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Der Pharmacia Lettre, 2016, 8 (7):179-182
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Study of MaASR1 Gene Expression from Dwarf Cavendish Banana under Salinity Treatment

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ABSTRACT

Environmental stresses including salinity are viewed as the most important limiting factors in crop production. The genetic improvement of banana is important in order to design more productive hybrids. Most of the molecular techniques are based on tissue culture. ASR protein located in both cytoplasm and nucleus and it is functioning as transcriptional regulator. Most of the ASR genes are up-regulated under different environmental stress conditions and during fruit ripening. ASR1 protein is a highly charged low molecular weight plant protein that is regulated by salt- and water-stresses and generated by abscisic acid. In this study, we obtained the full-inducing gene ABA, which is called MaASR1, from banana leaves based on cDNA and method of RT-PCR from a single clone Dwarf Cavendish cultured in MS medium treated with 250 mM of NaCL for 0, 6, 12, 24, and 48 hours, and studied it with agarose gel electrophoresis. The study indicated that the expression of MaASR1 gene in banana leaves is induced under salt-stress and the high expression of MaASR1 will improve plant tolerance to salinity stress. Further studies on identifying the direct target genes of MaASR1 using chip method and the cellular mechanisms of MaASR1 in the nucleus and plasma membrane, as well as further studies on the gene expression of ABA/stress-responsive and their effects on salinity and structure and function of abscisic acid receptors in salinity stresses, it would be easier to find the role and significance of MaASR1 in tolerance to salinity stress.

Keywords: MaASR1 gene, tissue culture, genetic improvement of bananas, salt-stress

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INTRODUCTION

Banana (Musa acuminate L.), which has an important role in agricultural economics, is a commonly consumed fruit. Banana tree has shallow roots and constant green covering crest that make it sensitive to inappropriate conditions such as freezing, dryness and high salinity [1][2][3][4]. Dwarf Cavendish cultivar belongs to the Cavendish group, which includes commercially important cultivars [5].Environmental stresses specially salinity are effective factors in production of agricultural crops [6][7]. Various studies on salinity stress has been previously done showing that the significant damage of salinity is slowing down the growth of plants [6][8]. Most of the modern plants will be damaged in 3000 PPM salinity and in 5000 PPM salinity there is no possibility to grow [8]. Genetic modification and selection of salt tolerance plants is an important and effective method to defense against salinity phenomena [9][10][11].

To decrease the negative response of environmental tensions such as salinity, plants carry self-regulating mechanisms [12][13]. The main and major response of plants against salinity includes stress signals of abscisic acid (ABA), which receives and transfers the stress signals to a complex network of gene regulation, ABA biosynthesis control and transcriptional regulation [14]. Genes involving in reception and understanding of ABA and downstream transferring are still not well known, therefore understanding the molecular mechanisms of abiotic stress response to improve the genetically tolerance of banana is necessary [15][7].

Abscisic acid stress ripening (ASR1) is a low molecular weight (~ 16 kDa) protein that expresses in stress conditions such as desiccation, salinity and induction with abscisic acid [16]. ASR1 can act as chaperone, which prevents the folding of other cytosolic proteins in desiccation stress. Two groups of stress regulators proteins are involved in response to stress conditions: proteins that are involved in signal transduction and proteins that directly function in survival of plant under stress conditions [5][17].

MATERIALS AND METHODS

As we are studying different genes of the same plant, Dearf Cavendish banana, all methods of sample and media preparation, growth conditions control, sample transfers, RNA extraction and cDNA synthesis in this article are the same with our previously published article [18].

2.1 Implementation of salinity stress

Twenty plants in 5 groups that were having constant growth at three leaves stage were chosen to implement salinity stress. Solutions of 250 mMNaCl was prepared by addition of 14.62 g NaCl to 1 liter media. The sample banana plants were irrigated using these solutionsin 0 (control), 6, 12, 24 and 48 hours all the samples were collected separatelyand kept at -20 ˚C for RNA extraction and MaASR1 gene expression analysis.

2.2 Amplification of MaASR1 gene by RT-PCR (Real-Time PCR)

Primer Design: since the sequence of the MaASR1 gene is alreadyavailable, forward and reverse primers were designed as bellow:

maASR1-Forward: 5’-GATATACTCCGAGACAGCCTACT-3’
maASR1-Reverse: 5’-GACAAGCCAGCCTCAACTTA-3’

Amplification ofMaASR-1 gene: 50 µl PCR reaction mixture containing 5 µl buffer, 0.5 µldNTPs, 1.5 µl $MgCl_2$, 4 µl of each maASR1-Forward and Reverse primers, 2 µl of cDNA, 0.5 µl *Tag* DNA polymerase and 32.5 µl nuclease free water was prepared in PCR tube. The PCR mixture was subjected to a BIO-RAD thermo cycler machine under program of pre-denaturation at 95 °C for 3 min, 30 cycles of denaturation (95 °C; 30 s), annealing (50 °C; 30 s) and extension (72 °C; 1 min) followed by final extension at 72 °C for 7 minutes. The PCR product was analyzed by electrophorese on 3 % (w/v)agarose gel and visualized under UV light.

RESULTS AND DISCUSSION

3.1 Study of MaASR1 gene using RT-PCR

The cDNA of MaASR1 gene was synthesized by RT-PCR and results of expression of MaASR1 under 250 mMNaCl salinity at different time of 0, 6, 12, 24 and 48 hours are shown in figure 1. Based to the intensity of bands on agarose gel: at 0 hour(control)the band is less intense, which shows the partial expression of the MaASR1 gene in normal conditions (band a); after 6 hours of salinity stress the expression level of MaASR1 gene is increasing that shows a more intense band than control sample in agarose gel (band b); the band intensity of the sample with12 hours salinity is more intense that 0 and 6 hours (band c) showing that the expression of MaASR1 gene is increasing; the most intense band achieved after 12 to 24 hours salinity stress, which shows the maximum time of the plant tolerance against salinity tension. The sample of 48 hours stress shows a less intense band than 24 hours that indicates the expression of MaASR1 gene is decreasing.

It is clear from the results that the expression level of MaASR1 gene in control (0 hour) sample is partially expressed as normal conditions and by increasing the time under salinity stress, the expression level of MaASR1 gene is gradually increasing until the plant reaches the longest time (24 hours) of tolerance against the salinity stress. At 48 hours of salinity stress expression level of MaASR1 gene is decreasing, which means the plant is not able anymore to tolerate in salt tension.

3.2 Phenotypic response of banana plants to salinity stress

Apical meristem of 10 offshoot samples have been cut and were cultured for 60 days at establishment environment, after 40 to 45 days were transferred into proliferation environment, transferred into rooting medium for 20 to 25 days, were transferred to pot for to implement the salinity stress, were irrigated with Hoagland solution for adaptation with pot environment and finally were under salinity stress for different times to analyze the phenotypic response of the plants.

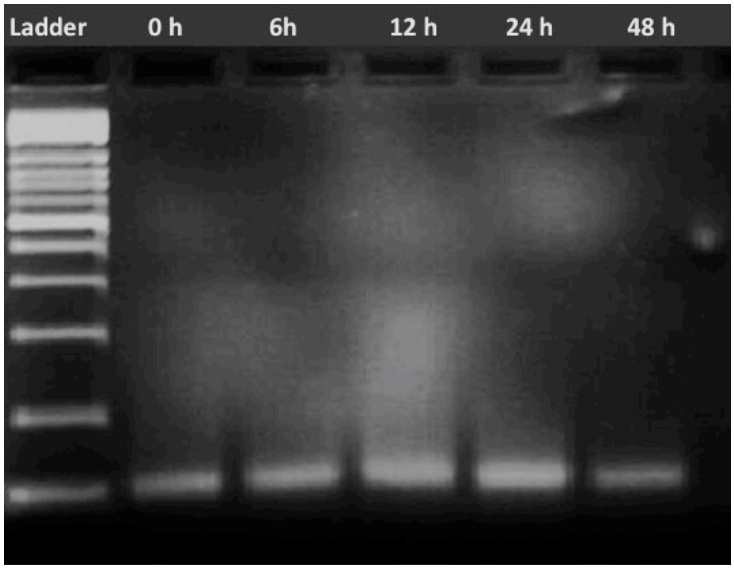

Figure 1: Analysis of the expression of MaASR1 gene in leaf tissues of Dwarf Cavendish banana at different times of salinity stress on 3% agarose gel. According to the intensity of bands, the highest level of MaASR1 gene expression achieved after 12 to 24 hours salinity stress.

By passing of time, obvious phenotypic changes were observed. At 0 time (control) leaves seemed green and bright and no phenotypic change was monitored. After 6, 12 and 24 hours of salinity stress, the leaves became brighter and greener than control sample, which shows increase in expression level of MaASR1 gene and also increase in tolerance of the plants against salinity tension (figure 2). By increasing the time of salinity stress to 48 hours the leaves became darker that shows the expression level of MaASR1 is decreased. The phenotype changesin banana plant confirm that the plant is not able to tolerate more than 24 hours insalinity of 250 mMNaCl and the growth, metabolism and finally productivity of the plant will be affected.

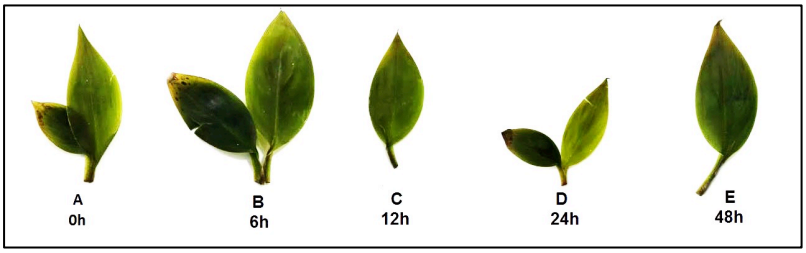

Figure 2: Phenotypic analysis of Dwarf Cavendish banana at different times (0, h, 12, 24 and 48 hours) under 250 mMNaCl salinity. After 24 hours under salinity tension young leaves of the plants became gradually dark

CONCLUSION

The global increasing of soil salinity brings the necessity to design novel mechanisms of plant tolerance. In order to produce stress resistant plants to increase the production of crops, it is important to analyze the expression of various genes involved in tolerance mechanisms. Outputs of this study are considerable in improvement of banana plant tolerance against salinity stress. Analysis of MaASR1 expression from Dwarf Cavendish banana, a commercially

important banana,provides applicable informationin understanding the tolerance ability of itin different time of salinity and also the results show the important role of MaASR1 gene in response to salinity stress. The Dwarf Cavendish banana was experimentedat different times (0, 6, 12, 24 and 48 hours) under 250 mMNaClsalinity and the results shows that the maximum time of tolerance against salinity is around 24 hours, in which the plant is showing higher expression level of MaASR1 gene but after this time it gradually decreased. The phenotype study also confirmed that at 12 and 24 hours after implementation of salinity stress the plant is in normal growth like the control sample but at more period of salinity the leaves became darker. The importance of MaASR1 genein adaptation of Dwarf Cavendish banana plants to newly environmental conditions is confirmed through both genotypic and phenotypic results, which is in agreement with previous studies. Furthermore it could be concluded that Dwarf Cavendish banana is not able to tolerate the salinity stress for more than 24 hours. However, to comprehendthe mechanisms of stress response, studying of the MaASR1 gene alone is not enough but analysis and study of various genes of stress mechanisms to collect applicable data of plant adaptation will definitely open a new insight toward genetic modification of stress tolerance plants.

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