Development of measuring system for weak electrical potential in plants and its application

Li Guochen[1,2], Yu Haiye[1], Ma Chenglin[1]
(1. *School of Biological and Agricultural Engineering, Jilin University, Changchun* 130025, *China*;
2. *Engineering College, Shenyang Agricultural University, Shenyang* 110161, *China*)

Abstract: Plant potentials are ultra-weak signals, sometimes its amplitude is only about 10～20 μV [1,2], and the weak parts of potential are easily submerged by noising. This paper introduced the construction and technology in developing the measuring system for plant electrical potentials, the input impedance of the amplifier must be high enough ($\geq 10^{10}$～$10^{12}\Omega$) in order to measure the plant weak signals and ensuring high common mode rejection ratio (CMRR). In signal processing, wavelet transform was applied in decomposing and reconstructing based on multi-resolution wavelet analysis and in denoising based on cross-validation (CV) threshold value. Furthermore, the paper gives an application example in measuring plant potentials, the experiment shows that this system could effectively detect the change of plant potentials. Four-week old cucumber seedlings were stimulated by heat injury, the variation potential (VP) and action potential (AP) would be evoked, the AP varying amplitude can reach 40 mV; the AP changed very fast, its duration time is about 0.01～0.02 s and its amplitude often changes within 15 μV～30 mV.

Key words: plant potentials; measuring system; variation potential(VP); action potential(AP)
CLC number: TP274; TM93 **Document code**: A **Article ID**: 1002-6819(2005)08-0006-05

Li Guochen, Yu Haiye, Ma Chenglin. Development of measuring system for weak electrical potential in plants and its application[J]. Transactions of the CSAE, 2005, 21(8): 6-10.

0 Introduction

About 130 years ago, both Burden-Sanderson (1873) and Charles Darwin (1875) demonstrated the existence of electrical signals in plant[3], the bulk of experiments demonstrated that plant potentials consisted of variation potential (VP) and action potential (AP)[4-7]. The AP is self-perpetuating signal based on the activity of voltage-gated channels which respond to changes in membrane potential[8] (Davies, 1993); by contrast, the VP is a local change (in living cells) to either a hydraulic surge or chemicals transmitted in the dead xylem[9] (Stankovic B, 1997); In China, as for plant potentials, LOU Cheng-Hou (Academician, China Agricultura University) has researched the transmitting and inducing of mechanism[10,11]. Until now, in order to research plant signals, different measuring methods and materials have been used, and many papers presented that the plant potentials varied from 10 μV to 50 mV, but rarely discussed how to measure the signals more truly.

Based on the papers which described about the property of the plant electrical potentials[1,5-7,10,12,13], being of biological information, plant signals are very complicated, it belongs to low-frequency and unstable ultra-weak signal, and its amplitude and frequency are very different according to the kinds of plant and intensity of stimuli. Because there are unavoidable strong noises in measuring signals, effective measuring system is very important in order to avoid noise disturbing. The main purpose of this paper is to discuss technology of measuring system for plant weak signals and how to acquire the true information from the background noises.

1 System devices

Figure. 1 is a block diagram of measuring system for plant electric potentials. The weak plant signals, which were measured with electrodes, were acquired, stored and processed in computer through amplifying, filtering and A/D converting. Plant electrical potentials belongs to the biological source of high resistance and weak signal; In order to increase signal precision, the measuring technology also varies according to different measuring methods and environmental conditions. At present, there are mainly two types of electrodes used for measurement of the extra-cellular electric potential differences. The

---

Received date: 2004-07-26 Accepted: 2005-04-07
Foundation item: the Doctoral Program Foundation of State Education Ministry of China (No. 20010183016); Natural Science Foundation of Jilin Province (No. 20020657)

Biography: Li Guochen, Ph. D, School of Biological and Agricultural Engineering, Jilin University, 130025, Changchun; Associate professor, Shengyang Agricultural University, 110161, Shenyang, China.
Corresponding author: Yu Haiye, Ph. D, Professor, doctor adviser, Email: yhy@email.jlu.edu.cn

one is surface-contact felt-tip calomel electrodes which were oppressed to the surface of plant through electrolyte bridges[7,14,15], this measuring method is nondestructive to the plant, but the electrolyte tends to dry out and generally be used for short-term (12 h) monitoring; another is metal electrodes (Ag/AgCl, foil silk, silver) which were directly pierced the plant[12,16], although it has light-damage to plant, this method can be used for long-term (up to 7days[17]) recordings.

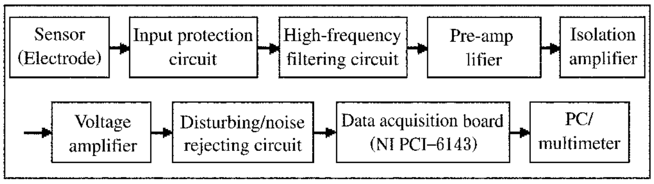


Fig. 1　Schematic diagram of measuring plant signals

The amplifier circuit is an important part of measuring system. As the plant signals are very weak and unstable, it is necessary to be amplified in order to detect the detail of the signals. The gain of amplifier cannot be too high because of the existence of polarization; therefore, this instrumentation circuit was adopted with two-level amplifier (preamplifier and voltage amplifier). The Fig. 2 was the inner circuit diagram and basic connections. The basic demand of preamplifier is high-impedance, high common-mode rejection and low offset voltage. INA114 was chosen to be preamplifier which is laser trimmed for very low offset voltage (50 μV max), drift (0.25 μV/℃), high common-mode rejection (115dB) and quiescent current is 3 mA maximum, its low-frequency noise is just 0.4 μV (frequency: 0.1～10 Hz).

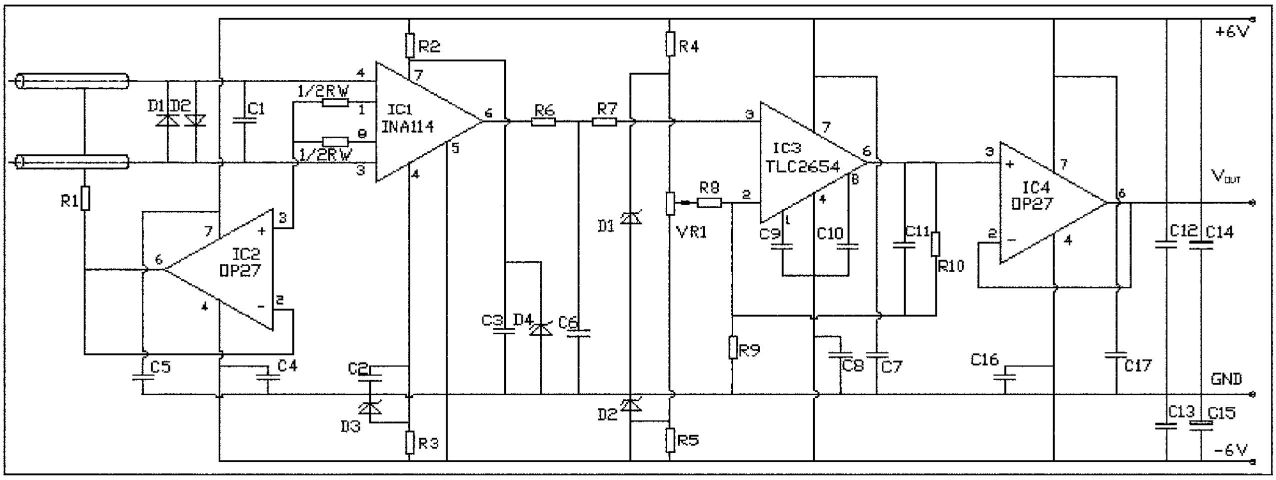


Fig. 2　Circuit diagram of instrumentation amplifier

The input impedance of the amplifier must be high enough to avoid low frequency distortion. According to different types of electrodes, the source impedances have great differences. As for surface-contact felt-tip calomel electrode, which has the effect of cut-in and surface touch, its source impedance can reach about $10^9\Omega$, the input impedance of differential amplifiers should be between 10^{12}～$10^{15}\Omega$; in this system, the electrodes are Ag/AgCl electrodes which directly pierced the plant, the source impedance is just about $10^6\Omega$(the plant was cucumber, the measuring distance between electrodes was 10～15 mm), therefore, $10^{10}\Omega$ input impedance is enough for this differential amplifier.

In order to minimize the power-line interference (50 Hz) and other background noises, high common mode rejection ratio (CMRR) is the main features of instrumental amplifier. This instrumentation was adopted with typical shield driver differential circuit which has better high common-mode rejection and is very suitable to measure weak signals.

2　Signal processing

2.1　Notching

Filtering and de-noising are the main matter of processing signals. This system adopted universal active filter-UFA42 (BB Corp.) for notching the 50 Hz power-line interference and an algorithm of orthonormal multi-resolution analysis of denoising.

Figure 3 is the configured as a 50 Hz notch filter; the notch frequency for the notch filter is set by the following calculations:

$$f_{NOTCH} = f_0 \tag{1}$$

f_0 is given by $f_0 = \frac{1}{R_F \cdot C \cdot 2\pi}$; $R_F = R_{f1} = R_{f2}$ and $C = C_1 = C_2$

The notch frequency is modified by simply changing the R_F resistors and/or adding external capacitors. The - 3dB bandwidth can be set by with the proper selection of external components R_{f1}, R_{f2} and R_q.

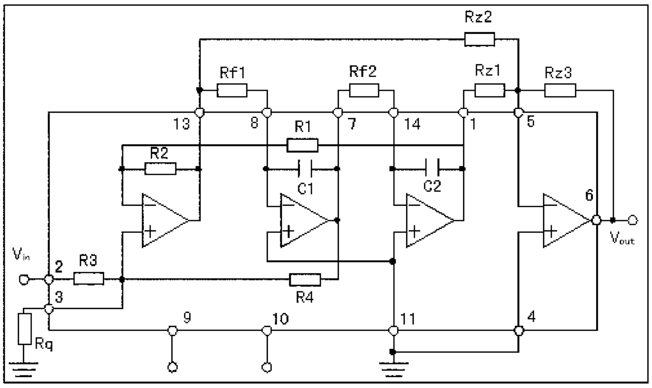


Fig. 3 UAF42 configured as a 50 Hz notch filter

2.2 Denoising

Wavelet transform is one of the most effective methods for denoising unstable signals, wavelets are a new family of basis functions, well localized in time-frequency domains. An instrumental signal-f can be presented as the sum of two components:

$$f = t + ns \tag{2}$$

Where t is the ideal signal and ns is the noise.

With multi-resolution wavelet analysis, the main steps of signal denoising are:

1) Decomposition of the signal,

2) Thresholding (elimination of small coefficients),

3) Reconstruction of the signal.

For the decomposition and reconstruction of the signal which length (N) equals an integer power of two ($N = 2^P$), the fast pyramid algorithm by Mallat can be applied. As for the choice of thresholding, the most popular one, known as ‘universal thresholding (ThU)’, is based on statistical properties of white Gaussian noise, but this definition of threshold value can lead to overestimation of the noise level. To overcome this problem, the cross-validation (CV) theory[18], which was developed to optimize the soft threshold value, is applied here; the following is the specific procedure:

1) The original data $y = (y_1, k, y_n)'$, $n = 2^J$ are set into 2 subsets of equal size, removing the odd-indexed y_i leaves even-indexed y_i which is re-indexed from $j = 1, k, 2^{J-1}$. These re-indexed data are used to construct a function to estimate $\hat{g}^e_\lambda$ using a particular threshold parameter-λ with soft/hard thresholding;

2) The even-indexed points are computed and an interpolated version of $\hat{g}^e_\lambda$ is formed:

$$\bar{g}^e_{\lambda,j} = \frac{1}{2}(\hat{g}^e_{\lambda,j+1} + \hat{g}^e_{\lambda,j}),\ j = 1, K, n/2 \tag{3}$$

3) Setting $\hat{g}^e_{\lambda,n/2+1} = \hat{g}^e_{\lambda,1}$; similarly, the interpolated version for odd-indexing is formed as:

$$\bar{g}^o_{\lambda,j} = \frac{1}{2}(\hat{g}^o_{\lambda,j+1} + \hat{g}^o_{\lambda,j}),\ j = 1, K, n/2 \tag{4}$$

4) The threshold level-λ is minimized by mean integrated squared error (MISE) between the interpolated wavelet estimators and the left points, the symbols are:

$$\hat{M}(\lambda) = \sum_{j=1}^{n/2} [(\bar{g}^e_{\lambda,j} - y_{2j+1})^2 + (\bar{g}^o_{\lambda,j} - y_{2j})^2] \tag{5}$$

5) The leave-out half cross-validation threshold is defined as:

$$\lambda_{\min} = \arg\min_{\lambda \geq 0} \hat{M}(\lambda) \tag{6}$$

In order to illustrate the performance of the proposed method, the denoising results of simulated signals are compared by using two methods of choosing threshold: CV and ThU thresholding, the denoising procedure was applied by using wavelet function-the 4th member of the Biorthogonal family. The result in Fig. 4 showed that the more detailed information was not lost by using CV thresholding, the reconstruction square error (RSE) between the denoised signal and the ideal was 67.71119; as for ThU, the result was 75.87545; seen from the result, the CV method is much better than the ThU method.

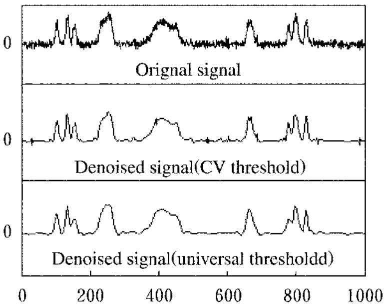


Fig. 4 Denoised signals with CV and ThU thresholding

3 Applications

3.1 Materials and methods

Figure 5 is the arrangement of measuring system, all electrophysiological measurements were conducted at a plant incubator (HP1500GS, Wuhan Ruihua instrument & equipment Co., Ltd), which was well shielded and in which the environmental factors (air temperature, humidity and the density of illumination) could be controlled. An IBM-compatible microcomputer with S series simultaneous sampling Multifunction DAQ-NI PCI-6143 (National Instruments) was interfaced through a multiplexed screw terminal accessory board with 0.2 mm non-polarizable reversible Ag/AgCl electrodes and used to record the digital data. Electrode outputs were passed through a custom-made high-impedance instrumental amplifier and low-pass filter (500 Hz) before the signals were acquired, frequency of scanning was 2000 samples/second. Ag/AgCl electrodes were inserted into the plant at different positions, the reference electrode was usually inserted in the stem or in the root of a cucumber, and the working electrode was inserted in the leaf or the upper part of the stem.

According to the description[7], the heat-wounding can evoke the AP and VP, therefore, the heat stimuli was applied in the experiment. The cucumber upper-leaf was wounded by passing a lit match for about 3 s underneath a region 3~4 cm^2. Cucumber (Changqing 1#, Jilin Province) plants were grown in a greenhouse for 35~40 days at 20~30℃, those of similar height (about 12 cm) and appearance were selected for the experiments and transferred into plant incubator.

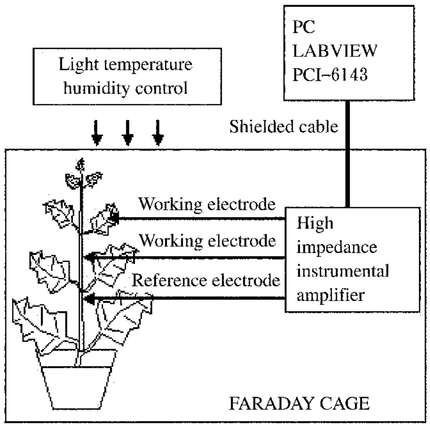
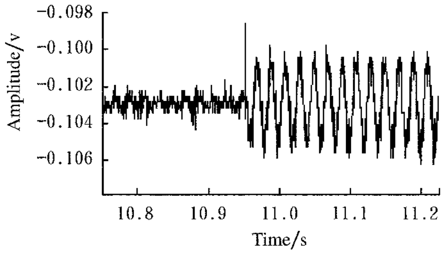


Fig. 5　Arrangment for measuring plant electrical signals

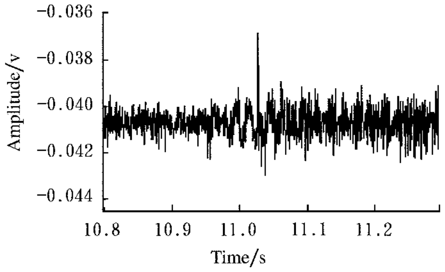
3.2　Results and discussion

The wave in Fig. 6a is the original signal which directly measured with DAQ (PCI-6143). Being without any proceeding, the wave consists of visible power-line interference (50 Hz). In the meantime, the wave in Fig. 6b is measured with the instrumental amplifier (including notching circuit), it is not interfered by the power-line (50 Hz), but it is still mixed with other noises (≤500 Hz).

Insertion of electrodes in plants evokes slow fluctuations of the variation potential in the phloem and action potential across the stem, after approximately 1.5 h, the variation potential stabilizes and action potentials disappear[16]. In Fig. 7a, the wave is the signal without being de-noised, the wave in Fig. 7b is the result of de-noised signal, which is clearer and it possesses 95.6% energy of original signal. The waves in Fig. 8 are the plant potentials evoked by heat wounding; the vertical line is the time point for heat simulation.

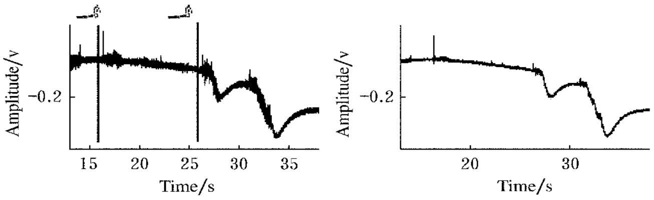


a. Original signals



b. Processed signals

Fig. 6　Processed signals and original signals



a. Un-denoised signals　　b. Denoised signals

Fig. 7　Un-denoised signals and denoised signals

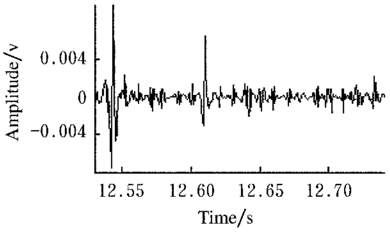


Fig. 8　Original signals and processed signals

Based on the property of AP and VP[11], generally, the VP change slowly and can be clearly observed in Fig. 7, its varying amplitude can reach 40 mV, and the VP visible fluctuation wave can last for about 5~6 minutes. Compared with the VP, VP belongs to high-frequency signals; the multi-resolution wavelet analysis can be used for decomposing the high-frequency part from the denoised signals. The wave in Fig. 8 is the high-frequency part which reconstructed with wavelet decomposition (4th member of the Biorthogonal family), the wave can clearly express the variation of VP, the duration is about 0.01~0.02 s, and its amplitude gradually drops off from 30～40 mV to 15 μV.

4 Conclusions

1) The custom-made instrument organically integrates the high-impedance preamplifier (INA114) and low-noise precision operational amplifier (OP27 and TLC2654), its circuit is adopted with typical shield driver differential circuit with better high common-mode rejection and is very suitable to measure weak signals

2) Using UFA42 notching filter and wavelet de-noising method based on CV thresholding, this system can effectively avoid power-line interference (50 Hz); multi-resolution wavelet analysis (4th member of the Biorthogonal family) can be used for decomposing and reconstructing the AP and VP.

3) The results of simulated and practical experiment show that the design of this measuring system can clearly detect the weak changes of AP and VP. Generally, under the heat stimulation, the AP varying amplitude can reach 40 mV; the AP changes very fast, its duration time is about 0 01～0 02 s and its amplitude often changes in 15 μV～30 mV.

[References]

[1] Zhao Zikai A study on ultra-weak electronic signal in plants[J]. Journal of China Institute of Metrology, 2002, 13(03): 243-257.

[2] Wang Lanzhou, Cao Wenxia, et al The determination of weak electrical signal in leaves of lycoris radiate [J]. Journal of Northwest Normal University, 2000, 36(2): 62-66.

[3] Eruc Davies New functions for electrical signals in plants [J]. New Phytologist, 2004, 161: 607-610.

[4] Wildon D C, Thain J F, Minchin P E H, et al Electrical signaling and systemic proteinase inhibitor induction in the wounded plant[J]. Nature, 1992, 360: 62-65.

[5] Zawadzki T, Davies E, Dziubinska H, et al Characteristic of action potentials in Heliantbus annuus L [J]. Physiological Plantarum, 1991, 83: 601-604.

[6] Volkov A G, Haack R A. Insect-induced bioelectrochemical signals in potato plants [J]. Bioelectrochemistry and Bioenergetics, 1995, 37: 55-60.

[7] Bratislav Stankovic, Eric Davies Both action potentials and variation potentials induce proteinase inhibitor gene expression in tomato[J]. FEBS Letters, 1996, 390: 275-279.

[8] Davies E. Intercellular and intracellular signals in plants and their transduction via the membrane-cytoskeleton interface[C]. Seminars in Cell Biology (4): 139-147.

[9] Stankovic B, Davies E. Wounding evokes rapid changes in tissue deformation, electrical potential, transcription and translation in tomato[J]. Plant and Cell Physiology (39): 268-274.

[10] Lou Chenghou, Hua Baoguang Plant signaling system [J]. Chinese Bulletin of Life Sciences, 2000, 12(2): 49-50.

[11] Lou Chenghou. Electrochemical wave transmission in plant[J]. *ACTA Biophysica AINICA*, 1996, 12(4): 739-745.

[12] Li Haishan, Cao Wenxia, Wang Lanzhou. Study on weak signal for *P. tabulaeformis and P. bungeana* [J]. Journal of Northwest Nomal University, 2000, 36(4): 77-81.

[13] Alexander G. Volkov. Green plants: electrochemical interfaces [J]. Journal of Electroanalytical Chemistry, 2000, 483: 150-156.

[14] Patrick Favre, Hubert Greppin, Robert Degli Agosti Repetitive action potentials induced in Arabidopsis thaliana leaves by wounding and potassium chloride application[J]. Plant Physiol Biochem, 2001, (39): 961-969.

[15] Wang Zhongyi, Chen Duansheng, Huang Lan. Plant physiological status monitoring system and its application in greenhouse[J]. Transactions of the CSAE, 2000, 16 (2): 101-104.

[16] Tatiana Shvetsova, John Mwesigwa, Alexander G. Volkov. Plant electrophysiology: FCCP induces action potentials and excitation waves in soybean [J]. Plant Science, 2001, (161): 901-909.

[17] András Koppán, László Szarka, Viktor Wesztergom. Annual fluctuation in amplitudes of daily variations of electrical signals measured in the trunk of a standing tree [J]. Life Sciences, 2003, 323: 559-563.

[18] Nason G P. Wavelet regression by cross-validation[J]. J R Soc Ser B: Methodological, 1996, 58(2): 463-479.

植物微弱信号测量系统的研究与应用

李国臣[1,2], 于海业[1], 马成林[1]

(1. 吉林大学生物与农业工程学院, 130025; 2. 沈阳农业大学工程学院, 110161)

摘　要: 植物电位信号属于微弱电信号, 研究表明, 它的幅度在十几 μV 到几十个mV 之间, 而这种微弱信号很容易淹没在噪声中。介绍了适用于微弱信号检测的一般方法, 并根据试验中所开发的植物电位信号采集与处理系统, 详细说明了以 INA114 为主要放大器所组成的前置放大器的结构、限波电路和基于CV 阈值的小波降噪算法。此外, 采用黄瓜幼苗(3～4片展开叶)为样本, 进行试验应用, 经试验可检测到黄瓜幼苗在热伤害下的植物电位信号, 其变异电位的变化较大, 最大可达40 mV 以上, 而动作电位的变化较快, 经小波重构的信号可明显地反映动作电位的变化位置和变化周期, 其周期变化范围通常在 0 01～0 02 s。

关键词: 植物电位信号; 测量; 变异电位; 动作电位