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Genetic transformation of chrysanthemum with cold regulated gene

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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 and was tested growth characterizes on low temperature

Materials and Methods

1. Materials

Plant - Chrysanthemum(*Dendranthema grandiflorum*). *Agrobacterium* strain - MP90/pBin19, cold regulated gene(BN115)

2. Methods

The infection was done by gene-gun and *Agrobacterium*-mediated methods for 10 min with 10 times dilution. The trasnforments was selected on media containing kanamycin 5.0mg/L and 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

The cold regulated gene(BN115) has been injected in Chrysanthemum leaf disc with use of Agrobacterium and gene-gun, and transgenic plants have been producedsuccessfully 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. In this diagram, PCR cyclesare 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). 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.

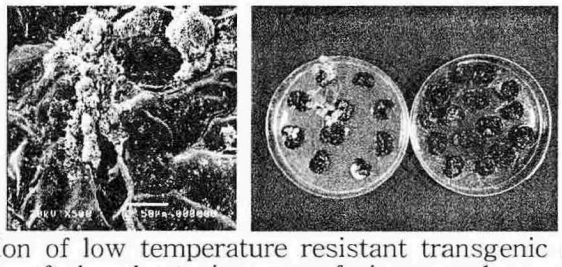

Fig. 1. Production of low temperature resistant transgenic chrysanthemum
A) Electron microscopic view of *Agrobacterium tumefacience* and on vacuum infiltrate/gene-gun at leaf disc through injury. B) Cold regulated gene(BN115) successfully transferred and callus formation on the selection medium.

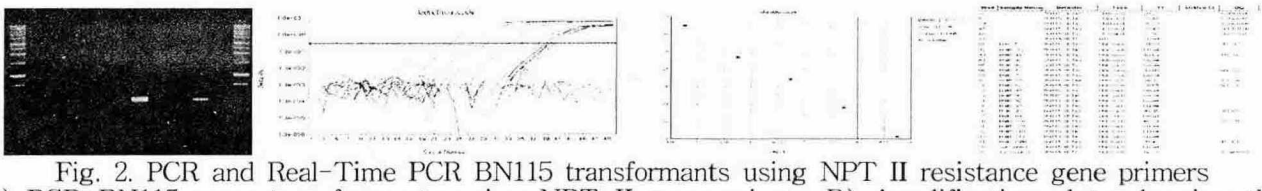

Fig. 2. PCR and Real-Time PCR BN115 transformants using NPT II resistance gene primers
A) PCR BN115 gene transforments using NPT II gene primer. B) Amplification plots showing the changes in fluorescence of Taqman prove plotted versus cycle number. C, D) Standard curve and Result showing Ct values plotted versus the log of the initial amount of genomic DNA.

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