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SPONTANEOUS ACTION POTENTIALS IN *LUPINUS ANGUSTIFOLIUS* L.

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Extracellular electrical potential measurements were applied to perform electrophysiological analyses of narrow-leaved lupin (*Lupinus angustifolius* L.). Spontaneous action potentials (SAPs) were investigated during three-day long Ag/AgCl electrode registration in the lupin stem under light/dark (LD), continuous light/light (CLL), and continuous very low light (CVLL). The average number of SAPs recorded during one day was approx. 5-7 per plant in the LD and CVLL conditions and on average approx. 9-20 SAPs per plant during the entire three-day measurements. No SAPs were recorded in the CLL conditions. The amplitude, half time of duration, velocity of propagation, time between two subsequent SAPs, and direction of propagation were described. Spontaneous APs propagated faster in CVLL (approx. 6 cm min^{-1}) than SAPs in the LD conditions (approx. 4 cm min^{-1}). In the CVLL conditions, spontaneous bursts of APs occurred more frequently (144 min. interval) than in the LD variant (233 min. interval). SAPs propagating from the roots and from the bottom of the stem towards the plant apex were the most common action potentials (85%). The spontaneous APs in lupin were visualized and drawn using a new informatics tool *SAP Tracker*. The hypothetical mechanism of SAP generation in intact lupin plants is discussed.

Keywords: action potentials, electrical signals, extracellular electrical potential measurements, narrow-leaved lupin, *Lupinus angustifolius*, spontaneous action potentials, ultradian

ABBREVIATIONS

AP – action potential
SAP – spontaneous action potential
LD – light/dark
CLL – continuous light/light
CVLL – continuous very low light

INTRODUCTION

Electrical signals in plants have recently attracted increasing interest from researchers (Bakshi et al., 2023; Brownlee, 2022; Feijó, 2023; Hagihara et al., 2022; Hedrich and Kreuzer, 2023; Huang and Hedrich, 2023; Iosip et al., 2023; Li et al., 2024; Pupkis et al., 2022; Scherzer et al., 2022; Yokawa et al., 2018). Nowadays, it has been 150 years

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since the discovery of electrical phenomena in leaves of the insectivorous plant *Dionaea muscipula* (Burdon-Sanderson, 1873). Remarkably, electrical signals, named action potentials (APs), have been best described in insectivorous plants and those that show rapid movements. While investigating this phenomenon, a question arose whether APs also occur in common plants which do not show rapid movements and, consequently, whether this phenomenon occurs commonly in all plants. It is already known that some plants that do not exhibit spectacularly fast movements are capable of generating APs as well. This issue was first addressed in a series of six of papers published 50 years ago on APs in *L. angustifolius* i.e., a representative of vascular plants showing no rapid movements. During approximately ten years of investigations, researchers Adam Paszewski and Tadeusz Zawadzki observed stimulus-induced action potentials (Paszewski and Zawadzki, 1973) and then described the strength-duration relation, the all-or-nothing law (Paszewski and Zawadzki, 1974), the refractory period (Paszewski and Zawadzki, 1976a), the application of thermal stimuli, and the excitation conduction pathways (Paszewski and Zawadzki, 1976b). Next, the excitation spreading in the stem, leaves, and root (Zawadzki, 1980) and the propagation of action potentials in the stem after the application of a mechanical block (Zawadzki and Trebacz, 1982) were described. It should be emphasized that the excitatory laws had been previously determined only for *Conocephalum conicum* and *Helianthus annuus*. These were the first studies of APs in lower and vascular plants showing no rapid movements. Subsequently, APs were described in sunflower and tomato plants (Dziubińska et al., 2001; Macedo et al., 2015; Stankovic et al., 1998; Zawadzki et al., 1991; Zawadzki et al., 1995). Long-distance electrical signals were also reported for *Arabidopsis thaliana* (Gao and Farmer, 2022; Gao et al., 2023; Kurenda et al., 2019), *Vicia faba* (Dziubińska et al., 2003), and *Populus* plants (Lautner et al., 2005). The aforementioned publications describe stimulus-induced APs only in a few plant species.

In addition to stimulus-induced APs, action potentials occurring in plants without any evident environmental stimuli, named spontaneous APs (SAPs), have also been reported. They have been described in *Helianthus annuus* L. (Stolarz and Dziubinska, 2017a; Stolarz and Dziubińska, 2017b; Zawadzki et al., 1995), *Solanum lycopersicum* L. (Macedo et al., 2015; Silva et al., 2021), and *Mimosa pudica* L. (Stolarz and Trębacz, 2021). The present study describes investigations of SAPs in *L. angustifolius* – a previously electrophysiologically well-characterized model legume plant.

Recently, automated sorting and computing of APs have been employed in neurobiological studies (Carlson and Carin, 2019; Nasiotis et al., 2019; Quian Quiroga, 2009; Rácz et al., 2020; Unakafova and Gail, 2019; Zhao et al., 2013). Special computer algorithms and computation methods are also developed to analyze electrical signals registered in plants (Li et al., 2024; Yao et al., 2023; Zhao et al., 2013). Moreover, modelling of APs in *Nitellopsis obtusa* is being continuously developed (Beilby and Al Khazaaly, 2017; Kisnieriene et al., 2019). The frequency of SAPs firing in plants described so far is extremely low; hence, we have devised a new way for browsing plant firing. Here, we used special algorithms implemented in an informatics application dedicated to long-term electrical potential recordings in *L. angustifolius* plants and named the *SAP Tracker* (*ST*).

L. angustifolius is a widely cultivated leguminous plant that has gained popularity as a crop over other lupins in the past few years due to its high grain yield and lower seed alkaloid content (Clements et al., 2014; Kroc et al., 2019). In addition to being cultivated for its protein-rich grain, this plant is employed in crop rotation practices with cereals in Mediterranean climates (McNeill and Fillery, 2008; Wijayanto et al., 2009). There are multiple benefits of legumes for agriculture sustainability (Stagnari et al., 2017); therefore, a better understanding of the physiology of lupin plants that host symbiotic bacteria is very important.

The aim of our work was to check whether narrow-leaved lupin generates SAPs in different lighting conditions and to build an IT tool for the presentation and graphical analysis of these electrical signals.

The present study showed the occurrence of SAPs in lupin plants. Our research was based on the method of long-term measurements of electrical potential changes and the new method for visualization of the results using informatics tools, i.e., the *ST* application. Spontaneous APs in the cyclic light-dark conditions and very low light in lupin plants have been revealed for the first time.

MATERIALS AND METHODS

EXPERIMENTAL PLANTS

The studies were carried out on 15-60-day-old *L. angustifolius* L. plants (Planta sp. z o.o. Tarnów, Poland) grown in a vegetation room in pots filled with garden soil (Fig. 1). They were watered with tap water. A 16:8-h light:dark (04:00–20:00) photoperiod was maintained. The intensity of white artificial light in the PAR range (Power Star HQT-T400W/OSRAM GmbH, Munich, Germany) for 16 h a day at the level of plant leaves was approximately 70 µmol m^{-2} s^{-1}. The vegetation room was air-conditioned; the temperature was 24 ± 1°C and humidity was 50–70%. Approximately 15-40-cm high plants were taken for the experiments (Fig. 1). The research was conducted from June to December.

ELECTROPHYSIOLOGICAL MEASUREMENTS: EXTRACELLULAR METHOD

The experiments were carried out in a laboratory room, in which constant environmental conditions were maintained. The electrophysiological measurements were performed in a Faraday cage. Lupin plants were placed in the Faraday cage in the morning at approx. 08:00, and extracellular electrodes were inserted into the stem. The measurement started at 12:00 and lasted for three days. Changes in the electrical potential were measured with two or four extracellular Ag/AgCl electrodes (silver wire, 0.2 mm diameter, World Precision Instruments) inserted across the lupin stem and interfaced with a multi-channel data acquisition system composed of a differential amplifier (ME-4600 Meilhaus, Germany) and RealView software (Abacom, Germany). During the preparation of the Ag/AgCl electrodes, the silver wire was electrolytically coated with silver chloride. A reference electrode of the same type as the measuring electrodes was placed in the pot soil (electrode ref.). The long-distance SAPs passed by electrodes 1, 2, 3, 4, and the reference basal electrode; therefore, the reference electrode was a relative reference electrode because the potentials passed through it (Stolarz and Dziubińska 2017a; Stolarz and Dziubińska 2017b). The frequency of sample recording was 1 Hz. The arrangement of the electrodes on the *L. angustifolius* plant is shown in Fig. 1. The SAP interval, i.e., the time spacing between two subsequent SAPs was calculated. The data were visualized and analyzed using the *SAP Tracker* (Institute of Biological Science, Maria Curie-Skłodowska University), Microsoft Office Excel (Microsoft Corporation), and RealView software (Abacom, Germany).

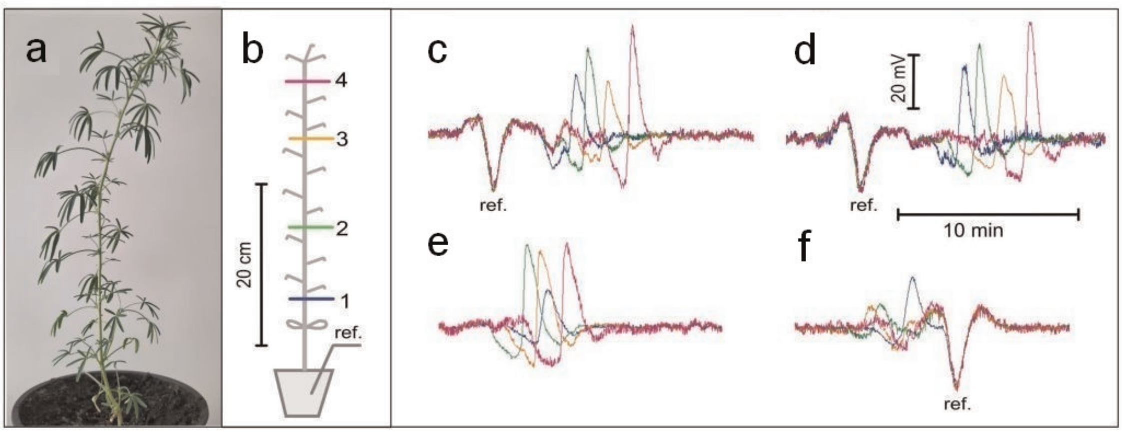

Fig. 1. Narrow-leaved lupin (*L. angustifolius* L.) plant and spontaneous action potentials (SAPs). (**a**) Example of an experimental lupin plant. (**b**) Scheme of the plant and electrode arrangement on the stem (1 – blue, 2 – green, 3 – orange, and 4 – pink lines represent measurement electrodes, ref: reference electrode in the pot with soil). (**c**, **d**) Examples of recordings of the most common SAPs propagating acropetally (**e**) acropetally – basipetally, and (**f**) very rare SAPs propagating basipetally. APs are registered by the reference electrode (inverted spike on all electrode records at the same time; the reference electrode is a relative reference electrode) and pass by electrodes 1, 2, 3, and 4. Data from the LD conditions. Detailed data of the parameters of SAPs are presented in Table 1, Supplementary Table S1, and Supplementary Table S2.

VARIANTS OF EXPERIMENTS

The measurements were carried out in three different light conditions: light/dark, 16 h of 25–40 µmol m^{-2} s^{-1} light/8 h of darkness (LD); continuous light, 25–40 µmol m^{-2} s^{-1} (CLL); continuous very low light, 5 µmol m^{-2} s^{-1} (CVLL) in a photosynthetically active radiation (PAR) range, white light (Power Star HQT-T400W/OSRAM GmbH, Munich, Germany). In the individual conditions, the following number of plants was measured: LD – 7 plants, CLL – 10 plants, and CVLL – 10 plants.

STATISTICAL ANALYSIS

The data set was first tested for normality using the Shapiro-Wilk test. When the data had a non-normal distribution, the non-parametric Mann-Whitney U test for pairwise analysis was used. The t-test for pairwise analysis was used when the data had a normal distribution. The level of statistical significance for all tests was set at $p < 0.05$. The data were analyzed using Statistica ver. 13 software (TIBCO Software Inc., 2017).

SAP TRACKER – NEW APPLICATION FOR SAP VISUALIZATION

The original recordings of single SAPs and SAP series in individual plants can be viewed using the *SAP Tracker* (*ST*) application. The application, user guide, and samples are available at http://circumnutation.umcs.lublin.pl/sap-tracker. Examples of SAPs and series of SAPs tracked by *ST* are shown in Fig. 1 and Fig. 2.

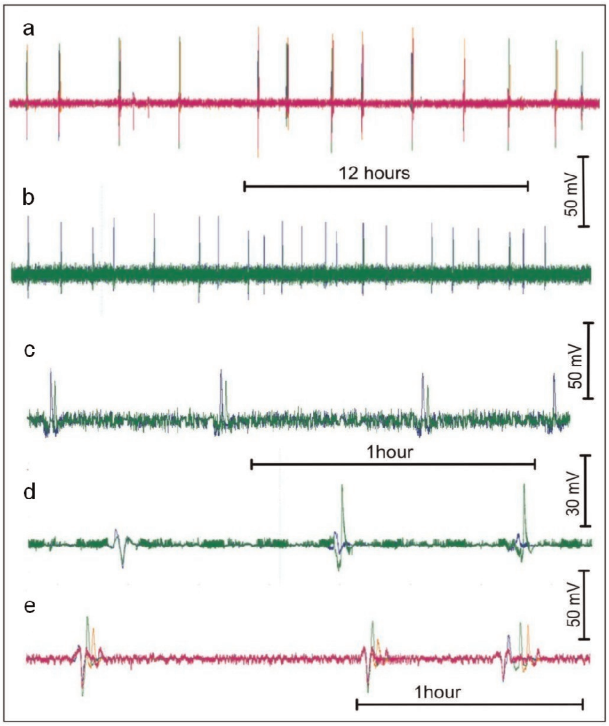

Fig. 2. Examples of series of spontaneous action potentials (SAPs) in the narrow-leaved lupin (*L. angustifolius* L.) plant. Series visualized by the *SAP Tracker* application. Electrical impulsation: (**a**) in the LD conditions, one day, (**b**) in the CVLL conditions, one day, (**c**, **d**, and **e**) examples of series having a more extended time scale to better show the APs (**c**, **d** – CVLL conditions, **e** – LD conditions). Examples of recordings of SAPs and series of SAPs can be viewed in the *ST* application (http://circumnutation.umcs.lublin.pl/sap-tracker)

RESULTS

OCCURRENCE OF SPONTANEOUS ACTION POTENTIALS

In the LD conditions, 63 SAPs were observed. The SAP parameters in the LD conditions are presented in detail in Tab. S1 (Supplementary material). In short, the average amplitude of SAPs was 30±1.4 mV with median 30 mV (n=63 SAPs), and their half-time was 38±2.5 seconds with median 31 seconds (n=62 SAPs). The velocity of propagation of SAPs in the LD conditions was 4.1±0.2 cm min^{-1} with median 3.6 cm min^{-1} (n=54). The average number of SAPs per plant per day was 4.5±1 with median 3.5 (n=14 days), and the average number of SAPs per plant per three days was 9.3±13.7 with median 9 (n=7 plants).

The three-day observation of ten plants indicated that the LL plants did not generate any SAPs.

Next, continuous very low light conditions (CVLL, 5 µmol m^{-2} s^{-1}) were applied. In these conditions, 138 SAPs, usually bursting in ultradian series, were observed. The registration of SAPs was carried out in ten plants, and three of them did not produce SAPs. Detailed SAP parameters in the CVLL conditions are presented in Table S2 (Supplementary material). In short, the average amplitude was 35.8±0.9 mV with median 36 mV (n=138 SAPs), and the half-time of these SAPs was 20±0.3 seconds with median 20 seconds (n=138 SAPs). The velocity of propagation of SAPs in the

CVLL conditions was 5.7±0.3 cm min^{-1} with median 4.5 cm min^{-1} (n=114). The average number of SAPs per plant per day was 6.9±1.3 with median 5 (n=20 days), and the average number of SAPs per plant per three days was 19.7±5 with median 15 (n=7 plants). Table 1 compares the SAP parameters in the LD and CVLL conditions.

SPONTANEOUS ACTION POTENTIALS ARE PROPAGATED ALONG THE STEM OF *L. ANGUSTIFOLIUS* L. – ACROPETAL, BASIPETAL, AND ACRO-BASIPETAL DIRECTION OF PROPAGATION

The occurrence of APs in intact plants was investigated in constant environmental conditions during multi-day experiments. The use of electrodes inserted into the stem allowed identification of the plant part where SAPs appeared and from which they propagated to the other parts of the plant. The direction of SAP propagation was established, and acropetal, basipetal, and acropetal-basipetal directions of propagation were registered (Table 1, Fig. 1, Tables S1 and S2).

In the LD conditions, almost 81% of SAPs propagated in the acropetal direction. Only 3% propagated in the basipetal direction, and the acropetal-basipetal direction of propagation was exhibited by approx. 16% of SAPs. In the CVLL conditions, almost 87% of SAPs propagated in the acropetal direction. Only 1% propagated in the basipetal direction, and the acropetal-basipetal direction of propagation was recorded in approx. 12% of SAPs. Approximately 81-87% of SAPs were generated in the lower part of the plant (roots and lower stem) and only approx. 1-3% occurred in the upper part of the plant.

Table 1. Parameters of spontaneous action potentials (SAPs) in *L. angustifolius* in different light conditions. The SAP parameter values were statistically compared between the LD and CVLL conditions. The SAP interval is the time spacing between two subsequent SAPs. Acropetal SAPs are SAPs propagating upwards the stem; basipetal SAPs are SAPs propagating downwards the stem; acropetal–basipetal SAPs are SAPs propagating upwards and downwards the stem. The different letters denote significant differences between the groups (within one variable). The level of statistical significance for all tests was set at $p < 0.05$. The p-value refers to the comparison of the SAP parameters in the LD and CVLL conditions. Values represent mean ± standard error.

SAPs parameters	**Light/dark (LD)**	**Very low light (CVLL)**	***p* value**
Number of plants investigated	7	10	–
% of plants with SAPs	100	70	–
Amplitude (mV)	30±1.4^{a} (n=63)	35.8±0.9^{b} (n=138)	normal distribution t-test *p* = 0.000384
Half-time (s)	38±2.5^{a} (n=62)	20±0.3^{b} (n=138)	non normal distribution Mann-Whitney U test *p* = 0.000000
Velocity of propagation (cm min^{-1})	4.1±0.2^{a} (n=54)	5.7±0.3^{b} (n=114)	non normal distribution Mann-Whitney U test *p* = 0.000104
SAPs interval (min)	233±28^{a} (n=56)	144±16^{b} (n=131)	non normal distribution Mann-Whitney U test *p* = 0.000016
Number of SAPs per day per plant	4.5±0.9^{a} (n=14 days)	6.9±1.^{a} (n=20 days)	non normal distribution Mann-Whitney U test *p* = 0.401
Number of SAPs per three days per plant	9.0±3.4^{a} (n=7 plants)	19.7±5^{a} (n=7 plants)	normal distribution t-test *p* = 0.101
Acropetal SAPs (%)	81	87	–
Basipetal SAPs (%)	3	1	–
Acropetal – basipetal SAPs (%)	16	12	–

ULTRADIAN SERIES OF SPONTANEOUS ACTION POTENTIALS – SAP INTERVALS

Spontaneous APs recorded in the lupin stem propagated over a distance of several to several dozen of centimeters in the acropetal, basipetal, or both directions simultaneously. Such long-distance impulses occurred with varying frequency. In the LD conditions (Table 1), the average interval between two subsequent SAPs was 233±28 minutes with median 146 minutes (n=56 SAPs). In the CVLL conditions, the average interval between two subsequent SAPs was 144±16 minutes, with median 88 minutes (n=131 SAPs). Both mean values of the time interval between subsequent SAPs in the series differed in a statistically significant way (p=0.0076). The most frequently observed series of SAPs appeared at the so-called ultradian intervals. This SAP interval ranged from several minutes to several hours, as described in Table S1 and Table S2. In the CVLL conditions, spontaneous bursts of APs occurred at a higher frequency than in the LD conditions. Examples of series of SAPs are presented in Fig. 2.

DISCUSSION

PARAMETERS OF SPONTANEOUS ACTION POTENTIALS

The experiments and results presented above clearly indicate the existence of spontaneous electrical excitability in *L. angustifolius* plants. The SAP parameters described above for the *Lupinus* plants are similar to the AP parameters obtained by electrical stimulation (Paszewski and Zawadzki, 1973). Despite the differences in the details of the measurement method, the amplitudes, half-times, and velocity of propagation were similar to those of electrically induced APs (Paszewski and Zawadzki, 1973). The half-time (20-38 s) in the *Lupinus* plant was two-fold longer than the half-time in *Mimosa pudica* SAPs (15-16 s), and the velocity of propagation was almost two-fold slower in *Lupinus* (4-6 cm min^{-1}) than in *Mimosa* (10 cm min^{-1}) (Stolarz and Trębacz, 2021). Recently, touch- or wounding-induced electrical signals in *Mimosa pudica* have been shown (Hagihara et al., 2022). They last approx. 1 second and have a velocity of propagation approx. 30 cm min^{-1} and an amplitude of 80 -150 mV. *Dionaea muscipula* APs also last approx. 1 second and have a velocity of approx. 600 cm min^{-1} and an amplitude of approx. 100 mV (Hedrich and Kreuzer, 2023). In lupin – the so-called ordinary plant – SAPs last longer and propagate slower than the stimuli-induced APs in highly specialized plants (*Mimosa*, *Dionaea*), which exhibit rapid movements.

DISTINCT DOMINANCE OF ACROPETALLY PROPAGATED SPONTANEOUS ACTION POTENTIALS

In *H. annuus* plants, more SAPs propagated in the basipetal direction (approx. 75%) than acropetally (approx. 25%) (Stolarz and Dziubińska, 2017b). In the experiments presented above, SAPs propagating acropetally in the *Lupinus* plants clearly dominated. It cannot be ruled out that the ultradian SAPs recorded by us, with the origin region of 85% of SAPs located in the lower part of the plant, are merely an element of the root response to soil conditions (for example, the content or lack of nutrient compounds, nodule bacteria, changes in pH and salinity). SAP signals could also be an element of electrical communication between plants growing naturally in soil (Johnson and Gilbert, 2015).

LIGHT CONDITIONS INFLUENCE THE OCCURRENCE OF SPONTANEOUS ACTION POTENTIALS

In this study, the number of SAPs observed in *Lupinus* depended on the light conditions. It is clearly visible that continuous light (CLL) completely silences the appearance of SAPs. This also indicates and confirms that APs in plants may be a signal related to light and dark perception. The significantly higher total number of SAPs in the CVLL and LD conditions than in the CLL conditions clearly shows that the appearance of SAPs is modulated by light conditions. The number of SAPs is modulated by external light conditions, which suggests increased excitability of plants in low light conditions. A similar regularity was demonstrated in earlier experiments with *H. annuus* and *Solanum lycopersicum* (Macedo et al., 2015; Silva et al., 2021; Stolarz and Dziubinska, 2017a; Stolarz and Dziubińska, 2017b; Zawadzki et al., 1995). Possibly, electrical signals and photosynthesis may also be interrelated (Fromm and Eschrich, 1993; Koziolek et al., 2004; Lautner et al., 2005; Sukhov, 2016; Szechyńska-Hebda et al., 2017).

ULTRADIAN SERIES OF SPONTANEOUS ACTION POTENTIALS

Research on the excitability of *L. angustifolius* conducted over 50 years ago confirmed the laws of excitability (Paszewski and Zawadzki, 1973; 1974; 1976a,b). Action potentials induced by an electrical stimulus confirmed the existence of the strength-duration relation, the all-or-nothing law, and the existence of a refractory period. The next step in electrophysiological testing should be focused on checking whether a frequency code exists. The SAPs analyzed in this study occurred sequentially with an ultradian interval. In the CVLL conditions, the interval was 144 minutes, and it was statistically significantly different from the 233-minute interval observed in the LD conditions (Table 1). This suggests that spontaneous impulsation may have a different frequency depending on external conditions; this may be referred to as a frequency code. The observed ultradian bursts with very low frequency of SAPs, a few SAPs per day, could be regarded as a frequency code of SAPs in lupin.

The rate of a few SAPs a day yields several dozen or even several hundred SAPs during the growing season. This number of SAPs may be significant and may indicate their participation in the processes of growth regulation and adaptation to environmental conditions. This may happen through the flows of calcium ions during SAP generation at specific intervals. Previous studies have shown that AP and Ca^{2+} signals are coupled in *Dionaea muscipula* and *M. pudica* (Scherzer et al., 2022; Huang and Hedrich, 2023; Hagihara et al., 2022). As a secondary messenger, calcium can regulate metabolism and physiological processes. The role of electrical potential changes and “calcium signature” are considered to be involved in the mechanism of symbiosis induction in legumes. SAPs in lupin may benefit root-legume symbiosis through Ca^{2+} ions that couple with electrical signals.

The above-presented results indicate that SAPs represent long-distance electrical signals, and there are universal signals suitable for frequency analysis in future investigations. The multi-day continuous 1 Hz measurements revealed the existence of spontaneous ultradian frequency impulsation (spiking). The appearance of many SAPs in plants verifies the assumption of the existence of frequency coding in plants.

Information may also be included in the direction of propagation. In *Lupinus*, most SAPs propagated acropetally, which may indicate that the information about the condition of the roots was transferred up the plant.

SAP TRACKER – NEW APPLICATION FOR SAP VISUALIZATION

The analysis of neuronal spiking in neurobiology is performed with the use of specialized algorithms. For such analyses in *Lupinus*, a new application, i.e., the *SAP Tracker* (*ST*), was designed for visualization of plant SAPs. The input data are .txt or .csv files. These files are time series modulated by an initial algorithm; then, such data are visualized by creation of plots. Various artefacts are recorded during many days of measurements with a sampling rate of 1 Hz. These artefacts are removed by preliminary algorithms. When searching and viewing SAPs, the user has both raw and smoothed electrophysiological recordings. In future, computer algorithms will be developed for searching the SAP signal in many-hour experiments and computation of SAP parameters.

HYPOTHETICAL MECHANISM OF SAP GENERATION IN INTACT *L. ANGUSTIFOLIUS* PLANTS

The APs described above are called spontaneous because the causes of their generation are not known. We can assume that they are generated as a result of changes in the concentration of substances causing depolarization inside the plant. Recently, a list of plant chemical compounds that cause depolarization of the cell membrane has been presented (Gao and Farmer, 2022). The plant-derived membrane-depolarizing elicitors include glutamate, K^+, sucrose, and glucose. It is likely that a spontaneous local increase or decrease in the concentration of the above chemical compounds may trigger SAPs.

The AP ion mechanism described so far in plants clearly demonstrates the involvement of a number of membrane proteins, such as ion channels and pumps (e.g., H^+-ATPase), in the generation of APs (Brownlee, 2022; Scherzer et al., 2022; Yao et al., 2023). Electrophysiological studies on *L. angustifolius* can also be combined with information about its genome. In *L. angustifolius*, genes related to membrane transport have been described: calcium pump, sucrose transporter, cation channels, and ATPase (Hane et al., 2017). Genome research together with electrophysiological studies will provide a better understanding of the physiology of this economically important symbiotic plant. Genes related to photoperiodism and flowering are being studied as well (Ksiazkiewicz et al., 2016), with a focus on an important nitrogen assimilation enzyme (Ksiazkiewicz et al., 2013).

CONCLUSIONS AND FUTURE PERSPECTIVES

It is expected that the above-presented investigation approach, i.e., multi-day extracellular measurement reinforced by informatics tools, will support studies of the role of long-distance propagated APs in plant growth in the future. After the recent discovery of the SAP phenomenon in *Mimosa pudica* (Stolarz and Trębacz, 2021), the current work is an attempt to answer the questions raised in the previous study: can environmental conditions shape spontaneous excitation and how common is this phenomenon among plants? The results presented above confirm the possibility of modulating spontaneous excitability by environmental conditions. Optimization of the hydroponic medium to enhance spontaneous excitation in plants holds promise for further research. In this regard, the role of nitrogen and potassium fertilization may be worth exploring. These studies also present *L. angustifolius* as another plant in which the SAP phenomenon occurs. Electrophysiological studies in other plant species are needed to elucidate the role of such signals in plant growth regulation. Further research including new environmental modifications and new species will answer the question of the significance of electrical firing in plant life.

AUTHORS' CONTRIBUTION

MS conceptualization, designing and conducting the experiments, data analysis, writing the manuscript, discussion and revision, reviewing the manuscript; EŁ software and user guide preparation, reviewing the manuscript, JB, MPS, PS data analysis, discussion and revision, reviewing the manuscript. All authors have read and agreed to the published version of the manuscript.

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