Thigmomorphogenesis: a complex plant response to mechano-stimulation

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Abstract
In nature, plants are challenged with hurricane winds, monsoon rains, and herbivory attacks, in addition to many other harsh mechanical perturbations that can threaten plant survival. As a result, over many years of evolution, plants have developed very sensitive mechanisms through which they can perceive and respond to even subtle stimuli, like touch. Some plants respond behaviourally to the touch stimulus within seconds, while others show morphogenetic alterations over long periods of time, ranging from days to weeks. Various signalling molecules and phytohormones, including intracellular calcium, jasmonates, ethylene, abscisic acid, auxin, brassinosteroids, nitric oxide, and reactive oxygen species, have been implicated in touch responses. Many genes are induced following touch. These genes encode proteins involved in various cellular processes including calcium sensing, cell wall modifications, and defence. Twenty-three per cent of these up-regulated genes contain a recently identified promoter element involved in the rapid induction in transcript levels following mechanical perturbations. The employment of various genetic, biochemical, and molecular tools may enable elucidation of the mechanisms through which plants perceive mechano-stimuli and transduce the signals intracellularly to induce appropriate responses.

Key words: ABA, auxin, brassinosteroids, calcium, ethylene, jasmonates, nitric oxide, ROS, thigmomorphogenesis, touch.

Introduction
When animals encounter adverse or life-threatening circumstances, they often react by relocating to a more favourable environment. Plants do not have this luxury of high mobility. They are non-motile organisms that are persistently challenged by a wide spectrum of environmental stresses. These stimuli can be extremely detrimental to plants if they had not evolved mechanisms to sense and respond to their dynamic surroundings (Liscum, 2002). Examples of challenges related to mechanical force include wind, physical barriers, and predation. Initially, plants have to sense these stimuli and subsequently launch appropriate responses by either avoiding obstacles, clinging to supporting structures, or producing toxic chemicals to fend off herbivorous predators. In 1881, Charles Darwin reported on mechano-stimulus-induced plant behaviour. In The power of movement in plants, Darwin described in detail directed plant growth in response to external stimuli (Darwin and Darwin, 1881), including how roots of many plant species reorient their growth direction upon making contact with barriers. Such observations were fascinating to Darwin and continue to be an active and intriguing area of research.

Mechanical perturbations are among the many environmental stimuli to which plants respond. Plants sense forces ranging from very intense and physically damaging to more subtle, moderate ones. Many studies have focused on plant responses to wounding, a tissue-damaging mechanical perturbation often used to simulate insect and microbe attacks. Plants sense and respond to mechanical stimuli immediately, as well as over time, by synthesizing an array of phytohormones and other chemicals in addition to expressing defence-related genes that decrease herbivore ability to colonize, feed, and/or reproduce (Green and Ryan, 1972; Karban and Baldwin, 1997; Chen et al., 2005; Chehab et al., 2006, 2008). Similar responses occur in plants stimulated by more subtle mechanical cues including touch. In addition to the production of the phytohormones and expression induction of
defence-related genes, touched plants also respond physically to the stimulus, but with varying degrees depending on the plant species examined. Some responses are very rapid and highly noticeable due to the presence of specialized cells which constitute part of the plant touch response machinery (Braam, 2005). For example, touching *Mimosa pudica* will cause leaf folding within 1 s (Fig. 1) and disturbing the trigger hairs on a Venus’ Fly Trap leaf will cause the trap to close within the same time frame.

**Thigmomorphogenesis**

Plants without specialized sensory cells also respond to mechanical perturbations. However, they react slowly over time by altering their morphology as well as their growth rate. Salisbury (1963) reported that repeatedly touching the leaves of young cocklebur plants caused a 30% inhibition in growth in addition to an increase in the rate of leaf senescence. Mark Jaffe was the first to introduce the term ‘thigmomorphogenesis’ to describe these mechanically-induced responses (*thigma* is the Greek word for touch) (Jaffe, 1973). Thigmomorphogenesis in higher plants is generally a slow response occurring over time and, unlike the responses of *Mimosa* or the Venus Fly Trap, touch-induced morphological changes are not readily apparent immediately after the stimulus (Jaffe, 1973). Among many plant species, a common thigmomorphogenetic response includes a decrease in shoot elongation coupled to an increase in radial expansion (Telewski and Jaffe, 1986; Braam and Davis, 1990; Braam, 2005). An example of thigmomorphogenetic changes displayed by *Arabidopsis* is shown in Fig. 2. Other responses include alterations in chlorophyll content, hormone levels, biotic and abiotic stress resistance, pithiness, flowering time, senescence, and stomatal aperture (Biddington, 1986). Some thigmomorphogenetic alterations cause strengthening whereas others result in the relaxation of the perturbed tissue; both responses are thought to help plants cope or withstand repeated force stresses (Grace, 1977; Glidden, 1982; Jaffe et al., 1984; Telewski, 1995; Biddington, 1986; Telewski and Jaffe, 1986). Therefore, thigmomorphogenesis may be an adaptive response to help plants improve resistance to mechanical perturbations (Grace, 1977; Glidden, 1982; Telewski and Jaffe, 1986). Interestingly, different tissues show variations in their magnitude of responses to mechanical stimuli. Specifically, young tissues have a stronger thigmomorphogenetic response than older ones (Biddington, 1986). A possible explanation is that young tissues may be more fragile and susceptible to stresses and thus must respond strongly to survive harsh environmental stimulations.

Although thigmomorphogenesis is generally perceived as a slow response to mechanical perturbation, very fast physiological responses, not observable to the naked eye, have been reported. For example, Jaffe (1976) detected changes in electrical resistance within a few seconds of touch. Furthermore, Jaeger et al. (1988) documented the inhibition of phloem transport within 2 min following mechanical perturbation.

**Cellular signalling**

Plant thigmomorphogenetic responses to mechanical stimulations have been reported to be saturable (Beryl and Mitchell, 1977), dose-dependent (Jaffe, 1976), and systemic, i.e. the stimulus and its subsequent response translocate from plant regions directly stressed to non-disturbed distal regions (Erner et al., 1980). Furthermore, perturbed plant symptoms of altered morphology and growth rate can be mimicked or antagonized by applications of specific chemical compounds (Erner and Jaffe, 1982; Boyer et al., 1983; Biro and Jaffe, 1984). Altogether, these data suggest that plant responses to mechanical perturbations are mediated by signalling molecules. Hormones, secondary messengers, nitric oxide (NO), reactive oxygen species (ROS), as well as lipid-derived metabolites have been implicated as potential signalling factors. These factors and the potential implications in plant responses to mechanical perturbations are discussed here.

**Calcium**

Calcium (Ca^{2+}) is a universal signal transduction molecule. Its signalling capabilities are implicated in plant responses.
to diverse stimuli. Plant cells cannot tolerate the levels of Ca\(^{2+}\) found outside the cells (10 to 3 M), so cytosolic Ca\(^{2+}\) is kept at low concentrations (<1 µM) by active removal to the extracellular space or to intracellular organelles such as the endoplasmic reticulum (ER) and vacuole. Cells capitalize on the consequent Ca\(^{2+}\) gradient across these membranes and can rapidly and efficiently generate cytosolic Ca\(^{2+}\) influx through the opening of Ca\(^{2+}\) channels present in the plasma membrane (Ding and Pickard, 1993) and intracellular organelar membranes, such as that of the ER and vacuole (Knight et al., 1992; Klusener et al., 1995).

The immediate, transient, and dose-dependent increase in cytosolic Ca\(^{2+}\) following mechanical perturbations has led scientists to propose that Ca\(^{2+}\) may play a role in plant sensing and subsequent responses to such stimuli (Toriyama and Jaffe, 1972; Knight et al., 1991, 1992; Trewavas and Knight, 1994; Batiza et al., 1996; Legue et al., 1997; Calaghan and White, 1999; Berridge et al., 2000; Fasano et al., 2002). For example, Allen et al. (1999) reported that mechanically stimulating Arabidopsis roots causes a transient and local increase in intracellular Ca\(^{2+}\). Ca\(^{2+}\) sensors have also been implicated in gravitropic and mechanical stresses (Sinclair et al., 1996), further suggesting the involvement of Ca\(^{2+}\) as a second messenger in the roots’ signalling mechanisms in response to a range of stimuli (reviewed by Fasano et al., 2002). Intriguingly, the intracellular Ca\(^{2+}\) levels in animal cells also increase following mechanostimulation. Opening of stretch-activated Ca\(^{2+}\) channels has been proposed to be responsible (Sachs, 1986). Similar channels have been identified on plant plasma membranes (Gens et al., 2000). Calcium-induced calcium release channels are also present on vacuolar membranes (Ward and Schroeder, 1994). Mechanically stimulated transgenic plants expressing aequorin, a Ca\(^{2+}\)-dependent bioluminescent protein, show rapid and transient intracellular Ca\(^{2+}\) increases derived from internal pools (Knight et al., 1992). However, recent studies support a role for plasma membrane stretch-activated channels in plant responses to mechano-stimulations (Hayashi et al., 2006). One possibility is that, following mechanical perturbations, small bursts of Ca\(^{2+}\) may enter the cytosol from the extracellular space through plasma membrane stretch-activated channels; the resulting overall increase in Ca\(^{2+}\) may be below the level of aequorin detection. However, these Ca\(^{2+}\) bursts may be sufficient to gate calcium-induced calcium release channels, causing the influx of large amounts of Ca\(^{2+}\) from intracellular stores.

Increases in intracellular Ca\(^{2+}\) need to be sensed to function in signal transduction. Braam and Davis (1990) discovered an intimate connection between Ca\(^{2+}\) sensor proteins and mechano-stimulation. Arabidopsis plants mechanically perturbed by wind, rain or touch induce the expression of at least four touch (TCH) genes within 10–30 min post-stimulation. Three of the TCH genes encode potential Ca\(^{2+}\) sensors: calmodulin (CaM) and calmodulin-like (CML) proteins. More recently, Lee et al. (2005) performed a comprehensive transcriptome analysis in Arabidopsis and identified 12 CML genes whose expression is up-regulated in response to touch. Consistent with the possibility that CaM and CMLs may have roles in touch responses, inhibitors of CaMs and possibly a subset of CMLs block mechano-stimulated thigmomorphogenetic responses (Jones and Mitchell, 1989). Therefore, CaMs as well as CMLs are potential sensors of cytosolic Ca\(^{2+}\) changes induced by mechanical perturbations. Following increases in intracellular Ca\(^{2+}\), these proteins may be activated by binding Ca\(^{2+}\) and, consequently, modulate target enzyme activities to mediate the appropriate physiological responses.

Our laboratory has previously performed a genome-wide analysis to identify and classify Arabidopsis CaM and CML genes. The presence of 50 CMLs and 7 CaMs has been reported (McCormack and Braam, 2003; McCormack et al., 2005). Some of the encoded products have altered Ca\(^{2+}\) binding EF-hand motifs. For example, CaM1 potentially has four functional EF hands whereas CML1, CML28, CML13, and CML12 have 1, 2, 3, and 6 predicted EF-hand motifs. For example, CaM1 potentially has four functional EF hands whereas CML1, CML28, CML13, and CML12 have 1, 2, 3, and 6 predicted EF hands, respectively (McCormack and Braam, 2003). EF-hand motifs are central to the function of Ca\(^{2+}\) sensors. Modifications in such a vital region of the protein may reflect potential variations in binding affinity for Ca\(^{2+}\). One possibility is that the intracellular Ca\(^{2+}\) levels may selectively activate specific CaMs and/or CMLs depending on their Ca\(^{2+}\) affinity. As a consequence of Ca\(^{2+}\) binding, CaMs and/or CMLs are predicted to undergo an alteration in structure (Babu et al., 1985; Wriggers et al., 1998, Delk et al., 2005; Tsai et al., 2007) and reveal hydrophobic surfaces with which they interact with target proteins. McCormack and colleagues further compiled data from Expressed Sequence Tag (EST) databases as well as from 1900 Arabidopsis Affymetrix microarray chip experiments and concluded that these potential Ca\(^{2+}\) sensor encoding genes are expressed under different stimuli and have distinctive developmental as well as spatial expression patterns (McCormack and Braam, 2003; McCormack et al., 2005). For example, CaM1, 2, 3, and 4 are expressed in siliques and leaves.
whereas CaM1 transcripts are in roots. CML7, 8, and 9 are expressed in flowers, leaves and developing siliques; CML10 is expressed in leaves (Ling et al., 1991; Perera and Zielinski, 1992; Gawienowski et al., 1993; Ling and Zielinski, 1993; Zielinski, 2002; McCormack and Braam, 2003; McCormack et al., 2005). CML23 and CML24, which encode proteins that are 78% identical, are both expressed in root tissue; however, CML23 is more restricted to the outer epidermal regions and the extreme root tip; whereas CML24 expression is absent from the distal tip but is in the meristematic and elongation zones (Delk et al., 2005; Tsai et al., 2007). Furthermore, Chiaisson et al. (2005), using GUS staining analyses, showed that exogenous application of methyl jasmonate (MeJA) on Arabidopsis plants results in activating the promoter of CML39 but not that of CML37 or CML38. Therefore, the differences in the predicted CaM and CML structures, in addition to the variations in their transcript expression patterns, indicate that these Ca2+ sensors are likely to have evolved distinct in vivo functional roles, some of which might be involved in tissue-, developmental-, and/or dose-specific thigmomorphogenetic responses to mechno-stimulations.

Jasmonates

Jasmonates (JAs) are a family of cyclopentanone derivatives synthesized from linolenic acid via the octadecanoid pathway. These lipid-derived metabolites, which include jasmonic acid (JA), its methyl ester (MeJA), and 12-oxo-10,15-phytodienoic acid (12-OPDA) have been implicated in plant thigmomorphogenetic responses to mechno-stimulations. Recently, Tretner et al. (2008) reported that touch-treated Medicago truncatula plants show an increase in chlorophyll content and stunted shoot growth in addition to an increase in biomass. These thigmomorphogenetic alterations reflect similar phenotypes observed in Arabidopsis cem1 mutant plants producing constitutively high levels of JA and 12-OPDA (Ellis et al., 2002), Stelmach et al. (1998) have also reported that application of coronatine, a 12-OPDA analogue, on Phascolus vulgaris elicits physiological changes reminiscent of thigmomorphogenesis. Furthermore, mechanically impeding root growth causes temporary inhibition of root elongation coupled to an increase in JA (Staswick, 1992; Berger, 2002; Yan et al., 2007). JAs are also implicated in sensing and subsequent.coiling of the Bryonia dioica tendrils (Weiler et al., 1993), a physiological response produced by touch. Intracellular MeJA levels in coiling tendrils are more elevated compared with those from already coiled ones. Furthermore, the application of MeJA, or its precursor 12-OPDA, on B. dioica also elicits a tendril response (Weiler et al., 1993).

What might trigger the increase in the jasmonate levels following such perturbations? Touch causes an increase in lipoxygenase (LOX) transcripts as well as those of other genes constituting part of the JA biosynthetic pathway (Mauch et al., 1997; Lee et al., 2005; Tretner et al., 2008). LOX is responsible for the production of jasmonate precursors from free polyunsaturated fatty acids. As previously discussed, mechno-stimulation also increases cytosolic Ca2+ levels. Therefore, the elevated Ca2+ levels may be responsible for the localization of phospholipase D (PLD) to membranes. Membrane-associated PLD is active and releases free polyunsaturated fatty acids from membrane phospholipids (Vick, 1993; Ryu and Wang, 1996; Creelman and Mullet, 1997). These unesterified fatty acids are acted upon by LOX to initiate the jasmonate biosynthetic pathway. This scenario provides a potential mechanism for the increase in MeJA and OPDA levels in response to mechno-stimulations (Stelmach et al., 1998; Chehab et al., 2008). A similar mechanism has been reported in animal cells whereby Ca2+ stimulates eicosanoid biosynthesis by activating LOX and phospholipase (Taylor et al., 1990).

Altogether these findings suggest that subsequent to mechanical stimulations, JAs might be playing a role in the transduction pathway, thus coupling the stimulus to the observed thigmomorphogenetic responses. However, to identify the specific function(s) of these lipid-derived metabolites in the touch-induced responses, it is necessary to perform further investigations. Mutants defective in their ability to synthesize JAs such as aos (Park et al., 2002; Chehab et al., 2008) and opr3 (Stintzi and Browse, 2000) or that are insensitive to these compounds, such as coi1 (Feys et al., 1994), will be valuable in further investigating the role(s) JAs play in the mechno-responsive pathway.

Ethylene

Ethylene is a gaseous hormone that regulates a variety of plant processes including aspects of metabolism and development as well as defence (Ecker and Davis, 1987; Abeles et al., 1992; Bleecker and Kende, 2000). Ethylene is the hormone that has been studied for longest with respect to its involvement in plant responses to touch (Mitchell and Myers, 1995). Thigmomorphogenetic changes mimic those that occur subsequent to ethylene exposure (Goeschl et al., 1966; Brown and Leopold, 1972; Jaffe and Biro, 1979; Erner and Jaffe, 1983; de Jaegher et al., 1987; Abeles et al., 1992; Telewski, 1995). For example, frequently rubbing tomato plant internodes causes bending of the petiole, whereas repeatedly touching or physically impeding the growth of bean plants results in a significant increase in stem diameter as well as stunted growth (Jaffe, 1973, 1976), all of which are thigmomorphogenetic characteristics resembling aspects of ethylene exposure. Ethylene release also increases in many different plant species following mechanical perturbation (Goeschl et al., 1966; Brown and Leopold, 1972; Robitaille and Leopold, 1974; Biro and Jaffe, 1984; Takahashi and Jaffe, 1984; Telewski, 1995; Onguso et al., 2006). Physically impeding maize roots results in an increase in root ethylene production coupled to aerenchyma formation (Sarquis et al., 1991; He et al., 1996). Furthermore, dark-grown pea plants produce large amounts of ethylene as they push up through glass beads compared to unimpeded plants (Goeschl et al., 1966). Goeschl et al. (1966) also found that ethylene application on pea plants acts as a substitute for mechanical stimulation and elicits a thigmomorphogenetic dwarf
response. Intriguingly, 1-aminocyclopropane-1-carboxylate synthase (ACS), encoding a key enzyme in the ethylene biosynthetic pathway, is rapidly up-regulated following mechanical stimulation (Biro and Jaffe, 1984; Botella et al., 1995; Arteca and Arteca, 1999). If increased ACS expression leads to enhanced ethylene production, this transcriptional change may be an important regulatory step in thigmomorphogenesis induction.

A key question to answer is whether ethylene plays an early transduction role in the mechanical perturbation response. Whereas transcriptional induction of mechano-responsive genes occurs within 5–30 min post-stimulation, ethylene production peaks at ∼2 h in beans (Jaffe and Biro, 1979) and 9 h in Pinus taeda (Telewski and Jaffe, 1986) following stimulation. This delay in ethylene production suggests that ethylene is unlikely to be a primary factor in the mecano-response signal transduction pathway (Boyer et al., 1983; Biro and Jaffe, 1984, 1986; Biddington, 1986; Johnson et al., 1998).

Inhibitor and mutant studies are valuable in order to reveal potential roles for ethylene in mechano-responses. Mutants defective in the ethylene response pathway show touch-inducible decreases in elongation growth (Johnson et al., 1998; Coutand et al., 2000). Therefore, the ethylene pathway is not required for this particular response following mechano-perturbations (Johnson et al., 1998; Coutand et al., 2000). Furthermore, increases in touch-inducible expression of at least the subset of genes examined still occur in the ethylene response mutants (Johnson et al., 1998). Therefore, although aspects of thigmomorphogenesis, such as increases in radial expansion, may be regulated through ethylene, many features of plant touch responses are independent of this phytohormone’s regulation.

The in vivo interactions of ethylene are complex, as ethylene can cross-talk with other growth regulators, such as auxin (Rahman et al., 2001), abscisic acid (Beaudoin et al., 2000; Ghassemian et al., 2000), cytokinins (Cary et al., 1995), and gibberellins (Rahman et al., 2000). Therefore, a concerted action of ethylene, in addition to other signalling molecules, may constitute the machinery that is important for the full morphological responses observed in plants subjected to mechanical perturbation.

Abscisic acid

Abscisic acid (ABA) is another plant hormone that regulates stress responses and developmental processes (Giraudat, 1995). The in vivo accumulation of ABA retards and/or suppresses plant growth, phenotypes also observed in thigmomorphogenesis. The mechanical perturbation of different plant species results in thigmomorphogenetic responses coupled with the accumulation of ABA (Jeong and Ota, 1980; Erner and Jaffe, 1982). For example, mechano-stimulation of bean plants results in increased ABA levels followed by growth retardation (Erner and Jaffe, 1982). A similar growth response is observed subsequent to exogenous ABA application (Erner and Jaffe, 1982). Furthermore, mechanically stimulated rice plants have closed stomata and are more drought resistant as compared to untreated plants. The authors attributed these developmental and physiological changes to elevated in vivo ABA levels (Whitehead, 1962; Weyers and Hillman, 1979).

Although some evidence suggests that ABA is involved in the plant thigmomorphogenetic responses, further experiments are needed to unravel ABA function in touch-induced changes. ABA-insensitive and biosynthetic mutants are powerful tools to reveal potential role(s) for ABA in plant responses to mechanical force.

Auxin

The functional role of auxin in plant tropisms has been an active area of research for at least the past eight decades (Esmon et al., 2006; Lucas et al., 2008). Auxin has been implicated in plant thigmomorphogenetic responses. Mechanical stimulation of soybean and pea plants reverses auxin-promoted shoot elongation (Victor and Vanderhoef, 1975; Mitchell, 1977). Furthermore, Hofinger et al. (1979) demonstrated that auxin, normally present in the lower internodes of the wild cucumber, Bryonia, disappears when the plants are mechanically stimulated. A major mechanism for auxin turnover is peroxidase-mediated oxidative decarboxylation (Normanly et al., 1997; Ostin et al., 1998). An increase in the peroxidase activity occurs in mechanically perturbed plants (Hofinger et al., 1979; Boyer et al., 1979). Therefore, Boyer and colleagues proposed that peroxidase-driven catabolism of auxin is stimulated in the lower internodes of Bryonia upon the mechanical application of force (Boyer et al., 1979).

The potential Ca2+ sensor, CML12, first identified as TCH3 (Braam and Davis, 1990; Braam, 1992; Sistruk et al., 1994; Antosiewicz et al., 1995) interacts with and regulates the activity of pinoid (PID) (Benjamins et al., 2003). PID is a serine/threonine protein kinase that potentially acts as a switch in regulating the activity of the PIN family of auxin regulators (Bennett et al., 1996; Christensen et al., 2000; Benjamins et al., 2003; Friml et al., 2004). This relationship between a Ca2+ sensor encoded by a touch-inducible gene and a protein involved in auxin transport represents an intriguing connection between auxin signalling and thigmomorphogenesis.

Brassinosteroids

Brassinosteroids (BRs), comprising brassinolide as well as other related compounds, have been implicated in regulating vegetative growth, seed germination, as well as other physiological processes (reviewed by Haubrick and Assmann, 2006). BRs have also been linked to plant thigmomorphogenesis. Exposing Arabidopsis plants to 24-epibrassinolide, a highly active BR (Mandava, 1988), results in the up-regulation of TCH4 (Xu et al., 1995; Iliev et al., 2002). TCH4 encodes a xyloglucan endotransglycosylase/hydrolase (XTH) (Xu et al., 1995; Purugganan et al., 1997; Campbell and Braam, 1998; Rose et al., 2002), an enzyme predicted to have a role in cell wall modification.
Touch-inducible genes, such as TCH4 (also known as XTH22), that may affect cell wall properties have been suggested to play a role in Arabidopsis thigmomorphogenesis (Xu et al., 1995; Purugganan et al., 1997). Recently, Arteca and Arteca (2008) reported that the application of brassinolide in combination with auxin on Arabidopsis plants results in the increased production of ethylene, a phytohormone suggested to play a role in aspects of thigmomorphogenesis. Therefore, the 24-epibrassinolide-mediated induction of TCH4/XTH22 expression, as well as the involvement of brassinolide in increasing the production levels of ethylene, suggest the involvement of BRs in thigmomorphogenesis.

Nitric oxide

Nitric oxide (NO) is involved in regulating various developmental and physiological processes in plants, including seed germination, cell differentiation, transition to flowering, and senescence (Stamler et al., 1992; Leschem, 1996; Keeley and Fotheringham, 1997; Gouvea et al., 1997; Kojima et al., 1998; Van Camp et al., 1998; Beligni and Lamattina, 2000; He et al., 2004). NO is also important in plant defence responses against invading pathogens (Dangl, 1998; Durner and Klessig, 1999; Ma et al., 2008). The biosynthesis and function of NO has been well studied in animals. NO production in animal cells is regulated by Ca2+/CaM. The mechanism of NO production in plants remains unclear (Zemojtel et al., 2006; Moreau et al., 2008). However, Garees et al. (2001) reported that mechanically stressing Arabidopsis cells results in high NO production. Since mechano-stress causes intracellular Ca2+ fluctuations as well as transcriptional induction of CaM and CML genes in plant cells, it suggests that, similar to animal cells, these Ca2+ sensors may also have roles in NO production/regulation in plants. The coincidence of ROS and Ca2+ changes following mechano-stimuli suggests that these molecules may be functionally linked and that both may play a role in plant touch responses.

It is unlikely that a single signalling factor controls the full suite of the plant touch responses. More likely, it is the concerted action of ROS, Ca2+, and plant hormones that function interdependently to generate the observed touch-inducible morphological and physiological responses.

Identifying touch-inducible genes

Thigmomorphogenesis, characterized by the many physiological and morphological changes that occur with mechano-stimulation, probably requires alterations in gene expression. Braam and Davis (1990) first reported the unexpected existence of touch-inducible genes in plants. A differential cDNA library screen led to the discovery of touch-inducible (TCH) genes, genes that are rapidly up-regulated in expression following touch and other forms of mechano-stimulation (Braam and Davis, 1990). TCH1 encodes one of the Arabidopsis CaMs (CaM2) (Braam and Davis, 1990; Lee et al., 2005), TCH2 and TCH3 encode CML proteins (CML24 and CML12, respectively) (Braam and Davis, 1990; Sistrunk et al., 1994; Khan et al., 1997; McCormack and Braam, 2003; Delk et al., 2005; McCormack et al., 2005). Our laboratory has previously shown that CML24, also known as TCH2, is transcriptionally induced following touch (Braam and Davis, 1990). Tsai et al. (2007) report that Arabidopsis CML24 mutants have aberrant NO levels. More recently, Ma et al. (2008) show that applying the CaM antagonist (W7) to Arabidopsis plants impairs NO generation in the innate immune response. A CML24 loss-of-function mutant is also defective in pathogen-induced NO accumulation (Ma et al., 2008); thus the function of CML24 is required for appropriate NO production and accumulation in the innate response to pathogens. Altogether, these findings implicate CML24 in transducing Ca2+ signals important for regulating NO accumulation.

Reactive oxygen species

Plants subjected to various environmental stresses, including mechanical stress, accumulate hydrogen peroxide (H2O2) and superoxide (O2−), reactive oxygen species (ROS) (Legendre et al., 1993; Yahraus et al., 1995; Legue et al., 1997; Gus-Mayer et al., 1998; Bergey et al., 1999; Bolwell, 1999; Orozco-Cardenas and Ryan, 1999; Jih et al., 2003). For example, the mechanical stimulation of Mesembryanthemum crystallinum leaves causes an increase in the in vivo levels of H2O2 (Slesak et al., 2008). Furthermore, the application of physical stress on soybean cell cultures results in elevated ROS levels (Yahraus et al., 1995). Cells may utilize ROS as signalling molecules to regulate the expression of genes (Van Breusegem et al., 2001). Recently, Mori and Schroeder (2004) reported that ROS may control Ca2+-permeable channel activity, thus suggesting a role for ROS in intracellular Ca2+ regulation. As previously discussed, Ca2+ potentially plays an important role in mediating the touch-induced responses following mechanical stimulation. The coincidence of ROS and Ca2+ changes following mechano-stimuli suggests that these molecules may be functionally linked and that both may play a role in plant touch responses.
proteins are perhaps not expected because Ca\textsuperscript{2+} sensors are thought to be regulated post-translationally through the perception of cytosolic Ca\textsuperscript{2+} changes. Thus, both transcriptional and post-translational regulation mechanisms are likely to impact CaM and CML functions.

In addition to potential Ca\textsuperscript{2+} sensor genes, genes encoding cell wall modification enzymes are also highly represented among those up-regulated in expression by touch stimulation. Nearly half of the 33 XTH genes are induced in expression by touch. XTHs catalyse the cleavage and religation of xyloglucan polymers in the plant cell wall, thus possessing the potential to modify wall architecture (Campbell and Braam, 1998; Steele et al., 2001). Xyloglucans hydrogen bond to cellulose microfibrils, thus cross linking them. The cellulose/xyloglucan structure provides cell wall integrity (Rose et al., 2002). Plant cell shape, size, and form are largely determined by the plant cell wall. Thus, enzymes that regulate wall architecture are central to the determination of plant morphology. Altered expression of cell-wall modifying genes, such as XTHs, in response to mechanical perturbation may, therefore, be critical for the consequent morphological changes that manifest in thigmomorphogenesis.

The third most represented gene class among touch-regulated genes is the one with potential roles in disease resistance (Lee et al., 2005). JA signalling as well as JA-related compounds play roles in plant defence responses by activating defence-related genes (Chehab et al., 2008; Howe and Jander, 2008). Therefore, the up-regulation of these defence-relevant genes in mechano-stimulated plants might be due to the increased production of molecules involved in the defence-related signal transduction pathway. Furthermore, the expression induction of about 10% of kinase and transcription factor genes following touch suggests an active role for the kinase transduction pathways and downstream transcriptional activities in launching the appropriate responses to such perturbations (Lee et al., 2005).

As stated previously, roots also respond to mechanical stimulations. In an attempt to identify the genes specifically involved in such underground stresses, Kimber and colleagues performed expression profiling studies on mechanically perturbed roots (Kimbrough et al., 2004). Out of 22 744 genes present on the Affymetrix GeneChip, 1696 had altered transcript abundance. Interestingly, 96% of these genes are also transcriptionally regulated by gravitropism (Kimbrough et al., 2004). This observation is consistent with evidence that suggests the sharing of common mechano-transduction elements between gravitropic- and mechano-responses (Mullen et al., 2000; Moseyko et al., 2002; Massa and Gilroy, 2003). Genes specifically induced following root mechanical perturbations are only 26 in number. The presence of such root-induced mecano-specific regulated genes that are distinct from those controlled by gravity might serve initially to perceive the distinct mecano-perturbation events and possibly initiate a general signal transduction pathway shared by gravitropic stimulations ultimately to result in root reorientation.

Recently, Walley and colleagues performed whole genome microarray analyses to monitor Arabidopsis gene expression changes within 5 min post-wounding, a stimulus that shares mechanical properties with touch (Walley et al., 2007). The aim of this work was to begin identifying the primary stress signal transduction pathway components following mechanical perturbation. Approximately 160 Rapid Wound Responsive (RWR) genes were identified. Analyses of sequences 5' to predicted translational start sites revealed an over-represented Rapid Stress Response Element (RSRE) motif. This motif, when used with a minimal transcriptional promoter, is sufficient to confer an in vivo rapid transcriptional response to a wide range of stresses including wounding. Touch was not investigated. However, transgenic plants with a multimerized RSRE-containing 5' region driving LUCIFERASE (LUC) also show an apparent touch response. Plants that were not wounded but to which a drop of water, oligouronides (OGA), or insect regurgitate was added, show a response higher than the background but lower than when plants are mechanically wounded. Furthermore, 23% of the upstream regions of touch-inducible genes reported by Lee et al. (2005) contain the RSRE cis-element (EW Chehab and J Braam, unpublished results). The presence of the RSRE in touch-inducible genes suggests that this motif might also play a role in the signal transduction of mechano-stimulation leading to gene expression changes.

Comparison of the RWR genes with the transcript profile of the touch-induced genes obtained by Lee et al. (2005) reveals 47 common ones (EW Chehab and J Braam, unpublished results). These common genes include ones involved in the ethylene pathway, such as ACC SYNTHASE 6 and ETHYLENE RESPONSE FACTORS (ERF2 and ERF4), auxin signalling such as AUXIN RESPONSE PROTEIN gene (ARP), and finally Ca\textsuperscript{2+} signalling such as TCH3 (CML12), CALCIUM-DEPENDENT PROTEIN KINASE 32 (CDPK32) and CML38. These data indicate that there is an overlap in genes up-regulated in expression by touch and wound stimuli. Thus, the initial response to touch or wounding may be mediated through similar signal transduction factors and machinery, potentially involving Ca\textsuperscript{2+} and/or phytohormones. However, the distinctive gene expression patterns between the two microarrays might be attributed to the magnitude of the stimulus, with touch being milder than wounding, and/or to the temporal expression difference following stimulation.

As previously discussed, many genes have been suggested through microarray analyses to play roles in the plants’ responses to mechano-stimulation. Recently, Perrin et al. (2007) used activation tagging to identify the microtubule-associated WAVE-DAMPENED 2 (WVD2) protein as potentially involved in Arabidopsis thigmomorphogenesis. Plants constitutively expressing WVD2 exhibit phenotypes characterized by short and thick stems and roots as well as reduced cell elongation. The transgenics also show a more dramatic thigmomorphogenetic response to touch compared with the wild type.

Many touch-inducible genes are also increased in expression following other stimuli, such as darkness, temperature extremes, and hormones (Braam and Davis, 1990; Braam,
1992; Sistrunk et al., 1994; Antosiewicz et al., 1995; Xu et al., 1995; Polisensky and Braam, 1996; Delk et al., 2005; Lee et al., 2005). Although these stresses do not appear to share mechanical properties with those of touch or wounding, these stimuli induce expression of similar sets of genes. For example, Lee et al. (2005) reported that ~53% of the touch-inducible genes showing at least a 2-fold increase in response to touch are also up-regulated by darkness. TCH2 (CML24) transcripts, for example, increase in abundance by touch, heat, and cold (Braam and Davis, 1990; Braam, 1992; Polisensky and Braam, 1996). Consistent with the RNA behaviour, transgenic Arabidopsis plants with the putative TCH2 regulatory region regulating LUC reporter expression have increased luminescence following not only touch stimulation, but also exposure to cold or heat (Fig. 3). Arabidopsis transgensics containing the ubiquitin regulatory region driving LUC expression as controls show no significant changes following similar stimuli (data not

![Fig. 3. Activity of TCH2 promoter in response to touch, cold and heat. Soil-grown plants were imaged for 5 min prior to receiving stimuli (0 min). Plants were then given treatments of cold (4 h at 4 °C), heat (1 h at 37 °C), or a brief touch. Control plants were treated identically except for the application of the stimulus. Imaging following stimulation began 5 min after placing plants into the camera chamber and 5 min exposures were acquired every 6 min over 3.5 h. The first image after stimulus (5 min), the average ‘Peak’ activity image (107 min for cold, 53 min for heat, and 83 min for touch), and the final image (209 min) are shown. Pseudo-colouring of images was performed on commonly scaled images for each stimulus in WinLight (Berthold). A scale bar representing levels of activity from low (blue) to high (pink) is shown at the right.](image-url)
shown). These data indicate that temperature stresses, in addition to mechanical ones, can cause an increase in TCH2 regulatory region activity. However, the LUC reporter experiment illustrates that expression characteristics, more specifically spatial patterning, is distinct for the different stimuli. Whereas the touch and heat responses are strongly prominent in the plant apex, cold-induced expression is more widely distributed with luminescence detected throughout the rosette leaves. The finding that distinct stimuli result in shared gene expression responses suggests that either the mechanisms of perception of these diverse stimuli are shared or that the signal transduction pathways activated by distinct receptors converge at some step prior to gene regulation.

**A possible mechano-sensing network**

How do plant cells sense touch? One hypothesis suggests that intracellular pressure may play a role in perceiving mechano-stimulation (Morris and Homann, 2001). A mechano-stimulus received at the cell wall may cause alterations in the intracellular pressure. This pressure stimulus may lead to the translocation of subcellular organelles (Gus-Mayer et al., 1998), thus transducing the stimulus to a cellular alteration with the potential for downstream effects. This hypothesis is corroborated by Sato et al. (1999) who observed that stimulation of a leaf with a glass capillary induces chloroplasts to migrate away from the site of contact, possibly due to changes in the intracellular turgor. Nuclei have also been observed to move closer to the site of micro needle contact with the cellular surface as well as to the distorted cell wall (Kennard and Cleary, 1997; Gus-Mayer et al., 1998).

Jaffe et al. (2002) propose a second hypothesis which suggests that changes in membrane surface tension transduce the touch stimulation into stretch-activated channel activity. In addition to membrane stretching, touch may result in perturbation of connections among the cell wall, plasma membrane, and the cytoskeleton, thus conducting the extracellular stimulus into an intracellular signal. The plant plasma membrane has specialized extension regions called the Hecthian strands composed of actin microfilaments, microtubules, endoplasmic reticulum as well as RGD (Arg-Gly-Asp)-containing peptides (Lang et al., 2004). These peptides are part of integrin-like proteins proposed to connect the plasma membrane to the cell wall via the cytoskeleton (Lang and Gunning, 2000; Lang et al., 2004). The stretching and relaxation of the cell membrane in response to mechanical stimulation of the cell wall is thought to perturb the cell wall-plasma membrane-cytoskeleton network and relay this information to the interior of the cell. The presence of stretch-activated channels as well as calcium-induced calcium release channels (Edwards and Pickard, 1987; Ding and Pickard, 1993; Ward and Schroeder, 1994) in plants suggests the possibility that mechano-perturbations cause increases in intracellular Ca\(^{2+}\) due to gating of these channels. These intracellular Ca\(^{2+}\) increases are possibly sensed by Ca\(^{2+}\) sensor proteins, such as CaMs and CMLs, which have the potential to bind these ions and subsequently interact with their target enzymes to activate the downstream signal transduction machinery leading to the mechano-induced responses characterized by thigmomorphogenesis.

A substantial amount of work remains before unraveling the signal transduction mechanism(s) through which plants sense and respond to mechanical stimuli. Bioinformatics, genetics, biochemistry, physiology, as well as molecular approaches will aid the elucidation of this intriguing plant communication between its external world and its intracellular microenvironment.

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