SWP analysis R script

Note: Consistent annotation is crucial for the proper execution of this script.

The SWP is characterized by three phases: initial hyperpolarization, depolarization, and a secondary hyperpolarization. Each phase is defined by multiple parameters (see Figure X). These parameters can be analyzed from a single trace. To automate the determination of six different parameters, we developed the R script **SWPanalyzer.Rmd**, which is available on GitHub [here](https://github.com/jucbca/SWP-data_analysis).

The script is designed to handle data obtained using the LabScribe software. It can analyze recordings from up to two plants, with one electrode on the wounded leaf and another on a non-wounded leaf.

**How to Annotate Files:**

All relevant experimental information (e.g., genotype, leaf number, treatments) should be saved in the electrode label that records on leaf 8. Organize this information according to the format specified in the script description.

**How to Export .txt Files for Analysis:**

Once the recording is saved in LabScribe, export it to a .txt file by selecting **File > Export**. In the window that appears, check the box for “Include time of day.” The sampling rate can be set to either 1 to 100 Hz, in any case the script down samples the data to 1 Hz. Gather all files in a single folder, naming the folder after the recording date using the format “yymmdd”. The specific name of each file is irrelevant to the script. The folder(s) containing recordings from different days will serve as the initial input for the script.

**How to Use the Script:**

To run the script, create a new project in RStudio by selecting **File > New Project**. In the window that opens, choose **New Directory > New Project**. Enter a name for the folder (e.g., “home”) and select a path where it will be created. This “home” folder will contain the project file (home.Rproj). Place all “yymmdd” folders containing the exported data into this “home” folder. Open the script by selecting **File > Open** and choosing the downloaded **SWPanalyzer.Rmd** file. Read the description and follow the instructions provided in the script.

The first four sections of the script cover the minimum analysis required. After running these sections successfully, the following output files will be generated in the “home” folder:

* A “genotype.csv” file for each genotype measured, containing all recorded traces.
* A “genotypeSummary.csv” file with the calculated parameters for each leaf of each plant.
* A “genotype-yymmdd” folder containing plots of each leaf of each plant, with overlays of the calculated parameters. These are called “#.Lxx.pdf”. Where # is the number of that plant and xx is the leaf number assigned (08 or 13).
* A violin plot for each parameter comparing results between genotypes (“yymmdd-parameter.pdf”).
* A “Mean-genotype-Lxx.pdf” file for each genotype, featuring a mean trace with an overlaid ±SEM of all traces. The traces included in this mean plot can be manually selected in Section 5.

**General Troubleshooting:**

* **Section 1:** Ensure you run this section every time the project is opened.
* **Section 2:**  
  **Problem:** Different folders are created for the same genotype.  
  **Solution:** Ensure the spelling of the genotype is consistent across all electrode labels. Variations in spaces, capital letters, or dashes will cause the script to identify them as different genotypes (e.g., Col0, col0, Col 0, Col-0).
* **Section 3:**  
  **Problem:** A trace generates aberrant parameters, causing Chunk 3.2 to stop running.  
  **Solution:** Re-run Chunk 3.2 and remove the problematic trace. This trace cannot be analyzed due to poor recording quality.
* Review of quantified parameters:

After running the script, make a visual inspection of the parameters quantified. This can be done by checking the “#.Lxx.pdf” images generated for every trace. If a parameter is wrongly quantified (SUPPLEMENTARY), you can trace it back to the “genotypeSummay.csv” by the date, the # and the leaf, and exclude it of further statistical analysis.

