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In vitro Propagation of Plumed Thistle (*Cirsium japonicum* DC. cv. Teraoka) by Vertical Shoot-split Method

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In vitro propagation of plumed thistle (*Cirsium japonicum* DC.) cv. Teraoka was achieved by subculturing vertically split shoot-axis followed by division of growing axillary buds on Murashige and Skoog (MS) based medium with 0, 0.44 or 4.4 μM N^6-benzyladenine (BA). A proliferation rate of 2.9 shoots per 16 day-interval was achieved on the medium with BA. Shoots obtained by this method could be rooted in the frequency of 90% on MS basal medium. Plantlets were readily acclimatized on the soil and flowered normally in the greenhouse.

Plumed thistle (*Cirsium japonicum* DC.) native to Japan is a perennial plant with deep red flowers[1] and the cultivar, Teraoka grown in Osaka Pref., is popular for cut flowers because of its long life and glossy and less spiny leaves[2]. Conventional propagation of this plant is vegetatively conducted by division because seedlings do not produce duplicates due to genetic segregation. Division produces only a limited numbers of plants per year, which is inefficient. Previously we reported efficient *in vitro* propagation methods by node culture and division of axillary buds in *Salvia leucantha*[3] and *Verbena tenera*[4]. In a preliminary experiment, however, node culture by traverse stem-sectioning was not applicable for multiplication of plumed thistle because shoot-apex and axillary buds did not elongate stems at the initial culture on Murashige and Skoog (MS)[5] based medium with or without N^6-benzyladenine (BA). Therefore, on subculture for multiplication, we devised vertical shoot axis split method to increase the axillary bud growth.

To date, there have been no data on *in vitro* propagation of *Cirsium spp*. The objective of this study was to develop *in vitro* propagation methods of plumed thistle by splitting the shoot axis and dividing axillary buds. Shoot segments about 0.5 cm in length at the top were excised from greenhouse-grown plants (cv. Teraoka) and rinsed with tap water for 30 sec. They were then surface-disinfected with a solution of sodium hypochlorite (active chlorine, 0.5%) containing 0.1% Tween 20 (v/v) for 10 min. and rinsed twice in sterile water. After basal leaves were removed, shoot-tip explants about 0.3 cm long were excised for the initial cultures. Explants were placed into test tubes (2 cm diam, 15 cm tall) containing 15 m*l* agar-gelled medium and were sealed with aluminum foil. The basal culture medium consisted of MS major salts[5] and FeEDTA and Ringe and Nitsch minor elements and vitamins[6] and 2% sucrose. The medium was gelled with 0.8% agar (Wako Pure Chemical Co., Tokyo) after adjusting the pH to 5.6 with NaOH. The basal medium was supplemented with 0, 0.44 or 4.4 μM BA. The cultures were incubated at 25±1 °C with a 16 h photoperiod provided by cool-white fluorescent lamps (4000 lux). Twenty explants per each BA concentration medium were cultured.

Twenty-four days after culture initiation, explants had expanded 3-5 leaves regardless of BA concentration. Axillary bud growth occurred only on BA supplemented media. Any apical and axillary buds cultured on the medium with or without BA did not elongate stems although leaves developed and expanded. Therefore, traverse stem-sectioning could not be achieved. For further multiplication (the first subculture) of the shoots on the basal medium, shoot axis was vertically split into two sections (microcuttings) so that one section included a shoot apex plus axillary buds with leaves and the other section included only axillary buds with leaves. The former sections were subcultured on the same basal medium to continue growth of the shoot apex; the latter sections also were subcultured on the same basal medium which resulted in growth of axillary buds by release of apical dominance. Shoot splitting was not conducted for the explants with less than 4 leaves because such small explants did not always include shoot axis with nodes but included only leaves. Such explants were transferred to the fresh basal medium without splitting. Axillary buds grown on the media with BA were divided from the apical buds, and both apical and axillary buds with expanding leaves (microcuttings) were cultured on media with the corresponding BA concentrations. A total of 4 subcultures were conducted at about 16 day interval. During subcultures, a few axillary buds on the basal medium spontaneously initiated growth and expanded leaves.

All the data of initial- and four subcultures are analyzed by regression analysis and single degree-of-freedom contrast (**Table 1**). Length of the longest leaf decreased with increasing BA concentrations (**Fig. 1**). Numbers of apical and axillary buds which initiated growth as observed by leaf

Table 1. *In vitro* propagation of plumed thistle by repeated split of shoot axis and division of axillary buds[1].

BA (μM)	Length of the longest leaf (cm)	Number of apical and axillary buds with expanding leaves	Number of microcuttings[2]
0	4.5±1.0	1.2±0.4	1.5±0.5
0.44	2.5±0.8	2.6±0.8	2.9±1.0
4.4	1.5±0.5	2.9±1.3	2.9±1.3
Significance[3]			
Linear	**	**	*
Quadratic	**	**	**
Contrast (μM)[3]			
0 vs. 0.44	**	**	**
0 vs. 4.4	**	**	**
0.44 vs. 4.4	**	NS	NS

[1] Mean±SD of all the data of initial culture plus four subcultures.

[2] Split shoots, apical buds and divided axillary buds.

[3] NS,*,**Nonsignificant, or significant at $P<0.05$ or 0.01, respectively.

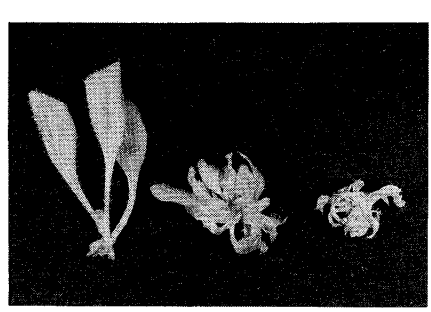

Fig. 1 Shoots cultured for 14 days on the MS medium with 0, 0.44 or 4.4 μM BA (left, middle and right, respectively).

development and expansion were significantly greater on the medium with 0.44 and 4.4 μM BA, compared to those on medium without BA. Thus, the greatest numbers of microcuttings were obtained on the media with BA mainly due to increased numbers of growing axillary buds by release of apical dominance. Shoot splitting for some explants on the basal medium increased the number of microcuttings but it did not reach the number of microcuttings on the media with BA. Although number of microcuttings was the same between the medium with 0.44 and 4.4 μM BA, leaf growth on the former medium was better (**Table 1** and **Fig. 1**). Thus, adding of 0.44 μM BA to the basal medium was suitable for multiplication of microcuttings. If cultures are transferred at about 16 day intervals, 22 subcultures are hypothetically possible per year with a single explant and each subculture produces 2.9 fold new microcuttings. This multiplication rate is by far more rapid than conventional *ex vitro* division. The chance of somaclonal variation would likely be low, because multiplication was conducted only by growth of axillary buds[7].

For rooting, shoots with 4-5 leaves were transferred to the same basal medium supplemented with 0, 0.49 or 4.9 μM indolebutyric acid (IBA). Twenty two days after transfer, shoots rooted well regardless of IBA concentration. High rooting frequency (90%) and root number (4.0±2.4, mean± SD) per shoot were obtained on the basal medium. Increasing IBA concentrations propotionally decreased root length. Thus, the addition of IBA to the basal medium was not necessary for rooting.

Twenty rooted microcuttings were randomly taken from the medium with 0 or 0.49 μM IBA and planted in a plastic tray containing sandy loam and covered with a plastic film to retain moisture. They were watered daily and fertilized twice weekly with 15K-8P-17K water soluble fertilizer (2 g/l). Light conditions were the same as for the *in vitro* multiplication stage but the temperature was lowered to 20 °C to reduce the incidence of fungus and bacterial contamination. Rooted plantlets were acclimatized by gradually opening the film day by day; it was completely uncovered after 20 days. All plantlets were established without any signs of water stress. They were then transferred to a 23,940 cm^3 wooden box containing 2 part sandy loam: one part bark (v/v) and grown in a greenhouse where the temperature was maintained at about 30/22 °C (day/night). Four months after growing in the greenhouse, 17 out of 20 plants flowered normally without any signs of morphological variation (**Fig. 2**). The remaining 3 plants were still too small to flower.

Thus, *in vitro* propagation of plumed thistle could be accomplished from shoot-tip culture and division of axillary bud on MS medium supplemented with BA. In addition, splitting shoot axis may be a useful technique to increase the axillary buds growth when stem elongation is poor and

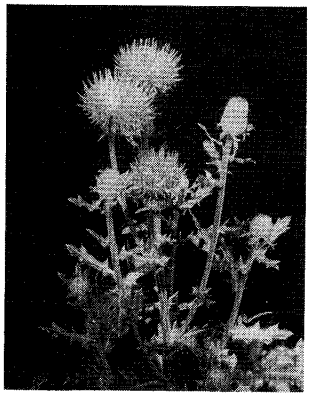

Fig. 2 A flowering plant obtained by *in vitro* culture; 4 months after transfer to soil (cultivated in a greenhouse).

traverse stem sectioning is difficult on the subcultures.

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《和文要約》

寺岡アザミ(*Cirsium japonicum* DC. cv. Teraoka)の シュート縦断法による *in vitro* 増殖

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寺岡アザミ(*Cirsium japonicum* DC. cv. Teraoka)の *in vitro* 増殖 を，0, 0.44 および 4.4 μM BA を含む MS 基本培地上で，茎を縦断して 継代培養することで行った．BA 添加培地で腋芽の成長の促進がみられ，16 日間隔で 2.9 倍のシュート増殖率が得られた．この方法で得られたシュートの 90% は MS 基本培地で発根した．これらは土壌条件下で容易に順化活着し，温室内で正常に開花した．