Chapter 4

Rice, a monocot grass shows thigmonorphogenesis and various physiological adaptations in response to touch.

Chapter 4:

Rice, a monocot grass shows thigmonorphogenesis and various physiological adaptations in response to touch.

4.1 Abstract:

Rice (*Oryza sativa*), is a monocot crop plant. There are very few reports on effect of mechanical stress on growth of monocot plants. In this chapter we have focused on studying effect of regular mechanical stress on morphological, physiological and anatomical adaptations of rice seedlings. Regular mechanical stress in the form of touch suppressed overall growth of leaves and root of rice seedlings. An immediate increase in levels of reactive oxygen species and decrease in chlorophyll content was observed upon touch stimuli. We have also found that mechanical stress induces expression of jasmonic acid responsive genes. Present study provides evidences of various adaptation to touch stimuli in monocot plant rice.

4.2. INTRODUCTION:

Rice (*Oryza sativa*), is an annual grass which is grown for its edible seeds. Rice is a major staple food of Asian, American and African population (Pillai and Patlavath 2020). It takes five months from the day of sowing to harvest final crop. During cultivation, the rice crop has constant threat from many insect pests namely brown plant hopper, leaf folder, stem borer, grain sucking insect etc (book chapter). Chemical pesticides are used to control these pest attack. However, only one percentage of the sprayed pesticide reaches the harmful pests. On other hand many beneficial insects are killed by these chemical pesticides. With increase in awareness of harmful effects of chemical pesticides and the benefits of organic products, farmers are switching to organic farming. Organic rice cultivation includes practice of natural substances and eco-friendly techniques for growing crop and pest management. Thus, a lot of research is being done in the area of integrated pest management to develop new chemical free tools and techniques for pest control.

Mechanical stress like touch treatment induces reactive oxygen species (ROS), expression of jasmonic acid/ ethylene responsive genes and defense response genes. Adaptations to mechanical stress also provides cross-tolerance to biotic and abiotic stress. Studies in Arabidopsis shows that regular touch treatment enhanced resistance against necrotrophic fungi, *Botrytis cinerea.* In lettuce crop regular touch treatment reduced feeding by cabbage looper pest, *Trichoplusia ni* (Chehab et al 2012). This resistance to pest and fungi was found to be jasmonic acid dependent. Other crop plants namely maize, potato, tomato, wheat, mentha, mulberry etc have been studied for mechanical stress induced cross tolerance to biotic and abiotic stress (Markovic et al. (2014, 2019), Keller and Steffen (1995), Suge (1980), Si et al. (2019), Ghalkhani et al. (2020), Current knowledge of morphological adaptations and gene expression analysis is being applied in agriculture for development of new methods of pest management. In present study, we have made first attempt to identify adaptations of rice seedlings to regular mechanical stress in the form of touch. We have found regular touch treatment to rice seedlings leads to reduced growth and chlorophyll content. Touch treatment enhanced ROS production and expression of JA responsive gene, *OsJAZ8* and *OsWRKY42*. Overall, this is a preliminary study in a monocotyledon plant, *Oryza sativa,* rice plant which display similar thigmomorphogenic responses as reported for dicotyledonous plants.

4.3. MATERIAL AND METHODS:

4.3.1. Plant growth and treatments:

Oryza sativa indica cultivar Taichung native-1(TN-1) seeds were obtained as research gift from Dr. Ramesh V. Sonti, National Institute of Plant Genome Research (NIPGR), India. TN1 seedlings were grown under laboratory conditions. For this seed were sterilized using sterilization solution (90% ethanol; 0.1% SDS) and washed multiple times with water. Seeds were directly germinated over filer paper in petri plates in dark at 28^{o}C for 2 days. Germinated seeds were transferred to soil and grown in light under laboratory conditions. Mechanical stress experiments were carried out on two- weeks-old seedlings. Plants were touched with fingers at 24 hrs intervals for fifteen days (Chehab et al 2012). Seedlings were monitored for morphological changes.

4.3.2. Detection of ROS production:

The accumulation of reactive oxygen species (ROS) in leaves was detected by staining with DAB (Daudi *et al* 2012). Leaves were harvested at different time point after touch treatment (0, 15, 30, 60 min) and immersed in DAB solution in test tubes. The leaves were left in the staining solution for 8-12 hr in dark by covering the tubes with aluminium foil (since DAB is light-sensitive). Following the incubation, the DAB solution was replaced with bleaching solution and kept in boiling water bath for 15 min. After complete removal of chlorophyll, the bleach solution was replaced with fresh bleaching solution. Leaves were directly visualized and photographed. The intensity and the amount of the brown DAB precipitate was semi-quantified from multiple images using Image J software. The average value obtained from 3-5 leaves from different plants were compared for each sample. Similar three independent experiments were performed.

4.3.3. Chlorophyll estimation:

For chlorophyll estimation, touched and untouched leaves (n=5) were harvested after 15 days of touch treatment. The leaves were weighed and then crushed in 95% acetone. The absorbance was measured at 663nm and 645 nm using Spectrophotometer (Eppendorf), and the absolute chlorophyll content was calculated using formulas (Arnon 1949):

Chlorophyll a = $11.75\ A_{662.6} - 2.35\ A_{645.6}$

Chlorophyll b = $18.61\ A_{645.6} - 3.96\ A_{662.6}$

Total= Chlorophyll a + Chlorophyll b

The average value obtained from three seedlings was compared for each sample. Three such independent experiments were performed.

4.3.4. Bioinformatic analysis of TCH genes:

The cDNA sequence of Arabidopsis TCH genes namely *AtTCH-1(CAM2), AtTCH-2* and -*AtTCH-3* were obtained from TAIR using accession no. AT2G41110.2, AT5G37770.1 and AT2G41100.1 respectively. For the three Arabidopsis TCH genes, the orthologues in rice were identified using the BLASTn algorithm at Rice genome annotation project (RGAP) website (http://rice.uga.edu/) (Altschul 1997). The rice genes with highest identity (>50%) were further.

4.3.5. RT-PCR and Quantitative PCR analysis:

The expression of predicted TCH gene orthologues from rice were studied using qPCR at thirty minutes after touch treatment. Primers were designed using Quant prime tool (Table 3.2). For this total RNA was extracted from control and touch treated plant tissue using TRIzol reagent (Thermo Fisher Scientific). Briefly the entire seedling was frozen in liquid nitrogen and crushed in TRIzol. For cDNA synthesis 1 μg of the total RNA was subjected to single strand synthesis using Oligo dT primer (Invitrogen). The cDNA was diluted ten times and used for qPCR analysis using SYBR green/ROX Master Mix (Thermo Fisher Scientific) on the 7900 HT sequence detection system (Applied Biosystem). *OsGAPDH* was used as internal control. The fold change in expression for two JA responsive genes (*OsJAZ*8 & *OsWRKY42*) were compared between untouched (control) and touched seedling was calculated using the $2^{-\Delta\Delta Ct}$ formula (Livak and Schmittgen, 2001). Such three replicates were used for calculating the average fold change.

4.3.6. Statistical analysis:

All the experiments were repeated in more than three independent biological replicates. For significance, all the data were analysed with Student's *t*-test for independent means using Microsoft Excel software.

4.4. RESULTS:

4.4.1. Regular mechanical stress suppresses growth of rice seedlings

To understand effect of touch on growth of rice seedlings, touch treatment was performed on one-week-old seedlings (two leaf stage). Rice seedlings (n=20 seedlings) were given mild touch twice a day for fifteen days and were monitored for phenotypic changes (Figure 4.1). A significant reduction in total growth was observed in all three replicates (Figure 4.2a). As rice is a monocot plant, the shoot and leaves are not distinguishable. So, we measured fresh weight of whole seedlings, above soil portion (leaves) and below soil portion (root). The total weight of entire seedling, leaves and adventitious root were found to be significantly lower in regularly touched plants as compared to the control plants (Figure 4.2 b) ($p<0.05$). This experiment was performed three times and each experiment had three sets (n=20) for control and test. Similar results were observed in all experiments.

4.4.2. Regular mechanical stress induces production of ROS in rice seedlings

One week old rice seedlings were given mechanical stress in the form of touch and collected at different time (0, 15, 30, 60 minutes) for assaying ROS production. Collected rice leaves were immediately processed for DAB staining and photographed using stereo microscope. Using Image-J software the brown precipitate was quantitated from all the leaf images. An increase in ROS production was observed after mechanical stimuli ($p<0.05$) (Figure 4.3a-b). This experiment was performed three times and each experiment had three sets (n=5) for each time point. Similar results were observed in all experiments.

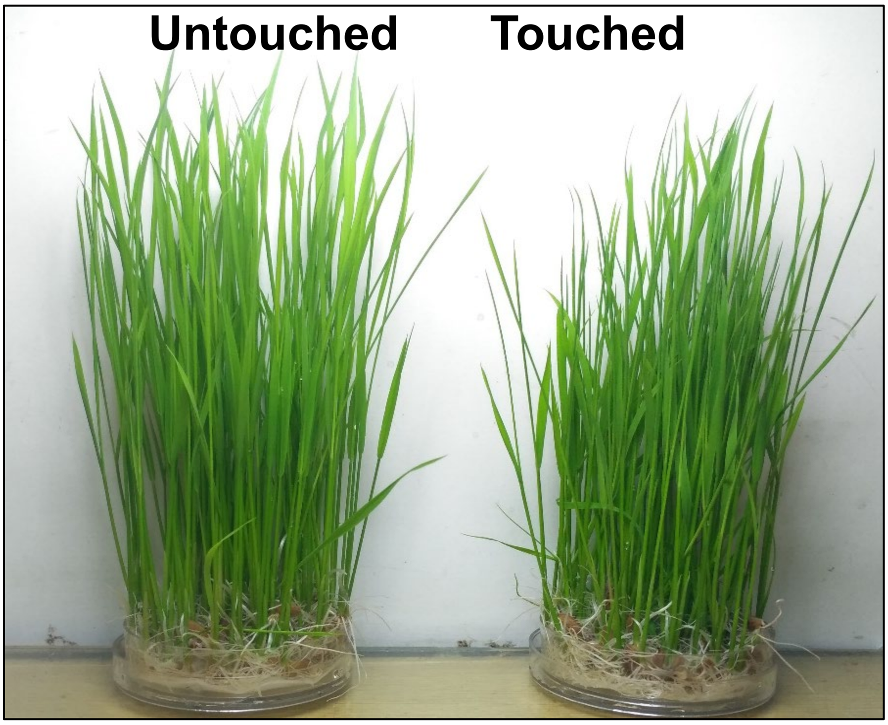


Figure 4.1: Regular touch treatment suppressed growth rice seedlings:

For assaying effect of touch on plant growth, one-week-old rice seedlings (n=15-20) were given touch treatment for 15 days.

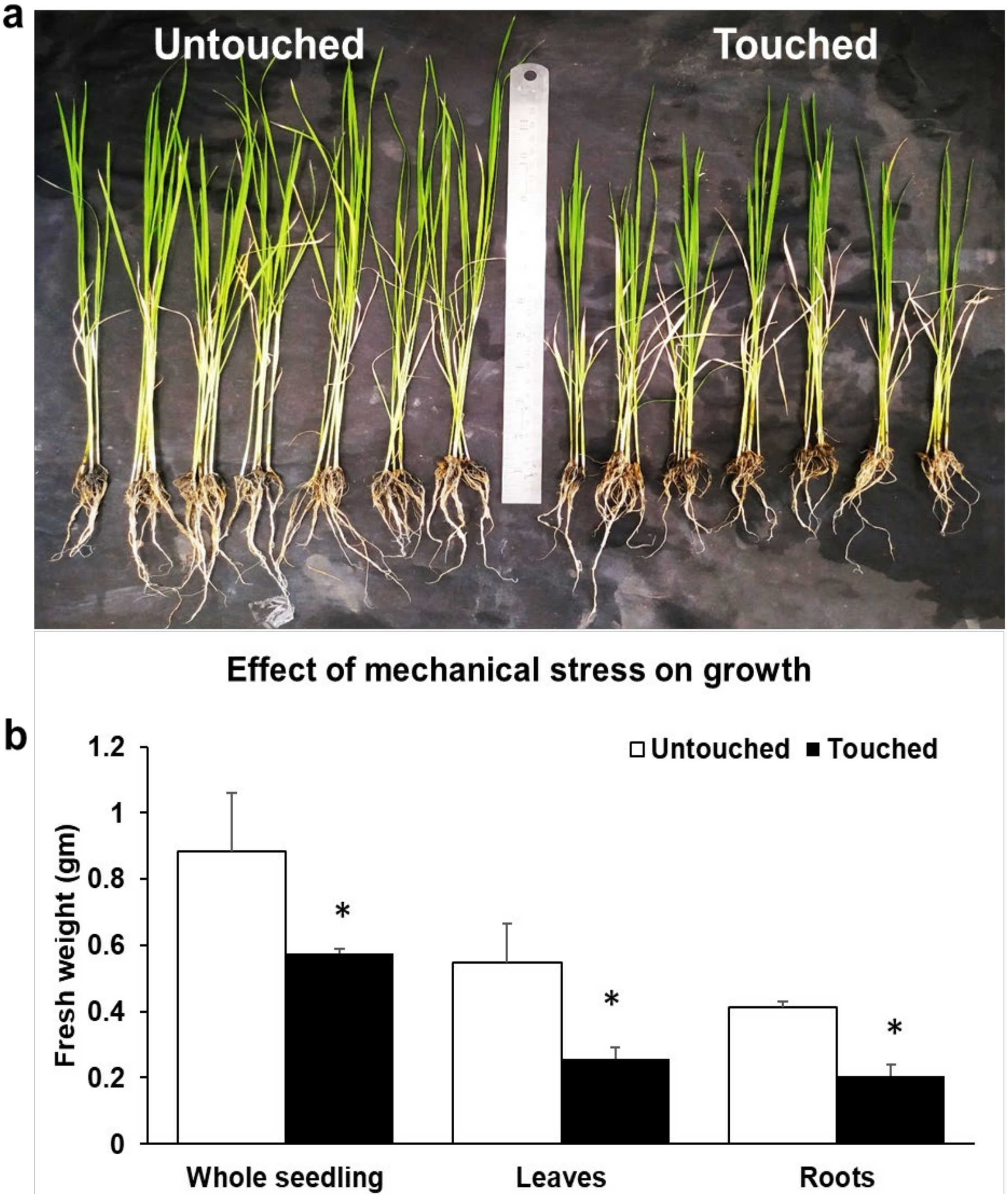


Figure 4.2: Regular Touch treatment suppresses stem, root and leaf growth in rice seedlings. *For assaying effect of touch on plant growth, one-weeks-old rice seedlings (n=15-20) were given touch treatment for 15 days and were observed for effect on shoot and root growth as shown in (a). (b)The fresh weights of whole seedlings, leaves and roots were measured for both touch treated and untreated seedlings. All the above data was analysed using Student's T-test. The asterisk on the bar indicates significant differences with a p-value less a p-value less than 0.05. Similar results were observed in three independent experiments.*

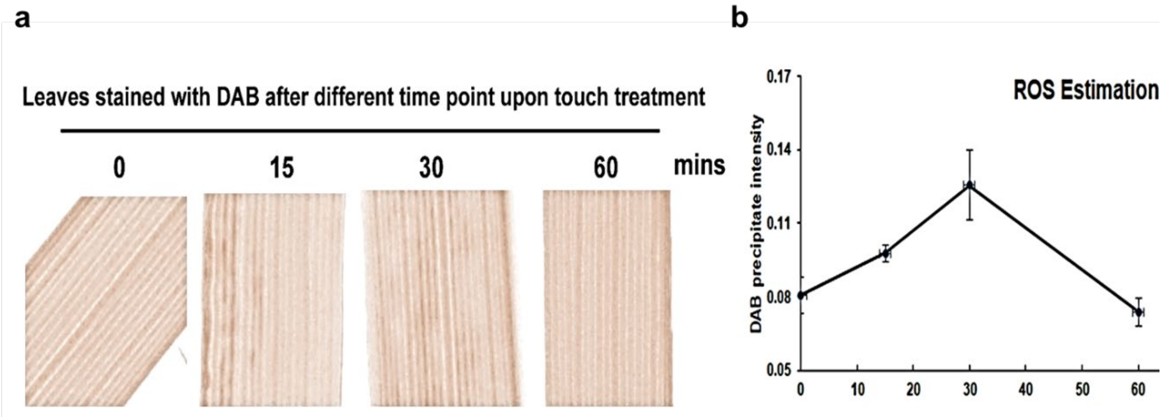


Figure 4.3: Touch treatment induces ROS production in rice seedlings*: For assaying ROS production, one-weeks-old seedlings were given touch stimuli and harvested at different time points (0,15, 30, 60, 120 minutes). (a) Leaves were stained using DAB and photographed. (b) The brown precipitate formed after reaction of DAB with ROS was quantitated using ImageJ software. Similar results were observed in three independent experiments.*

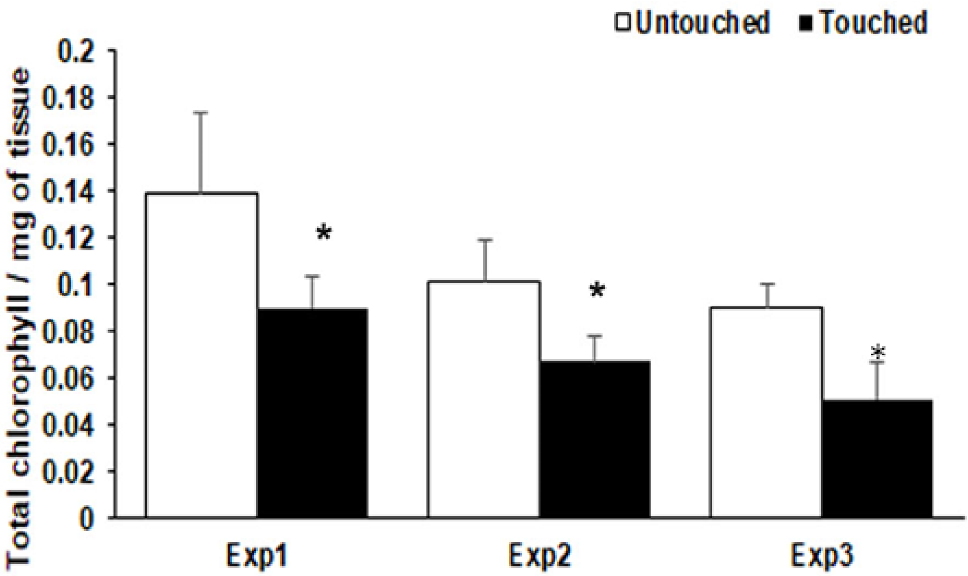


Figure 4.4: Touch treatment alters chlorophyll a/b ratio: *For assaying effect of touch on chlorophyll content, leaves were harvested after 15 days of touch treatment. The total chlorophyll was extracted in acetone and estimated using spectrophotometer. The above data was analysed using Student's T-test. The asterisk on the bar indicates significant differences with a p-value less than 0.05. Similar results were observed in three independent experiments.*

4.4.3. Regular mechanical stress reduces total chlorophyll content in rice seedlings:

For estimation of total chlorophyll leaves were harvested after fifteen days of mechanical stress treatment. The total chlorophyll content was measured using spectrophotometer (Arnone 1949). The total chlorophyll content for touched plants were found to be significantly reduced as compared to that of control rice seedlings (p<0.5) (Figure 4.4). This experiment was performed three times and each experiment had three sets (n=20) for control and test. Similar reduction in chlorophyll content was observed in all

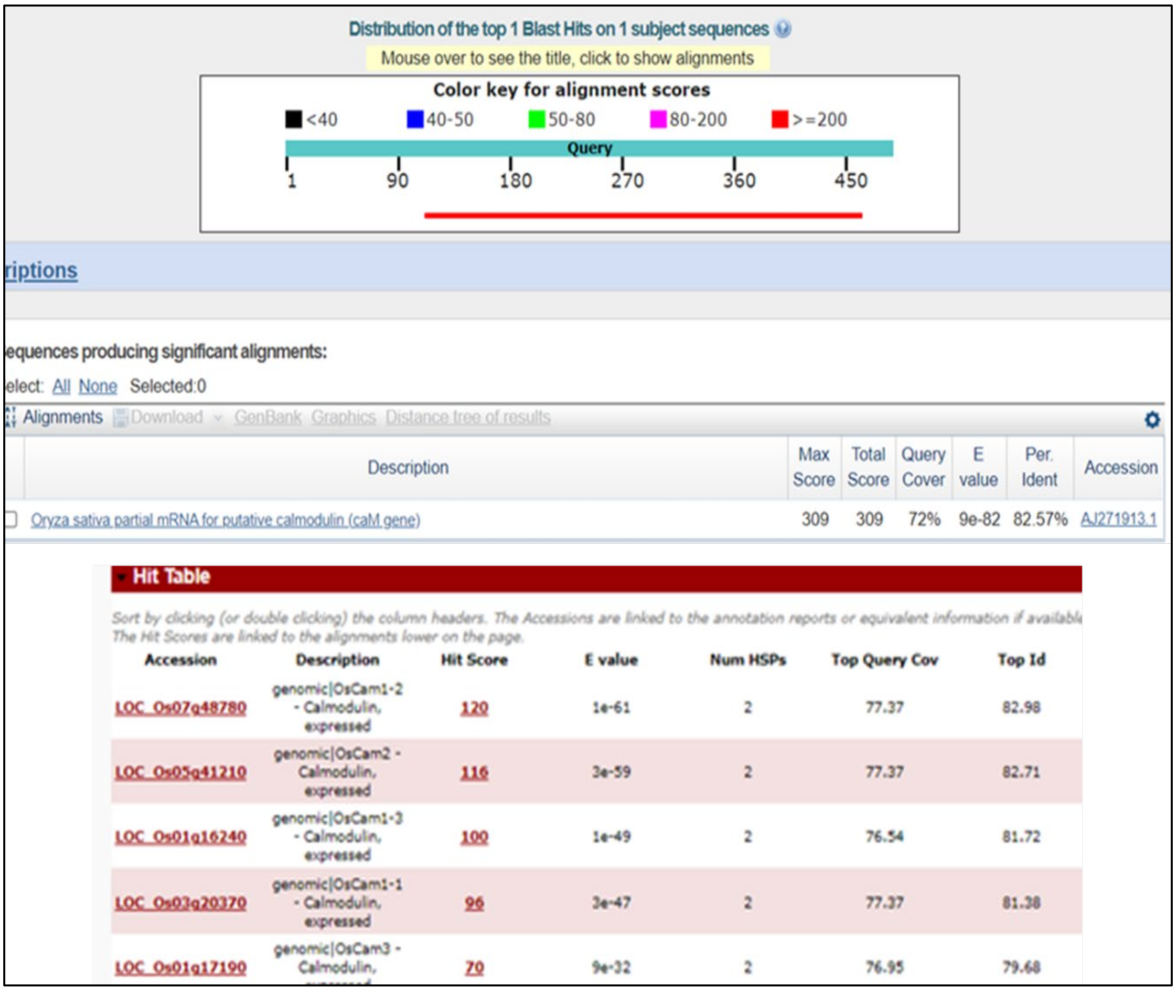


Figure 4.5: Identification of orthologues for TCH1 gene: *The Arabidopsis TCH1 gene orthologues in rice identified using the BLAST analysis. (a) shows the output for AtTCH1sequence search in NCBI and (b) shows the output in rice genome database, RGAP.*

experiments.

4.4.4. Identification of Arabidopsis TCH gene orthologues in rice

Touch responsive genes are expressed after mechanical stress in Arabidopsis. Among the touch induced genes *AtTCH1*, *AtTCH2* and *AtTCH3* are found to be expressed under different forms of mechanical stress (). In order to study the molecular response of mechanical stress in rice we have attempted to identify these TCH genes orthologues in rice using bioinformatic analysis. For this, FASTA sequence of the Arabidopsis TCH-1, -2, and -3 were individually subjected to BLAST analysis in NCBI data base. For *AtTCH1* cDNA sequence a single orthologue was identified Using NCBI tool 82% identity (Figure 4.5a). We subjected the same sequence for BLAST analysis in RGAP website where many orthologues were identified. We designed primer for top three hits with high score namely

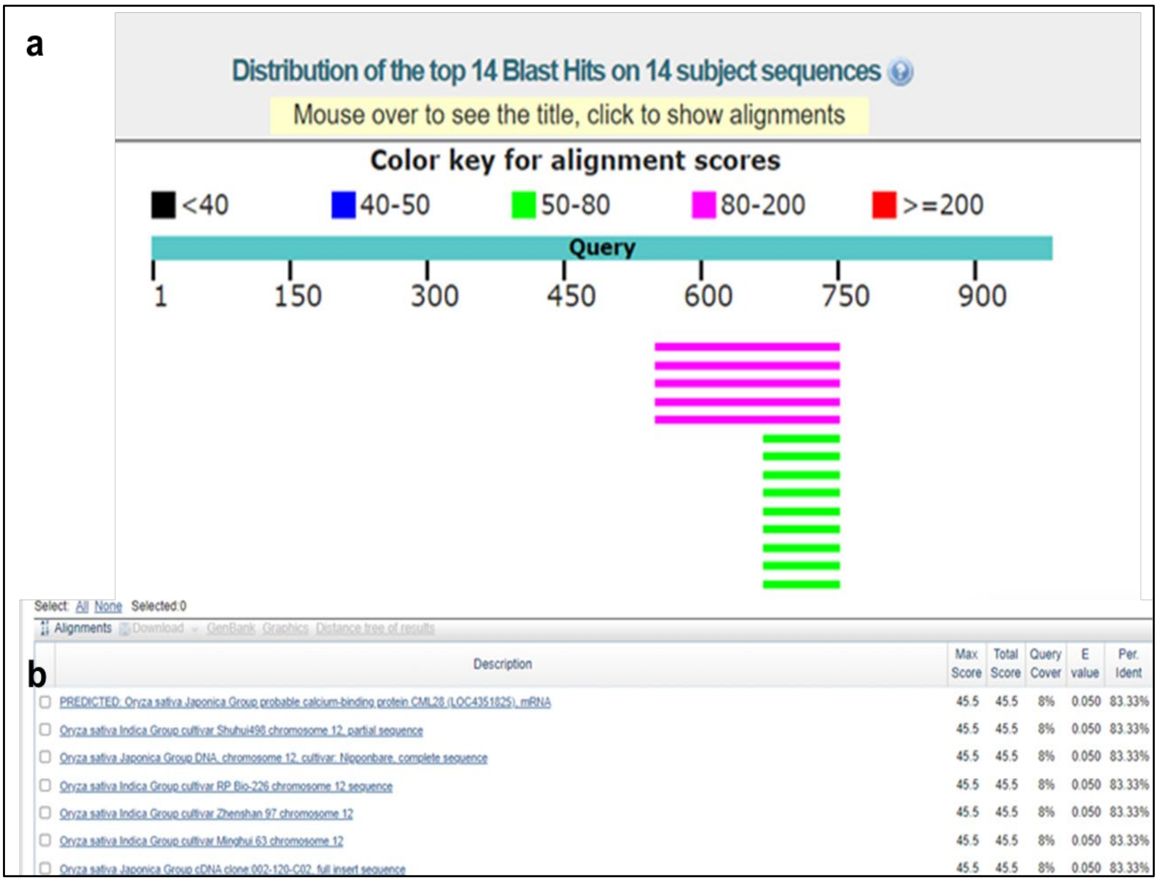
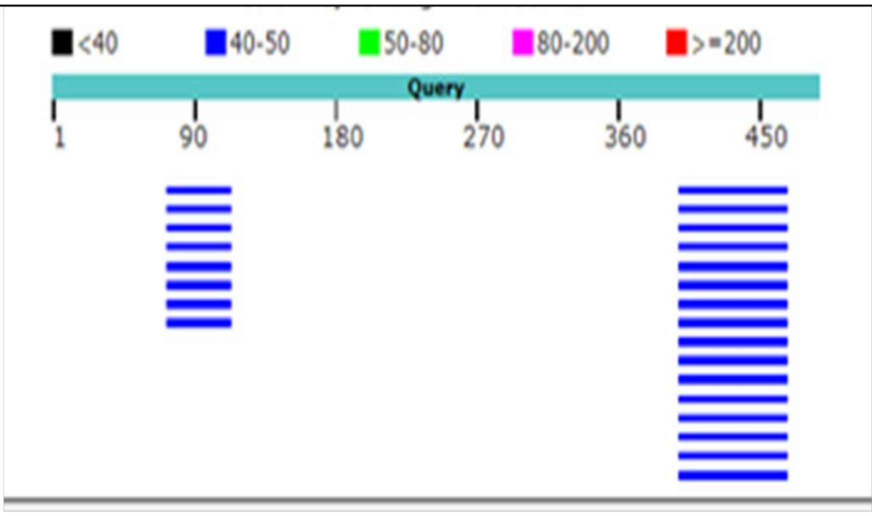


Figure 4.6: Identification of orthologues for TCH2 gene: *The Arabidopsis TCH2 gene orthologues in rice were searched using the BLAST analysis. No gene with more than 50% identity were found in the search output.*

(*OsCaM1-2, OsCaM3* and *OsCaM1-3*) (Figure 4.5b). For *AtTCH2 and -3*, no orthologues were obtained in rice gene database with more than 50% identity (Figure 4.6 and 4.8). The search for TCH2 genes identified hits with only 8% query coverage which had alignment only in small region of the query from 500th to 750th base (Figure 4.6). Similarly, the search for TCH3 genes identified hits with only 20% query coverage which had alignment with two small regions of the query (Figure 4.7) The putative *TCH1* orthologues were studied for gene expression upon touch treatment in rice.

4.4.5. The identified three putative TCH1 orthologues are not expressed after mechanical stimuli in rice seedling:



Description	Max Score	Total Score	Query Cover	E value	Per. Ident
PREDICTED: Oryza sativa Japonica Group dihydrofolate reductase-like (LOC4344343), misc_RNA	104	104	20%	9e-20	76.24%
Oryza sativa (indica cultivar-group) cDNA clone:OSIGCEA030M14, full insert sequence	104	104	20%	9e-20	76.24%
Oryza sativa clone 15079 calmodulin (CaM) mRNA, complete cds	104	104	20%	9e-20	76.24%
Oryza sativa Japonica Group cDNA clone:J023117O08, full insert sequence	104	104	20%	9e-20	76.24%
O.sativa gene encoding calmodulin	104	104	20%	9e-20	76.24%
Oryza sativa Indica Group cultivar Shuhui498 chromosome 7, partial sequence	73.1	73.1	8%	3e-10	83.13%
Oryza sativa Japonica Group DNA, chromosome 7, cultivar: Nipponbare, complete sequence	73.1	73.1	8%	3e-10	83.13%
Oryza sativa Indica Group cultivar RP Bio-226 chromosome 7 sequence	73.1	73.1	8%	3e-10	83.13%
Oryza sativa Indica Group cultivar Zhenshan 97 chromosome 7	73.1	73.1	8%	3e-10	83.13%

Figure 4.7: Identification of orthologues for TCH3 gene: *The Arabidopsis TCH3 gene orthologues in rice were searched using the BLAST analysis. No gene with more than 50% identity were found in the search output.*

The gene expression of the above identified putative TCH1 gene orthologues (*OsCaM1-2, OsCaM3* and *OsCaM1-3*) was studied by quantitative PCR at 30 and 60 minutes in rice seedlings. At both the time points 30 and 60 minutes after touch treatment the Ct values of all three genes for touched and untouched plants were comparable no change in expression was found (data not shown).

4.4.6. FASTA Sequences of Rice TCH genes used for Bioinformatic Analysis

Arabidopsis TCH genes sequence mentioned in Chapter 2 (Material and methods: FASTA) was used to identify following rice TCH gene orthologue.

>LOC_Os07g48780 (OsCam1-2)

GACAAGGACGGCGATGGTTGCATCACAACCAAGGAGTTGGGAACTGTCATGCGTTCACTA
GGGCAGAACCCAACGGAAGCTGAGCTCCAGGACATGATCAACGAGGTTGATGCTGATGGC
AATGGAACCATTGATTTTCCTGAGTTTCTCAATCTGATGGCTCGCAAGATGAAGGACACT
GATTCAGAGGAAGAACTCAAGGAGGCCTTCCGGGTGTTTGACAAGGACCAAAATGGCTTC
ATCTCCGCTGCTGAGCTCCGCCATGTGATGACAAATCTTGGCGAGAAGCTAACTGACGAG
GAGGTGGATGAGATGATCCGTGAGGCTGATGTTGATGGTGATGGTCAGATAAACTATGAG
GAGTTTGTGAAGGTCATGATGGCCAAGTGA

>LOC_Os05g41210 (OsCam2)

ATGGCGGACCAGCTCACCGACGAGCAGATCGCCGAGTTCAAGGAGGCGTTCAGCCTCTTC
GACAAGGACGGCGACGGTTGCATCACTACTAAGGAGCTTGGAACCGTGATGCGGTCCCTT
GGTCAGAACCCAACTGAGGCGGAGCTGCAGGACATGATCAACGAGGTTGATGCTGATGGC
AATGGGACCATTGACTTCCCAGAGTTCCTGAACCTGATGGCGAAGAAGATGAAGGATACC
GACTCTGAGGAGGAGCTCAAGGAGGCCTTCCGTGTGTTTGACAAGGACCAGAACGGTTTC
ATCTCGGCTGCTGAGCTCCGCCACGTCATGACCAACCTTGGTGAGAAGCTGACCGACGAG
GAAGTCGACGAGATGATCCGTGAGGCTGACGTCGATGGCGATGGCCAGATCAACTACGAG
GAGTTCGTTAAGGTCATGATGGCCAAGTGA

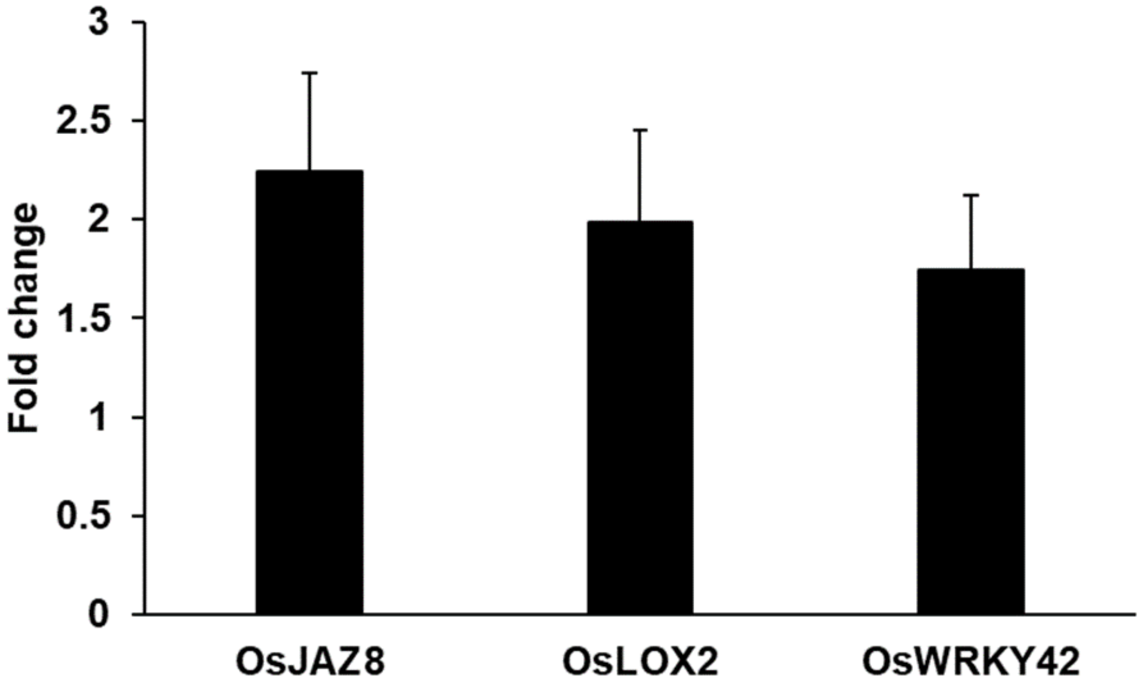


Figure 4.8: Mechanical stress induces expression of JA responsive genes in rice seedlings
The expression of the JA responsive genes was studied by quantitative PCR. For this experiment rice leaves were given touch treatment and collected after 60 minutes. The graph represents relative fold change which was calculated over untouched leaves. OsGAPDH was used as internal control. The graph represents the mean of three set of replicates and error bar represents standard deviation.

4.4.7. Mechanical stress induced expression of JA responsive genes in rice seedlings.

In Arabidopsis, it was reported that mechanical stress induces expression of many JA responsive genes. In order study role of JA mediated response in rice under mechanical stress, we tested expression of three JA marker genes namely *OsJAZ8, OsLOX2* and *OsWRKY42* which is a rice transcription factor expressed in rice under various biotic and abiotic stress conditions. The expression of all the above genes were tested using quantitative PCR at 60 minutes after giving mechanical stress. The expression of *OsJAZ8*, *OsLOX2* and *OsWRKY42* were found to be increased by approximately 1.5-fold as compared to the untouched controls.

Table 4.1: List of rice gene primer sequences used for qPCR analysis

Primers used for qPCR analysis		
OsJAZ8 F	GAAGGCTCAACAGCTGACCATAT	Jha et al., 2010
OsJAZ8 R	TTGGTGGACGGGAAGTTCTC	Jha et al., 2010
OsLOX2 F	TGGAGGGCATAACTTGGTCGAG	Jha et al., 2010
OsLOX2R	TGCGTAGTCTTCTACCGTAATCCG	Jha et al., 2010
OsWRKY42F	ACGACTATTCGTGGCGCAAG	Pillai et al 2018
OsWRKY42R	CTTGTAGTACCCGCGTGGATAAG	Pillai et al 2018
OsGAPDHF	GAGTATGATGAGTCGGGTCCAG	Jha et al., 2010
OsGAPDHR	ACACCAACAATCCCAAACAGAG	Jha et al., 2010
OsCaM3F	GCGACGGTTGCATCACTACTAAGG	This work
OsCaM3R	TCAGTTGGGTTCTGACCAAGGG	This work
OsCaM1-2F	GATGGTTGCATCACAACCAAGGAG	This work
OsCaM1-2R	GTTGGGTTCTGCCCTAGTGAAC	This work

4.5 DISCUSSION:

Plants undergo various physiological and anatomical changes that help them to adapt and survive mechanical stress condition (Telewski Jaffe 1986). Regular mechanical stress in the form of touch suppressed overall shoot growth of *C. cajan* seedling (chapter 2). Consistent touch treatment had a similar effect on overall growth of rice seedlings. The growth of leaves and adventitious roots of rice seedlings were suppressed upon regular touch treatment.

In response to biotic and abiotic environmental stress, plants accumulate reactive oxygen species which act as immediate mobile signalling molecules (Miller et al., 2009; Choi et al., 2016). 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). Mechanical stimuli in tomato and soybean results in increased levels of ROS

(Yahraus et al 1995). In rice seedlings, level of ROS was detected at 15 minutes after touch stimuli which gradually increased and reached a peak at 30 minutes.

The ratio of Chl-a/b is reported to change depending upon the external environment and internal cellular conditions like light intensity, nitrogen availability, salinity, drought and oxidative stress (Gamon et al., 1990). Studies performed in several plant species indicates mechanical stress either increases or decreases the total chlorophyll content of the leaves (Biddington and Dear man 1985, Mitchell et al 1975, Porter et al 2009). In rice seedling, the total chlorophyll content was significantly reduced upon touch treatment

Gene expression studies in Arabidopsis indicates changes in expression of many CaMs and CML molecules within 30 min of touch stimulation (Braam and Davis 1990; Lee et al 2005). In Arabidopsis, Braam and co-workers have extensively worked on few of the touch induced CaM and CML also as known as TCH genes (Braam and Davis 1990). In our work we have attempted to identify rice orthologues of three TCH genes namely *AtTCH1* (calmodulin; CaM2), *AtTCH2* (CML24) and *AtTCH3* (CML12). Rice is a monocot plant which is evolved after dicot plants like Arabidopsis and Cajanus cajan. The identified orthologue of TCH1 gene showed no change in expression level upon touch stimuli in rice leaves. It has been reported that mechanical stress leads to increase in levels of a plant stress hormone, jasmonic acid (JA) and induces the expression of JA responsive genes which suppresses plant growth (Lee et al, Chehab et al 2012, Chehab et al 2008). Studies in Arabidopsis indicate that treatment with JA inhibits growth of plant (Pillai et al 2018). Thus, we tested levels expression few JA responsive genes viz, *OsLOX2, OsJAZ8* and *OsWRKY42*. The expression of all three JA responsive genes were induced at 30 minutes after touch treatment. This indicates involvement of JA responsive genes in both dicot and monocot plants.

4.6. CONCLUSIONS:

In present study on rice, we have found that regular mechanical stress induces various morphological and physiological adaptations. The touch induced phenotypes identified in present study can be used as marker for mechanical stress in rice for future studies.

4.7 REFERENCES:

1. Markovic D, Colzi I, Taiti C, Ray S, Scalone R, Gregory Ali J, Mancuso S, Ninkovic V. 2019. Airborne signals synchronize the defenses of neighboring plants in response to touch. Journal of Experimental Botany 70, 691–700.
2. Markovic D, Glinwood R, Olsson U, Ninkovic V. 2014. Plant response to touch affects the behaviour of aphids and ladybirds. Arthropod-Plant Interactions 8, 171–181.
3. Si T, Wang X, Huang M, Cai J, Zhou Q, Dai T, Jiang D. 2019. Double benefits of mechanical wounding in enhancing chilling tolerance and lodging resistance in wheat plants. Plant Biology 21, 813–824.
4. Suge H. 1980. Dehydration and drought resistance in Phaseolus vulgaris as affected by mechanical stress. Reports of the Institute for Agricultural Research Tohoku University 31, 1–10.
5. Keller E, Steffen KL. 1995. Increased chilling tolerance and altered carbon metabolism in tomato leaves following application of mechanical stress. Physiologia Plantarum 93, 519–525.
6. Ghalkhani E, Hassanpour H, Niknam V. 2020. Sinusoidal vibration alleviates salt stress by induction of antioxidative enzymes and anatomical changes in Mentha pulegium (L.). Acta Physiologiae Plantarum 42, 39.

7. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods*, *25*(4), 402-408.
8. Pillai, S. E., & Patlavath, R. (2015). Touch induced plant defense response. *J. Plant Biol. Res*, *4*(3), 113-118
9. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research*, *25*(17), 3389-3402.
10. Arvidsson, S., Kwasniewski, M., Riaño-Pachón, D. M., & Mueller-Roeber, B. (2008). QuantPrime–a flexible tool for reliable high-throughput primer design for quantitative PCR. *BMC bioinformatics*, *9*(1), 1-15.
11. 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
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. 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
14. Biddington, N. L. (1986). The effects of mechanically-induced stress in plants—a review. Plant growth regulation, 4(2), 103-123.
15. Pillai, S. E., & Patlavath, R. (2015). Touch induced plant defense response. *J. Plant Biol. Res*, *4*(3), 113-118
16. Chehab, E. W., Eich, E., & Braam, J. (2009). Thigmomorphogenesis: a complex plant response to mechano-stimulation. *Journal of experimental botany*, *60*(1), 43-56.

17. Braam, J. (2005). In touch: plant responses to mechanical stimuli. *New Phytologist*, *165*(2), 373-389.

18. Chehab, E. W., Eich, E., & Braam, J. (2009). Thigmomorphogenesis: a complex plant response to mechano-stimulation. *Journal of experimental botany*, *60*(1), 43-56.

19. Li, Z. G., & Gong, M. (2011). Mechanical stimulation-induced cross-adaptation in plants: an overview. *Journal of Plant Biology*, *54*(6), 358.

20. Porter, B. W., Zhu, Y. J., Webb, D. T., & Christopher, D. A. (2009). Novel thigmomorphogenetic responses in Carica papaya: touch decreases anthocyanin levels and stimulates petiole cork outgrowths. *Annals of botany*, *103*(6), 847-858.

21. 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.

22. Biddington, N. L. (1986). The effects of mechanically-induced stress in plants—a review. *Plant growth regulation*, *4*(2), 103-123.

23. 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.

24. Ślesak, I., Ślesak, 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*(2), 127-137.

25. Lee, D., Polisensky, D. H., & 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.

26. Pillai, S. E., & Patlavath, R. (2015). Touch induced plant defense response. *J. Plant Biol. Res*, *4*(3), 113-118.

27. Biddington, N. L., & Dearman, A. S. (1985). The effect of mechanically induced stress on the growth of cauliflower, lettuce and celery seedlings. *Annals of Botany*, *55*(1), 109-119.

28. Chehab, E. W., Yao, C., Henderson, Z., Kim, S., & Braam, J. (2012). Arabidopsis touch-induced morphogenesis is jasmonate mediated and protects against pests. *Current Biology*, *22*(8), 701-706.

29. Daudi, A., & O'Brien, J. A. (2012). Detection of hydrogen peroxide by DAB staining in Arabidopsis leaves. *Bio-protocol*, *2*(18), e263-e263.

30. Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology*, *24*(1), 1.

31. Telewski, F. W., & Jaffe, M. J. (1986). Thigmomorphogenesis: field and laboratory studies of Abies fraseri in response to wind or mechanical perturbation. *Physiologia Plantarum*, *66*(2), 211-218.

32. Choi, W. G., Hilleary, R., Swanson, S. J., Kim, S. H., & Gilroy, S. (2016). Rapid, long-distance electrical and calcium signaling in plants. *Annual Review of Plant Biology*, *67*, 287-307.

33. Van Breusegem, F., Vranová, E., Dat, J. F., & Inzé, D. (2001). The role of active oxygen species in plant signal transduction. *Plant science*, *161*(3), 405-414.

34. Yahraus, T., Chandra, S., Legendre, L., & Low, P. S. (1995). Evidence for a mechanically induced oxidative burst. *Plant Physiology*, *109*(4), 1259-1266.

35. Mitchell, C. A., & Myers, P. N. (1995). Mechanical stress regulation of plant growth and development. *Hort. Rev*, *17*, 1-42.

36. Gamon, J. A., Field, C. B., Bilger, W., Björkman, O., Fredeen, A. L., & Peñuelas, J. (1990). Remote sensing of the xanthophyll cycle and chlorophyll fluorescence in sunflower leaves and canopies. *Oecologia*, *85*(1), 1-7.

37. Braam, J., & Davis, R. W. (1990). Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. *Cell*, *60*(3), 357-364.

38. Pillai, S. E., Kumar, C., Patel, H. K., & Sonti, R. V. (2018). Overexpression of a cell wall damage induced transcription factor, OsWRKY42, leads to enhanced callose deposition and tolerance to salt stress but does not enhance tolerance to bacterial infection. *BMC plant biology*, *18*(1), 1-15.

39. Chehab, E. W., Kaspi, R., Savchenko, T., Rowe, H., Negre-Zakharov, F., Kliebenstein, D., & Dehesh, K. (2008). Distinct roles of jasmonates and aldehydes in plant-defense responses. *PloS one*, *3*(4), e1904.