Low Temperature Storage of In Vitro Shoots of Japanese Persimmon (*Diospyros khaki*)

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In vitro storage at low temperatures was applied to the shoot segments of Japanese persimmon (cultivars Fuyu and Nishimurawase) that were proliferated through shoot tip culture. At 2C storage temperature, preconditioning of the shoot segments on a medium of 60 g litre-1 sucrose showed high viability. In the case of 10C storage, however, preconditioning on a medium of 15 g litre-1 sucrose maintained the best viability. The shoot segments of 'Nishimurawase' survived for 30 weeks at 10C, while those of 'Fuyu' lived for 12 weeks at the same storage temperature. The results of this work establish the possibility of an in vitro gene bank for Japanese persimmon.

INTRODUCTION

The maintenance of valuable plant genotypes of woody fruit trees in an orchard is costly and involves a considerable investment in labour, land area, and materials. The use of in vitro repositories for the maintenance of valuable plant genotypes offers a number of advantages but subculturing can be costly. The use of low temperatures for long-term storage of cell and organ cultures has been applied successfully to a number of species (Mullin and Schlegel, 1976; Monette, 1986; Borkowska; 1990) and reduces the cost of subculturing. Using in vitro techniques we have established and proliferated 102 cultivars (Fukui et al., 1990) of Japanese persimmon (*Diospyros khaki*). The objective of the present study was to determine the suitability of storage at low temperatures on cultures of Japanese persimmon.

MATERIALS AND METHODS

Shoot segments of Japanese persimmon (cultivars Fuyu and Nishimurawase), produced by shoot-tip culture (Fukui et al., 1989) on a modified MS medium (½ N, 1 µM zeatin, 30 g 1itre^{-1} sucrose, 8 g litre^{-1} agar), were used in the following experiments.

Experiment 1: Effects of Preconditioning on the Viability of Shoot Segments After Cold Storage. For preconditioning, the shoot segments of 'Fuyu' were cultured on a basal medium (½ N, 1 µM zeatin, 8 g litre^{-1} agar) containing 15, 30, or 60 g litre^{-1} sucrose for 6 weeks at 25C with a 16 h photoperiod under 3000 lx. Shoot segments cut from the preconditioned shoots were placed on the basal medium containing 15 or 30 g litre^{-1} sucrose, and were stored for 6 weeks at 2 or 10C in the dark. Upon removal from storage the explants were transferred onto the basal medium with 30 g litre^{-1} sucrose, and were examined for viability by culturing them for 6 weeks at 25C with a 16 h photoperiod under 3000 lx.

Experiment 2: Low Temperature Storage of Shoot Segments. Shoot segments of 'Fuyu' and 'Nishimurawase' were preconditioned for 6 weeks at

25C with a 16-h photoperiod under 3000 lx on the basal medium with 15 g litre^{-1} sucrose. The preconditioned shoot segments were placed on the basal medium with 30 g litre^{-1} sucrose and stored for 42 weeks at 10C in the dark. After 6, 12, 18, 30, and 42 weeks of cold storage, the shoot segments were removed and cultured at 25C with a 16-h photoperiod under 3000 lx and checked for viability after 6 weeks.

RESULTS

Effect of Preconditioning on Viability During Cold Storage. Table 1 shows the effect of preconditioning on viability after cold storage. The number of elongated shoot explants after storage at 2C increased with increasing sucrose concentration during preconditioning. The number of brown or dead explants decreased as the sucrose concentration was increased. Therefore, the viability in storage at 2C increased as the sucrose concentration increased. However, after storage at 10C, the presence of a high sucrose concentration for preconditioning was an inhibiting factor because it increased the number of explants without growth. Therefore, while a sucrose concentration of 30 g litre^{-1} during storage at 2C enhanced the viability of explants as compared with the 15 g litre^{-1} sucrose concentration, there was no effect under 10C storage. The explants preconditioned at a 15 g litre^{-1} sucrose concentration and stored at 10C on the media with 15 or 30 g litre^{-1} sucrose showed the least mortality and highest viability.

Table 1. Effect of preconditioning on viability after cold storage.

Storage temp. (°C)	Sucrose conc. (g litre^{-1})		No. of shoot elongated explants	No. of explants without growth	No. of browning or dead explants	Shoot length (mm)
	Precondi-tioning	Storage				
2	15	15	3	0	17	12.0[1]
		30	5	1	14	12.2
	30	15	9	0	11	10.6
		30	12	2	6	8.8
	60	15	12	1	7	10.9
		30	18	1	1	13.7
10	15	15	19	0	1	11.3
		30	19	0	1	12.8
	30	15	14	2	4	9.5
		30	17	3	0	7.1
	60	15	15	4	1	9.2
		30	12	5	3	7.6

[1] Average length of elongated shoot from viable explant.

Low Temperature Storage. With 'Nishimurawase', all of the stored explants survived for up to 18 weeks. The survival of the shoots declined substantially after

storage for 30 weeks and fell to 15% after 48 weeks of storage. Survivals with 'Fuyu' was 100% up to 6 weeks of storage; declined to about 80% during 12, 18, and 30 weeks in storage; and eventually fell to 15% after 48-weeks storage.

Storage duration had a marked effect on the growth of shoots. Figure 1 shows the viability after culture for 6 weeks at 25C followed by 0, 6, 12, 18, 30, and 42 weeks in storage. The viability of 'Nishimurawase' explants was higher than that of 'Fuyu'. The shoot length after 12 weeks of storage was 19 mm in 'Nishimurawase', which was almost the same as the shoot length from non-stored explants. The viability after 18-weeks storage declined gradually and the explants after 42 weeks of storage had no viability. The shoot length of 'Fuyu' declined with longer storage, and explants stored over 18 weeks had no viability.

DISCUSSION

The in vitro storage of pathogen-free plantlets at low temperature has been established in several plant species (Monette, 1986; Borkowska, 1990). In strawberries, for example, more than 50 cultivars have been maintained for up to 6 years at 2C (Mullin and Schlegel, 1976). The temperature for in vitro storage was different for each plant species. For strawberries (Mullin and Schlegel, 1976) and sour cherries (Borkowska, 1990), 4 to 5C was favourable for long-term storage,

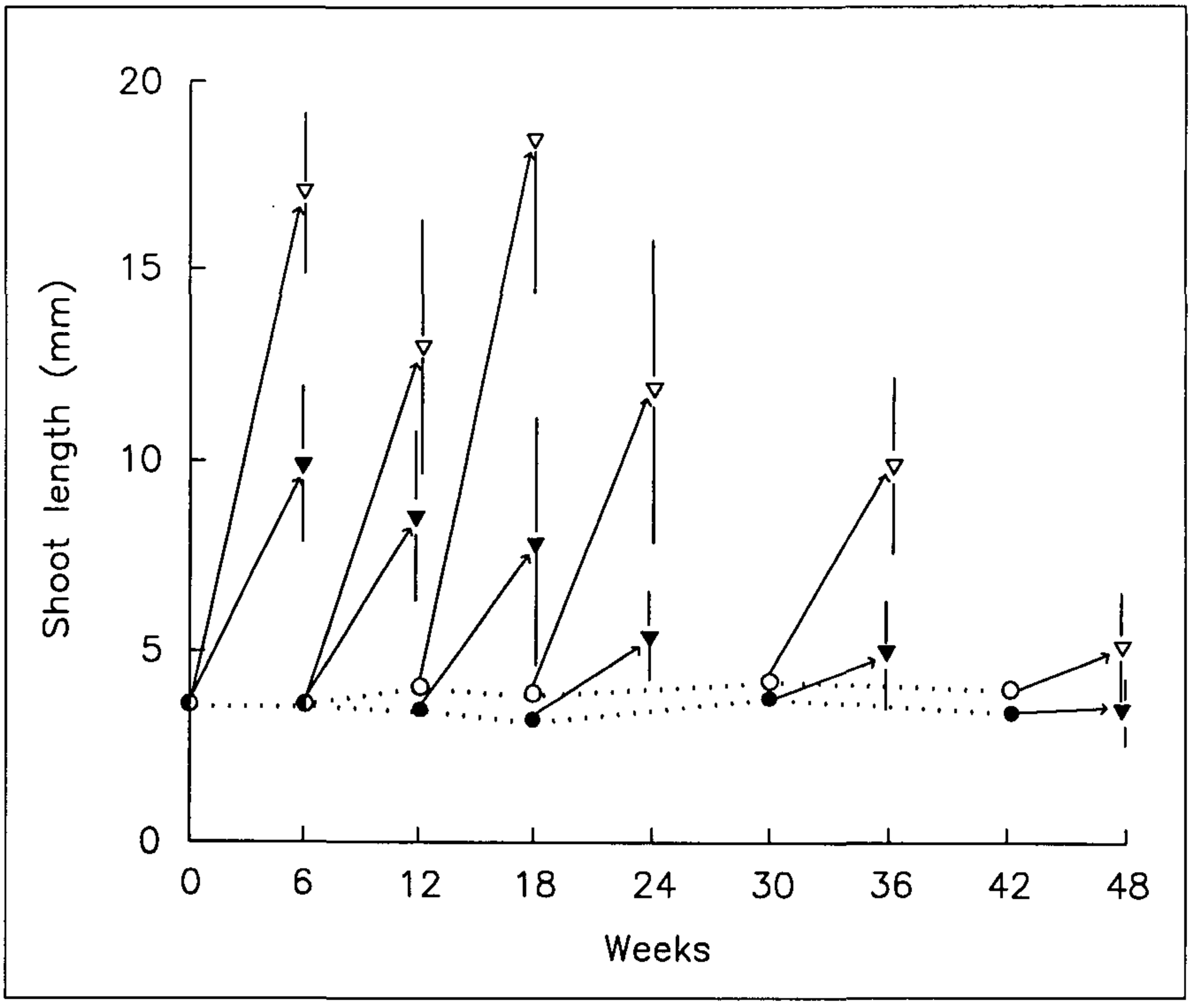

Figure 1. Shoot length of Japanese persimmon cv. Fuyu (•) and cv. Nishimurawase (○) shoot segments stored in vitro at 10C, and shoot elongation of Fuyu (▼) and Nishimurawase (▽) by culturing for 6 weeks at 25C after storage.

while 8C was best for kiwifruit. In this study, the shoot segments of Japanese persimmon survived at 10C and browned at 2C. This difference in appropriate storage temperatures might be related to the cold hardiness of each plant, and Japanese persimmon might have little cold hardiness. Bannier and Steponkus (1972) reported that the cold hardiness of in vitro plantlets was influenced by preconditioning. In the present study, the viability of Japanese persimmon after storage at 2C increased as the sucrose concentration increased during preconditioning. The preconditioning for the increase of cold hardiness, therefore, was effective in Japanese persimmon. We attempted the low temperature storage of Japanese persimmon cultivars Fuyu and Nishimurawase. The viability of 'Nishimurawase' was maintained at a high level for up to 30 weeks, but the viability of 'Fuyu' declined after 18 weeks. The bud-burst date of 'Nishimurawase' is earlier than that of 'Fuyu' and 'Nishimurawase' shows cold resistance in orchards. Therefore, we concluded that 'Nishimurawase' had a higher cold hardiness in vitro in comparison with Fuyu. A cultivar difference of viability in low-temperature storage was also observed in pears (Moriguchi et al., 1990). Monette (1986) succeeded in the long term storage of the shoot segments of kiwifruit, which grew a little during storage. In this study, the explants of Japanese persimmon exhibited no growth during storage at 2C or 10C. When a storage temperature over 10C (for example 15C) was applied, the explants grew a little and exhibited a high viability after long term storage.

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