Development of in Vitro Root System from Microcuttings of Fruit Trees©

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Success of micropropagation (in vitro propagation) depends on the establishment of four stages: establishment of explants, multiplication of shoots, rooting of microcuttings, and acclimatization of micropropagules to ex vitro environment (Murayama et al., 1989). During the acclimatization, loss of micropropagules directly affects multiplication rate. Hence, there are many reports aiming to prevent them from wilting by controlling the humidity, light, carbon dioxide, and so on. However, most microcuttings of fruit trees, that is, woody plants, are difficult-to-root and need treatment with auxins in order to root. A treatment with a high concentration of auxin often results in the weakening of microcuttings, and many of them do not survive during acclimatization even if they have roots and grow in a suitable environment.

Japanese persimmon (*Diospyros kaki* Thunb.) is one of the fruit trees whose micropropagules are difficult to acclimatize (Tao and Sugiura, 1992). The number of adventitious roots from the microcutting is usually one and the roots do not have secondary roots (Fig. 1). These micropropagules have high mortality during the acclimatization and grow poorly after the acclimatization even if they survive. As a result, it takes a long time for them to increase in size in the nursery. On the other hand, most in vitro herbaceous micropropagules have many roots with secondary roots and grow well after the acclimatization. They have many root apical meristems where cytokinin is biosynthesized. Consequently, they are certainly rich in cytokinin, which induces cell division. Active cell proliferation probably causes the easiness of acclimatization of in vitro herbaceous micropropagules with many roots.

Figure 1. In vitro roots from microcuttings of 'Jiro' Japanese persimmon.

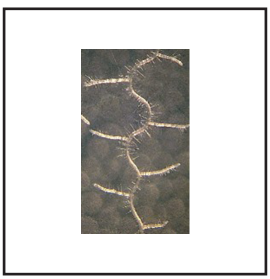

Figure 2. Lateral roots emerging on the curve tops of a waving primary root of *Arabidopsis thaliana.*

Figure 3. Microcuttings of Japanese chestnut seedlings. (Left; the vermiculite-containing and gellan gum-solidified medium. Right; the medium without vermiculite.)

Increasing the number of adventitious roots (root apical meristems) from microcuttings of a difficult-to-root fruit tree may be achieved by a treatment with a very high concentration of auxin. However, the treated microcuttings may be damaged severely. Increasing the number of lateral roots of a microcuttings is an alternative, but how can one achieve it? The model plant, *Arabidopsis thaliana*, was the first plant genome to be sequenced and is a popular tool for understanding the molecular biology of many plant traits. When seeds were germinated on an agar-solidified and inclined medium, their primary roots displayed wavy patterns (Okada and Shimura, 1990). De Smet et al. (2007) showed that a primary root displaying wavy patterns had more lateral roots than those growing straight and most of the lateral roots emerged on the curved tops of them (Fig. 2). Is it possible to apply this phenomenon to adventitious roots from microcuttings of a difficult-to-root fruit tree for increasing the number of root apical meristems. Generally, as a result of gravitropic response, adventitious (primary) roots from microcuttings grow downwards and vertically in a rooting medium (Fig. 1).

A vermiculite-containing and gellan-gum-solidified medium was developed for the improvement of in vitro rooting of microcuttings of hybrid walnuts (Jay-Allemand et al., 1992). The rooting percentages of microcuttings of Shinano walnut (*Juglans regia* L.) in the vermiculite-containing and gellan gum-solidified medium, namely ½ DKW medium (Driver and Kuniyuki, 1984) plus vermiculite (4:5, v/v), were higher than those in the medium without vermiculite (Tetsumura et al., 2002). Moreover, more than half of the rooted microcuttings had lateral (secondary) roots, which were not produced from the roots in the medium without vermiculite. Rooting of the microcuttings of Japanese chestnut (*Castanea crenata* Sieb. et Zucc.) seedlings was also improved by the vermiculite-containing and gellan-gum-solidified medium (Tetsumura and Yamashita, 2004). During the acclimatization, the micropropagules rooting in vermiculite-containing gellan gum-solidified medium survived better than those in the medium without vermiculite and grew faster after the acclimatization. The micropropagules in the vermiculite-containing gellan-gum-solidified medium had dark green leaves, while those in the medium without vermiculite had pale green leaves (Fig. 3). It may be considered that the primary root grew avoiding the vermiculite, curved irregularly, and differentiated the secondary roots on the curve tops. As a result, a well-developed root system, which may help the survival during the acclimatization, was completed (Tetsumura and Yamashita, 2004). In this study, the vermiculite-containing medium without gellan gum, namely the vermiculite-plus-liquid medium, was also used for the rooting experiments. However, the percentages of rooting and survival of the microcuttings in the medium was lower, because they became weak in the medium and one third of them died after 30 days in the culture.

After treating with 1.25 mM IBA solution for 5 sec, we inserted microcuttings of ‘Taishuu’ Japanese persimmon in the four types of rooting medium: ½ MS (½ N) medium plus vermiculite (4 : 5, v/v) solidified with 0.2% gellan gum (4 : 5 medium), ½ MS (½ N) medium plus vermiculite (6 : 5) solidified with gellan gum (6 : 5 medium), ½ MS (½ N) medium plus vermiculite (8 : 5) solidified with gellan gum (8 : 5 medium), and ½ MS (½ N) medium solidified with gellan gum (medium without vermiculite). Although the rooting percentage and survival of the microcuttings inserted in the 4 : 5 medium, the same ratio as used in the walnut and chestnut studies (Tetsumura et al., 2002; Tetsumura and Yamashita, 2004), were

Table 1. Effect of medium on rooting of 'Taishuu' Japanese persimmon microcuttings after 40 days in culture.

Medium[z]	Survival (%)[y]	Rooting (%)[y]	Length of roots (mm)[y]	No. of roots[yx]	Secondary roots (%)[yw]
4 : 5	27±4	4±2	26±16	1.0	0±6
6 : 5	87±6	31±4	35±6	1.3±0.1	12±6
8 : 5	93±2	24±2	32±5	1.3±0.1	20±11
1 : 0	100	22±4	21±5	1.0	0±6

Means were derived from 5 replicates of 9 microcuttings each.

[z] ½ MS (½ N) medium : vermiculite (v/v). All media were solidified with 0.2% gellan gum.

[y] Mean ± SE.

[x] Number of roots per rooted microcutting.

[w] Number of rooted microcuttings forming secondary roots/Number of rooted microcuttings.

lower than those in the medium without vermiculite, the lower ratio of vermiculite improved the survival (Table 1). The rooting percentage of the 6 : 5 medium was higher than that of the medium without vermiculite, and some micropropagules rooting in the vermiculite-containing and gellan-gum-solidified medium had secondary roots (Table 1). All of the micropropagules rooting in the 6 : 5 medium survived, whereas only two-thirds of those rooting in the medium without vermiculite did. The vermiculite of the 6 : 5 and 8 : 5 media sank in the solidified media and the media were not porous, although the 4 : 5 medium was porous. It may be difficult for the microcuttings of 'Taishuu' Japanese persimmon to survive in the porous medium in which those of walnut and chestnut survived well.

Figure 4. Roots from microcuttings of 'Bluecrop' highbush blueberry inserted in the rooting medium without vermiculite.

The response of highbush blueberry (*Vaccinium corymbosum* L.) microcuttings to the types of rooting medium varied with the cultivars tested (Table 2). Generally, microcuttings of blueberry are easy-to-root and do not need treatment with auxins in order to root. Their roots have many secondary roots and develop well in the rooting medium without vermiculite (Fig. 4), and almost all of the micropropagules survived during the acclimatization. It seems likely that there is no need to add vermiculite to the rooting medium for rooting of the microcuttings having high rooting abilities. A preliminary test showed that microcuttings from a dwarfing rootstock for Japanese persimmon developed secondary roots in the rooting medium without vermiculite and were acclimatized easily.

We have introduced that the vermiculite-containing gellan-gum-solidified medium improved rooting of microcuttings collected from the fruit trees having low rooting ability and their roots formed secondary roots, which may helped their survival during the acclimatization. Although it is necessary to examine what ratio of rooting medium to vermiculite is the best for a genotype, you may try the rooting medium if you want to acclimatize its micropropagules which are difficult-to-root and reluctant to be acclimatized.

Table 2. Effect of medium on rooting of microcuttings from three cultivars of highbush blueberry after 60 days in culture.

Medium	'Berkeley'		'Bluecrop'		'Earliblue'	
	Rooting (%)	Degree of root system [z]	Rooting (%)	Degree of root system [z]	Rooting (%)	Degree of root system [z]
Gellan gum[y]	44	4.0	17	4.0	83	3.7±0.2[x]
Gellan gum + vermiculite	92	1.9±0.3	67	2.6±0.4	100	2.9±0.2
Vermiculite	92	3.1±0.2	90	3.1±0.3	75	3.4±0.2

Means were derived from 12 microcuttings each.

[z] Degree of the root system per rooted shoot (from poor, score 1, to well-developed, score 4), following the ranking system presented by Suzuki et al. (1992).

[y] All media were saturated with ½ MW medium without plant growth regulators.

[x] Mean ± SE.

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