Mass Propagation of *Smilax oldhami* Miq. by Tissue Culture

T. Yamamoto
Minami-Kyushu University, Takanabe, Miyazaki 884 Japan

H. Oda
Miyazaki Prefectural Forestry Research and Instruction Center, Saigo, Miyazaki 883 Japan

A donor plant was produced aseptically by culturing a nodal segment of an in vivo *Smilax oldhami* Miq. plant. From the donor plant, segments of nodes, internodes, and roots were taken and cultured on a modified Murashige and Skoog (MS) basal medium. Hormonal effects on shoot formation and rooting were investigated. The rate of propagation from the internodal segment was higher than that from the other two types of explants. It could be concluded from the data in the present culture system that more than 75 plantlets were obtained from one nodal segment of an in vivo plant.

INTRODUCTION

Smilax oldhami Miq. is a perennial plant of the liliaceae family. In Japan, the plant grows indigenously in forest regions from Kyushu to the northern districts and is called "shiode". The young leaves and shoots of the plant are used as a fried food and as boiled greens with dressing for its fine flavour. Propagation of the plant from seeds is difficult. Recently, we reported methods for mass propagation of the plant by tissue culture (Yamamoto and Oda, 1992). Somatic embryos, induced directly on the surface of leaf explants, were considered to be effective for the mass propagation of the plant. However, embryogenesis was sporadic and it took about six months to occur. The present paper describes the in vitro regeneration of shoots from seedling shoot and root explants.

MATERIALS AND METHODS

A donor plant was produced aseptically by culturing a nodal segment (3 mm in length) taken from an in vivo plant. The methods for sterilization and composition of medium were same as those described previously (Yamamoto and Oda, 1992). From the donor plant in vitro segments of node, internode (10 mm in length), and root (10 mm in length) were taken and placed on each medium. Nodal segments were cultured on 1/2 Murashige and Skoog (MS) medium without hormones. Internodal explants were cultured on MS medium supplemented with BA (6-benzyladenine) (1 mg/l) and/or 2,4-D (2,4-dichlorophenoxyacetic acid) (1 mg/l) for two weeks, then transferred to hormone-free medium. Root segments taken from proximal, median, and distal parts of the root, were cultured on MS medium containing cytokinin and auxin. Hormones used were BA, KIN (kinetin), ZEA (zeatin), NAA (naphthaleneacetic acid), IBA (indolebutyric acid), and 2,4-D. The

concentrations of these hormones were adjusted to 0.1 mg/l, because it had been already reported that low concentrations of hormones were effective for shoot formation from root segment (Lazzeri and Dunwell, 1984a; b; 1986; Chen et al., 1987; Dubois et al., 1990). Cultures were kept at 25°C and 16-h photoperiod. For acclimatization, the regenerated plants were transferred to pots (9 cm in diameter) containing vermiculite.

RESULTS AND DISCUSSION

Axillary buds were easily induced from lateral meristems within a short period. After 17 days of culture on 1/2 MS medium without hormones, the ratio of the explants with shoot to those cultured was 0.92, and cytokinins such as BA and KIN had little effect on axillary bud induction.

Table 1. Effect of BA and 2,4-D on shoot formation from internodal segments.

BA (mg/l)*	2,4-D (mg/l)*	Explant with shoot %	Number of shoots per explant
0	0	28	0.5
0	1.0	100	8.2
1.0	0	24	0.2
1.0	1.0	21	0.2

Each value was scored after 100 days in culture.
* These hormones were supplied for the initial two weeks of culture.

As shown in Table 1, the highest shoot formation from the internodal segments was achieved by the treatment of 2,4-D for an initial two weeks of culture, and the average number of shoots per explant was 8.2. The shoots regenerated were regarded as adventitious shoots formed directly from the explants. The effects of 2,4-D on shoot formation were more favourable than those of BA and NAA reported previously (Yamamoto and Oda, 1992).

Table 2. Shoot formation from root segment.

	Explants with shooting (%)		Number of shoots per explant	
	Days in culture			
Root segment	35	70	35	70
Proximal	30	80	1.0	4.9
Median	30	70	0.6	4.9
Distal	0	20	0	0.4

The root segments were placed on the various media with nine combinations of cytokinins and auxins. The formation of embryogenic callus was observed only for combinations of BA and NAA. The data for shoot formation are shown in Table 2. The frequency of shoot formation was highest in the proximal region of the original root. This is consistent with the observations for *Brassica oleracea* and *B. napus* (Lazzeri and Dunwell, 1984a; Sharman and Thorpe, 1989). After 70 days in culture the number of the shoots regenerated averaged 3.4 for the three segments.

Table 3. Ratio of the shoots rooted to those cultured in rooting medium.

Type of explant	NAA in rooting medium (mg/l)	Days of culture in rooting medium	
		30	45
Node	0	0.0	0.1
	0.5	0.8	0.8
Internode	0	0.6	0.9
Root	0	0.7	1.0

Table 3 shows the ratio of the shoots rooted to those cultured in rooting medium. A marked effect of NAA was observed on the rooting of shoots induced from nodal segments. In a previous experiment, it was observed that auxins were necessary for the rooting of shoots induced from nodal segments, and that NAA was better than IAA (indoleacetic acid) and IBA (Fukuda et al., 1990). On the other hand, shoots regenerated from internode and root explants easily rooted on 1/2-strength MS medium without auxins. The rooting ratios for shoots regenerated from internodal and root segment were 0.9 and 1.0 after 45 days of culture, respectively.

Figure 1. Smilax plantlets after acclimatization.

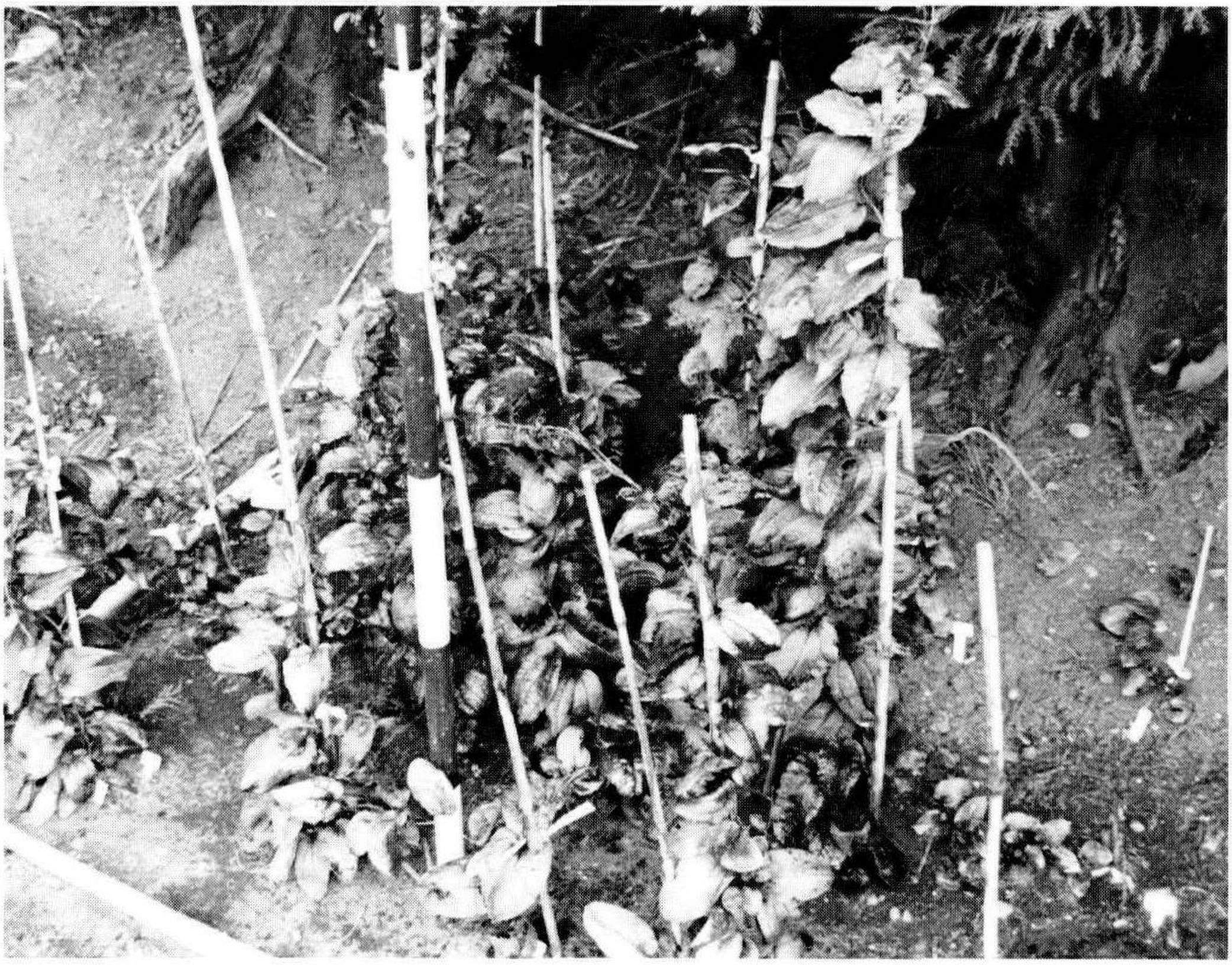

Figure 2. Growth of the plants propagated by the tissue culture.

The number of plantlets produced per explant (P) is an important index for planning practical propagation. When the propagation was carried out from one type of explant, the rate of propagation P can be expressed by the following equation: $P = S \times R \times f \times A$, where S is the number of shoots regenerated per explant, R is rooting ratio, f is the ratio of the plants available for acclimatization to those regenerated in vitro, and A is the acclimatization rate. From our research and experience, we consider values for f and A of 0.9 and 1.0, respectively, to be correct. As shown in Table 4, the rate of propagation from the internodal explant was higher than those from the other two explant types.

Table 4. Rate of propagation from node, internode, and root explants.

Type of explant	Number of shoots formed (S)	Rooting (R)	Rate of propagation (P)	Number of explants* (N)	(P × N)
Node	0.9	0.84	0.70	6	4.2
Internode	8.2	0.93	6.90	5	34.5
Root	3.4	1.0	3.06	12	36.7

P = S R f A (f = 0.9, A = 1.0) The sum of (P × N) = 75.4

* The explants were taken from an in vitro donor plant.

The number of explants obtained from the donor plant in vitro (N), and the product of N and P are also shown in Table 4. The value, N × P, corresponds to the number of plantlets produced per N explants of a type taken from the donor plant. Accordingly, the product N × P of 75.4 can be regarded as the total number of plantlets produced from a nodal segment of an in vivo plant, indicating the rate of propagation from the explant in the present culture system. This is a rough estimation, because some shoots were subsequently regenerated after cutting off the shoots formed before. Taking this point into consideration, it could be concluded that in the present culture system more than 75 plantlets were obtained from one nodal segment. Consequently, if the 75 plants regenerated in vitro are used as new donor plants, more than 75^2 plantlets will be obtained several months later. The plants propagated by this culture system have grown normally in soil for more than three years. Figures 1 and 2 show the plantlets acclimatized and the plant growing in the soil, respectively.

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