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EFFECTS OF LOW TEMPERATURE ON *CHRYSANTHEMUM SHIWOGIKU* VAR. *KINOKUNIENSE IN VITRO* CONSERVATION

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Abstract

The effect of low temperature condition on wild *Chrysanthemum shiwogiku* var. *in vitro* conservation was investigated, and its plant regeneration hereditary stability was detected using its sterile seedling. The results show that the test tube young plant grows rapidly, and its preservation time is short at a temperature at 25±2°C, at condition of 12 h/d, photoperiod at 2000 ~ 3000 lx, and all die after 180 days. However, the sterile seedling grows slowly under low-temperature, and its preservation time extends to 360 days. The sterile seedling surviving rate was above 96%. The data indicate that the 4°C low temperature is advantageous to *Chrysanthemum* plantlets preservation. After preservation, the recovered plantlets grow well and show no differences in morphology and isoenzyme zymogram of peroxidase, ISSR-PCR compared with the control. In addition, the results show that low temperature *in vitro* plantlets maintain genetic stability.

Compared with the conventional method of plant germplasm conservation, slow-growing *in vitro* possesses many advantages, such as less space required, less danger from insect and disease contamination, faster propagation when requirements of production, and lower expenses as well (Bestoso 2006, Gopal 2010).

It is the most commonly used method to reduce cultivating temperatures that plant cultures grow slowly for preservation. The principle of low temperature plant germplasm conservation is that all the activities of life weaken significantly or even reduce to nothing with the decrease of temperature. The survival rate of tube plantlets increases with decreasing temperature within certain limits of temperature.

So far, the research that the growth rate of plantlets was retarded for long time storage by decreasing temperature at home and abroad, is involved in main plant species including grape (Cohen 2012, pear (Hu 2009), kiwi fruit (Seleznyova 2001, (strawberry, Koehler 2012), dendrobium, Shi 2000, taro (Murakami 2007, balsamine Lan 2004 chrysanthemum (Zhang 2012).

But a growing number of studies demonstrate that different plants or even the same plants with different genotypes are with different sensitive to low temperature, and studies on related wild daisy conservation of germplasm *in vitro* have not yet been reported around the globe.

In this study, the effects of low temperature on wild daisy *Chrysanthemum shiwogiku* var. *kinokuniense in vitro* conservation were investigated preliminarily, in the hope of providing data and information for its conservation of germplasm *in vitro*. The results show that every physiological character of the *Chrysanthemum shiwogiku* growing at lower temperature can be reversed under the room temperature. The data suggest that the wild germplasm, even or domesticated resources do not degenerate under appropriate low temperature.

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Chrysanthemum shiwogiku var. *kinokuniense* was native to Japan that distributes in Wakayama and Mie prefecture counties, and this species is highly tolerant to various environmental stresses, particularly salt stress (Guan 2010). It was introduced into the Chrysanthemum Germplasm Resource Preserving Centre, Nanjing Agricultural University, China (32°05' N, 118°90' E) in 2006. Experiments were performed in Fuyang Teachers' College and the flower genetic breeding laboratory of Nanjing Agricultural University from March 2010 to August 2013 for three times.

Sprouting crural buds (stem sections half of which leaf length and leaf stalk length were cut out) were first washed by water to remove surface dust, after that the buds were rinsed under running water for 10 min. The plants were blotted on filter paper to soak the water, cleansed with 70% ethanol for surface sterilization, washed twice with sterile water, and then immersed in a 1% mercuric chloride solution for 7 min. Then the buds washed with sterile water for four times, the test material stem segments with two internodes, the rest of which petioles were removed. According to the directional growth movement, the stem segments were inoculated onto 1/2 MS supplemented with 30 g/l sucrose and 6.5 g/l agar). After 20 days, the germination axillary buds were transferred to MS supplemented with0.5 g/l BA, 0.1 g/l NAA, 30 g/l sucrose, and 6.5 g/l agar. The stem segments could continue to grow.

After 30 days of culture, two sterile seedling internodes, growing vigorously with a very uniform stand, were used as experimental materials. Basal culture medium was MS (pH 5.8) supplemented with 0.5 mg/l BA, 0.1 mg/l NAA, 30 g/l sucrose, and 6.5 g/l agar. The culture conditions were light intensity with 2000 ~ 3000 lx, 12 hrs/ days photoperiod. There were three types of temperature treatments: (1) Treatment at room temperature (25±2)°C all the time as Control; (2) Pre-cultured at room temperature (25±2)°C for 10 days, and then conserved at 4°C (Treatment I); (3) Conserved at 4°C always (Treatment II). Among them, internodes at 4°C temperature were carried out in the growth chambers that can automatically and accurately control temperature, light time, relative humidity, and photosynthetically active radiation. Each group experiment material was pre-incubated under the respective condition for 5 days, in order to remove traces of pollutants. Seven bottles are randomly selected from each treatment, four seedlings in each bottle. The survival rate and plant height of test-tube plantlets were observed and counted every month. When all of the internodes and leaf in the plants turned yellow, they were regarded as the loss of life.

Table 1. Effects of different temperature treatments plant survival rate *in vitro* conservation.

Temperature treatment	Survival rate (%)					
	60 d	120 d	180 d	240 d	300 d	360 d
Control	100	28.57	0	0	0	0
Treatment I	100	100	100	100	97.86	85.17
Treatment II	100	100	100	100	100	96.15

Control: Conserved at 25 ± 2 always. Treatment I: Pre-cultured at 25 ± 2 for 10 days, and then conserved at 4°C; Treatment II: Conserved at 4°C always.

Plants at 25±2°C grew much faster than those in treatments I and II. For example, plants at 25±2°C was nearly full of the bottle after 60 days of culture, while the height of plants in treatments I and II was around one third of the height of plants at 25±2°C. After 120 days of culture, the bottle was full of plants at 25±2°C, but most of leaves became yellow and the survival rate was only 28.57%, (Table 1). In contrast, plants in treatments I and II were still very small after

120 days of growth, but they grew vigorously and the survival rate was 100% (Table 1). All the plants at 25 ± 2°C withered up and died after 180 days of culture (Table 1), whereas the survival rate was still 100% in treatments I and II (Table 1). The survival rates of plants were 85.17 and 96.15% in treatments I and II, respectively, after 360 days of growth, and the plants grew vigorously with a very uniform stand (Table 1). A part of plants grew abnormally in treatment I.

Table 2. Proliferation rate and height of *in vitro* plantlets after 120 days of conservation during different temperature treatments.

Temperature treatment	Mean proliferation rate(times)	Mean height (cm)
Control	2.25 ± 0.21a	7.33 ± 0.39a
Treatment I	2.19 ± 0.38a	1.53 ± 0.24b
Treatment II	2.00 ± 0.35a	1.18 ± 0.15b

Means within the same column followed by the same letter are not significantly differently at 0.05% level. Control: Conserved at 25 ± 2°C always; Treatment I: Pre-cultured at 25 ± 2 for 10 days, and then conserved at 4°C; Treatment II: Conserved at 4°C always.

Morphological characteristics and proliferation rate of plants were recorded in different temperature treatments at different culture time (Tables 2). Height of plants grown at 25 ± 2°C was significantly higher than that of those grown in treatment I and II, but there were no significant differences in proliferation rate among the control and two treatments (Table 2). In addition, there was no significant difference in plant height between treatment I and II (Table 1).

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References

Bestoso F, Ottaggio L, Armirotti A, Balbi A, Damonte G, Degan P, Mazzei M, Cavalli F, Ledda B and Miele M 2006. *In vitro* cell cultures obtained from different explants of *Corylus avellana* produce taxol and taxanes. BMC Biotechnol. **6**: 45.

Gopal J Chauhan NS 2010. Slow growth *in vitro* conservation of potato germplasm at low temperature. Potato Research **53**: 141-149.

Cohen SD, Tarara JM, Gambetta GA, Matthews MA and Kennedy JA 2012. Impact of diurnal temperature variation on grape berry development, proanthocyanidin accumulation, and the expression of flavonoid pathway genes. JEB **63**: 2655-2665.

Hu CX, Wang L and Tang J 2009. Effect of low temperature on physiological indicators of nanguo-pear. J Shenyang Agric. University **40**: 349-352.

Seleznyova A and Greer DH 2001. Effects of temperature and leaf position on leaf area expansion of Kiwifruit (*Actinidia deliciosa*) shoots: Development of a modelling frame work. Annals of Bot. **88**: 605-615.

Koehler G, Wilson RC, Goodpaster JV, Sonsteby A, Lai XY, Witzmann FA, You JS, Rohloff J, Randall SK and Alsheikh M 2012. Proteomic in cold tolerance. Plant Physiol. **159**: 1787-1805.

Shi YZ, Pan RC, Wang XJ, Ye QS and Guo LR 2000. *In Vitro* conservation of dendrobium offiicinale at low temperature. Chinese J. Biol. **6**: 326-330.

Murakami K, Kumabe H and Kimura M 2007. Effect of harvesting time and low-temperature treatment on germination of corm in taro (Colocasia esculenta Schott). Horti. Res. (Japan) **1**: 125-129.

Lan Qinying, Yin Shouhua, He Huiying and Zhang Yanjun 2004. *In vitro* conservation of *Impatiens mengtzeana*. Acta Bot. Boreali-Occidentalia Sinica **24**:146-148.

Zhang FH, Wang YJ, Liu T, Li L and Li TX. 2012. Effects of low temperature phosphine fumigation on postharvest quality of white chrysanthemum 'Dabaiju'. Scientia Horticulturae **142**: 92-97.

Guan ZY, Chen SM, Wang YY and Chen FD 2010. Screening of salt-tolerance concentration and comparison of salt-tolerance for *chrysanthemum* and its related taxa. Chinese J. Ecol. **29** : 467-472.

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