



Use of differential display for the identification of touch-induced genes from an ethylene-insensitive *Arabidopsis* mutant and partial characterization of these genes

Thitinun Chotikacharoensuk, Richard N. Arteca^{*}, Jeannette M. Arteca

Department of Horticulture, The Pennsylvania State University, 103 Tyson Building, University Park, PA 16802, USA

Received 26 October 2005; accepted 22 December 2005

KEYWORDS

Calcium;
Ethylene;
Jasmonates;
Touch;
Wounding

Summary

Touch has been shown to affect plant growth and development and ethylene has been shown to have similar effects. However, the mechanisms responsible for touch-induced responses remain unclear. Differential display PCR was used to identify touch-regulated genes from 3-week-light-grown ethylene-insensitive *etr1-3 Arabidopsis* (Columbia ecotype) mutant plants. The differential display PCR screening process yielded 32 cDNA fragments. Subsequent screening of the 32 fragments using northern analysis yielded three touch-inducible clones (A8A, G5A and G7F). These three cDNA were then used to screen a cDNA library. A 1.2 kb fragment for *OPR3* was obtained from A8A screenings. This cDNA fragment encodes 12-oxophytodienoate-10, 11-reductase (OPR), an enzyme in the jasmonic acid biosynthetic pathway. *OPR3* was found to be induced by touch, wounding, methyl jasmonate (MeJA), NaCl and CaCl₂ while ethylene and darkness had no effect. A 2 kb cDNA encoding a calcium-dependent protein kinase (*CDPK32*) was obtained with G5A screenings. *CDPK32* was shown to be induced by touch, wounding, NaCl and darkness while ethylene and MeJA had little or no effect. A 1.4 kb cDNA encoding a novel protein was recovered from the cDNA library screenings with a G7F fragment. This cDNA had some sequence similarity to *GDA1* and was designated *GDL* for *GDA1*-like cDNA. *GDL* was activated by touch, wounding, MeJA, NaCl and CaCl₂ while there was no induction with ethylene and darkness. Using differential display PCR we have successfully been able to identify three clones that are inducible by touch and not by ethylene.

© 2006 Elsevier GmbH. All rights reserved.

Abbreviations: CaCl₂, calcium chloride; CDPK, calcium-dependent protein kinase; GDA, G2 pea dark accumulated gene; GDL, *GDA*-like; MgCl₂, magnesium chloride; MeJA, methyl jasmonate; NaCl, sodium chloride; OPR, 12-oxophytodienoate-10, 11-reductase

^{*}Corresponding author. Tel.: +814 863 2252; fax: +814 863 6139.

E-mail address: rna@psu.edu (R.N. Arteca).

Introduction

The ability of plants to respond to mechanical forces (touch) was initially described by Darwin in 1880. Since this time it has been shown that plants are very responsive to touch in a variety of experimental systems. Today there are several excellent reviews in this area of research (Lloyd, 1942; Biddington, 1986; Edwards and Pickard, 1987; Simons, 1992; Trevawas and Knight, 1994; Slack, 2000; Jaffe et al., 2002; Braam, 2005). The discovery of touch-induced genes began in an indirect manner as part of a study using differential cDNA screening techniques to identify gibberellin-induced genes. In this study Braam and Davis (1990) indirectly identified five *Arabidopsis* genes (*TCH*), which were induced by several stimuli including: touch, wind, rain, wounding and darkness. It has been reported that developmental responses of plants to mechanical stress are dose dependent (Jaffe, 1976), saturable (Beryl and Mitchell, 1977), and that a stimulus from the plant parts that have been touched moves to those that have not (Erner et al., 1980). In addition, many touch symptoms can be mimicked or antagonized by chemical agents (Erner and Jaffe, 1982; Boyer et al., 1983; Biro and Jaffe, 1984). All of the previously mentioned reports support that mechanical stress responses in plants are hormonally mediated. The plant hormone ethylene has received the most attention of all of the plant hormones with respect to its involvement to mechanical growth regulation (Mitchell and Myers, 1995). Ethylene is a gaseous plant hormone that is produced by all higher plants and is involved in numerous aspects of plant growth, development and senescence (Abeles et al., 1992). In fact, similar alterations in growth have been shown to result from either touch or exogenous applications of ethylene (Mitchell and Myers, 1995). Ethylene gas has been reported to be a by-product of physically restricting dark-grown pea seedlings and as a substitute for physical impedance in terms of eliciting the dwarfing response (Goeschl et al., 1966). Epinasty in tomato plant leaves can be induced by touch stimulation of internodes (Jaffe, 1973), an effect that is well documented to be caused by ethylene (Abeles et al., 1992). Mechanical wounding has been reported to increase the activity of ACC synthase, an important regulatory enzyme in the ethylene biosynthetic pathway (Biro and Jaffe, 1984). Both ethylene and ACC have been shown to increase dramatically following touch stimulation (Biro and Jaffe, 1984) and treatments with inhibitors of ethylene biosynthesis and action inhibitors block many of the symptoms of touch (Boyer et al., 1983;

Jaffe, 1984; Jaffe and Forbes, 1993). The relationship between touch and ethylene at the transcriptional level was first documented in 1995 using mung beans (Botella et al., 1995). A gene for ACC synthase (*AIM-1*) was found to be rapidly induced by touch. This relationship between touch and ethylene has also been demonstrated in *Arabidopsis*. *ACS6*, a touch-responsive gene encoding a sixth ACC synthase gene in *Arabidopsis* has since been reported to be rapidly stimulated by touch and ethylene (Arteca and Arteca, 1999). Since the pioneering work of Braam and Davis (1990) there have been many touch-inducible genes identified (Braam, 2005; Lee et al., 2005). Examples of touch-induced genes that are also induced by ethylene are *TCH3* and *TCH5*, (Braam and Davis, 1990), *ACS6*, *ACO2* and others (Van Zhong and Burns, 2003). Expression of touch-induced genes range from 5 to 30 min after touch stimulation with *ACS6* being one of the genes up-regulated the fastest of those reported in the literature (Arteca and Arteca, 1999), thereby providing further evidence that other factors such as ethylene could be involved in the induction many of the touch-inducible genes.

Although there is a substantial amount of physiological and biochemical evidence available that ethylene is involved in touch-induced responses as previously mentioned, Johnson et al. (1998) provided evidence that ethylene is not involved in touch-induced responses in *Arabidopsis* plants. Using the *Arabidopsis* ethylene-insensitive mutants *etr1-3* and *ein2-1*, they showed that wind treatments delayed flowering, decreased inflorescence growth while up-regulating *TCH* gene expression. In addition, they showed that vibration enhanced hypocotyl elongation in these mutants. However, there were differences in sensitivity between *etr1-3* and *ein2-1* with respect to their response to wind and vibration. In a study by Binder et al. (2004) it has been shown that ethylene receptors have a redundant function during the ethylene-induced reduction in growth and that no single receptor null mutation altered responses to ethylene. *ein2* knock-down mutants have also been identified in petunia (Shibuya et al., 2004) confirming *ein2* functions in plant systems other than *Arabidopsis*. One main difference between petunia and *Arabidopsis* mutants is that the petunia *ein2* is regulated by ethylene suggesting a level of control over ethylene responses that has not yet been detected or possible does not exist in *Arabidopsis*. Based on the current literature the nature of ethylene's involvement in touch-induced responses remains unclear. Ethylene production and up-regulation of *ACS6* are among the earliest responses to mechanical stimulation, however it appears that

Download English Version:

<https://daneshyari.com/en/article/2057368>

Download Persian Version:

<https://daneshyari.com/article/2057368>

[Daneshyari.com](https://daneshyari.com)