

## Identification, Cloning and Characterization of a Thioredoxin *h* (*VvTrxh10*) Gene Isolated from Grape (*Vitis vinifera L.*) cv. Yaquti Berry Tissue

Mussavi<sup>1</sup>, S, S., Haddad<sup>2\*</sup>, R., Garousi<sup>2</sup>, Gh. and Hosseini<sup>2</sup>, R.

### **Abstract**

Total RNA was extracted from grape (*Vitis vinifera L.*) cv. Yaquti berry tissue to characterize a thioredoxin *h* gene (*VvTrxh10*). A cDNA library was synthesized using reverse transcription polymerase chain reaction (RT-PCR). Then, the *VvTrxh10* gene was amplified, isolated and cloned in a pUC19 vector plasmid. Nucleotide sequence analysis revealed that the cloned cDNA expressed thioredoxin and contained a single open reading frame of 345 bp encoding a protein of 114 amino acid residues. Predicted protein sequence analysis showed that this gene contains a nongeneral catalic site RCGLC, characteristic tryptophan (W) and potential structural motif involving cell-to-cell taransfer (MAEE) in N-terminal. Phylogenetic and alignment studies revealed that such isoform belongs to the subgroup I from *h* thioredoxins group. Moreovere, relevant predicted protein exhibited a high similarity with the other plant thioredoxins *h* gene in the NCBI gene bank.

**Keywords:** Grapevine, Thioredoxin *h*, Cloning, Sequence analysis, Yaquti cultivar

### **References**

- Balmer, Y., Vensel, W. H., Cai, N., Manieri, W., Schurmann, P. and Hurkman, W. J. 2006. A complete ferredoxin/thioredoxin system regulates fundamental processes in amyloplasts. *The National Academy of Sciences of the USA* 8: 2988-2993.
- Birnboim, H. C. and Doly, J. 1979. A rapid alkaline procedure for screening recombinant plasmid DNA. *Nucleic Acid Research* 7: 1513-1525.
- Buchanan, B. B. 1991. Regulation of the CO<sub>2</sub> assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. *Archives of Biochemistry and Biophysics* 288: 1-9.
- Cazalis, R., Pulido, P., Aussénac, T., Perez-Ruiz, J. M. and Cejudo, F. J. 2006. Cloning and characterization of three thioredoxin *h* isoforms from wheat showing differential expression in seeds. *Journal of Experimental Botany* 57: 2165-2172.
- Cha, M. K., Kim, H. K. and Kim, I. H. 1995. Thioredoxin-linked 'thiol peroxidase' from periplasmic space of *Escherichia coli*. *Journal of Biological Chemistry* 270: 28635-28641.
- Chew, E., Lu, J., Bradshaw, T. D. and Holmgren, A. 2008. Thioredoxin reductase inhibition by antitumor quinols: a quinol pharmacophore effect correlating to antiproliferative activity. *Medical Nobel Institute for Biochemistry* 6: 2072-2083.
- Chibani, K., Wingsle, G., Jacquot, J., Gelhaye, E. and Rouhier, N. 2009. Comparative genomic study of the thioredoxin family in photosynthetic organisms with emphasis on *Populus trichocarpa*. *Molecular Plant* 2: 308-322.
- Cho, M. J., Wong, J. H., Marx, C., Jiang, W., Lemaux, P. G. and Buchanan, B. 1999. Overexpression of thioredoxin *h* leads to enhanced activity of starch debranching enzyme (pullulanase) in barley grain. *PNAS* 96: 14641-14646.
- Cohen, G., Yanko, M., Mislovati, M., Argaman, A., Schreiber, R., Av-Gay, Y. and Aharonowitz, Y. 1993. Thioredoxin-thioredoxin reductase system of *Streptomyces clavuligerus*: sequences, expression, and organization of the genes. *Journal of Bacteriology* 175: 5159-5167.
- Cohen, S. N., Chang, A. C. Y. and Hsu, L. 1972. Nonchromosomal antibiotic resistance in bacteria genetic transformation of *Escherichia coli* by R-factor DNA. *Proc, Natl. Acad. Sci.* 69: 2110-2114.
- Cramer, G. R. and Cushman, J. C. 2002. Chardonnay and cv. Cabernet Sauvignon and their responses to water deficit and salinity. *Genome Research*. 12: 231-240.
- Da Silva, F. G. 2005. Characterizing the grape transcriptome. Analysis of expressed sequence tags from multiple *vitis* species and development of a compendium of gene expression during berry development. *Journal of biological chemistry* 10: 510-521.
- Decottignies, P., Schmitter, J. M., Jacquot, J. P., Dutka, S., Picaud, A. and Gadal, P. 1990. Purification, characterization and complete amino acid sequence of a thioredoxin from a green alga *Chlamydomonas reinhardtii*. *Archives of Biochemistry and Biophysics* 280: 112-121.
- Droux, M., Jacquot, J. P., Miginiac-Maslow, M., Gadal, P., Huet, J. C., Crawford, N. A., Yee, B. C. and Buchanan, B. B. 1987. Ferredoxin-thioredoxin reductase: an iron-sulfur enzyme linking light to enzyme regulation in oxygenic photosynthesis. Purification and properties of the enzyme from cyanobacterial species. *Archives of Biochemistry and Biophysics* 252: 426-439.

1. Graduate in MSc of Agricultural Biotechnology, Department of Agricultural Biotechnology, Imam Khomeini International University, Qazvin, IR of Iran.

2. Assistant Professor, Department of Agricultural Biotechnology, Imam Khomeini International University, Qazvin, IR of Iran, P. O. Box: 34149-288 Tel: 0281- 8371165, Fax: 3780073.

\*: Corresponding author

- Foerster, H. T. 2007. *Arabidopsis thaliana* col Pathway: thioredoxin pathway. Molecular Biology 4: 1357-1363.
- Gelhaye, E., Rouhier, N., Gerard, J., Jolivet, Y., Gualberto, J., Navrot, N., Ohlsson, P., Wingsle, G., Hirasawa, M., Knaff, D. B., Wang, H., Dizengremel, P., Meyer, Y. and Jacquot, J. 2004. A specific form of thioredoxin *h* occurs in plant mitochondria and regulates the alternative oxidase. PNAS 101: 14545-14550.
- Hagglund, P. H., Bunkenborg, J., Maeda, K. and Svensson, B. 2008. Identification of thioredoxin disulfide targets using a quantitative proteomics approach based on isotope-coded affinity tags. Proteome Research. 12: 5270-5276.
- Hall, M., Mata-Cabana, A., Akerlund, H. E. and Florencio, F. J. 2010. Thioredoxin targets of the plant chloroplast lumen and their implications for plastid function. Proteomics 10: 572-583.
- Hashemy, S. I., Ungerstedt, J. S., Zahedi, A. F. and Holmgren, A. 2006. Thioredoxin reductase and ribonucleotide reductase. Journal of biological chemistry 281: 10691-10697.
- Heyman, J. A., Cornthwaite, J. and Foncerrada, L. 2010. Frames using topoisomerase imediated ligation genome-scale cloning and expression of individual open reading. Genome Research 9: 383-392.
- Juarez, D. J., McClure, B., Vazquez, S. S., Guevara, G. A., Leon, M. P., Marquez, G. J. and Cruz, G. F. 2006. A novel thioredoxin *h* is secreted in *Nicotiana alata* and reduces S-RNase *in vitro*. Biology Chemistry 6: 3418-3424.
- Juttner, J., Olde, D., Langridge, P. and Baumann, U. 2008. Cloning and expression of a distinct subclass of plant thioredoxins. European Journal of Biochemistry 267: 7109-7117.
- Kyte, J. and Doolittle, R. F. 1982 A simple method for displaying the hydrophobic character of a protein. Journal Molecular Biology 157: 105-132.
- Lepisto, A., Kangasjarvi, S., Luomala, E. M., Brader, G., Sipari, N., Keranen, M., Keinanen, M. and Rintamaki, E. 2009. Chloroplast NADPH-thioredoxin reductase interacts with photoperiodic development in *Arabidopsis*. Plant Physiology 149: 1261-1276.
- Li, Y. C., Ren, J. P., Cho, M. J., Zhou, S. M., Kim, Y. B., Guo, H. X., Wong, J. H., Niu, H. B., Kim, H. K., Morigasaki, S., Lemaux, P. G., Frick, O. L., Yin, J. and Buchanan, B. B. 2009. The level of expression of thioredoxin is linked to fundamental properties and applications of wheat seeds. Molecular Plant 3: 430-441.
- Maeda, K., Hagglund, P., Finnie, C., Svensson, B. and Henriksen, A. 2008. Crystal thioredoxin *h* isoforms HvTrxh1 and HvTrxh2 reveal features involved in protein recognition and possibly in discriminating the isoform specificity. Protein Science. 6: 1015-1024.
- Pulido, P., Cazalis, R. and Cejudo, F. J. 2009. An antioxidant redox system in the nucleus of wheat seed cells suffering oxidative stress. Plant Journal 1: 132-145.
- Reid, K. E., Niclas, O., James, S., Fred, P. and Steven, T. L. 2006. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biology 6: 27-35.
- Sandalova, T., Zhong, L., Lindqvist, Y., Holmgren, A. and Schneider, A. 2001. Three-dimensional structure of a mammalian thioredoxin reductase: Implications for mechanism and evolution of a selenocysteine-dependent enzyme. PNAS 98: 9533-9538.
- Serrato, A. J., Guilleminot, J., Meyer, Y. and Vignols, F. 2008. *AiCXXS*: atypical members of the *Arabidopsis thaliana* thioredoxin *h* family with a remarkably high disulfide isomerase activity. Physiology Plant 3: 611-622.
- Shahpiri, A., Svensson, B. and Finnie, C. 2008. The NADPH-dependent thioredoxin reductase/thioredoxin system in germinating barley seeds: gene expression, protein profiles, and interactions between isoforms of thioredoxin *h* and thioredoxin reductase. Plant Physiology 146: 789-799.
- Shahpiri, A., Svensson, B. and Finnie, C. 2009. From proteomics to strutural studies of cytosolic/mitochondrial-type thioredoxin systems in barely seeds. Molecular Plant 1-12.
- Sheng, Z. W., Fen, L. Y., Wu, Z. S. and Bao, X. X. 2007a. Changes in proteins within germinating seeds of transgenic wheat with an antisense construct directed against the thioredoxin. National Engineering Research Center for Wheat 1: 18-24.
- Sheng, Z. W., Fen, L. Y., Wu, Z. S. and Bao, X. X. 2007b. Effects of antisense-thioredoxin *s* gene on expression of endogenous thioredoxin *h* gene in transgenic wheat seed. National Engineering Research Center for Wheat 4: 325-332.
- Stein, M., Jacquot, J. P., Jeannette, E., Decottignies, P., Hodges, M., Lancelin, J. M., Mittard, V., Schmitter, J. M. and Miginiac, M. 1995. *Chlamydomonas reinhardtii* thioredoxins: structure of the genes coding for the chloroplastic *m* and cytosolic *h* isoforms; expression in *Escherichia coli* of the recombinant proteins, purification and biochemical properties. Plant Molecular Biology 28: 487-503.
- Velasco, R., Dematte, L. and Toppo, S. 2006. Coping with an heterozygous genome: the Pinot Noir whole genome sequencing. Molecular Plant 10: 619-628.
- Wang, X. L., Wayne, B. L., Yuan, Y. X., Wang, Y., Wei, Y., Theodore, A. C., Lopez, B. L., Liu, H. and Liang, M. 2010. Methylglyoxal increases cardiomyocyte ischemia/reperfusion injury via glycative inhibition of thioredoxin activity. Physiology Endocrinology Metabolism 10: 473-481.
- Zhong, L. and Holmgren, A. 2000. Essential role of selenium in the catalytic activities of mammalian thioredoxin reductase revealed by characterization of recombinant enzymes with selenocysteine. Journal of Biological Chemistry 275: 18121-18128.

To look at the figures and tables, please refer to the Persian text (pages: 17-26= ۱۷-۲۶).