



## Tansley review

# In touch: plant responses to mechanical stimuli

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## Summary

**Key words:** *Arabidopsis*, mechanical stimuli, mechano-responses, *TCH* genes, touch.

Perception and response to mechanical stimuli are likely essential at the cellular and organismal levels. Elaborate and impressive touch responses of plants capture the imagination as such behaviors are unexpected in otherwise often quiescent creatures. Touch responses can turn plants into aggressors against animals, trapping and devouring them, and enable flowers to be active in ensuring crosspollination and shoots to climb to sunlit heights. Morphogenesis is also influenced by mechanical perturbations, including both dynamic environmental stimuli, such as wind, and constant forces, such as gravity. Even individual cells must sense turgor and wall integrity, and subcellular organelles can translocate in response to mechanical perturbations. Signaling molecules and hormones, including intracellular calcium, reactive oxygen species, octadecanoids and ethylene, have been implicated in touch responses. Remarkably, touch-induced gene expression is widespread; more than 2.5% of *Arabidopsis* genes are rapidly up-regulated in touch-stimulated plants. Many of these genes encode calcium-binding, cell wall modifying, defense, transcription factor and kinase proteins. With these genes as tools, molecular genetic methods may enable elucidation of mechanisms of touch perception, signal transduction and response regulation.

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## I. Introduction

From the violence of tree strangling and insect trapping to the elegance of roots navigating through barriers in the soil, responses to mechanical perturbation are integral features of plant behavior. Although some plants have very specialized touch-response machinery and rapid and highly noticeable behaviors, touch responses of other plants may occur slowly over time and are often therefore not easily recognized or appreciated. Yet probably all plants sense and respond to mechanical forces. Indeed, cellular responses may be critical for fundamental processes such as turgor regulation, cellular expansion and morphogenesis. The mechanistic bases of touch perception and inter- and intracellular signaling are not well understood. It is also unclear whether the widely diverse responses are related at either the perception or response level or whether the mechanisms and machinery used by single cells to respond to mechanical perturbations such as turgor fluctuations are related to those used at the organ or tissue level to react to externally applied mechanical forces.

For excellent additional information, I refer readers to wonderful reviews written both long ago and more recently (Darwin, 1880, 1893, 1906; Lloyd, 1942; Biddington, 1986; Edwards & Pickard, 1987; Simons, 1992; Trewavas & Knight, 1994; Slack, 2000; Jaffe *et al.*, 2002). Here, I describe briefly some diverse touch responses in higher plants. In addition, I report on aspects of genomic responses to mechanical perturbations and the potential of genetic approaches to this intriguing area of biology.

## II. Fast and fascinating – thigmonasty and thigmotropism

Rapid responses to touch responses are generally thigmotropic or thigmonastic (thigma is the Greek word for touch). Tropic and nastic responses are distinguished by the influence of the stimulus vector on the direction of movement. Tropic responses occur in a direction determined by the placement or direction of the stimulus, such as the climbing behavior exhibited by some mechano-sensitive vines. In contrast, nastic responses are movements, such as the folding up of *Mimosa pudica's* leaflets, which occur in a direction largely independent of the direction of the stimulus.

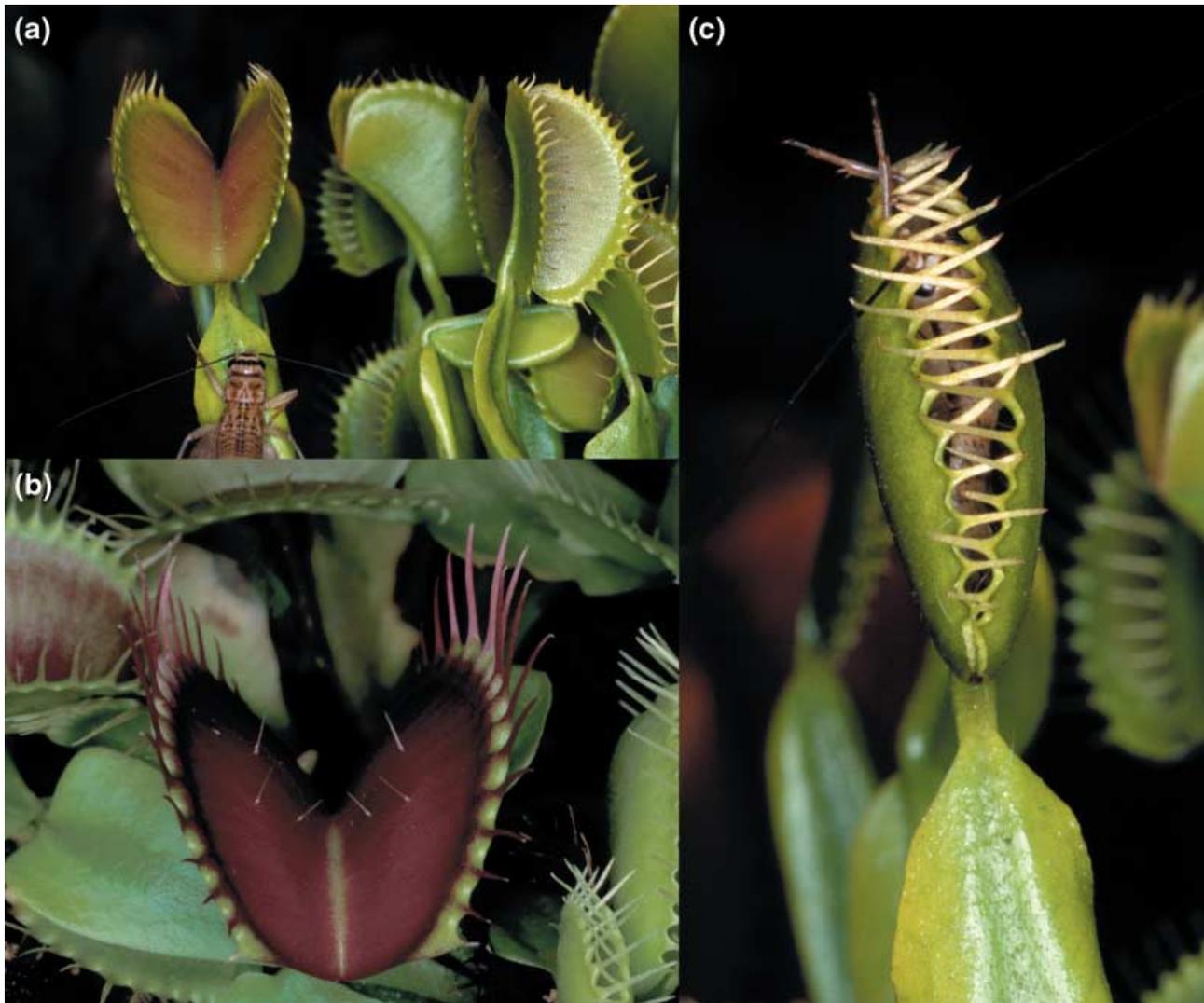
### 1. Carnivorous plants

Venus' Flytrap (*Dionaea muscipula*) accomplishes one of the more spectacular thigmonastic movements resulting in a swap of the role of predator with animals (Simons, 1992). Indeed, Darwin referred to it as 'one of the most wonderful in the world' (Darwin, 1893). The specialized bi-lobed leaves with needle-shaped tines on the leaf margins are generally spread open as invitations to visitors (Fig. 1a). When an unsuspecting insect crawls along the ventral leaf surfaces and bumps into

the three small trigger hairs (Curtis, 1834) (Fig. 1b), inter-cellular electrical signals are generated (Burdon-Sanderson, 1873; Jacobs, 1954; Jacobson, 1965; Simons, 1981) and are thought to trigger differential enlargement of lobe cells (Fagerberg & Allain, 1991), resulting in trap closure in less than a second. Generally, multiple stimulations of one or more of the trigger hairs are required for a response. This first stage of closure interlaces the tines trapping the prey inside the barred cage (Fagerberg & Allain, 1991) (Fig. 1c). Smaller insects, perhaps not worth the energy of further trapping and digestion, may be free to escape through the bars, but larger insects struggle to get free (Darwin, 1893). The insect's further agitation and excretion of nitrogenous compounds, although not always necessary for complete closure (Fagerberg & Allain, 1991), may enhance the subsequent sealing of the lobes around the doomed animal and the secretion of acids and digestive enzymes that kill and digest the nascent meal (Robins & Juniper, 1980). Spatially complex and dynamic changes in cell expansion among distinct tissue layers and sublobe domains effect each stage of the trap closure (Fagerberg & Allain, 1991). This carnivory provides nitrogen supplements to the Venus' Flytrap, enabling them to live in nitrogen-poor environments.

Distinct touch-induced carnivorous behavior is demonstrated by *Drosera rotundifolia*, a species examined in detail by Darwin (Darwin, 1893). The mucilage on the ends of the *Drosera's* tentacles glisten in the sunlight (Fig. 2a,b) and give the plant its common name 'Sundew'. Over 100 tentacles can cover the surfaces of its modified leaves. This showy appearance attracts insects, which upon alighting on the tentacle surfaces become trapped in the gluey substance. The tactile sensitive tentacles detect the presence and further movements of the insect as it struggles to be released. In response, neighboring tentacles bend towards the ones directly stimulated by the prey's presence. Together the tentacles can generate a cup-like indentation that encloses the prey within (Fig. 2c,d). Both thigmonastic and thigmotropic growth-based tentacle movements are involved (Lloyd, 1942). Differential growth may be controlled by changes in auxin levels or sensitivity, as exogenously applied indole-3-acetic acid (IAA) can elicit similar growth behaviors and IAA transport blockers can inhibit touch-induced movements (Bopp & Weber, 1981). The mechano-sensitivity of the tentacles is remarkable, being able to detect a piece of human hair weighing less than a microgram (Darwin, 1880) and yet they fail to respond to the water droplets or even the force of heavy rains (Darwin, 1880; Darwin, 1893). How the plant distinguishes between stimuli unrelated to food, such as rain and wind, from the perturbations of insects is unclear.

The behavior of the bladderwort, *Utricularia*, which may have the most forceful prey capture mechanism among plants, is described in detail by Lloyd (Lloyd, 1942). *Utricularia* have worldwide distribution in aquatic and moist terrestrial environments. Lloyd's writing reveals a particular reverence for this creature and its traps that 'present an astounding

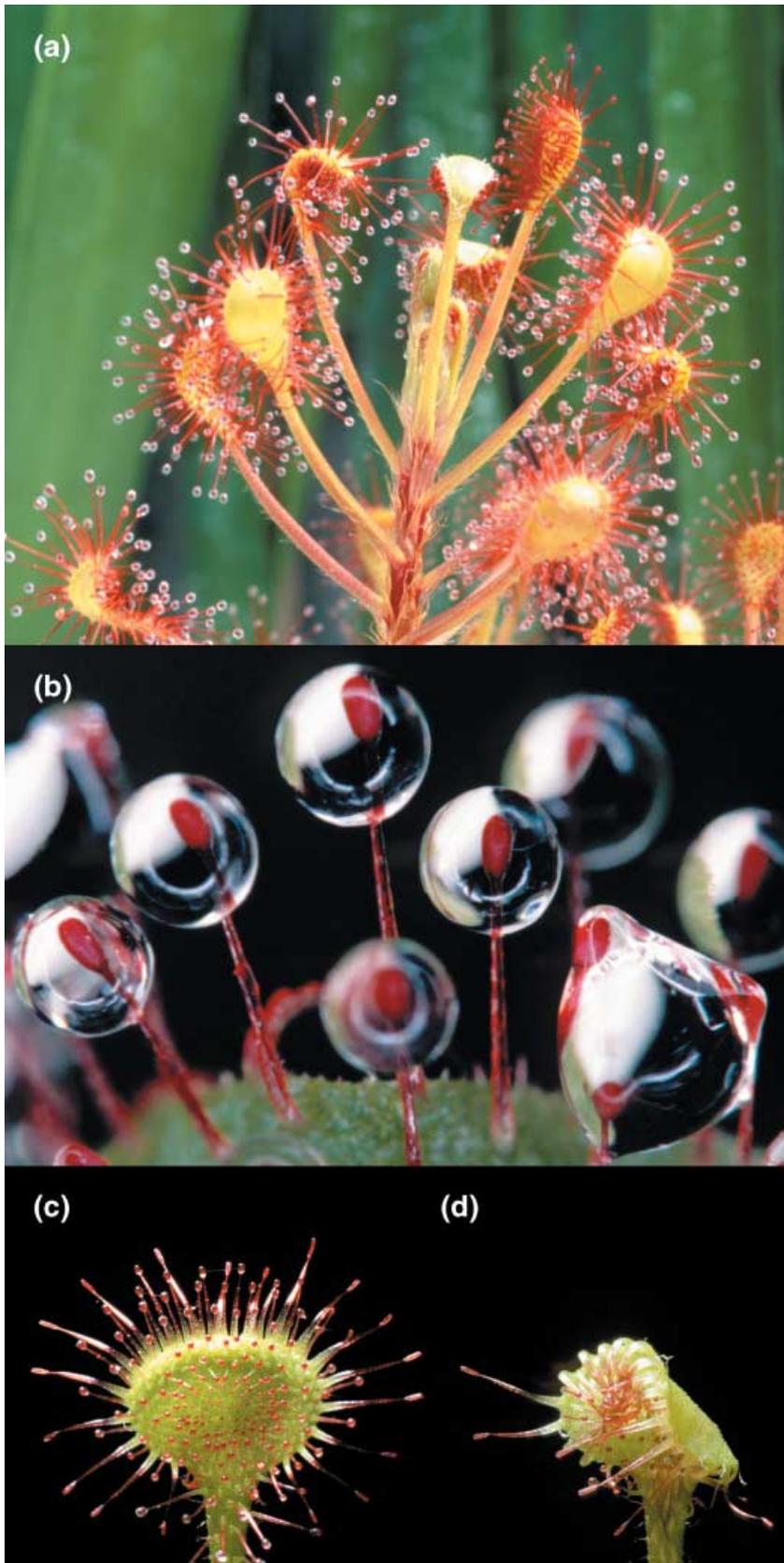


**Fig. 1** *Dionaea muscipula* (Venus' Flytrap). (a) Potential prey nears an open trap. (b) Close-up of trap showing trigger hairs. (c) Interlaced tines trap prey in first phase of trap closure. Photos by Barry Rice, PhD; <http://www.sarracenia.com/galleria/galleria.html>.

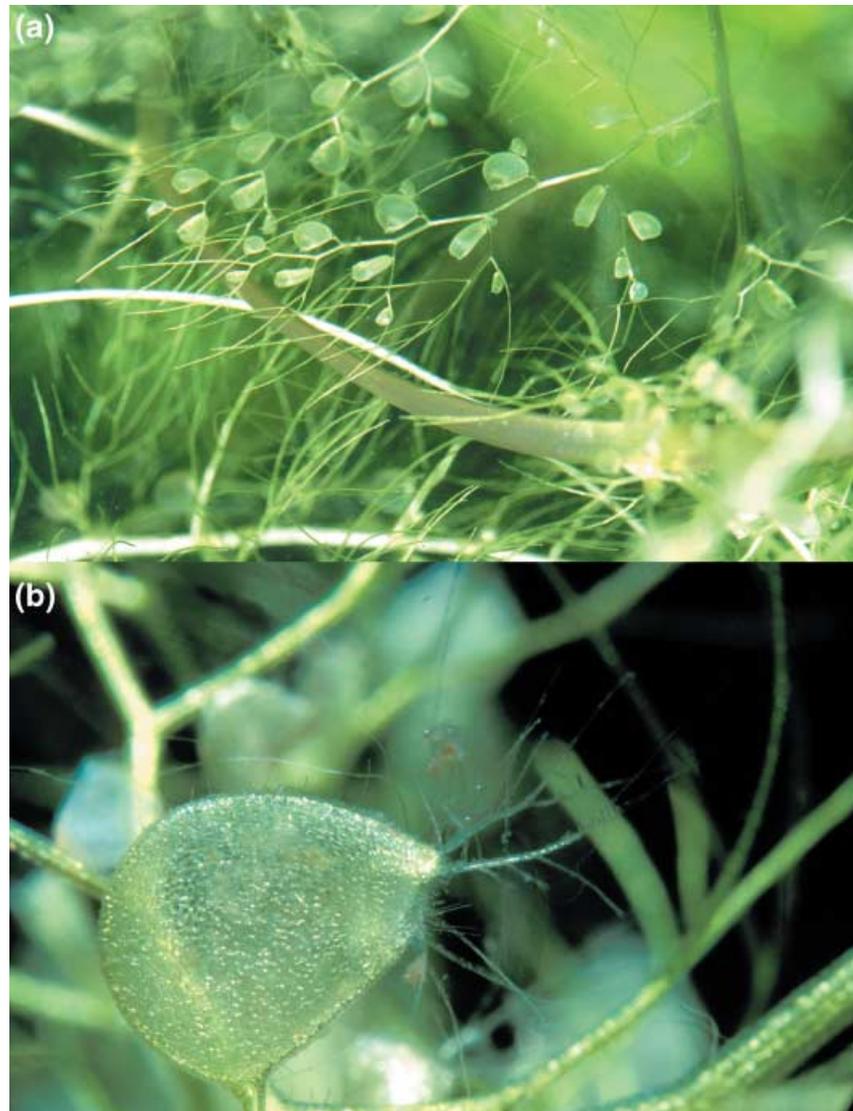
degree of mechanical delicacy depending on a fineness of structure scarcely equaled elsewhere in the plant kingdom'. The *Utricularia* trap, attached via a stalk to the rootless plant body (Fig. 3a), is a thin-walled hollow sac with a watertight trapdoor. When the trap is set, the outer walls are concave and the chamber is under negative hydrostatic pressure. Various appendages, some with mucilage-secreting glands, have roles in luring prey to the trap door entrance and others near the bottom of the door are the touch-sensitive triggers (Fig. 3b). Small creatures, such as water fleas, are examples of those that fall prey to *Utricularia* traps. Within 30 ms of tactile detection, the trap door bursts open, the sac walls move outward to take on a slightly convex shape and, as a result, there is a tremendous suction of water into the bladder. All accompanying creatures are swept in with the current. The trapdoor closes and the meal takes place.

## 2. The sensitive plant

*Mimosa pudica*, known as the sensitive plant, and some of its relatives are other well-known thigmonastic plants. A touch stimulation results in a very rapid folding up of the small leaflets composing the doubly compound leaves (Fig. 4a,b). Remarkably, the touch response is not restricted to the stimulated leaflet, but can propagate to all the neighboring leaflets of the leaf (Simons, 1981; Malone, 1994). A stronger stimulus, resulting in wounding, can elicit folding behavior at distantly located leaf petioles (Simons, 1981; Malone, 1994). The rapid folding of the leaflets may serve to both scare away potential predators and give the appearance of a less voluminous meal. Alternatively, the leaf movements may be a mechanism to expose protective thorns (Eisner, 1981).



**Fig. 2** *Drosera* (Sundew). (a) Open and folded tentacle-laden leaves of *D. madagascariensis*. (b) Close up of mucilage-covered tentacle tips. (c) Open leaf before touch stimulation. (d) Folding leaf following touch stimulation. Photos by Barry Rice, PhD; <http://www.sarracenia.com/galleria/galleria.html>.



**Fig. 3** *Utricularia* (Bladderwort).  
(a) Numerous *U. foliosa* bladder traps.  
(b) *U. inflata* side view with tentacles visible  
at right near trap door. Photos by Barry Rice,  
PhD; <http://www.sarracenia.com/galleria/galleria.html>.

The thigmonastic *Mimosa* leaflet closure is mechanically similar to the rhythmic movements of related species, such as *Samanea saman* (Satter *et al.*, 1990). Both of these folding leaf movements result from a loss of turgor from extensor cells of pulvini, specialized motor organs located at the bases of the leaflets and petioles. Opposite in position from the extensors are the flexors. When the extensors lose turgor, the flexor cells stretch. Together these reversible cell volume and shape changes enable elaborate leaflet and petiole movements.

The touch-induced extensor cell volume changes in *Mimosa* may be initiated by a triggered unloading of sucrose from the phloem, resulting in the lowering of apoplastic water potential (Fromm & Eschrich, 1988). Extensor cell turgor and volume loss occur through the passive transport of  $K^+$  and  $Cl^-$  ions and the concomitant water movement driven by osmosis (Simons, 1981; Fromm & Eschrich, 1988). Strong

electrochemical gradients enabling rapid ion movements across plasma membranes are set up by proton pumps (Roblin, 1982), and *Mimosa* motor cells have an abundance of  $H^+$ -ATPase proteins (Fleurat-Lessard *et al.*, 1997), perhaps to accommodate a requirement for high pump activity. The osmotically driven cellular water loss is extensive, with up to a 25% volume change, and rapid, occurring within 1 s (Fleurat-Lessard *et al.*, 1997b). Such a fast and dramatic loss of water from pulvinar cells is likely facilitated through transporters such as aquaporins or solute-water cotransporters (Fleurat-Lessard *et al.*, 1997a,b; Morillon *et al.*, 2001; Moshelion *et al.*, 2002). The actin cytoskeleton may also be involved; inhibitors that affect actin can block movements (Fleurat-Lessard *et al.*, 1993), and movements are correlated with decreased actin tyrosine phosphorylation (Kameyama *et al.*, 2000).



**Fig. 4** *Mimosa pudica* (Sensitive Plant). (a) The doubly compound leaves of *Mimosa pudica* open before stimulation. (b) The folding up of leaflets after touch stimulation. Photos by Barry Rice, PhD; <http://www.sarracenia.com/galleria/galleria.html>.

How pulvini located far from the initial site of stimulation receive signals is uncertain. Action potentials, likely traveling through companion cells and phloem parenchyma (Fromm & Eschrich, 1988; Fleurat-Lessard *et al.*, 1997a), may be responsible for touch-stimulated leaflet folding within a single leaf (Houwinck, 1935). Electrical signals, called slow wave potentials or variation potentials, are also detected systemically after wounding. However, these systemic signals have properties inconsistent with action potentials in part because they can travel through dead tissue. Indeed, the electrical phenomena detected in wounded *Mimosa* and other plants may not be propagating signals but static responses to hydraulic pressure changes that move through the xylem (Malone, 1994; Stahlberg & Cosgrove, 1997). Xylem pressure alterations can distribute pressure radially to epidermal cell layers and trigger local epidermal cell membrane depolarizations (Stahlberg & Cosgrove, 1997).

Thus, it may be that hydraulic pressure, not the detected electrical changes, serves as the transmission mechanism for distant wound responses. There is also evidence for chemical signals in *Mimosa*. As early as 1916, a role was proposed for a chemical signal, Ricca's factor, that could travel through a water-filled tube connecting severed stem tissues (Ricca, 1916) to signal distant leaf folding in response to wounding. More recently, substances that can induce leaf closing have been isolated and characterized (Schildknecht & Meier-Augenstein, 1990; Ueda *et al.*, 2001), adding evidence for a potential role of chemical signaling in the touch-responsive leaflet folding behavior of *Mimosa*.

### 3. Touch-sensitive tendril coiling and vine climbing

Many plants utilize a sense of touch to achieve vertical height with the goal of rich sun exposure while avoiding the energy

expenditure of developing a supporting trunk. These plants can reach out to grasp sturdy objects and then use them as supports by coiling around them.

Tendrils are often modified leaves or stems (Jaffe & Galston, 1968), appearing as long graceful fingers capable of coiling. The distal portions of tendrils are highly touch-sensitive. Indeed, some tendrils may have greater touch sensitivity than humans. Simons reports that a 0.25 mg thread drawn along a tendril can be enough to evoke a response (Simons, 1992), and Darwin documented tendril responses to stimuli in the range of 1–5 mg (Darwin, 1906). Touch stimulation leads to a rapid onset of tip coiling, often within seconds, that enables a secure association with an object. Some species have thigmonastic tendrils and others are thigmotropic, depending upon whether the direction of contact coiling is determined by the structure of the tendril or the direction or point of touch stimulation (Jaffe & Galston, 1968). The touch sensitivity of tendrils shares a selective sophistication with that of *Drosera*. Application of water droplets causes no coiling response; therefore tendrils avoid nonproductive coiling in rainstorms (Jaffe & Galston, 1968). Furthermore, coiling that occurs in response to transient stimulation are often reversed by uncoiling (Jaffe & Galston, 1968).

Some species undergo a second round of tendril coiling between the tip coiling at the support and the attachment of the tendril to the main body of the plant. This secondary coiling behavior, called free coiling (Jaffe & Galston, 1968), serves to bring the climbing plant closer to its support. Once coiling has occurred, wall changes such as lignification are often induced to stiffen the coils and prevent unwinding (Jaffe & Galston, 1968).

Tendril coiling results from differential growth. The earliest responses include a contraction of ventral cells and an expansion of dorsal cells. Sustained coiling then follows with a differential rate of cell expansion across the ventral and dorsal surfaces (Jaffe & Galston, 1968).

Elmar Weiler and colleagues have found that an increased level of octadecanoids, in particular 12-oxo-phytodienoic acid (OPDA), correlates with touch-induced *Bryonia dioica* tendril coiling (Stelmach *et al.*, 1998; Blechert *et al.*, 1999) and that OPDA, and related analogs that cannot be converted to jasmonic acid (JA), are sufficient to cause tendril coiling with physiologically relevant kinetics in the absence of a mechanical stimulus (Weiler *et al.*, 1993, 1994; Blechert *et al.*, 1999). Octadecanoid pathway signaling may not be universally involved in touch-induced tendril coiling, however, because exogenous OPDA or JA is not sufficient to induce pea tendril coiling (Brosché & Strid, 2000; Engelberth *et al.*, 2001). In *B. dioica*, octadecanoid signaling leads to IAA accumulation (Stelmach *et al.*, 1999), which itself is an active inducer of tendril coiling (Jaffe & Galston, 1968; Jaffe, 1985; Weiler *et al.*, 1993). The central role of IAA in differential growth leading to tropic behaviors is well established (Friml, 2003), so it is not surprising that IAA may have a major role in tendril coiling.

In contrast, ethylene, whose levels increase in octadecanoid- or IAA-treated plants, is not required for the coiling response because coiling still occurs when ethylene synthesis is blocked (Weiler *et al.*, 1993).

Alamethicin (ALA), derived from a parasitic plant fungus and capable of forming voltage-dependent ion channels, can induce tendril coiling in both *B. dioica* and *Pisum* (Engelberth *et al.*, 2001). Because this effect of ALA occurs even when octadecanoid synthesis is inhibited, it may be that ALA-induced membrane depolarization can trigger tendril coiling, perhaps through alterations in IAA accumulation, transport or action (Engelberth *et al.*, 2001).

The *Monstera* vine has an unusual technique for finding a host tree to climb. It travels by growing along the ground in search of the darkness of a tree's shadow; this movement towards darkness is called skototropism (Strong & Ray, 1975). The vine completes its journey when the vine apex senses arrival at the host tree by the resulting touch encounter. A dramatic change in growth direction then occurs. The *Monstera* vine begins an upward climb of its host tree (Strong & Ray, 1975), and leaf and stem development changes in anticipation of increased light and productive photosynthesis.

The strangler fig (*Ficus costaricensis*) has a more sinister interaction with host trees (Putz & Holbrook, 1986; Putz & Holbrook, 1989). Roots of the epiphyte form grow downward from its host tree and, in addition to wrapping around the trunk, roots that touch each other fuse together (Fig. 5). As the roots continue to grow and fuse, they eventually encircle and prevent further growth of the host trunk. Further differentiation of the figs' roots result in such constricting pressure that it damages and eventually kills the host in a manner befitting its name. In this way, the strangler remains alone without competition from its former host.

#### 4. Bold flowers

Many flowers have evolved touch-sensitive organs, with examples of thigmonastic and thigmotropic stamen filaments, petals and pistils among many plant species. These behaviors generally are focused on preventing self-pollination or successfully depositing pollen on insects or hummingbirds. Self-pollination is avoided by a variety of touch-responsive mechanisms. For example, some stigmas bend toward the petals in response to an insect crawling down towards its anthers, apparently to avoid contact with the pollen-laden visitor as it departs (Simons, 1992). Stamens of other species avoid accidental pollination by bending away from the stigma during circadian-regulated flower closure (Jaffe *et al.*, 1977). More commonly, touch-sensitive stamens bend over to dab pollen on visiting insects (Simons, 1992). Some touch-responsive flowers have spring-loaded mechanisms that trigger rather explosive responses (Simons, 1992). One fascinating example is the behavior of the dimorphic flowers of *Catsetum*. Male flowers are poised to respond to visitors that contact antennae



**Fig. 5** *Ficus costaricensis* (Strangler Fig). Strangler fig roots surrounding host tree trunk. Photo by Tim Skyrme; <http://www.artzend.com>.

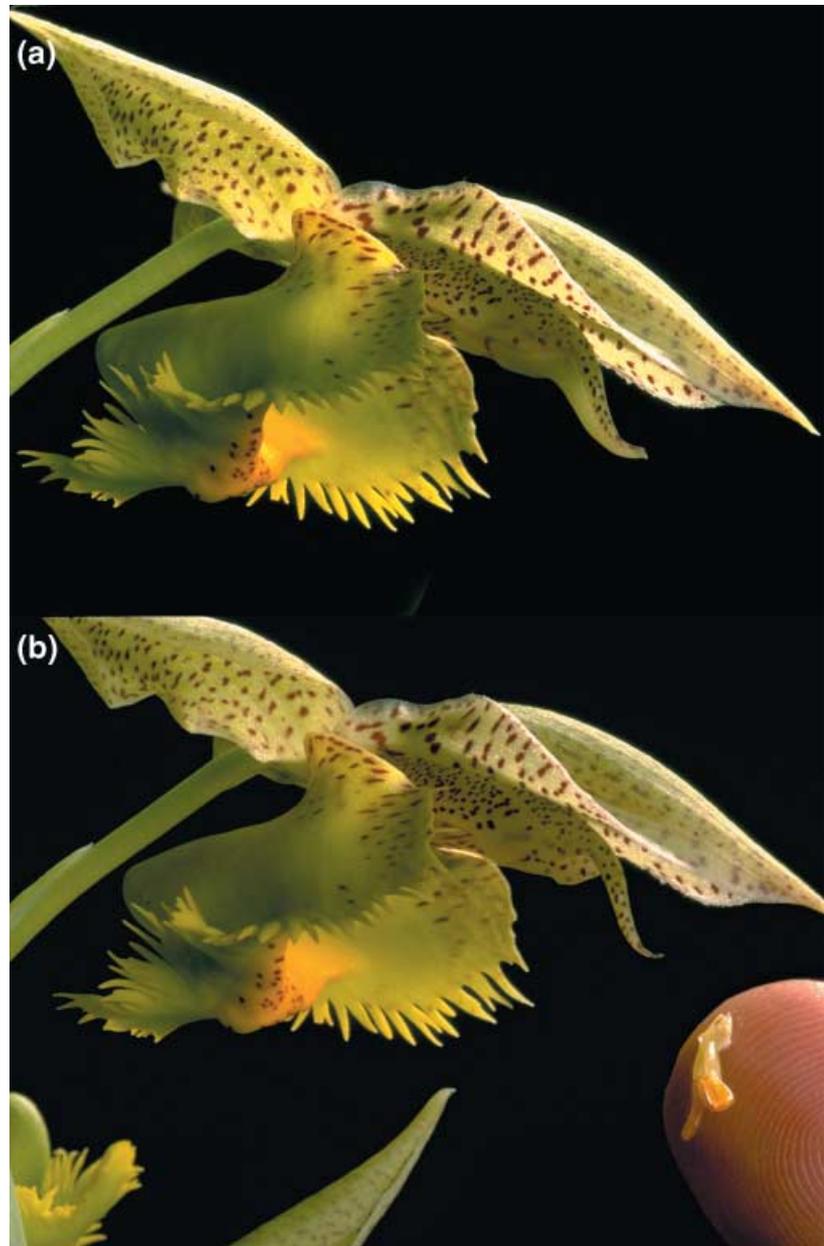
in the center of the flower by the release of stamen filaments held under tension by petals (Fig. 6a,b). The force by which a sticky disc with pollen sacs hits the chosen pollinator can be strong enough to knock a bee from the flower (Simons, 1992). This experience and the burden of the attached large pollen sac can be so traumatic that the bee will carefully evaluate potential future visiting sites and strongly prefer female flowers rather than the explosive male ones (Romero & Nelson, 1986). In this way, male *Catasetum* flowers may have evolved to compete for exclusive pollination rights of female flowers (Romero & Nelson, 1986).

### 5. The struggle of roots

Darwin (Darwin, 1880) contemplated the fate of roots as they grow through the soil encountering many obstacles, such as

stones, in their paths. He observed that when roots are blocked in their downward growth pattern by an obstacle, such as a plate of glass, root tips flatten, take on an oblique shape and turn nearly 90° to take on a new direction of growth over the surface of the glass. Darwin hypothesized that the root apex is touch-sensitive and that contact results in a transmission of a signal to change growth at more proximal root regions. He also subjected young root apices to local mechanical stimulation through the placement of small, flat materials such as sandpaper to the sides of root tips. He observed a loss in gravitropic behavior as thigmotropic growth resulted in movement in a direction away from the point of contact.

More recently, Gilroy and colleagues have reported similar avoidance behavior in *Arabidopsis* roots (Massa & Gilroy, 2003). When *Arabidopsis* roots encounter glass barriers, gravitropism



**Fig. 6** Male *Catasetum* orchid flowers before and after triggered release of pollen sac. (a) The pollen sac is held within the pointed anther cap. (b) When the trigger is touched, the sticky pollen sac is fired at the intruder. Photo by Greg Allikas; <http://www.orchidworks.com>.

appears to be compromised and thigmotropism occurs instead. The touch-stimulated roots neglect their propensity to grow towards gravity vector and instead grow away from the perceived point of mechanical contact. The interaction of touch and gravity may occur at the subcellular level; touch can delay one of the earliest known graviresponses, the falling of starch granules in the root columella cells (Massa & Gilroy, 2003).

This mechanical stimulus avoidance response of roots has been proposed to be an important component of the wave-like turning growth of *Arabidopsis* roots on dense agar plates

held at an incline (Okada & Shimura, 1990). Under these growth conditions, the roots form in the pattern of a sinusoidal wave as a consequence of alternating directions of differential cell expansion in the elongation zone and twisting of tip cells. Mutants defective in this elegant growth patterning have provided evidence of the importance of polar auxin transport and microtubule function for proper waving behavior (Okada & Shimura, 1990; Bennett *et al.*, 1996; Garbers *et al.*, 1996; Deruere *et al.*, 1999; Thitamadee *et al.*, 2002; Nakajima *et al.*, 2004; Sedbrook *et al.*, 2004).



**Fig. 7** Repetitive touch stimulation leads to a delay in flowering and an inhibition of inflorescence elongation in *Arabidopsis*. The plants on the right were touched twice daily; the plants on the left are untreated controls. Photo by Dereth Phillips, Rice University.

### III. Subcellular touch-induced movements

Touch responses also occur at the cellular level; organelles move in directed fashion in mechanically perturbed cells. For example, stimulation with a glass capillary induces chloroplast migration away from the point of contact (Sato *et al.*, 1999). This behavior is dependent upon the activity of gadolinium- and lanthanum-sensitive functions (Gd and La), suggestive of a role for stretch-activated channels, and is dominant to light-induced responses (Sato *et al.*, 2001).

Nuclear migration is also affected by a mechanical perturbation to the cellular surface; nuclei move closer to the site of microneedle contact and induced distortion of the cell wall (Kennard & Cleary, 1997; Gus-Mayer *et al.*, 1998).

### IV. Thigmomorphogenesis

In contrast to the generally rapid thigmonastic and thigmotropic responses of plants or organs specialized to respond to externally applied mechanical stress, gradual morphogenetic alterations in response to stimuli such as touch and wind are common, if not universal, among higher plants. These touch-induced morphogenetic changes occur slowly over time and are therefore often not readily apparent or appreciated; however, these responses can be quite dramatic (Fig. 7). Mark Jaffe, who has carried out systematic analyses of plant growth responses to mechanical perturbations over the past 30 year, coined the term 'thigmomorphogenesis' to describe the touch-induced developmental response of nonspecialized plants (Jaffe, 1973). Biddington (Biddington, 1986) summarizes in detail many of the diverse aspects of thigmomorphogenesis and how responses vary in different species. Briefly, the most

common features of shoot thigmomorphogenesis among many different plant species are a decrease in elongation growth and an increase in radial expansion. These growth changes are likely adaptive, enabling plants to withstand additional mechanical force stresses (Telewski & Jaffe, 1986). Touch-induced morphogenesis changes are correlated with increased production of strengthening tissue and improved resistance to mechanical perturbation induced damage (Jaffe *et al.*, 1984; Biddington, 1986; Telewski & Jaffe, 1986). Some species increase tissue rigidity whereas others have higher flexibility in response to mechanical perturbation (Biddington, 1986; Telewski, 1995).

The earliest detectable physiological touch responses include electrical resistance changes within seconds (Jaffe, 1976) and localized phloem blockages within 1 to 2 min (Jaeger *et al.*, 1988) after stimulation. Younger tissues show greater magnitude responses than older tissues (Biddington, 1986). Long-distance signaling is also likely because growth alterations are not limited to regions that are directly stimulated but are also found at sites not directly stimulated (Erner *et al.*, 1980; Biddington, 1986; Depege *et al.*, 1997; Coutand *et al.*, 2000). In addition to overall growth effects, thigmomorphogenesis can include other changes that are often variable among species. Other processes often affected by mechanical perturbation include flowering time, dormancy, senescence, chlorophyll content, drought resistance, abscisic acid levels, low temperature resistance, pithiness, stomatal aperture and pathogen resistance (Biddington, 1986).

Thigmomorphogenesis may have evolved as a response to wind. Trees allowed to sway in wind tend to have reduced height and greater trunk girth than those tightly staked to prevent movement (Jacobs, 1954). These thigmomorphogenetic-like

growth alterations may be advantageous. Upon freedom from staking, trees suddenly allowed to sway in the wind are more likely to experience wind damage (Jacobs, 1954). Severe wind combined with cold and drought stress can dramatically affect tree form. Krummholz, descriptively referred to as knee forest or twisted wood (Yulsman, 1999), may be an extreme manifestation of thigmomorphogenesis. Krummholz displays an overall stunted phenotype that favors expansion of growth and branching in a leeward direction (Yulsman, 1999).

### 1. The role of strain and feedback mechanisms

Without specialized organs, such as the trigger hairs of Venus' Fly Trap and *Utricularia*, to monitor mechanical perturbations, how do nonspecialized plants perceive such stimuli? Recent experiments indicate that the perception of longitudinal strain is critical, and feedback mechanisms play important roles in determining tissue responses to mechanical perturbation. There is a strong correlation between the degree of longitudinal strain experienced and the extent of the thigmomorphogenetic response (Coutand *et al.*, 2000). Consistent with a central role for strain in inducing thigmomorphogenetic changes, transgenic tobacco plants with xylem of reduced tensile stiffness undergo enhanced xylem development and attain overall stem stiffness comparable to wild type (Hepworth & Vincent, 1999). Enriched production of supporting tissue, such as the xylem cylinder in tobacco stems (Hepworth & Vincent, 1999), may be a mechanism of responding to tissue material properties and their propensity to experience strain. Thus, plants may compensate for defects in wall components by generating a greater abundance of strengthening tissues. This evidence of feedback from wall properties to wall biogenesis is reminiscent of the findings that cellulose-deficient mutants have increased wall pectin content (His *et al.*, 2001), and that inhibition of lignin synthesis results in enhanced cellulose accumulation (Hu *et al.*, 1999). Thus, plants appear to have multiple mechanisms of adapting to wall deficiencies and compensating for potential structural integrity challenges.

Thigmomorphogenesis may not only result in response to externally applied mechanical stress that results in strain. Progressive plant growth itself is sufficient to cause thigmomorphogenetic changes, perhaps by increased weight-triggered tissue strain. For example, increased xylem production occurs in *Arabidopsis* in response to the augmented weight associated with increased height (Ko *et al.*, 2004); enhanced cambium differentiation can also be mimicked by addition of weight to immature inflorescences (Ko *et al.*, 2004). Direct compressive forces applied to callus cells can induce cambium-like development (Lintilhac & Vesecky, 1984; Barnett & Asante, 2000). Just as bone and muscle mass development increase in correlation to the amount of force experienced (Frost, 1992; Goldspink, 1999), tissue strain in plants may induce growth and differentiation to contribute to the reinforcement of

structural tissue integrity. Similar reinforcement must occur at branching points. In this way, plants adapt to morphogenetic and environmental changes, enabling them to maintain continued growth even in windy or otherwise mechanical stress-rich environments.

How the mechanical perturbation caused by strain is sensed at the cellular level is not defined, although two models of mechano-perception at the plasma membrane and/or cell wall have been proposed. As in animal and bacterial cells, plant membranes have stretch-activated channel activity that may be responsible for triggering ion flux changes in response to a mechanical membrane perturbation (Falke *et al.*, 1988; Cosgrove & Hedrich, 1991; Ding & Pickard, 1993; Garrill *et al.*, 1994; Kikuyama & Tazawa, 2001).

An alternative possibility is that proteins that link the extracellular matrix to the plasma membrane and/or cytoskeleton may act as mechano-receptors similarly to how integrins function in animal cells (Wayne *et al.*, 1992; Ingber, 1998; Jaffe *et al.*, 2002; Hayashi & Takagi, 2003). It is also possible that channel activities and tethered transmembrane proteins collaborate to sense cellular mechanical strains and trigger ion fluctuations as intracellular second messengers in plants, as proposed for animal cells (Mano & Driscoll, 1999; Mobasher *et al.*, 2002).

### 2. Involvement of hormones and cellular signaling in thigmomorphogenesis

A number of inter- and intracellular signaling components, including hormones and potential second messengers, have been implicated in touch-induced alterations in plant morphogenesis. At this point, evidence strongly implicates these potential signals as being responses to mechanical stimulation and thus may trigger physiological responses. However, the primary signal controlling the full suite of plant mechano-responses, if such a signal exists, has not yet been identified.

Intracellular calcium ( $\text{Ca}^{2+}$ ) has long been implicated as an important second messenger in mechano-signaling and response in both animal and plant cells. Very rapid cellular  $\text{Ca}^{2+}$  increases are detected in mechanically perturbed cells and tissues (Batiza *et al.*, 1996; Calaghan & White, 1999). Transgenic plants expressing the *Aequoria* jellyfish gene encoding aequorin, a  $\text{Ca}^{2+}$ -dependent luminescent protein, have proven to be valuable tools for monitoring  $\text{Ca}^{2+}$  fluctuations in response to many different kinds of stimuli (Knight, 2000). In particular, aequorin transgenic plants have demonstrated a rapid intracellular  $\text{Ca}^{2+}$  increase in response to touch or wind stimuli (Knight *et al.*, 1991) and have provided evidence that mechano-responsive  $\text{Ca}^{2+}$  increases are derived from internal stores (Knight *et al.*, 1992). The finding that mechanical stimuli, thought to cause organellar membrane stretching, also can evoke organellar  $\text{Ca}^{2+}$  release in *Nitella flexilis* cytoplasmic extracts (Kikuyama & Tazawa, 2001) suggests that mechano-perturbations can be directly sensed by subcellular membrane-bound compartments.

Reactive oxygen species (ROS) are also important signals in plant morphogenesis and responses to stimuli (Mori & Schroeder, 2004). ROS have been detected immediately after a mechanical perturbation (Legendre *et al.*, 1993; Yahraus *et al.*, 1995; Legue *et al.*, 1997; Gus-Mayer *et al.*, 1998). Coincidence of ROS and Ca<sup>2+</sup> changes in many plant behaviors and the evidence for Ca<sup>2+</sup> channel regulation by ROS suggests that these two cellular signals may be interdependently generated and functionally linked. Generation of ROS may be responsible for regulating Ca<sup>2+</sup> channel gating (Mori & Schroeder, 2004).

For many years, ethylene has been proposed to function in aspects of thigmomorphogenesis. Exogenous application of ethylene can result in morphological and physiological changes that resemble aspects of thigmomorphogenesis (Goeschl *et al.*, 1966; Brown & Leopold, 1972; Jaffe & Biro, 1979; Erner & Jaffe, 1983; de Jaegher *et al.*, 1987; Telewski, 1995). Ethylene production occurs after mechanical stimulation of plants (Goeschl *et al.*, 1966; Biro & Jaffe, 1984; Takahashi & Jaffe, 1984). Mutant and some inhibitor studies, however, suggest that although ethylene may have a role in radial expansion aspects of thigmomorphogenesis, ethylene production and/or response are not required for the reduction in elongation growth induced by mechanical perturbation (Boyer *et al.*, 1983; Biro & Jaffe, 1984; Biddington, 1986; Boyer *et al.*, 1986; Johnson *et al.*, 1998). These results and similar studies on tendril coiling (Weiler *et al.*, 1993) are consistent with the idea that ethylene production may be one of the responses to mechanical perturbations, but ethylene is unlikely to be the primary signaling molecule that controls all touch responses.

Octadecanoids have been shown to effect *B. dioica* tendril coiling (Weiler *et al.*, 1993; Weiler *et al.*, 1994) and therefore may have additional touch-response roles. Intriguingly, mechanical stimulation results in an increase in OPDA levels in *P. vulgaris* internodes, and treatment with the OPDA analog, coronatine, can elicit physiological changes reminiscent of thigmomorphogenesis (Stelmach *et al.*, 1998).

## V. Discovery of the *Arabidopsis* TCH genes

Control experiments serendipitously revealed the existence of touch-inducible genes in plants (Braam & Davis, 1990). These genes, originally called the TCH genes and isolated by differential cDNA library screening, are strongly and rapidly up-regulated in expression in response to touch (Braam & Davis, 1990). The TCH genes were identified by their dramatic mRNA accumulation in plants sprayed with gibberellins. However, further northern analyses revealed that TCH expression increased in plants sprayed with a variety of hormones, for example, auxin, cytokinin and abscisic acid, and indeed by a spray of simply water (Braam & Davis, 1990). Finally, a simple mechanical stimulation, administered by touching the rosette leaves and bending them down or

back and forth, was found to be sufficient to elicit a dramatic enhancement of TCH expression (Braam & Davis, 1990). The differential cDNA screening technique tends to identify preferentially genes with high expression levels and indeed the handful of TCH genes proved to be only the tip of the iceberg. Over the past 14 years, many other genes have been discovered, often unintentionally, to have mechano-stimulus-inducible expression (Ling *et al.*, 1991; Perera & Zielinski, 1992; Gawienowski *et al.*, 1993; Botella & Arteca, 1994; Botella *et al.*, 1996; Mizoguchi *et al.*, 1996; Oh *et al.*, 1996; Royo *et al.*, 1996; Shirsat *et al.*, 1996; Eldick *et al.*, 1997; Mauch *et al.*, 1997; Gilmour *et al.*, 1998; Arteca & Arteca, 1999; Gadea *et al.*, 1999; Hirsinger *et al.*, 1999; Tatsuki & Mori, 1999; Müssig *et al.*, 2000; Oufattole *et al.*, 2000; Lee *et al.*, 2005).

## VI. Microarray identification of touch-inducible genes

To gain insight into the prevalence of touch-inducible gene expression, a genome-wide search for *Arabidopsis* genes sharing TCH regulatory properties was undertaken (Lee *et al.*, 2005). Hybridization of the 22 810 genes represented on the Affymetrix DNA chip (Affymetrix, Santa Clara, CA, USA) revealed that 589 genes, over 2.5% of the genome, are up-regulated at least twofold in expression within 30 min of a touch stimulation (Lee *et al.*, 2005). One hundred and seventy-one genes have reduced expression (Lee *et al.*, 2005).

TCH1 encodes one of the *Arabidopsis* calmodulins, CAM2, TCH2 and TCH3 encode calmodulin-like (CML) proteins, CML24 and CML12, respectively, and TCH4 encodes a xyloglucan endotransglucosylase/hydrolase (XTH22) (Braam & Davis, 1990; Sistrunk *et al.*, 1994; Xu *et al.*, 1995; Khan *et al.*, 1997; Rose *et al.*, 2002; McCormack & Braam, 2003). Thus, it is not unexpected that Ca<sup>2+</sup>-binding proteins and cell wall modifying enzymes would be among the functions found by microarray analyses to be potentially up-regulated by touch. Indeed, of the 48 CML and 33 XTH genes included on the chip, 19 CMLs and 12 XTHs show greater than twofold up-regulated expression in touched plants (Lee *et al.*, 2005). Genes encoding other putative Ca<sup>2+</sup>-binding proteins, arabinogalactan proteins, pectin esterases, cellulose synthases, expansins and extensins also have touch-inducible expression (Lee *et al.*, 2005). Interestingly, despite the high sequence identity among the seven CAM genes (McCormack & Braam, 2003), only one CAM gene, CAM2/TCH1, was identified by microarray analysis to be touch-inducible (Lee *et al.*, 2005).

Less expected, perhaps, is the finding that genes implicated in disease resistance compose the third most represented functional class of touch-inducible genes. Further investigation into a potential relationship between mechanical perturbation responses and plant disease resistance is necessary. Expression levels of over 10% of the genes encoding kinases and transcription factors are increased in touch-stimulated plants (Lee

*et al.*, 2005), indicating that many kinase transduction pathways and additional transcriptional activities may be impacted by a simple touch perturbation.

## VII. Regulation of *TCH* gene expression

The signaling pathways and transcriptional mechanisms controlling touch-regulated gene expression are not well understood. Intracellular  $\text{Ca}^{2+}$  fluctuations and protein phosphorylation may play roles in touch-regulated gene expression (Braam, 1992; Wright *et al.*, 2002). A functional ethylene response pathway, however, is not required for *TCH* regulation (Johnson *et al.*, 1998).

Intriguingly, expression of the original *TCH* genes is inducible not only by stimuli that appear to share mechanical properties, such as touch and wounding, but also by darkness, temperature extremes and some growth promoting hormones (Braam & Davis, 1990; Braam, 1992; Sistrunk *et al.*, 1994; Antosiewicz *et al.*, 1995; Xu *et al.*, 1995; Polisensky & Braam, 1996). This latter group of stimuli might be predicted to be unrelated in properties, and thus in perception mechanisms, to touch. However, microarray data indicate that the seemingly distinctive stimuli of touch and darkness evoke transcript abundance changes that are highly similar. Indeed, over half of the touch-inducible genes identified by microarray analysis resemble the *TCH* genes in that they are also up-regulated by darkness (Lee *et al.*, 2005). Of the 60 genes found to be up-regulated more than tenfold by touch, 56 are also up-regulated in darkness-treated plants (Lee *et al.*, 2005). These findings are consistent with the hypothesis that the diverse stimuli capable of inducing *TCH* expression may share the common property of being capable of causing mechanical perturbations, perhaps at a cellular level, and thus indirectly inducing *TCH* expression (Braam, 2000).

How might the stimuli known to up-regulate gene expression indirectly result in mechanical perturbations? Darkness-induced stomatal aperture regulation and humidity changes could result in local cellular turgor changes (Braam, 2000). Alternatively, darkness and the growth-promoting hormones, IAA and epi-brassinolide that increase expression of at least a subset of the *TCH* genes (Antosiewicz *et al.*, 1995; Xu *et al.*, 1995) may lead to enhanced growth through cellular expansion. Transient cellular turgor alterations may occur as walls are loosened during cell expansion (Braam, 2000). As individual cells embedded in tissues undergo expansion, forces can be transmitted to neighboring cells, thus propagating cell-to-cell mechanical stimuli (Ding & Pickard, 1993). Sudden temperature shifts may directly affect membrane properties and transmembrane transport activities, and thus trigger cellular turgor alterations. These potential turgor variations may be sensed at the wall, membrane or even intracellular organelles and may lead to activation of intracellular mechano-transduction pathways that regulate *TCH* expression. Consistent with the idea that there is commonality between touch and darkness,

Stankovic and colleagues found that a shift to darkness results in membrane depolarizations similar to that evoked by wounding (Stankovic *et al.*, 1998).

The idea that the diverse stimuli that regulate *TCH* expression may share perception and/or signaling mechanisms is consistent with the finding that a regulatory region consisting of 102 basepairs from 5' untranscribed *TCH4* sequences is sufficient to confer touch, dark, cold, heat and epi-brassinolide regulation (Iliev *et al.*, 2002). A related sequence from the cold- and touch-inducible *CBF2* gene has also been shown to have mechano-responsive regulatory activity (Zarka *et al.*, 2003). These initial studies aimed at defining touch-inducible regulatory elements will be aided by the recent identification of hundreds of additional *Arabidopsis* genes that share this regulatory behavior (Lee *et al.*, 2005). Such elements will be useful tools in employing genetic approaches to investigating plant responses to mechanical perturbation. For example, we are screening for mutants defective in appropriate regulation of a luciferase gene driven by the defined *TCH4* regulatory region in an effort to define functions and identify genes required for perception and response to touch, heat and other environmental stimuli.

The *TCH* genes also show developmental expression patterns. Two possible correlations can be made. *TCH* expression is often seen at sites of potential mechanical strain and/or increased growth. For example, *TCH::reporter* transgenes are expressed and *TCH* proteins accumulate at branching points, the root-shoot junction, elongating hypocotyls and roots, and developing trichomes (Sistrunk *et al.*, 1994; Antosiewicz *et al.*, 1995, 1997; Xu *et al.*, 1995). Furthermore, *TCH2* and *TCH4* expression levels are increased in inflorescence stems when increased weight applied at the apex results in enhanced secondary growth (Ko *et al.*, 2004). Thus, *TCH* regulation occurs not only in response to externally applied stimuli, but also may be induced by mechanical strains that might be generated through morphogenesis (Xu *et al.*, 1995; Campbell & Braam, 1999; Braam, 2000). The developmental expression data are consistent with the idea that transient turgor changes at the cellular level may be sufficient to trigger *TCH* regulation.

Finally, because plant cells maintain turgor and maintenance of turgor is highly regulated, it is likely that plants deal constantly with changes in mechanical stress at the cell surface, both in response to stimuli (like water deficit) and throughout morphogenesis, for example, during the process of cell expansion. Furthermore, it is possible that the machinery and mechanisms used by plants to perceive and respond to external environmental stress may be related to processes used at the cellular level to control fundamental events such as cell expansion. That is, the mechanical force of turgor pressure may be used as a regulatory event to control cellular enlargement and growth. How mechano-perception and response mechanisms of nonspecialized plants may be related to those enabling carnivory, climbing, active pollination and root-avoidance behaviors is unknown. Thus, understanding the mechanisms

of perception and response are likely fundamental to understanding plant biology.

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