Varietal Differences in Rooting Ability of In Vitro Subcultured Japanese Persimmon Shoots

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Summary

In vitro rooting ability was investigated in 95 Japanese persimmon (*Diospyros kaki* Thunb.) cultivars and three other species by repeatedly subculturing shoots which were treated with a synthetic rooting hormone. Of the *D. kaki* cultivars, the 25 which failed to root were categorized as not having any potential to root. The remaining 70 which rooted to varying degree were classified into three groups, namely, low-rooting ability (31 cvs.), medium-rooting ability (23 cvs.), and high-rooting ability (16 cvs.). A relationship between rooting ability and tree vigor was noted, that is, the vigorous cultivars had better rooting percentages than had those which were less vigorous. The pollination constant and astringent (PCA) type cultivars were difficult to root. Japanese persimmons were classified into three types based on the relation between number of roots and the longest root.

Introduction

There are more than 1,000 Japanese persimmon cultivars; their fruit, shoot, and flowering characteristics have been described in a report by the Hiroshima Experimental Research Station (7). Recently, they were classified by isozymes (12, 13) and by their ethanol productivity and tannin content (10).

Propagation of Japanese persimmon by soft- and hard-wood cuttings has not been too successful. However, clonal propagation by shoot tip culture (1, 4, 11) has been successfully accomplished in recent years, provided shoot tips are subcultured repeatedly (3, 6, 11). Nevertheless, varietal differences in rooting ability have appeared (3, 6). The rooting ability in a species or variety is promoted by auxin and/or rooting cofactors in the shoots which are controlled genetically. These inherent characteristics are supposed to be stable in vitro; they function physiologically, so that they are used to classify organogenetic potential in rice (14), Chinese cabbage (8), wheat (9), and Japanese persimmon (5).

In this study, shoot apices from a large population of *D. kaki* and three other species were as-

sayed in vitro for their rooting ability and classified accordingly.

Materials and Methods

Shoot tips excised from current growth of 95 Japanese persimmon (*Diospyros kaki* Thunb.) cultivars and three other species, *D. japonica*, *D. lotus* (male plant), and *D. virginiana* (male and female plant) were cultured in Murashige and Skoog's medium containing half-strength nitrogen (1/2 N-MS) plus 10^{-5} M zeatin, 3% sucrose and 0.7% agar (4, 5). Subsequently the shoots were repetitively subcultured every six weeks in a fresh medium for 27 months. Shoots were treated with α -naphthyl acetamide powder (containing 0.40% α -naphthyl acetamide) to induce rooting (3), and then cultured on the 1/2 N-MS medium containing 3% sucrose and 0.7% agar (zeatin free). Data were obtained from 10 shoots per cultivar and the different *Diospyros* species 6 weeks after culturing.

Results and Discussion

No roots were obtained in 25% of the kaki cultivars (Table 1); they were classified as "difficult-to-root". The percentage of shoots of the remaining cultivars which formed roots ranged from 10 to 100%. Of these cultivars, 31 had rooting percentage of less than 30%, and these were classified as

Table 1. Variations in rooting observed in 95 Japanese persimmon cultivars and three *Diospyros* species.

Cultivars and species	No. of rooted shoots	No. of roots per rooted shoots	The longest root length (mm)	Fruit type ^z	Tree vigor ^y
Aburatsubo	3/10	1.3±0.6 ^x	10.0± 4.4 ^x	PVNA	4
Akagaki	6/10	2.2±1.5	11.3± 9.6	PVNA	5
Ama Hyakume	4/10	2.3±1.3	8.0± 2.4	PVNA	7
Ama Yotsumizo	1/10	2.0	15.0	PVNA	6
Aosa	3/10	1.7±0.6	13.3± 5.5	PCA	6
Aoso	1/10	2.0	12.0	PCA	7
Atago	0/10	—	—	PCA	7
Beni Emon	7/10	1.3±0.5	13.6± 9.6	PVA	5
Benigaki	7/10	3.4±1.1	42.9±13.2	PCA	6
Beni Hagakushi	5/10	1.8±0.8	9.8± 7.2	PCA	7
Da Mo Pan	0/10	—	—	PCA	7
Dojo Hachiya	4/10	3.0±0.8	28.8±17.7	PCA	5
Ebo	3/10	1.7±0.6	9.5±10.0	PVA	5
Eboshi	8/10	2.9±1.2	29.1±13.6	PVA	6
E Goshō	10/10	1.8±0.8	8.8± 5.9	PVNA	3
Emon	0/ 9	—	—	PVA	5
Fujiwara Goshō	2/ 7	2.0	13.0± 1.4	PCNA	4
Fuyu	9/10	2.1±0.8	28.9±11.3	PCNA	6
Hagakushi	2/10	2.0±0.1	19.1±15.6	PCA	7
Hegurogaki	6/10	4.8±2.8	41.2±13.8	PVNA	5
Hiratanenashi	9/10	2.0±0.9	36.4±30.2	PVA	7
Houtoku	0/10	—	—	PVA	4
Ibogaki	1/10	1.0	6.5	PCA	4
Ichidagaki	2/10	1.0± 0.1	15.5± 3.5	PCA	5
Ichiryo	6/10	1.5±0.8	23.8± 6.4	PCA	6
Ikutomi	4/10	2.0±1.4	28.6±18.3	PCNA	7
Inasa	4/10	3.0±1.4	45.0±31.1	PCA	7
Inayama	0/10	—	—	PCA	6
Iwasedo	0/ 8	—	—	PCA	7
Izaemon	0/10	—	—	PVA	5
Jisha	0/10	—	—	PCA	5
Kawabata	0/ 7	—	—	PCA	5
Konashiba	4/10	1.5±0.6	17.3±11.6	PVNA	4
Koshu Hachiya	4/10	3.0±2.8	11.5± 9.5	PCA	5
Koshumaru	1/10	2.0	13.0	PVA	5
Koshushi	1/10	4.0	40.0	PCA	7
Kubo	8/10	2.4±1.4	12.5± 7.1	PVNA	7
Kubogata Obishi	3/10	1.7±0.6	19.0± 1.0	PVNA	4
Kurogaki	5/ 9	1.6±0.5	15.2± 3.7	PVNA	3
Kyara	0/10	—	—	PVNA	7
Mammoth	3/10	4.3±3.2	45.0±22.1	PCNA	—
Mikagemaru	7/10	1.7±0.8	24.0±16.6	PCA	6
Mikatani Goshō	2/10	2.5±2.1	30.0±12.7	PVNA	7
Mizuta Fuyu	2/ 8	1.5±0.7	21.0± 1.4	PVNA	5
Monpei	7/10	2.1±0.9	23.3±15.1	PVA	6
Mushiroda Goshō	0/10	—	—	PCNA	2
Nagano Goshō	0/10	—	—	PVNA	5
Nagatorogaki	0/10	—	—	PCA	5
Nage Eboshi	0/10	—	—	PCA	5
Nishimurawase	9/10	2.3±1.3	26.0±13.4	PVNA	4
Oshoro	6/10	3.3±3.3	9.7± 6.4	PVNA	6
O Yotsumizo	0/10	—	—	PCA	5
Rendaiji	0/10	—	—	PVNA	4
Saijo	1/10	1.0	5.5	PCA	7

(continued)

Sakushu Mishirazu	5/10	2.0±1.2	21.8±14.5	PVA	4
Sanja	3/10	2.0±1.7	14.7± 6.1	PCA	3
She Gu Shi	7/11	3.0±2.0	82.4±25.9	PCA	4
Shidaregaki	1/10	1.0	5.0	PCA	—
Shiga tanenashi	1/10	2.0	22.0	PCA	6
Shin Hachiya	2/10	3.5±3.5	17.5±13.4	PVA	4
Shinpei	0/10	—	—	PCA	7
Shiro Nippon	6/10	2.5±1.0	11.0± 5.1	PCA	6
Shirotodamashi	4/10	1.5±1.0	11.0± 6.5	PCA	5
Shogatsu	6/10	1.3±0.5	18.1±13.2	PVNA	5
Shoro	8/10	3.1±1.7	22.1± 5.5	PVNA	6
Shuren	3/10	3.3±2.5	55.0±11.0	PCA	4
Suruga	6/10	1.3±0.5	12.2± 8.4	PCNA	6
Takeda	9/10	2.8±1.1	15.8±10.5	PCA	6
Tateishi	6/10	1.3±0.5	11.5± 9.6	PCA	5
Tenjin Gosho	0/10	—	—	PCNA	4
Tenryubo	1/10	3.0	11.0	PVNA	4
To Gosho	3/10	2.3±1.5	29.7±12.9	PCNA	—
Tohachi	7/10	3.4±1.3	21.0± 7.2	PVNA	5
Toyoichi	6/10	2.2±1.0	24.3±22.9	PVNA	7
Toyooka	10/11	2.5±1.2	8.6± 5.2	PVNA	7
Tsukiyo	2/10	1.5±0.7	35.7±21.9	PVNA	5
Tsunomagari	0/10	—	—	PCA	3
Tsurunohashi	0/10	—	—	PCA	5
Tsurunoko	2/10	1.0	15.0±12.7	PVA	3
Wakamiya	1/10	8.0	27.0	PCA	4
Wasamatsuo	0/10	—	—	PCA	5
Wase Jisha	0/10	—	—	PCA	5
Watarizawa	0/10	—	—	PCA	5
Yamato	0/10	—	—	PCA	7
Yamatogaki	8/10	1.6±0.7	14.6± 8.2	PVNA	5
Yamato Hyakume	7/11	2.1±1.2	11.1± 4.8	PVA	6
Yamato Gosho	8/10	1.8±1.0	21.6±12.6	PCNA	7
Yashima	3/10	1.0±0.0	7.7± 3.2	PVNA	5
Yokono	4/10	2.0±1.2	13.0±11.9	PCA	6
Yonsungaki	1/10	1.0	7.0	PCA	6
Yoshimoto Gosho	0/10	—	—	PCNA	5
Yoshino	4/10	1.3±0.5	15.3±13.4	PVA	4
Yotan	2/10	1.0±0.0	30.5± 2.1	PVNA	4
Yotsumizo	2/10	2.0±1.4	8.5± 6.4	PCA	6
Yukineri	2/10	1.0±0.0	16.0± 8.5	PVNA	5
<i>D. lotus</i> (♂)	2/ 8	11.5±9.2	11.0± 2.8	—	—
<i>D. japonica</i>	4/10	2.5± 1.0	20.8±12.0	—	—
<i>D. virginiana</i> (♂)	6/10	8.8±5.6	34.0±17.4	—	—
<i>D. virginiana</i> (♀)	10/10	9.4±4.0	19.5±11.5	—	—

^z PCNA shows pollination constant and non-astringent type. PVNA shows pollination variant and non-astringent type. PVA shows pollination variant and astringent type. PCA shows pollination constant and astringent type.

^y The following value of tree vigor is based on the investigation at Hiroshima Prefecture (7), 2; very peak, 3; weak, 4; slightly weak, 5; medium, 6; slightly vigorous, and 7; vigorous.

^x The mean ± standard deviation.

“cultivars of low rooting ability”. *D. lotus* (male plant) was included in these group. Twenty-three cultivars had rooting percentages ranging from 40

to 60; they are considered “cultivars of medium rooting ability”. This group includes *D. japonica* and male plants of *D. virginiana*. The 16 cultivars

and the female plant of *D. virginiana*, in which more than 70% of the shoots rooted are classified as the high rooting ability group. Of all the cultivars and *Diospyros* species examined, 42.2% were in the medium and high rooting groups.

Soft- and hard-wood cuttings of Japanese persimmon taken from current or biennial shoots have been very difficult to root. However, in this experiment, shoots of many cultivars produced roots in vitro. In general, shoots in the juvenile phase, that is, shoots of young seedlings and water sprouts arising from the trunk near the soil surface are known to root readily. Franclet et al. (2) suggested that the degree of rejuvenation increased by repetitive subculturing. Therefore, the shoots propagated in vitro might revert the juvenile phase, because repetitive subculturing on the medium containing zeatin reverted shoots from the adult phase to the juvenile phase.

Successful in vitro rooting has been accomplished in 3 Japanese persimmons: 'Hiratanenashi' (11), 'Gailey' (1) and 'Nishimurawase' (6). In this experiment, a rooting percentage of 90% was attained with 'Nishimurawase' and 'Hiratanenashi' which agree with the findings of the previous reports.

We also found a relation between rooting ability and tree vigor in this trial. The classification of tree vigor was based on the investigations of growth of current shoots at the Hiroshima Experimental Research Station (7), where the cultivars were ranked in vigor from 1 to 7. Twenty-three cultivars were ranked between 2 to 4 in vigor; their mean rooting percentage was 29.5. Thirty-nine vigorous cultivars which were ranked from 6 to 7 had a mean rooting percentage of 41.4. From these data, we concluded that the vigorous cultivars had a much better rooting ability than less vigorous ones.

Sixteen of the 25 cultivars which failed to root were the pollination constant and astringent (PCA) type (Table 1). Of the 43 PCA cultivars assayed, 30, 10, and 3 were classified as having; low (< 30%), medium (40 to 60%), and high-rooting ability (more than 70%), respectively. It was concluded that the PCA type cultivars were difficult to root. Of the pollination variant and astringent (PVA) type, 7, 3, and 4 cultivars were classified as being low-, medium-, and high- rooting ability, respectively, whereas of the 38 pollination variant

Fig. 1. Distribution of root length and number of roots. 1: *Diospyros lotus* (δ), 2: *D. virginiana* (φ), 3: Wakamiya, 4: *D. virginiana* (δ), 5: Hegurogaki, 6: Koshushi, 7: Mammoth, 8: Benigaki, 9: Inasa, 10: Shuren, 11: She Gu Shi.

and non-astringent (PVNA) type, 19, 10, and 9 were grouped as low-, medium-, and high-rooting ability, respectively.

Number of roots in Japanese persimmon was about two except for 'Wakamiya' (Table 1), and that of *D. lotus* and *D. virginiana* was about ten. It was observed that the roots of *D. kaki* seedlings (Japanese persimmon) are composed of a tap root and few lateral roots, whereas the roots of *D. lotus* seedlings are composed of many lateral roots and fine roots. Therefore, the root system in orchard may have relation to the number of roots developed in vitro.

Fig. 1 shows a relation between number of roots and length of the longest root. 'Wakamiya', *D. lotus* and *D. virginiana* produced many roots which were obviously different from other Japanese persimmons. 'Benigaki', 'Hegurogaki', 'Inasa', 'Koshushi', 'Mammoth', 'She Gu Shi' and 'Shuren' had long roots and comparatively large numbers of roots, therefore these cultivars were distinguishable from the other Japanese persimmons.

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培養したカキのシュートからの発根能力の品種間差異

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摘 要

カキ95品種とマメガキ, アメリカガキ, リュウキュウマメガキを用い, *in vitro* での発根能力を調査した。供試したシュートは, 茎頂培養後窒素成分を1/2にしたMS培地に zeatin を 10^{-5} M 添加した培地で2年間継代培養したもので, 発根処理として α -naphthyl acetamide 粉末 (0.4%含有) を処理した。供試品種のうち, 25品種はまったく発根が認められず, 31品種が

30%以下の低い発根性を示した。また, 23品種は40~60%の発根性を示し, 16品種は70%以上の高い発根能力がみられた。樹勢が強い品種は発根能力が高いものが多かった。完全渋ガキ (PCA) には発根能力の低いものが多かった。発根数と根長との関係から供試品種を3群に分類することができた。