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Fertilization strategies as a tool to modify the organoleptic properties of raspberry (*Rubus idaeus* L.) fruits

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ABSTRACT

Raspberry cultivation is increasing all over the world, being these fruits highly appreciated not only for their taste but also for their high content in bioactive compounds. However, the short shelf-life of these fruits limits their fresh consumption and industrial use to a defined time frame. For this reason, setting agronomic strategies aimed at extending the fruit shelf-life represent a prerequisite to ensure the presence of high-quality raspberries on the market for a prolonged time. Therefore, aim of this work was to assess the influence of different nutritional regimes (over-fertilization with NH_4^+, Si or B) on the firmness and the potential extension of the shelf-life as well as on the quality of raspberry fruits.

Raspberry canes (*Rubus idaeus*) cv. 'Lagorai Plus' were grown in a high tunnel plastic greenhouse and starting from middle July to September raspberry fruits were collected. The over-fertilization with NH_4^+, Si or B lead to higher yields, increased colour indexes, firmness, sucrose content and sweetness indexes. Furthermore, the fertilizations led to an improved shelf-life due to a significant reduction of fruit darkening and fruit weight loss, which was particularly evident at the end of the incubation.

The results of this work highlighted that the modification of the nutritional status of raspberries by adding either Si, B or NH_4^+ could be helpful in obtaining fruits with improved qualitative parameters. In particular, an increase in the sweetness index and firmness of fruits as well as an extension of the shelf-life could be beneficial for the attractiveness of consumer and also for the possibility of using fruits in the industrial transformations. Moreover, considering the role of Si and B in human health, the bio-fortification programs can become particularly interesting, thus opening the way for new market perspectives of these fruits in addition to the traditional ones.

1. Introduction

Raspberries (*Rubus idaeus* L.) are cultivated in many countries worldwide, but particularly in Eastern Europe and in the USA (Giovanelli et al., 2014) having a significant economic relevance for farms (Hristov et al., 2009). As well as other berries, the global production of these fruits is increasing, being highly appreciated by consumers not only for their good taste but also for their high content in health beneficial compounds (Rao and Snyder, 2010). Indeed, many studies have shown that raspberries are a major source of antioxidants because of their high concentration in polyphenolic compounds such as anthocyanins, flavonols, catechins, ascorbic acid and ellagic acid derivatives (Beekwilder et al., 2005; Deighton et al., 2000; Moyer et al., 2002; Sariburun et al., 2010). In particular, these compounds can scavenge free radicals (Zhang et al., 2012) helping the prevention of chronic disease and some forms of cancer (Beattie et al., 2005; Ross et al., 2007; Seeram, 2008).

Moreover, beside their positive effect on human health, phenolic compounds can also considerably affect the shelf-life and postharvest quality of the berries (Hodges and DeLong, 2007; Khanizadeh et al., 2009). In fact, the seasonality of the raspberry cultivation and the fast senescence characterizing these fruits after the harvest (i.e. short shelf-life) limits their fresh consumption and industrial use to a defined time frame (Han et al., 2004). Postharvest life of red raspberries represents a main issue for this fruit crop since it is only limited to a few days (generally 2–3 days from picking) (Giovanelli et al., 2014). This is mainly due to the loss of firmness and their susceptibility to fruit rot (Khanizadeh et al., 2009), caused by their structural fragility and their high respiration rate (Haffner et al., 2002). As a consequence, fresh fruits are only available in the ripening seasons and are mostly

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consumed locally. For this reason, setting agronomic strategies aimed at extending the fruit shelf-life (increasing its conservation and storability without impacts on the nutritional value) can have an interest not only in terms of more prolonged presence in the market of high-quality raspberries, but also from the economy point of view of a specific production area.

It is widely demonstrated that mineral nutrition has a thorough impact on both fruit yield and quality (Álvarez-Fernández et al., 2003; Pestana et al., 2010; Tomasi et al., 2014; Valentinuzzi et al., 2015) as well as shelf-life (Dalla Costa et al., 2011). In this respect it is interesting to mention that very recently a relationship between anthocyanins in strawberries and nitrogen (N) fertilization has been reviewed by Jezek et al., (2018). An additional example can be represented by boron (B) that, when applied in field cultivation both on soil and on leaves, not only increased tomato weight by improving fruit set, but also improved fruit firmness and shelf-life (Davis et al., 2003). Moreover, Wojcik, (2005) showed an increased berry strength in raspberries cv. Polana fertilized with B. This phenomenon was related by the authors to a possible increase in the number of berry drupelets; in fact, Robbins and Moore, (1990) observed that raspberries with a higher number of drupelets were firmer than those of a similar size with fewer large drupelets. In addition to N and B, it is also interesting the case of silicon (Si). Although its beneficial effects have been extensively proven, Si is usually omitted from the composition of nutrient solutions (Gottardi et al., 2012). Indeed, its supplementation increases significantly plant fitness with a resulting enhancement of agricultural production (Montesano et al., 2016; Savvas and Ntatsi, 2015). Furthermore, Stamatakis et al., (2003) observed an increase in total solid solutes, vitamin C and fruit firmness in tomatoes by adding K_2SiO_3 to the hydroponic system. Moreover, Si has shown to prolong the shelf-life of strawberry fruits (usually few days) harvested from plants supplemented with bio-available Si (Babini et al., 2012). However it should be noted that fruit yield and its quality are not exclusively related to the presence or absence of a specific nutrient/element, but also to the nutrient form supplied with the fertilizers. In this context, N represents a particular example. In fact, it is well demonstrated that a nitrate-based fertilization affects rhizosphere pH values in a totally opposite way than an ammonium-based one (Hinsinger et al., 2003). In this latter case, the ammonium-induced acidification of the soil surrounding the roots affects consistently the availability of some other nutrients (e.g Fe, Thomson et al., 1993), influencing therefore the whole nutrient acquisition process of roots. These phenomena, pretty well described for some crops such as strawberry, are still quite unknown in raspberry, particularly when fruit quality parameters are considered.

Therefore, in the present work the effect of N (ammonium), B or Si availability on the firmness and an extension of the shelf-life of raspberry fruits, has been assessed. To this purpose, growth medium (ammonium) or foliar (B or Si) fertilizations were applied to the plants. At harvest, quality parameters (colour, sweetness index, sugar, organic acid contents) and the bioactive compound (phenolic compounds) concentration of raspberry fruits were assessed and related to the overall fruit quality as a function of the different nutritional treatments.

2. Materials and methods

2.1. Plant growth

After a storage period between −1 °C and 1.5 °C raspberry floricanes (*Rubus idaeus*) cv. 'Lagorai Plus' were transplanted at the end of May to 6.5-L pots containing coconut fiber and transferred into a high tunnel plastic greenhouse for the growing and fruiting period. 200 cane plants per treatment were utilized.

The experiment was laid out in a randomized block design, with five replicates per treatment. Each block consisted of 40 cane plants and thirty centrally located plants per block were used to collect vegetative and qualitative parameters.

Control plants (Control) were fertigated with the following nutrient solution (NS): NO_3^- 6 mM, $H_2PO_4^-$ 1 mM, SO_4^{2-} 1.5 mM, NH_4^+ 0.6 mM, K^+ 3 mM, Ca^{2+} 4 mM, Mg^{2+} 4 mM, Fe^{2+} 30 µM, Mn^{2+} 20 µM, Zn^{2+} 8 µM, B 12 µM, Cu 1.75 mM, Mo 0.75 mM.

For a second set of plants ($+NH_4^+$) NH_4^+ was added to the NS once a week, while for a third set (+Si) Si (K_2SiO_4 3 mM) was added to the NS during the whole growing period. The fourth set of plants (+B) was fertigated as + NH_4^+ and treated foliarly with 1.5 mg H_3BO_3 plant^{-1} (Wojcik, 2005) applied 4 times during the growing season (2 weeks before flowering, at flowering, 3 and 6 weeks after flowering). The solution was characterized by an average Electric Conductivity (EC) of 2000 µS cm^{-1} and a pH = 5.5. Harvest season started on end of July and ended in September.

2.2. Measurement of plant growth and fruit productivity

During the growing period, SPAD index of fully expanded leaves was determined using a portable chlorophyll meter SPAD-502 (Minolta, Osaka, Japan). Measurements were carried every two weeks on both basal and apical leaves (at least two per plant), and five SPAD measurements were taken per leaf and averaged. At the end of the harvest season, raspberry plants were collected separating lateral braches from the cane and fresh weight (FW) of both biomasses was measured.

At harvest, total fruit yield, yield per plant (kg FW plant^{-1}), average fruit yield (g FW) and number of discarded fruits were assessed.

2.3. Characterizazion of fruit quality

Raspberry fruits were harvested once at least 80% of the fruit surface showed a red coloration. The color index (CI) to express the intensity of red color was determined using a portable tristimulus colorimeter (Chroma Meter CR-400, Konica Minolta Corp., Osaka, Japan) and calculated as $CI = 100 \times a/(L \times b)$ with higher values expressing a more intense red color (Tezotto-Uliana et al., 2014). Total soluble solid contents, expressed in Brix degrees (°Bx), were determined using a digital refractometer (Atago, Tokyo, Japan) on fresh extracted fruit juice. Titratable acidity (TA) was determined as previously described (Valentinuzzi et al., 2015). Raspberry firmness was assessed on fresh fruits with a penetrometer (model PCE-FM200; PCE Instruments, Southampton, UK) equipped with a cylindrical probe 3 mm in diameter and expressed in Newton (N).

2.4. Raspberry extracts

Freeze-dried raspberry samples were ball-milled (model MM400; Retsch, Haan, Germany) to obtain a homogeneous powder; the ground samples were extracted with methanol (HPLC grade, Merck, Darmstadt, Germany) using a 1:10 extraction ratio. The mixtures were then sonicated for 30 min in a thermostatic bath and centrifuged at 14000xg for 30 min at 0 °C; finally, the supernatant was collected and filtered through a 0.2 µm nylon filter (Phenomenex Inc., USA).

2.5. Organic acid and sugars analyses

The separation of both organic acids and sugars was performed by HPLC using a cation exchange column Aminex 87-H column (300 × 7.8 mm, 9 mm, Bio-Rad) using an isocratic elution with 10 mM H_2SO_4 as mobile phase at a flow rate of 0.6 mL min^{-1}. Organic acids were detected at 210 nm with a Waters 2998 photodiode array detector (Waters Spa, Italy), whilst sugars were detected by a refractive index detector (Waters Spa, Italy). Standard acids and sugars were prepared as individual stock solutions and then combined to give diluted reference standards. Organic anions and sugars were identified by comparing retention times of unknowns to pure compounds. Standards were purchased from Sigma–Aldrich (St. Louis, MO, United States). Sweetness index (SI) was calculated as in Mahmood et al., (2012).