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Proline and light as quality enhancers of rocket (*Eruca sativa* Miller) grown under saline conditions

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ABSTRACT

Stress conditions experienced during growth may affect plant responses during post-harvest storage and eventually determine the overall quality of commercial products. In this context, we hypothesized that foliar applications of proline during the growth cycle and light exposure during post-harvest storage could be two important modulators of yield and quality parameters of rocket plants exposed to NaCl stress. Dry matter percentage increased upon NaCl treatment. However, fresh weight loss during storage did not change over time as a consequence of salt stress. High salinity (100 mM NaCl) moderately reduced both leaf nitrate (14%) and nitrite (3%) contents. Lipophilic (LAC) and hydrophilic (HAC) antioxidant activities also decreased by 10% at the highest salinization (average of two growth cycles). In contrast, during storage, LAC decreased whereas HAC increased. Proline applications reduced the leaf nitrate content during storage by 16%, increased carotenoids and chlorophyll contents in salinized plants and also increased the ascorbate leaf concentration in both salinized and non-salinzed plants. Light storage enhanced fresh weight loss in contrast to dark storage. However the exposure to light reduced leaf nitrate levels by 7% (average of two growth cycles) and contributed to maintain high leaf ascorbate concentrations over time.

The combined control of plant physiological responses to environmental stressors and post-harvest storage parameters may affect the nutritional profile of fresh rocket and consequently should be considered to define standard production protocols to improve the nutritional qualities of commercial produce.

1. Introduction

There is an increasing interest of consumers towards quality aspects of fresh fruits and vegetable, with a particular attention to their content in health promoting compounds (Liu, 2003; Brandt et al., 2004). The production chain has promptly reacted to this market trend by launching a broad range of quality products with high nutritional value, such as selenium enriched potatoes (Turakainen et al., 2008) and high lycopene tomatoes (Canene-Adams et al., 2005), and functional properties specific to the prevention of several diseases (Rao and Agarwal, 1999). Many species of the *Brassicaceae* family have a relative rapid growth cycle and high content of phytonutrients including vitamin C, fiber, flavonoids, carotenoids and glucosinolates, which make them particularly suitable for the preparation of ready-to-eat products that best respond to these market needs (Barbieri et al., 2009; Winkler et al., 2007).

In recent years, the technology for the production of leafy vegetables has developed in parallel with soilless cultivation, which allows to effectively control plant growth and nutritional requirements (Gruda, 2009). Nevertheless, the full potential of these cultivation systems is rather underexploited since our knowledge of key physiological processes that can be functionally targeted to enhance the biosynthesis of specific metabolites is still limited. The tissue concentration of high-value metabolites, such as antioxidant compounds, is constitutive of certain species or cultivars but can also be increased/decreased by environmental stresses that would elicit the biosynthesis/catabolism of stress responsive molecules (De Pascale et al., 2001).

A thorough understanding of the functional biology of plant responses to different cultivation systems, environmental constraints and/or controlled imposition of different stressors is therefore pivotal to identify effective strategies to improve both the efficiency of the entire production process and products quality (Maggio et al., 2003). In this respect, an increasing salinization of the irrigation water is forcing farmers of arid and semi-arid regions to implement innovative techniques to preserve crop yield and quality while coping with the degradation of this major resource (Debaeke and Aboudrare, 2004). Over the last few decades, in parallel with breeding and biotechnological strategies to improve plant salinity tolerance (Maggio et al., 2003), several techniques to ameliorate plant performance in saline environments have been proposed. These include seeds/seedling priming (Azooz, 2009), pre-

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exposure to moderate stresses (Friedman et al., 2006), applications of stress metabolites that could be recognized and/or integrated by plants as components of stress-induced adaptation responses (Ashraf and Foolad, 2007). Foliar applications of osmoprotective molecules such as proline and glycinebtaine have been proven to have beneficial effects on plants exposed to salt stress (Ali et al., 2007). The actual mechanism through which these molecules exert their protective function is not fully understood. Most likely they accumulate in the cytoplasm and contribute to cellular osmotic adjustment, which is necessary to overcome hyperosmotic stress (Yoshiba et al., 1997). Alternatively, osmoprotectants may act as stress signals that elicit a series of protective responses, including the accumulation of antioxidants and other high-value metabolites that contribute to stress adaptation (Maggio et al., 2002a). While the application of these molecules has been demonstrated to improve yield of salt stressed crops (Ashraf and Foolad, 2007), it is largely unknown its effect on quality parameters of the commercial products. Nevertheless, the link between stress adaptation and accumulation of high-value metabolites may have a critical significance in agricultural productions (De Pascale et al., 2001). In some areas of Sicily, salinity of the irrigation water is a fundamental quality determinant of typical tomato productions such as those of the Pachino region (Restuccia et al., 2003).

The accumulation of antioxidative molecules during plant growth (pre-harvest) may also have important implications during post-harvest transportation and storage (Huang et al., 2007). Leafy vegetables are relatively perishables products that should be consumed within a few days, since their shelf-life is of approximately 7–10 days (Barbieri et al., 2009; Winkler et al., 2007). Upon harvest, leaves tend to lose their turgidity and crispness as a consequence of water loss and activation of wound-induced chemical and enzymatic reactions that lead to tissue oxidation. These modifications will alter the vegetables nutritional profile since antioxidant molecules can be either newly synthesized in response to wounding (Leja et al., 2001) or recruited to detoxify Reactive Oxygen Species (ROS) (Lee and Kader, 2000). Consequently, a total control of the growth determinants that may define the nutritional profile of fruit and vegetable is essential to valorize both products and production systems.

Rocket (*Eruca sativa* Miller) is rather sensitive to salinity (Salama et al., 1981), with a considerable genetic variability for this trait (Ashraf, 1994), and responds well to hydroponic cultivation (D'Anna et al., 2003). Here we hypothesized that foliar applications of proline may ameliorate the performance of rocket plants and enhance the accumulation of functional metabolites under saline stress. In addition, we considered the indirect effects of proline treatments on tissue accumulation of nitrates and nitrites, whose levels are particularly important in leafy vegetables. We finally evaluated the combined effect of pre- and post-harvest variables to identify critical steps from *farm-to-fork* that may affect the accumulation of nutritionally valuable metabolites and the overall quality of fresh vegetables.

2. Materials and methods

2.1. Growth conditions and post-harvest treatments

Two independent experiments were performed in spring (Experiment 1) and summer (Experiment 2) 2008. Rocket plants were grown in unheated greenhouses at the University of Naples Federico II (40°49′N; 14°15′E; 30 m above sea level) using a floating system (De Pascale et al., 2008). Rocket seeds were directly sown in 0.25 m^2 polystyrene trays, filled with a 1:1 (v/v) mixture of local soil (42.3% sand; 23% loam; 34.7% clay) and peat that floated continuously in 4 m^2 containers (0.3 m high) filled with 1000 L of aerated nutrient solution, which was replaced every week. In the spring growth cycle, rocket seeds were sown on March 18, plants were harvested on April 24 (39 Days After Sowing – DAS), while in the summer cycle, seeds were placed in the tray on June 5 and plants were harvested on July 2 (27 DAS). In both experiments, at the stage of 4 leaves, root systems had well grown and expanded below the polystyrene trays and were fully submerged in the nutrient solution. Therefore the soil–peat mixture had a nutritional function only during seed germination and at early stages of seedlings development, after which the small amount of soil (mostly sand) had only a physical support function, since the root systems were not in contact with it. This procedure was used to avoid further stress caused by transplanting the young seedlings after germination. The final plant density was 100 plants m^{-2}.

The composition of the nutrient solution used in all experiments was N-NO_3 14 mM; N-NH_4 6 mM; Cl 3.0 mM; PO_4^{3-} 3.5 mM; S 6.0 mM; Ca 5.0 mM; Mg 3.7 mM; K 10.5 mM; Na 2.2 mM; B 0.02 mM; Fe 0.04 mM. At 26 DAS (first cycle) and 19 DAS (second cycle), leaves were sprayed with a solution of 20 mM proline in distilled water. This concentration was chosen based on published literature (Heuer, 2003; Ben Ahmed et al., 2010) and to avoid osmotic stress. Control plants were sprayed with distilled water only. Twenty-four hours after the proline treatment, NaCl was added to each 4 m^2 growing unit (2 per salinity level) to a final concentration of 25, 50 and 100 mM NaCl. Overall, each growing unit contained fourteen polystyrene trays, each containing 24 plants. Seven of the fourteen trays were sprayed with proline whereas the remaining seven were sprayed with distilled water (proline control plants). Two days before harvest, a second proline treatment was applied to the plants. For each growing cycle, the experimental design was a randomized block with two replications.

2.2. Analytical procedures

Marketable and non-marketable yields were recorded at harvest. In Experiment 1 (spring cycle), intact leaves were accurately washed three times with sterile water, while in Experiment 2 (summer cycle), leaves were washed with a solution of ascorbic acid (150 mg L^{-1}). In both cases cut leaves were air-flow dried on a strainer. The leaves were subsequently kept in plastic boxes. The containers were wrapped with a multistrate film (PET 12+ COEX/EVOH/PE 95), highly impermeable to gases and water vapour, with permeability to O_2 < 5 dm^3 m^{-2} d^{-1} bar^{-1} at 23 °C and 0% U.R. and to H_2O < 5 g m^{-2} d^{-1} bar^{-1} at 38 °C and 100% U.R. (Gruppo Fabbri S.P.A., Modena, Italy).

For each growing cycle, the plastic containers were kept at 4 °C for 6 days, under two storage regimes: (1) dark conditions (control); (2) daily 8-h exposure to low light intensity (PPFD = 16 μmol m^{-2} s^{-1}). Leaf samples were collected on days 0, 2, 4 and 6 of storage for fresh and dry (after drying at 60 °C) weight measurements. The dry matter percentage (DM%) and weight loss were thus calculated.

Nitrates and nitrites were measured with a spectrophotometer (HACH DR/2000, Hach Co., Loveland, CO, USA) on dried and ground tissue samples after cadmium reduction following the procedure of Sah (1994).

Two different cation assays were utilized to measure the antioxidant activity of the hydrophilic (HAC) and lipophilic (LAC) fractions on liophilized leaf samples. The antioxidant activity was measured on the water-soluble fraction using the DMPD [N,N-dimethylpphenylenediamine] method and expressed as mM of ascorbic acid (ASA)/kg^{-1} of dry weight (Fogliano et al., 1999). The lipophilic fraction was measured with the ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] method performed as described by Pellegrini et al. (1999) and expressed as mM of Trolox for kg^{-1} of dry.