International Journal of Plant & Soil Science
13(1): 1-9, 2016; Article no.IJPSS.28999
ISSN: 2320-7035

SCIENCEDOMAIN ***international***
www.sciencedomain.org

Pre Harvest Calcium Treatment under Selected Poly Films Improves Leaf Chlorophyll Content in Rose Cut Flower

G. Oloo-Abucheli[1,2*], J. N. Aguyoh[3] and G. Liu[2]

[1]Department of Plant Sciences, Chuka University, P.O.Box 109-60400, Chuka, Kenya.
[2]Department of Crops, Horticulture and Soil Sciences, Egerton University, P.O.Box 536, Egerton, Kenya.
[3]Department of Agriculture and Environmental Studies, Rongo University College, P.O.Box 1023-40404, Rongo, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Author GOA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JNA managed the literature searches, analyses of the study performed the spectroscopy analysis and author GL managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2016/28999
Editor(s):
(1) Genlou Sun, Professor, Biology Department, Saint Mary's University, 923 Robie Street, Halifax, Nova Scotia, B3H 3C3, Canada.
Reviewers:
(1) Ilham Zahir, University Sidi Mohamed Ben Abdellah, Morocco.
(2) Isaac Kojo Arah, Ho Polytechnic, Ghana.
(3) Rafael Julio Macedo Barragán, Universidad de Colima, Mexico.
Complete Peer review History: http://www.sciencedomain.org/review-history/16402

Original Research Article

Received 17th August 2016
Accepted 16th September 2016
Published 30th September 2016

ABSTRACT

Quality is a key attribute in marketing of cut flowers. A number of factors may determine quality in cut flowers, among them being the pre-harvest conditions under which the plant was grown. This experiment was designed to investigate the effect of light transmission through selected poly films, rose cultivar and calcium foliar feed on leaf chlorophyll content. The study site was at an elevation of 2238 m above sea level with average maximum and minimum temperature ranges from 19℃ to 22℃ and 5℃ to 8℃ respectively. The area receives a total annual rainfall ranging from 1200 to 1400 mm. The experimental design was split split plot laid down in a Randomized Complete Block Design (RCBD) with poly films forming the main plot treatments. Data collection involved use of Watch dog data logger mini weather station to determine Photosynthetically active radiation among

**Corresponding author: E-mail: opetugrace@yahoo.co.uk;*

other spectrum properties. Light transmission was evaluated using UV-1800 Shimadzu spectrophotometer. It was observed that leaf chlorophyll content increased with increase in calcium concentration in the foliar feed. Interactive effect was observed between the calcium foliar feeds and the poly film covers on stem firmness. The obtained results were consistent in both trials however the stem firmness was varied from one flush to the other. Effect of temperature caused by varying light intensity on chlorophyll content is also discussed. The results obtained show impact of environmental and nutritional factors on rose cut flower quality.

Keywords: Calcium; temperature; rose cultivar; chlorophyll content.

1. INTRODUCTION

Calcium plays a crucial role as a regulator of growth and development in plants [1,2] however, its movement through the phloem vessels is very limited [3]. It is known to strengthen the plant cell wall [4,5] and improve plant structural strength where it accumulates as calcium pectate and binds the cells together. In addition the mechanical strength of the stems is highly correlated with the content of the secondary cell wall components such as cellulose, hemi-cellulose and lignin [6]. The mode of application of calcium as a fertilizer determines its availability in the plants, together with the prevailing weather conditions.

Generally, the soil may have sufficient quantities of calcium yet the plant shows deficiency mainly in young tissues with new growth [3] a special case implicated to poor translocation of this nutrient. Different ways have been tested to mitigate the problem and has been shown that application of foliar fertilizer significantly affect plant growth and quality [7,8]. Fertilizer utilization is 10 times efficient when applied as a foliar feed compared to similar a mounts applied directly to the soil [9,10]. Foliar application increases availability of nutrients to the plant and stimulates biological activities resulting to positive changes in plant growth and development [11]. Plant nutrient availability is influenced by the inherent characteristics for example different plant cultivars have different demand for calcium in their developmental processes [12] making it difficult to establish general optimal levels of calcium in plants. Furthermore, the environmental and edaphic factors play a crucial role, for example deviation of soil temperature from normal and soil pH from the neutral, affects availability of nutrients [13].

Rose cut flower is mainly a greenhouse enterprise and the use of coloured poly films has very profound influence on spectrum properties, which affect light transmission in turn influencing plant growth and development. Light transmission through such poly films depends on the concentration of the cladding and other additives during the treatment process. Concentration of the dye for example increases far red light absorption of the poly film [14,15]. These differences in light transmission bring about variations in greenhouse microclimate mainly temperature and relative humidity which impacts on nutrient absorption and general plant morphogenesis.

Leaf chlorophyll content is influenced by a number of factors mainly temperature, light and cultivar which is inherent. The effect of these environmental factors on pigment levels may occur either singly or synergistically [16]. Anthocyanin concentration increases under low and decreases at elevated temperatures, implying that temperature affects plant pigmentation [17]. The influence of air temperature on carotenoid concentration in spinach was studied and reported to decrease with an increase in air temperature [18]. Low temperature (20℃) resulted in significantly higher anthocyanins than higher temperature of 30℃ [19]. Chlorophyll one of the main plant anthocyanins is equally affected by temperature. Therefore, the objective of this study was to evaluate the effect of selected poly films and pre harvest calcium application on leaf chlorophyll content of rose flower cultivars.

2. MATERIALS AND METHODS

2.1 Planting Material, Experimental Design and Treatment Application

Top grafted plants of two rose cultivars were purchased from the commercial propagator (Stokman Rozen limited) in Naivasha. The research was carried out under a split- split plot experiment laid down in a completely randomized block design. The main treatment involved poly film covers with different colours denoted as; G_0 = UV-A clear (control), G_1= IR

504 (green tint) and G_2 = UV- A 205/N (Yellow tint) of similar gauge 200 microns. The greenhouse was divided into three sections of $44M^2$. Each section was covered with a different poly film cover as described above and replicated three times. The sub-plot treatment included two rose cultivars; Red calypso and Furiosa. Calcium was applied to the sub-sub plots and the application rate was based on the manufacturer's specification (2.5 ml/L) as shown: the blank T_0 = Distilled water, T_1 = CalMax® 1.25 ml/L (half the recommended rate),, T_2 = CalMax® 2.5 ml/L (recommended rate), T_3 = CalMax® at the rate of 3.75 ml/L (50% higher) and T_4= CalMax® at the rate of 5.00 ml/L (double the recommended rate).

Soil treatment was done following land preparation using Sodium methylaminomethanedithioate at the rate of 0.12 ml/m^2 (The application rates as per the product specification) was applied through drip lines and plots were left undisturbed for 21 days. Plots were aerated after three weeks and germination test carried out to a certain depletion of chemical residues before planting. Planting was done 14 days later after achieving 95% germination in all main plot treatments. The plants were grown in double rows spaced at 30 cm x 20 cm to accommodate 10 plants per square meter. Management activities involved hosing, fertigation, bending, weeding, de-suckering and general plant cleaning.

2.2 Data Collection and Analysis

The transmission and absorbance properties of the selected poly film covers were evaluated in the laboratory. Measurements were carried out on samples taken before and after the installation of the poly films on the greenhouse structure. A strip of the poly film was cut from the extra ends of greenhouse. The strip was carefully cut in a rectangular shape and locked in a jig before insertion in the cuvette holder to ensure an upright position perpendicular to the source of light. Care was taken to ensure that the light beam from the spectrophotometer entered through the outer surface of the poly film and left through the inner surface. Transmission of active radiation was evaluated using UV-1800 Shimadzu spectrophotometer.

Non destructive measurement of foliar chlorophyll was done using Chlorophyll content meter (CCM- 200 plus; Opti-Sciences, Tyngsboro, MA). Where by 3 readings were taken from the 3^{rd}, 4^{th} and 5^{th} developed leaves. Mean value were determined and recorded. The collected data was subjected to analysis of variance (ANOVA) using JMP and SAS statistical package (SAS Inst., Inc., Cary, NC) at P ≤ 5%. Where there were treatment differences, mean separation was done using Tukey's procedure.

Split-split plot mathematical model was fitted for analysis as shown below:

$$X_{ijk} = \mu\ldots + B_i + G_{j\,+}(BG)_{ij} + C_{k\,+}GC_{jk} + (GC)_{jk} + T_l + GT_{jl} + CT_{kl} + GCT_{jkl} + (GCT)_{jkl} + e_{ijkl}$$

Where;

Xijk	= Response of rose plants
μ	= the overall experiment mean
G_i	= the main plot treatment effect
B_j	= the block effect
$(BG)_{ij}$	= the main plot error (error a)
C_k	= the subplot treatment effect
GC_{ik}	= Interaction effect of the j^{th} poly film type and the k^{th} rose cultivar effect
$(GC)_{jk}$	= the sub-plot error (error b)
T_l	= calcium foliar feed treatment effect
$(GT)_{jl}$	= the treatment interaction effect of poly flim and calcium foliar feed
(CT)kl	= the treatment interaction effect of varieties and calcium foliar feed
(GCT)ikl	= the treatment interaction effect of j^{th} poly film, the k^{th} rose varieties and l^{th} calcium foliar feed
eijk	= the sub subplot error (error c)
i, k, l	= a particular treatment
j	= a particular block

3. RESULTS AND DISCUSSION

3.1 Micro Climate under the Greenhouse Poly Film Covers

Poly film type had significant effect on the transmission of photosynthetically active radiation (PAR). There was significantly high transmission under the UV-A clear poly film compared to UV-A 205/N and IR504. Generally, light transmission through the different poly films increased with increase in wavelength. At low wavelength of 400 nm, the transmission under UV-A clear poly film was 35% compared to 54.8% recorded at 680 nm. Similarly for poly films UV-A 205/N and IR 504 7.9% and 8.1% light transmission was recorded at 400 nm, while at 680 nm the percent light transmission was 33% and 31% respectively (Fig. 1). Mean temperature values of 41℃, 35.2℃ and 32.8℃ were recorded at 1200 hrs of the day and 12.3℃, 13.1℃ and 12.1℃ at 1600 hrs under UV-A clear, IR-504 and UV-A205/N covers. During the day UV-A clear recorded the highest temperature compared to UV-A 205/N, while the mean night temperatures between the two poly films were not significantly different. Although, IR504 poly film recorded the lowest mean day temperature it had the highest mean night temperature (13.1℃) of 0.8℃ and 1.0℃ greater than UV-A clear and UV-A 205/N respectively.

3.2 Leaf Chlorophyll Content

Generally, high temperature under UV-A clear as characterized by high transmittance impacted negatively on the total leaf chlorophyll content (Fig. 2). Chlorophyll content was significantly high under IR504 poly film cover compared to UV-A 205/N and UV-A clear. High temperature has been reported in other studies to impact on plant growth and development. According to Hutin et al. [20] they observed that when the photosynthetic complexes absorb excess light, reactive oxygen species were generated in the chloroplasts causing damage in the systems of photosynthetic pigments. Comparison made on the effect of chlorophyll by temperature and nutrition in another study showed that chlorophyll content especially chlorophyll a is affected more by variations in season compared to the strength of the nutrient solution [21] implying that the effect of temperature on chlorophyll supersedes plant nutrition at any given time. It is possible that the plants developed mitigation strategies to protect the destruction of the photosynthetic apparatus under this high temperature. Several changes occur within the plant cell under high temperature conditions, among them being the closure of the stomata. Once the stomata close the photosynthetic activities are affected and assimilation of CO_2 is also affected hence reduction in the chlorophyll content among other physiological processes.

Chlorophyll content was also observed to vary with the prevailing weather condition, it was high in trial II compared to trial I. Generally, the overall mean day temperature was low in trial II compared to trial I, showing the relationship between temperature and leaf chlorophyll. A study done by Gilmore and Marilyn [22] to establish which type of chlorophyll is sensitive to which type of environmental conditions established that Chl-b was lower than Chl-a at low temperature indicating the sensitivity of Chl-b to environmental changes. The effect of greenhouse microclimate on Chl-a and Chl-b may vary in magnitude depending on the environmental factor that dominates. Clear observation noted here is that chlorophyll was more affected by high solar radiation as opposed to temperature alone. This observation is in agreement with Lopez et al. [23] who observed low chlorophyll content in *Amaranthus hypochondriacus* leaves under full sunlight. Chlorophyll reduction under full sunlight in this particular study was thought to be a mechanism to reduce photo-induced damage.

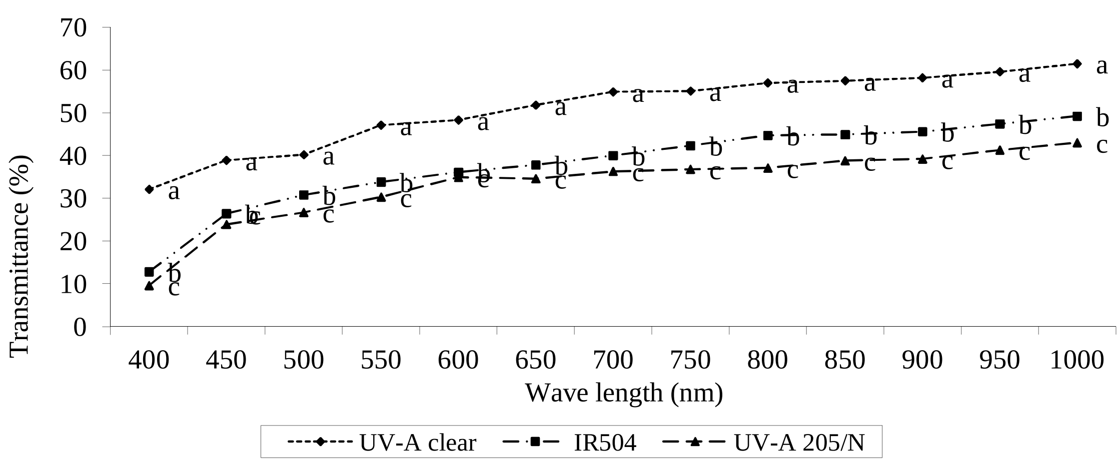

Fig. 1. Effect of selected poly films on light transmission in the PAR and infra red wavelength regions before installation

Where a, b and c denotes mean separation with a being significantly higher than b and c

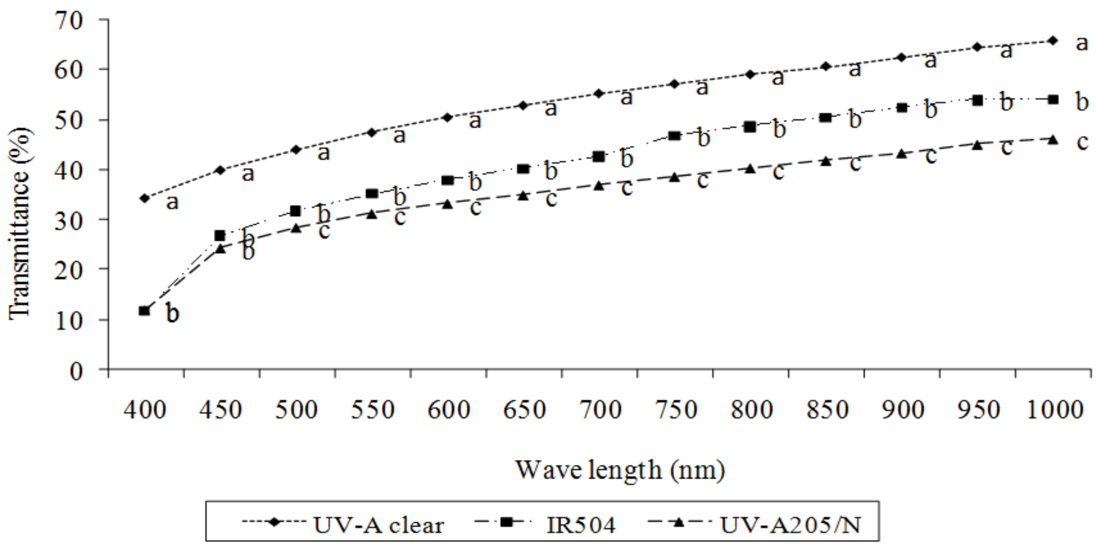

Fig. 2. Effect of selected poly films on light transmission in the PAR and infra red wavelength region after installation

Values presented are averages over 24 months period. Where a, b and c denotes mean separation with a being significantly higher than b and c

The amount of solar radiation absorbed by a leaf is basically a function of the foliar concentration of photosynthetic pigments [24] which may differ from one plant species to the other. Chlorophyll content determines the photosynthetic potential of the plant and can drastically affect crop yield. Different authors present varying arguments about the effect of temperature on leaf chlorophyll, with most findings describing that chlorophyll increases with increase in temperature as observed in tomatoes Gupta et al. [25] and grasses [22]. Leaf chlorophyll content in the current study was observed to be low under the control poly film cover that exhibited very high solar radiation and temperature. This could be attributed to the mechanism to survive the excess temperature by reducing the capabilities of leaf absorbance of solar radiation.

3.3 Calcium Foliar Feed and Leaf Chlorophyll Content

Chlorophyll content was significantly affected by the application of calcium foliar feed. Chlorophyll content under the experimental plots treated with 2.5 ml/L, 3.75 ml/L and 5 ml/L of calcium foliar feed was statistically high compared to 1.25 ml/L and the control (Fig. 3). The results of trial II were inconsistent to trial I, leaf chlorophyll content was high under T4 which was statistically similar to T3. In trial II unlike trial I calcium foliar feed level T4 was higher than T2, T1 and the control experiment (T0). Leaf chlorophyll content under T1 treatment was significantly higher than the T0 but lower than the preceding treatments with higher calcium concentration in both trials I and II.

Low and high calcium concentration have been reported to influence plant growth negatively [7]. In addition according to Gupta et al. [25] low and high calcium concentrations were observed to decrease plant biomass production and fruit yield of bitter gourd. According to this study of Gupta et al. [25] they established that calcium concentration influenced chlorophyll a and chlorophyll b separately. They also established that Chl a and Chl b were high (1.67 and 0.81 mg g-1 fresh weight) at 4.0 mM of calcium than 8.0 mM (1.19 and 0.497 mg g-1 fresh weight) respectively. This observation is in consonance with the results of the current study where higher calcium concentration denoted as T3 and T4 had no significant effect on chlorophyll concentration compared to T2. However, lower concentration of T1 and the control had significantly lower chlorophyll content (48.67 and 45.31 respectively). In another study to evaluate the effect of season verses nitrogen nutrition on pigment dynamics in tomato it was observed that chlorophylls were affected more by the season and less by the strength of the nutrient solution [21]. The role of calcium in cell division and expansion in addition to its role in gaseous exchange through regulation of stomata could be implicated in this study. Consequently, where calcium concentration was low, leaf chlorophyll content was also observed to be low.

Combined treatment of red calypso and calcium foliar feed at an application rate of 3.75 ml/L had the highest leaf chlorophyll content. Low chlorophyll content (40.18f) was recorded under the cultivar Furiosa with the control experiment that had no calcium compared to Red calypso under similar treatment (Table 1). There was significant variation in the chlorophyll content indicating plant difference in response to growth conditions. The difference in chlorophyll content observed amongst the different cultivars is due to genetic variability in plants. This finding supports Daymond and Hardley, [26] who observed differences in chlorophyll content among different cacao clones. This observation could in part be attributed to the potential of different cultivars to acclimate to greenhouse growth factors at specific levels, with every cultivar having its own optimal level.

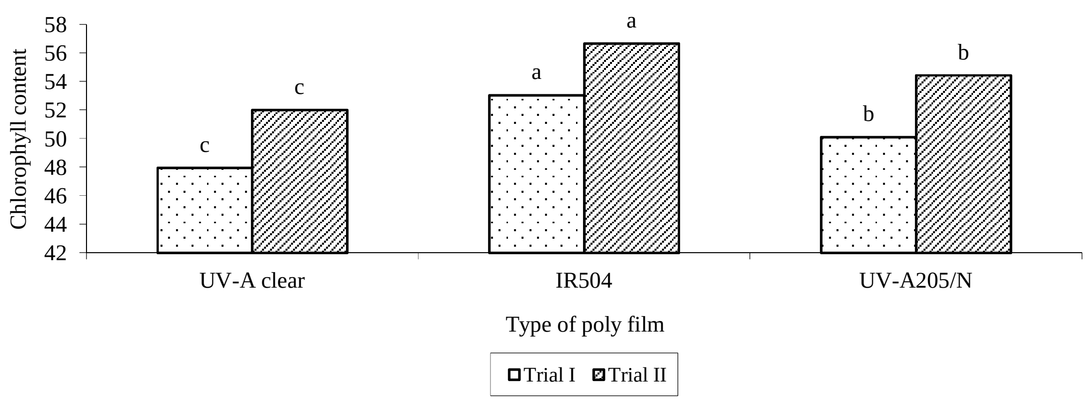

Fig. 3. Effect of greenhouse cover on the rose leaf chlorophyll content (trial I&II)
Where a, b and c denotes mean separation with a being significantly higher than b and c

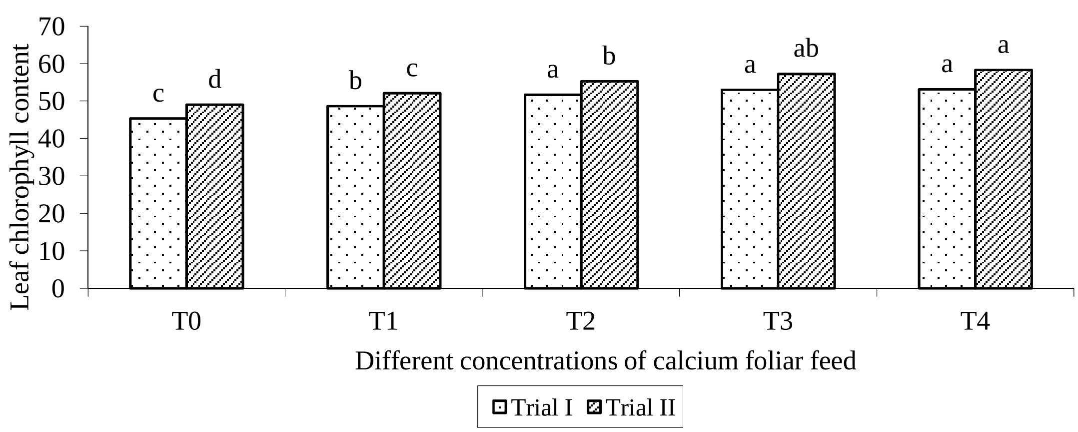

Fig. 4. Effect of calcium levels on leaf chlorophyll content (Trial 1 & 2)
Where T0 is a control experiment and T1, T2, T3 and T4 are calcium treatments at the rate of 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5 ml/L respectively

Table 1. Effect of calcium foliar feed and rose cultivar interaction on leaf chlorophyll content

Calcium/Variety	Trial 1				
	T0	**T1**	**T2**	**T3**	**T4**
C1	49.81cd[1]	51.79bc	55.89a	56.23a	54.43ab
C2	40.81f	45.56e	47.51de	50.12cd	51.57bc
	Trial 2				
C1	52.52 de[1]	54.59bcd	57.26ab	60.59a	57.89ab
C2	45.39f	49.56f	53.32cd	55.96bcd	56.6bc

[1]Means followed by the same letter(s) along the column for different calcium foliar feed levels by cultivars are not significantly different at 5% level of significance according to Tukey's Studentized Range (HSD) Test. Where T0 is a control experiment and T1, T2, T3 and T4 are calcium treatments at the rate of 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5 ml/L respectively. C1 and C2 are rose cultivars Red calypso and Furiosa

Table 2. Effect of cover and calcium interaction on the leaf chlorophyll content

Cover and calcium	Trial I			Trial II		
	UV-A clear	**IR504**	**UV-205/N**	**UV-A clear**	**IR504**	**UV-205/N**
0	41.68f[1]	48.38de	45.87ef	46.2f[1]	51.62de	49.05ef
T1	45.92ef	52.00abcd	48.13de	49.88ef	55.43abcd	50.90def
T2	49.62cde	54.90ab	50.58bcd	52.08cde	58.97ab	54.82bcd
T3	51.20bcd	55.98a	52.35abcd	56.53abc	59.63a	58.65ab
T4	51.37bcd	53.95abc	53.68abd	55.35abcd	57.67ab	58.75ab

[1]Values in the column followed by different letter (s) are significantly different at 5% level of significance for selected poly film covers and along the rows for calcium foliar feed according to Tukey's Studentized Range (HSD) Test. Values are the means of the treatments (n = 3) Where T0 is a control experiment and T1, T2, T3 and T4 are calcium treatments at the rate of 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5 ml/L respectively

Leaf chlorophyll content was high under the IR504 poly film and calcium at concentration 3.75 ml/L. Under the control experiment of poly film UV-A clear and no calcium foliar feed, leaf chlorophyll content was significantly low compared to IR504 cover. Generally the leaf chlorophyll content was low during trial I compared to trial II. This could be due to temperature differences which was the only varying factor in the study. Leaf chlorophyll content was statistically similar in all calcium treated experimental units under the IR504 poly film. There was little variation in temperature recorded under the IR504 poly film hence the chlorophyll content did not vary. Under the UV-A clear and UV-A 205/N poly films chlorophyll was high under T2, T3 and T4 while T1 and T0 were statistically lower. This implies that under lower temperature low concentrations of calcium had effect compared to similar concentrations under high temperature. High temperature under the UV-A clear poly film is likely to have initiated stomata closure leading to reduced photosynthesis activities and consequently low chlorophyll content.

4. CONCLUSION

Cut flower quality is affected by growth environment under the poly film greenhouse covers. High chlorophyll content was observed under the IR504 poly film, implying that the coloured poly films in this study served the role of reducing greenhouse temperature and had better quality cut flowers compared to the clear poly film. Leaf chlorophyll content varied with the prevailing weather condition, it increased with decrease in temperature during the second trial and decreased with increase in temperature during the first trial. Red calypso and calcium foliar feed at an application rate T3 (3.75 ml/L) recorded the highest leaf chlorophyll content in both trials I and II. Treatment combination of calcium foliar feed at application rate 3.75 ml/L and IR504 poly film cover significantly improved leaf chlorophyll content negating the use of higher calcium concentration. In this study it can be concluded that, leaf chlorophyll content vary with temperature, cultivar and calcium concentration.

ACKNOWLEDGEMENT

We wish to thank Egerton University, Stokman Rozen Limited Kenya and National Commission of Science, Technology and Innovation (NACOSTI) for facilitating this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Harper JF, Breton G, Harmon A. Decoding CA^{2+} signals through plant protein Kinases. Annual Rev. Plant Biol. 2004;55:263-288.
2. Asfanani M, Davarynejad EH, Tenranifar A. Effect of preharvest calcium fertilization on vase life of rose cut flowers cv, alexander. Acta Horticulturae. 2008;804.
3. Hepler PK. Calcium: A central regulator of plant growth and development. Plant Cell. 2005;17(8):2142-2155.
4. Vorwerk SS, Somerville, Somerville C. The role of plant cell wall polysaccharide composition in disease resistance. Trends in Plant Science. 2004;9(4):203-209.
5. Luiz AM, Finger FL, Batista UG. Pre harvest calcium sulfate applications affect vase life and severity of gray mould in cut roses. Scientia Horticultutae. 2005;103(3): 329-338.
6. Liao LX, Peng YH, Ye QS. Neck bending phenomena in cut gebera flowers. Acta Horticulturae. 2003;30:110-112.
7. Mengel K, Kirkby EA. Principles of plant nutrition. 5th ed*.,* Kluwer Academic Publishers, Dordrecht; 2001.
8. Yildirim E, Guvenc MI, Karatas TA. Effect of foliar urea application on quality, growth, mineral uptake and yield of broccoli (*Brassica oleracea* L., var. *italica* 120. Plant Soil Environment. 2007;53(3):120–128.
9. Regina LR, Pritts MP. Dynamics of nutrient uptake from foliar fertilizers in red ruspberry (*Rubu idaeus* L.). American Society of Horticultural Science. 1996; 121(1):158-163.
10. Naoya C, Kouhei Y, Shunji J. Differences in mobility of calcium applied to the aboveground parts of broad bean plant (*Vicia faba* L.). Soil Science and Plant Nutrition. 2007;53(3):286-288.
11. Nadlhoffer KJ. The potential effects of nitrogen deposition on fine root production in forest ecosystem. New Phytologist. 2000;147(1):137-139.
12. Alvarez-Sanchez ME, Maldonado RT, Mateos RG, Vangas GA, Anyala JR, Zavala FE. Calcium supply in the development and nutrition of *Asiatic lilium*. Agrociencia. 2008;42(8):881-889.
13. Jing Z, Dong Y, Xie X, Li X, Zhang X, Shen X. Effect of annual variation in soil PH on avaliable soil nutrients in pear orchards. Acta Ecologica Sinica. 2011;31(4):212-216.
14. Shumin L, Rajapakse NC, Young RE, Ryu O. Growth responses of chrysanthemun and bell pepper transplants to photoselective plastic films. Scientia Horticulturae. 2000;84(3-4):215-225.
15. Shumin L, Rajpakse NC. Far-red light absorbing photoselective plastic films affect growth and flowering of chrysanthemum cultivars. Hort Science. 2003;38(2):284-287.
16. Kleinhenz MD, French DG, Gazula A, Scheerens JC. Variety, shading and growth stage effect on pigment concentrations in lettuce grown under contrasting temperature regimes. HortTechnology. 2003;13:677-683.
17. Riyuan CH, Liu Q, Huang G, Huang D. Changes of chlorophyll and anthocyanin content and related enzyme activities of flower stalk in chinese kale under different light intensities. Acta Horticulturae. 2008; 769:103-111.
18. Lesfrud MG, Kopsell DA. Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. HortScience 2005;40(7):2026-2030.
19. Yamane T, Jeong S, Goto-Yoimamoto N, Koshita Y, Kobayashi S. Effect of temperature on anthocyanin biosynthesis in grapes berry skins. American Journal of Viticulture and Enocology. 2006;57(1):54-59.
20. Hutin C, Nussaune L, Moise N, Kloppstech K, Havaux M. Early light-induced proteins protect arabinoses from photo-oxidative stress. Proceedings of National Academy of Science. 2003;100(8):4921–4926.
21. Darawsheh M, Bouranis D. Season Vs. nutrition-dependent fruit loading: Effect on pigment dynamics of tomato leaves. Journal of Plant Nutrition. 2006;29(3):699-715.
22. Gilmore A, Marilyn CB. Protection and storage of chlorophylls in over-wintering evergreens. Proceedings of National Academy of Science USA. 2000;92:2273–2277.
23. Lopez YN, Alejandro BL, John DF, Eulogio PB. Light intensity and activity of trypsin inhibitors in amaranth leaves and seeds. Revista Fitotecnia Maxicana. 2004;27(2): 127–132.

24. Richardson DA, Shane PD, Graeme BP. An evaluation of non invasive methods to estimate foliar chlorophyll content. New Phytologist. 2002;153:185-194.
25. Gupta S, Gopal R, Singh MV. Growth and physiological changes in bitter gourd plants growth with variable calcium supply and sand culture. Journal of Plant Nutrition. 2007;30:2051-2059.
26. Daymond AJ, Hardley P. The effect of temperature and light integral on early vegetative growth and chlorophyll fluorescence of four contrasting genotypes of cacao (*Theobroma cacao*) Ann. Appli. Biol. 2004;245:257-262.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16402