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PLANT TISSUE CULTURE

Effects of different healing agents on acclimatization success of *in vitro* rooted Garnem (*Prunus dulcis* × *Prunus persica*) rootstock

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Abstract

Continuing productivity of the acclimatization stage of plantlets means successful micropropagation. Due to the free water and high humidity in the culture container environment, poorly developed cuticle results in rapid water loss and drying of plantlets with watery stems and leaves, incomplete stomata, and large intercellular space. When plantlets are exposed to an environmental condition different from the culture medium, problems, such as rapid water loss and drying, may be encountered, and the survival rate of plantlets *in vitro* may be reduced. The aim of this study was to reduce the stress factors that occur during the acclimatization phase. For this reason, various healing agents have been used. Some of these compounds are ascorbic acid (AsA), salicylic acid (SA), and sodium nitroprusside (SNP). In the study, the response of AsA (100.0 and 200.0 mg L^{-1}), SA (100.0 and 200.0 mg L^{-1}), and SNP (100.0 and 200.0 µM) applications on growth parameters (survival rate (%), node count (pieces per plant), count of leaves (pieces per plant), shoot length (mm), and shoot diameter (mm)) and physiological variable (Soil Plant Analysis Development (SPAD)) were examined. The AsA100 (100.0 mg L^{-1} ascorbic acid), AsA200 (200.0 mg L^{-1} ascorbic acid), and SNP100 (100.0 µM sodium nitroprusside) applications resulted in an improvement in node count, leaf count per plant, shoot length, and shoot diameter parameters. The highest shoot length (60.50 ± 7.81 mm) and node count (16.83 ± 1.15 pieces per plantlet) were achieved with the AsA100 application. The maximum stem diameter (2.31 ± 0.37 mm) was determined with the SNP100 application. There were no statistically significant differences found in the survival rate, leaf count, and SPAD parameter. The current study determined that AsA, SA, and SNP applications were effective in regulating several growth parameters in Garnem plantlets and in reducing acclimation stress thereby facilitating adaptation to external conditions.

Keywords Acclimatization · *In vitro* · Healing agent · Stress

Introduction

The use of rootstocks holds great importance in fruit cultivation. The use of rootstocks is imperative to counteract adverse soil conditions. Rootstocks have various effects on the variety. Utilizing rootstocks cannot only enhance fruit yield and quality but also promote the growth and development of the variety.

Rootstocks are divided into two categories based on their method of acquisition: clonal and seedling rootstocks. Rootstocks obtained from seeds demonstrate a heterozygous structure. Hence, clonal rootstocks are preferred based on this reasoning. Clonal rootstocks can be propagated through different methods, such as cutting and biotechnology. Clonal rootstocks carry the characteristics of the parent plant. Therefore, micropropagation is preferred in cultivating to obtain a uniform plant structure

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(Nimbolkar *et al.* 2016; Sharma *et al.* 2020; Ak *et al.* 2021). Garnem rootstock is a widely used clonal rootstock for the *Prunus* species. This rootstock was obtained through the crossbreeding of the Spanish almond variety, Garfi, as the female parent, and the North American peach, Nemared, as the pollen donor. Garnem rootstock demonstrates good compatibility in grafting with plum, peach, almond, and nectarine. It is tolerant to drought, and weak, calcareous, and heavy soils. This rootstock can be propagated through both cutting and tissue culture methods (Felipe 2009; Erbas 2011; İpek 2015; Kankaya *et al.* 2021).

Micropropagation is a technique developed for rapid multiplication and holds significant commercial potential in obtaining disease-free plants of high-quality standards (George and Debergh 2007; Chandra *et al.* 2010; Saskin *et al.* 2022). However, one of the disadvantages of micropropagation is its costliness, the requirement for intensive labor, and the low survival rate upon transfer of the *in vitro* propagated plant to external conditions. The most important factor limiting the success of the micropropagation method is the acclimatization process of the obtained plants. During the acclimatization phase, high losses occur due to different factors, such as light intensity, temperature, and water stress (Kumar and Rao 2012). The primary reasons for this are water loss after the transfer of plants, planting shock, various pathogenic attacks, weak photosynthesis, and similar factors occurring during the post-transfer period (Krishna *et al.* 2005; Kara *et al.* 2022). The plant is adversely affected by such stressful situations.

Various healing agents have been used against stress factors in the acclimatization phase. One of these healing agents, sodium nitroprusside, which is a nitric oxide donor, is widely used in various plant tissue culture methods because it positively affects plant growth. SNP also has positive effects against abiotic and biotic stresses. When used *in vitro*, it increases shoot and root formation. Additionally, it yields positive results in callus formation, organogenesis, transformation, and embryogenesis, and it also helps against browning (Sundararajan *et al.* 2022).

Salicylic acid is found in all plant groups. It belongs to the group of plant growth regulators. It reverses the effects of abscisic acid, which is effective in leaf shedding. The main roles of salicylic acid include influencing plant growth, ion uptake, flowering, important enzyme activities, photosynthesis rate, and thermogenesis (Raskin *et al.* 1990; Popova *et al.* 1997; Yusuf *et al.* 2013; Rady *et al.* 2015).

Salicylic acid promotes seed germination and root development. Salicylic acid is directly effective against the negativities caused by various abiotic stresses (drought, salinity, and heavy metal). Roots can change the root structure by reacting to these stress factors in various ways. With the application of SA, the effects that occur according to the degree of stress are largely eliminated (Bagautdinova *et al.* 2022).

Ascorbic acid is an organic acid with antioxidant properties. It is one of the most important antioxidant molecules. It directly affects plant growth and development positively. It provides cell division and expansion (Zhang *et al.* 2016). AsA plays an important role in plant physiological processes because it is a cofactor for various enzymes. Ascorbic acid (AsA) provides protection to the plant against photosynthesis and environmental stresses (Shah *et al.* 2019; Smirnoff and Wheeler 2000; Çoban and Aras 2022).

Research has also reported that plant losses occur during the acclimatization process in plants propagated *in vitro* (Pospóšilová *et al.* 1999; Kara *et al.* 2022). The high water content of plantlets propagated *in vitro* and the fact that stomata are not fully developed make the adaptation process difficult. The same situation occurs in Garnem plantlets. While some studies focused on the regeneration of Garnem rootstock, the resulting plantlets were transferred to external conditions. However, the acclimatization of Garnem rootstock has not been specifically examined, and growth-supporting exogenous applications have not been tried (Erfani *et al.* 2017; Ak *et al.* 2021). In the present study, different healing agents and concentrations were tried to improve the process. With the use of these healing agents, the aim is to prevent the losses that can occur during the transfer of plantlets to the external environment, to increase the success of survival in the acclimatization stage, and to obtain healthier plants.

The principle of success in plant tissue culture is to enhance the survival rate of *in vitro* propagated plantlets. Based on this notion, this study investigated the effects of foliar spray application of 100.0 mg L^{-1} and 200.0 mg L^{-1} doses of SA, 100.0 mg L^{-1} and 200.0 mg L^{-1} AsA, and 100.0 and 200.0 µM SNP on the acclimatization stage of *in vitro* propagated Garnem plantlets.

Material–methods

Plant material and treatments In the research, shoots obtained from the Garnem rootstock collection plot belonging to the Department of Horticulture, Faculty of Agriculture, Harran University, Sanliurfa, Türkiye, were utilized. Shoots taken during the active growth phase of the Garnem rootstock were propagated using the node culture method in the Plant Tissue Culture Laboratory of the Department of Horticulture, Faculty of Agriculture, Harran University. Garnem rootstock used for the *Prunus* species was one of the clonal rootstocks commonly used for plum, peach, and almond species. This rootstock was strong and tolerant to drought and calcareous heavy-textured soils (İpek 2015; Kankaya *et al.* 2021). Three replications were constructed

with five plants in each replication, based on random plots. Shoots were prepared as single nodular cuttings. For surface sterilization, they were kept in a sterile cabinet (vertical air flow) (Nuve, Ankara, Türkiye) for 1 min in 70% ethyl alcohol and then in 12 min 10.0% sodium hypochlorite solution (Domestos, Unilever, Türkiye, and 5.0% active ingredient); and at the end of these processes, the cuttings were rinsed three times with sterile distilled water (Yalçin-Mendi *et al.* 2003; İpek 2015). After surface sterilization, the cuttings were treated with 3.0% sucrose (Merck, Darmstadt, Germany), 0.4 mg L^{-1} GA_3 (Merck, Hohenbrunn, Germany), and 2.0 mg L^{-1} BAP (Acros Organics, Morris Plains, New Jersey), and 1.0 mg L^{-1} Plant Preservative Mixture (PPM) (Plant Cell Technology, Washington, DC) to prevent bacterial contamination of explants, and they were transferred to jars containing Murashige and Skoog (MS) (Sigma-Aldrich®, St. Louis, MO) (Murashige and Skoog 1962) medium solidified with 0.6% agar (Duchefa, 2003 RV Haarlem, Netherlands). After emergence of the nodes, shoots were transferred to MS rooting medium containing 1.5 mg L^{-1} IBA (Merck) (Kara and Yazar 2020; Kara *et al.* 2022). The explants were kept in the climatic chamber with a temperature of 18 to 28 °C and white fluorescent lamps (4000 lx) (Koninklijke Philips Electronics N.V., Netherlands) as the light source with 16 h of light and 8 h of darkness.

Healthy shoots (approximately 4 to 5 cm in length) with more than three nodes from the multiplied shoot clumps were used for root regeneration. Garnem plantlets, after completing root formation in the rooting medium (1.5 mg L^{-1} IBA), were removed from the nutrient medium, and their roots were cleaned. The plantlets were transferred to cups (produced by a local manufacturer) with a volume of 450 mL and a diameter of 95 mm, containing aquarium sand (produced by a local manufacturer). These transferred plantlets were placed in a climatic chamber with conditions set at 65% relative humidity and a temperature of 24 ± 1 °C (white fluorescent tubes (produced by a local manufacturer), 100 µmol m^{-2} s^{-1} irradiance) after the transfer. In the study, AsA (100.0 mg L^{-1} and 200.0 mg L^{-1}), SA (100.0 mg L^{-1} and 200.0 mg L^{-1}), and SNP (100.0 µM and 200.0 µM) chemicals and concentrations were used (Zangani *et al.* 2023). AsA (Sigma-Aldrich®), SA (Merck), and SNP (Merck) chemicals were dissolved in distilled water until they reached a homogeneous structure. Subsequently, they were transferred into spray bottles (Fig. 1). The prepared solutions were applied as a spray to the plantlets undergoing acclimatization at 5-d intervals for a total period of 50 d. The control group of plants received a spray of distilled water.

Survival rate, node count, shoot length, count of leaves per plant, shoot diameter, and SPAD (Soil Plant Analysis Development) measurements The survival rate (%), node count (node per plant), count of leaves per plant (leaves per plant), shoot length (mm), stem diameter (mm), and SPAD parameters were examined. The survival rate was determined as the percentage ratio of the number of live plants to the total number of plantlets taken for acclimatization. Node count and leaf count were determined by counting the nodes and leaves present on the plantlets. Shoot diameter and shoot length were measured in millimeters using calipers (Kara *et al.* 2022). Chlorophyll content of the 3rd and 4th leaves of all shoots was measured by a chlorophyllmeter (SPAD-502, Minolta, Tokyo, Japan) (Bayraktar *et al.* 2016; Fang *et al.* 2018).



Figure 1. The appearance of Garnem (*Prunus dulcis*×*Prunus persica*) plantlets propagated under in *in vitro* conditions during the acclimatization process. (*A*) General view of plantlets obtained from Garnem (*Prunus dulcis*×*Prunus persica*) rootstock in *in vitro* conditions. (*B*) Keeping the plantlets transferred to aquarium sand under controlled conditions. (*C*) Applications are made by spraying.

Data Analysis Data were subjected to statistical analysis using the analysis variance (ANOVA) technique (Gomez and Gomez 1984). To determine significant differences among the treatments, Tukey multiple comparison test (with a significance level of 0.05) was used. JMP Pro 13 software was used for Tukey multiple comparison test. Hierarchical clustering analysis (HCA) and principal component analysis (PCA) were conducted *via* the Software R (Version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria).

Results and discussion

Garnem rootstock plantlets some growth parameters and physiological variable The growth parameters (survival rate (%), node count (nodes per plant), leaf count (leaves per plant), shoot length (mm), shoot diameter (mm)) and the physiological variable SPAD were significantly affected by the applications (Table 1). Plantlets subjected to AsA100 application showed the highest node count and shoot length parameters. On the contrary, plantlets subjected to individual control and SNP200 applications exhibited the lowest levels in growth parameters (survival rate, node count, leaf count, and shoot diameter). Generally, healing agent applications to plantlets exposed to external stress in the acclimatization stage improved the levels of growth parameters, and the AsA100, AsA200, and SNP 100 healing agent individual treatments showed the best performance compared to SNP200 and SA200 applications.

Survival rate (%) The micropropagation method provided clonally efficient and fast production. One of the factors affecting the success of this method was the survival rate in the acclimatization stage.

In plants obtained under *in vitro* conditions, the leaf water content is high due to elevated humidity in the container. In addition, the cuticle layer and stoma pores are not fully developed. Therefore, in the process of transferring to external conditions, plantlets lose water through transpiration and the survival rate of plantlets decreases (Shiwani *et al.* 2022). Based on this idea, beneficial applications aimed at improving plant quality during the acclimatization phase may contribute to the enhancement of the process and shorten the adaptation period. One of the critical processes during acclimatization was the survival rate. There was no statistically significant difference in the survival rates of plants in this study. The highest survival rate was seen in the AsA200 application with 53.33%, and the lowest survival rate was observed in the AsA 100 (40.00%) and SNP200 application with 40.00% (Table 1). In the study conducted by Vasar (2001), the effect of ascorbic acid and citric acid on the acclimatization of *Prunus avium* L. plantlets was examined, and the highest survival rate was recorded in the plantlets sprayed with ascorbic acid. Similar studies also observed positive effects of various beneficial agents applied during the acclimatization phase on the survival rates of plantlets obtained under *in vitro* conditions (Johkan *et al.* 2008; Kara *et al.* 2022).

Node count (nodes per plant) There was a statistically significant difference in the number of nodes in the plants. While the highest number of 16.83 nodes per plant was obtained from the AsA100 application, the lowest number, 8.83 nodes per plant, was determined in the AsA200 treatment (Table 1). AsA played an important role in plant stress tolerance. Many studies revealed that ascorbic acid played an important role in improving plant tolerance to abiotic stress (Shalata and Neumann 2001; Toksoy and Dogru 2021). The acclimatization phase of plantlets grown under *in vitro* conditions is a very important step in which plantlets are exposed to a high mortality rate. Ascorbic acid is an antioxidant that can inhibit the oxidation process that occurs in the plant under adverse conditions. In addition to its antioxidant role, ascorbic acid also plays a role in cell

Table 1. Effect of healing agents applied to Garnem (*Prunus dulcis*×*Prunus persica*) plantlets during the acclimatization stage on vegetative properties

Treatments	Survival rate (%)	Nodes per plant	Leaves per plant	Shoot length (mm)	Stem diameter (mm)	SPAD*
Control	40.00 ± 0.00	9.33 ± 0.29b	8.33 ± 1.26	39.72 ± 6.62abc	1.68 ± 0.10ab	35.90 ± 5.88
SNP100	46.67 ± 11.55	12.83 ± 2.36ab	11.67 ± 2.52	20.33 ± 6.77c	2.31 ± 0.37a	28.42 ± 5.56
SNP200	40.00 ± 0.00	10.00 ± 2.29 b	6.00 ± 0.50	32.37 ± 2.41bc	1.34 ± 0.20b	31.75 ± 2.93
AsA100	40.00 ± 0.00	16.83 ± 1.15a	10.17 ± 2.08	60.50 ± 7.81a	1.80 ± 0.21ab	21.78 ± 1.35
AsA200	53.33 ± 11.55	8.83 ± 2.08b	8.50 ± 4.77	37.44 ± 6.79abc	1.92 ± 0.19ab	18.47 ± 6.25
SA100	46.67 ± 11.55	11.50 ± 1.00b	12.17 ± 1.61	52.22 ± 14.07ab	1.98 ± 0.21a	23.40 ± 10.04
SA200	46.67 ± 11.55	11.83 ± 1.89ab	11.00 ± 2.29	42.08 ± 12.12abc	1.86 ± 0.11ab	32.29 ± 10.78

SNP100 sodium nitroprusside 100.0 µM, *SNP200* sodium nitroprusside 200.0 µM, *AsA100* ascorbic acid 100.0 mg L^{-1}, *AsA200* ascorbic acid 200.0 mg L^{-1}, *SA100* salicylic acid 100.0 mg L^{-1}, *SA200* salicylic acid 200.0 mg L^{-1}

*SPAD, Soil Plant Analysis Development

division, elongation, and shoot regeneration (Babbar *et al.* 2010; Das and Srivastav 2015). In the present study, it was determined that ascorbic acid played an important role in increasing the number of nodes. In the studies carried out on vegetable species, it was determined that the seedling growth, the number of branches, and the number of nodes were positively affected (Sajid and Aftab 2009; Gaafar *et al.* 2020; Çoban and Aras 2022).

Number of leaves per plant (leaves per plant) Plants treated with SA100 showed higher leaf count (12.17 leaves per plant) than control plants (Table 1). These results supported the findings of Rady *et al.* (2015) who reported an increase in the number of leaves of *Phaseolus vulgaris* L. plants treated with SA. There was no statistically substantial difference between the applications in terms of the number of leaves. Salicylic acid affects many biochemical and physiological responses in plants, acts as an important signaling molecule, and has various effects on tolerance to environmental stresses (Nazar *et al.* 2011; Aras 2018). When the effects of SA were examined, positive improvements were observed in different studies. In a study conducted by Yenilmez (2016), there was a significant difference in the number of leaves of SA applied at different doses to some vine rootstocks in saline conditions, and improvements were noted.

Shoot length (mm) In *in vitro* conditions, humidity in culture vessels was high, and lighting was low compared to the conventional situation. The decrease in plant growth rate under stress during the adaptation process of these plantlets to external conditions was probably due to the high water and moisture content in leaves and other tissues. For this reason, when transferred from *in vitro* conditions to *ex vitro* conditions, the plant cannot immediately adapt to external conditions and losses increase (Munns 2002; Ejaz *et al.* 2012). As demonstrated by the growth parameters examined in this study, exogenous application of AsA significantly increased the growth of Garnem plants under stress conditions that occurred during adaptation. Plants treated with AsA100 showed higher shoot length (60.50 mm) than control plants. A statistically significant difference was found between the treatments in terms of shoot lengths (Table 1). It is known that AsA plays a direct role in shoot development of woody plants (Bilska *et al.* 2019). In Hassan *et al.* (2021), it was reported that AsA that was applied exogenously from the leaves in barley plants grown in saline conditions increased plant growth. The results supported this study (Gaafar *et al.* 2020).

Shoot diameter (mm) The stem diameter was found to be statistically significant among the treatments. The shoot diameter was highest in the SNP100 treatment with 2.31 mm compared to the control (Table 1). Exogenous SNP application in plants is one of the strategies to improve plant photosynthetic performance and stress tolerance. It can be concluded that the increased endogenous nitric oxide with the application of SNP alleviates the negative effects on growth and photosynthesis under stress conditions (Li *et al.* 2022). Studies on appropriate nitric oxide concentration have also reported that it contributes to DNA concentration. Therefore, nitric oxide promotes cell division and growth (Bai *et al.* 2012; Novikova *et al.* 2017). It is thought that increasing stem diameter is directly proportional to the role of nitric oxide in cell division and growth (Tan *et al.* 2013).

SPAD As a result of the applications, there was no statistical difference between the SPAD values. The highest value was obtained from the control (35.90) application. However, among the conducted applications, it was determined that the SA200 (32.29) application yielded results closest to the control. This result can be explained by the salicylic acid application increasing the chlorophyll content in the plant (Gautam and Singh 2009).

Hierarchical clustering analysis (HCA) HCA is a clustering method that determines order in groups of samples and between groups taking into account the order of importance. The result of the HCA is usually presented in a dendrogram, which is a graph showing the arrangement and relationships of the samples in tree form. HCA was performed using six different parameters in line with the applications made in the study (Fig. 2). In the heatmap resulting from the analysis, applications were divided into groups as I, II, III, IV, and V. The applications were situated as follows: control and SNP200 in group I, AsA100 in group II, SA100 and SA200 in group III, SNP100 in group IV, and AsA200 in group V (Fig. 2). HCA of the six physiological parameters considered as a result of the applications was divided into four groups: A, B, C, and D. The grouping included the following: in cluster B, shoot length and node count; in cluster D, leaf count and shoot diameter; survival rate in cluster C; and the physiological variable SPAD in cluster A. During the adaptation of the plantlets obtained under *in vitro* conditions to the external environment, parameters, such as node count, shoot length, survival rate, count of leaves, and shoot diameter, were adversely affected by the hardening stress. An improvement in these values was observed with the effect of AsA100, SA100, AsA200, and SNP100. While node count and shoot length parameters were in the B cluster, as a result of the AsA100 application, it had the highest value. Finally, the survival rate parameter was placed in cluster C, and the highest value was detected in the AsA200. It has been reported by studies that exogenous ascorbic acid applications have a positive effect on cell growth and division, differentiation, and metabolism in plants as an antioxidant

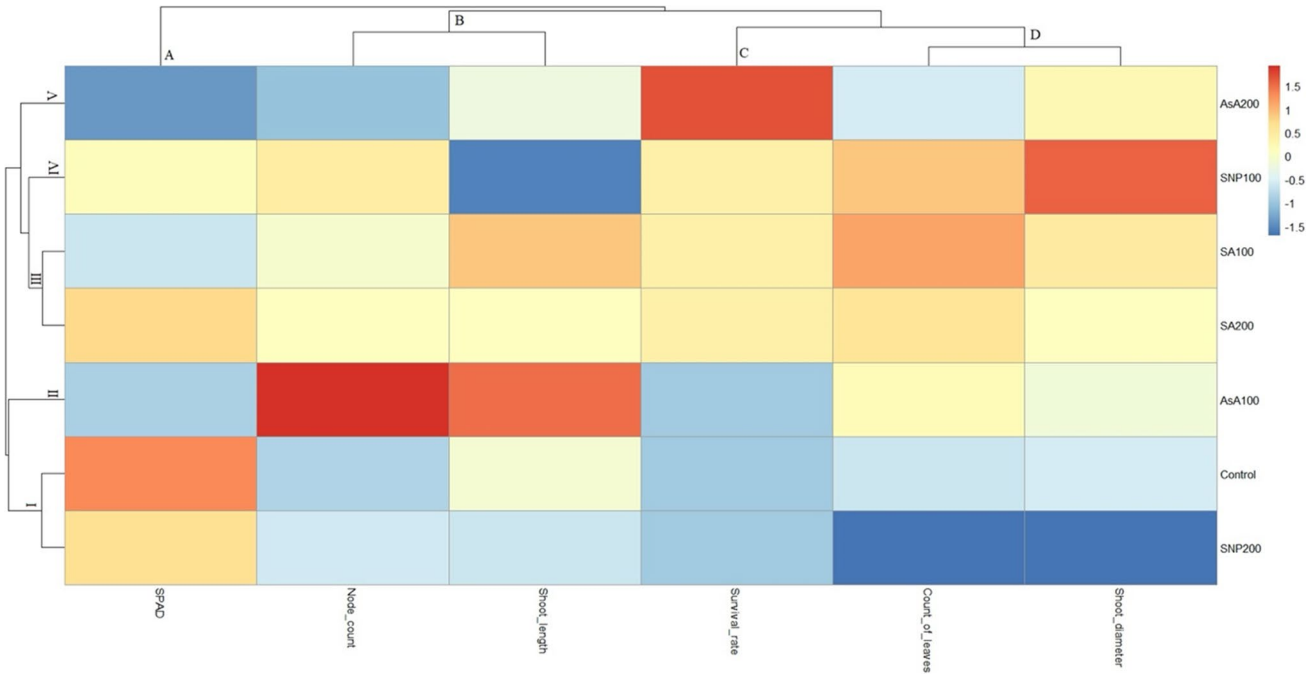


Figure 2. Heat map for evaluated morphological traits under control and treatment conditions in the Garnem (*Prunus dulcis*×*Prunus persica*) rootstock. *I*, *II*, *III*, *IV*, and *V* represent the groups of the treatments studied. *A*, *B*, *C*, and *D* represent the groups of parameters studied. SNP100 (sodium nitroprusside 100.0 µM), SNP200 (sodium nitroprusside 200.0 µM), AsA100 (ascorbic acid 100.0 mg L^{-1}), AsA200 (ascorbic acid 200.0 mg L^{-1}), SA100 (salicylic acid 100.0 mg L^{-1}), and SA200 (salicylic acid 200.0 mg L^{-1}).

(Athar *et al.* 2009; Xu *et al.* 2015; Hafez and Gharib 2016). In the present study, the positive increase in node count and shoot length parameters in the AsA100 application and the survival rate parameters in the AsA200 application were parallel to similar studies (Sajid and Aftab 2009; Xu *et al.* 2015; Seleem *et al.* 2021). SNP induces carbohydrate metabolism in plants through consumption of soluble sugars by increasing photosynthesis and carbohydrate anabolism (Ghadakchiasl *et al.* 2017; Chavoushi *et al.* 2019). This increased metabolic activity may be a possible reason for the observed improvement in shoot diameter from growth parameters (Fig. 2).

Principal component analysis (PCA) PCA was used to represent the current variation of the dataset obtained from the applications made in the study (Fig. 3). Variables observed in the iteratively calculated PCA were largely explained as PC1 (71.7%) and PC2 (27.3). When the analysis map was examined, survival rate, shoot diameter, and parameters for SNP100 and AsA200 applications were in the same group, and their values that were greater than 1 made the applications meaningful. It has been observed that the parameters shoot length, node count, and count of leaves were in the same direction as the SA100 application. However, it was determined that the SPAD parameter, unlike the survival rate, number of nodes, number of leaves, shoot length, and shoot diameter, was in the opposite group with the control and SNP200 applications. When looking at the PCA map of the applications, SNP100 and AsA100 applications had a significant effect on the parameters; SNP200 applications, which were closer to the control, were negatively affected (Fig. 3). It was determined that AsA100, SNP100, and AsA200 applications had a positive effect on the growth parameters measured in Garnem plantlets (Fig. 3). Using molecules that have been shown to have protective functions against stresses may help reduce the negative effects of plants adapted to external environments under *in vitro* conditions. Such healing substances are powerful antioxidants that protect cells from damage caused by abiotic stresses. These antioxidants (AsA, SA, and SNP) play a key role in plant growth, development, and defense responses (Smirnoff 1996; Hemavathi Upadhyaya *et al.* 2010; Kumar *et al.* 2011).

Conclusions

The micropropagation method plays an important role in obtaining a large number of plants as well as clonal propagation. The tissue culture method plays a role in the development of new varieties and the preservation of plant gen resources. Plant losses during acclimatization of valuable plants obtained by this method adversely affect the sustainability of

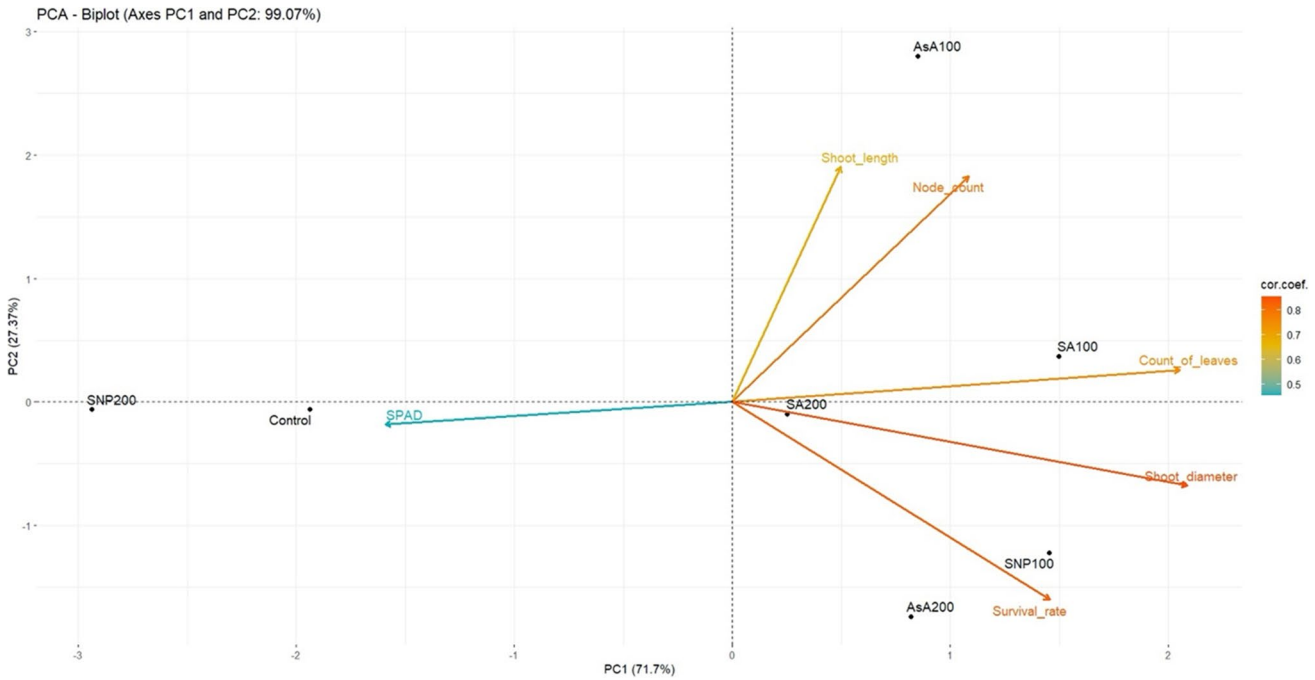


Figure 3. A loading plot of all the detected variables included in PCA (principal component analysis) for the physiological parameters in Garnem (*Prunus dulcis* × *Prunus persica*) rootstock. SNP100 (sodium nitroprusside 100.0 µM), SNP200 (sodium nitroprusside 200.0 µM), AsA100 (ascorbic acid 100.0 mg L^{-1}), AsA200 (ascorbic acid 200.0 mg L^{-1}), SA100 (salicylic acid 100.0 mg L^{-1}), and SA200 (salicylic acid 200.0 mg L^{-1}).

micropropagation. Therefore, minimizing plant loss during acclimatization is the most important step in making plant tissue culture sustainable. Due to low ambient humidity and high lighting, plants cannot adapt to external conditions right away when transferred from *in vitro* to *ex vitro*. Research supports the positive effects of healing agents on stress reduction. In the present study, it was determined as a result of observations that exogenous applications improved the vegetative development of the plant (shoot length, number of leaves, and stem diameter) positively and accelerated the adaptation process to external conditions. The count of nodes (16.83 pieces per plant) and shoot length (60.50 mm) increased positively with AsA (100 mg L^{-1}) while the stem diameter (2.31 mm) showed a positive increase with SNP (100 µM) applications in vegetative development. For subsequent acclimatization studies, AsA100 and SNP100 applications are recommended. Improving the acclimatization process of the Garnem rootstock is crucial, as it serves as a significant source of plant material for almond cultivation, which is widely used in the provinces of Sanliurfa and Adiyaman. Additionally, the Garnem rootstock is used in research conducted at numerous universities throughout Türkiye.

Author contribution Investigation and conceptualization, B.E.A., N.S., H.E. (Heydem Ekinci); methodology, N.S., H.E. (Heydem Ekinci); software and formal analysis, N.S., H.E. (Heydem Ekinci); writing-original, writing-review and editing, and draft preparation, H.E., B.E.A., B.D.D and N.S.; visualization H.E. (Heydem Ekinci). All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval This original article does not include any studies with human participants or animals by any of the authors.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

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