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Study of Musa DHN-1 Gene Expression from Banana Cultivar of Dwarf Cavendish under Salinity Treatment

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ABSTRACT

Soil salinity is an increasing problem, which causes stress response in plants and ultimately prevents the full expression of the genetic potential of the plants. In response to stressing factors, there are various genes with positive regulation, which can reduce the effects of stress and lead to adaptation and tolerance of plants. Dehydrins are proteins that affected by water stress, low temperature, salinity and abscisic acid (ABA) and they accumulate in vegetative organs, which suggests a protective role of this group of proteins in terms of water restrictions. Because of shallow roots and constant green covering crest, bananais very sensitive to the conditions that lead to lack of water. Banana meristem cultivation for is applicable in banana cultivation and transformation agendas. In this study, to investigate the MusaDHN-1 gene expression under salt stress conditions, apical meristems of banana, Dwarf Cavendish, were cultured in MS environment and after 4 months, Samples were transferred to pots for applying stress, and for 10 days with concentration of 0mM (control), 25mM, 50mM, 100mM, 150mM, NaCl were irrigated. RNA was extracted from leaf tissue samples, and the level of MusaDHN-1 gene expression was examined by RT-PCR method. The results showed high gene expression of MusaDHN-1 in terms of phenotypic and genotypic in conditions of salinity stress is involved in the plant tolerance and consistency. Studying the expression level of MusaDHN-1 gene is not the solution individually, but identifying a set of the co-expression genes and pattern study of their collective changes in response to different levels of stress are of particularly importance and also monitoring MusaDHN-1 gene expression level in other tissues such as roots, stems, and the impact of it on ions and osmolytes such as proline, could gives a better understanding of responses to stress conditions.

Keywords: Salinity stress, MusaDHN-1, Dwarf Cavendish banana, gene expression

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INTRODUCTION

Banana (*Musa acuminate L.*) is a big one-year herbaceous monocot plant that grows in tropical and semitropical regions and it is one of most popular fruits that consumed freshly. Since banana has shallow roots and constant green covering crest, it is very sensitive to conditions that lead to lack of water. Understanding the stress tolerance mechanisms in banana to increase the quality and quantity of production of this commercially important fruit is a valuable and applicable research [1,2].

The phenomenon of soil salinity is increasing by irrigation, poor drainage, progressing the sea in coastal areas, and salt accumulation in desert areas and etc. In Iran almost 50 % of under cultivation grounds, because of locating in dry and semi-dry region, are facing the problem of soil salinity [3]. Soil salinity is created through ionic, osmotic

and oxidative tensions associated with nutrition disorders in restricting areas of the crops and garden growth, which ultimately prevents the full expression of the genetic potential of the plants [4,5,6,7,8]. Stresses in plants could decrease the quality of products that finally deflate the price of products and cause lose of millions dollars [9]. Generally, the salinity of soil affects all main process of plants such as growth, photosynthesis, protein synthesis and metabolism of lipids and energy and finally decreases the growth and amount of the product and even cause death of the plants [10, 11, 12,13].

In response to stress factors there are several genes with positive controlling that can reduce the impact of stress and adapt the cells with new environmental conditions and increase the tolerance of plants. Naturally in response to these tensions there are different signal paths cooperating with each other to fight and tolerate against them [9]. Therefore plants are applying physiological, biochemical and cell and molecular processes to response environmental tensions and adapt themselves with new conditions [14]. This molecular and biochemical responses not only depend on length of tension period but also depend on growth stage and morphological and anatomical parameters of the plants [15]. By reception and recognition of stress in cellular level, different signaling pathways will start to change the environmental tension to an appropriate biochemical response; each of them expressing particular genes related to stress respond [16]. The products of these genes will function in signaling as well as response to tensions [17]. Thus the product of these genes could be classified in two groups; the first group includes proteins involving in stress tolerance such as chaperons, water channel proteins, proteins of late fetal stage, enzymes for biosynthesis of osmolytes, antitoxin enzymes and enzymes that change membrane lipids, the second group includes protein factors that are functioning in gene regulation and signaling in response to tensions such as protein kinases, enzymes involved in phospholipid metabolism and transcription factors [17,18].

MusaDHN-1, a novel stress-inducible SK3-type dehydringene, is identified in banana and its function as tolerating factor in stress conditions has been previously studied [19]. In this article the expression pattern of MusaDHN-1 gene, under stress on different concentration of salinity in Dwarf Cavendish banana is studied.

MATERIALS AND METHODS

2.1 Plant sample preparation

Ten offshoots of Dwarf Cavendish banana were collected from Bahoukalat research center Chabahar. Offshoots were cut in 10 centimeter height and 5 centimeter diameter, washed truly and transferred to a 500 milliliter Erlenmeyer.

Sterilization: the offshoots were washed in 70 percent ethanol for 1 minute then transferred into 20 percent whitex for 20 minutes. The offshoots were washed with double distilled water for 5 minutes (3 times repeat). Finally all of sterilization steps were repeated again.

2.2 Preparation of solutions and MS growth medium

One liter macronutrients solution was prepared supplementing KNO_3 (9.5 g), NH_4NO_3 (8.25 g), $MgSO_4$ (1.85 g), KH_2PO_4 (0.85 g), $CaCl_2$ (2.2 g) and one liter micronutrients solution was prepared supplementingH_2BO_3 (310 mg), $MnSO_4$ (845 mg), $ZnSO_4$ (430 mg), Na_2MoO_4(12.5 mg), $CuSO_4$(12.5 mg), $CaCl_2$(12.5 mg), KI (41.5 mg).

One liter nutrition solution supplemented with 50 ml macronutrients, 5 ml micronutrients and 3 µl EDTA was prepared. The components of 1 liter growth mediums are indicated in Table 1.

Table 1: Composition of growth mediums

Material	Type of growth medium		
	Establishment	**Proliferation**	**Rooting**
Macronutrients	50 ml	50 ml	50 ml
Micronutrients	5 ml	5 ml	5 ml
Folic Acid	1 ml	1 ml	1 ml
Vitamins	1 ml	1ml	1ml
Myoinositol	30 g	100 mg	100 mg
Sugar	30 g	30 g	30 g
Agar	80 g	-	80 g
Active Charcoal	-	-	2 g
Adenine	80 mg	-	-
BAP	2 ml	5 ml	3 ml
NAA	-	-	0.25 ml
IAA	3 mg	-	-

** The pH of growth medium was measured at 5.70 and the prepared growth medium was autoclaved for sterilization.*

2.3 Environmental conditions of the growth room
The growth room of the samples was prepared at temperature of 26-28 ˚C, 70 % of humidity and 16 hours light/8 hours darkness.

2.4 Sample transferring to different growth media
Establishment media: autoclaved establishment medias were transferred into sterile glass and leave them for one day to let their water for evaporation. The offshoots were cut to reach apical meristem and then transferred into confirmation medium in glasses. The samples were transferred into growth room and were sub-cultured (the dry and black parts of samples were cut and were cultured in new medium)every two weeks.

Proliferation media: the sterile proliferation medias were transferred into sterile glasses, the samples were transferred from establishment medium to proliferation medium and located in growth room for 40 to 45 days and were sub-cultured (samples were cleaned and new offshoots were separately cultured) very week.

Rooting media: autoclaved rooting medias were transferred into sterile glass, the proliferated samples were transferred to rooting medium and all the samples were located in growth room for 20 to 25 days.

2.5 Transferring the samples to pots and implementation of salinity tension
A mixture of peat and perlite was autoclaved for sterilization and filled into pots as soil. Rooted banana samples were extracted from rooting medium, washed, transferred into pots and irrigated with nutrition solution. *In vitro* banana plant were grown at 28 ˚C, 70 % humidity and at cycle of 16 hours light versus 8 hours darkness. In vitro plants with constant growth at three leaves stage were chosen and 20 plants in 5 groups were selected for salinity stress. To implement the salinity, each group of samples was irrigated using solutions of 0.0, 25, 50, 100 and 150 mM NaCl for 10 days and then all the samples were stored at -20 ˚C for further RNA extraction.

2.6 RNA extraction
The RNA of plants was extracted from leaves using RNX-Plus kit according to manufacturer's instructions. Because of hydroxyl group in the structure of RNA, it is sensitive and not stable and the process of converting RNA to cDNA should be done quickly.

2.7 Amplification of MusaDHN-1 gene by RT-PCR (Real Time PCR)
cDNA synthesis: Six microliter of RNA was transferred into 0.2 ml tube and 4 µl of a master mix containing 1 µl random hexamers, 1 µldNTPs and 2 µl nuclease free water, was added, centrifuged for 10 seconds, incubated at 65 ˚C for 5 minutes to unfold the DNA clamp and then transferred into ice bath. Ten microliter of cDNA synthesis mixture (2µl of 10X buffer M-MulV, 0.5 µl of M-MulV reverse transcriptase and 7.5 µl nuclease free water) was added to RNA tube and centrifuged for 10 seconds then it was subjected to PCR program (25 ˚C for 10 min, 42 ˚C for 60 min and 85 ˚C for 5 min). After completion of PCR the samples were centrifuged and stored at -20˚C for further applications.

Primer Design: as the sequence of the MusaDHN-1 gene is already known, forward and reverse primers were designed as follow:

MusaDHN1-Forward: 5'-CACAGTCTCTCAGCATCTTCTC-3'
MusaDHN1-Reverse: 5'-CTCTAGCTCGGTATGTACTCCT-3'

Amplification ofMusaDHN-1 gene: 50 µl PCR reaction mixture containing 5µl buffer, 0.5 µldNTPs, 1.5 µl $MgCl_2$, 4 µl of each MusaDHN1-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 was run on BIO-RAD thermo cycler under the temperature program of predenaturation 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 for7 minutes. The PCR product was electrophoresed on 3 % (w/v) agarose gel visualized under UV light.

RESULTS AND DISCUSSION

3.1 Expression level of MusaDHN-1 gene
The RT-PCR results the expression ofMusaDHN-1 under different samples at 0, 25, 50, 100 and 150 mMNaCl are illustrated in figure 1. According to the bands intensity on agarose gel: the control sample is showing a less intense band that indicates a minimal expression of the MusaDHN-1 gene in absence of salinity stress (band a); the expression level of MusaDHN-1 gene for plants under 25 mMNaClstress is higher than other fractions (band b) that indicates increasing in MusaDHN-1 gene expression; the intensity of the band at 50 mMNaCl is less that 25

mMNaCl salinity (band c) showing that the expression level is decreased compare to 25 mMNaCl salinity; also for 100 and 150mMNaCl salinity the band is less intense that indicates less expression of MusaDHN-1 gene.

Therefore the results confirm that the MusaDHN-1gene in control (0.0 mMNaCl) sample is partially expressed and by entering the plant to stress condition (25 mMNaCl) the expression of MusaDHN-1 gene is increased and then for 50, 100 and 150 maMNaCl it is gradually decreased. As the plant is not able to tolerate more than 25 mMNaCl salinity, at higher NaCl concentrations (50, 100 and 150 mM) the expression level of MusaDHN-1 gene and therefore the tolerance ability of the plant will decrease. The higher expression level of MusaDHN-1 gene at 25 mMNaCl indicates the optimum salinity for the productivity and growth of the plant.

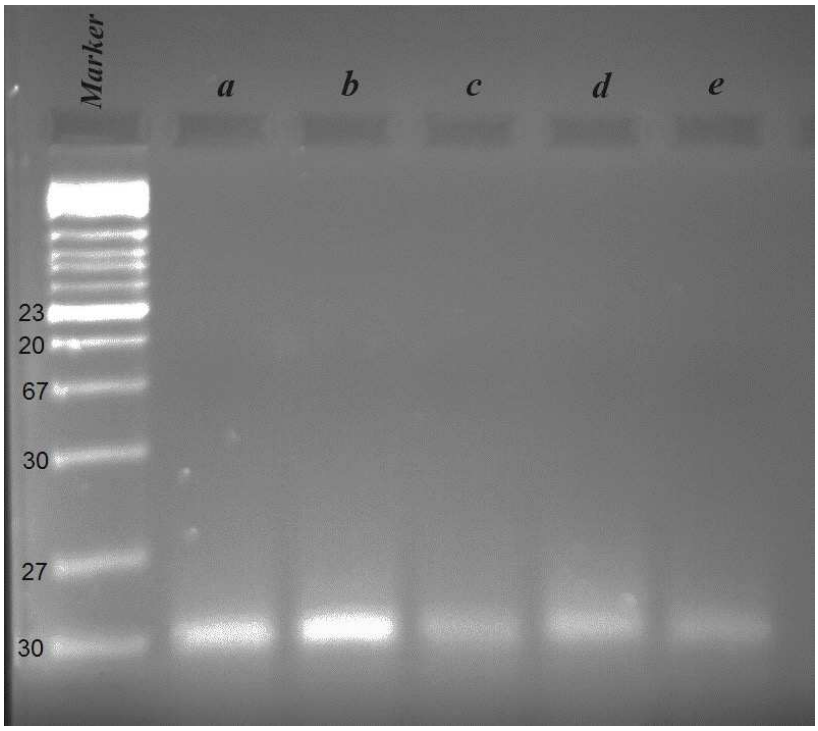

Figure 1: Analysis of MusaDHN-1 gene expression in leaf tissue of Dwarf Cavendish banana under different salinity stress. The results of RT-PCR run on 3 % agarose gel. Lane a: control; lane b: 25 mMNaCl salinity; lane c: 50 mMNaCl salinity; lane d: 100 mMNaCl salinity and lane e: 150 mMNaCl salinity

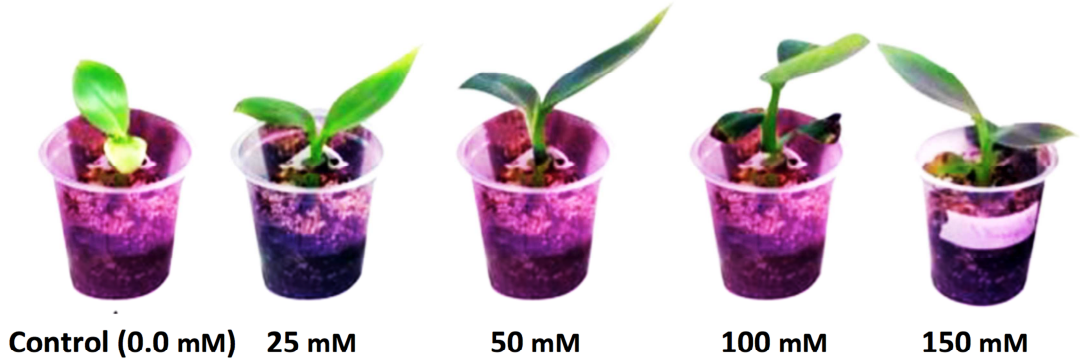

Figure 2: Phenotypic response of Dwarf Cavendishbanana plant to salinity tensions at 0.0 mM NaCl (control), 25mM NaCl, 50 mM NaCl, 100mMNaCl and 150 mM NaCl. At concentrations more than 25 mM NaCl, young leaves of the plants became dark and elders obviously dried.

3.2 Phenotypic response to salinity

The 10 offshoot samples that were cut to culture their apical meristem, was cultured for 60 days at establishment environment, transferred for 40 to 45 days into proliferation environment, transferred into rooting condition, were

transferred to pot for 10 days for implementation of salinity stress and were irrigated with Hoagland solution to be adapted with pot environment and finally were under salinity stress for 10 days to evaluate the phenotypic response of the plants. After 10 days obvious changes is monitored on salinity stressed samples. In control and sample with 25 mMNaCl salinity the leaves seemed green and bright and no phenotypic change was observed. But for 50, 100 and 150 mMNaCl salinity the leaves seemed dark and some of them dried, which shows obvious changes in phenotypic response of the plants (Figure 2). The gradual changes in phenotype of banana plant at higher salinity than 25 mMNaCl confirms that the plant is not able to tolerate any higher NaCl concentration and the growth, metabolism and product of the plant will be affected. By increasing the salt concentration the leaves are getting darker and the photosynthesis and therefore fruit production of the plant will be affected.

CONCLUSION

By increasing of soil salinity, the study on characterizing novel mechanisms to produce stress resistance plants and increase the efficiency of crops becomes more important than before. Results of this study are considerable in explanation of tolerance mechanism of banana plant against salinity. Study on expression of MusaDHN-1 gene from Dwarf Cavendishbanana presented applicable information about understanding the tolerance level of this plant and about the important role of MusaDHN-1 gene in response to salinity tension. The Dwarf Cavendishbanana was stressed in different salinity (0.0 mM, 25 mM, 50 mM, 100 mM and 150 mM of NaCl) and it is found that the plant is showing higher expression level of MusaDHN-1 gene at 25 mM NaCl and then gradually decreased at higher concentrations. The phenotype study also showed that at 25 mM NaCl the plant is in normal response like the control sample but at higher concentrations the young leaves seemed darker and the elders started drying. Both genotypic and phenotypic results confirms that expression of MusaDHN-1 gene is important in adapting of the Dwarf Cavendishbanana in new stress conditions, which is in agreement with previous studies. In addition it is obtained that this type of banana is able to tolerate up to 25 mM NaCl salinity. However, it should be considered that studying of the MusaDHN-1 gene alone is not the solution to explain the stress response mechanisms but evaluation and recognition of cluster of genes involved in tension response will definitely provide better and more understanding.

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