

## Revisiting the physiology of ascent of sap in plants: legendary experiment of J. C. Bose

Jagadish Chandra Bose observed rhythmic electrical oscillations or pulsations in living cells of the innermost layer of cortex, and linked them to upward pumping of water into the xylem<sup>1</sup>. Bose measured bioelectric potentials in Indian telegraph plant *Desmodium* (Bon Charal or forest churl) using the cresograph, a self-invented instrument, that consisted of an electric probe, a galvanometer, an electric dry cell and a thin copper wire. He connected the galvanometer to one point of a potted plant and the probe to another point of the plant, and slowly inserted the probe into the stem. The galvanometer showed momentary deflection for a longer period after the probe reached the innermost layer of the cortex. Bose observed a physiological motif and interlinked the measured pulsations in cellular electric potentials with oscillations in cell turgor pressure<sup>2</sup>. Bose argued that all plants have a universal electro-mechanical oscillation that may be described as a kind of vegetable heartbeat. However, contemporary scientists in the West were not successful in repeating the experiment, and denounced his finding<sup>3</sup>. For example, George James Peirce, a professor of plant physiology at Stanford University, USA, stated, 'The trouble with Bose, as I see it with my Occidental eyes and my American mind, is that while his curiosity is directed to biological phenomena, his mind is inadequately equipped with the information and habits necessary for accurate study, and his reflections are addressed to philosophical problems. He is practical-minded to the extent of using self-recording apparatus in his laboratory..., but his ambitions exceed his capacities...' <sup>4</sup>. Interestingly, present-day scientists believe that the signals, in the form of potential difference, recorded by Bose are real<sup>5</sup>. Gensler and Diaz-Munoz<sup>6</sup>, and Gensler and Yan<sup>7</sup> recorded characteristic potential-time fluctuations in crop plants and linked the signals to plant condition, water status, atmospheric changes and time of day. However, there is no obvious explanation for the oscillations in electric potential measured by Bose, and mainstream opinion questions the role of living cells as well as electro-mechanical pulsations in the ascent of sap<sup>2</sup>. Nonethe-

less, the signal measured by Bose has been connected to voltage difference across the plant cell wall due to a complex mechanism involving movement of sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) ions between the cells<sup>8</sup>. Further, electrical signal induced by release of calcium ( $\text{Ca}^{2+}$ ) in the cell cytoplasm, known as action potential, is generated by external stimuli that act as abiotic stress to plants. Action potential involves abrupt depolarization, activation of chlorine ( $\text{Cl}^-$ ) and  $\text{K}^+$  channels, and results in water,  $\text{Cl}^-$  and  $\text{K}^+$  efflux<sup>9,10</sup>, transitory loss of turgor pressure<sup>11</sup> and transitory contraction of the cell<sup>12</sup> causing rapid change in polarity that moves from cell to cell along the phloem<sup>8,13</sup>. This motif ( $\text{Ca}^{2+}$  influx, and  $\text{K}^+$  and  $\text{Cl}^-$  efflux, contraction and turgor change) is fundamental to the osmotic machinery<sup>14</sup> and mirrors the electro-mechanical 'pulsations' of Bose. Toko *et al.*<sup>15</sup> showed oscillatory action potentials along the root of higher order plants through theoretical modelling and experiment. Recently, Choi *et al.*<sup>16</sup> have shown a travelling wave of elevated  $\text{Ca}^{2+}$ , running from the root tip upwards along the shoot of a plant, similar to the experiment of Bose. The authors have demonstrated the role of salt stress in systematic signalling, where sodium chloride ( $\text{NaCl}$ ) applied to the root tip of a plant triggers  $\text{Ca}^{2+}$  wave that propagates through cortical and endothermal cells to distal shoot tissues<sup>16</sup>.  $\text{Ca}^{2+}$  simultaneously activates a protein called Toppore Channel 1 (TPC1) and carries the signal through TPC1 between cells and from root to shoot<sup>16</sup>. The ion flow further generates potential difference in the plant body. We hypothesize that the experiment of Bose involving the measurement of potential difference in plants was successful due to the presence of naturally occurring Na in the water (River Ganga water or groundwater) that he used as a medium for the experiment, where Na acted as an abiotic stress to the plants. The objective of the present study was to repeat the classic experiment of Bose with modern instrumentation and to investigate the role of Na, present in the water, in the success of his experiment.

Bose most likely conducted his experiment at Bose Institute or Presidency

College (presently Presidency University) in Kolkata where he served as a faculty. Kolkata lies in the lower deltaic plain of River Ganga<sup>17</sup> and the river flows close (<4 km) to both places. The climate of the area is predominantly influenced by the northeast and southwest monsoons, with an average annual rainfall of about 1650 mm (ref. 18). The groundwater geochemistry is controlled by the underlying geology<sup>17</sup> that is primarily constituted of Quaternary fluvial sediments comprising a succession of clay, silt, sand and gravel<sup>18</sup>.

Groundwater samples were collected from a hand-pumped tube well in close vicinity of Presidency University, Bose Institute and adjacent areas. Water from River Ganga, supplied by the Kolkata Municipal Corporation, was collected from a roadside tap close to Bose Institute. Water samples were collected in pre-cleaned, 250 ml polypropylene bottles and filtered using 8  $\mu\text{m}$  Whatman 40 filter paper. Water was filled up to the brim of bottles which were stored in a cool dark place until analysis. The pH of water (Table 1) was measured using a pre-calibrated Systronics 335 digital pH meter (SYSTRONICS, India). Sodium concentration in the samples was measured using a Perkin-Elmer AAnalyst 100 Flame Atomic Absorption Spectrometer. Table 1 provides results of the analyses.

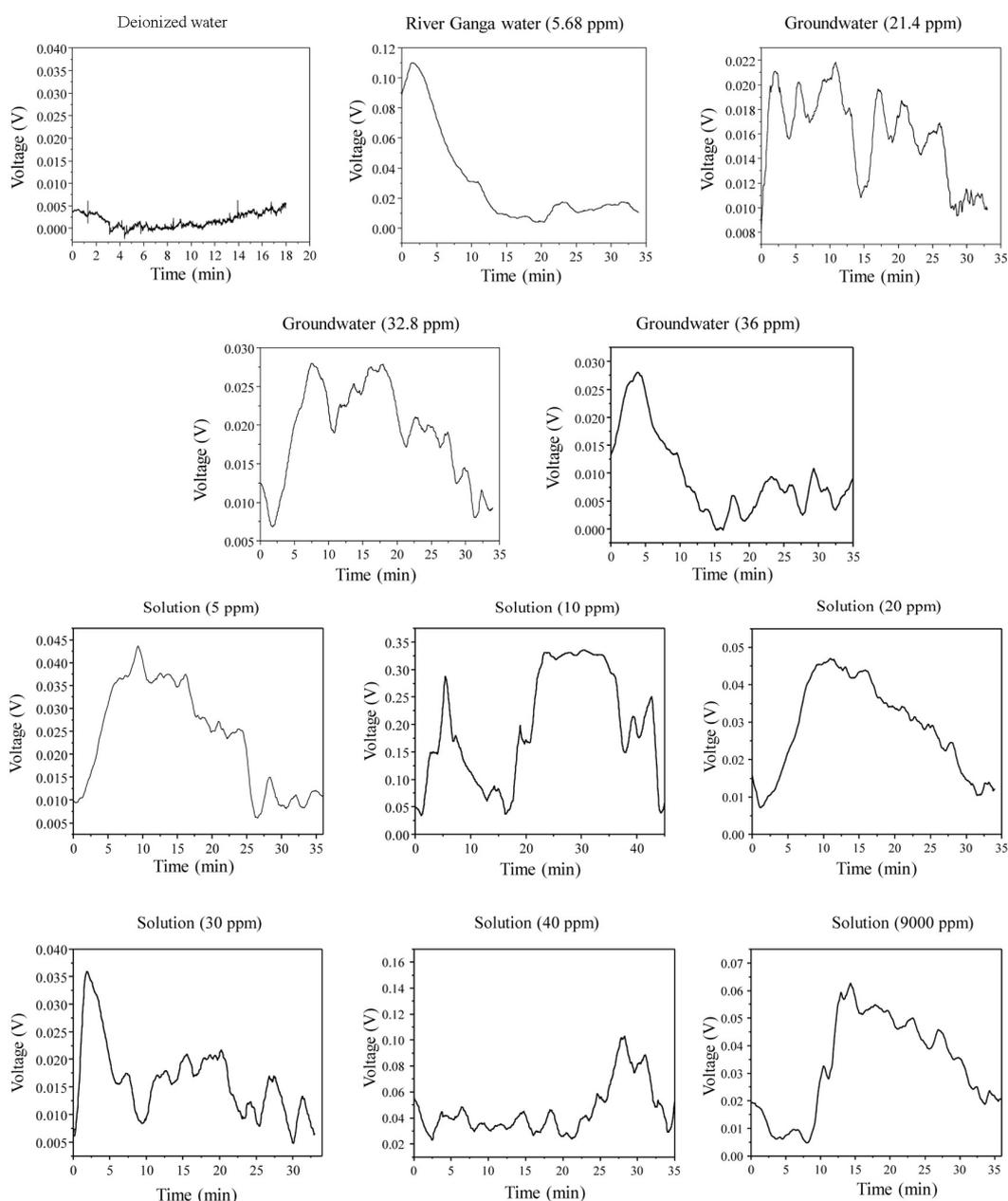
Twelve young plants of the species *Desmodium gyrans* (L.f.) DC. (family: Fabaceae) were used for the experiment. Roots of the plants were kept immersed in the experimental medium (deionized water, natural water (groundwater and river water) and in Na solution of concentrations, i.e. 5, 10, 20, 30, 40 and 9000 ppm) for 30 min. The voltage difference (Figure 1) was measured using a source meter (Keithley 2450, USA), which is a source measure unit instrument with a capacitive touch screen user interface. The experiment was conducted at constant room temperature (28°C).

Potential difference measured across two points on the stem of the plants ranged between 6.38 and 47.4 mV (Table 2). We could not compare our results with the experiment conducted by Bose, because he did not report results in absolute scale<sup>1</sup>. Moreover, we did not observe

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**Table 1.** pH and Na concentration in groundwater at College Street and adjoining areas in Kolkata, and Barasat, a suburb on the outskirts of Kolkata

Location	Water type	Latitude	Longitude	pH	Na (mg l <sup>-1</sup> )	Study
Heramba Das Lane, Kolkata	Ground water	22°34'44.6"	88°21'59.6"	6.4	21.4	Present
College Street, Kolkata	Ground water	22°34'38.9"	88°21'40.9"	6.1	36	Present
Banerjee Lane, Bowbazar, Kolkata	Ground water	22°34'8.9"	88°21'49.5"	6.3	32.8	Present
Keshab Chandra Sen Street, Kolkata	River Ganga water	22°34'43.9"	88°21'57"	6.7	5.68	Present
Humaipur, Barasat	Ground water	—	—	6.5	13.3	Chaudhury <i>et al.</i> <sup>20</sup>
Berpol, Barasat	Ground water	—	—	6.5	24	Do
Choughoria, Barasat	Ground water	—	—	7.5	19.6	Do
Ioibra, Barasat	Ground water	—	—	6.5	38.3	Do
Gobra, Barasat	Ground water	—	—	7.0	22.3	Do
Deara, Barasat	Ground water	—	—	7.0	23	Do
Matiagacha, Barasat	Ground water	—	—	6.5	22	Do



**Figure 1.** Voltage versus time graph measured in the plant using the source meter. Values in parentheses are concentration of Na.

**Table 2.** Relationship between potential difference and Na concentration measured using groundwater and Na solutions of different concentrations

Sample type	Location	Na (mg l <sup>-1</sup> )	Potential difference (mV)
River Ganga water	Keshab Chandra Sen Street	5.68	47.4
Solution	–	5	41.18
Solution	–	10	42.11
Solution	–	20	33.34
Ground water	Heramba Das Lane	21.4	31.97
Solution	–	30	36.36
Ground water	Banerjee Lane, Bowbazar	32.8	30.46
Ground water	College Street	36	27.13
Solution	–	40	23.08
Solution	–	9000	25.66
Deionized water	–	–	6.38

any rhythmic electrical oscillations or pulsations in our experiment, as reported by Bose<sup>1</sup>. This is not surprising because rhythmic electrical oscillations have not been observed by later workers<sup>2</sup>. Table 2 and Figure 1 show the relationship between potential difference and Na concentration. The lowest value (6.38 mV) of potential difference was recorded using deionized water, where Na concentration was below detection limit. The plant did not become stressed due to the absence of Na, and generated only resting potential (voltage difference between inside and outside of the cell<sup>8</sup>). Action potential was generated with the introduction of Na in the experimental medium. Interestingly, the highest value of the action potential (47.4 mV) was recorded at Na concentration of 5.68 ppm (River Ganga water). Action potential roughly decreased with increasing Na concentration (from 5 to 40 ppm) (Table 2). The observed decrease in action potential most likely be caused by the production of excess Ca<sup>2+</sup> that creates a barrier and restricts ion flow between and within the plant cells. Plants produced a low signal when Na concentration increased to 9000 ppm, as the solution becomes saturated with Na making the plant stabilized to the external abiotic stress. Our results suggest that the presence of Na in natural water available in Kolkata has played a significant role in the success of Bose's experiment. We

conclude with a statement in *Handbuch der Pflanzenphysiologie*<sup>19</sup> (*Encyclopedia of Plant Physiology*) on the significance of Bose's experiments; 'Unfortunately Bose's theoretical views and his emotional style of reporting have generated what may be an excessive scepticism concerning the validity of his observations.'

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SUPRIYO KUMAR DAS<sup>1,\*</sup>  
 DEBASISH DUTTA<sup>2</sup>  
 SARANYA NASKAR<sup>2</sup>  
 SNIGDHA PALCHAUDHURY<sup>1</sup>  
 RABINDRANATH GAYEN<sup>2</sup>  
 ABHIJIT DEY<sup>3</sup>

<sup>1</sup>Department of Geology,

<sup>2</sup>Department of Physics, and

<sup>3</sup>Department of Life Sciences,  
 Presidency University,

College Street 86/1,  
 Kolkata 700 073, India

\*For correspondence.

e-mail: sdas.geol@presiuniv.ac.in