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Isolation of an osmotin-like protein gene from strawberry and analysis of the response of this gene to abiotic stresses

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Summary

A strawberry genomic clone containing an osmotin-like protein (OLP) gene, designated *FaOLP2*, was isolated and sequenced. *FaOLP2* is predicted to encode a precursor protein of 229 amino acid residues, and its sequence shares high degrees of homology with a number of other OLPs. Genomic DNA hybridization analysis indicated that *FaOLP2* represents a multi-gene family. The expression of *FaOP2* in different strawberry organs was analyzed using real-time PCR. The results showed that *FaOLP2* expressed at different levels in leaves, crowns, roots, green fruits and ripe red fruits. In addition, the expression of *FaOLP2* under different abiotic stresses was analyzed at different time points. All of the three tested abiotic stimuli, abscisic acid, salicylic acid and mechanical wounding, triggered a significant induction of *FaOLP2* within 2—6 h post-treatment. Moreover, *FaOLP2* was more prominently induced by salicylic acid than by abscisic acid or mechanical wounding. The positive responses of *FaOLP2* to the three abiotic stimuli suggested that strawberry *FaOLP2* may help to protect against osmotic-related environmental stresses and that it may also be involved in plant defense system against pathogens.

Introduction

Plants respond to pathogen invasions or severe environmental stresses by the activation of several different mechanisms. One of the most important mechanisms is the accumulation of pathogenesis-related (PR) proteins. Osmotin and osmotin-like proteins (OLPs) belong to the thaumatin-like

Abbreviations: ABA, abscisic acid; OLP, osmotin-like protein; ORF, open reading frame; PR protein, pathogenesis-related protein; SA, salicylic acid; SAR, systemic acquired resistance; TLP, thaumatin-like protein

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protein (TLP) or PR-5 family (Van Loon and Van Strien, 1999). Osmotin was originally identified as the predominant protein accumulated in NaCl-adapted tobacco cell cultures (Singh et al., 1985). Many studies have demonstrated that the expression of OLPs can be activated by microbial infections and by a variety of abiotic stress factors (reviewed in Velazhahan et al., 1999).

Although the biological functions of OLPs have not yet been fully established, some have been shown to act as antifungal proteins in vitro. For example, tobacco osmotin was reported to cause spore lyses and growth inhibition of *Phytophthora infestans* (Abad et al., 1996). Grape osmotin exhibited inhibition of the hyphal growth of *Botrytis cinerea* (Salzman et al., 1998). Moreover, studies have demonstrated that over-expression of PR-5 proteins in transgenic plants conferred enhanced resistance to pathogens (reviewed in Velazhahan et al., 1999; Velazhahan and Muthukrishnan, 2003). However, the molecular mechanism that accounts for the antifungal activity of PR-5 proteins is still not clear. A mechanism involving membrane permeabilization was proposed by Abad et al. (1996). Another study conducted by Yun et al. (1998) demonstrated that osmotin could subvert target cell signal transduction pathway to increase its cytotoxic efficacy. A more recent study showed that tobacco osmotin-induced apoptosis in *Saccharomyces cerevisiae*, which was correlated with intracellular accumulation of reactive oxygen species and was mediated via the RAS2/cAMP pathway (Narasimhan et al., 2001).

In addition to their antifungal activities, OLPs have also been indicated in other developmental and physiological functions, including roles in flower formation and fruit ripening (Neale et al., 1990; Salzman et al., 1998), protections against osmotic stress (Zhu et al., 1995) and antifreeze activities (Hon et al., 1995). Furthermore, some TLPs were shown to either bind β-1, 3 glucans or possess glucanase activity (Grenier et al., 1999; Osmond et al., 2001).

Strawberry is a member of the Rosaceae family, which consists of more than 3000 species (Baumgardt, 1982). This plant family includes many important fruit crops such as apples, pears and raspberries. Thus far, relatively few studies have been reported on PR protein genes or PR proteins of the Rosaceae family members. No studies have been reported on the expression of any PR genes in response to any abiotic stresses in strawberry plants. In fact, to our knowledge, only two reports have been published thus far on the effect of abiotic stresses on PR genes in the Rosaceae family—one on a TLP gene in apple plants and the other on two TLP genes and one chitinase gene in peach plants (Ruperti et al., 2002; Kim et al., 2003). In a previous study conducted by our laboratory, we reported the isolation of the first OLP gene from strawberry (Wu et al., 2001). Here, we report the cloning and characterization of another strawberry OLP gene. Moreover, we report the response of this OLP gene to abscisic acid (ABA) and salicylic acid (SA), which are the signal molecules implicated in plant response to osmotic-related stresses and pathogenic invasion, respectively (Skriver and Mundy, 1990; Gaffney et al., 1993). The expression of this OLP upon mechanical wounding is also investigated, since wounding is a common damage occurring to plants as a result of abiotic or biotic stresses.

Materials and methods

Plant material

Dormant strawberry (*Fragaria ananassa* Duchesne) plantlets were purchased from Nourse farms (Deerield, MA). The plantlets were planted into 9 cm^2 containers (Kord, Ontario, Canada) that contained a soil mix [bark, peat moss, and perlite (7:2:1, v/v/v)] mixed with dolomitic lime (4.7 $kg\,m^{-3}$). Approximately 5 g of Osmocote-plus fertilizer (15-9-12; Scotts-Sierra, Marysville, OH, USA) was spread on top of each container. The plants were grown in Percival growth chambers (Percival Scientific, Boone, IO, USA, Model AR-60L) at 26/18 °C (day/night) and an 11-h photoperiod. General Electric (T32T8SP41) lamps were used delivering irradiance of 8 $W\,m^{-2}$. The relative humidity was kept at 60–70%. The plants were watered with distilled water approximately every other day. For abiotic stress study, the plants were treated with abiotic stimuli in about 10–14 days after planting. For analysis of gene expression in different organs, samples were collected from leaves, roots and crowns of strawberry plants at 1 month after planting. In addition, samples were collected from green fruits of plants at 5 month after planting and from ripe red fruits about 2 week later.

Isolation of DNA and RNA

Total nucleic acids were extracted from different strawberry organs according to the method described by Manning (1991). For genomic DNA preparation, RNA was removed from the total nucleic acid sample by treating with RNase A at a