Effect of pre-cooling treatment, 1-methylcyclopropene (1-MCP) and controlled atmosphere (CA) on vase life of cut spray carnation flowers

Chairat Burana

Faculty of Innovation Agricultural Management, Panyapiwat Institute of Management, Nonthaburi, Thailand
E-mail: chairatbur@pim.ac.th

***Abstract*— The vase life of 'Light Pink Barbara' (LPB) and 'Boundee' (BD) after treatment with pre-cooling at 5°C for 48 h (dark condition), 1-MCP treatment by EthylBloc® (EB) sachet for 6 h and CA (CO_2 20% and O_2 5% at 5°C for 12, 24 and 48 h) has been investigated. All treatments decreased ethylene production in both cultivars 'LPB' and 'BD'. Pre-cooling treatment prolonged the vase life of 'LPB' but induced senescence in 'BD'. The vase life of both cut spray carnations were prolonging after by 1-MCP treatment in the form of EB. CA treatments prolonged the vase life of 'LPB' cut single carnation flowers while decreased vase life of 'BD'. The vase life of multiple flowers on a stem of 'LPB' was prolonged by pre-cooling, 1-MCP and CA for 12 h treatments. Treatments with CA for 24 h and 48 h enhanced the senescence in 'LPB'. The vase life of multiple flowers on a stem of 'BD' prolonged by 1-MCP treatment, but reduced by pre-cooling and all CA treatments. These finding suggest that CA treatment was useful to prolong the vase life and maintain the quality of 'LPB' during transportation and display.**

***Index Team* — Carnation, Vase life, Controlled Atmosphere, 1-MCP, Senescence**

I. Introduction

Carnation (*Dianthus caryophyllus* L.) is one of the most important commercial cut flowers in many countries around the world. The vase life of cut flower is the main determinant of ornamental cut flower value commercially [1]. In carnation, loss of qualities such as early sleepiness (in-rolling of the petals), wilting, unbloomed florets (blasting), and yellowing of leaf are major problems during the postharvest phase. Variation in the postharvest life is effect of differences in autocatalytic production of endogenous ethylene, stress-induced ethylene production and exogenous ethylene sensitivity [2]. 1-Methylcyclopropene (1-MCP, EthylBloc) is an ethylene analog was shown to be very effective inhibitor of ethylene action in ornamentals, fruits and vegetables [3]. Recently, 1-MCP has been commercial application for reduce the ripening process, maintain quality and extending the postharvest shelf life in a wide range of plant products [4].

Although controlled atmosphere (CA) has been used commercially for a long time, the mode action of low level of O_2 and high level of CO_2 in delaying plant senescence in fruits and vegetables [5]. However, very few reports on the effects of CA on the quality, production of ethylene and the vase life of spray type cut carnation. The aim of the present research was to determine the effects of pre-cooling treatment, 1-MCP and CA condition on extending the vase life of spray type cut carnation.

II. Materials and Methods

A. Plant materials

Cut flowers of carnations (*Dianthus caryophyllus* L.) cultivars 'Light Pink Barbara' (LPB) and 'Boundee' (BD); spray type carnations were used in this research. The potted carnation plants were cultivated and held under a natural day-length condition in a greenhouse (20°C minimum and 30°C maximum) in Utsunomiya University Tochigi prefecture Japan. The flowers at the commercial stage of flowering, at the first flower out of 6-8 flower buds on all stem were mostly open, were harvested in the morning. All cut stems were transfer to laboratory within 1 h. All multiple cut flower stems were prepared to have totally five or six flowers and buds. Removing the immature tight buds on each stem has to operate.

In the experiments with single flowers, the open flowers stage V (Fig. 1.) was the stage which their outermost petals had just reached right angles to the stems. Each treatment had five replicate flowers.

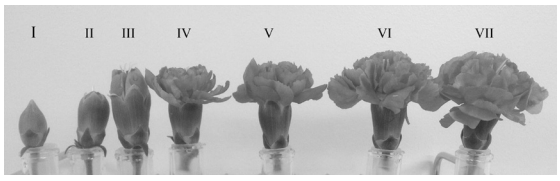

Fig. 1. Stages of carnation flower

B. Pre-cooling method

Cut carnation flowers were placed into the temperature controlled storage room at 5˚C and 75±5% relative humidity (RH) under the dark condition for 48 h.

C. 1-MCP Treatment using EthylBloc® (EB) sachet

The cut carnation flowers were place in test tube contained with distilled water, all flowers treated with 4 EthylBloc® (EB) sachets (a.i., 0.014%) in the 50×28×28 cm plastic boxes at 20˚C and 75±5% RH for 6 h.

D. CA treatments

Cut carnation flowers stem were held in the bottles contained with 50 mL distilled water. All bottles were hold in CO_2 incubator (CO_2 multi gas incubator WMI 165-R, ASTEC, Fukuoka, Japan) generated CO_2 concentrations to 20% and O_2 5% at 5°C and 75±5% RH for 12, 24 and 48 h, respectively.

E. Measurement of ethylene production

Individual flowers with stem length for 10 cm were prepared for measurement. The flowers were contained in the plastic bottles size 750 mL (one flower each bottle), and left at 20 °C for 1 h. Gas samples (l-mL) were taken from the sampling port on the bottle's lid. Ethylene concentration was determine by gas chromatography with a flame ionization detector (model GC-15 A, Shimadzu, Kyoto, Japan) using an activated alumina column (2.0m×3.0mm I.D., Shinwa Chemical Industries Ltd. Kyoto, Japan) according to Yamane et.al (2007) [6]. Data were expressed per gram flower fresh weight.

F. Statistical analysis

Data were analyzed with the JMP (SAS Institute Inc., Cary, NC, USA) statistical software program using Student's *t*-test and ANOVA. Data were tested by Tukey-kramer test for mean separation among treatments when ANOVA was significant ($P < 0.05$).

III. Results and discussion

A. Effect of short-term CA on the production rate of ethylene in cut flowers

In control flowers, ethylene production was low during the first 4 days then sharply increased and reached a maximum at day 6 (14.78 $nLC_2H_4 \cdot g^{-1} \cdot h^{-1}$) and decreased thereafter. Treated flowers shown ethylene production at low level throughout the experiment period (Fig. 2a). These results show that the treatments inhibited ethylene of 'LPB'. Treatment with CA for 12 and 24 h stimulated ethylene production of 'BD'. Stimulation was stronger in CA 12 h than those 24 h treatments and reached maximum at day 2. The climacteric peak of CA 12 h and 24 h treatments were 143.55 and 47.91 $nLC_2H_4 \cdot g^{-1} \cdot h^{-1}$, respectively (Fig. 2b).

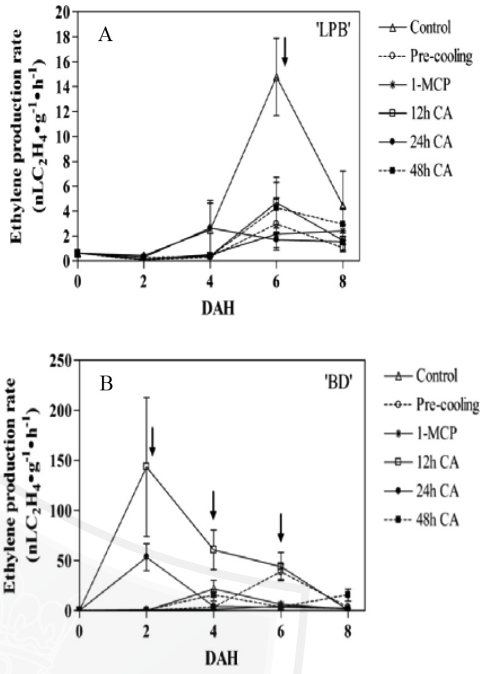

Fig. 2. Ethylene production cut carnation flowers 'LPB' (A) and 'BD' (B) during vase life. After treatments, all flowers were kept under 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PPFD, 12h) at 75±5% RH and 20°C. The vertical bar indicates Standard Error (SE) (n=5).

Previous study noted that the CA with high CO_2 treatments inhibit the production of ethylene result to maintained the quality and delaying the initiation of senescence [7]. Treating fruits and vegetables with high level of CO_2 can have beneficial effects on inhibited the production of ethylene. It is due to high level of CO_2 can compete with ethylene on binding sites at the ethylene receptor [8]. In earlier experiments, point to another site of inhibition by CO_2. In case of tomato, high levels of CO_2 suppress the expression of ethylene-dependent and ethylene-independent ripening-associated genes [9]. However, high CO_2 conditions result to anaerobic respiration, causing a severe deterioration of the overall appearance of plant species [10].

Numerous experiments on various cut flowers reported that 1-MCP inhibited effects of exogenous ethylene, such as wilting, petal or flower abscission and other senescence symptoms [11-12].

It is believed that 1-MCP molecules bind permanently to the ethylene binding protein (EBP) at the ethylene receptors site in carnation tissue [13]. The combination of 1-MCP and high level of CO_2 condition may be a feasible technique to extend the postharvest shelf life of mint [14], similar to the findings in the present study. The primary responses to CA storage (high CO_2 and low O_2) has also been shown reduced the respiration rate (i.e. O_2 uptake), which can be prolonged the storage life, vase life and reduced degradation rate of soluble pectin [15]. The second responses, important beneficial reactions

include a reduction ethylene synthesis and perception, reduce chlorophyll degradation, reduce cell wall degradation, and reduced phenolic oxidation.

It has been reported that 1-MCP inhibits ethylene biosynthetic enzymes such as ACO and ACS. It has also been reported that continuous CO_2 treatment and 1-MCP inhibit the accumulation of mRNA of ethylene biosynthesis genes [16-17]. In this study, pre-cooling, 1-MCP, high CO_2 concentrations in CA inhibited the production of ethylene, suppress the expression of ethylene biosynthesis genes including *DcACS2*, *DcACS3*, and *DcACO1* in the gynoecium and petal tissues of carnation florets [18]. There is strong evidence in carnation flowers that the activities of ACC synthase and ACC oxidase enzymes higher when the production of ethylene increased [19-20].

B. Effect of short-term CA storage on vase life of cut single carnation flowers

The treatments were not significantly prolonged the vase life of cut single flowers 'LPB' (Fig. 3a). However, 1-MCP prolonged the vase life of 'BD', while CA treatments decreased the vase life by exhibited senescence of cut single flowers 'BD' (Fig. 3b) In earlier experiments, high level of CO_2 prior to storage could have injury symptoms on cucumbers when stored at low temperatures [21]. The negative metabolic responses to low O_2 condition included aroma biosynthesis in fruit including apple, banana, peach and other agricultural commodities [22].

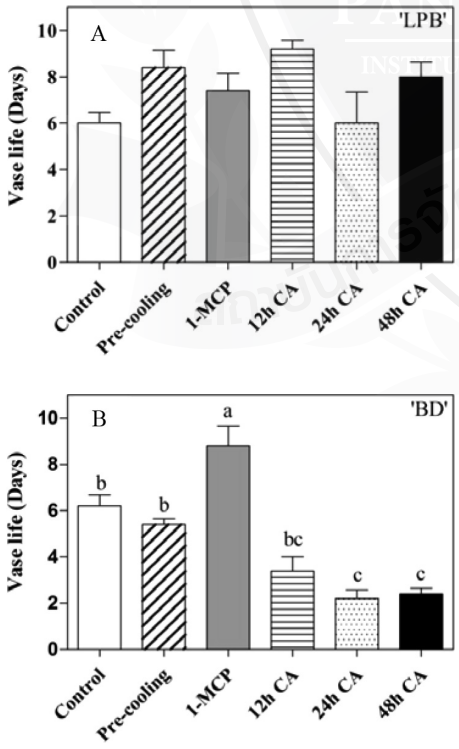

Fig. 3. Effect of the treatments on the vase life (days) of cut single carnation flowers 'LPB' (A) and 'BD' (B). After treatments, all flowers were kept under 15 μmol·m^{-2}·s^{-1} (PPFD, 12h) at 75±5% RH and 20°C. The vertical bar indicates Standard Error (SE) (*n*=5).

C. Effect of short-term CA on vase life of multiple flowers on a stem

The multiple flowers on a stem of cut carnations shown negative effected when exposed to high CO_2 for long times. Flowers of 'LPB' treated with pre-cooling, 1-MCP and 12 h CA shown the vase life longer than that in the control (Fig. 4a). However, the CA treatment for 24 and 48 h exhibited disorder symptom like-skin burning at sepals of flower buds. From Fig. 4., pre-cooling 1-MCP, CA 12 and 24 h treatment prolonged the vase life of 'BD' longest 1-MCP (17 days). Treatment with CA 48 h. induced disorder symptom like-skin burning at sepals of 'BD'. These results related with previous studies, 1-MCP treatment prevented abscission of Pelargonium [23], *Alstroemeria* L. and *Antirrhinum majus* L. [24]. Controlled Atmosphere (high level of CO_2 condition) inhibits ethylene production [25] and delay senescence of carnation [26]. However, the effect of CA also depended on CO_2 and O_2 concentration, plant verities and duration of treatment.

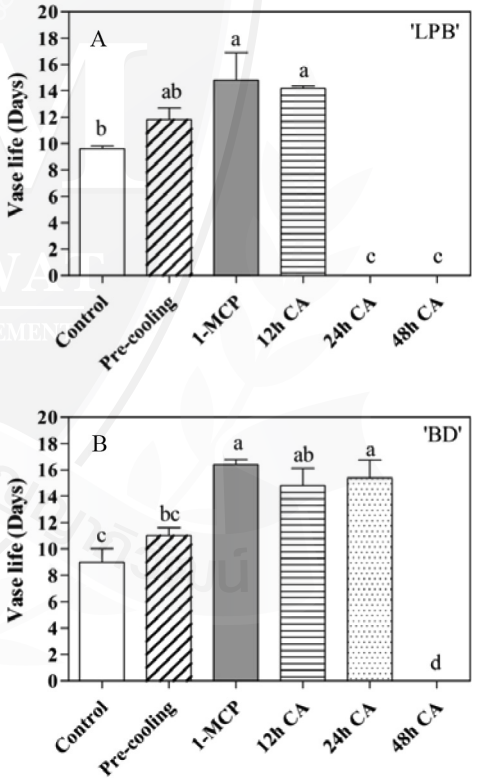

Fig. 4. Effect of the treatments on the vase life (days) of multiple flowers on a stem 'LPB' (A) and 'BD' (B). After treatments, all flowers were kept under 15 μmol·m^{-2}·s^{-1} (PPFD, 12 h) at 75±5% RH and 20°C. The vertical bar indicates Standard Error (SE) (*n*=5).

IV. Conclusion

The results suggest that CA treatment for 12 h was useful to prolong the vase life and maintain the quality of 'LPB' during transportation and display. Understanding the role of CA in flower senescence may lead to development of both chemical and physical

techniques to delay the senescence of flower that are economically detrimental, and extend the postharvest life of cut spray carnation flowers. However, the concentration of CO_2 and O_2, plant verities and duration of treatment should be considered.

Acknowledgment

The authors wish to deeply thank Prof. Dr. Kenji YAMANE, Horticulture Laboratory, Faculty of Agriculture, Utsunomiya University, Tochigi Prefecture, Japan, for supplying plant materials, technical assistance and kind advice.

References

[1] H. Nukui, S. Kudo, A. Yamashita and S. Satoh., "Repressed ethylene production in the gynoecium of long-lasting flowers of carnation 'White Candle': role of gynoecium in carnation flower senescence," *J. Exp. Bot*, vol. 55, no. 397, pp. 641-650. 2004.

[2] S. Satoh, "Ethylene production and petal wilting during senescence of cut carnation (*Dianthus caryophyllus*) flowers and prolonging their vase life by genetic transformation," *J. Japan. Soc. Hort. Sci,* vol. 80, no. 2, pp 127-135*,* 2011.

[3] E. C. Sisler and M. Serek, "Inhibitor of ethylene responses in plants at the receptor level: recent developments," *Physiol. Plant,* vol. 100, no. 3, pp. 577-582, July. 1997.

[4] S. M. Blankenship and J. M. Dole, "1-Methylcyclopropene: a review," *Postharvest Biol. Technol*, Vol. 28, pp. 1-25, 2003.

[5] A. A. Kader, "Biochemical and physiological basis for effects of controlled and modified atmosphere on fruits and vegetables," *Food Technol,* vol. 40, no. 5, pp. 99-104, 1986.

[6] K. Yamane, A. Inotsume, Y. Wada, A. Shimizu and M. Hayashi, "Effects of inhibitors on indoor quality and longevity in potted carnations," *Acta Hortic*, vol. 755, no. 755, pp. 191-196, Dec. 2007.

[7] S. Mayak and D. R. Dilly, "Altering the sensitivity of carnation flowers (*Dianthus caryophyllus*): Effect of abscisic acid and carbon dioxide on ethylene production," *Plant Physiol*, vol 58, no. 5, pp. 663-665, Nov. 1976.

[8] S. P. Burg and E. A. Burg, "Molecular requirements for the biological activity of ethylene." *Plant Physiology*, vol. 42, pp. 144-152, July. 1967.

[9] C. Rothan, S. Duret, C. Chevalier and P. Raymond, "Suppression of ripening-associated gene expression in tomato fruits subjected to a high CO_2 concentration," *Plant Physiol*. vol. 114, no.1, pp. 255-263, May. 1997.

[10] D. Zagory and A. A. Kader, "Quality maintenance in fresh fruits and vegetables by controlled atmospheres In Jen, J. (Ed.), Quality factors of fruits and vegetables," *Amer. Chem. Soc., USA,* vol. 405, no. 405, pp. 174-188. 1989.

[11] F. G. Celikel and M. S. Reid, "Postharvest handling of stock (*Matthiola incana*)." *Hort Science*, vol. 37, no. 1, pp. 144-147. 2002.

[12] K. Yamane, Y. Yamaki, and N. Fujishige, "Effects of exogenous ethylene and 1-MCP on ACC oxidase activity, ethylene production and vase life in Cattleya alliances J. Japan," *Soc. Hort. Sci,* vol. 73, no. 2, pp. 128-133. 2004.

[13] M. Serek, E. C. Sisler and M. S. Reid, "Novel gaseous ethylene binding inhibitor prevents ethylene effect in potted flowering plants." *J. Amer. Soc. Hort. Sci.*, vol. 119, no. 6, pp. 1230-1233, 1994.

[14] D. Kenigsbuch, D. Chalupowicz, Z. Aharon, D. Maurer and N. Aharoni, "The effect of CO_2 and 1-methylcyclopropene on the regulation of postharvest senescence of mint, *Mentha longifolia, L.,*" *Postharvest Biol. Technol*, vol. 43, no.1, pp. 165-173, Jan. 2007.

[15] S. J. Kays, "Postharvest physiology of perishable plant products." *Van Nostrand Reinhold, N.Y.1997* (Exon Press (1997), pp. 532.

[16] W. Owino, O. R. Nakano, Y. Kubo and A. Inaba, "Differential regulation of genes encoding ethylene biosynthesis enzymes and ethylene response sensor ortholog during ripening and in response to wounding in avocado," *J. Amer. Soc. Hort. Sci,* vol. 127, no. 2, pp. 520-527, 2002.

[17] S. Shiomi, M. Yamamoto, R. Nakamura and A. Inaba, "Expression of ACC synthase and ACC oxidase genes in melons harvested at different stages of maturity." *J. Japan Soc. Hort. Sci.,* vol. 68, no. 1, pp. 10-17, 1997.

[18] K. Tanase et al., "Transcriptome analysis of carnation (Dianthus caryophyllus L.) based on next-generation sequencing technology," BMC Genomics, vol. 13, no. 1, 2012.

[19] H. Wang and W. R. Woodson, "Reversible inhibition of ethylene action and interruption of petal senescence in carnation flowers by norbornadiene.," *Plant Physiol.*, vol. 89, no. 2, pp. 434-438, 1989, Feb. 1989.

[20] H. Wang, A. S. Brandt, and W. R. Woodson, "A flower senescence-related mRNA from carnation encodes a novel protein related to enzymes involved in phosphonate biosynthesis," *Physiology*, Vol. 96, no. 3, pp. 1000-1001, July. 1991.

[21] I. L. Eak, "Effect of modified atmospheres on cucumber at chilling and non-chilling temperature" *Proceeding of the American Society for Horticultural Science*, vol. 67, pp. 473, 1956.

[22] J. P. Matthesis and J. K. Fellman, "Impacts of modified atmosphere packaging and controlles atmosphere on aroma, flavor and quality of horticultural commodities," *HortTechnol,* vol. 10, no. 3, pp. 507-510, 2000.

[23] A. A. Cameron and M. S. Reid, "1-MCP block ethylene-induced plant abscission of Pelargonium peltatum but the ehhect is traneient," *Postharvest Biology and Technology*. vol. 22, pp. 169-177, 2001.

[24] M. Serek, E. C. Sisler and M. S. Reid, "Effect of 1-Mcp on the vase life and ethylene response of cut flowers," *Plant Growth Regulation,* vol. 16, no. 1, pp. 93-97, 1995.

[25] M. Uota, "Carbon dioxide suppression of ethylene-induced sleepiness of carnation blooms," *J. Am. Soc. Hort*, *Sci.* vol. 94, pp. 598-601, 1969.

[26] R. Nichols, "The response of carnation to ethylene," *J.Hort*. *Sci,* vol. 43, no. 3, pp. 335-349, 1968.

Chairat Burana is a lecturer and researcher in the Faculty of Innovative Agricultural Management, Panyapiwat Institute of Management. He was born on 5th June 1983 at Sisaket Province. He received the B.Sc. (Agricultural Science) from Mahidol University, Thailand. He obtained his M.Sc. (Postharvest Technology) international program from King Mongkut's University of Technology Thonburi (KMUTT) Bangkok Thailand. During his Master's, He has a research exchange student at a laboratory of horticulture, the Faculty of Agriculture, Utsunomiya University, which was supported by Japan Student Services Organization (JASSO) Scholarship. His research topic was "Effects of 1-methylcyclopropene (1-MCP), Modified Atmosphere Packaging (MAP) and Intermittent temperatures on the Display Quality and Display Life of Potted Carnation". In 2013, he has presented his work at the national and international conferences. Additionally, He has received a research award called "Dr. Adel A. Kader Award for Young Scientists" at XI Controlled & Modified Atmosphere Research International Conference in 2013, held in Trani, Italy. He received his Ph.D. in Bio-production Science from Tokyo University of Agriculture and Technology, Tokyo, Japan in 2014. During Ph.D. course he has supported by Japanese Government (MEXT) Scholarship and Tsuji Asia Foundation Scholarship. His research interests are Postharvest Technology: maintain the qualities deterioration and handling systems for Agricultural products. He is a member of the International Society for Horticultural Science (ISSH) and Japanese Society for Horticultural Science (JSHS).