Journal of Applied Botany and Food Quality 86, 212 - 216 (2013), DOI:10.5073/JABFQ.2013.086.029

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Drought tolerance and water status of bean plants (*Phaseolus vulgaris* L.) as affected by citric acid application

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(Received June 05, 2013)

Summary

Enhancement of drought tolerance of plants is a crucial concern in arid and semi-arid regions. Using safe and environmentally-friendly tools and treatments for this purpose is needed to overcome the problems of water shortage with particular emphasis on sustainable resource management and environmental protection. This study investigated the water status and drought tolerance of beans. Bean plants (*Phaseoulus vulgaris* L.) were treated with citric acid (0.5, 1, 1.5 and 2 g/L) as a foliar application prior the exposition to drought stress conditions. Physiological changes, such as leaf temperature, relative water content (RWC) and chlorophyll content of leaves, were recorded in response to citric acid application. The results revealed that the water status of bean plants under drought stress conditions was improved by citric acid application, indicated by higher RWC of leaves compared to control plants. The most effective level in this respect was 1.5 g/L. A similar trend was observed with total chlorophyll content of leaves. In addition, plant growth, productivity and quality parameters were significantly improved by application of citric acid compared to control plants. The possible roles of citric acid on water status and drought tolerance of bean plants are discussed.

Introduction

Bean plants (*Phaseolus vulgaris* L.) are important protein source world-wide. However, they are sensitive to drought conditions. Growth and productivity of bean plants are extremely affected by water stress (Millar and Gardner, 1972). Moreover, according to El-Tohamy et al. (1999) water stress resulted in a decline of leaf water potential, stomatal conductance, photosynthesis rate and all growth, productivity and quality parameters of bean plants.

Finding relatively safe tools and treatments to overcome the negative effects of drought stress or improve drought tolerance of sensitive plants could be of great value especially under arid and semi-arid conditions where shortage of water becomes a limiting factor for plant growth and productivity.

Recently, there is a tendency to explore the relationships between citric acid accumulation in plants and improvement of drought tolerance. For example, Levi et al. (2011) found that the accumulation of some organic acids including citric acid could contribute to superior capacity of some lines of cotton to cope with drought. Merewitz et al. (2012) indicated that the enhanced drought tolerance of some drought tolerant lines of creeping bentgrass was associated with the maintenance of accumulation of several metabolites including the organic acids that are mainly involved in the citric acid cycle. They concluded that the accumulation of these metabolites could contribute to improved drought tolerance. Other authors, emphasized the importance of citric acid for improving quality of fruits (such as tomato, pear and citrus fruits) and its accumulation is evident under mild water stress (Morinage and Sykes, 2001; Nahar and Gretzmacher, 2002; Papadopoulus et al., 2004; Kang et al., 2009; and Iwasaki et al., 2011). Moreover, Sun and Hong (2011) reported that exogenous citric acid significantly increased internal citric acid concentration in plant tissues and improved plant growth and stress tolerance during exposure to saline stress conditions. On the other hand, Darandeh and Hadavi (2011) indicated that foliar spray of citric acid during growth stage significantly increased post-harvest vase life of the cut *Lilium* flowers. It seems that this compound is able to be taken up by plant cells, although the leaf surface is covered with cutin. Recently, Burkhardt et al. (2012) studied the stomatal penetration by aqueous solutions and their results clearly confirmed the stomatal uptake of aqueous solutions.

However, investigations concerning the effect of citric acid on water status of other sensitive plants such as bean plants are scarce in the literature. The present study aimed to explore the effects of citric acid on drought tolerance and water status of bean plants subjected to drought conditions.

Materials and methods

The experiments were carried out at the National Research Center during two successive seasons 2011 and 2012. Seeds of snap bean (*Phaseolus vulgaris* L.) cv. 'Bronco' were sown in 5 liter pots (filled with peat moss) in the second week of February for both seasons. At the 3rd leaf stage, bean plants were sprayed with citric acid at four levels (0.5, 1, 1.5 and 2 g/L). One day after treatments, plants were subjected to drought stress by withholding water for 7 days. Control plants were also subjected to drought stress for the same period of time and not sprayed with citric acid (control 1), while an additional treatment of non-stressed plants were installed (control 2). The plant in control 2 were not treated by citric acid and kept at field capacity during the entire experiments. After stress period, all stressed-plants were re-watered and kept at field capacity for post stress observations on vegetative growth, generative development and yield as well as some quality parameters of pods. All plants were fertilized by using a nutrient solution (15-5-25, NPK) during the entire experiments.

The following parameters were recorded:

Physiological parameters: The second fully expanded leaves were used for the following measurements at the end of drought stress period: Total chlorophyll content of leaves was measured using TYS-A chlorophyll Meter (Zhe Jang Top Instrument Co. LTD., China), RWC of leaves according to Turner (1981), and leaf temperature measurements by infrared thermometer (DT 8500-Cheerman, China).

Post stress growth, quality and productivity observations: (plant fresh weight and branches number per plant) were recorded 60 days after sowing for both seasons. Productivity measurements (weight of pods and pods number per plant) were recorded as well as pod characteristics (length and diameter).

Statistical analysis: The experiments were established as complete randomized block design with 4 replicates and analysis of variance was calculated according to Snedecor and Cochran (1967). Least significant difference (L.S.D.) at 5% was used to compare the means for different treatments.

Results and discussion

Effects of citric acid on leaf temperature of bean plants subjected to drought stress

Previous investigations indicated that leaf or canopy temperature can be used as a cheap, fast and not-destructive screening tool for estimating drought tolerance among different plants such as maize (LIU et al., 2011) and rice (HIRAYAMA et al., 2006). TALEBI (2011) studied the effect of drought stress on canopy temperature and total chlorophyll content on durum wheat and found that the genotypes with high yield in well-watered condition had also low canopy temperature and high chlorophyll content. He concluded that wheat genotypes with a low canopy temperature can maintain high transpiration and photosynthetic rate as well as produce a high yield under moisture-stressed conditions.

Leaf temperature of bean plants under drought stress as well as non-stressed plants for both seasons is illustrated in Fig. 1. Generally, although plants treated by citric acid had lower leaf temperature than control 1 plants, these differences were not significant between treatments. Only non-stressed plants (control 2) showed significantly lower leaf temperature compared to other treatments. This was probably because, plants of this treatment were able to maintain higher transpiration rate, by which the leaf temperature was reduced. Generally, the leaf temperatures of the second season were higher than the first season for all treatments, and this was related to the climatic variations between both seasons.

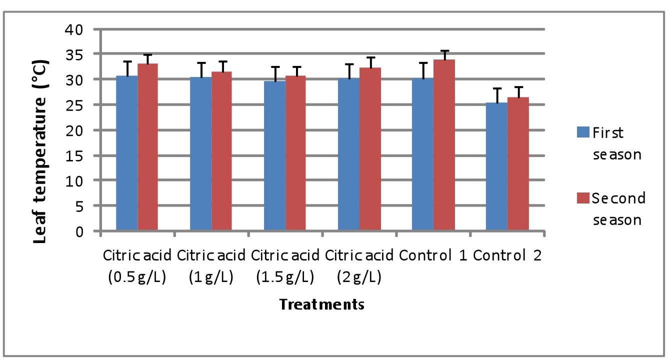

Fig. 1: Effect of different levels of citric acid on leaf temperature of bean plants subjected to drought stress conditions .Control 1: plants not treated by citric acid, but stressed. Control 2: plants not treated by citric acid and kept at field capacity during the entire experiments. Vertical bars present LSD value at 5%.

Effects of citric acid on chlorophyll content of leaves from bean plants subjected to drought stress

Chlorophyll content as an indicator of leaf photosynthesis is one of the criteria to access drought tolerance of plants. KHAYATNEZHAD and GHOLAMIN (2012) evaluated the effect of drought stress on this parameter and stress tolerance of different maize cultivars (*Zea mays* L.) and found that different genotypes had different chlorophyll content and finally different resistance to drought stress. Also, ARJENAK et al. (2012) indicated that chlorophyll content (and RWC) made difference between resistance and susceptible genotypes and thus, this attributes can be used as screening tool for drought tolerance in wheat. According to SUN and HONG (2011), when citric acid was exogenously applied significantly improved the plant growth under stress conditions and also induced defense mechanisms by increasing the activities of antioxidant enzymes. DARANDEH and HADAVI (2011) found that foliar application of citric acid during growth stage significantly increased post-harvest vase life of the cut *Lilium* flowers. In addition, the authors indicated that although any direct effect of citric acid on chlorophyll content was missing, the interaction effect between citric acid and malic acid on chlorophyll content was significant.

In here presented study, plants sprayed with citric acid showed a higher total chlorophyll content compared to control for both seasons (Fig. 2). The most effective concentration was 1.5 g/L of citric acid. Citric acid seems to be a substance contributing to osmotic adjustment during drought stress and helps minimizing the injury caused by dehydration to plant tissues, which is indicated by higher chlorophyll content in leaves. Although the mechanism behind this effect is not clearly explained, probably plays here the stomatal penetration by aqueous solutions a role. The results of BURKHARDT et al. (2012) supported the relevance of stomatal uptake of substances, as indicated by the different behaviors of adaxial/abaxial leaf sides. Moreover, a pH decrease in the apoplastic continuum with related effects on Fe and chlorophyll contents or distributions has been reported by KOSEGARTEN et al. (2001) and DARANDEH and HADAVI (2011).

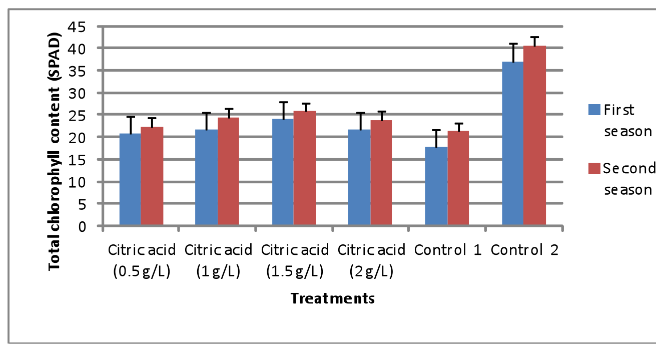

Fig. 2: Effect of different levels of citric acid on total chlorophyll content of leaves of bean plants subjected to drought stress conditions. Control 1: plants not treated by citric acid, but stressed. Control 2: plants not treated by citric acid and kept at field capacity during the entire experiments. Vertical bars present LSD value at 5%.

Effects of citric acid on water status of bean plants subjected to drought stress

Relative water content (RWC) in leaves is an indicator of plant water status and is markedly affected by drought stress. The RWC of non-stressed plants (watered at field capacity for the entire experiments, control 2) is indicated in Fig. 3. The plants of this treatment represented the normal water status of plants and showed the highest RWC (see the comparison between the control 1 and control 2, for both seasons). Interestingly, higher RWC were maintained also by citric acid applications at the end of drought stress period, especially at the level of 1.5 g/L, while control plants showed the lowest RWC in leaves of bean plants. This is in agreement with the statement of KRAMER (1983), who showed that the solutes involved in osmotic adjustment in response to drought stress vary, but consist of inorganic ions, carbohydrates and organic acids. MEREWITZ et al. (2012) observed an accumulation of several metabolites during drought conditions, including organic acids that were mainly involved in the citric acid cycle. The authors concluded that the accumulation of these metabolites could contribute to improved plant drought tolerance (in this case of creeping bentgrass) due to their roles in the stress response pathways such as stress signaling, osmotic adjustment, and respiration for energy production. LEVI et al. (2011) also indicated that the improvement of drought tolerance of some cot-

ton lines could be related to organic acid accumulation including citric acid. In addition, PECKMANN and HERPPICH (1998) indicated that increased net citric acid accumulation in response to drought of *Aptenia cordifolia* may result from the larger inhibition of the citrate degrading system relative to citrate synthesis. Moreover, HERPPICH and PECKMANN (1997) studied water relations and malic and citric acid accumulation in response to a short-term drought (10 days) in *Aptenia cordifolia* and found that while water potential was reduced with decreasing water content, bulk leaf pressure potential remained constant due to active osmotic adjustment. The authors reported that the changes in tissue osmotic content, which were fully reversible upon rewatering, resulted largely from variations in citrate content.
On the other hand, the investigation of SUN and HONG (2011) indicated that exogenous citric acid improved internal citric acid and helped improving stress tolerance of *L. chinensis* plants during saline stress conditions. The results from our study indicate that citric acid improved plant water status (as indicated by higher RWC) during drought stress and thus improved drought tolerance of bean plants. In accordance with here cited literature, the contribution of citric acid in improving water status maybe due to its role in osmotic adjustment during drought conditions. WILLIAMSON and MILBURN (1995) found also that citric acid treatment resulted in the highest RWC in stems of *Acacia amoena* in relation to water stress and indicated the involvement of citric acid in increasing hydraulic conductance.

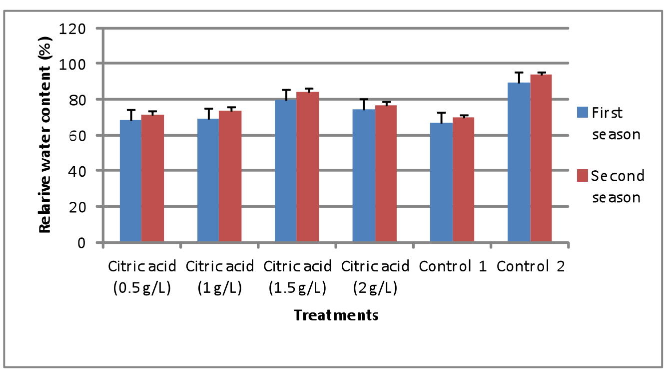

Fig. 3: Effect of different levels of citric acid on relative water content of leaves of bean plants subjected to drought stress conditions. Control 1: plants not treated by citric acid, but stressed. Control 2: plants not treated by citric acid and kept at field capacity during the entire experiments. Vertical bars present LSD value at 5%.

Effects of citric acid on post stress growth, quality and productivity of bean plants subjected to drought stress

In order to regulate water loss and prevent further dehydration, plants respond to drought stress by changes in their morphology, e.g. by decreasing leaf area, plant height and plant weight, and other plant parameters (GRUDA and SCHNITZLER, 2000). This is in agreement with the results of here presented study. Plant fresh weight and the number of branches per plants were reduced when the stress situation was induced, which is well documented by comparison of control 1 with control 2 for both seasons (Tab. 1 and 2). Not only the vegetative growth, but also the productivity and the quality of bean plants subjected to drought stress were negatively affected (Tab. 1 and 2). However, the data clearly revealed that foliar application of citric acid ameliorated the negative effects of drought compared to control. Vegetative growth parameters, expressed as fresh weight of plants and branches number per plant, generative development, expressed as number and weight of pods, as well as the quality of pods, expressed as length and pod diameter were improved by using of acid citric applications. These effects were significant especially at the level of 1.5 g/L of citric acid, being in accordance with results of physiological parameters. Increasing the citric acid concentration to more than 1.5 g/L led not to better effects for all parameters measured. Moreover, both seasons had the same trend concerning the effects of the treatments. The relatively better growth and productivity in the second (Tab. 2) than the first season (Tab. 1) could be related to climatic variations of the seasons. As it was mentioned before, leaf temperatures of the second season were higher than the first season (Fig. 1), due to relatively warmer climate conditions during the growth period that in turn was much favorable for the enhancement of bean growth and productivity.
The improvement of growth, productivity and quality of drought-stressed bean plants in response to citric acid application can be explained by the enhancement of water status during drought stress due to osmotic adjustment. As it was shown before, the physiological responses indicated a better water status especially at the level of 1.5 g/L of citric acid (Fig. 3). Changes in metabolites accumulation during drought and their relation to osmotic adjustment were discussed by SILVENTE et al. (2012), who indicated that metabolic changes in response to drought conditions play a significant role in the adjustment of metabolism and physiology of the soybean varieties. SUN and HONG (2011) showed that plant growth was significantly improved by citric acid application during saline stress conditions. According to authors, citric acid seem to be an important component of the stress response in *L. chinensis*.

Tab. 1: Effect of different levels of citric acid on vegetative growth, generative productivity and pod characteristics of bean plants subjected to drought stress conditions (first season). Control 1: plants not treated by citric acid, but stressed. Control 2: plants not treated by citric acid and kept at field capacity during the entire experiments.

Treatments	Plant fresh weight (g/plant)	Branches number/plant	Number of pods/plant	Pod length (cm)	Pod diameter (cm)	Fresh weight of pods (g/plant)
Citric acid (0.5 g/L)	165.53	4.67	13.67	7.50	0.67	57.60
Citric acid (1 g/L)	178.77	6.67	14.67	8.17	0.70	58.93
Citric acid (1.5 g/L)	243.12	7.67	16.33	9.81	0.77	64.13
Citric acid (2 g/L)	207.47	6.33	15.33	9.17	0.70	61.00
Control 1	162.00	4.00	13.33	7.33	0.63	54.50
Control 2	314.50	10.00	33.67	12.17	1.00	116.67
L.S.D. at 5 %	15.30	1.82	2.18	2.12	0.20	9.70

Tab. 2: Effect of different levels of citric acid on vegetative growth, generative productivity and pod characteristics of bean plants subjected to drought stress conditions (second season). Control 1: plants not treated by citric acid, but stressed. Control 2: plants not treated by citric acid and kept at field capacity during the entire experiments.

Treatments	Plant fresh weight (g/plant)	Branches number/plant	Number of pods/plant	Pod length (cm)	Pod diameter (cm)	Fresh weight of pods (g/plant)
Citric acid (0.5 g/L)	170.70	6.4	16.0	7.9	0.75	58.80
Citric acid (1 g/L)	180.82	7.3	16.4	9.7	0.78	62.93
Citric acid (1.5 g/L)	245.61	9.7	18.8	11.6	0.90	69.93
Citric acid (2 g/L)	210.02	7.9	16.7	10.5	0.80	64.20
Control 1	167.07	5.1	14.6	7.6	0.69	56.83
Control 2	321.04	13.0	37.0	12.3	0.97	120.67
L.S.D. at 5 %	15.71	1.33	2.37	1.88	0.14	7.52

It can be concluded that citric acid application helped improving water status of bean plants exposed to drought stress conditions and subsequently improved drought tolerance. Citric acid can be applied during or before an expected drought period for improving drought tolerance of bean plants. Using citric acid in combination with other technological tools, such as e.g., the application of drip irrigation, can minimize the problems of water shortage with particular emphasis on sustainable resource management and environmental protection. Under the conditions of Egypt spraying at a level of 1.5 g/L of citric acid is recommended.

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