Mechanostimulation: a promising alternative for sustainable agriculture practices

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HAL Id: hal-03244326
https://hal.inrae.fr/hal-03244326v1

Submitted on 1 Jun 2021

REVIEW PAPER

Mechanostimulation: a promising alternative for sustainable agriculture practices

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Abstract

Plants memorize events associated with environmental fluctuations. The integration of environmental signals into molecular memory allows plants to cope with future stressors more efficiently—a phenomenon that is known as 'priming'. Primed plants are more resilient to environmental stresses than non-primed plants, as they are capable of triggering more robust and faster defence responses. Interestingly, exposure to various forms of mechanical stimuli (e.g. touch, wind, or sound vibration) enhances plants' basal defence responses and stress tolerance. Thus, mechanostimulation appears to be a potential priming method and a promising alternative to chemical-based priming for sustainable agriculture. According to the currently available method, mechanical treatment needs to be repeated over a month to alter plant growth and defence responses. Such a long treatment protocol restricts its applicability to fast-growing crops. To optimize the protocol for a broad range of crops, we need to understand the molecular mechanisms behind plant mechanoresponses, which are complex and depend on the frequency, intervals, and duration of the mechanical treatment. In this review, we synthesize the molecular underpinnings of plant mechanoperception and signal transduction to gain a mechanistic understanding of the process of mechanostimulated priming.

Keywords: Defence response, mechanoperception, nucleus, priming, stress tolerance, sustainable agriculture, thigmomorphogenesis.

Introduction

Although they are sessile, plants are present in the most challenging ecosystems of the planet. One of the keys to this evolutionary success is the ability of plants to perceive a wide variety of biotic and abiotic signals associated with their environment. The integration of environmental signals not only determines the instantaneous response of plants to their fluctuating environment but also determines their future responses. Put more simply, plants learn from the past, which makes them battle-ready for future events. Biological mechanisms in which exposure of plants to environmental signals makes them more

resilient to future events are termed 'priming' (Conrath *et al.*, 2015; Mauch-Mani *et al.*, 2017). Priming favours two biological functions in particular: resistance to pathogens and physiological adaptation to the physical environment. For instance, exposure to sublethal temperature enhances the thermotolerance of plants, whereas moderate salt treatment enhances the plant's immunity to bacterial pathogens (Singh *et al.*, 2014; Ling *et al.*, 2018). Priming is based on the plant's memorization of past events. The memory of plants is mainly a molecular memory, which is a direct consequence of the irreversibility of metabolic reactions (Barbacci *et al.*, 2015). Transduction of signals perceived by the naive plant leads to various molecular alterations, including transient or sustained expression of defence-related genes, signalling proteins, and transcription factors, as well as increased levels of pattern-recognition receptors, alteration of chromatin states, and changes in protein conformation (Conrath *et al.*, 2015; Mauch-Mani *et al.*, 2017). Consequently, cells of the naive plant become more sensitive to the signal and switch to a primed state. The molecular memory that forms the basis of the primed state of plants can last over a wide range of times, from a few hours to several generations (Lämke and Bäurle, 2017). As a result, primed plants respond to subsequent triggering events (i.e. exposure to a second environmental stressor) more efficiently. Compared to direct defence responses in naive (i.e. non-primed) plants, primed plants have some advantages, such as low fitness costs, sturdy and faster defence responses, better performance, and tolerance to multiple stresses (Martinez-Medina *et al.*, 2016). Thus, primed plants can respond to a stressful environment in a more adaptive way than non-primed plants. However, in a stable and favourable environment, the somatic memory may fade, and reallocation of resources occurs to maximize plant growth (Crisp *et al.*, 2016).

Defence priming of plants has been gaining attention as it offers a promising solution for crop protection. Methods of enhancing plant resistance, especially quantitative disease resistance (Roux *et al.*, 2014) to pathogens, or of increasing favourable physiological traits would efficiently complement expensive genetic modification of crops and would limit time-consuming breeding processes. In particular, it could be an attractive alternative in low-economy countries. Methods that have been developed to date are based on molecules associated with the plant–pathogen interaction. Various natural and synthetic chemicals, such as salicylic acid (SA), jasmonic acid (JA), abscisic acid, azelaic acid, pipecolic acid, β-aminobutyric acid, proline, benzothiadiazole, and hydrogen peroxide, are used to prime plants and enhance stress tolerance (Savvides *et al.*, 2016). For example, the application of β-aminobutyric acid enhances salt and drought stress tolerance, as well as resistance against the pathogenic fungus *Peronospora parasitica* and the bacterium *Pseudomonas syringae*, in Arabidopsis (Zimmerli *et al.*, 2000; Jakab *et al.*, 2005). Nevertheless, the chemical-priming approach is neither environmentally friendly nor cost effective. The uncontrolled deposition of these chemicals with the aim of increasing plant protection deeply modifies plant ecosystems and microbiota, which affects soil fertility and crop yields. These major drawbacks make the intensive use of the chemical-priming approach questionable in the context of sustainable agriculture. An alternative approach is the use of beneficial microbes (i.e. plant–growth–promoting rhizobacteria and fungi) for priming, which can overcome the problems associated with chemical priming (Mauch-Mani *et al.*, 2017). However, using biological control agents for large-scale farming requires high-efficiency formulations, which depend on multiple factors (Keswani *et al.*, 2016). The complexity of these formulations could be a limitation in using beneficial microbes for large-scale agriculture. Interestingly, the perception and transduction of mechanical signals can also induce stress tolerance in plants. In this context, the so-called thigmo-priming method ('thigmo' means 'touch' in Greek) could be a promising alternative for sustainable agriculture, as it is a cost-effective and immediately applicable method. In this article, we discuss various aspects of thigmo-priming, with an emphasis on the molecular mechanisms of plant mechanoperception and signal transduction.

Mechanostimulation improves plant performance in stressful environments

Plants have evolved a sophisticated molecular mechanism to respond to myriad environmental cues, including mechanical ones such as gusts of wind, being touched, raindrops, the pressure of penetrating hyphae, or the buzzing of bees. Mechanoperception is not limited to carnivorous plants (e.g. Venus flytrap and sundew) or *Mimosa pudica*, which possess specialized sensory cells and respond conspicuously to mechanical signals (Braam, 2005). Mechanoperception and transduction also play central roles in complex core mechanisms such as morphogenesis (Hamant *et al.*, 2008) and proprioception (the self-perception involved in postural regulation; Moulia *et al.*, 2006; Bastien *et al.*, 2013) in every plant. In addition, plants exposed to repeated external mechanical stimulation exhibit thigmomorphogenesis (Jaffe, 1973), which, in extreme cases, leads to severe alterations of the plant morphology, such as dwarfism, pithiness, delayed flowering, and reduction in stomatal aperture (Chehab *et al.*, 2009, 2011). Moreover, it has been reported to greatly alter the biomechanical and structural traits in a wide range of plant species, from an aquatic macrophyte to trees (Kern *et al.*, 2005; Paul-Victor and Rowe, 2011; Schoelynck *et al.*, 2015; Gladala-Kostarz *et al.*, 2020). Mechanical stimulation (e.g. touching, bending, brushing, wind, and sound vibration) causes transient molecular and physiological changes in plants such as calcium ion (Ca^{2+}) spiking, the generation of reactive oxygen species (ROS), the induction of antioxidant enzyme activity, hormonal modulation, altered gene expression, and reorganization of the cytoskeleton (Chehab *et al.*, 2009, 2011; Mishra *et al.*, 2016).

During the past decade, several experiments have suggested that exposure to repetitive mechanical stimulation can substantially increase the stress resilience and fitness of plants (Table 1, Fig. 1A). For instance, periodic bending (twice daily for 4 weeks) of Arabidopsis leaves enhanced resistance against the necrotrophic fungus *Botrytis cinerea* and the herbivore *Trichoplusia ni* in a JA–dependent manner (Chehab *et al.*, 2012). Similarly, another study showed that gently rubbing the leaves of Arabidopsis plants can induce resistance against *B. cinerea* in a dose-dependent manner (Benikhlef *et al.*, 2013). Ca^{2+} spiking, enhanced ROS production, and increased cuticle permeability were also observed in the rubbed Arabidopsis leaves (Benikhlef *et al.*, 2013). Wind-stimulated bean plants showed enhanced resistance against the fungus *Colletotrichum lindemuthianum*, coupled with higher lignin content and increased activities of cinnamyl-alcohol dehydrogenase and peroxidase enzymes than in control plants (Cipollini, 1997). In the same study, reduced egg production and population growth of spider mites (*Tetranychus urticae*) were noticed on wind-stimulated (twice daily for 7–10 days) bean plants, indicating that mechanical stimulation can affect host plant selection by a pest (Cipollini, 1997). Repeated brushing (1 min daily for 6 days) of the leaves of maize and bean plants also reduced settling of *Rhopalosiphum padi* and *Aphis fabae* aphids, respectively, on the mechanostimulated plants (Markovic *et al.*, 2014). Interestingly, a preliminary study showed that touch treatment of maize leaves generates volatile signals that activate chemical defence responses in non-touched neighbouring plants (Markovic *et al.*, 2019). It has also been demonstrated that exposure to mechanical waves such as sound can significantly increase the plant defence response. For instance,

exposure to airborne vibrations of 1000 Hz at 80 dB invigorates the SA–mediated defence response in Arabidopsis against *B. cinerea* and reduces the speed of disease progression (Choi *et al.*, 2017). Plant responses to mechanostimulation are not only restricted to disease resistance but are also evident as enhanced abiotic stress tolerance. For instance, mechanostimulated tomato plants gained chilling tolerance by maintaining higher photosystem II efficiency and showed less visible damage after cold stress treatment (Keller and Steffen, 1995). Similarly, sound vibration–treated Arabidopsis plants showed a higher survival rate under drought stress and significant up-regulation of abiotic stress-responsive genes compared with plants that had not been exposed to the sound vibration treatment (López–Ribera and Vicient, 2017a).

Mechanostimulation of plants is also proving to be a powerful technique for controlling plant lodging by acting on the reinforcement of the aerial parts as well as root anchoring. Until now, significant progress has been made to reduce the threat of lodging by introducing dwarfing genes and developing semi-dwarf varieties. However, further improvement is required to increase plant resilience to severe storms associated with global climate change. Unfortunately, many lodging-resistant varieties are already near the critical height range (i.e. the minimum crop height for optimum yield), and the production of shorter plants would reduce the yield of crops (Berry, 2012). To circumvent this challenge, mechanostimulation could be a promising approach for managing lodging risk to achieve high crop yields. For example, mechanostimulated wheat plants show increased lignin content and improved mechanical strength as well as lodging resistance (Si *et al.*, 2019). A complementary

Table 1. Exposure to mechanical stimuli improves defence response and stress tolerance in various plants

Mechanostimulus	Plant	Stressor	Environment	References
Bending	Arabidopsis	Fungus (*Botrytis cinerea*)	Controlled	Chehab *et al.* (2012)
Rubbing	Arabidopsis	Fungus (*Botrytis cinerea*)	Controlled	Benikhlef *et al.* (2013)
Sound vibration	Arabidopsis	Fungus (*Botrytis cinerea*)	Controlled	Choi *et al.* (2017)
Sound vibration	Arabidopsis	Fungus (*Ralstonia solanacearum*)	Controlled	Jung *et al.* (2020)
Rubbing	Strawberry	Fungus (*Botrytis cinerea*)	Controlled	Tomas-Grau *et al.* (2018)
Wind	Bean	Fungus (*Colletotrichum lindemuthianum*)	Controlled	Cipollini (1997)
Bending	Arabidopsis	Herbivore (*Trichoplusia ni*)	Controlled	Chehab *et al.* (2012)
Wind	Bean	Herbivore (*Tetranychus urticae*)	Controlled	Cipollini (1997)
Brushing	Bean	Aphid (*Aphis fabae*)	Controlled	Markovic *et al.* (2014)
Brushing	Maize	Aphid (*Rhopalosiphum padi*)	Controlled	Markovic et al. (2014, 2019)
Brushing	Maize	Ladybird (*Coccinella septempunctata*)	Controlled	Markovic *et al.* (2014)
Brushing	Potato	Aphid (*Macrosiphum euphorbiae*)	Controlled	Markovic *et al.* (2016)
Brushing	Potato	Aphid (*Myzus persicae*)	Controlled	Markovic *et al.* (2016)
Brushing	Tomato	Cold	Controlled	Keller and Steffen (1995)
Rubbing/stroking	Bean	Drought	Controlled	Suge (1980)
Weight loading/pressing	Wheat	Cold	Semi-field	Si *et al.* (2019)
Sound vibration	Arabidopsis	Drought	Controlled	López-Ribera and Vicient (2017a)
Sound vibration	*Mentha pulegium*	Salt stress	Controlled	Ghalkhani *et al.* (2020)
Weight loading/pressing	Wheat	Lodging	Semi-field	Si *et al.* (2019)
Rubbing, brushing, shaking	Mulberry	Lodging	Field	Tateno (1991)
Wind	*Brachypodium distachyon*	Lodging	Controlled	Nam *et al.* (2020)
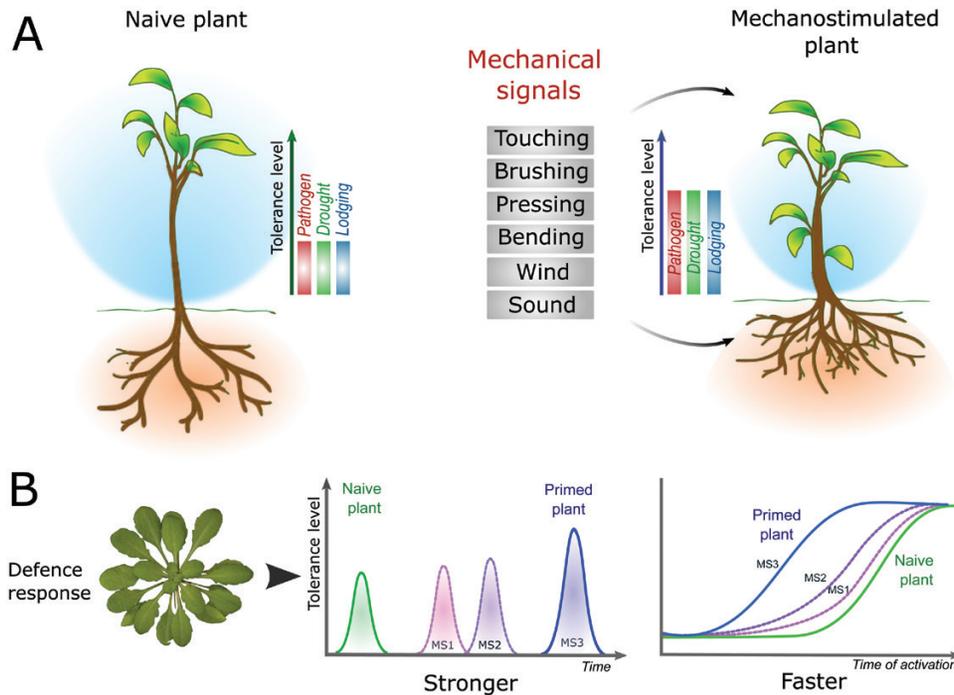
Fig. 1. (A) Repeated exposure to mechanical stimulation alters the morphology of plants, and mechanostimulated plants exhibit greater stress tolerance than naive (i.e. non-stimulated) plants. (B) The success of thigmo-priming depends on the frequency, interval, and duration of the mechanical treatment. Here, for instance, only plants mechanostimulated three times (MS3) show a stronger and faster defence response compared with naive plants and plants mechanostimulated once or twice (MS1 and MS2, respectively).

way to decrease lodging risk is to strengthen the anchoring of the roots in the soil. Interestingly, the application of mechanical treatments to the aerial parts of a plant can cause changes to the root system architecture and biomechanical properties, consequently increasing the anchorage strength and resistance to deflection of sunflower and Sitka spruce roots, respectively (Stokes *et al.*, 1997; Goodman and Ennos, 1998). This phenomenon could be exploited to protect crops from root lodging. Densification of crops in greenhouse conditions favours leaf expansion and branch elongation, which in turn increases lodging risk. Therefore, limiting stem elongation and maintaining compactness is a challenge for the horticulture industry; the currently available approaches to overcome this problem are laborious pruning or the application of chemical growth regulators. Here, too, mechanical stimulation can be useful for improving mechanical and structural traits of plants. For instance, mechanostimulated rose plants showed increased branching and compactness in greenhouse conditions (Morel *et al.*, 2012). This technique can be utilized for large-scale farming under field conditions such as 'mugifumi'. This is a crop-management method practised in Japan from the 17th century, whereby wheat and barley plants are compressed physically by treading them. As a result, treated plants show more tillers, longer spikes, reduced lodging, higher yield, and induced root growth (Iida, 2014). Altogether, these studies illustrate the potentially wide application of mechanostimulation in improving stress tolerance and favourable agricultural traits.

Thigmo-priming: a promising strategy to improve agricultural productivity

The increase in tolerance to abiotic and biotic factors after mechanostimulation may be due to an active process such as the triggering of signalling pathways common to several factors or a passive process related to the modification of phenotypic traits. At this stage, molecular details explaining how mechanostimulation activates plant defences remain elusive, despite an impressive gain of resistance to pathogens that has been obtained. Nonetheless, the available studies indicate the potential uses of mechanical cues to prime plants for improved stress tolerance. To confirm this, key characteristics (i.e. memory, low fitness costs, sturdy and faster defence responses, better performance, and tolerance to multiple stresses) of priming responses need to be evaluated in future studies. Defence priming achieved through repetitive mechanostimulation can be termed 'thigmo-priming'. Thigmo-priming may have some advantages over chemical priming. It offers the possibility of more cost-effective and environmentally friendly approaches, which are easy to implement, use, and maintain. Most importantly, thigmo-priming may alleviate the risk of molecular crosstalk associated with chemical priming. Therefore, chemical priming is a more suitable approach in the prevention of a specific risk than to confer resistance to a broad range of stresses. For instance, SA-mediated defence priming might not be suitable against necrotrophic fungi, as resistance against

necrotrophs generally depends on the JA and ethylene–mediated pathway (Glazebrook, 2005). Similarly, JA-mediated priming might not be effective against biotrophic fungi, as resistance against biotrophs is SA–dependent (Glazebrook, 2005). Interestingly, mechanostimulated plants show cross-tolerance against various biotic and abiotic stresses. Transcriptomic and hormone analyses have shown that the expression of a broad range of defence–related genes and the concentration of various plant hormones can be modulated by mechanostimulation (Lee *et al.*, 2005; Chehab *et al.*, 2009; Pomiès *et al.*, 2017; Van Moerkercke *et al.*, 2019). These results suggest that mechanical stimulation has the potential to prime plants against a broad range of stresses. Diverse mechanical stimuli delivered at various doses can be used to prime a plant (Table 1, Fig. 2). However, the same mechanical treatment may not be easily applicable to all plants. For example, bending treatments are suitable for tree species, whereas mechanical treading can be easily applied to rice or wheat. Similarly, sound vibration treatments cannot feasibly be applied in field conditions; however, they can be used for priming seeds. It has been observed that sound vibration treatment can accelerate the germination of seeds of many plant species, for example, Arabidopsis, rice, and cucumber (Takahashi *et al.*, 1991; Uchida and Yamamoto, 2002; López–Ribera and Vicient, 2017*b*).

So far, plant responses to mechanostimulation have been mostly studied in controlled environments (Table 1), which did not take account of natural conditions, where plants constantly encounter a range of mechanical cues. Thus, the question arises whether it is possible to thigmo–prime plants under field conditions. It has been hypothesized that plants can fine-tune their
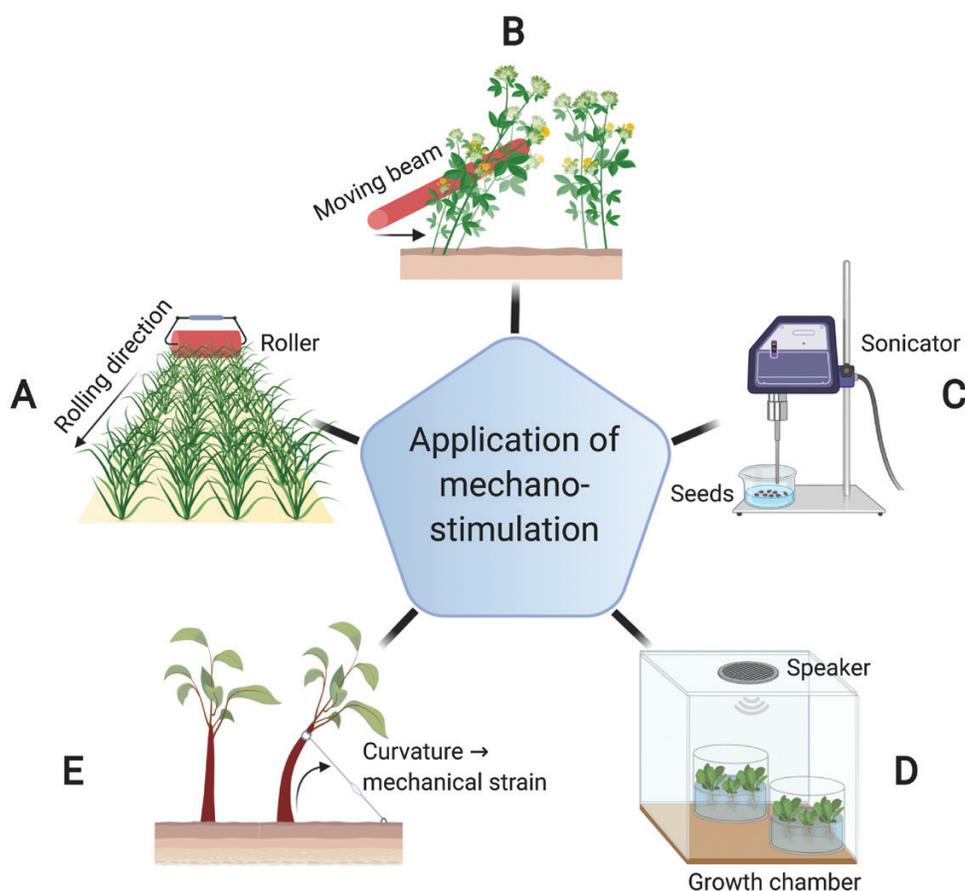
Fig. 2. Examples of mechanostimulation methods. (A) Crop plants can be compressed by rolling a cylinder over the young plants, as described by Si *et al.* (2019). Application of the right amount of pressure at the appropriate growth stage is imperative for an optimal effect. (B) Plants can be mechanostimulated by creating variation in the curvature of plant structures, as demonstrated by Morel *et al.* (2012). A soft plastic tube or steel bar can be used for this purpose, depending on the rigidity of the plants. (C) Imbibed seeds can be treated with ultrasound, as shown by López-Ribera and Vicient (2017b), before sowing. (D). Methods based on airborne sound waves, such as plant acoustic frequency technology (PAFT), as described by Meng *et al.* (2012), are usually used in greenhouses. A reason behind this is that the sound intensity drastically attenuates over distance. However, this could be a promising technology for the vertical farming industry. (E) Plants can sense the mechanical signals generated by variations in stem curvature. Thus, young trees can be mechanostimulated by bending them to different extents through controlled external pulling, as described by Bonnesoeur *et al.* (2016). Although there are limitations to the large-scale application of this technique, it might be promising in urban areas to protect ornamental trees from wind damage or for maintaining favourable agricultural traits (e.g. stunted growth, increased branching) of fruit trees. Readers are directed to dedicated reviews, which provide more information about PAFT (Hassanien *et al.*, 2014) and mechanical growth regulation (Börnke and Rocksch, 2018).

response to frequent and low-intensity mechanical cues (e.g. daily wind) to avoid costly investment in redirecting growth, and this is also considered as an important strategy of plants for acclimation to strong mechanical forces (e.g. storms). In this context, a recent study shows that beech trees can filter out chronic lower-intensity wind signals and respond only to intense ones (Bonnesoeur *et al.*, 2016). This result, along with the findings of another system modelling of single-cell electro-physiological data, indicates the existence of mechanosensing thresholds in plants (Moulia *et al.*, 2015), which help them to avoid overreacting to each mechanical perturbation. Certainly, further studies are warranted to strengthen this hypothesis. However, this result indicates that knowledge of local climatic conditions is imperative in order to apply mechanostimulation with an appropriate intensity in field conditions.

From an agricultural point of view, the use of thigmo-priming requires some trials to determine the optimal param-eters of stimulation for crop protection, such as the intensity of mechanical treatment, the number of repetitions, the time between treatments, and the appropriate growth stage at which to apply stimulation. The success of thigmo-priming can be assessed by measuring the kinetics of the plant defence re-sponse upon exposure to an environmental stress (Fig. 1B). According to the traditional mugifumi protocol, mentioned earlier, crops with a long seedling stage (e.g. winter wheat and barley) are treated with a series of mechanostimulations re-peated two to four times per growing season over a period of 3–4 months (Iida, 2014). Interestingly, proper intervals be-tween repeated mechanostimulations determine the success of mugifumi. Such a lengthy period of treatment is not suitable for fast-growing crops and vegetables. To modify the protocol for a wider selection of crop species, we will need to under-stand the molecular mechanisms of thigmo-priming, which remain largely unknown. A molecular analysis (e.g. of the kin-etics of expression of mechanoresponsive genes) could pro-vide a rapid and useful tool for identifying mechanosensitive crop varieties and optimizing treatment parameters. For basic research, it would be relevant to identify the molecular links between mechanoperception and plant resistance, especially the quantitative disease resistance that is activated against, for instance, attacks from necrotrophic fungi (Roux *et al.*, 2014; Mbengue *et al.*, 2016).

Perception and transduction of mechanical signals

At this point, the intriguing question is: how do plants per-ceive mechanical stimuli? Various molecular players involved in plant mechanoperception and signalling—from ion chan-nels to receptor kinases—have been identified (as summarized in Fig. 3A). Among them, mechanosensitive-ion (MS) chan-nels are considered to be a major class of mechanoreceptors. MS channels are transmembrane proteins that sense membrane

tension and convert the mechanical force to ion flux in the cell. So far, three types of plant MS channels have been iden-tified: MS channel of small conductance-like (MSL), Mid1-complementing activity family (MCA), and OSCA. MCAs and OSCAs are Ca^{2+}-permeable channels, whereas MSLs show a preference for anions (Basu and Haswell, 2017; Frachisse *et al.*, 2020). Arabidopsis has 10 MSL proteins, which are lo-calized across organelles: MSL8/9/10 are localized in the plasma membrane, while MSL2/3 are plastid localized and MSL1 is mitochondrion localized (Basu and Haswell, 2017). There are two MCA proteins in Arabidopsis, both of which are localized in the plasma membrane (Nakagawa *et al.*, 2007; Yamanaka *et al.*, 2010). The stretch activation of Arabidopsis MSL1, MSL8, MSL9, MSL10, and MCA1 proteins has been demonstrated through electrophysiological analysis (Haswell *et al.*, 2008; Furuichi *et al.*, 2012; Hamilton *et al.*, 2015; Lee *et al.*, 2016). With 15 members, Arabidopsis OSCA is the lar-gest family of MS channels identified in plants to date. Thus far, mechanosensitive gating of six proteins of this family has been identified through electrophysiological analysis; among these proteins, OSCA1.1, OSCA1.2, and OSCA3.1 are prominent (Yuan *et al.*, 2014; Murthy *et al.*, 2018). Recently, DEK1 pro-tein, which is believed to be a part of a rapidly activated Ca^{2+} MS channel activity (RMA), has been identified (Tran *et al.*, 2017). However, the molecular identity of RMA is unknown.

The physical force exerted by mechanical stimulation can cause damage to the cell wall, which may trigger cell wall damage (CWD) and/or cell wall integrity (CWI) signalling in plants. Plasma-membrane-localized receptor-like kinases (RLKs) play dominant roles in CWD/CWI signalling (Seifert and Blaukopf, 2010). Plant RLKs recognize damage-associated molecular patterns (DAMPs) that are produced by plants during pathogen attacks and induce a series of intracellular signalling events such as the MAPK cascade, ROS burst, and Ca^{2+} spiking (He *et al.*, 2018). It has been hypothesized that RLKs play vital roles in plant mechanotransduction, although evidence is limited. The Arabidopsis genome encodes more than 600 RLKs (Seifert and Blaukopf, 2010). Nonetheless, until now, the involvement of only one RLK (FERONIA, which belongs to a *Catharanthus roseus* RLK1-like subfamily) in plant mechanical signal transduction has been established. *feronia* loss-of-function mutants show impaired ion signalling, reduced expression of mechanoresponsive genes, and altered root growth responses to mechanically challenging environ-ments (Shih *et al.*, 2014). It has been noted that extracellular ATP (eATP), a DAMP signalling molecule, can also trigger CWD/CWI signalling during the stress response (Tanaka *et al.*, 2014). A preliminary study has shown that mild touch treatment can induce eATP release in the Arabidopsis root and shoot, and the heterotrimeric G-protein complex is in-volved in this process (Weerasinghe *et al.*, 2009). Interestingly, transcriptomic analysis revealed that mechanostimulation up-regulates a large number of Arabidopsis and poplar genes encoding cell-wall-modifying enzymes (e.g. *TCH4*, encoding
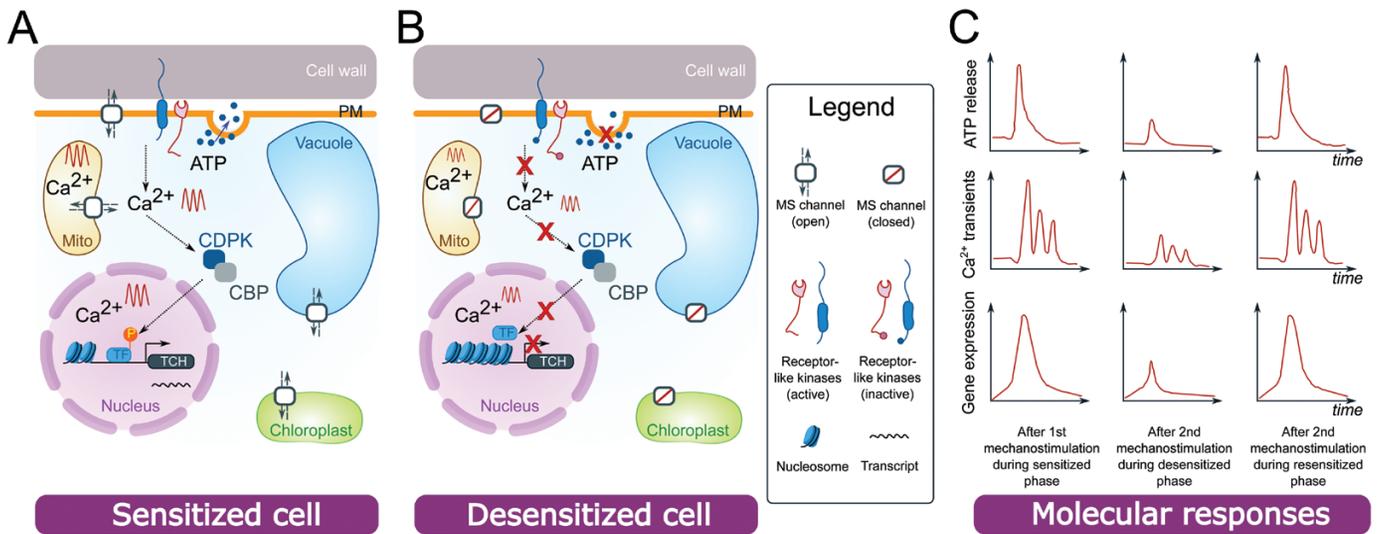
Fig. 3. Hypothetical model showing the effects of repetitive mechanostimulation on the mechanosensitivity of plant cells. (A) Plasma membrane (PM)-bound mechanosensors (e.g. MS channels, receptor-like kinases) perceive mechanical cues, which subsequently trigger cytosolic Ca^{2+} spiking. Extracellular ATP accumulates in response to mechanical stimulation, and this also contributes to a major part of Ca^{2+} signalling. MS channels localized in chloroplasts and mitochondria (Mito) can be activated through cytoskeletal reorganization or a biochemical cascade during the mechanotransduction process. As a consequence, mechanostimulation generates a transient increase in Ca^{2+} in organelles. Calcium-dependent protein kinases (CDPKs) and calcium-binding proteins (CBPs) activate various target proteins, which ultimately convert Ca^{2+} signals into transcriptional responses. Additionally, mechanostimulation causes favourable epigenetic modifications at mechanoresponsive loci (e.g. *TCH*), which trigger their transcription. P, phosphate; TF, transcription factor. (B) Mechanical stimulation desensitizes plant cells to successive episodes of mechanostimulation for a certain period, which attenuates Ca^{2+} signalling, extracellular ATP release, and the expression of mechanoresponsive genes. Mechanostimulated cells can be desensitized due to the inactivation of MS channels or modifications of other mechanoreceptors. In addition, epigenetic modifications at mechanoresponsive loci can cause the formation of heterochromatin and block transcription. The desensitization phase can be maintained in the long term by modulating the chromatin states. (C) Kinetics of ATP release, Ca^{2+} transients, and expression of mechanoresponsive genes after the first and second mechanostimulation during the sensitized, desensitized, and resensitized phases.

a xyloglucan endotransglucosylase/hydrolase) (Lee *et al.*, 2005; Pomiès *et al.*, 2017). It is most likely that these cell-wall-modifying enzymes take part in the CWD/CWI signalling process through altering the microstructure of the cell wall upon mechanostimulation. Another recent study showed that MS channels (e.g. MCA1, MSL2, and MSL3) contribute to CWD-induced signalling in Arabidopsis (Engelsdorf *et al.*, 2018). Collectively, these studies suggest that a profound analysis of CWD/CWI signalling is required to unravel the molecular underpinnings of mechanosignalling in plants.

Ca^{2+} regulates numerous signalling pathways, including the mechanoresponse. Ca^{2+}-permeable MS channels are involved in mechanosensitive gating of Ca^{2+} (Frachisse *et al.*, 2020). In addition, FERONIA plays an important role in triggering mechanostimulated Ca^{2+} signalling in Arabidopsis (Shih *et al.*, 2014). Ca^{2+} spiking immediately after mechanical stimulation is one of the upstream signalling events in the plant that triggers rapid protein phosphorylation through calcium-dependent protein kinases (CDPKs) and calcium-binding proteins (CBPs). CDPKs and CBPs play important roles in converting Ca^{2+} signals into transcriptional responses. Recently, a phosphoproteomic analysis of touch-treated plants showed the importance of the phosphorylation pathway in mechanosignalling (Wang *et al.*, 2018). In summary, plants

perceive mechanical signals either through directly activated MS channels or by RLK-mediated signalling, which ultimately turns on the signature cellular responses such as Ca^{2+} spiking, eATP release, and oxidative burst. For more dedicated reviews on plant mechanoperception, readers are directed to Hamant and Haswell (2017), Ackermann and Stanislas (2020), and Bacete and Hamann (2020).

Molecular underpinnings of thigmomorphogenesis

Unravelling the molecular mechanisms behind plant mechanoperception and thigmomorphogenesis is imperative for the successful application of thigmo-priming methods in agriculture. Unfortunately, how mechanoreceptors are involved in thigmomorphogenesis has remained elusive. An attempt to unravel the mechanisms has been made by investigating the role of MSLs in thigmomorphogenesis. Surprisingly, no phenotypic difference was observed in touch- and wind-stimulated *mslΔ5* (*msl4;msl5;msl6;msl9;msl10*) compared with wild-type Arabidopsis plants (Haswell *et al.*, 2008). This observation suggests that the integration of mechanical signals into a thigmomorphogenic response is more complex

than cellular mechanosensing alone. Other experimental findings, such as the strong touch-inducible expression of mechanoresponsive genes in the *aos* mutant (which is impaired in thigmomorphogenesis), also corroborate this inference (Chehab *et al.*, 2012). Thus far, the role of only one MS channel in root thigmomorphogenesis has been clearly established: MCA1 is required for sensing the hardness of growing media by the Arabidopsis primary root and for subsequent responses (Nakagawa *et al.*, 2007). A growing body of evidence suggests that thigmomorphogenesis depends on the plant's hormone status. For instance, a reduced concentration of bioactive gibberellin (GA) and the induction of the GA-inactivating enzyme *GA2ox7* causes thigmomorphogenesis in Arabidopsis (Lange and Lange, 2015). Another study has indicated the importance of JA biosynthesis and/or signalling genes (*AOS*, *JAR*, and *COI1*) in touch-mediated growth alterations in Arabidopsis (Chehab *et al.*, 2012). In addition, a recent study shows that the JA-activated transcription factors MYC2/MYC3/MYC4 are important for thigmomorphogenesis in Arabidopsis (Van Moerkercke *et al.*, 2019). The roles of three phosphoproteins—MAP kinase kinase (MKK) 1 and 2, and a novel protein named TREPH1—in the process of thigmomorphogenesis have been established (Wang *et al.*, 2018). A delayed bolting phenotype was not observed in touch-treated *treph1-1*, *mkk1*, and *mkk2* mutants. Some molecular players behind root thigmomorphogenesis have also been identified. For example, in rice, the typical root-curling phenotype in response to a physical barrier is regulated by OsHOS1, an E3-ubiquitin ligase (Lourenço *et al.*, 2015). In addition, OsRMC, a negative regulator of JA signalling, also negatively regulates root curling in rice (Lourenço *et al.*, 2015). The same study also demonstrated that the straight root phenotype of the transgenic RNAi::*OsHOS1* line can be reverted by the application of JA. This result indicates that JA signalling plays a pivotal role in root thigmomorphogenesis. However, the majority of these identified molecular players are believed to act in the lower part of the signalling cascade. Clearly, further research is warranted to unravel the upstream signalling mechanism behind thigmomorphogenesis.

Plants 'remember' exposure to mechanostimulation

The application of excessive and uncontrolled mechanical treatment to plants increases the risk of wounding, which may reduce the optimal effect of thigmo-priming due to the use of cellular energy for damage recovery. Moreover, wounding will predominantly activate JA/ethylene signalling pathways, which may affect the broad stress-tolerance phenomenon of primed plants. Thus, both the number and the intensity of mechanical treatments are believed to be crucial in the thigmo-priming process. In most cases, plants need repetitive mechanical stimulations over a period for successful thigmomorphogenesis, but

this may not be an ironclad rule. A single transient bending treatment is sufficient to alter radial growth and gene expression in poplar plants for up to 3 days (Coutand *et al.*, 2009; Pomiès *et al.*, 2017). Furthermore, studies on poplar have shown that the first mechanostimulation desensitizes the plant to the following treatments, and an interval of a few days between two successive bending events is necessary to restore full mechanosensitivity. For example, the expression of *PtaZFP2*, an early mechanoresponsive gene, was markedly weaker after the second bending (applied 24 h after the first) of the poplar stem (Martin *et al.*, 2010). The same study also showed that an interval of around 5 days between two successive bending events is necessary to recover the full capacity for the induction of *PtaZFP2* expression. This process has a global impact on the transcriptional response; transcriptome analysis showed that when two bending treatments are applied with a 24 h interval to a poplar stem, 96% of the mechanoresponsive genes remain less responsive or non-responsive after the second bending relative to the first bending (Pomiès *et al.*, 2017). A recent study showed that this process has a global impact on transcription in Arabidopsis as well: mechanostimulated expression of a large number of Arabidopsis genes gradually reduced after repetitive touch treatments at 0.5, 12, and 24.5 h (Xu *et al.*, 2019). These findings indicate that plants do not respond to each mechanical perturbation with the same intensity, which could be due to a desensitization phenomenon (Fig. 3B). Interestingly, the diameter growth response of poplar plants was reduced only after three or four rounds of repetitive treatments (Martin *et al.*, 2010), which suggests that plant memory ensures commitment to permanent acclimation only when the stress is constant or reoccurring. Besides effects on growth and gene expression, attenuation of Ca^{2+} spiking and eATP release in response to repetitive mechanostimulation have also been noted (Fig. 3C). For example, ATP release in Arabidopsis roots can be diminished if re-touching occurs within 9.3 min of the initial stimulation (Weerasinghe *et al.*, 2009). Similarly, three instances of repetitive touch stimulation applied at 5 min intervals attenuates cytosolic Ca^{2+} spiking in root cap cells (Legué *et al.*, 1997). This study also showed that repetitive touch stimulation separated by 10 min intervals cannot attenuate Ca^{2+} release. Another study reported that exposure to five to six bursts of wind makes *Nicotiana* seedlings refractory to further wind stimulation, and a recovery period of around 60 s is required for seedlings to regain sensitivity and show cytosolic Ca^{2+} elevation (Knight *et al.*, 1992). Taking these findings together, it is clear that the plant's response to mechanical stimulation is complex, as it depends on the treatment frequency, interval, and duration.

At this point, the intriguing question is how plants count and memorize the number of mechanical stimulations they have received. It has been noted that Arabidopsis plants lacking the G-protein complex do not show a pronounced refractory period for touch-induced ATP release compared with the wild type (Weerasinghe *et al.*, 2009). This suggests that G-protein-coupled signalling plays an important role in regulating the

mechanosensitivity of plants. It is most likely that there are many other regulators/sensors, and this needs to be explored further. However, the refractory period of upstream mechanoresponses such as eATP release and cytosolic Ca^{2+} spiking is very short (1–10 min), which may not be sufficient to maintain desensitization for a long time (e.g. 5 days in poplar). Although many hypotheses exist to explain the prolonged desensitization phase (Leblanc-Fournier *et al.*, 2014; summarized in Fig. 3), the involvement of epigenetic regulation, which plays a central role in plant development and stress responses, is the most promising one. The mechanism of plant memory formation is just beginning to unfold, and to date, several molecular factors mediating epigenetic memory in plants primed by stressors (e.g. heat, cold, salt, and pathogen infection) have been identified (Lämke and Bäurle, 2017). Nonetheless, information about epigenetic regulation during plant responses to mechanical stimulation is scarce. So far, two candidates have been identified that strongly establish the link between thigmomorphogenesis and epigenetic regulation. These are Arabidopsis *SDG8* (which encodes a histone lysine methyltransferase) and *VIP3* (which encodes part of the RNA polymerase II-associated factor 1 complex, Paf1). Both *sdg8* and *vip3* mutants showed perturbation in the thigmomorphogenic response (Cazzonelli *et al.*, 2014; Jensen *et al.*, 2017). It has also been demonstrated that SDG8 and VIP3 are required to maintain active histone marks (H3K4me3 and H3K36me3) for up-regulating mechanosensitive loci such as *TCH3* and *TCH4* (Cazzonelli *et al.*, 2014; Jensen *et al.*, 2017). These results indicate that epigenetic modifications may play critical roles in thigmo-priming through memorizing and regulating the cascade of signalling events after touch treatment.

Epigenetic modifications predominantly take place in the nucleus, and it has been noted that mechanostimulation can induce structural changes in nuclear envelope proteins and chromatin in animal and yeast cells. Unfortunately, no conclusive evidence is available yet to show that mechanical signals alter gene expression in plants by directly affecting nuclear shape and stiffness. However, a recent study shows that mechanical signals induced by hyperosmotic stress (which modifies cell wall mechanical tension) increase nuclear stiffness and the expression of mechanoresponsive genes in the Arabidopsis root (Goswami *et al.*, 2020). In addition, the role of the nuclear envelope proteins GIPs in the mechanical shielding of nuclei and the expression of mechanoresponsive genes has been demonstrated through transcriptome analysis of a *gip1gip2* mutant (Goswami *et al.*, 2020). Mechanostimulated nuclear–cytoplasmic shuttling of VIP1 protein (a bZIP transcription factor) has also been observed in Arabidopsis roots (Tsugama *et al.*, 2016), which indicates the putative involvement of the nuclear pore complex in the mechanotransduction process. Interestingly, mechanical signals induced by treatment with a hypotonic solution cause nuclear–cytoplasmic shuttling of VIP1, which requires Ca^{2+} signalling but not Ca^{2+}-permeable MCA channels (Tsugama *et al.*, 2018). Moreover, pH-dependent Ca^{2+} spiking was observed in isolated Arabidopsis nuclei upon mechanostimulation

(Xiong *et al.*, 2004), which indicates that the plant nucleus possesses an independent mechanosensing machinery and it can be triggered without the cell wall–plasma membrane–cytoskeleton (CWPMC) physical interface. The same study also showed that repeated mechanical perturbation causes a slight reduction in the amplitude of nuclear Ca^{2+} spiking (Xiong *et al.*, 2004), which corroborates the desensitization of gene expression phenomenon. Collectively, these results suggest the importance of the plant nucleus in mechanotransduction. Nonetheless, how the external mechanical force propagates through the CWPMC interface to the nucleus and facilitates chromatin remodelling remains elusive in plants. It has been noted that plant organelles are sensitive to physical touch. Nuclei of tobacco leaf epidermal hair cells showed oriented movement toward sites of mechanical stimulation applied through a microneedle (Qu and Sun, 2007). Similarly, gentle touching of Arabidopsis cotyledons by a microneedle causes the rapid reorganization of actin microfilaments, endoplasmic reticulum, and peroxisomes in epidermal cells (Hardham *et al.*, 2008). This subcellular reorganization may transduce external mechanical signals inside the nucleus through a biophysical interaction between the nucleoskeleton and cytoskeleton, which ultimately causes chromatin compaction and epigenetic regulation.

Future perspectives and conclusions

First, the following aspects need to be considered to identify optimal thigmo-priming parameters: the frequency of repetitive mechanical stimulation, the time interval between treatments, and selection of the growth stage, plant organ, and season for applying mechanostimulation. In order to do this, a dose-dependent response to mechanostimulation should be more precisely evaluated. At present, in experiments studying the relationship between mechanostimulation and plant defence mechanisms, the treatment dose is quantified in terms of the number of exposures to the mechanical stimulus. To properly evaluate the plant responses, the intensity of the mechanical signals should also be quantified. In recent years, numerous interdisciplinary approaches involving biologists and physicists have made it possible to quantify physical parameters depending on the type of mechanostimulation and according to the size of the organ being stimulated (Moulia *et al.*, 2015). Such a biomechanical approach could thus help in predicting the intensity of the perceived signal and choosing the most appropriate method for applying the mechanical load.

A better understanding of the molecular mechanisms behind plant mechanoperception is also imperative, as molecular responses are often rapid and easy to quantify. Thus, molecular analysis would be a highly promising approach for quantifying the effect of repetitive mechanical stimulation and the time interval between treatments rather than measuring

the response at the plant or organ level. The discovery of mechanosensors might make it possible to use them as molecular markers to select different varieties of plants that are hypersensitive to mechanical stimulation as well as suitable for thigmo-priming. In this context, as suggested in this review, it would be necessary to study the role of organelles in mechanosignalling. So far, plant mechanosensing research has concentrated on plasma-membrane-bound mechanosensors. However, recent developments have suggested that other organelles, such as the nucleus and mitochondria, play critical roles in mechanoperception and signal transduction in plants. For instance, transcriptome analysis with mitochondrial function/signalling mutants (25 single, 3 double, and 2 triple mutants) has shown that impaired mitochondrial function can strongly affect the expression of touch-responsive genes (Xu *et al.*, 2019). Moreover, in Arabidopsis, a surge in mitochondrial Ca^{2+} was noted upon mechanostimulation (Logan and Knight, 2003). Thus, inter-organelle Ca^{2+} signalling and the contribution of organelles to the cytoplasmic Ca^{2+} concentration upon mechanostimulation need to be investigated.

Enhanced stress tolerance in mechanostimulated plants can be the result of cross-tolerance and/or priming. It is thus imperative to analyse the key characteristics of priming responses in future studies. Transgenerational memory is another beneficial characteristic of stress priming. Interestingly, a preliminary study showed transgenerational resistance against *Helicoverpa zea* caterpillars in mechanically wounded tomato plants (Rasmann *et al.*, 2012). Whether mild mechanostimulation is capable of generating transgenerational memory and conferring stress tolerance needs to be evaluated. In summary, a better understanding of thigmomorphogenesis is required to facilitate the thigmo-priming process and translate it into agronomic benefits. Fundamental new knowledge on the underpinnings of plant mechanosensing would enable adaptation of the method to a broad range of crops by optimizing treatment intensity, frequency, and intervals.

Acknowledgements

Author contributions

Conceptualization: RG and NLF; writing—original draft: RG; writing—review and editing: RG, AB, and NLF.

Conflict of interest

The authors declare no competing interests.

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