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ORIGINAL ARTICLE

# Micropropagation of Promising Jujube (*Ziziphus jujuba* Mill.) Genotypes

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**Abstract** In the present study, two selected jujube genotypes (20-C-51 and 20-C-52) were used. The effects of different growth regulator combinations, carbon sources (sucrose, glucose and fructose) and silver nitrate concentrations on *in vitro* propagation of jujube were investigated. The highest percentage of explants that produced shoots (100 %) and the number of shoots per explant (5.5) were obtained on MS medium supplemented with 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP + 0.1 mg $L^{-1}$ IBA + 0.3 mg $L^{-1}$ $GA_3$. Different amounts of carbon sources and silver nitrate did not increase the percentage of explant that developed in to shoots and the number of shoots per explant. The highest rooting percentage (76.7 %) was obtained on MS and half-strength MS media supplemented with 2.0 mg $L^{-1}$ IBA.

**Keywords** Jujube · Micropropagation · Thidiazuron · Benzylaminopurine

## *In vitro*-Vermehrung vielversprechender Genotypen der Chinesischen Jujube (*Ziziphus jujuba* Mill.)

**Zusammenfassung** Die vorliegende Untersuchung wurde mit zwei ausgewählten Genotypen (20-C-51 und 20-C-52) der Chinesischen Jujube (Chinesische Dattel) durchgeführt. Dabei wurden die Wirkungen von zwei unterschiedlichen Zusammensetzungen an Wachstumsregulatoren, bestehend aus Kohlenstoff-Quellen (Saccharose, Glucose und Fructose) und Silbernitrat-Konzentrationen, auf die *in vitro*-Vermehrung der Chinesischen Jujube geprüft. Prozentual die höchste Ausbeute an Pflanzenspross produzierenden Explantaten (100 %) und die höchste Anzahl an Pflanzensprossen pro Explantat (5,5) wurde auf einem MS-Medium erreicht, das mit 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP + 0.1 mg $L^{-1}$ IBA + 0.3 mg $L^{-1}$ $GA_3$ angereichert war. Verschiedene Konzentrationen an Kohlenstoffen und Silbernitrat verbesserten den prozentualen Anteil der Pflanzenspross produzierenden Explantaten und die Anzahl der Sprosse pro Explantat nicht. Die höchste Bewurzelungsrate (76,7 %) wurde auf voll- oder halbkonzentriertem MS Medium, angereichert mit 2.0 mg $L^{-1}$ IBA, erreicht.

**Schlüsselwörter** Jujube · *In vitro*-Vermehrung · Thidiazuron · Benzylaminopurin

## Introduction

Jujube is an important food crop for people living in some countries such as China and India. Nevertheless, Turkey has a genetic variation for this species in some of its regions and is grown in limited amount. Jujube fruits are generally consumed as dried but a small portion is used for fresh consumption in Turkey. Roots, leaves, seeds, barks and fruits of jujube have been widely used as sources of crude drugs in traditional medicine in the world (Belford 1994; Li et al. 2005). Flowers of jujube have a high-quality nectar and their leaves are being consumed as tea (Zhao et al. 2008). The selection of this unique species for fruit quality or any other characteristic, is pertinent for its economic use. Con-

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sidering the growing of alternative crops in Turkey, it is of great importance to start a program for the improvement of jujube based on the genetic diversity occurring among natural population of Civril-Denizli region of Turkey.

The propagation of the selected jujube genotypes is especially important. Seed propagation does not provide genetic stability, and some specific characteristics can be lost. Vegetative propagation can be achieved by conventional vegetative propagation methods such as rooting of cuttings or micropropagation. Some propagation techniques such as green wood cutting (Shen et al. 1992; Shi and Xin 2003), hard wood cutting and layering (Assareh et al. 2005) have been performed for jujube propagation. However, micropropagation is more suitable than other vegetative propagation methods for jujube (Assareh and Sardabi 2005). Several micropropagation techniques such as somatic embryogenesis (Kim et al. 2006), organogenesis (Gu and Zhang 2005) and shoot tip culture (Sudhersan et al. 2001; Danthu et al. 2004) have been carried out for *in vitro* propagation of jujube. Among these methods, shoot tip culture is used for jujube clonal propagation (Sudhersan et al. 2001; Sudhersan and Hussain 2003). Nevertheless, the development of a protocol for an efficient *in vitro* propagation of other cultivars from adult trees is necessary.

In the present study, we describe a reliable and reproducible method to propagate adult trees of two jujube genotypes, 20-C-51 and 20-C-52 through shoot proliferation. Additionally, the effects of silver nitrate and carbon sources on *in vitro* propagation of jujube were determined.

## Materials and Methods

The experiment was conducted in 2007 and 2008. Shoots from adult plants of 20-C-51 and 20-C-52 genotypes (Ecevit et al. 2008) were used to obtain the explants used in the study.

### *In Vitro* Shoot Propagation

To determine the best plant growth regulator (PGR) combinations for micropropagation of jujube, young shoots of jujube genotypes were collected on May 17. The shoots were sterilized by immersion in 3.00 % (v/v) sodium hypochlorite solution for 18 min, followed by three rinses in sterile distilled water for 5 min. Shoot apices (about 0.5 cm) were used as explants to establish the cultures. Explants were incubated for 3 weeks in test tubes (Fig. 1a) and subcultured for 4 weeks in Erlenmeyer flasks on MS (Murashige and Skoog 1962) medium consisting of 0.5, 1.0 and 2.0 mg $L^{-1}$ benzyl amino purine (BAP) or thidiazuron (TDZ) and 0.01, 0.1 and 0.5 mg $L^{-1}$ indole-3-butyric acid (IBA). Efficient proliferation was not provide on MS medium consisting plant growth regulator combinations stated above. Therefore, combinations of TDZ and BAP were tested on MS medium at concentrations of 0.1, 0.3, 0.5 and 1.0 mg $L^{-1}$ with 0.1 mg $L^{-1}$ IBA and 0.3 mg $L^{-1}$ $GA_3$. MS basal medium without PGR was used as control and two subcultures were made at 4 weeks intervals. The percentage of explants forming shoots and number of shoots per explant were determined at the end of the subcultures.

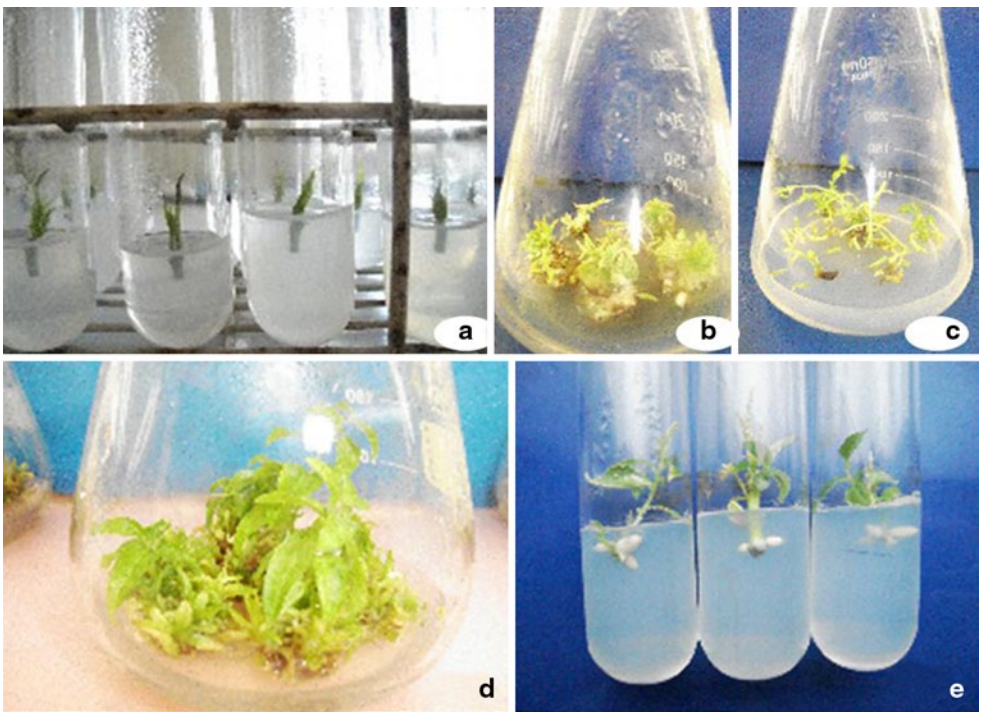


**Fig. 1** *In vitro* propagation of jujube genotypes **a** Explants cultured in test tubes, **b** *In vitro* shoot formation but not development on MS medium containing TDZ as cytokinin, **c** No proliferation on MS medium containing BAP as cytokinin, **d** Successful shoot proliferation on MS medium containing both TDZ and BAP as cytokinin, **e** Rooting of shoots on MS medium containing 2.0 mg L−1 IBA

### The Effects of Carbon Sources on Propagation

To determine the best carbon source and its dose for micropropagation of jujube, MS medium supplemented with 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP + 0.1 mg $L^{-1}$ IBA + 0.3 mg $L^{-1}$ $GA_3$ was used. For this purpose, sucrose, fructose and glucose were added in 20, 30, 40 and 50 g $L^{-1}$ doses to MS medium described above. Two subcultures were made at 4 weeks intervals. The percentage of explants forming shoots and the number of shoots per explant were determined at the end of the subcultures.

### The Effects of Silver Nitrate on Propagation

To determine the best silver nitrate dose for micropropagation of jujube, MS medium supplemented with 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP + 0.1 mg $L^{-1}$ IBA + 0.3 mg $L^{-1}$ $GA_3$ was used. For this purpose, silver nitrate was added in doses of 0.5, 1.0, 2.0 and 4.0 mg $L^{-1}$ to MS medium described above. Two subcultures were made at 4 weeks intervals. The percentage of explants forming shoots and the number of shoots per explant were determined at the end of the subcultures.

### Rooting of Microcuttings

Micropropagated shoots (15–20 mm) of jujube genotypes were transferred to MS and half-strength MS media supplemented with 0.5, 1.0, 2.0 and 4.0 mg $L^{-1}$ IBA and NAA. Cultures were incubated in a room at 25 ± 1 ºC with 16 h photoperiod for 3 weekls. The percentage of rooted shoots and the number of roots per shoot were determined at the end of 3 weeks.

All media were supplemented with 3 % (w/v) sucrose and 0.6 % agar (w/v) (Merck Co.), and the pH was adjusted to 5.7 before autoclaving at 121 ºC for 15 min. Different carbon sources and their doses were used in the treatment studied "the effects of carbon sources on propagation". Cultures were incubated in a room at 25 ± 1 ºC with 16 h photoperiod.

### Statistical Analyses

The experiments were repeated twice. Each treatment consisted of three Erlenmeyer flasks with five explants per flask. Data were subjected to ANOVA using Minitab software (MINITAB Inc.) and the means were separated by Duncan's Multiple Range test ($P < 0.05$). The percent data were transformed into angle values.

## Results and Discussion

### *In Vitro* Shoot Propagation

In the present study, MS medium supplemented with BAP alone and TDZ alone as a cytokinin was used for *in vitro* propagation of jujube. MS medium was successfully used for micropropagation of jujube in previous studies (Kim and Lee 1988; Yan et al. 1990; Rathore et al. 1992; Fougat et al. 1997; Wu et al. 2004; Sudhersan and Hussain 2003). Shoot formation was observed on the medium containing TDZ alone but shoots were very short and unhealthy (Fig. 1b). In addition, when the medium supplemented with BAP alone as a cytokinin was used, new shoot regeneration from explants was not observed and the explants turned yellow on the medium (Fig. 1c). Therefore, MS medium supplemented

**Table 1** The effect of plant growth regulator combinations on *in vitro* propagation of jujube

| Plant growth regulators combinations | 20-C-51 jujube genotype | | | | 20-C-52 jujube genotype | | | |
|---|---|---|---|---|---|---|---|---|
| | The percentage of explant forming shoots | | Number of shoots per explant | | The percentage of explant forming shoots | | Number of shoots per explant | |
| 0.1 mg $L^{-1}$ TDZ + 0.1 mg $L^{-1}$ BAP[a] | 60.0 | cd[b] | 2.33 | c | 66.9 | bc | 2.73 | de |
| 0.1 mg $L^{-1}$ TDZ + 0.3 mg $L^{-1}$ BAP | 93.8 | a | 5.07 | ab | 86.7 | ab | 4.67 | ab |
| 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP | 86.7 | ab | 5.50 | a | 100.0 | a | 5.50 | a |
| 0.1 mg $L^{-1}$ TDZ + 1.0 mg $L^{-1}$ BAP | 66.4 | bc | 3.43 | bc | 86.8 | ab | 3.97 | bc |
| 0.3 mg $L^{-1}$ TDZ + 0.1 mg $L^{-1}$ BAP | 73.5 | bc | 2.13 | c | 66.7 | bc | 2.40 | d–f |
| 0.3 mg $L^{-1}$ TDZ + 0.3 mg $L^{-1}$ BAP | 60.0 | cd | 3.07 | c | 53.5 | cd | 3.20 | cd |
| 0.3 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP | 53.6 | cd | 2.70 | c | 20.0 | e | 1.50 | ef |
| 0.3 mg $L^{-1}$ TDZ + 1.0 mg $L^{-1}$ BAP | 46.6 | cd | 1.73 | c | 26.5 | de | 2.00 | d–f |
| 0.5 mg $L^{-1}$ TDZ + 0.1 mg $L^{-1}$ BAP | 46.7 | cd | 2.23 | c | 40.0 | c–e | 1.40 | f |
| 0.5 mg $L^{-1}$ TDZ + 0.3 mg $L^{-1}$ BAP | 53.5 | cd | 2.00 | c | 20.5 | e | 2.13 | d–f |
| 0.5 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP | 40.7 | cd | 2.17 | c | 33.3 | de | 2.10 | d–f |
| 0.5 mg $L^{-1}$ TDZ + 1.0 mg $L^{-1}$ BAP | 26.7 | d | 1.73 | c | 46.4 | c–e | 2.60 | d–f |

[a]All media also containing 0.1 mg $L^{-1}$ IBA and 0.3 mg $L^{-1}$ $GA_3$

[b]Values (within each column) followed by the same letter are not significantly different at $p < 0.05$ level according to Duncan's multiple range test

with both TDZ and BAP as a cytokinin was used for *in vitro* micropropagation of jujube. TDZ and BAP were more effective to produce shoot from explants when used together (Fig. 1d). Table 1 presents the percentage of explant produced shoots and the number of shoots per explant obtained on MS medium containing both BAP and TDZ as cytokinin. Besides, the combinations of BAP and TDZ, 0.1 mg $L^{-1}$ IBA and 0.3 mg $L^{-1}$ $GA_3$ were added to all media.

The percentage of explant produced shoots and the number of shoots per explant differed significantly. While the percentage of explant produced shoots ranged from 26.7 to 93.8% for 20-C-51 and 20.0–100% for 20-C-52, the number of shoots per explant ranged from 1.73 to 5.50 for 20-C-51 and 1.40 to 5.50 for 20-C-52. The highest percentage of explant produced shoots and the highest number of shoots per explant were obtained from the media supplemented with 0.1 mg $L^{-1}$ TDZ + 0.3 mg $L^{-1}$ BAP and 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP for both jujube genotypes. The highest number of shoots per explant was 5.5 for both jujube genotypes. Similar results were also reported by Rathore et al. (1992). Micropropagation of jujube was achieved on MS medium supplemented with 0.5 mg $L^{-1}$ BAP (Kim and Lee 1988) or 1.0 mg $L^{-1}$ BAP (Yan et al. 1990; Fougat et al. 1997) in previous studies. However, the same doses of BAP were not effective on micropropagation of jujube in our study. Jiang et al. (2004) reported that BAP and TDZ were more effective on micropropagation of jujube when used together. Zeatin and kinetin were also used for micropropagation of jujube by other researchers (Wu et al. 2004).

## The Effects of Carbon Sources on Propagation of Jujube

Effects of carbon sources on micropropagation of jujube are given in Table 2. The carbon sources and their doses significantly affected the percentage of explants produced shoots and the number of shoots per explant in both jujube genotypes. In general, sucrose was more effective than glucose and fructose on micropropagation of jujube genotypes. Sucrose as a carbon source was successfully used for micropropagation of jujube or other species (Rathore et al. 1992; Onay 2000; Jiang et al. 2004; Assareh and Sardabi 2005). Glucose and fructose decreased the percentage of explants produced shoots and the number of shoots per explant in both jujube genotypes. The percentages of explants produced shoots and the number of shoots per explant in both jujube genotypes were 100 and 5.1 on the MS media containing 30 g $L^{-1}$ sucrose for 20-C-51 and 40 g $L^{-1}$ sucrose for 20-C-52.

## The Effect of Silver Nitrate on Propagation of Jujube

The silver nitrate doses did not increase the percentage of explants produced shoots and the number of shoots per explant. However, 4 mg $L^{-1}$ silver nitrate decreased the percentage of explants produced shoots and the number of shoots per explant in the study (Table 3). The highest percentage of explants produced shoots was obtained from control for 20-C-51 (100%) and 0.5 mg $L^{-1}$ silver nitrate for 20-C-52 (100%), but significant differences were not observed. The highest number of shoots per explant was obtained from 2.0 mg $L^{-1}$ silver nitrate for 20-C-51 (5.4) and 0.5 mg $L^{-1}$ silver nitrate for 20-C-52 (5.9).

Silver nitrate treatments incerase the internal polyamines due to its effects of decreasing ethylene biosynthesis in plants. Polyamines increase the cell division and development in prokaryotic and eukaryotic organisms (Bais et al. 2000). Therefore, silver nitrate was used to increase the shoot induction in jujube. However, silver nitrate doses did not increase the percentage of explants produced shoots

**Table 2** The effect of carbon sources on *in vitro* propagation of jujube

| Carbon sources | 20-C-51 jujube genotype | | | | 20-C-52 jujube genotype | | | |
|---|---|---|---|---|---|---|---|---|
| | The percentage of explant forming shoots | | Number of shoots per explant | | The percentage of explant forming shoots | | Number of shoots per explant | |
| 20 g $L^{-1}$ sucrose[a] | 93.3 | a[b] | 4.4 | ab | 80.0 | ab | 3.9 | a–c |
| 30 g $L^{-1}$ sucrose | 100 | a | 5.1 | a | 73.6 | a–c | 4.2 | ab |
| 40 g $L^{-1}$ sucrose | 73.5 | bc | 2.7 | b–d | 100 | a | 5.1 | a |
| 50 g $L^{-1}$ sucrose | 80.5 | b | 4.2 | a–c | 66.7 | a–d | 2.9 | b–e |
| 20 g $L^{-1}$ glucose | 66.7 | b–d | 3.0 | b–d | 73.6 | a–c | 3.7 | a–d |
| 30 g $L^{-1}$ glucose | 66.7 | b–d | 4.1 | a–c | 60.0 | b–d | 3.4 | a–d |
| 40 g $L^{-1}$ glucose | 53.6 | b–e | 3.0 | b–d | 60.0 | b–d | 3.0 | b–e |
| 50 g $L^{-1}$ glucose | 40.2 | de | 1.5 | d | 53.5 | b–d | 2.0 | c–e |
| 20 g $L^{-1}$ fructose | 60.0 | b–e | 1.3 | d | 26.7 | cd | 1.9 | de |
| 30 g $L^{-1}$ fructose | 66.8 | b–d | 2.6 | cd | 46.9 | b–d | 2.8 | b–e |
| 40 g $L^{-1}$ fructose | 46.7 | c–e | 2.1 | d | 40.5 | b–d | 1.4 | e |
| 50 g $L^{-1}$ fructose | 33.3 | e | 1.3 | d | 20.0 | d | 2.1 | c–e |

[a]Media also containing 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP + 0.1 mg $L^{-1}$ IBA + 0.3 mg $L^{-1}$ $GA_3$

[b]Within each column, values followed by the same letter are not significantly different at $p < 0.05$ level according to Duncan's Multiple Range test

**Table 3** The effects of silver nitrate on *in vitro* propagation of jujube

| Doses of silver nitrate | 20-C-51 jujube genotype | | | 20-C-52 jujube genotype | | | |
|---|---|---|---|---|---|---|---|
| | The percentage of explant forming shoots | | Number of shoots per explant | The percentage of explant forming shoots | | Number of shoots per explant | |
| Control[a] | 100 | a[b] | 5.1 | 93.3 | ab | 5.2 | ab |
| 0.5 mg $L^{-1}$ $AgNO_3$ | 86.8 | ab | 4.3 | 100 | a | 5.9 | a |
| 1.0 mg $L^{-1}$ $AgNO_3$ | 93.8 | ab | 5.2 | 94.5 | ab | 5.5 | a |
| 2.0 mg $L^{-1}$ $AgNO_3$ | 86.7 | ab | 5.4 | 80.0 | bc | 4.7 | ab |
| 4.0 mg $L^{-1}$ $AgNO_3$ | 73.5 | b | 4.2 | 66.4 | c | 3.8 | b |

[a]Media also containing 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP + 0.1 mg $L^{-1}$ IBA + 0.3 mg $L^{-1}$ $GA_3$

[b]Values (within each column) followed by the same letter are not significantly different at $p < 0.05$ level according to Duncan's Multiple Range test

**Table 4** The effects of different IBA and NAA consentrations on rooting of jujube

| Treatments | 20-C-51 jujube genotype | | | | 20-C-52 jujube genotype | | | |
|---|---|---|---|---|---|---|---|---|
| | Rooting shoots (%) | | Number of roots per rooted shoot | | Rooting shoots (%) | | Number of roots per rooted shoot | |
| *½ MS* | *0* | *f*[a] | | | *0* | *g* | | |
| ½ MS + 0.5 mg $L^{-1}$ IBA | 33.3 | b–e | 1.8 | a–e | 26.7 | d–f | 1.6 | |
| ½ MS + 1.0 mg $L^{-1}$ IBA | 60.0 | ab | 2.0 | a–c | 70.0 | a | 1.8 | |
| ½ MS + 2.0 mg $L^{-1}$ IBA | 76.7 | a | 2.3 | ab | 76.7 | a | 2.4 | |
| ½ MS + 4.0 mg $L^{-1}$ IBA | 40.0 | a–d | 1.5 | b–e | 46.7 | a–d | 1.5 | |
| ½ MS + 0.5 mg $L^{-1}$ NAA | 26.7 | b–e | 1.2 | de | 13.3 | e–g | 1.5 | |
| ½ MS + 1.0 mg $L^{-1}$ NAA | 23.3 | b–e | 1.7 | a–e | 33.3 | b–e | 1.9 | |
| ½ MS + 2.0 mg $L^{-1}$ NAA | 46.7 | a–c | 1.8 | a–e | 53.3 | a–c | 2.0 | |
| ½ MS + 4.0 mg $L^{-1}$ NAA | 13.3 | d–f | 1.0 | e | 53.3 | a–c | 2.1 | |
| *MS* | *0* | *f* | | | *0* | *g* | | |
| MS + 0.5 mg $L^{-1}$ IBA | 40.0 | a–d | 1.6 | a–e | 10.0 | fg | 1.5 | |
| MS + 1.0 mg $L^{-1}$ IBA | 36.7 | b–d | 1.8 | a–d | 46.7 | a–d | 2.0 | |
| MS + 2.0 mg $L^{-1}$ IBA | 76.7 | a | 2.0 | a–c | 66.7 | ab | 2.0 | |
| MS + 4.0 mg $L^{-1}$ IBA | 46.7 | a–c | 2.4 | a | 56.7 | a–c | 2.0 | |
| MS + 0.5 mg $L^{-1}$ NAA | 6.7 | ef | 2.0 | a–c | 13.3 | e–g | 1.5 | |
| MS + 1.0 mg $L^{-1}$ NAA | 23.3 | b–e | 1.4 | c–e | 46,7 | a–d | 2.1 | |
| MS + 2.0 mg $L^{-1}$ NAA | 40.0 | a–d | 1.7 | a–e | 50.0 | a–d | 1.8 | |
| MS + 4.0 mg $L^{-1}$ NAA | 20.0 | c–f | 1.8 | a–d | 26.7 | c–f | 1.8 | |

[a]Values (within each column) followed by the same letter are not significantly different at $p < 0.05$ level according to Duncan's Multiple Range test

and the number of shoots per explant in jujube. Aygün and Dumanoğlu (2007) reported that silver nitrate increased the shoot regeneration from leaf discs of quince.

## Rooting of Microcuttings

Rooting of 20-C-51 and 20-C-52 jujube genotypes were given in Table 4. Root induction frequency was significantly different among treatments. In general, the highest rooting percentage and the number of roots per rooted shoot were obtained from half strength MS and full strength MS media containig 2 mg $L^{-1}$ IBA (Fig. 1e). Half strength MS medium was relatively more effective than full strength MS medium on rooting induction of jujube genotypes. In addition, the media suplemented with IBA induced higher rooting of microcuttings than those containing NAA. The highest rooting percentage was 76.7 in both jujube genotypes on half strength MS medium containing 2.0 mg $L^{-1}$ IBA. The highest number of roots per rooted shoot was 2.4.

IBA was successfully used for rooting of jujube microcuttings in previous studies (Wu et al. 2004; Sudhersan and Hussain 2003; Du et al. 1997). Wu et al. (2004) reported that jujube microcuttings were rooted at 90% on half strength MS medium containing 0.8 mg $L^{-1}$ IBA. In addition, half strength MS medium containing 2.0 mg $L^{-1}$ IBA or 1.0 mg $L^{-1}$ IBA + 0.05 mg $L^{-1}$ IAA induced the rooting of jujube microcuttings (with 99–100% and the number of roots per rooted shoots at 5.3) (Du et al. 1997).

As a result, MS media supplemented with benzyl amino purine (BAP) alone and thidiazuron (TDZ) alone was not effective on *in vitro* micropropagation of jujube. TDZ and BAP were more effective to produce shoot from explants when used together. The highest percentage of explants that produced shoots and the number of shoots per explant were

obtained from MS medium supplemented with 0.1 mg $L^{-1}$ TDZ + 0.5 mg $L^{-1}$ BAP + 0.1 mg $L^{-1}$ IBA + 0.3 mg $L^{-1}$ $GA_3$. Different amounts of carbon sources and silver nitrate did not increase the percentage of explant that developed in to shoots and the number of shoots per explant. The highest rooting percentage (76.7%) was obtained from MS and half-strength MS media supplemented with 2.0 mg $L^{-1}$ IBA.

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