Effects of low temperature treatment on plantlet growth and development of *Asparagus officinalis* L. *in vitro*

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Abstract: The effects of low temperature on growth and development of asparagus were investigated using 'Mary Washington 500 W' plantlets. The explants of single node were cultured on MS medium with the combination of 0.05 mg/l NAA and 0.05 mg/l BA. After the culture at 20°C for 4 weeks, the explants were treated with the low temperature treatment of 5°C for 1, 2, 4, 6 or 8 weeks. After the treatment, they were cultured at 20°C again.

When asparagus plants were cultured at low temperature for 1 or 2 weeks, plantlet growth and development were suppressed even at 20°C. After they were cultured at 5°C for 4 weeks, plantlet growth and development recovered at 20°C. Whenever the low temperature treatment continued longer than 4 weeks, the growth and development of plantlets did not recover.

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Key words: asparagus, low temperature, growth and development, *in vitro*

Introduction

In early spring, new spears sprouted and developed into shoots. When shoots grew well, new roots and buds at crown were initiated and their formation continued until winter (Haynes, 1987). Shoot emergence is terminated and bud growth is stopped by low temperature in fall. Culpepper•Moon (1939) reported that very little or no growth of asparagus will occur below 40 F (4.44°C). Krug (1996) reported that low temperature plays a dominant role in controlling the seasonal growth cycle of asparagus. Many reports suggested that asparagus crowns stay dormant during the winter months (Matusbara 1980; Takatori, 1985; Pressmen *et al*, 1989). Although low temperature is viewed as the regulator of growth and development of asparagus, the effects of low temperature on growth and development of asparagus was not studied yet.

The effects of low temperature on growth and development of asparagus may be evaluated by measurement of buds growth rate after plants have been exposed to temperature favorable for growth. However, the major obstacle to study this purpose is the limit of growth chambers used for the temperature control. To solve this problem the experiment was designed to deal using *in vitro* plantlets.

In vitro plantlets can be regenerated from a single cell of protoplast isolation (Guangyu *et al*, 1997), or from embryogenic calli (Levi and Sink, 1991; Kohmura *et al*, 1994; Li and Wolyn, 1996). In many reports plantlets were regenerated from organ cultures (Yang and Clore, 1973; Matsubara and Clore, 1974; Yang, 1977; Chin, 1982; Desjardins *et al*, 1987; Slabbert *et al*, 1990; Conner *et al*, 1992). In organ cultures, plantlets were generally regenerated from stem explants with nodes or the other explants on MS medium with auxin and cytokinin. However, the effects of low temperature on growth and development of asparagus plantlets were not yet investigated well.

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Therefore, this report aimed to investigate the effects of low temperature on growth and development of asparagus plantlets *in vitro*.

Materials and Methods

Material preparation

The stem explants of 5 mm long with a single node were cut from 8-10 cm long stock shoots and were used as material in this experiment.

Culture medium

Murashige and Skoog's (MS) medium was supplemented with 0.05 mg/l NAA and 0.05 mg/l BA, 3% sucrose, adjusted pH to 7.75 and added 0.2% gellangum. One explant was cultured in 10 ml MS medium in 25×150 mm test tube.

Temperature treatment

As was shown in Table 1, three experiments were dealt to investigate the effects of low temperature on growth and development of asparagus.

In the first experiment, the explants were cultured at 20°C for 4 weeks. Then, they were cultured at the treatment of 5°C for 2, 4 or 6 weeks as a low temperature treatment. After the treatment they were cultured at 20°C again. The other explants were cultured at a constant temperature of 15, 20 and 25°C as a control.

In the second experiment, the explants were cultured at the same treatment condition as the first experiment and the treatment of 5°C for 8 weeks was added.

In the third experiment, the explants were cultured at the same treatment condition as the first experiment and the treatment of 5°C for 1 and 8 weeks were added.

Table 1 Temperature treatment

Experiment	Before treatment		Treatment		After treatment	
	(°C)	weeks	(°C)	weeks	(°C)	weeks
First	20	4	5	2	20	10
	20	4	5	4	20	10
	20	4	5	6	20	10
	15	4	15	6	15	10
	20	4	20	6	20	10
	25	4	25	6	25	10
Second	20	4	5	2	20	10
	20	4	5	4	20	10
	20	4	5	6	20	10
	20	4	5	8	20	10
	15	4	15	8	15	10
	20	4	20	8	20	10
	25	4	25	8	25	10
Third	20	4	5	1	20	10
	20	4	5	2	20	10
	20	4	5	4	20	10
	20	4	5	6	20	10
	20	4	5	8	20	10
	15	4	15	8	15	10
	20	4	20	8	20	10
	25	4	25	8	25	10

Twelve explants per treatment were used. The explants were cultured at 16 h in light condition with the intensity of 60 μmM $m^{-2}s^{-1}$ cool white fluorescence.

Measurement

Shoot length and the number of shoots per plantlet were measured every 2 weeks in the first and the second experiment and every 5 days in the third experiment. Amount of shoot elongation was calculated from the difference of shoot length before and after the treatment. The increased number of shoots per plantlet was calculated from the difference of shoot number before and after the treatment.

Results

Fig. 1 showed shoot length per plantlet at constant temperature at 15, 20 and 25°C. In the first experiment (Fig. 1-A), there was the tendency that shoot length at 25°C was higher than that at 20°C and 15°C. In the second experiment (Fig. 1-B), shoot length at 20°C was lower than at 25°C at the beginning but became similar afterward. In the third experiment (Fig. 1-C), shoot length at 25°C was similar to that at 20°C and that at 15°C was the lowest.

Fig. 2 showed the number of shoots per plantlet at 15, 20 and 25°C. There was the similar tendency that the

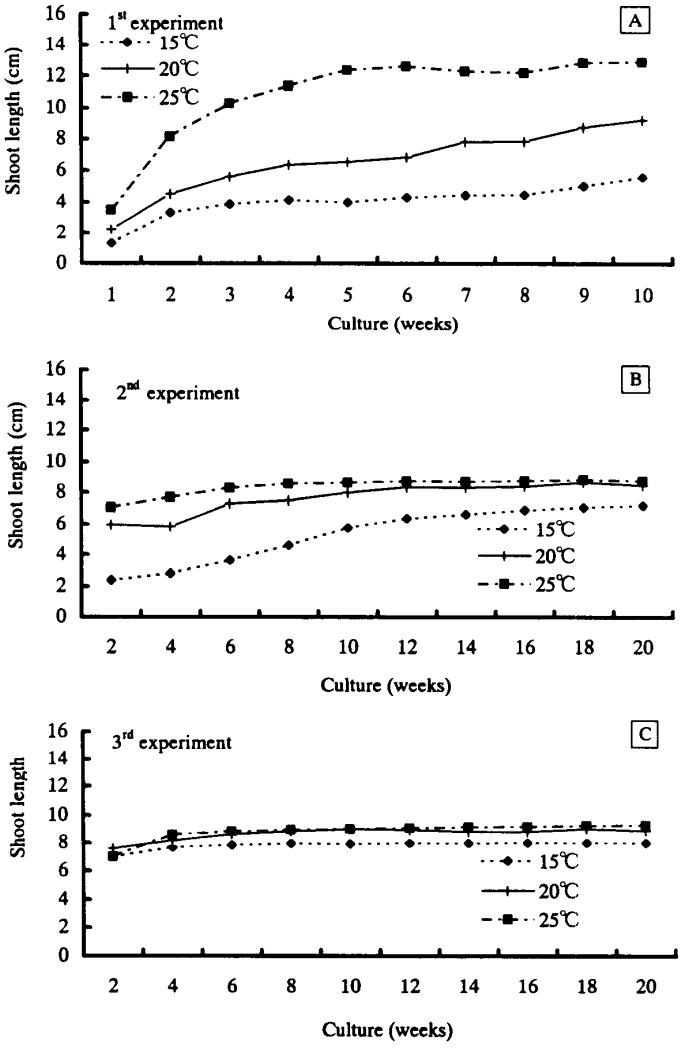

Fig. 1 Shoot length per plantlet cultured at constant temperature,
A: The first experiment
B: The second experiment
C: The third experimen

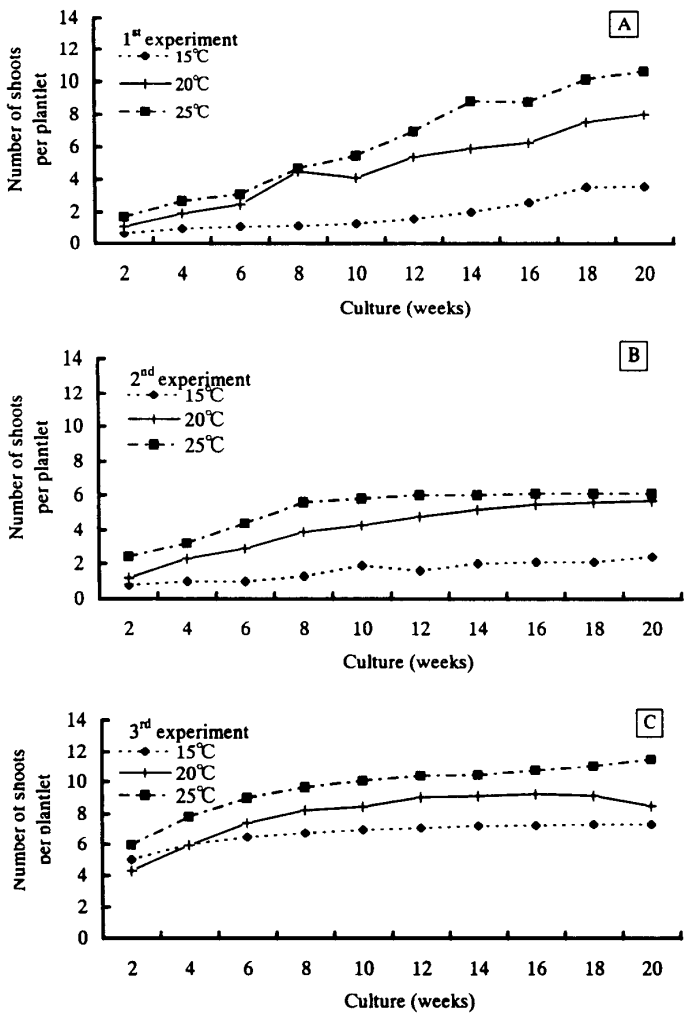

Fig. 2 The number of shoots per plantlet cultured at constant temperature,
A: The first experiment
B: The second experiment
C: The third experiment

number of shoots per plantlet at 25°C was higher than that at 20°C and 15°C in all repetitions. In the first experiment (Fig. 2-A), the number of shoots per plantlet at 25°C were similar to that at 20°C at the beginning but became higher afterward (Fig. 2-A). In the second experiment (Fig. 2-B), the number of shoots per plantlet at 20°C were lower than that at 25°C at the beginning but it became similar afterward. In the third experiment (Fig. 2-C). The number of shoots per plantlet at 20°C was lower than that at 15°C at the beginning but increased higher afterward.

Fig. 3 showed the effects of low temperature treatment on shoot elongation. In the first experiment (Fig. 3-A), shoot elongation was suppressed even at 20°C after the explants were cultured at the treatment of 5°C for 2 weeks. It was recovered at 20°C after the explants were cultured at 5°C for 4 weeks. When the treatment of 5°C continued to 6 weeks, shoot elongation was not recovered. The similar tendency of shoot elongation was shown in the second experiment (Fig. 3-B). When they were cultured at 5°C for 8 weeks, shoot length slightly increased at 20°C at the beginning and stopped elongating afterward. The third experiment showed a similar tendency (Fig. 3-C). After the explants were cultured at 5°C for 1 or 8 week, shoot elongation did not recover even at 20°C.

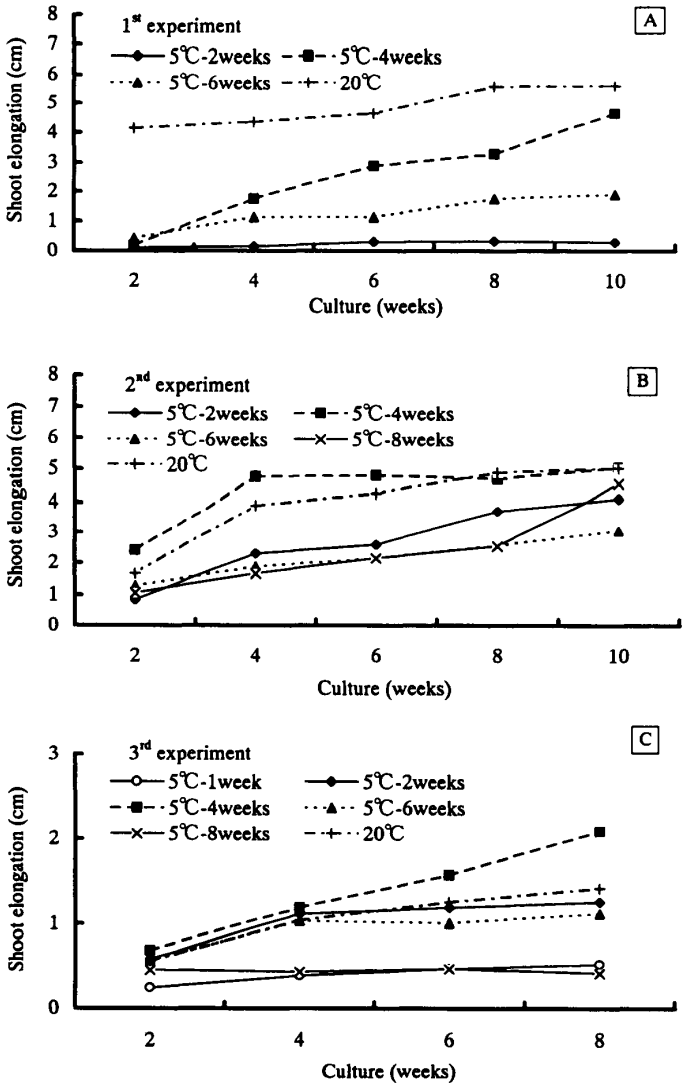

Fig. 3 Effects of low temperature treatment of 5°C on shoot elongation
A: The first experiment
B: The second experiment
C: The third experiment

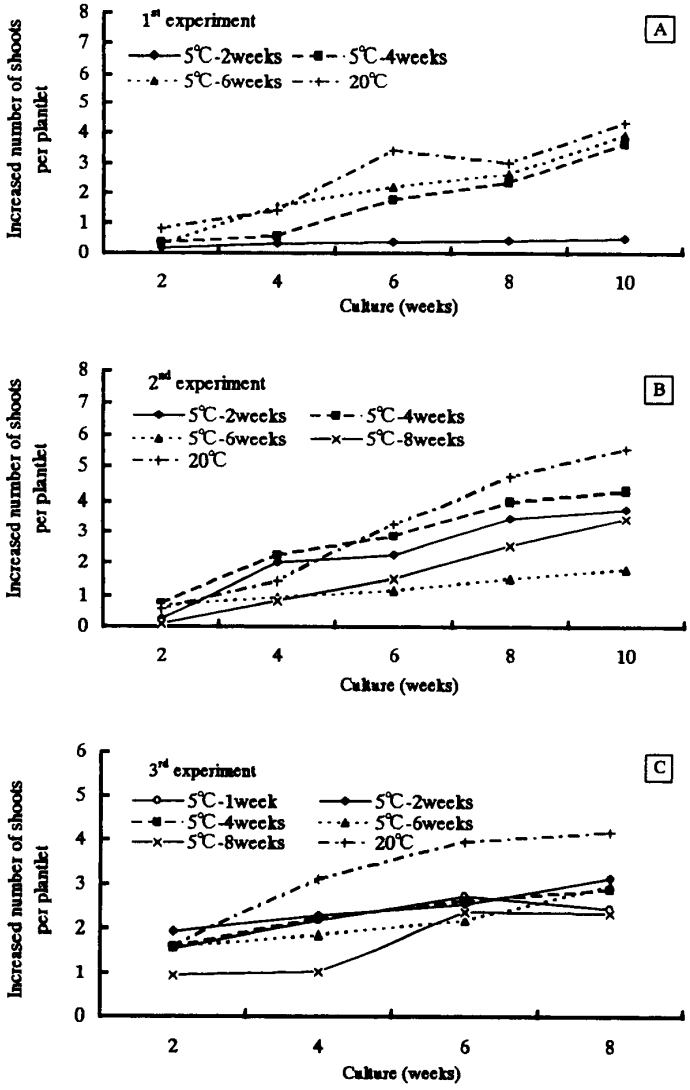

Fig. 4 Effects of low temperature treatment of 5°C on increased number of shoots
A: The first experiment
B: The second experiment
C: The third experiment

Fig. 4 showed the effects of low temperature treatment on the increased number of shoots. In the first experiment (Fig. 4-1), the number of shoots per plantlet did not increase even at 20°C after the explants were cultured at 5°C for 2 weeks. When the explants were cultured at 5°C for 4 or 6 weeks, the number of shoots per plantlets increased at 20°C.

In the second experiment (Fig. 4-B), the number of shoots per plantlet did not increase even at 20°C after the explants were cultured at 5°C for 2 weeks. After the explants were cultured at 5°C for 4 weeks, the number of shoots per plantlets increased at 20°C. When the treatment of 5°C continued to 6 or 8 weeks, the number of shoots per plantlet did not increase. In the third experiment (Fig. 4-C), the number of shoots per plantlet did not increase at 20°C after the explants were cultured at 5°C for 1, 2 or 4 weeks. When the explants were cultured at 5°C for 6 or 8 weeks, the number of shoots per plantlet did not increase at the beginning but slightly increased afterward.

Discussion

Shoot length and the number of shoots at the constant temperature of 25°C corresponded to the experiment by Hasegawa *et al* (1973) that reported a maximum plant formation in asparagus shoot apex culture at a constant 27°C temperature. In this experiment, the number of shoots per plantlet at 25°C was higher than that at 20°C but shoot length became similar.

The results in this experiment showed the tendency that plantlets growth and development were suppressed even at 20°C after they were cultured at the low temperature treatment for 1 or 2 weeks. After asparagus plantlets were cultured at the low temperature treatment for 4 weeks, the growth and development of asparagus were recovered at 20°C. Whenever the low temperature was longer than 4 weeks, the growth and development of asparagus were not recovered. Kurz *et al* (1989) reported the similar tendency in yellow cedar embryos that the treatment of 5°C for 2 weeks through 8 weeks resulting in a progressive increase in the mean number of shoot formed and a decline at 5°C for 12, 16 and 20 weeks.

Carew *et al* (2001) suggested that the less chilling the plants receive, the greater the depth of dormancy appeared to be and consequently the slower the rate of growth. This corresponded to the results in this experiment that shoot elongation at the treatment of 5°C for 4 weeks was higher than that at 5°C for 1 and 2 weeks. However, there was a decline of shoot elongation at the treatment of 5°C for 6 and 8 weeks. Moreover, shoot injury could be observed on some plantlets after they were cultured at the low temperature treatment. The long period of 5°C may lead to a decline of shoot length. Kozai *et al* (1987) suggested that the plantlets cultured under *in vitro* conditions are sensitive to environmental stress.

In a natural condition, the low temperature suppressed bud sprouting and elongation of sprouted buds. In the deciduous fruit tree, a minimum amount of chilling (4 to 10°C) was required for release from bud dormancy and initiation of growth (Young, 1990). In this experiment, the effect of low temperature on shoot elongation was investigated but bud sprouting was not. Therefore the results in our experiment can not describe the dormant character of asparagus well. To investigate bud sprouting the experiment may be applied by cutting plantlets shoots before they were cultured at the low temperature treatment.

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In vitro でのアスパラガスの生長と発育におよぼす低温処理の影響

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摘要：低温処理がアスパラガスの生長と発育に及ぼす影響を 'Mary Washington 500 W' を用いて調査した。一節をもつ外植体を0.05 mg/l NAA と0.05 mg/l BA 含む MS 培地で培養した。20°Cで 4 週間培養後，5 °Cで 1，2，4，6，8 週間培養した。処理の後は再び20°Cで培養した。

外植体が低温に 1 あるいは 2 週間処理された時，植物の生長と発育は20°Cに戻しても抑制された。外植体が低温に 4 週間処理されると，生長と発育は20°Cで回復した。4 週間以上低温処理が続くと，外植体の生長と発育は回復しなかった。