Functional analysis of myb transcription factor gene under abiotic stress in *Brassica juncea* L. Coss. & Czern.

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ABSTRACT

Abiotic stress, especially drought and salinity adversely affect the growth, development and productivity of crops worldwide. Plant tolerance to environmental stress depends upon activation of cascade of molecular network involved in stress perception, signal transduction, expression of specific stress related genes and metabolites. Abiotic stress tolerance is a quantitative trait involving many genes with cumulative effects. The identification of stress inducible regulatory genes offers promise to develop abiotic stress tolerant plants since these have profound ability to regulate metabolic steps associated with stress response, leading to cellular homeostasis, detoxification and recovery of growth. There is a growing interest in use of regulatory gene as an effective way of developing stress tolerant genotypes by modulating gene expression. The present study was undertaken for functional analysis of *Myb*, a transcription factor gene under drought and salt stress in Indian mustard. Two weeks old Indian mustard (vars. RH0116 & CS 52) seedlings were grown on MS medium without growth regulators and given salt and drought stress treatments followed by RNA isolation using trizol reagent. The primers were designed using *AtMyb2* gene sequence of *Arabidopsis thaliana* based on conserved DNA binding domain of *Myb* gene. Semi-quantitative RT-PCR was conducted to study *myb* expression under stress conditions. A 250 bp transcript was observed within 15 minutes of salinity stress which was maximum at 30 minutes irrespective of salt concentration in CS-52, a salt tolerant genotype. In RH0116, *myb* expression was only observed in seedlings exposed to drought and salinity stress for one hour. The *myb* transcripts were missing in non-treated and rehydrated seedlings. The cDNA was cloned and sequenced. It showed similarity to *Arabidopsis myb* genes involved in stomatal conductance. It demonstrates the role of *myb* gene in coordinated regulation of stress tolerance.

INTRODUCTION

Plants are continuously exposed to a myriad of abiotic and biotic stresses, which limit growth and productivity. Responses and adaptation under abiotic stress require differential gene expression, which is regulated by specific Transcription factors (TFs). TFs are proteins with a DNA domain that binds to the *cis*-acting element present in the promoter of a target gene (Saibo et al., 2009). As multiple stress responses are necessary for plant to endure severe stress conditions, the engineering of a single gene is not sufficient but it is possible for a single TF to control the expression of multiple target genes. There is growing interest in use of regulatory genes to develop stress tolerant genotypes by modulating gene expression. Transcriptome profiling and gene expression studies are prerequisite for identification of *myb* transcription factor gene which have role in stress tolerance (Chen et al. 2005). *Brassica juncea* cv. CS-52 is a highly salt tolerant genotype released by Central Soil Salinity Research Institute Karnal, India while RH-0116 genotype is drought tolerant, developed by CCS Haryana Agricultural University, Hisar. Comparative *myb* gene expression can help in knowing its role in drought and salt tolerance. Further it is important to know if *myb* can modulate expression of other stress related genes.

MATERIALS AND METHODS

Fourteen days old *Brassica juncea* cv. RH0116 and CS-52 seedlings, germinated on MS medium (Murashige and Skoog, 1962) without growth regulators, at 25^{0}C and 16 hours light and 8 hours dark photoperiod, were subjected to salinity stress (100 mM, 200 mM, 300 mM and 400 mM NaCl) and drought stress (Mannitol 100 mM, 200 mM, 300 mM and 400 mM) treatments and stressed seedlings were used for total RNA isolation using Trizol reagent (Supriya, 2006). The total RNA was used for studying *myb* gene expression in *Brassica juncea* cv. CS-52 under salinity and drought stresses. Primers were designed from conserved DNA binding domain from *AtMYB2* (NCBI Acc. No. AK229140.1/D14712) of *Arabidopsis thaliana* and were used to study myb gene expression through RT-PCR. The cDNA synthesis was carried out using total RNA (5µg) as template, 0.2 µg of random hexamer primer and DEPC treated autoclaved water in 25 µl reaction mixture and were incubated at 90°C for 5 minutes in Eppendorf thermocycler and immediately put on ice. RNase inhibitor (40 units), dNTPs mix (2 mM), 1X RT buffer, 200 units of RT (Novascript III RNase H minus) were added to the

template-primer mixture and incubated in thermocycler for 60 minutes at 42°C for cDNA synthesis. The reaction was ended by incubating the mix at 70°C for 10 minutes to stop the activity of RT enzyme. The synthesized cDNA was then further used for the *myb* gene amplification. BjActin primers were used for the amplification of actin gene, which acted as positive control in our experiment. For *myb* gene amplification 2 µg of template, 1X PCR buffer, 0.4 mM dNTPs mix, 2 mM $MgCl_2$, 1 µM primer (reverse and forward), 5 units of Taq DNA polymerase, 1.5 µl of DMSO and x µl of DEPC treated water were used for PCR. PCR amplification was performed using a programme of 36 cycles of following steps: denaturation at 92°C for 1 minute, primer annealing at 62°C for BjActin and 64°C for BjMyb for 1 minute, extension at 72°C for 1 minute and final extension for 10 minutes. The amplified product was analysed on 1.5% w/v agarose gel.

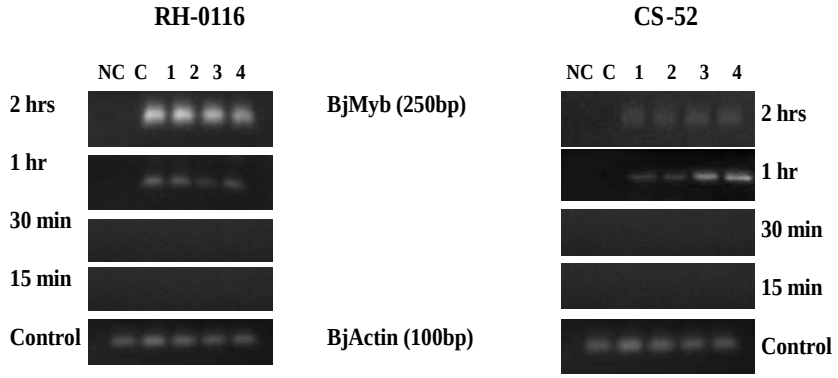
Table 1: Sequences of the primers used for *myb* gene expression in *Brassica juncea* cv. CS-52

Sr. No.	Primer	Sequence
1.	BjActin-F	5'TGG CAT CAC ACT TTC TAC AA 3'
2.	BjActin-R	5'CAA CGG AAT CTC TCA GCT CC3'
3.	BjMyb-F	5'CTG GTA AGA GTT GTA GAT TAA G3'
4.	BjMyb-R	5'CTC GGC ATC CAA ACA TTT CTC3'

RESULTS AND DISCUSSION

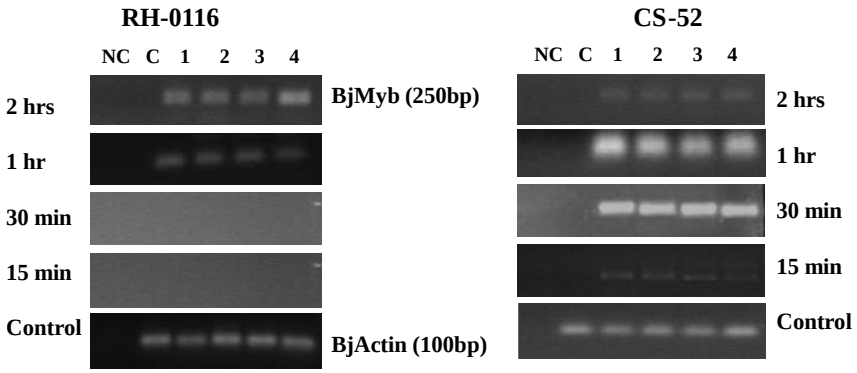
On exposure to salinity stress, *myb* induction was observed within 15 minutes of exposure in CS52 with an increase in its expression up to 30 minutes and followed by decline in *myb*

BjMyb* gene expression under mannitol induced drought stress in *Brassica juncea



NC-Negative Control,C- Control,1- 100 mM Mannitol, 2- 200 mM Mannitol,3- 300 mM Mannitol 4- 400 mM Mannitol

BjMyb* gene expression under NaCl induced salinity stress in *Brassica juncea



NC-Negative Control,C- Control, 1- 100 mM NaCl,2- 200 mM NaCl,3- 300 mM NaCl, 4- 400 mM NaCl

expression up to 1 hour and 2 hours. When *Brassica juncea* cv. CS-52 seedlings were exposed to drought stress (Mannitol 100mM, 200mM, 300mM and 400mM), *myb* expression was observed only at 1 hour drought stress exposure. In RH0116 cultivar, myb gene expression was induced when salt/ drought stress was given for one hour. Myb transcripts were not observed in non-treated seedlings of both the cultivars. Also these transcripts disappeared when seedlings were rehydrated for two hours.

The partial cDNA showed similiarity to Arabidopsis myb gene which controlled stomatal conductance under abiotic stress. The study showed the differential *myb* expression under different abiotic stresses (salinity and drought) in *Brassica juncea*.

Myb gene expression can be successfully studied using reverse transcription making it as an invaluable method for gene expression analysis (Maxfeild *et al*., 2005). Sunil kumar (2007) also used RT PCR for studying *myb* expression in *Brassica juncea* and observed early induction of these transcripts in response to salinity stress. The late induction of *myb* in *Brassica juncea* cv. CS-52 under drought is due the fact CS-52 is a salinity tolerant variety and may a different myb gene is induced under drought stress. In case of *Brassica carinata var.* HC212 also, expression of *myb* transcription factor was observed after exposure to 1 hour of drought (air drying) treatment (Supriya, 2006).
The study shows that *myb* (transcription factor) gene (homologous to drought inducible *AtMyb2)* is induced in *Brassica juncea* in response to abiotic stress (salinity and drought) only as no *myb* transcripts were observed in untreated seedlings and their disappearance upon rehydration. Myb transcripts under salinity and drought may also be different as these are induced at different times under these treatments. cDNA from these two treatments were cloned, sequenced and showed their involvement in stomatal conductance under abiotic stress.

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