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Research Article

Electrical Potential Responses to Dry/Wet, Light/Dark and Circadian Rhythm Conditions

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Abstract

The electrical response of plants to different environmental stimuli like soil water content and dark-light cycles can be measured and quantitatively related to the intensity of the triggering sources. A restricted soil water flow due to soil dryness can trigger water stress in plants and water stressed and unstressed in dark and light manifest different electrical responses. Important physiological information of plant can be obtained through processing and analysis of the signal. Real time plant electrical response measurements during day-night cycles, day/light conditions and at different conditions of soil water availability can be used as tools to assess early plant stress conditions. In this work, electrical potential differences are monitored continuously using Ag/AgCl micro-electrodes inserted at two positions of the spinach plant i.e. the leaf area and the stem-stalk interface. This research relates to the response of spinach plant electric signal to the soil water availability, light/dark conditions and circadian rhythm. The standard deviation of the spinach plant under light condition was 31.09, under dark condition 36.97, under wet condition 29.48 and under dry condition 37.68. The peak values of the spinach plant in micro-voltage for light, dark, wet and dry conditions were 104.7, 120.6, 44.3 and 90.7 respectively as shown in table under the Results section. The amplitude of the signal in the transition from day to light, of the plant under water stress and day/light cycles can used to reflect the status of the plant. The system was able to measure and analyze changes in electrical signals in plants in response to environmental changes.

Keywords

Electric Signal, Circadian Rhythm, Abiotic Changes, Day-Night Cycles, Water Availability, Spinach Plant

1. Introduction

Plants respond to external stimuli by generating electrical signals [8, 19, 26, 30]. Research has shown that factors like water stress, irrigation, and light can influence these electrical signals in various plants [9, 12]. For instance, in spinach plants, researchers found changes in electrical potential (EP) between the base of the stem and the leaves when they experienced drought, irrigation, and the daily cycle of light and dark [21, 22]. Similarly, in avocados, shifts in EP between the stem base and leaf petiole linked to reduced soil water content were tied to a drop in stomatal conductance, suggesting that stomatal closure might be triggered by these electrical signals [22]. Additionally, [3] and [6] noted a reversal in the Pp (sensor output) curves in olive trees as they approached the plasmolytic point during water stress, which could serve as a valuable indicator

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for assessing water stress levels in these trees.

Plants have their own biological clocks and circadian rhythms that rely on electric signals, allowing them to keep track of day and night [27]. While the biological clock is sensitive to light, many of the circadian responses to day and night can persist even in constant light or darkness, at least for a while [29]. This circadian clock acts as an internal timer with a 24-hour cycle, closely tied to the light-dark rhythm [29]. It plays a crucial role in regulating various aspects of plant physiology, such as the movement of leaves, organs, and stomata, as well as growth and signaling [34]. Additionally, the circadian clock helps coordinate plant development at both the tissue and cellular levels [11], manages plant immunity [17, 25], and contributes to plant defense mechanisms [10], especially in response to abiotic stresses [24, 25]. Therefore, the electric signals associated with circadian rhythms could serve as indicators of a plant's health and physiological state [3].

Researchers suggest that action and variation potentials are key physiological traits in plants, allowing them to communicate within their tissues and organs. This ability is crucial for adapting and responding to changes in their environment [7, 9, 12, 15, 28]. When the external environment shifts, the plant's electrical signals undergo significant changes. These variations can provide insights into the plant's physiological condition [32]. Given the strong link between environmental factors and electrical signals in plants, monitoring these signals can effectively track changes in the surrounding environment [33].

These reactions can be tracked and observed in real time, potentially acting as early warning signs for both biotic and abiotic stress conditions. [23] found that prolonged water restriction in plants influences electrical potential (EP) trends. Environmental factors play a significant role in how plants behave electrically, causing changes in EP slopes even when there's no water stress, which allows for early detection of water stress directly in the plants [13]. By using physiological sensors, we can monitor the early signs and recovery patterns of water stress, providing continuous and non-invasive measurements that help with efficient irrigation planning throughout the year. The aim of this research was to explore how extracellular electrical potential (EP) variations in spinach plants are affected by different water conditions (both drought and irrigation) as well as the daily shifts between light and dark.

2. Materials and Method

2.1. Plant Material

Spinach plant was grown in the green house for four weeks was used in this study. The spinach plant was planted in soil with manure and fertilizer in plastic containers.

2.2. Method; Electrodes and Circuitry

Electrical potentials were monitored continuously for every experiment. Two measuring microelectrodes were inserted to the root-stock and the leaf area of the plant. Electrodes were constructed using similar technique to that described by [14, 16, 18, 20]. These micro-electrodes consisted of silver wire 0.3 mm diameter and coated with a Silver Chloride (AgCl) film, which was achieved by applying a voltage of 2.5 V in a Hydrogen Chloride (HCl) solution for 30 s as done by [14, 16, 18, 20].

$Ag + Cl^- – AgCl + electron$

The wire was then inserted in a hypodermic needle 0.6 mm in diameter filled with a solution of 3.5 M KCl. Both needle ends were sealed with a solid polyethylene coating.

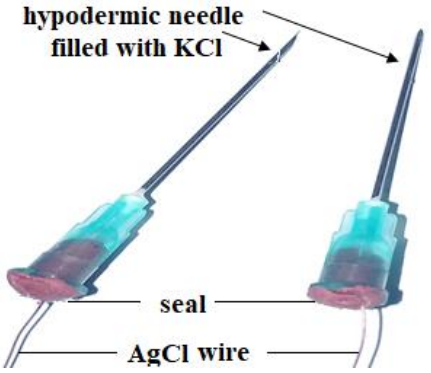


Figure 1. *Locally fabricated electrodes.*

An op-amp voltage amplifier was designed and fabricated to practically measure minute electrical signals. Its circuit diagram is as shown in Figure 2(a). It was made using an LF 356N op-amp which is an excellent amplifier for low noise applications using either high or low source of impedance. Other components included a capacitor (1 μF) and resistors (10 KHz and 100 KHz) all components soldered in a Printed Circuit Board (PCB) and housed in a wooden compartment as shown in Figure 2. The output and the input of the kit is via BNC connectors.

Thus, the data acquisition system was made of a voltage amplifier and an oscilloscope. The signal from the spinach plant was fed into the voltage amplifier via connector 4 in Figure 2b, powered via connector 1, connector 3 the reference, to the lock-in amplifier via connector 2 and finally to a computer for visualization and to the oscilloscope via connector 2. The data from the computer was recorded and analyzed using the Excel (a snapshot of the excel sheet is shown in appendix 1). The tabulated results were represented by graphs.

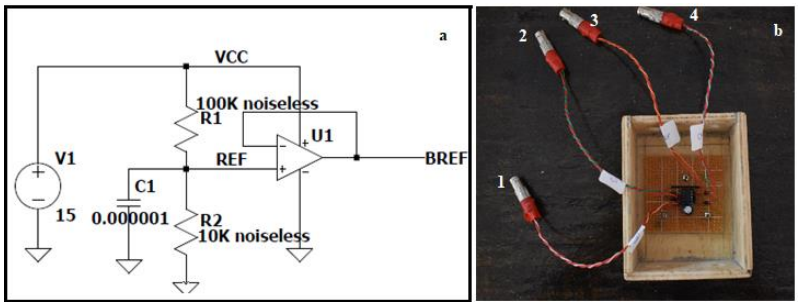


Figure 2. *Fabricated voltage amplifier (a) its circuit and (b) designed and fabricated as a housed kit.*

2.3. Experimental Set-up

For the first experiment, the spinach plant was kept under light and dark conditions for two hours and dark conditions for another two hours for four consecutive days. An EP measurement was done every 10 seconds to verify the electrical behaviour of spinach plant under light and dark conditions. Secondly, the spinach plant was kept under the normal ambient light conditions. Water availability was restricted for 3 days and irrigated on the third day. EP measurement was done every 10 seconds for three days to relate the soil water availability conditions to spinach plant electrical behaviour. Thirdly the spinach plant was kept under constant natural and dark conditions. This was done for two successive days to determine the circadian rhythm of EP in spinach grown under normal conditions. Finally, all the three experiments were repeated in faraday cages and EP measurements measured.

3. Results

3.1. Spinach Electric Signal Under Light/Dark Conditions

This study using the fabricated measuring kit with special Ag/AgCl electrodes (shown in § 2.2) measured the Electric signals in micro voltage of a spinach plant in the lab under light/dark conditions, first outside and secondly inside a faradays cage.

3.1.1. Electric Signal Outside Faraday's Cage

The spinach plant was first measured in presence of light for 90 minutes, followed by another 90 minutes in dark and finally light again for 90 minutes. The resulting micro voltage is as shown in Figure 3(a-c) and analysis in Table 1. The replicates of this experiment produced similar results and are shown in appendix (I).

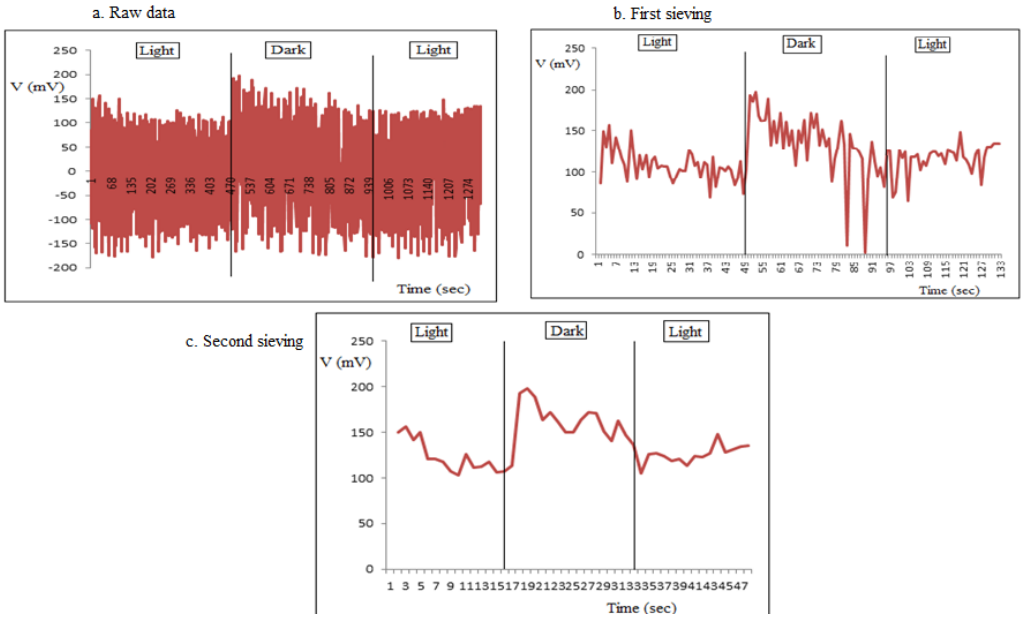


Figure 3. *Effect of light/dark conditions on spinach outside the faraday cage (a) the raw data from the measurement kit with a digital lock-in using Ag/AgCl electrodes. (b) The data that results from first sieving using the excel Index match formula (=INDEX(B: B, MATCH(E2, D: D, 0)) to get maximum values of the EP and (c) The data after second sieving using excel formula (=INDEX(F: F, MATCH(I2, H: H, 0)).*

The figures show the effect of light/darkness on EP behaviour. After an initial period of 2 hours in the sun at noon, plants were introduced into a dark room before being exposed again to sunlight. The peak values in this graph characterize the changes in the amplitude of the electrical signal in response to changing light/dark condition. There is more variation in peak amplitude from light to dark, indicating that electrical potential was affected by the change. The maximum amplitude of the signal is higher darkness 174.385275 than light 120.456079. The EP was higher at darkness than light.

Table 1. *The corresponding statistical parameters of the electric signals outside the faradays cage.*

		Minimum (mV)	Maximum (mV)	Mean (mV)	Peak (mV)	Std (mV)	Mean square (mV)
1	Light	22.483144	127.167	77.3429	104.683856	31.08875	8.798481
2	Dark	53.808353	174.385275	118.9683	120.576922	36.97064	10.90726
3	Wet	126.02537	170.300264	162.95	44.274894	29.47704	12.76519
4	Dry	163.203199	253.972769	200.5082	90.740779	37.67583	14.1601

Statistics in the table above show that the standard deviation in dry and dark conditions is larger than in light and wet, which corresponds to the fact that the amplitude of the electric signal is fluctuating more in those conditions.

3.1.2. Inside Faraday's Cage

Faraday Cage has been shown to improve plant measurements by reducing background electrical noise [1]. The experiment in section 3.1.1 was repeated inside a faradays cage made of iron mesh wire as shown in Figure 4.

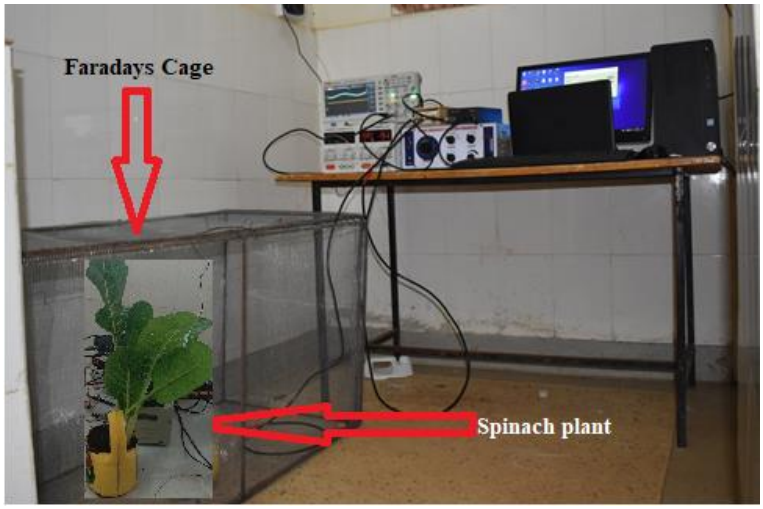


Figure 4. *Electrical potential measurement set up, with the spinach plant inside a faraday cage.*

The faradays cage was first tested on its accuracy of cancelling out electric/magnetic fields using a teslameter. The magnetic outside the faraday cage was found to be 0.07 T and 0.02 T inside the faraday cage. From the data the faraday cage was found to cancel electric/ magnetic fields by 71.43% i.e.

Difference in magnetic field 0.07-0.02=0.05

Total cancellation = (0.05/0.07) *100

=71.43%

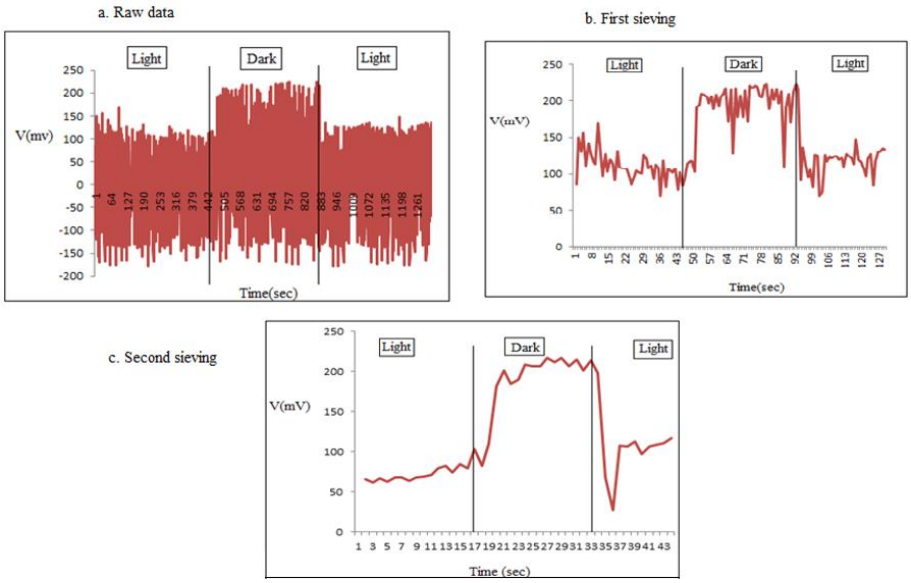


Figure 5. *Effect of light/dark conditions on spinach inside a faraday cage. (a) Raw data, (b) after first sieving and (c) after second sieving.*

3.2. Dry and Wet Conditions

The study also quantified the spinach ES under wet conditions and dry (water stressed) conditions inside and outside the Faraday's cage.

3.2.1. Outside Faraday's Cage

Water availability was restricted for three consecutive days and the spinach plant irrigated on the third day. The EP in spinach plant was measured from day 1 for 90 minutes each day for the three days and after irrigation on the third day. The resulting micro-voltage as shown in Figure 6(a-c) and analysis in Table 1. The replicates of this experiment produced similar results and are shown in appendix (II).

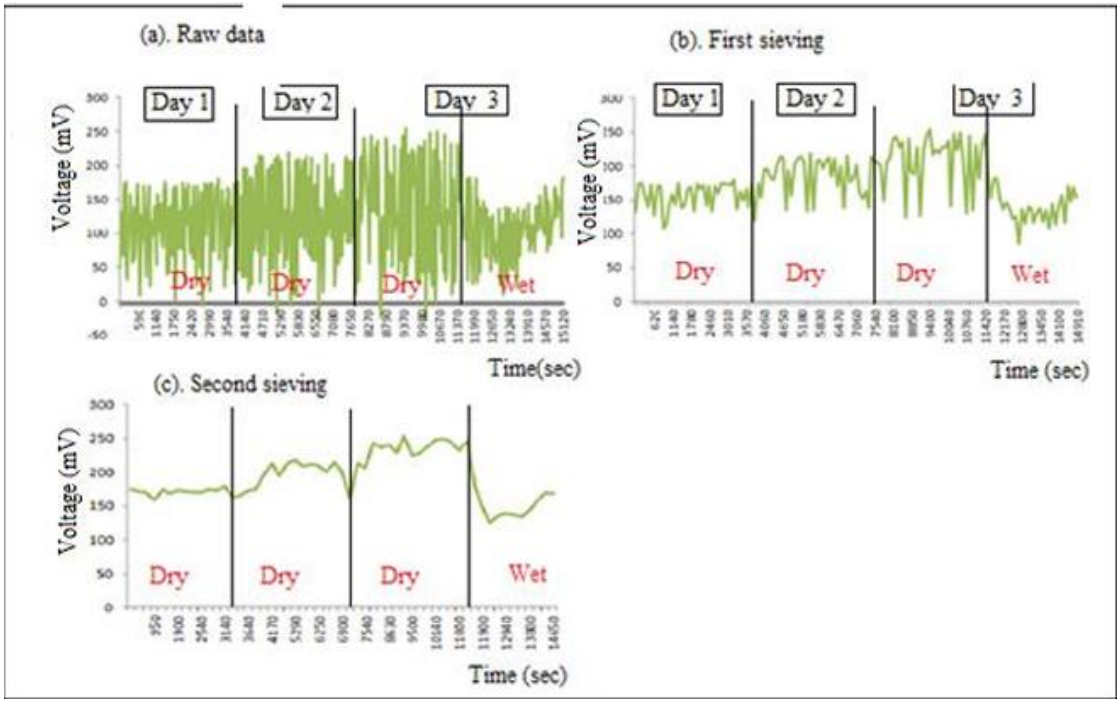


Figure 6. *Effect of irrigation on EP behaviour of spinach plant.*

Figure 6(a) the raw data from the measurement kit with digital lock-in using Ag/AgCl electrodes. Figure 6(b), the data that results from first sieving and 6(c) the data after second sieving using excel index match formula as in section 3.1.1. The spinach plants showed an interesting electrical response when faced with water scarcity. As the plants dry out, there was a noticeable rise in both their maximum and minimum daily electrical potential (EP) values. Specifically, the maximum EP values jumped from 174 mV at the start of the water restriction period to 232 mV by the end. This suggested that the water stress was at its peak during that time. On the flip side, the minimum EP values started at 158 mV when the non-irrigated period began and climbed to 232 mV just before the plants were watered again. After three days of being deprived of water, the addition of water led to a significant change in EP. The irrigation applied on day three resulted in a marked decrease in EP, indicating that the plants had managed to bounce back from the water stress and were beginning their recovery process after being rehydrated.

3.2.2. Inside Faraday's Cage

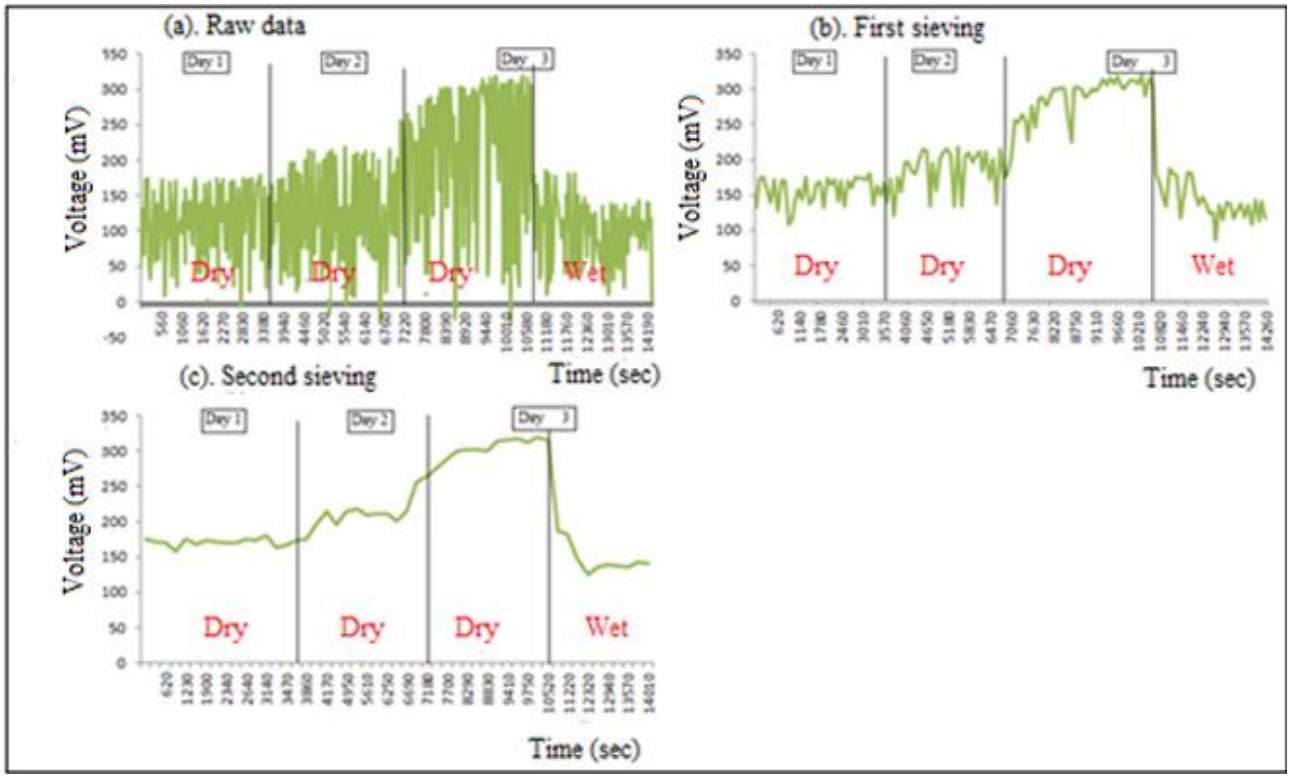


Figure 7. *Effect of irrigation inside the Faraday Cage.*

3.3. Circadian Rhythm

3.3.1. Outside Faraday's Cage

The EP in spinach plant was measured for 20 hours starting at 3.00 P.M. up to 11.00 A.M the next day. The experiment was carried out first with lights off throughout the night and secondly with lights on throughout the night. The resulting micro-voltage is as shown in Figure 8(a-d). The replicates of this experiment produced similar results are shown in appendix (III).

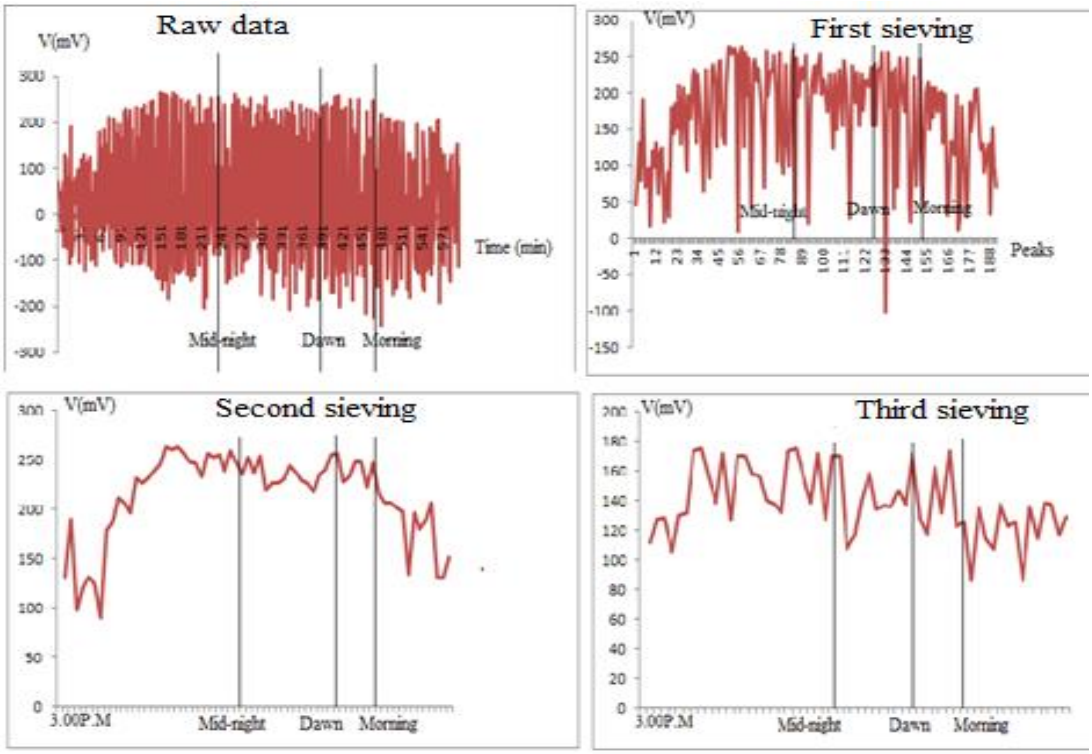


Figure 8. *Electrical potential responses to the circadian cycle for spinach plant.*

Figure 8(a-c) shows the data results when lights are off throughout the night and 8(d) when the lights are on. Figure 8(a) the raw data from measurement kit with a digital lock-in using Ag/AgCl electrodes. 8(b) the data results from first sieving and 8(c) the data after second sieving using excel index match formula as in section 3.1.1 and 3.1.2 above.

A diurnal pattern was observed in Figure 8, in which there were changes in EP between day and night. The experiment was carried out from 3.00 p.m. Immediately following the sunset there was a rapid increase in the EP values from 130.143761 mV to 173.173371 mV. After midnight, the rate of this increase slowed down and EP decreased to 136.719172 mV. Also, a small increase in EP values was detected few hours before daybreak to 172.170494 mV.

3.2.2. Inside Faraday's Cage

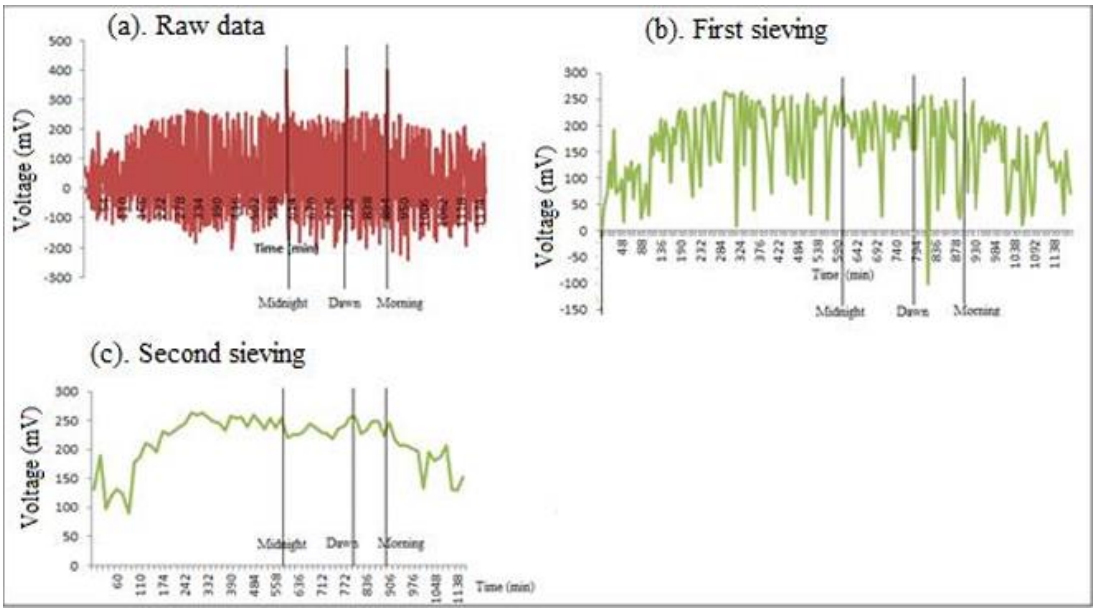


Figure 9. *Electrical potential responses to the circadian cycle inside a Faraday Cage.*

4. Discussion

Environmental changes generate bioelectric potentials in plants [4]. Previous experiments with *Salix Viminalis L.* [8] and *Zea mays* [7] showed that the electrical potentials are generated in response to different stimuli in the roots affecting leaf gas exchange. As a result of changes in the root zone, such as soil drying, a transmissible signal generated at the root and transported to the leaf would be expected to stimulate stomatal opening and closing. In this study with the spinach plant, the EP was measured for different stimulus. In the first experiment, placing plants in the dark resulted in an increase in the EP whereas illuminating plant previously in the dark resulted in a decrease in the EP. The effect of light and dark to electrical activity may be related to membrane polarization, which occurs in the leaf due to photosynthesis [31]. Also, [2] reported an effect of light and dark on the plasma membrane, electric excitation on H+ fluxes and photosynthesis in characean cells. In the second experiment short-term drought resulted in a significant increase in the EP. The graphs show that EP values gradually increased by the cumulative effect of water restriction. Similar results were reported by [7]. Plant water stress recovery after a short drought period, evaluated using its EP behaviour, indicates that the recovery process after rehydration does not start instantaneously after water is restored to the plant substrate. This behaviour was also clearly documented by [23]. There is also clear indication that the EP is gradually increased by the cumulative effect of water restriction.

The third experiment shows EP oscillatory behaviour during the whole experiment period, showing a clear circadian cycle. These fluctuations in EP observed in spinach plant appeared to correspond to the time of day (morning, afternoon or night) and were therefore presumably affected by diurnal changes in ambient light and vapour pressure deficit (VPD). A similar phenomenon was observed in another study with avocado, blueberry, lemon and olive trees [12]. Effects of sunset daybreak and water application are clearly reflected as fast changes in the EP [12]. Explanations of this behaviour may be related to circadian rhythms in plants [5].

Results reported in this work indicate that communication within plants may occur as signals between plant organs that sense natural or man- generated environmental modifications of plant organs showing a physiological response. Plants respond to external stimuli through different morphological and physiological changes [7]. Results presented in this paper indicate that light/light and soil water availability induce important electrophysiological changes in spinach plant organs. According to this work, continuous measurements of EP can be used as real-time communication mechanism of the plant physiological responses, for early detection of stress in the spinach. This will enable proper and automatic variation of the plant micro-environment in order to optimize the plant production.

5. Conclusion

Data presented in this paper represent further evidence of plant electrical response to environmental stimuli, specifically water availability, dark/light condition and circadian rhythm. The response to simulated drought and darkness was a decrease in voltage, whereas the response to soil wetting and exposure of spinach plant to light was an increase in voltage. Thus, the potential in spinach plant may exist to use stem to leaf voltage differences as a phytomonitoring technique for measuring spinach plant response to soil water content, dark/light and the circadian rhythm.

Abbreviations

Ag	Silver
AgCl	Silver Chloride
Ag/AgCl	Silver/Silver Chloride
BNC	Bayonet Neill–Concelman Connector
Cl^-	Chloride Ion
EP	Electrical Potential
ES	Electrical Signal
H^+	Hydrogen Ion (Proton)
HCl	Hydrogen Chloride
KCl	Potassium Chloride
Op-amp	Operational Amplifier
PCB	Printed Circuit Board

Author Contributions

Ndung'u Ruth Wairimu: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft

Kamweru Paul Kuria: Methodology, Resources, Supervision, Validation, Writing – review & editing

Conflicts of Interests

The authors declare no conflicts of interest.

Appendix

Appendix I: Excel Snapshot of the Tabulations of the EP from Spinach Plant

	A	B	C	D	E	F	G	H	I	J
1	Time(Sec)	V(mV)	Helper 1	Helper 2	PEAK No.	Peak V	Helper 1	Helper 2	PEAK No.	Peak V
2	0	-77.690942	0	0	1	12.94222	0	0	1	85.64949
3	10	-35.73786	0	0	2	85.64949	1	1	2	149.491
4	20	12.942216	1	1	3	36.00659	0	0	3	130.6073
5	30	-39.05336	0	0	4	149.491	1	2	4	156.3234
6	40	85.649485	1	2	5	19.60787	0	0	5	110.95
7	50	-109.223072	0	0	6	87.79974	0	0	6	141.7346
8	60	36.006587	1	3	7	130.6073	1	3	7	128.9649
9	70	-64.364703	0	0	8	108.4359	0	0	8	116.7615
10	80	-119.389979	0	0	9	15.43903	0	0	9	108.2535
11	90	149.49096	1	4	10	115.9756	0	0	10	88.32668
12	100	-88.931599	0	0	11	156.3234	1	4	11	149.7366
13	110	19.607873	1	5	12	16.39388	0	0	12	117.7158
14	120	-22.716128	0	0	13	101.0981	0	0	13	90.85075

Figure 10. *Excel snapshot of the tabulations of the EP from Spinach plant in column B, for the time in seconds in column A. Column C and D shows helper values (help to identify peak values) computed with the formula, =N(AND(B2>B3, B2>=B1)) and =SUM (C$2:C2, D1)*C2 respectively. Column E shows the peak number and column F shows the first sieved peak values of the EP in mV. Using replica formula, column G, H and I help to sieve for the second time, the peak values in mV shown in Column J. Respective graphs were plotted using relevant column values.*

Appendix II: Electric Signal Under light/Dark Condition Outside the Faraday Cage

Trial 2

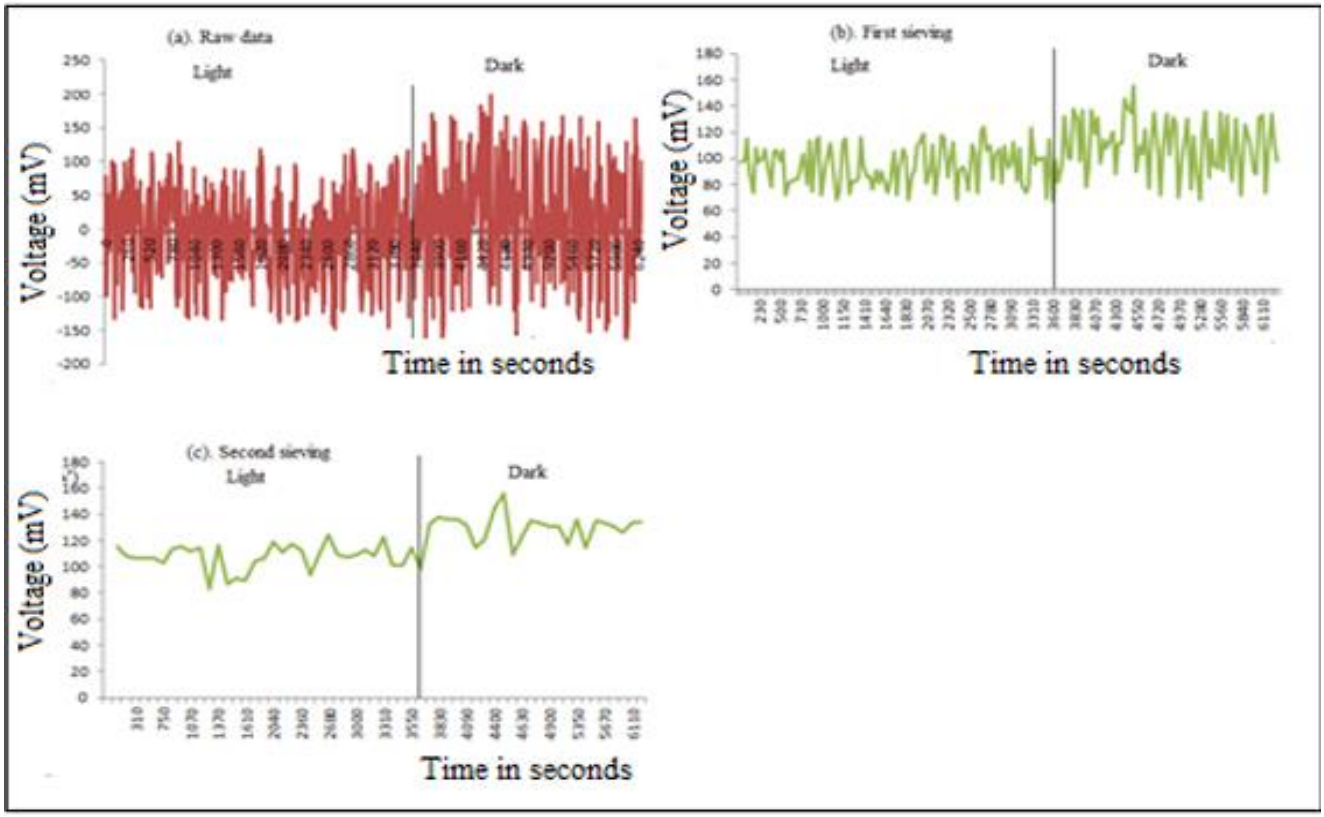


Figure 11. *Effect of light / dark on EP behaviour of spinach plant outside the faraday cage*

Trial 3

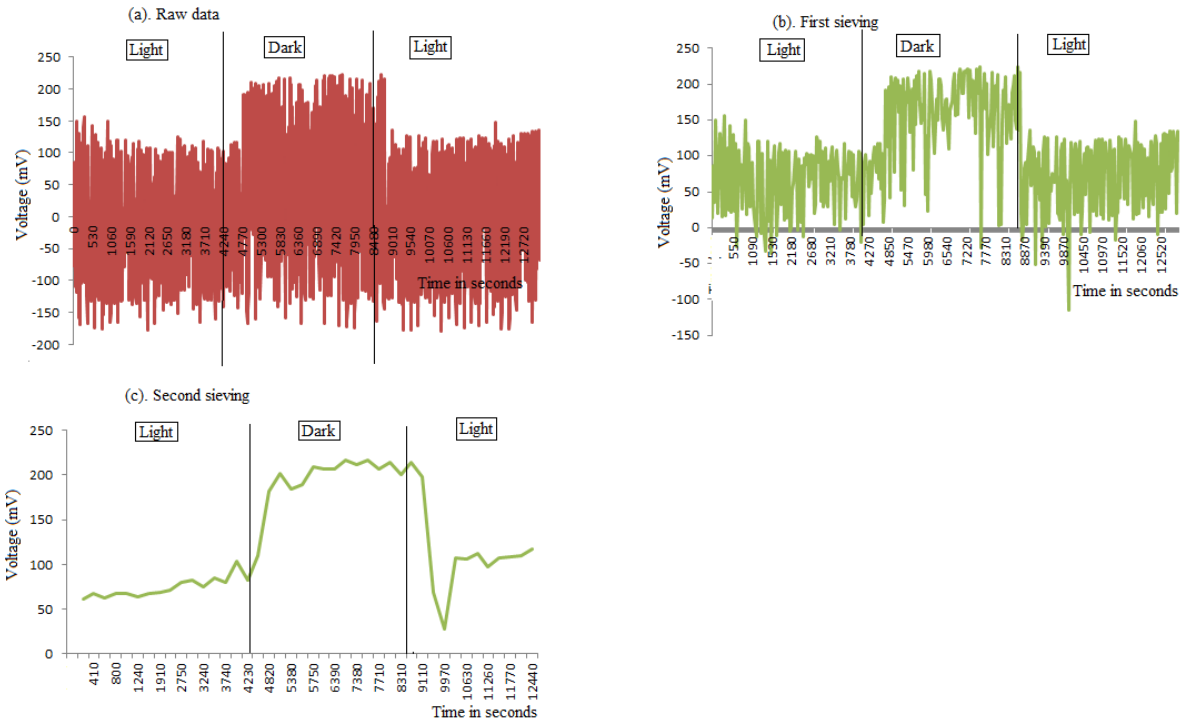


Figure 12. *Effect of light / dark on EP behaviour of spinach plant outside the faraday cage.*

Appendix III: Electrical Signal Under Light/ Dark Inside the Faraday Cage

Inside the faraday cage

Trial 2

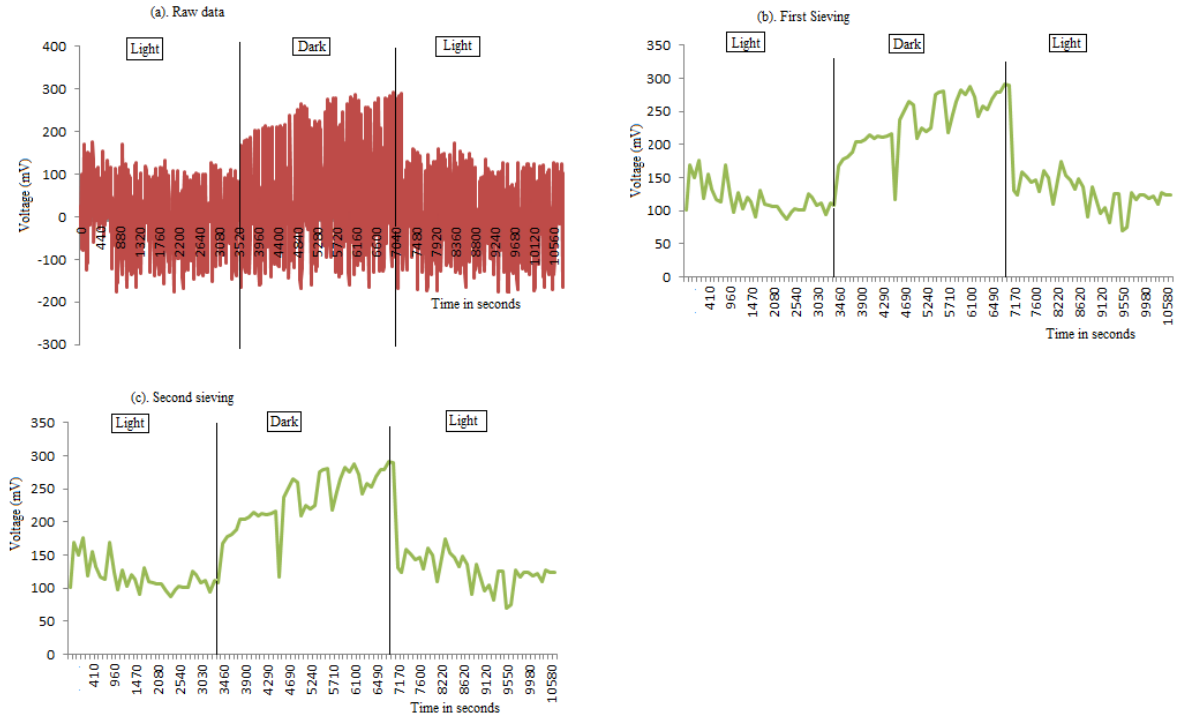


Figure 13. *Effect of light / dark on EP behaviour of spinach plant inside the faraday cage.*

Appendix IV: Electric Signal Under Watering and Lack of Watering Outside a Faradays Cage

Trial 2

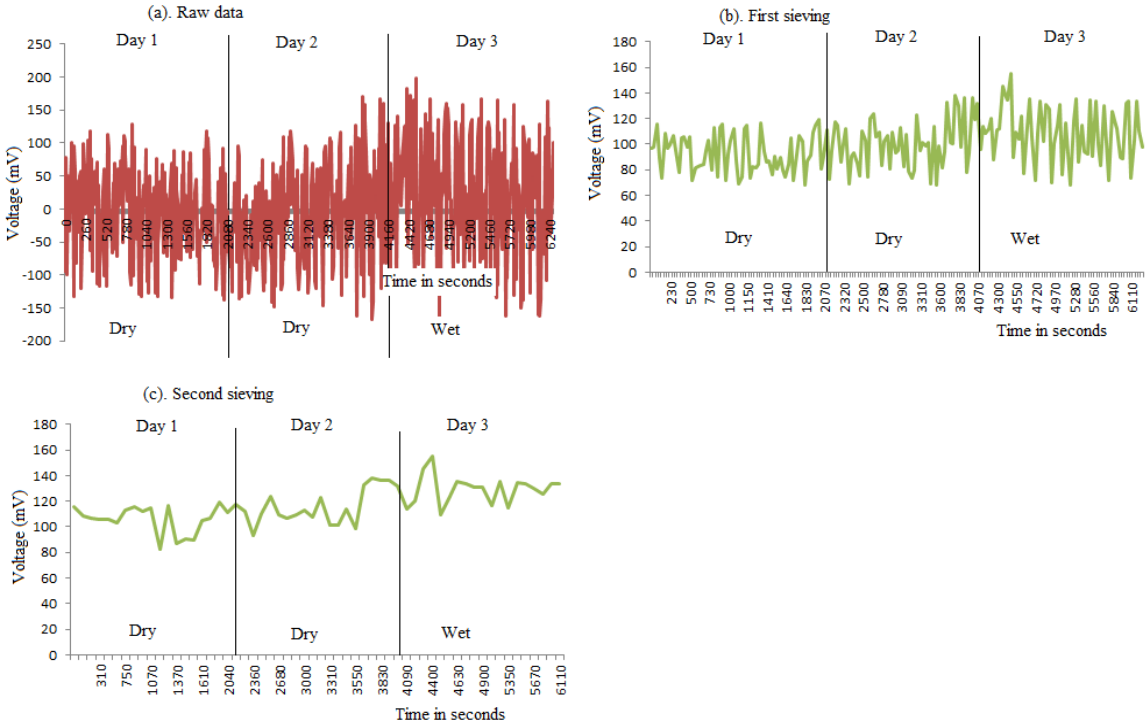


Figure 14. *Effect of irrigation on EP behaviour of spinach plant outside the faraday cage.*

Trial 3

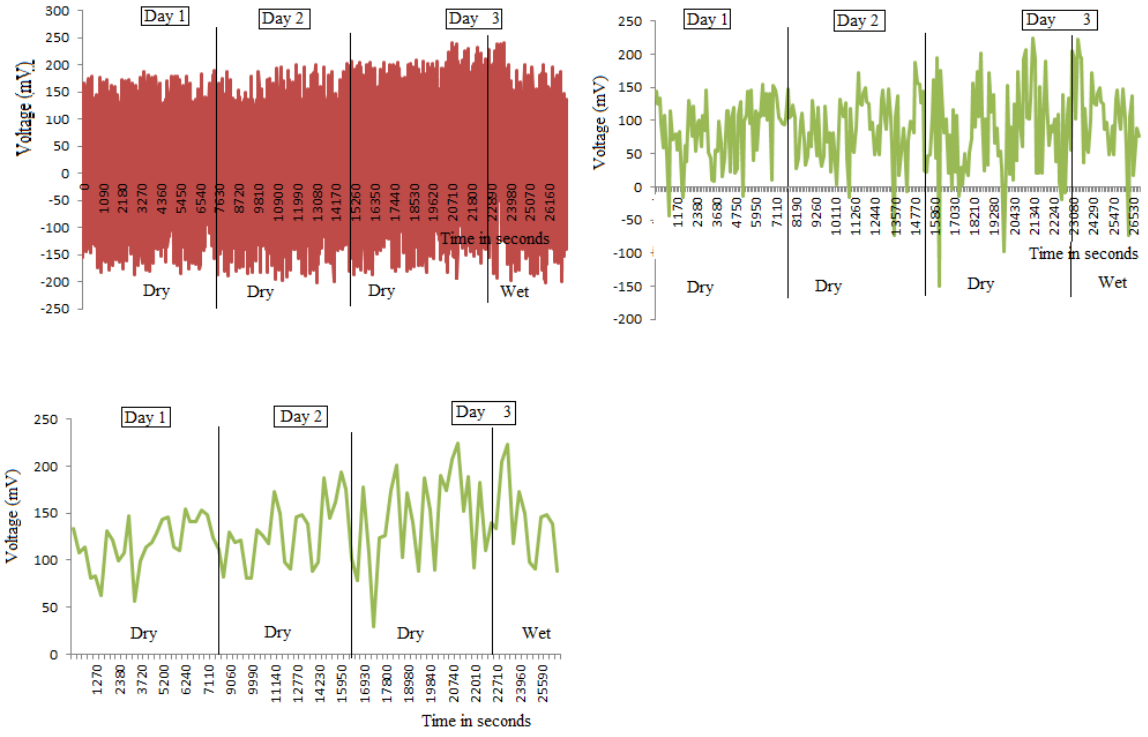


Figure 15. *Effect of irrigation on EP behaviour of spinach plant outside the faraday cage.*

Appendix V: Electric Signal Measured Under Dry (Not Watered) and Wet (Watered) Inside a Faraday Cage

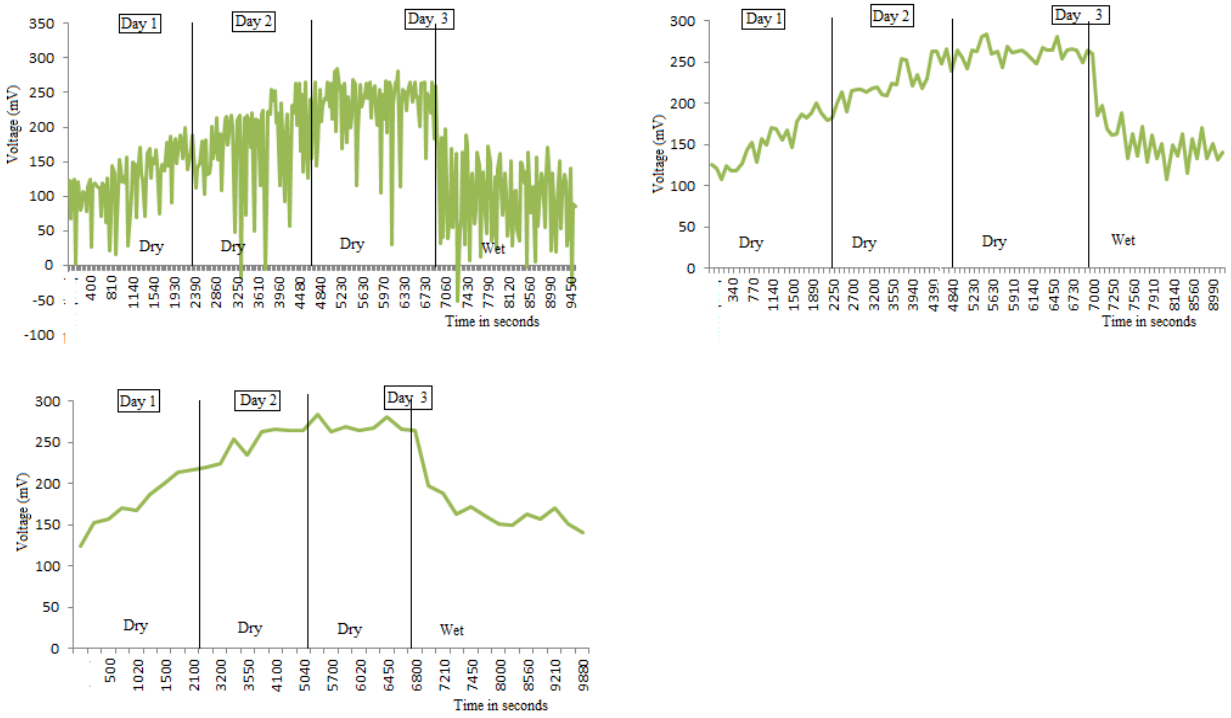


Figure 16. *Effect of irrigation on EP behaviour of spinach plant inside the faraday cage.*

Appendix VI: Electric Signal Showing Circadian Rhythm Outside the Faraday Cage

Electric Signal showing Circadian Rhythm outside the faraday cage
Trial 2: Outside faraday cage

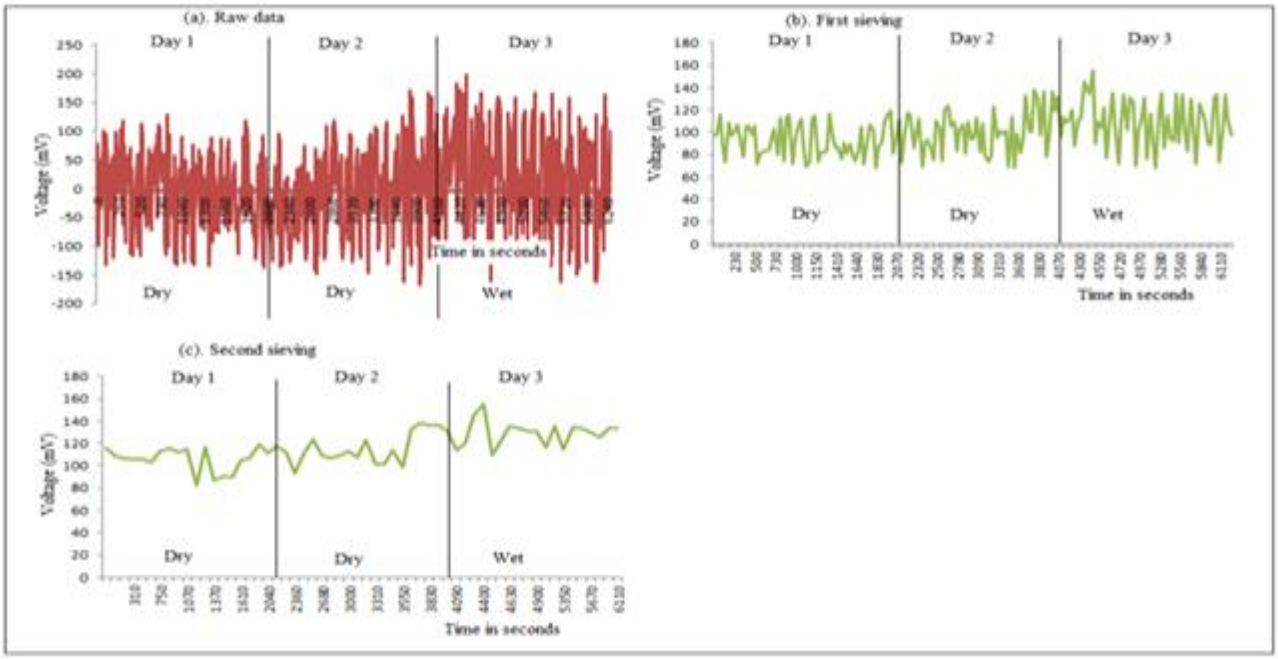


Figure 17. *Electrical potential response to the circadian rhythm outside the faraday cage.*

Trial 3: Outside the faraday cage

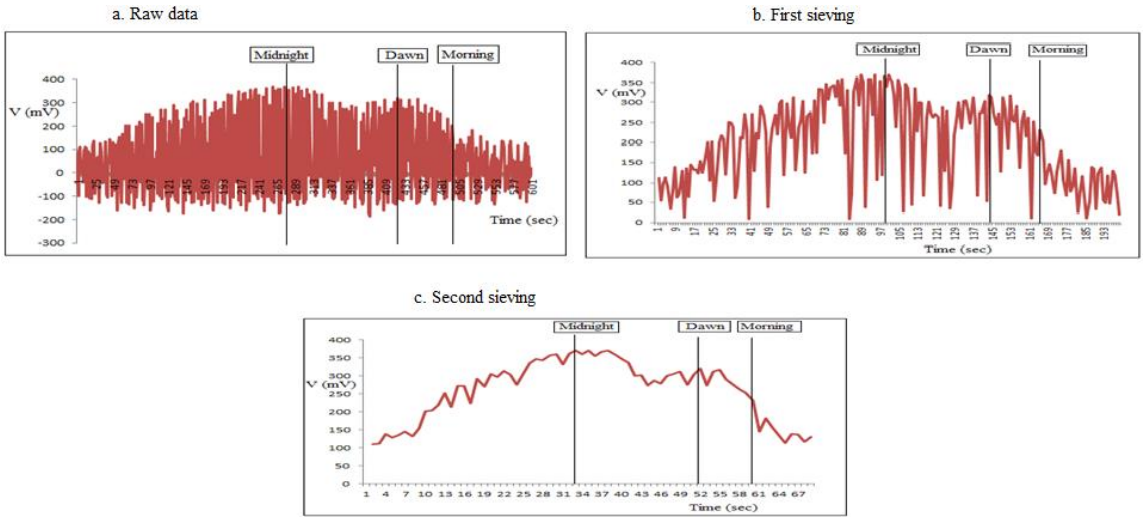


Figure 18. *Electrical potential response to the circadian rhythm outside the faraday.*

Appendix VII: Circadian Rhythm Inside the Faraday Cage

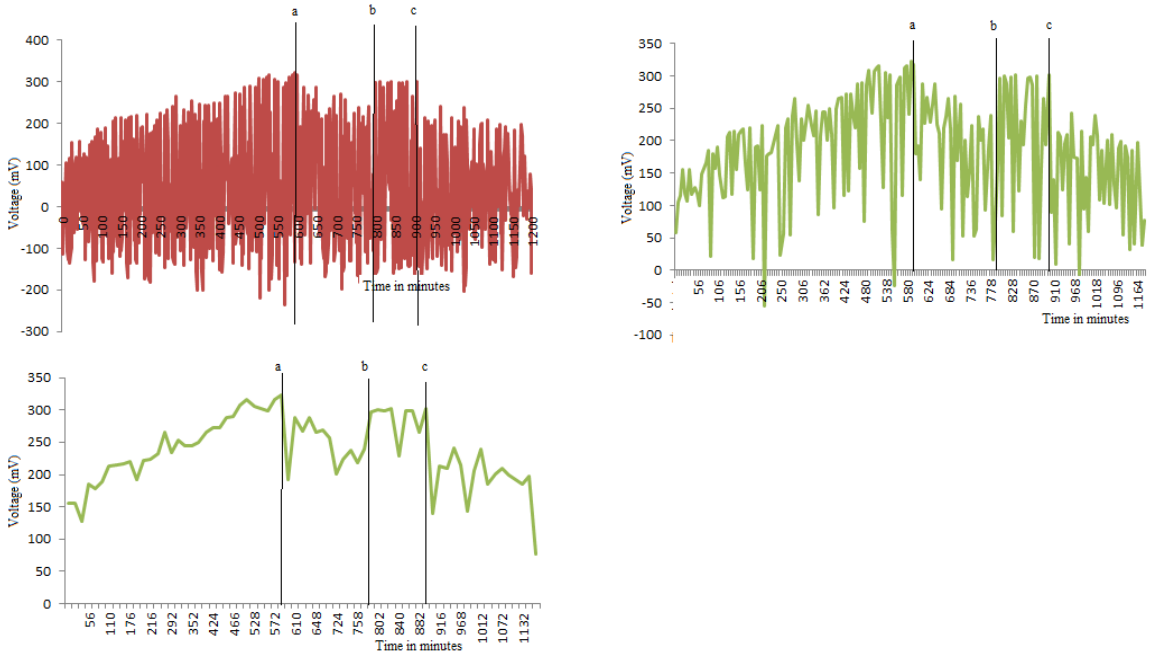


Figure 19. *Electrical potential response to the circadian rhythm inside the faraday cage.*

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