Note

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Optimum Concentrations of Benzyladenine and Naphthaleneacetic Acid for Shoot Cultures of Potted Carnations

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Abstract

The shoot culture of potted carnation plants exhibited a poor growth on a medium used previously for the shoot culture of non-potted carnations. In the present study, we determined suitable concentrations of N^6-benzyladenine (BA) and 1-naphthaleneacetic acid (NAA) to improve the growth of shoot cultures of potted carnation plants. The optimum concentrations of BA and NAA applied together for *in vitro* shoot cultures of potted carnation plants were 0.4 and 0.1 mg l^{-1}, respectively. The optimum concentration of BA was much lower than that (1 mg l^{-1}) reported for the culture of most non-potted carnation plants.

Key words: auxin, cytokinin, *Dianthus caryophyllus*, plant growth regulator, shoot culture.

Abbreviations

BA, N^6-benzyladenine; NAA, 1-naphthaleneacetic acid.

Ethylene is a primary plant hormone involved in the senescence of cut carnation flowers (Reid and Wu, 1992). Currently, preservatives that inhibit the synthesis or action of ethylene such as silver thiosulfate anionic complex (STS) (Veen *et al.*, 1979) and 1,1-dimethyl-4-(phenylsulfonyl)semicarbazide (DPSS) (Midoh *et al.*, 1996) are used to lengthen the vase life of cut carnation flowers. The chemicals are administered to carnation flowers through vascular transport by immersing the cut stem end in the preservative solutions. On the other hand, transgenic carnation plants with suppressed production or action of ethylene were generated in the past years; e.g., the lines transformed with a carnation 1-aminocyclopropane-1-carboxylate (ACC) oxidase cDNA in antisense orientation (Savin *et al.*, 1995) or in sense orientation (Kosugi *et al.*, 2000), and the line harboring an *Arabidopsis thaliana etr1-1* allele capable of rendering ethylene insensitivity in carnation (Bovy *et al.*, 1999). Cut flowers of these transgenic lines have a prolonged vase life compared with those of the non-transgenic plants.

Carnations are used as ornamental plants in the form of potted plants as well as in the form of cut flowers. Potted carnation plants usually grow in a dwarf form. In a preliminary study, we found that the longevity of each flower of potted carnation cultivars was generally shorter than that of flowers of non-potted carnation plants. The short longevity probably causes a short display time of the plants, which is the sum of flowering period of each flower. The preservatives for cut carnation flowers described above cannot be used for potted carnation plants. Therefore, the generation of transgenic plants is thought to be a promising way to lengthen the display time of potted carnation plants.

In the generation of transgenic carnations including those described above, *Agrobacterium*-mediated transformation has been widely used (Zuker *et al.*, 1998). In addition, some other methods were reported for the transformation of carnations; a microprojectile bombardment (Zuker *et al.*, 1995) and a combination of microprojectile bombardment and *Agrobacterium*-mediated transformation (Zuker *et al.*, 1999).

Previously, using *Agrobacterium*-mediated transformation, Kosugi *et al.* (2000) succeeded in the generation of a transgenic carnation line whose flowers produced ethylene in a negligible amount during the senescence period and had a vase life longer than flowers of the non-transformed parent cultivar (cv. Nora). Then we tried to apply this strategy to generate transgenic potted carnation plants with a lengthened display time, but we found difficulty in propagating a sufficient amount of

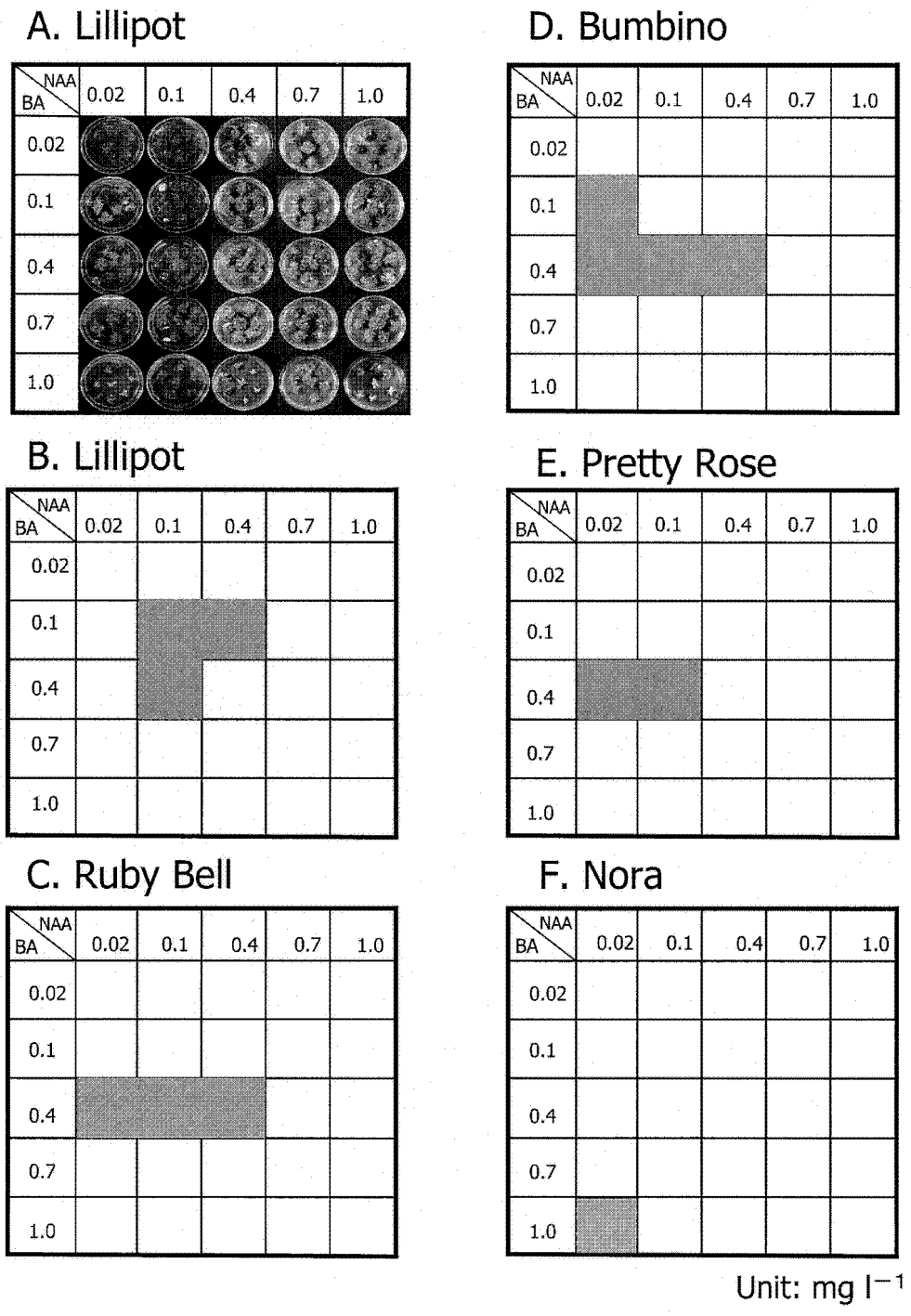

Fig. 1 Effects of BA and NAA at different concentrations on proliferation of cultured shoots of potted carnation plants.
(A) Small shoot clusters with three leaflets of cv. Lillipot were cultured for 4 weeks on MS medium supplemented with 3% sucrose, 0.25% agar and combinations of BA and NAA at given concentrations. The unit for BA and NAA concentrations is mg l^{-1}. (B – F) Optimal concentrations for potted carnation cvs Pretty Rose, Bumbino, Lillipot and Ruby Bell, and for non-potted cv. Nora (Kosugi *et al.*, 2000).

shoot explants that could be used for transformation experiments. That is, the shoot culture of potted carnation plants exhibited a poor growth on Murashige and Skoog (MS) medium supplemented with 0.25% agar, 3% sucrose, 1 mg l^{-1} N^6-benzyladenine (BA) and 0.02 mg l^{-1} 1-naphthaleneacetic acid (NAA), which was used previously for the shoot culture of cv. Nora carnation (Kosugi *et al.*, 2000). In the present study, we determined suitable concentrations of BA and NAA to improve the growth of shoot cultures of potted carnation plants.

Lateral shoots of about 8 cm in length from cvs Bumbino, Ruby Bell and Pretty Rose, and micorpropagated shoots of about 10 cm in height

from cv. Lillipot were obtained from Sakata Seed Co., Kakegawa Research Center (Kakegawa, Japan). All of the cultivars are currently being used as potted carnation plants. The shoots of these cultivars were cultured on the MS-based medium according to the method of Firoozabady *et al.* (1995). All cultures were incubated at 23±1℃ with a 16-h photoperiod (40 μmol m^{-2} s^{-1}, cool white fluorescent light) and subcultured at 4-week intervals for several months. Then, small shoot clusters with three leaflets were placed on the MS medium, supplemented with combinations of BA and NAA at different concentrations, and grown for 4 weeks under the conditions mentioned above. The concentrations tested were 0.02, 0.1, 0.4, 0.7 and 1.0 mg l^{-1} for both BA and NAA. The index of optimal concentrations for BA and NAA was the size of shoot clusters of 4-week cultures. Since all the four cultivars exhibited similar growth profiles in shoot cultures after a 4-week cultivation, only the photographs of cv. Lillipot are shown in **Fig. 1A**. Shoot clusters proliferated best at 0.1 mg l^{-1} BA applied together with 0.1-0.4 mg l^{-1} NAA (in the following shown as 0.1 BA / 0.1-0.4 NAA) as well as at 0.4 BA / 0.1 NAA (**Fig. 1B**). In these cases, the diameter of shoot clusters was larger than 2 cm after a 4-week cultivation. Shoot clusters on the media containing BA at 0.7 and 1 mg l^{-1} grew poorly and a considerable number of leaflets became yellow. Shoot clusters placed on the media containing NAA and BA at a ratio of five or more exhibited poor growth and formed adventitious roots in a considerable number of shoot clusters.

Similarly, cv. Pretty Rose proliferated well at 0.4 BA / 0.02-0.1 NAA, cv. Bumbino at 0.4 BA / 0.02-0.4 NAA as well as at 0.1 BA / 0.02 NAA, cv. Ruby Bell at 0.4 BA / 0.02-0.4 NAA (**Fig. 1C-E**). The cultured shoots of four potted carnation cultivars generally proliferated well at 0.4 BA / 0.1 NAA concentration. The combination of BA and NAA at these concentrations was different from that used for cv. Nora, which was successfully cultured on MS medium supplemented with 0.25% agar, 3% sucrose, and 1.0 BA / 0.02 NAA (**Fig. 1F**, Kosugi *et al.*, 2000). The latter combination of BA and NAA concentrations was adopted from the report by Firoozabady *et al.* (1995), who cultured carnation shoots of cv. Improved White Sim and six other cultivars successfully. Moreover, van Altvorst *et al.* (1995) cultured 30 cultivars on MS-based medium containing 1.0 BA / 0.1 NAA. In the previous works the optimum concentrations of BA and NAA for shoot culture of non-potted carnations were 1.0 and 0.02-0.1 mg l^{-1}, respectively. The difference between potted and non-potted carnations in the requirement of BA may result from the difference in the contents of plant hormones between the two categories of carnation plants. The potted carnations generally have a dwarf stature, which may be related to hormone contents, such as lower GA content and/or higher ABA content, than in non-potted carnations. The effects of combinations of these plant hormones remain to be tested for further optimization of the growth of shoot cultures of potted carnation plants. The present findings should help to supply us with a sufficient amount of shoot clusters for genetic manipulation of potted carnation plants.

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