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ORIGINAL PAPER

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EFFECT OF PLANT HORMONES ON MICROPROPAGATION POTENTIAL OF SUPERIOR STRAWBERRY GENOTYPES AND THEIR PARENTS VIA SHOOT-TIP CULTURE

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ABSTRACT

In this study, the effects of different hormones and their doses on the micropropagation and rooting of two strawberry cultivar candidates (291 and 299) selected from last step of breeding program carried out by Horticulture Department of Cukurova University (Adana, Turkey) and their parents (Rubygem and Kaşka cultivars developed by Horticulture Department of Cukurova University) were investigated using shoot-tip culture. Nodal segments of strawberry runners were sterilized through keeping in 7% sodium hypochlorite solution containing 1-2 drops of Tween-20 for 10 minutes. Five different Murashige ve Skoog (MS) nutrient media combinations containing 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ) at the ratios 0.75 and 1.50 mg L^{-1} were tested (Medium 1: MS + 30 g L^{-1} sucrose, 7 g L^{-1} agar, Medium 2: MS + 30 g L^{-1} sucrose, 7 g L^{-1} agar + 0.75 mg L^{-1} BAP, Medium 3: MS + 30 g L^{-1} sucrose, 7 g L^{-1} agar + 1.50 mg L^{-1} BAP, Medium 4: MS + 30 g L^{-1} sucrose, 7 g L^{-1} agar + 0.75 mg L^{-1} TDZ, Medium 5: MS + 30 g L^{-1} sucrose, 7 g L^{-1} agar + 1.50 mg L^{-1} TDZ). During the study, darkening, infection and rooting rates (%), shoot number per plant in both first and second subculture, root number per plant and root length (cm) parameters were examined. Use of four genotypes/cultivars and five nutrient media, this study was planned according to the factorial design of randomized plots with three replications. JMP package program and LSD test were used for statistical evaluation of data obtained. It was found that the media including TDZ was generally more effective than BAP in terms of shoot proliferation in both first and second subculture. The number of shoots per explant varied from 0 to 4.18 shoots per explant in the first subculture. Regarding to mean of genotypes and media, Kaşka cultivar (2.53 shoots per explant) and medium 5 (2.88 shoots per explant) gave the best results, respectively. In the second subculture, the number of shoots per explant changed between 0.33 and 4.83 shoots per explant. According to mean of genotypes and media, Kaşka cultivar (3.23 shoots per explant) and medium 4 (3.63 shoots per explant) were found to be the best material and medium, respectively. Regarding to rooting parameters, it was determined that the hormone-free MS medium was highly efficient. The number of root per *in vitro* plant varied from 0 to 30 roots per *in vitro* plant. According to mean of genotypes and media, Rubygem cultivar (9.60 roots per *in vitro* plant) and medium 1 (22.30 roots per *in vitro* plant) gave the best results, respectively. Root lenght changed between 0 and 5.04 cm. Regarding to mean of genotypes and media, the best values were obtained from Kaşka cultivar (2.10 cm) and medium 1 (3.89 cm), respectively. The value of 0 obtained from rooting parameters refers to *in vitro* plants that can not be rooted. Infection rate was low in both subcultures. Especially in the first subculture, it was observed only in the genotype 299 cultured in the medium 3. In the second subculture, infection was detected at low rates in the genotypes 291 and 299 and Rubygem cultivar cultured media 2, 3 and 5. No darkening was observed in the explants cultured in both subcultures. Based on genotypes, Kaşka cultivar developed in the Horticulture Department of Cukurova University (Turkey) was found to be superior in terms of both shooting and rooting criteria. Achieving successful results of this cultivar will speed up its use in breeding studies. Based on the study result and literature, it was concluded that the selection of the appropriate hormone type and dose are genotype-dependent.

Key words: *Fragaria*, tissue culture, bred line, *in vitro* proliferation, BAP, TDZ

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INTRODUCTION

Strawberries are among the most preferred fruits due to their rich vitamin content and unique aroma. According to TÜİK, 61 137 hectares of strawberry production is established in 2019 in the Mediterranean region of Turkey alone. This means that 370–400 million strawberry plants are needed for strawberry cultivation in this region [Turkish Statistical… 2020]. The selection of suitable cultivars and quality plant material, which is the first step of successful production in agriculture, is a well-known fact in many plant species. Plant diameter is a very important quality parameter in strawberry cultivation. As reported by Paydaş Kargı and Sarıdaş [2012], A+ plants with diameters greater than 15 mm should be used. Further on, selection of cultivars resistant or tolerant to soil-borne diseases and production of these cultivars in disease-free areas are important for productivity. In addition to the requirements of certified plant production, shoot-tip and meristem culture studies have been carried out for large scale, disease and virus free plant production in recent years [Haddadi et al. 2010, Taşkın et al. 2013, Capocasa et al. 2019]. One of the most important factors affecting the yield and fruit quality in strawberry cultivation is the use of healthy plant material. Runner plants are normally used in plant material production. A soil-borne disease that may occur in the plant production area can be spread easily by vegetatively propagated plant materials and yield is significantly lost in cultivation by using this plant material. In this context, meristem culture is an important tissue culture method that is used effectively in obtaining diseases and virus-free plants. If no virus or disease-free plants are needed, shoot tip culture can be an important alternative production method for fast, controlled and partially clean plant production.

There are many supplementing with relevant literature items. While some of these studies used meristems [Adams 1972, Nishi and Ohsawa, 1973, Torres 1989, Rattanpal et al. 2011, Quiroz et al. 2017], shoot tips were preferred in some of them [Bhatt and Dhar 2000, Haddadi et al. 2010, Moradi et al. 2011, Ghasemi et al. 2015, Jhajhra et al. 2018]. The effects of different factors such as genotype, nutrient medium, hormone type, concentration and combination on micropropagation success were tested in these studies. For the shoot formation and development, use of cytokinins such as BAP, Kinetin (KIN), 1-Naphthaleneacetic acid (NAA) and isopentenyl adenine (2ip) is quite common in literature. Usage of TDZ is also available in some studies, however, it seems less common [Passey et al. 2003, Yonghua et al. 2005, Adak et al. 2009, Haddadi et al. 2010].

The objectives of this study are as follows: (i) investigation of micropropagation capacities of two strawberry cultivar candidates that reached the final stage of breeding studies and Rubygem and Kaşka cultivars used as parents obtaining them via shoot-tip culture (ii) determining the effects of two different doses of BAP and TDZ on shoot formation, development and rooting in this process.

MATERIALS AND METHODS

This study was carried out in Prof. Dr. Saadet Büyükalaca Tissue Culture Laboratory and research areas of Cukurova University (Adana, Turkey) between 2017 and 2018 years. In the experiment; two strawberry cultivar candidates (genotypes 291 and 299), selected as productive, flavoured, good colored and with hard flesh from the individuals obtained by crossing breeding (Rubygem × Kaşka) and their parents (Rubygem and Kaşka cultivars) were used as plant material (Fig. 1).

Shoot formation and proliferation

The nodal segments taken from runners of strawberry plants used in the experiment were kept for 10 minutes in 7% sodium hypochlorite solution containing 1–2 drops of Tween-20 in sterile bench for sterilization. Afterward, the plant material was rinsed by sterile water at least 4–5 times. Shoot-tips were extracted from the nodal segments under microscope by using sterile forceps and scalpels in the sterile bench and were placed into glass jars containing nutrient medium. All materials used during culture (nutrient media, glass jars, water bottles, forceps-scalpels, papers) were sterilized using autoclave at 121°C temperature and 1.2 kg/cm^2 pressure for 15 minutes. Hormone-free Murashige and Skoog (MS) [1962] medium was used as nutrient medium during shoot formation. After obtaining shoots from shoot-tips (about 4–5 weeks later), micropropagation was applied to these shoots. In this

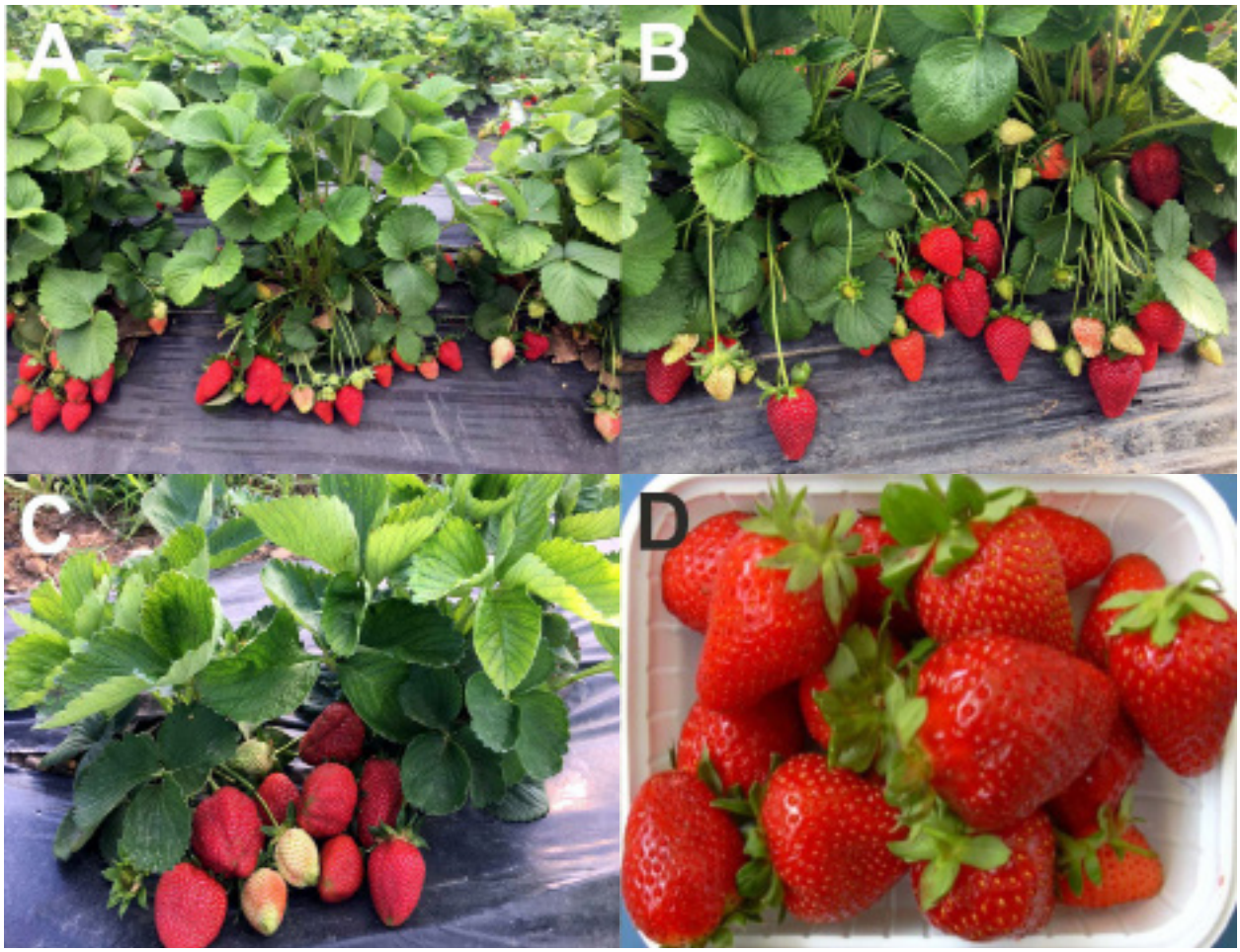

Fig. 1. Strawberry genotypes and cultivars used in this study (A) genotype 291 (B) genotype 299 (C) Rubygem cultivar (D) Kaşka cultivar

step, different concentrations of BAP and TDZ added to MS medium were tested (Tab. 1). Study plan was presented in Table 2.

Shoots developed from shoot-tips were incubated in growth chamber of tissue culture laboratory at specific conditions (25 ±2°C temperature, 3000 lux light, 16 hours light and 8 hours dark photoperiod). Shoots were cultured about 4–5 weeks in the first micropropagation media and then they were transferred to second micropropagation media. Similarly, they were cultured about 4–5 weeks in the second ones.

The following parameters were examined during the micropropagation stage:

Darkening ratio (%): The ratio of the darkening explants as a result of oxidation to the total explants.

Infection rate (%): The ratio of infected explants due to bacterial or fungal contamination to the total explants.

Number of shoots per explant: The average value of the total number of shoots produced by each explant.

Rooting and acclimatization of *in vitro* plants

Root length and root number of the *in vitro* plants developed were measured (about 5–4 weeks later from transferring to the rooting media) and then the plantlets were transferred to the trays for acclimatization (Fig. 2). The acclimatization was carried out step by step. Steps; loosening lid of the jars on the first day, opening the half of the lid on the second day, completely opening the lid of the jar on the third day, removing the *in vitro* plants from the nutrient medium

Table 1. Content of the nutrient media used in the study

Medium No	MS g L^{-1}	Sucrose g L^{-1}	Agar g L^{-1}	BAP mg L^{-1}	TDZ mg L^{-1}
Medium 1	4.4	30	7	–	–
Medium 2	4.4	30	7	0.75	–
Medium 3	4.4	30	7	1.50	–
Medium 4	4.4	30	7	–	0.75
Medium 5	4.4	30	7	–	1.50

Table 2. Work plan used in this study

Steps	Process	Time (weeks)	Nutrient medium used
Step 1	Shoot formation	About 4–5 weeks	Hormone free MS medium
Step 2	First subculture	About 4–5 weeks	Five different media including BAP and TDZ at different doses
Step 3	Second subculture	About 4–5 weeks	Same nutrient media with step 2
Step 4	Rooting	About 4–5 weeks	Same nutrient media with step 2
Step 5	Acclimatization	About 2 weeks	

and immersing them into water containing fungicide (Captan, Captan 48.9%, relatives derivatives 1.1%, other ingredients 50%) and planting into trays containing 1 : 1 sterile peat: perlite mixture. The first irrigation was done with water including fungicide (Captan, Captan 48.9%, relatives derivatives 1.1%, other ingredients 50%) (Fig. 3). At the end of the second weeks, the number of living plants was determined.

The following parameters were examined:

Rooting rate (%): The ratio of rooting *in vitro* plants to the total number of plants.

Root number per *in vitro* plant: The average number of roots per *in vitro* plant.

Root length: The average length of the roots reached during culture (cm).

Statistical analysis

This study was established according to the factorial design of randomized plots with four genotypes (Rubygem, Kaşka, 291, 299) and five nutrient media (two different doses of two hormones). The experiment was planned as three replications. Three jars were used for each repetition and each jar contained one explant. A total of 3 × 3 × 4 × 5 = 180 plants were studied in each subculture. The data obtained were subjected to variance analysis in JMP package program and differences between the means were determined by LSD test. Comparisons that yielded $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$ were considered to be statistically significant.

RESULTS

In this study, fresh runners of strawberry genotypes and cultivars grown under high tunnels were collected and shoot tips were extracted from nodal segments, then planted into MS nutrient medium in the tissue culture laboratory after their sterilization. While no darkening was observed in shoots developed from the shoot tips, different infection rates were determined to depend on the genotypes. While the highest infection rate was observed in Kaşka cultivar with 36%, the lowest infection level (24.3%) was found in the genotype 291. Healthy shoots obtained were transferred to the nutrient media containing different doses of BAP and TDZ (Tab. 1). Results of individual and combined effect of genotype and growing media on shoot development in first subculture were shown in Table 3. Based on data, the mean of the shoot number per ex-

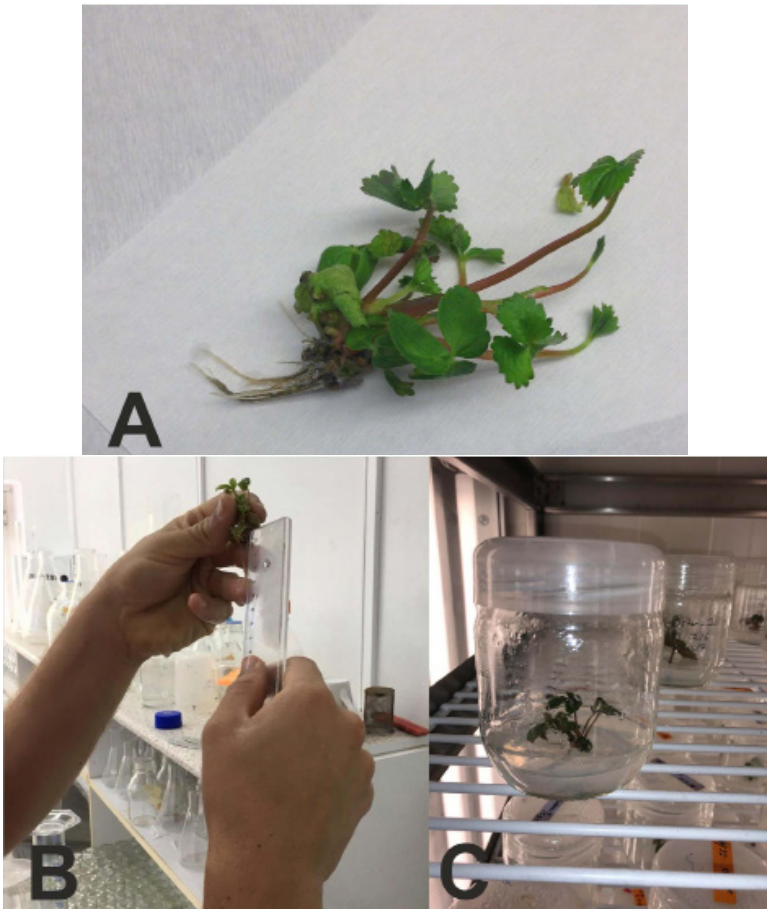

Fig. 2. *In vitro* strawberry plants at rooting step (A) rooted *in vitro* plants (B) measurement of root length of plants (C) Rooted plants in the jars

plant was found to be 2.53 in the Kaşka cultivar and it was followed by the genotype 291 (2.0 shoots/explant) nested in the same group statistically. The mean of the lowest number of shoots per explant (0.99) was determined in the genotype 299. At the same time, growing media significantly affected shoot number developed. As expected, hormone-free media showed the lowest proliferation. The media contained TDZ was more efficient than BAP contained in terms of shoot numbers. Increasing hormone doses generally increased the shoot number of plant materials except genotype 299 which showed high sensitivity to higher dose of these two different hormones. When the interaction values were examined, it was found that statistically significant differences and the responses of genotypes to hormones were quite different. The highest number of shoots per explant was determined as 4.18 in the genotype 291 in the medium 5, whereas the lowest value (0 shoots/explant) was observed at the same medium for genotype 299. At the same time, the maximum number of shoots (2.67 shoots/explant) was determined in the medium 4 including lower dose of TDZ for this genotype. The result not only showed the role of hormone type on shoot number but also indicated that the importance of hormone dose.

At the end of the first subculture, infection rates of media and genotypes are shown in Table 4. No infection was detected in any combinations except genotype 299 transferred to medium 3. In the combination of this medium with this genotype, infection was detected in 36.2% of shoots. This may be related to the sensitivity of this genotype.

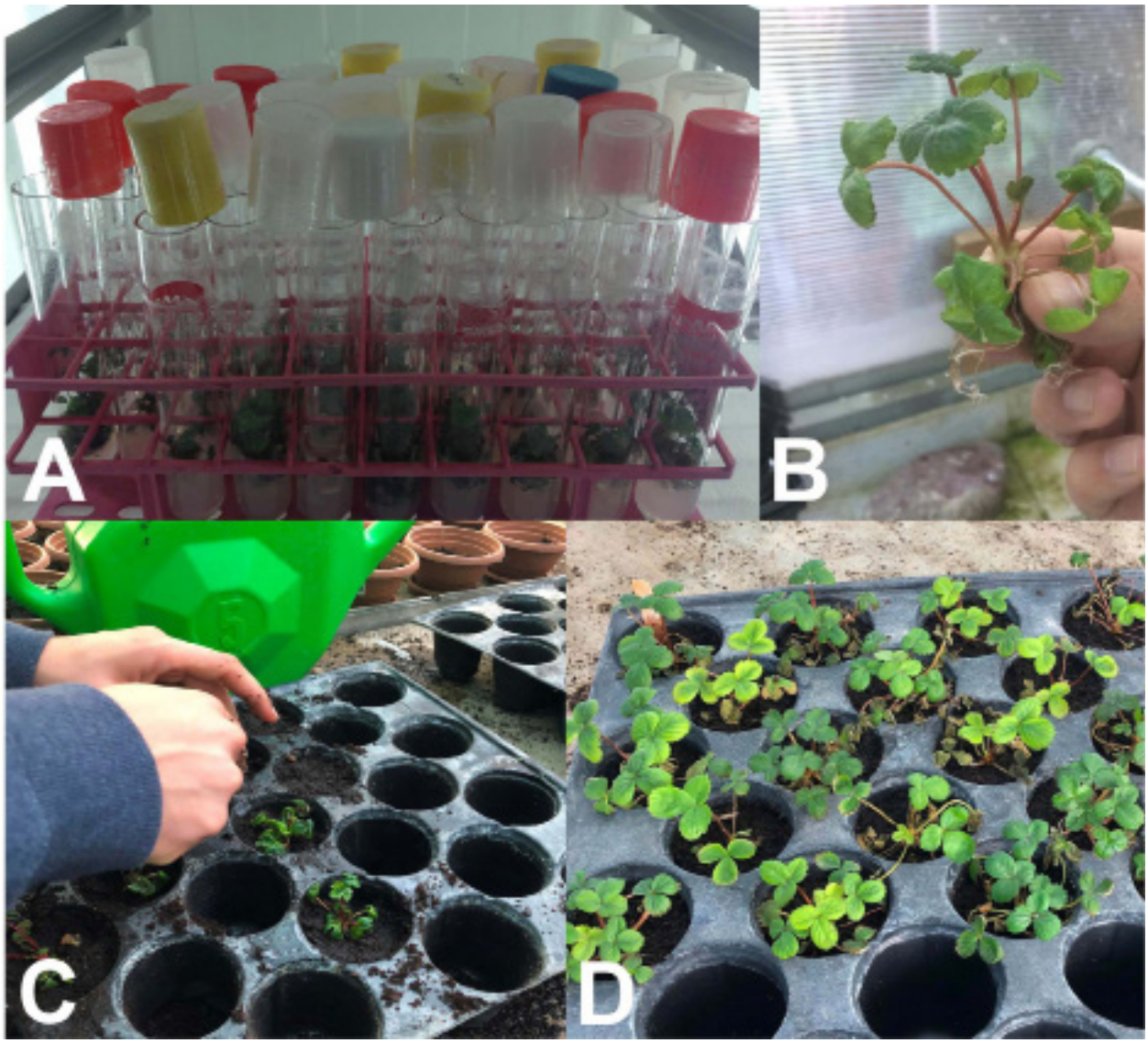

Fig. 3. Acclimatization of *in vitro* strawberry plants (A) opening lids of the jars on the second day (B) removing the *in vitro* plants from the nutrient medium (C) planting into trays containing 1 : 1 sterile peat: perlite mixture (D) acclimatized of *in vitro* plants

Similar responses were observed between genotypes and nutrient media in the second subculture in terms of the number of shoots per explant. Kaşka cultivar produced the highest value with 3.23 shoots/explant and was followed by the genotype 291 (3.00 shoots/explant) and Rubygem cultivar (2.90 shoots/explant) which nested in the same statistical group. At the same time, the lowest value was recorded in the genotype 299 with 2.32 shoots/explant. While the highest shoot number was obtained from medium that contained lower level TDZ (0.75 mg L^{-1}), it was in the same statistical group with medium 5 and medium 2 having 3.54 and 3.38 shoots/explant, respectively. As seen in the first subculture, the lowest value was determined in control medium (medium 1). The interaction was observed between genotypes and nutrient media in the second subculture in terms of the number of shoots

Table 3. The number of shoots per explant in the first subculture of strawberry genotypes/ cultivars transferred to different nutrient media

Medium	Strawberry genotypes/ cultivars				Mean of medium
	Kaşka	Rubygem	291	299	
1	1.00 e-i	1.50 c-h	1.06 e-i	0.42 ghi	0.99 C
2	2.17 b-e	0.83 f-i	1.17 d-i	1.50 c-h	1.42 BC
3	2.33 bcd	2.07 b-e	2.00 b-f	0.38 hi	1.70 B
4	3.00 ab	2.17 b-e	1.61 c-g	2.67 bc	2.36 A
5	4.17 a	3.17 ab	4.18 a	0.00 i	2.88 A
Mean of genotypes	2.53 A	1.95 B	2.00 AB	0.99 B	
LSD genotype***= 0.54, LSD medium*** = 0.61, LSD genotype × medium*** = 1.22					

Differences between averages showed by different letters are statistically important
N.S. – non significant; *P < 0.05, **P ≤ 0.01, ***P ≤ 0.001

Table 4. Infection rates in the first subculture of strawberry genotypes/ cultivars transferred to different nutrient media (%)

Medium	Strawberry genotypes/ cultivars				Mean of medium
	Kaşka	Rubygem	291	299	
1	0 B	0 B	0 B	0 B	0 B
2	0 B	0 B	0 B	0 B	0 B
3	0 B	0 B	0 B	36.2 A	9.04 A
4	0 B	0 B	0 B	0 B	0 B
5	0 B	0 B	0 B	0 B	0 B
Mean of genotypes	0 B	0 B	0 B	7.23 A	
LSD genotype*** = 0.12, LSD medium*** = 0.14, LSD genotype × medium*** = 0.28					

Differences between averages showed by different letters are statistically important
N.S. – non significant; *P < 0.05, **P ≤ 0.01, ***P ≤ 0.001

per explant and significant differences were detected (Tab. 5). Therefore, it is clear that responses of the genotypes to hormone type and concentrations are significantly different. For example, while the highest number of shoots per explant was determined in the nutrient media containing BAP for Rubygem cultivar, it was found that the number of shoots per explant in the media containing TDZ was lower than the control medium (medium 1). While the Rubygem cultivar responded positively to BAP in terms of the number of shoots, no change was observed with the use of TDZ. Again in the genotype 299, not only the hormone type but also the concentration had a significant effect. While the lowest number of shoots per explant was obtained in the medium containing high dose of BAP, the highest number of shoots (3.33 shoots per explant) was determined for this genotype in the medium 2 containing lower dose of BAP.

As seen in the first subculture, there was no darkening in any of the media of second subculture. The infection rate in the second subculture is shown in Table 6. Differences between all factors and their interactions were found to be significant statistically. When genotypes are evaluated within themselves, genotype 299 was the most sensitive with 21.1% infection rate and followed by genotype 291 nested in the different statistical groups with 19.2%. While a low level of infection (3.2%) was observed in the commercial cultivar Rubygem, there was no infection in our local cultivar Kaşka. The reason for not encountering any

Table 5. The number of shoots per explant in the second subculture of strawberry genotypes/ cultivars transferred to different nutrient media

Medium	Strawberry genotypes/ cultivars				Mean of medium
	Kaşka	Rubygem	291	299	
1	1.00 gh	2.67 def	1.00 gh	1.78 fg	1.61 C
2	3.33 bcd	4.17 abc	2.67 def	3.33 bcd	3.38 A
3	2.50 def	3.33 bcd	2.50 def	0.33 h	2.17 B
4	4.83 a	2.00 efg	4.50 a	3.17 cd	3.63 A
5	4.50 a	2.33 def	4.33 ab	3.00 de	3.54 A
Mean of genotypes	3.23 A	2.90 A	3.00 A	2.32 B	
LSD genotype*** = 0.49, LSD medium*** = 0.55, LSD genotype × medium*** = 1.09					

Differences between averages showed by different letters are statistically important
N.S. – non significant; *P < 0.05, **P ≤ 0.01, ***P ≤ 0.001

Table 6. Infection rates in the second subculture of strawberry genotypes/ cultivars transferred to different nutrient media (%)

Medium	Strawberry genotypes/ cultivars				Mean of medium
	Kaşka	Rubygem	291	299	
1	0.0 e	0.0 e	0.0 e	0.0 e	0.0 D
2	0.0 e	0.0 e	0.0 e	38.7 c	9.7 C
3	0.0 e	0.0 e	16.0 d	67 b	20.8 B
4	0.0 e	0.0 e	0.0 e	0.0 e	0.0 D
5	0.0 e	16.0 d	80 a	0.0 e	24.0 A
Mean of genotypes	0 D	3.20 C	19.2 B	21.1 A	
LSD genotype*** = 0.66, LSD medium*** = 0.73, LSD genotype × medium*** = 1.47					

Differences between averages showed by different letters are statistically important
N.S. – non significant; *P < 0.05, **P ≤ 0.01, ***P ≤ 0.001

infection in the Kaşka cultivar may be due to the resistance to diseases of local genotypes used in the breeding of this cultivar. It is known that one of its parent is quite resistance to diseases. In genotype 299, infection was also observed in the first subculture. Probably, as we have mentioned earlier, this genotype is probably sensitive to diseases.

In tissue culture studies, micropropagated *in vitro* plants must be rooted sufficiently before transferring to the field. During the study, effect of the nutrient media used for shoot development on parameters related to rooting such as rooting rate, number of roots and root length were also investigated without using a special rooting medium due to the potential of easy rooting of strawberry species. All *in vitro* plants of all genotypes and cultivars were rooted under hormone-free medium (medium 1) (rooting rate: 100%, 100%, 48.3%, 100% and 51.7% in the media 1–5 of Kaşka cultivar respectively; 100%, 15%, 74%, 0% and 100% in the media 1–5 of Rubygem cultivar respectively; 100%, 40%, 33.2%, 66% and 0% in the media 1–5 of genotype 291 respectively and; 100%, 60%, 0%, 0% and 0% in the media 1–5 of genotype 299 respectively). Besides of increasing effect of BAP and TDZ on shoot proliferation, these were negatively influenced to root formation. Another important parameter on rooting is

the root number. The data related to root number are shown in Table 7. Similar to the rooting rate, the highest root number (22.3 roots per *in vitro* plant) was obtained only in medium 1 without hormone. Although all other media are in the same statistical group, it was found that the medium containing lower dose of BAP resulted with higher number of roots than the medium containing higher dose of BAP. On the contrary, higher number of roots was obtained in the medium containing higher dose of TDZ than the one with lower dose of TDZ. It shows that root number is affected not only by the hormone types but also by their doses. Significant differences were found when genotypes were compared within themselves. The highest number of roots per *in vitro* plant was observed in Rubygem cultivar with 9.60 roots and it was followed by the genotype 291 nested in the same statistical group with 9.00 root numbers per *in vitro* plant. The lowest root number was recorded in genotype 299 with 4.27 roots per *in vitro* plant. The interaction between the factors evaluated in the study was found to be significant. The interaction of the genotypes/cultivars with medium 1 was at the top with between 18.30 and 30.0 roots per *in vitro* plant, while the effect of hormone type and dose on the number of roots was determined to be different. The high dose of TDZ (1.5 mg L^{-1}) increased significantly the root number compared to the low dose of TDZ in the Rubygem and Kaşka cultivars, while responses of the cultivars to doses were found to be different in the nutrient media containing BAP. It was determined that low dose of both hormones increased the root number in the genotype 291. Although both doses of TDZ inhibited the root formation in the genotype 299, it was determined that while rooting was observed in the low dose of BAP, no root recorded in the high dose of BAP (1.5 mg L^{-1}).

Although the interaction between the factors in the study was found to be significant, it was very important to catch the maximum number of roots per *in vitro* plant with hormone-free nutrient medium in all genotypes examined.

The results of root length are shown in Table 8. Differences between root length in culture media were found to be statistically significant. Similar to the root ratio and the number of roots, the highest root length was determined in the hormone-free medium. Increased dose of both hormones reduced root length, although there was no significant difference. The differences between root length values in the genotypes/ cultivars were found to be statistically insignificant and the results varied between 1.09 cm (genotype 299) and 2.10 cm (Kaşka cultivar). When the interaction of the investigated factors was examined, it was found that the differences were insignificant and root length values ranged between 0 and 5.04 cm.

When rooting rate, number of roots and root length values were evaluated together, the cytokinin group hormones had negative effects on these parameters

Table 7. The number of root per *in vitro* plant of strawberry genotypes/ cultivars transferred to different nutrient media

Medium	Strawberry genotypes/ cultivars				Mean of medium
	Kaşka	Rubygem	291	299	
1	21.00 ab	20.00 ab	30.00 a	18.30 bc	22.30 A
2	4.67 de	4.33 de	4.33 de	3.00 de	4.08 B
3	1.67 e	6.67 de	2.00 e	0.00 e	2.58 B
4	1.67 e	0.00 e	8.67 cde	0.00 e	2.58 B
5	12.70 bcd	17.00 bc	0.00 e	0.00 e	7.42 B
Mean of genotypes	8.33	9.60	9.00	4.27	
LSD genotype = N.S., LSD medium*** = 5.03, LSD genotype × medium*** = 10.01					

Differences between averages showed by different letters are statistically important
N.S. – non significant; *P < 0.05, **P ≤ 0.01, ***P ≤ 0.001

Table 8. Root length values of strawberry genotypes/cultivars transferred to different nutrient media (cm)

Medium	Strawberry genotypes/ cultivars				Mean of medium
	Kaşka	Rubygem	291	299	
1	5.04	2.02	3.97	4.56	3.89 A
2	1.89	0.64	2.26	0.86	1.41 B
3	1.37	1.67	1.08	0.00	1.03 B
4	0.90	0.00	2.32	0.00	0.80 B
5	1.32	1.25	0.00	0.00	0.64 B
Mean of genotypes	2.10	1.11	1.93	1.09	
LSD genotype = N.S., LSD medium*** = 1.09, LSD genotype × medium = N.S.					

Differences between averages showed by different letters are statistically important
N.S. – non significant; *P < 0.05, **P ≤ 0.01, ***P ≤ 0.001

as expected. Although auxin group plant hormone is needed for rooting in many fruit species, it is clearly determined that sufficient and desired rooting can be achieved with hormone-free nutrient medium in this study.

DISCUSSION

There are many studies related to micropropagataion of strawberries in literature. Shoots of wild strawberry (*Fragaria indica*) having a single node were cultured on MS medium including different BA and NAA combinations by Bhatt and Dhar [2010]. At the end of their study, combination of 4 µM BA with 0.1 µM NAA was found to be the most successful combination in terms of shoot proliferation with 22.3 shoots per explant. Shoot number per explant varied from 2.0 to 22.3. In addition, some experiments were carried out to find the best cytokinin (supplemented as BA, KIN, 2ip at 4 µM concentration) for shoot proliferation. BA was found to be better than KIN and 2ip with 22.3 shoots per explant. It was followed by KIN with 13.3 shoots per explant and by 2ip with 9.3 shoots per explant. Haddadi et al. [2010] suggested an efficient protocol for propagation of strawberry Camarosa cultivar. They used shoot tips of *in vitro* plants and cultured them in the MS medium including 2, 4, 8 µM TDZ and 0, 4, 9, 18, 27 µM BAP for shoot induction. The most successful combination for shoot number was MS with 2 µM TDZ and 4 µM BAP.

In a study carried out by Moradi et al. [2011], nodal segments obtained from *in vitro* plants were used for propagation of *Fragaria* in nutrient media having different combinations of plant growth regulators. The highest bud number was recorded in the nutrient medium supplemented only 1.5 mg L^{-1} KIN (in the BAP and KIN combinations) and in the medium contained only 0.1 mg L^{-1} BAP and 0.1 mg L^{-1} BAP and 0.2 mg L^{-1} IBA (in the BAP and IBA combinations). Ghasemi et al. [2015] investigated the most effective plant growth regulators for *in vitro* shoot regeneration and root induction of wild strawberry (*F. viridis* Duch.). For this aim, shoot tips were cultured in MS medium including B5 vitamins, 2.2 µM BA, 0.5 µM IBA and 0.3% activated charcoal for two weeks. The explants were further transferred to the same main medium having different concentrations of BA (2.2, 4.4, 8.8, 13.3, 17.7 µM), TDZ (0.04, 0.45, 1.1, 2.2, 4.4 µM) and IBA (0.5 and 2.5 µM) for five weeks. For shoot regeneration, stipules were cultured in MS medium containing BA (2.2, 4.4, 8.8, 13.3, 17.7 µM) and IBA (0.5 and 2.5 µM) for four weeks. Shoots were transferred to MS medium without hormone, with 0.13 µM-0.55 µM GA_3 and with 0.14 µM-0.58 µM GA_3-0.88 µM BA for elongation. As rooting medium, ½ MS supplemented with 0, 1, 2.5, 3.7, 5.7 µM IBA and 0, 1.1, 2.7, 4, 5.4 µM NAA was used. The combinations for shoot regeneration from stipule and proliferation were 8.8 µM BA-2.5 µM IBA with average 6.3 shoots per explant and 2.2 µM TDZ and 0.5 µM IBA with

14 shoots per explant, respectively. The maximum root number was recorded in the medium supplemented 1 µM IBA and it was followed by the other IBA doses. Cappelletti et al. [2016] investigated regeneration efficiency of Calypso and Sveva cultivars of *Fragaria* × *ananassa*. For obtaining *in vitro* plants for regeneration studies, MS medium including 3% sucrose, 0.25 mg L^{-1} BA and 7.5 g L^{-1} agar was used and different combinations of TDZ, BA and IBA were tested in regeneration studies. At the end of study, combinations of 0.5 mg L^{-1} TDZ-0.02 mg L^{-1} 2,4-D and 3 mg L^{-1} BA-0.2 mg L^{-1} IBA were found to be best in the cultivars Calypso and Sveva, respectively. Jhajhra et al. [2018] cultured nodal segments of strawberry (*Fragaria* × *ananassa* Duch.) on MS including different concentrations of hormones (BA, NAA, GA_3, adenine sulfate and coconut water). The maximum number of shoots was obtained from MS medium containing 1 ppm BA, 0.1 ppm NAA, 1 ppm adenine sulfate and 150 mL coconut water with 5.46 shoots per explant together with maximum number of leaves with 8.93 shoots per explant.

In our experiments, number of shoots per explant ranged from 0 (medium 5, genotype 299) to 4.18 (medium 5, genotype 291) in the first subculture. As an interesting result, although medium 5 including 1.50 mg L^{-1} TDZ had the highest shoot number per explant in the cultivars Kaşka and Rubygem and the genotype 291 (4.17, 3.17 and 4.18, respectively), performance of same medium was too weak in the genotype 299. In this genotype, medium 2 including 0.75 mg L^{-1} BAP was the best with 1.50 shoots per explant. However, we can clearly say that genotype 299 has lower micropropagation potential than the others (Tabs. 2, 4). Regarding to second subculture, shoot number per explant varied between 0.33 (medium 3, genotype 299) and 4.83 (medium 4, Kaşka cultivar). Medium 4 including 0.75 mg L^{-1} TDZ showed better performance in the cultivar Kaşka (4.83) and genotype 291 (4.50). Rubygem cultivar and genotype 299 were successful in a different medium (medium 2 supplemented 0.75 BAP with 4.17 and 3.33, respectively). Similarly to our results, Bhatt and Dhar [2010] reported that shoot number per explant varied from 2.0 to 22.3 and 4 µM BA-0.1 µM NAA was found to be the best combination in the wild strawberry (*Fragaria indica*). Ghasemi et al. [2015] recorded shoot proliferation between 1 and 14 shoots per explant in wild strawberry (*F. viridis* Duch.). The highest data was obtained from combination of 2.2 µM TDZ with 0.5 µM IBA. Jhajhra et al. [2018] found maximum number of shoots in MS medium containing 1 ppm BA, 0.1 ppm NAA, 1 ppm adenine sulfate and 150 mL coconut water with 5.46 shoots per explant in the strawberry (*Fragaria* × *ananassa* Duch.). The positive effect of cytokinins on shoot formation and development is already known. Bhatia and Sharma [2015] reported that shoot-tip contains apical meristem, leaf primordia about 1 cm in length, unexpanded leaves at different development stages and nutrient media containing cytokinin are used obtaining shoots from shoot-tips. They explain formation of shoots as a result of suppression of the apical meristem with cytokinins. Bhatia [2015] stated that the shoot-tip culture is a commonly used method for clonal propagation and suggested BAP (5–10 µM) and KIN (0.1–10 µM) for shoot development as stimulator. Based on this fact, we compared the effects of BAP and TDZ for shoot development in this study. Our outcomes show that TDZ was more effective than BAP in terms of shoot formation. Although similar results are seen in the literature, BAP is still used predominantly. The possible reason may be related to TDZ being more expensive than BAP. It is also one of the popular hormones that are usually kept in stock for other studies in tissue culture laboratories.

Root formation is one of the most important steps of tissue culture studies. Many fruit species require extra hormone, which contains auxin derivatives, for completing tissue culture process. Rooting rate changed between 0 and 100%. Root number per *in vitro* plant varied between 0 (medium 5 for genotype 291, medium 3, 4 and 5 for genotype 299) and 30 (medium 1, genotype 291). Similar to the rooting rate, medium 1 was the best in terms of root number and followed by the medium 5 containing higher dose TDZ (Tab. 6). Result of root length per *in vitro* plant ranged from 0 (medium 5 for genotype 291, medium 3, 4 and 5 for genotype 299) to 5.04 (medium 1, Kaşka cultivar). Medium 1 and Kaşka cultivar were found to be the best medium and plant material in terms of root length among all tested media and genotypes/cultivars

(Tab. 7). Bhatt and Dhar [2010] tested NAA, IBA and NAA + IBA at 0.5, 1 and 2 µM concentrations for rooting. They suggested 1 µM NAA and recorded root number per explant between 4.3 and 14.3. Haddadi et al. [2010] also recommended 1 µM NAA for root number. However, 1 µM IBA and hormone-free medium showed better performance than others in root percentage and root length, respectively. Based on these studies and other studies carried out in this subject, the positive effect of auxins on rooting is supported by the literature. Similar to the results of Karim et al. [2015], hormone-free medium was found to be the best related to rooting criteria. It may be related that different role of auxin and cytokinin group hormones on root formation.

Acclimatization success was approximately 100% in our research. It was 70% and 90% in the studies carried out by Bhatt and Dhar [2010] and Haddadi et al. [2010], respectively.

CONCLUSION

Micropropagation and rooting success of two strawberry cultivar candidates (genotypes 291 and 299) selected as productive, flavoured, good colored and hard-flesh from the individuals obtained by crossing breeding and their parents (Rubygem and Kaşka) were determined using two plant hormones (TDZ and BAP) at different concentrations (0.75 and 1.5 mg L^{-1}). Shoot-tip culture was used in the *in vitro* experiments. As a result of this study, genotypic difference was obviously observed. While Kaşka cultivar was found as the best for both shooting and rooting criteria, genotype 299 was the weakest for both of them. Kaşka is a cultivar developed by our department (Horticulture Department of Cukurova University, Adana, Turkey). This cultivar is important for breeding because it is enriched in terms of aroma. Its high *in vitro* proliferation and rooting potential may be quite useful in future breeding projects aimed use this cultivar as parent. Among the five nutrient media employed, those including TDZ were better than media including BAP related to shoot development. For rooting criteria, hormone-free medium was found to be particularly successful. Based on findings obtained from this study and the existing literature, each strawberry genotype requires special hormone type and concentration to reach the best *in vitro* proliferation and rooting performance.

Literature and our outcomes about hormone types and concentrations show that responses are based on strawberry genotype and species. For that reason, new procedures would be required for every single strawberry genotype in order to obtain more efficient results in tissue culture.

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