Chapter 2

Exposure to regular mechanical stress induces multiple physiological changes in *Cajanus cajan* (L) Millsp

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2.1 ABSTRACT:

Perception of mechanical stress helps plant to better adapt and survive in the constantly changing environment. Present study is focused on studying morphological, physiological and anatomical adaptations of pigeon pea seedlings (*Cajanus cajan*) to touch stimuli. Regular mechanical stress in the form of touch suppressed overall growth of shoot, number of leaves and root of *C. cajan*. In order to cope with the mechanical stress, the plant stem had enhanced lignification of xylem tissue and increased compactness of tissue. An immediate surge in levels of reactive oxygen species was observed upon touch stimuli. We have also found two novel effects of mechanical stress- touch treatment affects the nyctinasty movements of the leaves and nodule development in roots. Present study provides evidences of various adaptation to touch stimuli in the least studied legume, *Cajanus cajan.*

2.2. INTRODUCTION

Plants have evolved strategies to perceive mechanical stress in order to sense changes in their environment. In nature, plants can perceive mechanical perturbations during raining, blowing of wind, grazing by herbivore animals, touching of neighbouring plants, snowing and even landing of insects (Jaffe and Brio 1979, Braam 2005, Chehab et al 2009, Li and Gong 2011). At cellular level, the mechanical stress exerted on cell-wall due to turgor pressure, wounding and damage to cell wall layers can also be sensed by a plant cell (Heil et al 2009). Prolonged mechanical stress result in several morphological, physiological, biochemical and anatomical adaptations some of which are common and a few are unique to plant species. Morphological changes observed in response to mechanical stress is also known as thigmomorphogenesis (Jaffe 1973). Regular mechanical stress leads common adaptations like stunted shoot and root growth and altered flowering period (Braam 2005, Chehab 2009). At tissue levels mechanical stress leads to increased compactness of cells, reduced cell size and enhanced lignin deposition (Porter et al 2009, Meng et al 2006). Biochemical analysis after touch treatment shows alternations in chlorophyll ratio, levels of plant hormones, change in intracellular calcium levels and ROS production (Biddington 1986, Allen et al 1999, Slesak 2008). Studies indicate that these anatomical and morphological adaptations help the plant to withstand and cope with mechanical stress.

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

C. cajan (Pigeon pea) is a perennial shrub legume grown for its edible pod. It is the sixth most consumed pulse in the world majorly cultivated in semi-arid geographical regions of Asia and Africa (Patlavath et al 2019). *C. cajan* is one of the neglected and least studied plants for crop improvement. The whole genome of *C. cajan* has been recently sequenced to boost the research on this plant and support the regional farmers of the poor regions of the world (Varshney R K et al 2012). Our research in *C. cajan* indicates that regular touch treatment suppresses overall plant growth, enhances lignin deposition, elevates levels of reactive oxygen species (ROS) and reduces chlorophyll a/b ratio. Also, the properties common to legume family like nyctinasty movement of leaves and root nodule development, were negatively affected by regular touching. Overall, present study on *C. cajan* explores the adaptations of this crop plant to mechanical stress which are common and unique as compared to other plant species studied till now.

2.3. MATERIAL AND METHODS:

2.3.1. Plant growth and treatments:

Cajanus cajan seed variety ICP-2376 was obtained as research gift from Dr. Rajeev K. Varshney, Pigeon Pea breeding centre- ICRISAT, India. *C. cajan* seedlings were grown under laboratory conditions. For this seed were sterilized using 90% ethanol with 0.1% SDS and directly germinated over water-soaked cotton in petri plates. The germinated seeds were transferred to pots containing soil pre-treated with antifungal agent (RocketG). Seedlings were watered on every alternate day. Touch induced experiments were carried out on two-weeks-old, two leaf stage seedlings. Plants were given touch stress at every 24 hr intervals for 15 days. Gentle touch was applied by fingers as mentioned by Chehab et al, briefly the leaves were bend back and forth ten times (Chehab et al 2012). Plants were observed for morphological changes in terms of root and shoot length, number of compound leaves and root nodules.

2.3.2. Anatomical study:

In order to study anatomical changes, transverse section of stem and root nodules were studied. For this plant tissue were collected from control and test plants after 15 days of touch treatment. Fresh sections were stained with safranin and observed under 10X magnification with a light microscope. Plant root nodules were fixed immediately in formaldehyde-acetic acid (FAA) with 70% ethanol fixative solution followed by stocking in 70% ethanol and steady dehydration in Tertiary-butyl alcohol (TBA) solution (Johansen 1940). Samples were embedded in paraffin and sectioned using rotary microtome (Lecica RM 2035). The sections were stained with safranin and fast green. Samples were observed under stereo zoom microscope (Olympus) and images were captured using Zeiss camera.

2.3.3. 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.

2.3.4. 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.

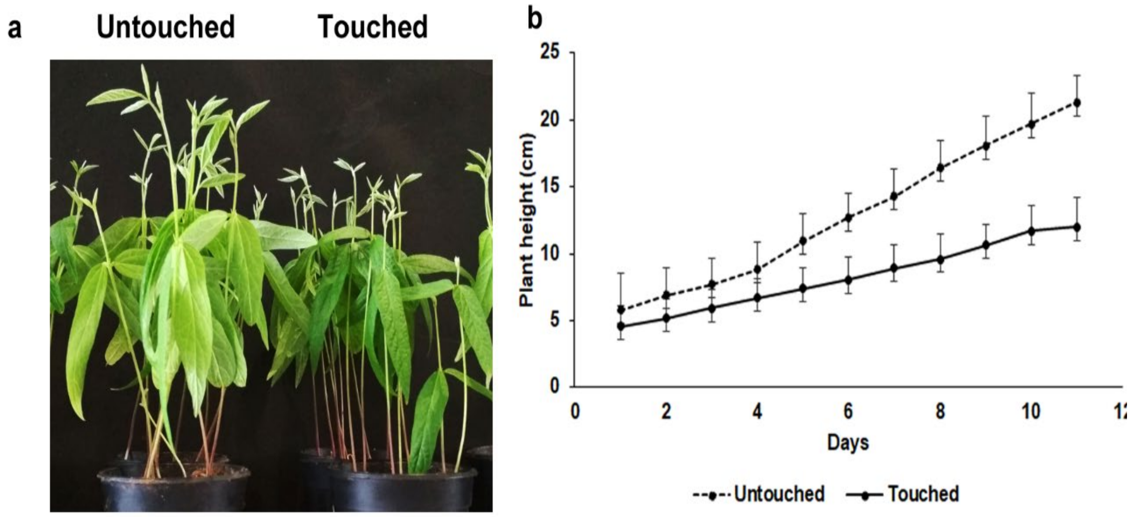

Figure 2.1: Regular touch treatment suppresses plant growth: *For assaying effect of touch on plant growth, two-weeks-old C. cajan seedlings (n=15-20) were given touch treatment for 15 days. Height of plant was measured on every alternate day.*

2.3.5. 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. For root nodule analysis multiple mean values were compared using Annova followed by Tukey Karmer's test.

2.4. RESULTS:

2.4.1. Regular touch treatment suppresses overall growth of *C. cajan*

In order to study touch induced morphological changes in *C. cajan,* we performed touch treatment on two-weeks-old seedlings (two leaf stage). Plants (n=15-20 seedlings) were given gentle touch twice a day for 15 days and were observed for morphological changes (Figure 2.1). A significant reduction in shoot height was observed upon touch stimuli (Figure 2.2 a-b and d) ($p<0.05$). The length of first internode from soil surface and second internode were also measured for both touched and untouched plants (Figure 2.2d).

Both the internode lengths were shorter in touched plants as compared to untouched plants. Moreover, the second internode length was highly reduced as compared to the first internode in the touched plants. The tap root length was also significantly reduced upon regular touch stimuli as compared to the controls (Figure 2.2 c) ($p<0.05$). Seedlings that received regular touch stimuli had reduced number of compound leaves (Figure 2.2 e). The population of touched plants had more number of plants with only two compound leaves while the population of untouched plants had more number of plants with 3-4 compound leaves (Figure 2.2 e). This experiment was repeated more than three times and similar results were observed.

2.4.2. Touch treatment alters anatomy of stem tissue:

Regular touch treatment for 15 days increased the stiffness (strength) of the stem in *C. cajan* seedlings which was experienced while performing the experiment. Thus, microscopic studies of the stem were performed to understand the anatomical changes. Microscopic examination of the fresh stem that received touch treatment for 15 days showed increased compactness in the tissue as compared to the control plants (Figure 2.3a-b). The hypodermal region of the touched stem had reduced number of cell layers (3-4 layers) as compared to untouched stems which depicted 6-7 layers of hypodermal cells. Moreover, the size of the hypodermal cells was smaller than that of the control. In order to study the differences in lignin deposition, microtome sections of the stem samples were stained with safranin and fast green stains. Stem sections of touched plants showed thicker lignified xylem vessels and xylem fibres as compared to that of the untouched plants (Figure 2.3c-d). Also, the number of xylem fibres were more in touched stems. The cortical cells of the stem of touched plants were smaller in size, flattened and compactly arranged as compared to loosely arranged, larger and oval to round shaped cells of untouched stems. Anatomical studies with fresh tissue sample were repeated three times while microtome sectioning was performed once with 5-10 replicates of touched and untouched plants. Similar observations were made in all the replicates.

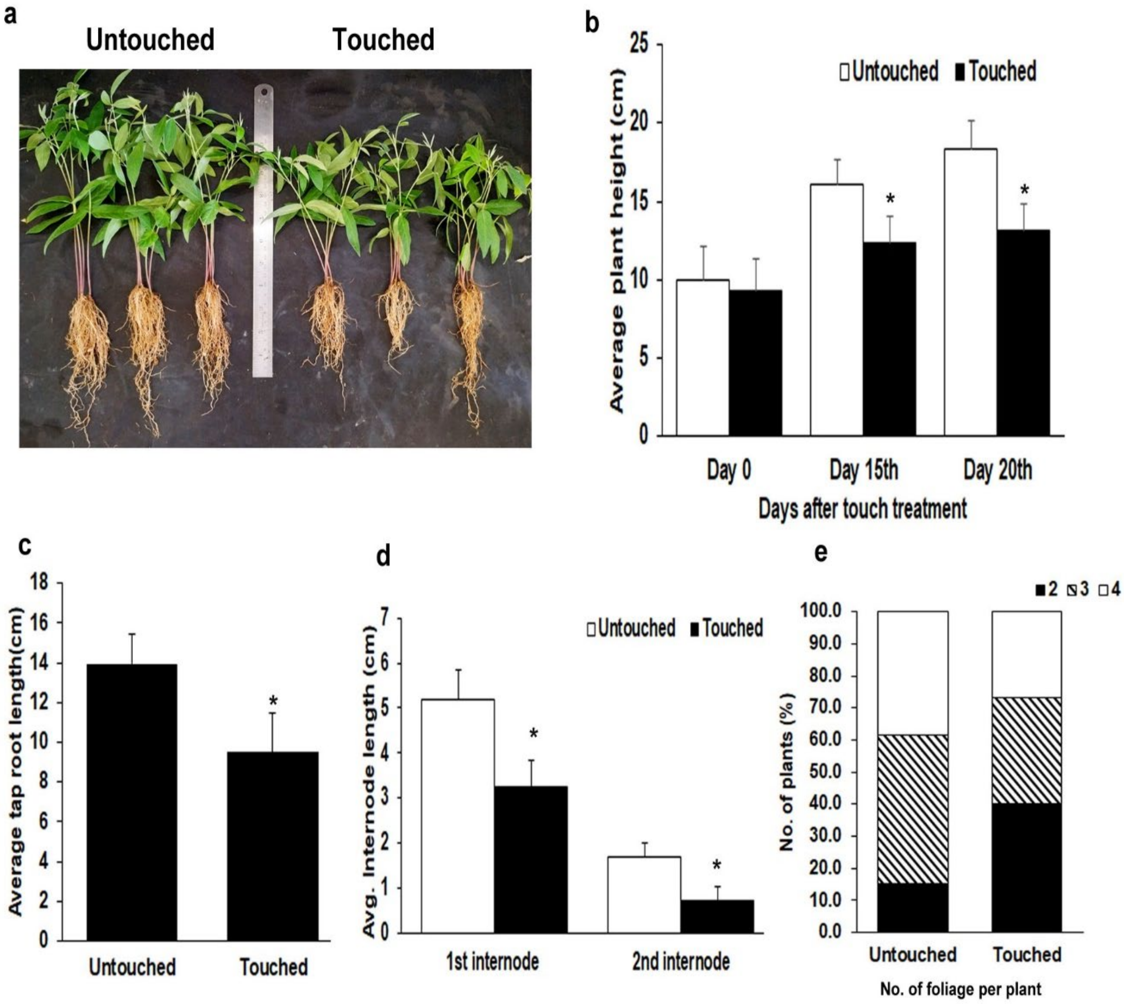

Figure 2.2: Regular Touch treatment suppresses stem, root and leaf growth in C. cajan seedlings.

For assaying effect of touch on plant growth, two-weeks-old C. cajan seedlings (n=15-20) were given touch treatment for 20 days and were observed for effect on shoot and root growth as shown in (a). (b)The shoot lengths were measured for both touch treated and untreated seedlings upon 0, 15 and 20 days of touch treatment. (c) For studying effect on root growth, the length of tap root was measured upon 20 days of treatment. (d) Internode length of stems were measured on 20th day starting from node at soil level as the first internode and the next towards apex as the second internode. (e) for leaf growth the number of compound leaves were counted for each plant. All 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.

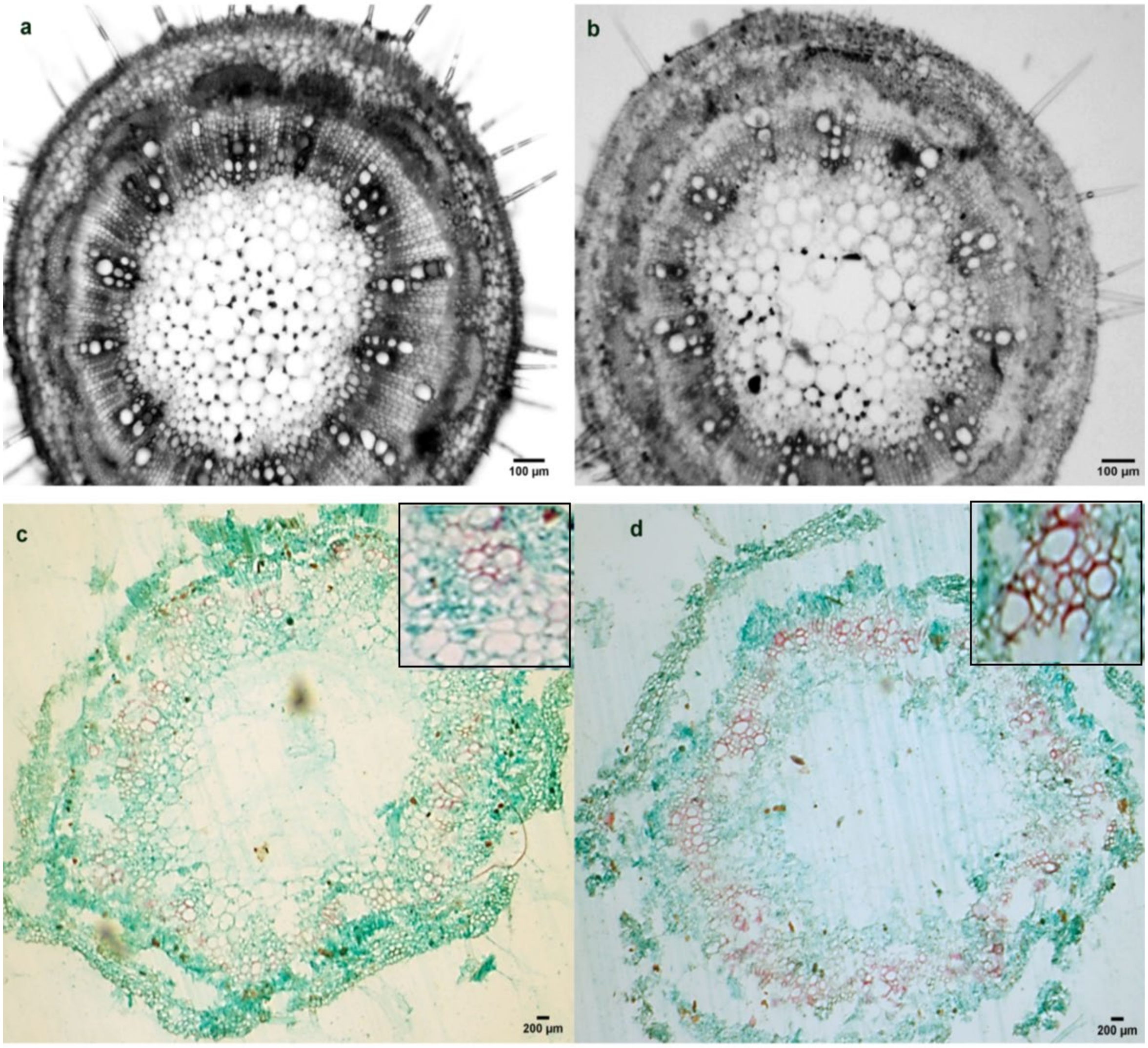

Figure 2.3 : Microscopic examination of C. cajan stem indicates increased compactness and thickness of cells.

For anatomical studies, initially fresh stem sections were taken and examined under 10X magnification of light microscope after 15 days of touch treatment. a and b are transverse sections taken from internode region of untreated and touch treated C. cajan seedlings respectively. (c) and (e) are safranin and fast green stained tissue sections of touch untreated and treated seedlings examined under 5X magnification. The figure c and d show the thick safranin-stained (red) sclerenchyma tissue of the respective microtome sections.

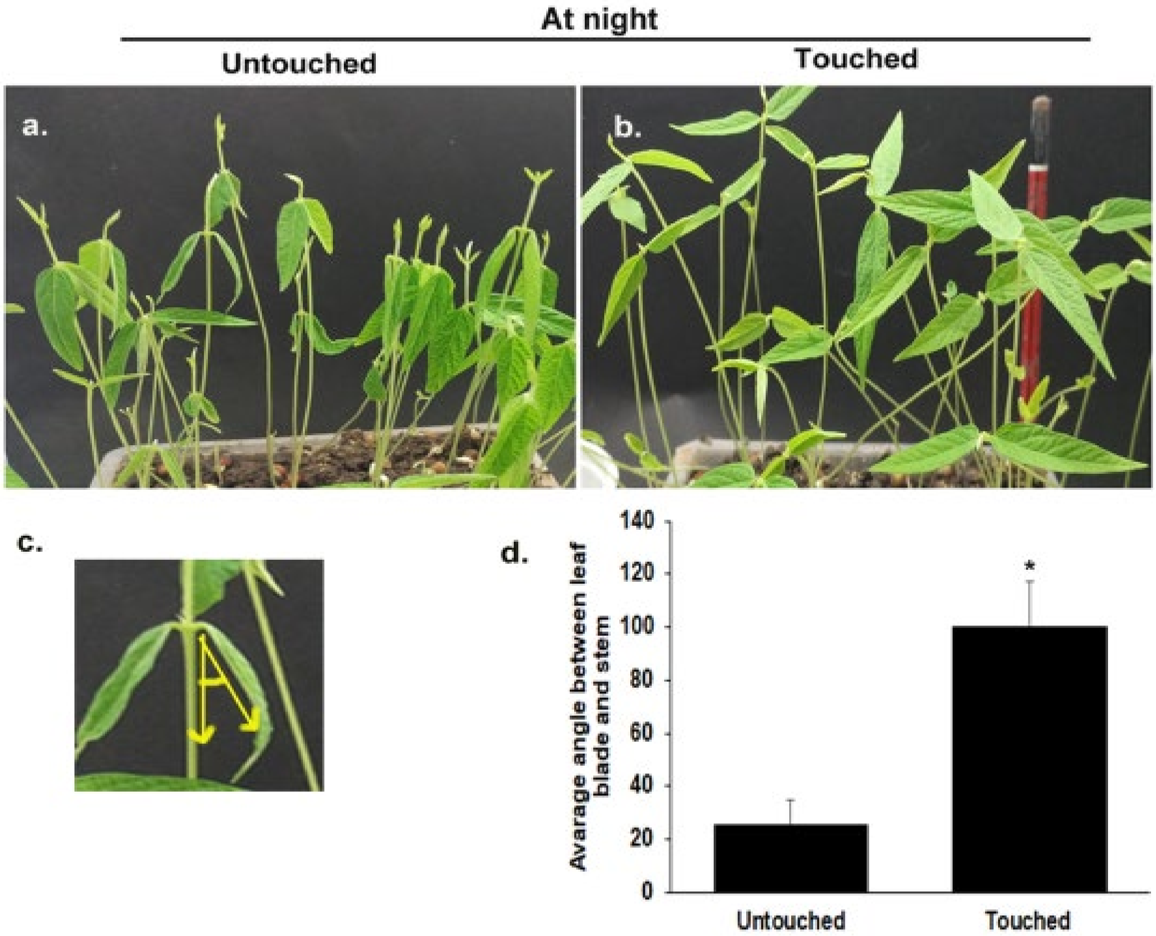

Figure 2.4: Nyctinasty movements of C. cajan leaves are altered upon touch treatment.
Two-weeks-old C. cajan seedlings (n=15) were given touch treatment. (a) shows the control seedlings (untouched) and (b) shows the seedlings that were given touch treatment. The leaves of touched plants remained upright when the leaves of control plants were dropped down at 9.00 PM at night. (c) The nyctinasty movement was quantitated by measuring the angle between the leaf blade and the stem using ImageJ tool. 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.

2.4.3. Touch treatment alters nyctinasty movement of leaves in *C. cajan*

Leguminous plant species distinctly display the phenomenon of sleeping by dropping their leaves during the Sunset, this is also known as nyctinasty movement. Regularly touched *C. cajan* seedlings became nonresponsive to the day and night cycle. Plants that were regularly touched (Figure 2.4 a) failed to drop their leaves at night as perceived by the untouched plants (Figure 2.4 b). The nyctinasty movement of leaves were

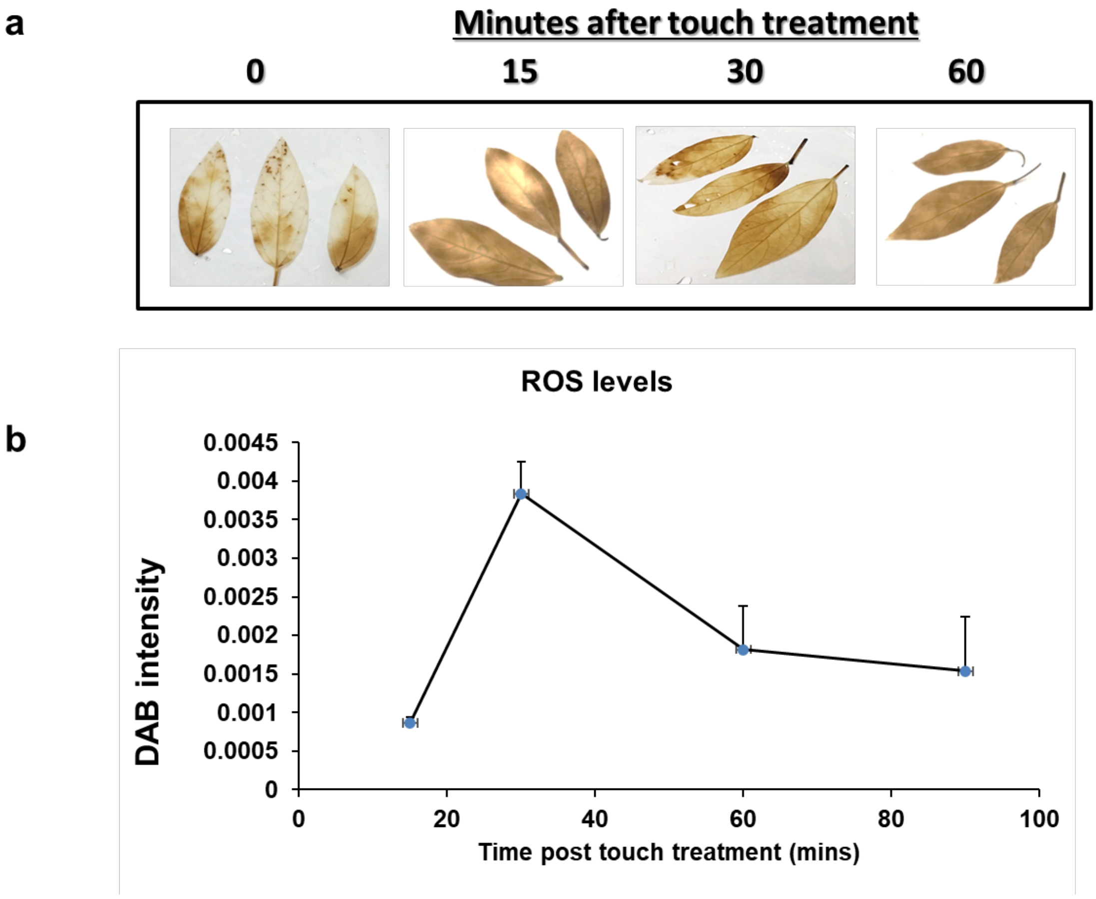

Figure 2.5: Touch treatment induces ROS production: *For assaying ROS production, two-weeks-old seedlings were given touch stimuli and harvested at different time points (15, 30, 60, 120 minutes). (a) Leaves were stained using DAB and photographed. (b) The brown precipitate formed after reaction of DAB with ROS was quantitated using ImageJ software. Similar results were observed in three independent experiments.*

quantified by measuring the extent of leaf bending. For this, the angle between the stem and the leaves (n=20) were measured using ImageJ tool from the images of plants captured after Sunset (Figure 2.4 c). The leaves of touched plants remained upright. A significant difference ($p<0.05$) was observed in the bending of leaves between the touched and untouched plants after sunset (Figure 2.4 d). Similar observations were obtained in three independent experiments.

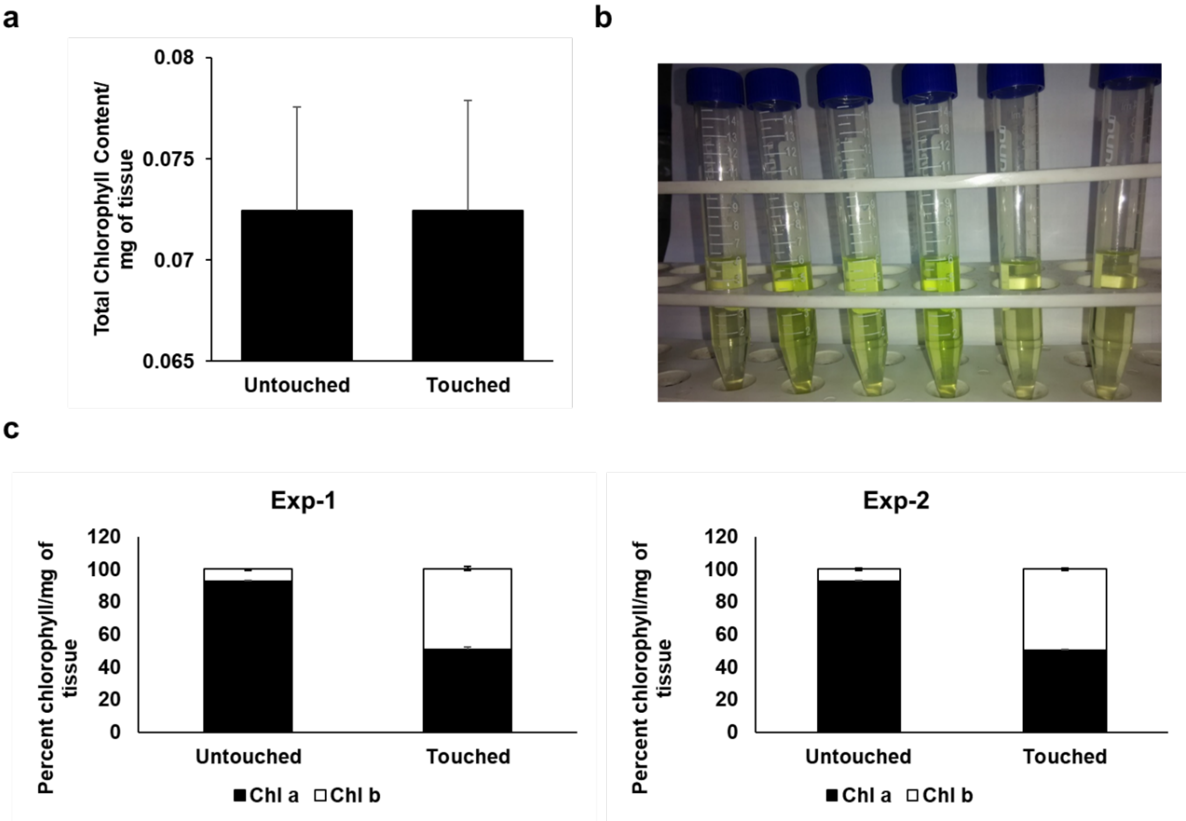

Figure 2.6: Touch treatment alters chlorophyll a/b ratio*: For assaying effect of touch on chlorophyll content, leaves were harvested after 15 days of touch treatment. The total chlorophyll was extracted in acetone (a-b) and estimated using spectrophotometer. The chlorophyll-a and -b was calculated and expressed as percentage of total chlorophyll per milligram of fresh weight (c). 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 two independent experiments.*

2.4.4. Touch stimulus induces ROS production in *C. cajan.*

Production of reactive oxygen species is an early response that is observed under different stress conditions. Thus, we tested ROS production upon touch stimuli in *C. cajan*. For this, leaves were given touch stimuli and harvested at different time points (15, 30, 60, 120 minutes) for detection of ROS production. Leaves were stained using DAB and photographed. The brown precipitate formed by DAB after reaction of with ROS was quantitated using ImageJ software. A significant increase in ROS level was observed after touch stimuli ($p<0.05$) (Figure 2.5a-b). The ROS level reaches maximum at 30 minutes and eventually normalised. Each experiment had three biological replicates for each time points and three such independent experiments were performed and similar results were obtained.

2.4.5. Regular touch treatment alters chlorophyll content

Under stress conditions, plants show alterations in total chlorophyll content. As we observed suppression in plant growth and induction of ROS production upon touch stimuli, we also studied effect of regular touch treatment on chlorophyll content in *C. cajan*. For chlorophyll estimation leaves were harvested after 15 days of regular touch treatment. The total chlorophyll content was measured spectrophotometrically using Anrone's method (Arnon 1949). The total chlorophyll content remained unchanged. However, when we calculate the ratio of chlorophyll-a and -b, we found a significant reduction in the value of Chla/b in the touch treated plants ($p<0.5$) (Figure 2.5 c). In the regularly touched plants, the level of Chlorophyll-b was found to be increased by approximately 7-fold as compared to the levels in untouched plants. Each experiment had three biological replicates and two such independent experiments were performed and similar results were obtained.

2.4.6. Touch treatment affects root nodule development in *C. cajan*

Plant members of leguminous (Fabaceae) family are known for their symbiotic association with *Rhizobium* sps. in the form of root nodules. Multiple environmental factors like heat, temperature, light intensity, soil pH, nutrient deficiency, fertilizer, heavy metals, pesticides etc. affect root nodule formation and development (Zahran 2001). In order to study the effect of touch on development of root nodules, the number of root nodules per plant was counted after 15 days of regular touch treatment. The total number of root nodules

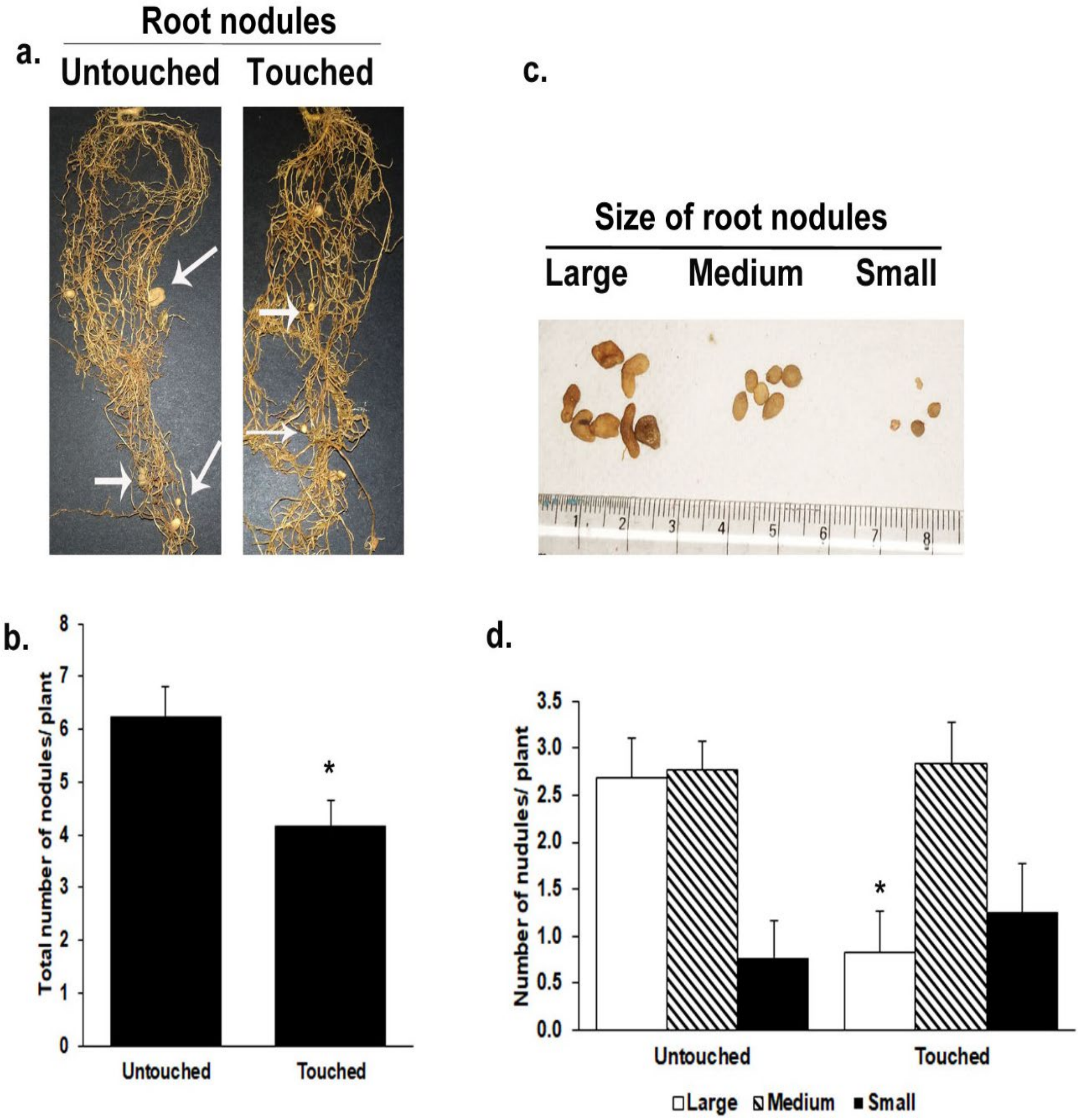

Figure 2. 7: Regular touch stimuli alters root nodule development in C. cajan.
The number of root nodules per plant (N=15-20) was counted on 15th day after regular touch treatment. The frequency of different sized nodules was classified as large sized nodules (0.7-1.0 cm) medium sized (0.6 to 0.4 cm) and small sized nodules (>0.3 cm). 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.

were less in touched plants as compared to the untouched plants (Figure 2.6 a-b). The frequency of different sized nodules in touch treated and untreated plants were also calculated (Figure 2.6 c-d). The untouched plants had more number of large sized nodules (0.7-1.0 cm) and fewer small sized nodules (>0.3 cm) while the touched plants had significantly($p<0.05$) a smaller number of large sized nodules and more of small sized nodules per plant (Figure 2.6 d). Similar observation was obtained in biological replicates. In order to understand the progression of bacterial infection in the nodules, we performed anatomical study of *C. cajan* root nodules. The microtome sections of root nodules from untouched plants showed clear epidermal cells, cortex, vascular bundles and the central infected region (Figure 6a-d). The infected region showed mixed distribution of large infected with clear unstained nucleus (blue coloured; IC) and unstained uninfected cells (UC). In regularly touched plants, the cells in the centre of the nodule (cortical cells) appeared flaccid and compactly arranged (Figure 2.7c-d). The number of infected cells were counted per unit area using ImageJ tool. The root nodule of touched plant had more number of infected cells as compared to that of untouched plants (Figure 2.7e). Conversely, the average size of the infected and uninfected cells in root nodule of touched plants were smaller than that of the untouched plants (Figure 2.7f).

2.4.7. Touch treatment enhances pod development in *C. cajan*

Touch treated and untouched plants were is planted in fields for further growth. Both types of plants were planted in same field and allowed to grow under same conditions. After six-seven months the number of branches per plant were counted (Figure 2.9a.). The plants started flowering at same period. The number of pods per plant were counted (Figure 2.9b.). This experiment had n=20 replicates of each touched and untouched plants. This experiment was performed once.

2.5. DISCUSSION:

2.5. 1. *C. cajan* shows Thigmomorphogenic adaptations to mechanical stress

Upon exposure to prolonged mechanical stress, plants undergo various physiological and anatomical changes that help them to adapt and survive the stress condition (Telewski Jaffe 1986). Similar to previously reported studies we have found that regular mechanical stress in the form of touch suppressed overall shoot growth of *C. cajan* seedling. This also affected the lateral development of compound leaves. In nature, roots constantly experience mechanical stimuli which leads to reduced root size (Potocka, I., & Szymanowska-Pułka, J. 2018). Consistent touch treatment had a similar effect on the growth of tap root in *C. cajan*. Touch treatment for 15 days lead to increased stiffness of stem *C. cajan* which is an adaptation to cope with the perceived mechanical stress. In many woody plants it has been reported that plants exposed to mechanical stress show increased hypocotyl diameter and increased lignin deposition in order to augment the mechanical strength of the stem (Porter et al 2009). Touch treated *C. cajan* plants displayed increased compactness in cortical region and increased number of xylem fibres which adds to the mechanical strength of the stem. Also enhanced lignification of xylem tissue increases the rigidity of the stem.

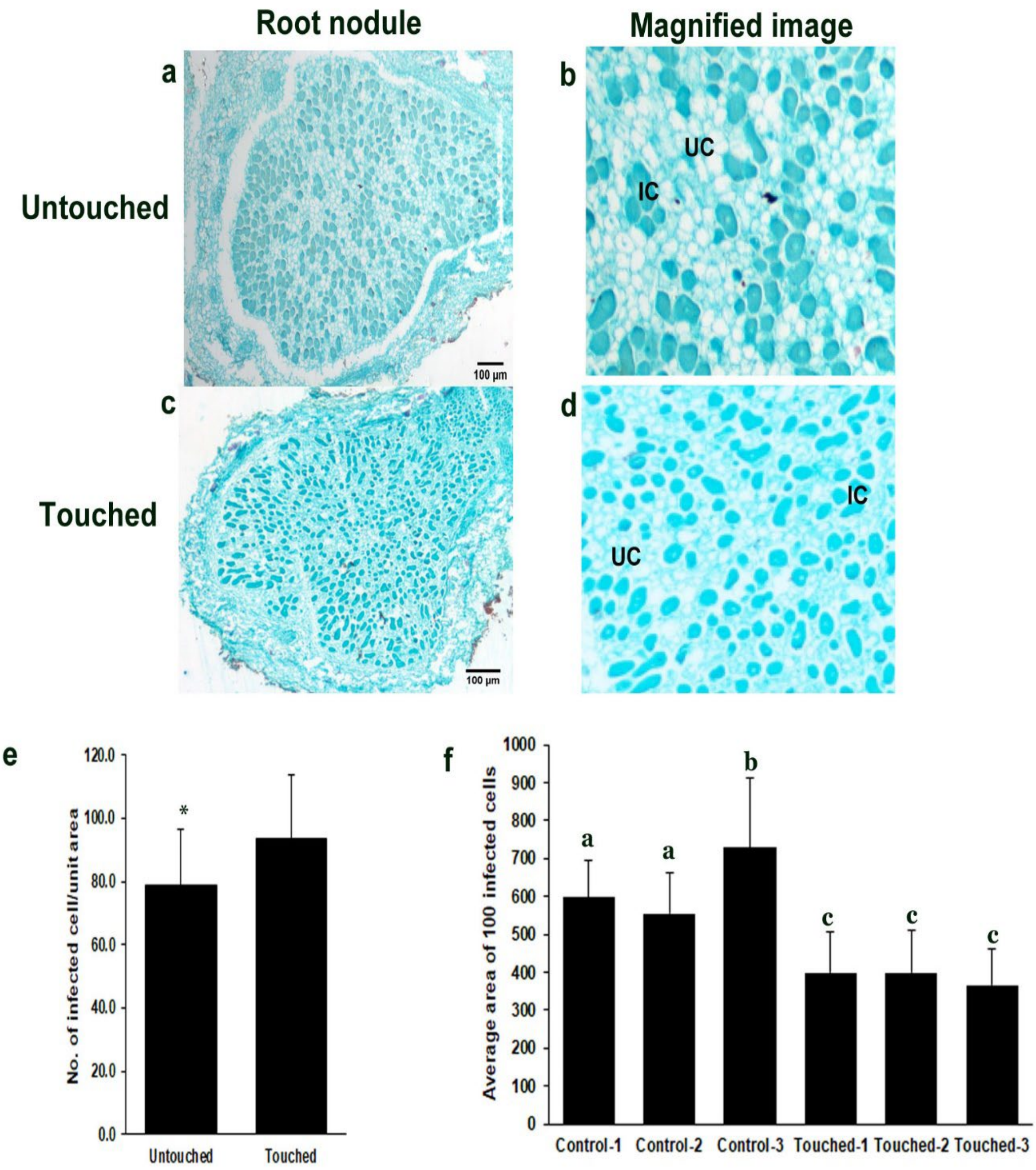

Figure 2.8: Touch treatment alters anatomy of root nodule in C. cajan.

Root nodules of both touch treated and untreated seedlings were processed and microtome sections of the nodules were stained with fast green. Blue coloured cells are Rhizobium infected cells (IC) and only border stained cells are uninfected cells (UC) (a and c). figure b and d are magnified images. The nodules were examined under 5X magnification. The number of infected cell per unit area and size of cell were counted using Image J tool (e and f). The data in (e) was analysed using Student's T-test. For comparision of multiple mean values were compared using Annova followed by Tukey Karmer's test (f). The asterisk on the bar indicates significant differences with a p-value less than 0.05.

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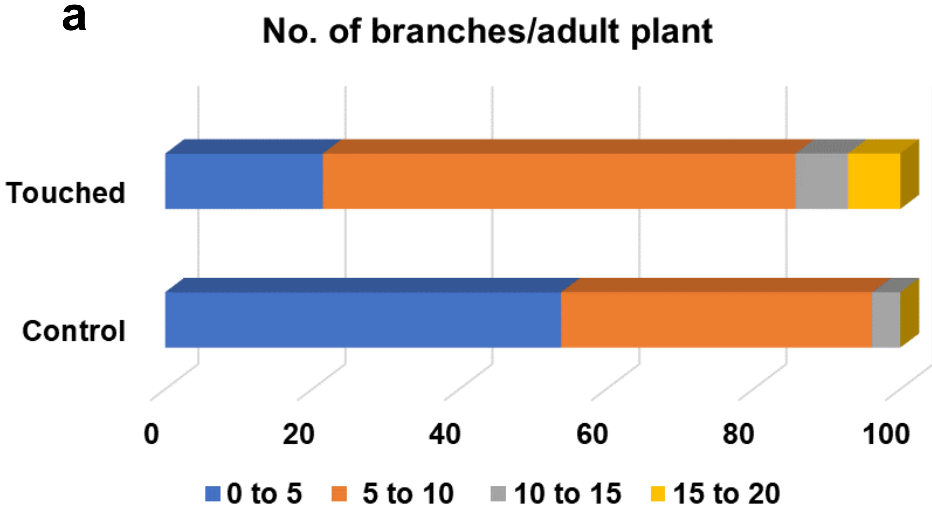

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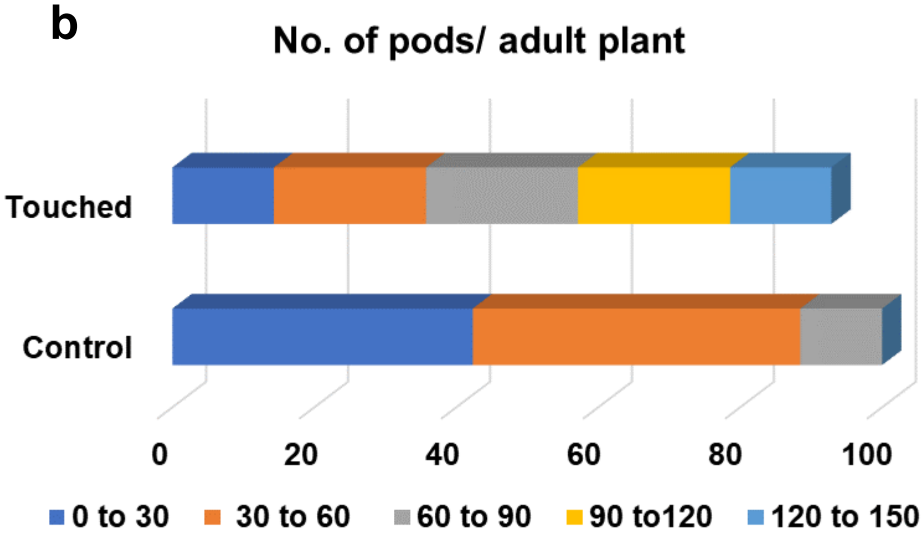

Figure 2.9: Effect of touch on seedlings affects growth in adult plants

Regularly touched and untouched plants (n=20) were planted in field. The plants were regularly watered and monitored. The data represented here was noted after pod development in both the touched and untouched plants.

2.5.2. Regular mechanical stress affects the sleep cycle in *C. cajan*.

Nyctinasty is a circadian behaviour displayed by leguminous plant species in which the plant oscillates its leaves in response to day and night cycle (PV Minorsky 2019). Leaves of *C. cajan* seedlings bend vertically down from the petiole at the sunset and raises back to horizontal position during the sunrise. *C. cajan* plants that received regular mechanical stress failed to drop their leaves after sunset. The increased stiffness in the tissue as an adaptation to mechanical stress may hinder the nyctinasty process in regularly touched *C. cajan*. In *Samanea saman,* change in cellular volume in the motor cells of the abaxial and adaxial sides of leaf base regulates their nyctinasty movement (Oikawa, et al 2018). However, it remains unanswered how regular mechanical stress alters nyctinasty movement of *C. cajan* leaves.

2.5.3. Touch stimuli immediately elevates levels of ROS

In response to biotic and abiotic environmental stress, plants accumulate reactive oxygen species and reactive nitrogen species which act as key secondary messengers or 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 *C. cajan*, level of ROS was detected at 15 minutes after touch stimuli which gradually increased and reached a peak at 30 minutes. Excess free radicals can also cause severe intracellular damage. Hence synthesis and scavenging of ROS is a tightly regulated process. It is observed that the level of ROS is brought down after attaining a threshold level. Similar phenomenon is observed in *C. cajan*, where the ROS level starts dropping after 30 minutes of touch treatment.

2.5.4. Regular touch treatment alters chlorophyll composition of *C. cajan*

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 *C. cajan,* the total chlorophyll content remains unchanged upon regular touch treatment. However, the Chl-a/b ratio was found to be significantly reduced upon touch treatment. 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). Under normal light conditions, the levels of Chl-a are higher than that of Chl-b. Chl-a is more sensitive to degradation under oxidative stress (Kasajima, 2017). In *C. cajan* upon touch treatment, the level of Chl-a was lower than that of Chl-b. We hypothesize that the reduction of Chl-a could be due to oxidative damage caused by the increased ROS that elevates after touch treatment. In order to compensate the loss of Chl-a, the touch treated plants may increase the level of Chl-b to restore the light capturing function for photosynthesis.

2.5.5. Regular mechanical stress suppresses root nodule development in *C. cajan*.

C. cajan is a legume plant which can associate symbiotically with *Rhizobium* bacteria and fix atmospheric nitrogen within root nodule. Our observations indicate that regular mechanical stress reduces root nodule initiation which is seen as reduced number of root nodules in touch treated plant. Also, we observe a significant reduction in large sized root nodules in touch treated plants which reveals that mechanical stress is affecting overall growth of the root nodule. The anatomy of the root nodule was also found to be altered by mechanical stress. The infected cells of touch treated root nodules were flaccid, smaller in size and compactly arranged. Root nodules of Common bean (*Phaseolus vulgaris* L.)

depicted similar anatomical changes under water stress (Ramos et al 2003). Legume plants showing altered root nodule structures and poor nodule development under salt stress also display reduced shoot growth (Cordovilla, M. P et al 1995). The symbiotic association between legumes and N_2 fixing bacteria depends on the exchange of carbon source, the photosynthate, produced by the plant. The N_2 fixation is an energy consuming process, this energy is obtained from the photosynthate supplied to the bacterium by the plant (K. J. Kunert1 et al 2016). We propose that under mechanical stress the *C. cajan* plants show reduced shoot and leaf development which must be directly affecting the energy supply chain to the nitrogen fixing bacteria, thus affecting the nodule growth and development.

How does regular touch treatment affect plant growth is not clearly defined? The hypothesis is 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). Studies in Arabidopsis indicate that treatment with JA inhibits growth of plant root (Pillai et al 2018). Another study in Arabidopsis indicates that touch induced JA also promotes gibberellic acid (GA) catabolism (Lange and Lange 2015). GA is a key plant hormone vital for plant growth and cell elongation. Reduced levels of GA causes suppression of cell growth as seen after prolonged mechanical stress. We propose that touch mediated reduced plant growth in *C. cajan* could be mediated by altered levels of these two plant hormones. How does plant perceive touch- the exact mechanism is not yet clear? However, it is proposed that touching plant increases levels of ROS which in turn may also lead to activation of calcium channels in plants (Mori et al 2004). This triggers expression of touch responsive gene which are calcium binding proteins, JA synthesis and responsive genes and many other defense regulating genes (Jaffe et al 2002, Chehab et al 2008). These genes eventually are responsible for the morphological and anatomical changes that we observe. Our studies in *C. cajan* indicates existence of similar conserved responses.

Additionally, effect of mechanical stress on nyctinasty and root nodule development are novel findings of current study. In *Medicago truncatula,* a common gene *MtLYH* regulates nyctinasty as well as nitrogen fixation in the root nodule (Yiming Kong et al 2020). Plants with loss of function of *MtLYH* genes, displayed irregular nyctinasty and reduced number of root nodules. In our study we observe that mechanical stress disturbs the nyctinasty movement in *C. cajan* and root nodule development. This indicates that mechanical stress could be affecting a common regulator/ function similar to *MtLYH*. However, more detailed biochemical study is needed to understand- How mechanical stress affects these two processes in legume plants?

2.6. CONCLUSIONS:

In present study on *C. cajan*, we have found that regular mechanical stress induces various morphological, anatomical and physiological adaptations. The touch induced phenotypes identified in present study can be used as marker for mechanical stress in *C. cajan* for future studies. This study lays foundation for our future field level research on effect of mechanical stress on productivity and yield of pigeon pea.

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