A testimony of inter-plant communication through electrophysiological signal analysis

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Plant responses to changes in environment are allied with electrical excitability, signaling and are observed by their electrophysiological signals. Similarly communication in between various plants is noticed by continuous monitoring of their electrophysiological signals at same instant comparatively. Signal acquisition is done with help of BIOPAC® MP36 set up.

Two plants of different species connected to two different channels at a time clearly visualized the amplitudes and frequencies with which plants pulsed during their sensory perception, communication and adaptation to eventual stimuli. The variation in signal potentials of plants depend upon intensity of stimulus perceived either from environment or neighboring plant. They communicate with each other through electrical signals using air as a medium; they are conscious enough to aware neighborhood about hazards and threats. This paper presents in detail the potential and frequency variations that plants illustrate during Inter-plant communication.

Highlighted Conclusion
The meticulous sensory perception of plants when they endure mechanical, heat and chemical stimuli clearly revealed that plants are highly sensitive to environmental changes. The communication in plants is not only intra-specific but from experimental results it is concluded that plants are good enough in stress/danger perception and self-defense clearly inter-plant communication exists.

Many scientists belonging to different times and cultures (e.g., Burdon-Sanderson 1873, Darwin 1875, Bose 1926, etc.) have clinched a belief that plants have much more intricated abilities than they are commonly perceptible. Instigated by a consistence with Grover Cleve Backster’s theory of ‘Primary perception’ that plants have ability to sense and respond to environmental stimulus, the signal transmission in plants is not only intra-specific but their inter-specific communication escorts to a term Plant Intelligence. Plants do not have a brain or neuronal network, but reactions within signaling pathways may provide a bio-chemical basis for learning and memory in addition to computation and problem solving. Plants have ability to distinguish between light and dark. They communicate using air, roots as the transmission media, they are conscious enough to aware neighborhood about hazards and threats. Conscious energy exists in everything; it depends on the matter that utilizes it. Merely matter is all energy pulsating with different frequencies and amplitudes. The frequencies and amplitudes with which plants pulsate can be characterized by monitoring the (Electrophysiology) electrical signals of plants. It is evident that electrical signals play a major role in many plant processes like photosynthesis (Kozielk et al. 2004), water intake (Davies et al. 1991), communication (Muller et al. 2006), detailed sensory perception (Roblin 1985, Mancuso 1999, Rhodes et al. 1996), respiration (Dziubinska et al. 1989), learning and memorizing the eventual stimuli etc. Electrocardiogram and Electroencephalogram (ECG & EEG) are also categorized as Electrophysiological recordings of human beings. Action Potential (AP) and Variation Potential (VP) are two different electrical signals observed in plants. The electrical pulse propagating rapidly with a constant velocity by maintaining constant amplitude is Action Potential (Fromm 2006). Variation Potential (slow wave) shows variation in the electrical pulse either to a long range or a short range depending upon the intensity of the stimulus provided (Stahlberg et al. 2006). Its velocity
and amplitude depend upon xylematic pressure and velocity decreases with increasing distance from site of generation (Davies and Murray 2004, Davies 2006).

In plants the ion translocation takes place by xylem and phloem tissues. They play a significant role in electric signal transmission entire the sap. Phloem is outermost tissue consisting of conducting cells greatly supportive in measuring the movement of Action Potential. The AP propagates with constant amplitude along the length of cell membrane when phloem is stimulated. (Davies and Murray 2004, Fromm and Lautner 2005, Fromm 2006). The Variation Potential is quite similar to the concept of event related potentials in humans. It is variation in membrane potential that moves along xylem which arises from increase in plant cell turgor pressure due to events like rain, wounds, burning, organic excision etc.

The electric signal parameters vary on type of stimulus applied and properties of plant species. The measurement of changes occurred in Action Potential and Variation Potentials due to environmental changes like light intensity, soil humidity, temperature, wind speed, heat stimulation, mechanical stimulation, and salt levels are more helpful for analyzing plant physiology . The potentials are dissimilar in different plants with respect to changes in day and night, but time taken to complete one particular depolarization and repolarization phase of a plant is constant which is 20 ms.

Two major techniques used in measurement of plant’s electrical activity are invasive and non-invasive methods. Invasive techniques involve making a cut or split in plant tissue for signal acquisition. Non-invasive techniques involve in surface measurements by inserting thin metal electrodes minimally into plant tissue or just placing sticking gel electrodes over the leaves of plant. These electrodes consist of conducting gel which is viscous in nature and acts as a liquid medium to determine the electrical activity just beneath the plant tissue. The electrodes are placed at respective positions on plant and connected by insulated cables to a signal acquisition system and activity of plant is observed on a monitoring system.

The main objective of this work is to observe electrical activity of two different plants when they are put far and together, signal transreceiving capabilities between them, variations in signal potentials by human touch, heat and localized chemical product application at the same time. The application of stimulus to one Plant created disturbance in potential of next one has been observed.

MATERIAL AND METHODS

The electrophysiological activity of plants is recorded in Biomedical signal Processing lab by using computer aided BIOPAC® MP36 signal acquisition set up. The basic parameters like humidity and PH level of soil are measured using Durable Pro 4 in 1 soil survey instrument which are maintained constant throughout the experiment. Atmospheric parameters like sunlight intensity and soil temperature are slightly varied with respect to day and night phenomena. Two Plants used in this study are normal house plants commonly named as Ti plant (Cordyline fruticosa) and Arrowhead vine (Syngonium podophyllum). Cordyline fruticosa (Plant 1) is of 45 cm tall, woody plant bearing 10 to 12 lance shaped leaves which emerge pinkish red and turn to dark green when matured. Syngonium podophyllum (Plant 2) is of 40 cm tall bushy plant bearing 25 to 30 leaves which emerge heart shaped, dark green with cream variegation and mould to arrowhead shape when matured. Temperature is maintained 28-30 °C during day and 18-20 °C during night. Atmospheric Humidity is 30-60% during day and 80-100% during night.

Acquisition set up used in this experiment BIOPAC® MP36 includes both hardware and software for acquiring physiological signals (life-science data) and its analysis. A simple model of acquisition set up where Plant 1 connected to channel 1, Plant 2 connected to channel 2 and their electrode positioning is shown in Figure 1. Solid gel electrodes containing 4% of NaCl are used. SS2LB connector with 9 pin DIN at one end connected to respective channel in MP36 and 3 pinch leads at other end connected to electrodes placed near shoot, root and ground of particular plants. Simple cable connectors for power input and standard interfacing cables like USB to connect with computer are used. The computer requires the application software BSL PRO 3.7.3 to run the acquisition. It allows multiple channel (4 analog) measurements at a time. The two plants Cordyline fruticosa and Syngonium podophyllum are connected to two distinct channels where recording can be monitored at same time as shown in Figure 1. The length of acquisition and rate at which data is recorded can be controlled. The physiological data like slow moving temperature data also fast moving Action Potential can be easily measured. The ion mechanism (electrical activity) beneath the plant tissue is continuously sent to MP36 by conducting gel electrodes through the probes to respective channels. The incoming analog signals are taken by acquisition unit and converted to digital signals that can be effortlessly processed by the computer. Phenomena of ion exchange during plant’s normal activity is expressed in Figure 2.
RESULTS AND DISCUSSION

The experimental set up was used for performing several experiments. The data was monitored for 24 hours in regular intervals of time where specific samples were selected to present in this paper. The two plants Cordyline fruticosa and Syngonium podophyllum were applied 1 liter of water at 6.00 am on Day 1. Initiating from 7.00 pm on Day 1 (28th October 2016) till 7.00 pm on Day 2 (29th October 2016) the potentials of normal physical activity of plant like photosynthesis and starch division, utilization were visibly monitored. The electrical characteristics of recorded data and biological parameters maintained for measurement are shown in Table 1. All parameters that affect the plant potential were maintained nearly constant. Observations from Figure 2 show the increase in amplitudes of plants from night to day (till sunrise) and gradual decrease from day to night (till sunset). The frequency response of plants throughout day and night is constant (50 hz) without any change. The time taken for...
The completion of one repetitive event (one cycle) is 20 milliseconds. The change in frequency of plant potential is occurred only when uneventful stimulus is being applied to plants. The variations in velocity of ion flow and charge concentration in plant cell due to changes in the gradual increase and decrease of photon energy is the reason for day and night amplitude variations.

Table 1. Electrical characteristics of Plants 1 & 2 under maintained biological parameters, (P1) denotes touch stimulus applied on Plant 1, (P2) denotes touch stimulus applied on Plant 2.

<table>
<thead>
<tr>
<th>Biological Characteristics</th>
<th>(Day 1) 7:00 pm</th>
<th>(Day 1) 10:30 pm</th>
<th>(Day 2) 6:00 am</th>
<th>(Day 2) 10:30 am</th>
<th>(Day 2) 3:30 pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Temperature of soil</td>
<td>24°C</td>
<td>20°C</td>
<td>18°C</td>
<td>26°C</td>
<td>28°C</td>
</tr>
<tr>
<td>2) pH level of soil</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>3) Intensity of sunlight</td>
<td>&lt;500 lux</td>
<td>&lt;500 lux</td>
<td>&gt;500 lux</td>
<td>10000 to 25000 lux</td>
<td>5000 to 10000 lux</td>
</tr>
<tr>
<td>4) Humidity of soil</td>
<td>5 to 10 %</td>
<td>10 to 20 %</td>
<td>5 to 10 %</td>
<td>5 to 10 %</td>
<td>10 to 20 %</td>
</tr>
</tbody>
</table>

The positive and negative peaks in normal activity of plant are due to polarization of plant cell. Three types of polarizations are noticed to be called as depolarization, repolarization & hyperpolarization. Depolarization in a plant cell is allowing Na+ ions from outside the cell to inside the cell through cell wall, where the concentration of positive charge inside the cell increases and positive peak is obtained. This potential or ion concentration due to depolarization phase is termed as Action Potential. Repolarization in plant cell allows K+ ions to flow from inside the cell to outside the cell, where the concentration of negative charge inside the cell increases and negative peak is obtained. This potential or ion concentration due to repolarization phase is termed as resting potential. The phenomena of action to resting potential and resting to Action Potential in a plant is as shown in Figure 3. The positive and negative peak variations in normal activity of a plant at different timings are as shown in Figure 2. Hyperpolarization occurs when a plant cell is subjected to stress (application of stimuli) either manually or environmentally.

The propagation of Action Potential is clearly the variation of charge concentration in plant cells along the length of phloem. In Figure 4 the response of Plants on application of mechanical stimulus (Human touch) when two Plants 1 & 2 are placed at a distance of 90 cm is shown. Figure 4(a) shows the amplitude of Plant 1 increased to 30 mv on human touch at 11.30pm and there is no variation in the potential of Plant 2. The amplitude increased to 41 mv during day as shown in Figure 4(b) there is no variation in Plant 2’s electric potential. Figure 4(c) describes variations in potentials of plants to during sunset, the amplitudes of plant are very low when compared to amplitudes in day but even though Plant 2 showed variation to applied stimulus (human touch) where Plant 1 has no variation. The changes in electrical potentials in Plants 1 & 2 when the distance between them is maintained 10-20 cm is shown in Figure 5. Figure 5(a) describes the change occurred in normal activity of Plant 1 when the touch stimulus is applied on Plant 2. At 11.40 pm Plant 2 had showed the amplitude of 50mv and at the same time within in few milliseconds delay Plant 1 had showed 30 mv amplitude. There is no connection between the two plants, both are connected to two different channels but the condition of Plant 2 is being perceived by Plant 1 (Inter-plant communication Eavesdropping) and potential variations are shown.
Figure 3. (a) Normal activity of Plants 1 & 2 at 7.00 pm, (b) Normal activity of Plants 1 & 2 at 10.30 pm, (c) Normal activity of Plants 1 & 2 at 6.00 am, (d) Normal activity of Plants 1 & 2 at 10.30 am and (e) Normal activity of Plants 1 & 2 at 3.30 pm.
Figure 4. At a distance of 90 cm: (a) Response of Plants 1 & 2 when stimulated by human touch at 11.30 pm, (b) Response of Plants 1 & 2 when stimulated by human touch at 10.30 am and (c) Response of Plants 1 & 2 when stimulated by human touch at 4.00 pm.
5(a) Soil temperature – 20°C, Humidity –10 to 20%, Sunlight intensity - < 500 lux, PH level of soil – 6.5
At a distance of 10cm when Plant 2 is stimulated by human touch Plant 1 showed variation at 11.40 pm.

5(b) Soil temperature – 20°C, Humidity –5 to 10%, Sunlight intensity – 10000 to 25000 lux, PH level of soil – 6.5
At a distance of 10cm when Plant 1 is stimulated by human touch Plant 2 showed variation at 10.40 am.

5(c) Soil temperature – 20°C, Humidity –10 to 20%, Sunlight intensity -5000 to 10000 lux, PH level of soil – 6.5
At a distance of 10cm when Plant 2 is stimulated by human touch Plant 1 showed variation at 4.10 pm.

Figure 5. At a distance of 10 cm: (a) Response of Plants 1 & 2 when stimulated by human touch at 11.40 pm, (b) Response of Plants 1 & 2 when stimulated by human touch at 10.40 am and (c) Response of Plants 1 & 2 when stimulated by human touch at 4.10 pm.
Figure 6. At a distance of 10 cm: (a) Response of Plants 1 & 2 when stimulated by chemical at 11.00 am, (b) Response of Plants 1 & 2 when stimulated by heat at 11.05 am, (c) Response of Plants 1 & 2 when stimulated by chemical at 11.15 am and (d) Response of Plants 1 & 2 when stimulated by heat at 11.20 am.

Initially when the plants were put far (Distance 90 cm) then the second species showed no difference in their potentials but when put together (Distance 10 cm) it does. In Figure 5(b) stimulus is applied on Plant 1 and amplitude recorded is 60 mv at 10.40 am where Plant 2 have shown 30 mv amplitude at the same time. The frequency of signal is not varied by human touch stimulus in this particular species of plants. There might be variation in signal frequency with respect to the applied touch stimulus for other species of plants because the velocity of Action Potential varies based upon their properties and carrying salt concentrations. In Figure 5(c) when
Plant 2 is stimulated the amplitudes 0.5 mv and 0.4 mv variation is shown in Plant 1 also. The application of heat and chemical stimuli on Plants had shown hyperpolarization in electric potential. In Figure 6(a) when the localized chemical product (contains 95% Ethyl alcohol, Isopropyl myristate, Cyclomethicone, Diethyl Phthalate, Tricosan) is sprayed on leaves of Plant 1 then there is a peak in Action Potential and delay in repolarization phase but there is no variation in potential of Plant 2 at 11.00 am. After 5 minutes gap the leaf of Plant 1 is burnt slightly (heat stimulation) at the tip then Plant 1 has peak in its potential of 10mv amplitude and a delay in returning to resting potential with a negative peak of -7.5 mv amplitude. The frequency of signal at that time is varying in between 10-30 hz. In Figure 6(c) the chemical product is applied on Plant 2 at 11.15 am then the potential of Plant 2 varied due to hyperpolarization of ions, here noticeable thing is amplitude of Plant 1 also increased at the same time as if it was making self-defense but hyperpolarization is not observed. The respective changes which are obtained in amplitudes and frequencies of the signal are clearly shown in Table 1. In Figure 6(d) the leaf of Plant 2 is burnt at 11.20 am and the potential variations are noticed in both the plants as shown.

All the variations in electric potentials last for some particular period of time (say in milliseconds) and tend back to normal state. The time taken to a Variation Potential to tend back to normal state depends on intensity and type of stimulus applied.

The physiological data that explain various electrical responses in plants with respect to various stimuli manually and environmentally are hypothesized. Few studies on plant–plant communication, particularly concentrate on medium of communication where exchange of information or electrical signaling takes place either through soil beneath or through volatiles chemicals by means of air (Baldwin and Schultz 1983). Revision to previous studies on plant–insect interactions the communications between the plants in terms of self-defense have extensively contributed for understanding inter-plant communication (Van der Putten et al. 2001), their responses to various stimuli applied have clearly visualized the potential change in neighboring plants.

Action Potential, Resting Potential and Variation Potential define a physiological response that informs far-away cells about eventual stimuli; light utilized during photosynthesis can also be termed as a stimulus (Fromm and Lautner 2005, 2007). The transformations of day and night effect the electric potentials of plants; this concept can be linked to membrane polarization that take place in a leaf during day time for performing photosynthesis (Whitmarsh 2004). Electrical potentials during night time fluctuated in a greater range in terms of amplitude when compared to the electrical potentials during day time. These observations noted for considered species may be related to the intensity of sap flow depending upon their life processes and can also be represented periodically in charge and time (Gilbert et al. 2006). It is also stated that transformation of daylight and darkness effect the plasma membrane, electric stimulation of H⁺ fluxes and photosynthesis in plant cells (Bulychev and Kamzolkina 2006). The leaves of a poplar tree when stimulated to heat have provoked the electrical signals that move along the length of tree through adjoining leaves where the rate of CO₂ uptake and the quantum yield of electron transport are temporarily reduced (Fromm and Lautner et al. 2005). This provoked changes in one plant are noticeable by the plant which is next to it; Understanding the type of volatile chemicals that are responsible to create the potential variation or a kind of awareness in the neighbor plant may help better to explain the type of interaction between them. All types of plants may or may not generate volatile signals it depends upon the type and properties of plants. Even if the plant is able to generate the volatile signals depending upon the measure, the transmitting range of signal may or may not reach the neighbor plant.

CONCLUSION

The new method of acquisition has provided a better physiological database that is more helpful to analyze time period, frequency of electrogenic ion transport systems and corresponding voltage dependent kinetics in Plants. The detailed sensory perception of plants to mechanical, heat, chemical stimuli makes clear that plants are highly sensitive to environmental changes. The communication in plants is not only intra- specific but from experimental results it is concluded that plants are good enough in stress/danger perception and self-defense clearly inter-plant communication exists. It is known that inter-plant communication uses roots as a transmission media in higher plants but here reported results show that plants are using air as a medium of transmission.

Future scope

Plants are purely potential (energy) beings with highly perceptible capabilities (sensing capabilities) that transform the photon energy, water energy into continuous propagating electric potentials for its utilities. If the range of potentials and frequencies with which the potentials are propagating are defined clearly then several applications
can be designed using high sensing capabilities of plants (Biosensors). In fields continuous monitoring of electrical potentials help to identify different types of stress observed in plants like over watering stress, drought stress, over nutrient stress, temperature stress etc. The air borne communication in plant reveals their transreceiving capabilities signifying that they can be used as antennas in wireless communications. The further progress in this research can be focused on 'Creating the ways for utilization of potential existed in plants' which may lead to innovations making this world Eco-friendly.

References


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