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Full Length Research Paper

The establishment of a slow-growth conservation system *in vitro* for two wild lily species

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There are abundant resources of wild lily in China. To achieve a sustainable use for these resources, a slow-growing preservation system should be well established. Therefore, to get an optimal storage condition, tube seedlings of *Lilium davidii* and *Lilium longiflorum* were used as explants cultured on nine different media at 24 and -2°C separately, which included: 1/4 or full Murashige and Skoog medium (MS) with 3, 6 or 9% sucrose, 1/4 or 1/2 MS with 1.0 or 3.0 mg/L abscisic acid (ABA). During the storage, the spout growth, and multiplication rate were measured every month. Results show that the growth restriction degrees for both species were related to the four factors (MS content, the concentration of sucrose and ABA, temperature). The inhibitory effect was more obvious at -2°C. At 24°C, the medium should be replaced every three to four months; otherwise the contamination rate would rise with the extension of storage time. At -2°C, the 1/4 MS with 9% sucrose or 3.0 mg/L ABA (M3A) were more effective, especially the latter one. The M3A played the greatest inhibitory effect on height growth of the two lily species, on which both species had been conserved on the original medium for more than 15 months. The tube seedlings conserved for 15 months could turn to normal plantlets after re-growth for one month which showed no obvious difference in morphology. 87.5% plantlets planted in peat could survive. In conclusion, the promising conservation condition was M3A at -2°C which was suitable for mid-long term preservation of lily germplasm resources.

Key words: Lily, wild species, slow-growth, conservation.

INTRODUCTION

There are rich resources of wild lily species in China (Fu et al., 2002). In recent years, lily resources' habitats have been suffering more and more serious destruction, and many wild species have become endangered species. So, germplasm conservation is very important as a source of genetic variation for breeding, research, and to prevent rare species from becoming extinct. Currently, the main ways of lily germplasm protection are the use of seed storage, field planting and tissue culture. Using seeds for preservation would break up unique genetic combinations, and the features would show segregation. Growth cycle from seed to flowering is too long, in addition to *Lilium formolongi*, normally takes two to four years. Field planting is costly and vulnerable to natural environment. Collections of bulb crops are usually maintained in the field or greenhouse by yearly planting, harvesting and storing the bulbs, leading to high investments of labor and space and risking losses caused by diseases (Towill, 1988; Withers, 1991). The maintenance of a germplasm collection *in vitro* has several advantages over storage of bulbs, as it requires small amounts of space and reduces the risk of losses caused by diseases (Towill, 1988). Since 1975, Henshaw and Morel proposed the preservation of plant germplasm *in vitro* for the first time; this technology had been highly valued (Villalobos and Engelman, 1995).

There are three types of *in vitro* conservation: normal *in vitro* culture preservation, cryopreservation and slow growth *in vitro* conservation. At room temperature (normal preservation), germplasm materials need frequent subculture (Peschke et al., 1992). In order to solve

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this problem, the slow growth conservation and cryo-preservation (that is, storage in liquid nitrogen, -196°C) have been developed. The basic work to achieve long-term preservation is the establishment of *in vitro* regeneration system. The greatest advantage of these two preservation methods is maintaining the material at a state of continued growth, while some material can be removed for other research (Mantell and Smith, 1983). Lily meristems have been successfully cryopreserved, by slow freezing (Bouman and De Klerk, 1990) and vitrification (Matsumoto et al., 1995). However, cryo-preservation requests high experiment technique, and the system is complicated operation.

Slow growth *in vitro* may be obtained by low temperature, osmotic stress (Grout, 1991; Withers, 1991), a low concentration of nutrients (Engelmann, 1991), growth inhibitor, or low oxygen content (Bonnier et al., 1996). Besides, temperature, sucrose and abscisic acid (ABA) are noticed to be the main factors involved in dormancy development (Aguettaz et al., 1990; Delvallée et al., 1990; Kim et al., 1994; Djilianov et al., 1994).

Low temperatures are often used to create conditions of minimal growth (Grout, 1991; Withers, 1991). In the commercial production of bulbs, the temperature of -2°C is commonly used to store lily bulbs (Beattie and White, 1993). Bulbs of Asiatic hybrids, Oriental hybrids and *Lilium longiflorum* could be stored in moist peat at temperatures between -1.5 and -2°C for one year (Beattie and White, 1993; Boontjes, 1983). Little research has been done in lily conservation *in vitro*. Right now, Bonnier and Van Tuyl (1997), and Chen et al. (2006) had carried out some relevant research. The preservation effects were different on different species and cultivars. Collections *in vitro* (Asiatic hybrids, Oriental hybrids) survived 28 months of storage under 25 and -2°C, whereas at -2°C, *L. longiflorum* and *Lilium henryi* survived six months of storage, but died during prolonged storage (Bonnier and Van Tuyl, 1997). Storage at a lower temperature than -2°C would further minimize growth conditions and would therefore increase maximum storage duration. However, freezing tolerance of lily should be increased to reduce the risk of freezing injury (Bonnier, 1997). Freezing tolerance can be increased by cold-acclimatization, abscisic acid, partial dehydration, or low atmospheric pressure (Halloy and Gonzales, 1993; Hincha, 1994; Lång et al., 1994; Mantyla et al., 1995). In general, -2°C is the lowest temperature for lily preservation.

The adjustment of medium contents (the type and composition of the base medium, regulators. carbon source type and content) can prolong the storage time. Lowering the content in mineral elements of culture medium [1/2 or 1/4 Murashige and Skoog medium (MS)] and/or high sucrose concentration (6 to 9%) gave reduction in sprout growth of lily, thus extending the storage duration (Bonnier and Van Tuyl, 1997; Chen et al., 2006). In other crops, such as the protocorms of *Cymbidium* sp., *Dendrobium chrysanthum* and *ochreatum*, high sucrose concentrations allowed the conservation at low temperature (Homes et al., 1982; Tandon and Sharma, 1986).

The growth inhibitors also play some effects on slow growing. Ancymidol (α-cyclopropyl-α[4-methoxyphenyl]-5-pyrimidinemethanol) is a substituted pyrimidine with potent plant growth regulatory activity, specifically inhibiting a series of oxidations in plant tissue. The potato microplants *in vitro* could be persisted through a 16-months culture period (Debabrata et al., 2001). The addition of mannitol significantly reduced the growth of *Colocasia esculenta* and *Xanthosoma brasiliense* shoots (Staritsky et al., 1985). However, the growth of lily shoots cultured in medium supplemented with 10 to 50 gL^{-1} mannitol or 10 to 40 mgL^{-1} trimethy1ammonium (CCC) was not significantly restricted (Chen et al., 2006).

ABA is a growth retardant which was used to reduce the growth of shoots of potato, grape, persimmon and date plum (Westcott, 1981; Roca et al., 1982; Li et al., 1992; Ai and Luo, 2004). However, these authors indicated that ABA was detrimental to some varieties. For *Lilium* oriental hybrids 'Siberia', *in vitro* shoots could be conserved in the media supplemented with 1.0 to 3.0 mgL^{-1} ABA for more than nine months at normal temperature of 20±1°C (Chen et al., 2006).

Up to now, the method of slow-growth conservation *in vitro* has been used to conserve germplasm of potato (*Solanum tuberosum*) (Roca, 1975), sweet potato (*Ipomoea btatas*) (Sigueńas, 1987), Andean root and tuber crops (Toledo et al., 1994), and lily (*Lilium* L.) genotypes (Asiatic hybrids, Oriental hybrids, *L. longiflorum* and *L. henryi*) (Bonnier and Van Tuyl, 1997); therefore, it is a very promising method. However, this method also has some problems yet to be resolved. On one hand, there are rich lily resources in China, and some resources are endangered, it is necessary to preserve resources *in vitro.* Furthermore, different genotypes had different requirements on storage conditions. Right now, there was no report about the effect of MS content and ABA at -2°C on lily conservation. In this paper, the MS content, the concentration of sucrose and ABA combined with low temperature (-2°C) and normal temperature (24°C) were used for *Lilium davidii* and *L. longiflorum*. The effects of culture conditions on bud growth and further survival rate after planting in soil were investigated.

MATERIALS AND METHODS

Establishment of *in vitro* regeneration system

L. davidii and *L. longiflorum* were used for experiment with bulb scales between 14 and 16 cm. Clean scales were rinsed with tap water for 40 min, then surface-sterilized for 30 s in alcohol, immersed in 20%(v/v) NaOCl for 15 min, washed three times with sterile water in the clean benches. Bulb scale segments of about 5 × 5 mm were cultured on MS-medium (Murashige and Skoog, 1962) with 3% sucrose, 6 gl^{-1} purified agar (Sigma), 1.5 mgl^{-1} 6-benzylaminopurine (6-BA) and 0.2 mgl^{-1} naphthaleneacetic acid

Table 1. The combinations of basal media and sucrose concentrations for the preservation at 24 and -2°C.

Code	MS	Sucrose (w/v) (%)	Agar (gL^{-1})
1	1/1	6	6.0
2	1/1	9	6.0
3	1/4	6	6.0
4	1/4	9	6.0
5(CK)	1/1	3	6.0

MS, Murashige and Skoog medium.

Table 2. The combinations of basal media and ABA concentrations for the preservation at -2°C.

Code	MS	Sucrose (w/v) (%)	ABA (mgL^{-1})	Agar (gL^{-1})
5(CK)	1/1	3	0.0	6.0
6	1/2	3	1.0	6.0
7	1/2	3	3.0	6.0
8	1/4	3	1.0	6.0
9	1/4	3	3.0	6.0

MS, Murashige and Skoog medium.

(NAA) at a pH of 5.8 in Erlenmeyer flask with a volume of 80 ml. The bottle was wrapped around with a polyethylene film. The scale segments were cultured with their abaxial side on the medium at 24°C with 16-h day light at an intensity of 1500 lx. After nine to 12 weeks, regenerated plantlets were excised and given various treatments in Erlenmeyer flask with a volume of 80 ml containing 40 ml medium for *in vitro* conservation experiments.

Treatments

Tube seedlings of *L. davidii* and *L. longiflorum* were used as explants cultured on two groups of media (Tables 1 and 2) and the fifth medium was the control. The sprout growth was measured before treatment. Table 1 shows five combinations of basal media and sucrose concentrations. The materials were divided into two groups placed on the same media. One-half of the tube seedlings were placed at 24°C (16 h/d, 1500 lx). The other one-half of the tubes were placed at 2°C for four weeks acclimatization, and then transferred to -2°C in the dark. 45 plantlets were used for each treatment with three replications.

Five combinations of basal media plus different concentrations of ABA are listed in Table 2. The tubes were placed at 2°C for four weeks acclimatization, and then transferred to -2°C in the dark. 45 plantlets were used for each treatment with three replications.

Sprout growth

The seedling height was measured every month. At normal temperature, the increment of sprout growth was obvious in the first three months, while under the low temperature, it was not conspicuous. The majority of sprouts had reached the top of the tube at normal temperature after five to six months. Therefore, we selected two periods of three and six months for detailed analysis at 24°C. The data were analyzed using the SPSS13.0.

Growth after storage

The bulblets on nine different media at -2°C after 15 months storage were placed on restoration medium (MS+NAA 1.0 mgL^{-1} + 30 gL^{-1} Sucrose + 6 gL^{-1} Agar) at 24°C with 16-h day light at an intensity of 1500 lx for two weeks, then they were taken from Erlenmeyer flask and planted in moist peat at 20°C in the greenhouse (Bonnier and Van Tuyl, 1997). The bulblets stored at 24°C were first placed at 4°C for six weeks to break dormancy (Langens-Gerrits et al., 2003), then planted in moist peat at 20°C in the greenhouse. The survival rate was measured.

RESULTS

The effects of basal media and sucrose concentrations on the preservation at room temperature (24°C) and low temperature (-2°C)

After 3 months storage at 24°C, the growth of two species was restricted to varying degrees by adjusting basal medium and sucrose concentration. There were no apparent external morphological changes for two species.

The influences of various media on two species were different (Tables 3 and 4). The growth of seedling cultured in medium supplemented with 3% sucrose (the control) was not apparently restricted. Lowering the content of mineral elements (1/4 MS) and/or adding high sucrose concentrations (6 to 9%) had an obvious restraint effect. The average increment of treatment groups was less than that of control group and the difference was very

Table 3. The effects of different basal media and sucrose concentrations on the sprouts growth of *in vitro* lilies after three months conservation at 24°C.

Species	Growth increment (mm)				
	MS with 3%sucrose(CK)	MS with 6% sucrose	MS with 9% sucrose	1/4 MS with 6% sucrose	1/4 MS with 9% sucrose
L. Davidii	85.9±3.6eE	82.6±2.0dD	21.5±1.9cC	12.8±1.4bB	6.5±0.6aA
L.longiflorum	85.7±2.7dD	72.3±6.4cC	23.9±2.7bB	2.7±0.7aA	1.9±0.3aA

The values are mean ± SD; different lower-case and capital letters in the same line represent the significant difference at $p < 0.05$ and $p < 0.01$ respectively.

Table 4. The effects of different basal media and sucrose concentrations on the sprouts growth of *in vitro* lilies after three months conservation at -2°C.

Species	Growth increment (mm)				
	MS with 3%sucrose (CK)	MS with 6% sucrose	MS with 9% sucrose	1/4 MS with 6% sucrose	1/4 MS with 9% sucrose
L. Davidii	9.0±0.9cB	2.6±0.8aA	3.0±0.8abA	3.6±1.2abA	3.7±0.8bA
L. Longiflorum	3.7±1.1cB	3.2±1.0bcB	2.9±0.8bB	3.6±1.0cB	1.6±0.5aA

The values are mean ± SD; different lower-case and capital letters in the same line represent the significant difference at $p < 0.05$ and $p < 0.01$ respectively.

Table 5. Duncan test of sucrose effect after three months conservation at 24°C.

Concentration of sucrose (%)	*L. davidii*		*L. longiflorum*	
	Mean increment (mm)	Duncan test	Mean increment (mm)	Duncan test
9	13.6	aA	12.9	aA
6	47.7	bB	37.5	bB
3	85.9	cC	85.7	cC

Table 6. Duncan test of sucrose effect after 3 months conservation at -2°C.

Concentration of sucrose (%)	*L. davidii*		*L. longiflorum*	
	Mean increment(mm)	Duncan test	Mean increment(mm)	Duncan test
9	2.3	aA	3.4	aA
6	3.4	bB	3.1	aA
3	3.7	bB	9.0	bB

significant. The inhibition effects increased using high sucrose concentrations (6 to 9%) (Tables 5 and 6). The combination of 1/4 MS nutrients and 9% sucrose gave the highest reduction in sprout growth. The increment of *L. davidii* was 6.5 mm for three months storage. The bulblets swelled significantly, but seldom propagated small bulblets. For *L. longiflorum*, the increment was 1.9 mm, meanwhile, the seedlings propagated many small bulblets during the storage. Besides, the bulb enlargement would occur.

Compared with the conservation at 24°C, the sprout growth of treatments at -2°C was more significantly reduced (Tables 3 and 4). Through the variance analysis, the effects of different sucrose concentration were significant. However, the effects were not similar for two species (Tables 5 and 6). At -2°C, the inhibitory effect for *L. davidii* was more obvious with 9% sucrose, which was significant with media with 3 and 6% sucrose. While for *L. longiflorum*, the effects of media with 6 and 9% sucrose were notable with the media plus 3% sucrose. (Tables 5 and 6) With the extension of storage, the sprout grew slowly.

The results indicate that the appropriate concentration of sucrose was 9% for *L. longiflorum*, while 6 to 9% for *L. davidii* (Table 8). However, the combination of low nutrient and high sucrose could play an important role in

Table 7. The effects of different basal media and sucrose concentrations on the sprouts growth of *in vitro* lilies after 6 months conserved at -2°C.

Species	Growth increment (mm)				
	MS with 3%sucrose (CK)	**MS with 6% sucrose**	**MS with 9% sucrose**	**1/4 MS with 6% sucrose**	**1/4 MS with 9% sucrose**
L.davidii	60.7±12.0dD	21.8±6.0bAB	29.5±8.3cBC	30.1±8.4cC	14.7±3.5aA
L.longiflorum	20.5±3.9cC	14.3±2.9bB	9.9±2.8aA	21.5±8.2cC	8.8±1.5aA

Table 8. Duncan test of different sucrose concentrations after 6 months conserved at -2°C.

Concentration of sucrose (%)	Mean increment (mm) (*L.davidii)*	Duncan test	Mean increment (mm) *(L. longiflorum)*	Duncan test
9	22.1	aA	9.4	aA
6	26.0	aA	17.9	bB
3	60.7	bB	20.5	bB

Table 9. The effects of different basal media and ABA concentrations on the sprouts growth of *in vitro* lilies after three months conserved at -2°C.

Species	Growth increment (mm)				
	MS with ABA (0 mgL^{-1})	**½ MS with ABA (1.0 $mg.L^{-1}$)**	**½ MS with ABA (3.0 mgL^{-1})**	**¼ MS with ABA(1.0 mgL^{-1})**	**¼ MS with ABA (3.0 mgL^{-})**
L. davidii	9.0±2.6dD	6.3±1.7cC	4.4±0.9bB	1.7±0.4aA	0.6±0.2aA
L. longiflorum	3.6±1.1cC	1.2±0.3aA	4.3±1.3dC	2.7±0.8bB	1.3±0.4aA

restriction conservation. At -2°C, the sprout growth was only 14.7 mm for *L. davidii* and 8.8 mm for *L. longiflorum* after six months by using 1/4 strength MS medium with 9% sucrose (Tables 7 and 8), it was significantly reduced. The promising preservation medium was ¼ MS with 9% sucrose for *L. davidii,* while ¼ or full MS with 9% sucrose for *L. longiflorum* (Table 7). For long-term preservation of lily germplasm resources, 1/4MS with 9% sucrose may be in common use.

The effects of basal media and ABA concentrations on the preservation at low temperature (-2°C)

There were remarkable differences in lily preservation between different treatments of ABA and nutrient concentrations. After three months storage at -2°C, the growth of two species was restricted to varying degrees by adjusting basal medium and ABA concentrations, the difference between groups and control group was significant (Tables 9 and 10). The lower nutrient content combined with increasing concentration of ABA had apparent inhibiting effect on sprout growth. Moreover, for *L. davidii*, the effect was more significant on the low nutrient medium with 3.0 mgL^{-1} ABA. It was generally similar for *L. longiflorum.* Different treatments showed no obvious difference in morphology after storage.

As prolonging storage, the effect was more significant for the low nutrient medium and the high ABA concentration from 1.0 to 3.0 mg/L. Through the duncan tests, the results show that the nutrient medium and ABA played a parallel restraint role in sprout growth after three months conserved (Table 10). After six months storage, the change of plantlet height of *L. longiflorum* was smaller than that of *L. davidii*, the effects of ABA from 1.0 to 3.0 mg/L were not significant (Tables 11 and 12). For *L. davidii*, the restricted effects were very significant for different concentrations of ABA and the nutrient medium. The promising preservation medium was 1/4 MS with 3.0 mgL^{-1} ABA.

The effects of preservation for the two species

With the extension of storage duration, the restraining effect of low temperature on lily growth decreased, moreover, sprout growth had an increase, especially the control (Figure 1). The growth of *L. davidii* began to accelerate at four months, at six months it was more obvious; condition of 1/4 basal media with 9% sucrose could effectively retard the sprout growth of lily, the mean height was still maintained at about 20 to 30 mm, in addition to a few of leggy seedlings. The increment of *L. longiflorum* was smaller than that of *L. davidii.* Besides,

Table 10. Duncan test of ABA and MS concentration effects after 3 months conserved at -2°C.

Species	ABA concentration (mgL^{-1})	Mean increment (mm)	Duncan test	MS concentration	Mean increment (mm)	Duncan test
	3.0	2.5	aA	1/4MS	1.1	aA
L. davidii	1.0	4.0	bB	1/2MS	5.3	bB
	0	9.1	cC	MS	9.0	cC
	3.0	1.9	aA	1/4MS	2.0	aA
L. longiflorum	1.0	2.9	bB	1/2MS	2.7	bB
	0	3.7	cC	MS	3.7	cC

Table 11. The effects of different basal media and ABA concentrations on the sprouts growth of *in vitro* lilies after 6 months conserved at -2°C.

Species	Growth increment (mm)				
	MS with ABA (0 mgL^{-1})	1/2MS with ABA (1.0 mgL^{-1})	1/2MS with ABA (3.0 mgL^{-1})	1/4MS with ABA (1.0 mgL^{-1})	1/4MS with ABA (3.0 mgL^{-1})
L. davidii	60.7±12.0cC	22.1±4.9bB	8.8±2.3aA	9.2±2.3aA	5.3±1.0aA
L. longiflorum	20.5±3.9dC	6.3±1.6bB	8.6±2.5cB	6.6±1.6bB	3.5±0.8aA

Table 12. Duncan test of ABA and MS concentration effects after six months conserved at -2°C.

Species	ABA concentration (mgL^{-1})	Mean increment (mm)	Duncan test	MS concentration	Mean increment (mm)	Duncan test
	3.0	7.1	aA	1/4MS	7.2	aA
L. davidii	1.0	15.7	bB	1/2MS	15.5	bB
	0	60.7	cC	MS	60.7	cC
	3.0	6.0	aA	1/4MS	5.0	aA
L. longiflorum	1.0	6.5	aA	1/2MS	7.5	bB
	0	20.5	bB	MS	20.5	cC

by using ABA, more remarkable effect can be obtained (Figures 1B and 2B). The results show that ABA may be a promising growth retardant to preserve lily germplasm.

Growth after storage

The tube seedlings conserved for 15 months could turn to normal plantlets after re-growth for one month which showed no obvious difference in morphology compared with control. The mean re-growth percentage after 15 months storage at -2°C on 1/4 strength MS medium plus 3.0 mg/L ABA was 87.5% for *L. davidii*, and 62% for *L. longiflorum.*

DISCUSSION

Varying degrees of the growth restriction for both species were related to the four factors (MS content, the concentration of sucrose and ABA, temperature). Compared with other conservation measures, storage at -2°C on 1/4 MS medium plus 3.0 mg/L ABA resulted in more apparently inhibitory effect. As to *L. longiflorum*, the data measurements and recodes were ended after nine months treatment, for the seedlings had grown to the top of the bottle. While *L. davidii* was growing rapidly, the control had grown to the mouth of the bottle after six months. Although the growth was accelerated in six months, the tube seedlings could still be preserved on the medium. Furthermore, the materials on 1/4 MS medium plus 3.0 mg/L ABA had the highest survival rate of re-growth after 15 months conservation at -2°C. Actually, results show that the lily genotypes managed to survived, even with some slight pollution during the preserving process, after the storage as long as 24 months under the condition of -2°C on 1/4 MS medium plus 3.0 mg/L ABA .

At 24°C, bulb growth and leaf formation occurred

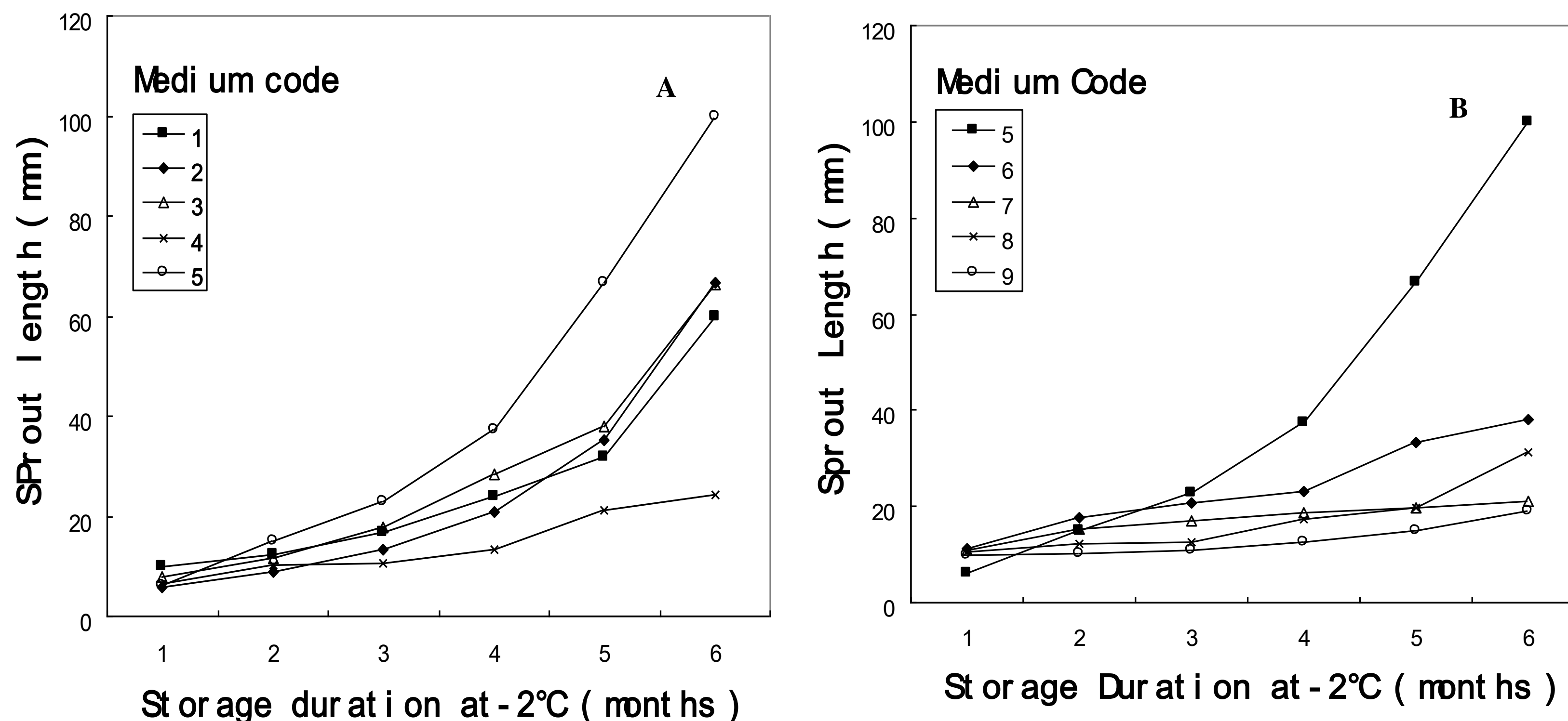

Figure 1. The effects of different media on the growth of *L. davidii* (A and B) stored at -2°C.

during prolonged storage. Due to consumption, the medium should be replaced once around every three to four months, especially the media with high concentration sucrose that promoted the bulb growth and accelerated the consumption. After five to six months storage, sprouts in some tubes had reached the top of the tube and the contamination rate rose with the extension of storage duration. Therefore, at normal temperature, the method of adding high concentration of sucrose and reducing macro element was suitable for mid-short term preservation of lily germplasm resources and could promote bulb growth and propagation.

At -2°C, based on investigation, we found that the adaptability to temperature and preservation effect were different between genotypes. The spout length of *L. davidii* increased rapidly (Figure 1) since the fourth month at the temperature of -2°C, which can be explained by the fact that dormancy was broken at this temperature; nevertheless, it could still keep on being preserved in this condition. However, the growth of seedlings of *L. longiflorum*, was slow (Figure 2). In addition, Langens-Gerrits et al. (2001) found that dormancy of *L. longiflorum* 'Snow Queen' was induced *in vitro* at 15°C, declined with increasing culture temperature. Moreover, the duration of the cold treatment needed for complete removal of dormancy was as long as two weeks at 5°C in Snow Queen. Therefore, it might be due to low temperature (5 to 15°C) which induced dormancy of *L. longiflorum.* At the condition of -2°C it was still dormant, or its growth was effectively delayed. *L. longiflorum* was more restricted to low temperature than *L. davidii* (Table 3), which could reflect their different origins. *L. davidii* originates from mountainous areas of Southwestern China (Abdullah and Ahmad, 1980; Anderson, 1988) where it grows in climate with moderately cold winters and warm summers. Wet and dry seasons are obviously in this region. However, *L.*

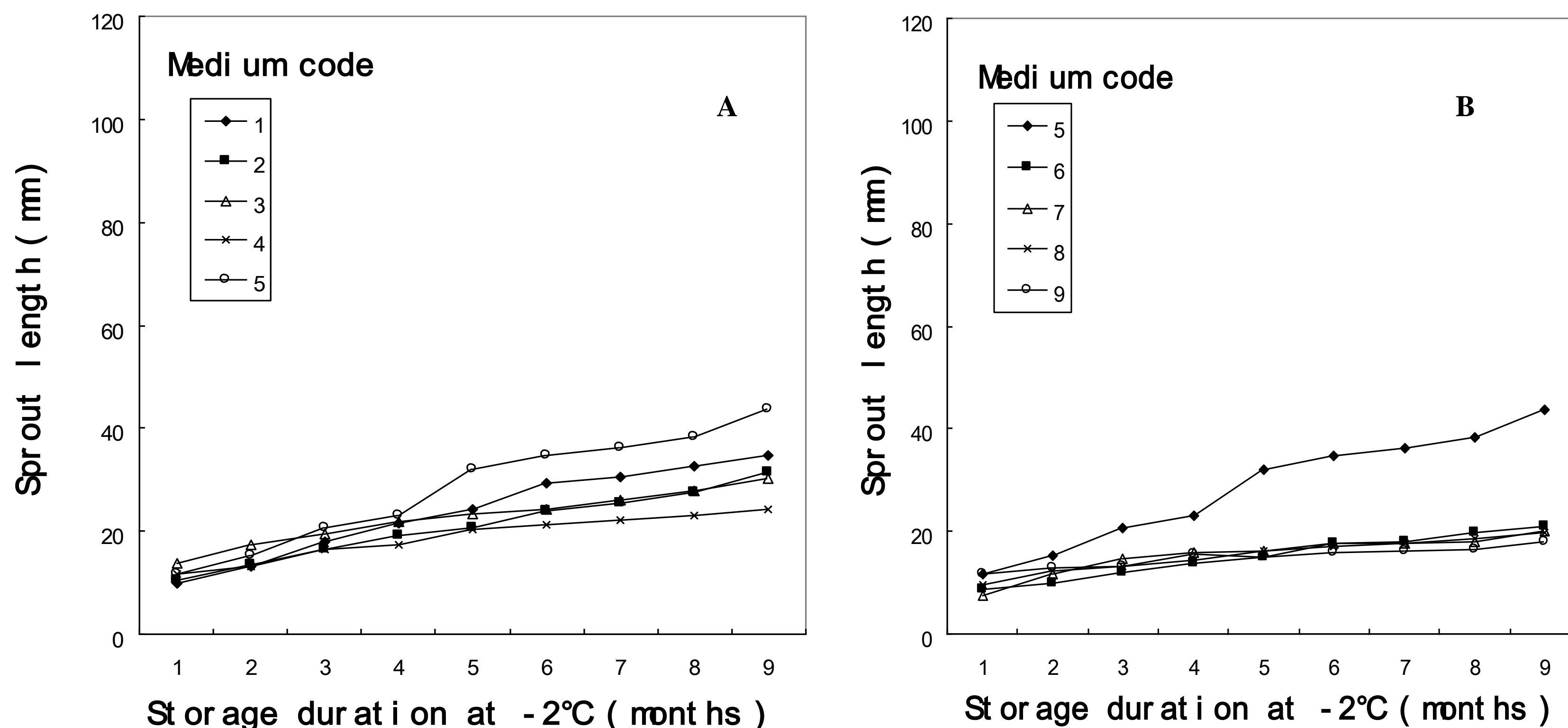

Figure 2. The effects of different media on the growth of *L. longiflorum* (C and D) stored at -2°C. The nine media, including: 1, MS medium plus 6% sucrose; 2, MS medium plus 9% sucrose; 3, 1/4 MS medium plus 6% sucrose; 4, 1/4 MS medium plus 9% sucrose; 5, MS medium plus 3% sucrose (the control); 6, 1/2 MS medium plus 3% sucrose and ABA (1.0 mgL^{-1}); 7, 1/2 MS medium plus 3% sucrose and ABA (3.0 mgL^{-1}); 8, 1/4 MS medium plus 3% sucrose and ABA (1.0 mgL^{-1}); 9, 1/4 MS medium plus 3% sucrose and ABA (3.0 mgL^{-1}); sprout length was measured every month. Each data point is the average sprout length of 45 plantlets.

longiflorum is a maritime plant of tropical nature growing in full sun with torrid summer and warm winter (Miller, 1993; Stoker, 1933). According to these facts, we can probably draw the conclusion that *L. longiflorum* is more sensitive to the low temperature, which gives rise to the growth stagnation during storage.

Previously, Bonnier and Van Tuyl (1997) reported that the seedlings of *L. longiflorum* could be stored at -2°C for six months. Later, the damage was aggravating, and died during prolonged storage. However, our results show that *L. longiflorum* could be conserved for more than 15 months, though some plantlets died during storage. On the one hand, the acclimatization period was two weeks in Bonnier and Van Tuyl's work, which was four weeks in our test. It suggests that the acclimatization period of four weeks could be sufficient to acclimatize bulbs to avoid further sprout growth of the bulblets, as well as reducing damage at low temperature. On the other hand, ABA as a growth inhibitor can enhance the ability to adapt to the stress of plantlets. Exogenous supply of ABA could inhibit sprout growth and leaf formation. Besides, ABA promotes tuberization and bulb formation in lily (Kim et al., 1994). Consequently, carbohydrate was placed into a storage organ which enhances the adaptability of seedlings to low temperature by osmotic stress. Another possible reason is that exogenous ABA may induce or enhance dormancy for some genotypes to a certain extent. ABA is considered to be the principal dormancy-

inducing agent. For various seeds and buds, it has been shown that ABA plays a role in dormancy development (Hole et al., 1989; Suttle and Hultstrand, 1994). Furthermore, young bulblets were very sensitive to the hormone (Djilianov et al., 1994).

During storage, some *in vitro* seedlings presented in the shape change which showed a certain degree of instability, such as generating base bulbs, turning into purple color, which were the physiological changes produced by environmental stimulation under stress conditions, rather than genetic variation. The physiological changes can be reversed through recovery culture. The feasibility of preservation method was preliminarily validated at the protein level (Chen et al., 2006). However, due to the physiological status of different test materials, it is necessary to examine and analyze their preservation and regeneration of plant germplasm from molecular level, to get the optimal slow-growth conservation *in vitro* technology.

Conclusion

It is concluded that the promising conservation conditions was the 1/4 MS with 3.0 mg/L ABA at -2°C which was suitable for mid-long term preservation of lily germplasm resources.

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REFERENCES

Abdullah ZN, Ahmad R (1980). Effect of ABA and GA3 on tuberization and some chemical constituents of potato. Plant Cell Physiol. 21: 1343-1346.

Aguettaz P, Paffen A, Delvallée I, Van Der Linde P, De Klerk GJ (1990). The development of dormancy in bulblets of *Lilium speciosum* generated *in vitro* I. The effects of cultureconditions. Plant Cell Tissue Org. Cult. 22: 167-172.

Ai PF, Luo ZR (2004). Conservation of *in vitro* shots of persimmon and date plum by slow growth and genetic stability of recovered planflets. Acta Hortic. Sin. 31(4):41-46.

Anderson NO (1988). The distribution of the genus *Lilium* with reference to its evolution. In: Montgomery JA (ed.). The Lily Yearbook of the North American Lily Society. Friesen. Sons. Altona, Canada. pp. 1-18.

Beattie DJ, White JW (1993). *Lilium* - hybrids and species. In: DeHertogh A & Le NardM (eds) The Physiology of Flower Bulbs Elsevier, Amsterdam-London-New. pp. 423-454.

Bonnier FJM, Jansen RC, Van Tuyl JM (1996). Long term lily scale bulblet storage: effects of temperature and storage in polyethylene bags. Ann. Appl. Biol. 129: 161-169. Bonnier FJM (1997). Long term storage of clonal material of lily (*Lilium* L.). pp. 123.

Bonnier FJM, Van Tuyl JM (1997). Long term *in vitro* storage of lily: effects of temperature and concentration of nutrients and sucrose. Plant Cell Tiss. Org. Cult. 49: 81-87.

Boontjes J (1983). The use of freezing and the danger of freezing damage for long-term storage of lily bulbs. Bulb Research Centre, Lisse, in Dutch, 53: p. 10.

Bouman H, De Klerk GJ (1990). Cryopreservation of lily meristems. Acta Hort. 266: 331-336.

Chen H, Chen XL, Chen LQ, Lu XX (2006). Studies on germplasm conservation of Lily (Lilium L.) by restricting growth method. Acta Hortic. Sin. 33(4): 779-789.

Debabrata S, Swarup KC, Prakash SN (2001). Slow-growth conservation of potato microplants: efficacy of ancymidol for long-term storage *in vitro*. Euphytica, 117: 133-142.

Delvallée I, Paffen A, De Klerk GJ (1990). The development of dormancy in bulblets of *Lilium speciosum* generated *in vitro* II. The effect of temperature. Physiol. Plant. 80: 431-436.

Djilianov D, Gerrits M, Ivanova A, Van Onckelen H, De Klerk GJ (1994). ABA content and sensitivity during the development of dormancy in lily bulblets regenerated *in vitro*. Physiol. Plant. 91: 639-644.

Engelmann F (1991). *In vitro* conservation of tropical plant germplasm-a review. Euphytica, 57: 227-243.

Fu LG, Chen TQ, Lang KY (2002). In: High Plants of China. Qingdao Publishing House, Qingdao, CN. pp. 118-133.

Gerrits MM, Kim KS, De Klerk GJ (1992). Hormonal control of dormancy in bulblets of *Lilium speciosum* cultured *in vitro*. Acta Hortic. 325: 521-527.

Grout WV (1991). Conservation *in vitro*. Acta Hort. 289: 171-178.

Halloy S, Gonzalez JA (1993). An inverse relation between frost survival and atmospheric pressure. Arctic Alpine Res. 25: 117-123.

Hincha DK (1994). Rapid induction of frost hardiness in spinach seedlings under salt stress. Planta, 194: 274-278.

Hole DJ, Smith JD, Cobb BG (1989). Regulation of embryo dormancy by manipulation of abscisic acid in kernels and associated cob tissue of *Zea mays* L cultured *in vitro*. Plant Physiol. 91: 101-105.

Homes J, Dubus F, Bourdon JL (1982). Cold storage of plant tissue cultures. In: A. Fujiwara (Ed). Proc. 5th Intl. Cong. Plant Tissue Cell Cult. Tokyo, pp. 801-802.

Kim K, Davelaar E, De Klerk GJ (1994). Abscisic acid controls dormancy development and bulb formation in lily plantlets regenerated *in vitro*. Physiol. Plant. 90: 59-64.

Langens-Gerrits MM, Nashimoto S, Croes AF, De Klerk GJ (2001). Development of dormancy in different lily genotypes regenerated *in vitro*. Plant Growth Regul. 34: 215-222.

Langens-Gerrits MM, Miller WBM, Croes AF, De Klerk GJ (2003). Effect of low temperature on dormancy breaking and growth after planting in lily bulblets regenerated *in vitro*. Plant Growth Regul. 40: 267-275.

Lång V, Mantyla E, Welin B, Sundberg B, Palva ET (1994). Alterations in water status, endogenous abscisic acid content, and expression of rab18 gene during the development of freezing tolerance in *Arabidopsis thaliana*. Plant Physiol. 104: 1341-1349.

Mantell SH, Smith H (1983). In: Plant Biotechnology. Cambridge University press, Cambridge, UK. pp. 168-218.

Mantyla E, Lång V, Palva ET (1995). Role of abscisic acid in drought-induced freezing tolerance, cold acclimation, and accumulation of LT178 and RAB18 proteins in *Arabidopsis thaliana*. Plant Physiol. 107: 141-148.

Matsumoto T, Sakai A, Yamada K (1995). Cryopreservation of *in vitro*-grown apical meristems of lily by vitrification. Plant Cell Tissue Org. Cult. 41: 237-241.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Miller WBM (1993). *Lilium longiflorum*. In: De Hertogh A and Le Nard M (eds), The Physiology of Flower Bulbs. Elsevier, Amsterdam. pp. 391-422.

Peschke VM, Phillips RL (1992). Genetic implication of somaclonal variation in plants. Adv. Genet. 30: 41-75.

Roca WM (1975). Tissue culture research at CIP. Am. Potato J. 52(9): 281.

Roca WM, Rodriguez J, Roa J, Mafla G (1982). Tissue culture for the conservation and international exchanges of germplasm. In: Fujiwara A (Ed). Proc. 5th Intl. Cong. Plant Tissue and Cell Cult. Tokyo. pp. 771-772.

Sigueńas C (1987). Propagacióny conservación in vitro de dos cultivares de camote (Ipomoea batats Lam). Thesis. La Molina, National Agrarian University. Lima, Peru. p. 108.
Staritsky G, Dekkers AJ, Louwaars NP, Zandvoort EA (1985). *In vitro* conservation of aroid germplasm at reduced temperatures and under osmotic stress. In: Withers LA & Alderson PG (Eds). Plant Tissue Culture and its Agricultural Applications, Butterworths, pp. 277-284.
Stoker F (1933). The environment of lilies in nature. In: Chittender JF (ed.), The Lily Yearbook of the Royal Horticultural Society. Benham & Co, Colchester, UK. pp.11-57.
Suttle JC, Hultstrand JF (1994). Role of endogenous abscisic acid in potato microtuber dormancy. Plant Physiol. 105: 891-896.
Tandon P, Sharma J (1986). Regeneration of *Dendrobium* from cold preserved protocorms. In: Somers DA, Gengenbach BG, Biesboer DD, Hackett WP & Green CE (Eds). Abstr. 6th Intl. Cong. Plant Tissue Cell Cult. Minneapolis, p. 425.
Toledo J, Arbizu C, Hermann M (1994). *In vitro* international collection of ulluco, oca, mashua and yacon. Abatracts of Ⅷ International Congress of Andean agriculture systems. Valdivia, Chile. In Spanish. p.1.
Towill LE (1988). Genetic considerations for germplasm preservation of clonal materials. Hort. Sci. 23: 77-97.
Villalobos VM, Engelman F (1995). Exsitu conservation of plant germplasm using biotechnology. Biotechology, 11(4): 375-382.
Westcott RJ (1981). Tissue culture storage of potato germlasm. 2. Use of growth retardents. Potato Res. 24: 343-352.
Withers LA (1991). *In-vitro* conservation. Biol. J. Linnaean Soc. 43: 31-42.