Preliminary results toward understanding drought resistance in *Acantholimon albanicum*

Fabjana Lakuriqi,[1] Ariola Bacu,[1] Mariella M. Finetti-Sialer[2]
[1] Department of Biotechnology, Faculty of Natural Science, University of Tirana, Albania
[2] Institute of Biosciences and Bioresources, CNR, Bari, Italy

Abstract

Drought is one of the most important environmental stresses that influences metabolism and growth of plant.The genetic basis of the molecular, cellular and developmental responses to drought involve many gene functions regulated by water availability. In this paper we describe the first attempts toward the identification of the drought-responsive genes in the endemic species *Acantholimon albanicum.* Total RNA was extracted from leaves using protocols based on TRIzol Reagent from Ambion and the other using Total RNA Mini Kit from Geneaid. Spectrophotometric readings showed differences in RNA quantity and quality as well as gel electrophoresis, determining the second protocol as more efficient in RNA quality. cDNA was synthetized using SuperScript III First-Strand Synthesis System for RT-PCR. Six pair of specific primers were tested for amplifying sequences of a DREB gene reported to be responsible for plant response to abiotic stresses.
Two sets of them resulted more effective in amplifying the target-sequences.

Keywords: endemic plant species, drought responsive genes, RT-PCR

Methods and Materials

Plant material was collected in July 2014, in Boboshticë, Korça region (N 40^o 32. 740', 20^o 46. 846'E), altitude 1046.2 m-1128.6 m, during flowering stage. Leaves from different individuals of twelve different plants were mixed and kept in 2 ml tubes with RNA later.
Total RNA was extracted from leaves using two protocols, using TRIzol Reagent from Ambion and the other one using Total RNA Mini Kit from Geneaid. Spectrophotometric readings were performed using Nanodrop 1000 3.8.1. cDNA was synthetized using SuperScript III First-Strand Synthesis System for RT-PCR.
Six pair of primers were designed using the Primer3 software available at NCBI to test their specificity in amplifying sequences of a DREB gene reported to be responsible for plant response to abiotic stresses.
Results were obtained using 1.2% agarose gel electrophoresis.

Results

Sample ID	TRIzol (Ambion)		Kit (Geneaid)	
	ng/µl	260/280	ng/µl	260/280
D1	242.80	1.51	15.46	1.67
D2	254.35	1.73	13.97	1.77
D3	374.28	1.54	12.44	1.60
D4	510.51	1.21	14.44	1.64
D5	570.40	1.19	20.73	1.79
H1	209.81	1.57	33.77	1.61
H2	73.79	1.30	29.16	1.65
H3	125.46	1.31	22.13	1.55
H4	152.18	1.43	20.30	1.66
H5	834.80	1.14	9.88	2.03
H6	208.57	1.36	25.63	1.58
H7	1533.17	1.08	17.79	1.67

Table 1. Comparison of spectrophotometric readings using two different RNA extraction protocols

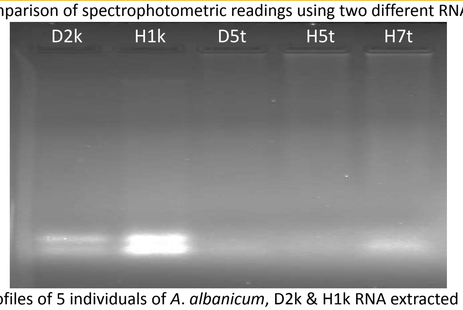

Figure 1. RNA profiles of 5 individuals of *A. albanicum*, D2k & H1k RNA extracted with total RNA Mini Kit (Geneaid); D5t, H5t, H7t RNA extracted with TRIzol (Ambion)

Primers	Sequence (5'- 3')	Product length (bp)
P3	For: AGATCCCTTGAGCGAGGAGT Rev: TCGGTTTGAGTGGTGTAGCC	294
P4	For: TCAGATCCCTTGAGCGAGGA Rev: AGCGGCCTTACCTCTTTTCC	804
P5	For: CGCCTAAACCCACCAAGCTA Rev: CGAAGGTTCCAAGCCAAAGC	111
P6	For: GGAAAAGAGGTAAGGCCGCT Rev: ATTCCGGTGACGAAGACGAC	132
P7	For: AGATCCCTTGAGCGAGGAGT Rev: CGAAGGTTCCAAGCCAAAGC	572
P8	For: AGATCCCTTGAGCGAGGAGT Rev: ATTCCGGTGACGAAGACGAC	914

Table 2. Primers sequences

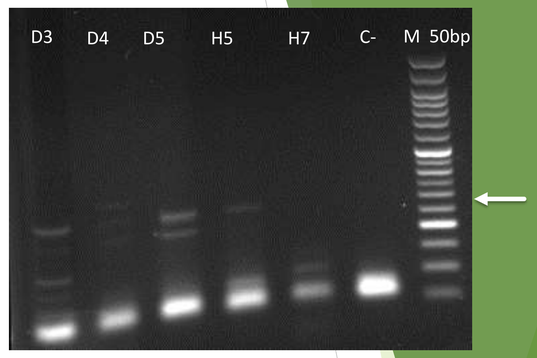

Figure 2 Amplification products of P3 primer

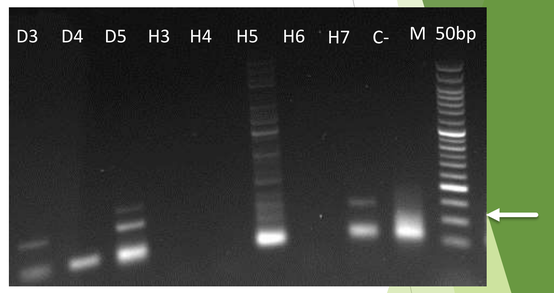

Figure 3. Amplification products of P5 primer

Conclusions

- Spectrophotometric readings and gel electrophoresis showed differences in RNA quantity and quality, determining Geneaid Total RNA Mini Kit protocol more efficient in RNA quality.
- In this preliminary data, two pairs of primers succeded in amplifying the target sequence related to drought resistance in plants.
- As far as we know this is the first report of molecular analysis for this endemic species.

Acknowledgments

Research work founded by Scienze per la Diplomazia, multi-sector training program. Agreement subscribed between Italian National Council of Research and Ministry of Foreign Affairs (CNR- MAE).

Contact

Fabjana Lakuriqi
Faculty of Natural Science, University of Tirana, Albania
Email: fabjanaa@hotmail.com

References

1. Charu Lata and Manoj Prasad (2011): Role of DREBs in regulation of abiotic stress responses in plants. Journal of Experimental Botany
2. Chaves M., Maroco J., Pereira J., (2003): Understanding plant responses to drought- from genes to the whole plant.
3. Hanson A., Rathinasabapathi B., Rivoal J., Burnet M., Dillon M., Gages D., (1994): Osmoprotective compounds in the Plumbaginaceae: A natural experiment in metabolic engineering of stress tolerance.
4. K.V. Madhava Rao, A.S. Raghavendra, K. Janardhan Reddy (2006): Physiology and molecular biology of stress tolerance in plants. Chapter 1& 2, 12-50
5. M. L. Zhou, J. T. Ma, J. F. Pang, Zh. L. Zhang, Y. X. Tang, and Y. M. Wu (2010): Regulation of plant stress response by dehydration responsive element binding DREB transcription factors. African Journal of Biotechnology Vol. 9, 54
6. Parvaneh Rahdari and Seyed Meysam Hoseini (2012): Drought Stress: A Review, International Journal of Agronomy and Plant Production. Vol., 3 (10)
7. Rahdari P., Hoseini S., (2012): Drought stress: A review.
8. Qiaoying Ban, Guifeng Liu, Yucheng Wang (2011): A DREB gene from *Limonium bicolor* mediates molecular and physiological responses to copper stress in transgenic tobacco. Journal of Plant Physiology, 168, 449–458