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Short Communications

Cloning of rd29A-CBF3 gene constructs in binary vector (pCAMBIA0390) for improving high altitude agriculture during cold stress*

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A number of environmental factors such as cold, chilling, drought, salinity, high temperature, anoxia, high and low light intensity and nutrient imbalance, collectively called abiotic stresses, negatively affect the processes associated with biomass production and grain yield in plants (Wang *et al*. 2003). Among other abiotic stresses, low temperature stress is an important factor limiting the production and geographic distribution of many horticultural species. Even in areas considered suitable for cultivation of given species, yield decline and crop failure frequently occur as a result of aberrant chilling temperature. Plants vary greatly in their ability to withstand low temperature stress. As an adaptation strategy, most plants native to the temperate climates develop chilling tolerance after prior exposure to cold, non-freezing temperatures, a phenomenon called acclimatization (Xin and Browse 2000). Many biochemical and physiological changes are known to occur during cold acclimation. With the onset of low temperature, putative temperature sensors at the cellular membrane generates stress signals which are transmitted and amplified through many steps that include Ca^{+2} signaling and a stepwise kinase/ phosphatase chain reaction termed the kinase cascade. The message eventually reaches the nucleus and regulators of gene expression called transcription factors, which acts as master switches to regulate the expression of groups of genes, resulting in an increase of proteins and other organic molecules that protect the cells from freezing damage. The plants response to cold acclimation is clearly complex and diverse, and the actual biochemical and physiological changes are still poorly understood at the molecular level (Knight and Knight 2001).

The CBF3 gene is a transcription factor that binds to CRT/DRE element, in the promoter region of the cold regulated (COR) genes and regulate their expression in response to both low temperature and water deficit (Gilmour *et al.* 1998). CBF3 belong to EFR (Ethylene Responsive Element Binding Factor)/AP2 family of transcription factors (Riechmann *et al*. 2000, Agarwal *et al*. 2006). In *Arabidopsis*, three DREB1/CBF namely, DREB1B/CBF1, DREB1A/ CBF3, and DREB1C/CBF2 have been isolated. It has been possible to engineer cold tolerance in transgenic plants by manipulating the expression of CBFs (Stockinger *et al*. 1997). Using the transformation technology, single dominant agronomical characteristics, such as tolerance to cold can be introduced into crops in a short time without changing desirable genes as conventional breeding technique is time consuming and often accompanied by changes in other desirable characteristics (Dunwell 2000, Wang *et al*. 2003). CBF3 plays a crucial role in providing tolerance to multiple stresses and displays overlapping responses to different stress conditions. CBF3 controls expression of stress responsive genes via ABA-independent pathways in both abiotic and biotic stress (Agarwal *et al*. 2006). The constitutive expression of the genes by strong constitutive promoters such as 35S CaMV may have serious implication with respect to energy loss and other deleterious effects. Over expression of CBF3 with stress inducible rd29A promoters was useful for improving drought, cold and salt stress without any penalty on growth (Kasuga *et al*. 1999).

pCAMBIA0390 vector DNA containing rd29A-CBF1 gene constructs (Gupta *et al*. 2009) were double digested with *BamH1* and *Sac1* restriction endonucleases and purified by using gel extraction kit from Qiagen (USA). cDNA of CBF3 gene (630 bp) was amplified from genomic DNA of *Arabidopsis thaliana* by PCR using the synthetic DNA oligomers (EF 5´ CGGGATCCGGAGTGCGCT TCTGATCAATGAACTC 3´ and ER 5´ AGAGCTCCGG AGGCTGAATAACTCCATAACGATACG 3´ as primers containing *BamH1* and *Sac1* restriction endonucleases sites. Amplified cDNA of CBF3 were double digested with *BamHI*

*Short note

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and *SacI* restriction endonucleases and purified by using gel extraction kit from Qiagen (USA). Double digested cDNA of CBF3 gene (630 bp) under the control of stress inducible rd29A promoter (686 bp) and Nos terminator (253 bp) has been cloned in T-DNA of pCAMBIA0390 vector between *BamHI* and *SacI* restriction end nuclease site (Fig 1). Cloning of rd29A-CBF3 gene construct in pCAMBIA0390 vector has been confirmed by restriction and PCR analysis. CBF3 gene constructs cloned in pCAMBIA0390 (Fig 2) and nptII gene containing pCAMBIA2200 vector DNA was transformed separately into the Agrobacterium strain LBA4404 by electroporation method as described by Sambrook and Russell (2001). Transformed *Agrobacterium* colonies were further confirmed by restriction and PCR analysis for the presence of CBF3 and nptII gene and subsequently used for genetic co-transformation of vegetable crops.

It is concluded that the production of cold-tolerant transgenic crops containing rd29A-CBF3 gene constructs would increase productivity and production in high altitude regions during cold stress.

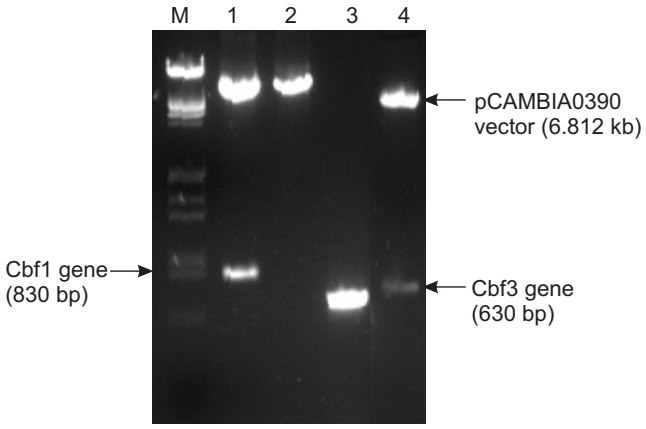

Fig 1 Cloning of rd29A-CBF3 gene constructs in pCAMBIA0390 vector.

LaneM- Lamda DNA (double digested with *EcoRI*/*HindIII*). Lane 1- Cloned pCAMBIA0390 (rd29A-CBF1 gene constructs) double digested with *BamH1* and *SacI* restriction endonucleases. Lane 2- Purified pCAMBIA0390 vector (6.812 kb) (double digested with *BamH1* and *SacI*). Lane 3- Purified cbf3 gene (630 bp) double digested with *BamHI* and *SacI*. Lane 4- Cloned pCAMBIA0390 (rd29A-CBF3 gene constructs) double digested with *BamH1* and *SacI*.

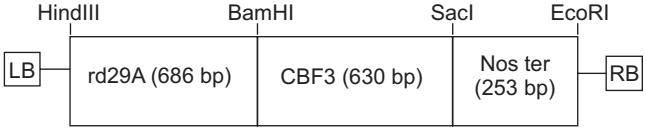

Fig 2 T-DNA of pCAMBIA0390 vector containing rd29A-CBF3 gene constructs

SUMMARY

CBF3 gene is rapidly induced upon exposing plants to low temperature, followed by expression of CBF-targeted genes, the CBF regulon, resulting in an increase in plant freezing tolerance. It has been possible to engineer cold tolerance in transgenic plants by manipulating the expression of CBFs. In this attempt CBF3 gene has been isolated from *Arabidopsis* and cloned in binary vector (pCAMBIA0390) under the control of stress inducible rd29A promoter. CBF3 gene cloned pCAMBIA0390 and nptII gene containing pCAMBIA2200 vector DNA was transformed into the *Agrobacterium* strain LBA4404 that could be used to generate cold-tolerant transgenic plants for improving high altitude agriculture during cold stress.

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