Chapter 1

Introduction to Plant Responses to Touch

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1.1. ABSTRACT

Perception of touch makes plant more sensitive to changes in the environment. Touch induced responses involve common players like calcium sensors, reactive oxygen species, nitric oxide, phytohormones and induce various morphological, biochemical and physiological changes. Touch induced cellular adaptations provides cross protection against biotic and abiotic stress. This chapter provides update of overall progress in research to understanding mechanical stress induced changes in plant.

1.2. TOUCH RESPONSE IN PLANT

Animals have sensory organs to perceive fluctuations in their environment and under situation of threat, animals escape away from the threat using their mobile ability. Unlike animals, plants lack mobility and live sedentary life. Thus, they have evolved different alternate mechanisms to perceive changes in their environment. In nature, plants experience mechanical stress in many different forms. Biotic stress such as landing of insects, herbivory by pest or animal is sensed as mechanical stress. For example landing of adult moths on the leaf of tomato plant alters cellular gene expression in its trichomes (Peiffer *et al* 2009). Abiotic stress such as wind, rainfall, hailstorm exert mechanical stress on plants. At cellular level, the mechanical stress exerted on cell-wall due to turgor pressure, wound and damage to cell wall layers can also be sensed by plant cell as mechanical stress (Heil *et al* 2009). Recent studies indicates that plants can sense the touching of leaves and stems of plants in their vicinity (Markovic *et al* 2019). For all the above stimuli, plant immediately respond by changing the biochemical status and transcriptional profile of the cell. Interestingly, mechanical perturbations induce expression of many molecules which are also known to enhance plant immunity and tolerance to biotic and abiotic stress (Chehab *et al* 2012).

Since 1880s, mechanical stress in the form of touch has been studied in plants. Charles Darwin had published his research about touch induced responses in insectivorous plants (Darwin C. 1880). During late 80s and early 90s extensive research was carried out on understanding the mechanism by which plants sense touch in the ‘touch-me-not plant’ - *Mimosa pudica* and carnivorous plants. In late 90s, the research was more diversified and aimed to understand touch induced cellular responses. This gave an insight to the molecules that are involved in touch induced responses (Braam & Davis 1990; Botella *et al* 1994; Botella *et al* 1996; Depege *et al* 1997). Mechanical stress induced morphological changes has been studied in many other plants namely Arabidopsis, *Cucurbita*, *Pisum*, wheat, pine, beans, lettuce, cauliflower, *Populus*, celery, *Carica papaya, Zea maize, Acacia koa* and *Nicotiana sp.* (Jaffe 1973, Biddington and Dearman 1985, Pruyn *et al* 2000, Porter *et al* 2009). In this chapter we have reviewed various systemic and cellular responses studied in plant system in response to mechanical stimuli.

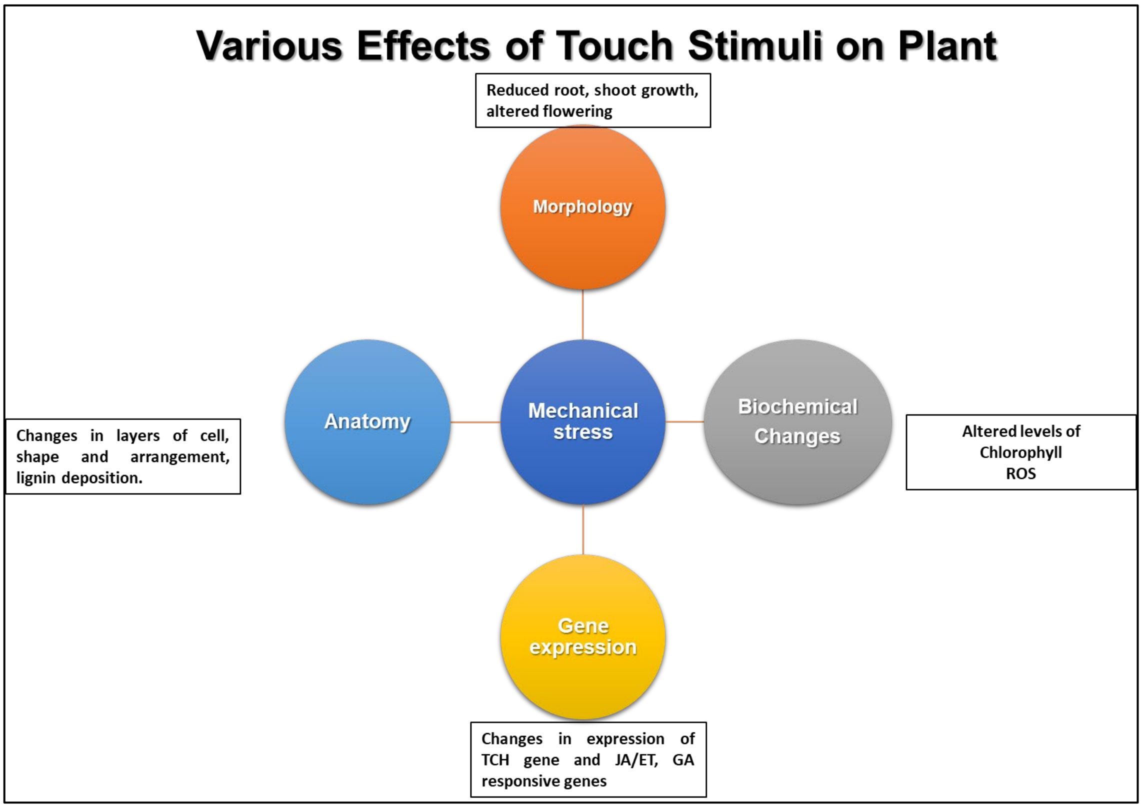

Figure 1.1: *The response to mechanical stress varies from plant species to species but the figure here represents few observations that are most common in most species.*

1.3. RECEPTORS FOR TOUCH:

Epidermal hairy structure, trichomes are sensitive to touch of insects and herbivores (Wagner 1991; Goertzen and Small 1993; Shepherd and Wagner 2007). Trichomes are present on the surface of aerial parts of plant like stem and leaves (Graham *et al* 1985; Peiffer *et al* 2009). Plant raises an early defense response just upon sensing touch of moth by increasing the number of glandular trichomes and enhancing the expression of proteinase inhibitor-2 (PIN2) which would prevent further attack by the moth or its larvae that may be released later from its eggs. Touch in absence of wound is sensed by trichomes and an anti-herbivore response is induced. Another example of plant sensors to touch is the touch sensitive hair-like leafy structures present on the surface of Venus-fly which helps them in catching their prey (Forterre *et al* 2005). The molecular receptor for touch, a mechanosensor at cellular level, is yet to be discovered.

1.4. THIGMOMORPHOGENESIS:

Regular touch treatment causes several morphological, physiological, biochemical and anatomical adaptations. Morphological changes observed in response to regular touch is termed as thigmomorphogenesis (Jaffe 1973). Mechanical stress leads to common morphological changes like suppressed shoot and root growth, shorter internode length and altered flowering period etc. (Braam 2005, Chehab 2009). Studies in Arabidopsis has shown that regular touch delays inflorescence development and further reduced the rosette diameter of leaves (Chehab *et al* 2012). The plant response after touch stimuli varies from species to species. Regularly touched papaya plants exhibited higher lignin deposition and reduced anthocyanin production in the petiole (Porter *et al* 2009). In tobacco, touching of stem increased vegetation (Anten 2005). Anatomical studies indicate increased compactness of cells, reduced cell size and enhanced lignin deposition in their petiole (Porter *et al* 2009, Meng *et al* 2006). These anatomical adaptations increase the mechanical strength of the plant and help the plant to withstand prolonged mechanical stress. Biochemical studies show that the chlorophyll ratio, plant hormones profile, calcium levels, and levels of the reactive oxygen species is altered upon mechanical stress (Biddington 1986, Allen *et al* 1999, Slesak 2008). These anatomical, biochemical and morphological adaptations aid the plant to withstand and cope with the constant mechanical stress.

1.5. INTRACELLULAR SIGNALLING MOLECULES

1.5.1. Reactive oxygen species (ROS)

Reactive oxygen species (ROS) and nitric oxide (NO) are elevated in response to biotic and abiotic stress conditions (Leshem 1996; Gus-Mayer *et al* 1998; Durner and Klessig *et al* 1999; Van Breusegem *et al* 2001). ROS accumulates within few seconds of mechanical stimuli which may also be involved in regulation of early gene expression (Chehab *et al* 2009, Van breusegem 2001). It has been shown that mechanical stress or touch in tomato and soybean results in increase in levels of ROS (Yahraus *et al* 1995; Depege *et al* 2000). Herbivore attack on tomato leaves results in increase in levels of H_2O_2 which later induces expression of anti-herbivory protein, PIN2 in the trichomes (Peiffer *et al* 2009). Mechanical stress induced ROS can serve multiple functions. Elevated ROS promotes strengthening of plant cell wall and it is also involved in direct killing of pathogens. ROS also functions as signalling molecule which can indirectly alter gene expression profile of plant cell (Heath 2000; Lam *et al* 2001). Touch stimulated ROS production can lead to activation of calcium channels in plants triggering calcium mediated intracellular signalling (Mori *et al* 2004).

1.5.2. Calcium:

Under normal conditions, the cytoplasmic calcium levels are maintained low by sequestering the ions inside cellular organelles like endoplasmic reticulum (ER). In response to external stimuli, the sequestered calcium ions from ER or extracellular spaces are released out in the cytoplasm triggering a chain of cellular signal transduction. Calcium reflux is well studied in associated with induction of plant immune response during plant pathogen interaction (Blume *et al* 2000; Grant *et al* 2000). Mechanical stimuli to Arabidopsis roots caused an increase in levels of intracellular calcium (Allen *et al.*1999). Transgenic plant, a calcium dependent bioluminescent protein, aequorin shows rapid and transient increase in the intracellular calcium levels upon mechanical stimulation (Knight *et al.*, 1992). Mechanical stimuli can activate plasma membrane stretch induced calcium channels in plant (Hayashi *et al.*, 2006). Moreover, in most living organism including animals, mechanical stimuli like touch and wound cause changes in cytoplasmic calcium (Cosgrove and Hedrich 1991; Batiza *et al* 1996; Calaghan and White 1999). As many different stimuli induce increases in intracellular Ca2+ concentration, the plant may distinguish the signals in terms of frequency, duration, amplitude, and spatial distribution of the signal and then interpret into different stimuli specific responses (Webb *et al.* 1996). Ca^{2+} signaling are generally mediated by calcium sensors; calmodulin (CaM) and calmodulin like-molecules (CML). The touch induced CaM and CML are termed as TCH genes (Braam and Davis 1990)

1.5.3. Touch responsive gene (TCH)

Expression of touch inducible genes (*TCH*) were first reported in 1990 (Braam and Davis 1990). Transcriptional profiling in Arabidopsis shows that expression of many CaM and CML molecules is induced within 30 min of touch stimulation (Braam and Davis 1990; Lee *et al* 2005). These involve calmodulin proteins, TCH1 (CaM2) and CML proteins like TCH2 and TCH3 respectively. In Arabidopsis, only TCH1 was found to be up-regulated out of seven CaM molecules found in the genome of this plant (Lee *et al* 2005). Likewise, 20 CML genes were differentially expressed out of 48 CML encoded in the Arabidopsis genome. Similarly, in *Acacia koa*, mechanical stimuli induced expression of the genes for calcium signalling within 10-60 min after mechanical stress (Ishihara *et al* 2017). This signifies the importance of Calcium signalling in the mechanical stress mediated cellular signalling. Studies done in Arabidopsis touch insensitive (ths1) mutant, indicates requirement of a gene *VERNALIZATION INDEPENDENCE 3 (VIP3)* which with other proteins cause chromatin remodelling (histone H3K36 trimethylation) at the *AtTCH3* and *AtTCH4* loci for fast touch-induced transcription of these two genes (Jensen *et al* 2017). *TCH4* gene also known as *XTH22* (xyloglucan endotransglucosylase/ hydrolase) is involved in cell wall modifications in Arabidopsis and is required for thigmomorphogenesis (Xu *et al*., 1995; Purugganan *et al*., 1997). This indicates that *TCH* genes may act as key upstream molecules in touch mediated cellular signalling.

1.5.4. Plant hormones

Various phytohormones like jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), auxin, brassinosteroids and gibberellic acid (GA) are reported to be involved in touch induced plant response. Each of these plant hormones are involved in regulation of different developmental processes and stress responses.

Jasmonic acid (JA) and its derivatives are involved in regulation of many different biotic and abiotic stress responses (Bennett and Wallsgrove 1994; McConn *et al* 1997). The levels of JA and JA responsive genes are found to be elevated upon touch stimuli (Koo and Howe 2009). The expression of Lipoxygenase, a JA biosynthetic enzyme is also found to be up regulated upon touch stimulation (Mauch *et al* 1997; Lee *et al* 2005). Mechanical stress studies performed on Arabidopsis JA mutants confirm that the touch induced thigmomorphogenesis is JA dependent and involves JA-Isoleucine (JA-Ile) mediated signalling. It appears that plant triggers JA response over touch stimulation and gets ready for subsequent herbivore attack. Morphological changes observed after mechanical stimulations are similar to those observed after ethylene treatment in tomato (Jaffe and Biro,

Table 1.1: List of genes studied in detail in touch induced response.

Genes	**Function**	**Reference**
LOX (lipoxygenase)	JA biosynthesis	Mauch *et al*., 1997; Lee *et al*., 2005
1-aminocyclopropane-1-carboxylate synthase (ACS)	ET biosynthesis	Biro and Jaffe, 1984; Botella *et al*., 1995; Arteca and Arteca, 1999
Pinoid (PID)	serine/threonine protein kinase	Benjamins *et al*., 2003
TCH1 (CaM2)	Calcium binding protein	Braam and Davis, 1990; Lee *et al*., 2005
TCH2 (CML24)	Calcium binding protein	Braam and Davis, 1990;
TCH4 /xyloglucan endotransglucosylase/ hydrolase (XTH)	Cell wall modification	Xu *et al*., 1995; Purugganan *et al*., 1997
Touch-Regulated Phosphoprotein1 (TREPH1)	Chromatin modification	Wang *et al* 2018
ZmCPK11	Calcium dependent protein kinase	Szczegielniak *et al* 2012

1979). Ethylene production was quantitated in beans and pine plants (Jaffe and Biro,1979; Telewski and Jaffe, 1986). However, studies indicate that ethylene could be involved quite later in the signalling event as ethylene mutant plants showed touch induced morphological changes. Cell size growth and elongation is regulated by GA hormone. The touch induced morphological changes are associated with reduced levels of GA. Recent studies indicate that the touch induced suppression of growth is due to expression of a gene, AtGA2ox7, which codes for an enzyme involved in gibberellin breakdown in Arabidopsis (Lange and Lange 2015). Treatment of plants with BR induces expression of TCH4 genes in Arabidopsis (Xu *et al.*, 1995; Iliev *et al.*,2002). Similarly, indirect evidences exist for involvement of auxin. PIN2 is a common molecule expressed after touch stimuli and auxin treatment (Chehab *et al* 2009).

1.5.5. Other signalling molecules:

With increasing interest in the research for understanding thimomorphogenesis, more and more molecules are being added to the list of touch induced molecules. In Arabidopsis, phosphoproteomic analysis after touch treatment shows 24 differentially phosphorylated polypeptides. Among these, TOUCH-REGULATED PHOSPHOPROTEIN1 (TREPH1) is rapidly phosphorylated after touch treatment and it is required for touch induced delay in flowering (bolting) in Arabidopsis (Wang *et al* 2018). Plant-plant communication occurs through release and perception of volatile organic compound. Touch experiments in maize plants shows that within minutes after touch the plant emits specific volatile compounds that primes neighbouring plants against herbivory (Markovic *et al* 2019).

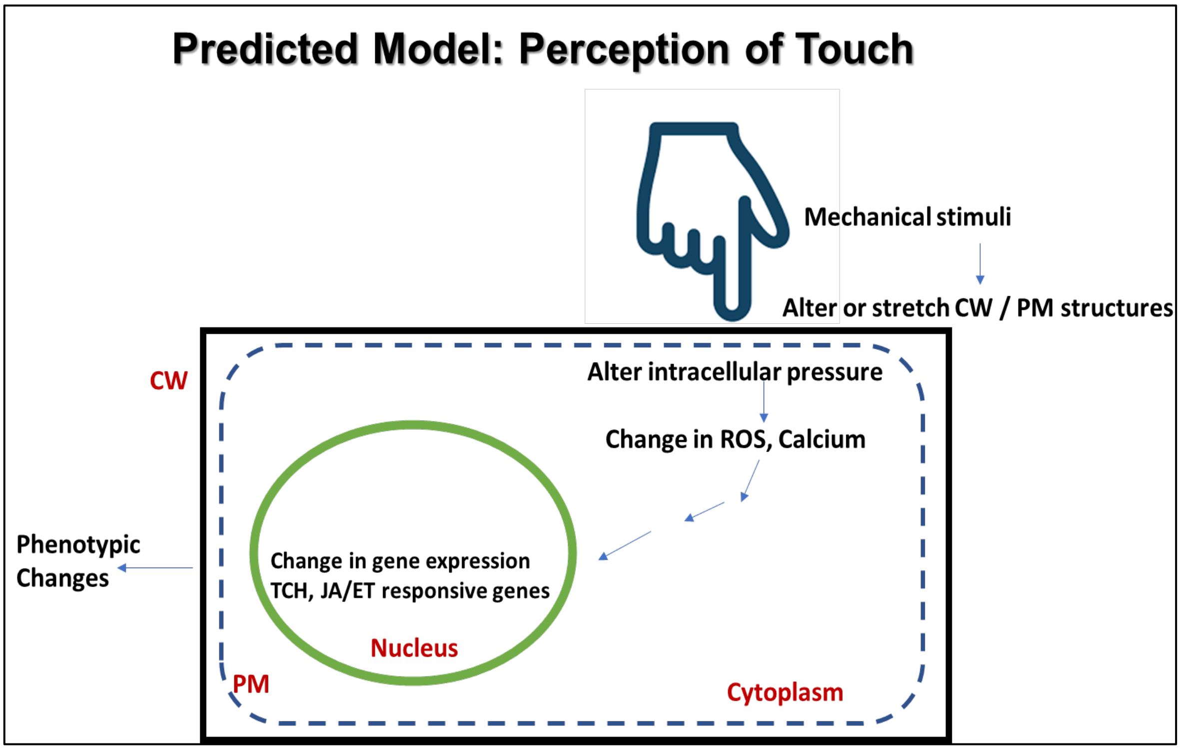

Figure 1.2: Model for Plant perception of touch:

Key words: CW: cell wall; PM: plasma membrane

1.6. HOW DOES PLANT CELL PERCEIVE TOUCH?

There are many hypotheses proposed for plant perception of touch. External pressure may change internal cytosolic pressure which is perceived by the cell (Morris and Homann, 2001). Another hypothesis suggests that external pressure may alter the association between cell wall and plasma membrane structure leading to opening of ion channels (Jaffe *et al* 2002). Research provides evidence that stretching of plasma membrane is recognised by membrane associated receptors. It is proposed that similar receptors may be involved in recognition of mechanical stress (Edwards and Pickard, 1987; Ding and Pickard, 1993; Ward and Schroeder, 1994). In figure 1.2 we have summarised the hypothesis. Perception of touch leads to calcium influx/ ROS and cascade of signalling events which ultimately leads to change in gene expression. This change leads to physiological modifications which helps in adaptation to mechanical stress.

1.7. Application of mechanical stress for tolerance to stress

Current knowledge of morphological adaptations and gene expression analysis is being applied in agriculture and pest management. Mechanical stress induced morphological changes has been studied in many crop plants namely *Cucurbita*, *Pisum*, wheat, lettuce, cauliflower, *Populus*, celery, *Carica papaya* and *Nicotiana* (Jaffe 1973, Biddington and Dearman 1985, Pruyn *et al* 2000, Porter *et al* 2009). Thigmomorphogenesis is being studied in economically important plants with an aim to identify novel traits. For example, ornamental plants are maintained short for aesthetic value by giving regular mechanical stimuli (Börnke, F., & Rocksch, T. 2018). Regularly touched papaya plants exhibited higher lignin deposition and reduced anthocyanin production in the petiole (Porter *et al* 2009). In tobacco, touching of stem increased vegetation (Anten 2005). As mechanical stress induces expression of defense response genes, touch stimuli is also being studied for development of environment friendly techniques for pest/ disease control in plants (Catherine Coutand, 2020). Regular touching enhanced resistance against necrotrophic fungi, *Botrytis cineria* in Arabidopsis and reduced feeding by cabbage looper pest, *Trichoplusia ni* on lettuce crop (Chehab *et al* 2012).

1.8. SCOPE OF PRESENT STUDY

Interestingly, different plant species display different adaptations in response to mechanical stimuli, some of which are unique to particular plant species. In present study, we have made first attempt to identify morphological, anatomical, biochemical and physiological adaptations of a dicotyledon crop plant *Cajanus cajan* (Pigeon pea) and a monocotyledon crop plant *Oryza sativa* (rice) to regular mechanical stress in the form of touch. The touch induced phenotypes identified in present study can be used as marker for mechanical stress for future studies in both the crop species. This study lays foundation for our future field level research on effect of mechanical stress on productivity and yield of the crop.

1.9. REFERENCES

1. Allen, G. J., Kwak, J. M., Chu, S. P., Llopis, J., Tsien, R. Y., Harper, J. F., & Schroeder, J. I. (1999). Cameleon calcium indicator reports cytoplasmic calcium dynamics in Arabidopsis guard cells. The Plant Journal, 19(6), 735-747.
2. Anten, Niels PR, Raquel Casado-Garcia, and Hisae Nagashima. "Effects of mechanical stress and plant density on mechanical characteristics, growth, and lifetime reproduction of tobacco plants." The American Naturalist 166, no. 6 (2005): 650-660.
3. Arteca JM, Arteca RN. 1999. A multi-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase (ACS6) in mature Arabidopsis leaves. Plant Molecular Biology 39, 209–219
4. Batiza A F, Schulz T and Masson P H 1996 Yeast respond to hypotonic shock with a calcium pulse; Journal of Biological Chemistry 271(38) 23357-23362

5. Benjamins R, Ampudia CS, Hooykaas PJ, Offringa R. 2003.PINOID-mediated signaling involves calcium-binding proteins. Plant Physiology 132, 1623–1630.
6. Bennett R N and Wallsgrove R M 1994 Secondary metabolites in plant defense mechanisms; New Phytologist 127(4) 617-633
7. Biddington, N. L. (1986). The effects of mechanically-induced stress in plants—a review. Plant growth regulation, 4(2), 103-123.
8. Biddington, N. L., and A. S. Dearman. "The effect of mechanically induced stress on the growth of cauliflower, lettuce and celery seedlings." Annals of Botany 55, no. 1 (1985): 109-119.
9. Blume B, Nürnberger T, Nass N, Scheel D 2000 Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley; Plant Cell 12 1425-40
10. Botella J R, Arteca J M, Somodevilla M, Arteca R N 1996 Calcium-dependent protein kinase gene expression in response to physical and chemical stimuli in mungbean (Vigna radiata); Plant Molecular Biology. 30 1129–1137
11. Botella J R, Arteca R N 1994 Differential expression of two calmodulin genes in response to physical and chemical stimuli; Plant Molecular Biology 24 757–766
12. Braam J, Davis R W 1990 Rain-, wind- and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis; Cell 60 357–364
13. Biro R, Jaffe MJ. 1984. Thigmomorphogensis: ethylene evolution and its role in the changes observed in mechanically perturbed bean plants. Physiologia Plantarum 62, 289–296
14. Calaghan S C and White E 1999 The role of calcium in the response of cardiac muscle to stretch; Progress in biophysics and molecular biology 71(1) 59-90

15. Chehab E W, Kaspi R, Savchenko T, Rowe H, Negre-Zakharov F, Kliebenstein D, and Dehesh K 2008 Distinct roles of jasmonates and aldehydes in plant-defense responses; PLoS One 3(4) e1904
16. Chehab E W, Kim S, Savchenko T, Kliebenstein D, Dehesh K, and Braam J 2011 Intronic T-DNA insertion renders Arabidopsis opr3 a conditional jasmonic acid-producing mutant; Plant Physiol. 156 770–778
17. Chehab E W, Yao C, Henderson Z, Kim S and Braam J 2012 Report Arabidopsis Touch Induced Morphogenesis Is Jasmonate Mediated and Protects against Pests; Current Biology 22(8) 701–706
18. Cosgrove D J and Hedrich R 1991 Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of Vicia faba L.; Planta 186(1) 143-153
19. Cunha C, Carvalho A, Esposito A, Bistoni F, Romani L 2012 DAMP signaling in fungal infections and diseases; Front. Immunol. 3 286
20. Dangl J. 1998 Innate immunity. Plants just say NO to pathogens; Nature 394 525–527
21. Darwin C 1880 The Power of Movement in Plants; London: William Clowes and Sons, Ltd.
22. Depege N, Thonat C, Coutand C, Julien J-L, Boyer N 1997 Morphological responses and molecular modifications in tomato plants after mechanical stimulation; Plant Cell Physiology 38 1127–1134
23. Depege N, Varenne M and Boyer N 2000 Induction of oxidative stress and GPX-like protein activation in tomato plants after mechanical stimulation; Physiologia Plantarum 110 209–214

24. Du L, Ali G S, Simons K A, Hou J, Yang T, Reddy A. S. N and Poovaiah B W 2009 Ca2+/calmodulin regulates salicylic-acid-mediated plant immunity; Nature 457(7233) 1154-1158

25. Durner J and Klessig D F 1999 Nitric oxide as a signal in plants; Current Opinion in Plant Biology 2 369–374

26. Ferrari S, Savatin D V, Sicilia F, Gramegna G, Cervone, F, and Lorenzo G D 2013 Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development; Front. Plant Sci 4 Article 49

27. Forterre Y, Skotheim J M, Dumais J and Mahadevan L 2005 How the Venus flytrap snaps; Nature 433(7024) 421-425

28. Garces H, Durzan D and Pedroso M C 2001 Mechanical stress elicits nitric oxide formation and DNA fragmentation in Arabidopsis thaliana; Annals of Botany 87 567–574

29. Goertzen L R and Small E 1993 The defensive role of trichomes in black medick (Medicago lupulina, Fabaceae); Plant Systematics and Evolution 184 101–111

30. Graham J S, Pearce G, Merryweather J, Titani K, Ericsson L H and Ryan C A 1985 Wound-induced proteinase inhibitors from tomato leaves. II. The cDNA-deduced primary structure of pre-inhibitor II; Journal of Biological Chemistry 260 6561–6564

31. Grant M, Brown I, Adams S, Knight M, Ainslie A and Mansfield J 2000 The RPM1 plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death; Plant Journal 23 441–450

32. Gus-Mayer S, Nation B, Hahlbrock K, Schmelzer E 1998 Local mechanical stimulation induces components of the pathogen defense response in parsley; Proceedings of the National Academy of Sciences, USA 95 8398–8403

33. Hayashi T, Harada A, Sakai T, Takagi S. 2006. Ca2+ transient induced by extracellular changes in osmotic pressure in Arabidopsis leaves: differential involvement of cell wall-plasma membrane adhesion. Plant, Cell and Environment 29, 661–672.

34. Heath M C 2000 Nonhost resistance and nonspecific plant defenses; Current opinion in plant biology 3(4) 315-319

35. Heil, Martin. "Damaged-self recognition in plant herbivore defence." Trends in plant science 14, no. 7 (2009): 356-363.

36. Heo W D, Lee S H, Kim M C, Kim J C, Chung W S, Chun H J, Lee K J, Park C Y, Park H C, Choi J Y and Cho M J 1999 Involvement of specific calmodulin isoforms in salicylic acid-independent activation of plant disease resistance responses; Proceedings of the National Academy of Sciences, USA 96 766–771

37. Iliev EA, Xu W, Polisensky DH, Oh MH, Torisky RS, Clouse SD, Braam J. 2002. Transcriptional and post-transcriptional regulation of Arabidopsis TCH4 expression by diverse stimuli. Roles of cis regions and brassinosteroids. Plant Physiology 130, 770–783.

38. Ishihara, Kazue L., Eric KW Lee, and Dulal Borthakur. "Thigmomorphogenesis: changes in morphology, biochemistry, and levels of transcription in response to mechanical stress in Acacia koa." Canadian Journal of Forest Research 47, no. 5 (2017): 583-593.

39. Jaffe MJ. 1973. Thigmomorphogenesis: the response of plant growth and development to mechanical stimulation. Planta 114, 143–157

40. Jensen, Gregory S., Kateryna Fal, Olivier Hamant, and Elizabeth S. Haswell. "The RNA polymerase-associated factor 1 complex is required for plant touch responses." Journal of experimental botany 68, no. 3 (2017): 499-511.
41. Knight MR, Campbell AK, Smith SM, Trewavas AJ. 1991. Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. Nature 352, 524–526
42. Koo A J and Howe G A 2009 The wound hormone jasmonate; Phytochemistry 70(13) 1571-1580
43. Lange, Maria João Pimenta, and Theo Lange. "Touch-induced changes in Arabidopsis morphology dependent on gibberellin breakdown." Nature Plants 1, no. 3 (2015): 1-5.
44. Lam E, Kato N, and Lawton M 2001 Programmed cell death, mitochondria and the plant hypersensitive response; Nature 411(6839) 848-853
45. Lecourieux D, Ranjeva R and Pugin A 2006 Calcium in plant defence-signalling pathways; New Phytologist 171(2) 249-269
46. Lee D, Polisensky D H and Braam J 2005 Genome-wide identification of touch-and darkness-regulated Arabidopsis genes: a focus on calmodulin-like and XTH genes; New Phytologist 165(2) 429-444
47. Leshem Y Y 1996 Nitric oxide in biological systems; Plant Growth Regulation 18 155–159
48. Levine A, Pennell R I, Alvarez M E, Palmer R and Lamb C 1996 Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. Current Biology 6 427–437

49. Meng, S. X., Lieffers, V. J., Reid, D. E., Rudnicki, M., Silins, U., & Jin, M. (2006). Reducing stem bending increases the height growth of tall pines. Journal of Experimental Botany, 57(12), 3175-3182.

50. Maffei, M. E., Mithöfer A, and Boland W 2007 Before gene expression: early events in plant–insect interaction; Trends Plant Sci 12 310–316

51. Markovic, Dimitrije, Ilaria Colzi, Cosimo Taiti, Swayamjit Ray, Romain Scalone, Jared Gregory Ali, Stefano Mancuso, and Velemir Ninkovic. "Airborne signals synchronize the defenses of neighboring plants in response to touch." Journal of experimental botany 70, no. 2 (2019): 691-700.

52. Ma W, Smigel A, Tsai Y C, Braam J and Berkowitz G A 2008 Innate immunity signaling: cytosolic Ca2+ elevation is linked to downstream nitric oxide generation through the action of calmodulin or a calmodulin-like protein; Plant Physiology 148 818–820

53. Mauch F, Kmecl A, Schaffrath U, Volrath S, Gorlach J, Ward E, Ryals J and Dudler R 1997 Mechanosensitive expression of a lipoxygenase gene in wheat; Plant Physiology 114 1561–1566

54. McConn M, Creelman R A, Bell E and Mullet J E 1997 Jasmonate is essential for insect defense in Arabidopsis; Proceedings of the National Academy of Sciences 94(10) 5473-5477

55. Mori I C and Schroeder J I 2004 Reactive oxygen species activation of plant Ca^{2+} channels. A signaling mechanism in polar growth, hormone transduction, stress signaling, and hypothetically mechanotransduction; Plant Physiology 135 702–708

56. Nürnberger T, Brunner F, Kemmerling B, and Piater L 2004 Innate immunity in plants and animals: striking similarities and obvious differences; Immunol Rev. 198 249-266

57. Peiffer M, Tooker J F, Luthe D S, and Felton G W 2009 Plants on early alert: glandular trichomes as sensors for insect herbivores; The New Phytologist 184(3) 644–56
58. Porter, Brad W., Yun J. Zhu, David T. Webb, and David A. Christopher. "Novel thigmomorphogenetic responses in Carica papaya: touch decreases anthocyanin levels and stimulates petiole cork outgrowths." Annals of botany 103, no. 6 (2009): 847-858.
59. Pruyn, Michele L., Benjamin J. Ewers III, and Frank W. Telewski. "Thigmomorphogenesis: changes in the morphology and mechanical properties of two Populus hybrids in response to mechanical perturbation." Tree Physiology 20, no. 8 (2000): 535-540.
60. Purugganan MM, Braam J, Fry SC. 1997. The Arabidopsis TCH4 xyloglucan endotransglycosylase. Substrate specificity, pH optimum, and cold tolerance. Plant Physiology 115, 181–190.
61. Slesak I, Slesak H, Libik M, Miszalski Z. 2008. Antioxidant response system in the short-term post-wounding effect in Mesembryanthemum crystallinum leaves. Journal of Plant Physiology 165, 127–137
62. Shepherd R W and Wagner G J 2007 Phylloplane proteins: emerging defenses at the aerial frontline?; Trends in Plant Science 12 51–56
63. Szczegielniak, Jadwiga, Lidia Borkiewicz, Blanka Szurmak, Elżbieta Lewandowska-Gnatowska, Małgorzata Statkiewicz, Maria Klimecka, Jarosław Cieśla, and Grażyna Muszyńska. "Maize calcium-dependent protein kinase (ZmCPK11): local and systemic response to wounding, regulation by touch and components of jasmonate signaling." Physiologia Plantarum 146, no. 1 (2012): 1-14.
64. Tsai YC, Delk NA, Chowdhury NI, Braam J. 2007. Arabidopsis potential calcium sensors regulate nitric oxide levels and the transition to flowering; Plant Signal Behavior 6 446–454

65. Van Breusegem F, Vranova E, Dat J F, Inze´ D 2001 The role of active oxygen species in plant signal transduction; Plant Science 161 405–414

66. Xu W, Purugganan MM, Polisensky DH, Antosiewicz DM, Fry SC, Braam J. 1995. Arabidopsis TCH4, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. The Plant Cell 7, 1555–1567.

67. Yahraus T, Chandra S, Legendre L, Low P S 1995 Evidence for a mechanically induced oxidative burst; *Plant Physiology* **109** 1259–1266

68. Wagner G J 1991 Secreting glandular trichomes: more than just hairs; Plant Physiology 96(3) 675-679

69. Wang, Kai, Zhu Yang, Dongjin Qing, Feng Ren, Shichang Liu, Qingsong Zheng, Jun Liu *et al*. "Quantitative and functional posttranslational modification proteomics reveals that TREPH1 plays a role in plant touch-delayed bolting." Proceedings of the National Academy of Sciences 115, no. 43 (2018): E10265-E10274