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ESTABLISHING AN EFFECTIVE PROTOCOL FOR MICROPROPAGATION OF MULLBERRY (*Morus nigra* L.)

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ABSTRACT

Murashige and Skoog (MS) medium supplemented with plant growth regulators composed of different concentrations were used *in vitro* rapid and mass multiplication of nodal explants obtained from shoots of black mulberry (*Morus nigra* L.) seedlings grown in a fully controlled greenhouse. Eighteen different concentration of 6-benzylaminopurin, thidiazuron (TDZ), kinetin (KN) GA_3 and naphthalene acetic acid (NAA) as plant growth regulators were used. In contrast to expectation, combinations of (TDZ 1.0 mg L^{-1} + GA3 0.25 mg L^{-1}) and (TDZ 2.0 mg L^{-1} + GA_3 0.25 mg L^{-1}) were found to give positive results in shoot proliferation and plant formation at the end of the study. In the rooting study (NAA 0.5 mg L^{-1} + KN 0.1 mg L^{-1}) and (NAA 0.5 mg L^{-1} + KN 0.2 mg L^{-1}) treatment showed positive results.

Key words: *in vitro*, MS medium, micropropagation, *Morus nigra* L.

INTRODUCTION

Mulberry (*Morus* spp. L.) is a deciduous plant which belongs to the *Moraceae* family, consists of 68 different species and adapted to tropical, subtropic and temperate climate zone for thousands of years [Özgen et al. 2009]. Among these species, white mulberry (*Morus alba*) spread from Southwest China, red mulberry (*Morus rubra*) from North America and black mulberry (*Morus nigra*) from Iran to the whole world [Aljane and Sdiri 2016]. Economical mulberry cultivation started with silkworm (*Bombyx mori* L.), which is an animal that eats leaves of mulberry most, cultivation [Zaki et al. 2011]. Due to the use of mulberry leaves in the silkworm industry in China, India and Brazil, mulberry cultivation is carried out in these countries with an area of 626.000, 28.000 and 38.000 ha, respectively [Singhal 2009]. Mulberry tree is used in making paper and some musical instruments as well as in the furniture industry. There are not only some bunch of mulberry species widely used in landscape architecture as ornamental plants but also some fast-growing mulberry species that are located at the garden edges as fence plants [Gunes and Cekic 2004]. Apart from these, mulberry (*Morus spp*. L.) is consumed as fresh, molasses, fruit juice, as well as its flavoring and coloring properties in pastry and ice cream industries. Besides, it has been used in the health sector as a sweetener and an aromatic substance in pharmaceutical production recently. Antioxidant properties of blackberry fruits are another popular subject that has been intensely focused on by both customer and researcher [Aras et al. 2019, Aljane and Sdiri 2016]. Black mulberry (*Morus nigra* L.) fruit is a source of many different vegetative nutrients such as phenolic substances, flavonoids (lutein, zeaxanthin, beta carotene and alpha-carotene), ascorbic acids and mineral substances – iron, copper, magnesium [Gundesli et al. 2019, Okatan 2020].

Mulberry (*Morus spp*. L.) is generally produced with cutting and grafting. Although grafting on white

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mulberry (*Morus alba* L.) in production, abnormal bulges and rootstock incompatibilities are encountered at the grafting point due to the difference in growing strength. Researches for the production of black mulberry (*Morus nigra* L.) with cutting or tissue culture has gained speed these days. Although it can be rooted directly in the field or rooting environments in cutting production, cutting quality and environmental conditions have been an important factor in the possibility of rooting. Rooting success may drop significantly in mulberry cuttings that are not taken into rooting in proper environments and conditions. Besides, propagation with cutting is not considered affordable and economical for a high number of mass production since it depends on the season [Desai et al. 2018, Attia et al. 2014]. Mass and standard quality mulberry (*Morus* spp. L.) production using biotechnological methods are possible with propagation *in vitro* conditions [Sajeevan et al. 2011]. Mulberry (*Morus* spp. L.) is a plant that is problematic *in vitro* production. However, it depends on the genotype, characteristic of the explant source and the combination of hormones added to the medium [Feyissa et al. 2005]. In the beginning, *Morus alba* was produced from the axillary bud *in vitro* conditions by Ohyama [1970], and then different studies were carried out on this subject [Balakrishnan et al. 2009, Rao et al. 2010a, 2010b, Lalitha et al. 2013]. Different results were obtained from different explant sources such as axillary bud or nodal explant [Sajevan et al. 2011, Zaki et al. 2011], hypocotyl and cotyledon [Bhatnagar et al. 2001], leaf [Raghunath et al. 2013] *in vitro* conditions [Desai 2018]. It has been reported that effective shoot proliferation was obtained from MS medium supported with BAP (1.5 mg L^{-1}) + NAA (0.5 mg L^{-1}) in proliferation studies of explants which is taken from nodal segments [Zaki et al. 2011]. Zaki et al. [2011] reported that the addition of NAA to the MS medium during rooting is more effective than IAA and IBA and that the BAP (1.0 mg L^{-1}) + NAA (2.0 mg L^{-1}) MS medium has a more positive effect on the rooting performance.

This study was carried out to create mass production protocols *in vitro* conditions and solve the rooting problems in cutting production by using the nodal segment as the explant source, which has recently been increasing in economic importance in the Eastern Mediterranean Region and investigated micro-propagation possibilities with biotechnological methods.

MATERIALS AND METHODS

This study was conducted in East Mediterranean Transitional Zone Agricultural Research of Institute Tissue Culture Laboratories in 2019 to develop the mass micro-production protocol of black mulberry (*Morus nigra* L.).

Plant material, preparation of explant and explant sterilization. The explant sources of the study are the shoots of the 4–5-year-old black mulberry (*Morus nigra* L.) plant, which was obtained by selection breeding in the garden of the East Mediterranean Transitional Zone Agricultural Research of Institute, and taken from the seedlings grown in a fully controlled greenhouse. Branches, 10–15 cm tall and have nodal segments, taken from these seedlings were brought to the laboratory after their leaves were cut. Physically concentrated dirt and microorganisms on the tissue were tried to be removed by shaking with liquid soap in a container filled with water for 30 minutes in the laboratory. Subsequently, the explants taken into the sterile cabinet were subjected to 70% of ethanol for 30 seconds and washed three times with sterile distilled water to prevent the toxic effect of ethanol. In the next step, the explants were rinsed with sterile pure 3 times after waiting for 20 minutes in a 30% (2.5% sodium hypochlorite) commercial hypo solution containing a few drops of Tween-80 (Tween 80, Sigma-Aldrich, USA). Irregular and unnecessary parts damaged tissues in aseptic conditions were removed from these sterilized explants in the sterile cabinet. Afterward, the upper parts of explants that containing one bud were cut with a number 3 scalpel in a way that the explants were 1,5–2 cm long was implanted in the medium.

Culture conditions. Shoot induction medium was prepared according to the standard protocol of MS [Murashige and Skoog 1962], containing MS salts, vitamins, sucrose (3%, w/v) and agar (0.8%, w/v). After adding specific PGRs to the medium, pH was adjusted to 5.6–5.8. The nutrient medium was boiled and transferred to 375 ml culture bottles with transparent cover as 65 ml of medium in order to distribute the agar homogenously. Afterward, the caps were closed and the culture bottles were sterilized in an autoclave at 121°C

and 1 atm pressure for 15 minutes. These sterilized explants were transferred to nutrient media and culture bottles (all the *in vitro* cultures) were incubated in the plant culture room at 16/8 hour light/dark cycle and 40 µmol m^{-2} s^{-1} light intensity with 25 ±2°C temperature. MS media without PGRs were used as a control treatment (T0) for all experiments.

Shoot proliferation and multiplication. The sterilized explants were transferred to MS medium containing 4 different specific PGRs – 6-benzylaminopurine (BAP), thidiazuron (TDZ), kinetin (KN), *α*-naphthalene acetic acid (NAA) – for production of multiple shoots after the surface sterilization was completed. Explants were cultured in medium supplemented with BAP (1.0, 1.5 mg L^{-1}), TDZ (1.0, 1.5 mg L^{-1}), kinetin (1.0, 1.5 mg L^{-1}), NAA (0.15, 0.3 mg L^{-1}) – Table 1. All the *in vitro* cultures were kept in the medium for 60 days in media containing these different specific PGRs for shoot proliferation and multiplication.

Rooting and acclimatization. Well developed and proliferated shoots obtained after shoot multiplication were transferred for root induction in rooting medium containing full strength MS fortified with 0.5, 1.0 mg L^{-1} NAA, 1.0, 1.0 mg L^{-1} BAP, 1.0, 2.0 mg L^{-1} TDZ and 1.0, 2.0 mg L^{-1} KN. Four week old plants transferred to greenhouse conditions were successful-

Table 1. Effects of 6-benzylaminopurine (BAP), thidiazuron (TDZ), kinetin (KN) and *α*-naphthalene acetic acid (NAA) combinations in different doses on black mulberry (*Morus nigra*) shoot proliferation, shoot number, shoot length, leaf number and plant weight

Treatments	Plant growth regulators (mg L^{-1})					Average number of shoot initiation (%)	Average number of number of shoot (shoot/explant)	Average length of shoot (cm)	Average number of leaf (leaf/explant)	Plant weight (g)
	BAP	TDZ	KN	GA_3	NAA					
TSP-0	–	–	–	0.25	–	53.64 ±1.01^{i}	1.51 ±0.08^{kl}	1.92 ±0.11^{def}	2.93 ±0.25^{h}	0.77 ±0.38^{f}
TSP-1	1.0	–	–	0.25	0.15	71.52 ±0.58^{g}	2.00 ±0.10^{hij}	1.64 ±0.25$^{f–i}$	3.42 ±0.14^{g}	2.00 ±0.11^{bc}
TSP-2	1.0	–	–	0.25	0.30	82.99 ±1.01^{cd}	2.30 ±0.30^{gh}	1.56 ±0.25$^{f–i}$	4.65 ±0.25^{cd}	2.06 ±0.25^{d}
TSP-3	2.0	–	–	0.25	0.15	67.13 ±0.58^{h}	1.76 ±0.46^{ijk}	3.03 ±0.06^{a}	4.70 ±0.40^{cd}	2.10 ±0.36^{bc}
TSP-4	2.0	–	–	0.25	0.30	73.54 ±1.55^{g}	2.40 ±0.17^{gh}	1.50 ±0.10$^{g–j}$	3.00 ±0.30^{h}	2.16 ±0.15^{bc}
TSP-5	–	–	1.0	0.25	0.15	51.27 ±1.55^{j}	1.33 ±0.29^{klm}	2.93 ±0.12^{a}	4.46 ±0.44^{d}	1.86 ±0.42^{bcd}
TSP-6	–	–	1.0	0.25	0.30	80.29 ±0.58^{e}	3.15 ±0.13^{e}	1.76 ±0.29^{efg}	4.07 ±0.12^{e}	1.85 ±0.10^{bcd}
TSP-7	–	–	2.0	0.25	0.15	46.89 ±1.55^{k}	1.30 ±0.17^{lm}	2.43 ±0.21^{bc}	3.82 ±0.43^{f}	2.30 ±0.11^{b}
TSP-8	–	–	2.0	0.25	0.30	65.44 ±1.01^{h}	1.58 ±0.38^{jkl}	2.05 ±0.38^{cde}	3.14 ±0.15^{h}	1.10 ±0.36^{ef}
TSP-9	–	1.0	–	0.25	0.15	86.02 ±1.55^{b}	4.00 ±0.30^{bc}	2.30 ±0.30^{bcd}	5.44 ±0.41^{b}	1.71 ±0.06^{cd}
TSP-10	–	1.0	–	0.25	0.30	82.65 ±1.01^{cd}	3.10 ±0.17^{e}	1.16 ±0.38^{j}	3.87 ±0.15^{ef}	1.43 ±0.10^{de}
TSP-11	–	2.0	–	0.25	0.15	76.91 ±1.01^{f}	2.56 ±0.25^{fg}	1.16 ±0.38^{j}	4.84 ±0.38^{c}	2.05 ±0.35^{bc}
TSP-12	–	2.0	–	0.25	0.30	81.98 ±1.55^{de}	2.98 ±0.33^{ef}	1.30 ±0.30^{ij}	4.04 ±0.25^{ef}	1.90 ±0.15^{bcd}
TSP-13	1.0	–	–	0.25	–	88.39 ±0.58^{a}	3.63 ±0.35^{cd}	1.36 ±0.15^{hij}	3.92 ±0.11^{ef}	1.01 ±0.24^{ef}
TSP-14	2.0	–	–	0.25	–	86.03 ±1.01^{b}	3.33 ±0.29^{de}	1.71 ±0.20$^{e–h}$	5.21 ±0.38^{b}	2.18 ±0.26^{bc}
TSP-15	–	–	1.0	0.25	–	71.85 ±1.55^{g}	2.06 ±0.21^{hi}	3.00 ±0.26^{a}	4.61 ±0.12^{cd}	3.06 ±0.55^{a}
TSP-16	–	–	2.0	0.25	–	45.20 ±2.92^{k}	1.05 ±0.09^{m}	1.78 ±0.20^{efg}	2.65 ±0.29^{i}	0.85 ±0.31^{f}
TSP-17	–	1.0	–	0.25	–	89.73 ±1.55^{a}	4.73 ±0.23^{a}	2.66 ±0.15^{ab}	7.14 ±0.15^{a}	2.86 ±0.32^{a}
TSP-18	–	2.0	–	0.25	–	88.72 ±1.55^{a}	4.26 ±0.31^{b}	2.96 ±0.12^{a}	7.30 ±0.29^{a}	3.03 ±0.45^{a}
$LSD_{0.05}$	–	–	–		–	2.24	0.42	0.38	0.22**	0.46**

Differences between the means were showed with different letters within the same column.
TSP – treat shoot proliferation.

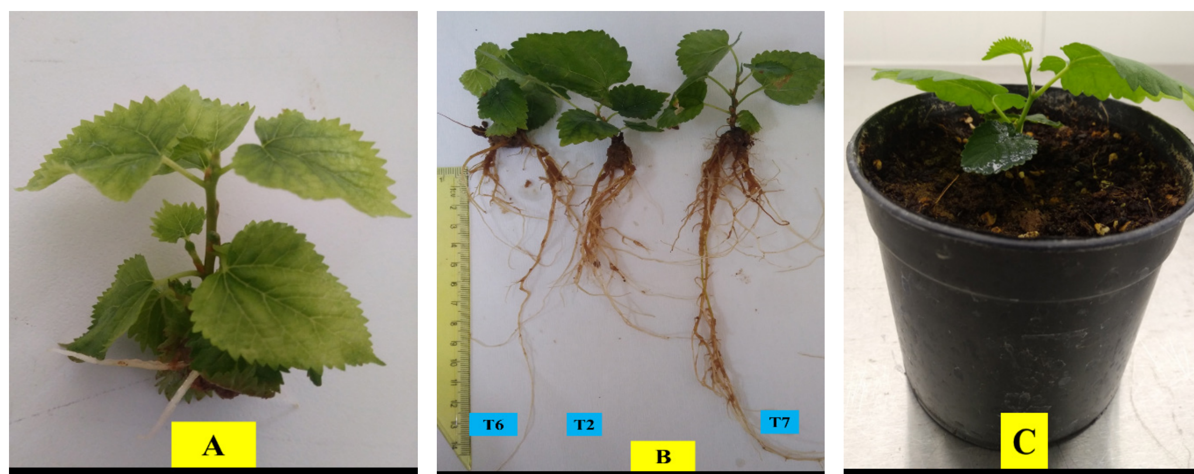

Fig. 1A. Rooted explant. **B.** Different effects of TR-6, TR-2 and TR-7 treatment on rooting. **C.** Acclimatized Black Mulberry plant mixed with soil, sand and peat (2 : 1 : 1)

ly primary hardened in poly bags using a mixture of soil : sand : peak (2 : 1 : 1) – Figure 1. The acclimatized plants were transplanted to the big pots.

Statistical analyses and evaluations of data

Completely randomized design with 3 replications was used in this experiment (3 plots × 25 explant each treatment). The data obtained from the study was subjected to variance analysis in proper statistical program. The data were analyzed by JMP 7 statistical software from SAS (V7; SAS Institute Inc. Cary, NC, USA) and all the analytical values were average of three replications. The significant differences were compared by the honestly significant difference (HSD) the Tukey test executed at 5% level of probability. The means ± the standard error (SE) were calculated from three independent experiment

RESULTS

Nodal explants inoculated to MS growth medium supplemented with plant growth regulators were found to have a high shoot proliferation rate compared to the control medium (Tab. 1). It was observed that the shoot proliferation rate of 50% and above in all combinations. Shoot development in explants started to be observed at the end of the second week of inoculation. The highest explant shoot proliferation rates were obtained from TSP-17 (89.73%) and TSP-18 (88.72%) treatment combined with TDZ (1.0 mg L^{-1} and 2.0 mg L^{-1}) and GA_3 (0.25 mg L^{-1}). It was found that shoot proliferation decreased when GA_3 (0.25 mg L^{-1}) and NAA (0.15 mg L^{-1}) were added to the increasing doses of KN (1.0 mg L^{-1} and 2.0 mg L^{-1}). Nodal explants gave the highest shoot proliferation values as 88.39%, 86.03%, 89.73% and 88.72%, respectively, when they inoculated in medium supplemented with TDZ (1.0 mg L^{-1} and 2.0 mg L^{-1}) and BAP (1.0 mg L^{-1} and 2.0 mg L^{-1}). It was revealed in the study that there were also significant differences ($P < 0.001$) between treatment in terms of the average number of shoots. It was determined that the highest shoot numbers per explant were obtained from two different doses of TDZ (1.0–2.0 mg L^{-1}) as 4.73 and 4.26. The lowest values were obtained in combinations of KN (1.0–2.0 mg L^{-1}) doses with GA3 (0.25 mg L^{-1}) and NAA (0.15 mg L^{-1}) in the number of shoots per explant as it seemed in plant shoot proliferation. It was observed that the treatment used in the study affected changes between the shoot lengths significantly ($P < 0.001$). The combination of BAP (2.0 mg L^{-1}), GA_3 (0.25 mg L^{-1}) and NAA is effective in the highest shoot length (3.03 cm), while two different doses of TDZ (1.0 – 2.0 mg L^{-1}) in combinations with GA_3 and NAA had the lowest shoot length. The results of the study showed that TDZ (1.0–2.0 mg L^{-1}) and GA_3 (0.25 mg L^{-1}) combination had a positive ef-

Table 2. Effects of 6-benzylaminopurine (BAP), thidiazuron (TDZ), kinetin (KN) and *α*-naphthalene acetic acid (NAA) combinations in different doses on black mulberry (*Morus nigra*) average rooting, average number of root and average length of root

Treatments	Plant growth regulators (mgL^{-1})				Average rooting (%)	Average number of root (root/explant)	Average length of root (cm)
	BAP	TDZ	KN	NAA			
TR-0	–	–	–	–	88.34 ±0.65^{bcd}	6.00 ±0.10^{e}	13.10 ±0.35^{c}
TR-1	0.1	–	–	0.5	88.34 ±1.72^{bcd}	6.66 ±0.14^{d}	17.20 ±0.26^{a}
TR-2	0.1	–	–	1.0	89.09 ±1.72^{bc}	7.00 ±0.26^{d}	8.73 ±0.58^{f}
TR-3	0.2	–	–	0.5	95.64 ±0.86^{a}	7.76 ±0.25^{c}	11.43 ±0.51^{de}
TR-4	0.2	–	–	1.0	85.34 ±1.12^{cde}	4.76 ±0.32^{g}	14.80 ±0.52b
TR-5	–	–	0.1	0.5	97.70 ±1.12^{a}	8.30 ±0.31^{b}	11.00 ±0.30^{e}
TR-6	–	–	0.1	1.0	93.21 ±1.12^{ab}	8.10 ±0.43^{bc}	11.83 ±0.31^{d}
TR-7	–	–	0.2	0.5	98.07 ±2.34^{a}	9.20 ±0.26^{a}	15.23 ±0.46^{b}
TR-8	–	–	0.2	1.0	80.85 ±1.12^{ef}	4.33 ±0.29^{g}	6.10 ±0.43^{h}
TR-9	–	0.1	–	0.5	8.33 ±7.23^{g}	0.50 ±0.44^{i}	0.31 ±0.28^{j}
TR-10	–	0.1	–	1.0	6.66 ±5.86^{g}	0.36 ±0.32^{i}	0.23 ±0.21^{j}
TR-11	–	0.2	–	0.5	5.66 ±4.93^{g}	0.29 ±0.21^{i}	0.20 ±0.20^{j}
TR-12	–	0.2	–	1.0	5.00 ±4.36^{g}	0.23 ±0.25^{i}	0.21 ±0.18^{j}
TR-13	0.1	–	–	–	78.98 ±2.34^{f}	2.66 ±0.29^{h}	2.16 ±0.29^{i}
TR-14	0.2	–	–	–	83.10 ±1.12^{cdef}	4.83 ±0.29f^{g}	7.33 ±0.29^{g}
TR-15	–	–	0.1	–	82.35 ±0.65^{def}	4.66 ±0.21^{gh}	7.47 ±0.13^{g}
TR-16	–	–	0.2	–	86.84 ±0.65^{cde}	5.33 ±0.14^{f}	9.00 ±0.43^{f}
TR-17	–	0.1	–	–	9.00 ±7.81^{g}	0.63 ±0.55^{i}	0.36 ±0.32^{j}
TR-18	–	0.2	–	–	9.00 ±7.94^{g}	0.63 ±0.55^{i}	0.31 ±0.26^{j}
$LSD_{0.05}$					6.24	0.36	0.56

Differences between the means were showed with different letters within the same column.
TR – treat rooting.

fect on the average number of leaves per explant and the average explant weight (Tab. 1).

Healthy and developed (~10 mm) explants were transferred to MS medium fortified plant growth regulator. Roots were measured at the end of 4th week of explants transferred to the medium. At the end of the study, it was seen that 18 different treatments had a statistically significant effect ($P < 0.001$) on rooting values in black mulberry (*Morus nigra L.*) – Table 2. The best rooting values were obtained from TR-7 and TR-5 treatments consisting of KN (0.1–0.2 mg L^{-1}) and NAA (0.5–1.0 mg L^{-1}) combinations as 98.7% and 97.7%, respectively. The lowest rooting percentages were found in TR-10 and TR-11 treatments, which are combinations of TDZ (0.1 mg L^{-1}) + NAA (1.0 mg L^{-1}) and TDZ (0.2 mg L^{-1}) + NAA (0.5 mg L^{-1}), respectively. According to the results, the same treatments (TR-5 and TR-7) had a positive effect on the average number of roots per explant (8.30 and 9.20 per plant, respectively). The average number of roots and root length per explant was minimum in TR-11 and TR-12 treatment. Mean root length per explant was observed in TR-1 (BAP 1.0 mg L^{-1} and NAA 0.5 mg L^{-1}) treatments (17.20 cm).

DISCUSSION

In recent years, micro-reproduction of different cytokinin and doses (BAP, zeatin, KN, TDZ) added to the medium in tissue culture studies have been

successfully provided by using stem segment, shoot tips, nodal segment and the axillary bud of explants in mulberry – *Morus* spp. [Wulandari and Harjosudirjo 2019]. This study aims to reveal the most economical ways of producing black mulberry (*Morus nigra* L.), which is known for its delicious fruits and has recently gained an economic value in the pharmacological and cosmetic sector, by using developing biotechnological methods. At the end of the study, it has been seen that there are important differences in shoot proliferation, daughter plant formation and rooting stages as in other studies [Sajeevan et al. 2011, Zaki et al. 2011, Desai et al. 2018] on this subject. Although our highest shoot proliferation rate result (89.73%) is slightly higher than the values obtained by Saajevan et al. [2011] – 85.67%, and Zaki et al. [2011] – 80%, it can be accepted that they are similar in general. In studies conducted in different *Morus* species, Akram and Aftab [2012], Chitra et al. [2014] and Niratker et al. [2015] determined alike plant shoot proliferation rates (85.6%, 90% and 80% respectively) with our study. This study and literature data on this subject show that shoot proliferation rates of the *morus* species give very good results *in vitro* conditions.

TDZ is a plant growth regulator known for obtaining positive results *in vitro* propagation of some hard-to-reproduce woody plants such as *Cercis canadensis* var. *alba* L. [Yusnita et al. 1990], muscadine grape [Gray and Benton 1991] and *Quercus robur* L. [English oak; Chalupa 1988]. In our study, the best results in shoot proliferation and daughter plant formation were determined by using TDZ. In *in vitro* propagation treatment, low doses of TDZ added to the nutrient medium lead shoot proliferation in the shoots with the prolongation and expansion of cells with the promotion of cell division [Guney 2019, Debnath 2005, Wulandari and Harjosudirjo 2019]. In recent studies on this subject, Saajevan et al. [2011] stated that the combination of TDZ with NAA is more effective in shoot proliferation compared to the combination of BAP (from the cytokinin group) with NAA in *Morus alba*. However, high doses of TDZ added to the nutrient medium limit the shoot elongation [Debnath 2005]. Sajeevan et al. [2011] reported that low doses of TDZ treatment positively affect the formation of daughter shoot in the micro-propagation of *Morus alba*. At the same time, Raghunath et al. [2013] noticed in their study that different concentrations of TDZ showed positive results in shoot formation in *Morus indica*. In this context, it can be seen that the combination of TDZ and NAA gives good results in rooting parameters during the rooting stage. In recent studies on *Morus alba* [Saajevan et al. 2011] and *Morus indica* [Raghunath et al. 2013], it is reported that TDZ gives better results in different mulberry species. The average number of shoots per explant varied between 4.73–1.51 and these results were higher than the study of Akram and Aftab [2012] (0–4.7) and Niratker et al. [2015] (0.35–3.78), although were slightly lower than the study of Saajevan et al. [2011] (1.33–7.33) and Desai et al. [2018] (3.00–6.75). Within the light of these findings, it can be said that the values of daughter shoots obtained from our study are parallel with the recent studies.

IBA, NAA, IAA and 2,4-D are among the most used auxin group plant growth regulators *in vitro* rooting studies in *Morus* species [Anis et al. 2003]. As a result of the study carried out, it has been revealed that NAA is more effective in rooting mulberry (*Morus spp* L.) *in vitro* among these plant growth regulators added to the MS medium [Anuradha and Pullaiah, 1992]. On the other hand, Chitra and Padmaja [2005] reported that 2,4-D is more effective than the use of NAA *in vitro* white mulberry (*Morus alba* L.) rooting. In another study, Attia et al. [2014] reported that IAA usage did not show the expected effect in rooting mulberry (*Morus spp* L.) *in vitro*, and the treatments of MS + 2 mg L^{-1} IBA provided rooting around 70%. In recent studies, it was noted that rooting rates in all *Morus* species *in vitro* have very different values between 30% and 92% [Kakarla and Rama 2014, Attia et al. 2014]. It was seen in our study that combinations of NAA and KN have a positive effect as a 98.07% rooting rate, and it is considered to be a very good result compared to the literature data on this subject. Zaki et al. [2011] found the highest rooting rate of 80% in the treatments of NAA at the dose of 0.5 mg L^{-1} in the micro-propagation of *Morus nigra* L. (black mulberry), although Akram and Aftab [2012] were able to achieve the highest rooting values of 85.6% with IBA at a dose of 2 mg L^{-1} in *Morus macroura* (white mulberry). In general evaluation of our study, rooting values in all treatment were 80% and above. It is understood from these results that the rooting rates obtained from our study will be considered as positive.

CONCLUSION

This study was carried out to establish an effective and economical mass micropropagation protocol of black mulberry (*Morus nigra*), which has recently gained economic importance with its different features and is being sought in the sapling market. The study was completed in three stages as shoot proliferation, shoot reproduction and rooting. At each stage, 18 different plant growth regulator combinations were used, one of which was control. In the shoot reproduction study, it was revealed that the use of TDZ only yielded very positive results compared to the literature, although micro-propagation results of black mulberry (*Morus nigra*) were positive in combinations with BAP, TDZ, and KN with GA_3 and NAA. In the rooting study, higher rooting values were obtained in combinations created by adding Kinetin and BAP to NAA. As a result, very good results were taken from TSP-17 (TDZ 1.0 mg L^{-1} + GA_3 0.25 mg L^{-1}) and TSP-18 (TDZ 2.0 mg L^{-1} + GA_3 0.25 mg L^{-1}) treatment in plant shoot proliferation and micro shoot reproduction study, and from TR-5 (NAA 0.5 mg L^{-1} + KN 0.1 mg L^{-1}) and TR-7 (NAA 0.5 mg L^{-1} + KN 0.2 mg L^{-1}) treatment in rooting study. It is understood that it is very important to use this fast technique in the reproduction of economically important plant species in agriculture on a commercial scale.

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