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Research article

# Silicon beneficial effects on yield, fruit quality and shelf-life of strawberries grown in different culture substrates under different iron status



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#### ABSTRACT

The silicon application either as foliar or to the radicular system of strawberry plants was investigated. Fortuna strawberry plants were grown in two different substrates (coconut fibre and organic substrate) under optimal (20  $\mu$ M) or low (5  $\mu$ M) iron (Fe) conditions. During the study, crop parameters including leaf area, SPAD and fruit yield were measured. At harvest, fruit quality and post-harvest shelf-life were evaluated. Results indicated that "Fortuna" strawberries plants had a poor development in coconut fibre and excellent growth and yield in the organic substrate. In the coconut fibre substrate, no differences in foliar area, fruit diameter, colour, pH and shelf-life were observed related to the Si addition under deficient Fe conditions, but an increased in weight and the firmness of the fruits, as well as in fructose content was shown. However, when 20  $\mu$ M Fe were supplied, the root application of Si significantly increases: protein, mineral and sugar content, as well as fruit shelf-life by an average of 1.5 days. Likewise, the radicular silicon application to the organic substrate considerably improved yield, fruit diameter, fruit weight, glucose and fructose fruit content and the firuit shelf-life without causing distinguishable chemical or physicochemical changes. In summary, Si application to Fortuna strawberries through the roots could be a good solution to increase fruit quality and yield and to increase benefits from the agronomical point of view. Further studies in other strawberry varieties and dose rates will allow knowing with better precision how the radicular application of silicon contributes to yield and fruit shelf-life.

#### 1. Introduction

The essential roles of silicon (Si) in plant systems have been extensively studied by several plant biologists for years, yielding the definition of Si as a quasi-essential or beneficial element for plants (Castellanos-González et al., 2015). Plants absorb Si as silicic acid [Si (OH)4], at pH below 9 (most of the agricultural soils). Therefore, all plants grown in soil contain some Si in their tissues (Hernández-Apaolaza, 2014). Silicon achieves functions in regulating the physiological, biochemical, and antioxidant metabolism in plants to alleviate abiotic and biotic stresses (Aleshin, 1988; Hodson and Sangster, 1988; Ordeñana, 2002; Gonzalo et al., 2013; Pascual et al., 2016; Castellanos-González et al., 2015; Carrasco-Gil et al., 2018). Silicon also helps in the formation of organic defence compounds through the alternation of gene expression (Aleshin, 1988; Hodson and Sangster, 1988; Ordeñana, 2002), or relieving diseases such as *Botrytis* or *Spodoptera* (Snyder et al., 2007).

In this sense, authors such as Miyake and Takahashi (1986) studied the effect of  $SiO_2$  (50 mg/L) in strawberries, observing that it favoured the development of the plant and produced fruits of greater weight than those without application. On the other hand, Matichenkov (1990) considered that to obtain benefits against the problems caused by pests and diseases the concentrations of Si in the plant tissues should be high (4-8 Tm/ha). Recently, Moreno-Guerrero et al. (2017) got similar results in strawberries applying high rates of Si (2.5 and 5.0 g/L) obtained from SiO<sub>2</sub> and CaSiO<sub>3</sub>. However, not all the studies carried out to reveal the beneficial effects of silicon. For example, Lieten (2000) found that high concentrations (150 mg/L) of silicon as K<sub>2</sub>SiO<sub>3</sub> in the irrigation water or in the nutrient solution increase the albinism in strawberry fruits because of the decrease in anthocyanins. The silicon forms added in these above mentioned studies were, or a slightly soluble form like the SiO<sub>2</sub> or, heavily increased nutrient solution or soil pH at the rhizosphere (silicates), which promoted the precipitation of metals as oxyhydroxides, diminishing their availability to plants and consequently given some nutritional imbalances.

On the other hand, iron deficiency is a very important plant nutritional disorder worldwide, especially for plants grown in calcareous soils. Iron deficiency symptoms have been profusely described as

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interveinal leaf yellowing or even leaf necrosis, and crop yield and quality could be severely reduced. Several hypotheses have been proposed to explain the effect of Si on Fe nutrition, Carrasco-Gil et al. (2018) showed that a different Si effect was expected depending on plant Fe status. Under Fe sufficiency, Si supply increased Fe root plaque formation under calcareous conditions, decreasing Fe concentration inside the root and therefore activating the Fe acquisition strategies. Under Fe deficiency, Si treated plants absorbed Fe from the plaque more rapidly than non-Si treated plants, due to the previous activation of Fe deficiency strategies. Moreover, Fu et al. (2012) concluded that Si increased Fe transport from root to shoot suggesting that the increased expression of Si transporters after Si addition might influence Fe uptake and translocation and will benefit Fe nutrition under deficiency conditions. But Si influence on iron deficiency might depend on the plant species treated.

The aim of this work is to show, for the first time, the effect of silicic acid (H<sub>4</sub>SiO<sub>4</sub>) supply, either to leaves or roots of strawberry plants; since previously, only studies had been carried out through the addition of different Si compounds (SiO<sub>2</sub>, CaSiO<sub>3</sub>, K<sub>2</sub>SiO<sub>3</sub> and K<sub>2</sub>SiO<sub>4</sub>). Additionally, the effect of Si in conditions of Fe deficiency and sufficiency is provided. The application of silicates to the nutrient solution implies an increase of solution pH, decreasing the absorption of Fe and other micronutrients, which will precipitate into the growth media. Silicic acid should be added at the optimal pH for each plant species growth. Moreover, this compound is very soluble and ready to absorb by plants; in contrast to other less soluble compounds added (i.e. SiO<sub>2</sub>, slags, etc). As mentioned, this compound (H<sub>4</sub>SiO<sub>4</sub>) is more soluble and easier to absorb by plants than the others, and do not alter the pH of the growing media. Also, this effect will be studied under conditions of deficiency and sufficiency of Fe because of the existing interaction between silicon and iron. Finally, due to the trend in the use of coconut substrate in fertigation crops, this effect will be analysed in two types of substrates (coconut fibre and organic substrate) to check the adaptation of this variety to both substrates.

#### 2. Material and methods

#### 2.1. Plants growth

The strawberry plantlets of variety "Fortuna" (Fragaria ananasa Duch. var. Fortuna) were provided by Solyfres S.L. (Huelva). Selection of this variety was based on their precocity, productivity and balance throughout the production season. Strawberries plantlets were planted on December 9, 2017 in Náquera (Valencia, Spain) and fruits were collected between February-July 2018. During this period the minimum average temperatures ranged was 6-13 °C and the maximum temperatures 15-22 °C. The accumulated rainfall was 214 mm (AEMET, 2019).

The substrates used were coconut fibre (FC) (typically used for growing strawberries) and a commercial organic substrate (SO). The selection was based on their physicochemical composition and on the preferential used by growers.

The coconut fibre (FC) substrate (Slab Rhizo Slab of U-Gro®) main components were 40% of coconut peat, 30% of fibres, 30% of chips and endomycorrhizas. Its physicochemical main characteristics were pH 5.5-6.5; electrical conductivity (EC) (1: 1.5): < 0.6 mS/cm; water holding capacity: 60%; organic matter content (% dry weight): 94.2; P: 1.10 mg/L; K: 37.0 mg/L; Fe: 0.282 mg/L; Mn: 0.004 mg/L; Zn: 0.028 mg/L; Cu: 0.001 mg/L. Each substrate bag contained 15 L of the substrate.

The organic substrate selected (COMPO® Substrate Huerto Urbano®) was a commercial organic substrate (SO). Its main components were high-quality tundra (degree of decomposition H3-H8), vegetable waste compost, lime and organic fertilizer (guano and horn flour). The pH of the substrate was 5.0-6.5; salt content: < 3.0 g/L; nitrogen: 80-400 mg/L; phosphate: 100-500 mg/L; potassium oxide:

300-900 mg/L; Cu: 1.43 mg/kg; Fe: 365.97 mg/kg; Mn: 127.88 mg/kg; Zn: 0.35 mg/kg. Each substrate bag contained 20 L of the substrate.

Because of iron low concentration in coconut fibre substrate (FC) and in order to test if, the Fe status may affect fruit growth and development and the shelf-life of the harvested strawberries, different Fe concentrations were applied to the FC grown strawberry plants. For that purpose, three iron treatments were tested: Fe0 (no Fe in the nutrient solution), Fe5 (5  $\mu$ M of Fe in the solution = iron deficiency) and Fe20 (20  $\mu$ M of Fe in the solution = iron sufficiency). While that in the organic substrate (SO) no iron was applied due to its presence in the substrate composition. Iron solutions were prepared by dissolving a Fe (III)-EDDHA commercial product with a declared 4.8% of Fe as FeEDDHA (Ferrilene® 4.8 Valagro®) in the water at final concentrations of 5 and 20  $\mu$ M.

For both, Fe deficiency and sufficiency plants, silicon was added either to the leaves (L) or to the roots (R) of the plants maintaining for each Fe concentration a control (without silicon application). The Si source was SiO<sub>4</sub>H<sub>4</sub> and was freshly prepared as described by Nikolic et al. (2007) passing Na<sub>2</sub>SiO<sub>3</sub>·5H<sub>2</sub>O (Sigma-Aldrich, Germany) throughout a column containing cation-exchange resin in its H<sup>+</sup> form (Amberlite IR 120<sup>+</sup>, Sigma-Aldrich, Germany). The silicon was applied at a concentration of 1.5 mM both in plants with foliar and root treatments, differing only in the way of application. In root treatments, Si concentration was calculated according to the volume of the substrate bag to achieve the final 1.5 mM and was applied by irrigation, while Si was applied as a spray in foliar treatments in a similar amount that when applied to the roots. The application of Si was carried out in three vegetative stages: inflorescence emergence, flowering and development of fruit. Si was applied when two of the plants per treatment had inflorescence or flowering while during fruit development Si was applied when fruits had 1 mm of size in two of the plants.

Both substrates were irrigated daily with a macronutrients solution by using a fertigation system with a 1000 L tank, while iron and the rest of the micronutrients were applied manually with a syringe once a week in FC plants only. The final nutrient solution composition were: KH<sub>2</sub>PO<sub>4</sub> 0.25 mM, Ca(NO<sub>3</sub>)<sub>2</sub> 5 mM, MgSO<sub>4</sub> 1.25 mM, K<sub>2</sub>SO<sub>4</sub> 1.75 mM, KCl 0.25 mM, H3BO3 25 µM, MnSO4 1.25 µM, ZnSO4 1.5 µM, CuSO4 0.5 μM, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> 0.025 μM (Valentinuzzi et al., 2015; Mimmo et al., 2017). The pH, conductivity and concentrations of the 1000 L tank solution were measured every 2 weeks.

Substrates and treatments were distributed randomly in the field. For each treatment (substrate bag), four plants were planted and each plant was considered as independent replicate. In summary, a total of 10 treatments and 4 replicates (Table 1) to perform a good statistical analysis of the data were carried out.

#### 2.2. Determination of crop parameters

The foliar area measurement was carried out using the image analysis program (ImageJ, UTHSCSA Image Tool software). Images of each plant at first harvest time were taken by treatment (n = 4). The scale

Table 1				
Description of the treatments	applied to	the	strawberry	plants.

Substrate	Fe status	Abbreviation	Treatment
Coconut fibre (FC)	Fe0 Fe5	FC-0-C FC-5-C FC-5-L	Fe 0 μM (-Si) Fe 5 μM (-Si) Fe 5 μM + Si foliar
	Fe20	FC-5-R FC-20-C	Fe 5 $\mu$ M + Si radicular Fe 20 $\mu$ M (-Si)
Organic substrate (SO)	50	FC-20-L FC-20-R SO-C	Fe 20 $\mu$ M + Si foliar Fe 20 $\mu$ M + Si radicular Control (-Si)
organic substitute (00)		SO-L SO-R	Si foliar Si radicular

was set using the relationship between pixels and the known distance. Then, the threshold was measured applying the default algorithm and the foliar area quantified.

SPAD index was measured (portable chlorophyll meter; SPAD-502, Minolta, Japan) weekly. The SPAD value of each leaf per plant was taken and the mean value was obtained per plant. Finally, to analyse the evolution of the index per plant (n = 4) the difference between the last measurement and the first one was calculated.

The total number of harvested fruits was counted per plant and treatment (n = 4). Later, fruits were separated from the plant to carry out quality and shelf-life analyses. For this purpose, the first strawberry harvested was destined for shelf-life assessment, the second for quality, the third for shelf life and so on. This fruit distribution allows having enough fruits for both analyses coming from the same inflorescence.

#### 2.3. Fruit physicochemical analysis

Physical properties were determined from three fruits harvested randomly at the first strawberry production on natural moisture content (n = 3). In order to understand our results three marketable fruits belonging to the same variety with an extra diameter ( $\geq 25$  mm) were subjected to the same analyses and used as commercial control.

The pH of the strawberries was measured with a 50 53 T electrode coupled to a Crison pH 25 + penetration pH meter (Barcelona, Spain). Fruits linear dimensions–length and diameter were measured by a micrometre with an accuracy of 0.01 mm. The weight was taken using an analytical balance. For greater precision, all measurements were carried out in triplicate.

The colour of the fruits was determined at three diverse locations by using a Minolta colorimeter (Chromameter CR-400/410. Konica Minolta. Japan) after standardization with a white calibration plate ( $L^* = 96:9$ ;  $a^* = -0.04$ ;  $b^* = 1.84$ ). The colour was collected using CIE- $L^*a^*b^*$  uniform colour space (CIE-Lab) where  $L^*$  indicates lightness,  $a^*$  indicates hue on a green (-) to red (+) axis, and  $b^*$  indicates hue on a blue (-) to yellow (+) axis.

Firmness was measured using a universal testing machine TA-XT-Plus Texture Analyses (Stable Micro Systems Ltd, Godalming, UK) equipped with a 5 kg load cell and a 2 mm aluminium cylindrical probe. Samples were cut with a scalpel in 10 mm slices. The penetration measurement was estimated from the yield by placing each slice perpendicularly in the equipment and compressing it at a speed of 1 mm/s.

#### 2.4. Fruit chemical composition and fruit shelf-life

The chemical properties of the strawberries were determined according to ICC corresponding standard methods (AOAC, 1984) from three randomly fruits harvested at first strawberry production per treatment and from three marketable fruits classified as premium quality ( $\geq$  25 mm wide) considered as control (n = 3).

The mineral content was calculated by weight difference after calcination of the berries samples in a muffle furnace at 850 °C for 8 h. The nitrogen content was estimated by the Kjeldahl method and converted to protein content by using the conversion factor 6.25. Soluble sugar (fructose and glucose) contents were quantified by HPAEC (High-performance anion-exchange chromatography). Soluble sugars from fruits were extracted with three times of 80% (v/v) aqueous ethanol. HPAEC analysis was carried out according to Benavent-Gil and Rosell (2017). Fructose and glucose were quantified on the basis of peak areas and comparison with a calibration curve obtained with the corresponding standards.

A shelf-life study of strawberries was conducted by storing three selected fruits per treatment in a fresh site with controlled light for their conservation (n = 3). During storage, visual decay of fruits was evaluated until the marketing requirements were considered poor for each treatment. The number of days was counted and the mean value was obtained by treatment.

#### 2.5. Statistical analysis

The data reported are the mean of replicates and expressed as a mean  $\pm$  standard deviation. Analysis of variance and Duncan's multiple range tests were used to achieve statistical analysis with a significance level of 0.05 on all results, using SPSS v.24 software (Stat-Packets statistical analysis software, SPSS Inc., Chicago, IL). Pearson correlation coefficient (*r*) and P-value were used to indicate correlations and their significance using Statgraphics Centurion XV software (Bitstream, Cambridge, N). Principal component analysis (PCA) was also performed to determine the number of principal components that significantly discriminated samples (P < 0.1). This analysis describes the information of a set of variables observed using a set of smaller variables (principal components) that are linear combinations of the starting variables obtaining a diagram of dispersions in terms of similarity and order of importance.

#### 3. Results and discussion

#### 3.1. Crop parameters

Leaf area growth determines light interception and is an important parameter in determining plant productivity (Koester et al., 2014). The analysis of leaf area did not show differences among treatments with and without Si application (Fig. 1) in plants grown in coconut fibre substrate (FC). As expected, plants grown in sufficient Fe conditions (Fe20) had a higher area (158.6 pixels) than Fe5 and Fe0 (110.2 and 52.5 pixels, respectively), although no significant differences have been found. On the other hand, the substrate type used, clearly affected the leaf area of the strawberry plants, as plants grown in the organic substrate (SO) showed a significantly higher leaf area than plants grown in FC substrate. Moreover, plants grown in the SO substrate with both Si treatments, foliar spray (L) and applied to the nutrient solution (R), considerably increased their leaf area than plants grown in SO control without Si supply. These results show that leaf (L) or root (R) Si application in FC plants does not cause any effect on their leaf area, although it helps when they are grown in SO. López-Pérez et al. (2005) proved that strawberry plants grown in FC had lower leaf area than ones grown in soil. However, Martínez et al. (2017) observed that strawberry plants of "Sabrina" variety presented a higher leaf area when grown in FC, which shows that each variety presents its optimal conditions and know each variety requirements is a key point to obtain an optimal development. The tested Fortuna variety seemed to increase its productivity, in terms of leaf area index, in the SO substrate and the Si supplied (foliar or root addition) increased it considerably.

A parameter related to leaf area was the photosynthesis rate, also related to the chlorophyll content of the plants. Plants grown in the FC substrate without and with 5  $\mu M$  of Fe showed a SPAD index decreased (data not shown) as expected, due to the Fe chlorosis assessment. When Fe was added to the nutrient solution (FC-20), an increase of SPAD index has been detected. No significant differences were found between both Si applications (L and R) and its respective control in the FC cultures in deficient or sufficient Fe conditions. On the contrary, for the SO substrate (Fig. 2), an increase in SPAD index along the growth period when Si was added to the root system was observed, but no improvement due to Si addition to the leaves has been detected. Wang and Galletta (1998) reported in Earliglow strawberry, that the foliar applications of Si as potassium silicate increased chlorophyll content and enhanced plant growth. The silicon solution pH used by these authors was adjusted with phosphoric acid to pH 5.5, and Tween 20 was added as surfactant. They have tested several Si doses from 0 to 17 mM, and control solutions with P or K and without Si was also tested as foliar sprays. The sprays were applied after runoff. At pH 5.5 silicon from K<sub>2</sub>SiO<sub>3</sub> may be mostly precipitated as SiO<sub>2</sub>, so the results shown by these authors may greatly differ from the ones presented in this work due to the uncertain Si concentration used by them. Wang and Galletta



Fig. 1. Effect of foliar and radicular Si application on leaf area (average of pixels per plant) in organic substrate (SO) and coconut fibre (FC) under conditions of deficiency (5  $\mu$ M) and sufficiency (20  $\mu$ M) of Fe and a control without Fe addition. The difference between letters denotes significant differences between treatments according to the Duncan test (P  $\leq$  0.1).

(1998) observed an increased chlorophyll content when Si was sprayed at concentrations of 4.25 and 8.50 mM, but higher concentrations did not seem to improve this parameter. All the referred authors used higher Si concentrations, but the uncertainty of which concentration has been used due to the precipitation of Si from other Si sources than  $H_4SiO_4$ , at the low pH required typically for foliar sprays to avoid plant damage, may mask the effects of this addition.

Moreover, when fruit yield at first flowering was analysed, significant differences between treatments in the average number of fruits produced in FC were observed (Table 2). In both Fe treatments (Fe5 and Fe20) the non-silicon application increased the number of fruits. However, the higher number of fruits were produced by Si application in SO where Si-R plants increased the fruits up to 7.0 followed by SO-L (5.5) and SO–C (4.2). This is highly related to the ability to produce more photosynthates related with the higher chlorophyll content inferred by the SPAD index (Fig. 2). Miyake and Takahashi (1986) observed a similar behaviour in runner strawberry plants (Fragaria x ananassa Duchesne cv. Hokowase) grown in solutions containing 50 ppm SiO<sub>2</sub> and without Si. Treatments were then divided into three series: (i) plants continuously subjected to 50 ppm SiO<sub>2</sub> treatment (referred to as + Si + Si), (ii) plants subjected to the 50 ppm SiO<sub>2</sub> treatment after initial silicon-free treatment (- Si + Si), and (iii) plants continuously deprived of Si (- Si–Si). The total amount of fruits produced was much higher in the plants with the + Si + Si and the -Si + Si



SPAD evolution in SO plants

Fig. 2. Effect of foliar (L) and radicular (R) Si application on the amount of chlorophyll in organic substrate (SO). The difference between letters denotes significant differences between treatments according to the Duncan test ( $P \le 0.05$ ).

#### Table 2

Physicochemical strawberry quality characteristics. Abbreviations used: coconut fibre substrate (FC), organic substrate (SO), non-silicon application (C), silicon application by leaf treatment (L) or silicon application by root treatment (R). Numbers following FC substrate abbreviation are referred to the iron concentration ( $\mu$ M) applied.

Treatment	Weight (g)	Diameter (mm)	Firmness (g)	Colour	рН
FC-0-C	2.11 ± 1.11 a	13.55 ± 4.10 a	491 ± 36 g	32,778 ± 12,992 ns	$3.86 \pm 0.01 \text{ ns}$
FC-5-C	3.21 ± 1.03 ab	16.06 ± 2.94 ab	169 ± 54 de	34,660 ± 7113	$4.01 \pm 0.49$
FC-5-L	$6.03 \pm 1.79 \text{ bcd}$	14.56 ± 3.54 ab	$339 \pm 12 \text{ f}$	36,172 ± 282	$4.04 \pm 0.67$
FC-5-R	7.89 ± 3.19 de	21.21 ± 6.15 bd	134 ± 94 be	36,101 ± 10,545	$3.90 \pm 0.27$
FC-20-C	6.96 ± 1.62 cd	24.09 ± 2.45 cd	104 ± 33 bd	38,885 ± 10,927	$3.87 \pm 0.16$
FC-20-L	3.87 ± 0.98 ac	15.73 ± 3.52 ab	150 ± 25 ce	40,291 ± 2046	$3.81 \pm 0.11$
FC-20-R	8.16 ± 1.71 de	18.56 ± 0.73 ac	192 ± 19 e	34,694 ± 1090	$4.12 \pm 0.94$
SO-C	5.55 ± 0.73 b d	25.46 ± 3.84 ce	73 ± 13 ab	38,046 ± 9890	$3.81 \pm 0.26$
SO-L	6.04 ± 0.63 b d	26.90 ± 6.26 de	86 ± 11 ac	36,832 ± 2846	$3.24 \pm 0.01$
SO-R	$10.92 \pm 3.17 \ e$	32.15 ± 6.14 e	35 ± 8 a	$33,546 \pm 6051$	$3.85~\pm~0.68$

Values followed by different letters within a column denote significant differences (P < 0.05). ns: no significant differences.

treatments than in the plants with the -Si-Si treatment. Likewise, the fertility of the pollen of the Si-free cultured plants was much lower than that of the plants with an application of Si either continuous or after an initial phase -Si. These authors also reported an interesting fact, which was that in the flowering stage, the pH of the culture solution decreased remarkably (from 5.5 to 4.2-4.0 within a day) in all of the plants where Si application had not been supplied. The same phenomena were already observed in the flowering stage of Si-free cultured tomato, cucumber, and soybean plants (Miyake and Takahashi, 1978, 1983, 1985). The cause of the decrease of the pH, which could not be ascribed to an unbalanced uptake of cation/anion, requires further investigation. The unfavourable influence of the decrease of the pH on the growth of strawberry plants may be negligible in the case of the hydroponic experiment done by these authors because the pH of the culture solution was adjusted every day. Moreover, in general, the growth of strawberry plants is not significantly affected by pH above 4.0. However, in the case of the FC substrate, which is almost inert and without any buffering characteristics may alter the final strawberry production, in that substrate the higher production has been obtained in the plants without Si, so this decrease in the pH may alter fruit formation and Si could make this effect more remarkable. The SO is supposed to have some buffer capacity due to the functional groups of the organic matter, which may protect the plants from this pH decrease. The Si source used also may contribute to this effect. Silicic acid activity in soils is not affected by pH (Lindsay, 1979) in a range of pH between 4.0 and 9.5, but silicates are completely soluble at pH around 11. Below this pH, Si precipitates as SiO<sub>2</sub> or silica gel, so when Si was added as a silicate a decreased in the pH may contribute to precipitate more Si, and other nutrients may co-precipitate with it, and being deprived of the nutrient solution. This fact requires further study to be confirmed.

These results refer to that Si application in plants grown in FC does not provide any improvement in production under the experimental conditions tested while the application of Si to the roots of plants grown in the organic substrate (SO) increases the leaves area, the SPAD index and consequently the average production per plant. Several reasons could explain this behaviour. Reis et al. (2007) obtained similar results when observed that Si application (L and R) increased production and calibre of fruits, maybe because of Si favoured the phosphorus absorption by plants due to the molecular similarity between the anionic forms ( $H_2PO_4^-$  and  $H_3SiO_4^-$ ). Furthermore, Korndörfer et al. (2010) observed that Si promoted the formation of a double layer of silica that reduces transpiration by stomata, limiting the loss of water and favouring greater production. In strawberry, Miyake and Takahashi (1986) indicated that silicon also helps to increase pollen fertility and also increases the production of fruit, as confirmed for this variety in this experiment.

#### 3.2. Fruit physicochemical analysis

The effect of the different treatments under study was evaluated in the physicochemical properties of the fruit. Data collected including weight, diameter, firmness, pH and colour are summarized in Table 2. Clear significant differences were obtained from the diameter analysis between plants grown in FC or SO (Table 2). Strawberries grown in FC substrate had a similar diameter for all treatments (Fe0, Fe5 and Fe20). Only one exception was observed, the FC-20-C treatment which fruits were bigger and getting sizes close to the "extra" diameter of the commercially available fruits (market: 36.92 mm). This implied that Fe sufficient non-Si treated plants presented the highest diameter when grown in coconut fibre. All fruits harvested from SO plants presented higher diameters than the berries classified as "extra diameter"  $(\geq 25 \text{ mm})$ . Strawberries grown in SO substrate without Si addition and with Si added to the leaves had a similar diameter that FC-20-C plants, but the root Si treated berries (SO-R) showed a similar diameter than the strawberries commercially available (market control). The observed diameter in the fruits was similar to those previously reported (Cecatto, et al. 2016; Maheshgowda et al.).

According to the fruit weight (Table 2), in FC substrate under Fe deficiency conditions the root application of Si seemed to increase the berries weight, but under Fe sufficiency the Si addition to the nutrient solution gave similar results than no Si supply. So a no clear effect of Si addition has been detected. In respect to fruits grown in the SO substrate, a significant increase in fruit weight has been observed for plants with a root Si supply. These observations are in agreement with the literature (Adak, et al. 2018; Ayesha, et al. 2011), which highlights the influence of growth media on the fruit quality parameters. The firmness analysis (Table 2) showed that SO fruits were softer than FC fruits but these fruits (SO) had a similar firmness to marketable fruits (Table 2). The observed firmness in the current study was comparable to that previously reported (Zeliou, et al. 2018). Ouellette et al. (2017) observed that Si is not translocated to strawberry fruits, and thus it not affect fruit firmness or quality. Besides diameter, weight and firmness, the effect of the type of substrate, the iron and concentration and the silicon application by different ways was studied in the colour of the fruits. Among the different physical parameters of the fruits, their colour has been stated as an important quality parameter which appeals to the customers. In line with previous (Ayesha, et al. 2011), none of the variables studied had a significant influence on the fruit colour. Indeed, all fruits exhibited similar colour than that observed for the marketable ones (34.79  $\pm$  3.41). This trend was also found in the pH values. For marketable fruits, the value of pH was 3.89  $\pm$  0.38. Zeliou et al. (2018) also reported pH values of strawberry (Fragaria ananasa Duch. var. Fortuna) in Greece ranging around 3.65  $\pm$  0.14.

These results showed that cultivation of the variety Fortuna on FC substrate provided lower yields of strawberries, an increase of fruits firmness (three times higher than the commercial ones), with lower

#### Table 3

Percentage (% DW, dry weight) of minerals, proteins, glucose and fructose obtained in strawberries. Abbreviations used: coconut fibre substrate (FC), organic substrate (SO), non-silicon application (C), silicon application by leaf treatment (L) or silicon application by root treatment (R). Numbers following FC substrate abbreviation are referred to the iron concentration ( $\mu$ M) applied.

Treatment	Minerals (%)	Proteins (%)	Glucose (%)	Fructose (%)
FC-0-C	$0.96 \pm 0.00 \text{ g}$	$2.00 \pm 0.35 \text{ ef}$	111.60 ± 1.34 i	$14.62 \pm 0.02 e$
FC-5-C	$0.59 \pm 0.01 \text{ d}$	$1.44 \pm 0.09 \text{ cd}$	129.57 ± 0.03 j	$0.15 \pm 0.07 \ a$
FC-5-L	0.98 ± 0.01 g	$2.19 \pm 0.31 \text{ f}$	108.83 ± 0.06 h	$15.92 \pm 0.12 \text{ f}$
FC-5-R	0.48 ± 0.03 c	$1.38 \pm 0.01 \ cd$	37.22 ± 0.28 c	$3.43 \pm 0.21 \text{ b}$
FC-20-C	0.44 ± 0.00 b	1.55 ± 0.03 cd	31.87 ± 1.40 b	$3.45 \pm 0.14 \text{ b}$
FC-20-L	0.70 ± 0.01 e	1.70 ± 0.07 de	40.94 ± 1.03 d	$4.07 \pm 0.03 c$
FC-20-R	$0.93 \pm 0.01  \mathrm{f}$	2.27 ± 0.44 f	72.16 ± 1.21 e	$12.94 \pm 0.04  d$
SO-C	0.32 ± 0.02 a	$1.24 \pm 0.13 \text{ bc}$	8.78 ± 0.39 a	$0.22 \pm 0.21 \ a$
SO-L	0.44 ± 0.00 b	0.83 ± 0.19 a	85.82 ± 0.03 f	$12.71 \pm 0.28 \text{ d}$
SO-R	$0.45 ~\pm~ 0.00 ~\mathrm{b}$	$0.98 \pm 0.08 \text{ ab}$	91.89 ± 0.12 g	$48.45 \pm 0.16 \text{ g}$

Values followed by different letters within a column denote significant differences (P < 0.05).

diameter and similar weight of the berries, than plants cultivated on an organic substrate. No clear effect of Si addition to this substrate was observed. However, silicon application to the irrigation solution of plants grown in the organic substrate improves the berries quality reaching the parameters of the marketable fruits. No Si addition or Si foliar sprays addition reduced significantly fruit yield, diameter, weight and increased firmness of the berries. So these berries will not achieve the "extra characteristics" to be sold at a high price.

#### 3.3. Fruit chemical composition

Higher content of minerals and proteins are desirable since both components are related to body health, strengthen bones and muscles, cardiovascular health or regulation of heart rate, among others (Pérez and Zamora, 2002; Gonzá;lez-Torres et al., 2007). So, the effect of the type of substrate, the iron concentration and the silicon application by different ways was also studied through the fruit chemical composition. Data collected are reported in Table 3. The statistical analysis indicated that the substrate employed had a significant (P < 0.05) influence on minerals, protein and glucose content. The silicon application significantly affects the mineral and fructose content, while iron only has a significant (P < 0.05) effect on the glucose content of the fruits.

From Table 3, it can be noticed that the different treatments employed modified the fruits chemical composition. For the marketable fruits, the mineral content was 0.19  $\pm$  0.016%. The treatments used in the present study caused a mineral content increased in all cases compared to marketable fruits. Overall, mineral content was higher in fruits harvested from plants grown in FC than those harvested from plants grown in SO. The iron concentration and silicon application had a significant effect on this parameter. For both Fe status, Si addition increased the mineral content of the berries, under Fe deficiency the foliar application was preferred and under Fe sufficiency, the root application gave a higher mineral content. When fruits grown in SO were compared, the silicon application led to higher mineral concentration than that observed in their respective control. However, both L and R treatment reached similar values. For protein component, differences in content values were also observed. The protein content in the marketable fruits was 0.83  $\pm$  0.04%, which was similar to that found in the fruits grown in SO. However, higher protein content was observed in the fruits grown in FC. Also, it is worth to see that protein content increased in the treatment that combines iron deficiency (5 µM of Fe in the solution) along with silicon application by leaves treatment (FC-5-L sample). Meanwhile, to reach similar protein content with iron sufficiency conditions (20 µM of Fe in the solution), the silicon had to be applied by roots treatment (FC-20-R sample). However, both treatments led to similar values than that observed in FC-0-C sample. These results contradict the results from Hajiboland et al. (2017) in strawberry or Liu et al. (2017) in rice where both shown that Si application increased the protein composition in strawberries. However, Islam and Sha (1969)

observed that Si application decreased the protein contents on rice plants. Higher content of minerals and proteins are desirable since both components are related to body health, strengthen bones and muscles, cardiovascular health or regulation of heart rate, among others (Pérez and Zamora, 2002; Gonzá;lez-Torres et al., 2007).

Flavour, which is an important factor for the strawberry quality, was determined by the sugars content, among others. In this regard, fruits mainly contain glucose and fructose. The glucose and the fructose content of marketable fruits were 128.36  $\pm$  0.03% and 0.35  $~\pm~$  0.48%, respectively. All the treatments result on a decrease in glucose content, except for control fruits grown in SO (Table 3). Among fruits grown in FC, a remarkable glucose content decrease was observed as iron concentration increase. Zargar et al. (2015) observed the same antagonism between iron and sugars because of higher iron concentration reduce the expression of sugar transporters. Moreover, Jarosz (2014) in tomatoes and Lin et al. (2016) in Arabidopsis got same results. Additionally, when the iron deficiency was considered, the silicon application by L treatment showed higher glucose content. However, for the iron sufficiency conditions, the silicon applied by R treatment revealed higher glucose content. Even with that, the major glucose content was observed for FC-5-C sample, followed by FC-0-C and FC-5-L samples, respectively. For fruits grown in SO, the silicon application significantly increased the glucose content. Valentinuzzi et al. (2017) observed a glucose content increase after the Si biofortification in strawberry fruits (Fragaria  $\times$  ananassa 'Elsanta'). Authors also reported that this increase depended on a large extent on the Si concentration. Moderate-high glucose content was observed by R treatment than that observed for L treatment. A similar trend was observed for fructose content. The highest fructose content was found for fruits grown in SO with silicon application by R treatment (SO-R sample). A higher fructose content provides more energy to the body to metabolize and transform into glucose (Coral et al. 2012).

#### 3.4. Fruits shelf-life

No differences in duration of days between strawberries harvested from Fe5 treatments were obtained. Moreover, these results were similar than ones obtained from marketable harvested fruits (Fig. 3). However, when Fe20 conditions were analysed, the radicular application of Si (FC-20-R) significantly increases the fruit shelf-life by an average of 1.5 days compared with commercial fruits (Fig. 3).

Finally, the SO fruits shelf-life study indicates that Si addition significantly increased shelf-life of the strawberries (Fig. 3). When SO fruits with both Si applications were compared with marketable ones Si applications showed clear differences having a shelf-life of 1.5–2.0 days longer (Fig. 3). In all treatments appeared *Botrytis cinerea* Pers.:Fr. which is the main post-harvest disease in strawberries. Poovaiah et al. (1988) and recently, Moreno-Guerrero et al. (2017) also observed the improvement produced in *B. cinerea* when Si was applied because of its



Fig. 3. Average of shelf-life (days) of fruits harvested by treatment and substrate (FC, SO and market) until the loss of marketable value. The difference between letters denotes significant differences between the treatments according to the Duncan test ( $P \le 0.1$ ).



**Fig. 4.** PCA score and loading biplot of samples and variables (crop parameters, physicochemical characteristics and chemical composition of fruits) obtained by principal component analysis. Abbreviations used: coconut fibre substrate (FC), organic substrate (SO), non-silicon application (C), silicon application by leaf treatment (L) or silicon application by root treatment (R). Numbers following FC substrate abbreviation are referred to the iron concentration (µM) applied.

accumulation in the epidermal tissue having an efficacy of 60.1–72.6% after 15 days of treatment.

## 3.5. Correlations between crop parameters, chemical, physicochemical and shelf-life

The characterization of each treatment was established by the analysis of main components (PCA) (Fig. 4). The PCA dimensions 1 and 2 (Dim1 and Dim2) showed a significant variability of 87.05% within the main components. Parameters of diameter, foliar density, SPAD,

shelf-life, weight and yield within SO treatments (SO–C, SO-L and SO-R) were related while colour, hardness, pH, glucose, minerals and proteins had relation with FC treatments (Fe0, Fe5 and Fe20). Therefore, PCA allowed discriminating between substrates and treatments.

The correlation matrix established that the most important marketable parameters were closely related (Table 3). For example, diameter and shelf-life were directly linked with foliar density, SPAD and yield because of good growth conditions increase the photosynthesis rate and contribute to get bigger fruits size (Raven, 1983; Wang and Galleta, 1998; Silva et al., 2013) and fruits with more disease resistance thanks to Si application which create a double layer of protection (Reis et al., 2007; Silva et al., 2013; Moreno-Guerrero et al., 2017).

#### 4. Conclusions

To conclude, "Fortuna" strawberries plants had a poor development in coconut fibre and an excellent growth and yield in organic substrate. Although no clear differences have been shown under Fe deficiency conditions, the Si addition to the roots of the plants grown in coconut fibre substrate with an optimal Fe nutrition increased shelf-life of the berries, mineral, protein, glucose and fructose contents but no yield improvement has been observed. Likewise, the radicular silicon application to the organic substrate considerably improved yield, fruit diameter, fruit weight, glucose and fructose fruit content and the fruit shelf-life without causing distinguishable chemical or physicochemical changes. In summary, Si application to Fortuna strawberries through the roots could be a good solution to increase fruit quality and yield, and to increase benefits from the agronomical point of view. Further studies in other strawberry varieties and dose rates will allow to know with better precision how the radicular application of silicon contributes to yield and fruit shelf-life.

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#### Contribution

The work is an original research carried out by our group, Dr. Peris did the field work and write the ms, Dr. Benavent did the nutritional parameters analysis work and I contribute to the experimental design, data analysis, correct the ms, and coordinate the work. All of us agree with the contents of the manuscript and its submission to the journal.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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