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THE EFFECTS OF POTASSIUM SILICATE AS A COMPONENT OF NUTRIENT MEDIUM FOR SELECTED IN VITRO CULTURES OF *PRUNUS* AND *CORYLUS* GENERA

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Abstract

Even though silicon is frequent compound in soil, its use in plant nutrient media is rare. Based on known physiological role and up to now performed studies it seems that silicon has a good potential to improve growth characteristics of *in vitro* cultivated plants. Before practical application, however, it is always necessary to assess the optimal conditions of application with regard to the fact that plant reactions to different chemicals added to nutritional media can vary on the species or even cultivar level. The presented study evaluate effects of potassium silicate used in in vitro cultivation media on growth parameters of *Prunus persica* × *Prunus davidiana* 'Cadaman,' *Prunus* × *amygdalopersica* 'GF 677' and *Corylus avellana* 'Tonda di Giffoni' genotypes, which are frequently subject of commercial in vitro multiplication.

In fact, four different concentrations of potassium silicate was added to the multiplication media, control medium was left silicon-free. Three different characteristics were observed during cultivation – number of new shoots per explant, weight of a new plant and length of new shoots, from which number of new shoots per explant was considered the most important factor.

In all cases a positive effects of potassium silicate on the condition and other growth parameters of treated cultures were observed. In 'Cadaman' culture significant growth changes appeared on media with 20 $mg.l^{-1}$ potassium silicate, which can be recommended for future applications. For 'GF 677' the best results were obtained on media with 2 $mg.l^{-1}$ which can be recommended for improving condition and number of shoots on new plants. For hazelnut genotype 'Tonda di Giffoni' best results were obtained on media with 10 $mg.l^{-1}$ of potassium silicate, but also use of 5 $mg.l^{-1}$ of potassium silicate significantly improved growth parameters. Generally, presented study provides important and practically useful insights into the practical use of silicon in cultivation media designated for commercial in vitro micropropagation.

Keywords: silicon, micropropagation, tissue culture, in vitro, *Prunus, Corylus,* media

INTRODUCTION

Silicon is one of the most abundant elements in soil and it has important role in stress-related responses in plants. This microelement is a part of adaptive processes of plants when they're exposed to variety of both biotic and abiotic stress factors (Ma, 2004).

Regarding the use of silicone in tissue cultures, currently it is usually neglected in cultivation media and only few reports using silicone supplements exist. It was described that silicon can improve growth characteristics of micropropagated plants and their morphology, although the molecular and biochemical bases of silicon actions on organogenesis remain unknown (Sahebi *et al.*, 2016). Decrease in an occurrence of hyperhydricity and oxidative browning of shoots in plant tissue cultures enriched by silicon was also reported (Sahebi *et al.*, 2016; Colombo *et al.*, 2016). This behaviour confirmed also study based on the dispersive X-ray study of *Cotoneaster wilsonii* leaves (Sivanesan *et al.*, 2011), where no trace of silicon in the hyperhydric leaf samples was observed, while in non-hyperhydric plants silicon was found. Therefore, the hyperhydricity problem could be theoretically decreased through silicon supplementation to the *in vitro* medium (Sivanesan *et al.*, 2011).

Lišková *et al.* (2016) found that addition of silicate prevents shoots necrosis and supports leaf and shoot growth in *Harpagophytum procumbens*. Avestan *et al.* (2015) described positive effects of silicate on *in vitro* cultures of apple tree rootstock 'MM106', addition of silicon ions in media increase weight, length and number of new shoots.

Known physiological role of silicone and previously performed studies suggest that use of silicon as a supplement in cultivation media have a potential to improve growth characteristic of *in vitro* cultivated plants. Of course, there are other possibilities to improve growth parameters by using other chemicals such as phytohormones, however, these compounds are usually significantly more expensive compared to very cheap potassium silicate and their use would distinctly increase costs if applied in commercial *in vitro* propagation. Nevertheless, the precise evaluation of the effects of different silicon concentrations and sources on the growth of respective types of cultures is necessary before extensive use in cultivation media. The reason is that plant reactions to different chemicals added to nutritional media can vary on the species or even cultivar level (Sivanesan and Park, 2014). Thus, the objective of presented study is to evaluate the effect of potassium silicate in cultivation media for three genotypes, which are quite frequently the subject of *in vitro* micropropagation – rootstock *Prunus persica* × *Prunus davidiana* 'Cadaman', rootstock *Prunus* × *amygdalopersica* 'GF 677' and hazelnut cultivar *Corylus avellana* 'Tonda di Giffoni. With regard this aim, the impact of four different concentration of potassium silicate on three growth parameters was evaluated with a focus on number of newly created shoots per explant, and an optimal concentration of potassium silicate for cultivation of individual genotypes was established.

MATERIALS AND METHODS

Biological Material

For experiment three different types of cultures were chosen: *Prunus* × *amygdalopersica* (Weston) Rehder cv. 'GF 677' (in USA sometimes named 'Paramount'), *Prunus persica* × *Prunus davidiana* cv. 'Cadaman', and *Corylus avellana* L. cv. 'Tonda di Giffoni'.

All plant material was provided by VITROTREE BY BATTISTINI s. r. o. as already established *in vitro* cultures.

Prunus × *amygdalopersica* (Weston) Rehder cv. 'GF 677' was chosen because it is one of the most widely used rootstocks for peaches in Europe, it can be also used as rootstock for almonds (Wilkinson, 2005).

Second culture was *Prunus persica* × *Prunus davidiana* cv. 'Cadaman', sometimes called 'Avimag'. It originates in Hungary and is used as rootstock for both peaches and almonds as well (J. Wilkinson, 2005).

As a third hazelnut culture (*Corylus avellana* L. cv. 'Tonda di Giffoni') was used, which is in high demand and known for their low multiplication coefficient, from Italy (Wilkinson, 2005). This cultivar, due to its round nuts and good processing quality, belongs between the most recognized ones and is preferred in new plantings (Petriccione *et al.*, 2010).

Cultivation and Concentrations of Potassium Silicate in Media

Experiment took place in plant tissue culture laboratory of Faculty of Horticulture in Lednice, Czech Republic. Silicon was added as anhydrous, water-soluble potassium silicate with level of purity ≥ 99%. All cultures were placed in the cultivation room, where temperature is kept on 24 °C, with 18/6 hours light/dark cycle and light intensity 36 W.

For cultivation of 'GF 677' and 'Cadaman' LP medium with 0.4 $mg.l^{-1}$ benzylaminopurine and 0.01 $mg.l^{-1}$ naphthaleneacetic acid was used as control. For hazelnut cultivation of cv. 'Tonda di Giffoni' DKW/Juglans medium with 1 $mg.l^{-1}$ meta-Topoline was used as control. These media were further modified with addition of potassium silicate in 2 $mg.l^{-1}$, 5 $mg.l^{-1}$, 10 $mg.l^{-1}$ and 20 $mg.l^{-1}$.

All cultures were transplanted to tested media for 4 weeks. In this phase plants adapt to new media with different concentrations of silicon, so their growth parameters weren't affected by previous cultivation. After this, plants were cut into explants and transferred to new media with the same compositions as in adapting step.

All variants were subsequently let to grow in cultivation room for another 4 weeks (6 weeks for hazelnut plants). After this time growth parameters were measured and at the same time the plants were cut again and transplanted to a new media for repetition of the experiment. The experiment was performed three times to prove its repeatability. From every plant-medium combination (0 mg.l^{-1}, 2 mg.l^{-1}, 5 mg.l^{-1}, 10 mg.l^{-1} and 20 mg.l^{-1} of silicon concentration) there were ten containers set up, each containing ten explants. Together there were 50 containers for each tested cultivar (150 containers for each repetition of the experiment).

Growth parameters consisted from fresh weight of the explants, number of established plantlets and their length measurement. After weighing on laboratory scale in laminary box the plant was divided to separate shoots, which were then counted up and measured by using graph paper.

Statistical Procedures

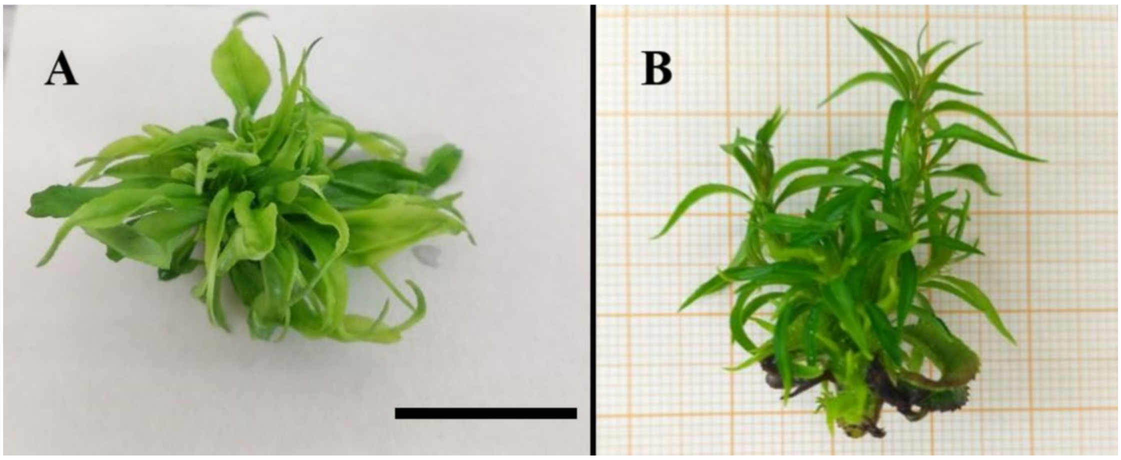
Kolmogorov-Smirnov test was performed for every set of plant data to prove or reject H_0. When H_0 was rejected on $p < 0.05$, the alternative hypothesis, that at least one combination is statistically different, was accepted. As the data were from other than normal distribution, the analysis, which counts with average, couldn't, be used. Because of that, Kruskal-Wallis test was chosen for the analysis. POST-HOC test based on Kruskal-Wallis test was used to find which concentrations of potassium silicate cause significantly different results.

RESULTS AND DISCUSSION

Effect of the Potassium Silicate Supplement on Growth Factors of *Prunus persica* × *Prunus davidiana* cv. 'Cadaman'

The cultures on control media without potassium silicate grew readily, some hyperhydrous shoots and small amount of leaf deformities were observed. As can be seen in Tab. I, media variations with 2, 5 and 10 mg.l^{-1} of potassium silicate didn't differ from the control significantly, just light increase of callus and root occurrence could be spotted. The weight of plants, number of new shoots or their length wasn't affected up until the concentration 10 mg.l^{-1}.

Significant difference in number of shoots was found on media with 20 mg.l^{-1} potassium silicate (Fig. 1), where increase occurred not only in shoot number, but as well in plant weight and length of shoots (Tab. I). This observation is quite remarkable, as increased number of shoots per explants usually reduces their length. In contrary, a study of da Silva *et al.* (2020) didn't find potassium silicate effective for improving growth traits of *Gerbera.* This could be caused by use of higher amount of potassium silicate in this study, where the effective amount of potassium silicate (20 mg.l^{-1} representing approx. 0.13 mM concentration) was 20× higher than the most concentrated treatment for *Gerbera* in

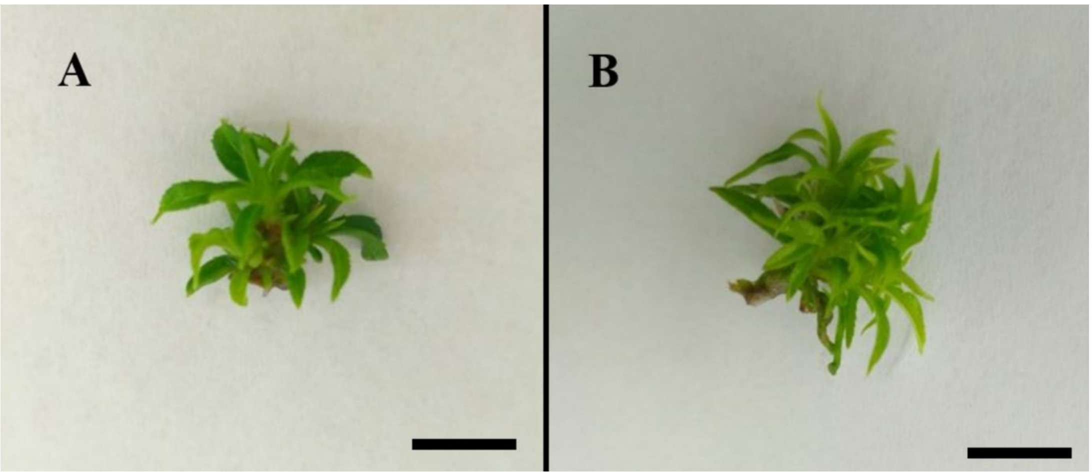


1: *A – Photo of ´Cadaman´grown on control media, B – Photo of ´Cadaman´grown on media with 20 mg.l^{-1} of potassium silicate. The increase in shoot length and their numbers was evident. Bar = 1 cm.*

I: *Average weight, number of shoots and shoot length of cv. ´Cadaman*

	Cadaman		
	Weight [g]	Number of shoots	Length [mm]
Control	0.4	4.8	9.4
2 mg.l^{-1} silicate	0.5	5.0	10,0
5 mg.l^{-1} silicate	0.5	4.5	9.8
10 mg.l^{-1} silicate	0.5	4.6	9.6
20 mg.l^{-1} silicate	0.6	5.9*	11.9

* statistically different, $p < 0.05$



2: *A – Photo of ´GF 677´grown on control media, B – Photo of ´GF 677´grown on media with 2 mg.l-1 ofpotassium silicate. The increase of number of shoots can be observed on picture B. Bar = 1 cm.*

II: *Average weight, number of shoots and shoot length of cv. ´GF 677´*

	GF 677		
	Weight [g]	Number of shoots	Length [mm]
Control	0.2	2.4*	6.8
2 mg.l-1 silicate	0.4	5.2*	6.5
5 mg.l-1 silicate	0.3	4.3	7
10 mg.l-1 silicate	0.3	3.5	6.7
20 mg.l-1 silicate	0.2	2.8	6.3

* statistically different, $p < 0.05$

da Silva's *et al.* study. As the best results were obtained on the media with highest tested concentration of potassium silicate, it is possible that results would be positively affected by adding even more silicon to media or using different sources. In a study on grape, concentration of potassium up to 2 mM were used, and the highest number of shoots was found on the plants fed with 2 mM potassium silicate (Ghadakchi *et al.*, 2019).

In terms of visual evaluation, plants on media with 20 mg.l-1 potassium silicate grew vigorously, although the long shoots often developed shoot necrosis. Phenomenon of vitrification was also observed in plants on this media.

Effect of the Potassium Silicate Supplement on Growth Factors of *Prunus* × *amygdalopersica* cv. *'GF 677'*

As can be seen in Tab. II, all variants with potassium silicate showed higher values in plant weight and number of shoots than a control group. Statistical analysis proved that results obtained on control differs from all other groups in term of number of new shoots, and at the same time that the results obtained on the media with 2 mg.l-1 differs from all the variants excepting variant 3 (5 mg.l-1 of potassium silicate). However, contrary to 'Cadaman', best results were obtained on media with lowest concentrations of potassium silicate, 2 and 5 mg.l-1, while media with 2 mg.l-1 of potassium silicate was better for supporting proliferation (Fig. 2, Tab. II). In the study on *Tagetes patula* by Sivanesan *et al.* (2010) it is shown that silicon can cause decrease in plant height. In study conducted by Sivanesan and Jeong (2014) they found out that he addition of the proper amount of silicon (3.6 mM) to the culture medium resulted in an increase in *Ajuga multiflora* height, while 7.2 mM of silicon supplementation led to shorter plants. Such divergence in ability of silicon to increase or decrease plant height is highly dependent on plant genotype (Sivanesan and Park, 2014). This could be caused by silicon toxicity as described in study of Mantovani *et al.* (2020), where toxicity of silicon was registered on orchids in much higher concentrations than presented study.

A common trend of decreasing growth factors with increasing concentration of potassium silicate can be seen in Tab. II. At the concentration of 10 mg.l-1, the weight of plants, number and length of shoots decreased noticeably, and at the concentration of 20 mg.l-1 the results were close to the control group. These results can indicate lower tolerance of *Prunus* × *amygdalopersica*, or specifically cv. 'GF 677' to silicon.

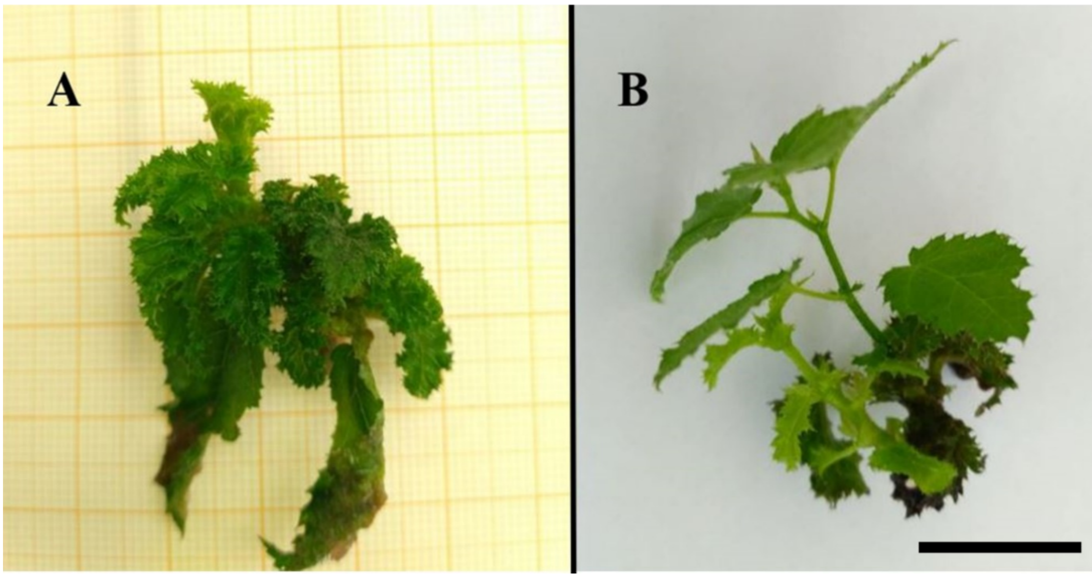
Effect of the Potassium Silicate Supplement on Growth Factors of *Corylus Avellana* L. cv. 'Tonda di Giffoni'

In hazelnut cultures the comparison between different potassium silicate concentrations isn't completely unambiguous, but all media with potassium silicate gave higher values for number of shoots than a control group. Statistical analysis proved that results obtained on control media differs from all other silicon-treated groups in regard of number of newly created shoots per explant. (Fig. 3A, Tab. III). Regarding the most important multiplication aspect, number of newly created shoots, the media with 10 $mg.l^{-1}$ of potassium silicate provided the best results (Fig. 3B). Almost the same results were however obtained on media with half of the concentration, 5 $mg.l^{-1}$ of potassium silicate. Plants on medium with 20 $mg.l^{-1}$ potassium silicate produced the longest shoots, which, though, resulted in reduced proliferation.

Hazelnuts cultures showed unusually fast growth on media combining both meta-Topoline and potassium silicate. It was possible to reduce the length of cultivation from usually used eight weeks to six weeks only, so this variant expressed interesting properties from the point of view of cultivation time shortening if used within commercial micropropagation.

Variability of Optimal Concentration of Silicone in Cultivating Media

The study confirms that addition of potassium silicate into cultivation media can have positive effects on plant weight, number and length of shoots on chosen plant genotypes, which was previously observed by other authors in different species of plants (Lišková *et al.*, 2016; Sivanesan and Jeong, 2014; Máthé *et al.*, 2012). Positive effect was noticed in all tested cultures, however it has also been confirmed that optimal concentration of silicon in media can vary even within the same plant species (Soares *et al.*, 2011; Soares *et al.*, 2013). For example, the inclusion of calcium silicate at 0.5 and 2.0 $mg.l^{-1}$ to the MS medium differently stimulates the growth of native (*Brassavolva perrinii*) and hybrid (*Laelia cattleya* 'Culminant Tuilerie' × *L. cattleya* 'Sons Atout Rotunda') × (*Brassolaelia cattleya* 'Startifire Moon Beach') orchid plants, respectively (Soares *et al.*, 2012). Lim *et al.* (2012) also reported that the effect



3: *A – Photo of ´Tonda di Giffoni´grown on control media, B – Photo of ´Tonda di Giffoni´ grown on media with 10 $mg.l^{-1}$ of silicon. The increase in number of shoots and improved condition of plants can be observed on picture B. Bar = 1 cm.*

III: *Average weight number of shoots and shoot length of hazelnut cultivar cv. ´Tonda di Giffoni´*

	Tonda di Giffoni		
	Weight [g]	Number of shoots	Length [mm]
Control	0.3	1.5*	17.6
2 $mg.l^{-1}$ silicate	0.4	1.6	17.4
5 $mg.l^{-1}$ silicate	0.7	2.0	16.7
10 $mg.l^{-1}$ silicate	0.5	2.1	15.8
20 $mg.l^{-1}$ silicate	0.6	1.8	21.1

* statistically different (lower), $p < 0.05$

of potassium silicate on the growth traits of begonia are mainly dependent on the cultivars.

As culture 'GF 677' respond best to the lowest tested concentration (2 mg.l^{-1}) while on the control media the plants didn't grow as good, this might suggest that even lower concentrations of silicon, similar to those of which Zhuo (1995) or Rodrigues *et al.* (2017) found as most suitable, could be tested for this cultivar. The decreasing growth parameters with increasing concentrations can indicate lowered tolerance of this genotype to potassium silicate.

Unusually higher concentrations were used in the work of Soundararajan *et al.* (2015). They described that for *Dianthus caryophyllus* cultures best results were obtained on media supplemented with 50 mg.l^{-1} of silicon, *Salvia splendens* plants improved growth traits under concentration of 50 and 100 mg.l^{-1} potassium silicate (Soundararajan *et al.*, 2013). Lim *et al.* (2012) used even 300 mg.l^{-1} of silicon for *Begonia semperflorens* plants with success. As 'Cadaman' plants reacted positively to highest concentration (20 mg.l^{-1} potassium silicate), it might be also hypotehtised that optimal potassium silicate concentration in the cultivation media for Cadaman would be even higher.

CONCLUSION

Use of potassium silicate proved to be beneficial in all three tested plant species and can be recommended to improve *in vitro* cultivation of *Prunus* × *amygdalopersica* cv. 'GF 677', *Prunus persica* × *Prunus davidiana* cv. 'Cadaman' and *Corylus avellana* cv. 'Tonda di Giffoni'. These cultivars are often propagated by *in vitro* techniques commercially and even small improvements described in this study can lead to important amelioration of economical parameters for commercial micropropagation. Potassium silicate shoved the ability to increase number of new shoots in tested cultures, which is especially useful for cultures which have low propagation coefficient (hazelnut).

The optimal concentration of potassium silicate was different for every tested culture. For *Prunus persica* × *Prunus davidiana* cv. 'Cadaman', recommended amount of potassium silicate in media is 20 mg.l^{-1}, and for *Prunus* × *amygdalopersica cv. 'GF 677'* media with 2 mg.l^{-1} of potassium silicate. For *Corylus avellana* L. cv. 'Tonda di Giffoni' media with 5 mg.l^{-1} of potassium silicate could be recommended for practical use.

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