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In vitro Storage of *Chrysanthemum morifolium* at Room Temperature

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The vegetatively propagated-plants need much labor and cost to maintain important clones in the field. It was already reported[1] that shoot tips of chrysanthemum, lily or strawberry could be stored *in vitro* at −2℃ for half or one year. Recently, Fukai *et al.* (1988)[2] reported that chrysanthemum plantlets could be stored *in vitro* at 10℃ by suppressing shoot growth on treatment with growth retardant or deletion of sucrose from the culture medium. In either −2℃ or 10℃, low temperature storage needs refrigirating equipment and maintenance cost. Here, the author has investigated *in vitro* storage of chrysanthemum at room temperature to save these costs.

In the first experiment, shoots of *Chrysanthemum morifolium* 'Otomezakura' were harvested on Aug. 30, 1985, and after sterilization with a diluted solution of sodium hypochlorite (0.8% active chlorine) for 10 min, the shoot tips were trimmed to approximately 2 mm in length, and placed in the test-tubes (2 cm ϕ, 12 cm in height) containing 15 ml of 0.8% agar solidified medium. The nutrient medium consisted of one quarter strength of MS major elements,[3] FeEDTA, Nitsch and Ringe minor elements and vitamines[4] and 0.1 mg/*l* N^6-benzylaminopurine (BA) as a growth regulator. The sucrose concentration adopted was 2, 4 and 6%. Nine shoot tips were cultured for each treatment. The test tubes were capped with aluminiumfoil. The whole cultures were covered with a plastic bag to minimize desiccation and placed on the laboratory table near the window facing the north without direct sunlight. The room temperature during storage period (Aug. 30, 1985-Jan. 21, 1986) fluctuated approximately from 30℃ to 10℃. Light intensity was between 1,000-4,000 lux in the daytime. Four weeks after culture, all the shoots grew well with leaves open. Eleven weeks after culture, 44-66% of the shoots were surviving, but the rest showed leaf-yellowing, resulting in senescent death. The survived shoots continued to grow (**Fig. 1**), and some of them reached the top of the test tubes and again grew downward. On the termination of storage (Jan. 21, 1986), about 5 months after culture, 55% of the shoots in 2% sucrose medium, 44% in 4% sucrose medium and 33% in 6% sucrose medium were survived (**Table 1**).

In the second experiment, a longer storage was tested on the same medium where the sucrose concentration was fixed to 2%, which was the best in the first experiment. Aluminium foil cap was tightened with double layers of rubber bands to minimize desiccation and fungous contamination of the medium for the longer period of culture. Twenty five shoot tips of 'Otomezakura' were cultured on April 28, 1986, in the same manner as the first experiment. The uncontaminated 21 shoots were stored in the room condition. The temperature and light intensity during the storage fluctuated similarly as the first experiment. On Jan. 8, 1987, about 8 months after culture, 32% of the cultured shoots were surviving. One year and four months (Sept. 1, 1987) after culture, only 4 shoots (19%) were survived. The culture medium was almost depleted, and some test tubes (less than 5) were lost with fungous contamination during the storage period. On Sept. 1, 1987, the survived 4 shoots were cut into 20 sections at the internode, and each node section with a single leaf was re-cultured in the same fresh medium

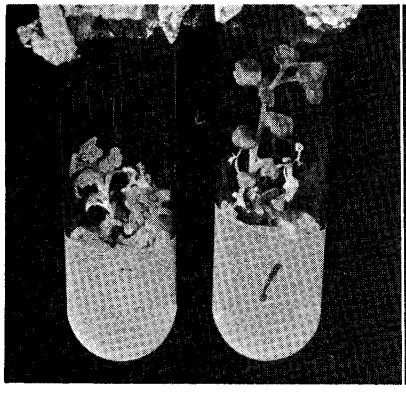

Fig. 1. Shoots of chrysanthemum in *in vitro* storage at room-temperature (4 months after culture.)

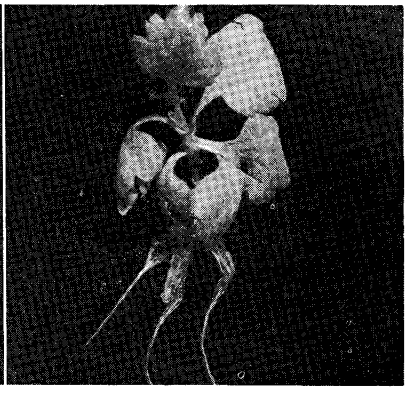

Fig. 2. Rooted plantlet after one year storage at room-temperature.

Table 1. Effect of sucrose concentration on the surviving rate (%) of chrysanthemum shoots in *in vitro* storage at room-temperature.

Sucrose concentration (%)	Storage weeks after culture (date)		
	4 (Sept. 26)	11 (Nov. 13)	20 (Jan. 21)
2	100	66	55
4	100	44	44
6	100	66	33

except which a full strength of MS major elements were used. The axillary shoots elongated from all the nodes and grew well under the same room conditions. The surviving rate was 60% on April 9, 1988. One year after culture (Sept. 1, 1988), 35% of the shoots were still surviving under the room conditions. Forty five % showed yellowing of the whole shoots indicating complete death. The rest 20% was lost with fungous contamination. The survived 7 shoots had 8 nodes in average (8.7±5.1, Avg. ±SD), suggesting that each shoot could be multiplied 8 times by node culture again. In fact, each node produced one axillary shoot when nodal section was cultured in the same medium (data not shown). Three weeks after culture, the axillary shoots (1-2 cm in length) were transferred to MS medium supplemented with 0.1 mg/*l* indolebutylic acid to induce roots. One month after culture, the rooted plantlets were taken out of the test tubes (**Fig. 2**) and cultured on the porous soil medium under the room conditions (20℃ in average). The plantlets are now growing well.

In a preliminary experiment of storage at room temperature in 1985, treatments suppressing shoot growth were tested. The supplements of 1 and 10 mg/*l* abscisic acid or application of high osmosis (−2.5 and −5.0 bars) with polyethyleneglycol in the culture medium suppressed shoot growth but also accelerated senescent yellowing 3 months after culture. Meantime, untreated control shoots gradually became yellow from the lower leaves and 6 months after culture, 83% of them reached senescent death. Thus, suppressing shoot growth was not effective in case of storage at room temperature unlike that at low temperature. Therefore, supplement of growth promotors was investigated. BA, which activates shoot growth, was adopted in this experiment. In fact, by BA treatment, 35% of chrysanthemum shoots survived for one year (2nd experiment). By subculturing the nodal section of the survived shoots, the clone could be maintained for another one year. Here, at least, three or four times of the required shoot number should be subcultured each year, because a loss by senescence and contamination

must be taken into account. Thus, this *in vitro* storage at room temperature seems to be convenient and economical since only one subculture each year is enough to maintain an important clone or cultivar.

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References

1) Hosoki, T., T. Asahira, 1978. Abstr. Japan. Soc. Hort. Sci. Autumn Meet., p. 370-371.
2) Fukai, S., M. Morii, M. Oe, 1988. Plant Tissue Cult. Lett., **5**: 20-25.
3) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
4) Ringe, F., J. P. Nitsch, 1968. Plant Cell Physiol., **9**: 639-652.

≪和文要約≫

キクの *In vitro* 室温貯蔵

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キクの茎頂部を，BA を添加した培地に植え付け，室温・自然光下に 8 カ月間置いたところ，約 1/3 のシュートが生存していた．節培養を行うことで新しいシュートが伸長し，さらに 1 年間室温貯蔵したところ，約 1/3 のシュートが保存できた．以上より 1 年 1 回の継代培養で，キクの長期室温貯蔵が可能となった．