been conducted on dual cropping rice with *Azolla* (Watanabe et al., 1977; Venkataraman, 1981; Roger and Ladha, 1992; Roger, 1996; Prasanna et al., 2003). For example, studies by Cissé and Vlek (2003) has determined that cultivating rice with *Azolla filiculoides* and urea showed an increase in uptake of nitrogen from 40% without *Azolla* to 57% with *Azolla*. This was mainly

because nitrogen is fixed biologically by *Azolla filiculoides* and then made available to rice (Ladha et al., 2000; Cissé and Vlek, 2003). Roger and Ladha (1992) showed that *Azolla–Anabaena* symbiosis produced between 20 - 30 kg.ha<sup>-1</sup>N where by 70% was obtained from the atmosphere and this was used successfully to cultivate rice. The yield was shown to improve after applying *Azolla*, which amounted to 1.8 - 3.9 t.ha<sup>-1</sup> above the amounts, produced by the control. This method achieved results similar to that of applying 60 kg.ha<sup>-1</sup> of urea (Ventura and Watanabe, 1993). Experiments carried out by Wagner (1996) with *Azolla nilotica* on rice achieved 19 - 103% increase in yield and a significant increase in plant height and number of tillers per plant. *Azolla* is known to increase the soil fertility by increasing the N, organic carbon, available phosphorus and improve the soil structure (Van Hove, 1989; Singh and Singh, 1990; Satapathy, 1993; Thangaraju and Kannaiyan, 1993).

Additional studies conducted by Cissé and Vlek, (2003) resulted in *Azolla* being able to increase the nitrogen recovery by the rice by 43.5%. This was mainly due to the extra N contributed by the *Azolla–Anabaena* symbiosis. Furthermore, it was also established that nitrogen was continuously replenished by the *Azolla* throughout the growth period as opposed to chemical N fertilizer, which is only available and when applied.

Plant physiological studies involving the application of different inorganic N sources and concentrations have indicated negative effects (Peters and Mayne, 1974; Rowell et al., 1977; Manna and Singh, 1990). For example by Rowell et al. (1977), which involved combining NH<sub>4</sub>-N in high concentrations exceeding 300 mg/L with *Azolla* in paddy fields, resulted in reduced nitrogen fixing ability. Other reports showing the effect of chemical N fertilizer such as urea and nitrate resulted in *Azolla* retaining its N fixing ability (Peters and Mayne, 1974), whereas experiments with urea by Manna and Singh (1990) showed decreases in their biomass.

Singh (1979) mentions that results that are more favourable are attained when *Azolla* is added as part of the planting medium as opposed to cultivating *Azolla* alongside the rice. He showed that by incorporating *Azolla* into the planting medium, an average of 6.1 - 54.1 kg.ha<sup>-1</sup> of nitrogen was attained, which surpassed the medium treated with 4.8 - 5.6 kg.ha<sup>-1</sup> of fertilizer. A report by Alimagno and Yoshida

(1977) showed that fields treated with urea showed values of 2.3 - 5.7 kgN.ha<sup>-1</sup> as opposed to the *Azolla* fields which contained 18.5 - 33.3 kgN.ha<sup>-1</sup>.

From the above case studies, *Azolla–Anabaena* symbiosis has positively affected rice cultivation in the traditional and scientific experiments. Therefore, this information provides encouraging insight on the possible uses of *Azolla–Anabaena* symbiosis on other food crops where nitrogen fertilization is needed for their success.

#### **1.4.2 Possible use of *Azolla* in vegetable production**

Studies conducted on the use of *Azolla* on other agricultural crops besides rice have produced different results (D'Souza, and Bourke, 1986). These crops mainly include plants that thrive in waterlogged soils (Wagner, 1997).

Field experiments conducted on taro (*Colocasia esculenta*) showed that adding *Azolla* to the mud resulted into yield increases (Teckle-Haimanot, 1995; Onwueme, 1999). For example, Teckle-Haimanot, 1995 reported a 54 - 87.3% increase in taro yield by co-cultivating *Azolla* and taro. In other studies, positive increases in yield were recorded by mono-cropping *Azolla* with wheat in a rice-wheat cropping cycle (Kolhe and Mittra, 1990). For example, Mahapatra and Sharma (1989) showed that applying *Azolla* to wheat resulted in a 56 - 69% increase in yield as opposed to the control treatments. Similar results were documented by Marwaha et al. (1992) in which the fresh fronds of *Azolla* applied to wheat-increased yield favourably. In mung beans, mixing *Azolla* into the soil resulted into yield increases (Van Hove, 1989). The application of *Azolla* in banana and sugarcane also significantly promoted growth (Mohamed, 2005; James, 2000). Therefore, with proper planning and designing, a system could be developed to utilize the *Azolla–Anabaena* symbiosis in supplying N to crops grown in hydroponic cultures.

#### **1.4.3 Translocation of N fixed from *Azolla* into water**

The association between *Azolla–Anabaena* symbiosis has shown to fix moderate quantities of N into the water and surrounding soil of rice paddies (Pharm, 1971; Tran and Dao, 1973; Becking, 1976; Roger and Reynaud, 1979). Mian and Stewart (1985) and Rains and Talley (1979) mention that N produced from *Azolla* is released in two ways: viz decomposition and leaching. Release of N through decomposition is

initiated by incorporating the biomass of *Azolla* into the planting medium where microbiological activity breaks it down and hence releasing N into available form (Lumpkin, 1987a). Leaching occurs while the *Azolla* is actively growing and fixing nitrogen in association with the cyanobacteria, whereby, N is transferred or leached into the surrounding aqueous solution. Based on this background, the decomposition or leaching of N from *Azolla* could be ideal for supplying N in a hydroponic system. The decomposed or leached nutrient can be directed to the cultivated crop contained within the system.

### **1.5 The effect of N from nitrogen fixation from *Azolla filiculoides* on growth and development of associated crops**

Nitrogen is a major element required for the physiological process within the plants. This element enhances the metabolic processes, which in turn influences growth patterns and nutritional content (Fernandes and Pereyra, 1995), fruiting (Golomb and Goldschmidt, 1987; Conradie, 1992; Brown et al., 1995) and root growth (Caldwell et al. 1981). Studies conducted on leaf vegetables by Ter Steege et al. (1999) showed that the relative growth rate (RGR) increased when more nitrogen was absorbed by the plants due to higher application rates. Conversely, nitrogen fertilizers might affect root growth unfavourably. Studies by Caldwell et al. (1981) showed that root growth was decreased when N levels were increased.

Nitrogen fertilizers are supplied in either organic or inorganic forms. Numerous studies conducted on dual cropping rice with *Azolla* concluded that N uptake of the rice was positively influenced (Watanabe et al., 1989a, b; Roger and Ladha, 1992; Ladha et al., 2000; Cissé and Vlek, 2003). Studies conducted by Talley and Rains, (1980) showed that by cultivating *Azolla* alongside rice resulted in a positive increase in plant growth probably due to the nitrogen fixed by *Azolla*.

The photosynthetic capacity is directly related to the nitrogen content in the plants (Evans, 1989). Physiologically nitrogen plays a key role in electron transport and photophosphorylation activities during photosynthesis (Terashima and Evans, 1988). This means that the amount of nitrogen content in the growth media will directly affect the rate of photosynthesis as higher uptake of N by plants will induce an increase in photosynthetic rate (Paul and Driscoll, 2008). Absence of nitrogen will result in N deficiency and may induce a decrease in photosynthetic rate in plants

(Bottrill et al., 1970; Evans and Terashima, 1987). Nitrogen supplied by *Azolla* in hydrocultures could lead to the increase in the photosynthetic capacity of the cultivated plants.

Chlorophyll content in plants is strongly correlated with N supply, and can be used as a parameter to indicate the N content in plants (Schepers et al., 1992; Blackmer and Schepers, 1995). Nitrogen in plant leaves is stored mainly in the chloroplasts, an important component in the formation of chlorophyll (Evans, 1989). Studies conducted on rice by Tadahiko, (1997) show that 80% of the N stored in healthy leaves was allocated to the chloroplasts and this reflected higher chlorophyll content in the foliage. Mechanistically, N deficient plants show yellow discoloration due to decrease in chlorophyll content (Zhouping et al., 2000). Studies on Tomato conducted in hydroponic system showed that N deficiency induced increased production of carbohydrates and phosphoesters but a decreased organic and amino acids that are important components in chlorophyll formation (Urbanczyk-Wochniak and Fernie, 2004). Therefore, *Azolla filiculoides* and *Anabaena* symbiosis seems to have the potential of producing mineral N that is essential for plant development.

## 1.6 Conclusion

From what has been reviewed the *Azolla–Anabaena* symbiosis presents promising prospects in its use in hydroponics mainly due to its ability to fix N at a considerable rate, and possibilities of it leaching to the nutrient solution where its positive influence on the crops grown in hydroponic systems could be realised. More insight must be gathered on its effectiveness and viability of its use to supply N on other crops, mainly those that do not thrive in an aqueous environment but could be cultivated in modified hydroponic systems.

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## **CHAPTER TWO**

### **PROBLEM STATEMENT, AIMS, HYPOTHESIS AND OBJECTIVES**

## 2.1 PROBLEM STATEMENT

In modern vegetable production in glasshouses, the use of high quantities of mineral nitrogen fertilizer increases costs of production hence lowering profit margins. Furthermore, over application of nitrogen fertilizers may result into water pollution. Cheaper alternative sources of nitrogen may be required to address this problem. The aquatic water fern *Azolla filiculoides* in association with *Anabeana azolloae* may provide a suitable solution as it can fix atmospheric nitrogen, release it into hydroponic systems and make it available to plants.

## 2.2 AIM

This study aims at cultivating *Beta vulgaris subsp. cycla* 'FORDHOOK GIANT' using different combinations of Hoagland's solution and *Azolla* in hydroponic cultures.

## 2.3 HYPOTHESIS

The growth and development of *Beta vulgaris subsp. cycla* 'FORDHOOK GIANT' (Swiss chard) will be influenced by the nitrogen fixed by *Azolla filiculoides* and *Anabeana azollae* symbiosis.

## 2.4 OBJECTIVES OF THE RESEARCH

### 2.4.1 MAIN OBJECTIVE

Assess *Beta vulgaris subsp. cycla* 'FORDHOOK GIANT' exposed to different combinations of Hoagland's solution and *Azolla* in hydroponic cultures.

### 2.4.2. SPECIFIC OBJECTIVES:

- 1) Assess the effect of different combinations of Hoagland's solution and *Azolla filiculoides* on the uptake of nitrogen and other nutrients in *B. vulgaris* grown in hydroponic cultures.
- 2) Evaluate the effect of different combinations of Hoagland's solution and *A. filiculoides* on photosynthesis and chlorophyll content of *B. vulgaris* grown in hydroponic cultures.

- 3) Assess the effect of different combinations of Hoagland's solution and *A. filiculoides* on growth and development of *B. vulgaris* grown in hydroponic cultures.

## CHAPTER THREE

### EFFECTS OF DIFFERENT COMBINATIONS OF HOAGLAND'S SOLUTION AND *AZOLLA FILICULOIDES* ON NUTRIENT UPTAKE IN *BETA VULGARIS* SUBSP. *CYCLA* 'FORDHOOK GIANT' GROWN IN HYDROPONIC CULTURES

**EFFECTS OF DIFFERENT COMBINATIONS OF HOAGLAND'S SOLUTION AND AZOLLA FILICULOIDES ON NUTRIENT UPTAKE IN BETA VULGARIS SUBSP. CYCLA 'FORDHOOK GIANT' GROWN IN HYDROPONIC CULTURES**

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### **3.1 ABSTRACT**

This study involved the evaluation of nutrient uptake (N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B) in the shoots and roots of *Beta vulgaris subsp. cycla* 'FORDHOOK GIANT' grown in different hydroponic nutrient solutions. The objective was to assess the effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the uptake of nitrogen and other nutrients in *B. vulgaris* grown in hydroponic cultures. Treatments were made up of 1) Hoagland's minus N solution as the 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. The best nutrient uptake was acquired by the full Hoagland's solution, followed by the *Azolla* plus full Hoagland's solution. *Azolla* plus Hoagland's minus N solution produced better results than the control (Hoagland's minus N solution), but lower than the other treatments. Nitrogen content in the *A. filiculoides* plus Hoagland's minus N treatment was more than double in shoots and roots collected during harvest 1 and 2, suggesting *Azolla filiculoides* and

*Anabeana* – symbiosis could be used as a possible source of nitrogen fertilizer for *B. vulgaris* in the hydroponic systems.

**Keywords:** nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), boron (B), concentration.

**Abbreviations:** mg = milligrams; mg / L= milligram per liter; PVC = polyvinylchloride

### 3.2 INTRODUCTION

The assimilation of nitrogen and other essential nutrients such as N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B are affected by the form of nutrients applied into the medium (Cox and Reisenauer, 1973; Reddy et al., 1989; Pahlsson, 1992; Lu et al., 2005). Most macro and micro nutrients used in crop productivity may be supplied from chemical fertilizer. For example, the chemical nitrogen is widely used in agriculture and contributes to considerable part of production costs (Kumarasinghe and Eskew, 1995, Awodun, 2008). Nitrogen can also be generated from biological processes such as those involving the symbiotic relationship between plants and bacteria. The biological nitrogen such as one from *Azolla filiculoides* and *Anabaena azollae* relationship is a renewable source which is finally released into the ecosystem through leaching or decomposition (Mian and Stewart, 1985; Rains and Talley, 1979).

In rice cultivation, nitrogen from *Azolla* and *Anabaena* symbiosis has shown significant effects with respect to growth and development of rice (Talley and Rains, 1980; Watanabe et al., 1989a, b; Roger and Ladha, 1992; Ladha et al., 2000; Cissé and Vlek, 2003). The successes in using the *Azolla–Anabaena* symbiosis in rice have opened doors on the possible uses of this symbiotic relationship to contribute to the N economy in the hydroponic cultivation systems. In the terrestrial systems, the evidence of direct N transfer from the legume to the crops growing in the close proximity has been documented (Ndakidemi and Dakora, 2007)

Therefore, nitrogen fixed from *Azolla* and *Anabaena* symbiosis could be manipulated to supply or supplement N in crops growing in the hydroponic systems. This could possibly be achieved by cultivating both *Azolla filiculoides* and the target crop in a hydroponic system designed to accommodate both plant species. As *Azolla–Anabaena* symbiosis is able to grow in N deficient conditions (Wagner, 1997), plants cultivated in nitrogen deficient conditions could possibly benefit from nitrogen fixed by the *Azolla* and *Anabaena* symbiosis and then leaked into the hydroponic medium.

This research was conducted with the objective of assessing the effect of different combinations of Hoagland's solution and *Azolla filiculoides* on the uptake of nitrogen and other nutrients in *B. vulgaris* grown in hydroponic cultures.

### **3.3 MATERIAL AND METHODS**

#### **3.3.1 EXPERIMENTAL**

The nitrogen and nutrient content of *B. vulgaris* was assessed at week 4 and 8 into the experiment. A mechanically ventilated greenhouse situated at the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa was used to accommodate the system. A hydroponic system depicted by Roberto, (2000) assembled on a four block experiential design was applied. Treatments incorporated four PVC pipes bearing 20 plants and a resultant 80 plants in totality. A submersible water pump circulating nutrient solution at 3500 L / hr in a 70 L reservoir. A, B, C, D lettering was used to label the treatments and 1 to 20 to differentiate between the plants.

The cultivation of *B. vulgaris* and *Azolla filiculoides* was facilitated by using a hydroponic system constructed from PVC piping (Roberto 2000). *B. vulgaris* was placed in the PVC piping using net baskets. *Azolla filiculoides* was exposed to the nutrient solution by floating in the reservoir. The use of the apparatus ensured that *Azolla* and *B. vulgaris* were exposed to the nutrient solutions allocated to each treatment. *Azolla filiculoides* was ascertained from the flora identification division at CPUT and *B. vulgaris* from a local garden centre in Cape Town.

One week prior to the planting of *B. vulgaris*, *Azolla filiculoides* was inoculated into 2 of the treatments. A 7 day period allowed for N to be leached into the nutrient solution while *Azolla filiculoides* established. A spatial arrangement of 40 cm was used for *B. vulgaris*. Two nutrient solutions were constituted a full Hoagland's solution and a Hoagland's minus N nutrient solution (Hershey 1994; Hershey 1995). Macro elements were made up by 2 Mole  $\text{KNO}_3$ ; 2 Mole  $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ ; 1 Mole  $\text{NH}_4\text{NO}_3$ ; 1000 mg / L Fe - EDTA; 2 Mole  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ ; 1 Mole  $\text{KH}_2\text{PO}_4$  and the minor elements 0.86 g  $\text{H}_3\text{BO}_3$ ; 1.81g  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ; 0.22 g  $\text{ZnSO}_4 \times 4\text{H}_2\text{O}$ ; 0.051 g  $\text{CuSO}_4$ ; 0.09 g  $\text{H}_3\text{MoO}_4 \times \text{H}_2\text{O}$  depicted the full Hoagland's solution. A commixture of 0.05 Mole  $\text{CaH}_2\text{PO}_4$ ; 0.01 Mole  $\text{CaSO}_4 \times 2\text{H}_2\text{O}$ ; 0.5 Mole  $\text{K}_2\text{SO}_4$ ; 1 Mole  $\text{Mg}_5\text{O}_4$ ; 1000 mg / L Fe - EDTA; 1 Mole  $\text{KH}_2\text{PO}_4$  as macro elements and 0.86 g  $\text{H}_3\text{BO}_3$ ; 1.81 g  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ; 0.22 g  $\text{ZnSO}_4 \times 4\text{H}_2\text{O}$ ; 0.051 g  $\text{CuSO}_4$ ; 0.09 g  $\text{H}_3\text{MoO}_4 \times \text{H}_2\text{O}$  as micro elements made up 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.

### 3.3.2 NUTRIENT ANALYSIS

The, P, K, Ca, Mg Fe, Cu, Zn, Mn, and B content in plant tissue were measured by ashing 1 g of powdered sample in a ceramic crucible at 500°C overnight. The ash was then placed in 5 ml of 6 M HCl and placed in an oven at 50°C for 30 min. 35 ml of deionised water was then added. Whatman no. 1 filter paper was used to filter the sample. The ICP was used to determine the nutrient concentration in plant extracts (Giron 1973). The product of nutrient uptake ( $\text{mg} \cdot \text{plant}^{-1}$ ) was determined as nutrient concentration ( $\text{mg} \cdot \text{g}^{-1}$ , data not shown) and the weight of the dry plant part matter ( $\text{g} \cdot \text{plant}^{-1}$ ).

### 3.3.3 STATISTICAL ANALYSIS

A One-Way analysis of variance (ANOVA) was used to analyse the data. 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).

### 3.4 RESULTS

#### 3.4.1 Effect of different combinations of Hoagland's solution and *A. filiculoides* on macro nutrient uptake in shoots of *B. vulgaris*

Results of the effects of different combinations of Hoagland's solution and *A. filiculoides* on macro nutrient content in shoots of *B. vulgaris* collected during harvest 1 are shown in Table 1. Significant differences ( $P \leq 0.001$ ) between treatments were noted in the macro nutrient uptake (N, P, K, Ca, Mg and Na). For all nutrients, the control treatment (Hoagland's minus N solution) had the lowest uptake per plant, then followed by the treatment with *Azolla* plus Hoagland's minus N solution, *Azolla* plus full Hoagland's solution and the highest in the full Hoagland's solution treatment. Some similarities were also observed in the uptake of N, K, Mg and Na between the control and *Azolla* plus Hoagland's minus N solution. A comparison between the full Hoagland's solution and *Azolla* plus full Hoagland's solution treatments showed some similar outcomes in N uptake. During harvest 1, the full Hoagland's solution resulted into significant uptake of K, Ca, Mg and Na compared with the *Azolla* plus full Hoagland's solution treatments.

In the analysis of macro nutrients in the second harvest of the shoots, significant ( $P \leq 0.001$ ) uptake were also observed in N, K, Ca, Mg and Na (Table 1). The full Hoagland's solution treatment resulted into the highest uptake of N, P, K, Ca, Mg and Na. This was succeeded by the *Azolla* plus full Hoagland's solution and *Azolla* plus Hoagland's minus N solution treatments and finally the control (Hoagland's minus N solution). Similarities were noted in the uptake of N, K, Ca, Mg and Na when *Azolla* plus Hoagland's minus N solution treatment was compared with the control (Hoagland's minus N solution). However, figures from the *Azolla* plus Hoagland's minus N solution treatment were numerically higher than those of control but were not statistically significant.

### **3.4.2 Effect of different combinations of Hoagland's solution and *A. filiculoides* on macro nutrient uptake in roots of *B. vulgaris***

In the first harvest, root contents of N, P, K, Ca, Mg and Na of *B. vulgaris* were significantly affected by different treatments (Table 2). The full Hoagland's solution treatment had the highest concentrations of K. The *Azolla* plus full Hoagland's solution, and full Hoagland's solution had the highest and comparable uptakes of N, P, K, Ca, Mg and Na. The *Azolla* plus Hoagland's minus N treatment succeeded these and the lowest uptake was found in the control treatment (Hoagland's minus N solution). Similarities between the control (Hoagland's minus N solution) and *Azolla* plus Hoagland's minus N solution were recorded where N, K, and Na were similar, but *Azolla* plus Hoagland's minus N solution had a higher mean. The *Azolla* plus full Hoagland's solution, and full Hoagland's solution had similar in N, K and Na values, but the *Azolla* plus Hoagland's minus N solution was higher in P, Ca and Mg.

In the second harvest, except P, the macro nutrient concentrations in root of *B. vulgaris* were significantly affected by different treatment combinations (Table 2). The full Hoagland's solution treatment had higher nutrients uptake in their roots relative to all other treatments. Roots collected from the *Azolla* plus full Hoagland's solution achieved the second highest nutrient uptake and this was followed by the *Azolla* plus Hoagland's minus N solution treatment. Least uptake values were found in the control (Hoagland's minus N solution) treatment.

### **3.4.3 Effect of different combinations of Hoagland's solution and *A. filiculoides* on micro nutrient uptake in shoots of *B. vulgaris***

The shoot micro nutrient uptake of *B. vulgaris* (Table 3) in harvest 1 show that the full Hoagland's solution had the highest uptakes, then preceded by *Azolla* plus full Hoagland's solution and *Azolla* plus Hoagland's minus N. The lowest uptakes were produced by the control (Hoagland's minus N solution) treatment. Comparing *Azolla* plus Hoagland's minus N solution with the control, similarities were noted in Fe and B uptake, but the shoot uptake of Mn, Cu and Zn were significantly different between the two treatments. The comparison between *Azolla* plus Hoagland's minus N solution and *Azolla* plus full Hoagland's solution showed some similarities in the

uptake of Mn, Fe and Zn. However, the uptake of Cu and B was significantly different between the two treatments.

The micro nutrient uptake (except Zn) in the shoots of *B. vulgaris* during the second harvest (Table 3) was significantly different. Shoots from full Hoagland's solution treatment attained the highest significant uptake of Mn, Fe, Cu, and B, followed by *Azolla* plus full Hoagland's solution. The control and *Azolla* plus Hoagland's minus N solution produced similar results with respect to the uptake of Mn, Fe, Cu, and B.

#### **3.4.4 Effect of different combinations of Hoagland's solution and *A. filiculoides* micro nutrient uptake in roots of *B. vulgaris***

During first harvest, the micro nutrients in roots of *B. vulgaris* showed significant differences between the treatments (Table 4). The *Azolla* plus full Hoagland's solution produced roots with the highest uptake of Fe, Cu, Zn and B. Mn was highest in the full Hoagland's solution treatment. The second best uptake of Fe, Cu, Zn and B was recorded in the full Hoagland's solution. With the exception of Zn, the *Azolla* plus Hoagland's minus N solution and the control produced comparable results for the uptake of Mn, Fe, Cu, and B.

In the second harvest, significant ( $P \leq 0.001$ ) treatment effects were observed in the uptake of Mn, Fe, Cu, Zn and B in roots of *B. vulgaris* (Table 4). The full Hoagland's solution and *Azolla* plus full Hoagland's solutions were comparable with higher uptake values and were significantly different from the *Azolla* plus Hoagland's minus N solution and the control (Hoagland's minus N solution). The *Azolla* plus Hoagland's minus N solution and the control (Hoagland's minus N solution) were not significantly different on their uptake patterns although the *Azolla* plus Hoagland's minus N solution had higher uptake numerical values.

### **3.5 DISCUSSION**

In this study, different combinations of Hoagland's solution and *A. filiculoides* affected the uptake of macro and micro nutrients in shoots and roots of *B. vulgaris* (Table 1-4). As expected, the best nutrient uptake (N, P, K, Ca, Mg Na, Mn, Fe, Cu,

Zn and B) was observed in the full Hoagland's solution treatment which contained all the required nutrients in balanced formulation, including nitrogen. The second best nutrient uptake for N, P, K, Ca, Mg Na, Mn, Fe, Cu, Zn and B was recorded in the *Azolla* plus full Hoagland's solution. It is likely that in this setting there was competition for nutrients between *B. vulgaris* and *Azolla*. The same nutrients needed by *B. vulgaris* in this treatment were also utilized by the *Azolla* to meet their internal physiological functions. Lumpkin (1987b) indicated that *Azolla* required a mix of nutrients to sustain its activities, a phenomenon which might have affected the uptake of macro and micro nutrient in shoot and roots of *B. vulgaris*. The *Azolla* plus Hoagland's minus N produced the third highest uptake of macro and micro nutrients in shoots and roots: probably due to *Azolla* releasing nitrogen in its surrounding environment and therefore influencing the uptake of other nutrients in the nutrient solution. Roger and Ladha, (1992), Ladha et al., (2000) and Cissé and Vlek, (2003) indicated that when *Azolla* was dual cropped with rice the assimilation of nutrients especially nitrogen was positively influenced.

It has been acknowledged that *Azolla* is able to fix atmospheric nitrogen into usable forms and release it to its surrounding environment (Rains and Talley, 1979; Mian and Stewart, 1985; Obreht et al., 1997). The *Azolla* plus Hoagland's minus N produced encouraging N uptake results compared with the control treatment. In most case, the N uptake in the *Azolla* plus Hoagland's minus N was 2-3 fold in shoots and roots of *B. vulgaris* compared with the control treatment (Tables 1-2), suggesting a possible N leakage into the hydroponic system. A similar improvement from the fixing partner to the cereal counterpart was reported by Ndakidemi and Dakora (2007). These instances of numerical increase in N uptake in *B. vulgaris* organs clearly suggests the need for further experimentation on the Nitrogen fixed by the *Azolla* and its availability in the hydroponic systems.

Finally, these results give some insights into the potential of using *Azolla filiculoides* as a nitrogen fertilizer in the hydroponic culture of *B. vulgaris*. This is the only report in literature to assess the effectiveness of different combinations of Hoagland's solution in combination with *Azolla* in the hydroponic technologies. The execution of a more efficacious hydroponic system, which is able to hold a greater amount of

*Azolla* and successfully promote the growth and nitrogen fixation of *Azolla* for sustainable crop production in hydroponic cultures, warrants more research.

### 3.6 ACKNOWLEDGEMENTS

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**Table 1:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the uptake of macronutrients in shoots of *B. vulgaris* during the first and second harvest.

Treatment	Shoot Macro nutrient content first harvest (mg per plant)					
	N	P	K	Ca	Mg	Na
Control( Hoagland's minus N solution)	44.8±12.3c	70.1±15.0c	211.8±55.1c	21.4±4.1d	16.3±1.8c	18.3±4.8c
<i>Azolla</i> plus Hoagland's minus N solution	132.4±10.4c	280.6±56.4a	629.7±114.3c	164.5±37.0c	102.5±20.2c	47.4±7.8c
<i>Azolla</i> plus full Hoagland's solution	1539.3±90.6b	132.4±17.3bc	2453.8±313.0b	411.0±55.9b	377.0±49.0b	190.8±18.6b
Full Hoagland's solution	2812.1±178.2a	220.0±25.9ab	4650.7±285.4a	638.1±45.4a	702.4±57.2a	428.9±52.3a
One - Way ANOVA (F-Statistic) Rep	171.4***	7.9***	83.9***	45.1***	62.9***	44.4***
Treatment	Shoot Macro nutrient content second harvest (mg per plant)					
	N	P	K	Ca	Mg	Na
Control( Hoagland's minus N solution)	4.1±0.5c	13.1±4.3a	27.6±3.2c	4.8±1.0c	3.7±0.6c	1.8±0.4c
<i>Azolla</i> plus Hoagland's minus N solution	11.4±0.9c	28.4±2.9a	54.7±3.4c	11.3±1.5c	8.9±1.3c	3.4±0.5c
<i>Azolla</i> plus full Hoagland's solution	645.8±79.8b	45.4±1.9a	926.1±75.2b	144.2±14.3b	141.2±17.9b	66.6±12.3b
Full Hoagland's solution	1137.6±190.8a	328.6±217.1a	1800.4±132.8a	297.9±34.7a	294.7±37.2a	127.1±17.0a
One - Way ANOVA (F-Statistic) Rep	28.1***	1.9NS	121.8***	54.0***	44.3***	32.3***

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.

**Table 2:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the uptake of macronutrients in roots of *B. vulgaris* during the first and second harvest.

Treatment	Root Macro nutrient content first harvest (mg per plant)					
	N	P	K	Ca	Mg	Na
Control( Hoagland's minus N solution)	6.0±1.6b	1.3±0.2c	4.9±0.8b	0.8±0.4c	1.1±0.3c	0.3±0.0c
<i>Azolla</i> plus Hoagland's minus N solution	16.3±1.2b	4.2±1.2bc	14.8±2.7b	4.6±0.8bc	4.8±0.8b	1.0±0.2bc
<i>Azolla</i> plus full Hoagland's solution	51.0±6.5a	9.3±1.8a	37.0±4.5a	32.2±7.5a	9.0±1.4a	1.5±0.3a
Full Hoagland's solution	49.1±11.8a	5.2±1.3b	39.8±8.6a	16.0±3.8b	6.5±1.0ab	1.3±0.4a
One - Way ANOVA (F-Statistic) Rep	11.3***	7.3**	11.2***	11.0***	11.9***	5.0**
Treatment	Root Macro nutrient content second harvest (mg per plant)					
	N	P	K	Ca	Mg	Na
Control( Hoagland's minus N solution)	6.0±0.5c	3.3±0.4b	8.5±1.1c	3.1±0.4b	2.1±0.2b	0.5±0.1b
<i>Azolla</i> plus Hoagland's minus N solution	15.0±4.5c	3.3±1.1b	9.8±3.4c	3.3±0.9b	3.0±0.8b	0.6±0.2b
<i>Azolla</i> plus full Hoagland's solution	183.5±29.6b	31.1±3.2a	104.6±11.2b	31.8±35.9a	23.4±4.5a	3.4±0.5a
Full Hoagland's solution	272.2±18.3a	33.0±3.7a	147.2±23.8a	33.0±6.4a	24.6±2.7a	3.0±0.4a
One - Way ANOVA (F-Statistic) Rep	55.3***	44.1***	27.5***	44.0***	22.0***	23.5***

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.

**Table 3:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the uptake of micronutrients in shoots of *B. vulgaris* during the first and second harvest.

Treatment	Shoot Micro nutrient content first harvest (mg per plant)				
	Mn	Fe	Cu	Zn	B
Control( Hoagland's minus N solution)	0.684±0.199c	3.994±2.315b	0.009±0.006c	0.363±0.095b	0.219±0.069c
<i>Azolla</i> plus Hoagland's minus N solution	3.123±0.621b	1.821±0.231b	0.067±0.013a	1.936±0.345a	0.547±0.090c
<i>Azolla</i> plus full Hoagland's solution	3.864±0.702b	4.186±0.465b	0.038±0.002b	1.574±0.318a	2.035±0.238b
Full Hoagland's solution	9.963±1.008a	8.205±0.518a	0.057±0.003ab	2.202±0.294a	3.012±0.328a
One - Way ANOVA (F-Statistic) Rep	32.2***	4.8**	12.0***	12.0***	38.4***
Treatment	Shoot Micro nutrient content second harvest (mg per plant)				
	Mn	Fe	Cu	Zn	B
Control( Hoagland's minus N solution)	0.107±0.035b	0.460±0.064c	0.002±0.001b	0.046±0.014a	0.022±0.004c
<i>Azolla</i> plus Hoagland's minus N solution	0.111±0.015b	0.848±0.031c	0.004±0.001b	0.113±0.012a	0.050±0.003c
<i>Azolla</i> plus full Hoagland's solution	1.454±0.098b	16.650±2.120b	0.022±0.007a	0.821±0.010a	1.018±0.144b
Full Hoagland's solution	5.523±1.703a	28.997±3.219a	0.027±0.055a	1.479±0.930a	1.508±0.128a
One - Way ANOVA (F-Statistic) Rep	9.0***	50.9***	9.3**	2.1NS	58.1***

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

**Table 4:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the uptake of micronutrients in shoots of *B. vulgaris* during the first and second harvest.

Treatment	Root Micro nutrient content first harvest (mg per plant)				
	Mn	Fe	Cu	Zn	B
Control( Hoagland's minus N solution)	0.081±0.023c	1.060±0.340b	0.004±0.002b	0.027±7.187c	0.007±0.001c
<i>Azolla</i> plus Hoagland's minus N solution	0.255±0.041bc	2.773±0.468b	0.019±0.002b	0.138±26.967b	0.022±0.004c
<i>Azolla</i> plus full Hoagland's solution	0.386±0.036ab	9.590±1.947a	0.058±0.010a	0.243±29.545a	0.101±0.019a
Full Hoagland's solution	0.567±0.142a	4.237±0.719b	0.015±0.002b	0.732±18.199a	0.060±0.013b
One - Way ANOVA (F-Statistic) Rep	7.2**	11.7***	21.4***	17.6***	13.3***
Treatment	Root Micro nutrient content second harvest (mg per plant)				
	Mn	Fe	Cu	Zn	B
Control( Hoagland's minus N solution)	0.254±0.056b	0.438±0.045b	0.006±0.000b	0.124±0.040b	0.014±0.001b
<i>Azolla</i> plus Hoagland's minus N solution	0.296±0.099b	0.640±0.116b	0.030±0.008b	0.252±0.018b	0.020±0.004b
<i>Azolla</i> plus full Hoagland's solution	1.401±0.235a	4.727±0.685a	0.229±0.022a	1.524±0.145a	0.167±0.033a
Full Hoagland's solution	2.478±0.363a	5.525±0.445a	0.079±0.004a	0.660±0.048a	0.160±0.014a
One - Way ANOVA (F-Statistic) Rep	22.3***	41.8***	69.0***	63.5***	21.7***

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

## **CHAPTER FOUR**

### **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**

**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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#### **4.1 Abstract**

The assessments of photosynthetic rate, stomatal conductance, evapotranspiration, intercellular CO<sub>2</sub> 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 showed that photosynthetic rate, evapotranspiration, intercellular CO<sub>2</sub> 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<sub>2</sub> concentration, chlorophyll a, chlorophyll b

**Abbreviations:** DMSO = dimethyl sulphoxide; DWC = deep water channel; L / hr = litres per hour; mg / L<sup>-1</sup> = milligrams per litre; N = nitrogen; nm = nanometres; PVC = polyvinylchloride

## 4.2 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 (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 via biological and chemical nitrogen fertilizers (Roger and Reynaud, 1982; Kumarasinghe and Eskew, 1995). Chemical nitrogen fertilizers 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). *Anabaenas* occurs extracellularly and fix 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 *Azolla filiculoides* on photosynthesis processes and chlorophyll content in *B. vulgaris* grown in hydroponic cultures.

### **4.3 MATERIAL AND METHODS**

#### **4.3.1. EXPERIMENTAL**

The physiological responses photosynthesis and chlorophyll concentration of *B. vulgaris* was measured throughout a 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 PVC pipe deep water channel (DWC) hydroponic system on a four block experimental 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 / hr 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. filiculoides* was introduced to 2 of the systems 1 week earlier to that of the *B. vulgaris*. A 1 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; Hershey 1995). Macro elements were characterized by 2 Mole  $\text{KNO}_3$ ; 2 Mole  $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ ; 1 Mole  $\text{NH}_4\text{NO}_3$ ; 1000 mg/L Fe-EDTA; 2 Mole  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ ; 1 Mole  $\text{KH}_2\text{PO}_4$  and minor elements constituted 0.86 g  $\text{H}_3\text{BO}_3$ ; 1.81 g  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ; 0.22 g  $\text{ZnSO}_4 \times 4\text{H}_2\text{O}$ ; 0.051 g  $\text{CuSO}_4$ ; 0.09 g  $\text{H}_3\text{MoO}_4 \times \text{H}_2\text{O}$  portrayed the full Hoagland's solution. Combination of 0.05 Mole  $\text{CaH}_2\text{PO}_4$ ; 0.01 Mole  $\text{CaSO}_4 \times 2\text{H}_2\text{O}$ ; 0.5 Mole  $\text{K}_2\text{SO}_4$ ; 1 Mole  $\text{MgSO}_4$ ; 1000 mg/L Fe-EDTA; 1 Mole  $\text{KH}_2\text{PO}_4$  as macro elements and 0.86 g  $\text{H}_3\text{BO}_3$ ; 1.81 g  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ; 0.22 g  $\text{ZnSO}_4 \times 4\text{H}_2\text{O}$ ; 0.051 g  $\text{CuSO}_4$ ; 0.09 g  $\text{H}_3\text{MoO}_4 \times \text{H}_2\text{O}$  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.

#### 4.3.2 MEASUREMENT OF PHOTOSYNTHESIS

A portable photosynthesis system LCpro+ 1.0 ADC, (Bioscientific Ltd., 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 09.00-11.00 am from the apparatus included: photosynthetic rate, stomatal conductance, intercellular carbon dioxide concentration and evapotranspiration rate.

#### 4.3.3 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 hours at 4 °C. 3 ml of DMSO was added to dilute the extraction to 10ml. 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 formulae developed by Arnon (1949).

$$\text{Chl}_t = 20.2D_{645} + 8.02D_{663}$$

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

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

#### 4.3.4 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).

#### 4.4 RESULTS

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

Table 1 portrays weekly results from measurements of the photosynthetic rate of *B. vulgaris*. Results show that the treatment with *Azolla* plus full Hoagland's solution produced significantly ( $P \leq 0.001$ ) higher photosynthetic rate when compared with the control where the photosynthetic 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, which recorded significantly ( $P \leq 0.001$ ) higher photosynthetic rate in weeks 1, 4 and 8. In week 2, no significant differences were noted between all other treatments relative to the control. In week 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 general, 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).

#### **4.4.2 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 differences between treatments were observed in weeks 2, 3, 5 and 8. However significant differences were noted in weeks 1 ( $P \leq 0.001$ ), 4 ( $P \leq 0.01$ ), 6 ( $P \leq 0.001$ ) and 7 ( $P \leq 0.01$ ). At 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 stomatal conductance was 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 week 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 stomatal conductance in week 6 and 7 but was very similar to that of *Azolla* plus full Hoagland's solution in week 7.

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

The evapotranspiration rate of *B. vulgaris* is documented in Table 3. The results showed 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 week 3-8 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).

#### **4.4.4 Effects of different combinations of Hoagland's solution and *A. filiculoides* on intercellular CO<sub>2</sub> concentration of *B. vulgaris***

The intercellular CO<sub>2</sub> concentration *B. vulgaris* in Table 4 shows no significant results in 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<sub>2</sub> 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<sub>2</sub> concentrations were recorded in the *Azolla* plus Hoagland's minus N solution and full Hoagland's solution treatments.

#### **4.4.5 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* presented in Table 5 showed 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.

#### **4.5 DISCUSSION**

Results in Tables 1-5 show that photosynthetic rate, evapotranspiration, intercellular CO<sub>2</sub> 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, followed by *Azolla* plus full Hoagland's solution and *Azolla* plus Hoagland's minus N solution treatment. The control (Hoagland's minus N solution) had lowest values for these parameters. 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) and chlorophyll formation (Evans, 1989; Schepers et al., 1992; Blackmer and Schepers, 1995). In photosynthesis, nitrogen is crucial in the functioning of 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, intercellular CO<sub>2</sub> concentration and chlorophyll formation, a phenomenon which was also observed in the present study.

The *Azolla* plus Hoagland's solution minus N treatment alone in this study displayed improved photosynthesis, evaporative transpiration, intercellular CO<sub>2</sub> concentration and chlorophyll content. *Azolla* 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<sub>2</sub> concentration and chlorophyll content at different measurements dates.

Lastly, these results give convincing information into *A. filiculoides* as a possible nitrogen fertilizer in the hydroponic cultivation of *B. vulgaris* and other food crops (Figure 1). To the best of my 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.

#### **4.6 ACKNOWLEDGEMENTS**

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**Table 1:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the photosynthetic rate 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±0.1c	0.2±0.0a	0.0±0.3b	0.3±0.1b	0.3±0.0b	0.3±0.0c	0.3±0.0c	0.2±0.0c
<i>Azolla</i> plus Hoagland's minus N solution	0.4±0.0c	0.2±0.1a	0.0±0.3b	0.4±0.0b	0.5±0.0b	0.5±0.1b	0.5±0.1c	0.6±0.0b
<i>Azolla</i> plus full Hoagland's solution	0.9±0.1b	0.5±0.1a	0.1±0.6a	1.0±0.1a	1.0±0.1a	1.1±0.0a	1.1±0.1b	0.8±0.0b
Full Hoagland's solution	1.3±0.0b	0.6±0.2a	0.1±0.7a	1.1±0.0a	0.9±0.0a	1.2±0.0a	1.6±0.1a	1.2±0.2a
One - Way ANOVA (F-Statistic) Rep	45.4***	3.1NS	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.

**Table 2:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the stomatal conductance 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±0.003c	0.000±0.000a	0.003±0.003a	0.000±0.000c	0.003±0.003a	0.003±0.003c	0.003±0.003c	0.003±0.003a
<i>Azolla</i> plus Hoagland's minus N solution	0.010±0.000bc	0.003±0.003a	0.003±0.003a	0.005±0.003bc	0.010±0.000a	0.010±0.000b	0.010±0.000b	0.010±0.000a
<i>Azolla</i> plus full Hoagland's solution	0.025±0.006b	0.005±0.003a	0.005±0.003a	0.013±0.003a	0.013±0.006a	0.010±0.000b	0.013±0.003ab	0.010±0.004a
Full Hoagland's solution	0.055±0.003a	0.005±0.005a	0.008±0.003a	0.010±0.000ab	0.013±0.003a	0.180±0.003a	0.018±0.003a	0.013±0.003a
One - Way ANOVA (F-Statistic) Rep	27.9***	0.58NS	0.85NS	8.4**	1.7NS	12.0***	8.3**	2.6NS

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

**Table 3:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the evapotranspiration 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±0.025c	0.050±0.006a	0.128±0.023b	0.093±0.006c	0.090±0.023c	0.090±0.013c	0.110±0.019c	0.108±0.020b
<i>Azolla</i> plus Hoagland's minus N solution	0.098±0.010bc	0.060±0.018a	0.105±0.024b	0.140±0.015b	0.173±0.010ab	0.158±0.017b	0.200±0.013b	0.228±0.009a
<i>Azolla</i> plus full Hoagland's solution	0.178±0.015b	0.113±0.015a	0.110±0.011b	0.150±0.020b	0.145±0.033bc	0.170±0.007b	0.183±0.009b	0.155±0.025b
Full Hoagland's solution	0.23±0.000a	0.095±0.028a	0.293±0.044a	0.270±0.004a	0.220±0.014a	0.255±0.009a	0.268±0.005a	0.213±0.008a
One - Way ANOVA (F-Statistic) Rep	13.4***	2.5NS	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, 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 4:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the intercellular CO<sub>2</sub> 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±5.0a	243.0±12.0ab	206.0±33.6a	217.3±44.7a	140.3±57.0a	188.0±39.8bc	221.5±51.4a	207.3±41.2a
<i>Azolla</i> plus Hoagland's minus N solution	262.0±12.0a	281.0±8.9a	207.8±23.2a	218.0±6.6a	244.5±9.1a	261.5±5.1a	249.3±6.4a	248.5±4.1a
<i>Azolla</i> plus full Hoagland's solution	257.3±7.5a	194.0±31.1b	167.3±27.6a	183.5±23.3a	150.5±33.7a	137.0±20.3c	143.8±20.3a	214.8±17.1a
Full Hoagland's solution	276.0±3.9a	215.3±19.9b	218.8±21.7a	217.0±4.9a	223.5±9.7a	208.8±9.0ab	219.5±9.0a	245.3±22.0a
One - Way ANOVA (F-Statistic) Rep	1.5NS	3.6**	0.7NS	0.4NS	2.4NS	5.1**	2.6NS	0.7NS

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:** Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on the chlorophyll concentration of *B. vulgaris*

Treatment	Total chlorophyll concentration	Total chlorophyll a concentration	Total chlorophyll b concentration
Control (Hoagland's solution minus N)	0.3±0.1d	0.3±0.1c	-0.1±0.0c
<i>Azolla</i> plus Hoagland's minus N solution	1.2±0.0c	0.9±0.0b	0.3±0.1b
<i>Azolla</i> plus full Hoagland's solution	2.5±0.1b	2.2±0.1a	0.3±0.0b
Full Hoagland's solution	2.8±0.0a	2.2±0.1a	0.5±0.1a
One - Way ANOVA (F-Statistic) Rep	531.***	161.7***	25.0**

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.



**Figure 1:** Comparison between a control (left side) and *Azolla* plus Hoagland' minus N (right side)

## CHAPTER FIVE

### 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

## **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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### **5.1 ABSTRACT**

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 weeks. 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 the 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

**Abbreviations:** BNF = biological nitrogen fixation; kg.ha<sup>-1</sup> = kilograms per hectare; L = litres; L / hr = litres per hour; LECA = light expanded clay aggregate; m<sup>2</sup> = metres square; PVC = polyvinylchloride; t.ha<sup>-1</sup> = tonne per hectare

## 5.2 INTRODUCTION

Physiological processes within plants are greatly affected by 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, mineral fertilization is the chosen method of supplying nitrogen to ensure sustained plant yields (Anjana et al., 2007). 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 to replace or supplement the mineral fertilizers.

*A. 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; Obrecht 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 - 162 kg.ha<sup>-1</sup> 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 - 103% increase in rice yield due to a substantial increase in plant height and number of tillers per plant. This positive increase is what is required by a biological N fertilizer, somewhat similar to mineral 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 from the atmosphere into the hydroponic system and make it available to plants. 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* grown in hydroponic cultures.

## 5.3 MATERIAL AND METHODS

### 5.3.1 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 was 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 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 / hr 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 – 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 was 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 the 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) and Hershey (1995). Macro elements composed of 2 Mole  $\text{KNO}_3$ ; 2 Mole  $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ ; 1 Mole  $\text{NH}_4\text{NO}_3$ ; 1000 mg / L Fe-EDTA; 2 Mole  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ ; 1 Mole  $\text{KH}_2\text{PO}_4$  and minor elements made up of 0.86 g  $\text{H}_3\text{BO}_3$ ; 1.81 g  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ; 0.22 g  $\text{ZnSO}_4 \times 4\text{H}_2\text{O}$ ; 0.051g  $\text{CuSO}_4$  ; 0.09 g  $\text{H}_3\text{MoO}_4 \times \text{H}_2\text{O}$  represented the full Hoagland's solution. Whereas the minus Hoagland's solution was prepared from 0.05 Mole  $\text{CaH}_2\text{PO}_4$ ; 0.01 Mole  $\text{CaSO}_4 \times 2\text{H}_2\text{O}$ ; 0.5 Mole  $\text{K}_2\text{SO}_4$ ; 1 Mole  $\text{MgSO}_4$ ; 1000 mg / L Fe-EDTA; 1 Mole  $\text{KH}_2\text{PO}_4$  as macro elements and 0.86 g  $\text{H}_3\text{BO}_3$ ; 1.81 g  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ; 0.22 g  $\text{ZnSO}_4 \times 4\text{H}_2\text{O}$ ; 0.051 g  $\text{CuSO}_4$ ; 0.09 g  $\text{H}_3\text{MoO}_4 \times \text{H}_2\text{O}$  as minor elements depicted the minus N Hoagland's solution. The treatments included 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.

### 5.3.2 MEASUREMENT OF GROWTH AND DEVELOPMENT

Growth and development of *B. vulgaris* was determined by measuring the 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. Measurements of growth were done at 2, 4, 6, 8 weeks after planting. Total leaf number was determined by counting the developing and developed leaves. Plant colour was visually assessed on a scale of 1 - 5 where 1 represented a pale yellow colour and 5 typified a dark green colour, these weekly 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 hours at 60°C to ascertain the dry mass.

### 5.3.3 STATISTICAL ANALYSIS

Data collected were 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).

## 5.4 RESULTS

### 5.4.1 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 showed that by applying *Azolla* plus Hoagland's minus N solution resulted in significant ( $P \leq 0.001$ ) increase in height as opposed to the control (Hoagland's minus N solution) treatment in week 2, 4, 6 and 8. Significant ( $P \leq 0.001$ ) 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). Plants growing in treatment with full Hoagland's solution achieved the highest height, followed by those in *Azolla* plus full Hoagland's solution. Overall, all the treatments depicted a significantly ( $P \leq 0.001$ ) 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.

### 5.4.2 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

( $P \leq 0.01$ ) 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 leaves and 2.8 leaves for the control (Hoagland's minus N solution).

#### **5.4.3 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 ( $P \leq 0.001$ ) 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).

#### **5.4.4 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* are shown in Table 4. Significant differences were observed between the treatments for shoot, root and total fresh weight. During harvest 1 and 2, full Hoagland's 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 3<sup>rd</sup> 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 differences in fresh weight yield 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.

#### **5.4.5 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 mass of dry shoots, roots and whole plant were produced in the the full Hoagland's solution. This was closely followed by the *Azolla* plus full Hoagland's solution.

### **5.5 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 (Figure 1-3). The nutrient solution containing *Azolla* plus Hoagland's minus N solution showed a difference in growth relative to the control (Hoagland's minus N solution) which had no nitrogen and no *Azolla* (Fig. 1). The most favourable results were achieved by the solutions containing full Hoagland's solution (Fig. 3) and *Azolla* plus full Hoagland's solution (Fig. 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 Mitra, 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 ( $\text{CaNO}_3$ ), potassium nitrate ( $\text{KNO}_3$ ) and ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) which are readily available for absorption (Hershey, 1994; Hershey, 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 - 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 - 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 my knowledge, this is the only study in literature to evaluate 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 of *Azolla* and effectively promote the growth and nitrogen fixation from *Azolla*-*Anabaena* symbiosis for effective crop production in hydroponic cultures.

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

Values presented are means ± SE. \*\*\* = significant at  $P \leq 0.001$ . 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±0.3b	5.0±0.0b	2.8±0.9c	2.8±0.6b
<i>Azolla</i> plus Hoagland's minus N solution	5.5±0.5ab	5.8±0.6b	4.5±1.0bc	3.8±0.9b
<i>Azolla</i> plus full Hoagland's solution	5.5±0.3ab	5.5±0.5b	6.8±0.9ab	8.3±0.6a
Full Hoagland's solution	6.3±0.5a	8.0±0.4a	8.5±0.5a	10.3±0.9a
One - Way ANOVA (F-Statistic) Rep	2.4NS	8.7**	9.1**	22.8***

Values presented are means  $\pm$  SE. \*\*: \*\*\* = significant at  $P \leq 0.01$ ,  $P \leq 0.001$  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.

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

Treatment	Week2	Week4	Week6	Week8
Control (Hoagland's minus N solution)	2.3±0.1d	2.5±0.0b	2.5±0.0d	2.5±0.0c
<i>Azolla</i> plus Hoagland's minus N solution	3.0±0.0c	2.9±0.1b	3.1±0.1c	3.3±0.1b
<i>Azolla</i> plus full Hoagland's solution	3.8±0.1a	3.8±0.1a	5.0±0.0b	5.5±0.0a
Full Hoagland's solution	3.4±0.1b	4.0±0.2a	5.4±0.1a	5.5±0.0a
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.

**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±0.4c	2.4±0.6b	3.9±1.0d	2.1±0.2c	4.9±3.2c	7.0±0.7c
<i>Azolla</i> plus Hoagland's minus N solution	4.2±0.3c	8.5±1.2a	12.8±1.4c	4.9±0.3c	8.5±6.1c	13.4±1.0c
<i>Azolla</i> plus full Hoagland's solution	25.9±1.4b	11.6±1.9a	37.5±2.4b	123.1±16.6b	52.5±39.2b	175.5±19.8b
Full Hoagland's solution	48.3±2.6a	8.7±0.9a	57.0±3.0a	268.5±33.2a	77.5±50.0a	346.0±38.5a
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±0.1c	0.2±0.1c	0.4±0.1c	0.5±0.1c	0.4±0.0b	0.9±0.1b
<i>Azolla</i> plus Hoagland's minus N solution	0.9±0.1b	0.7±0.1c	1.6±0.2c	0.8±0.0c	0.6±0.1b	1.5±0.1b
<i>Azolla</i> plus full Hoagland's solution	1.5±0.2a	3.8±0.2b	5.3±0.3b	16.7±2.1b	4.7±0.7a	21.4±2.6a
Full Hoagland's solution	1.2±0.2ab	5.3±0.6a	6.6±0.7a	29.0±3.2a	5.5±0.4a	34.5±3.5a
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.



**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).

## **CHAPTER SIX**

### **GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION**

## 6.1 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

Greenhouse production of vegetables is limited by various constraints; amongst them being the use of expensive mineral nitrogen fertilizers. Nitrogen fertilizers can be supplied from biological sources such as those involving *Azolla–Anabeana* symbiosis. Results from this study conducted in the glasshouse have shown that different combinations of Hoagland solution and *Azolla filiculoides* enhanced *Beta vulgaris* growth through improved plant length, leaf numbers, leaf colour and plant biomass. Furthermore, the photosynthesis components such as photosynthesis rate, evapotranspiration rate, intercellular CO<sub>2</sub> concentration and chlorophyll content were also affected by the treatment combinations. The uptake of macro and micro nutrients (N, P, K, Ca, Mg Na, Mn, Fe, Cu, Zn and B) in shoots and roots of *B. vulgaris* were further influenced.

As shown in this study, *Azolla* plus Hoagland's minus N treatment produced significant growth in *B. vulgaris*. This was also associated with improved nitrogen content in *B. vulgaris*. It is postulated that, probably, *Azolla* released the fixed nitrogen in its surrounding environment and therefore making it available to *B. vulgaris* hence improving N uptake and growth. Generally, this implies that there was a synergistic effect from *Azolla–Anabeana* symbiosis. More studies to understand the mechanisms involved in improving the plant growth are recommended.

In small scale settings, where farmers are encouraged to construct simple structures for producing vegetables in closed environments, the use of this technology may be a step towards doubling their vegetable yields. However, appropriate measures must be taken to address the issue of developing an efficient system to co-cultivate the *Azolla* and vegetable concurrently in the hydroponic cultures. If designed well, this may result into greater economic returns to farmers, as this technology is simple, cheap and sustainable.

Lastly, these results give convincing information into *Azolla filiculoides* and *Anabeana* symbiosis as a possible nitrogen fertilizer in the hydroponic cultivation of *B. vulgaris* and other food crops. It is recommended to develop and execute a more efficient hydroponic cultivation method, where a more prominent amount, growth and nitrogen fixation of *Azolla* can be assessed and promoted for the effective vegetable production in hydroponic cultures.

**CHAPTER 7**  
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