

## Mechanical touch responses of *Arabidopsis TCH1-3* mutant roots on inclined hard-agar surface\*\*

Guodong Zha, Bochu Wang\*, Junyu Liu, Jie Yan, Liqing Zhu, and Xingyan Yang

Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, China

Received April 20, 2015; accepted October 7, 2015

**Abstract.** The gravity-induced mechanical touch stimulus can affect plant root architecture. Mechanical touch responses of plant roots are an important aspect of plant root growth and development. Previous studies have reported that *Arabidopsis TCH1-3* genes are involved in mechano-related events, however, the physiological functions of *TCH1-3* genes in *Arabidopsis* root mechanoresponses remain unclear. In the present study, we applied an inclined hard agar plate method to produce mechanical touch stimulus, and provided evidence that altered mechanical environment could influence root growth. Furthermore, *tch1-3 Arabidopsis* mutants were investigated on inclined agar surfaces to explore the functions of *TCH1-3* genes on *Arabidopsis* root mechanoresponses. The results showed that two *tch2* mutants, *cml24-2* and *cml24-4*, exhibited significantly reduced root length, biased skewing, and decreased density of lateral root. In addition, primary root length and density of lateral root of *tch3* (*cml12-2*) was significantly decreased on inclined agar surfaces. This study indicates that the *tch2* and *tch3* mutants are hypersensitive to mechanical touch stimulus, and *TCH2* (*CML24-2* and *CML24-4*) and *TCH3* (*CML12-2*) genes may participate in the mechanical touch response of *Arabidopsis* roots.

**Key words:** mechanical touch stimulation, *TCH1-3* genes, root growth, *Arabidopsis*

### INTRODUCTION

Mechanical stimulus experienced by roots induces continuous root response as roots are growing in soil (Gleeson *et al.*, 2012). In natural environment, the main sources of mechanical stimulation for plants are wind, touch, rain and obstacle (Coutand, 2010). Higher plant roots change their growth patterns in response to these mechanical stimuli (Okada *et al.*, 1990). When root tips encounter obstacles in soil, they avoid the obstacles by changing the direction

of their growth (Monshausen and Gilroy, 2009b). Besides, the positive gravitropism and touch stimuli can affect many growth patterns of plant roots on the surface of agar, such as waving, skewing, helix and circumnutation (Chehab *et al.*, 2009; Migliaccio *et al.*, 2013; Okada and Shimura, 1990; Silverberg *et al.*, 2012). Therefore, exploring the cellular and molecular basis of roots mechanoresponses is of great importance for fundamental plant biology and agricultural practice.

Gravity-induced touch stimulus can give rise to two characteristic root growth patterns of *Arabidopsis*, waving and skewing, when *Arabidopsis* seedlings grow on impenetrable and inclined agar surface (Okada and Shimura, 1990; Rutherford and Masson, 1996). This altered growth behaviour is proposed to be a touch-induced resistance based on gravity perception or response and may enable the fine navigation ability of plant roots during growth through soil (Massa and Gilroy, 2003). In addition, root branching can also be affected by mechanical forces (Richter *et al.*, 2009). Previous studies have reported that lateral roots emerge from the convex side of a curved root, whether the curvature is formed by waving or physical bending (Ditengou *et al.*, 2008; Richter *et al.*, 2009). Despite these many mechanical-induced root growth behaviours, the molecular mechanisms of mechanoresponse remain largely unknown (Monshausen and Gilroy, 2009a,b).

In order to explore the molecular mechanisms of mechanoresponses in *Arabidopsis* root, Braam and Davis (1990) isolated four touch-induced *TCHs* (touch inducible genes). Among the four genes, *TCH1-3* genes encode Calmodulin (CaM) and Calmodulin-like (CML)

\*Corresponding author e-mail: wangbc2000@126.com

\*\*This work was supported by the fund of National Natural Science Foundation of China (No. 11172337), 2012-2015.

proteins, which suggests potential involvement of  $\text{Ca}^{2+}$  and potential  $\text{Ca}^{2+}$  receptors in *Arabidopsis* mechano-responses (Braam and Davis, 1990). Although recent reports implicate intracellular  $\text{Ca}^{2+}$  as the second message in plant mechano-transduction pathways leading to appropriate mechanoresponses, such as primary root elongation and lateral root initiation (Monshausen *et al.*, 2009; Richter *et al.*, 2009), mechano-related functions of *TCH1-3* genes have not been investigated.

The effects of numerous genes on mechanoresponses of roots have been demonstrated (Braam, 1992; Braam and Davis, 1990; Lee *et al.*, 2005), however, the functions of *TCH1-3* in the mechanoresponses are still unclear. Thus, in the present work, we took advantage of root length, skewing degree, and the density of lateral root (DLR) assays to explore the potential mechanoresponses functions of *TCH1-3* genes in *Arabidopsis* root behaviours using a reverse genetic approach. We found that the *tch1-3* mutants roots tested showed distinct phenotypic consequences on inclined agar surface. This study indicated that *tch2* and *tch3* mutants were hypersensitive to increased levels of mechanical stimuli, namely the normal function of the *TCH2* (*CML24-2* and *CML24-4*) and *TCH3* (*CML12-2*) might be necessary for *Arabidopsis* primary root in response to mechanical touch stimulus.

#### MATERIAL AND METHOD

The wild-type (WT) *tch1* (*cam2-1* and *cam2-3*), *tch2* (*cml24-2* and *cml24-4*) and *tch3* (*cml12-2*) *Arabidopsis* mutants were used in this study (Tsai *et al.*, 2007). *Arabidopsis* seeds were surface-sterilised with 75% ethanol for 1 min and half-strength bleach for 10 min. Sterilized seeds were cold-treated at 4°C for 2 days before being sown on a growth medium containing half-strength Murashige and Skoog and 1.5% (w/v) sucrose, and solidified with 1.2% (w/v) agar (Sigma, product No. A4675), pH 5.8. The plates were sealed with micropore tape and grown at 22°C with a 16 h light/8 h dark cycle before imaging.

In the present study, mechanical touch stimuli were performed as previously described (Okada and Shimura, 1990). The 2 days cold-treated *Arabidopsis* seeds were sown on the agar surface of the growth medium (each plate with 8 seeds in a row). The plates were placed in racks where they were maintained at 0, 15, 30, 45, and 60° angles from the gravity vector (Fig. 2A), and then incubated at 22°C with a 16 h light/8 h dark cycle before imaging.

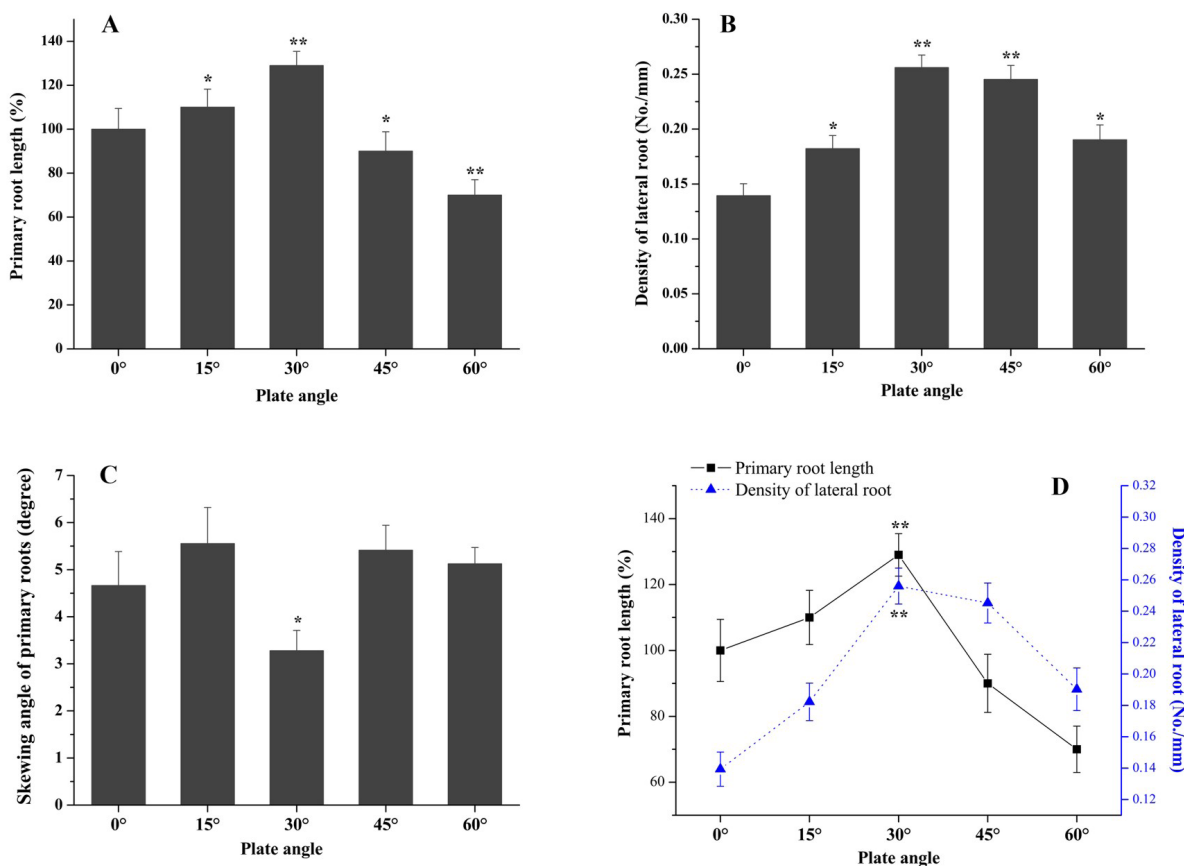
On inclined agar plates, the hard agar surface presents a barrier that mechanically impedes the root, while at the same time downward growth in response to gravity causes the root tip to continuously push against the agar surface. The resulting mechanical cues influence the primary root growth and development and primary root tip direction, as well as the lateral root formation. To test the mechano-response behaviour of *tch1-3* mutants under mechanical

stimuli, we took the average length and skewing angle of primary roots and lateral root density into consideration. The primary root length and root skewing were measured after 7 days of cultivation. Photographic images were taken above each plate with an Olympus FE-320 digital camera. Primary root length (mm) was defined as the actual length starting from the shoot-root junction to the root tip. Root skewing degree was defined as the angle between the gravitational vector and the growth vector starting from the shoot-root junction to the root tip. The positive value was referred to left skewing, while the negative value was referred to right skewing. Density of lateral root (No./mm) was customarily calculated as the ratio between the number of lateral roots and the total length of the primary root after 12 days of cultivation.

Projections of microscopy and all digital measurements were performed with Image J 1.46 software (NIH, USA), and all statistical analyses were performed by IBM® SPSS® Statistics software (IBM, Armonk, NY, USA), and all figures were plotted with Origin 8.5.1 (Origin-Lab Corporation, USA). Statistical analysis was carried out using Student *t* test.

#### RESULTS

In our study, to explore the effects of mechanical touch stimulus on root growth and development, we had to establish an effective mechanical stimulus approach. To this end we chose to cultivate wild-type *Arabidopsis* seeds on 15, 30, 45, and 60° inclined and vertically positioned agar plates. Roots growing along the surface of agar were mechanically stimulated by contact between the root tip and the agar surface, and the level of mechanical touch stimulus could increase with the plate angle (Okada and Shimura, 1990; Thompson and Holbrook, 2004). Quantitative analysis showed that mechanical touch stimulus significantly stimulated primary roots elongation at 30° from the vertical (Fig. 1A). However, the high level of touch stimulation (30° <  $\theta$  < 60°) would inhibit primary roots elongation. Furthermore, as seen in Fig. 1B, mechanical touch stimulus could promote lateral root growth and development, and the DLR was significantly increased at 30° from the vertical. As the characteristic root growth pattern on the surface of agar plate, the skewing growth pattern was reported by numerous studies (Monshausen and Gilroy, 2009b; Oliva and Dunand, 2007; Qi and Zheng, 2013; Wang *et al.*, 2011). In our experiments, the results showed that primary roots exhibited slight, insignificant left skewing at 30° from the vertical (Figs 1C and 3A), indicating that the roots growth direction was along the gravitational vector on inclined plates. Those results show that cultivating seedlings on inclined agar surface is sufficient to develop a mechanical touch stimulus, and at 30° inclined condition the mechanical force was more capable to affect both the primary root elongation and direction and



**Fig. 1.** The wild-type roots response to various angles of hard agar surface (0, 15, 30, 45, and 60° from the vertical). A – average length of primary root, B – density of lateral root, C – skewing angle of primary root, D – both primary roots and lateral roots exhibit significant discrepancies at 30° from the vertical compared to the other groups. Data are means  $\pm$  SD ( $n > 40$ ); \* $p < 0.05$ , \*\* $p < 0.01$ , t test.

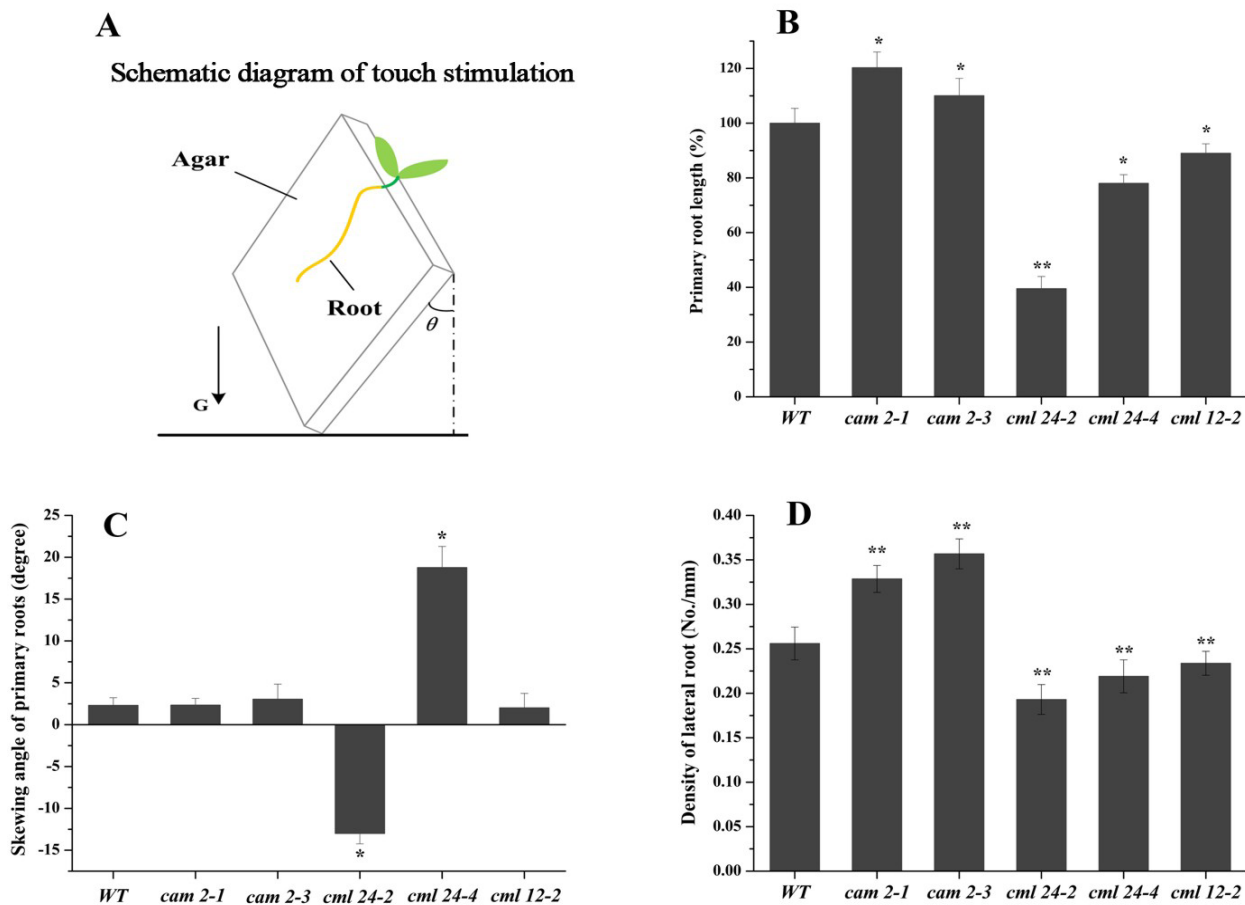
the lateral root formation. Taken together, we selected the 30° from the vertical cultivating environment for subsequent experiments.

According to the results above, we found that the effects of touch stimulus on root growth and development were different, and significant discrepancies were exhibited at 30° from the vertical (Fig. 1D). Therefore, to assess *tch1* (*cam2-1* and *cam2-3*), *tch2* (*cml24-2* and *cml24-4*), and *tch3* (*cml12-2*) responses to mechanical touch stimulation, root growth was analyzed in seedlings growing at 30° from the vertical. When grown on inclined plates, *tch1-3* mutant roots displayed indistinguishable waving patterns from those of WT (Fig. 3), but they were apparent (Okada and Shimura, 1990). Quantitative analysis revealed that primary root length of *tch1* (*cam2-1* and *cam2-3*) mutants was significantly longer ( $p < 0.05$ ) than that of WT (Fig. 2B). However, the primary roots elongation of *tch2* (*cml24-2* and *cml24-4*) and *tch3* (*cml12-2*) was significantly inhibited ( $p < 0.05$  or 0.01) on the inclined agar plates

(Fig. 2B). These suggested that *CML24* and *CML12* were involved in promoting primary root elongation in response to mechanical stimulus.

In addition, the root skewing degree of *tch1-3 Arabidopsis* primary roots exhibited the left- or right-skewing phenotypes in response to mechanical touch stimulus (Figs 2C and 3). However, two functionally disrupted points mutant lines for *tch2*, *cml24-2* and *cml24-4* exhibited significantly right- and left-skewing behaviour relative to that of WT, respectively (Figs 2C, 3E, 3F). In our experiments, all the primary roots presented the waving growth pattern when growing on inclined agar plates, while primary roots of *tch2* mutants appeared to skew towards one side, suggesting that *CML24* and *CML12* inhibited primary root growth direction under mechanical stimuli.

The lateral root is one of the most important organs for plant growth and development. The DLR is widely used in current literature, representing an estimation of overall biomass of the root system (De Smet *et al.*, 2012; Dubrovsky *et al.*, 2006). To explore the effects of mechanical touch stimulus on lateral root growth and development of *tch1-3*



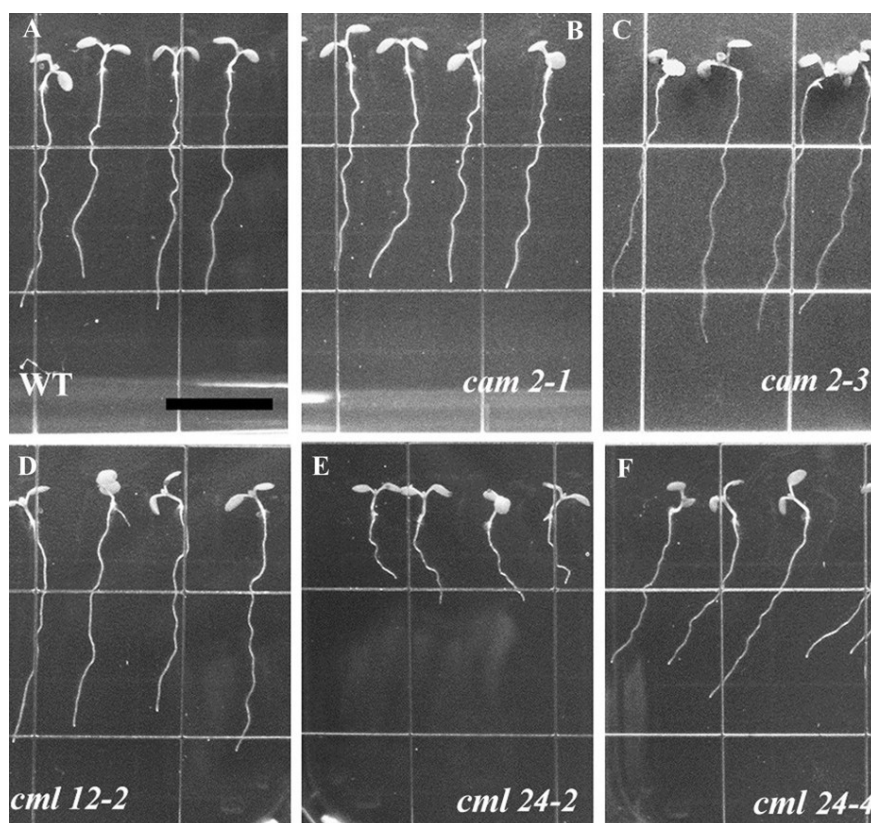
**Fig. 2.** Effects of mechanical touch stimulus on root growth of *tch1-3* mutants. A – schematic diagram of touch stimulation; plates maintained at 0, 15, 30, 45, and 60° angles ( $\theta$ ) between the root growth axis and the gravity vector; B, C, and D – represent primary root length, root skewing and DLR of *tch1-3* and WT on inclined plates, respectively. In panel (C), positive values refer to left skewing, while negative values refer to right skewing. Explanations as in Fig. 1.

mutants, we analyzed the DLR of all mutants after 12-day cultivation. Our results showed that mechanical touch stimulus could increase the DLR of WT at 30° from the vertical (Fig. 1B). However, quantitative analysis showed that the DLR of *tch2* (*cml24-2* and *cml24-4*) and *tch3* (*cml 12-2*) were significantly ( $p < 0.01$ ) decreased (Fig. 2D) on inclined plates (30°), indicating that *CML24* and *CML12* were involved in mechano-touch responses of lateral roots. Remarkably, the DLR of *tch1* (*cam2-1* and *cam2-3*) was significantly ( $p < 0.01$ ) increased at 30° from the vertical (Fig. 2D), so it was possible that the *TCH1* gene defect had no effect on mechanical stimulation of lateral root formation, or it suppressed periodically lateral root formation to some extent; all these need further verification.

#### DISCUSSION

Although more and more reports demonstrate the fundamental function of mechanostimulus in the regulation of plant growth and development, the molecular basis for

plant mechanoresponses is still largely unknown (Chehab and Eich, 2009; Coutand, 2010; Monshausen *et al.*, 2009; Monshausen and Gilroy, 2009a; Telewski, 2006). In the present study, we investigated the touch mechanoresponses of *Arabidopsis* roots, and found that the touch stimulus could affect both the primary root and lateral root behaviour. To further explore the effect of touch stimulus on regulating root growth, *tch1-3 Arabidopsis* mutants were used to reveal the potential functions of *TCH1-3* genes in *Arabidopsis* root mechanoresponses by reverse genetic approach (Tamura *et al.*, 2010). In our mechanical stimulation model, the force acting on roots is a complex resultant, the force arising from the contact between the root tip and the agar surface being beyond quantification. In our experiments we just selected a fixed inclined angle (30° from the vertical), which would develop a sustainable and stable resultant force on roots. Under this condition, we discussed the function of *TCH1-3* in response to mechanical stimuli.



**Fig. 3.** Growth patterns of agar surface-grown primary roots of *tch1-3* mutants and WT *Arabidopsis*. Examples of primary roots of 7-days-old WT (A), *cam2-1* (B), *cam2-3* (C), *cml12-2* (D), *cml24-2* (E) and *cml24-4* (F) grown on agar surfaces. Note the similar waving growth of all the genotypes, and that *cml24-2* roots skew significantly to the right and *cml24-4* roots skew significantly to the left (Bar scale in A-F=10 mm).

Gravity-induced touch stimulus can result in primary root waving and skewing on hard agar surface (Oliva and Dunand, 2007). Although waving and skewing are two growth behaviours usually occurring at the same time, skewing can also be found without obvious waving (Yuen *et al.*, 2005). Our observation also supports the uncoupling of these two behaviours (Fig. 3). All the mutants tested in this work exhibited similar waving behaviours to those of the WT. However, evidence supporting the conclusion that *tch2* root response to mechanical cues comes from the analysis of mechanically stimulated roots growing on tilted agar plates and other mechanostimulus (Braam, 1992; Henry-Vian *et al.*, 1995; Sistrunk *et al.*, 1994; Wang *et al.*, 2011). We have found that *tch2* roots growing on inclined plates appear left- and right skewed compared with those of WT (Fig. 2C, 3E, 3F). These results suggest that the activities of *CML24-2* and *CML24-4* could repress touch-induced root phenotypes. Furthermore, the increased repressions observed in *cml24-2* and *cml24-4* mutants are greater than that of WT, indicating that the *tch2* root is hypersensitive to an increase in mechanical stimulus, and the *tch2* mutants roots make use of a positive regulatory pathway that activates skewing in response to touch stimu-

lus. In other words, the activities of *CML24-2* and *CML24-4* could regulate the *Arabidopsis* root growth behaviour in response to mechanical stimulus.

Noteworthy is the fact that the loss of *CML24* and *CML12* activities has opposing effects on root length. In touch stimulated *tch2* and *tch3* mutant roots, average length of primary roots is shorter while skewing is enhanced, indicating that *CML24* and *CML12* activities promote the elongation and inhibit the skewing of primary roots responding to mechanical cues. This phenomenon could explain the opposing effects of *CML24* and *CML12* on touch-stimulus, and gravity-mediated growth is as follows: the proteins can reduce skewing and promote elongation indirectly, by reinforcing gravity responses when mechanically stimulated (Gleeson *et al.*, 2012). Besides, from Fig. 2 we believe that the sensitivity of *CML12* to mechanical stimulus is possibly lower than that of *CML24*, because the remarkable defective mechanoresponses behaviour of *cml24* is more distinct than that of *cml12*. However, the specific mechanisms need further verification.

The lateral root could improve the capability of the root system to acquire nutrients and water. In addition, the DLR is one of the most important indexes to evaluate the lateral root development (De Smet *et al.*, 2012; Dubrovsky *et al.*,

2006). Previous studies have reported that the lateral root is conducive to plant response to mechanostimulus, especially the mechanical bending (Ditengou *et al.*, 2008; Hamant *et al.*, 2008; Richter *et al.*, 2009). In our experiments, the appropriate mechanical touch stimuli (at 30 and 45° from the vertical) could significantly stimulate ( $p < 0.01$ ) the lateral root development of WT (Fig. 1B), which was consistent with that mentioned above. Moreover, the DLR of *cml24* and *cml12* are lower than those of the others (Fig. 2D), indicating that *CML24* and *CML12* are involved in mechano-touch responses of lateral roots.

Although we did not discover the significant defective mechanoresponses behaviour of *tch1* in our work, the functional redundancy of *CAMs* and *CMLs* family may remedy the defective function of the deficiency of *TCH1* (Delk *et al.*, 2005; McCormack and Braam, 2003; McCormack *et al.*, 2005). Taken together, the normal function of the *TCH* 1-3 genes may be necessary for *Arabidopsis* primary root mechanoresponse in the present study, especially *TCH2* (*CML24-2* and *CML24-4*) and *TCH3* (*CML12-2*), and the potential roles of *CAMs* and *CMLs* in mechano-related physiological events will also be an important future research direction.

#### CONCLUSIONS

1. The 30° from the vertical cultivating condition is the most favourable and efficient to produce a sustainable and stable mechanical touch stimulus in the growing root system of *Arabidopsis*.

2. *TCH2* (*CML24-2* and *CML24-4*) and *TCH3* (*CML12-2*) genes are involved in the response to mechanical stimuli in *Arabidopsis* root system, while the role of *TCH1* in root system mechanoresponses seems to be not important, which still needs further exploration.

#### REFERENCES

- Braam J., 1992.** Regulated expression of the calmodulin-related TCH genes in cultured *Arabidopsis* cells: induction by calcium and heat shock. Proc. National Academy of Sciences, 89(8), 3213-3216.
- Braam J. and Davis R.W., 1990.** Rain-induced, wind-induced, and touch-induced expression of calmodulin and calmodulin-related genes in *arabidopsis*. Cell, 60(3), 357-364.
- Chehab E.W., Eich E., and Braam J., 2009.** Thigmomorphogenesis: a complex plant response to mechano-stimulation. J. Experimental Botany, 60(1), 43-56.
- Coutand C., 2010.** Mechanosensing and thigmomorphogenesis, a physiological and biomechanical point of view. Plant Sci., 179(3), 168-182.
- De Smet I., White P.J., Bengough A.G., Dupuy L., Parizot B., Casimiro I., Heidstra R., Laskowski M., Lepetit M., Hochholdinger F., Draye X., Zhang H., Broadley M.R., Peret B., Hammond J.P., Fukaki H., Mooney S., Lynch J.P., Nacry P., Schurr U., Laplaze L., Benfey P., Beeckman T., and Bennett M., 2012.** Analyzing lateral root development: how to move forward. Plant Cell, 24(1), 15-20.
- Delk N.A., Johnson K.A., Chowdhury N.I., and Braam J., 2005.** *CML24*, regulated in expression by diverse stimuli, encodes a potential Ca<sup>2+</sup> sensor that functions in responses to abscisic acid, daylength, and ion stress. Plant Physiology, 139(1), 240-253.
- Ditengou F. A., Tealea W.D., Kochersperger P., Flittner K.A., Kneuper I., van der Graaff E., Nziengui H., Pinosa F., Li X., Nitschke R., Laux T., and Palme K., 2008.** Mechanical induction of lateral root initiation in *Arabidopsis thaliana*. Proc. Nat. Acad. Sci. USA, 105(48), 18818-18823.
- Dubrovsky J., Gambetta G., Hernandez-Barrera A., Shishkova S., and Gonzalez I., 2006.** Lateral root initiation in *Arabidopsis*: developmental window, spatial patterning, density and predictability. Annals Botany, 97(5), 903-915.
- Gleeson L., Squires S., and Bisgrove S.R., 2012.** The microtubule associated protein END BINDING 1 represses root responses to mechanical cues. Plant Sci., 187, 1-9.
- Hamant O., Heisler M.G., Jonsson H., Krupinski P., Uyttewaal M., Bokov P., Corson F., Sahlín P., Boudaoud A., Meyerowitz E.M., Couder Y., and Traas J., 2008.** Developmental patterning by mechanical signals in *Arabidopsis*. Science, 322(5908), 1650-1655.
- Henry-Vian C., Vian A., Davies E., Ledoigt G., and Desbiez M.O., 1995.** Wounding regulates polysomal incorporation of hsp70 and *tch1* transcripts during signal storage and retrieval. Physiologia Plantarum, 95(3), 387-392.
- Lee D., Polisenky 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.
- Massa G.D. and Gilroy S., 2003.** Touch modulates gravity sensing to regulate the growth of primary roots of *Arabidopsis thaliana*. Plant J., 33(3), 435-445.
- McCormack E. and Braam J., 2003.** Calmodulins and related potential calcium sensors of *Arabidopsis*. New Phytologist, 159(3), 585-598.
- McCormack E., Tsai Y.C., and Braam J., 2005.** Handling calcium signaling: *Arabidopsis* CaMs and CMLs. Trends Plant Sci., 10(8), 383-389.
- Migliaccio F., Tassone P., and Fortunati A., 2013.** Circumnutation as an autonomous root movement in plants. Am. J. Bot., 100(1), 4-13.
- Monshausen G.B., Bibikova T.N., Weisenseel M.H., and Gilroy S., 2009.** Ca<sup>2+</sup> regulates reactive oxygen species production and pH during mechanosensing in *Arabidopsis* roots. Plant Cell, 21(8), 2341-2356.
- Monshausen G.B. and Gilroy S., 2009a.** The exploring root – root growth responses to local environmental conditions. Current Opinion Plant Biology, 12(6), 766-772.
- Monshausen G.B. and Gilroy S., 2009b.** Feeling green: mechanosensing in plants. Trends in Cell Biol., 19(5), 228-235.
- Okada K. and Shimura Y., 1990.** Reversible root-tip rotation in *Arabidopsis* seedlings induced by obstacle-touching stimulus. Science, 250(4978), 274-276.
- Oliva M. and Dunand C., 2007.** Waving and skewing: how gravity and the surface of growth media affect root development in *Arabidopsis*. New Phytologist, 176(1), 37-43.
- Qi B. and Zheng H., 2013.** Modulation of root-skewing responses by KNAT1 in *Arabidopsis thaliana*. The Plant J., 76(3), 380-392.

- Richter G.L., Monshausen G.B., Krol A., and Gilroy S., 2009.** Mechanical stimuli modulate lateral root organogenesis. *Plant Physiology*, 151(4), 1855-1866.
- Rutherford R. and Masson P.H., 1996.** *Arabidopsis thaliana* sku mutant seedlings show exaggerated surface-dependent alteration in root growth vector. *Plant Physiology*, 111(4), 987-998.
- Silverberg J.L., Noar R.D., Packer M.S., Harrison M.J., Henley C.L., Cohen I., and Gerbode S.J., 2012.** 3D imaging and mechanical modeling of helical buckling in *Medicago truncatula* plant roots. *Proc. National Academy of Sciences*, 109(42), 16794-16799.
- Sistrunk M.L., Antosiewicz D.M., Purugganan M.M., and Braam J., 1994.** *Arabidopsis tch3* encodes a novel Ca<sup>2+</sup> binding-protein and shows environmentally-induced and tissue-specific regulation. *Plant Cell*, 6(11), 1553-1565.
- Tamura W., Hidaka Y., Tabuchi M., Kojima S., Hayakawa T., Sato T., Obara M., Kojima M., Sakakibara H., and Yamaya T., 2010.** Reverse genetics approach to characterize a function of NADH-glutamate synthase1 in rice plants. *Amino Acids*, 39(4), 1003-1012.
- Telewski F.W., 2006.** A unified hypothesis of mechanoperception in plants. *Am. J. Bot.*, 93(10), 1466-1476.
- Thompson M.V. and Holbrook N.M., 2004.** Root-gel interactions and the root waving behavior of *Arabidopsis*. *Plant Physiology*, 135(3), 1822-1837.
- Tsai Y.-C., Delk N.A., Chowdhury N.I., and Braam J., 2007.** *Arabidopsis* potential calcium sensors regulate nitric oxide levels and the transition to flowering. *Plant Signaling Behavior*, 2(6), 446-454.
- Wang Y., Wang B., Gilroy S., Chehab E.W., and Braam J., 2011.** CML24 is involved in root mechanoreponses and cortical microtubule orientation in *Arabidopsis*. *J. Plant Growth Regulation*, 30(4), 467-479.
- Yuen C.Y.L., Sedbrook J.C., Perrin R.M., Carroll K.L., and Masson P.H., 2005.** Loss-of-function mutations of ROOT HAIR DEFECTIVE3 suppress root waving, skewing, and epidermal cell file rotation in *Arabidopsis*. *Plant Physiology*, 138(2), 701-714.