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ORIGINAL ARTICLE

Effectiveness of silicon sources for in vitro development of gerbera

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Abstract

Each species cultured in vitro has specific nutritional requirements on the culture medium. The use of silicon in culture can provide benefits to plants, such as increase of tolerance to water stress, increase of photosynthetic capacity and reduction in transpiration. These effects may be quite relevant for in vitro growth and development of plant and during acclimatization. However, the effectiveness of this nutrient may vary depending on the used sources. The aim of this study was to verify the ideal concentration of the MS culture medium and to evaluate the to evaluate the different sources as well as the function of silicon for in vitro development and acclimatization of gerbera plantlets. Shoots (4–6 cm) of gerbera (*Gerbera jamesonii* cv. Jaguar Cream) cultured in vitro were inoculated in MS medium with different concentrations of salts (MS ¼, MS ½, MS ¾, MS) supplemented with different silicon sources (potassium silicate, sodium silicate, calcium silicate and silicic acid) at concentrations of 0 (control), 0.25, 0.5, 0.75, and 1 mg L^{-1}. The use of MS ¼ medium and the supplementation of 0.25 g L^{-1} calcium silicate provided better shoot development. The acclimatization rate was not affected by the origin of plants treated in vitro by different silicon sources, their concentrations, and the used culture medium. Thus, the use of silicon is beneficial for in vitro development of gerberas, however, the effectiveness depends on the used source.

Key message

In vitro shoots of *Gerbera jamesonii* cv. Jaguar Cream regenerate better, shoots are taller andhealthier, and root more effectively when are cultured on MS 1/4 supplemented with 0.25 mg L^{-1} calciumsilicate.

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Keywords *Gerbera jamesonii* · Ornamental plants · Silicate · Acclimatization

Introduction

Gerbera jamesonii Bolus is one of the most popular ornamental plant worldwide used both as cut flower and potted plant. Some cultivar show excellent agronomic characters such as color, floral diameter, stem length, and vigor, which make this plant of commercial importance. the "Jaguar Cream", with white flowers, is one of the most planted worldwide planted cultivar. Conventionally, multiplication is done through seeds or rhizome cuttings however the multiplication of Gerbera, with improved agronomic traits, has been achieved by using tissue culture methods (Minerva and Kumar 2012).

The use of micropropagation techniques allows obtaining, in a short time, a large number of plants with superior quality (Al-Khayri and Naik 2017; Silva et al. 2018). Therefore, it is a widely used technique for the commercial propagation of gerbera and other commercial ornamental species, such as orchids (Chugh et al. 2009), anthurium (Desai et al. 2015), zantedeschia (Kulpa 2016; Chang et al. 2003), *Portulaca grandiflora* (Cruz et al. 2019) among others largely produced.

However, in vitro cultured plants or explants have specific nutritional requirements, requiring nutrient media supplemented with different concentrations of essential and beneficial elements, organic constituents and energy sources (Sa et al. 2016; Sá et al. 2018; Ayub et al. 2019; Timoteo et al. 2019). For gerbera, several studies testing culture media have already been developed (Nhut et al. 2007, Bhatia et al. 2009; Cardoso and Teixeira da Silva 2012), but the use of low concentrations of culture media salts has not yet been found in the literature. These studies are important to determine the lower consumption of culture medium in order to reduce the expenses of gerbera micropropagation.

Silicon, although is not usually included in culture medium formations, stood out among the mineral element beneficial for plants. For instance, its storage in the epidermis of leaves forms a mechanical barrier of protection. This accumulation of silicon in transpiration organs promotes the formation of a cuticle-silicon double layer, providing greater tolerance to water stress (Yoshida et al. 1962; Malavolta 2006).

For in vitro development, silicon may affect the growth and development of plants (Braga et al. 2009; Lim et al. 2012; Soares et al. 2013; He et al. 2013; Hartley 2015), the formation and regeneration of adventitious shoot (Sahebi et al. 2016). It is also reported to increase the frequency of shoot induction and the average number of shoots per explant (Sivanesan and Jeong 2014), and promote greater induction and root development (Luz et al. 2012; Soares et al. 2013; Sahebi et al. 2016), besides increasing the plantlet survival in acclimatization (Ziv 2010; Asmar et al. 2013).

Silicon can be supplemeted to media in the form of several different sources, such as calcium, potassium and sodium silicate, and silicic acid. However, there is no information on the effectiveness of this element based on the use of varied sources. There is also no information on the effect of this nutrient on in vitro development of gerberas.

The aim of the present study was to determine the ideal concentration of the MS basal mrdium and to evaluate the effcincy of different silicon sources and their different concetration on in vitro growth and development of gerbera.

Material and methods

Inicial establishment of the gerbera

Gerbera jamesonii cv. Jaguar Cream already established in vitro, were sectioned in two-leaf explants measuring 4–6 cm long. The plantlets were subcultured and inoculated in modified nutrient medium (Murashige and Skoog 1962) without the addition of pyridoxine and nicotinic acid and the addition of 10 mg L^{-1} Mio inositol and 1 mg L^{-1} thiamine. The medium was added with 30 g L^{-1} sucrose and solidified with 6 g L^{-1} agar with the pH adjusted to 5.8 ± 0.1. Glass vials containing 50 mL of culture medium were used.

Sterilization of the culture media was performed by autoclaving at 121 °C and pressure of 1.05 kg cm^{-2} for 20 min. The plantlets were inoculated in a laminar flow chamber under sterile conditions and transferred to the growth room for 45 days with 16 h photoperiod, 25 ± 2 °C temperature and 43 µmol m^{-2} s^{-1} photon irradiance.

Culture medium concentrations for in vitro growth and development of gerbera

Plantlets of *Gerbera jamesonii* cv. Jaguar Cream with 60 days, which were already established in vitro, after 3 multiplication, were sectioned in shoots with two leaves measuring between 4 and 6 cm and inoculated in MS medium (Murashige and Skoog 1962) at concentrations of 25% (MS ¼), 50% (MS ½), 75% (MS ¾), and 100% (MS) of their salts, supplemented with 30 g L^{-1} sucrose, and solidified with 6 g L^{-1} agar.

Silicon sources and concentrations for in vitro growth and development of gerbera

In another experiment, gerbera shoots with two leaves, measuring between 4 and 6 cm and inoculated in MS medium with ¼ of salts, supplemented, before the sterilization, with different silicon sources dissolved in distilled water before the sterlization [potassium silicate (K_2SiO_3) (Sigma-Aldrich,

USA), calcium silicate ($CaSiO_3$) (Sigma-Aldrich, USA), sodium silicate (Na_2SiO_3) (Sigma-Aldrich, USA) or silicic acid (H_4SiO_4) (Sigma-Aldrich, USA)] at concentrations of 0 (Witness), 0.25; 0.5; 0.75 and 1 g L^{-1}, amounting a 4×5 factorial. Two plants per vials were arranged in 10 replicates for each treatment. The medium was added with 30 g L^{-1} sucrose and solidified with 6 g L^{-1} agar.

General conditions for sterilization and incubation

The pH of all media, 50 mL of which was included to each 300 mL flask, were adjusted to 5.8 ± 0.1, with 50 mL of culture medium at each 300 mL flask. Sterilization of culture media was performed by autoclaving them at temperature of 121 °C and pressure of 1.2 atm for 20 min.

Plants were inoculated in 10 replications, with two plants per vial and transferred to growth room with 16 h photoperiod, temperature of 25 ± 2 °C and irradiance of 58 µmol photon m^{-2} s^{-1}.

The characteristics evaluated after 45 days of culture were: number of shoots, shoot length of the highest shoot (cm), number of leaves, and number of roots.

Silicon sources in gerbera acclimatization

After development, the plants were acclimatized by transferring to tubes containing the commercial Plantmax® substrate. The tubes were covered with plastic bags to maintain humidity, which were gradually withdrawn every seven days, until the total withdrawal, on the 21st day. The plants were kept in a acclimatization room under 12 h photoperiod and irradiance of 60 µmol photon m^{-2} s^{-1}. After 30 days, the survival rate of plantlets (%) was evaluated.

Statistical design

All experiments were conducted in a completely randomized design (CRD) and the data were analyzed via Scott-Knott test by the SISVAR® statistical software (Ferreira 2014).

Results and discussion

Culture medium concentrations for in vitro growth and development of gerbera

Gerbera shoot culture at MS medium with 50% of salts (MS ½) and 25% of salts (MS ¼) resulted in higher shoot formation (10.83 and 10.79 shoots/plants, respectively) (Fig. 1a), with higher length of shoots (6.39 and 6.56 cm, respectively) (Fig. 1b) and higher number of leaves (3.34 and 3.32 leaves/plants, respectively) (Fig. 1c). Considering the root formation, a higher number was observed only at MS ¼, with an average of 3.56 roots/plants (Fig. 1d), indicating that it is possible to reduce salt concentrations of the culture medium during rooting stage, which besides providing optimal results, also makes the process more economically viable.

The MS culture medium is the most commonly used. The modification of this medium, as well as the use of its dilution, has been recommended not only for crop adequacy but also for cost saving (Vieira et al. 2009; Silva et al. 2016).

Silicon sources and concentrations for in vitro growth and development of gerbera

When evaluated the effect of the addition of silicates to the culture medium, the use of calcium silicate showed generally a higher shoot formation when compared to the other silicon sources (Fig. 2). The use of silicic acid also provided an increase in the number of shoots, even when used up to 0.75 g L^{-1}. The effectiveness was reduced at higher concentrations. However, the use of calcium silicate in all tested concentrations provided a discrepant increase in the number of shoots compared to the other concentrations and, above all, with the other tested sources.

In contrast, the use of sodium silicate and potassium silicate did not favor the formation of the number of shoots in gerbera explants, with results similar or inferior to the control (Fig. 2). It was also observed that the increase of potassium silicate concentrations affected positively the formation of new orchid shoots (Soares et al. 2008; Alves et al. 2016).

By evaluating the plant height, it was observed that the use of different concentrations and different silicon sources resulted in a small increase in its length in relation to the control. But when compared the different tested sources, silicic acid and sodium silicate showed to be less effectiveness (Fig. 3).

Moreover, it was verified that the use of calcium silicate at lowest concentration (0.25 g L^{-1}) resulted in the formation of higher number of leaves. In contrast, the other silicon sources formed a number of leaves similar or inferior to the control (Fig. 4).

It was also observed that the increasing concentrations of calcium silicate also increased root formation. Conversely, when tested the sodium silicate, potassium and silicon acid sources, the root development was affected negatively (Fig. 5).

Thereby, the use of calcium silicate, besides the formation of a larger number of shoots and with higher height, provided a larger number of leaves, confirming the non-occurrence of etiolation. Therefore, it is proven that a careful selection of silicon source to be used is fundamental for the effectiveness of results.

In orchids, the effect of silicon on growth is dependent on the species and source. The use of calcium silicate increased shoot size in *Brassavola perrine* and *Laelia* hybrid (Pasqual

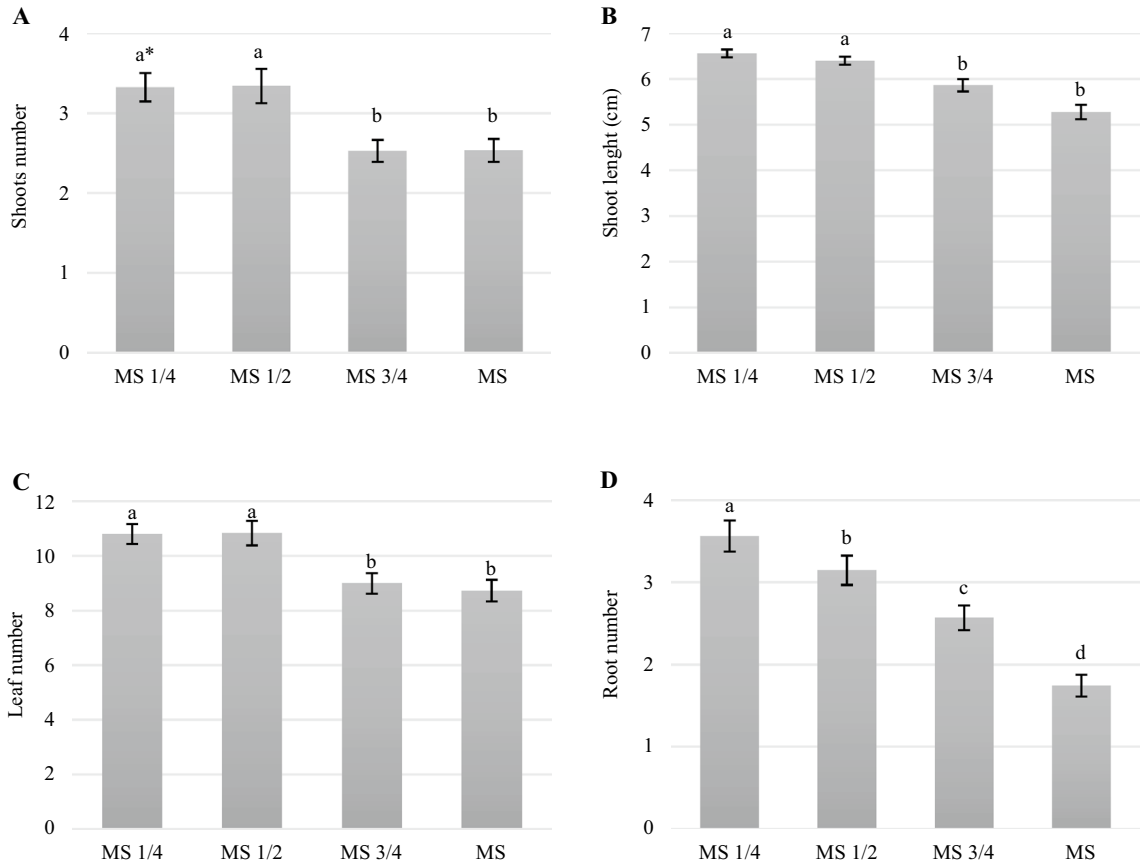

Fig. 1 Number of shoots (**a**), shoot length (**b**), number of leaves (**c**) and number of roots (**d**) formed in gerbera explants cultured in vitro at different concentrations of MS culture medium. *Letters differ among themselves within concentrations of MS culture medium

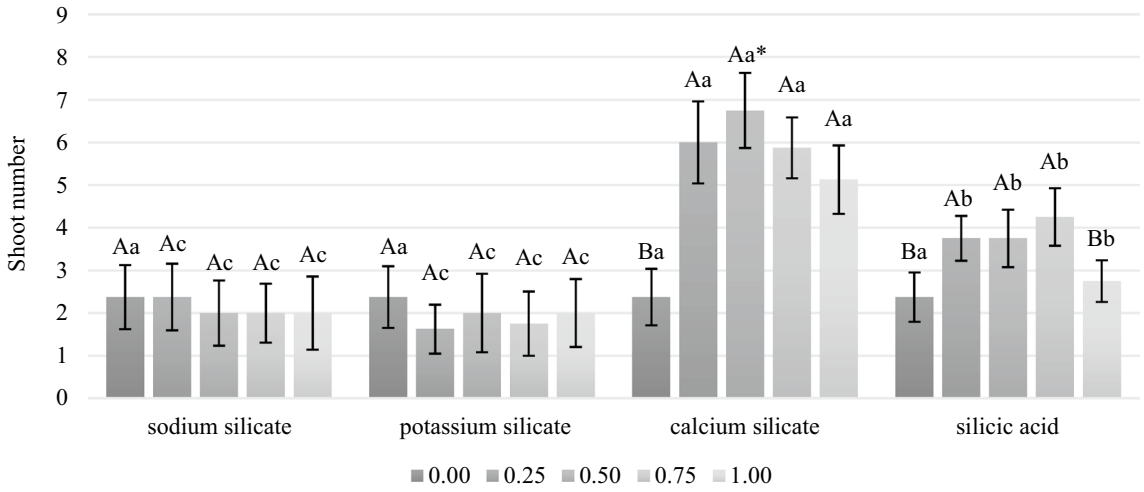

Fig. 2 Number of shoots formed in gerbera explants cultured in vitro at medium supplemented with different sources and concentrations of silicon (sodium silicate, potassium silicate, calcium silicate and silicic acid). *Capital letters differ among themselves within the silicon sources (sodium silicate, potassium silicate, calcium silicate and silicic acid) and lowercase letters differ among themselves within silicate concentrations (0.0; 0.25; 0.5; 0.75 and 1 g L^{-1})

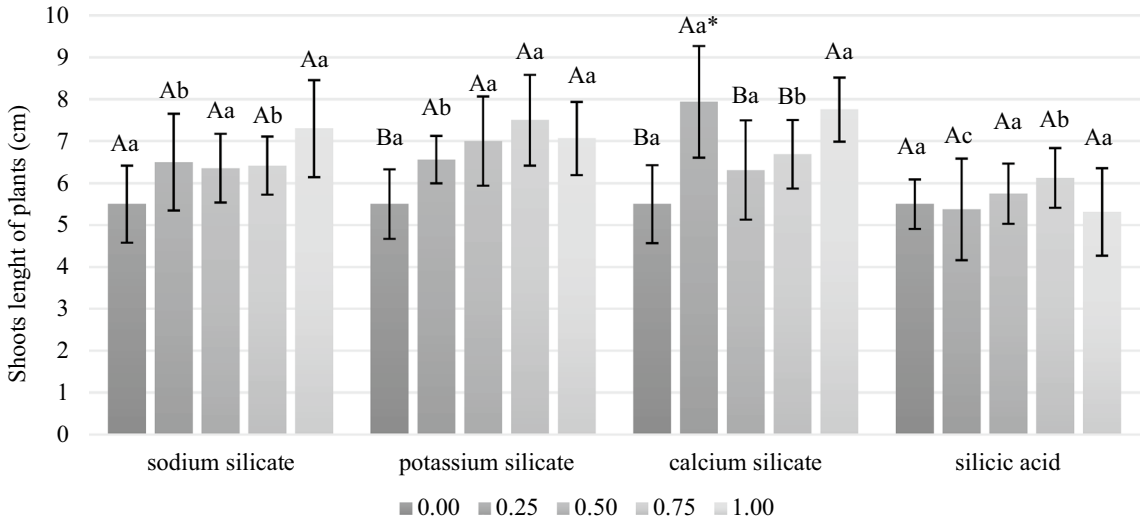

Fig. 3 Shoot length of plants (cm) formed in gerbera explants cultured in vitro at medium supplemented with different sources and concentrations of silicon (sodium silicate, potassium silicate, calcium silicate and silicic acid). *Capital letters differ among themselves within the silicon sources (sodium silicate, potassium silicate, calcium silicate and silicic acid) and lowercase letters differ among themselves within silicate concentrations (0.0; 0.25; 0.5; 0.75 and 1 g L^{-1})

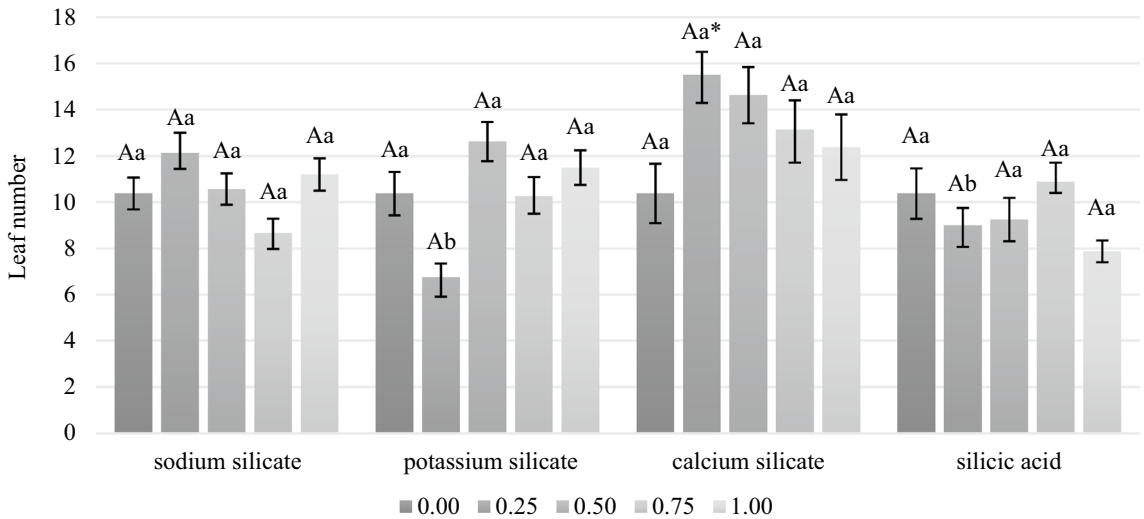

Fig. 4 Number of leaves formed in gerbera explants cultured in vitro at medium supplemented with different sources and concentrations of silicon (sodium silicate, potassium silicate, calcium silicate and silicic acid). *Capital letters differ among themselves within the silicon sources (sodium silicate, potassium silicate, calcium silicate and silicic acid) and lowercase letters differ among themselves within silicate concentrations (0.0; 0.25; 0.5; 0.75 and 1 g L^{-1})

et al. 2011), while the use of sodium silicate inhibited the growth of *Hadrolaelia lobatta* x *Hadrolaelia purpurata aço* (Soares et al. 2008). These authors, however, did not test the effects of different sources, therefore it is not possible to determine if there are different behaviors when the source is different.

In general, the use of calcium silicate has been shown to be the best silicon source for the plant, favoring the higher growth and development of gerbera plantlets. The use of sodium silicate and potassium silicate did not affect the development. Considering that the shoot development is fundamental, the use of 0.25 and 0.5 g L^{-1} is very effective,

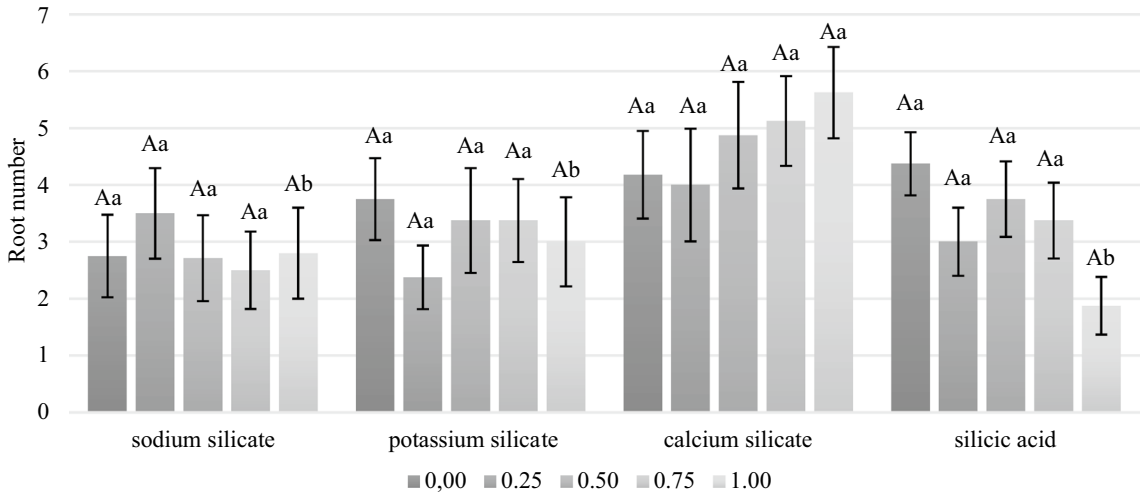

Fig. 5 Number of roots formed in gerbera explants cultured in vitro at medium supplemented with different sources and concentrations of silicon (sodium silicate, potassium silicate, calcium silicate and silicic acid). *Capital letters differ among themselves within the silicon sources (sodium silicate, potassium silicate, calcium silicate and silicic acid) and lowercase letters differ among themselves within silicate concentrations (0.0; 0.25; 0.5; 0.75 and 1 g L^{-1})

providing a significant increase in the number of shoots with good size and good root development.

The efficiency of the use of calcium silicate source may be related to the supply of calcium in association with silicon to gerbera plantlets, which plays an important role in cell signaling, besides maintaining plasmalemma integrity (Reddy 2001; Aranda-Peres et al. 2009) and being an important component of cell wall (Schroeder et al. 2001).

The silicon absorption in plants is preferably performed by the silicic acid form (Epstein 1999; Assis et al. 2018). However, our results showed that the use of sources other than silicic acid, calcium silicate for instance, might be more efficient in increasing the effective growth of gerbera plantlets.

Some studies have demonstrated the efficiency on the use of some silicon sources, but at a fixed concentration (Asmar et al. 2013, 2015; Luz et al. 2012) and others performed tests at different concentrations, but using only one or two sources (Colombo et al. 2016; Soares et al. 2012). Thus, the silicon efficiency can only be confirmed if different sources at different concentrations were tested. In our opinion, the silicon efficiency can be confirmed the most accurately if different sources of silicon are tested at the different concentrations.

The use of silicon sources such as calcium and potassium silicate may have favored the growth and development of gerbera plantlets by providing calcium and potassium in addition to silicon. When MS medium concetration was tested, the increase of the nutrient concetration did not favor the development and growth of gerbera plantlets. We can thus infer that increased growth of gerbera plantlets was favored by the supply of silicon.

During the acclimatization process, no significant differences were observed in the different silicon sources for the survival rate (Fig. 6). A high survival rate was observed in all silicon sources, with an overall average of 86% of plantlet survival (Fig. 7).

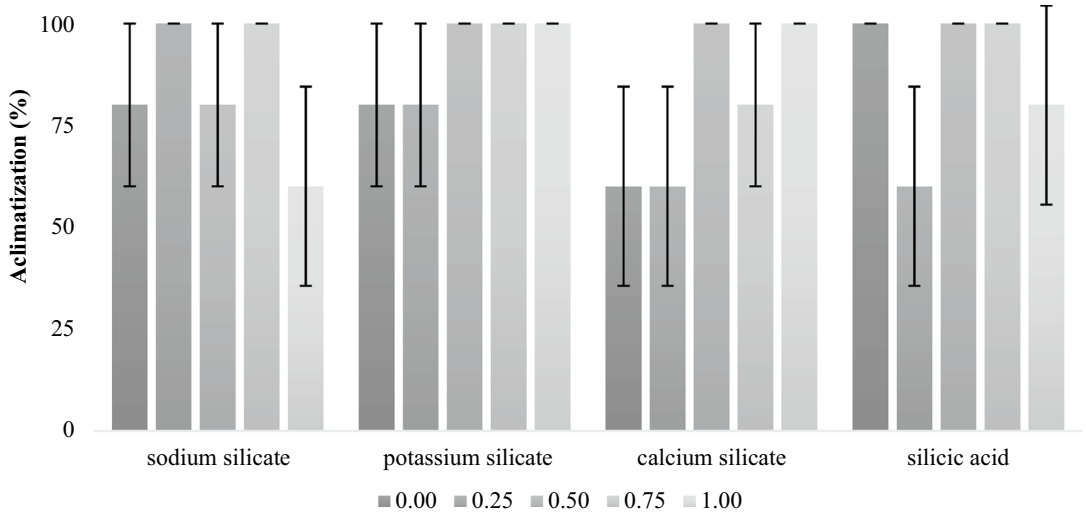

Fig. 6 Acclimated plantlets of gerbera cultured in vitro at medium supplemented with different sources and concentrations of silicon (sodium silicate, potassium silicate, calcium silicate and silicic acid)

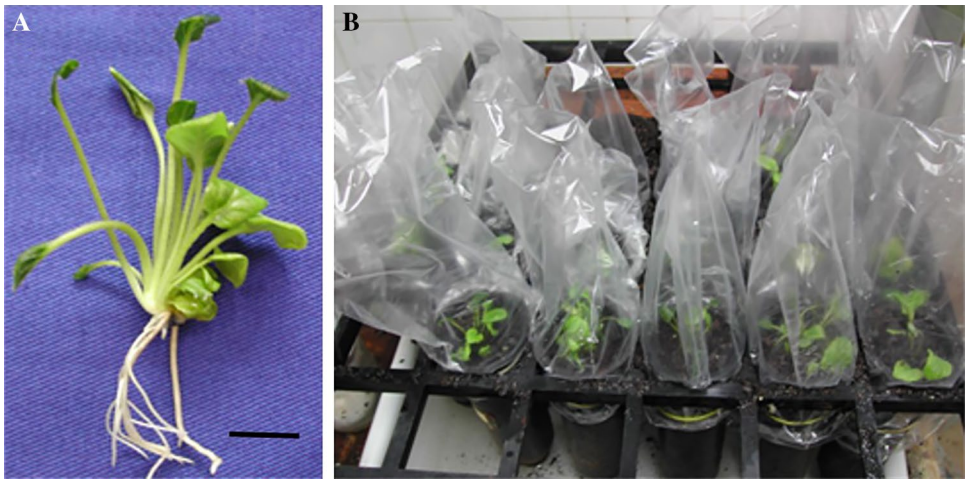

Fig. 7 Plantlets of gerbera cultured in vitro at medium supplemented with 0.25 mg L^{-1} calcium silicate (**a**) and plantlets during the acclimatization (**b**). Bar = 1 cm

Conclusions

Our results suggest that in vitro gerbera shoots regenerate and even root better on MS 1/4, and shoot regeneration is even further enhanced when 0.25 mg L^{-1} calcium silicate is added to the multiplication medium.

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Author Contributions SDPC: main author of the manuscript, performing all analysis, tabulating data and discussing the results with the other authors. PPDO: main researcher, responsible for the laboratory where the experiment was executed. HRRC, PJMP, RMV and PR: assisted in the implementation of tests, tabulation and interpretation of data and construction of related tables and charts. Authors also assisted with writing and discussion of all results found.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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