Electrophysiological Signal Study: Measuring, Analyzing, and Classifying Plant Responses

Bhoumik Patidar, Birudugadda Srivibhav
Department of Computer Science And Engineering, IIT Gandhinagar
Project Supervisor: Prof. Subramanian Sankaranarayanan,
Department Of Biological Sciences And Engineering, IIT Gandhinagar

Abstract

This interdisciplinary study integrates biology, electrical engineering, and computational methods to explore plant electrophysiology. The project involved growing *Nicotiana tabacum* plants under controlled conditions, measuring variation potentials post-injury, attempting a signal processing circuit, and employing data acquisition techniques. Despite circuit challenges, extensive data collection and pre-processing enabled the development of two distinct models. Model 1 accurately detects injury onset, achieving 96.77% accuracy, while Model 2 delineates variation potential duration with 96.39% accuracy. End-to-end testing on unseen data validated the algorithm's success in pinpointing injury onset and variation potential durations. The project emphasizes the synergy of multiple disciplines in studying plant electrophysiology.

Introduction

- Within the realm of plant electrophysiology, variation potentials (VPs) stand as distinctive electrical responses observed in plants. These VPs indicate rapid alterations in the electrochemical state of plant cells, revealing transient changes in membrane potentials.
- VPs manifest as a rapid electrical signal triggered by various stimuli such as mechanical damage, light, or environmental stresses. This unique response is considered a crucial part of the plant's defence mechanisms, aiding in rapid signalling for initiating stress responses or wound healing.
- Understanding VPs contributes significantly to uncovering the intricacies of plant signalling pathways and stress responses. In this project, we delve into the computational analysis of electrophysiological signals from *Nicotiana tabacum* plants to detect and interpret these variation potentials, aiming to elucidate their role and significance in plant electrophysiology.

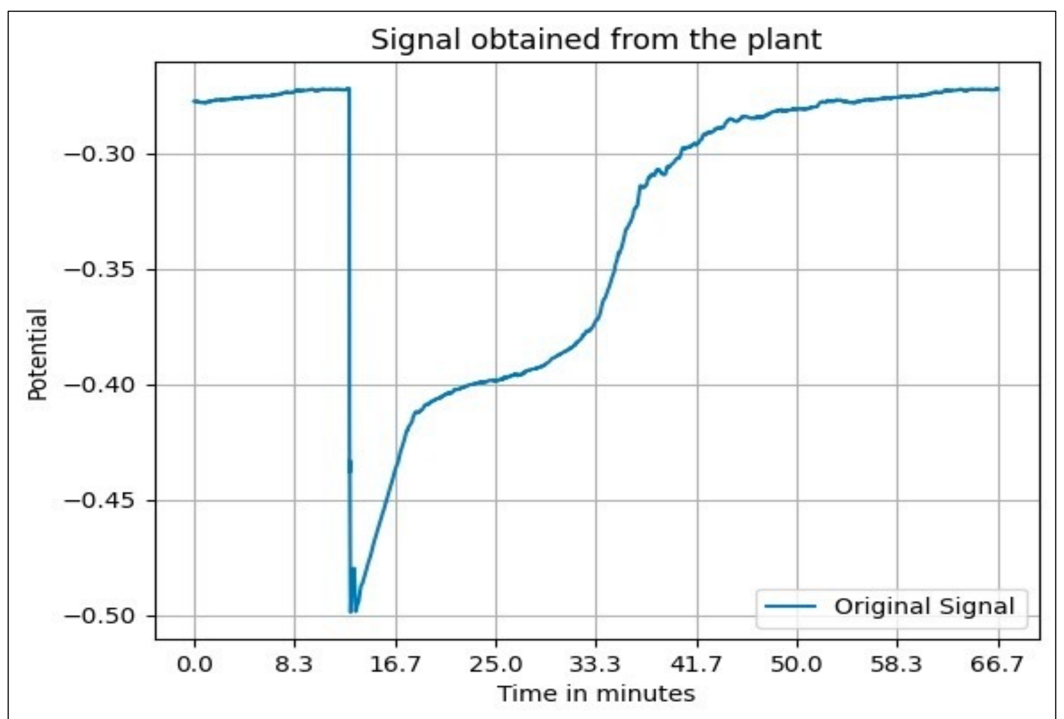


Figure 1. Plant electrical signal generated on wounding. The potential present here is not the actual value as invasive methodology is an indirect measure.

Experimental Setup

The study began with the cultivation of *Nicotiana tabacum* plants in a controlled laboratory environment, ensuring consistent and diverse data collection. The setup involved 32 plants segregated into two batches, each containing 16 plants, with a two-month interval between their initiation to encompass varied age ranges within the dataset.

•**Soil Preparation:** A precise mix of perlite, soilrite, vermiculite, and soil facilitated optimal plant growth, maintaining a healthy environment.

•**Sterilization Process:** Thorough soil sterilization at 120 degrees Celsius and pot disinfection with ethanol ensured a controlled, microorganism-free growth setting.

•**Electrode Readiness:** Ag/AgCl electrodes, critical for signal measurement, were meticulously prepared, allowing precise electrical recordings near the leaf base upon injury.

Signal Measurement:

Electrodes were strategically inserted near the leaf base, and another was grounded. Upon wounding, electrical recordings were captured using Ag/AgCl electrodes, which accurately measured plant electrical signals, notably the variation potentials induced by leaf injury.

Circuit Design

•**Design Objective:** The aim was to create a signal filtering and amplification circuit for precise signal processing from the probes.

•**LTSpice Simulation:** The initial design phase involved creating and simulating the circuit in LTSpice. The circuit was constructed to filter and amplify weak signals for better processing.

The layout aimed to achieve a total amplification of 72 times and effective signal filtering.

PCB Prototyping Attempt

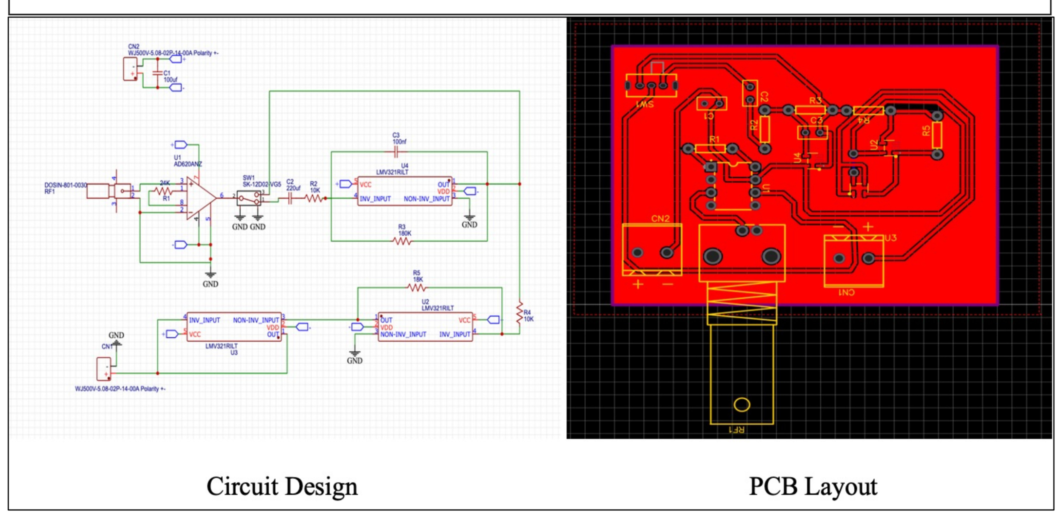
•**PCB Prototyping:** After the simulation phase, the design progressed to PCB prototyping using EasyEDA.

•**Circuit Phases**

- **AD620 Amplifier:** Provided 2x signal amplification.
- **Frequency Filtering and Amplification:** Filtered within range and applied 18x amplification.
- **Additional Amplification Stages:** Improved signal quality through two extra stages.
- **Buffer Phase:** Ensured impedance matching and signal preservation pre-digitization.

•**Outcome and Analysis:** Despite a successful simulation in LTSpice, the physical circuit encountered significant noise issues, rendering it non-functional. Probable reasons for failure included component tolerances, interference, inadequate grounding, and suboptimal PCB layout.

•**Conclusion:** The implemented circuit failed due to noise interference despite performing well in simulation. This emphasized the significance of addressing noise-related challenges in circuit design for precise signal processing.



Circuit Design PCB Layout

Data Acquisition And Processing

•**Methodology:**

- *Electrode Connection:* Soldered electrodes facilitated reliable analogue signal transmission.
- *NI DAQ 6009 Device:* Wired electrodes directly interfaced as analogue inputs, managed and interpreted using the NIDAQMX library in Python.

•**Signal Handling:**

- *NIDAQMX Library:* Leveraged for acquisition, processing, and interpretation of analogue signals, ensuring streamlined handling and storage of electrophysiological data. Sampling rate is 100 samples per second.
- *Variation Potential Signal Description:* Exhibits a weak amplitude, reaching a peak of a few tens of millivolts. Records surface potential drop post-plant injury, normalizing within 15 to 45 minutes.
- *Data Description:* Over 50 hours of electrical signal data were collected, comprising 36 variation potential measurements, equivalent baseline data, and additional noise data.

•**Pre-processing Steps:**

- *Digital Data Filtering:* Employed a series of filtering steps, including low-pass Butterworth, median, and Savitzky-Golay filters to enhance signal quality, reducing noise for model input.

Algorithm Overview

Aim: The primary goal of this algorithm is twofold: first, to pinpoint the time of injury onset, and second, to delineate the duration of the variation potential**.**

Exploratory data analysis: Visual analysis and statistical measures were employed to distinguish variation potential signals from baseline and noise data, providing insights crucial for subsequent classification modelling.

This algorithm comprises two distinct models, each addressing a specific phase in the plant signal:

Model 1: Detection of Wounding Time

•**Data Preparation:** Segments of 500 samples with wounding (35) and healthy plant (68) examples.

•**Features:** Extracted max-min difference, last value variation, variance, and IQR. Applied feature scaling.

•**Model Training:** SVM using RBF kernel for identifying sudden drops.

Model 2: Detection of Recovery Phase

•**Data Preparation:** Segments (5000 elements) with recovery (6681) and non-recovery (5410) samples.

•**Features:** Extracted time gap from wounding, statistical measures. Applied feature scaling.

•**Model Training:** SVM for recovery phase detection.

	Model - 1 Performance	Model - 2 Performance
Accuracy	96.77%	96.39%
Recall	100%	97.35%
Precision	92.31%	96.26%
F1-score	96.0%	96.80%
Cross-Validation	94.48%	96.44%

Model Workflow

Part 1: Detection and Confirmation of Wounding Time

•**Model 1 Analysis:**

- Analyze 500-sample segments to detect wounded signal segments.

•**Wounding Confirmation Conditions:**

- Condition 1: Identifies detected segments as wounded, followed by 20 non-wounded segments.
- Condition 2: Validate the subsequent five segments as recovery phase segments using Model 2.

•**Wounding Time Determination:**

- If both conditions are met, confirm the segment as wounded, recording its timestamp as the wounding time.

Part 2: Detection of Recovery Phase Duration

•**Model 2 Analysis:**

- Analyze 5000-length segments to discern recovery phase segments.

•**Recovery Phase Duration Determination:**

- Utilizes identified wounding time as the start of the recovery phase.
- Sequentially evaluates 5000-length segments from the wounding time to identify recovery phase duration.
- Marks recovery phase ends when five consecutive 5000-length segments are classified as 'not recovery phase' by Model 2.

•**Fallback Procedure:**

- If initial conditions for confirming wounding are not met, iterate through subsequent 500-length segments to find a wounded segment for recovery phase start detection.

Overall Model Performance

End-to-End Testing on Unseen Data

•**Overall Algorithm Performance:** The algorithm excelled on unseen signal data, accurately pinpointing injury onset and delineating recovery time ranges.

•**Task 1 Success Rate:** Achieved a flawless 100% accuracy in identifying injury onset.

•**Task 2 Success Rate:** Displayed good precision in predicting variation potential duration, ensuring effective recovery phase delineation.

•**Overall Success Metric:** The algorithm's robustness in detecting injury onset and outlining variation potential durations on unseen data was prominently demonstrated through the successful execution of both tasks.

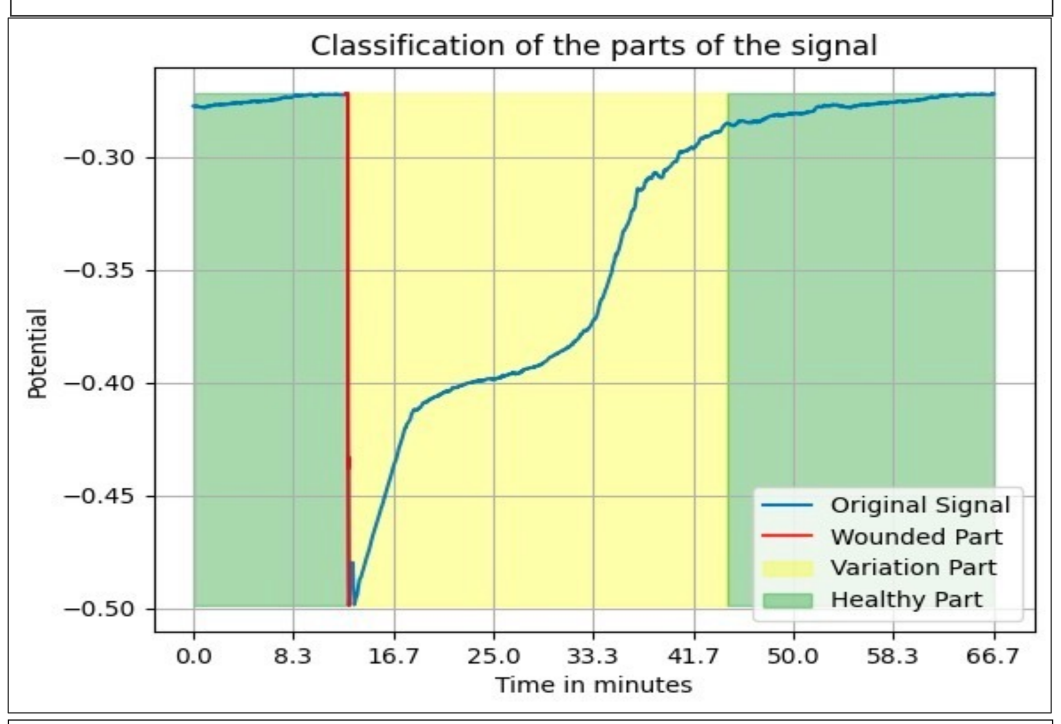


Figure 2. Image shows the classification of the segments done by the algorithm

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