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Effect of pre-harvest foliar application of citric acid and malic acid on chlorophyll content and post-harvest vase life of *Lilium* cv. Brunello

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Citric acid is a regular ingredient in many vase solution formulations but pre-harvest use of citric acid is a novel method in vase life extension of cut flowers, which is reported on tuberose earlier. In order to verify previous result, and check for possible substitution of citric acid by malic acid, the current research was designed. Citric acid (0, 0.075, 0.15% w/v) and malic acid (0, 0.075, 0.15% w/v) were used in a factorial design with three replications. Foliar sprays were applied two times during growth period of *Lilium* plants. The results point out that 0.15% citric acid alone had increased vase life from 11.8 in control treatment to 14 days ($\alpha < 0.05$). The interesting finding was the effect of citric acid on bulbil weight, which was decreased from 9 g in control to 1.5 g in treatment containing combination of 0.075% citric acid and 0.075% malic acid. Malic acid while having no direct effect on pre-mentioned traits surprisingly increased the chlorophyll content significantly. The interaction effect between citric acid and malic acid on vase life and chlorophyll content proved significant and was evident in results, both as antagonistic and synergistic in various traits.

Keywords: *Lilium*, organic acids, foliar nutrition, vase life

INTRODUCTION

Lilies are distinguished by having large and attractive flowers. They are among the six most economically important major geniuses of bulbous plants (Dole and Wilkins, 1996, 1999). Lilies are produced both as potted and cut flowers and are used in landscaping (Dole and Wilkins, 1999). Organic acids are source of both carbon skeleton and energy for cells and are used in the respiratory cycle and other biochemical pathways, therefore can influence on the cut flower's vase life (da Silva, 2003). Citric acid is a regular ingredient in many vase solution formulations that acts as a *pH* regulator that reduces bacterial proliferation and enhances the water conductance in xylem of cut flowers (Goszczynska and Rudnicki, 1988; van Doorn, 2010). Citrate and malate are among the intermediate organic acids in Krebs cycle which produces cellular energy by oxidative phosphorylation (Wills et al., 1981). Citrate complex is one of the mobile forms of iron inside the plant so it plays an important role in iron transport inside plants (Hell and Stephan, 2003). Iron (Fe) availability in the apoplast of leaf mesophyll tissues is controlled by *pH* and organic acids (Abadía et al., 2002). Fe III has to undergo a reduction by a ferric chelate reductase (FCR) before entering the cells such as citric and malic acids (Rombolà et al., 2001). In leaves moderately affected by chlorosis the regreening effect was induced by citric acid (Álvarez-Fernández et al., 2004). Malate is the organic acid with a diverse role in plant from osmotic balance of vacuole (Lüttge and Ball, 1979), central role in *pH* regulation (Wedding, 1989), and as an energy source for plant mitochondria (Casati et al., 1999).

The positive effect of pre-harvest citric acid sprays on post-harvest longevity of cut flowers is first reported recently on tuberose (Eidyan, 2010). As the highest applied concentration of citric acid in the previous report had a beneficial effect on vase life, in the current study, we applied up to 50% higher concentration of citric acid on *Lilium* to explore its potential effect on extension of post-harvest vase life this time on a different plant. In the current study, we tried to evaluate the effectiveness of malic acid, as well as citric acid. Because citric acid readily converts to malic acid in Krebs cycle, we expected to see similar effects by malic acid as well. This would help us to get closer to the mechanism of action of citric acid by comparing its effect to malic acid.

MATERIALS AND METHODS

The experiment was conducted in experimental greenhouse of the Agriculture Faculty (Islamic Azad University, Karaj Branch). Three levels of both citric acid (0, 0.075, and 0.15% w/v) and malic acid (0, 0.075, and 0.15% w/v) were applied as foliar sprays. The experimental design was a 3×3 factorial arrangement of treatments with three replications. Each replication consisted of one pot containing one plant resulting total of 27 plants which were arranged in nine treatments in a completely randomized design. Both malic acid (Fluka, ≥99.5% Pure) and citric acid (Sigma-Aldrich, 99%) were obtained from local distributors.

The bulbs of *Lilium* × *elegans* Thunb. "Brunello" Asiatic hybrid lily were planted in pots filled with a growing medium composed of peat moss, sand, and garden soil (2, 2, 1 by volume) on February 5, 2009 and first time irrigated by a solution containing 5,000 ppm benomyl and 2,000 ppm diazinon. The plants were irrigated twice a week. To avoid any interference with results, no fertilizer was used during experiment period. Shoots emerged on February 12 and 13, 2009, and 15 days later, the flower buds were observed. The spray solutions were prepared based on distilled water and a

Table 1 | Effect of pre-harvest foliar application of citric acid and malic acid on plant growth, chlorophyll content, and vase life of *Lilium*.

Citric acid (CA; % w/v)	Malic acid (MA; % w/v)	Stalk height (cm)	Floret (*n*°)	Floret diameter (cm)	Pedicle length (cm)	Chlorophyll content (SPAD reading)	Stem diameter (cm)	Vase life (days)	Bulbil (*n*°)	Bulbil weight (g)
TREATMENTS MEAN COMPARISON (NUMBERS REPRESENT THE MEAN)										
0	0	72.6^{a}	5^{a}	9.2^{a}	10.6^{a}	24.3^{f}	1^{a}	11.8^{b}	13^{a}	9^{a}
	0.075	61.1^{a}	5^{a}	8.9^{a}	10.7^{a}	27.3^{cd}	1^{a}	13.7^{a}	25^{a}	7.6^{ab}
	0.15	63^{a}	4.7^{a}	9.2^{a}	9.6^{a}	31.5^{a}	1.1^{a}	13.8^{a}	27.3^{a}	9.1^{a}
0.075	0	60.8^{a}	4.7^{a}	9.5^{a}	9.5^{a}	25.2^{ef}	1.1^{a}	13.3^{a}	18.7^{a}	5.4^{ab}
	0.075	70.4^{a}	5^{a}	9.3^{a}	10.1^{a}	29.8^{ab}	1^{a}	12.2^{b}	9^{a}	1.5^{b}
	0.15	60.7^{a}	5^{a}	8.7^{a}	8.7^{a}	25.5^{def}	1^{a}	12.3^{b}	8^{a}	1.9^{b}
0.15	0	59.8^{a}	5^{a}	9.3^{a}	9.5^{a}	25.2^{ef}	1.1^{a}	14.3^{a}	12^{a}	3.3^{ab}
	0.075	60.3^{a}	4.7^{a}	8.7^{a}	8.6^{a}	26.6^{cde}	1^{a}	13.7^{a}	12^{a}	2.5^{b}
	0.15	63.3^{a}	5^{a}	8.8^{a}	9.7^{a}	28.1^{bc}	1^{a}	14^{a}	13.3^{a}	4.8^{ab}
***F*-TEST PROBABILITIES (FACTOR EFFECTS)**										
Citric acid		ns	ns	ns	ns	ns	ns	0.000	ns	0.003
Malic acid		ns	ns	ns	ns	0.000	ns	ns	ns	ns
Citric acid*malic acid		ns	ns	ns	ns	0.000	ns	0.001	ns	ns

ns = non-significant ($\alpha \geq 0.01$). Means in each column followed by similar letters are not significantly different (Duncan's test, $\alpha < 0.05$).

few drops of Tween 20 was added to each 500 ml of solution. All solutions contained 0.1% glycerin, as well. The plants were hand-sprayed until the spray liquid dripped freely from the foliage. First spray applied on April 9, 2009 and the second one made 8 days later.

The chlorophyll content was measured by a portable chlorophyll meter (SPAD-502, Minolta Camera Co., Ltd., Japan) at the time of harvest by reading and averaging of the SPAD value from a vein-less part of six leaves from all parts of plants. The flower stems were harvested when the buds reached at puffy stage showing color. To evaluate post-harvest performance, cut flowers were placed in a vase solution containing 10% w/v sucrose, 150 ppm hydroxyquinoline sulfate, and 50 ppm silver nitrate. The flower's vase life was determined for all 27 cut flowers, as the number of days after harvest until floret's wilting. The other recorded parameters after harvest included flowering stalk height, stem diameter, floret diameter and number, bulblet number, and weight.

Data were analyzed by analysis of variance using SPSS 16.0 software (IBM Inc.) and means were separated at the 5% level using Duncan's test.

RESULTS AND DISCUSSION

Malic acid spray increased chlorophyll content significantly. Chlorophyll content was highest in plants treated with 0.15% malic acid alone with SPAD reading of 31.5 compared with control (24.3). Except one case, all factor-levels containing malic acid had significantly higher chlorophyll content compared with control (see **Table 1**). Succinate is the precursor molecule used as carbohydrate skeleton for chlorophyll biosynthesis, which is readily converted to malate in Krebs cycle. We could assume that added malate could retard this conversion leading to succinate accumulation, which could speed up other processes that depend on succinate metabolism like chlorophyll biosynthesis.

Citric acid spray reduced marginal mean weight of bulbils per flower stalk significantly (see **Table 1**, *F-test probabilities* section). The mean comparison among factor-levels revealed that the highest bulbil weight per flower was observed in CA0 + MA0.15 and control (see **Table 1**). As is readily deducible from data presented in **Table 1**, the sum of citric acid and malic acid concentrations has played the deciding role in decrease of the bulbil weight.

Foliar spray of citric acid during growth stage increased post-harvest vase life of the cut *Lilium* flowers significantly (see **Table 1**, *F-test probabilities* section). The mean comparison among factor-levels pointed out that the highest vase life of 14.3 days reached by CA0.15 + MA0 which was significantly higher than the control treatment with 11.8 days. Malic acid has increased the vase life when applied alone but an antagonistic effect was evident between presence of both acids in some combinations (see **Table 1**).

Our results confirm earlier reported by Eidyan (2010). However, in the present experiment the malic acid showed a similar effect as well but not significant statistically (see **Table 1**, *F-test probabilities* section). On the other side, any direct effect by citric acid on chlorophyll content was missing and a significant cross talk between them was noticed, which suggests that they do not simply substitute each other in function and effect on metabolism (see **Table 1**). This cross talk could be tracked in bulblet weight as an effect on carbohydrate partitioning to bulbils by reducing their weight.

The inductive effect of citrate on production of citrate by microorganisms is previously reported (Cantino and Goldstein, 1967), which could be the case in plants, as well. The role of citrate as the substrate for biosynthesis of fatty acids (Ratledge, 2004), might describe part of responses by enforcing cell membranes with extra fatty acid production. Use of specific inhibitors of the mitochondrial tricarboxylate and dicarboxylate carriers had caused reduced fat accumulation due to restraint of citrate export from

mitochondria (Kajimoto et al., 2005). This membrane enforcement could be the cause for the noted increase in vase life. We assume that there is a cross talk between regulatory effects of both substances, which could have practicable applications in regulating some of the plant growth and development responses.

We conclude that we could confirm earlier report by Eidyan regarding the effect of pre-harvest application of citric acid on extension of post-harvest longevity of cut tuberose here on *Lilium*, as well. This could be an affordable means of post-harvest extension of vase life especially in a situation that there is lack of proper facilities to insure efficient post-harvest handling. Understanding the mechanism of actions of these substances could give us a better strategy for design of further experiments in this regard.

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