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Effects of humic acid derived from sediments on the postharvest vase life extension in cut chrysanthemum flowers

Hong-mei Fan, Tian Li, Xia Sun, Xian-zhi Sun, Cheng-shu Zheng*

State Key Laboratory of Crop Biology, College of Horticulture Science and Engineering, Shandong Agriculture University, No. 61, Daizong Street, Tai'an 271018, Shandong, PR China

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ABSTRACT

Previous research has shown that humic acid can extend the vase life of cut flowers. However, the mechanisms responsible for this effect are unclear. In this study, the physiological mechanisms of foliar humic acid fertilizer on cut chrysanthemum flower postharvest vase life were investigated. Seedlings of chrysanthemum were sprayed with the same volume of distilled H_2O, inorganic NPK fertilizer and organic foliar humic acid fertilizer every 15 days (15, 30, 45, 60 days after transplanting).

The results showed that foliar application of humic acid improved the chlorophyll content, the net photosynthetic rate (P_n), contents of soluble sugars and soluble protein in the leaves of chrysanthemum, and increased the flower size, fresh weight, vase life, activities of antioxidant enzymes, and decreased the malondialdehyde (MDA) content in cut chrysanthemum flowers.

It was concluded that the responses of the foliar humic acid fertilizer on postharvest vase life extension of cut chrysanthemum flowers could be related with the higher chlorophyll content, P_n, contents of soluble sugars and soluble protein in the leaves, the greater flower size, fresh weight, activities of antioxidant enzymes, and the lower MDA content in cut chrysanthemum flowers.

1. Introduction

Chrysanthemum (*Chrysanthemum morifolium* R.) cut flowers and play an important role in the florist trade (Zhang et al., 2013). Since beauty is the main reason why cut flowers are sold, much effort has been made to lengthen flower lifespan (Arrom and Munné-Bosch, 2012; Asrar, 2012; Gul and Tahir, 2013). Senescence is a programmed process that does not occur in all floral organs at the same time. According to their specific biological function, petals are the first tissues showing signs of senescence (Arrom and Munné-Bosch, 2012). Currently, postharvest senescence is a major limitation to the marketing of cut flowers, as shown by petal in-rolling and discoloration due to reactive oxygen species (ROS) after harvest (Trippi and Paulin, 1984). Considerable effort is needed therefore to develop postharvest handling to suppress ROS and extend vase life of cut flowers. Many studies have been conducted to extend flower longevity with scientific and technological advances (Halevy and Mayak, 1979, 1981; Van Doorn, 1997; Scariot et al., 2014).

Humic acid (HA) is the fraction of naturally occurring organic materials commonly found in soils, sediments and natural waters, which derive from the decomposition of plant and animal residues (Bandiera et al., 2009). Some authors have proposed that humic acid promotes photosynthesis, respiration (Heil, 2005) and chlorophyll content (Xu et al., 2012), thus improving plant carbohydrate contents, which will directly influence the quality and life of flowers. Cordeiro et al. (2011) have also reported that humic acid has effects on antioxidative defense mechanisms, reporting the stimulation of catalases (CAT) and generation of ROS. Garcíaa et al. (2012) reported that humic acid could play a major role in resisting oxidative stress by enhancing antioxidative activity and improving membrane stability.

These facts suggest that humic acid could be beneficial for postharvest quality, if applied preharvest to the chrysanthemum plant. Therefore, the present study was conducted to investigate the mechanisms by which preharvest applied humic acid could influence postharvest quality and vase life from the viewpoint of chlorophyll content, photosynthesis, contents of soluble sugars and soluble protein in the leaves, and the flower size, fresh weight,

* Corresponding author. Tel.: +86 13695386893.
E-mail addresses: fanhongmei890815@163.com (H.-m. Fan), zcs@stau.edu.cn, zcs@sdau.edu.cn, 2225424644@qq.com (C.-s. Zheng).

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antioxidative capacity and MDA content in cut chrysanthemum flowers.

2. Materials and methods

2.1. Humic acid extraction

Compost derived humic acid was obtained in a valley filled with sediments of plant and animal residues in China (Hohhot, Inner Mongolia). The compost was extracted using an alkali/acid fractionation procedure (Valdrighi et al., 1996). The compost was digested in 0.1 N KOH (1:10 w/v) for 24 h at 25 °C. The undigested bulk residue was then separated from the solute fraction by centrifugation at 8000 rpm for 20 min followed by filtration through a glass wool layer. The filtered supernatant was then acidified at pH 2.0 with 6.0 N H_2SO_4 and maintained in the dark at 25 °C for 24 h in order to obtain flocculation of humic acid. Finally, the humic acid was collected by centrifuging at 8000 rpm for 20 min, and resuspended in 0.1 N KOH.

2.2. Growth conditions of plants, humic acid application

The experiment was conducted in a greenhouse at Horticultural Station of Shandong Agricultural University, located in Tai'an, Shandong (35°38′ N, 116°02′ E). Cutting seedlings of chrysanthemum (*C. morifolium* cv. Jinba) of similar height and diameter were cultivated on July 17th, 2013. Rooted seedlings were transplanted into plots with 150 plants per plot (10 m^2) on August 1st, 2013 with a relative humidity (RH) 65–75%, temperatures 18–25 °C, and the mean daily photosynthetically active radiation (PAR) 1000 Mol m^{-2} day^{-1}. On August 16th (15 days after transplanting), the seedlings were sprayed with the same volume of distilled H_2O (Control), inorganic NPK fertilizer (N:P_2O_5:K_2O = 16:6:20) at 0.3% (w/v) concentration and foliar humic acid (FHA) fertilizer at 1:600 (v/v) diluted concentration respectively every 15 days (15, 30, 45, 60 days after transplanting). Each treatment was replicated three times in a randomized complete block design. The concentration of the FHA used was determined from a preliminary experiment. The concentration of the NPK fertilizer was determined on the total content of NPK in the 1:600 (v/v) diluted FHA fertilizer at the optimum concentration range used in the production to ensure that any differences in flower life and quality responses were humic acid-mediated.

2.3. Measurements of chlorophyll content in the leaves of chrysanthemum

The 4th–5th fully expanded leaves from the top of the chrysanthemum were selected at 0, 15, 30, 45, 60 DAS (days after treatment). The leaves were soaked in 80% acetone for 12–24 h, and then centrifuged at 5000 rpm for 10 min. The supernatant was collected. Then the absorbance of the supernatant was read at 645 and 663 nm, respectively. The content of chlorophyll was calculated according to the equation: $20.2A_{645} + 8.02A_{663}$ (Lichtenthaler and Lester Packer, 1987).

2.4. Measurements of the P_n in the leaves of chrysanthemum

The net photosynthetic rate (P_n) was measured from 9:00 a.m. to 11:00 a.m. at 0, 15, 30, 45, 60 DAS on the 4th–5th fully expanded leaves from the top of the chrysanthemum, using a CIRAS-2 infrared gas analyzer (PP-System, Hitchin, UK) with a Parkinson's Automatic Universal Leaf Cuvette equipped with 2.5-cm^2 area cuvette inserts. Environmental conditions inside the cuvette were set as follows: PAR = 1000 μmol m^{-2} s^{-1}, leaf temperature = 25 °C, CO_2 = 450 ppm.

2.5. Measurements of the contents of soluble sugars and soluble protein in the leaves of chrysanthemum

The leaves of chrysanthemum were selected at 0, 15, 30, 45, 60 DAS for measurement. Soluble sugar and soluble protein measurements were followed the procedures that were described by Frohlich and Kutscherah (1995). Soluble sugar was extracted with anthrone. Samples (0.30 g) of fresh leaves were put into test tubes with 10 mL distilled water and sealed. The tubes were incubated in a water bath at 90 °C for 30 min, then the tubes were removed and the volume set at 25 mL. 0.5 mL supernatant was collected and mixed with 1.5 mL distilled water, 0.5 mL anthrone and 5 mL concentrated sulfuric acid. The mixed solution was read at 620 nm for soluble sugars measurement.

Soluble protein was extracted with coomassie brilliant blue. Samples (0.50 g) of fresh leaves were ground in a mortar with 5 mL phosphate buffer solution and then transferred into centrifuge tubes. The solutions were centrifuged at 5000 rpm for 15 min and the supernatant extracted. 1 mL supernatant was mixed with 5 mL coomassie brilliant blue and then read at 595 nm for soluble protein measurement.

2.6. Vase life test

Flowers were collected at the pre-opening stage (October 28th) with a similar maturity, then re-cut in water to about 75 cm stem length, and finally inserted into vases with same volume of distilled water and renewed every day throughout the holding time. There were three flowers per flask and 20 flasks per treatment. Throughout the vase period, the flowers were held in a room at 22 ± 1 °C and 65 ± 3% of relative humidity (RH) and an 8 h light period per day under irradiance of 13 W m^{-2} above the flowers using fluorescent tubes.

2.7. Measurements of the flower size, fresh weight and vase life

Flower size was defined as the maximum width of each flower and measured with a ruler every two days. Fresh weight of the capitulum was measured every two days throughout the vase life period. The average vase life was assessed to be terminated when 80% flowers had senesced, which was characterized by loss of turgor followed by petal wilting.

2.8. Measurements of the antioxidant enzymes activities in the cut chrysanthemum flowers

Petals were selected from each treatment at 0, 2, 4, 6, 8 days of inserting for measurement. 0.5 g of flower tissue was suspended in 5 mL of ice-cold HEPES buffer (25 mM, pH 7.8) containing 0.5 mM EDTA and 2% PVP. The homogenate was centrifuged at 4 °C and 5000 × *g* for 15 min and the resulting supernatants were used for the determination of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) (Ramiro et al., 2006). The determination of SOD activity was performed at 560 nm following Hwang et al. (1999). One unit of SOD activity was defined as the amount of enzyme that causes a 50% inhibition of the rate of nitroblue tetrazolium reduction. POD activity was determined at 470 nm by measuring peroxidation of hydrogen peroxide with guaiacol as an electron donor (Chance and Maehly, 1955). CAT activity was assayed at 240 nm by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules (Beers and Sizer, 1952).