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In vitro propagation of stone fruit rootstock cultivar ´*Evrica 99*´ and its influence on some phytochemical traits of fresh apricot fruit

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ABSTRACT

Background: The cultivation of stone fruits is of primary importance in Armenia. Their fruits contain antioxidants, fiber, potassium, vitamin A, C, E, minerals, etc., which have a beneficial effect on human health and prevent many diseases. The concentration of those components varies depending on ecological factors, cultivar, rootstock, cultural practices, etc. Clonal rootstocks are important for increasing orchard density, tree uniformity, and high yields, and they can also affect fruit quality. *In vitro* culture is a valuable method for rapid propagation of high-quality, virus free plant material.

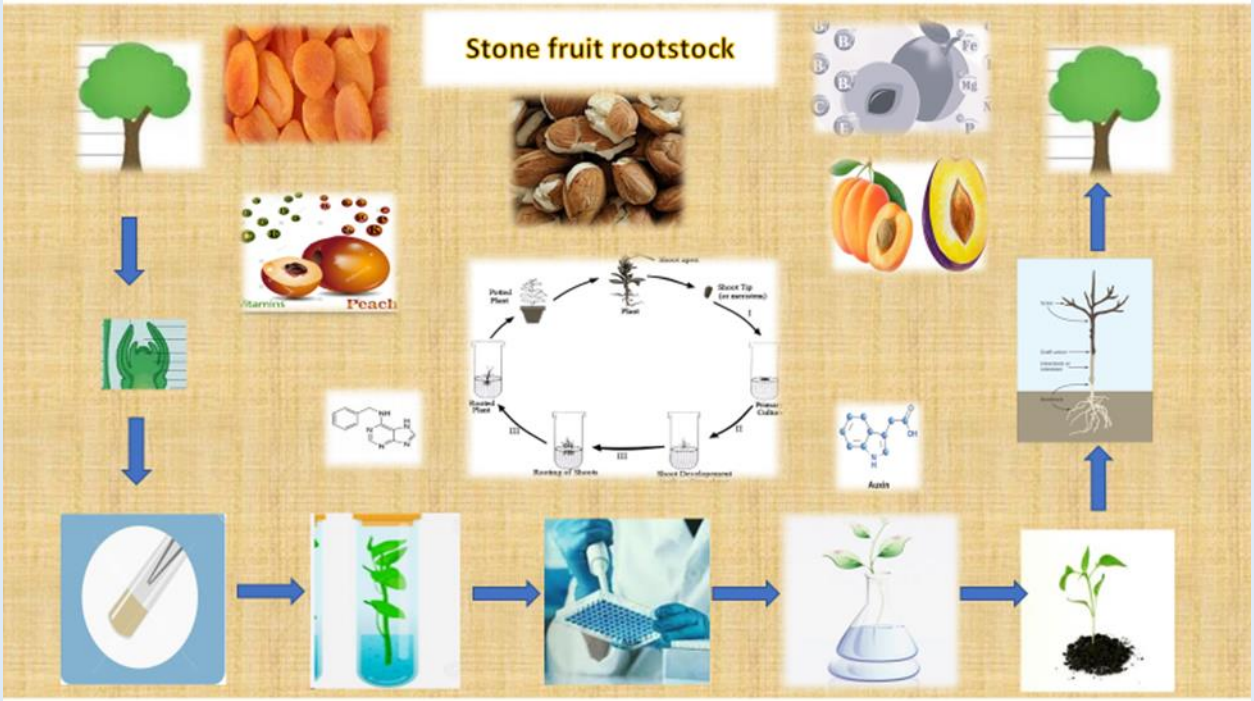
Objective: The purpose of this study was to develop *in vitro* production technology for the stone-fruit rootstock cultivar *´Evrica 99'*, and to determine if rootstocks affect some fresh fruit traits of *´Yerevani´* and *´Sateni´* apricot cultivars.

Methods: The shoot apical meristem and lateral bud served as explants for shoot regeneration. Different sterilizing agents at various periods of exposure were used for the explant surface sterilization. Various concentrations of phytohormones, both individually and in combinations, were employed for in vitro regeneration and rooting of plants. The titratable acidity (TA), dry matter (DM), vitamin C, mineral content, total carotenoids (TC), and sugar contents were evaluated in fresh fruit.

Results: The most optimal option for explant surface sterilization was the gradual application of calcium hypochlorite [Ca $(ClO)_2$] (2.0% solution, exposure time 10 min) and ethanol (70% solution, exposure time 20 s), as a result of which we had 75.5% survival rate of explants. The efficient medium for *in vitro* shoot regeneration was MS supplemented with 6-Benzylaminopurine (BAP) 0.8 mg/l, Kinetin (Kin) 0.2 mg/l, and Gibberellic acid (GA_3) 1.0 mg/l. The half-strength Murashige and Skoog (MS) medium containing 0.8 mg/l indole-3-butyric acid (IBA) was optimal for *in vitro* rooting. Rooted plants were successfully adapted with a survival rate of 85.0%. The defined method can be successfully used for *'Evrica 99'* cultivar micropropagation. The results obtained showed that fruit quality strongly depended on both the varieties and the rootstock tested.

Conclusion: In the current study, an alternative *in vitro* propagation technology for rootstock cultivar *´Evrica 99'* was developed by direct organogenesis, enabling mass-scale production of virus-free plants that is suitable for commercial purposes as well. The apricot cultivars *´Yerevani´* and *´Sateni´* grafted on the virus-free rootstock cultivar *´Evrica 99´* showed higher fruit quality traits, which are essential for human health and diet.

Keywords: *´Evrica 99´,* functional foods, *in vitro* regeneration, micropropagation, plant growth regulators, stone fruit rootstock, tissue culture.



INTRODUCTION

Apricots (*P. armeniaca* L.), peaches and nectarines (P. *persica*), plums (*P. salicina*), sweet cherries (*P. avium*), almonds (P. *dulcis*), and sour and tart cherries (P. *cerasus*) are stone fruits and the main members of the genus Prunus (family *Rosacea*) [1]. Stone fruit cultivation is one of the leading sectors of Armenian agriculture. According to the National Food Balance of the Republic of Armenia in 2021, 46.6 kg of stone fruits (apricot, peach, sour cherry, cherry, and plum) were consumed per capita (51.2 kcal/day). Currently, agriculture plays a decisive role in human nutrition. Consumers view food as a way

to improve their health and well-being, and manufacturers are actively responding by introducing new products to meet these needs [2]. Studies have shown that stone fruits are a rich source of biologically active compounds including carotenoids and flavonoids, phenolic compounds, vitamins A, C, and E and minerals that have antioxidant potential [3–4]. They also possess antimicrobial, anti-mutagenic, anti-inflammatory, cardio stimulating, anti-cancer, and antioxidant properties, among others [5–6]. Stone fruits are consumed fresh, dried, canned, or used to make liqueurs, brandy, and wines. Gaining considerable attention over the years due to their nutritional value and positive impact on health [7–8], they serve as an outstanding functional food. These fruits are a large source of metabolites other than carbohydrates and amino acids; therefore, interest in their study is increasing for researchers. The concept of functional foods first emerged in Japan in the mid-1980s [9]. Functional products play an important role in maintaining a healthy lifestyle and reducing risk factors for various diseases [10]. In accordance with the Functional Food Center, the term "functional food" refers to natural or processed foods that contain biologically active compounds in specific, useful, non-toxic quantities that support optimal health, lower the risk of chronic or viral diseases, and manage their symptoms. These health benefits have been clinically proven and confirmed using precise biomarkers [11]. In recent years, functional foods have seen a marked increase in popularity. This is largely attributed to the rise in health-conscious consumers who are searching for food products that offer more than just fundamental nutritional value [12–15]. The rising prevalence of chronic health conditions, including obesity, diabetes, and hypertension, has heightened interest in functional foods [16].

Stone fruit rootstocks are important in increasing the productivity of grafted cultivars. They are broadly utilized because of their special capabilities to control tree size, orchard uniformity, resistance to stress factors that affect the scion cultivar, quantity, and quality of fruits [17]. Rootstock are commercially propagated either through seeds or vegetative methods. Producing rootstock through seeds leads to segregation; thus, it is impossible to obtain uniform plants and maintain the characteristics of the plant [18–19]. Propagation by cuttings does not guarantee healthy plants. Prunus species are affected by several pathogens, including viruses and viroids [20]. Tissue culture is the most promising method for obtaining disease-free plant material in sufficient quantities, and it is widely used for numerous Prunus rootstocks [21–26]. *Evrica 99´* is a medium-sized clonal rootstock of stone fruit crops: apricot, plum, and peach, which was created at the Crimean experimental breeding station via crossing cherry plum Sapa (*Microcerasus pumila* x P. *salicina*) with cherry plum Otlichnitsa (P. *cerasifera*) by authors G.V. Eremin, V.F. Gavrish, and V.G. Eremin. It is particularly resistant to lack of moisture and high temperatures, which has been strongly observed over the past 5-7 years. It is resistant to heavy, dense, waterlogged soils, excess lime, bacterial cancer, root rot, and nematodes. The root system is quite winter-hardy and does not form root shoots in the garden [27].

The aim of this study was to develop an efficient protocol for *in vitro* propagation of the stone-fruit rootstock cultivar *´Evrica 99´* and to evaluate whether some chemical fresh fruit traits are affected by rootstocks.

MATERIALS AND METHODS

In vitro propagation of the stone-fruit rootstock cultivar *´Evrica 99´* was studied from 2012–2014 at the Scientific Center of Agrobiotechnology, ANAU.

Plant material: The stone-fruit rootstock cultivar *´Evrica 99´* was selected for *in vitro* studies. Plant explants were obtained from the nursery of the private enterprise ´Hovsepyans Farm´, Lenughi community, Armavir region,

Republic of Armenia (coordinates: latitude: ***40°7'33" N,*** longitude: ***43°57'58" E***, height: 960 m above sea level). The planting material for rootstock cultivar *´Evrica 99´* was imported to Armenia from the Crimean Experimental Breeding Station, a VIR branch. The shoot culture was established by the actively growing main and lateral shoots.

Surface sterilization of explants: After removing the expanded leaves, the shoots around 15 cm long were carefully cleaned for 15-20 minutes using soap and tap water. Shoots about 3.0 cm long with an apex or node were surface sterilized using two disinfectants: 2.0% Ca (ClO)2 (exposure time 10, 15, and 20 minutes) and 70% ethanol for 20 seconds. Three combinations of disinfectants were used. In each sterilization treatment, 20 explants were used, and the test was replicated three times.

Shoot regeneration and rooting in vitro: The medium used for regeneration was MS, 1962 [28], to which various plant growth regulators (PGRs) were added, namely BAP, Kin, and GA_3, with different amounts fluctuating from 0.2 to 1.0 mg/l either separately or in combination. After 35 days of in vitro culture, the shoot length, shoot number induced from a single nodal or apical explant, and the percentage of regeneration were assessed. Regenerated shoots (approximately 1.5–2.0 cm in length) were grown on MS½ enhanced with three different types of auxins, namely IBA, Indole-3-Acetic Acid (IAA), and α-Naphthaleneacetic Acid (NAA), in varying amounts ranging from 0.5–1.5 mg/l. In each treatment, 15 explants were used, and the trial was carried out a total of three times.

Culture conditions: The pH of the nutrient medium was adjusted to 5.7. The cultures were maintained under 25 ± 2^0 C, 16 light/8 dark photoperiods, a light intensity of 2500-3000 Lux provided by cool fluorescent lamps, and 70% humidity.

Acclimatization stage: *in vitro* plantlets with well-growing roots were transferred into plastic pots with a mixture of garden soil, perlite, and biofilms in equal proportions. Plastic bags were placed over the pots to keep the humidity at 25 ± 20 °C. 15 days later, the bags were removed. The percentage of adapted plants that survived after a month was determined by dividing the number of surviving plants by the total number of transplanted plants and multiplying the result by 100%.

Statistical analysis: The *mean ± standard error* (SE) of three independent experiments was used to present the results. The student's *t-test* was used (from Graph Pad software) to detect significant
differences between mean values.

Field trial and biochemical analysis: The biochemical properties of apricot fruits of *´Yerevani´* and *´Sateni´* cultivars grafted on different rootstocks were studied. Rootstocks used were 1) stone fruit rootstock cultivar *´Evrica 99´* propagated *in vitro*; 2) stone fruit rootstock cultivar *´Evrica 99´* propagated by cuttings; and 3) seedling rootstock obtained from the local wild apricot genotype.

The field trials were carried out in 2014–2019 at ´Hovsepyans farm´. In the spring of 2014, the grafted plants were transferred to the field. The standard agrotechnical practices were carried out, including pruning, thinning, and fertilizing. Fresh and ripe fruits were collected at the stage of ripeness (during 2017–2019). The chemical characteristics of the fruits were measured immediately after harvest. Sugars were analyzed using a modified method as described by Melgarejo et al., 2000 [29]. To determine the titratable acidity (TA) as citric acid, 10 g of fruit pulp was diluted

with 100 ml of distilled water. The titration was carried out using a 0.1 N sodium hydroxide solution, with the subsequent addition of 2–3 drops of phenolphthalein. Ascorbic acid (vitamin C) was analyzed by the iodine titration method. To determine dry matter, 5 g of pulp was placed at 60°C in a hot air oven until a constant weight was reached. The method described by AOAC in 2005 [30] was used for mineral analysis. Total carotenoids (TC) were measured using Rodriguez-Amaya's (2004) methodology [31]. The analysis was conducted with three replications in a randomized block design experiment. ANOVA was employed for the statistical analysis of the obtained data.

RESULTS

The effect of different sterilizing agents at various exposure times on explant contamination, mortality, and survival after 18 days from culture initiation is shown in Figure 1.

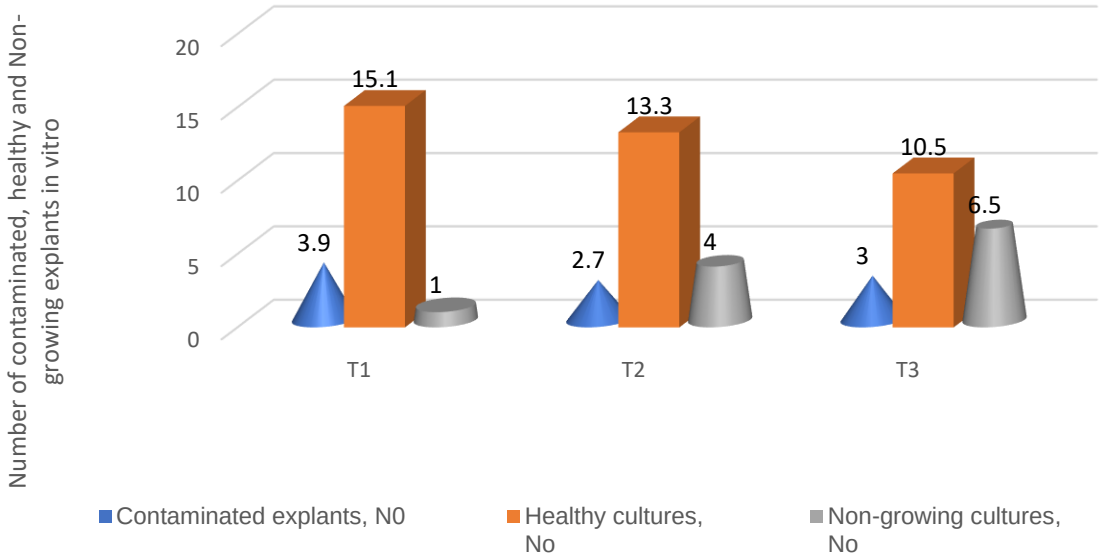


Figure 1. Effect of surface sterilizer, different exposure times on contamination, mortality and explant survival. **T_1.** 2.0% Ca $(ClO)_2$ 10 min + 70% ethanol 20 s, **T_2.** 2.0% Ca $(ClO)_2$ 15 min + 70% ethanol 20 s, **T_3.** 2.0% Ca $(ClO)_2$ 25 min + 70% ethanol 20 s.

Out of the sterilization treatments tried during culture establishment, treatment T1. (2.0%) (w/v) Ca $(ClO)_2$ 10 min + 70% (v/v) ethanol solution 20 sec was found to be the best for surface sterilization, with a 75.5% (15.1 healthy cultures) rate of explant survival. Shoot regeneration from the explant was observed in all treatments containing PGRs. Based on the results, the frequency of shoot regeneration was recorded in the range of 54.5–92.6% for both types of explants. No shoots were observed in the control. The highest frequencies of 92.6% and 87.6% of shoot regeneration were observed on the MS medium enhanced with 0.8 mg/l BAP, 0.2 mg/l Kin, and 1.0 mg/l GA_3 from apical and nodal explants, respectively. The mean number of shoots/explants showed statistically significant variations (P 0.05) among treatments (Fig. 2).

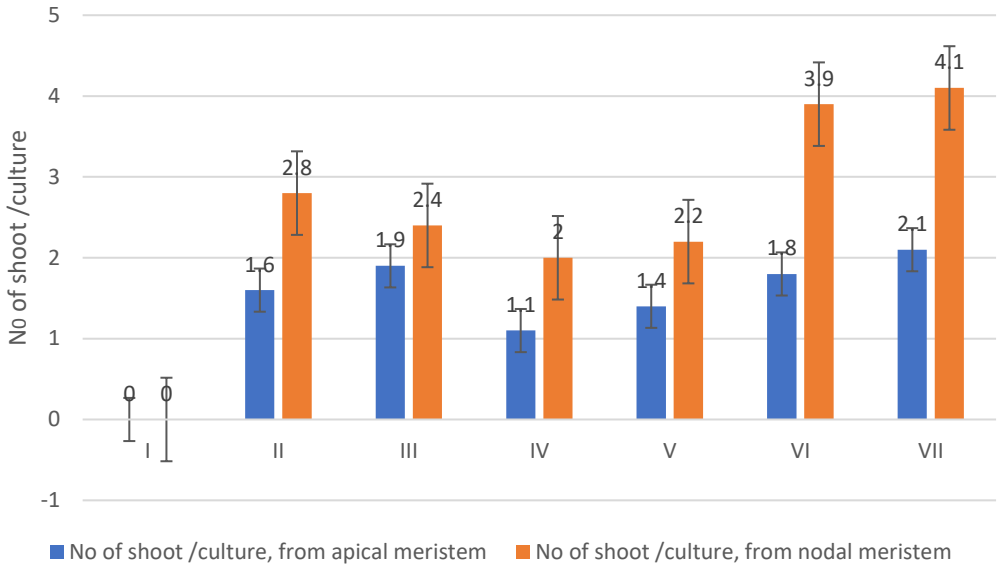


Figure 2. Effect of PGRs on the number of shoots treatment: I) 0; II) BAP 0.8 mg/l, III) BAP 1.0 mg/l, IV) Kin 0.8 mg/l, V) Kin 1.0 mg/l,VI) BAP 0.8 mg/l + Kin 0.2 mg/l, VII) BAP 0.8 mg/l + Kin 0.2 mg/l + $GA_3$1.0 mg/l

The mean shoot number per apical and nodal explant ranged 1.1–2.1 and 2.0–4.1, respectively. The addition of 1.0 mg/l GA_3 to MS medium supplemented with 0.8 mg/l BAP and 0.2 mg/l Kin increased the number of shoots (duration of culture: 5 weeks), and the number of shoots produced from nodal meristems was higher than those derived from apical meristems (4.1 and 2.1, respectively). Shoot length ranged from 1.1–3.2 cm formed from the apical meristem and 1.9–3.5 cm from the axillary bud (Fig. 3). Shoot length decreases with an increase in cytokinin concentration from 0.8 mg/l to 1.0 mg/l. The longest micro-shoots per apical meristem and nodal bud were accordingly 3.2 cm and 3.5 cm obtained in MS medium enhanced with a combination of BAP 0.8 mg/l, Kin 0.2 mg/l, and GA_3 1.0 mg/l PGRs.

The impact of various amounts of three types of auxins on the rooting of stone fruit rootstock cultivar ´*Evrica* 99´ is shown in Table 1.

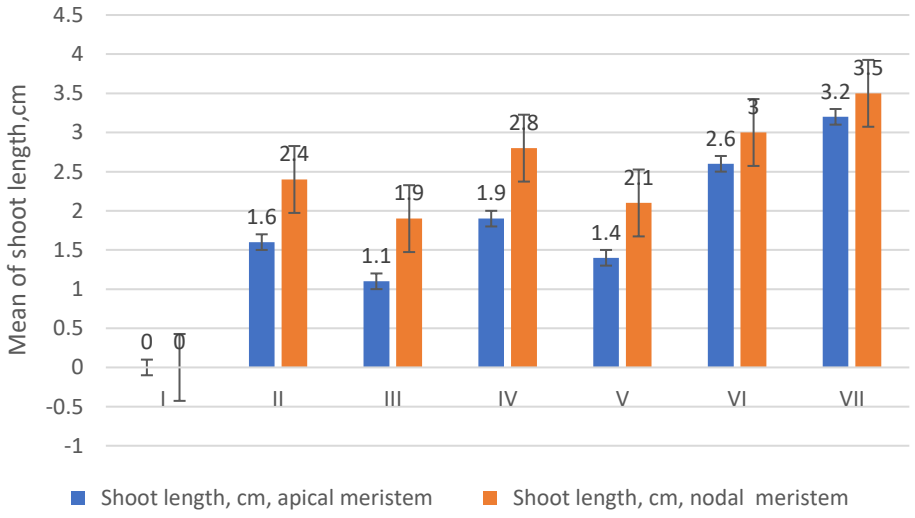


Figure 3. Effect of PGRs on the shoot length Treatment: I) 0; II) BAP 0.8 mg/l, III) BAP 1.0 mg/l, IV) Kin 0.8 mg/l, V) Kin 1.0 mg/l, VI) BAP 0.8 mg/l + Kin 0.2 mg/l, VII) BAP 0.8 mg/l + Kin 0.2 mg/l + GA_3 1.0 mg/l

Table 1. Influence of various auxin concentrations on the rooting of stone rootstock variety *´Evrica 99´* after 30 days of culture

Treatment Auxins, mg/l	Rooting, %	N_0 of roots/shoot (Mean ± SE)	Root length, cm (Mean ± SE)
-	-	-	-
0.5 IBA	74.0	4.3 ± 0.3^{c}	5.1 ± 0.4^{d}
0.8 IBA	100.0	7.0 ± 0.4^{a}	9.0 ± 0.4^{a}
1.0 IBA	78.0	3.6 ± 0.3^{de}*	4.8 ± 0.3^{d}
1.5 IBA	56.6	2.1 ± 0.4^{f}*	1.3 ± 0.5^{h}
0.5 IAA	67.0	2.5 ± 0.3^{f}	4.1 ± 0.3^{e}
0.8 IAA	85.0	3.9 ± 0.3^{d}	6.2 ± 0.3^{c}
1.0 IAA	92.0	5.4 ± 0.4^{b}	7.0 ± 0.4^{b}
1.5 IAA	77.0	2.2 ± 0.3 *	2.1 ± 0.2^{g}
0.5 NAA	88.0	2.7 ± 0.3^{f}	4.7 ± 0.2^{d}
0.8 NAA	96.0	5.1 ± 0.3^{b}	5.6 ± 0.3^{c}
1.0 NAA	71.0	3.1 ± 0.2^{e}*	3.2 ± 0.3^{f}
1.5 NAA	65.0	1.4 ± 0.3^{g}*	1.2 ± 0.2^{h}

Note: * (with callus)

Means after same letter in column are not statistically significant with a $p \leq 0.05$. The results were represented the average no of root, root length as *mean ± standard error (SE)* of 3 repeated experiments.

The MS/2 medium supplemented with 0.8 mg/l IBA showed the highest rate of rooting (100%) and was followed by MS/2 + 0.8 mg/l NAA (96%). The results showed that MS/2 medium supplemented with 0.8 mg/l IBA had the highest mean number of roots per shoot (7.0±0.4) and higher root length (9.0±0.4 cm), while nutrient medium supplemented with 1.5 mg/l NAA had the lowest number of roots (1.4±0.3) and lowest root length (1.2±0.2 cm). Callus formation was observed when IAA in the medium was 1.5 mg/l, and both IBA and NAA - 1.0 and 1.5 mg/l. The *in vitro*-rooted plants were effectively adapted to the plastic pots containing a soil mixture of garden soil, perlite, and biohumus in equal amounts (85% survival rate) and then transferred to the greenhouse.

The biochemical parameters of the fruits of apricot cultivars *´Yerevani´* and *´Sateni´* grafted on the rootstock *´Evrica 99´* (1. propagated in vitro; 2. propagated by cuttings) and the apricot seedling rootstock were compared during three harvesting seasons (2017–2019) at the stage of readiness for consumption (Table 2). As shown in Table 2, the range of the total sugar content per 100 grams was 11.46 to 14.85 g. Sucrose accounted for over sixty percent of the total sugars, with a content range of 7.28 to 9.72, while fructose constituted about 15% of the total sugars, and its content varied from 1.63 to 2.40 g/100 g. The dry matter content ranged from 17.41% to 20.80%. Vitamin C content ranged from 6.84 to 8.60 mg/100 g. The differences between rootstocks for the mean TA and carotenoids values were insignificant at $p \leq 0.05$, viz., the rootstock had no effect on the level of TA and carotenoids in apricot fruits.

Table 2. Some phytochemical traits in fruits of two apricot cultivars grafted on different rootstocks

Parameters	Cultivar + Rootstock					
	´Yerevani´ virus-free *´Evrica 99´*	*´Yerevani´* *´Evrica 99´*	*´Sateni´* virus-free *´Evrica 99´*	*´Sateni´* *´Evrica 99´*	*´Yerevani´* apricot seedling	*´Sateni´* apricot seedling
Vitamin C (mg/100g)	8.06±0.01	7.16±0.02	8.60±0.01	7.48±0.01	6.84±0.01	8.02±0.03
Total carotenoids (mg/100 g ß-carotene)	0.88±0.04	0.84±0.01	0.95± 0.01	0.93±0.01	0.84±0.03	0.91±0.05
Sucrose (g /100 g)	7.92±0.1	7.28±0.2	9.72±0.5	8.81±0.3	7.28±0.3	8.38±0.2
Glucose (g/100g)	2.88±0.1	2.55±0.7	3.18±0.6	3.10±0.2	2.96±0.4	2.66±0.1
Fructose (g /100 g)	2.05±0.2	1.63±0.2	2.40±0.12	2.16±0.1	1.99±0.17	2.02±0.11
Titratable Acidity:(g /100 g FW)	0.67±0.01	0.65±0.02	0.40±0.01	0.41±0.02	0.66±0.01	0.40±0.02
Dry matter, (%)	17.95±0.12	17.41±0.32	20.80±0.30	19.40±0.7	18.05±0.17	19.01±0.50

The total content is the sum of glucose, sucrose and fructose. The highest content of sugar, dry matter, and vitamin C in fresh fruits was observed in the *´Sateni´* cultivar, grafted onto the virus-free rootstock *´Evrica 99',* with the following values accordingly: 14.85 g/100g, 20.80%, and 8.60 mg/100g. The data on chemical parameters of the fresh fruit of apricot are presented in Table 3. The mineral composition of fruits from the *´Yerevani´* and *'Sateni'* cultivars, grafted onto different rootstocks differed significantly *($p < 0.05$)*.

Table 3. Mineral contents in fresh fruits of two apricot cultivars grafted on different rootstocks

Minerals	**Cultivar + Rootstock**					
	´Yerevani´* virus-free *´Evrica99	***Yerevani´ ´Evrica 99´***	***´Sateni ´* virus-free *´Evrica 99´***	***´Sateni ´ ´Evrica 99´***	***´Yerevani´* apricot seedling**	***´ Sateni ´* apricot seedling**
Potassium, (K)	259.1±5.30	241.2±4.01	271.3±3.15	263.3±2.25	236±4.46	253.3±4.05
Phosphorus, (P)	24.1±1.25	20.84±1.25	25.8±1.05	21.6±1.60	20.7±1.35	21.7±1.35
Calcium, (Ca)	12.8±0.20	12.1±0.20	13.4±0.10	13.0±0.10	11.9±0.10	13.1±0.10
Magnesium, (Mg)	10.6±0.10	10.1±0.10	11.5±0.10	11.1±0.08	10.1±0.07	10.9±0.06
Sodium, (Na)	1.34±0.04	1.19±0.05	1.43±0.05	1.37±0.03	1.02±0.05	1.31±0.04

Minerals	Cultivar + Rootstock					
	´Yerevani´ virus-free *´Evrica99*	*Yerevani´ ´Evrica 99´*	*´Sateni ´* virus-free *´Evrica 99´*	*´Sateni ´ ´Evrica 99´*	*´Yerevani´* apricot seedling	*´ Sateni ´* apricot seedling
Iron, (Fe)	0.63±0.02	0.57±0.02	0.67±0.01	0.61±0.02	0.58±0.01	0.59±0.02
Zinc, (Zn)	0.27±0.01	0.21±0.02	0.28±0.01	0.24±0.0 2	0.25±0.06	0.23±0.05
Manganese, (Mn)	0.075±0.01	0.072±0.01	0.088±0.01	0.085±0.01	0.071±0.01	0.080±0.01
Copper, (Cu)	0.067±0.01	0.064±0.01	0.078±0.01	0.074±0.01	0.061±0.01	0.069±0.01

The results were given in mg per 100 g

According to an analysis, the amount of minerals in the fruit differs, and potassium was the most abundant in all the samples. The amount of potassium varied from 241.2 to 271.3 mg/100 g, followed by P (20.7–25.8 mg/100 g), Ca (11.9–13.4 mg/100g), Mg (10.1–11.5 mg/100 g), Na (1.02-1.43 mg/100 g), Fe (0.57–0.67 mg/100 g), Zn (0.21-0.28 mg/100 g), and Mn (0.071–0.088 mg/100 g). Copper was contained in the smallest amounts in the samples, ranging from 0.061 to 0.078 mg/100 g.

DISCUSSION

The study investigated the factors influencing the micropropagation of the stone fruit rootstock cultivar 'Evrica 99.' Additionally, it explored the variations among the utilized rootstocks in terms of their impact on certain phytochemical traits in fresh apricot fruit. The process of *in vitro* propagation of rootstock can be separated into four stages: initiation of aseptic culture, proliferation of explants, rooting of micro-stems, and acclimatization. Microbial contamination of explants is a serious limiting factor for *in vitro* culture, and the elimination of contamination is one of the basic requirements for tissue culture success. For surface sterilization, it is necessary to use compounds that are toxic to microorganisms but not toxic to plants. Surface sterilization of explants is necessary to make them free of contaminants [32]. Two different types of sterilizers were used in this study: ethanol and Ca $(ClO)_2$. The results indicated that an extended exposure time to calcium hypochlorite led to a reduction in infection for the T2 and T3 treatments. However, concurrently, there was an increase in non-growing cultures, possibly due to the toxic effects on the explants. Plant regeneration *in vitro* is a process in which explants undergo cellular division and differentiation and then form tissues and organs throughout the growth period [33-34]. The achievement of plant regeneration depends on numerous things, namely on explant types [35], nutrients [36], PGR types, their concentrations and combinations [37–42], the age of the mother plant from which the explant is separated [43], the season of explant separation, temperature, illumination [44], etc.

Explants of *´Evrica 99´* were grown on MS nutrient medium added with varied doses of BAP and Kin single or in conjunction with 1.0 mg/l GA_3 for *in vitro* growth of the explant. Sasidharan and Jayachitra [45] observed a significant shoot regeneration rate (98.51%) from the apical bud of *Enicostema* axillare cultured in nutrient medium MS, which included BAP 1.0 mg/l in conjunction with 0.2 mg/l Kin, which is consistent with the results we have obtained. In the current study, *in vitro* plant regeneration occurred through direct organogenesis from nodal and apical explants. Numerous studies have proven Prunus spp. micropropagation through direct organogenesis [46–47]. Explants in medium without the addition of PGRs did not exhibit any response in terms of

the formation of shoot buds. This is consistent with the study by Lakho et al. (2023), which showed that pineapple explants did not grow in nutrient media without growth regulators [48]. BAP treatments registered a better response for the formed shoots per explant than kinetin treatments. This is in conformity with the observation by Ahmadpour et al., (2023) in *Hyoscyamus niger* [49]. Among BAP and Kin concentrations, Kin was more effective than BAP in increasing shoot length, and along with an increase in cytokinin concentration, shoot height decreased in this study. Shoots produced by nodal meristems had a longer mean length than shoots produced by apical meristems.

Our results showed that 1.0 mg/l GA3 in conjunction with BAP and Kin induced the elongation of the shoot (Fig. 3). The positive effect of GA_3 on shoot elongation has been reported in other studies [50–51]. This study found no root establishment of micro-shoots in PGRs-free nutritional media. As IBA and NAA concentrations increased from 0.5 mg/l to 0.8 mg/l and IAA concentrations increased from 0.5 mg/l to 1.0 mg/l, the average number of roots and their length increased. This result is consistent with those reported by other researchers regarding the essential role of auxins in plant propagation by cuttings [52–56]. IBA was found to be the most effective *in vitro* rooting regulator of all three auxins studied, in agreement with results from other studies (57–58). The results revealed that as the amount of auxin in the culture medium increased, the formation of calluses at the base of micro-shoots occurred. This finding aligns with the study conducted by Ghan et al., 2021 [59], where it was observed that surpassing an NAA concentration of 0.75 mg/l led to a progressive increase in callus induction and root induction in the medicinal plant *Solanum torvum* Sw. The data of our research (Table 2) showed a rich nutritional and functional composition in terms of sugars (11.46–14.85 g/100 g), ascorbic acid (6.84–8.60 mg/100 g), and carotenoids (0.84–0.95 mg/100 g). The sugar, dry matter, and vitamin C content of the fruits from the grafted cultivars were found to vary significantly depending on the rootstocks used.

The presence of sucrose, the natural form of carbohydrates, can provide a stable energy supply, which makes apricots an excellent ingredient in high-energy functional products [60]. Vitamin C plays a crucial role determining both antioxidant capacity and post-harvest quality of the yield [61-62]. Being a powerful functional component of food, vitamin C has many applications in health care [63]. The vast majority of ascorbic acid, also known as vitamin C, in human meals comes from fruits and vegetables [64-65]. The cultivars 'Yerevani' and 'Sateni' demonstrated the highest Vitamin C content when grafted onto the virus-free 'Evrica 99' rootstock. In contrast, when 'Sateni' was grafted onto an apricot seedling, it exhibited the lowest Vitamin C content. However, even when 'Yerevani' was grafted onto an apricot seedling, it maintained a relatively high Vitamin C content. This highlights the influence of both the rootstock and the specific cultivar on Vitamin C levels.

DM content in apricots affects how well they can be processed and transported and if they are good for drying [66]. Both the *´Yerevani´* and *´Sateni´* cultivars exhibit varying DM contents when grafted onto different rootstocks. This suggests that the choice of rootstock can significantly influence the DM content of the cultivar. Interestingly, the rootstocks do not have a significant effect on the quantity of carotenoids and TA in fruits. However, they do influence other fruit quality traits such as sugar content, DM, vitamin C, and overall chemical composition. These characteristics are intrinsically linked to the bioactive compounds found in fruits, which are widely recognized for their numerous health benefits [67-70]. When grafted on virus-free *´Evrica 99´* rootstock, both *´Yerevani´* and *´Sateni´* cultivars had a higher sugar content, as well as vitamin C and DM in fruits, compared to the fruits of the same cultivars grafted on the same rootstock cultivar propagated by cutting. In all cases,

the ´Sateni´ cultivar had higher sugar, DM, TC, and vitamin C content compared to the *´Yerevani´*. Conversely, the TA amount was higher in the fruits of the *´Yerevani´* cultivar compared to the *´Sateni´*. The TC content was higher in the *Sateni´* cultivar compared to *´Yerevani´*.

The study reveals that the fruits of the studied apricot cultivars contain significant amounts of essential minerals and that their amount is affected by the selected rootstock. Essential minerals found in apricots, such as potassium, calcium, and magnesium, are known to have various health benefits. These minerals contribute to the overall nutritional value of apricots, making them a functional food [71]. In general, the cultivars grafted on the virus-free 'Evrica 99' rootstock exhibited superior fruit quality. However, notable variations were observed among the 'Evrica 99' rootstock propagated by cuttings and the seedling rootstock, particularly concerning the impact on the mineral content in apricot cultivars' fruits. From the comparative study of mineral elements in the fruits of studied apricot cultivars grafted on *´Evrica 99´* (propagated by cuttings) rootstock and on apricot seedling rootstock, it was found that the fruits of *´Yerevani´* had a higher content of Na, those of *´Sateni´* had a higher content of Ca, and both *´Yerevani´* and *´Sateni´* cultivars had a higher content of K, respectively, when using the *´Evrica 99´* rootstock propagated by cuttings. By influencing the fruit quality traits, rootstocks indirectly affect the concentration of bioactive compounds, thereby impacting the health benefits derived from the fruits.

This research presents a novel approach to plant propagation, utilizing *in vitro* propagation technology for the stone fruit rootstock cultivar *´Evrica 99´* via direct organogenesis. This method serves as an innovative alternative to traditional propagation techniques. Furthermore, we conducted an analysis of the biochemical properties of fresh apricot fruits from the *´Yerevani´ and ´Sateni´* cultivars, which were grafted onto the virus-free *´Evrica 99´* rootstock. The results were compared with those from the same rootstock propagated by cuttings and with the seedling rootstock, which is commonly used. Future research directions could include field studies under varying climatic conditions to evaluate potential changes in both the quality and the functional food properties of the fruit. It would also be beneficial to consider other influential factors, such as yield and disease resistance. This could enhance the quality of the resulting produce, both in terms of yield and nutritional value.

CONCLUSION

The successful technology of micropropagation for the rootstock cultivar *´Evrica 99'* has been developed, which will provide an effective way to produce healthy planting material from this economically important rootstock. The study revealed that the selection of appropriate and high-quality rootstock plays a major role in increasing the fruit quality traits of grafted cultivars. The cultivars grafted on the virus-free rootstock had a richer profile of nutrients, containing biologically active compounds and minerals. The work has practical and theoretical significance. By enhancing the phytochemical traits through rootstock selection, we could potentially enhance these health benefits, further solidifying apricots' status as a functional food. As there is a growing interest in healthy, quality, and useful fruits, this result can be beneficial for both growers and consumers.

Abbreviations: BAP: 6-Benzylaminopurine, DM: Dry matter, GA3: Gibberellic Acid, IAA: Indole-3-Acetic Acid, IBA: Indole-3-Butyric Acid, Kin: Kinetin, MS: Murashige and Skoog; NAA: α-Naphthaleneacetic Acid; PGRs: Plant Growth Regulators; TA: titratable acidity (DM); TC: total carotenoids.

Authors Contributions: GS and GM designed this study, GM carried out the experimental part of tissue culture,

GS and GM carried out filed experiment, GS carried out biochemical analysis, GS and GM read and agreed with the final version of the manuscript.

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