RESULTS AND DISCUSSION

In the 8H and 12H treatments, the growing points were between the flower cluster initiation and flower cluster differentiation stages. The flower cluster initiation stage was dominant in treatment 8H and the flower cluster differentiation stage was dominant in treatment 12H. In treatment 16H, aII of the growing points formed flower clusters (Table 1). All of the seedlings bolted in treatment 16H. The leaf length, root length, and shoot fresh mass were not significantly different among the treatments, while the shoot fresh weight and shoot and root dry weight were greater in treatment 16H than those in treatments 8H and 12H.

In conclusion, the short photoperiod treatment retarded flower bud development of *S. oleracea* seedlings. This result could be beneficial when producing seedlings for transplanting, with the aid of artificial light.

The Growth and Development of Cut-flower Rose Cultivars in Shoot-Tip Culture

Z. Lin and S. Hatasa
United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu 501-1193

H. Fukui and Y. Asano
Faculty of Agriculture, Gifu University, 1-1 Yanagido, Gifu 501-1193

S. Matsumoto
Faculty of Education, Gifu University, 1-1 Yanagido, Gifu 501-1193

The shoot-tip culture and propagation in vitro of roses for cut-flower production was developed in order to test their resistance to crown gall. Terminal buds were taken from the shoots after they had grown 1 cm following flower harvest. These buds grew well and culturing in May was found to be the most suitable season for *Rosa* 'Carl Red'. After the in vitro culturing of 25 cut-flower cultivars, MS medium containing BAP and GA_3 was found to give the best results. The most suitable medium for maximum viability, leaf number, lateral shoots, and maximum shoot length was in most cases the same one for a given cultivar.

INTRODUCTION

The selection of resistant rose cultivars to crown gall disease by in vivo inoculation has been reported on (Boelema, 1969; Ohta, 1993). However, climate, soil conditions and plant growth affect this method.

Recently, tissue culture techniques have been applied to plant breeding (Toyoda et al., 1989; Isizawa et al., 1992; Chatani et al., 1996).

We have developed an in vitro inoculation method for testing the resistance of roses to crown gall disease (Zhou et al., 1996). For this method, culturing and propagating plant cultivars in vitro is important. Therefore, we have propagated 20 types of rose

rootstocks by shoot-tip culture and the propagation of these rootstocks has been standardised (Zhou et al., 1997). In this paper, we report on the multiplication of 25 cut-flower rose cultivars and their growth and development in shoot tip culture.

MATERIALS AND METHODS

Experiment 1: The Collection Season, Method of Shoot-Tip Use and Selection of the Medium. The cut-rose cultivar *Rosa* 'Carl Red', grown in the greenhouses of Gifu Takahashi Rose Garden Ltd., in Gifu City, Japan, was used in this experiment.

1) The 2nd, 3rd, 5th, and 7th nodal segments with an axillary bud were excised from the flowering shoots.
2) When the bud from below the cut had grown 1 cm, the segment with a terminal bud was cut off.
3) When the shoot from below the cut had grown 3 cm, the segments with a terminal bud and an axillary bud were cut off. These segments with shoot tips were surface sterilized for 10 min in a 1% (v/v) sodium hypochlorite solution containing 0.01% (v/v) Tween-20 and rinsed three times with sterile distilled water. Under sterile conditions, the shoot tips (1 mm) were cut out from these shoot segments, and put into Murashige and Skoog (MS) medium containing 3% sucrose and 0.2% Gelrite.

Factorial combinations of three gibberellic acid (GA_3) concentrations (0,0.1 μM and 1.0 μM) and three 6-benzylaminopurine (BAP) concentrations (0.1 μM, 1.0 μM, and 10 μM) were used, giving nine treatments. The pH of each medium was adjusted to 5.7 with 0.1 N NaOH and HCl before the addition of Gelrite. The medium was then autoclaved at 120C for 15 min. The cultures were kept at 25C with a 16-h light period for 6 weeks. This experiment was repeated three times, in May and Sept. 1993 and in Jan. 1994.

Experiment 2: The Growth Habit of the Cultivars. Twenty-five cultivars of cut-flower rose cultivars that had been grown in greenhouses in Godo Town in Gifu Prefecture were used in this experiment. In May 1994, the shoot tips were excised from the 5th segments on the flowering shoots and put into MS medium containing 3% sucrose, 0.2% Gelrite, and 10 μM BAP. Two GA_3 concentrations were used, 0.1 and 1.0 μM. The pH of each medium was adjusted to 5.7 with 0.1 N NaOH and HCl before the addition of Gelrite. All media were autoclaved at 120C for 15 min.

RESULTS

Table 1. Growth of shoot-tip cultures by different culture season.

	Culture date		
	September	January	May
Survival rate	70	67	78
Leaves number	0.4	0.3	0.8
Leaves length (mm)	10.5	9.3	11.9

Table 2. Growth of shoot-tip cultures from different bud positions.

	Axillary buds of different node-order on flowering shoots				Elongated shoots (1 cm) after harvesting	Elongated shoots (3 cm) after harvesting	
	2nd	3rd	5th	7th	terminal bud	terminal bud	axillary bud
Survival rate (%)	73	69	78	76	86	70	60
Leaves number	0.2	0.2	0.3	0.3	0.9	0.4	0.4
Leaves length (mm)	11.0	12.2	9.5	10.5	12.3	10.7	9.3

Table 3. Effect of different concentrations of BAP and GA_3 on the growth of shoot tip cultures.

	BAP (μM)			GA_3 (μM)		
	0.1	1.0	1 0	0	0.1	1.0
Survival rate (%)	57	68	93	81	75	65
Leaves (number)	0.6	0.4	0.1	0.3	0.4	0.4
Leaves length (mm)	12.3	9.5	7.0	10.0	12.0	11.0

Experiment 1. For all buds, the growth and development of shoot tips cultured in May were better than those cultured in September and January. The survival rate of shoot tips cultured in May was highest at 78%, whereas the survival rates for September and January were 67% and 70%, respectively (Table 1). The elongation of shoot tips was not found on any cultures. All cultures had induced leaves and the length of these leaves was about 10 mm. The growth of shoot tips collected from different positions is given in Table 2. The terminal buds that had been cut out from the elongated shoots (1 cm) after flower harvest, grew well on all media, with a survival rate of 86%, the leaves were 12.3 mm long and the average leaf number per plantlet was 0.9. The shoot tips excised from axillary buds on the elongated shoots (3 cm) after flower harvest had a survival rate of 60% and a 9.3 mm leaf length. For the flowering shoots, the lateral buds from the upper two segments grew better than those from the lower two segments. Table 3 shows the growth and development of cultures in media containing different concentrations of BAP and GA_3. The viability was high, with 93% survival rate on the media containing 10 μM BAP, but viability was only 57% on media containing 0.1 μM BAP. On media without GA_3, the viability was high (81%), and as the GA_3 concentration increased the viability dropped. The leaf number and the maximum leaf length were no different among media containing different concentrations of GA_3, but they were affected by BAP concentrations. On media containing 0.1 μM BAP, the mean leaf number was 0.6 and the maximum leaf length was 12.3 mm, but these were lower on the other media.

Experiment 2. In primary culture, the shoot tips of 20 cultivars had high viability (over 90%), while 5 cultivars had a viability of 70% to 85%.

Leaves were induced on all of the cultivars. Two or more leaves were produced on 10 of the cultivars. The shoot tips of 60% of all cultivars had elongated and those of 36% had developed lateral shoots. After the third subculture, all of the cultivars were growing well and the growth and development habit of those cultivars was steady. Among the hybrid tea cultivars, 14 had long shoots (10 to17 mm), while the four miniature rose cultivars had shoot lengths under 10 mm. Most cultivars had 10 to 25 leaves, but 'Bekola', Aalsmeer Gold® rose; 'Kardinal', Kardinal® rose; 'Meikola' Livia™ rose; and 'Meihaitoil', Concerto® rose had under 10 leaves. 'Kawamoblue', Purple Rain® rose, and 'Intermoto', Joy® rose had more than three lateral shoots, but Aalsmeer Gold® rose and Kardinal® rose had only 1.4 lateral shoots. The MS medium with BAP and GA_3 was suitable for the shoot tip culture of these cut-flower rose cultivars (Table 3). The most suitable medium for the

maximum viability, leaf number, lateral shoot number, and shoot length was the same in most cases for a given cultivar, but differed between cultivars.

DISCUSSION

From our experiments, it is clear that the position of the buds and their season of collection affect the growth and development of cultures in vitro.

Normally, roses grown in greenhouses during summer became dormant because their growth and development was affected by high air temperatures.

After the plants resumed growth in September, their vitality was lower. In winter, the intensity of solar radiation is lower and the photoperiod is shorter and so the growth of plants is inhibited, leading to dormancy.

For this reason, the growth and development of these shoot tips was inhibited after they were cultured in vitro. However, in May the growth and development of the plants was active and shoot tip vitality high.

For buds taken from different positions, the growth and development in vitro is different. Bressan et al. (1982) reported that the shoot apex of a rose stem functions to regulate the growth and development of the lateral buds subjacent to it even after those buds have been cultured in vitro. From our results it was also observed that the shoot apexes, on the 1-cm and 3-cm shoots that formed after flower harvest, showed high viability, with more and longer leaves. These shoot apexes developed more rapidly than lateral buds subjacent on the elongated 3-cm shoot. The regulatory effect of the shoot apex has been described (Zieslin et al., 1976, 1978) and it is thought that this apical dominance is the result of a number of inhibitors, including auxin and abscisic acid (Zieslin et al., 1978). The MS media used in this experiment was without auxin and abscisic acid.

Therefore, our interpretation is that the buds on the shoots contained different levels of these inhibitors. The buds in acropetal and basipetal positions on the stem were the most inhibited, while those from the middle section of the stem developed most rapidly. The inhibition of development of buds obtained from nodes at the base of the stem may be due to the proximity of these buds to the main stem or trunk of the rose. These buds are under the control of the shoot apex of the main stem (Bressan et al.,1982). In our experiment, the buds in the 2nd, 3rd, 5th, and 7th segments were used, but the uppermost buds were not used, because they are very small.

The results showed that the buds in the basipetal position grew the slowest, while the buds in 7th and 5th sections, those in the middle of the flowering shoot, grew well.

Rout et al. (1989) reported that the growth and development of cultures in vitro of *R.* 'Landorain' were the best on an MS medium with BAP and GA_3. Our experimental results also showed that MS media with BAP and GA_3 was suitable for the shoot-tip culture of these rose cultivars. The most suitable medium for the maximum viability, number of leaves, number of lateral shoots per plantlet, and the maximum shoot length, was in most cases the same one for a given cultivar, but it was different between the cultivars. For some cultivars, the most suitable medium for shoot elongation was different than that for the formation of leaves and lateral shoots. The growth of shoot tips in vitro is different because of the physiological and genetic differences of different rose cultivars (Cai et al., 1984). Our interpretation is that this different reaction to BAP and GA_3 may be due to the special physiological and genetic characteristics of these cultivars.

ACKNOWLEDGEMENT

We thank Dr. T. P. McGonigle for his suggestions for the research.

LITERATURE CITED

Boelema, B.H. 1969. Resistance of rose rootstock to crown gall (*Agrobacterium tumefaciens*). Neth. J. Plant Pathol. 75:147-150.

Bressan, P.H., Y.J. Kim, S.E. Hyndman, P.M. Hasegawa, and **R.A. Bressan** 1982. Factors affecting in vitro propagation of rose. J. Amer. Soc. Hort. Sci. 107: 979-990.

Cai, J., M.Y. Cai, and **D.L. Qian** 1984. Induction of multiple shoots and rapid propagation of clones of China rose (*Rosa chinensis*). Plant Physiol. Comm. 5:37-38. (In Chinese).

Chatani, K., H. Toyoda, K. Yoshida, and **K. Koreeda** 1996. Selection of powdery mildew-resistant regenerants obtained from leaf explant-derived calli of *Rosa hybrida*. J. Japan. Soc. Hort. Sci. 65:381-385.

Ishizawa, Y., H. Kyoutani, and **K. Nishimura** 1992. Establishment of testing methods of resistance to crown gall in peach rootstocks. J. Japan. Soc. Hort. Sci. 61:(Suppl.1)154-155. (In Japanese).

Ohta, K. 1993. Studies on crown gall and hairy root of flower crops. Technical Bulletin No.16 (In Japanese).

Rout G.R., B.K. Debata, and **P. Das** 1989. In vitro mass-scale propagation of *Rosa hybrida* cv. Landara. Curt. Sci. 58:876-878.

Toyoda, H., K. Shimizu, K. Chatani, N. Kita, Y. Matsuda, and **S. Ouchi** 1989. Selection of bacterial wilt-resistant tomato through tissue culture. Plant Cell Rept. 8: 317-320.

Zhou, L., H. Fukui, and **S. Matsumoto** 1996. In vitro inoculation test for resistance to crown gall disease on roses. Comb. Proc. Intl. Plant Prop. Soc. 46:750-754.

Zhou, L., K. Suzuki, T. Naruse, H. Fukui, and **S. Matsumoto** 1997. Specific differences in shoot tip culture of roses. Comb. Proc. Intl. Plant Prop. Soc. 47:645-647.

Zieslin, N., H. Haaze, and **A.H. Halevy** 1976. Components of axillary bud inhibition in rose plants. II. The effect of bud position on degree of inhibition. Bot. Gaz. 137:297-300.

Zieslin, N., H. Spiegelstein, and **A.H. Halevy** 1978. Components of axillary bud inhibition in rose plants. IV. Inhibitory activity of plant extracts. Bot. Gaz. 139:64-68.