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Research Article

The Effects of Ortho Silicone Applications on the Acclimatization Process of Grapevine Rootstocks

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ABSTRACT

Micropropagation is a tool for large-scale reproduction of planting material for viticulture sustainability. Successful micropropagation depends on the continued productivity of plantlets during the acclimatization phase. Due to high humidity in the culture container and free water in the environment, it causes rapid water loss and drying of plantlets with watery stems and leaves, poorly developed cuticle, large intercellular space, and incomplete stomata. Acclimatization of *in vitro* grown plantlets is often difficult. Silicon is gaining in importance as a useful tool in coping with multiple stress factors in different plant species, due to its contribution to the formation of the cuticle of plants, its mechanical resistance to biotic and abiotic stress, and its contribution to the flow of water through stomata and plant surfaces. In this study, the effects of 500 and 1000 µL ortho silicon applications at the acclimatization stage on *in vitro* propagated plantlets of 41B, 110R and Fercal grapevine rootstocks were evaluated by examining their survival rates. In the *in vitro* propagation process, rooting rates were listed as Fercal (64%) and 110R (32%) and 41B (28%) according to rootstocks, while root numbers were listed as Fercal (11.8), 41B (8.5), 110R (3.6). Genotypic differences were determined in the effects of silicon on plantlets in the acclimation process. In 110R and Fercal control plantlets, all plantlets were lost during acclimatization. Of the plantlets treated with 1000 µL SiO_2, 110R 66% Fercal 88% and in 41B, the control, 500 and 1000 µL SiO_2 applied plantlets survived 100%, 66% and 66%, respectively. 1000 µL SiO_2 dose was more effective on survival rates than 500 µL applications. In subsequent studies, it was found that 1000 µL SiO_2 applications could be used for practical success in grapevine genotypes that had problems in acclimatization and in other *in vitro* propagation studies.

1. Introduction

Grapevine (*Vitis* spp) is one of the most widely grown species worldwide due to its economic importance (Reynolds 2017). Since its widespread use for the production of wine, table grape and dried grapes, and the beneficial effects of grape metabolites on health, the grapevine is the focus of attention in plant science and a model woody species in plant biotechnology (Yancheva et al. 2018). Commercial grape varieties and grapevine rootstocks are propagated by cuttings and grafting techniques, which require long growing times and are labour intensive. Like other asexual propagated plant species, grapevines are often infected with more than one virus, which reduces crop quality and yield (Choi et al. 2008). Propagation with tissue cultures has recently been applied practically in viticulture to obtain high quality plantlets (Barlass and Skene 1978; Mukherjee et al. 2010; Eftekhari et al. 2012; Jin et al. 2013). In some plant species, the success of the plants propagated in tissue culture is low in greenhouse conditions and then in the stages of transporting them to the open field. This is mainly because of transplant shock, excessive water loss, pathogen attack, poor photosynthesis, etc. This is due to the inability to tolerate different types of stress, such as in plantlets under such stressful conditions, various plant processes such as CO_2 assimilation, chlorophyll biosynthesis and water relationships are altered or severely affected (Krishna et al. 2005). Similarly, *in vitro* grapevine propagation studies, a significant part of plantlets cannot maintain their vitality during the acclimation stage to natural environmet (Faulks and Mudge 1988; Kara and Yazar 2020). In previous studies to reduce the problems that occur in the acclimatization process of grapevine and some other plant species, arbuscular mycorrhiza applications (Krishna et al. 2005),

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changing the ambient humidity and plantlet age (Thomas 1998), changing *in vitro* environmental conditions and nutrient content (Kadleček et al. 2001), abscisic acid (Pospíšilová et al. 1998), humic acid and endophytic bacteria applications (Baldotto et al. 2010) were tested.

Bio-stimulators are defined as materials containing one or more active substances and/or microorganisms and are attracting more attention. These substances improve nutrient uptake by plants, tolerance to abiotic and biotic stress (Calvo et al. 2014), and also increase the activity of rhizosphere microorganisms and soil enzymes, as well as stimulate hormone production and photosynthesis (Fageria et al. 2009). Silicon (SiO_2) is one of the most popular nanoparticles (NP) materials in the group of synthetic bio-stimulators (Le et al. 2014). SiO_2 stimulates the natural immune systems, growth and development of plants and increases tolerance to adverse environmental conditions. SiO_2 and its derivatives have beneficial effects on many plant species under both biotic and abiotic stresses. The effects of SiO_2-containing Opytsil applications were investigated in the control of some diseases in plants (Hasan et al. 2020), *in vitro* drought stress, in terms of improving physiological and biochemical properties and coping with water stress of plants (Sacala 2009), and its positive effects were noted.

In this study, the effects of ortho silicon (200 g SiO_2 dm^{-3}) in SiO_2 liquid form (Niewiadomska et al. 2020) applied as a foliar spray at doses of 500 and 1000 µL to three grapevine rootstock plantlets during the acclimation period were investigated.

2. Materials and Methods

In the study, the shoots from Fercal, 110R and 41B rootstocks in the grapevine rootstock plot belonging to the Selcuk University were cultured as a node culture in the tissue culture laboratory.To obtain suitable material from the grapevine rootstocks grown in the rootstock collection plot, explants were taken during the active development period (Gray and Benton 1991; Di Genova et al. 2014) and single-node micro cuttings were prepared. The study was set up in a randomized plot design with 25 nodes in each plot with 3 replications. For surface sterilization (in a vertical airflow sterile cabinet), the micro cuttings were soaked in 70% ethanol for 2 minutes and then in 12% sodium hypochlorite (NaOCl) solution for 15 minutes and then rinsed 3 times with sterile distilled water. Micro cuttings were placed in jars containing Murashige and Skoog (MS) medium (2% sucrose, 0.7% agar) after surface sterilization (Murashige and Skoog 1962). 1 mg L^{-1} BAP was added to the medium at the beginning and shoot formation stages, and 1 mg L^{-1} IBA was added at the rooting stage. Plant preservative mixture (ppm) was added at a dose of 1 ml L^{-1} to prevent bacterial growth in explants (Kara and Yazar 2020). The explants in the culture medium were placed on shelves with a light source at 3000 lux m^{-2} illumination intensity in the climate room (25±1°C), developed in a 16/8 hour light/dark photoperiod (Notsuka et al. 2000).

The root regions of the plantlets, which were transferred to the rooting medium after the shoot development and whose rooting development were completed, were washed in warm water and treated with fungicide and planted in containers with ReeFlowers Iceland Black Sand (Kara and Yazar 2020).

During the acclimatization phase, 500 and 1000 µL SiO_2 doses were applied to the plantlets as a foliar spray for thirty days, repeated five days intervals. Control plantlets were sprayed with pure water only.

2.1. Measurements and statistical analysis

At the beginning of culture, the rate of shooting on 30th day was determined as percent. Rooting rates of plantlets (%) and root numbers per plantlet (pieces plantlets^{-1}) were determined in the rooting medium (Mukherjee et al. 2010). One-way analysis of variance of the data obtained from the experiment was performed in a randomized plot design at SPSS 22 statistical program, and compared with Duncan's multiple comparison test at $p<0.05$ significance level (Yue et al. 2017).

3. Results and Discussion

Shooting rate (%)

In vitro micropropagation of Fercal, 110R and 41B rootstocks yielded 100% (Figures 2), rooting differences between rootstock genotypes were insignificant. Contamination is considered a major problem in *in vitro* micropropagation and may cause irreversible damage to the shoot growth medium (Bhojwani and Razdan 1996; Eftekhari et al. 2012). In this study, the optimization of the surface sterilization and the growth-promoting quality of the initial nutrient medium enabled the shooting rate to be 100%.

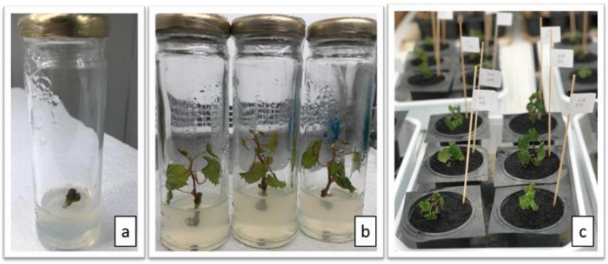

Figure 1
a) explants taken into node culture, b) shoot development stage, c) plantlets transferred to acclimatization medium

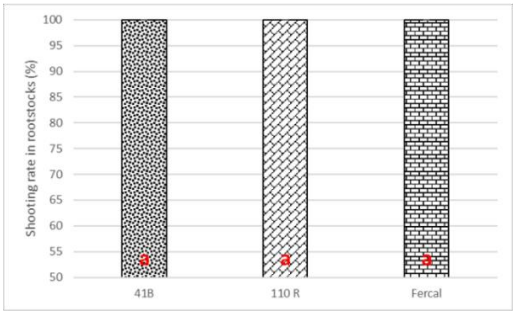

Figure 2
Growth rates (%) of rootstocks in *in vitro* rootstock culture

Contamination is considered a major problem in *in vitro* micropropagation and may cause irreversible damage to the shoot growth medium (Bhojwani and Razdan 1996; Eftekhari et al. 2012). In the study, the optimization of the surface sterilization and the growth-promoting quality of the initial nutrient medium enabled the shooting rate to be 100%.

Rooting rate (%)

The difference between rooting rates of rootstocks was significant ($p<0.05$). Although the rooting rates of the plantlets transferred to the rooting medium varied according to the rootstocks, the highest rooting was determined in Fercal (64%), followed by 110R (32%) and 41B (28%) rootstocks (Figure 3).

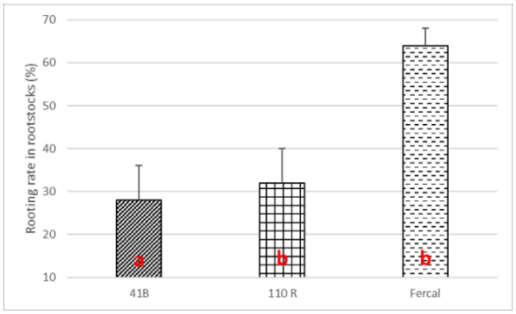

Figure 3
In vitro rooting rate (%)

The good rooting and grafting rate of Fercal grapevine rootstock was also reported in previous studies (Laucou et al. 2008). Rooting rates of 41B and 110 R rootstocks are generally low and therefore, silver nanoparticles (Kara et al. 2021), brassinosteroids (Uzunoğlu and Gökbayrak 2018), symbiotic microorganisms (Kara and Başevli 2012), electric current (Kök 2018) and hot water treatment (İşçi et al. 2019) is being tried to be increased as various applications.

Number of roots (pieces/plantlet)

Root numbers in plantlets grown in rooting medium were listed as Fercal (11.8), 41B (8.5) and 110R (3.6) (Figure 4). The differences between root numbers of rootstocks were significant ($p<0.05$). Rooting abilities among rootstocks were related to their genetic origins, and the number of roots per plantlets increased in parallel with the rooting rate in Fercal rootstock. Similarly, rooting rates of 41B and 110R rootstocks were low and root numbers were also low, which was considered a genotypic relationship (Galet 1979; Uzunoğlu and Gökbayrak 2018)

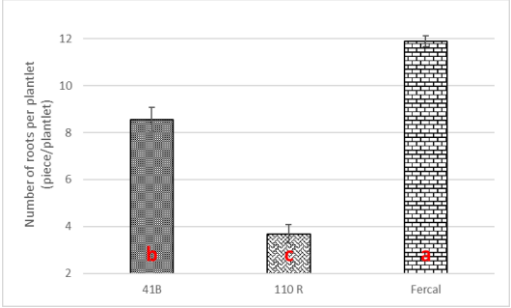

Figure 4
Number of roots (pieces/plantlet)

Survival rates at the end of acclimation (%)

The survival rate of plantlets of 41B rootstock was 66.6% in 500 µL and 1000 µL applications, and 100% in the control, with SiO_2 applications during the acclimatization phase (Figure 5). In 110R rootstock, plantlet survival at the end of acclimatization was the lowest in the control, followed by 500 µL (33.3%) and 1000 µL (66.6), respectively (Figure 4). Similarly, plantlets in the control group did not survive in Fercal rootstock. Many plantlets survived at 500 µL and 1000 µL treatments (88.8%) compared to control (Figure 4). In 41B, in 500 µL and 1000 µL applications, contamination occurred in some plantlets during the acclimatization phase. For this reason, the plantlets could not maintain their viability and a lower survival rate was obtained compared to the control. A similar result was reported by Barreto and Nookaraju (2007).

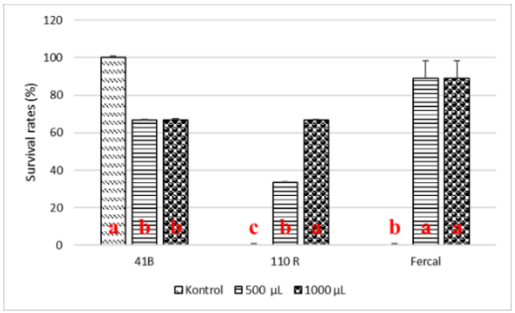

Figure 5
Survival rates (%) of plantlets after acclimatization

Successful micropropagation depends on the sustainability of plantlets at the acclimatization stage. Since plantlets have watery stems and leaves, poorly developed cuticles, large intercellular spaces and incompletely formed stomata due to high humidity in the culture container and free water in the environment, when plantlets are exposed to natural environmental conditions, the problem of drying out is encountered. For this reason, acclimatization of plantlets *in vitro* is often difficult (Savvas et al. 2009; Kamenidou et al. 2010; Whitted-Haag et al. 2014; Lešnik et al. 2017). In a previous studiy report that SiO_2 strengthens the cell walls of plants and makes them more resistant to adverse conditions, including low temperature (Ma and Yamaji 2006). It is thought that 500 µL and 1000 µL SiO_2 applications increase cell endurance and maintain the vitality of plantlets. SiO_2 can affect the metabolism and physiological functions of plantlets, especially under stress conditions. SiO_2 is involved in the creation of mechanical or physical barriers in cell walls and intercellular spaces within the cell, reducing the water demand of plants and limiting water loss due to transpiration (Radkowski et al. 2018). At the same time, SiO_2 regulates osmosis, provides water balance in plants and positively affects plantlets (Sacala 2009). It is thought that 500 µL and 1000 µL SiO2 applications contribute to the healing of the cuticle layer in poorly developed leaves with the application of Fercal and 110R rootstocks to the plantlets in the acclimatization stage. This is supported by data from applications compared to control (Figure 4). In the current study, it was determined that SiO_2

applications showed a satisfactory feature as it limited the stress on plantlets exposed to acclimatization stress and positively affected plant vitality. It can be concluded that SiO_2 can produce various metabolites that cause a decrease in transpiration, increase the rate of photosynthesis, affect stomatal conductivity, increase the chlorophyll content and photochemical efficiency of the leaves.

4. Conclusion

The results of this study showed that SiO_2 applications can be an important tool in dealing with the problems experienced during the acclimatization phase. It is thought that there is a need for further studies examining metabolic functions that will enable us to better understand the interactions between SiO_2 application and plant responses.

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