Regeneration of Sweet Pepper (*Capsicum annuum* L.) from Axillary Bud Induction in Vitro

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Donor plants of 10 cultivars of sweet pepper were produced in vitro from seeds. Having been excised from the donor plant, nodal segments were placed on a modified half-strength Murashige and Skoog (MS) medium. The axillary shoots induced from the explants were transferred to the rooting medium. Benzyladenine (BA) in the medium for culturing of donor plant was not effective on the induction of axillary buds from the nodal segments. Rooting of the axillary shoot was promoted by 0.2 mg/liter indoleacetic acid (IAA) in the medium. Cultivar difference in potential for plant regeneration was observed, which was mainly attributable to the difference in rooting ability among these cultivars. The plants propagated by nodal segment culture developed normally in the greenhouse as well as those grown from conventional seedlings.

INTRODUCTION

Sweet pepper is widely used as an important fruit vegetable. In Japan, the amount of its production has been increasing, and has now reached 200,000 tons per year. Most growers use the seeds of a F1 hybrid cultivar which are expensive. Moreover, they have to nurse the seedlings for transplanting in a greenhouse. By using tissue culture two advantages are expected. One is supplying a good cultivar which is genetically stable. The other is labour saving for growing seedlings in a greenhouse. Up to now, several investigations have been reported on plant regeneration of sweet pepper from various explants, cotyledon (Gunay and Rao, 1978; Sripichitt et al., 1987), hypocotyl (Fári and Czákо, 1981), embryo (Agrawal and Chandra, 1983), and leaf disc (Phillips and Hubstenberger, 1985). In general, axillary bud induction from the node explants is often used in some horticultural crops. In this case, promotive effects of benzyladenine (BA) on axillary bud induction have been reported (Kantharajah and Dodd, 1990; Ni and Wetzstein, 1990; Xiao-Shan Shen et al., 1990). In our preliminary experiment using sweet pepper, it was also observed that 0.5 mg/liter BA in the medium for culturing of donor plant had positive effect to some extent on plant regeneration from the node explant (Yamamoto et al., 1991). In sweet pepper, however, the effect of BA has to be ascertained using a number of cultivars. The present paper describes the effect of BA in the medium for culturing of donor plant (BA pre-treatment) on organogenesis of nodal explants, cultivar differences in potential for plant regeneration, and the growth of the regenerated plants in greenhouse.

MATERIALS AND METHODS

Ten cultivars of sweet pepper used are shown in Table 1. After being sterilized

with 1% sodium hypochlorite solution, seeds of these cultivars were placed on a half strength MS medium without hormones. After two weeks plantlets which germinated were transferred to a medium with 0.5 mg/liter BA or without BA,

Table 1. Axillary bud induction and rooting of ten *Capsicum* cultivars.

Cultivars	BA pretreatment (mg/liter)	Bud induction (%) 3 (Days)	10 (Days)	Rooting (%) 15 (Days)	30 (Days)[1]
Kyounami	0	33.3	94.3	18.2	37.5
	0.5	53.8	93.5	17.2	31.2
Hokuto	0	64.2	94.0	22.4	33.8
	0.5	57.4	93.6	10.6	19.1
Tokahikari-D	0	19.0	95.2	7.5	33.3
	0.5	37.5	94.6	10.7	16.1
Tosakotobuki	0	39.1	95.7	0	39.1
	0.5	33.3	77.8	0	7.4
Tosokatsura	0	45.0	95.0	0	30.3
	0.5	34.3	91.0	2.9	5.7
TM-5	0	33.3	97.2	2.8	20.8
	0.5	43.0	84.8	5.1	22.8
Shinsakigake	0	41.5	80.5	2.4	19.5
	0.5	43.2	75.7	0	5.4
Golden Bell	0	56.8	91.9	0	2.7
	0.5	26.7	46.7	0	0
Wonder Belle	0	10.0	33.3	0	3.3
	0.5	13.6	27.3	0	4.5
California Wonder	0	12.5	33.3	0	0
	0.5	0	18.8	0	0
Mean	0	35.5	81.0	5.3	22.0
	0.5	34.3	70.4	4.7	11.2
S.D.	0	17.9	25.6	8.3	15.2
	0.5	17.5	28.8	6.2	10.5
C.V.	0	0.50	0.32	1.57	0.69
	0.5	0.51	0.41	1.32	.94

S.D. standard deviation; C.V. coefficient of variation.
[1] Days of culture in the rooting medium.

and cultured for two successive weeks. Nodal segments taken from the donor plants were placed on the half strength MS medium. Axillary shoots induced from the explants were cultured on the rooting medium. Cultures were kept at 25C and 16 h photoperiod. After acclimatization for one week the regenerated plants were grown in pots (18 cm in diameter). When the plants attained about 30 cm in height, these plants were transplanted to the soil in the greenhouse. The growth period in the greenhouse was approximately 10 months.

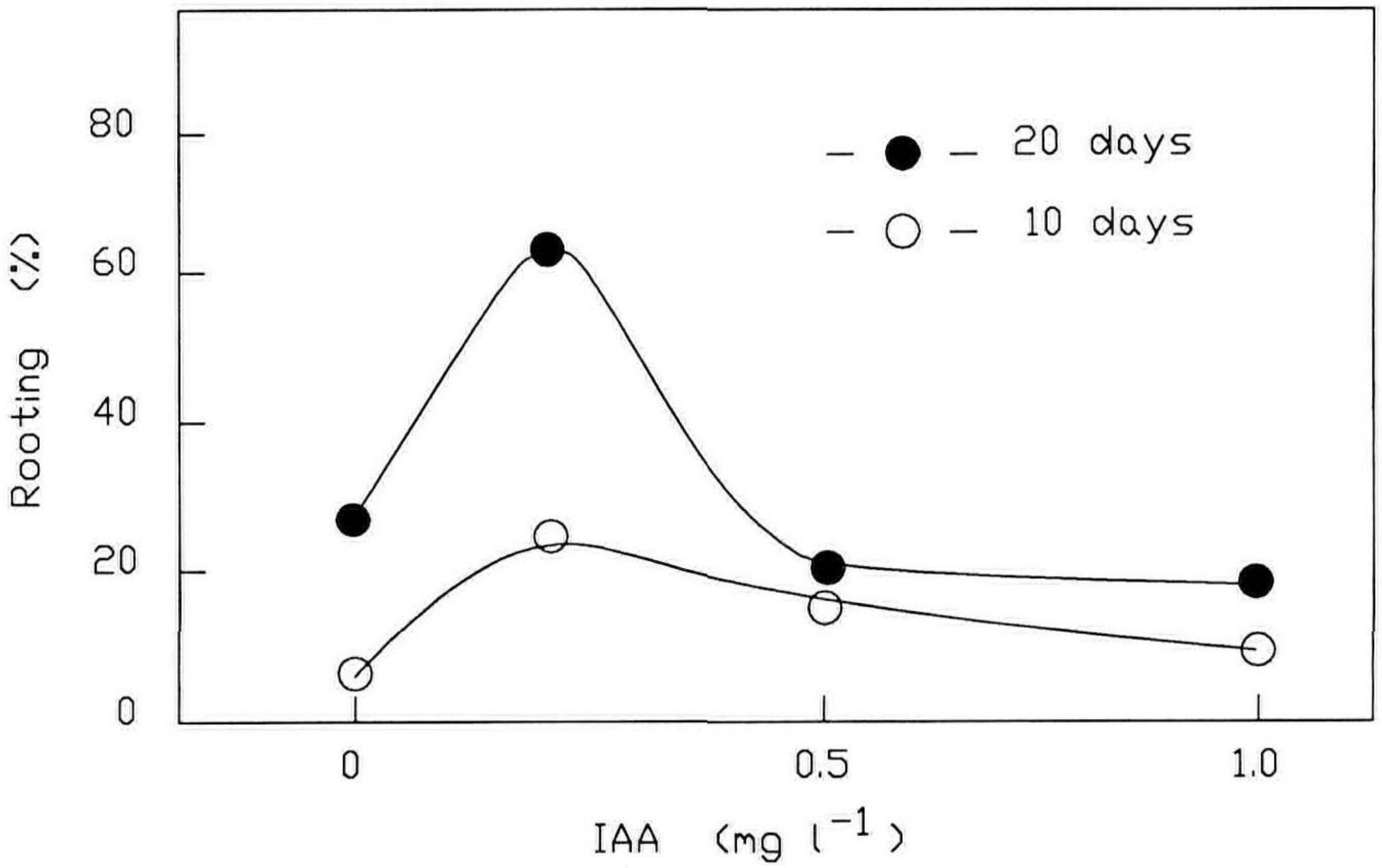

Figure 1. Effect of IAA concentration on rooting of axillary shoots.

RESULTS AND DISCUSSION

Table 1 shows axillary-bud induction and rooting from the node explants of 10 cultivars. After 20 days of culture without BA more than 80% of axillary-bud induction was observed in the eight cultivars except for Wonder Belle and California Wonder. The rooting of Golden Bell, Wonder Belle, and California

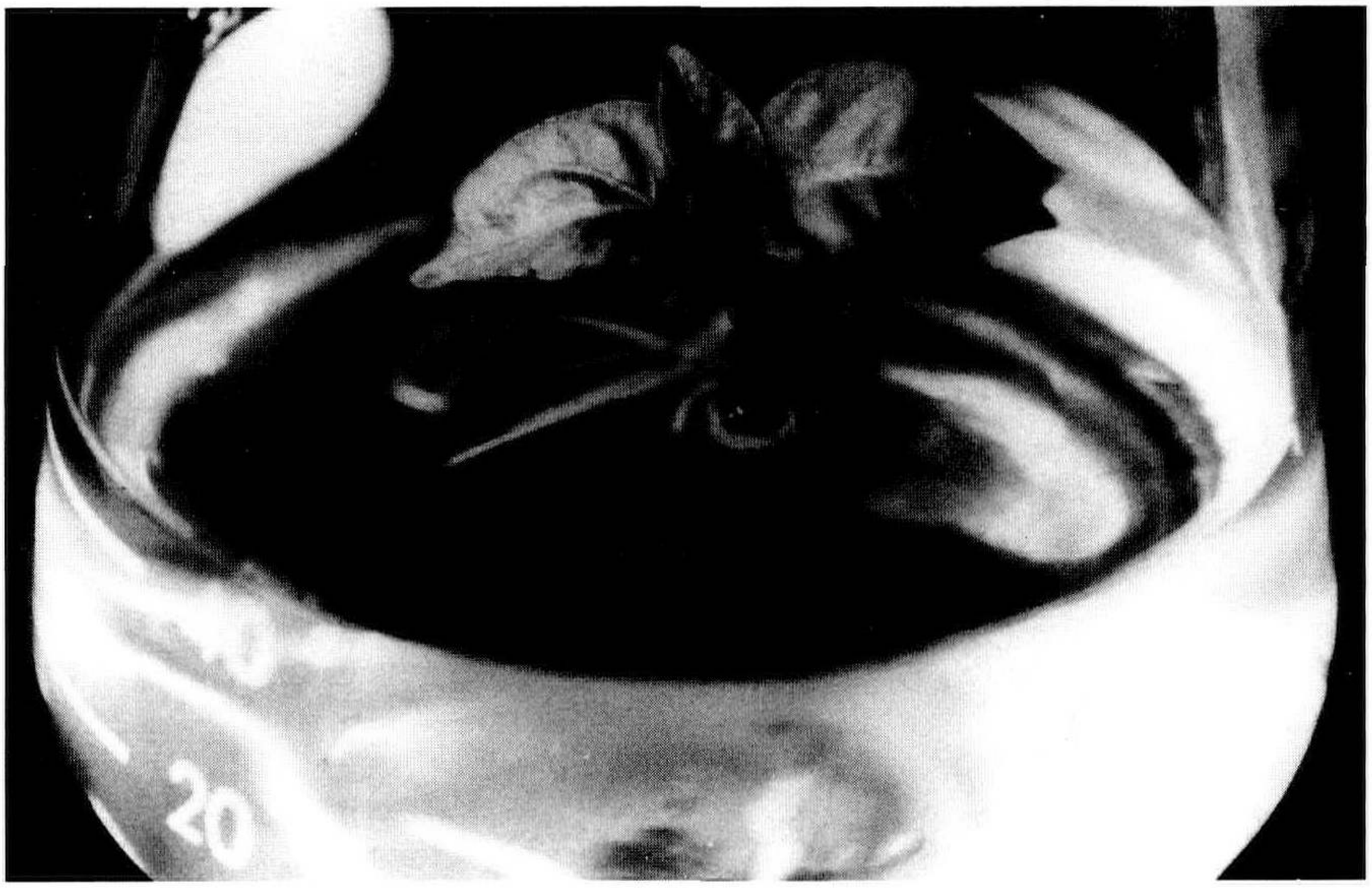

Figure 2. A plant regenerated by nodal segment culture.

Figure 3. Growth of the regenerated plants in greenhouse

Wonder were very low compared with the other seven cultivars. On the whole, BA had little effect on the axillary-bud induction but a decreasing effect on the rooting. The coefficients of variation for rooting were considerably higher than those for axillary-bud induction. This indicates that cultivar differences in rooting are larger than that in axillary-bud induction. The results can be explained from the difference in the mode of organogenesis between the axillary-bud induction and the rooting. The former is considered to be outgrowth of bud primordia which had been already formed on axillary meristem. In contrast the rooting is a new differentiation occurring during the in vitro culture. From the data shown in Table 1 it can be also concluded that the 10 cultivars can be divided into three groups with regard to potential for plant regeneration which is mainly attributable to the difference in rooting ability. 'Kyounami', 'Hokuto', 'Tokahikari-D', 'Tosakotobuki', and 'Tosokatsura' have higher potential for plant regeneration, while the lowest were 'Golden Bell', 'Wonder Belle' and 'California Wonder'. TM-5 and 'Shinsakigake' are intermediate. Figure 1 shows the effect of indoleacetic acid (IAA) concentration on rooting of axillary shoots. The rooting was maximum in the half-strength MS medium supplemented with 0.2 mg/litre IAA. A typical example for the plant regenerated by the nodal segment culture is shown in Figure 2. At this stage, the plants were transferred to small pots in a plastic box for acclimatization. After acclimatization for one week, the plants were grown in the pots (18 cm in diameter). When the plants attained about 30 cm in height, these were transplanted to soil in the greenhouse. Figure 3 shows the regenerated plants grown two months after transplantation in the greenhouse. The plants propagated by the nodal segment culture developed comparable to those grown from conventional seedlings in the greenhouse.

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