

# Use of Chlorophyll Meter for Nitrogen Management and Recommendation of Optimum Nitrogen Concentration in Soilless Culture of Lily

# Sasan Aliniaeifard<sup>1\*</sup>• Seid Jalal Tabatabaei<sup>2</sup>

Young Researchers Club of Science and Research Campus of Islamic Azad University, Iran
Faculty of Agriculture, Tabriz, university Box 51664, Tabriz, Iran
Corresponding author: \* Sasan13603@yahoo.com

## ABSTRACT

Nitrogen (N) is one of the most important essential elements that affects plant growth and has the highest concentration among mineral elements; a shortage of N causes more adverse effects than other mineral elements. To achieve optimum N concentration in nutrient solution through the use of a chlorophyll meter in the soilless culture of lily, four N concentrations (100, 200, 300, 400 mg  $L^{-1}$ ) were applied to lily (*Lilium longiflorum*) cv. 'Pink perfection'. Chlorophyll index and leaf number increased as N concentration increased. The increase in leaf number was gradual. Higher concentrations of N in nutrient solution induced an increase in shoot length. The chlorophyll meter is a simple way to monitor N concentration at different stages of lily growth.

Keywords: chlorophyll index, growth stage, leaf number, nutrient solution, shoot length

# INTRODUCTION

Nitrogen (N) is the fourth most abundant element in plant structures. Its constitutes less than 0.1% of the earth's crust but makes up about 80% of the atmosphere, mostly as  $N_2$  (Buchanan *et al.* 2000). N plays a pivotal role in the inorganic nutrition of plants and hence in determining growth (Mengel 1994; Marschner 1997). It is also an essential component of amino acids, nucleic acids, cofactors and other common metabolites (Buchanan *et al.* 2000) and is a major component of chlorophyll (Wang *et al.* 2004). The characteristic yellow color of N-starved plants (chlorosis) reflects their inability to synthesis adequate amounts of green chlorophyll (Chl) under N-limited conditions (Buchanan *et al.* 2000; Taiz and Ziger 2002). In addition, several plant hormones which are required for plant growth contain N or are derived from nitrogenous precursors (Buchanan *et al.* 2001).

Liquid fertilization is able to provide nutrition at appropriate levels for both root and shoot growth, but the root: shoot ratio may decrease with high fertilizer concentrations (Catanzaro *et al.* 1998). Greenhouse soilless systems for lily production have many advantages over soil production, including providing the ideal physical structure for moisture retention and aeration, avoidance of soil-borne pests and diseases, and superior control over plant nutrition. However, selection of the right media type and composition is vital to success in lily.

The SPAD-502 Chl meter is used for simple, rapid, and non destructive estimation of Chl contents in tomato leaves (Guimarães *et al.* 1999). It shows relative content of leaf Chl based on transmission of light from two different wavelengths (Aliniaeifard *et al.* 2007). The use of a Chl meter to evaluate plant N status in real time is suitable for precision agriculture and canopy greenness and might serve as a useful diagnostic tool to assess plant N demand (Wiesler *et al.* 2002).

The objective of this study was to evaluate the feasibility of using the SPAD-502 Chl meter for N management in lily plant under soilless greenhouse conditions. In addition, recommendations of optimum N concentration in nutrient solution for lily were determined.

## MATERIALS AND METHODS

#### Plant material and growth conditions

Bulbs of lily (*Lilium longiflorum*) cv. 'Pink perfection' produced in the Tabriz University greenhouse were cultured in pots (5 L) containing a mixture of perlite: sand (3: 1, v/v) for soilless culture. For better drainage, the bottoms of pots were notched and placed in saucers. At the beginning of experiment, bulbs were irrigated by water and after bulb germination half-strength Hoagland's solution (Hoagland and Arnon 1950) was used for daily nutrition of lily bulbs. The pots were kept in the greenhouse at 70-80% relative humidity and  $30 \pm 3$  and  $20 \pm 3^{\circ}$ C in the day and at night, respectively.

#### **Experiments and treatments**

Lily bulbs with the same size (average of 3 cm in diameter) were chosen for experiment. The experiment was carried out with 100, 200, 300, 400 mg  $l^{-1}$  N which was added to half-strength Hoagland's solution. Four different N solutions were prepared in 200-L containers and were used for daily nutrition of plants. The experiment was conducted based on a completely randomized block design and each treatment was replicates four times. Leaves were counted, and shoot length was measured 30, 60 and 80 (in the time of flowering) days after the culture of bulbs. The Chl index of plant leaves was measured using a Chl meter (SPAD-502, Tokyo, Minolta, Japan) at the same time intervals. The latter measurements were assessed on 10 leaves from each plant, at the same time, from 7:00 to 9:00 am, immediately after irrigation with nutrient solution.

#### Statistical analysis

Data was analyzed by analysis of variance (ANOVA) using SAS 8.2 software and treatment means were compared using the least significant difference (LSD) test at P < 0.05.



Fig. 1 Effects of different concentrations of N in nutrient solution on chlorophyll index 30 (A), 60 (B) and 80 (C) days after bulb culture in soilless culture of lily.

#### RESULTS

Chl index in lily leaves was significantly affected by N concentration in nutrient solution. The highest and lowest



Fig. 2 Correlation between N concentrations in nutrient solution and chlorophyll index in soilless culture of lily.



Fig. 3 Relationship between chlorophyll index and time after bulb culture in soilless culture of lily.



Fig. 4 Effects of different concentrations of N in nutrient solution on leaf number 30 (A), 60 (B) and 80 (C) days after bulb culture in soilless culture of lily.

Chl indices were obtained in 400 and 100 mg  $l^{-1}$  N in nutrient solution, respectively (**Fig. 1**). As depicted in **Fig. 1A-C** the Chl index was induced as N concentration increased in nutrient solution at 30, 60 and 80 days after bulb culture, respectively. A significant correlation between N concentration in nutrient solution and Chl index is depicted in **Fig. 2**. Chl index increased from 30 to 80 days after bulb culture (**Fig. 3**).

Most and fewest leaves were observed in 400 and 100 mg  $l^{-1}$  N in nutrient solution, respectively (**Fig. 4A-C**). For leaf number, there were significant differences between 100 and 200 mg  $l^{-1}$  N with 300 and 400 mg  $l^{-1}$  N in nutrient solution (**Fig. 4A-C**). There was a significant correlation



Fig. 5 Correlation between N concentrations in nutrient solution and leaf number in soilless culture of lily.



Fig. 6 Relationship between leaf number and time after bulb culture in soilless culture of lily.

between N concentration in nutrient solution and leaf number (**Fig. 5**). The number of leaves significantly increased from the first to the second month after bulb culture while at the time of flowering (80 days after bulb culture) leaf number increased slowly (**Fig. 6**). The number of leaves at the time of flowering increased 37 and 7% more than at 30 and 60 days after bulb culture, respectively.

Shoot length was significantly affected by different N concentrations in nutrient solution. Minimum shoot length was observed in 100 mg  $I^{-1}$  N, although, in the case of shoot length, there were no differences between 200, 300 and 400 mg  $I^{-1}$  N in nutrient solution (**Fig. 7A-C**). The correlation between different N concentrations in nutrient solution and shoot length is depicted in **Fig. 8**. The shoot length increased from the first to the second month after bulb culture, whereas at the time of flowering (80 days after bulb culture) shoot growth increased very slowly (**Fig. 9**). At the time of flowering, the length of lily shoots induced 59 and 6% at 30 and 60 days after bulb culture, respectively.



Fig. 8 Trend between N concentrations in nutrient solution and shoot length in soilless culture of lily.



Fig. 7 Effects of different concentrations of N in nutrient solution on shoot length 30 (A), 60 (B) and 80 (C) days after bulb culture in soilless culture of lily.

#### DISCUSSION

In this study, a higher N concentration induced the Chl index, leaf number and shoot length in lily. Canopy biophysical parameters such as N content, above-ground biomass, green leaf area index, net ecosystem  $CO_2$  exchange (Gitelson *et al.* 2006), absorbed photosynthetic active radiation (Vina and Gitelson 2005) and yield (Walters 2003) have been related to canopy Chl content. Chl content has been suggested as the community property most directly relevant



Fig. 9 Relationship between shoot length and time after bulb culture in soilless culture of lily.

to the prediction of productivity (Dawson et al. 2003). Hawkins et al. (2009) proved that SPAD-502 is an effective tool for non-destructive estimation of total foliar Chl content across a range of plant ages, growing conditions, and genotypes for Lindera melissifolia populations and the ecologically widespread congener L. benzoin. Ku and Hershey (1996) fertigated poinsettias (Euphorbia pulcherrima) with 0, 100, 200 and 300 mg l<sup>-1</sup> N and subjected each of these treatments to differing leaching fractions (LF): 0, 0.2, and 0.4. The poinsettias fertigated at 200 and 300 mg  $l^{-1}$  N were darker green than those fertigated at 100 mg  $l^{-1}$  N. N accounted for major variations in tomato (Lycopersicon esculentum) transplant height, leaf number and area, and stem diameter, as N concentration increased, both root and shoot growth increased (Melton and Dufault 1991). Ciganda et al. (2009) found that non-destructive measurements of a single leaf can be used to accurately estimate total Chl content in a maize canopy. Van Iersel et al. (1998) compared growth responses of impatiens and petunia, the shoot dry weight of petunia increased in a linear manner with increasing N rates, the shoot dry weight of the impatiens responded in a manner similar to lily in our experiment and peaked at 336 mg l N, the next highest N rate. Thomas et al. (1998), working with container-grown freesias (Freesia x hybrida), found that fertilization with N increased foliage production, particularly leaf number, plant height and fresh weight. Melton and Dufault (1991) studied fertilizer impacts on tomato (Lycopersicon esculentum) transplant growth. They found that shoot dry weights increased in a linear manner with increasing N rates. Huang et al. (2008) suggested that the planting of a variety insensitive to high N input such as LYP9 would lead to over-application of fertilizer-N by rice farmers if knowledge-intensive N management technology were not used. Campos and Reed (1993) reported enhanced growth of Spathiphyllum and Dieffenbachia after small increases in nutrient concentration. Our results were slightly different from Whipker et al. (1999), who studied the effect of fertilizer concentration on the growth of double impatiens (Impatiens walleriana). They found that both impatiens cultivars examined had a larger diameter after being fertilized for 9 weeks with 100 mg l<sup>-1</sup> N in comparison with those plants fertilized with 50 or  $200 \text{ mg l}^{-1} \text{ N}$ .

Chl meters have been used to make N fertilizer recommendations in wheat to improve yield (Denuit et al. 2002) and quality (Lopez-Bellido et al. 2004). It seems possible to find better relationships between the readings of Chl meters in advanced crop stages and the grain N concentration at maturity (Lopez-Bellido et al. 2004). Approaches based on N contents in leaves have been used to increase N fertilizer use efficiency. An N management program can be attained by suitable evaluation of the plant's N status (Coltman 1988; Smith and Loneragan 1997) which is usually accomplished by quantitative analysis of the N concentration in the plant dry matter. Alternatively, quick procedures had been proposed such as the leaf greenness determination by a hand-held device - Minolta SPAD-502 meter (Guimarães et al. 1999; Sandoval-Villa et al. 1999). As several authors have shown a relationship between Chl and N content in plant leaves exists (Scheepers et al. 1992; Sexton and Carol 2002; Wang et al. 2004); thus, Chl content can be used as an alternative measure of plant N status (Fontes 2001).

#### CONCLUSIONS

We found a significant relationship between N concentration in soilless culture of lily and growth parameters, chlorophyll index and time. Meanwhile, a Chl meter can be utilized for N management at different periods of lily growth.

#### ACKNOWLEDGEMENT

The authors appreciate the technical assistance of Mrs. M. Yousefi.

#### REFERENCES

- Aliniaeifard S, Tabatabaei SJ, Hajilou J, Chaparzadeh N (2007) Effects of some antistress agents on tolerance of olive to salinity stress. In: *Fifth Iranian Congress of Horticulture*, 3-6 Sept. 2007, Shiraz. Iran 517 pp
- Buchanan BB, Gruissem W, Jones I (2000) Biochemistry and Molecular Biology of Plants, American Society of Plant Physiologists, Rockville, Maryland, USA, 1160 pp
- Campos R, Reed DW (1993) Determination of constant-feed liquid fertilization rates for Spathiphyllum 'Petite' and Dieffenbachia 'Camille'. Journal of Environmental Horticulture 11, 22-24
- Catanzaro CJ, Williams KA, Sauve RJ (1998) Slow release versus water soluble fertilization affects nutrient leaching and growth of potted chrysanthemum. *Journal of Plant Nutrition* 21, 1025-1036
- Ciganda V, Gitelson A, Schepers J (2009) Non-destructive determination of maize leaf and canopy chlorophyll content. *Journal of Plant Physiology* 166, 157-167
- Coltman RR (1988) Yield of greenhouse tomatoes managed to maintain specific petiole sap nitrate. *HortScience* 23, 148-151
- Dawson TP, North PRJ, Plummer SE, Curran PJ (2003) Forest ecosystem chlorophyll content: implications for remotely sensed estimates of net primary productivity. *International Journal of Remote Sense* 24, 611-617
- Denuit JP, Olivier M, Goffaux MJ, Herman JL, Goffart JP, Destain JP, Frankinet M (2002) Management of nitrogen fertilization of winter wheat and potato crops using the chlorophyll meter for crop nitrogen status assessment. Agronomie 22, 847-853
- Fontes PCR (2001) Diagnóstico do Estado Nutricional das Plantas, UFV, Viçosa, 122 pp
- Gitelson AA, Vina A, Verma SB, Rundquist DC, Arkebauer TJ, Keydan G (2006) Relationship between gross primary production and chlorophyll content in crops: implications for the synoptic monitoring of vegetation productivity. *Journal of Geophysics Research* **111**, D08S11
- Guimarães TG, Fontes PCR, Pereira PRG, Alvarez VHV, Monnerat PH (1999) Teores de clorofila determinados por medidor portátil e sua relação com formas de nitrogênio em folhas de tomateiro cultivados em dois tipos de solo. *Bragantia* 58, 209-216
- Hawkins TS, Gardiner ES, Comer GS (2009) Modeling the relationship between extractable chlorophyll and SPAD-502 readings for endangered plant species research. *Journal for Nature Conservation* 17, 123-127
- Hoagland DR, Arnon DS (1950) The water culture method for growing plants without soil. California Agricultural Experimental Station Circulation 374, 1-2
- Huang J, He F, Cui K, Buresh RJ, Xu B, Gong W, Peng SH (2008) Determination of optimal nitrogen rate for rice varieties using a chlorophyll meter. *Field Crops Research* 105, 70-80
- Ku CSM, Hershey DR (1996) Fertigation rate, leaching fraction, and growth of potted poinsettia. *Journal of Plant Nutrition* 19, 1639-1652
- Lopez-Bellido RJ, Shepherd CE, Barraclough PB (2004) Predicting postanthesis N requirements of bread wheat with a Minolta SPAD meter. *European Journal of Agronomy* 20, 313-320
- Marschner H (1997) Mineral Nutrition of Higher Plants (2<sup>nd</sup> Edn), Academic Press, London, 532 pp
- Melton RR, Dufault RJ (1991) Nitrogen, phosphorus, and potassium fertility regimes affect tomato transplant growth. *HortScience* 26, 141-142
- Mengel K (1994) Fe availability in plant tissues-Fe chlorosis on calcareous soils. Plant and Soil 165, 275-283
- Sandoval-Villa M, Wood CW, Guertal EA (1999) Ammonium concentration in solution affects chlorophyll meter readings in tomato leaves. *Journal of Plant Nutrition* 22, 1717-1729
- Scheepers JS, Francis DD, Vigil M, Below FE (1992) Comparison of corn leaf-nitrogen concentration and chlorophyll meter readings. *Communications in Soil Science and Plant Analysis* 23, 2173-2187
- Sexton P, Carrol J (2002) Comparison of SPAD chlorophyll meter readings vs. petiole nitrate concentration in sugarbeet. *Journal of Plant Nutrition* 25, 1975-1986
- Smith FW, Loneragan JE (1997) Interpretation of plant analysis: concepts and principles. In: Reuter DJ, Robinson JB (Eds) *Plant Analysis - An Interpretation Manual*, CSIRO Publishing, Collingwood, 33 pp
- Taiz L, Zeiger E (2002) Plant Physiology, Sinauer Associates Inc., Sunderland, MA, 623 pp
- Thomas M, Matheson S, Spurway M (1998) Nutrition of container-grown freesias. *Journal of Plant Nutrition* **21**, 2485-2496
- van Iersel MW, Thomas PA, Beverly RB, Latimer JG, Mills HA (1998) Nutrition affects pre- and posttransplant growth of impatiens and petunia plugs. *HortScience* **33**, 1014-1018
- Vina A, Gitelson AA (2005) New developments in the remote estimation of the fraction of absorbed photosynthetically active radiation in crops. *Geophysics Research Letters* 32, L17403
- Walters DT (2003) Diagnosis of nitrogen deficiency in maize and the influence of hybrid and plant density. In: North Central Extension-Industry Soil Fertility Conference, Des Moines, IA, Vol 19, pp 19-26
- Wang Q, Chen J, Li Y (2004) Nondestructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. *Jour-*

nal of Plant Nutrition 27, 557-569

Whipker BE, Dasoju S, Dosmann MS, Iles JK (1999) Effect of fertilizer concentration on growth of double impatiens. *HortTechnology* 9, 425-428

Wiesler F, Bauer M, Kamh M, Engels T, Reusch S (2002) The crop as indicator for sidedress nitrogen demand in sugar beet production – limitations and perspectives. *Journal of Plant Nutrition and Soil Science* **165**, 93-99