Plant Regeneration from in Vitro Stem Explants of *Dianthus spiculifolius*

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Plant regeneration from in vitro stem explants of *Dianthus spiculifolius*

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Key words: *Dianthus spiculifolius*, Stem, Regeneration.

Introduction *Dianthus spiculifolius* is a charming dianthus that has fine, grassy, blue-green foliage and much dissected flowers, all cleanly snipped as if someone has gone mad with the crimping sheers. The calyces below the flowers are colored pinkish purple. It is low that can be used as a kind of beautiful turf grass.
Up to now, there was no report about the tissue culture and plant regeneration of *Dianthus spiculifolius*. Our aim in conducting the present investigation was to attain an efficient system for regeneration in *D. spiculifolius*. It is suitable for screening gene-transformed *Dianthus spiculifolius* plants.

Materials and methods Plant material came from Beijing Liangxianglvjing Planting Center of Seedling and Wood. Cultures of *Dianthus spiculifolius* were established from young stem collected from greenhouse-grown plants. Murashige and Skoog (1962) medium supplemented with 3% (w/v) sucrose, 2, 4-D, 6-BA and NAA in various combination was used for callus initiation. KT in combination with 6-BA and IAA were used for shoot regeneration. Culture media were solidified with 0.8% agar and adjusted to pH 5.8 before autoclaving at 121℃ and 1.2-1.3 kg/cm^2 pressure for 20 min. Forty ml of medium was dispensed into 100 ml Erlenmeyer conical flasks. Ten flasks were prepared for each treatment and in each flask at least 2 explants were inoculated. Cultures were incubated in a growth chamber at a temperature of 26 ± 2℃ with light intensity of 250 umol. M^{-2} s-1 provided by fluorescent tube lights and incandescent bulbs. A photoperiod of 16 h was maintained with the help of photo thermal controller. Weekly observations were recorded.

Results The best callus initiation medium was MS + 2, 4-D (1.0mg/l) + 6-BA (0.5mg/l) + NAA (0.1mg/l). The callus was maintained by regular subculture every 3 weeks.
No shoot bud induction was observed in the cultures above. That may be the effect of 2, 4-D. Callus After the 3rd week of subculture, best response in terms of shoot formation was observed and shoot bud development in the callus became conspicuous on MS medium supplemented with KT (4.0mg/l) + 6-BA (0.2 mg/l) + IAA (0.02mg/l). The number of shoot buds increased as the culture period progressed to 7 weeks. After 7 weeks, almost the entire callus was converted into shoots. Callus sub cultured on medium containing 6-BA and IAA without KT did not show any organogenesis.
After 8 weeks, plantlets were potted in a soil-vermiculite mixture, covered with film for acclimatization, and subsequently transferred to the greenhouse.

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