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A study on variation in spatial voltage distribution pattern across tissue layers between non-excitable plant and excitable plant

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Understanding the mechanism of information processing in plants remains a challenging task even in the era of machine learning and artificial neural networks. Sir J.C. Bose had demonstrated through his experiments that the various modes of stimulation which effectively initiated nervous impulse in animals led to impulse generation in the excitable plant *Mimosa pudica* as well. In order to localize the tissue responsible for conduction of excitation in the petiole of Mimosa, Bose had constructed a specialized 'Electric Probe' (glass tip electrode). From this experiment, Bose found that there were different intensities of transmitted excitation in different tissue layers of the petiole. In this backdrop, an experimental research has been conducted to comparatively study the pattern of spatial voltage distribution across different tissue layers in both, a non-excitable plant *Alternanthera philoxeroides* (in stem) and an excitable plant *Mimosa pudica* (in petiole), by following experimental principles similar to that of Sir J. C. Bose. For the present experimental study, the electrical probes (glass tip electrode), similar to the one designed by J.C. Bose and the whole experimental setup has been constructed and developed completely in the laboratory. The results indicated a striking difference in the spatial voltage distribution pattern between the non-excitable and the excitable plant. Since Mimosa is an excitable plant having specialized mechanoreceptor cells, the change in spatial voltage distribution in the different layers of petiole, following excitation (uniform electrical stimuli) of a sub-petiole has been also studied, as an additional segment of the present research. In the present study a notable difference in the intensities of the transmitted excitation was also found upon electrical stimulation of one of the sub-petioles of the excitable plant *M. pudica*.

Keywords: *Alternanthera philoxeroides*, Electrophysiology, *Mimosa pudica*, Phloem, Spatial electrical property, Transmitted excitation

The electrical phenomena in plants attracted researchers since the 18th century[1]. In history, the initiation of research in the field of plant electrophysiology is marked by pioneering works of Bertholon, Burdon-Sanderson, Darwin, Sibaoka and J.C. Bose[1-4]. Extensive research concerning electrophysiological signals in plants was significantly developed by the end of 19th century when Acharya Jagadish Chandra Bose, the father of Biophysics, began his series of plant electrophysiological research. He made significant discoveries in the field of plant physiology and plant biophysics. It is also known that his research ideas and analysis were around 60 years ahead of his time[5]. He performed numerous experiments to study the electrical nature of conduction of various stimuli in plants. He also conducted extensive research on plant growth, plant movements and studied their nature of responses to various environmental and artificially generated stimuli[6]. Bose also showed that plants possess electromechanical pulse, memory and learning ability and are capable of exhibiting intelligent behaviour[7,8]. In a nutshell, J.C. Bose made a revolutionary attempt to correlate several features associated with the plant kingdom with identical characteristics found in the animal world[8]. In the recent past, some experimental and modelling research works have focused on determining the relationship between intracellular and extracellular potential in plant cells in order to clarify the characteristics of extracellular measurement by developing a multi-channel plant physiological signal recording system[9,10]. A recent review has documented the series of research conducted in the past few years concerning plant electrical signals from a novel, interdisciplinary perspective, which is needed to improve the efficient aggregation and use of plant electrical signal data and to expedite interpretation of

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plant electrical signals[11]. There are some extensive reviews concerning the characteristics of electrical signals in plants and their corresponding physiological significance and their role in plant communication[12,13]. Some present day research have focused on explaining how voltage-activated ($Ca2^{+}$) channels could fulfil a physiological function in non-excitable cells and tissues of animals[14,15.] Recent advances in the field of plant electrical signalling have revealed that in the model plant *Arabidopsis thaliana*, a family of 20 glutamate receptor-like proteins (GLRs) shares similarities to animal ionotropic glutamate receptors in sequence and predicted secondary structure[16-18]. Another recent study has documented isolation of a novel single-domain Na^{+}-selective voltage-gated channel in photosynthetic eukaryotes[19]. Some recent review works have highlighted Bose's revolutionary discoveries pertaining to plant electrical signals, propagation of action potential in plants and plant perception[20,21].

J.C. Bose had demonstrated through his experiments that the various modes of stimulation which effectively initiated nervous impulse in animals led to impulse generation in the excitable plant *Mimosa pudica* as well[22]. He conducted similar experiments using other excitable plants like *Neptuniaoleracea*, *Biophytum sensitivum*, *Averrhoa Carambola* and *Mimosa Sphegazzinii.* After establishing the fact that stimulation in Mimosa gives rise to an impulse like that of nervous tissue in animals he continued his experiments to precisely localize the position of conducting tissue (nerve like) in the petiole of the Mimosa plant. In order to localize the conducting tissue in the petiole of Mimosa, Bose constructed a specialized Electric Probe (glass tip electrode) which was insulated except at the tip. The probe was intruded gradually in a direction perpendicular to the diameter of the petiole. Bose periodically applied uniform stimuli (electrical) on the third sub-petiole of Mimosa due to its connection with the upper phloem. From this experiment, Bose found that there were different intensities of transmitted excitation in different tissue layers of the petiole[22].

In this backdrop, an experimental research has been conducted to comparatively study the pattern of spatial voltage distribution across different tissue layers in both, a non-excitable plant – the alligator weed *Alternanthera philoxeroides* ([von Martius] Grisebach) and an excitable plant – the sensitive plant *Mimosa pudica* (Linnaeus), by following experimental principles similar to that of Sir J.C. Bose[23]. Apart from this, as an additional part of the present study, an experiment has been conducted. Since Mimosa is an excitable plant having specialized mechanoreceptor cells, the change in spatial voltage distribution in the different layers of petiole, following excitation (uniform electrical stimuli) of a sub-petiole has been studied and compared to that of Bose's experimental findings on the localization of the transmitted excitation in different tissue layers of the petiole of *Mimosa*[22-24]. For the present experimental study, the whole electrophysiological set-up and the electrical probes (glass tip electrode), similar to the one designed by J.C. Bose, has been constructed in the laboratory. It is also worthwhile to mention that while such electrophysiological investigations are now mostly done at the cell level of plants, in the present study, the experiment has been restricted to electrophysiological study at the tissue level only.

Materials and Methods

Experimental design

In the study, the non-excitable plants *Alternanthera philoxeroides* were collected from the campus garden (Indian Statistical Institute, Kolkata) and the excitable plants *Mimosa pudica* (full grown plants) were procured from a local nursery. Before starting the experiments both, the non-excitable and the excitable plants were acclimatized in the laboratory conditions, inside a customized plant acclimatization chamber. In the present experiment, for studying spatial voltage distribution in the non-excitable plant a total of 5 *A. philoxeroides* plants were used. For studying the spatial voltage distribution in the excitable plant a total of 5 plant segments (stem with petiole and leaves) from 2 separate full grown *M. pudica* plants were used. During the experiment the mean recorded ambient temperature was 28°C. In the experiment, for studying the spatial voltage distribution pattern across the tissue layers, the sites of investigation were the stem of the non-excitable plant *A. philoxeroides* and the petiole of the excitable plant *M. pudica*[22]. After completion of the electrical study across the tissue layers, cross-sections of the stems and petioles were obtained from all the plants used in the experiment using a microtome constructed in the laboratory[25]. The cross sections were used for identifying the possible regions of the varying spatial

voltage across the different tissue layers of the stem and petiole and for correlating with the approximate depth of electrode tip insertion. The tissue cross sections of stem of *A. Philoxeroides* and petiole of *M. pudica* were observed under both, a standard commercial petrological microscope (model: Leica DMLP, available with the Geological Studies Unit, Indian Statistical Institute, Kolkata) and a specially designed multi-modal polarizing microscope constructed in the laboratory, for obtaining better structural details of the whole tissue cross sections[25]. The approximate depth from surface (epidermis) of each of the tissue layers of the stem and petiole of *A. philoxeroides* and *M. pudica*, respectively, were analysed using standard image analysis software. The cross section diameters of the different tissue layers of the stem and petiole were measured under the microscope at magnifications 25X and 100X, respectively.

In the present study, the whole experimental apparatus designed in the laboratory for studying the spatial voltage distribution pattern in the non-excitable and excitable plant consisted of three main parts: i) the electrodes (specialized glass-tip electrodes and the reference electrode), ii) the manual micro-manipulator system for insertion of the tip electrodes and iii) the electrical signal detecting system. The detector system was made up of a pre-amplifier, differential amplifier and linear amplifier. The high input impedance of the pre-amplifier ensured minimum voltage drop within the plant system while the signals were measured.

Designing the electrode

In the present study, the two types of electrodes – the reference electrode and the penetrating tip electrodes were constructed using the same material, *i.e*. copper[26]. The two different glass tip electrodes used in the study for the non-excitable (Fig. 1A) and excitable plant (Fig. 1B) were made up of glass capillary and fine copper wire. The electrode was encapsulated (except at the tip) within the fine glass capillary (Fig. 1C)[27,28]. The diameter of the exposed tip of the glass tip electrode used for studying the spatial voltage distribution of the stem of *A. philoxeroides*was 200 µM. Since, the diameter of petiole of *M. pudica* is much narrower than that of *A. philoxeroides*, a more fine copper wire of diameter 80 µM was used for preparing the glass-tip electrode for studying the spatial voltage distribution in the petiole of *M. pudica*. These tip electrodes constructed in the laboratory were based on the principles of the 'electric-probe' designed by J.C. Bose for his experiments[22]. The tip electrodes were designed in a way so that during the movement of the tip electrode, only its tip would come in contact with a particular tissue layer of the plant stem or petiole, at a time. The insertion of the electrode tip was controlled with the help of the manual micro-manipulator setup designed for the present experiment.

Micro manipulator system

In the present study, two separate micro-manipulator systems were constructed, one for the non-excitable plant (Fig. 2A) and the other for the excitable plant (Fig. 2B).

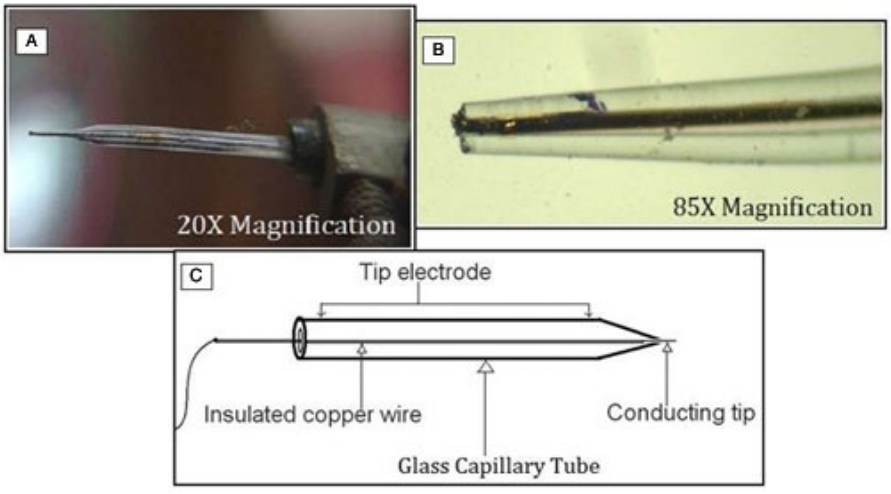


Fig. 1 — The Designed Electrical Probes (A) Microphotograph of the designed electrode for the non-excitable plant (tip diameter 200 µM); (B) Microphotograph of the designed electrode for the excitable plant (tip diameter 80 µM); and (C) Schematic diagram of the designed electrode with its various parts labelled

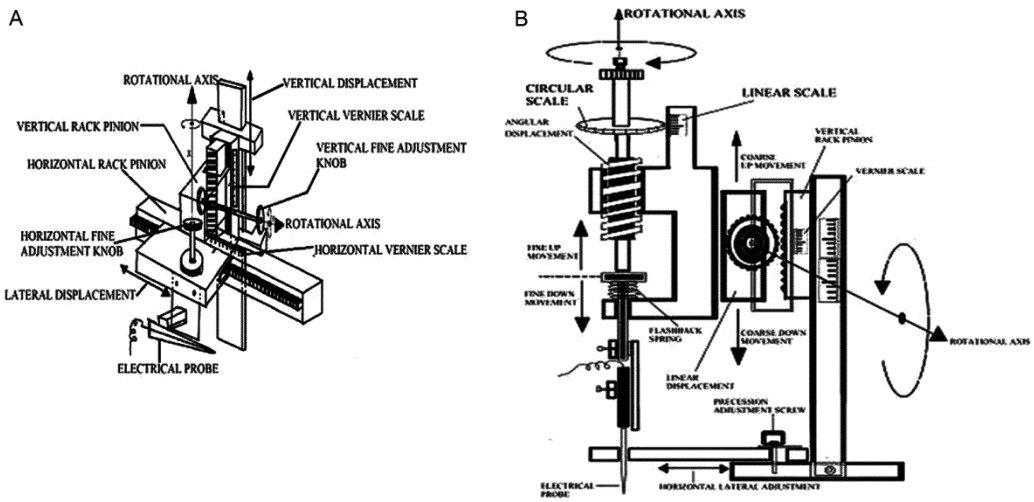


Fig. 2 — Schematic Diagram of the Manual Micro-manipulator Systems (A) Manual micro-manipulator system for the non-excitable plant; and (B) Manual micro-manipulator system for the excitable plant

Non-excitable plant: A two-dimensional mechanical displacement system was assembled using a rack-pinion set-up bearing Vernier scales, capable of moving along both X (vertical height along the stem) and Y (horizontal displacement across the stem) coordinates. This system was used to precisely insert the *tip electrode* into different layers of the stem of the non-excitable plant *A. philoxeroides*.

Excitable plant: Since the diameter of the petiole of the excitable plant *M. pudica* was much thinner, a highly sensitive micro manipulator system was specially designed for the excitable plant using a rack-pinion set-up bearing Vernier scales (coarse adjustment) and a modified screw gauge (fine adjustment). The measurement accuracy of this whole micro manipulator setup was 1/100 (0.01) mm. Thus, this micro manipulator system allowed highly controlled displacements of the tip electrode each time at a depth of around 50 µM along the Y axis having both coarse and fine adjustments. At the end of the tip electrode shaft, a precession attenuator was added for limiting the lateral displacements of the tip electrode and for maintaining proper axis of insertion of the electrode tip through the micro manipulator system.

Electrical signal detecting system

Electrophysiological signals in plants are weak electrical signals, which can fluctuate with the change of environment. Therefore, development of a customised amplifier-detection system is essential[29]. The circuit diagram of the above mentioned system has been described in (Fig. 3). Two pre-amplifiers (A1 and A2) were constructed using OP-AMP 741. As the stem and petiole of the plants contain water medium, the non-inverting inputs of the pre-amplifiers were found to suffer from some instability in voltage drop. Accordingly, to prepare the system for such an environment, both the electrodes were first dipped in water and both the non-inverting inputs were grounded with high resistance (1MΩ) so that the system became stable even in water medium. At this stage, the *reference electrode* and *tip electrodes* were connected to the A1 and A2 pre-amplifiers respectively (Fig. 3). The differential amplifier (B) and the linear amplifier (C) were also constructed using 741 OP-AMP. The amplifiers were arranged for proper voltage offset null. The differential amplifier (B) was adjusted for unity gain and the linear inverting amplifier (C) was set for a gain of magnitude ten by adjusting the potentiometer (as shown in Fig 3). Voltmeters V1 and V2 were connected to B and C respectively. The linearity of the amplifier system was calibrated each time before starting the experiment. The linearity (inverted polarity of the output was converted to original polarity) of the amplifier system has been graphically represented in (Fig. 4).

Experimental Procedure

At the time of experimentation, each of the plants, at a time, were mounted inside a 5 mL glass bottle containing tap water in order to maintain its physiological functioning. The probes that have been used to study the spatial electrical property of the plant tissues were directly connected to the pre-

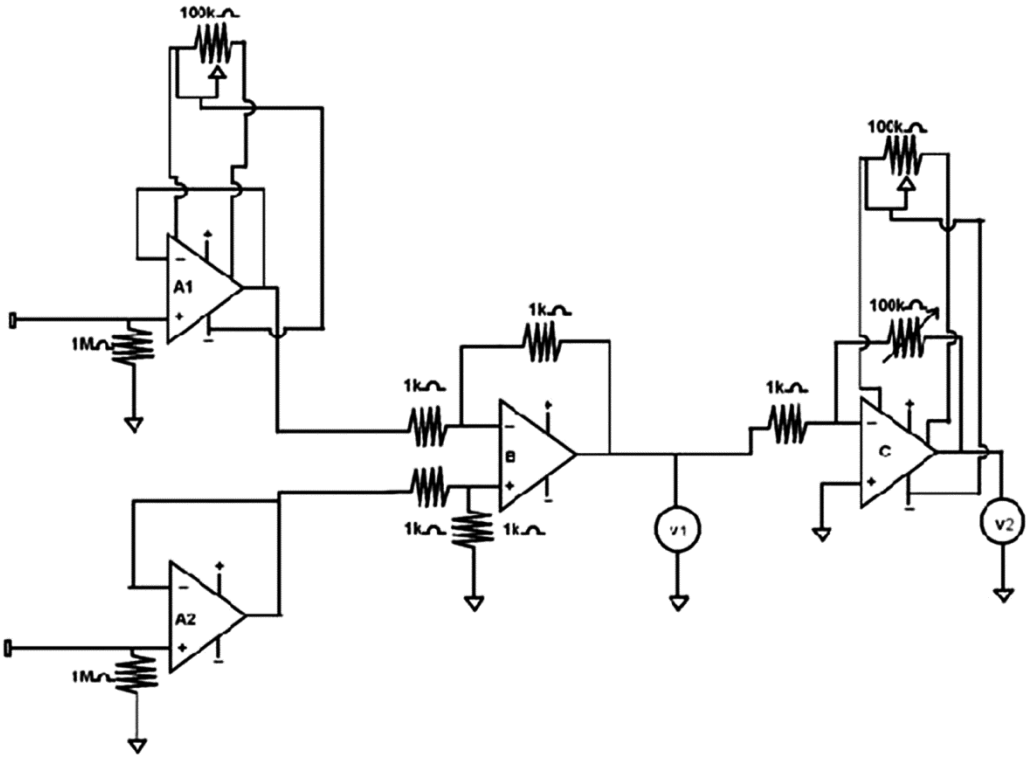


Fig. 3 — Circuit Diagram of the Electrical Signal Measuring System

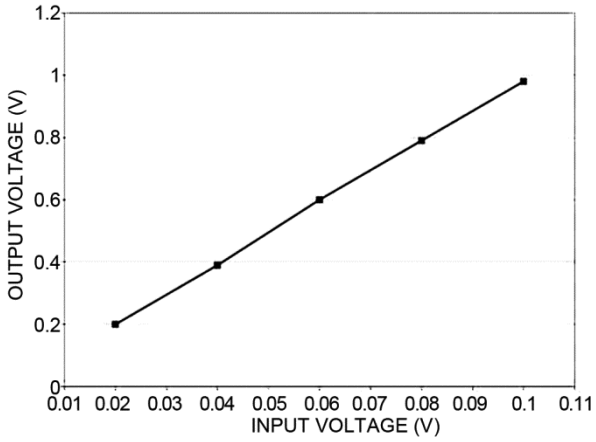


Fig. 4 — Linearity of the Amplifier at 10 times Gain

amplifiers (having high input impedance). It may be noted that the high input impedance of the pre-amplifier minimizes voltage drop within the plant system at the time of signal measurement through the amplifier. The output signal of the pre-amplifier was fed to the differential amplifier and finally the output signal of the differential amplifier was carried to the linear inverting amplifier. The adjustable gain of the linear amplifier provided a provision of proper handling of very low outputs, if any, from the differential amplifier. The changes were monitored through a digital voltmeter. The amplifier systems were calibrated with the aid of standard voltage sources. At the time of recording the spatial voltage, the polarity of the inverted output of the linear inverting amplifier was converted to its original polarity and the 10 times gain factor of the output voltage was converted to its original value (Original Output = Gain Output/10) in case of all the experimental readings. The values of the recorded spatial voltage were then subjected to ANOVA (Analysis of Variance) and student t test statistical analysis, calculated through Python 2.7.6 programming language. Using the manual micro-manipulator system, the glass electrode was inserted perpendicularly to the diameter of stem and petiole in a direction from epidermis to pith, (into the different tissue layers), in a step-wise manner and the reference electrode was placed by coiling around the stem outer surface. The surface electrode was fixed on the stem surface using an impedance matching solution prepared in the laboratory. The impedance matching solution was prepared using 0.9N NaCl solution, glycerine solution and Kaolin powder[16,30,31]. Following such directed tip insertion into the different tissue layers the corresponding changes in the digital voltmeter readings were recorded. In order to study

the effect of electrical stimulation on spatial voltage distribution pattern across the petiole tissue layers of the excitable plant, a uniform and pulastile electrical stimulus s (peak-peak voltage = 12.70 V, quantified through a digital oscilloscope – Tektronics TBS 2000 Series) was applied on the lower side of one of the subpetioles of *M. pudica* for a very brief period (make-break current for about ≤ 1sec)[1,24]. The tip electrode was intruded perpendicular to the diameter of the petiole using the designed micro-manipulator system by steps, of about 50µM at a time. The uniform electrical stimulus was applied each time the electrode tip reached a depth of 50 µM across the petiole tissue layers. The change in intensity and polarity of the spatial voltage at each of the tissue layers in this excited state was recorded accordingly. The configuration of the electrical signal detecting system for both, the non-excitable and excitable plant was maintained in the same order. The position of the surface electrode was the same for both the non-excitable and the excitable plant. The complete experimental set-ups for studying the spatial voltage distribution pattern in the non-excitable and excitable plant have been shown in (Fig. 5A & 5B) respectively. As mentioned before, owing to the thin diameter of the petiole of *M. pudica* it was difficult to visually monitor proper insertion of the electrode tip into the petiole tissue layers. To maintain the visual accuracy at the time of tip insertion, a 10X macro-lens set up was mounted in front of the junction of electrode tip and surface of the petiole (Fig. 5B).

Results

The relative diameter of the tissue layers under microscope and the corresponding values of the spatial potential difference, following insertion of tip of the electrode into the stem of the non-excitable plant *A. philoxeroides* and petiole of the excitable plant *M. pudica* have been represented in (Tables 1 & 2), respectively. From the result it was seen that in case

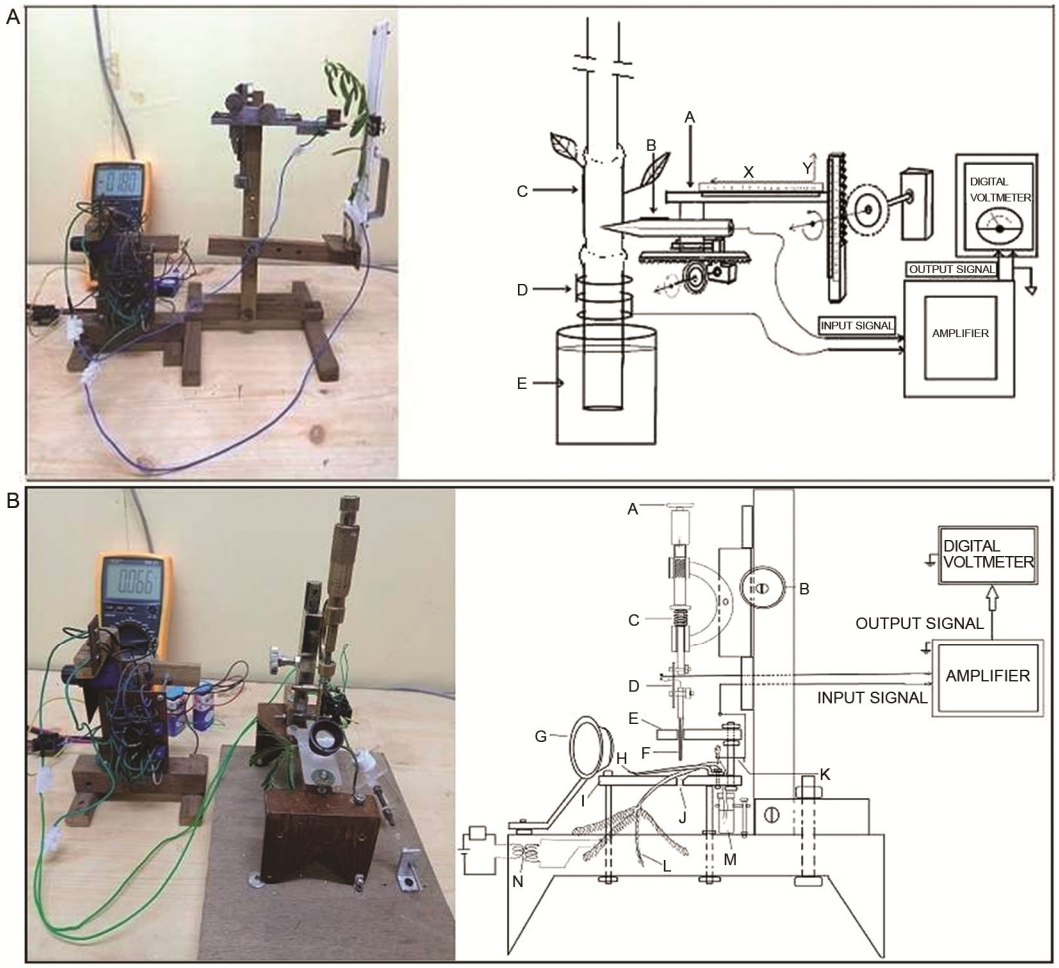


Fig. 5 — (A) The Complete Experimental Setup for the Non-Excitable Plant; and (B) The Complete Experimental Setup for the Excitable Plant

Table 1 — Tabular Representation of the Spatial Voltage Distribution across different tissue layers of the stem of the 5 non-excitable plants (*A. philoxeroides*)

Approximate depth of tip from stem surface (μM)	Values of Spatial Potential Difference (mV)					Mean±SD (mV)	Range (Max/Min) (mV)
0 (Surface/Epidermis)	9	13	8	8	12	10±0.023	13 /8
200 (Cortex)	15	22	13	14	16	16±0.035	22/13
500 (Vascular Bundle)	23	35	22	25	25	26±0.052*	35/22
900 (Pith)	13	15	19	13	15	15±0.024	19/13

*P <0.05

Table 2 — Tabular Representation of the Spatial Voltage Distribution across different tissue layers of the petiole of the 5 plant segments of the excitable plant *M. pudica*

Approximate depth of tip from petiole surface (μM)	Values of Spatial Potential Difference (mV)					Mean±SD (mV)	Range (Max/Min) (mV)
0 (Surface/Epidermis)	−7	−7	−6	−6	−6	−6.4±0.008	−7/−6
100 (Cortex)	−8	−8	−6	−5	−8	−7±0.013	−8/−5
450 (Vascular Bundle)	−8	−8	−6	−5	−9	−7.2±0.017	−9/−5
550 (Pith)	−7	−7	−6	−6	−9.6	−7.12±0.016	−9.6/−6

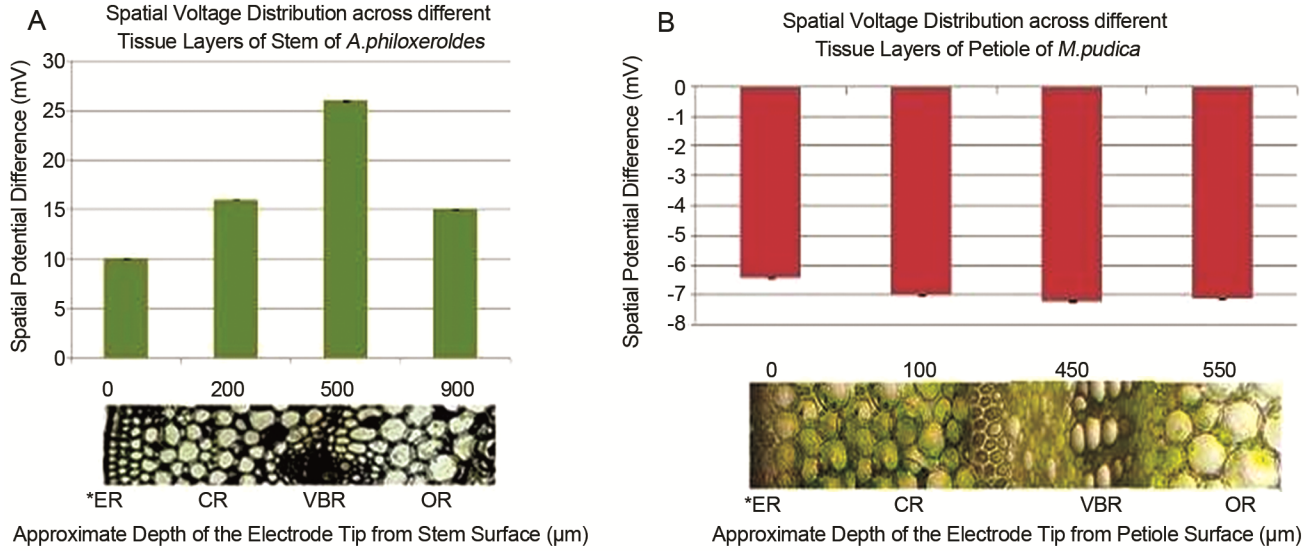


Fig. 6 — Graphical Representation of the Spatial Voltage Distribution across Different Tissue Layers of the Plants Following Insertion of the Tip-Electrode (A) – Spatial voltage distribution across different tissue layers of the stem of the non-excitable plant; and (B) – Spatial voltage distribution across different tissue layers of the petiole of the excitable plant *ER – Epidermal Region, CR – Cortical Region, VBR – Vascular Bundle Region, OR – Pith Region

of the non-excitable plant *A. philoxeroides* (Fig. 6A), the spatial voltage across the different tissue layers of the stem showed positive polarity. However, in case of the excitable plant *M. pudica* (Fig. 6B) the spatial voltage across different tissue layers of the petiole showed negative polarity. It was also evident from the result that in case of the non-excitable plant (Table 1 and Fig. 6A) there were significant differences (P <0.05) among the intensities of the spatial voltage across the various stem tissue layers. The region where the maximum intensity of spatial voltage appeared was around the vascular-cambium layer of the plant stem. The mean value of the maximum attained voltage in the vascular-cambium layer was +26 mV (Fig. 6A), which was significantly higher (P <0.05) than that of the other stem tissue layers. In contrast, in the case of the excitable plant *M. pudica*, there were no significant differences (Table 2 and Fig. 6B) in the intensities of the spatial voltage across the various tissue layers of the petiole, under resting state.

In case of the excitable plant *M. pudica*, a remarkable variation in both, the intensity and polarity of spatial voltage was observed when one of

Table 3 — Tabular Representation of the Spatial Voltage Distribution across different tissue layers of the petiole of the excitable plant *M. pudica* under excited (sub-petiole) and resting states

Approximate depth of tip from petiole surface (μM)	Mean±SD Values of Spatial Potential Difference (mV)	
	Resting State	Excited State
0 (Surface/Epidermis)	−6.4±0.008	−12.5±0.072
100 (Cortex)	−7±0.013	9.3±0.041
250(Phloem 1)	−6.4±0.015	18.3±0.075*
400 (Xylem)	−7.2±0.017	4±0.057
500(Phloem 2)	−7.8±0.02	22.5±0.147*
550(Pith)	−7.12±0.016	17±0.206

*P <0.05

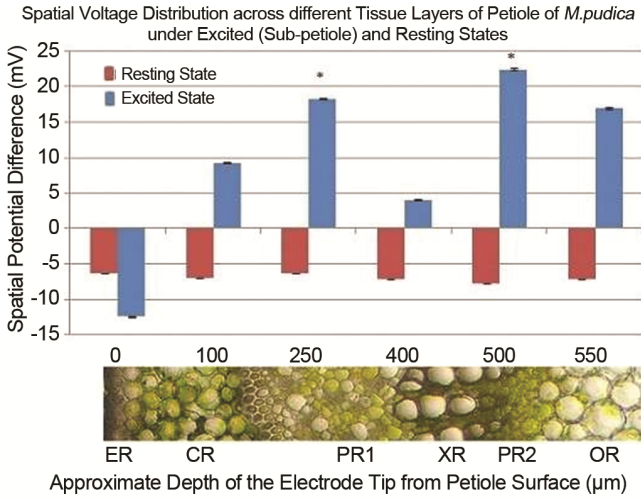


Fig. 7 — Graphical Representation of the Spatial Voltage Distribution across different Tissue Layers of the Petiole of the Excitable Plant under Resting State and Excited State

the sub-petioles was excited with a uniform and pulsatile electrical stimulus (peak-peak voltage = 12.70 V), in comparison to the resting state spatial voltage distribution pattern (Table 3 and Fig. 7). In the excited state condition, when the tip of the electrode entered the epidermal region (Fig. 7) there was a negative rise in the potential difference (–12.5 mV). However, under this excited state condition when the electrode tip reached the other inner tissue layers of the petiole, the polarity of the spatial voltage changed to positive (Fig. 7). In the cortical region, the potential difference value was +9.3 mV. When the electrode tip entered the outer phloem layer (phloem 1 region) there was a sudden rise in the positive voltage which showed a value of +18.3 mV (Fig. 7). However, when the tip electrode reached the xylem region there was a diminution in the positive voltage to +4 mV. Once the tip electrode entered the inner phloem layer (phloem 2 region) there was again a rise in the positive voltage which showed a value of +22.5 mV (Fig. 7). When the electrode tip reached the pith region the spatial voltage underwent a diminution to +17 mV. From the result (Fig. 7), it was also found that under this excited state, the spatial voltage values of the phloem 1 (+18.3 mV) and phloem 2 (+22.5 mV) regions were significantly different (P <0.05) from the spatial voltage values of the other tissue layers (epidermis, cortex, xylem) of the petiole.

Discussion

In the present research, the difference in the spatial voltage distribution pattern between the non-excitable plant *A. philoxeroides* and excitable plant *M. pudica*have been studied on the basis of two aspects – microscopic structural features and spatial electrical property. The structure function relationship between tissue structural arrangements of stem and petiole and the corresponding spatial electrical property have also been studied in detail. Since Bose had clearly stated in his experiments that the localization of conducting tissue transmitting excitation in the plant was definitely possible by means of the specialized ‘electric probe’, in the present set of experiments in order to study the difference in pattern of spatial voltage distribution across different tissue layers in the non-excitable and excitable plant, related type of electric probes (Fig. 1B) were constructed in the laboratory by following the design principles similar to that of J.C. Bose[22]. In the present study, the pattern of spatial voltage distribution (intensity and magnitude of spatial voltage) between stem tissue layers of non-excitable plant *A. philoxeroides* and petiole tissue layers of excitable plant *M. pudica* has been compared because Bose had explained through his experiments that in *M. pudica* the anatomical characteristics of the vascular bundle region, are the same in the stem as in the petiole[22].

Examination of Microscopic Structural Features:

The difference between microscopic tissue structural features of the stem of non-excitable plant *A. philoxeroides* and petiole of excitable plant *M. pudica* have been studied in detail. As per the microscopic examination it was found that the structural arrangement of the vascular bundle in case of the stem of non-excitable plant *A. philoxeroides*

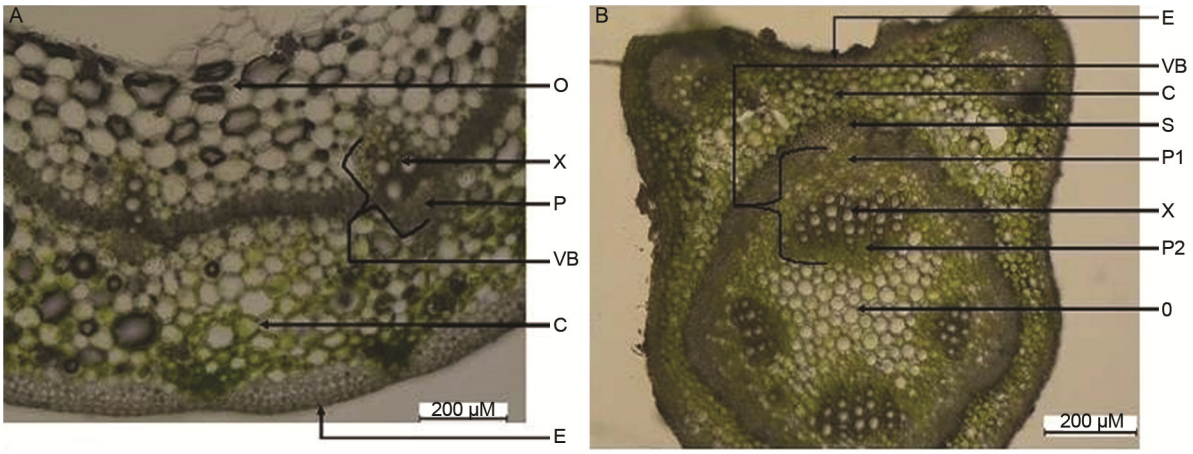


Fig. 8 — Microphotographs of the Tissue Cross-sections of the Plants (100X Magnification) (A) – Tissue cross section of the stem of the non-excitable plant *A. Philoxeroides*; and (B) – Tissue cross section of the stem of the excitable plant *M. pudica* E – Epidermis, C – Cortex, VB – Vascular Bundle, P – Phloem, P1 – Phloem1 (outer phloem), P2 – Phloem2 (inner phloem), X – Xylem, O – Pith, S – Sclerenchyma

(Fig. 8A) was strikingly different from that of the petiole of the excitable plant *M. pudica*(Fig. 8B). However, the epidermis, cortex and pith of both the non-excitable and excitable plants appeared structurally similar.

As observed from the stem-tissue cross sections under the microscopes used in the study, it was found that in *A. philoxeroides* the vascular contains a single phloem strand and xylem (Fig. 8A)[25]. However, in the case of the petiole of *M. pudica* (Fig. 8B) the vascular bundle consists of an outer phloem, xylem and inner phloem. This peculiarity of the structural arrangement in the petiole of *M. pudica* was similar to Bose's findings where he had shown that in the petiole of *M. pudica* there are two phloems in each vascular bundle – one external and the other internal to the xylem, thus forming a bi-collateral structure[21]. Another prominent tissue structural difference between *A. philoxeroides* and *M. pudica* was presence of a sclerenchyma tissue layer (Fig. 8(B)) surrounding the vascular bundles in the excitable plant *M. pudica*[22]. The total diameter of the stem of *A. philoxeroides*, as measured from the microscopic tissue cross sections, was in the order of 3600 µM. The total diameter of the petiole of *M. pudica*, as measured from the microscopic tissue cross sections was in the order of 1049µM. As observed from the microscopic tissue cross sections, the vascular bundle region of the petiole of *M. pudica* was much wider (Fig. 8B) owing to the presence of outer phloem-xylem-inner phloem bi-collateral arrangement, in comparison to the stem of *A. philoxeroides* having single phloem strand and xylem (Fig. 8A).

Analysis of spatial electrical property and the corresponding structure-function relationship

In the present study, the striking difference in the spatial voltage distribution pattern between the non-excitable plant *A. philoxeroides* and excitable plant *M. pudica* was based on the difference in the polarity of the spatial voltage across the different tissue layers of the stem and petiole, respectively. In the non-excitable plant *A. philoxeroides* (Fig. 6A), the polarity of the recorded spatial voltage across the different stem tissue layers was positive whereas, in the case of the petiole of the excitable plant *M. pudica* (Fig. 6B), the polarity was negative. It is meaningful to mention that this difference in the polarity of spatial voltage between the non-excitable and excitable plant as found in the present study was based on the positions of the surface electrode (reference electrode) and the tip electrode (Fig. 9) and the configuration of the electrical signal measurement system.

In the present study another notable difference between the spatial voltage distribution pattern of the non-excitable and excitable plant was observed. In the non-excitable plant the spatial voltage recorded across the different stem tissue layers, upon penetration of the electrode tip was significantly different (P <0.05) in each of the tissue layers of the stem (Fig. 6B), whereas, in the excitable plant *M. pudica* there was no significant difference in the spatial voltage across the different petiole tissue layers upon penetration of the electrode tip (Fig. 6B). The probable reason behind this difference in the pattern of spatial voltage distribution between the non-excitable and the excitable plant following insertion of the electrode tip

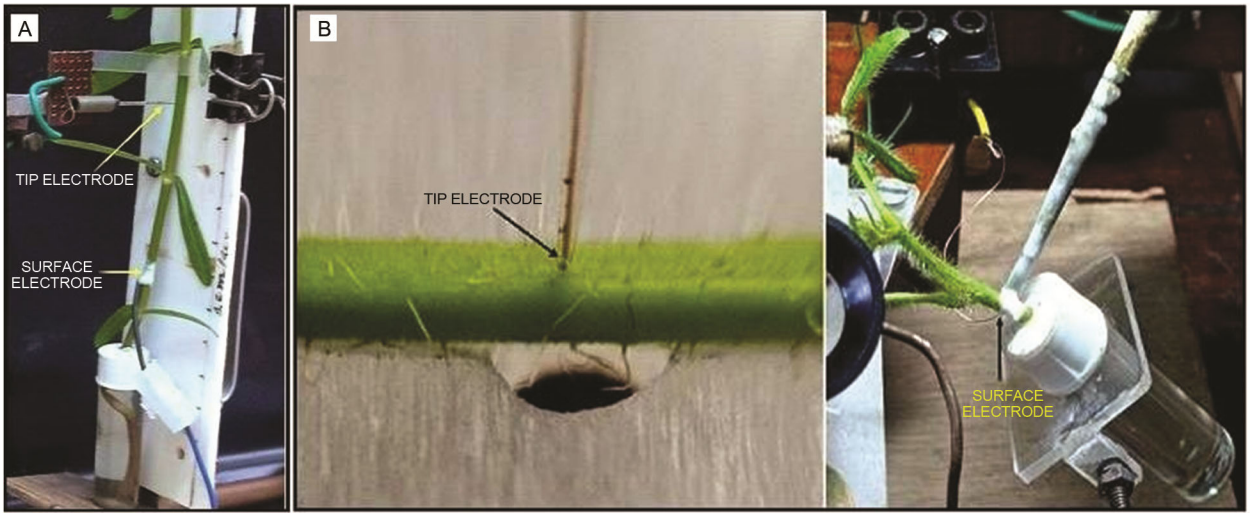
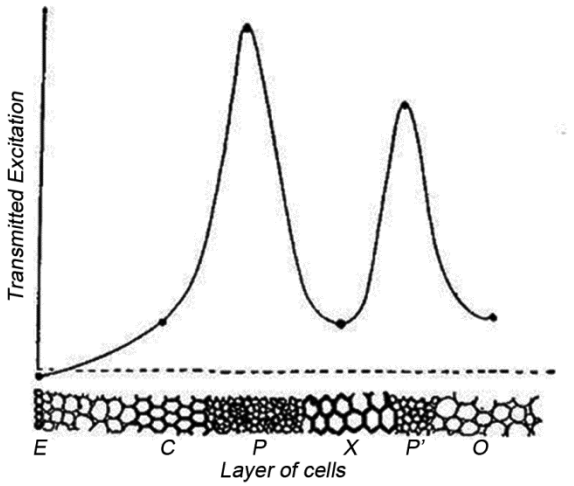


Fig. 9 — Photographic Images of the Positions of the Electrodes in the Non-excitable and Excitable Plant (A) – Electrode positions in the non-excitable plant: Tip electrode at stem, Surface electrode at a distant region of the stem; and (B) – Electrode positions in the excitable plant: Tip electrode at petiole, Surface electrode at stem

into each of the tissue layers was generation of stimulus-like responses across the different tissue layers of the stem of the non-excitable plant. Some recent electrophysiological studies have reported that in case of non-sensitive plants, insertion of electrodes into extracellular space, vessels or inner tissue layers of plants can generate electric potential (EP) responses due to disturbance of plant tissues[32,33]. Conversely, in the excitable plant *M. pudica* the reason behind no significant difference in the spatial voltage values recorded across the different tissue layers of the petiole following electrode tip insertion was possibly due to the fact that in the excitable plant *M. pudica* electrical potential induced rapid movements are generated only upon electrical or mechanical stimulation of the excitatory motor organ *i.e*., pulvinus located at the junction of the leaflet-rachilla, rachilla-petiole, and petiole-stem[1,34]. Recent experimental studies have reported that rapid movements in *Mimosa* are only possible due to the electrical or mechanical stimulation of the pulvini found at the base of leaflet, rachilla, and petiole[1,24]. Thus, in the present study under the resting state, *i.e*., in the absence of any form of mechanical or electrical stimulation of the pulvini there was no significant variation in the spatial voltage values recorded across the different tissue layers of the petiole (Fig. 6B). Research on electrical signalling of non-sensitive plants have shown that EP generated following insertion of electrode into plants may propagate as a systematic signal to distant parts of the plant[35]. A recent experimental study demonstrated that a system potential was triggered upon insertion of a glass (Ag/AgCl) microelectrode and this signal was propagated along the stele of the stem of the non-sensitive plant *Myriophyllum aquaticum*[36]. In the present experimental study, in case of the non-excitable plant *A. philoxeroides*, the maximum intensity of the spatial voltage following insertion of the glass-tip electrode into the stem was recorded in the vascular-cambium tissue layer of the stem (Fig. 6A) and upon analysis it was found that the spatial voltage in this region was significantly higher (P <0.05) in comparison to the spatial voltage of the other stem tissue layers. This result is similar to the findings of other experimental studies on long-distance electrical signalling in ordinary plants[37,38]. Recent research studies have reported that in plants the phloem tissue serves as the 'cable' for long-distance electrical signalling[39]. Another recent study has discussed the role of long-distance sucrose transport in phloem through a process called phloem loading. The study also provided insights on intra molecular interactions in Sucrose/H^+ Symporters (SUTs) and identified residues that are most likely involved in sucrose/H^+ symport[40].

Since *Mimosa* is an excitable plant, having specialized mechanosensory effector organs, as an additional segment of the present study, the effect of uniform and pulsatile electrical excitation (peak-peak voltage = 12.07 V) of a sub-petiole on the pattern of spatial voltage distribution was also studied[1]. In the present study, when one of the sub-petioles was stimulated with a uniform and pulsatile electrical



Curve showing the different intensities of Transmitted Excitation in the different tissuse.
E, epidermis; c, cortex; P, outer phloem; X, xylem; P', inner phloem; o, pith.

Fig. 10 — Scanned Image of the Curve showing the different intensities of Transmitted Excitation in the different tissues of the petiole of *M. pudica* obtained from J. C. Bose's book[22]

stimulus a notable and significant variation in both, the intensity and polarity of spatial voltage was observed (Fig. 7). This is contrary to that of the resting state spatial voltage distribution pattern (Fig. 6B) where no significant variation in the spatial voltage across different petiole tissue layers was found. Extensive research on electrical signalling in *M. pudica* and mechanical and electrical stimulation of plant movements have revealed that both mechanical and electrical stimulation of pulvinus of *M. pudica* can lead to the closing of a pinna and the falling down of the petiole indicating that the pulvinus plays an essential role in the generation of rapid movements[41-44]. These rapid movements are caused by different processes and components in the primary and secondary pulvini, which involve voltage-gated ion channels[45]. Some experimental studies conducted in the past have shown that application of an electric shock in the form of a threshold voltage of 25 V for a brief period induces fast petiolar movements and leaf closure in *M. pudica*[1]. In the present study, under excited state, when the electrode tip reached the outer phloem region (phloem 1 region) there was a prominent and sudden rise in the intensity of spatial voltage (Fig. 7), having a value of +18.3 mV. Conversely, when the tip electrode entered the xylem region the intensity of the spatial voltage reduced to +4 mV. However, when the electrode tip reached the inner phloem region (phloem 2 region) the intensity of the spatial voltage again increased to +22.5 mV. This pattern of spatial voltage distribution across the vascular bundle tissue layers under excited state (Fig. 7) is similar to the experimental findings of Bose's experiment on localization of the excitation-conducting tissue in the petiole of *M. pudica* (Fig. 10)[22]. From the results of the electrical method of exploration, Bose had found that there are two conducting phloems in each vascular bundle, one external and the other internal to the xylem forming a bi-collateral structure. Bose had also explained through his experiments that after electrical stimulation of a sub-petiole the phloem was invariably the best channel that conducted the transmitted excitation (Fig. 10)[22]. In the present study a similar pattern of results has been obtained. As evident from the results of the present experiment, on stimulation of a sub-petiole with uniform pulsatile electrical stimulus the maximum increase in the intensity of spatial voltage was recorded in the two phloem layers – outer phloem (phloem region 1) and inner phloem (phloem 2 region) resulting in the two graphical peaks, as visible in the (Fig. 7). Thus, it may be an indication of the fact that the phloem is the tissue responsible for conduction of excitation (electrical).

Conclusion

From the present study, it may thus be concluded that in both non-excitable and excitable plants the phloem is probably responsible for electrical signalling, as evident from the maximum intensity of the spatial voltage in the phloem regions (vascular cambium region) of both the plants studied. The present experimental study also indicated a difference in the pattern of the spatial voltage distribution between non-excitable and excitable plants following insertion of tip-electrode into each of the tissue layers. In the non-excitable plant *A. philoxeroides* following insertion of tip-electrode there was a significant difference in the intensities of the spatial voltage across stem tissue layers. The vascular-cambium region showed the maximum intensity of the spatial voltage whereas, in the excitable plant *M. pudica* there was no such difference in the intensity of the spatial voltage across the petiole tissue layers following insertion of tip-electrode. However, in the excited state *i.e.*, upon electrical stimulation of the sub-petiole of *M. pudica*, the two phloem regions (outer phloem and inner phloem) showed the maximum intensity of spatial voltage.

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Conflict of Interest

All authors declare no conflict of interest.

References

1 Volkov AG, Foster JC, Ashby TA, Walker RK, Johnson JA & Markin VS, *Mimosa pudica*: electrical and mechanical stimulation of plant movements. *Plant Cell Environ*, 33 (2010) 163.
2 Bose JC, *Response in Living and Non-Living* (Longmans Green & Co., London), 1902.
3 Sanderson BJ, Note on the electrical phenomena which accompany irritation of the leaf of *Dionœa muscipula*. *Proc R Soc Lond*, 21 (1873) 495.
4 Volkov AG, Signaling in electrical networks of the Venus flytrap (*Dionaea muscipula* Ellis). *Bioelectrochemistry*, 125 (2019) 25.
5 Raghavendra AS & Govindjee, Sir Jagadish Chandra Bose (1858–1937): A Pioneer in Photosynthesis Research and Discoverer of Unique Carbon Assimilation in *Hydrilla*. *In:* Raghavendra, A., Sage, R. (eds) *C4 Photosynthesis and Related CO2 Concentrating Mechanisms. Advances in Photosynthesis and Respiration* (Springer, Dordrecht), 32 (2010) 3.
6 Bhattacharyya P & Engineer M, *Acharya J.C. Bose A Scientist & A Dreamer* (Bose Institute, Kolkata), 1996.
7 Bose JC, *Life movement in plants* (Longmans Green & Co., London), 1931.
8 Shepard VA, At the roots of plant neurobiology: a brief history of the biophysical research of J.C. Bose. *Sci Cult*, 78 (2012) 196.
9 Zhao DJ, Wang ZY, Li J, Wen X, Liu A, Huang L, Wang XD, Hou RF & Wang C, Recording extracellular signals in plants: A modeling and experimental study. *Math Comput Model,* 58 (2013) 556.
10 Li T, Wang ZY, Zhao DJ, Huang L & Wang ZY, Development of a portable multi-channel system for plant physiological signal recording. *Inf Process Agric*, 3 (2016) 124.
11 Li JH, Fan LF, Zhao DJ, Zhou Q, Yao JP, Wang ZY & Huang L, Plant electrical signals: A multidisciplinary challenge. *J Plant Physiol*, 261 (2021) 153418.
12 Yan X, Wang Z, Huang L, Wang C, Hou R, Xu Z & Qiao X, Research progress on electrical signals in higher plants. *Prog Nat Sci*, 19 (2009) 531.
13 García SMÁ, Mendoza SM & Contreras MLM, Electrical signals as an option of communication with plants: a review. *Theor Exp Plant Physiol*, 33 (2021) 125.
14 Kaestner L, Wang X, Hertz L & Bernhardt I, Voltage-activated ion channels in non-excitable cells-a viewpoint regarding their physiological justification. *Front Physiol*, 9 (2018) 450.
15 Pitt GS, Matsui M & Cao C, Voltage-gated calcium channels in nonexcitable tissues. *Annu Rev Physiol*, 83 (2021) 183.
16 Wang PH, Lee CE, Lin YS, Lee MH, Chen PY, Chang HC & Chang IF, The glutamate receptor-like protein GLR3.7 interacts with 14-3-3ω and participates in salt stress response in *Arabidopsis thaliana*. *Front Plant Sci*, 10 (2019) 1169.
17 Gangwar SP, Green MN, Michard E, Simon AA, Feijó JA & Sobolevsky AI, Structure of the *Arabidopsis* glutamate receptor-like channel GLR3. 2 ligand-binding domain. *Structure*, 29 (2021) 161.
18 Mousavi SA, Chauvin A, Pascaud F, Kellenberger S & Farmer EE, Glutamate Receptor-Like genes mediate leaf-to-leaf wound signalling. *Nature*, 500 (2013) 422.
19 Helliwell KE, Chrachri A, Koester JA, Wharam S, Taylor AR, Wheeler GL & Brownlee C, A novel single-domain Na+-selective voltage-gated channel in photosynthetic eukaryotes. *Plant Physiol*, 184 (2020) 1674.
20 Minorsky PV, American racism and the lost legacy of Sir Jagadis Chandra Bose, the father of plant neurobiology. *Plant Signal Behav*, 16 (2021) 1818030.
21 Tandon PN & Bose JC, plant neurobiology. *Indian J Med Res*, 149 (2019) 593.
22 Bose JC, *The nervous mechanism of plants* (Longmans Green & Co., London), 1925.
23 Clements, D, Dugdale TM, Butler KL, Florentine SK &Sillitoe J, Herbicide efficacy for aquatic *Alternantheraphiloxeroides* management in an early stage of invasion: Integrating above-ground biomass, below-ground biomass and viable stem fragmentation. *Weed Res*, 57 (2017) 257.
24 Visnovitz T, Világi I, Varró P &Kristóf Z, Mechanoreceptor cells on the tertiary pulvini of *Mimosa pudica* L. *Plant Signal Behav*, 2 (2007) 462.
25 Roy S, Bhattacharya B, Bal B & Ghosh K, A microscopic study on scattering in tissue section of *Alternanthera philoxeroides* under polarized light. *J Biosci*, 46 (2021) 1.
26 Chong PL, Singh AK & Kok SL, Characterization of Aloe Barbadensis Miller leaves as a potential electrical energy source with optimum experimental setup conditions. *PLoS One*, 14 (2019) 0218758.
27 Cooper RL, Thomas MA, Vascassenno RM, Brock KE & McLetchie DN, Measuring Electrical Responses during Acute Exposure of Roots and Rhizoids of Plants to Compounds Using a Flow-Through System. *Methods Protoc*, 5 (2022) 62.
28 Senavirathna MD & Muhetaer G, Electrode insertion generates slow propagating electric potentials in *Myriophyllum aquaticum* plants. *Plant Signal Behav*, 15 (2020) 1734332.
29 Li T, Wang ZY, Zhao DJ, Huang L & Wang ZY, Development of a portable multi-channel system for plant physiological signal recording. *Inf Process Agric,* 3 (2016) 124.
30 Volkov AG, *Plant electrophysiology: methods and cell electrophysiology* (Springer, New York) 2012.
31 Domínguez MB, Rodríguez AA, Barrientos MM, Cervantes AP, Zamora AD & Hernández BA, Ghrelin and Leptin increase voltage-gated Na^+ and $Ca2^+$ currents in the RIN-m5f insulin-producing cell line. *Indian J Biochem Biophys*, *56*(2019) 93.
32 Hagihara T & Toyota M, Mechanical signaling in the sensitive plant *Mimosa pudica* L. *Plants*,9 (2020) 587.
33 Gibert D, LeMouël JL, Lambs L, Nicollin F & Perrier F. Sap flow and daily electric potential variations in a tree trunk. *Plant Sci*, 171 (2006) 572.

34 Senavirathna MDHJ & Asaeda T, Microwave radiation alters burn injury-evoked electric potential in *Nicotiana benthamiana*. *Plant Signal Behav*, 13 (2018) 1.

35 León J, Rojo E & Sánchez SJJ, Wound signalling in plants. *J Exp Bot*, 52 (2001) 1.

36 Senavirathna MD & Muhetaer G, Electrode insertion generates slow propagating electric potentials in *Myriophyllum aquaticum* plants. *Plant Signal Behav*, 15 (2020) 1734332.

37 Fromm J & Lautner S, Electrical signals and their physiological significance in plants. *Plant Cell Environ*, 30 (2007) 249.

38 Fromm J, Hajirezaei MR, Becker VK & Lautner S, Electrical signaling along the phloem and its physiological responses in the maize leaf. *Front Plant Sci*, 4 (2013) 239.

39 Hedrich R, Salvador RV & Dreyer I, Electrical wiring and long-distance plant communication. *Trends Plant Sci*, 21 (2016) 376.

40 Syamaladevi DP & Biswal B, Structural insights on Sucrose transport by *Oryza sativa* L. Sucrose/H^+ Symporter1 (OsSUT1) through refined sequence-template alignment based structural modelling. *Indian J Biochem Biophys*, 57 (2020) 283.

41 Wairimu NU, Kamweru P & Tuwei AK, Action and variation potential electrical signals in higher plants. *Afr J Biol Sci*, 3(2021) 1.

42 Volkov AG, Foster JC & Markin VS, Signal transduction in *Mimosa pudica*: biologically closed electrical circuits. *Plant Cell Environ*, 33 (2010) 816.

43 Mano H & Hasebe M, Rapid movements in plants. *J Plant Res*, 134 (2021) 3.

44 Sibaoka T, Physiology of rapid movements in higher plants. *Annu Rev Plant Physiol*, 20 (1969) 165.

45 Wang Y & Li H, Bio-chemo-electro-mechanical modelling of the rapid movement of *Mimosa* pudica. *Bioelectrochemistry*, 134 (2020) 107533.