Cape Peninsula
University of Technology

EFFECTS OF REGULATING HYDROPONIC SOLUTION TEMPERATURE ON PLANT GROWTH, ACCUMULATION OF NUTRIENTS AND OTHER METABOLITES

By

SINOVUYO NXAWE

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Supervisor: Prof. Patrick A. Ndakidemi
Co Supervisor: Assoc. Prof Charles Laubscher

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DECLARATION

I declare that this thesis is my own work. It is being submitted for the Master degree in Horticulture in the Department of Applied Sciences, Cape Peninsula University of Technology. It has not been submitted for any degree or examination at any other University.

Signed Date: December 2011

ABSTRACT

The experiment was conducted with the objectives of establishing effects of regulating hydroponic solution temperatures on the chlorophyll content and photosynthesis processes, accumulation of anthocyanins and flavonoids, nutrient uptake and growth and development of pregnant onion (*Ornithogalum longibracteatum* L.) in the glasshouse during winter periods in 2009 and 2010. The plants were exposed to four hydroponic solution temperatures (control (10 - 15°C), 26°C, 30°C and 34°C). The treatments were arranged in a complete randomized design.

Results from this study conducted in the glasshouse in 2009 and verified in 2010 showed that photosynthesis rate (A) and the gas exchange parameters [stomata conductance (gs), intercellular CO_2 concentration (Ci) and transpiration (E)] were significantly increased by elevating the hydroponic solution temperatures to 26-30°C compared with the control and then decreased significantly at 34°C. Furthermore, increasing hydroponics solution temperature from 26°C to 34°C significantly increased the levels of flavonoids and anthocyanins in roots, bulbs, shoots and flowers of *O. longibracteatum* in both years 2009 and 2010.

Warming of the hydroponic solution to 26, 30 and 34°C significantly increased the uptake of (N, P, K, Ca, Mg, S, Na Fe, Cu Zn, Mn and B and Mo) in organs of *O. longibracteatum* (root, bulbs shoot, and whole plant) in 2009 and verified in 2010. The control treatments 10/15°C (day/night) had the lowest uptake of most nutrients.

Results from the two years study also showed that plant growth parameters such as number of bulbs per plant, bulb circumference, flower stalk length, flower length, and dry and fresh weights of root, bulb, shoot and flower respectively were significantly increased by warming the

hydroponic solution. Elevating the hydroponic solution temperature to a range of 26 - 30°C induced best growth and produced the highest dry matter yield in *O. longibracteatum* under glasshouse conditions whereas further increase to 34°C resulted in reduced growth and yield.

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Last but not least the Lord above without Him I wouldn't have made it this far.

DEDICATION

I dedicate this thesis to my family especially those lost during my quest for knowledge.

Zanempi Peter Nxawe

Grandfather

(06 June 1918-26 March 2002)

Fezeka Yvonne Nxawe

Aunt

(28 September 1967-19 March 2011)

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S. Nxawe[1], P. A Ndakidemi[1, 2,] C.P. Laubscher[1].

[1]Faculty of Applied Science, Cape Peninsula University of Technology, Cape Town Campus, Keizersgracht, P.O. Box 652, Cape Town 8000, South Africa.

[2]Corresponding author

Tel: +27214603196

Fax: +27214603193

Email: NdakidemiP@cput.ac.za

Abstract

Water temperature can affect many physiological processes during plant growth and development. Temperatures below or above optimum levels may influence plant metabolic activities positively or negatively. This may include accumulation of different metabolites such as phenolic compounds, reactive oxygen species (ROS), nutrient uptake, chlorophyll pigment formation, and photosynthesis process and finally the growth and development of the plant. The optimum temperature of the growth medium can contribute to improving and optimising the ealier mentioned plant physiological processes. Information on how the temperature of hydroponic solution influences certain flowering plant production in glasshouses during the winter is limited. This review suggest the possible benefits of regulating temperatures in the hydroponic solution with the aim of optimising production of flower in the glasshouse during winter periods.

Keywords: Chlorophyll pigmentation, nutrient uptake, phenolic compounds, photosynthesis rate, reactive oxygen species, regulated temperature.

1.2 Introduction

Temperature is the major environmental factor that influences the vegetative growth processes in plants from the initial stages of development to flower formation (Roh and Hong, 2007). During growth, optimum temperature is required below and above which may impair plant development (Summerfield et al., 1989). Very low or very high temperatures in the growth environment may be detrimental to various metabolic processes in plant tissues such as nutrient uptake, chlorophyll formation and photosynthesis (Taylor and Rowley, 1971; Rhee and Gotham, 1981; Markwell et al., 1986). Studies have shown that, when temperatures are lowered, the nutrient uptake, chlorophyll pigmentation and photosynthesis rate are negatively affected. However, at optimum levels the metabolism rates in plants are improved (Taylor and Rowley, 1971; Macduff et al., 1986; Engels et al., 1992; Kurek et al., 2007) and increase the plant growth (Went, 1953; Gonzàlez-Meler et al., 1999; Frantz et al., 2004). Furthermore, stress due to very low temperature may induce plants to produce different species of reactive oxygen species (ROS): such as superoxide (O^{2-}), hydrogen peroxide (H_2O_2), oxygen (O_2) and HO (hydrogen oxide) which may ultimately culminate into oxidative stress, thus, damaging the plant cells. Generally, an increase or decrease in temperature above or below the optimum level is known to alter several physiological processes in plants and damage the plant cells, thus, altering the growth (Wahid, 2007; Yang et al., 2009).

The accumulation of other metabolites such as anthocyanins and flavonoids in plants may be influenced by temperature (Kleinhenz et al., 2003; Ling et al., 2007). Studies have shown that in several plants, increasing thermal stress slightly above or below the optimum range may induce the production and accumulation of phenolic compounds such as flavonoids and anthocyanins (Rivero et al., 2001; Taulavuori et al., 2004; Guy et al., 2008; Padda and Picha., 2008), a defensive mechanism employed by plants against this type of stress. In several plants, thermal regulation of hydroponic solution temperature may optimise the plant physiological processes mentioned earlier, thus, affecting the quality of the plant.

Due to a decrease in temperature, commercial growers experience a lower level of ornamental plant production during winter than in summer (Olivier, 1974; Mills et al., 1990). However, there is a high demand for flowers during winter season when temperatures are below optimum for flower production. During this period, the production levels are lower due to lowered temperature (Olivier, 1974; Mills et al., 1990). By modifying irrigation water temperature to optimum levels, specific ornamental plants can be grown hydroponically in greenhouse during winter period. Heating of hydroponic solution in greenhouse production has shown success in other parts of the world in a variety of crops (Moorby and Graves, 1980; Rovira, 2005; Kozai, 2006; Sethi and Sharma, 2007). This review exploits the potential of increasing production of flowers during winter season by regulating temperatures in the hydoponic solution to optimize plant growth.

1.3 Effects of regulating hydroponic water temperature on profiling of secondary metabolites production such as flavonoid and anthocyanins.

Phenolic compounds are the major molecules among plant secondary metabolites and they play a very important role in plant development (Ndakidemi and Dakora, 2003; Makoi and Ndakidemi, 2007). In the vacuole of a plant organ such as leaves and flowers, anthocyanins plays major role in flower colour and fragrance (Harborne, 1980; Schijlen et al, 2004). Anthocyanins and flavonoid accumulation in plants is influenced by environmental factors such as light, temperature, and other stress levels (Kleinhenz et al., 2003; Ndakidemi and Dakora 2003; Ling et al., 2007; Makoi et al., 2010). Like all other organisms, plants may exhibit the maximum rate of metabolite production at an optimum temperature for which they have adapted (Aldred et al., 1999).

It has been reported that cultivation of crops under cold temperature decreases metabolites as a results of low rate of yield (Van Der Ploeg and Heuvelink, 2005; Thakur et al., 2010). Studies have shown that, the accumulation of phenolic compounds such as anthocyanins

and flavonoid by plants in winter can differ in comparison to summer due to temperature variations (Mori et al., 2005; Olsen et al., 2008; Kassim et al., 2009). Different mechanisms are proposed. For example, variations in temperature may exert thermal stress on the plants tissues, consequently, interfering with the activity of the various plant enzymes and hence the production of metabolites. In this context, significant changes in phenolic compound metabolism may be affected by extended periods of low temperature which may result in chilling injury. Taulavuori et al. (2004) and Padda and Picha (2008) reported that, a plant exposed to low temperature resulted into increased content of phenolic compound in their tissues. Moreover, anthocyanins are highly water soluble and therefore produced under different stress levels, such as temperature stress.

Research evidence suggests that, plants may exhibit the maximum rate of metabolite production at a given optimum temperature. In most plants, increasing thermal stress slightly above the optimum range may induce the production and accumulation of metabolites such as flavonoids and anthocyanins (Rivero et al., 2001; Guy et al., 2008). Elevated temperatures above the optimum level similarly increases enzyme activity (Pearcy, 1977) and results in the production of different types of metabolites. The effect of thermal stress is often manifested by the appearance of physiological injuries into the plant tissues thus, resulting into the excessive production of secondary metabolites (Revero et al., 2001) a strategy used to protect the plant from further stress damage. To verify this, Wahid (2007) reported that, accumulation of anthocyanins in *Photinia* spp and aster (*Aster amellus*) flower were increased with exposure to high temperature. Other studies involving *Rehmannia glutinosa* have reported decreased content of phenolic compounds at very high temperatures (Chung et al., 2006).

Little information is available on the influence of hydroponic solution temperatures on the pigment formation for plants grown in the greenhouse conditions during winter. From this background, it is therefore important to establish the effects of temperature gradients on

metabolite production in plants grown in the hydroponic media with varied temperatures during winter period.

1.4 Effects of regulating hydoponic water temperature on reactive oxygen species in different plant tissues.

Reactive oxygen species (ROS) are warning signal for plants subjected to stress including cold stress (Nobuhiro and Ron, 2006). Reactive oxygen species such as superoxide (O^{2-}), hydrogen peroxide (H_2O_2), oxygen (O_2) and HO (hydrogen oxide) are toxic molecules producing oxidative damage to proteins, DNA and lipids which may finally affect plant growth and development (Ping et al., 2008). Excessive accumulation of ROS in plants occur when stress is severe and thus causing oxidative injury (Ling et al., 2007). It is likely that, ROS produced at low temperatures can cause damage to cellular components by disrupting metabolic function (Anderson et al, 1995). Some research evidence indicated that cold stress enhanced the transcription of protein and activity of different reactive oxygen species-scavenging enzymes in plants (Nobuhiro and Ron, 2006).

However, the exposure to low temperature may increase the amount of reactive oxygen species (Ping et al., 2008), an antioxidant strategic defence mechanism enabling plants to adapt in heat stressed environments. The ROS-scavenging mechanisms have an important role in protecting plants against temperature stresses (Miller et al., 2006).

Accordingly, ROS production is increased by oxidative stress under unfavourable environmental conditions such as those involving temperature changes to extreme limits (Gechev et al., 2006). The accumulation of ROS in plants can lead to many physiological injuries of tissues, loss of membrane integrity and chlorophyll bleaching (Xu et al., 2006; Liu and Pang, 2010). Furthermore, ROS is accredited for decreasing membrane stability and facilitate lipid peroxidation (Sairam et al., 2002).

Generally, most plants display their antioxidative enzyme activities at a temperature of 25°C (Peltzer et al., 2002). However, the exposure of plants to low temperature may increase the amount of ROS as an antioxidant strategic defence mechanism enabling plants to adapt in low temperature stressed environments (Ping et al, 2008). Studies conducted in low temperature environments revealed reductions in enzymatic activation energies due to production of ROS (Peltzer et al., 2002).

Many scholars have indicated that high temperature may enhance the production of ROS including singlet oxygen (O_2), superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (Liu and Huang, 2000; Suzuki and Mittler, 2006). These may cause lipid peroxidation and pigments membrane instability (Xu et al., 2006; Lopez-Vazquez et al, 2007), then negatively affecting plant metabolism and limiting growth and yield (Sairam and Tyagi, 2004). In heated environments, the protection against oxidative stress is an important component in determining the survival of a plant under heat stress (Gong et al., 1997; Dat et al., 1998). Assessing the accumulation of ROS in glasshouse plants grown under different hydroponic temperature regimes will enable us to understanding how ROS can affect growth and development of such plants grown under a controlled environment during winter period. Further research is necessary to establish the mechanisms involved in the production of antioxidants in cells exposed to heat stress.

1.5 Effects of regulating hydroponic water temperature on nutrient uptake and accumulation in plant tissues

Plant nutrients have a great potential for increasing yield and the capable of promoting plant growth (Ndakidemi and Semoka, 2006). Nutrient uptake and accumulation in plant tissues may be influenced by various environmental factors including temperature (Reay et al., 1999; Aðalsteinsson and Jensén, 2006). Calatayud et al. (2008) revealed that in most plant species, nutrient uptake by roots decrease at low temperatures. Temperatures of growth media may influence chemical reaction rates of nutrients in the solution, nutrient transport in

the medium, physiological aspects related to ion uptake rate, and functioning of soil microbial communities (Pregitzer and King, 2005). Therefore, it is of paramount importance to regulate hydroponics solution temperatures in situations whereby plants are grown in controlled environment during winter months. Optimizing temperature in the growth medium can be achieved by warming the nutrient solution (Morgan et al., 1980).

Studies have shown that, elevated temperatures increased nutrient uptake in cucumber (*Cucumis sativus* L.) and enhanced plant growth leading to significant increase in yield (Daskalaki and Burrage, 1998). Experiment involving Jojoba (*Simmondsia chinensis*) showed that, the uptake rate of N, P, K, Na, Fe, Mn and Zn were significantly reduced at low temperatures compared with those exposed to temperatures as high as 33°C (Reyes et al., 1977). Furthermore, nutrient concentrations in roots were similarly higher in plants grown at 33°C than at 21 or 27°C (Reyes et al., 1977)**.** Studies by Hood and Mills (1994) and Stoltzfus et al. (1998) showed that, increasing temperature from 15 to 29°C increased uptake of P, K, Ca, Mg, Fe, Mn, Zn and B and finally the plant growth. Nutrient uptake, especially N in pine (*Pinus sylvestris* L.) increased with increasing root temperature from 8°C to 16°C (Vapaavuori et al., 1992). In cucumber (*Cucumis sativus* L.), uptake of N, P, K, Ca, and Mg was increased when temperature was raised in closed hydroponic system from 12°C to 20°C (Daskalaki et al., 1998).

On the other hand, low temperatures are known to induce B deficiency and leaf damage in crop plants (Huang et al., 2005). For example in cucumber, low temperature of (10°C) doubled nitrate accumulation in the root zone when compared with the root zone temperatures of 18°C and 26°C (Kim et al., 2002). This was probably due to restricted mobility and volatilisation of nitrates at low temperatures (Thomas and Kissel, 1970), depending on the type of nutrients. Nutrient uptake for plants grown in glasshouse may be positively and adversely affected by manipulating the hydroponic solution temperature to the optimum level. Studies should therefore be conducted to establish the optimum temperatures to meet nutrient uptake demands of specific plants during winter season.

1.6 Effects of varying hydroponic water temperature on chlorophyll pigmentation.

Colour pigmentation in plants, especially the chlorophyll, is important to plant growth and development. The amount of chlorophyll formed in plants is strongly influenced by environmental factors including temperature changes (Hauvax and Lannoye, 1984; Tian et al., 1996; Shvarts et al., 1997; Yun et al., 1998; Kleinhenz et al., 2003). The influence of temperature on chlorophyll formation involves several mechanisms. At optimum temperatures, synthesis of metabolites such as carbohydrates may be enhanced, thus, leading to increased chlorophyll in the leaves (Reay et al, 1998; Al-Hamdani and Ghazal, 2009). Scientific evidence points out that, plant subjected to various levels of stress (high temperatures) can damage their cells and eventually affecting chlorophyll quality (López-Ayerra et al., 1998). Vu and Yelenosky (1987) reported that, the amount of chloroplast proteins tends to drop with increasing growth temperatures. The experiments involving testing of maize at various temperatures revealed that their exposure to higher temperatures triggered membrane damage and lowered the chlorophyll concentration in the plant tissues (Yang et al., 1996). In barley (*Hordeum vulgare* L.), other researcher (Ilík et al., 2000) reported plasmalema and chloroplast membrane damage, loss in cell permeability, thylakoids burst and the formation of condensed structures due to high temperature. Funamonto et al. (2003) also showed that in broccoli (*Brassica oleracea*); chlorophyll degradation by heat treatment was mainly due to the suppression of chlorophyll peroxidase activities in microsomes and cytosol.

Low temperature treatments may also affect chlorophyll quality in plants as the cells are subjected to cold stress (López-Ayerra et al., 1998). Studies have shown that orange trees (*Citrus sinensis* L. Osbeck) grown under low temperature contained less chlorophyll than those grown at high temperatures (Vu and Yelenosky, 1987). In spinach (*Spinacia oleracea* L.) lipid peroxidation and chlorophyll levels were reduced by cold temperatures (López-Ayerra et al., 1998) by a mechanism which involved shrinking and damaging of the elastic

cells due to cold stress. Generally, when plants are subjected to low temperature stress, the development of chlorotic bands on leaves may appear (Vu and Yelenosky, 1987). Under such circumstances, a decrease in chlorophyll content may be a consequence of oxidative stress which leads to chlorophyll deficiency (Bacelar et al., 2006). With regard to thermo regulation in hydroponic systems, no information is available on the influence of temperatures on the production of chlorophyll pigments in plants grown during the winter period.

1.7 Possible effects of regulating hydroponic water temperature regimes on the photosynthesis rate

Temperature is an important environmental factor to plants, which directly influences their photosynthetic functions (Vu and Yelenosky, 1987; Collatz et al., 1991; Williams and Black, 1993; Ling et al., 2007). An increase in temperature to optimum levels may favourably shift electron transport and make the plant to synthesise adequate metabolites such as carbohydrates thus, leading to optimum growth (Piere and Urs, 2005). It is well known that, warm temperature conditions affect plant growth structures including all physiological processes in plants such as membrane structure, stomatal conductance and protein synthesis. The low temperature effects on photosynthesis may include changes in stomatal and non-stomatal characteristics (Pearcy, 1977; Berry and Bjorkman 1980; Vu and Yelenosky, 1987; Vierling, 1991; Calatayud et al., 2008). Studies on olive plants showed that low temperature decreased photosynthesis and this was correlated to its influence on stomatal closure (Bacelar et al., 2006). Temperatures above the optimum levels may also damage various cell functions, as the photosynthesis process is very sensitive to heat stress (Piere and Urs, 2005). Similarly, photosynthesis can also be affected negatively by low root temperature (Calatayud et al., 2008).

According to Lambreva et al. (2005) stimulation of photosynthesis was observed at the growth temperature of 23°C but at 39°C the effects of elevated CO_2 on photosynthesis was

induced downward. Generally, increased temperature above the optimum limit may reduce photosynthetic rate (Wahid et al., 2007). For instance, in a study involving rice (*Oryza sativa* L.) plant, Mohammed and Tarpely (2009) indicated that high temperatures had negative effect on photosynthesis as well as various enzymes involved in the process. Uniformity of shoots and time to flower on plants is also increased by increasing photosynthetic photon flux (Quedado and Friend, 1978; Karlsson et al., 1989). However, information concerning the effects of hydroponics water temperature during winter on the photosynthesis rate on plants is rather limited in plants grown under glasshouse conditions. Therefore, it is important to document the influence of plants photosynthetic activities when exposed to hydroponic media of different temperature treatments. Such information could assist in developing adaptable hydroponics solution temperature for cultivating glasshouse plants with highly functional substances.

1.8 Effects of temperature changes in hydroponic water on plant growth and development.

Water temperature is an important growth factor that may influence plant development including plants growing in hydroponic system. Therefore, it is beneficial to study the optimum temperature requirements for different crops grown in climates with adverse winter conditions.

Water temperature plays a vital role in plant development (Chung et al., 2006). At optimum temperatures, water can nourish growth while at lower or high levels; plant growth can be negatively affected (He et al., 2002). In plants, water is required to maintain cell turgidity so as to ensure continuous column of moisture in the cells (Stewart and Dwyer, 1983; Noguchi and Terashima, 1997; Outlaw, 2003). It is also indispensable to the intracellular chemical processes that keep the plant growing (Outlaw, 2003; Yamori et al., 2006). Cold water may cause frost damage to plants by forming sharp-edged ice crystals, which puncture cell walls. Studies have shown that, at lower temperatures (10°C), flower abortion in different plant

occurred because pollen and ovule fertility were highly sensitive to cold temperature (Jakobsen and Martens, 1994; Dom´ınguez et al., 2005; Singh et al., 2008). In flower industry, these effects on flower physiology can lead to drastic reduction in economic yield (Diepenbrock, 2000; Thakur et al., 2010).

Temperature may also affect many other growth physiological processes at different developmental stages of the plant. Studies have shown that, most tender plants will grow well in temperatures ranging from 6°C to 24 °C and half-hardy plants from 10°C to 18 °C whereas hardy plants may survive in temperature range of 7 °C to 16 °C (Bubel, 2007; Gesch and Forcella, 2007). Therefore, when water temperatures drop below 6°C in such type of plants, thermal modifications can be essential to sustain growth.

The effects of temperature on vegetative growth and flower development of plant will vary depending on the growth stage of the plant (Selander and Welander, 1984). In a glasshouse experiment the effect of temperature on *Primula vulgaris* and showed that, an increasing temperature up to 18°C delayed flower opening and decreased the number of flowering shoots, whereas at a lower temperature (12°C) inhibition of flower development was overcome (Selander and Welander, 1984). In other studies involving *Aeschynanthus speciosus*, increasing the temperature from 12°C to 21°C resulted in higher percentage of flowering plants with increased number of leaves formed (Welander, 1984). In separate studies, number of days to flowering of *Centradenia inaequilateralis* and flower formation was significantly affected by increasing temperature (Tromp, 1984; Friis and Christensen, 1989; Zhu et al., 1997; Roh and Hong, 2007). Other studies conducted in the glasshouse to test the effect of temperature in *Primula vulgaris*, showed that increasing the temperature up to 18°C delayed flower opening and decrease the number of flowering shoots whereas at 12°C flower development was enhanced and the plant performed well (Selander and Welander, 1984; Roussopoulos et al.,1998). Similarly, studies on a different plant (*Chrysanthemum*) showed that increasing temperature from 14°C to 26°C delayed flowering for more than 30 days (Karlsson et al., 1989). In Passion fruits (*Passiflora edulis*), temperature ranging from 25°C to

30°C limited flowering, while temperature ranging from 10°C - 15°C reduced the yield of vegetative growth (Menzel et al., 1987). Therefore, it is important to establish other possible effects of regulating temperatures in the hydroponic solution on plant growth and development in the glasshouse during winter periods.

1.9 Conclusion

Temperature changes in hydroponic media temperature may affect development of plants. Most plants are unable to grow at sub optimum levels. When temperatures are not at optimum level, several physiological functions such as photosynthesis, chlorophyll formation and pigmentation, nutrient uptake, accumulation and synthesis of secondary metabolites in plants is affected. Thermo regulation of hydroponic solution in the glasshouse is a technique which can be used to optimise the production of flowers or flowering during winter periods.

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Chapter 2

2.0 Justification, research hypothesis and objectives of the study.

2.1 Justification

Production of plants such as *O. longibracteatum* in Western Cape during winter is difficult than in summer owing to lower temperatures experienced by growers during this period. In winter season, irrigation water from taps is always available at lower temperatures ranging between 0 - 10°C as compared to summer. Plant production in winter period in the greenhouse can be achieved by modifying temperatures in hydroponic solution to optimum levels through use of specialised heaters. Successes in using such systems in different plants has been reported (Moorby and Graves,1980; Welander, 1984; Sethi and Sharma, 2007)

2.2 Research hypothesis

Lower temperatures below the accepted limits in irrigation water in winter season may negatively impact the plant growth in different aspects. Increasing the temperature of irrigation water to optimum levels will positively influence growth, accumulation of metabolites and nutrients in O*. longibracteatum*.

2.3 Overall aim of the study

The aim of this study was to manipulate hydroponic temperatures to enhance the production of *O. longibracteatum* in the greenhouse.

2.4 Objectives of the study were to:

- Assess the effect of varying hydroponic solution temperature on chlorophyll synthesis and photosynthetic rate in *O. longibracteatum* in cold season.
- To investigates the effects of hydroponics solution temperature on the concentrations of anthocyanins and flavonoids in different tissues of *O. longibracteatum*
- To assess the influence of different temperature treatments in the hydroponic cultures on nutrient uptake and accumulation in the tissues of *O. longibracteatum*.

- To establishing effects of regulating temperatures in the hydroponic solution on the growth and development of *O. longibracteatum* in the glasshouse during winter periods.

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Chapter 3

3.0 Chlorophyll pigmentation and photosynthetic parameters in *Ornithogalum longibracteatum* L. as affected by varying temperatures in hydroponics solution.

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S. Nxawe[1], P. A Ndakidemi[1, 2,] C.P. Laubscher[1].

[1]Faculty of Applied Science, Cape Peninsula University of Technology, Cape Town Campus, Keizersgracht, P.O. Box 652, Cape Town 8000, South Africa.

[2]Corresponding author

Tel: +27214603196

Fax: +27214603193

Email: NdakidemiP@cput.ac.za

Abstract

The effects of different temperature regimes of hydroponic solution temperature on the chlorophyll pigmentation and photosynthesis of *O. longibracteatum* L. were determined in the greenhouse for 10 weeks in year 2009 and 2010. The plants were irrigated with hydroponic solution heated to various temperatures (26, 30 and 34°C) via pumps connected to 4 sets of water tanks each maintained at the experimental temperatures using Dolphin aquarium heaters. Unheated water supplied from the forth tank served as control. All the plants were supplied with 1mg/L nutrient solution of Hortical Calcium Nitrate (Hortical $Ca(NO_3)_2$) and changed at weekly intervals. After 2 -10 weeks of experimentation, data showed that chlorophyll a, chlorophyll b and total chlorophyll were significantly increased by elevating the hydroponic solution temperature from 26 - 30°C and started decreasing at 34°C compared with the control in both 2009 and 2010. Photosynthesis rate (A) and the gas exchange parameters [stomata conductance (gs), intercellular CO_2 concentration (Ci) and transpiration (E)] were significantly increased by elevating the hydroponic solution temperatures to 26-30°C compared with the control and then decreased significantly at 34°C. The findings from this study suggest that controlled production of *Ornithogalum longibracteatum* L. during winter seasons is possible by heating the hydroponic solution up to 30°C beyond which there was impaired chlorophyll formation and reduced photosynthesis.

Key words: Intercellular CO_2 concentration, photosynthesis rate, stomata conductance, transpiration.

3.1 Introduction

Ornithogalum longibracteatum L. is classified as a medicinal bulb used wildly in South Africa. The plant which is also commonly known as a pregnant onion is used as a medicinal plant by traditional healers (Kulkarni et al., 2005). From its potential as a medicinal plant, its on-farm and greenhouse cultivation is becoming popular. *O. longibracteatum* can be grown hydroponically in a greenhouse even during the adverse climatic conditions (Rosik-Dulewska and Grabda, 2002) provided that the harsh environmental factors are addressed. *O. longibracteatum* grows best at temperatures ranging from 22 to 27°C (Luria et al., 2002) and does not grow well in cold weather if temperatures are less than 11°C (Halevy et al., 1971).

Temperature is an important factor affecting physiological processes in plants including photosynthetic rate and chlorophyll synthesis (Lambreva et al., 2005; Calatayud et al., 2008). Like other growth processes in plants, temperature changes in the soil and air may have positive or negative impacts on leaf photosynthetic rate and the chlorophyll synthesis (Vu and Yelenosky, 1987). For example, some of the photosynthetic parameters such as stomata conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration (E) are known to be influenced by the changes in temperature (Drake et al., 1970; Pearcy, 1977; Haldimann and Feller, 2004). In plants such as tomato (*Lycopersicon esculentum* L.*)*, low temperature (1°C) reduced stomata conductance by 25% leading to decreased leaf chloroplast functioning (Martin et al., 1981). However, low temperature inhibits the rate of photosynthesis by approximately 60% as the result of the impairment of water oxidation mechanism (Martin et al., 1981). Other studies (Haldimann and Feller, 2004) have shown that increase in leaf temperature up to 45°C in oak (*Quercus pubescens* L.) plants reduced photosynthesis rate by 90%, and stomata conductance, but increased intercellular CO_2 compared with when it was 25°C.

According to Drake et al. (1970), high temperatures, ranging from 35 to 40°C, increased transpiration in leaves and low levels such as 5 and 10°C reduced transpiration of *Xanthium*

spp plants, ultimately affecting stomata conductance activities. In another study, temperatures above 25°C led to closure of stomata, thus reducing the transpiration in potatoes (*Solanum tuberosum* L.) a mechanism for adaptation to hot environment (Ku et al., 1977). In a study by Baig and Tranquillini (1980), it was reported that increasing temperature from 15 to 20°C increased transpiration rate in *Pica abies* and *Pinus cembra* to 65.8 % and 63.6% respectively. Similarly, when temperature was further increased to 25°C, transpiration was also increased to 146.3% and 196.7% respectively.

Research has revealed that at low temperatures (10^{o}C), the peroxidation activities in the chloroplast membrane were lowered due to inhibition of the metabolic processes in the leaves of coffee seedlings (Gonçalves de Oliveira et al., 2009). It was also showed that low temperature (8°C) significantly reduced the chlorophyll levels in spinach leaves (Spinacea oleraceae L.) (Lopez- Ayera et al., 1998). The findings of Ilík et al. (2000) strongly suggested that, an increase in temperature within the range of 25 to 75°C affect the chloroplast membrane which resulted to the burst of thylakoids and formed condensed structures in barley leaves. Temperature variations in the rooting zone are an important factor which may influence different metabolic processes in plants (Walker, 1969; Gur et al., 1972; Cooper, 1973; Sattelmatcher., 1990). For instance, it is suggested that higher temperatures in the rooting zone above the optimum range could result into excessive consumption of carbon assimilation in photosynthesis (Huang and Gao, 2000; Xu and Huang, 2000a, b, 2001; Liu and Huang, 2001). Furthermore, research has shown that the disturbance of carbohydrate metabolism in roots was a major primary factor responsible for growth inhibitionat high soil temperature (Du and Tachibana, 1994; Chung et al., 2002). Therefore, it is worth establishing if any stress factor such as variations in temperature of the hydroponic solution will impair the physiological function of the plant such as those involving chlorophyll formation and photosynthetic processes. The purpose of this study was to determine the effect of changes in hydroponic solution temperature regimes on chlorophyll synthesis and photosynthetic rate so as to establish the optimum temperature for the growth of *O. longibracteatum* in cold season.

3.2 Materials and methods

3.2.1 Site location and description

The experiment was conducted at the greenhouse of the Cape Peninsula University of Technology, Cape Town, South Africa during the winter season of 2009 and 2010. A steel table (2.5 m x 1 m) was used as a flat surface, black plastic container (50 L), leca clay pebbles were supplied by Horticultural Department of Cape Peninsula University of Technology (CPUT), Cape Town, South Africa. Four (4) plastic gutters (2 m x 0.6 m), 4 pumps, 20 ml black plastic pipe, cable tie and 3 Dolphin aquarium heaters were purchased from Builders Warehouse (Maitland, Cape Town), South Africa. Bulbs of pregnant onion (*O. longibracteatum*) used as planting material were obtained from the CPUT nursery.

3.2.2 Experimental design

A randomised complete block design, with four replicates, was conducted to study the effects of temperature on chlorophyll pigmentation and photosynthetic rate in *O. longibracteatum* L**.** Four white plastic gutters (2 m x 0.6 m) filled with leca clay pebbles were placed on a 2.5 m x 1 m steel table. Water was supplied to the leca pebbles through pumps projecting from 4 sets of black plastic containers (50 L) placed beneath the table. The water in the 3 containers was heated by using Dolphin aquarium heaters to maintain the temperatures at 26, 30 and 34°C respectively. Unheated water supplied from the forth container served as a control. Using the thermometer, the temperature ranged between 10 and 15°C (day/night) throughout the experiment period. *O. longebracteatum* bulbs were planted in each gutter (10 bulbs per gutter) and supplied with nutrient solution immediately after transplanting. The nutrient solution was prepared according to Ocean HYDROGRO (2009) and Ocean HORTICAL (2009) respectively. Nutrient solution supplied from the pumps was recirculated back to the

black plastic container (50L) through a 20 ml black plastic pipe. The plants were left to grow for the period of 10 weeks. To prevent concentration of nutrients in the clay pebbles due to evaporation, water was drained from the gutters and refreshed after every 2 weeks.

3.2.3 Determination of chlorophyll contents in plant leaves

Chlorophyll concentration was extracted from the third leaf of the growing tip of each plant in the gutters using dimethyl sulphoxide (DMSO), as described by Hiscox and Israelstam (1979). The strap-like leaves were cut into small pieces, and a 100 mg of the middle portion of the leaf tissue was placed in a 15 ml vial containing 7 ml DMSO and incubated at 4°C for 72 h. After the incubation, the extract was diluted to 10 ml with DMSO and 3 ml of extract was used to read the absorbance at 645 nm and 663 nm on a spectrophotometer (UV/Visible Spectrophotometer, Pharmacia LKB. Ultrospec II E) against DMSO blank. Chlorophyll levels were calculated using the following equations used by Arnon (1949) with a unit of $mg.L^{-1}$ and is given thus:

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

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

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

3.2.4 Measurement of photosynthesis in plant leaves

At 52 days after planting, photosynthesis, stomata conductance, intercellular CO_2 and evapotranspiration were measured in four young leaves (flag leaves) per gutter using a portable infrared red gas analyzer (LCpro+ 1.0 ADC, Bioscientific Ltd., Hoddesdon, Hertfordshire, UK). Measurements were made from 8 a.m to 11 a.m and from 2 p.m to 4 p.m for each replicate gutter per day. Leaves were allowed at least 5 min to acclimate to the light environment in the chamber. Under normal conditions, each measurement took approximately 2 min, which was the minimum time allowed for the readings to stabilize

before they were recorded. During measurements, the conditions in the leaf chamber were: photosynthetic photon flux density (PPFD) = 1100 µmol (quantum) $m^{-2}.s^{-1}$, relative humidity = 44%, leaf vapor pressure deficit = 1.83 kPa, flow rate = 400 µmol.s^{-1}, reference CO_2 = 400 ppm, and leaf temperature = 25^{o}C.

3.2.5 Statistical analysis

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

3.3 Results

3.3.1 Effect of temperature on chlorophyll content of leaves of *Ornithogalum longibracteatum*.

Table 3.1 shows the effect of four different temperature treatments on chlorophyll content in the leaves of *O. longibracteatum*. Results showed that, relative to the control treatment, increasing the water temperature to 26, 30 and 34°C significantly increased the levels of chlorophyll a, chlorophyll b and total chlorophyll in 2009 and 2010. For instance, at 30°C, the level of chlorophyll a, chlorophyll b and total chlorophyll were significantly higher compared with all the other treatments (Table 3.1). However, as the temperature was increased to 34°C, leaf chlorophyll content was significantly reduced compared with the other treatments in both 2009 and 2010. Although plants grown during 2010 season contained more chlorophyll than their counterparts grown in 2009, the influence of temperature showed similar trend across seasons.

3.3.2 Effect of temperature on photosynthesis and gas-exchange parameters of leaves of *Ornithogalum longibracteatum*.

There was significant difference on the photosynthesis rate (A) and the gas exchange parameters (E, Ci and Gs) at different temperature treatments during 2009 and 2010 (Tables 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7). Generally, the photosynthesis rate and the gas exchange parameters were significantly increased by elevating the hydroponic solution temperatures to 26, 30, and 34°C compared with the control. For example, results indicated that in week 2, 4, 6, 8 and 10 the photosynthesis rate (A) values were consistently increased by modifying the temperature to 26 and 30°C but decreased significantly at 34°C. Data from this study showed that raising the temperature beyond 30°C, the photosynthesis rate (A) started experiencing significantly negative effects and the values were significantly maximized at 30°C in both years.

The values for transpiration (E) recorded in weeks 2, 4, 6, 8 and 10 during 2009 and 2010 indicated that there was significant increase in this parameter when temperatures were raised to 26°C, 30°C and 34°C compared with the control. Relative to the control treatment, increasing temperature to 26°C in weeks 2, 4, 6, 8 and 10 significantly increased E values in the average range of 21 - 159% in 2009 and 30 - 136% in 2010 (Tables 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7). Furthermore, compared with the control increasing temperature to 30°C in weeks 2, 4, 6, 8 and 10 significantly increased E values with the average range of 134 to 206% in 2009 and 114 - 339% in 2009 and 2010 respectively. Generally, there were significantly reduction in E values when the hydroponic solution temperature was raised to 34°C compared with the 30°C treatment in both 2009 and 2010 (Tables 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7). The data showed that the best result for E was recorded in the 30°C treatment.

In this study, the intercellular CO_2 concentration (Ci) values were significantly increased by elevating the hydroponic solution temperatures to 26, 30, and 34°C compared with the control. Data collected in weeks 2, 4, 6, 8 and 10 showed that (Ci) values increased significantly when temperatures were raised from 26°C to 30 °C compared with the control during 2009 and 2010 (Tables 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7) and started decreasing at 34°C. For example at 26°C, Ci values increased significantly between 21 to 45% in 2009, and 24 to 73% in 2010. Raising temperature to 34°C, however, resulted into significant decrease in Ci values compared with 30°C treatments in both years. The overall results obtained during weeks 2, 4, 6, 8 and 10 showed that at 30°C, Ci was the highest. The average values ranging from 54% to 132% and 50 to 68% in 2009 and 2010 respectively.

The value of stomata conductance (Gs) increased significantly in weeks 2, 4, 6, 8 and 10 when temperature was increased to 26, 30 and 34°C compared with the control in 2009 and 2010 (Tables 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7). For example at 26°C, results observed during weeks 2, 4, 6, 8 and 10 showed significantly ($P \leq 0.05$) greater Gs values and ranged from 38 to 137% in 2009 and from 97 to 258% in 2010. During the two years of experimentation, best results for stomata conductance were obtained at 30°C where their valued were increased significantly between 89% and 300%, in 2009 and 369% and 611% in 2010. There was however, a significant decline in the levels of Gs when temperature was increased to 34°C.

3.4 Discussion

Optimum temperature in hydroponics plays a crucial role in plant growth and other plant physiological characteristics including chlorophyll content. Exposure of plants to high or low temperatures may damage the chlorophyll membrane structures leading to low chlorophyll content (Ilík, 2000). Likewise, extreme temperatures may either stop or denature enzyme activities leading to reduced rate of A, gs, Ci) and E (Pearcy, 1977; Camejo et al., 2005). Maintaining optimum hydroponics temperature may be one way to ensure optimum chlorophyll content and increased A, Gs, Ci and E in such plants. In this study, the

chlorophyll content in the leaves of *O. longibracteatum* showed that by increasing the hydroponics water temperature to 26 and 30°C significantly increased the levels of chlorophyll a, chlorophyll b and total chlorophyll compared with the control, chlorophyll a content was 108, 201 and 51% at 26, 30 and 34^{o}C respectively (Table 3.1). Similar trend was observed in 2010, where chlorophyll a content increased by 174, 297 and 41% by elevating temperatures to 26, 30 and 34^{o}C respectively over the control (Table 3.1). Greater accumulation of the chlorophyll content in the leaves of *O. longibracteatum* L. at 30°C suggests that there was no damage in its physical chemical properties and its functional organization. However, as the hydroponics water temperature was raised to 34°C, leaf chlorophyll content was significantly decreased. The reduced leaf chlorophyll content suggests that this temperature altered its physical and chemical properties and its functional organization. This confirms the findings of Taylor and Craig (1971), Ferrini et al. (1995) and Ilik et al. (2000).

Photosynthesis is considered as one of the most temperature sensitive processes, and may be completely inhibited by high temperatures above the optimum (Camejo et al., 2005). In this study, increasing the temperature from 26 to 30°C increased A, gs, Ci and E in the leaves of *O. longibracteatum* to the optimum level. That is the peak of these parameters was observed at 30°C. For example in the second week of 2009, the rate of photosynthesis was 118, 144 and 38% at 26, 30 and 34°C respectively and in 2010, photosynthesis rate was 63, 143 and 23% at 26, 30 and 34°C respectively over the control (Tables 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7). The increase in the rate of photosynthesis and the related parameters suggests that these temperatures were limiting A, Ci, Gs and E. The data also showed that as the temperature was increased to 34°C, A and the related parameters were significantly reduced (Tables 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7). The diminution in the rate of photosynthesis at 34°C may be ascribed to disruption of structure and function of chloroplasts, reduction of chlorophyll accumulation (Table 3.1), enzyme denaturation due to oxidative stress, stomata closing or increased respiration rate (Xu et al., 1995; Dekov et al., 2000). These findings suggest that the rate of photosynthesis in plants growing at 35°C depends not on stomatal

opening but on biochemical factors of an enzymatic nature. Previous reports have indicated that increased temperature beyond optimum (30°C) in citrus plant (Ribeiro et al., 2004; Hu et al., 2007) and self-rooted cv. Trebbiano grapevines (Ferrini et al., 1995) was the reason for decreased carboxylation efficiency. In another study, Ku and Edwards (1977) revealed that increasing temperature resulted not only in reduced internal CO_2 concentration in potatoes (*Solanum tuberosum* L.) but also the rate of photosynthesis was inhibited by 38%.

In conclusion, increasing hydroponics water temperature to 30°C, lead to significantly positive increase of chlorophyll content in *O longibracteatum*, greater photosynthesis rate, stomata conductance, intercellular CO_2 concentration and transpiration. However, increasing the temperature to 34°C resulted into possible structural and functional disruptions of chloroplasts and reduced chlorophyll accumulation leading to decreased rate of photosynthesis and related parameters in *O. longibracteatum.*

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Table 3.1: Effect of temperature on chlorophyll content in leaves of *O. longibracteatum* during 2009 and 2010.

Treatment	Chlorophyll a	Chlorophyll b	Chlorophyll total
		($mg.L^{-1}$)	
2009			
Control (10 - 15 ^{o}C)	2.25±0.15d	0.51±0.03d	2.77±0.14d
26^{o}C	4.68±0.17b	1.89±0.07b	6.57±0.22b
30^{o}C	6.77±0.25a	3.65±0.05a	10.42±0.24a
34^{o}C	3.39±0.16c	1.42±0.05c	4.81±0.17c
One - Way ANOVA (F-Statistic)			
Rep	109.63**	596.70**	271.07**
2010			
Control (10 - 15 ^{o}C)	2.13±0.20d	0.56±0.04d	2.69±0.22d
26^{o}C	5.83±0.25b	2.95±0.14b	8.78±0.28b
30^{o}C	8.45±0.37a	5.02±0.38a	13.46±0.69a
34^{o}C	2.99±0.17c	1.99±0.21c	4.98±0.31c
One - Way ANOVA (F-Statistic)			
Rep	123.38**	67.94***	126.33**

Values presented are means ± SE, n = 10. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P \leq 0.05$ according to Fischer least significance difference.

Table 3.2: Effect of temperature on photosynthesis and gas-exchange parameters of leaves of *O. longibracteatum* as measured from week 2 and 4 during 2009.

Treatments	WEEK 2				WEEK 4			
	A	E	Ci	Gs	A	E	Ci	Gs
	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$
Control (10-15 ^oC)	1.14±0.01d	0.38±0.01d	216.00±0.77d	0.02±0.00d	1.29±0.01d	0.52±0.03d	223.00±2.69d	0.03±0.00d
26^oC	2.48±0.10b	0.70±0.01b	261.30±5.19b	0.04±0.00b	2.63±0.05b	0.77±0.02b	274.60±5.01b	0.05±0.00b
30^oC	2.78±0.04a	0.89±0.02a	336.10±8.55a	0.06±0.00a	3.81±0.04a	1.30±0.07a	375.20±4.33a	0.07±0.00a
34^oC	1.57±0.02c	0.52±0.02c	240.70±7.51c	0.03±0.00c	2.29±0.02c	0.66±0.01c	248.10±4.68c	0.04±0.00c
One - Way ANOVA (F-Statistic)								
Rep	194.00**	156.89**	68.34***	74.73***	936.14**	75.16***	244.11**	49.47***

Values presented are means ± SE, n = 10. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P \leq 0.05$ according to Fischer least significance difference.

Table 3.3: Effect of temperature on photosynthesis and gas-exchange parameters of leaves of *O. longibracteatum* as measured from week 6 and 8 during 2009.

Treatments	WEEK 6				WEEK 8			
	A	E	Ci	Gs	A	E	Ci	Gs
	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$
Control (10 - 15 ^oC)	1.43±0.09d	0.62±0.00d	226.90±2.38d	0.03±0.00d	1.66±0.07d	0.74±0.01d	248.30±5.30d	0.04±0.00d
26^oC	3.38±0.07b	0.81±0.02b	292.70±1.51b	0.05±0.00b	3.29±0.05b	0.89±0.02b	333.60±6.12b	0.06±0.00b
30^oC	4.61±0.05a	1.59±0.05a	365.00±4.79a	0.07±0.00a	6.84±0.19a	1.91±0.02a	372.80±6.10a	0.08±0.00a
34^oC	2.33±0.07c	0.71±0.02c	266.30±7.70c	0.04±0.00c	2.59±0.08c	0.81±0.01c	273.30±8.31c	0.05±0.00c
One - Way ANOVA (F-Statistic)								
Rep	367.21**	219.00**	150.20**	58.64***	412.46**	1022.03**	74.58***	35.69***

Values presented are means ± SE, n = 10. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P \leq 0.05$ according to Fischer least significance difference.

Table 3.4: Effect of temperature on photosynthesis and gas-exchange parameters of leaves of *O. longibracteatum* as measured at week 10 during 2009.

Treatments	Week 10			
	A	E	Ci	Gs
	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$
Control (10 - 15 ^oC)	3.50±0.12d	0.69±0.08d	231.70±6.10d	0.04±0.00d
26^oC	5.39±0.09b	1.79±0.03b	335.60±2.25b	0.06±0.00b
30^oC	8.23±0.10a	2.12±0.02a	384.00±4.06a	0.08±0.00a
34^oC	4.59±0.12c	0.95±0.01c	284.90±3.39c	0.05±0.00c
One - Way ANOVA (F-Statistic)				
Rep	334.78**	228.89**	244.90**	88.22***

Values presented are means ± SE, n = 10. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P \leq 0.05$ according to Fischer least significance difference.

Table 3.5: Effect of temperature on photosynthesis and gas-exchange parameters of leaves of *O. longibracteatum* as measured during Week 2 and 4 in 2010.

Treatments	WEEK 2				WEEK 4			
	A	E	Ci	Gs	A	E	Ci	Gs
	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$
Control (10 - 15 ^oC)	1.15±0.01d	0.26±0.01d	154.90±7.17d	0.01±0.00d	1.19±0.02d	0.35±0.01d	227.70±4.74d	0.02±0.00d
26^oC	1.87±0.03b	0.42±0.02b	267.40±4.46b	0.03±0.00b	1.99±0.07b	0.82±0.07b	281.90±4.25b	0.06±0.00b
30^oC	2.79±0.03a	0.69±0.01a	359.50±7.68a	0.06±0.00a	3.30±0.06a	1.52±0.10a	351.30±11.49a	0.13±0.01a
34^oC	1.46±0.03c	0.35±0.02c	242.90±1.92c	0.03±0.00c	1.74±0.03c	0.57±0.02c	257.80±6.79c	0.04±0.00c
One - Way ANOVA (F-Statistic)								
Rep	743.57**	113.70**	211.35**	71.02***	329.39**	61.94***	50.69***	66.87***

Values presented are means ± SE, n = 10. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P \leq 0.05$ according to Fischer least significance difference.

Table 3.6: Effect of temperature on photosynthesis and gas-exchange parameters of leaves of *O. longibracteatum* as measured during Week 6 and 8 in 2010.

Treatments	WEEK 6				WEEK 8			
	A	E	Ci	Gs	A	E	Ci	Gs
	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$
Control (10 - 15 ^oC)	1.35±0.01d	0.41±0.01d	207.40±15.67d	0.03±0.00d	1.38±0.04d	0.50±0.02d	238.90±2.35d	0.03±0.00d
26^oC	2.55±0.09b	0.63±0.03b	334.00±16.31b	0.07±0.01b	3.07±0.09b	0.86±0.04b	308.00±9.61b	0.09±0.02b
30^oC	4.16±0.31a	0.88±0.03a	374.40±2.72a	0.15±0.01a	7.01±0.15a	1.54±0.06a	352.10±3.86a	0.17±0.01a
34^oC	1.89±0.02c	0.51±0.03c	258.80±12.23c	0.05±0.00c	2.35±0.11c	0.63±0.03c	267.30±1.99c	0.06±0.00c
One - Way ANOVA (F-Statistic)								
Rep	55.43***	69.15***	33.51***	97.51**	538.20**	152.33**	83.27***	42.57***

Values presented are means ± SE, n = 10. **; *** = significant at $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P \leq 0.05$ according to Fischer least significance difference.

Table 3.7: Effect of temperature on photosynthesis and gas-exchange parameters of leaves of *O. longibracteatum* as measured during Week 10 in 2010.

Treatments	Week 10			
	A	E	Ci	Gs
	µmol $CO_2.m^{-2}.s^{-1}$	$mmol.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol $H_2O.m^{-2}.s^{-1}$
Control (10 - 15 ^oC)	3.55±0.03d	1.35±0.03d	241.90±13.20d	0.03±0.00d
26^oC	5.77±0.05b	1.76±0.03b	352.50±5.14b	0.06±0.00b
30^oC	8.33±0.17a	2.59±0.09a	378.50±1.97a	0.18±0.01a
34^oC	4.30±0.03c	1.54±0.05c	320.10±9.94c	0.04±0.00c
One - Way ANOVA (F-Statistic)				
Rep	508.81**	96.43**	46.31***	362.44**

Values presented are means ± SE, n = 10. **; *** = significant at *P*≤0.01, *P*≤0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at *P*≤0.05 according to Fischer least significance difference.

Chapter 4

4.0 Effects of regulating hydroponics water temperature on profiling of flavonoid and anthocyanins in *Ornithogalum longibracteatum* L.

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S. Nxawe[1], P. A Ndakidemi[1, 2,] C.P. Laubscher[1].

[1]Faculty of Applied Science, Cape Peninsula University of Technology, Cape Town Campus, Keizersgracht, P.O. Box 652, Cape Town 8000, South Africa.

[2]Corresponding author

Tel: +27214603196

Fax: +27214603193

Email: NdakidemiP@cput.ac.za

Abstract

The effects of different temperature regimes of hydroponic solution temperature on profiling flavonoids and anthocyanins concentration in *Ornithogalum longibracteatum* L. were determined in the Cape Peninsula University of Technology (CPUT) laboratory in year 2009 and 2010. The plants were irrigated with hydroponic solution heated to various temperatures (26, 30 and 34°C) via pumps connected to 4 sets of water tanks each maintained at the experimental temperatures using Dolphin aquarium heaters. Unheated water supplied from the forth tank served as control. All the plants were supplied with 1mg/L nutrient solution of Hortical Calcium Nitrate (Hortical $Ca(NO_3)_2$) and changed at weekly intervals. After 60 days plants were sampled from each gutter and were carefully uprooted with their entire bulbs and root system, washed and divided into roots, bulbs, shoots, flowers. The plant parts were oven-dried at 65°C for 72 hrs, weighed, ground into a fine powder (0.85 mm) and stored prior to the bioassay for anthocyanins and flavonoids concentrations. The results showed that, compared with the control, increasing hydroponics water temperature from 26°C to 34°C significantly increased the levels of flavonoids and anthocyanins in roots, bulbs, shoots and flowers in 2009 and 2010. These results suggested that increasing temperatures from 26 to 34°C induced the accumulation of flavonoids and anthocyanins concentration in roots, bulbs, shoots and flowers in *O. longibracteatum.*

Key Words: metabolites, phenolic compounds

4.1 Introduction

Anthocyanins and flavonoids are a group of biologically active non-nutrients phenolic compounds ubiquitous in many plant species (Havsteen 1983; Schahidi and Naczk 1995). These secondary metabolites are not only important in plants growth and their physiological mechanisms but also are becoming increasingly important as antioxidants, food colorants, anti-allergenic, anti-inflammatory, anti-viral, anti-proliferative, anti-oxidative and anti-carcinogenic (Stavric 1994; Rice-Evans et al. 1996; Robards et al. 1999; Lillo et al., 2008). Furthermore, anthocyanins and flavonoids play an important role as materials for cell wall support, as colourful attractants for birds and insects during seed dispersal and plant pollination (Harborne; 1994), as defence mechanisms to plants against wounding, infection, excessive or harmful light as well as indicators of plant nutrient status (Bennet and Wallsgrove 1994, Dixon and Paiva 1995). However, these secondary metabolites have been reported to be affected by different biotic and abiotic environmental conditions such as non optimal temperature stress (Makoi and Ndakidemi, 2007; Guo et al., 2008). For example, it was reported that accumulation of secondary metabolites in plants cell walls (i.e. suberin and lignin) was attributed to low or freezing temperature stress (Treutter, 2005). Their accumulation enabled the plant to respond better to photoinhibition and therefore protect the plants from stress due to freezing and/or low temperatures (Solecka, 1997). In a study involving grapevine (*Vitis vinifera* L.), Braidot et al. (2008) reported that there was greater accumulation of secondary metabolites during cold temperatures compared with when the temperatures were constant. It is also well documented by Rivero et al. (2001) that the accumulation of secondary metabolites in tomato (*L. esculentum* L.) was due to high temperatures above

optimum range (such as 35°C) although Mori et al (2007) showed increasing degradation rate in anthocyanins.

O. longibracteatum, commonly known as pregnant onion, is widely used by traditional healers as a magical and medicinal plant in South Africa, and, is generally classified as a medicinal bulb (Kulkarni et al., 2005). The plant, which is commonly found growing in wild environments, is amongst the indigenous and horticultural plants with medicinal potential in South Africa. As a result of its potential medicinal value, its cultivation in the glasshouse hydroponically has recently gained momentum (Rosik-Dulewska and Grabda, 2002). Intensive cultivation of this plant hydroponically will not only increase production of this plant but also will be a potential source of income in urban areas (Larson et al., 1996). Medicinal plants have been reported to contain a plethora of secondary metabolites including anthocyanins and flavonoids. Although the effect of temperature on the growth of *O. longibracteatum* has been reported on different temperature ranges (Halevy et al., 1971; Luria et al., 2002), studies on the effect of hydroponics water temperature on the concentration of secondary metabolites are limited. Therefore, this study investigates the effects of hydroponics water temperature on the concentrations of anthocyanins and flavonoids which are probably believed to form the major part of its medicinal value.

4.2 Materials and methods

4.2.1 Site location and description

The experiment was conducted at the greenhouse of the Cape Peninsula University of Technology, Cape Town, South Africa from July 2009 and July 2010. A steel table (2.5

m x 1 m) was used as a flat surface, black plastic container (50 L), leca clay pebbles were supplied by Horticultural Department of Cape Peninsula University of Technology (CPUT), Cape Town, South Africa. Four (4) plastic gutters (2 m x 0.6 m), 4 pumps, 20 ml black plastic pipe, cable tie and 3 Dolphin aquarium heaters were purchased from Builders Warehouse (Maitland, Cape Town), South Africa. Bulbs of pregnant onion (*O. longibracteatum*) used as planting material were obtained from the CPUT Nursery and Hortical Ca $(NO_3)_2$ was obtained from Stark Ayres all in Cape Town, South Africa.

4.2.2 Experimental design

A completely randomized design, with four replicates, was conducted to study the effects of temperature on profiling of anthocyanins and flavonoids concentration in *O. longibracteatum.* Four white plastic gutters (2 m x 0.6 m) filled with leca clay pebbles were placed on a 2.5 m x 1 m steel table. Water was supplied to the leca pebbles through pumps projecting from 4 sets of black plastic containers (50 L) placed beneath the table. The water in each of the 3 tanks was heated by using Dolphin aquarium heaters to maintain the temperatures at 26°C (A), 30°C (B) and 34°C (C) respectively. *O. longibracteatum* bulbs were planted in each gutter (i.e. 10 bulbs per gutter) and supplied with nutrient solution (1 $mg.L^{-1}$ Hortical Ca $(NO_3)_2$) immediately after transplanting. Nutrient solution supplied from the pumps was re-circulated back to the black plastic container (50 L) through a 20 ml black plastic pipe. The plants were left to grow for the period of 10 weeks. To prevent concentration of nutrients in the clay pebbles due to evaporation, water was drained from the gutters and refreshed after every 2 weeks.

4.2.3 Preparation of plant materials for flavonoids and anthocyanins assay

At 60 days after planting, 10 pregnant onion (*O. longibracteatum*) plants were sampled from each gutter. The plants were carefully uprooted with their entire bulbs and root system, washed and divided into roots, bulbs, shoots, flowers. The plant parts were oven-dried at 65°C for 72 hrs, weighed, ground into a fine powder (0.85 mm) and stored prior to the bioassay for anthocyanins and flavonoids concentrations.

4.2.4 Determination of flavonoids and anthocyanins in plant parts

Concentration of anthocyanins and flavonoid were assayed according to Lindoo and Caldwell (1978) as well as Mirecki and Teramura (1984). In this method, 0.1g of grinded plant material was weighed and placed in a centrifuge tube. 10 mLs of acidified methanol (A-MeOH) prepared from a ratio of 79:20:1 (MeOH:H_2O:HCl) was added to the plant material. The solution was then incubated for 72 hrs in darkness. After the incubation period, the solution was filtered through Whatman paper No: 2. The absorbance of the clear supernatant fluid was measured spectrophotometrically at wavelengths (λ) of 300, 530, and 657 nm using A-Methanol as a standard (Mirecki and Teramura, 1984). The concentration of flavonoid compounds was expressed as:

$$Flavonoids\,(Abs\,g^{-1}\,DM) = Abs_{300}$$

Anthocyanins content was calculated as described in Lindoo and Caldwell (1978) as:

$$Anthocyanins\,(Abs\,g^{-1}\,DM) = Abs_{530} - \frac{1}{3}Abs_{657}$$

4.2.5 Statistical analysis

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

4.3 Results

4.3.1 Effect of hydroponics water temperature on flavonoids and anthocyanins in roots and bulbs of *O. longibracteatum.*

Table 4.1 shows the effect of temperature on anthocyanins and flavonoids concentration in roots and bulbs of *O. longibracteatum*. Compared with the control, increasing hydroponics water temperature from 26 to 34°C significantly increased the levels of flavonoids in roots and shoots in both years 2009 and 2010 (Table 4.1). For example the data showed that, increasing the hydroponics water temperature from 26 to 34°C, the levels of flavonoids in root parts significantly increased by 38 and 55% in 2009, and 57 and 98% in 2010. Similar trend was observed with the levels of anthocyanins when hydroponics temperature was increased from 26 to 34°C. The anthocyanins concentration increased by 13 and 107% in 2009 and 56 and 156% in 2010. Furthermore, changing hydroponics water temperature significantly influenced the levels of anthocyanins and flavonoids in bulbs and flowers of *O. longibracteatum* (Table 4.2). For instance, in bulbs, increasing the hydroponics water temperature from the control (10-15°C) to 26, 30 and 34°C significantly increased the concentration of flavonoids by

14, 25 and 42% in year 2009, and by 25, 67 and 92% in 2010. Results also showed that anthocyanins in the bulb was significantly increased by 34, 80 and 118% in 2009 and by 74, 124 and 153% in 2010 when hydroponics water temperature was increased from (10-15°C) to 26, 30 and 34°C.

4.3.2 Effect of hydroponics water temperature on flavonoids and anthocyanins in the shoots and flowers of *O. longibracteatum.*

There was significant effect of hydroponics water temperatures on the levels of anthocyanins and flavonoids in the shoots and flowers of *O. longibracteatum* (Tables 4.1 and 4.2). By increasing the hydroponics water temperature from 26 to 34°C, the levels of flavonoids in shoot parts significantly increased by 13 and 32% in 2009, and 26 and 51% in 2010. Similar trend was observed with the levels of anthocyanins when hydroponics temperature was increased from 26 to 34°C. This concentrations increased by 249 and 789% in 2009 and 63 and 744% in 2010 respectively. There was also significant effect of hydroponics water temperatures on the levels of anthocyanins and flavonoids in the flowers of *O. longibracteatum*. The levels of flavonoids increased by 20, 51 and 85% in 2009 and 31, 46 and 62% in 2010 when hydroponics water temperature was elevated to 26, 30 and then 34°C, respectively relative to control. Following similar trend as shown in the flavonoids concentration, the levels of anthocyanins in the flowers significantly increased by 47, 129 and 162% in 2009 and 63, 264 and 441% respectively when the hydroponics water temperature was increased to 26, 30 and 34°C relative to control (10-15°C).

4.4 Discussion

Phenolic compounds play an important role in protecting plants from abiotic and biotic stress conditions such as adverse temperature conditions (Solecka, 1997; Königshofer and Lechner, 2002). Changes in temperature have been reported to be one of the major environmental factors influencing the accumulation of flavonoids and anthocyanins in plants (Wahid et al, 2007; Joakola and Hohtola, 2010). Some studies have reported increased accumulation of flavonoids and anthocyanins with decreasing temperatures (Zhang et al., 1997; Solecka et al., 1999; Leng et al., 2000; Havaux and Kloppstech, 2001; Hasegawa et al., 2001; Romero et al., 2008 a and b). However, in this study, increasing the hydroponics solution temperature significantly elevated the accumulation of flavonoids and anthocyanins in *O. longibracteatum*. Results from this study showed that increasing temperatures to 26, 30 and 34°C significantly and progressively induced the accumulation of flavonoids in root, bulbs shoots and flowers in 2009 and in 2010 compared with the control. Similar to the above trend, the anthocyanins concentration in roots, bulbs shoots and flowers were also increased when temperature was elevated to 26, 30 and 34°C compared with the control. Comparison between organs showed that in *O. longibracteatum* more flavonoids were extracted from the flowers, followed by bulbs, roots and shoots. But, the anthocyanins concentration was generally higher in flowers, followed by shoots, bulbs and roots. In all instances, the data showed that greater concentrations of these biomolecules were significantly elevated in each organ when temperature in the hydroponic solution was increased to 34°C compared with all other treatments. Other researchers have similarly reported the induction of phenolic compounds in different plant species due to increased temperatures in the soil/hydroponic solution (Bilger et al., 2007; Mori et al, 2007) and attributed this to the strategy employed by plants in preventing them from heat stress (Chalker- Scott, 1999;

Rivero et al., 2001). Similar to our study, Wahid (2007), Rivero et al. (2001) and Guy et al. (2008) reported elevated accumulation of anthocyanins in *Photinia* spp, *Aster amellus* and *Lycopersicon esculatum* due to their exposure to high temperature. Other researchers working on red clover (*Trifolium pretense*), Faba bean (*Vicia faba* L.) also reported increased levels in phenolic compounds at elevated soil/hydroponic temperature (Nasar-Abbas et al., 2009; Saviranta et al., 2010). Based on these profiles, one could target specific organ(s) of *O. longibracteatum* for the extraction of either flavonoids or anthocyanins for special purpose(s) and at the specified environmental conditions such as those used in this study. This is due to the fact that the accumulation of flavonoids and anthocyanins in the tissues was stimulated by changes in the hydroponic solution temperatures.

In conclusion, temperature might affect the accumulation of flavonoids and anthocyanins in *O. longibracteatum*. In this study, results indicated that elevated temperature in the hydroponic solution increased the accumulation of flavonoids and anthocyanins in two years of experimentation. These bio-molecules are known to play important role in protecting the plants against stress and improving the medicinal value of the tissues. Future studies should focus on the qualitative aspects of the flavonoids and anthocyanins molecules produced at elevated temperatures such as those reported in this study.

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Table 4.1: Effect of temperature on flavonoids and anthocyanins (Abs.g^{-1} DM) in shoots and roots of *O. longibracteatum* as measured during 2009 and 2010.

	Roots		Bulbs		Roots		Bulbs	
	Flavonoids	Anthocyanins	Flavonoids	Anthocyanins	Flavonoids	Anthocyanins	Flavonoids	Anthocyanins
	2009				2010			
Control (10 - 15 ^{o}C)	1.70±0.10c	0.15±0.10c	12.55±0.42d	0.44±0.03d	1.36±0.03d	0.16±0.01d	11.16±1.32d	0.34±0.03d
26^{o}C	2.34±0.04b	0.17±0.02c	14.27±0.52c	0.59±0.05c	2.14±0.03c	0.25±0.01c	13.99±0.79c	0.59±0.03c
30^{o}C	2.65±0.08a	0.24±0.10b	15.70±0.58b	0.79±0.04b	2.37±0.04b	0.37±0.01b	18.61±0.41b	0.76±0.04b
34^{o}C	2.64±0.08a	0.31±0.10a	17.77±0.42a	0.96±0.04a	2.69±0.06a	0.41±0.02a	21.39±0.49a	0.86±0.04a
One - Way ANOVA (F-Statistic)								
Rep	33.96***	33.70***	20.66***	34.98***	176.17**	126.69**	30.14***	45.21**

**: $P \leq 0.01$; ***: $P \leq 0.001$. Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different by Least Significant Difference test at $P=0.05$.

Table 4.2: Effect of temperature on flavonoids and anthocyanins (Abs.g^{-1} DM) of bulbs and flowers of *O. longibracteatum* as measured during 2009 and 2010.

	Shoots		Flowers		Shoots		Flowers	
	Flavonoids	Anthocyanins	Flavonoids	Anthocyanins	Flavonoids	Anthocyanins	Flavonoids	Anthocyanins
	2009				2010			
Control (10 - 15 ^{o}C)	1.79±0.06c	0.37±0.03d	11.93±0.14d	0.34±0.02d	1.71±0.07d	0.16±0.01d	12.53±0.50d	0.27±0.00c
26^{o}C	2.02±0.05b	1.29±0.04c	14.37±0.18c	0.50±0.03c	2.16±0.01c	0.26±0.01c	16.39±0.18c	0.44±0.01c
30^{o}C	2.30±0.05a	2.23±0.02b	18.02±0.42b	0.78±0.03b	2.34±0.03b	0.68±0.04b	18.31±0.54b	0.98±0.01b
34^{o}C	2.37±0.08a	3.29±0.08a	22.03±1.27a	0.89±0.02a	2.58±0.04a	1.35±0.05a	20.34±0.45a	1.46±0.27a
One - Way ANOVA (F-Statistic)								
Rep	21.53***	682.78**	42.30***	97.94***	79.11***	338.20**	56.88***	15.64***

**: $P \leq 0.01$; ***: $P \leq 0.001$. Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different by Least Significant Difference test at $P=0.05$.

Chapter 5

5.0 Nutrient uptake in plant tissues of *Ornithogalum longibracteatum* L. as influenced by regulating the hydroponic solution temperature

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S. Nxawe[1], P. A Ndakidemi[2,*] C.P. Laubscher[1].

[1]Faculty of Applied Science, Cape Peninsula University of Technology, Cape Town Campus, Keizersgracht, P.O. Box 652, Cape Town 8000, South Africa,

[2]The Nelson Mandela African Institute of Science and Technology, Old Arusha - Moshi Rd, TENGERU, P.O. Box 447 ARUSHA. TANZANIA.

[*]Corresponding author

Tel: +255757744772

Email: ndakidemipa@gmail.com

Abstract

The effects of different hydroponic solution temperature on wintertime nutrient uptake of pregnant onion (*Ornithogalum longibracteatum*) were evaluated in the glasshouse experiment, in Cape Town, South Africa. The aim of the study was to assess the influence of different temperature treatments in the hydroponic cultures on nutrient uptake and accumulation in the tissues of *O. longibracteatum*. Plants were exposed to four hydroponic solution temperatures (control (10 - 15°C), 26, 30 and 34°C) during wintertime by the manipulation of temperatures using Dolphin aquarium heaters to maintain the temperatures at 26, 30 and 34°C respectively. For the control treatment, unheated water was supplied directly from the tap and using the thermometer, the temperature ranged between 10 - 15°C (day/night) throughout the experiment period. Results showed that warming of the hydroponic solution with Dolphin aquarium heaters to 26, 30 and 34^{o}C significantly increased the uptake of the following macronutrients and micronutrients (N, P, K, Ca, Mg, S, Na Fe, Cu Zn, Mn and B and Mo) in organs of *O. longibracteatum* (root, bulbs shoot, and whole plant) in 2009 and verified again in 2010. The control treatments 10 - 15°C (day/night) had the lowest uptake of most nutrients. The optimum uptake of most nutrients was achieved at 30°C. Further increase to 34°C resulted into the declining trend which in most cases was significantly lower than the 30°C treatment. It was concluded that lower winter temperature in the hydroponic solution can result in reduced nutrient uptake capacity and growth rate during winter season. Heating of the hydroponic solution temperature in the controlled environments is recommended during winter season for optimum growth *O. longibracteatum*

5.1 Introduction

Nutrient uptake and accumulation in plant tissues is very important for growth and development, and partly depends on their availability in the growth media. However, the uptake of these nutrients and their accumulation in plant organs may be affected by various environmental factors including exposure to high or low temperature, thus, affecting the overall plant performance (Reay et al**.,** 1998; Aðalsteinsson and Jensén, 2006). It is generally acknowledged that low temperatures are capable of decreasing nutrient uptake and accumulation in plants due to reduced metabolic activities which ultimately affects growth and development of the plant (Gavito et al., 2001). For instance, in cotton seedlings (*Gossypium hirsutum* L. cv Deltapine 70), uptake rate of N and P decrease at a low temperature of 12°C (Radin and Matthews, 1989). A study by Daskalaki and Burrage (1998) showed that increased nutrient uptake in cucumber (*Cucumis sativus* L.) was due to elevated temperature up to 28°C, leading to enhanced plant growth and yield. Some studies have similarly shown that increasing temperature from 15 to 29°C increased the uptake of P, K, Ca, Mg, Fe, Mn, Zn and B and finally the plant growth (Hood and Mills, 1994; Stoltzfus et al., 1998). Furthermore, nutrient uptake, especially N in pine (*Pinus sylvestris* L.) increased with increasing soil temperature from 8°C to 16°C (Vapaavuori et al., 1992). Raising temperature from 12°C to 20°C in a closed hydroponic system induced the uptake of N, P, K, Ca and Mg in *Cucumis sativus* L (Daskalaki et al., 1998).

Water temperature plays a vital role in plant development (Chung et al., 2006). At optimum temperature, water can nourish growth compared with low or high temperatures levels (He, et al., 2002). In plants, water is required to maintain cell turgidity to ensure continuous column of moisture in the cells (Stewart and Dwyer, 1983; Noguchi and

Terashima, 1997; Outlaw, 2003). Hence, growth media solution temperature is an important factor regulating nutrient uptake rate and thus, plant growth and development (Barrow and Shaw, 1975). Therefore, improving nutrient uptake under environmental stress condition through hydroponic water solution temperature management will not only determine the nutrient uptake but also will enhance better plant growth and development. This study assesses the influence of different temperature treatments in the hydroponic cultures on nutrient uptake and accumulation in the tissues of *O. longibracteatum*.

5.2 Materials and methods

5.2.1 Experimentation.

The experiment was conducted at the greenhouse of the Cape Peninsula University of Technology, Cape Town, South Africa at the beginning of the month of July 2009 and repeated in July 2010. A completely randomised design, with four replicates, was conducted to study the effects of temperature on chlorophyll pigmentation and photosynthetic rate in *O*. *longibracteatum*. Four (4) white plastic gutters (2 m x 0.6 m) filled with leca clay pebbles were placed on a 2.5 m x 1 m steel table. Water was supplied to the leca pebbles through pumps projecting from 4 sets of black plastic containers (50 L each) placed beneath the table. The water in the 3 containers was heated by using Dolphin aquarium heaters to maintain the temperatures at 26, 30 and 34°C respectively. Unheated water supplied from the fourth container served as control. Using the thermometer, the temperature ranged between 10-15°C (day/night) throughout the experiment period. *O*. *longibracteatum* bulbs used as planting material were obtained from the CPUT Nursery and were planted in each gutter (i.e. 10 bulbs per

gutter) and supplied with nutrient solution immediately after transplanting. The nutrient solution was prepared according to Ocean HYDROGRO (2009) and Ocean HORTICAL (2009) respectively. Nutrient solution supplied from the pumps was re-circulated back to the black plastic container (50 L) through a 20 mL black plastic pipe. The plants were left to grow for the period of 10 weeks. To prevent concentration of nutrients in the clay pebbles due to evaporation, water was drained from the gutters and refreshed after every week.

5.2.2 Preparation of plant materials for nutrient uptake and accumulation in plant tissues assay

At 70 days after planting, 10 pregnant onion (*O. longibracteatum*) plants were sampled from each gutter. The plants were carefully uprooted with their entire bulbs and root system, washed and divided into roots, bulbs and shoots. The plant parts were oven-dried at 65°C for 72 hrs, weighed, ground into a fine powder (0.85 mm) and stored prior to the bioassay for nutrient uptake and accumulation in plant tissues.

5.2.3 Measurement of nutrients in plant tissue

Measurements of P, K, Ca, Mg Fe, Cu, Zn, Mn, B and Al were determined by ashing 1 g of ground sample in a porcelain crucible at 500°C overnight. This was followed by dissolving the ash in 5 ml of 6 M HCl and placing it in an oven at 50°C for 30 min and 35 ml of deionised water was added. The mixture was filtered through Whatman no. 1 filter paper. Nutrient concentration in plant extracts was determined using the inductively coupled plasma mass spectrometry (ICP-MS) (Giron, 1973). Sulphur (S) was determined by wet digestion procedure using 65% nitric acid. In each case, 1g of milled plant

material was digested overnight with 20 ml of 65% nitric acid in a 250 ml glass beaker. The beaker containing the extract was then placed on a sand bath and gently boiled until approximately 1 ml of the extract was left. After that, 10 m of 4 M nitric acid was added and boiled for 10 min. The beaker was removed from the sand bath, cooled, and the extract washed completely in a 100 ml volumetric flask and the extract filtered through Whatman no. 2 filter paper. Sulphur in the sample was then determined (FSSA 1974) by direct aspiration on the calibrated simultaneous ICP-MS. Nutrient uptake (mg.plant^{-1}) was then calculated as the product of nutrient concentration (mg.g^{-1}, data not shown) and the weight of the plant part dry matter (g.plant^{-1}).

$$N_{uptake}\left(mg.plant^{-1}\right) = ON_{conc.}\left(mg.g^{-1}\ DM\right) \times O_{drymass}\left(g.plant^{-1}\right)$$

Where: N_{uptake} = *Microelement uptake,* ON_{conc} = *Organ nutrient concentration,* $O_{dry\ mass}$ = *Organ dry mass.*

Whole plant nutrient uptake (mg.plant^{-1}) was calculated as the sum of the uptake of individual organs (i.e.roots, bulbs, shoots and whole plant).

5.2.4 Statistical analysis

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

5.3 Results

5.3.1 Effect of varying hydroponics solution temperature on the uptake of macronutrients in roots of *O. longibracteatum* in 2009 and 2010.

There were significant differences on the uptake of N, P, K, S and Na by varying hydroponic solution temperatures in the roots, of *O. longibracteatum* in 2009 and 2010 (Table 5.1). Compared with the control, in the year 2009, the best uptake for all nutrients in the roots was recorded in a solution which was adjusted to 26^{o}C. Furthermore, increasing the temperatures to 34^{o}C significantly lowered the root uptake of N, P, K, S and Na compared with the control treatment. In the year 2010, elevating the hydroponic solution temperatures to 30^{o}C resulted into significantly greater uptake of N, P, K, S and Na relative to the control. Similar to the results obtained in 2009, with the exception of N, increasing the temperatures from 30^{o}C to 34^{o}C slightly reduced the uptake of all other macronutrients measured in roots.

5.3.2 Effect of varying hydroponics solution temperature on the uptake of macronutrients in bulbs of *O. longibracteatum* in 2009 and 2010.

The modification of hydroponics solution temperature from 10^{o}C to 34^{o}C significantly increased the uptake of N, P, K, Ca, Mg, S and Na in bulbs of *O. longibracteatum* in 2009 (Table 5.2). Similar trend appeared in bulbs grown in 2010 with an exception of K. In 2009, relative to the control treatment (10 - 15^{o}C) elevating the hydroponic temperature to 26, 30 and 34^{o}C significantly increased the uptake of all macronutrient in the bulbs. However, macronutrient uptake in the bulbs exposed within the temperature ranges of 26, 30 and 34^{o}C were not significantly different from each other. The result for

2010 showed significant progressive increase in the uptake of N, P, Ca, Mg, S and Na in bulbs of *O. longibracteatum* with increased hydroponic temperature from 10^{o}C to 34^{o}C. The greater uptake of these macronutrients was observed at each level of elevating the hydroponic solution temperature. The maximum uptake was recorded in temperatures 30 and 34^{o}C and was significantly superior to 10^{o}C and 26^{o}C, respectively.

5.3.3 Effect of varying hydroponics solution temperature on the uptake of micronutrients in roots of *O. longibracteatum* in 2009 and 2010.

The effect of varying hydroponic solution temperature on the uptake of micronutrients in roots of *O. longibracteatum* in 2009 and 2010 is shown in Table 5.3. Compared with the control, raising the temperature to 26, 30 and 34^{o}C significantly increased the uptake of Fe, Cu Zn and B in both 2009 and 2010. Generally, the greatest uptake in all micronutrients was observed at a temperature of 30^{o}C.

5.3.4 Effect of varying hydroponics solution temperature on the uptake of micronutrients in bulbs of *O. longibracteatum* in 2009 and 2010.

The uptake of Zn, Mn and B in 2009 and Fe, Zn, Mn and B in 2010 into bulb were significantly different in hydroponic solution temperature treatments (Table 5.4). In 2009 the uptake of Zn, Mn, and B were simila in hydroponic solution temperature of 26, 30 and 34 ^{o}C and significantly higher than the control. The highest uptake of Fe, Zn, Mn and Bo was observed in hydroponic solution temperatureof 30 and 34^{o}C, whereas control treatment had the lowest uptake of Fe, Zn, Mn and Bo in 2010.

5.3.5 Effect of varying hydroponics solution temperature on the uptake of macronutrients in shoots of *O. longibracteatum* in 2009 and 2010.

The effect of temperature changes on the macronutrients uptake in shoots of *O. longibracteatum* in 2009 and 2010 is shown in Table 5.5. Relative to control, increasing the temperatures to 26, 30 and 34^{o}C significantly enhanced the shoot uptake of N, P, K, Ca, Mg, S and Na in 2009 and 2010. Generally, comparing the control treatment with 30 and 34^{o}C in 2009, the uptake of N, P, K, Ca, Mg, S and Na increased steadily at each level of elevating the temperature. However, in the second year of experimentation the shoot uptake of N, P, K, Ca, Mg, S and Na were progressively increased at 26 and 30^{o}C and then slightly decreased at 34^{o}C treatment.

5.3.6 Effect of varying hydroponics solution temperature on the uptake of macronutrients in whole plant of *O. longibracteatum* in 2009 and 2010.

The whole plant uptake of macronutrients N, P, K, Ca, Mg, S and Na in *O. longibracteatum* were significantly altered by hydroponic solution temperature modifications in 2009 and 2010 (Table 5.6). Generally, control constently significantly had lower uptake for all the macronutrients than the other temperatures in 2009. Contrary to the above results, in 2010, raising the temperature above 30^{o}C significantly reduced the uptake of N, P and S but increased those of K, Ca, Mg and Na.

5.3.7 Effect of varying hydroponics solution temperature on the uptake of micronutrients in shoots of *O. longibracteatum* in 2009 and 2010.

The shoot micronutrient uptake (Fe, Cu, Zn, Mn and B) of *O. longibracteatum* in 2009 and 2010 were significantly increased (Table 5.7) by adjusting the temperatures to 26, 30 and 34^{o}C compared with the control. In 2009, increasing the hydroponic solution temperature to 26, 30 and 34^{o}C resulted into significant increased in the uptake of micronutrients. However, 26, 30 and 34^{o}C had similar uptake for the macronutrients. In 2010 the highest uptake was increased at 30^{o}C. The control, 26 and 34^{o}C had similar uptake of Fe and Cu whereas the control treatment and 26^{o}C had similar uptake of Zn, Mn and Bo but significantly lower than those of 34^{o}C.

5.3.8 Effect of varying hydroponics solution temperature on the uptake of micronutrients in whole plants of *O. longibracteatum* in 2009 and 2010.

The control treatment had similar uptake of Fe and Cu in the 34^{o}C hydroponic solution temperature in 2009 (Table 5.8). 26 and 30^{o}C which favoured uptake of Fe and Cu were also similar in 2009 for Zn, Mn, Bo, the control had lower uptake compare to the other treatments. Compared with the control, in 2009, increasing the temperature at all levels significantly increased the whole plant uptake of all micronutrients except that of Cu. The micronutrient uptakes in whole plants grown in 2010 were significantly elevated in the 30 and 34^{o}C treatments relative to the 26 and the control (10-15^{o}C) treatments except Fe which declined at 34^{o}C ydroponic solution.

5.4 Discussion

It was well established that low growth medium temperature may reduce root growth and nutrient uptake in plants (Lal, 1974; Engels and Marschner, 1990, 1992; Delucia et al., 1992; Pregitzer et al., 2000). In this study, warming of the hydroponic solution with Dolphin aquarium heaters to 26, 30 and 34^{o}C significantly increased the uptake of the following macronutrients and micronutrients (N, P, K, Ca, Mg, S, Na Fe, Cu Zn, Mn and B and Mo) in organs of *O. longibracteatum* (root, bulbs shoot, and whole plant) grown in the glasshouse in 2009 and verified again in 2010. The temperature changes in the growth medium have been reported to be one of the major environmental factors influencing the plant growth (Pregitzer and King, 2005) and the uptake and accumulation of nutrients in their tissues (Lee et al., 2007). Controlled studies with other plants have reported decreased uptake of nutrients with decreasing temperatures in the growth medium (Menzel et al., 1987), a concept which was also proved in our study. In general, nutrient uptake in most plants was sensitive to temperature changes (Lahav and Turner, 1985; Debusk and Reddy, 1987). Studies have shown that growth medium temperature may influence rate of chemical reactions, nutrient transport in the medium, and the important plant physiological aspects related to ion uptake and root growth (McMichael and Burke 1998; Pregitzer et al., 2000). Low temperatures below the optimum point in the growth medium may be associated with reduced rate of nutrient transport and hence their uptake. In this study, the optimum uptake of most nutrients was achieved at 30°C. Further increase to 34°C resulted into the declining trend which in most cases was significantly lower than the 30°C treatment. Similar to our study, Lal (1974) reported that increasing the soil temperature above 30°C significantly decreased the shoot and root growth and transpiration rate in maize, thus, decreasing the uptake of N, P, and K. Our

results were in line with the above mentioned facts from different authors that low or high temperatures in the hydroponic solution beyond optimum points reduced the root growth and decreased nutrient accumulation in different plant organs

In conclusion, lower winter temperature in the hydroponic solution resulted into reduced growth rate and poor nutrient uptake during winter season. Heating of the hydroponic solution to 30^{o}C temperature in the controlled environments is recommended during winter season for optimum nutrient uptake and the growth *O. longibracteatum*

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Table 5.1: Effect of hydroponics solution water temperature on the uptake of macronutrients in roots of *O. longibracteatum* grown in the greenhouse in 2009 and 2010.

Treatment	N	P	K	Ca	Mg	S	Na
				(mg.plant^{-1})			
2009							
Control (10 - 15 °C)	56.05±8.32b	8.91±1.22c	67.61±10.64b	5.14±0.57a	1.64±0.26a	4.58±0.53b	1.71±0.57c
26°C	216.17±43.60a	42.76±8.08a	229.99±40.13a	22.83±5.28a	6.60±1.70a	21.71±4.77a	29.37±7.31a
30°C	155.34±44.42ab	37.03±11.22ab	157.00±41.44ab	19.43±5.66a	6.54±1.86a	22.72±7.41a	20.77±8.11ab
34°C	90.29±32.16b	17.35±5.86b	98.57±37.88b	12.01±4.08a	3.08±0.96a	10.13±3.29ab	4.70±1.79b
One - Way ANOVA (F-Statistic)							
	4.05*	4.51*	4.19*	3.24ns	3.41ns	3.56*	5.64*
2010							
Control (10 - 15 °C)	74.55±25.88b	12.84±4.59b	84.16±29.26b	6.54±1.69a	2.11±0.53a	6.06±2.10c	2.67±1.61d
26°C	106.78±11.68ab	20.69±3.24ab	99.83±14.94b	10.41±0.52a	2.90±0.23a	11.08±0.89bc	13.57±3.15b
30°C	143.37±31.99a	35.82±9.82a	163.63±41.85a	17.00±4.05a	5.66±1.46a	22.49±6.12a	18.93±7.37a
34°C	153.66±29.66a	27.28±5.00a	156.76±33.55a	16.93±2.92a	4.26±0.94a	18.05±3.11ab	7.94±2.02c
One - Way ANOVA (F-Statistic)							
	4.93*	4.49*	4.62*	3.78ns	2.93ns	4.06*	4.78*

Values presented are means ± SE, n = 4. * = significant at $P \leq 0.05$, MSE = standard error of the mean. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 5.2: Effect of hydroponics solution water temperature on the uptake of macronutrients in bulbs of *O. longibracteatum* grown in the greenhouse in 2009 and 2010.

Treatment	N	P	K	Ca	Mg	S	Na
				(mg.plant^{-1})			
2009							
Control (10 - 15 ^{o}C)	57.39±12.02b	10.03±2.99b	57.72±12.78b	26.62±5.36b	8.78±1.98b	4.38±0.75b	1.63±0.22b
26^{o}C	239.56±44.72a	50.43±12.19a	237.28±35.80a	99.25±18.10a	32.07±6.63a	16.28±3.12ab	5.89±1.20a
30^{o}C	195.73±27.04a	40.82±5.47a	186.64±32.73a	92.50±18.54a	29.43±4.60a	19.51±3.98a	5.58±0.76a
34^{o}C	241.33±58.60a	56.98±13.69a	230.24±39.00a	108.33±21.85a	38.23±8.21a	27.56±7.21a	5.74±0.74a
One - Way ANOVA (F-Statistic)							
	4.76*	4.61*	6.86**	4.72*	4.79*	4.74*	6.44**
2010							
Control (10 - 15 ^{o}C)	82.53±28.06c	16.14±4.78c	101.66±29.52a	40.86±12.04c	12.54±4.09c	5.54±1.86c	1.49±0.63c
26^{o}C	188.21±28.04b	35.70±4.61b	158.85±23.27a	76.41±9.36b	25.08±3.47b	13.88±1.64b	5.54±1.86b
30^{o}C	337.76±33.13a	70.11±7.71a	321.21±43.92a	168.94±20.86a	50.92±5.90a	36.02±3.34a	8.02±1.28a
34^{o}C	459.01±191.03a	93.93±44.26a	436.97±201.49a	216.26±90.90a	67.69±30.93a	45.92±21.24a	12.97±5.04a
One - Way ANOVA (F-Statistic)							
	4.80*	4.34*	2.13ns	4.94*	3.43*	4.02*	5.98*

Values presented are means ± SE, n = 4. *; ** = significant at $P \leq 0.05$, $P \leq 0.01$ respectively, MSE = standard error of the mean. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 5.3: Effect of hydroponics solution water temperature on the uptake of micronutrients in roots of *O. longibracteatum* grown in the greenhouse in 2009 and 2010.

Treatment	Fe	Cu	Zn	Mn	B
			(mg.plant^{-1})		
2009					
Control (10 - 15 ^{o}C)	1.05±0.31b	0.08±0.01c	0.10±0.01c	0.31±0.09a	0.04±0.01b
26^{o}C	9.49±1.18ab	0.29±0.05a	0.91±0.21a	0.99±0.22a	0.18±0.04a
30^{o}C	13.85±5.60a	0.24±0.05ab	1.02±0.34ab	1.03±0.35a	0.20±0.06a
34^{o}C	7.15±2.96ab	0.13±0.05bc	0.33±0.13bc	0.61±0.21a	0.08±0.03ab
One - Way ANOVA (F-Statistic)					
	2.74*	4.68*	4.50*	2.08ns	3.56*
2010					
Control (10 - 15 ^{o}C)	1.73±0.75c	0.15±0.06b	0.14±0.06b	0.62±0.29a	0.05±0.01b
26^{o}C	4.66±0.74b	0.15±0.03b	0.44±0.07ab	0.59±0.11a	0.09±0.01b
30^{o}C	8.48±2.64a	0.27±0.09a	0.95±0.28a	1.04±0.27a	0.20±0.05a
34^{o}C	7.87±2.00a	0.23±0.04a	0.66±0.19ab	1.21±0.20a	0.13±0.03ab
One - Way ANOVA (F-Statistic)					
	3.24*	4.14*	3.84*	1.81ns	4.45*

Values presented are means ± SE, n = 4. * = significant at $P \leq 0.05$, MSE = standard error of the mean. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 5.4: Effect of hydroponics solution water temperature on the uptake of micronutrients in bulbs of *O. longibracteatum* grown in the greenhouse in 2009 and 2010.

Treatment	Fe	Cu	Zn	Mn	B
			(mg.plant^{-1})		
2009					
Control (10 - 15 ^{o}C)	0.09±0.01a	0.01±0.00a	0.12±0.04b	0.12±0.04b	0.04±0.01b
26^{o}C	0.26±0.08a	0.04±0.00a	0.52±0.12a	0.62±0.13a	0.12±0.02a
30^{o}C	0.49±0.15a	0.03±0.01a	0.49±0.06a	0.51±0.10a	0.12±0.02a
34^{o}C	0.67±0.24a	0.05±0.02a	0.60±0.10a	0.77±0.19a	0.16±0.04a
One - Way ANOVA (F-Statistic)					
	2.97ns	2.48ns	6.05**	4.84*	4.40*
2010					
Control (10 - 15 ^{o}C)	0.13±0.04b	0.02±0.00a	0.16±0.06c	0.23±0.08c	0.04±0.02c
26^{o}C	0.22±0.04b	0.03±0.00a	0.39±0.06b	0.51±0.06b	0.10±0.02b
30^{o}C	0.75±0.16ab	0.07±0.02a	0.96±0.12a	0.97±0.10a	0.21±0.03a
34^{o}C	1.27±0.48a	0.08±0.03a	1.12±0.55a	1.39±0.66a	0.31±0.14a
One - Way ANOVA (F-Statistic)					
	4.31*	2.58ns	5.64*	4.33*	4.76*

Values presented are means ± SE, n = 4. *; ** = significant at $P \leq 0.05$, $P \leq 0.01$ respectively, MSE = standard error of the mean. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 5.5: Effect of hydroponics solution water temperature on the uptake of macronutrients in shoots of *O. longibracteatum* grown in the greenhouse in 2009 and 2010.

Treatment	N	P	K	Ca	Mg	S	Na
				(mg.plant^{-1})			
2009							
Control	341.37±151.27c	41.10±15.35c	452.43±196.91c	110.18±47.68b	24.76±11.61c	13.00±5.40b	12.45±5.32b
26^{o}C	1025.36±217.70a	104.86±21.71b	1301.94±335.76b	423.07±110.96a	69.02±16.78b	37.80±8.29ab	37.73±6.40ab
30^{o}C	933.75±257.33a	96.85±27.52b	1055.80±323.96b	438.12±117.32a	70.43±19.88b	40.13±10.70ab	36.43±9.89ab
34^{o}C	1384.69±303.94a	150.20±30.31a	1747.43±410.65a	538.36±94.38a	103.81±20.86a	61.58±14.46a	64.25±16.58a
One - Way ANOVA (F-Statistic)							
	8.27**	23.36***	4.74*	3.69*	4.37*	3.75*	4.05*
2010							
Control	242.54±26.79c	108.57±14.60d	27.44±5.55b	2321.15±382.94b	330.88±45.27c	2989.63±527.54c	9.20±3.99b
26^{o}C	926.77±158.64b	507.29±60.69c	212.14±83.38ab	7901.95±1154.66b	1011.43±180.91bc	14626.90±2821.90bc	51.50±6.09a
30^{o}C	2086.54±318.10a	1470.57±181.55a	326.54±57.06a	31217.45±7399.64a	2723.55±699.39a	39368.08±6156.23a	62.68±8.97a
34^{o}C	1333.00±119.98b	891.46±55.96b	288.15±71.84a	29320.45±9610.75a	1592.35±147.77ab	22178.00±4174.79b	56.82±10.86a
One - Way ANOVA (F-Statistic)							
	16.81***	33.66***	4.59*	5.83*	7.53**	14.64***	9.42**

Values presented are means ± SE, n = 4. *; **, *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, MSE = standard error of the mean. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 5.6: Effect of hydroponics solution water temperature on the uptake of macronutrients in whole plant of *O. longibracteatum* grown in the greenhouse in 2009 and 2010.

Treatment	N	P	K	Ca	Mg	S	Na
				(mg.plant^{-1})			
2009							
Control	454.81±159.22b	60.04±16.59b	577.76±204.13b	141.95±50.52b	35.18±12.76b	21.96±5.83b	15.80±5.89b
26^{o}C	1481.09±235.91a	198.05±28.68a	1769.22±360.63a	545.16±113.14a	107.69±19.99a	75.79±12.79a	72.99±11.97a
30^{o}C	1284.81±243.87a	174.69±22.88a	1399.44±321.10ab	550.05±125.81a	106.40±22.53a	82.36±6.99a	62.77±5.54a
34^{o}C	1716.32±359.19a	224.53±43.08a	2076.24±440.78a	658.70±111.89a	145.11±27.89a	99.27±20.57a	74.69±17.41a
One - Way ANOVA (F-Statistic)							
	4.47*	6.04**	3.57*	4.74*	4.57*	6.67**	5.99**
2010							
Control	394.66±35.77d	136.63±13.54d	206.49±30.80c	2368.12±391.60b	345.36±44.88c	3000.82±527.40c	13.18±3.96b
26^{o}C	1201.41±160.18c	560.17±60.50c	458.87±70.18bc	7986.88±1159.63b	1038.91±182.98bc	14649.94±2819.92bc	67.16±7.61a
30^{o}C	2573.96±348.44a	1576.31±188.45a	807.88±125.18ab	31403.38±7415.34a	2780.05±705.09a	39426.00±6157.95a	91.38±16.32a
34^{o}C	1907.63±183.45b	1007.29±46.22b	842.95±198.44a	29549.92±9578.47a	1663.33±128.00ab	22238.56±4170.63b	76.19±8.10a
One - Way ANOVA (F-Statistic)							
	19.26***	36.68***	6.02**	5.91*	7.78**	14.68***	11.42***

Values presented are means ± SE, n = 4. *; **, *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, MSE = standard error of the mean. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 5.7: Effect of hydroponics solution water temperature on the uptake of micronutrients in shoots of *O. longibracteatum* grown in the greenhouse in 2009 and 2010.

Treatment	Fe	Cu	Zn	Mn	B
			(mg.plant^{-1})		
2009					
Control (10 - 15 ^{o}C)	0.57±0.23b	0.03±0.01b	0.33±0.13b	0.57±0.20b	0.34±0.16b
26^{o}C	2.66±0.58a	0.09±0.03a	1.31±0.30a	1.90±0.44a	1.12±0.29ab
30^{o}C	2.17±0.60ab	0.11±0.03a	1.48±0.41a	1.99±0.53a	1.33±0.35ab
34^{o}C	1.93±0.54ab	0.08±0.00a	1.45±0.26a	2.52±0.49a	2.04±0.47a
One - Way ANOVA (F-Statistic)					
	4.10*	3.33*	4.46*	3.67*	4.38*
2010					
Control (10 - 15 ^{o}C)	0.29±0.04b	0.01±0.00b	0.17±0.01c	0.32±0.01c	0.15±0.02b
26^{o}C	1.52±0.43b	0.06±0.02b	0.88±0.20bc	1.14±0.22bc	0.86±0.11b
30^{o}C	5.80±1.68a	0.17±0.03a	2.39±0.52a	3.36±0.45a	2.21±0.40a
34^{o}C	2.24±0.46b	0.08±0.03b	1.27±0.20b	1.82±0.29b	1.93±0.36a
One - Way ANOVA (F-Statistic)					
	6.97**	9.06**	9.67**	20.15***	12.08***

Values presented are means ± SE, n = 4. *; **, *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, MSE = standard error of the mean. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 5.8: Effect of hydroponics solution water temperature on the uptake of micronutrients in whole plant of *O. longibracteatum* grown in the greenhouse in 2009 and 2011.

Treatment	Fe	Cu	Zn	Mn	B
			(mg.plant^{-1})		
2009					
Control (10 - 15 ^{o}C)	1.71±0.24b	0.12±0.01c	0.55±0.14b	0.99±0.17b	0.42±0.17b
26^{o}C	12.41±1.44a	0.43±0.06a	2.74±0.47a	3.51±0.62a	1.42±0.31ab
30^{o}C	16.51±5.04a	0.39±0.03ab	2.98±0.23a	3.53±0.32a	1.64±0.31a
34^{o}C	9.75±2.85ab	0.26±0.07bc	2.38±0.43a	3.90±0.76a	2.28±0.50a
One - Way ANOVA (F-Statistic)					
	4.38*	9.13**	10.24**	6.53**	5.09*
2010					
Control (10 - 15 ^{o}C)	2.08±0.77c	0.17±0.07a	0.46±0.06c	1.15±0.28b	0.24±0.02b
26^{o}C	5.35±0.96bc	0.22±0.03a	1.63±0.17bc	2.13±0.15b	1.03±0.11b
30^{o}C	14.97±2.49a	0.51±0.13a	4.27±0.80a	5.37±0.70a	2.61±0.45a
34^{o}C	9.23±1.15b	0.33±0.07a	2.87±0.60ab	4.16±0.88a	2.34±0.30a
One - Way ANOVA (F-Statistic)					
	13.56***	3.45ns	10.32**	10.74**	16.09***

Values presented are means ± SE, n = 4. *; **, *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, MSE = standard error of the mean. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Chapter 6

6.0 Effects of temperature changes in hydroponic solution on growth and development of *Ornithogalum longibracteatum* L.

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S. Nxawe[1], P. A Ndakidemi[2,*] C.P. Laubscher[1].

[1]Faculty of Applied Science, Cape Peninsula University of Technology, Cape Town Campus, Keizersgracht, P.O. Box 652, Cape Town 8000, South Africa.

[2]The Nelson Mandela African Institute of Science and Technology, Arusha-Tanzania. P O Box 447, Arusha-Tanzania.

[*]Corresponding author

Email: ndakidemipa@gmail.com

Abstract

This experiment was conducted with the aim of establishing effects of regulating hydroponic solution temperatures on the growth and development of *O. longibracteatum* in the glasshouse during winter periods. The plants were exposed to four hydroponic solution temperatures (control (10 - 15°C), 26, 30 and 34°C). The treatments were arranged in a completely randomized design. After 10 weeks of experimentation, data showed that plant growth parameters such as number of bulbs per plant, bulb circumference, flower stalk length, flower length, and dry and fresh weights of root, bulb, shoot and flower were significantly increase by warming the hydroponic solution. Elevating the hydroponic solution temperature to a range of 26 - 30°C induced best growth and produced the highest dry matter yield in *O. longibracteatum* under glasshouse conditions. Elevated temperatures of 34°C resulted in diminished growth and yield.

Key words: bulb circumference, dry matter yield, flower length, root growth, shoot growth.

6.1 Introduction

Temperature has a significance influence on growth and yield development of plants (Midmore, 1988). As other environmental factors, temperature is known to affect many growth physiological processes at different developmental stages of the plant. Modifying temperature of growth medium at optimum levels may result into the maximum growth and yield of plants (Chung et al., 2006; Nxawe et al., 2009). This may improve root growth (Vogelezang, 1990), shoot (Gosselin and Trudel, 1984) and flowering patterns.

Recently, there has been growing interest of some pharmaceutical companies and certain researchers in traditional medicinal plants as a source of new commercial products including the pregnant onion (*O. longibracteatum*) (Verschaeve et al. 2004). This plant is bulbous plant which is widely used as a traditional medicine in rural areas of Eastern and Southern Africa for treating anti-inflammatory disorders (Mulholland et al., 2004; Koorbanally et al., 2006). The supply of this plant throughout the year is therefore very important. However, the production of *O. longibracteatum* during winter season in South Africa is limited by low temperatures. Therefore, manipulating the growing conditions in the greenhouse during winter period by modifying temperature of growth medium including that of hydroponic solution at optimum levels may result into the maximum growth and yield of the *O. longibracteatum*. Studies have shown that the modifications of temperature in the growth media during cold seasons affected plant growth and yield on greenhouse plants (Cooper, 1973; Nxawe et al 2010). It is well established that increasing root temperature accelerated the vegetative growth and root development. In their study, Pregitzer et al., (2000) reported that low temperature limited the enzyme root process and resulted into poor growth, nutrient uptake and respiration. Increasing root temperature accelerated the vegetative growth and root development. In another study by Dunlap (1986), warming of the soil at 21, 27 and 32°C with

muskmelons showed greater yield increase when root temperature was accelerated to its optimum level. Conversely, low temperature may limit or reduce crop yield which prevent vegetative growth (Seiler, 1998). Research evidence showed that at 6°C the growth of *Chrysanthemum morifolium* Ramat was significantly reduced resulting into poor rooting system and decreased fresh and dry weight (Mortensen, 1982). In another study, Gesch, (2007) documented that temperature below 0°C caused freezing injury to plants by developing sharp-edged ice crystals, which decreases cell size leading to plant physiological disorders associated with reduced growth. Other researchers (Jakobsen and Martens, 1994; Singh et al., 2008) found that at lower temperature (10°C), flower abortion occurred in different plant because pollen and ovule fertility were highly sensitive to cold temperature. In flower and pharmaceutical industries these effects on flower physiology can lead to drastic reduction in economic yield (Diepenbrock, 2000; Thakur et al., 2010). Therefore, it is anticipated that increasing the hydroponic water solution to its optimum temperature may enhance the glasshouse production of *O. longibracteatum* during winter periods through the modification of root zone temperature. This study was conducted with the objective of establishing effects of regulating temperatures in the hydroponic solution on the growth and development of *O. longibracteatum* in the glasshouse during winter periods.

6.2 Materials and methods

6.2.1 Site location and description

The experiment was conducted at the greenhouse of the Cape Peninsula University of Technology, Cape Town, South Africa from July 2009 and July 2010. The climate controlled greenhouse had temperatures ranging from 16 - 36°C during the days, and 10

- 18°C at night. The relative humidity of the glasshouse averaged 35%. There is a 40% Alunet shade cloth suspended 2 m above the ground of the glasshouse. The light intensities ranged from 030 lux to 600 lux, as measured by a Toptronic T630 light meter. Irrigation water was supplied from a Hager IP65 Water Filtration Plant de-ioniser, and had an average temperature of 16°C.

6.2.2 Supply of experimental materials

A steel table (2.5 m x 1 m) used as a flat surface, black plastic container (50 L), leca clay pebbles were supplied by Horticultural Department of Cape Peninsula University of Technology (CPUT), Cape Town, South Africa. Four (4) plastic gutters (2 m x 0.6 m), 4 pumps, 20 ml black plastic pipe, cable tie and 3 Dolphin aquarium heaters were purchased from Builders Warehouse (Maitland, Cape Town), South Africa. Bulbs of pregnant onion (*O. longibracteatum*) used as planting material were obtained from the CPUT Nursery and Hortical Ca $(NO_3)_2$ was obtained from Stark Ayres all in Cape Town, South Africa.

6.2.3 Experimental design

A completely randomized design, with four replicates, was conducted to study the effects of temperature on growth and development in *O. longibracteatum***.** Four white plastic gutters (2 m x 0.6 m) filled with leca clay pebbles were placed on a 2.5 m x 1 m steel table. Water was supplied to the leca pebbles through pumps projecting from 4 sets of black plastic containers (50 L) placed beneath the table. The water in each of the 3 tanks was heated by using Dolphin aquarium heaters to maintain the temperatures at 26, 30 and 34°C respectively. Unheated water supplied from the fourth container served as

control. Using the thermometer, the temperature ranged between 10 - 15°C (day/night) throughout the experiment period. *O. longibracteatum* bulbs were planted in each gutter (i.e. 10 bulbs per gutter) and supplied with nutrient solution (1 $mg.l^{-1}$ Hortical Ca $(NO_3)_2$) immediately after transplanting. Nutrient solution supplied from the pumps was re-circulated back to the black plastic container (50 l) through a 20 ml black plastic pipe. The plants were left to grow for the period of 10 weeks. To prevent concentration of nutrients in the clay pebbles due to evaporation, water was drained from the gutters and refreshed after every 2 weeks.

6.2.4 Collecting and analyzing data

After 10 weeks of transplanting, *O. longibracteatum* was determined by taking measurements of root mass, bulb mass, shoot mass, flower mass, bulb circumference, flower stalk length flower height (mm) and enumeration of leaf numbers at harvesting. Total root, bulb, shoot and flower mass (g) was determined by weighing. The bulb circumference was measured with a veneer calliper. Flower stalk length and flower length were measured with a ruler.

6.2.5 Statistical analysis

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

6.3 Results

6.3.1 Effect of hydroponics water temperature on yield in root mass (fresh weight) of *O. longibracteatum*

Table 6.1 shows the effect of four different temperature treatments on root mass of *O. longibracteatum*. Raising hydroponics' water temperature from 26 to 30°C significantly increased the root mass and decreases at 34°C compared with the control. Root mass significantly increased by 353, 790 and 180% in 2009 and by 273, 479 and 110% in 2010 by raising the temperature to 26, 30 and 34°C respectively relative to the control. In the heated system, maximum root mass was obtained at 30°C and was significantly decreased at 34°C in both years.

6.3.2 Effect of hydroponics water temperature on yield in bulb mass (fresh weight) of *O. longibracteatum*

The effect of hydroponic water temperature on bulb mass is shown in Table 6.1. The data showed that bulb mass of *O. longibracteatum* increased significantly when temperatures was raised from 26 to 34°C but the mass was significantly reduced at 34°C. For instance, increasing hydroponics' water solution temperature from 26, 30 and 34°C significantly increased the bulb mass by 273, 389 and 131% in 2009 and 268, 532 and 208% in 2010 compared with the control. From the heated treatments, the maximum bulb mass was obtained at 30°C and was significantly decreased at 34°C in both years.

6.3.3 Effect of hydroponics water temperature on yield in shoot mass (fresh weight) of *O. longibracteatum*

There was a significance effect on hydroponic water temperature on the shoot weights of *O. longibracteatum* (Table 6.1). Relative to the control treatment, results showed that increasing temperature to 26, 30 and 34°C significantly increased the shoot mass of *O. longibracteatum* by 1180, 1988, and 683% in 2009 and 215, 384 and 129% in 2010. Generally, in the heated treatments, shoot mass was significantly decreased when temperature was increase from 30 to 34°C. The data showed that best results for root mass were recorded at 30°C.

6.3.4 Effect of hydroponics water temperature on flower fresh weight of *O. longibracteatum*

There was a significance difference in flower mass of *O. longibracteatum* at different temperature treatments during 2009 and 2010. Flower mass was significantly increased by elevating hydroponic temperature to 26, 30 and 34°C compared with the untreated water or control. Relative to the control, increasing the hydroponics solution temperature from 26, 30°C and 34°C fresh flower weight increased by 479, 1091,316% in 2009 and 725, 981,532% in 2010 respectively. Generally, highest flower mass were recorded at elevated temperature of 30°C in both 2009 and 2010.

6.3.5 Effect of hydroponics water temperature on bulb circumference (mm), flower stalk length (mm) and flower length (mm) of *O. longibracteatum*

There was a significant increase in bulb circumference, flower stalk length and flower length when temperature was raised from 10^{o}C to 26, 30 and 34°C. Bulb circumference increased by 151, 238 and 87% in 2009 and by 41, 80 and 24% in 2010 respectively by increasing the temperature to 26, 30 and 34°C compared with the control. Similar trend was observed in flower stalk length. The flower length stalk increased significantly by 367, 481 and 54% in 2009 and in 2010 it increased by 417, 514 and 319% respectively. Flower stalk length was greater at 30°C but slightly increased at 34°C in both years relative to the control. As shown in Table 6.1, raising the hydroponic solution temperatures to 26 and 30°C significantly increased the flower length by 987% and 1560% but at 34°C it was decreases by -27% in 2009 compared with the control, while in 2010 raising temperatures to 26, 30 and 34°C resulted into increased flower length by 671, 1900 and 350% respectively.

6.3.6 Effect of hydroponics water temperature on number of bulbs of *O. longibracteatum*

Results (Table 6.1) showed that, relative to the control, increasing hydroponic water temperature to 26, 30 and 34°C significantly increased the number of bulbs. Hydroponics solution temperature of 30°C the number of bulbs was higher by 155%, followed by 77% at 26 °C and slightly increased by 25% in 2009 at 34°C compared with the control. In 2010, raising temperature to 34°C resulted into significant increases in number of bulblets by 21% compared with the control. However, greater increases in

number of bulbs of 99 and 216% occurred at the temperatures of 26 and 30°C respectively relative to the unheated control.

6.3.7 Effect of hydroponics water temperature on total dry weight (root, bulb, shoot and flower) of *O. longibracteatum*

Table 6.2 showed the effect of hydroponic water temperature on dry yield matter in roots, bulb, shoots and flowers of *O. longibracteatum*. Compared with the control, increasing the hydroponic temperatures to 26, 30 and 34°C significantly increased the dry yield matter yield of root, bulb, shoots and flowers. Dry root yields increased by 153, 408 and 70% in 2009 increased by 104, 236 and 104% by raising temperatures to 26, 30 and 34°C respectively. Similar trend was also observed in dry yield of bulbs. Raising temperature to 26, 30 and 34°C increased the bulb weight by 197,440 and 111% in 2009 and 215, 494 and 147% in 2010 respectively. Furthermore, increasing hydroponic water temperature influenced the dry matter yield of shoots by 376, 1026 and 182% in 2009 and by 393, 863 and 164% in 2010 relative to the control. Results also showed that elevating hydroponic solution temperatures to 26, 30 and 34°C significantly increased the dry matter yield in flowers by 963,1665 and 690% in 2009 and by 609,1100 and 500% in 2010 compared with the control . Generally, optimum dry matter yield was recorded at the elevated temperature of 30°C. However, when temperature was increased to 34°C dry matter yield in roots, bulbs, shoots and flowers started decreasing.

6.4 Discussion

The results from this study suggested that the optimum hydroponic solution temperature to grow *O. longibracteatum* under glasshouse conditions is 30°C. Most plant growth parameters such as number of bulbs per plant, bulb circumference, flower stalk length, flower length, and dry and fresh weights of root, bulb, shoot and flower were increased by warming the hydroponic solution. Relative to the control treatment, increasing the hydroponic temperatures to 26°C improved all plant growth parameters measured in this study to a certain degree. However, further increase to 30°C resulted into optimum plant growth in all parameters measured. Temperature above 30°C resulted into reduced growth and yield. In this study, the higher yields obtained with elevated hydroponic water temperature compared to the control suggests the great potential of this practice in inducing positive growth of *O. longibracteatum* and other related plants during cold winter season under the glasshouse conditions. As temperature is one of the factors which influence growth, heating of the hydroponic solution was important. It is evident that, elevated temperatures improved and accelerated chlorophyll production, net photosynthesis, and respiration rate in *O. longibracteatum* (Nxawe et al., 2011) which may have contributed to higher carbon fixation that was reflected in improving plant growth and the fresh and dry matter yield in different plant organs. Other researchers have proposed that increased growth at optimum temperature was due to optimization of various physiological process such as water (Kramer 1983) and nutrient (Engels, 1993) uptake.

On the other side, elevated temperatures of 34°C resulted in diminished chlorophyll synthesis and reduced photosynthesis, and respiration (Nxawe et al., 2011). This ultimately resulted in reduced growth and yield. Similar to this study, Xu and Huang

(2000), Huang et al., (2001), Lyons et al., (2007) reported reduced plant growth at elevated soil temperature above optimum in creeping bentgrass (*Agrostis palustris*) by reducing the fresh weight and number of roots per plant thus affecting the synthesis and transport of metabolites in the plant.

In conclusion, these results suggest that plant growth parameters such as number of bulbs per plant, bulb circumference, flower stalk length, flower length, dry and fresh weights of root, bulb, shoot and flower were increased by warming the hydroponic solution. Elevating the hydroponic solution temperature to a range of 26- 30°C induced best growth and produced the highest dry matter yield in *O. longibracteatum* under glasshouse conditions. Elevated temperatures of 34°C resulted in diminished growth and yield.

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Table 6.1:Effect of temperature on growth of *O. longibracteatum* (fresh mass) during 2009 and 2010.

Treatments	RM	BM	SM	FM	BC	FSL	FL	NoB
	g				mm			
Year 2009								
Control (10 - 15 ^{o}C)	28.7±2.4d	75.53±5.10d	54.84±8.73d	13.6±1.5d	24.0±0.6d	175.0±14.4d	37.5±4.3c	16.0±1.2d
26^{o}C	130.1±2.9b	281.84±5.23b	702.21±24.53b	78.7±6.3b	60.3±2.1b	817.5±114.8b	407.5±32.8b	28.3±1.8b
30^{o}C	255.3±13.9a	369.18±15.12a	1145.19±24.36a	162.0±14.4a	81.0±2.7a	1017.5±13.8a	622.5±47.9a	40.8±1.5a
34^{o}C	80.3±6.1c	174.77±9.32c	429.34±24.38c	56.6±6.7c	44.8±1.5c	270.3±27.0c	27.5±4.1d	20.0±2.0c
One - Way ANOVA (F-Statistic)								
	154.86***	176.71***	452.20***	52.96***	157.26***	47.31***	100.32***	44.28***
Year 2010								
Control (10 - 15 ^{o}C)	44.9±4.7d	56.3±15.5d	246.92±7.36d	7.9±0.9d	39.8±5.1d	167.5±4.3d	35.0±2.9d	14.5±1.5d
26^{o}C	167.5±5.8b	207.1±33.3b	777.55±32.77b	65.2±7.6b	56.3±4.5b	866.3±48.8b	270.0±37.2b	28.8±6.2b
30^{o}C	259.8±25.6a	355.6±13.9a	1195.99±80.70a	85.4±6.0a	71.8±2.7a	1029.0±17.8a	700.0±27.2a	45.8±4.4a
34^{o}C	94.3±13.3c	173.5±10.3c	565.16±20.70c	49.9±5.1c	49.3±4.9c	701.3±52.3c	157.5±20.3c	17.5±3.6c
One - Way ANOVA (F-Statistic)								
	39.48***	36.83***	78.55***	35.58***	9.31**	102.42***	131.82***	10.93***

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at **: $P \leq 0.01$; ***: $P \leq 0.01$. RM=Root mass, BM=Bulb mass, SM=Shoot mass, FM=Flower mass, BC=Bulb circumference, FSL=Flower stalk length, FL=Flower length, NoB=Number of bulblets

Table 6.2: Effect of temperature on growth of *O. longibracteatum* (dry mass) during 2009 and 2010.

Treatment	Root	Bulb	Shoot	Flower
		g		
2009				
Control (10 - 15 °C)	1.46±0.03d	1.77±0.16d	5.70±0.26d	0.49±0.02d
26°C	3.70±0.32b	5.26±0.45b	27.13±1.08b	5.21±0.28b
30°C	7.42±0.39a	9.55±0.21a	64.16±3.13a	8.65±0.36a
34°C	2.48±0.08c	3.74±0.42c	16.07±0.88c	3.87±0.24c
One - Way ANOVA (F-Statistic)				
	102.13**	96.07**	220.41**	173.73**
2010				
Control (10 - 15 °C)	1.63±0.09c	1.61±0.07d	5.13±0.44d	0.47±0.02d
26°C	3.33±0.22b	5.07±0.38b	25.29±1.64b	3.33±0.21b
30°C	5.48±0.34a	9.57±0.28a	49.41±2.30a	5.64±0.42a
34°C	3.33±0.25b	3.98±0.15c	13.56±0.99c	2.82±0.30c
One - Way ANOVA (F-Statistic)				
	41.56***	180.76**	161.62**	57.35***

Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at *$P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$.

Chapter 7

7.0 General Discussion and Conclusion

In South Africa, low temperatures may limit growth and production of certain crops during winter period. The cold environments may affect all stages of plant growth and specifically the chlorophyll production, photosynthesis, accumulation of plant metabolites such as flavonoids and anthocyanins, nutrient uptake and finally the dry matter yield of roots, shoots and flowers.

Results from this study conducted in the glasshouse in 2009 and verified in 2010 have shown that photosynthesis rate (A) and the gas exchange parameters [stomata conductance (gs), intercellular CO_2 concentration (Ci) and transpiration (E)] were significantly increased by elevating the hydroponic solution temperatures to 26-30°C compared with the control and then decreased significantly at 34°C. Furthermore, increasing hydroponics solution temperature from 26°C to 34°C significantly induced increased the levels of flavonoids and anthocyanins in roots, bulbs, shoots and flowers of *O. longibracteatum* in both years 2009 and 2010.

Warming of the hydroponic solution to 26, 30 and 34°C significantly increased the uptake of the following macronutrients and micronutrients (N, P, K, Ca, Mg, S, Na Fe, Cu Zn, Mn and B and Mo) in organs of *O. longibracteatum* (root, bulbs shoot, and whole plant) in 2009 and in 2010. The control treatments 10 - 15°C (day/night) had the lowest uptake of most nutrients.

Results from the two years study also showed that plant growth parameters such as number of bulbs per plant, bulb circumference, flower stalk length, flower length, and dry

and fresh weights of root, bulb, shoot and flower were significantly increase by warming the hydroponic solution. Elevating the hydroponic solution temperature to a range of 26-30°C induced best growth and produced the highest dry matter yield in *O. longibracteatum* under glasshouse conditions. Elevated temperatures of 34°C resulted in diminished growth and yield.

In conclusion, these findings suggest that controlled production of *O. longibracteatum* during winter seasons is possible by heating the hydroponic solution up to 30°C beyond which there was impaired plant growth in most parameters which were measured. More studies on other physiological growth characteristics of *O. longibracteatum* should be investigated to explore the required growth conditions during winter seasons in the controlled settings such as glasshouses.

Chapter 8

8.0 References

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