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Research Note

Genomic organization and expression of an osmotin-like gene in *Vitis vinifera* L.

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Summary: Southern analysis of genomic DNA indicated the presence of a multigene osmotin-like family in the grapevine genome. The expression of one of these genes that corresponds to the previously isolated pVVOSM1 cDNA is developmentally regulated showing high transcript levels in root, stem and leaf tissues of *in vitro*-grown plants, intermediate in calluses and berries and low in cell suspensions. Treatment of cell suspension cultures with abscisic acid (ABA), ethylene or NaCl resulted in high levels of expression of the pVVOSM1 gene suggesting regulation of the osmotin-like gene by the abiotic signals mentioned above.

Key words: Vitis vinifera, abscisic acid, ethylene, gene expression, osmotic stress, osmotin-like proteins.

Introduction: Osmotin was originally isolated from tobacco cell cultures adapted to an environment of low osmotic potential and numerous studies have implicated a putative function for osmotin and the related osmotin-like proteins in osmotic stress (Singh *et al.* 1989; LaRosa *et al.* 1992; Zhu *et al.* 1995). Based on similarities in amino acid sequences and in expression patterns, osmotin and osmotin-like proteins have been classified as members of the family 5 of pathogenesis-related (PR) proteins (Singh *et al.* 1989; Bol *et al.* 1990). Osmotin and osmotin-like proteins share significant sequence identity with the bifunctional α-amylase/trypsin inhibitor from maize, the sweet protein thaumatin and PR proteins (Singh *et al.* 1989; Casas *et al.* 1992). The expression of osmotin and osmotin-like genes has been linked to development and is induced by various stress conditions provoked by biotic or abiotic stimuli (LaRosa *et al.* 1992; Nelson *et al.* 1992; Zhu *et al.* 1995).

Although osmotin and osmotin-like proteins are now relatively well-known in annual species they have not been extensively studied in perennial plants. Recently, the nucleotide sequence of the full-length pVVOSM1 cDNA clone from grapevine was described (Loulakakis 1997). This clone shows significant sequence similarities to annual plant osmotin and osmotin-like proteins. The purpose of this study was to investigate the gene organization and the expression patterns of the gene, corresponding to pVVOSM1, in various grapevine tissues and in response to abscisic acid (ABA), ethylene and osmotic stress.

Materials and methods: Grapevine (*Vitis vinifera* L. cv. Sultanina) callus and cell suspension cultures were developed and maintained as described by Loulakakis and Roubelakis-Angelakis (1996). For induction experiments, filter-sterilized concentrated solutions of ABA, NaCl, sucrose and ethephon were added to culture medium 3 d after inoculation to final concentrations of 75 μM, 100 mM, 170 mM and 1 mM, respectively. In addition, leaf, stem and root tissues from *in vitro*-grown grapevine plants and berry tissue from greenhouse-grown plants were used in this work.

High molecular weight genomic DNA was extracted from leaves of *in vitro*-grown grapevine plants by a modified cetyltrimethyl ammonium bromide procedure (Lodhi *et al.* 1994). Genomic DNA was digested with the appropriate restriction enzymes, fractionated on 0.7 % agarose gels, blotted and hybridized at high stringency conditions as described by Loulakakis and Roubelakis-Angelakis (1996).

Total RNA extraction and northern blot analysis were performed as described by Loulakakis and Roubelakis-Angelakis (1996).

Results and Discussion: The genomic organization of the grapevine osmotin-like gene family was determined by Southern blot hybridization using the pVVOSM1 insert as a probe. As illustrated in Fig. 1, multiple hybridizing bands were revealed; 9 *Eco*RI, 5 *Eco*RV and 4 *Kpn*I genomic fragments hybridized. It must be noticed that the pVVOSM1 nucleotide sequence contains one internal *Eco*RI site but no *Eco*RV or *Kpn*I sites. These restriction data indicate that in grapevine there is a multigene osmotin-like family consisting of approximately 4 or 5 members. Thus, the grapevine osmotin-like gene family seems to be similar in complexity to the well characterized osmotin-like gene families of other plants (Casas *et al.* 1992; Zhu *et al.* 1995).

The insert of pVVOSM1 hybridized to a transcript of approximately 1.0 kb which showed varying abundance in the grapevine tissues tested (Fig. 2 A). Loading of RNA from different tissues was normalized with regard to ribosomal RNA (Fig. 2 B). High levels of expression of the pVVOSM1 gene were observed in root, stem and leaf tissues, intermediate in callus and berry tissues and low in cell suspensions at the stationary phase (12 d after inoculation). These results are in accordance with previous reports on the differential tissue specificity of osmotin and osmotin-like proteins from other plants. For example, tobacco osmotins are developmentally regulated in intact plants with higher levels of expression in roots (Singh *et al.* 1989; LaRosa *et al.* 1992; Nelson *et al.* 1992). The high transcript accumulation observed in grapevine leaves (Fig. 2) could be a characteristic of a particular stage of development of *in vitro*-grown plants.

The expression of the grapevine osmotin-like gene was further characterized by northern blot analysis of total RNA extracted from cell cultures treated with ABA, 2-chloroethylphosphonic acid (ethephon, an ethylene-releasing agent), NaCl and sucrose for 2 d. Loading of RNA was normalized with regard to ribosomal RNA (data not shown). As shown in Fig. 3, in the control cells transcript levels remained rather constant during this period. Culture of cells in the presence of ABA or ethephon resulted to increase steady-state mRNA levels that progressively accumulated for up to 48 h. In addition, NaCl- (Fig. 3) and sucrose-treated

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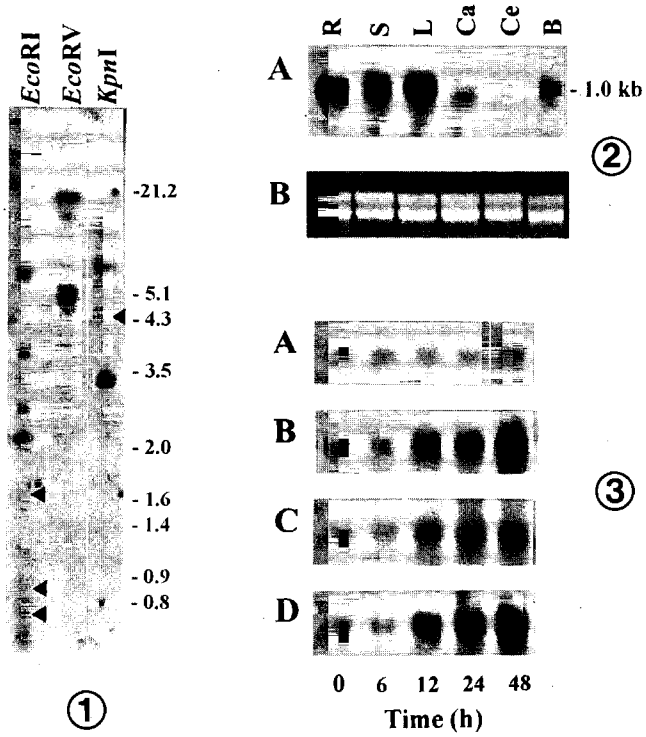

Fig. 1 (left): Southern blot analysis of grapevine genomic DNA. Blot of genomic DNA (10 µg per lane) digested with *Eco*RI, *Eco*RV or *Kpn*I was probed with the pVVOSM1 insert. Arrows denote the position of the more weakly hybridizing bands. The position of the size standards are indicated on the right in kb.

Fig. 2 (right, top): RNA gel blot analysis of osmotin-like transcript in different grapevine tissues. **A**. Blot of total RNA (20 µg per lane) isolated from roots, stems and leaves of *in vitro*-grown plants, calluses, cell suspensions at the stationary phase and immature berries. The blot was hybridized to ^{32}P-labeled pVVOSM1 cDNA. **B**. Ethidium bromide stained native agarose gel of the respective total RNAs. R, roots; S, stems; L, leaves; Ca, calluses; Ce, cells; B, berries.

Fig. 3 (right, bottom): Induction of osmotin-like mRNA level in grapevine cells by ABA, ethylene and NaCl. Cell cultures were treated with ABA (**B**) or ethephon (**C**) or NaCl (**D**) or an equal volume of water (**A**, control) for the indicated times. Blots of total RNA (20 µg per lane) were hybridized to ^{32}P-labeled pVVOSM1 cDNA.

cells (data not shown) accumulated the osmotin-like transcript 12 h after the low water potential treatment and reached high levels after 48 h. It must be noticed that the rate of accumulation of osmotin-like mRNA was similar between the 4 treatments tested. The above mentioned results indicate that expression of the grapevine osmotin-like gene is upregulated by all stress inducing factors that were tested.

Regulation of osmotin and osmotin-like gene expression appears to be very complex in annual plants (LAROSA *et al.* 1992; ZHU *et al.* 1995). These genes are developmentally regulated and also controlled by multiple signals including ABA, salicylic acid, virus infection, low water potentials, cold temperature, wounding and ethylene (SINGH *et al.* 1989; KONONOWICZ *et al.* 1992; LAROSA *et al.* 1992; ZHU *et al.* 1995). In addition, products of these genes have been shown to exhibit *in vitro* antifungal activity (WOLOSHUK *et al.* 1991). Thus, it was proposed that induction of osmotins by osmotic stress may merely reflect the activation of a general stress response of plants which evolved to defend pathogens (KONONOWICZ *et al.* 1992; LAROSA *et al.* 1992). The expression of grapevine osmotin-like gene is clearly induced by ABA and ethylene, the two plant hormones related to both, osmotic- and pathogen-induced stresses. However, further experiments are needed to determine the properties of this gene and the biological function of its product.

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BOL, J. F.; LINTHORST, H. J. M.; CORNELISSEN, B. J. C.; 1990: Plant pathogenesis-related proteins induced by virus infection. Ann. Rev. Phytopathol. **28**, 113-138.

CASAS, A. M.; NELSON, D. E.; RAGHOTHAMA, K. G.; D'URZO, M. P.; SINGH, N. K.; BRESSAN, R .A.; HASEGAWA, P. M.; 1992: Expression of osmotin-like genes in the halophyte *Atriplex nummularia* L. Plant Physiol. **99**, 329-337.

KONONOWICZ, A. K.; NELSON, D. E.; SINGH, N. K.; HASEGAWA, P. M.; BRESSAN, R. A.; 1992: Regulation of the osmotin gene promoter. Plant Cell **4**, 513-524.

LAROSA, P. C.; CHEN, Z.; NELSON, D. E.; SINGH, N. K.; HASEGAWA, P. M.; BRESSAN, R. A.; 1992: Osmotin gene expression is posttranscriptionally regulated. Plant Physiol. **79**, 138-142.

LODHI, M. A.; YE, G. N.; WEEDEN, N. F.; REISCH, B. I.; 1994: A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol. Biol. Reptr. **12**, 6-13.

LOULAKAKIS, K. A.; 1997: Nucleotide sequence of a *Vitis vinifera* L. cDNA (Accession No. Y10992) encoding for osmotin-like protein. (PGR97-064), Plant Physiol. **113**, 1464.

- -; ROUBELAKIS-ANGELAKIS, K. A.; 1996: Characterization of *Vitis vinifera* L. glutamine synthetase and molecular cloning of cDNAs for the cytosolic enzyme. Plant Mol. Biol. **31**, 983-992.

NELSON, D. E.; RAGHOTHAMA, K. G.; SINGH, N. K.; HASEGAWA, P. M.; BRESSAN, R. A.; 1992: Analysis of structure and transcriptional activation of an osmotin gene. Plant Mol. Biol. **19**, 577-588.

SINGH, N. K.; NELSON D. E.; KUHN D.; HASEGAWA P. M.; BRESSAN R. A.; 1989: Molecular cloning of osmotin and regulation of its expression by ABA and adaptation to low water potential. Plant Physiol. **90**, 1096-1101.

WOLOSHUK, C. P.; MEULENHOF, J. S.; SELA-BUURLAGE, M.; VAN DEN ELZEN, P. J. M.; CORNELISSEN, B.J.C.; 1991: Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. Plant Cell **3**, 619-628.

ZHU, B.; CHEN, T. H. H.; LI, P. H.; 1995: Activation of two osmotin-like protein genes by abiotic stimuli and fungal pathogen in transgenic potato plants. Plant Physiol. **108**, 929-937.