



## Mild salt stress improves strawberry fruit quality



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### ABSTRACT

Strawberry is one of the most popular fruits because of its shape, color and taste, and the presence of antioxidant compounds. Because severe abiotic stresses result in detrimental consequences to plant growth, the effect of cultivating under mild stress conditions has rarely been investigated. Therefore, we evaluated the effect of mild salt stress on yield and quality of strawberry fruit. Mild salt stress did not affect yield. The lower level of mild salt stress evaluated showed increased vegetative growth (24%), higher photosynthetic effectiveness, and increased activity of phenoloxidase (22%) and polyphenoloxidase (33%), as well as the accumulation of sucrose (5%) and anthocyanins (60%) in the fruit, compared to non-stressed plants. The higher level of mild salt stress increased root growth (30%), the activity of phenylalanine ammonia lyase (68%), and the accumulation of total phenolic compounds (14%), and total antioxidant activity (13%) in the fruit, compared to non-stressed plants. The only phenolic compound improved in these treatments was (+)- catechins. Both levels of salt stress affected the expression of genes involved in the phenylpropanoid and flavonoid pathways, cell wall disassembly and abscisic acid-related genes. Therefore, mild salt stress improves strawberry fruit quality.

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### 1. Introduction

To meet increasing food demands under the anticipated scenario of climatic change, it will be necessary to expand our agricultural systems to drier and more saline lands. Intensive production systems such as soilless culture, in which saline solutions supply nutrients to plants, have recently increased in use to address intensified food market demand. Moreover, due to the reduction in water resources available for crop production, there will be an increase in the use of poor quality irrigation water in the future (Huang et al., 2012; Krasensky & Jonak, 2012). Therefore, it is of great importance to evaluate the effect of salt stress on crop development.

Although severe abiotic stresses such as salt stress result in detrimental consequences to plant growth, mild stress conditions may be deliberately applied to improve the content of antioxidant compounds in the edible part of the plant, and stimulate plant adaptation to stress-prone environments (Kim, Fonseca, Choi, Kubota, & Kwon, 2008; Cogo et al., 2011; reviewed by; Ripoll

et al., 2014). The consumption of fruit and vegetables containing antioxidant compounds is positively associated with the prevention of several chronic diseases and the improvement of general health; therefore, efforts to increase the nutritional and functional quality of foods during plant cultivation, an approach known as biofortification, are of great interest (Messias, Galli, Silva, Schirmer, & Rombaldi, 2013; Zhu et al., 2013).

The strawberry (*Fragaria × ananassa* Duch.) is one of the most popular fruits because of its taste and well-recognized health-promoting properties due to the presence of potential functional compounds such as phenolic compounds (Giamperi et al., 2012). Its demand and availability in the market has widely increased, making this fruit a target of biofortification efforts. Strawberry plants are sensitive to salt stress, but are cultivated using fertilizers and water of inadequate quality for irrigation, resulting in a gradual buildup of salt in the soil (Jamalian, Gholami, & Esna-Ashari, 2013). Despite this fact, the effect of cultivating strawberry plants under mild salt stress conditions has rarely been investigated, especially the effects on fruit quality. Moreover, the molecular mechanisms underlying the effects of mild stress remain to be elucidated. Therefore, in the present study, we evaluated the effects of different levels of mild salt stress on several biochemical, physiological and molecular aspects of plant growth and the yield and quality of

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strawberry fruit, as well as the mechanisms underlying these effects.

## 2. Materials and methods

### 2.1. Experimental design and treatments

The experiment was conducted in a greenhouse at the Brazilian Agricultural Research Corporation (Embrapa Temperate Agriculture/Pelotas/RS/Brazil). Strawberry seedlings of the Camarosa cultivar were transplanted and grown in 9 L pots containing a mixture of soil (Ultisoil, 6.6 kg/pot) and vermiculite (2.2 kg/pot). The fertilizer was composed of urea, triple superphosphate and potassium chloride as sources of 267 kg/ha N, 619 kg/ha P<sub>2</sub>O<sub>5</sub> and 333 kg/ha K<sub>2</sub>O, respectively. Irrigation was performed by a drip irrigation system through daily dripping. The relative soil moisture was always maintained between 16 and 19% (soil saturation) with no water leaching.

The experimental design was completely randomized with three treatments and four replicates per treatment, as follows: C (control); L1 (stress level 1 – salt solution of 40 mmol/L NaCl in distilled water); L2 (stress level 2 – salt solution of 80 mmol/L NaCl in distilled water). For the salt stress treatments (L1 and L2), 50 mL of salt solution was applied once a week from the beginning of the flowering stage (105 days after transplanting – DAT) to the end of the crop cycle (190 DAT). The same volume of distilled water was applied in the C treatment.

Mature fruits (fully red, according to Jia et al. (2011)) were sampled at the end of the experiment, frozen in liquid nitrogen, and stored at –80 °C until analyzed. The experimental timeline is shown in Fig. 1.

### 2.2. Soil electrical conductivity

Soil electrical conductivity was determined using a conductivitymeter (Tecnal, TEC-4MPP model). The samples were diluted in distilled water (1:5 v/v) prior the quantification. These measurements were performed after two (measurement M1), seven (measurement M2) and ten (measurement M3) applications of the treatments (Fig. 1), and are presented in microsiemens per centimeter (μS/cm).

### 2.3. Photosynthetic parameters

The CO<sub>2</sub> assimilation rate of the plants was monitored with a portable gas exchange fluorescence system infrared gas analyzer

(IRGA) (Heinz Walz GmbH, GFS 3000 model), using 500 ppm of CO<sub>2</sub> and 800 ppm of light as parameters. Five measurements (M1 to M5) (Fig. 1) were performed during the crop cycle, at the same time of the day (from 11:00–14:00), and using new, fully developed leaves from three of the six plants in each replicate. The results are presented as mmol/m<sup>2</sup>/s.

### 2.4. Yield of fruit, fresh biomass and root biomass

For the determination of crop yield, fruits in the commercial stage of ripening (full red, according to Jia et al., 2011) were sampled and weighed throughout the crop cycle. Fresh plant biomass was determined at the end of the cycle by weighing the aboveground portion of the plant. The underground portion of the plant was also weighed to quantify root biomass. The results are expressed in grams per plant (g/plant).

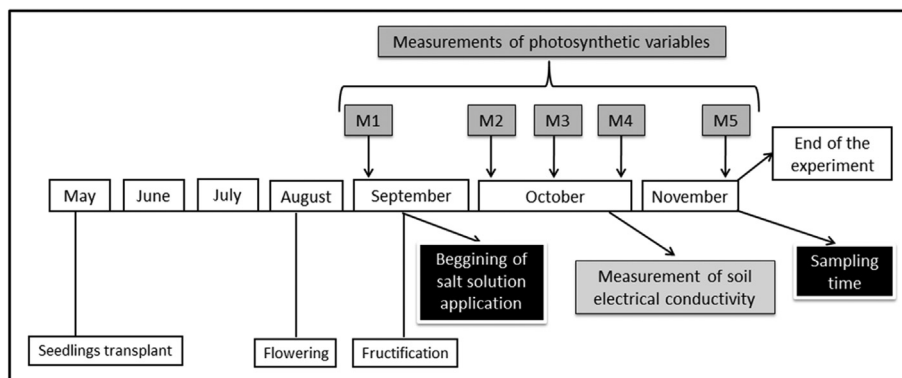
### 2.5. Content of sodium (Na) and chloride (Cl) in strawberry leaves

The content of Na and Cl was determined in dried leaves, according to the method described by Tedesco, Gianello, Bissani, Bohnen, and Volkweiss (1995). These measurements were performed in four biological replicates and three analytical replicates. The results are expressed as grams per kilogram of leaves (g/kg).

### 2.6. Quality traits of strawberry fruit

The method described by Nelson (1944) was used to quantify and reducing sugars in lyophilized strawberry fruit. Samples were subjected to an acid hydrolysis to determine the total content of sugars. The difference between the total content of sugars and the content of reducing sugars was considered to correspond to the content of sucrose. The results are expressed as g/kg. Total phenolic content was quantified following the method from Swain and Hillis (1959). The data are presented as grams of gallic acid equivalents per kilogram of fruit (g GAE/kg fruit). The total anthocyanins content in the strawberry fruit was determined according to Zhang, Pang, Yang, and Jiang (2004). The data are presented as grams of pelargonidin equivalents per kilogram of fruit (g PE/kg fruit). The methodology described by Brand-Williams, Cuvelier, and Berset (1995) and Arnao, Canoa, and Acosta (2001) was used to determine the total antioxidant activity. The data are presented as mmol/L of Trolox equivalents per grams of fruit (mmol/L TE g fruit<sup>-1</sup>).

Individual phenolic compounds were quantified from 0.5 g of the lyophilized sample suspended in 30 mL of methanol. Next,



**Fig. 1. Timeline of the experiment with strawberry plants.** Strawberry plants (Camarosa cv.) were cultivated under mild salt stress. The measurement of soil electrical conductivity and photosynthetic variables (M1 to M5) performed during the experiment are represented by light and dark gray squares, respectively. The beginning of the salt stress application and the sampling dates of fruit and leaves used in biochemical and molecular measurements are represented by black squares.

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