Artículo original
Biotecnología Vegetal Vol. 19, No. 3: 171 - 178, julio - septiembre, 2019
Instituto de Biotecnología de las Plantas. UCLV. MES.
eISSN 2074-8647, RNPS: 2154

Silicon in micropropagation of strawberry cv. 'Jonica'

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ABSTRACT
The addition of silicon (Si) to the culture media has shown beneficial action for the growth and development of seedlings of several species *in vitro* cultivated. This paper aimed to determine the effect of potassium silicate on the in vitro propagation of strawberry (*Fragaria* x ananassa Duch). Seedlings from Italian 'Jonica' strawberry obtained from shoots apex *in vitro* regenerated were cultured in glass vials containing 30 ml of the MS culture medium solidified with 6 g l^{-1} agar. Five concentrations of potassium silicate (0.0, 0.5, 1.0, 1.5 and 2.0 g l^{-1}) were added to the culture medium. The pH was adjusted to 5.8 ± 0.2 prior sterilization by autoclave. The experiment was maintained in a growth room with irradiance of 42 µmol m^{-2} s^{-1}, at 25 ± 2 °C and 16 hours photoperiod. The variables analysed were number of shoots, average length of shoots (cm), number of leaves, SPAD index and number of roots and average length of roots (cm). For most of the analysed variables, a decrease was observed as a function of the increase in Si concentration. Thus, for the micropropagation of strawberry cv. 'Jonica', the concentrations of Si tested did not show a beneficial effect.

Keywords: culture medium, multiplication, rooting, tissue culture

Silicio en la micropropagación de fresa cv. 'Jonica'

RESUMEN
La adición de silicio (Si) a los medios de cultivo ha demostrado una acción beneficiosa para el crecimiento y desarrollo de plántulas de varias especies cultivadas *in vitro*. Este artículo tuvo como objetivo determinar el efecto del silicato de potasio en la propagación *in vitro* de fresa (*Fragaria* x *ananassa* Duch). Las plántulas de fresa italiana 'Jonica' obtenida de brotes de ápice regenerados *in vitro* se cultivaron en viales de vidrio que contenían 30 ml del medio de cultivo MS solidificado con 6 g de agar l^{-1}. Se agregaron cinco concentraciones de silicato de potasio (0.0, 0.5, 1.0, 1.5 y 2.0 g l^{-1}) al medio de cultivo. El pH se ajustó a 5.8 ± 0.2 antes de la esterilización en autoclave. El experimento se mantuvo en una sala de crecimiento con irradiancia de 42 µmol m^{-2} s^{-1}, a 25 ± 2 °C y 16 horas de fotoperíodo. Las variables analizadas fueron el número de brotes, la longitud promedio de los brotes (cm), el número de hojas, el índice SPAD y el número de raíces y la longitud promedio de las raíces (cm). Para la mayoría de las variables analizadas, se observó una disminución en función del aumento en la concentración de Si. Por lo tanto, para la micropropagación de fresa 'Jonica', las concentraciones de Si analizadas no mostraron un efecto beneficioso.

Palabras clave: cultivo de tejidos, enraizamiento, medio de cultivo, multiplicación

INTRODUCTION

The main species of small fruits produced worldwide are the strawberry, the blueberry and the raspberry (Faostat, 2017). Within this group, the strawberries is the one with the greatest economic expression, being appreciated in several regions of the world (Oliveira *et al*., 2011). The emphasis on the production of the species is based not only on the economic return, but also, as an important source of vitamin C, anthocyanins

and antioxidant activities, which can contribute to disease prevention (Cocco *et al*., 2015).

The production and use of healthy seedlings are the most important factors for obtaining high quality fruits and better responses to the technologies used (Oliveira and Scivittaro, 2009). Thus, tissue culture techniques allow the production of a large number of plants with excellent phytosanitary and genetic quality in a shorter period of time, through several cycles of *in vitro* multiplication (Fonseca *et al*., 2013). The use of the micropropagation technique makes it possible to meet the demands of the consumer market by offering high quality seedlings to nurseries and homogeneous seedlings to farmers (Dias *et al*., 2014).

The culture media composition has often been modified to stimulate the growth and development of specific plant material (Sivanesan and Park, 2014). Among the nutritional components, silicon is considered an element that has not been much studied in the plant micropropagation. However, from the physiological point of view, for plants growth and development, silicon has shown beneficial effect on the increase the production of several crops (Gomes *et al.,* 2008). Culture medium supplemented with silicon can benefit seedlings cultured *in vitro*, raising the hemicellulose and lignin content and thus increasing the stiffness in the cell wall (Camargo *et al.,* 2007).

Thick cell wall presence is a mechanism of preformed structural resistance of hosts and may contribute to the restriction of plant colonization by phytopathogens (Pascholati, 2011). With this, silicon has provided promising results in the control of plant diseases (Domiciano *et al.,* 2010; Lima *et al.*, 2010).

This paper aimed to determine the efect of potassium silicate added to the MS medium (Murashige and Skoog, 1962) in the strawberry *in vitro* propagation.

MATERIAL AND METHODS

Trial was carried out at the Laboratory of Plant Micropropagation of Santa Catarina State University (UDESC / CAV) in Lages city, Santa Catarina State (SC), Brazil, in 2016.

Plant material

Strawberry seedlings originating from the regeneration of shoot apex *in vitro* and grown in MS medium were used as plant material.

In vitro culture

Plantlets approximately 1.5 ± 0.5 cm length were introduced into glass bottles containing 30 ml of the same medium MS with potassium silicate at five different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 g l^{-1}). The basal medium was composed of 30 g l^{-1} sucrose, 0.1 g l^{-1} myo-inositol and 0.5 g l^{-1} 6-benzylaminopurine (BAP) and the pH was adjusted to 5.8 before addition of 6 g l^{-1} of the solidifying agent (agar). Then, the vials containing the culture medium were sterilized by autoclave at 121 ºC and 1.2 atm for 20 minutes.

In a laminar flow chamber, seedlings were introduced into the culture medium and then transferred to a growth room at 25 °C (± 2 °C), photoperiod of 16 hours and light intensity of 27 µmol m^{-2} s^{-1} supplied by white fluorescent lamps.

After 45 days of culture, the following variables were evaluated:
a) number of shoots: obtained by separation and manual counting of shoots per explant
b) number of leaves: obtained by the separation and manual counting of leaves per explant
c) shoot length: measured from the neck of the plant to the upper end of the largest leaf, expressed in centimeters
d) SPAD index: quantified using the Minolta® SPAD-502-PLUS portable meter, which performs a sheet absorbance measurement in two regions of wavelength (red and near infrared regions) and from these two transmittances, the equipment calculates the SPAD value proportional to the amount of chlorophyll present in the leaf
e) number of roots: obtained by separation and manual counting of roots per plant, and
f) root length: measured from the neck of the plant to the end of all roots, expressed in centimeters.

Statistical analysis

Data were analysed for normality by the Shapiro-Willk test. Values that were not

normal were transformed into $\sqrt{x} + 0.1$, where $\sqrt{x}$ is the average obtained from each variable. They were then submitted to variance analysis and when significant by the F test, regression analysis was performed. In addition, Pearson correlation analysis was performed between the variables number of roots per explant and average length of shoots; also average length of roots and average length of shoots. The statistical program used for data processing was Sisvar (5.6 version).

RESULTS AND DISCUSSION

The concentrations of potassium silicate added to the culture medium had a negative influence on the number of shoots, mean length of shoots and number of leaves (Figure 1).

In the treatment with 2 g l^{-1} potassium silicate there was an approximately 80% reduction in the number of leaves when compared to the treatment without silicon. As observed in this study, the addition of potassium silicate to the MS culture medium also resulted in a lower number of leaves in the explants of strawberry cultivar 'Oso Grande' (Braga *et al*., 2009). Besides, studying two species of orchids (*Brassavola perrine* and *Laelia cattleya* Culminant 'Tuilerie' x *Laelia cattleya* Sons Atout Rotunda x *Brassolaelia cattleya* Startifire Moon Beach) Pasqual *et al*. (2011) reported that the number of leaves decreased with increasing concentrations of calcium silicate added to the culture medium. In the same way, Alves *et al.* (2017), verified that the increase of the concentrations of calcium silicate caused a reduction in the leaf area and in the height of the orchid aerial part.

According to Soares *et al*. (2008), the reduction in the number of leaves of plants that receive silicon may be a mechanism to reduce the perspiration. However, Soares *et al*. (2011) found greater numbers of leaves in *Cattleya loddigesii* when the specie was grown in Knudson C medium supplemented with 20 mg l^{-1} calcium silicate and 20 mg l^{-1} potassium silicate. The adjustment of the osmotic potential in plant cells is regulated by potassium, which also activates enzymes involved in respiration and photosynthesis (Taiz and Zeiger, 2009), so it is possible to attribute the increased leaf growth observed in this study to potassium and not just to silicon.

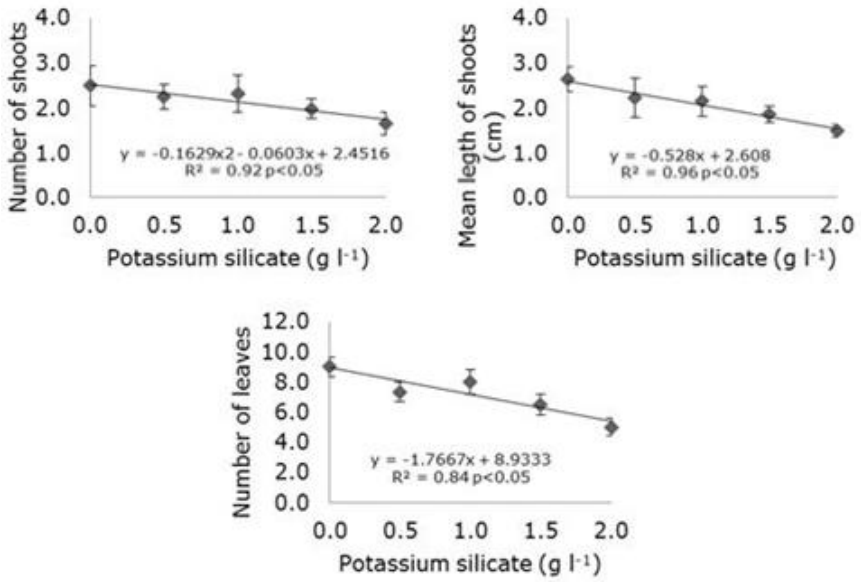

Figure 1. Number of shoots, mean shoot length (cm) and number of leaves as a function of the addition of different concentrations of potassium silicate to MS medium in strawberry cv. 'Jonica' *in vitro* propagation.

The absence of responses in relation to the analysed variables can be related to the determination of the optimum concentration and the silicon source used that varies according to the species or plant genotype (Soares *et al*., 2013). Another explanation is that nutritional excess, as in this case potassium, may have caused an imbalance leading to deficiencies or overaccumulation of nutrients (Malavolta *et al.*, 2006). Moreover, the negative results obtained with the addition of potassium silicate can be attributed to an unfavourable interaction between this and the other components of the medium. In this sense, according to Borgatto *et al*. (2002), *in vitro* growth of plants, organs, tissues and/or cells depends on the perfect interaction between the essential components of the culture medium, such as carbon sources and mineral nutrients, which are limiting factors for *in vitro* development.

The SPAD index had a polynomial regression curve response, where the inflection point was at 0.67 g l^{-1} of potassium silicate added to the nutrient medium (Figure 2).

The inclusion of 0.67 g l^{-1} of potassium silicate in the culture medium increased the chlorophyll levels, in the 'Jonica' strawberry seedlings, which are the green pigments specialized in light absorption (Taiz *et al.,* 2017). According to Takahashi (1995), the best foliar architecture provided by silicon allows greater penetration of light, greater absorption of CO_2 and decrease of excessive transpiration, which causes the increase of the photosynthetic rates. The beneficial action of silicon has been associated with several indirect effects, such as increased photosynthetic efficiency and chlorophyll content (Sivanesan and Park, 2014).

The linear regressions for the variables related to the root system of strawberry cv. 'Jonica' explants (Figure 3) indicated that the Potassium silicate concentrations added to the culture medium did not contribute to the increase of the variables analysed.

When the number of roots formed *in vitro* is increased, the root/culture medium contact area is also increased, reflecting a higher nutrients absorption (Soares *et al*., 2013). Thus, in the control treatment it was observed that the root number and mean root length were superior related to the other treatment (the control do not increase, just it was not affected by the silicate added), and the addition of any concentrations of potassium silicate decreased the means for these variables. A similar result was observed in coffee (*Coffea arabica* L.), where the addition of potassium silicate to the nutrient solution did not present promising results, since there was a decrease in the dry mass of the roots (Cunha *et al.,* 2012).

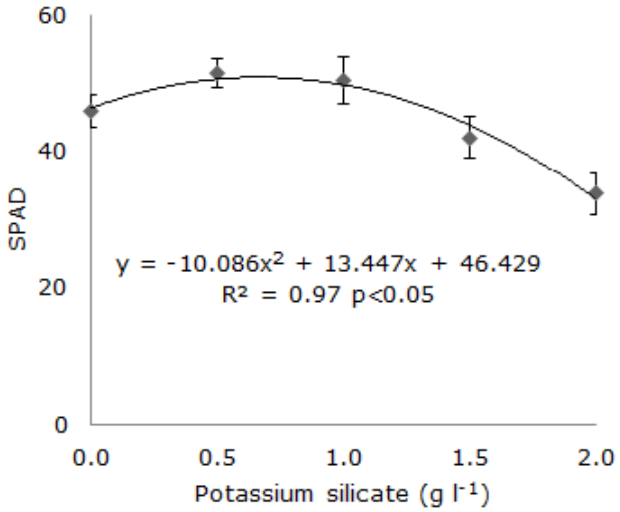

Figure 2. SPAD index as a function of the addition of different potassium silicate concentrations to the MS culture medium in strawberry cv. 'Jonica' *in vitro* propagation.

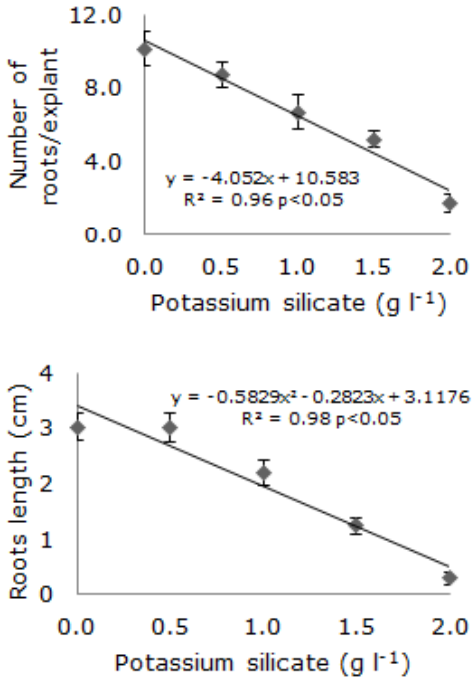

Figure 3. Number of roots and mean root length (cm) as a function of the addition of different potassium silicate concentrations to the MS culture medium in strawberry cv. 'Jonica' *in vitro* propagation.

Potassium silicate effects on *in vitro* root development of strawberry cv. 'Jonica' showed that under the conditions in which this experiment was performed, the number and length of roots decreased with increasing potassium silicate concentrations, indicating that potassium may have been over-offered. According to Malavolta *et al.* (1997) potassium is involved in the meristematic growth, since the phytohormones acting in this process are put into action by this mineral. When in excess it can inhibit the absorption of other ions, like Calcium and Magnesium, essential for the development of the roots. The lack of Magnesium would prevent the complete development of the roots and the lack of Calcium, besides limiting the growth of the roots leaves them with a darker colour (Nunes, 2016).

A trial carried out with the banana variety 'Apple' (*Musa acuminata*) shows that there was no significant effect of adding different sources of silicon to the MS culture medium on root mean length compared to the control treatment (Asmar *et al.,* 2011). Besides, silicate sources did not affect the *in vitro* development of orchids (*Cattleya forbesii)* and there was a decrease in the number and average root length of the plants (Colombo *et al*., 2016), in agreement with the results obtained in the present trial. The inhibitory effect on root formation due to the addition of potassium silicate to the nutrient medium may be associated with the concentration itself (George *et al.*, 2008), since all nutrients in excess cause a nutritional imbalance in both the seedling and the culture medium (Soares *et al*., 2013).

In studies of mineral nutrition, it is necessary to consider nutrients as a whole, because in the absorption process, one can exert influence on the other, given the possible interactions that may occur (Malavolta *et al.,* 1997). It is possible that the potassium silicate concentrations caused a nutritional imbalance in the plants inhibiting the nutrients absorption from the culture medium, thus, silicon could not exert beneficial effects on the growth and development of strawberry seedlings.

As roots are responsible for the uptake of water and nutrients, lower emission and lower growth of the root system generally reflect negatively on shoot growth rate (Colombo *et al.,* 2016). This response was assessed because the reduced development of the aerial part was correlated with the number of roots (r = 0.85) and with the average length of roots (r = 0.83) (Figure 4).

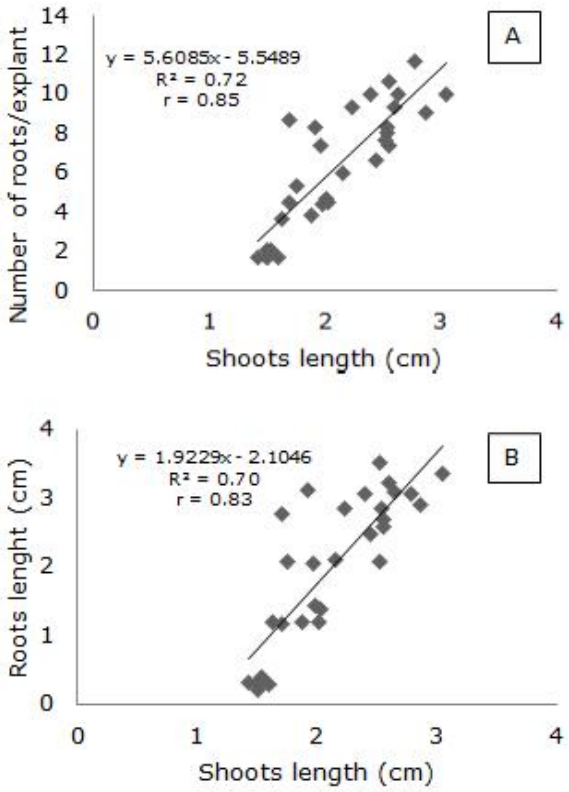

Figure 4. Pearson correlation between the number of roots per explant and the mean of shoots length (A) and between the mean root length and the mean shoot length (B) in strawberry cv. 'Jonica' explants culture with different potassium silicate concentrations in the *in vitro* medium culture.

In vitro growth of seedlings depends on the optimization of culture media that interact perfectly with essential components such as carbon source, mineral nutrients and growth regulators (Soares *et al*., 2013). The ideal silicon concentration required for plant growth and development varies between different genotypes within the same species and among plant species (Lim *et al.,* 2012). Due to the lack of data on the influence of silicon on the strawberry micropropagation it is necessary to carry out future trials in order to fill this deficiency of information. Based on the results obtained through this trial, it is not recommended to use potassium silicate for 'Jonica' strawberry *in vitro* multiplication.

CONCLUSIONS

The silicon concentrations used in this trial reduce the multiplication and rooting of 'Jonica' strawberry explants cultivated *in vitro*.

Conflict of interest

The authors declare that they have no conflict of interest.

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Recibido: 06-06-2019
Aceptado: 22-07-2019