Journal of AGROBIOLOGY

J Agrobiol **28**(2): 113–118, 2011
DOI 10.2478/v10146-011-0012-9
ISSN 1803-4403 (printed)
ISSN 1804-2686 (on-line)
http://joa.zf.jcu.cz; http://versita.com/science/agriculture/joa

ORIGINAL ARTICLE

Adventitious shoot regeneration from dormant buds of persimmon (*Diospyros kaki* Thunb.) cv. Hachiya

Zuzana Kochanová[1], **Naci Onus**[2], **Ján Brindza**[1]

[1]*Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Nitra, Slovakia*
[2]*Akdeniz University, Faculty of Agriculture, Department of Horticulture, Antalya, Turkey*

Received: 13th May 2011
Revised: 23rd September 2011
Published online: 31st January 2012

Abstract
The effects of plant hormones 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA) on adventitious shoot regeneration from dormant persimmon buds were studied. The object of the study was the persimmon cultivar Hachiya, one of the most important persimmon cultivars in the Mediterranean area and Asia. Shoot regeneration was evaluated 4, 6 and 8 weeks after initiating the hormone treatment. Average shoot length was measured after 8 weeks and was evaluated by LSD test. Except for the media without hormone supplement, there was a statistically significant difference among average values of shoot length of plants, grown on the tested media. The increase in BAP had an effect on shoot regeneration that was significant and more pronounced with the addition of IBA, especially to the MS (1/2 N) medium. The highest value of shoot regeneration (98%) was obtained on medium MS (1/2 N), supplemented with 5 μmol l^{-1} BAP and 1 μmol l^{-1} IBA, with the highest average shoot length 23.69 mm, measured 8 weeks after the experiment initiation. The results indicate that adventitious shoots can be successfully produced in persimmon cv. Hachiya, especially with the supplement of hormone BAP, which, according to our results, plays an important role in persimmon *in vitro* regeneration.

Key words: BAP; *Diospyros*; Hachiya; IBA; persimmon

✉ Zuzana Kochanová, Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia

zuzana.kochan@gmail.com

INTRODUCTION

Persimmon (*Diospyros kaki* Thunb.) is a tree, economically important for fruit production in many countries of the Mediterranean and in Asia. For instance, in Japan, persimmon is the fourth most important fruit (Yonemori 1997). In recent years persimmon production has increased

throughout most of Europe (Llacer and Badenes 2009) and Turkey (Ercisli and Akbulut 2009).

The persimmon cultivar Hachiya was bred in Japan (Badenes et al. 2003) and for a long time has been grown also in the USA (Hodgson 1939). Data about its production in Florida date back to 1887 (Knight 1987). Earlier studies on Hachiya were focused mainly on morphometrical characteristics (Celik and Ercisli 2008), harvest conditions (Pekmezci et al. 1997), conservation (Koyuncu et al. 2005), and genetic relationships to other persimmon cultivars (Badenes et al. 2003). Despite its importance, Hachiya has not been tested for *in vitro* propagation in earlier investigations.

The genus persimmon (*Diospyros* L.) is interesting for biotechnological research focused mainly on quality improvement and preservation of the cultivars popular among growers. Because of the small number of cultivars utilised, there has been a loss of persimmon genetic variability. In recent years, much attention has been paid to persimmon *in vitro* micropropagation (Tao and Sugiura 1992), and especially to cultivars Jiro (Tao et al. 1988) and Rojo Brillante (Bellini and Giordani 1997a).

An efficient and reproducible plant regeneration protocol is an important step in utilising the potential of the persimmon. However, a universal protocol for persimmon regeneration, applicable to all cultivars, has not been developed. Research focused on its development is of great importance (Bellini and Giordani 1997a).

In the present work, we report on a reproducible method for *in vitro* shoot regeneration of the persimmon cultivar Hachiya from dormant buds using 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA).

MATERIAL AND METHODS

Plant material

Persimmon plants (*Diospyros kaki* Thunb.) cv. Hachiya were cultivated in the Botanical Orchard of the Akdeniz University in Antalya, Turkey. Dormant buds were harvested in the middle of the day (around 12 o'clock) on January 28–29, 2009, from approximately 10 year old trees. Internal meristems of the buds, approximately 1 mm long, were isolated under a dissecting microscope and used as explants for *in vitro* cultures. A solution of 40% sodium hypochlorite was applied for the sterilisation of the surface of the meristems, lasting for 10 min and followed by three 10 min washes in sterile distilled water. A total of 600 viable dormant buds were used in the study.

Media and culture conditions

Modified MS medium with half-strength macronutrients KNO_3 and NH_4NO_3 (Duchefa) and MS medium (Murashige and Skoog 1962) (Sigma) with a saccharose (Guinama) content 30 g l^{-1}, supplemented with various concentrations of plant hormones, were used in the study. For determination of the optimal medium with cytokinin (BAP; 6-benzylaminopurine, Sigma) and auxin (IBA; indole-3-butyric acid, Merck) dormant buds of cv. Hachiya were cultured on 6 variants of media, as follows: medium A, MS (1/2 N); medium B, MS (1/2 N) plus 5 μmol l^{-1} BAP; medium C, MS (1/2 N) plus 5 μmol l^{-1} BAP plus 1 μmol l^{-1} IBA; medium D, MS; medium E, MS plus 5 μmol l^{-1} BAP; medium F, MS plus 5 μmol l^{-1} BAP plus 1 μmol l^{-1} IBA. In the study we focused on the comparison of the effects of two basal media, MS and MS (1/2 N), generally used for plant regeneration, and the influence of hormones BAP and IBA, (two of the most (relatively) inexpensive in the market) on Hachiya shoot proliferation. The hormone concentrations studied were tested to find out if low concentrations of the hormones are sufficient for best practice in relation to Hachiya.

The buds were cultured in 100 ml glass jars with screw top lids, each with 30 ml medium per jar, with the base of bud down to the culture medium. The media were sterilized in an autoclave at 120 °C for 20 min. Cultures were incubated in a growth chamber with 16/8 h day/night photoperiod, generally used for persimmon, and temperature 26 °C. One hundred replicates per each of 6 variants of the media were initiated and viability was recorded. Any contaminated material was discarded from the experiments and from analyses. Several factors were studied: the type of basal medium, the effect of growth regulators and the suitability of the persimmon cultivar Hachiya for *in vitro* shoot formation. The experiments were carried out in the laboratories of Akdeniz University in Antalya.

Statistical analyses

At the end of the culturing period, the percentage of regenerating buds and the length of shoots (less than 10 mm or more than 10 mm) regenerated by each bud after 4, 6 and 8 weeks were recorded. Shoots were defined as having an apex and leaves. Maximal shoot length was defined as the length of the longest leaf. Eight weeks after

initiating the experiment; average and standard deviation were determined from data on shoot length analysed using a 95.0% LSD test (program Statgraphics).

RESULTS AND DISCUSSION

In order to establish optimal conditions for the regeneration of adventitious shoots from cv. Hachiya persimmon dormant buds, we tested cytokinin BAP in combination with auxin IBA. Except for media A and D, which were both without hormone supplement, there was a statistically significant difference among average values of shoot length, produced on the rest of the media (Table 1).

Regeneration frequency of at least 50% was obtained with each of 6 tested variants of media (Table 1). Hormone BAP was effective in stimulating the regeneration of adventitious shoots. Better results were obtained with the basal medium MS (1/2 N) than with MS, confirming the the research of Xiangsheng et al. (1998). In the study on persimmon shoot regeneration, Tetsumura and Yukinaga (2002) successfully obtained a high percentage of shoot regeneration with the basal medium MS from cultivar Jiro, confirming the individual reactions of different persimmon cultivars to cultivation media and plant hormones (Bellini and Giordani 1997a). The present study was consistent with previous reports on using dormant buds as explants (Fukui et al. 1989, Bellini and Giordani 1997a, Xiangsheng et al. 1998, Mitrofanova and Mitrofanova 2004, Naval et al. 2009, XiaoNa and JunLian 2009).

Table 1. Percentage of persimmon dormant buds regeneration (%) and homogenous groups for tested media

Media	Basal medium	Growth hormone (μmol l^{-1})		Regeneration / Shoots longer than 10 mm (%)			Homogenous groups (95.0% LSD)
		BAP	IBA	after 4 weeks	after 6 weeks	after 8 weeks	
D	MS	0	0	52 / 18	52 / 22	48 / 0	a
A	MS (1/2 N)	0	0	54 / 0	53 / 0	50 / 0	a
E	MS	5	0	60 / 0	60 / 0	60 / 18	b
F	MS	5	1	63 / 20	63 / 42	63 / 60	c
B	MS (1/2 N)	5	0	95 / 54	93 / 70	93 / 91	d
C	MS (1/2 N)	5	1	98 / 50	98 / 75	97 / 97	e

BAP – benzylaminopurine; IBA – indole-3-butyric acid

The percentage of shoots produced by the buds, after 4, 6 and 8 weeks of culturing was similar, with only a small variation, increasing with prolonged time only in shoot length (Fig. 1, Fig. 2), and was appreciably higher, reaching the highest value, 98%, on medium MS (1/2 N), supplemented with 5 μmol l^{-1} BAP and 1 μmol l^{-1} IBA, with the highest average shoot length after 8 weeks, 23.69 mm (Fig. 2). Shoots with a length of less than 10 mm dominated in media MS and MS (1/2 N) without the supply of hormones.

From the results it is obvious that a combination of auxin (IBA) and cytokinin (BAP) was more appropriate for shoot regeneration on MS (1/2 N) than the use of cytokinin only; nevertheless, cytokinins are responsible for shoot regeneration in *in vitro* conditions. These results are in agreement with earlier experiments on cvs. Kaki Tipo (Bellini and Giordani 1997b) and Fuyu (Liu and Jia 2007).

Much attention is given to *in vitro* persimmon propagation because the seedlessness of the fruit is a highly desirable trait in persimmon. The same tissue culture conditions do not give the same results in every persimmon cultivar and shoot proliferation is largely influenced by genotype (Fukui et al. 1990). Tao and Sugiura (1992) suggested that more research is needed to develop a commercially feasible micropropagation method for persimmon. Although there is no universal regeneration protocol for all persimmon cultivars, it was successfully created for the Spanish cultivar Rojo Brillante (Bellini and Giordani 1997a). According to our results, cv. Hachiya can be cultivated *in vitro*, in contrast with cvs. Fuyu and Hana Gosho (Tetsumura 1997).

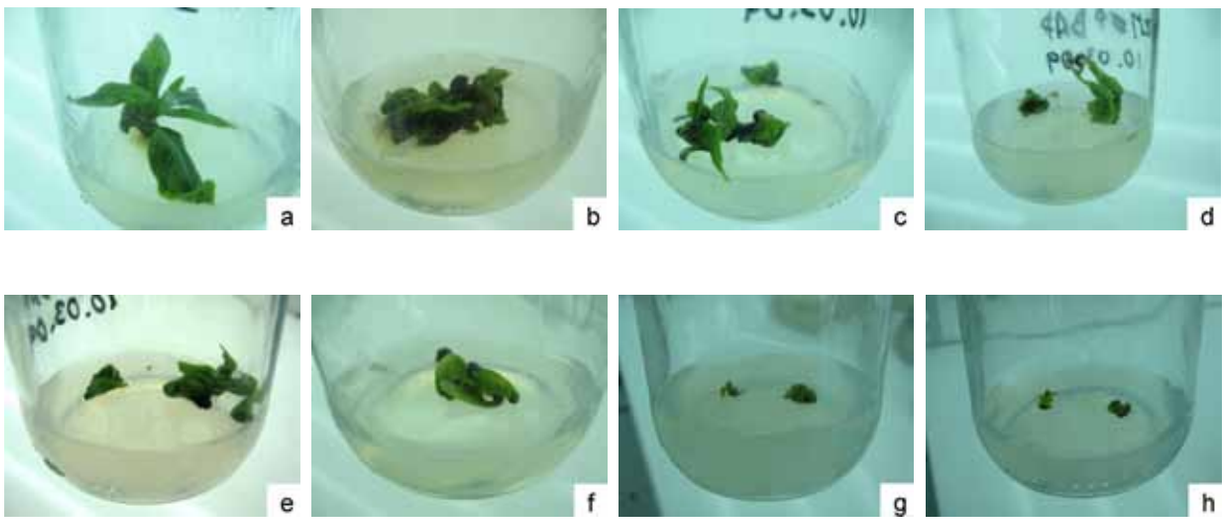

Fig. 1. Effect of BAP and IBA on persimmon cv. Hachiya regeneration, 8 weeks after the establishment of *in vitro* cultures on media: (a–b) MS (1/2 N) plus 5 µmol l^{-1} BAP and 1 µmol l^{-1} IBA; (c–d) MS (1/2 N) plus 5 µmol l^{-1} BAP; (e) MS plus 5 µmol l^{-1} BAP and 1 µmol l^{-1} IBA; (f) MS plus 5 µmol l^{-1} BAP; (g) MS (1/2 N); (h) MS

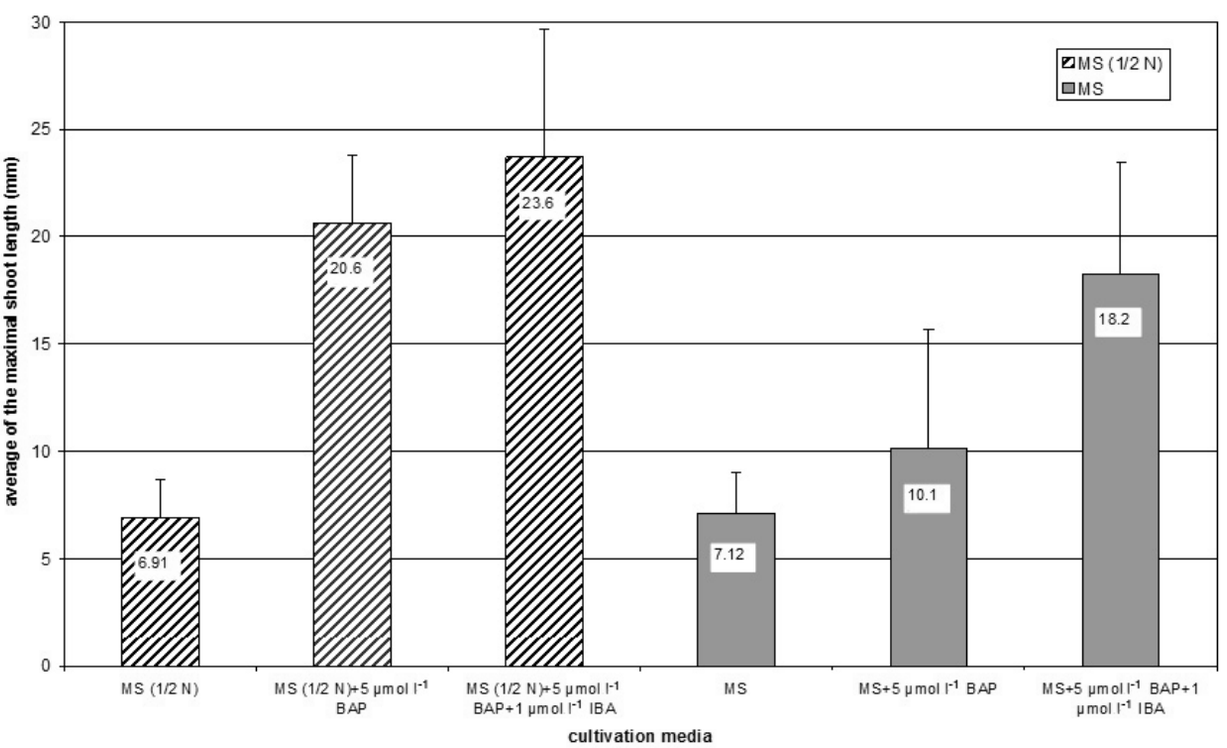

Fig. 2. Average value of regenerated shoot length of persimmon cv. Hachiya dormant buds, cultured on tested variants of media, 8 weeks after initiating the experiment

Dormant buds are preferred explantates for the propagation of persimmon *in vitro* (Fukui et al. 1989, Bellini and Giordani 1997a, Xiangsheng et al. 1998, Mitrofanova and Mitrofanova 2004, Naval et al. 2009, XiaoNa and JunLian 2009). Other explantates, tested in the past for persimmon *in vitro* regeneration, were leaf segments (Fukui et al. 1988, Choi et al. 2001, Mitrofanova and Mitrofanova 2004, Xiaoxin et al. 2004, Liu et al. 2006), eggs (Welgel and Hughes 1986), embryos, cotyledons (Mitrofanova and Mitrofanova 2004), hypocotyls (Sun et al. 2000), cambium of twigs (Yokoyama and Takeuchi 1981) and roots (Tetsumura and Yukinaga 2002). These types of plant material had a lower regeneration ability in comparison with buds, and therefore they were not accepted into practice. According to our results, dormant buds are appropriate expantates for the *in vitro* regeneration of the cultivar Hachiya.

Different plant hormones were tested for persimmon *in vitro* regeneration in past studies: zeatin (Cooper and Cohen 1985, Fukui et al. 1990, 1992, Liu et al. 2006, Naval et al. 2009), zeatin in combination with indole-3-acetic acid (IAA) (Xiangsheng et al. 1998, Sun et al. 2000, Tetsumura et al. 2001, Tetsumura and Yukinaga 2002, Xiaoxin et al. 2004, Liu and Jia 2007, XiaoNa and JunLian 2009), 6-benzyladenine (BA) (Mitrofanova and Mitrofanova 2004), 6-benzylaminopurine (BAP) (Fukui et al. 1989), and naphthaleneacetic acid (NAA) (Fukui et al. 1988).

Plant hormones BAP and IBA were chosen for our experiments, as relatively inexpensive hormones, less utilised for persimmon *in vitro* regeneration. Cooper and Cohen (1985) reported that only zeatin is a suitable hormone for persimmon *in vitro* shoot regeneration. Fukui et al. (1989) found that for cv. Nishimurawase BAP was less effective than zeatin, but only in the length of shoots. For shoot growth, similar concentrations of BAP and zeatin were required to induce shoot elongation, from 10^{-5} mol l^{-1} to 10^{-4} mol l^{-1}. Tetsumura (1997) reported the effects of different cytokinins on shoot proliferation and rooting of cvs. Fuyu and Hana Gosho persimmons. Shoots subcultured with 20 μmol l^{-1} BAP showed a substantially higher rooting percentage (more than 60%) than those subcultured with zeatin (less than 30%). Tetsumura (1997) recommended that BAP should be substituted for zeatin in the proliferation medium because zeatin is the most expensive cytokinin and shoots can effectively proliferate on the medium with BAP. Hormone IBA helps rooting of persimmon explants in *in vitro* conditions (Fukui et al. 1992), in contrast with *in vivo* conditions (Tetsumura and Yukinaga 1996). The support effect of IBA was confirmed for the cultivars Nishimurawase, Fuyu (Choi et al. 2001) and Jiro (Sun et al. 2000).

The influence of basal medium and hormones BAP and IBA on shoot regeneration should be considered in the development of a practical regeneration system, useful for the micropropagation of the persimmon cultivar Hachiya. The data clearly demonstrate that shoot regeneration is possible from *in vitro* cultured dormant buds of persimmon cv. Hachiya. This constitutes the first report for *in vitro* shoot regeneration in this cultivar.

Although the topic of persimmon *in vitro* shoot regeneration is relatively well developed, the cultivar Hachiya, popular in many countries, was tested from this aspect for the first time in the present study. The results of this work demonstrate that with the growth regulators BAP and IBA employed in the study, a relatively high frequency of shoots can be obtained. The effects of BAP and IBA on persimmon shoot regeneration are less well known in comparison with zeatin. While the hormones tested are cheaper than zeatin, the work is important from the economical point of view.

ACKNOWLEDGMENT

This work was supported by the Government of the Republic of Turkey in 2009.

REFERENCES

Badenes M, Garce A, Romero C, Romero M, Clave J, Rovira M, Llacer G (2003): Genetic diversity of introduced and local Spanish persimmon cultivars revealed by RAPD markers. Genet Resour Crop Ev 50: 579–585.

Bellini E, Giordani E (1997a): Germoplasm conservation and evaluation of *Diospyros kaki* L. within the European project "minor fruit tree species conservation". Acta Hort 436: 69–76.

Bellini E, Giordani E (1997b): *In vitro* culture establishment and shoot elongation of 'Kaki Tipo' (*Diospyros kaki* L.) dormant buds. Acta Hort 436: 129–134.

Celik A, Ercisli S (2008): Persimmon cv. Hachiya (*Diospyros kaki* Thunb.) fruit: some physical,

chemical and nutritional properties. Int J Food Sci Nutr 59: 599–606.
Choi JY, Kim HJ, Lee CH, Bae JM, Chung YA, Shin JS, Hyung NI (2001): Efficient and simple plant regeneration via organogenesis from leaf segment. In Vitro Cell Dev Biol Plant 37: 274–279.
Cooper PA, Cohen D (1985): Micropropagation of Japanese persimmon (*Diospyros kaki*). Plant Prop Soc 34: 118–124.
Ercisli S, Akbulut M (2009): Persimmon cultivation and genetic resources in Turkey. Acta Hort 833: 35–38.
Fukui H, Nishimoto K, Murase I, Nakamura M (1988): Somatic embryogenesis from the leaf tissues of continuously subcultured shoots in Japanese persimmon (*Diospyros kaki* Thunb.). Jpn J Breed 38: 465–469.
Fukui H, Sugiyama M, Nakamura M (1989): Shoot tip culture of persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Hort Sci 58: 43–47.
Fukui H, Nishimoto K, Murase I, Nakamura M (1990): Annual changes in responsiveness of shoot tip cultures to cytokinin in persimmon. J Jpn Soc Hort Sci 59: 271–274.
Fukui H, Nishimoto K, Nakamura M (1992): Varietal differences in rooting ability of *in vitro* subcultured persimmon shoots. J Jpn Soc Hort Sci 60: 821–825.
Hodgson RW (1939): Rootstocks for the oriental persimmon. Proc Amer Soc Hort Sci 37: 338–339.
Knight RJ, Jr. (1987): New tropical fruit crops of 1887 – a blueprint for today, and a swepstakes. Proc Fla State Hort Soc 100: 265–268.
Koyuncu MA, Savran E, Dilmacunal T, Kepenek K, Cangi R, Cagatay O (2005): Bazi trabzon hurmasi cesitlerinin sogukta depolanmasi (The cold storage of some persimmon cultivars). Akdeniz Univ J Fac Agr 18: 15–23.
Liu K, Jia C (2007): Study on tissue culture 'Fuyu' persimmon (*Diospyros kaki* Thunb.). J Baoding Univ Teach College 20, Doi: 1008-4584.0.2007-02-017.
Liu Y, Ma J, Tang X, Song C (2006): Study on the adventitious shoot regeneration of persimmon leaves. Hubei Agri Sci 45: 618–621.
Llacer G, Badenes ML (2009): Production of persimmon in Spain. Acta Hort 833: 39–42.
Mitrofanova IV, Mitrofanova OV (2004): Development of recipient system of woody subtropical plants *in vitro*. Acta Univ Latviensis Biol 676: 189–196.
Murashige T, Skoog F (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497.
Naval MM, Llacer G, Badenes ML, Giordani E (2009): Adventitious shoot regeneration from leaf explants of the persimmon (*Diospyros kaki* Thunb.) cv. 'Rojo Brillante'. Acta Hort 833: 183–186.
Pekmezci M, Erkan M, Gubbuk H (1997): The effects of harvest time, and method and duration of storage on quality of 'Hachiya' and 'Fuyu' persimmons. Acta Hort 441: 279–286.
Sun Q, Sun H, Liu Q, Shi Y (2000): Plant regeneration from hypocotyls of persimmon. Deciduous Fruits 11: 4–5.
Tao R, Sugiura A (1992): Micropropagation of persimmon (*Diospyros kaki* L.). Biotechnol Agr Forest 18: 426–440.
Tao R, Murayama H, Moriguchi K, Sugiura A (1988): Plant regeneration from callus cultures derived from primordial leaves of adult persimmon. Hort Sci 23: 1055–1056.
Tetsumura T (1997): Effect of types of cytokinin used for *in vitro* shoot proliferation of persimmon on the subsequent rooting of shoots. Acta Hort 436: 143–148.
Tetsumura T, Yukinaga H (1996): High-frequency shoot regeneration from roots of persimmon. Hort Sci 31: 463–464.
Tetsumura T, Yukinaga H (2002): Comparative rooting of shoot tips of four Japanese persimmon cultivars vs. shoots regenerated from roots cultured *in vitro*. Hort Sci 35: 940–944.
Tetsumura T, Tao R, Sagiura A (2001): Factors affecting rooting of Japanese persimmon hardwood cuttings. J Jpn Soc Hort Sci 70: 163–169.
Welgel RC, Hughes KW (1986): *In vitro* ovule culture of a seedless persimmon. J Hered 77: 213.
Xiangsheng K, Miaoxia Z, Yimin Z, Zhenyi W (1998): *In vitro* propagation of persimmon (*Diospyros kaki* Linn.). J Fruit Sci 14.
XiaoNa L, JunLian M (2009): Regeneration of root segment of persimmon cultivar Uenishwase in *in vitro* culture. China Fruits 4: 24–26.
Xiaoxin S, Guowiang D, Junlian M, Yi G, Lei W (2004): Callus formation and adventitious bud regeneration from leaves *in vitro* in persimmon (*Diospyros kaki*). J Fruit Sci 20.
Yokoyama T, Takeuchi M (1981): The induction and formation of organs in callus cultures from twigs of mature Japanese persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Hort Sci 49: 557–562.
Yonemori K (1997): Persimmon industry and research activities in Japan. Acta Hort 436: 21–32.