キク‘秀芳の力’における形質転換体獲得効率に及ぼす諸要因

古賀正明・平島敬太・中原隆夫（福岡県農業総合試験場）

Masaaki KOGA, Keita HIRASHIMA and Takao NAKAHARA (Fukuoka Agric. Res. Cent.) :

Factors of Transformation Effciency in Chrysanthemun ‘Shuhono-chikara’

‘Shuhono-chikara’ has been the predominant chrysanthemum cultivar in Japan in this quater of a century for it's beautiful floweres and plant form. However,this cultivar has a week point of being susceptible to fungal deseases. Transgenic plants, also having characters in original cuktivars, are shown to be useful for controlling the fungal diseases in some species. Aida et.al.[1] reported the gain of transgenic chrysanthemum ‘Shuhono-chilara’, but they did not refer to the transgormation efficiency. Therefore, we investigated the factors for the efficient gain of transformants in this plant.

1. Materials and Methods

Petioles of the in vitro chrysanthemum ‘Shuhono-chikara’ were cut into pieces of about 3mm in length and the pieces were immersed for 10 min in a suspension culture of *Agrobacterium tumefacience*, which was grown in YEB medium (pH5.2). Pieces of petioles were blotted on sterilized filter paper to remove excessive amount of bacterial suspension, and co-cultured with Agrobacterium on callus inducing medium (MS, Sucrose 30g/l, gellan gum 2g/l, BA 1mg/l, NAA 1mg/l, pH5.8) in the dark for 3 days at 25℃. Then, the culture was continued at 25℃ under continuous lighting at 6,000lux for 16 hr. The pieces of petioles were transferred to callus inducing medium containing 500mg/l of Claforan for removing *Agrobacterium*. Following culture of two weeks, 25mg/l of kanamycin was added to the medium for selection of transformed calli. Concentration of Claforan in the medium was reduced to 250, 100 and 0mg/l every two weeks. Kanamycin resistant calli, induced on the medium and showing green color, were cut from the pieces of petioles when they leached 3-5mm in diameter, and grown on the callus inducing medium containing no antibiotic in test tube (φ25mm ×100mm). The calli raised were transferred every 2-3 months onto regeneration medium (MS, Sucrose 30g/l, gellan gum 2g/l, BA 1mg/l, pH5.8). Regenerated shoots were planted in 1/2MS solid medium supplemented with 25mg/l of kanamycin and their rooting was confirmed. DNA of the rooted plants was extracted by PEG method and the presence of NPT Ⅱ gene was confirmed by PCR.

2. Results and Discussion

Antibiotic resistant calli were induced on callus inducing media containing 25mg/l of Kanamycin, but not on the ones supplemented with 30mg/l of geneticin. There was no effect of the supporting material of the media in co-culture on the induction of the Kanamycin resistant callus (Table 1). The induction efficiency of the Kanamycin resistant callus in combination with vector and *Agrobacterium* strain was higher in pSMAK251 and EHA101 than in pBI121 and LBA4404 (Table 2). Shoots regenerated from the kanamycin resistant calli on the media containing only 1mg/l of BA, in contrast to regeneration of no shoot on the media supplemented with 1mg/l of BA and NAA (Table. 3). Existence of NPT Ⅱ gene were confirmed in 12 of 14 plants regenerated from individual Kanamycin resistant calli.

Summarrizing these results, it is expected that about 9 transformants are able to be acquired from 100 pieces of petioles under present best conditions.

Reference

1) Aida, R., Tabei, Y., Hirai, M. and Shibata M., 1992. Japan. J. Breed. 42 (supple 2) : 270-271.

Table 1. Effects of supporting material in co-culture media and antibiotics on induction of antibiotic resistant calli

Supporting material in co-culture media	Antibiotic used	No.of petiole pieces tested	No.of induced antibiotic resistant calli (%)
gellan gum 2g/l	Kanamycin 25mg/1	200	7 (3.5)
filter paper	Kanamycin 25mg/1	200	4 (2.0)
gellan gum 2g/l	geneticin 30mg/1	200	0 (0)
filter paper	geneticin 30mg/1	200	0 (0)

a) Investigation was conducted on the 79th day after inoculation.
b) Vector and strain of *Agrobacterium tumefacience* were pBI121 and LBA4404.

Table 2. Effect of combination of vector and strain of *Agrobacterium* on induction of kanamycin resistantcalli

Vector /Strain of *Agrobacterium*	No.of petiole pieces tested	No.of induced kanamycin[a] resistant calli (%)
pBI121/LBA4404	250	27 (10.8)
pSMAK251/EHA101	200	164 (82.0**[b])

a) Investigation was conducted on the 150th day after inoculation.
b) Significant at 1% level in accordance with Fisher's exact probability test.

Table 3. Effects of kinds of plant growth regulators on plant regeneration from kanamycin resistant callus

Plant growth regulators BA	NAA	No.of calli[a] tested	No.of regenerated[b] calli (%)
mg/1	mg/1		
1	-	252	32 (12.6)
1	1	63	0 (0**[c])

a) Calli which were kanamycin resistant and grown on the media containing 1mg/1 of BA and NAA and no antibiotic were tested.During the test period,the calli were transferred to the same types of new media at 30-60 day intervals.
b) Investigation was conducted on the 180th day after transfer to test media.
c) Significant at 1% level in accordance with Fisher's exact probability test.
d) Half-strength MS media containing 30g/1 of sucrose and 2g/1 of gellan gum in test tubes covered with aluminium foil were used for the test.