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Balanced zinc nutrition enhances the antioxidative activities in Oriental lily cut-flower leading to improved growth and vase quality

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ABSTRACT

Zinc (Zn) being an activator of certain enzymes, can regulate antioxidative activities and enhance the vase-life of cut-flowers. This greenhouse study on zinc nutrition of Oriental lily was performed with four levels of zinc, 0, 3, 6 and 9 mg Zn kg^{-1} of soil media, applied after two weeks of lily bulbs plantation. Maximum plant height, internodal distance, and leaf area were recorded at 6 mg Zn kg^{-1} soil media. Floral attributes, viz., flower-bud diameter, flower diameter, number of flowers per plant, stalk length, stalk diameter, fresh and dry weight of nine flowers per treatment also showed the maximum response at 6 mg Zn kg^{-1}. Earlier flower initiation, extended vase-life, lesser weight loss of flower, greater membrane integrity, content of chlorophyll and protein were recorded at 6 mg Zn kg^{-1}. Photosynthetic rate, transpiration rate, stomatal conductance, relative water contents and net assimilation rate were higher at 6 mg Zn kg^{-1} dosage. Antioxidant enzymes, viz., SOD, POD and CAT in cut-flowers also remained at the maximum with this medium dose of zinc. This study concludes that zinc applied at 6 mg kg^{-1} is suitable dose for enhancing the plant growth, floral vase-life and quality of lily cut-flower.

1. Introduction

Zinc as a micronutrient, is well known for the better growth and high quality of cut-flowers (Hansch and Mendel, 2009). It acts as a metal component of various enzymes as functional, structural and regulatory co-factor. Contribution of Zn for protein synthesis, photosynthesis, cell division and maintaince of membrane integrity is recognized (Sarwar et al., 2013). Involvement of Zn in the production of biomass (Cakmak, 2008) and increment in the level of antioxidants within plant tissues is well documented (Luo et al., 2010). It plays crucial role in stomata regulation and ion balance in plant systems (Vazin, 2012). Role of Zn is well established in the vase-life improvement of cut-flowers by eliminating oxidative stress and per-oxidation of membrane lipids and protein (Saeed et al., 2013). In addition, Zn is critical for the synthesis of phyto-hormones such as auxin, abscisic acid, gibberellins and cytokinins (Imran et al., 2014).

Oriental lily (*Lilium* spp.) is an important bulbous flowering plant that belongs to the family Liliaceae (Addai, 2010). It is one of the leading cut-flowers ranking seventh in position all over the world (Saifullah et al., 2010) and there has a great market demand of lily due to its majestic long slender perfumed and showy flowers (Sajid et al., 2009). Oriental lily hybrid "Robina" has evolved by crossing the Orienpet lily × Mero Star cv. of Oriental lily. Market value of cut-flowers depends upon many factors as stalk length, thickness, number of flowers, freshness and vase-life. Therefore, more attention is being paid on quality along with its production. Commercial production of Oriental lily can generate not only good income but can also fetch foreign exchange through export.

Postharvest longevity of cut-flowers is a key factor that contributes the aesthetic and marketable value of the crop (Mansouri, 2012). Petals are the floral organs that determine the commercially acceptable aesthetic value of flowers. Therefore, much attention is required to improve all the biochemical and physiological processes that take place during petal development and senescence (Sood et al., 2006). Present study was undertaken for optimization of zinc dosage for the enhancement of floral, vase quality, biochemical characteristics and antioxidant enzymes activities in Oriental lily flower during vase-holding period.

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2. Materials and methods

This greenhouse trial was conducted with Oriental lily hybrid "Robina" bulbs (16–18 cm). Four levels of zinc, 0, 3, 6 and 9 mg kg^{-1} were applied from stock solution of zinc sulphate ($ZnSO_4$) (0.25% w/v) in earthen pots containing 10 kg soil media. Basal dosage of N-P-K was applied uniformly at 60–45–30 mg kg^{-1}, respectively in all the experimental pots. Soil had sandy loam texture, with pH, 7.2; EC, 0.31 dS m^{-1}; organic matter, 0.39%; total N, 0.023%; available P, 8.4 mg kg^{-1}; extractable K, 122 mg kg^{-1} and Zn, 0.84 mg kg^{-1} by using ammonium bicarbonate-diethylenetriamine pentaacetic acid (AB-DPTA) extract. Experiment was laid out in completely randomized design (CRD) with three replications. For post-harvest work, three lily stalks at colored bud stage were harvested and brought to post-harvest laboratory in water. Flower stalks were re-cut to the uniform size of 50 ± 5 cm under water and were placed in glass jars containing distilled water at 25 ± 2 °C, 70 ± 5% RH and 14 h illumination period. Following parameters were observed.

2.1. Plant growth and floral characteristics

Data on plant height, internodal distance, leaf area and number of leaves were recorded at harvesting stage. Days taken to flower initiation, flower-bud/flower diameter, number of flowers, flower stalk length/diameter, fresh and dry weight of flowers, were recorded in the field.

2.2. Vase quality parameters

Days taken to 50% and 100% flower opening, vase-life, and weight loss of flower were recorded during vase holding period. Flowers were dried in oven at 80 °C for 48 h. Weight loss of flowers was calculated as follows:

$$\text{Weight loss of flower (\%)} = \frac{\text{weight of fresh flower (g)} \times \text{weight of dried flower (g)}}{\text{weight of fresh flower}} \times 100$$

2.3. Membrane integrity

Membrane integrity was measured as described by Singh et al. (2008) with some modifications. Five flower petals, 1 cm of each treatment were put together into test tube containing 10 ml of distilled water. Initial electrolyte leakage was determined following incubation of flask at 25 °C for 3 h (hours). The solution was then autoclaved at 121 °C for 15 min before the final conductivity was measured. Membrane integrity was calculated as follows:

$$\text{Membrane integrity(\%)} = [1 - (\frac{\text{electrolyte leakage after 3h of incubation}}{\text{total electrolyte leakage}})] \times 100$$

2.4. Zinc content in plant and soil

Zinc concentration in plant leaves was determined on atomic absorption spectrophotometer (GBC 932 plus). Soil zinc content was determined by using AB-DPTA extract.

2.5. Biochemical attributes

Chlorophyll content was measured by SPAD 502 (Konica Minolta, Japan). Protein content in flowers was determined with the method as described by Bradford (1976).

2.6. Physiological characteristics

Photosynthetic rate, transpiration rate and stomatal conductance were determined according to the method described by Long and Bernacchi (2003). LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England) was used between the hours of 12:00 and 15:00 at the prevailing solar radiation on flowering stage.

2.7. Relative water content

Relative water content (RWC) was determined according to the method given by Bars and Weatherley (1962). Leaves were excised and fresh weight (FW) was immediately recorded then leaves were soaked for 4 h in distilled water at room temperature and turgid weight (TW) was recorded. After drying for 24 h at 80 °C, dry weight (DW) was recorded. The RWC was calculated as follows:

$$\text{RWC}(\%) = \left[\frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})}\right] \times 100$$

2.8. Net assimilation rate

Net assimilation rate (NAR) was calculated with the following formula as given by Gardner et al. (1985).

$$\text{NAR}\left(\text{gm}^{-2}\text{day}^{-1}\right) = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\text{InLAI}_2 - \text{InLAI}_1}{\text{LAI}_2 - \text{LAI}_1}$$

where, W = dry weight, t = Time and LAI = Leaf area index.

2.9. Preparation of enzymes extract

One gram sample of frozen flowers (in liquid nitrogen) was taken and grinded in pre-cold mortar and pestle for cell free enzyme extract. The tissues were suspended in 5 ml of 0.1 M KPO_4 buffer (pH 7.8) containing 0.5% triton and 0.2 g of polyvinylpyrrolidone (PVP). The mixture was centrifuged at 13,000 rpm for 30 min at 4 °C (Abbasi et al., 1998).

2.10. Superoxide dismutase assay

Superoxide dismutase (SOD) activity was determined using the method described by Dhindsa et al. (1981) with some modifications. Two sets of five cuvettes (3 ml) each containing 0, 50, 100, 200 or 300 ml of enzyme extract were added with 50 mM KPO_4 buffer (pH 7.8) and reaction mixture. One set of cuvettes kept in the dark (blank) while other set was placed under fluorescent lamps for 10 min. Absorbance of solution was read at 560 nm wavelength with spectrophotometer (Optima® 3000 plus).

2.11. Peroxidase assay

Peroxidase (POD) activity was assayed according to the method mentioned by Reuveni et al. (1992) with slight modifications. Assay mixture consisted of 1 mM H_2O_2, 0.1 mM guaiacol in 15 mM $NaKPO_4$ buffer (pH 6.0) and 200 μl crude enzyme extract. The POD activity was recorded at 470 nm as a change in the optical density (OD) over a 3 min period.

2.12. Catalase assay

Catalase (CAT) activity was assayed according to the method described by Luck (1965). Reaction was carried out by using two buffer solutions, one containing 50 mM KPO_4 buffer and other consisting of 12.5 mM H_2O_2 in 50 mM KPO_4 (pH 7.0). Reaction was started by adding 300 μl enzyme to each buffer in 3 ml cuvettes, and

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