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Monitoring system for electrical signals in plants in the greenhouse and its applications

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We have developed a multi-channel system for simultaneous monitoring of multiple environmental factors and electrical signals in cucumber plants in the greenhouse. The system includes a special sensor, which is both sensitive and reliable for long-term use for collecting electrical signals. In this work, coefficients of variability (CVs), Pearson's coefficient of correlation and intra-class correlation (ICC) procedures were applied to assess the reliability of the recorded signals. The signal amplitudes were reliable with coefficients of variation of less than 7.0% and ICCs of more than 69.3%. Using this system, we have proven that the electrical signals in plants respond to environmental changes under natural conditions in the greenhouse. The system could provide a long-term stable tool to measure and analyze the electrical signals in plants in greenhouses.

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1. Introduction

In recent years, several monitoring and controlling systems that take temperature, humidity, light and other environmental factors in the greenhouse into consideration have been developed as commercial devices (Aaslyng et al., 2003). Different physiological signals, such as stem sap flow and the difference in leaf temperature, have been chosen to serve as response-to-environment indicators (Herzog et al., 1997; Gratani et al., 2000). The electrical signal is an essentially critical signal that reflects the responses of a plant to the environmental changes (Martynenko, 2000), but its possible use for monitoring plant response to environmental changes has not been investigated to date.

Electrical signals in plants can be divided into three types: local electrical potential (LEP), action potential (AP) and variation potential (VP). LEP is a sub-threshold response induced by changes in environmental factors, e.g. soil, water, fertility, light, air temperature and humidity (Lou, 1996; Leng, 1998; Wang et al., 2001; Huang & Wang, 2002; Hu, 2003; Pyatygin et al., 2006). Although the LEP is only locally generated and is not transferred to other parts of a plant, it has a tremendous impact on the physiological status of the plant (Ren et al., 1993). In contrast, both AP and VP can transmit from the stimulated site to other parts of the plant (Davies, 1987; Mwesigwa et al., 2000; Davies, 2004). AP or VP transmissions in plants have been investigated by several researchers (Trebacz & Zawadzki, 1985; Stankovic et al., 1998; Szarek & Trebacz, 1999; Dziubinska et al., 2001, 2003; Pyatygin, 2008). However, plant electrical signals in response to stimuli were always recorded under laboratory conditions, and over short intervals (Vian et al., 1999; Fromm & Lautner, 2007). Obviously, these conditions do not match the

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Nomenclature

CV	coefficient of variability
μ	mean
σ	standard deviation.
ICC	intra-class correlation coefficient
δ_S^2	variance due to plant-to-plant variability
δ_D^2	variance due to day-to-day variability
$\xi[k]$	grey relation coefficient
$R_{0i}(x_0, x_i)$	grey relation grade
x_i	a factor sequence of the system
x_0	the reference sequence
$x_0[k]$	the reference series
$x_i[k]$	the compared series
i	number of factors
j	number of factors, but $j \neq i$
k	represents the number of data point, i.e. time point

natural environment of the solar greenhouse or the fields where the plants grow.

From an engineering perspective, there remain many difficulties and challenges in measuring plant electrical signals in the field or the greenhouse. Unlike laboratory experiments, acquiring plant electrical signals in the greenhouse or the field requires the consideration of several factors: (1) since the electrical signals in plants reflect the changes in multiple environmental factors, e.g. light, temperature and humidity, the repeatability of the signals should be investigated; (2) sampling of the electrical signals from plants in the greenhouse can suffer electromagnetic interference from apparatus running in the greenhouse without a Faraday cage, and thus the reliability of system should be estimated; (3) the methods for connecting electrodes to the plants should adapt to the growth of the plants; (4) the transfer method for the weak electrical signal must be considered as the distance from the detection site to the computer may be several metres.

The aim of our study is to establish a new signal monitoring system suitable for greenhouse applications, and then estimate the reliability and repeatability of the system using statistical indexes. Also, automatic grey relation algorithms were proposed to determine the relationship between the environmental variations and the electrical signals in plants in real time.

2. Monitoring system and methods

2.1. *The prototype of the monitoring system*

The monitoring system consisted of hardware and software with data acquisition and processing functions. According to the experimental requirements, the environmental factors in the greenhouse, including temperature, light intensity, humidity, and CO_2 concentration, were taken into account. The electrical signals in the plants and the leaf temperature were also recorded. All analog signals from the amplifiers are converted to digital signals and transferred using a data acquisition board, KH-9250H (Ke Hai Corporation), which allows 32-channel recording. KH-9250H provides high resolution, a wide gain range and any single channel can be sampled at 100 kHz. The signals were recorded as a text file using software (copyright 2006, BJ 4291) designed by us. To reduce the noise, the software also provides signal processing functions, e.g. smoothing, singular value decomposition.

2.1.1. *Selecting the sensors*

Environmental temperature and relative humidity are monitored using an integrated sensor (type: JWSB, accuracy of 0.5 °C, ColliHigh Sensor Technology Center); CO_2 concentration is detected by a CO_2 sensor (GMW22, Vaisala Corporation) with a measurement range of 0–2000 ppm and resolution of 1 ppm. The light intensity is measured by a PHT-II (range from 0 to 2000 $\mu mol\ m^{-2}\ s^{-1}$, Ecotron Corporation). In addition to these sensors, the sensor for measuring the electrical signals in plants consists of an electrode and a preamplifier. The electrode acts as an interface between the plant and the preamplifier, from which the electrical signals in the plant can be measured. There are two kinds of electrode: extracellular electrodes (surface contacted electrodes) and microelectrodes. Extracellular electrodes are used to measure the electrical signals in plants generated by multi-cellular actions. The microelectrodes with a tip diameter of less than 1 μm penetrate into a cell to detect the membrane potential at the cell level, which indicates the electrical behaviour of a few adjacent cells or of a single cell.

The extracellular electrodes are suitable for detecting bioelectrical activity in large groups of cells. Metal thread electrodes (Pt electrodes), Ag/AgCl electrodes and calomel electrodes are applied as the measuring electrodes. In this study, the electrodes were selected to measure the electrical signals in plants. The sensors applied in the greenhouse are selected based on the following facts:

(1) Considering the significant fluctuation in temperature between day and night, the temperature characteristics of the electrode must be stable.
(2) The electrodes used in the greenhouse should not be polarized and eroded.
(3) The input impedance of the preamplifier must be high ($>10^{10}\ \Omega$), and the temperature drift must be low, so the temperature coefficient of the input offset voltage is less than 10 $\mu V\ °C^{-1}$.

2.1.2. *Collecting electrical signals in plants in the greenhouse*

Electrical signals in plants were detected by a cotton thread soaked in an experimental solution (KCl 0.1 mM, $MgCl_2$ 0.1 mM, $CaCl_2$ 0.5 mM, Na_2SO_4 0.05 mM). One end of the cotton thread was covered with a soft plastic tube which contacted the stem surface of the plant, while the other end is soaked in a 25 ml volume chamber of the electrode supported with an adjustable holder, and the electrode is also inserted into a chamber containing the experimental solution, where the

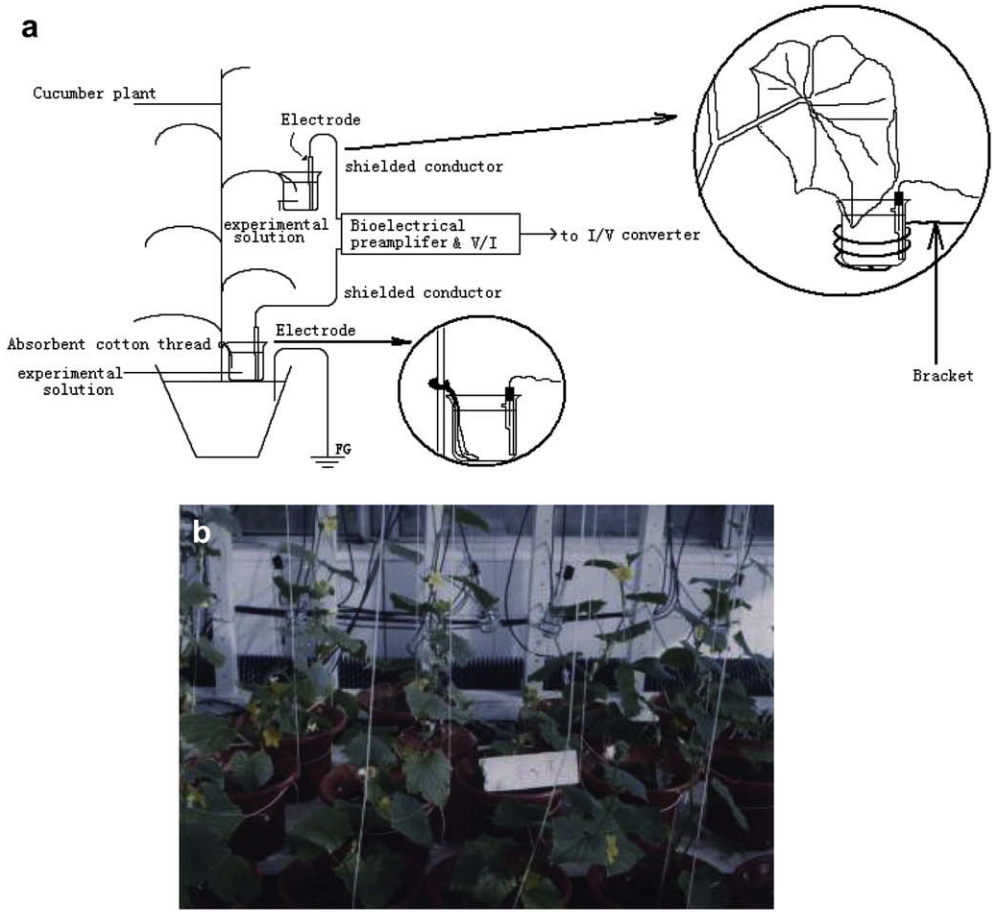

Fig. 1 – (a) Experimental setup for detecting electrical signals in plants. FG (frame ground, i.e. the earth). (b) Measuring the electrical signals in plants in the greenhouse.

solution was renewed every other day (Fig. 1(a)). The 4–5 mm^2 tip of the leaf is immersed in the solution. Fig. 1(b) shows the overall measurement profile for the physiological signals using the monitoring system in the greenhouse. By avoiding the influence of anoxia of the submerged cells on the measurements, the plant electrical signals were detected by relative measurement, and replacing one leaf for monitoring purposes will not affect the measurement trend.

The device designed to measure the signal is shown (Fig. 2). The signal from the electrode was transmitted into a CA3140 impedance converter, which is an integrated circuit operational amplifier that provides very high input impedance, very low input current, and high speed performance (Intersil Corporation), and is then connected to an AD620 amplifier (Fig. 3). After amplification, the amplitude range of the electrical signals was ±4000 mV. In the greenhouse, the distance from the detection site to the computer is 30 m, so a voltage to current converter (*V*/*I*) is used so that the signal could be transferred reliably. After the long distance transfer, the current signal is converted back into a voltage signal by an *I*/*V* converter.

2.2. *Data analysis of reliability*

To investigate the reliability of the monitoring system, statistical analyses are performed and acceptability criteria are defined in the following.

2.2.1. *Coefficient of variability*

Calculation of the coefficient of variability (CV) for the signal peak amplitude of the plants is given by:

$$CV = \frac{\sigma}{\mu} \tag{1}$$

where μ is the mean, and σ is the standard deviation.

CV indicates the reliability for recording of the electrical signal within plants. CV can be used as a first measure of pattern stability: CV ≈ 1 indicates a nearly random process; CV < < 1 reflects very high stability (Stanforth *et al.*, 2000).

2.2.2. *Pearson coefficients and paired* t*-test*

Pearson's coefficient of correlation is used for comparison between the measurements. Pearson correlation coefficients are used to assess the between-trials test–retest reliability (Ng & Richardson, 1996; McEvoy *et al.*, 2000). This method is applied to pairs of comparisons for measurements, e.g. T1–T2, T2–T3, and T1–T3 in our experiment (i.e. T1 means trial 1).

The paired *t*-test is sensitive to a consistent pairwise difference between the two sets of data and is useful for detection of the repeatability of the signals.

2.2.3. *Intra-class correlation coefficient (ICC)*

The classical statistical tool used to assess data repeatability or reliability is the intra-class correlation coefficient

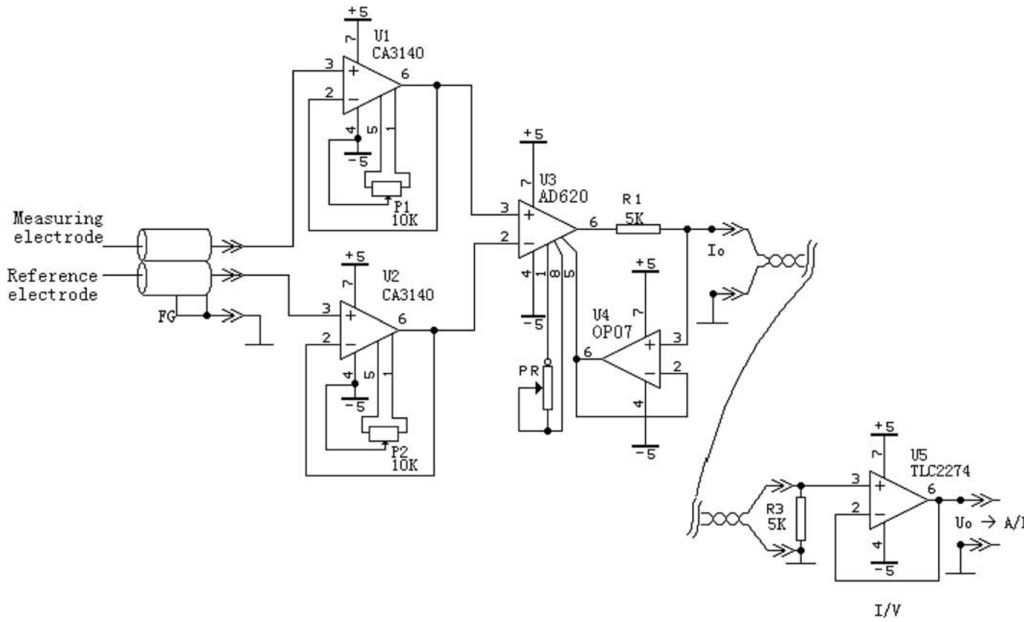

Fig. 2 – Preamplifier and voltage/current converter circuit for electrical signals in plants.

(ICC) (Bartko, 1966; Shrout & Fleiss, 1979), defined in this case as

$$ICC = \frac{\delta_S^2}{\delta_S^2 + \delta_D^2} \times 100\% \quad (2)$$

where: δ_S^2, variance due to plant-to-plant variability; δ_D^2, variance due to day-to-day variability; To compute the variances δ_S^2 and δ_D^2, analysis of variance (ANOVA) can be performed using statistical software, e.g. SPSS, Microsoft Excel. It is current practice to accept ICC values in the range 80–100% as excellent repeatability and good repeatability ICC values are in the 60–80% range, whereas values below 60% indicate poor repeatability (Bartko, 1966; Kollmitzer *et al.*, 1999).

Here, CV and Pearson coefficients are applied to investigate the reliability of electrical signal measurements under single stimuli over a day. ICC values indicate the day-to-day reliability of the system when the environmental temperature, humidity and light change.

2.3. *Grey relation method*

In a system that is complex and multivariate, the description of the relationships between various factors is difficult when based on classical statistics. Their analysis by classical statistical procedures may not be acceptable without large data sets to determine their distribution type and data satisfying certain mathematical criteria.

If a system has absolutely explicit information, it is a 'white' system. However, if a system's information is totally unknown, it is a 'black' system. In the real physical world, most systems are 'grey' rather than white or black. A grey system is a system in which the characteristics or parameters are partially known, but the exact value is not known. The grey relational space concept was proposed by Deng (1982), based on the combined concepts of system theory, space theory and control theory. It can be used to capture the correlations between the reference factor and other compared factors of a system (Wen & Chang, 1999). The purpose of grey relational analysis (GRA) is to search for primary relationships between the factors and to determine important factors that significantly influence defined objectives (Deng, 1989, 1992; Luo & Kuhell, 1993; Liang, 1999; Wang, 1999). Compared with traditional methods, there are two advantages in GRA: (1) no specific statistical data distribution is required; (2) large data samples are not needed. In this study, GRA is applied to evaluate the effects of environmental factors on electrical signals in plants and to determine the main factors. The grey relation coefficient $\xi[k]$, grey relation grade $R_{0i}(x_0, x_i)$ and grey relational ordering are defined as following.

Let $x = \{x_i | i \in I\}$ be a space sequence where $i = 1,2,\ldots m$. x_i is a factor of the system, and its value at the kth-entity in the sequence is $x_i[k]$, $k = 1,2,\ldots,n$. If we denote the reference sequence x_0 by (x_0 (1), x_0 (2),…, x_0 (n), i.e. the observed electrical signal in a plant), and the compared sequence x_i by (x_i (1), x_i (2),…, x_i (n), x_i, i.e. environmental factors), then the grey

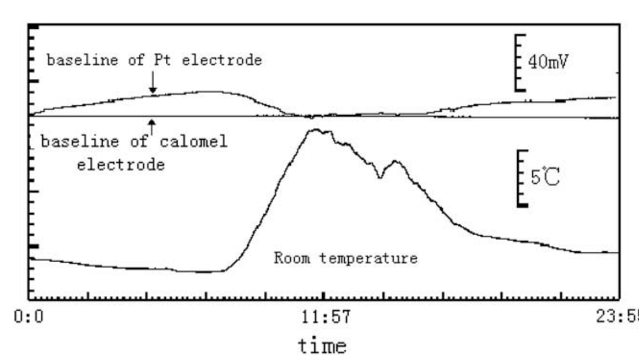

Fig. 3 – Changes of the baseline detected by the calomel electrode and the Pt electrode.

relational coefficient between x_0 and x_i at the kth-entity is obtained by Eq. (3).

The grey relational coefficient $\xi[k]$ indicates the relation grade between two series at a certain time point. The grey relation grade $R_{0i}(x_0, x_i)$ represents the degree whereby two series can be correlated. It describes the trend relationship between the reference series and a compared series in the system.

$$\xi_i[k] = \frac{\min_i \min_k |x_0[k] - x_i[k]| + \max_i \max_k |x_0[k] - x_i[k]|}{|x_0[k] - x_i[k]| + \max_i \max_k |x_0[k] - x_i[k]|}$$

$$R_{0i}(x_0, x_i) = \frac{1}{n}\sum_{k=1}^{n} \xi_i[k] \tag{3}$$

where i (=1,2,3,…,m) is the factor; k (=1,2,3,…n) is the time point; $x_0[k]$ is the reference series; $x_i[k]$ is the compared series; $|x_0[k]{-}x_i[k]|$ denotes the absolute difference between the two sequences; $\min_i \min_k |x_0[k]{-}x_i[k]|$ and $\max_i \max_k |x_0[k]{-}x_i[k]|$ are the minimum and maximum values of the absolute differences in all compared sequences, respectively.

One of the main purposes of GRA is to find the importance order of the factors based on the values of $R_{0i}(x_0, x_i)$ and identify the key factors affecting electrical signals in plants, so the rankings of the relational grades (grey relational ordering) are more significant than the values of grey relational grades between factors. If $R_{0i} > R_{0j}$, then the influence of x_i on x_0 is more predominant than that of x_j on x_0. If $R_{0i} = R_{0j}$, then the influence of x_i on x_0 is equal to that of x_j on x_0.

3. Plant materials

Cucumber plants (*Cucumis sativus* L.) were grown in a greenhouse under 14 h light/10 h dark period conditions. The air temperature varied from 15 to 30 °C during the day and 12 to 22 °C during the night; and the CO_2 concentration in air was 600 ± 200 ppm. The soil contains all the nutrients essential for the plants with 70% vermiculite and 30% humus soil. All measurements were performed on 51 to 54-day-old plants.

4. Results and discussion

4.1. *Characteristics of the electrode*

We compared the performance of the calomel electrode and the Pt electrode in detecting the signal baseline when the temperature changed (Fig. 3). The baseline of the calomel electrode fluctuated only by 1 mV within 24 h as the air temperature varied from 4 to 17 °C, while the baseline of the Pt electrode fluctuated by 11 mV under the same conditions. Hence, we concluded that the calomel electrode is more reliable for long-term measurements in the greenhouse.

4.2. *Reliability measures*

To verify the accuracy of the system, we compared the potential differences between the two electrodes at distances of 0.0, 1.5, 6.0 and 9.5 cm in the stem of cucumber detected by the designed circuit with that detected by the pH meter (PHB-1, SanXin Corporation). The results are shown in Table 1. There is no significant difference in output voltage between the designed circuit and the pH meter. Further experiments were performed on the plants to examine the reliability of the measurements.

4.2.1. *Reliability of electrical signals in plants induced by light*
Experiments were performed on five plants under the same conditions over a day. Each experiment consisted of three trials separated by light (3 min)/dark (15 min). The illumination photon flux density was 285 ± 12 μmol m^{-2} s^{-1}. Fig. 4 shows the experimental results for a plant during three trials. In a lightless environment at night, the temperature and relative humidity in the greenhouse did not change significantly, and the recorded plant electrical signals were stable and without serious fluctuations, but were changed by induction of the change in light/dark. In an environment with a certain light intensity, the recorded environmental temperature was increased by 0.3 °C at a speed of not more than 0.002 °C S^{-1}. Such slow temperature change did not induce a plant to produce electrical signals due to its physiological adaptability, and the environmental relative humidity only changed very slightly without inducing any change in the electrical signal, but the electrical signals induced by light/dark changes can be reproduced.

In this work, the signal amplitude of each recording as a feature was calculated from the original electrical signal in the plant by subtraction of the stable baseline. The mean ± S.D. of the signal amplitude of five plants induced by light/dark is provided in Fig. 5. The values of mean ± S.D. of the peak amplitude were estimated using the recording data within the duration of the response. Table 2 compares the mean ± S.D., CV, paired t-test and Pearson coefficients of the signals in these plants. Thus analysis of these parameters revealed that good reliability (CV ranged from 4.9 to 6.9% for

Table 1 – Comparison of the potential differences between two electrodes at a distance in the stem of cucumber plant obtained by designed circuit and a pH meter

		Distance between the two electrodes (cm)			
		0.0	1.5	6.0	9.5
Potential differences between two electrodes (mV)	PHB-1	0.0 ± 0.0	−3.0 ± 1.0	−18.0 ± 1.0	−22.0 ± 1.0
	Designed circuit	0.0 ± 0.0	−3.0 ± 1.0	−18.0 ± 1.0	−22.0 ± 1.0

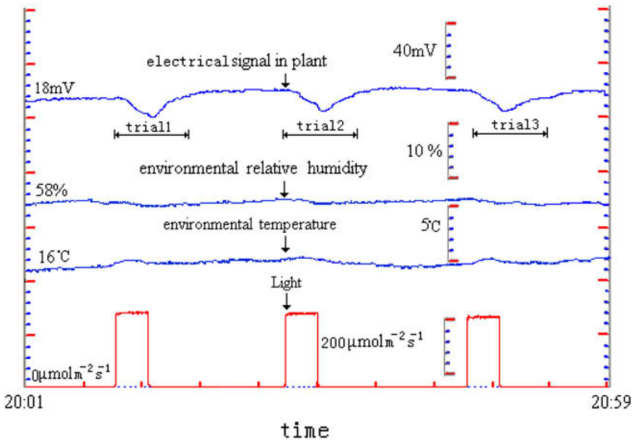

Fig. 4 – Electrical signal in plant evoked by change in light intensity. Three trials on the same plant are shown.

different plant trials) was found in a day under these experimental conditions. Light serves as the essential factor for the photosynthesis of plants. The capability of perceiving light variation is of significant physiological importance for plants. This shows that light can induce electrical signals in the leaf tissue of plants and the signals are reproducible under similar stimuli.

Light/dark change results in a depolarization or hyperpolarization. For large numbers of plant species, local non-propagating signals that depend on the stimulus strength were recorded (Elzenga *et al*., 1995; Krol & Trebacz, 1999). The amplitudes of these responses depended on the light intensity. A similar dependence was observed when the rate of potential changes following light/darkness was taken into account (Trebacz & Sievers, 1998). Our results from the plants in the greenhouse are in accordance with these studies under laboratory conditions.

4.2.2. *Reliability of electrical signals in plants induced by temperature*

Experiments were performed on five plants under the same conditions over a day. In order to have repeatable test conditions and minimize the influence of other factors, the test should be carried out at night under lightless environmental

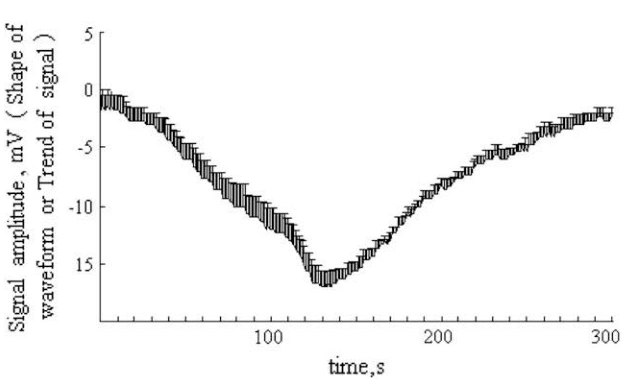

Fig. 5 – The mean ± S.D. of electrical signals amplitude of five plants induced by light/dark.

Table 2 – Comparison of the mean ± S.D., CV, paired *t*-test and Pearson coefficients of signals induced by light/dark in these plants (five plants) in a day

Peak amplitude Mean ± S.D. (mV)		
T1	T2	T3
−15.2 ± 0.8	−15.1 ± 0.9	−15.6 ± 1.1
Peak amplitude CV (%)		
T1	T2	T3
4.9	6	6.9
Signal amplitude Paired *t*-test ($\alpha = 0.05$)		
T1–T2	T2–T3	T1–T3
ns	ns	ns
Signal amplitude Pearson coefficient (during period of response: 300 s)		
T1–T2	T2–T3	T1–T3
0.98	0.96	0.95

T1 denotes trial 1, T2 denotes trial 2, T3 denotes trial 3; ns = not significant.

conditions and with a slight change of environmental relative humidity and temperature. The leaf was stimulated by local application of 0.11 cm^3 of an ice slice to the plant surface and the changes to its temperature and electrical signals were recorded simultaneously. Each experiment on a plant consisted of three trials. Fig. 6 shows the experimental results for one plant during three trials. In a lightless environment without change in the environmental temperature and humidity, the recorded plant electrical signal was stable and does not fluctuate significantly. When leaf temperature decreased at an initial rate of 0.05–0.07 °C S^{-1} from 16.3 ± 0.2 to 8.7 ± 0.3 °C, a repeatable change in the electrical signal was induced.

The mean ± S.D. of the signal amplitude induced by temperature of five plants is provided in Fig. 7. Table 3 compared the mean ± S.D., CV, paired *t*-test and Pearson coefficients of the signals to indicate the reliability values of the test–retest for selected plants. Because the correlation coefficient between trial 1 and trial 2 is similar to that between trial 2 and trial 3, and the results of the paired *t*-test are not significantly different ($\alpha = 0.05$ level), the effect of replacing either the leaf in this plant or the detection electrodes is similar. In general, the reliability values were better in these tests.

4.2.3. *Reliability of electrical signals induced by multiple factors*

In the greenhouse, although it is difficult to accurately control the environmental factors, the ICC can be calculated to verify the reliability of this monitoring system through the recorded changes in electrical signals under similar conditions of illumination, temperature and relative humidity within three consecutive days. There was no significant difference in the environmental factors over the 3 days ($\alpha = 0.05$ level). The ICC assessment was performed on five plants on three different days when all environmental factor changes took place in the

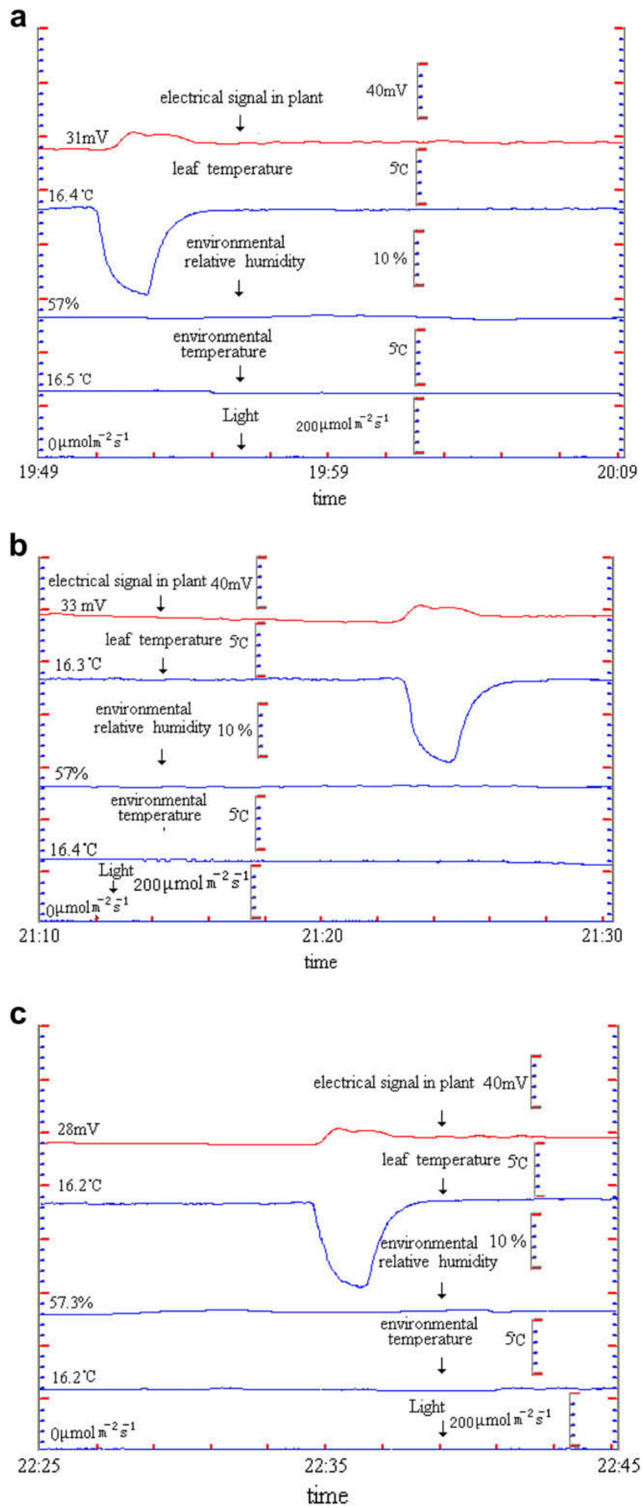

Fig. 6 – Electrical signal in plant evoked by change in leaf temperature; (a), (b), and (c) represent three recordings on three different leaves of the same plant, with different electrodes.

greenhouse (Fig. 8). In fact, a growing plant can respond to natural changes in the greenhouse environment with changes in its electric signal. At night, the greenhouse temperature changed by only 2.5 °C within 6 h and the environmental

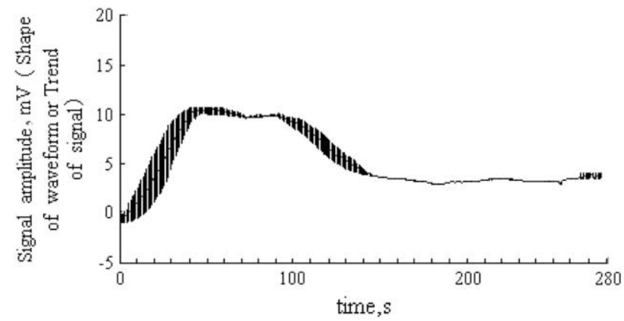

Fig. 7 – The mean ± S.D. of electrical signal amplitude induced by the temperature of five plants under the same experimental conditions.

relative humidity also changed slightly. This did not induce the fluctuation of the electrical signal, but the electrical signal fluctuated significantly and the 3 mV change during 00:00–6:00 was caused by the self-regulation of the plant. In the morning, the greenhouse temperature was increased by sunshine, the environmental relative humidity naturally decreased and the dark-to-light change induced a significant fluctuation of the electrical signal. After the sunshine reached a certain intensity, and when environmental temperature and relative humidity in the greenhouse were relatively stable, the plant would reach a new equilibrium state and its electrical signal did not fluctuate significantly any more due to its physiological adaptability, even under stronger light (sunshine) intensity (below a new threshold), while fluctuation of the electrical signal was induced by a light-to-dark change. Under certain conditions, any environmental factor can influence the plant electrical signals, but the recorded signals can still reflect the repeatability of the signal and the reliability of the system.

Table 3 – Comparison of the mean ± S.D., CV, paired t-test and Pearson coefficients and of signals induced by temperature in these plants (5 plants)

Peak amplitude Mean ± S.D. (mV)		
T1	T2	T3
10.3 ± 0.9	9.9 ± 0.7	10.2 ± 0.8
Peak amplitude CV (%)		
T1	T2	T3
3.8	5.1	4.9
Signal amplitude Paired t-test ($\alpha = 0.05$)		
T1–T2	T2–T3	T1–T3
ns	ns	ns
Signal amplitude Pearson coefficient (during period of response: 280 s)		
T1–T2	T2–T3	T1–T3
0.98	0.97	0.98

T1 denotes trial 1, T2 denotes trial 2, T3 denotes trial 3; ns = not significant.

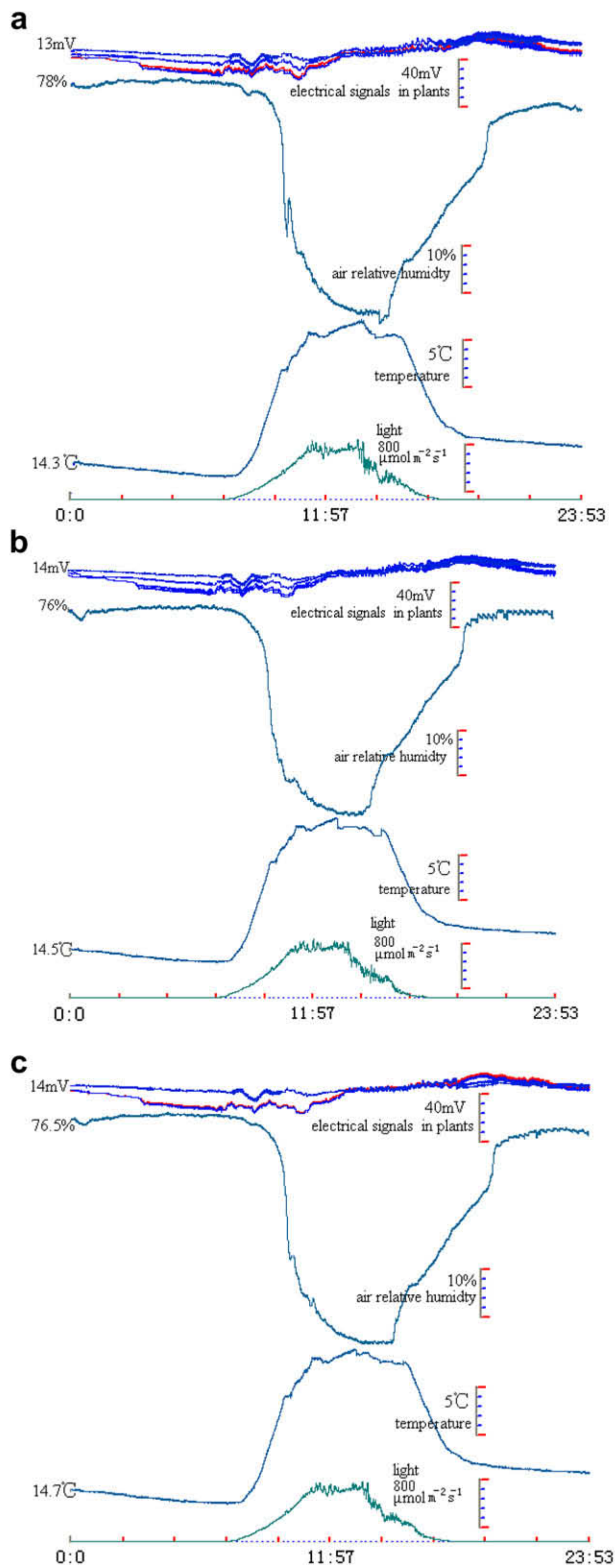

Fig. 8 – Recordings of five plants on three different days when the environmental factors change in the greenhouse. (a), (b) and (c) are the recordings from the 1st, 2nd and 3rd days respectively.

We compare the ICC value in different segments of time called S1(0:00–6:00), S2(6:00–12:00), S3(12:00–18:00) and S4(18:00–24:00). The reliability of electrical signal recording in plants was found to be good (ICC = 69.3%) to excellent (ICC = 86.6%) (Table 4). The variability in between-days reliability may be attributed to the inconsistency of variation of the recorded leaf on different days.

Under similar day-to-day experimental conditions, ICC demonstrated good test–retest reliability for these plants. From Table 4, it can be seen that the degree of reliability for measuring electrical signals in plants depends on the plant physiological status and time. In general, the obtained electrical signal in plants in a greenhouse revealed higher reproducibility over time.

4.3. *Electrical signals in plants under cold stress – a case study*

In order to evaluate the potential use of electrical signals in plants in a greenhouse, we performed measurements with our monitoring system from January 1999 to June 2002, and January to June in 2005, 2006 and 2008. Polygraphic tracing of the environmental factors by our multi-channel system showed that the electrical signals in cucumber plants were influenced by those factors. The traces of the environmental factors and the electrical signals in cucumber plants are shown in Fig. 9. The traces of the environmental factors, i.e. light intensity, environmental temperature, and air relative humidity, are shown in Fig. 9, in panels b–d, respectively.

Plants in a greenhouse are influenced by many environmental factors simultaneously. The recorded electrical signal reflects mixed changes of environment. Therefore, it is very important to find out the dominant environmental factor that affects the electrical signal. That is why we investigated electrical signals elicited by a combination of factors. Specifically, we tested the low environmental temperature with change in illumination, with a temperature range from 7.3 to 22 °C in the daytime and 11 to 13 °C at night (Fig. 9). The results show that low environmental temperature induced an obvious change in the electrical signals in the cucumber plants. The electrical signals were recorded from three different cucumber plants in the cold stress group (Fig. 9(a)). As shown in Fig. 9, the magnitude of the electrical signals in these plants declined with the descent of temperature from the critical point (10 °C), and varied following the temperature ascent.

The characteristics of the plant electrical signal in a greenhouse environment at night are discussed in Section 4.2.3. When sunshine appeared, the environmental temperature also gradually changed subsequently. During the period from 6:00 to 8:00, the electrical signal changed significantly, as the plant stomata opened for photosynthesis; then, the plant was at a new physiological dynamic equilibrium state, and thus the electrical signal did not fluctuate before the light intensity change speed and range reached a new threshold. This explains why the electrical signal did not change significantly during the period from 12:00 to 15:30, but when the environmental temperature decreased from 12.7 to 7.3 °C from 16:00 to 16:35, the electrical signal tended to decrease significantly. When the environmental temperature was

Table 4 – ICC for day-to-day reliability under greenhouse conditions

	δ_S^2 of plants, five plants				δ_D^2 of day-to-day, 3 days				ICC (%)			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Signal amplitude, mV	21	35	3.5	7	3.55	5.4	1.5	3.1	85.5	86.6	75	69.3

restored from 7.3 to 11.7 °C from 16:35 to 17:00, the electrical signal fluctuated. Under subsequent lightless conditions at a constant temperature of 11.7 °C, the electrical signal did not immediately return to the electrical potential level at the initial low temperature stage. This is because the duration of the low environmental temperature stress of 40 min affected the structure and function of the cell membrane in the plant leaf and their restoration requires a certain time period for self-regulation. Evidence at the cell level also showed that membrane activity and ion flow were restored through a dynamic process (Shabala & Shabala, 2002). Electrical signals on the plant surface were temporal and spatial superpositions of the electrical activity of the membranes in several cells, and thus the recorded signals did not restore immediately at the temperature restoration point. The recordings in 1 day show that three cucumber plants had similar responses when induced under the same environmental conditions.

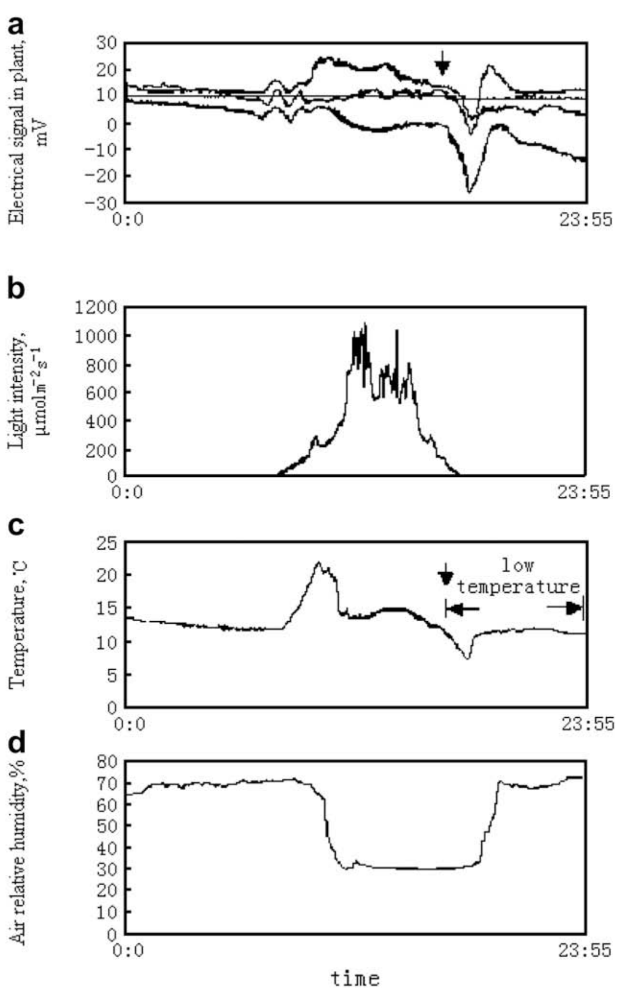

Fig. 9 – Electrical signals in plants in response to the lower temperature; (a) electrical signals, (b) light, (c) temperature, (d) relative humidity.

In addition, light intensity and relative humidity fluctuated with the change in environmental temperature (decreased and then restored). The grey relation grade for a certain time period was calculated and its results are shown in Table 5. In the function, the electrical signal was regarded as a reference sequence (x_0, i.e. a recording from Fig. 9(a)) and all environmental factors were collected simultaneously as a compared sequence (x_1, x_2, x_3, i.e. Fig. 9(b–d) representing environmental temperature, light, and relative humidity, respectively), and R-values (R_{01}, R_{02}, R_{03}) were ranked in a descending order. The factors with greater R-value were the main inducing factors for the change in the electrical signal in the time period. The relation grade order was $R_{01} > R_{03} > R_{02}$. The results show that during a period of low temperature, all environmental factors have an influence on the electrical signals in plants; however, temperature is the dominant (primary) factor affecting the electrical signals in plants.

Low-temperature-induced transmembrane potential changes in plants were reported previously in laboratory experiments (Opritov et al., 2002; Krol *et al.*, 2004, 2006; Opritov *et al.*, 2005; Vodeneev *et al.*, 2006). Electrophysiological studies have already revealed that ion transport depends on the cooling rate (dT/dt) (Minorsky & Spanswick, 1989). Bioelectric response of a plant stem cell was induced by gradual cooling at a rate of 1.0–1.3 °C min^{-1} from room temperature (20–23 °C) to 3–4 °C (Pyatygin *et al.*, 1992, 1999). However, when the cooling rate is below a certain minimum, the excitable threshold is never achieved. Under natural conditions in a greenhouse, plants will mostly experience very slow cooling rates (as in this work) and hence are likely to respond differently from treatments with sudden cooling.

Due to the potential use of bioelectrical phenomena for indicating the physiological condition of plants in agricultural fields, further investigations to analyze these signals and extract their features using statistical and signal processing methods are still necessary. A combination of different

Table 5 – Grey relation grade (indexed by *R*) between plant electrical signal and environmental factors under cold stress

Plant electrical signal and temperature (R_{01})	Plant electrical signal and relative humidity (R_{03})	Plant electrical signal and light (R_{02})
0.82 ± 0.03	0.70 ± 0.01	0.60 ± 0.01

electrophysiological techniques with modern signal processing methods (e.g. spectral analysis, time–frequency signal analysis or blind signal separation) may lead to determination of the physiological responses that are as yet not fully understood, and to interesting new discoveries.

5. Conclusion

Automatic measurements of the electrical signals in plants can be used effectively in the greenhouse for study of the influence of external stimuli on plants in real time. The results shown also demonstrated the between-days reliability of detection of the electrical signals in plants induced by environmental factors. We also present a grey relation algorithm to be calculated online for determination of the main stimulating factor inducing the plant electrical signals over a period of time, temperature or lighting. The acquisition system developed here can be used to monitor the relationships between the environmental factors and the plant responses, and to measure and analyze the plant physiological signals in the long-term in greenhouse applications.

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