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Effect of Different Silicone Sources and Concentrations on *in vitro* Micro Propagation of 140 Ru Grape Rootstock

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Abstract

Silicon, which is widely used in different fields, has been used in plant production in *vivo* and *in vitro* studies in recent years. Especially in *in vitro* studies, it is seen that its effect on plant growth and development has been examined. In this study, the effect of three different silicon sources and their four concentrations on micro-propagation of 140 Ru grape rootstocks was investigated. In the study, as explants one-node micro cuttings of rootstock and MS (Murashige and Skoog) as the nutrient medium were used. 1 mg L^{-1} BA (Benzyl Adenine) at the stage of obtaining shoots from cuttings and in the rooting stage, 1 mg L^{-1} IBA (Indole Butyric Acid) were added to the nutrient medium. At both stages, 0 (Control), 0.5, 1.0 and 2.0 mg L^{-1} doses of potassium, sodium and calcium silicate were added to the nutrient medium. Explant viability and mortality rate, shooting rate, plant length, node number, shoot fresh and dry weight, chlorophyll content (SPAD), root number, root length, root fresh and dry weight were examined to determine the effect of the applications. The variance analysis of the study was carried out according to the Two-Way Completely Randomized Experimental Design. According to the results, among the silicon sources, the highest shooting rate (84.40%) was found in the medium containing sodium silicate. The highest shoot fresh and dry weight (0.178 g and 0.026 g, respectively) and root fresh and dry weight values (0.213 g and 0.023 g, respectively) were obtained from potassium silicate. While the number of roots was 2.98 in the medium containing potassium, it was determined as 2.91 in the medium containing calcium silicate. Media containing 1 mg L^{-1} silicate was found to be more successful than 0, 05, 2 mg L^{-1} concentrations. The highest values recorded at the concentration were 4.49 cm in plant length, 7.44 in node number, 0.183 g and 0.028 g in shoot fresh and dry weight, respectively, 28.37 in SPAD value and 3.27 in root number. As a result of the study, it is concluded that adding 1 mg L^{-1} concentration of potassium, calcium and sodium silicate to the nutrient medium can be used in future studies related with in micro propagation.

Keywords: Grapevine, *In vitro*, Potassium silicate, Sodium silicate, Calcium silicate

Introduction

Plant tissue culture is the process of culturing plant cells, tissues and organs isolated under sterile conditions in suitable nutrient mediums (Babaoglu et al., 2001). The content of the nutrient medium and the culture conditions affect the growth and development of plant cells, tissues, and organs *in vitro*. Generally, organic nutrients, sugar, plant growth regulators and solidifier agents are added into plant tissue growth culture (Mansuroglu and Gurel, 2001; Sivanesan and Park, 2014).

Tissue culture is an effective technique used in micro propagation of herbaceous and woody plants. Micro propagation can be described as obtaining of the new plants from the plant parts in artificial nutrition culture and aseptic conditions. Micro-propagation of grapevine with tissue culture

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is widely performed worldwide. Shoot apical meristems and axillary buds are used as explants for grapevine genotypes *in vitro* culture (Gray and Benton, 1991; Diab et al., 2001; Banilas and Korkas, 2007; Alzubi et al., 2012). All grapevine genotypes give similar reactions for certain medium. Reaction degree shows momentous changes according to the genotype, culture medium and hormone. That's why, the most suitable micro-propagation protocol must be created for every genotype. Recently, it has been observed that studies have been carried out to be increased the success rate by adding different silicon (Si) sources to the nutrient medium in micro-propagation (Martins et al., 2019; Montovani et al., 2020).

According to some researchers, although silicon is not among the essential elements for plants (Ma, 2004; Soares et al., 2008), there are also studies reporting that many plants benefit from this element (Epstein, 1994) and that silicon plays a beneficial role in stimulating the growth and development of many plant species (Avestan et al., 2016; Sahebi et al., 2016; Costa et al., 2021).

According to Kadlecova et al. (2020), although silicon is an abundant compound in soil, it is rarely used in plant nutrient medium. Based on its known physiological role and studies done so far, it is observed that silicon has good potential to improve the growth properties of *in vitro* cultivated plants. However, before applying it in practice, it is necessary to determine the optimal application conditions by research, taking into account the fact that plant responses to different chemicals added to the nutrient medium may vary by species and even varieties.

It is submitted that adding silicon into plant tissue medium grows the morphogenetic potential of the plant cells, organs, and tissues. Many of the beneficial effects of silicon are attributed to accumulation of silicon on the cell walls of the roots, leaves and bodies (Ma and Yamaji, 2006)

It was reported, that adding silicon into culture medium increases the hardness of the cell wall and mechanical strength and decreases the transpiration by increasing hemicellulose and lignin content of the plants that are reproduced *in vitro* (Camargo et al., 2007). In many of studies, it was shown that silicon application is increased plant growth and yield under the biotic and abiotic conditions (Artyszak et al., 2015; D'Imperio et al., 2015; Hartly, 2015; Muneer and Jeong, 2015; Xu et al., 2015; Yin et al., 2016). Considering the reality that the effect of different silicates added to the nutrient medium may change according to plant species and even varieties. That's why, optimal application conditions should be re-evaluated for each case.

Under the satisfactory *in vitro* studies for addition of silicon, the plants relating to the orchid (Soares et al., 2011; Soares et al., 2012; Montovani et al., 2020), begonia and violet (Lim et al., 2012), clove (Manivanna et al., 2018), banana (Asmar et al., 2013a and 2013b; Asmar et al., 2015) and apple plants (Reezi et al., 2009; Avestan et al., 2016) are available.

Despite the effects described in various species, a limited number of studies (Mozafari et al., 2018) have been found examining the effects of adding Si to the culture medium on the micro-propagation of grapevine. Therefore, the study was planned to determine the effect of 0.5, 1.0 and 2.0 mg L^{-1} concentrations of potassium (K_2SiO_3), sodium (Na_2SiO_3), and calcium silicate ($CaSiO_3$) added as a silicon source to the Murashige and Scoog (MS) medium (Murashige and Skoog, 1962) in *vitro* on the micro-propagation of 140 Ru grapevine rootstock and aimed to eliminate the lack of information on this subject.

Materials and Methods

This study was conducted in Tissue Culture Laboratory of Department of Horticulture, Faculty of Agriculture, University of Cukurova in 2019.

Material

In the study, one node micro cuttings taken from actively growing shoots of 140 Ruggeri grapevine rootstocks grown in the Research and Application vineyard of Cukurova University, Faculty of Agriculture, Department of Horticulture were used.

Methods

Culture media preparation

After adding 30 g L^{-1} sucrose and silicates, additionally for shoot growing stage 1 mg L^{-1} BA (Benzyl Adenine) and for rooting stage 1 mg L^{-1} IBA (Indole Butyric Acid) was added to MS culture medium (Ducheva Biochemie, M0222.0050). pH of the medium was adjusted using 1N HCL and 1N KOH as 5.8 and then 8 g L^{-1} agar was added into media. Nutrient medium prepared was dispensed into the test tubes in diameter 2.5 cm and in length 15 cm as 10 mL. After the tubes were sterilized in autoclave at 121 ^oC and 1.05 kg cm^{-2} pressure for 15 minutes, they left to cool in a sterile cabinet.

Explant preparation and shoot obtaining

The tops of the shoots of 10 cm in active vegetation period were taken. After that, they were brought to laboratory and leaves on them were cleaned out and divided into one-node-cuttings These cuttings were disinfected for 20 minutes with using a solution containing 5% sodium hypochlorite (commercial bleach) and 1-2 drops of Tween 20 (Merck, 9005-64-5) (Mese and Tangolar, 2019). Afterwards, the explants were rinsed down in sterile cabinet 3 times with distilled water. After that, these explants were planted in the test tubes (25 mm x 150 mm) containing 10 mL MS medium that supplemented to 0, 0.5, 1.0 and 2.0 mg L^{-1} concentrations of K_2SiO_3 (Alfa Aesar, 1312-76-1), $CaSiO_3$ (Alfa Aesar, 10101-39-0) and Na_2SiO_3 (Sigma, 6834-92-0) and 1 mg L^{-1} BA for shoot obtaining stage of the experiment. These node explants were kept in the tubes for 4 weeks period.

Rooting of shoots

In the shoot obtaining stage, the shoots reached to the length of 2 cm and created 2-3 leaves were taken. These shoots were planted in the test tubes containing 10 mL MS medium that consists of 0, 0.5, 1.0 and 2.0 mgL^{-1} potassium, calcium and sodium silicate and 1 mg L^{-1} IBA concentrations. The shoots were cultured in the tubes for 45 days.

Culture conditions

Cultures were placed in a growth room with a temperature of 25 ± 1 ° C, a photoperiod of 16 hours, a luminous exposure of 3000-4000 lux (11000-15000 watts. m^{-2}). Lighting was provided by Cool daylight type TLD 36 w/54 fluorescent lamps.

Phytotechnical analyses

In the phase of shoot obtaining from the one-node microcutting, the explant viability, mortality and shooting rate were determined. These variables were calculated as follows:

Viability rate (%) = (no. of survived explants)/(no. of cultured explants) x 100

Mortality rate (%)= (no. of dead explants)/ (no. of cultured explants) x 100

Shooting rate (%) = (no. of sprouted explants)/(no. of survived explants) x 100

In the rooting phase of experiment, the number of node (or leaf) and root (n) were determined by counting. The length of shoots and roots (cm) were measured with a ruler.

Shoot and root fresh weights (g) were weighed, and then after drying in an oven at 65°C for 48 hours, their dry weights (g) were recorded by a digital scale.

The chlorophyll index (SPAD readings) was measured with a chlorophyll meter (SPAD 502, Konica Minolta, Osaka, Japan). The readings were determined on the two middle leaves of each plant.

Experimental design and statistical analysis

The research was planned according to the completely randomized experimental design of 3 replicates with 10 plants per repeat. The first factor was consisted of 3 Si sources (K_2SiO_3, $CaSiO_3$ and Na_2SiO_3);the second factor was composed of 4 concentrations (0, 0.5, 1.0 and 2.0 mg L^{-1}) of Si sources. Analysis of variance was performed to the obtained data using JMP (v.800, SAS Institute Inc., USA) statistical program based on SAS. For determination of different groups, LSD (Least Significant Different) was used at 0.05 level ($P \leq 0.05$). Angle transformation values were used for the variance analysis of the values obtained as percentages.

Results and Discussion

In the shoot obtaining stage, different Si sources had no significant effect on explants viability and mortality rates of 140 Ru rootstocks (Table 1). The highest shooting rate (84.40%) was obtained from sodium silicate and the lowest rates were in potassium and calcium silicate (72.12% and 73.86%, respectively) treatments. The effects of silicon doses were not significant on viability, mortality and shooting rates of explant. Interaction was not significant, either. The findings obtained at this phase, in which the roots were not formed, yet, suggested that the presence of roots is necessary for the silicon to be effective. The thought that silicon is an ineffective element on plant growth and development cannot be clearly supported at this stage in our opinion (El Fadl and Reda, 2014).

In the rooting stage of the research, the sources of silicon were not found important on shoot length and number of nodes. In spite of that, the effect was found to be significant in point of the plant fresh and dry weight values (Table 2). The highest shoot fresh and dry weight values were obtained from the medium containing potassium silicate (0.178 g and 0.026 g, respectively) and sodium silicate (0.159 and 0.024 g, respectively); the lowest values were obtained from media containing calcium silicate (0.139 g and 0.021 g, respectively) considering these properties. It is seen in Table 2 that the media containing 1 mg L^{-1} silicon concentration were more successful. In this concentration, the highest values were determined in shoot length 4.49 cm, in node number 7.44, in fresh and dry weight 0.183 g and 0.028 g, respectively.

No significant difference was found between the silicon sources in terms of SPAD index. In evaluation between silicon concentrations, the highest SPAD index (28.37) was taken from the medium containing 1 mg L^{-1} (Table 3).

Table 1. The effect of different silicon sources and concentrations on explant viability, mortality and shooting rate.

Sources of variation	Viability rate (%)	Mortality rate (%)	Shooting rate (%)
Si Source			
K_2SiO_3	95.02	4.98	72.12 b[x]
$CaSiO_3$	94.14	5.86	73.86 b
Na_2SiO_3	92.85	7.15	84.40 a
LSD 5%	NS	NS	7.36
p	0.244	0.764	0.023
Si Concentrations (mg L^{-1})			
Control	95.27	4.73	73.16
0.5	91.06	8.94	83.72
1.0	94.23	5.77	76.31
2.0	95.45	4.55	73.98
LSD 5%	NS	NS	NS
p	0.755	0.241	0.227
Interaction			
LSD 5%	NS	NS	NS
p	0.484	0.895	0.117

[x] Means within columns followed by the same letter/letters do not differ significantly from each other at $P \leq 0.05$ by by LSD multiple range test.
NS: Non Significant

Table 2. Effects of different silicon sources and concentrations on shoot growth and development.

Sources of variation	Shoot lenght (cm)	Node number (n)	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
Si Source				
K_2SiO_3	3.85	6.43	0.178 a[x]	0.026 a
$CaSiO_3$	3.61	6.75	0.139 b	0.021 b
Na_2SiO_3	3.90	7.14	0.159 ab	0.024 ab
LSD 5%	NS	NS	0.023	0.004
p	0.109	0.265	0.008	0.026
Si Concentrations (mg L^{-1})				
Control	3.49 bc	6.67 ab	0.151 b	0.023 b
0.5	3.35 c	6.17 b	0.143 b	0.022 b
1.0	4.49 a	7.44 a	0.183 a	0.028 a
2.0	3.82 b	6.81 ab	0.157 ab	0.023 b
LSD 5%	0.34	1.00	0.027	0.068
p	<0.0001	0.097	0.028	0.027
Interaction				
LSD 5%	0.59	1.73	0.046	0.007
p	<0.0001	0.204	0.005	0.005

[x] Means within columns followed by the same letter/letters do not differ significantly from each other at P≤0.05 by by LSD multiple range test. NS: Non Significant

Table 3. The effect of different silicon sources and concentrations on SPAD index

Sources of variation	SPAD index
Si Source	
K_2SiO_3	25.80
$CaSiO_3$	23.77
Na_2SiO_3	26.07
LSD 5%	NS
p	0.109
Si Concentrations (mg L^{-1})	
Control	22.76 b[x]
0.5	24.81 b
1.0	28.37 a
2.0	24.91 b
LSD 5%	2.72
p	0.003
Interaction	
LSD 5%	4.70
p	0.323

[x] Means within columns followed by the same letter/letters do not differ significantly from each other at P≤0.05 by by LSD multiple range test. NS: Non Significant

Contrary to our study, it has been stated by many researchers that the difference was important in the effect of silicate sources on plant height and number of nodes. Among these researchers, Asmar et al. (2011) tested the 1 g L^{-1} concentration of sodium, potassium, and calcium silicate in MS medium in the *in vitro* rooting stage of the Maçã banana plant, and it was observed that adding sodium silicate to the nutrient medium increased the shoot length and the fresh and dry weight of the shoots. Asmar et al. (2015) were determined in another study of them that 1 g L^{-1} concentration of calcium silicate which was applied in MS Medium in the *in vitro* culture, has increased the content of leaf chlorophyll of banana plants. It is seen that these results are in accordance with our data. Dias et al. (2017) have taken into culture Antorium's node explants with different sodium silicate concentrations (0.0, 0.5, 1.0 or 2.0 mg L^{-1}) in Pierik medium. They were determined that partly as same as our studies, the number of leaf and plant dry weight have increased in the plants in the medium added 0.5 mg L^{-1} and 2.0 mg L^{-1} sodium silicate. While in the same study (Dias et al., 2017), the highest chlorophyll a and b values were observed

in plants with 2.0 mg L^{-1} sodium silicate added, 1.0 mg L^{-1} concentration gave higher chlorophyll value in all three silicon sources. Rodrigues et al. (2017) in their study in sweet potato with 0.0, 0.5, 1.0 and 2.0 mg L^{-1} concentrations of potassium, calcium and sodium silicate, the maximum number of leaves (7.0 pieces) was determined. The longest shoots (4.02 cm) were determined from media containing 1 mg L^{-1} sodium silicate, and the highest plant fresh weights were determined in media containing potassium and calcium silicate (0.263 g and 0.284 g, respectively). While Soares et al. (2011) also reported that the addition of 5.0 mg L^{-1} K_2SiO_3 and 20.0 mg L^{-1} Na_2SiO_3 to modified Knudson C medium increased shoot length and number of shoots in orchid plants, Manivannan et al. (2018) observed that potassium silicate was more effective than calcium silicate on clove plant cultured *in vitro* in MS environment containing three different concentrations (0, 1.8 or 3.6 mM) of potassium and calcium silicate. El Fadl and Reda (2014) reported that in the dry date Bartamuda, fresh and dry weight increased in the presence of potassium and sodium silicate. Avestan et al. (2016) have obtained in their study on MM106 apple rootstock the highest plant fresh and dry weight and chlorophyll level from the mediums containing 100 mg L^{-1} nano-silicon. Lim et al. (2012) have reported that the highest plant fresh and dry weight and chlorophyll content in the varieties of begonia and violet were obtained from respectively 200 mg L^{-1} and 100 mg L^{-1} potassium silicate application. Braga et al. (2009) have determined that in their study in which they added 1 g L^{-1} doses of calcium, sodium and potassium silicate to MS medium in the micro propagation of strawberry plant, the fresh and dry weight of the shoots increased in sodium silicate presence, and adding silicate increased chlorophyll level. In the study of Soares et al. (2012), it was determined that the orchid plants have shown more growing in the artificial light application with 0.5 and 2.0 mg L^{-1} calcium silicate. As to in our studies, it was determined that 1.0 mg L^{-1} calcium silicate gives better result than the other concentrations. The idea has formed that the different results from this study and the studies mentioned above is probably due to the plant species studied and interaction of silicon sources.

In the studies of Martins et al. (2019) it was showed that the use of silicon was a good alternative to improve the photosynthetic pigment content and growth of plants propagated *in vitro*. SPAD values obtained in this study support the results of the researchers.

When the three different silicon sources used in the study were compared, the effects of the applications on the number of roots and the root fresh and dry weight were found to be significant. The highest root number was in the medium containing potassium (2.98) and calcium silicate (2.91). There was no significant difference between silicon sources in terms of their effects on average root length. In the root fresh and dry weight, potassium silicate (0.213 g and 0.023 g, respectively) was taken first place, calcium silicate (0.187 g and 0.019 g, respectively) second place, and sodium silicate (2.10 g, 0.152 g and 0.016 g, respectively) third place. The effect of concentrations used in the research was not significant on root length and fresh and dry weight of the roots. The highest root number value was seen in 1 mg L^{-1} dose (Table 4).

Table 4. Effects of different silicon sources and concentrations on root growth and development

Sources of variation	Root number (n)	Root length (cm)	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})
Si Source				
K_2SiO_3	2.98 a[x]	5.52	0.213 a	0.023 a
$CaSiO_3$	2.91 a	5.54	0.187 ab	0.019 ab
Na_2SiO_3	2.10 b	5.38	0.152 b	0.016 b
LSD 5%	0.51	NS	0.047	0.004
p	0.003	0.936	0.041	0.016
Si Concentrations (mg L^{-1})				
Control	2.18 b	5.84	0.182	0.019
0.5	2.67 b	5.19	0.179	0.018
1.0	3.27 a	5.80	0.197	0.021
2.0	2.53 b	5.09	0.177	0.019
LSD 5%	0.58	NS	NS	NS
p	0.007	0.441	0.863	0.584
Interaction				
LSD 5%	1.01	NS	0.094	0.008
p	0.002	0.212	0.0004	0.001

[x] Means within columns followed by the same letter/letters do not differ significantly from each other at P≤0.05 by by LSD multiple range test. NS: Non Significant

Lim et al. (2012) were also not found significant in their studies between the effect of potassium silicate at a concentrations of 0, 100, 200 or 300 mg L^{-1} added to the MS medium *in vitro* on the root length of the begonia plant. Contrary to our results, when Soares et al. (2011) cultured 1 cm long orchid seedlings by adding sodium silicate (0, 5, 10, and 20 mg L^{-1}) and potassium silicate (0, 5, 10, and 20 mL L^{-1}) and Knudson C medium in all possible combinations, they determined, that the maximum no. of roots and root length were realized in the medium added 20 mg L^{-1} sodium silicate and 5 mL L^{-1} potassium silicate. It is thought that the difference between our study and the findings of these researchers is due to quite high doses they used. Contrary to our data, Dias et al. (2017) have observed an increase in the root number of Anthurium plants in the nutrient medium supplemented with 1.0 mg L^{-1} sodium silicate. Soares et al. (2008) , who had taken results in contrary our findings, determined that there was a decrease in root fresh and dry weight of orchid plants with the increase in sodium silicate concentration. The fact that the silicon concentrations used here were higher than the concentrations used in this study and that the plant species were different indicates that the success changed according to the plant species and the source and concentration of silicon used. Mitani et al. (2005) also reported that the silicon accumulation in shoots varies significantly between plant species.

Conclusion

As result of the study, it has been determined that silicon may be added as supplementary source to the culture medium in the micro-propagation of grapevine. The results obtained regarding the different properties examined in our study showed that 1 mg L^{-1} concentration of potassium, followed sodium silicate to the nutrient medium during *in vitro* micro-propagation of the grapevine can be added successfully to increase plant quality. In subsequent studies, the trying of different silicon sources and concentrations would be beneficial to determine the reactions of other grapevine species and varieties.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

This study was derived from the Master Thesis of Sawsan Qasim LATEFF.

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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