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Genetic transformation of Chrysanthemum through *Agrobacterium tumefaciens*

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Objectives

We have tried to make improved cultivars of Chrysanthemum which is adapted to low temperature damage in the natural field using particle bombardment and vacuum assisted *Agrobacterium*-mediated transformations. The transgenic Chrysanthemum was selected in the media, PCR and Real-Time PCR screening methods.

Materials and Methods

1. Materials

Plant – Chrysanthemum(*Dendranthema grandiflorum*). *Agrobacterium* strain – MP90/pBin19(11,777bp), BN115 gene

2. Methods

Callus was obtained from leaves of Chrysanthemum and was maintained in MS+3%sucrose media containing phytohormone(1.0mg/L BA, 0.3mg.L^{-1} 2,4-D 1.0mg/). The infection was done by gene-gun and *Agrobacterium*-mediated methods for 10 min with 10 times dilution. The BN115 gene which was used for improving Chrysanthemum from low temperature damage was constructed in MP90/pBin19 that harbored gene for neomycin phosphotransferase gene(NPTII).

Results and Discussion

With the use of *Agrobacterium* and gene-gun, cold regulated gene(BN115) has been injected in Chrysanthemum leaf disc and transgenic plants have been produced successfully on the selection media containing phytohormone(Fig. 1). To determine the presence of the transferred cold regulated gene(BN115) in the transgenic Chrysanthemum, PCR-amplification indicated the presence of that gene(Fig.2A). Real-Time PCR for confirmation of the putative transgenic plants was established. The copy number of cold regulated gene(BN115) is extrapolated on the basis of a standard curve. Serial dilutions of known number of gene copies were in triplicates. In this diagram, PCR cycles are plotted against the fluorescence intensity. The cycle at which the fluorescence reaches a threshold cycle is inversely proportional to the starting amount of target DNA(Fig. 2B,C,D).

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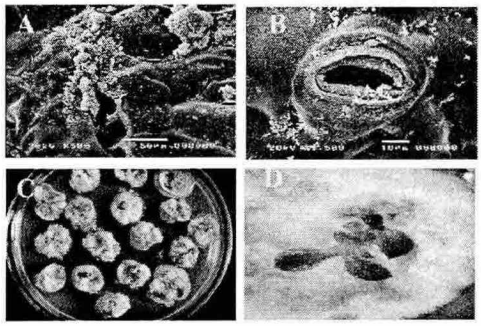
To checking whether over-expression of BN115 gene enhanced tolerance to cold stress, the growth of transgenic plant at each low temperature was better compared to wild-type plants.

Fig. 1. Production of low temperature resistant transgenic chrysanthemum



A,B) Electron microscopic view of *Agrobacterium tumefacience* and on vacuum infiltrate/gene-gun at leaf disc through injury. C) Cold regulated gene(BN115) successfully transferred and callus formation on the selection medium containing 5 mg/mL of Kanamycin. D) Cold regulated transgenic Chrysanthemum is growing on medium.

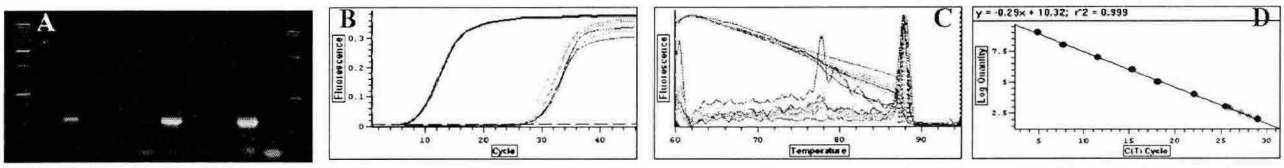


Fig. 2. PCR and Real-Time PCR BN115 transformants using NPT II resistance gene primers, indicating the presence of gene.

A) PCR BN115 gene transforments using NPT II gene primer. B,C) Amplification plots showing the changes in fluorescence of SYBR Green dye plotted versus cycle number. D) Standard curve showing C_t values plotted versus the log of the initial amount of genomic DNA.

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