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Effects of humic acid derived from sediments on growth, photosynthesis and chloroplast ultrastructure in chrysanthemum

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ABSTRACT

Humic acid (HA) is natural biological organic fertilizer derived from organic waste materials, which shows a highly promoting effect on plant growth and development. However, the mechanisms responsible for the promoting effect of humic acid on the growth and development are poorly understood. In this study, the physiological mechanisms of foliar humic acid (FHA) fertilizer on chrysanthemum growth and development from the viewpoint of photosynthesis and chloroplast ultrastructure were investigated. Seedlings of chrysanthemum were sprayed with the same volume of distilled H_2O (control), inorganic NPK fertilizer and organic foliar humic acid fertilizer every 15 days (15, 30, 45, 60 days after transplanting). The results showed that the morphological indices (stem diameter, fresh weights of shoots and roots, the root to shoot ratios, dry weights of shoots and roots, leaf area, flower diameter), the net photosynthesis rate, the chlorophyll fluorescence, the content of chlorophyll and the chloroplast ultrastructure of chrysanthemum improved obviously after foliar application of humic acid compared with those of the control and the NPK fertilizer. The promoting effect of the humic acid fertilizer showed an obvious dose-effect. The results from correlation analysis indicated that the responses of the foliar humic acid fertilizer on growth and development of chrysanthemum could be related with a constitutive increased net photosynthetic rate due to the high content of chlorophyll and the improved chloroplast ultrastructure.

1. Introduction

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). Research have shown that the effects of humic acid extracted from organic waste materials, like animal manure, food waste, paper-waste vermicomposts (Arancon et al., 2003a, 2006; Atiyeh et al., 2002; Canellas et al., 2010), aerated fermentation extracts of compost (Xu et al., 2012) and natural waters (Laglera et al., 2007, 2011; Rodr′ıguez and Nu˜nez, 2011) on plant growth and yield were stronger than commercial humic acid and inorganic fertilizer (Arancon et al., 2003a, 2004, 2006).

Many studies have reported the humic acid obtained from diverse origins could modify shoot and root growth (Arancon et al., 2003b; Atiyeh et al., 2002; Mora et al., 2012; Nardi et al., 2002; Valdrighi et al., 1996; Xu et al., 2012). Some authors proposed that humic acid promotes plant growth by acting as plant growth regulators and exertion of hormone-like activities (Arancon et al., 2006; Mora et al., 2010; Nardi et al., 2002; Pizzeghello et al., 2001). However, others suggested that humic acid plays an active role in stimulation of plant growth via promoting photosynthesis, respiration (Heil, 2005) and chlorophyll content (Xu et al., 2012).

Chrysanthemum (*Chrysanthemum morifolium* R.) is one of the most well-known traditional flowers (Li et al., 2013; Ren et al., 2013) and known as one of the fertilizer-sensitive plants. Improper fertilization can aggravate the contradiction of vegetative and reproductive growth, resulting in a decline in growth and ornamental quality (Kocamaza et al., 2006). Eyheraguibel et al. (2008) has reported that liquid humic extracts enhanced the whole plant growth including root, shoot and leaf biomass, and these effects were related to the high water and mineral consumption of plants. The ability of humic acid to improve plant growth has been well

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established in diverse plant species and growth conditions. However, the physiological mechanisms of foliar humic acid (FHA) fertilizer on chrysanthemum growth and development have not been well documented so far, and there is a need to gain more insight into the relationship between plant growth and FHA application. It is possible that growth and development promoting effect of the FHA fertilizer is closely related to improvement of photosynthetic characteristics and chloroplast ultrastructure.

To investigate this hypothesis, we studied the physiological mechanisms of the FHA fertilizer on chrysanthemum from the viewpoint of photosynthesis and chloroplast ultrastructure. To this end, we have investigated the changes in the morphological indices, the net photosynthetic rate, chlorophyll fluorescence, chlorophyll content and chloroplast ultrastructure of chrysanthemum sprayed with FHA fertilizer.

2. Materials and methods

2.1. Plant material and culture conditions

The experiment was conducted in a greenhouse at Horticultural Station of Shandong Agricultural University (Shandong, China). Cutting seedlings of chrysanthemum (*C. morifolium* cv. 'Jinba') of similar height and diameter were cultivated on July 17th, 2012. When rooted on August 1st, 2012, the seedlings were transplanted into plots with 45 plants per plot (3 m^2). NPK fertilizer ($N:P_2O_5:K_2O$ = 16:6:20) was applied into plots with 90 g per plot before transplanting. Plants were irrigated twice every week to ensure adequate substrate moisture. Inside the greenhouse, temperatures ranged between 18 and 25 °C, with a relative humidity (RH) 65%–75% and the mean daily photosynthetically active radiation (PAR) 1000 mol m^{-2} day^{-1}.

2.2. Treatments and experimental design

The spraying treatment was initiated on August 16th (15 days after transplanting). Chrysanthemum seedlings were sprayed with the same volume of distilled H_2O, 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. The humic acid was obtained in a valley filled with sediments of plant and animal residues in China (Hohhot, Inner Mongolia) and then extracted and purified. The spraying treatment was carried out 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 on the pre-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 growth and development responses were humic acid-mediated.

2.3. Measurements of the morphological indices

The stem diameter, fresh weights of shoots and roots, dry weights of shoots and roots, leaf area were measured both at the beginning and at the end of the experiment (August 16th and October 16th, respectively). Leaf area was measured by CI-203 Handheld Laser Leaf Area Meter (CID, Inc. Made in U.S.A.). The root to shoot ratio was equal to the fresh weight of root divided by the fresh weight of shoot. Flower diameter was measured at the blossom stage with a ruler.

2.4. Measurements of the P_n

The net photosynthetic rate (P_n) was measured from 9:00 a.m. to 11:00 a.m. at 0 (before spraying), 15, 30, 45, 60 DAS (days after treatment) respectively on the youngest fully expanded leaf, 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 chlorophyll fluorescence parameters

Fluorescence of 30 min dark-adapted leaf samples was measured from 9:00 a.m. to 11:00 a.m. at 0, 15, 30, 45, 60 DAS with a portable pulse amplitude modulation fluorometer (FMS-2, Hansatech, British). The fluorescence measurement on the leaf surface followed what was described by Schreiber et al. (1986) and Buwalda and Noga (1994). The leaf was exposed to the weak modulated measuring beam (<0.1 μmol m^{-2} s^{-1}) to measure initial fluorescence (F_o), when the PSII reaction centers were oxidized. Then, the leaf was exposed to an 800 ms saturation pulse of white light to produce a reduction of the PS II reaction center, at which point maximal fluorescence (F_m) was measured. Thirty seconds after the saturation pulse, continuous actinic light (100 μmol m^{-2} s^{-1}) was applied to estimate the steady-state fluorescence yield (F_s). Saturation pulses were then triggered every 20 s over a period of 300 s and the chlorophyll fluorescence corresponding to that correlated with maximal closure of PS II reaction center. The maximal fluorescence in the light adapted state (F'_m) was assessed for each pulse. The coefficients were defined according to Krall and Edwards (1992): $F_v/F_m = (F_m - F_o)/F_m$, $\Phi_{PSII} = F'_m - F_s/F'_m$, $NPQ = F_m - F'_m/F'_m$, where the maximal photochemical efficiency (F_v/F_m) represented maximum energy conversion efficiency of opening reaction center in dark-adapted leaves. The effective PSII quantum yield (Φ_{PSII}) was considered to represent proportion of the light absorbed by photosystem II used in photochemistry in light-adapted plant, whereas the non-photochemical quenching coefficient (*NPQ*) mainly represented non-photochemical quenching in the light-harvesting antenna of photosystem II.

2.6. Measurements of chlorophyll content

The leaves of chrysanthemum selected at 0, 15, 30, 45, 60 DAS were soaked in 80% acetone for 12–24 h, and then the chlorophyll was extracted. The absorbance 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).

2.7. Measurements of chloroplast ultrastructure

The chloroplast ultrastructure was measured at 60 DAS according to the method of Helliot et al. (2003), following modifications by Gao et al. (2010). After fixation with 4% glutaraldehyde for 6 h, leaves were post-fixed with 2% osmic acid at 4 °C for 6 h and then dehydrated with ethanol. When embedded in Spurr resin at 60 °C for 12 h, thin sections were cut from leaves with an LKB ultramicrotome and were picked upon 250-mesh grids. Cells were post-stained with uranyl acetate and lead citrate and observed in a transmission electron microscopy (JEM-1200EX; JEOL Ltd., Tokyo, Japan) at 80 kV. Chloroplasts per treatment were analyzed at magnifications of 10,000×.