World Applied Sciences Journal 18 (4): 580-583, 2012
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2012.18.04.1488

Expression of the *P5CS* Gene in Transgenic Versus Nontransgenic Olive (*Olea europaea*) under Salinity Stress

[1]*Maryam Farzaneh Behelgardy,*
[1]*Nasrin Motamed and* [2]*Ferdous Rastgar Jazii*

[1]School of Biology, College of Science, University of Tehran, Tehran, Iran
[2]National Institute of Genetic Engineering and Biotechnology (NIGEB). Shahrak-e-Pajoohesh, Kilometer 15, Tehran-Karaj Highway. Building A. P.O. Box 14965/161, Tehran, Iran

Abstract: Olive is an ever- green tree of the oleaceae family and of the genus *olea* , grown under a wide range of climates and soils all over the world. In Iran, 50 percent of all growing areas encountered by high salt concentration conditions and drought. Water deficit caused by drought and high salinity has been an important factor limiting crop productivity. To cope with these environmental stresses, application of molecular plant breeding is necessary. In response to osmotic stress elicited by condition of high salt, the expression of *P5CS* gene increased in olive plants. Transgenic and non transgenic olive plants were transferred to the solid ½ MS containing 200 mM NaCl and they were sub- cultured about 2 weeks, then total protein was extracted from fresh leaves of transgenic plants and non transgenic plants under osmotic stress condition and measured using of Bradford to subject to SDS-PAGE gel electrophoresis. To probe the effect of *P5CS* overexpression on salinity stress tolerance, polyclonal antibody developed in rabbit against P5CS protein was used. The result of immunological methods for specific detection of *P5CS* gene, strongly indicated P5CS up - regulation in stressed transgenic olive plants versus stressed non transgenic olive plants.

Key words: Osmotic stress · Olive · *P5CS* · Polyclonal antibody

INTRODUCTION

Plants are influenced by different environmental stresses. Osmotic stresses caused by drought, salinity and cold limit growth and crop productivity [1, 2]. One of the cellular responses to these tensions is accumulation of metabolites known as osmolytes including sugars such as sucrose and fructose, sugar alcohols such as glycerol and inositol, complex sugars such as trehalose and raffinose and amino acids such as proline and ectoine [3]. Proline as an important osmolyte plays a vital role in osmotic tolerance [3, 4]. The glutamic acid pathway is the main pathway in the cytoplasm leading to proline synthesis in higher plants especially under osmotic stress [5]. Proline is synthesised from Glu via two intermediates, glutamic -γ˜- semialdehyde (GSA) and Δ^1-pyrroline- 5-carboxylate (P5C). The first and the final steps of the proline biosynthesis are catalyzed by P5C synthetase (P5CS) and P5C reductase (P5CR), respectively [6]. The accumulation of proline induced by salinity in higher plants is a response to osmotic stress [7, 8]. Nanjo *et al*. (1999) used antisense transgenic *Arabidopsis* plants with a P5CS cDNA and showed that sensitivity to salinity stress enhanced in these plants and that P5CS played a key role in proline production, considering significantly lower level of proline in these antisense transgenic plants than wild-type plants [9]. Zhu *et al*. (1998) studied proline accumulation in transgenic rice plants with P5CS cDNA and proved stress-induced overproduction of the P5CS enzyme under salinity stress [10].

It has been also shown that salinity stress overproduces the P5CS enzyme in transgenic citrus rootstock *Carrizo citrange* and chickpea *Cicer arietinum* L. with *P5CS* cDNA [11, 12].

In the present study, we analyzed the expression level of the *P5CS* gene immunologically in the transgenic and control (nontransgenic) olive plants under osmotic stress.

Corresponding Author: Nasrin Motamed, School of Biology, College of Science, University of Tehran, Tehran, Iran. Tel: +021 61112472, 09123905218.

MATERIALS AND METHODS

To assess salinity stress-induced overproduction of the P5CS enzyme and proline accumulation, we used the olive plants (*Olea europaea*) transformed by Gheiratmand (2004) using *Agrobacterium tumefaciens* strain c58 (pGV3101) containing PBI-121-P5CS binary vector [13]. The transgenic plants (27-30 days old) cultured on the selective media and maintained in the greenhouse with 16 h light, 8 h dark cycle at 24±2°C temperature (See Gheiratmand, 2004) were transferred to the solid 1/2MS media containing 100 (seven plants) and 200 (seven plants) mM NaCl and also seven transgenic plants were transferred to the solid 1/2MS media without any salinity. Nontransgenic plants (27-30 days old) were also transferred from solid 1/2MS media (with 16 h light, 8 h dark cycle at 24±2°C temperature) to the solid 1/2MS media containing 100 (seven plants) and 200 (seven plants) mM NaCl. The transgenic and control plants were sub-cultured for 2 weeks and then total protein was extracted [14]. Equal quantity of total protein from both samples was subjected to SDS-PAGE gel electrophoresis. Free proline content was determined according to the procedures of Bates *et al*. (1973) [15]. Since the Pro contents vary from leaf to leaf and depend also on the age of the plant, plants and leaves with similar age and size were selected for proline assay. For immunodetection, protein extract were separated by 12.5% SDS-PAGE gel and transferred electrophoretically onto nitrocelloluse membrane in a solution of 25 mM Tris, 193 mM glycine and 20% (v/v) methanol. The level of P5CS enzyme was analyzed on immunoblots developed with antibodies raised in a rabbit against the synthetic peptide (Sigma-Genosys) corresponding to the 15 amino acids (EELDRSRAFARDVKR) of the AtP5CS N terminus representing the highly conserved amino acids of this enzyme [9]. Means of proline content were compared by ANOVA analysis and Duncan's Multiple Range Test at significant difference $P \leq 0.05$, using SPSS Package, ver. 15.

RESULTS AND DISCUSSION

On the solid 1/2 MS media containing 200 mM NaCl, the transgenic plants showed increase in growth compared with nontransgenic plants (Figure 1). Proline assay also detected that proline content of the transgenic plants was higher than that of nontransgenic plants in different concentrations of salinity (0, 100 and 200 mM NaCl). Proline content of transgenic and nontransgenic plants cultured in different media is shown in Figure 2. Significant differences of proline contents among control and transgenic plants are given in table 1, considering all differences were significant at significant difference $P \leq 0.05$. Western blotting using *P5CS* polyclonal antibody detected that in the tension medium containing 200 mM NaCl, the transgenic Plants accumulated higher content of P5CS protein than that in the control plants. A thicker protein band of 72 kDa belonged to the transgenic plants was detected (Figure 3).

Drought and salinity are important environmental factors cause osmotic stress in plants and reduce growth and crop yield [16]. Proline overproduction playing an important role for osmotic adment is a current response to salinity stress in many plants [3,5,10,-12, 17,18]. Khedr *et al*. (2003) stated that proline content of the desert plant *Pancratium maritimum* L. increased several times at 300 mM NaCl, enhancing tolerance to salt stress [19]. It has been shown that transgenic plants overexpressing P5CS accumulated higher proline compared with control plants under salinity stress [5, 10]. It sounds that under salinity stress, K^+ concentration in the cells decreases simultaneously with increase in proline content [20].

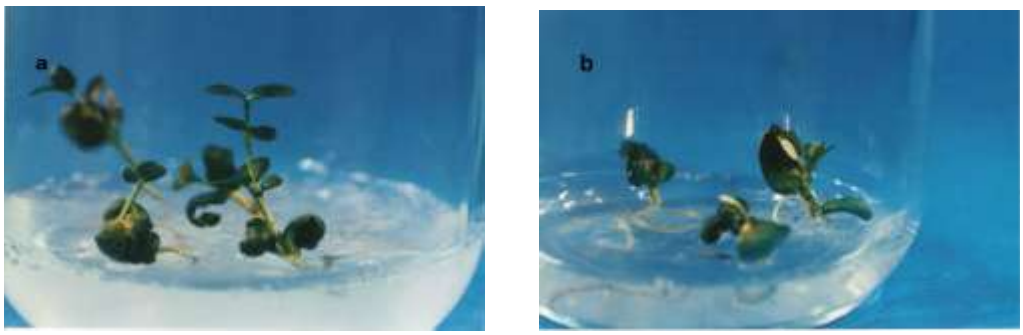

Fig. 1: Photographs of transgenic (a) and nontransgenic plants (b) in tension medium with 200 mM NaCl.

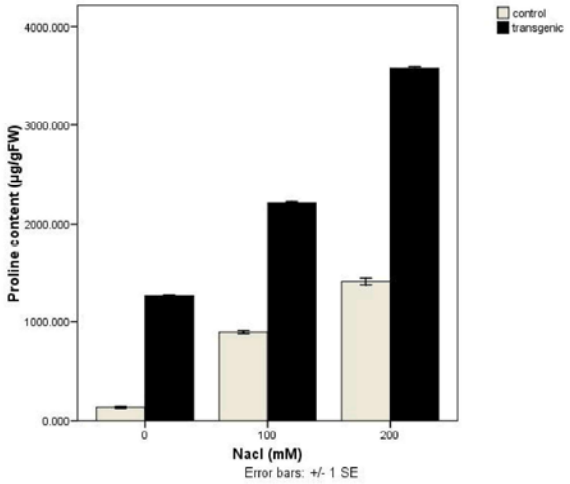

Fig. 2: Proline content in transgenic and nontransgenic olive plants.

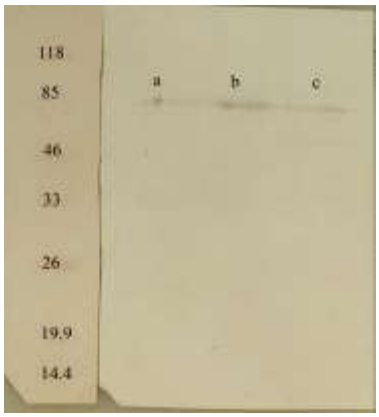

Fig. 3: Western blot of transgenic (b, 200 mM NaCl; c, non-stress medium) and nontransgenic plants (a, 200 mM NaCl)

Table 1: Free Proline content (µg g^{-1} FW) of control and transgenic olive plants in different salinity concentrations

NaCl mM	0	100	200
Control	137.09±8.21	893.87±15.76	1418.72±32.51
Transgenic	1274.08±9.53	2212.94±12.96	3574.80±15.18

Enhancement of tolerance to drought and salinity is one of the aims of genetic engineering manipulating responsible genes intervening in biosynthesis of low molecular weight metabolites or other molecules responsible for tolerance to drought tensions [5]. The present study proves that the transgenic olive plant can adapt to a variety of salinity conditions and be used in Iran agriculture, considering importance of this species in agriculture and that different parts of Iran are arid and semiarid.

REFERENCES

1. Boyer, J.S., 1982. Plant productivity and environment. Science, 218: 443-448.
2. Yoshiba, Y., T. Klyosue and T. Katagiri, 1995. Correlation between the induction of gene for Δ^1-pyrrolin-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress. Plant Journal, 7(5): 751-760.
3. Hasegava, P.M. and R.A. Bressan, 2000. Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology, 51: 463-99.
4. Solomon, A., S. Beer, Y. Waisel, G.P. Jones and L.G. Paleg, 1994. Effects of NaCl on the carboxylating activity of Rubisco from *Tamarix jordanis* in the presence and absence of proline- related compatible solutes. Plant Physiology, 90: 198-204.
5. Kavi-Kishor, P.B., Z. Hong, G.H. Miao, C.A.A. Hu and D.P.S. Verma, 1995. Overexpression of delta-1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiology, 108: 1387-1394.
6. Hare, P.D., W.A. Cress and J. Van Staden, 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. Journal of Experimental Botany, 50: 413-34.
7. Delauney, A.J. and D.P.S. Verma, 1993 . Proline biosynthesis and osmoregulation in plants. The Plant Journal, 4: 215-223.
8. Siripornadulsil, S., S. Traina, D.P.S. Verma and R.T. Sayre, 2002. Molecular mechanisms of proline-mediated to toxic heavy metals in transgenic microalgae. The Plant Cell, 14: 2837-2847.
9. Nanjo, T., M. Kobayashi, Y. Yoshiba, Y. Sanada, K. Wada, H. Tsukaya, Y. Kakubari, K. Yamaguchi-Shinozaki and K. Shinozaki, 1999. Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*. The Plant Journal, 18: 185-193.
10. Zhu, B., J. Su, M. Chang, D.P.S. Verma, Y.L. Fan and R. Wu, 1998. Overexpression of a Δ^1-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water and salt-stress in transgenic rice . Plant Science, 139: 41-48.
11. Molinari, H.B.C., C.J. Marur, J.C.B. Filho, A.K. Kobayashi, M. Pileggi, R.P. Leite Junior, L.F.P. Pereira and L.G. Esteves Vieira, 2004. Osmotic adment in transgenic citrus rootstock *Carrizo citrange* (*Citrus sinensis* Osb. x *Poncirus trifoliata* L. Raf.) overproducing proline. Plant Science, 167: 1375-1381.

12. Ghanti, S.K., K.G. Sujata, B.M. Vijay Kumar, N. Nataraja Karba, J. Janardhan Reddy, M. Srinath Rao and P.B. Kavi kishor, 2011. Heterologous expression of *P5CS* gene in chickpea enhances salt tolerance without affecting yield. Biologia Plantarum, 55(4): 634-640.
13. Gheiratmand, L., 2004. Studying the effects of transferring *P5CS* gene to olive embryo (cv. Zard) for improving its tolerance to salt stress. University of Tehran, M.Sc. thesis.
14. Tsugita, T., S. Kamatsu and H. Kajiwara, 1996. Two - dimensional electropheresis of plant proteins and standardization of gel patterns. Electrophoresis, 17: 855-866.
15. Bates, L., 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.
16. Bajaj, S., T. Jayaprakash, L. Li-Frei, T.H.D. Ho and R. Wu, 1999. Transgenic approaches to increase dehydration-stress tolerance in plants. Molecular Breeding, 5: 493-503.
17. Vendruscolo, E.C.G., I. Schuster, M. Pileggi, C.A. Scapim, H.B.C. Molinari, C.J. Marur and L.G. Esteves Vieira, 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. Journal of Plant Physiology, 164: 1367-1376.
18. Silva-Ortega, C.O., A.E. Ochoa-Alfaro, J.A. Reyes-Aguero, G.A. Aguado-Santacruz and J.F. Jime´nez-Bremont, 2008. Salt stress increases the expression of *P5CS* gene and induces proline accumulation in cactus pear. Plant Physiology and Biochemistry, 46: 82-92.
19. Khedr, A.H.A., M.A. Abbas, A.A.A. Wahid, W.P. Quick and G.M. Abogagallah, 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress, Journal of Experimental Botany, 54: 2553-2562.
20. Green way, H. and R. Munns, 1980. Machanism of salt tolerance in nonhalophytes. Annual Review of Plant Physiology, 31: 149-190.