Seed Science Research (2002) **12**, 17–29 DOI: 10.1079/SSR200194

Electrical impedance spectroscopy in relation to seed viability and moisture content in snap bean (*Phaseolus vulgaris* L.)

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Abstract

A method, electrical impedance spectroscopy (EIS), is introduced to study seed viability non-destructively. Snap bean (*Phaseolus vulgaris* L.) seeds were studied by EIS to determine the most sensitive EIS parameter(s) and the optimal range of moisture content (MC) for separation of viable and non-viable seeds. Hydrated seeds exhibited two impedance arcs in the complex plane at the frequency range from 60 Hz to 8 MHz, and impedance spectra of viable and non-viable seeds differed. The hydrated seeds were best-modelled by an equivalent electrical circuit with two distributed circuit elements in series with a resistor (Voigt model). Moisture content and seed viability had strong effects on the EIS parameters. The most sensitive EIS parameters for detecting the differences between viable and non-viable seeds were the capacitance $\log(C_2)$, the resistance R_2, the resistance ratio R_2/R_1 and the apex ratio, which all represent specific features of the impedance spectrum. The highest differentiation in the EIS parameters between the viable and non-viable seeds occurred in partially imbibed seeds between MC of 40 and 45% (fresh weight basis).

Keywords: bioimpedance, electrical impedance spectroscopy, seed ageing, seed quality, seed testing, seed viability

Introduction

Seeds are the propagules of higher plants, and high-quality seeds are essential for successful germination and seedling growth in agriculture and forestry. Seed quality encompasses several attributes of a seed lot, which include seed viability and vigour. Seed quality declines during storage, indicated by reduced germination due to ageing. Standard germination test methods are time consuming and also destructive to the seed sample. Research is needed to develop rapid and non-destructive methods to assess seed quality and to better understand mechanisms associated with viability loss.

Current technology used to predict seed viability prior to the completion of germination is limited. Vital staining methods are destructive and results are subjective (ISTA, 1996). Leakage of electrolytes and other solutes during the early phases of germination can be measured from intact seeds and provides rapid, objective data (AOSA, 1983). However, seeds are generally soaked (submerged) in water, resulting in a hypoxic environment that is injurious to certain species. Moreover, a semi-permeable layer in the seed coat of most species restricts leakage and thus confounds the relationship of leakage with seed quality (Taylor *et al*., 1997). Therefore, new methods that allow insight into cellular integrity and function should provide a biophysical basis for predicting seed quality. The method should be rapid (conducted within a 24-h period) and not be biased by the seed coat or seed-covering tissues. Furthermore, the test should be non-destructive and non-invasive so that tested seeds may be used for other studies or purposes.

Electrical impedance spectroscopy (EIS) has been applied in different fields of plant science. For example, it has been used to study cell membrane injury caused by stress factors such as heat and frost (Zhang and Willison, 1992; Zhang *et al*., 1993; Repo *et al*., 1994, 2000). In addition, EIS was used to study fruit ripening and mechanical treatment injuries (Cox *et al*., 1993; Harker and Maindonald, 1994; Varlan and Sansen, 1996). No comprehensive study of EIS has

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been published on seeds. One time-course study was reported on castor bean (*Ricinus communis* L.) in which changes in characteristic frequencies were recorded during germination (Ackmann and Seitz, 1984).

The objective of this study was to examine the effects of seed viability on EIS parameters, and to determine the most sensitive EIS parameter(s) to detect differences between viable and non-viable seeds. The effect of seed moisture content (MC) was studied as a variable to determine the optimal level for separation between viable and non-viable seeds. *Phaseolus vulgaris* (snap bean) seeds were used as a model system for all experiments.

Theory of electrical impedance spectroscopy

The technical background on the theory of impedance spectroscopy may be found in several papers (Ackmann and Seitz, 1984; Macdonald, 1987; Grimnes and Martinsen, 2000). In electrical impedance spectroscopy (EIS), alternating current (AC) of small amplitude is applied to a sample. This current is applied throughout a range of specified frequency points to produce a spectrum of measurements. Alternating current causes polarization and relaxation in the sample, leading to changes in amplitude and phase of the applied AC signal. These changes are dependent on the tissue properties of the sample. At low frequency, the current passes through the apoplastic space of the tissues where ions are the main current carriers. The cell membranes and other interfacial layers become conductive with increasing frequency, with the apparent current being dependent on the permittivity of the interfacial layers (Pethig and Kell, 1987). Accordingly, symplastic space is conductive at high frequencies. At high frequencies the symplastic and apoplastic resistance form a parallel circuitry. In general, the interfacial layers contribute capacitive properties, while the apoplastic and symplastic space contribute resistive characteristics of the sample (Zhang and Willison, 1991; Repo and Zhang, 1993).

Complex impedance is formed as a ratio of AC voltage and current. Complex impedance consists of real and imaginary parts, which form an impedance spectrum with frequency as the intrinsic variable. Typically, the spectrum of plant tissue is composed of one or two arcs in the complex plane shown on a Wessel diagram (Fig. 1), depending on the sample under study and the range of frequencies used (Ackmann and Seitz, 1984; Stout, 1988; Zhang and Willison, 1992; Repo and Zhang, 1993; Zhang *et al*., 1993; Repo *et al*., 1994; Repo and Pulli, 1996; Ryyppö *et al*., 1998). The data can be modelled with an electrical circuit. The complexity of the model depends on the tissue features. In the simplest case a lumped model may be used with discrete resistors and capacitors. In the case of unisotropic tissues, distributed models are more appropriate in which a reasonable number of unknown parameters may be estimated (Zhang and Willison, 1991; Repo and Zhang, 1993; Repo *et al*., 1994).

The proper model depends on the features of the impedance spectrum and on prior information of the structure of the study object. When two arcs exist with strongly depressed centres, i.e. the centres of the circles of the arcs are below the *x*-axis, the distributed model (Fig. 1) may be used. This distributed model is composed of two distributed elements in series with a resistor and has been termed double-DCE model (Repo *et al*., 1994) or Voigt model (Macdonald, 1987). The resistors (R, R_1 and R_2) of the model are obtained from the interceptions of the circles with the *x*-axis. The relaxation times (τ_1 and τ_2) characterize the location of the dispersion range on the frequency plane, and they are obtained from the apex of the arcs. At the apex, $(2\pi f)\cdot\tau = 1$ where f refers to a characteristic frequency. The coefficients ψ_1 and ψ_2 describe the distribution of the relaxation times τ_1 and τ_2, respectively. The total complex impedance of the model is:

$$Z = R + \frac{R_1}{1+\left(i\cdot\tau_1\cdot\omega\right)^{\psi_1}} + \frac{R_2}{1+\left(i\cdot\tau_2\cdot\omega\right)^{\psi_2}} \qquad (1)$$

where ω = angular velocity = $2\pi f$.

When we define

$$\tau_1 = C_1 R_1^{1/\psi_1} \qquad (2)$$

and

$$\tau_2 = C_2 R_2^{1/\psi_2} \qquad (3)$$

we may solve for 'pseudo-capacitance' values C_1 and C_2 (Jossinet *et al*., 1995; Repo *et al*., 2000).

Material and methods

Seeds and ageing

A single seed lot of 'Labrador' snap bean (*Phaseolus vulgaris* L.) was provided by Asgrow Seed Co., Twin Falls, ID, USA. The lot was obtained in 1997 and was not treated with chemicals or biological treatments. The seed stocks were stored at 5°C and 30% relative humidity, and seed samples were withdrawn as needed. The two seed qualities used in this study were obtained from this same seed lot. Seeds were first equilibrated in a chamber maintained at 70% relative humidity using a solution of glycerol and water at a specific gravity of 1.166 (Forney and Brandl, 1992). The chamber was a custom-designed Plexiglas enclosure (0.029 m^3) containing a circulation fan and made in a way as to maximize the surface area of the solution. Seeds in screen trays were placed

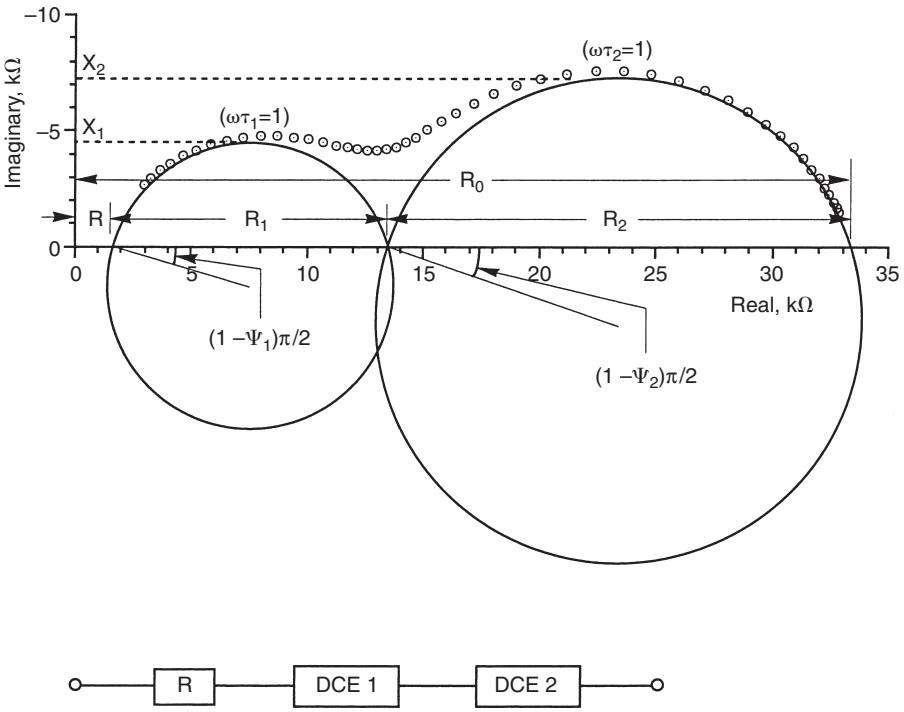

Figure 1. An impedance spectrum of a bean seed (dots in the upper part) shown as a Wessel diagram and the corresponding equivalent electrical model (lower part). The model is formed of two distributed circuit elements (DCE) in series with a resistor (R) (for mathematical expression of the complex impedance of the circuit, see equation (1)). The graph shows the interpretation of the model parameters: R, R_1, R_2 and R_0 are resistances, τ_1 and τ_2 are relaxation times, ψ_1 and ψ_2 are distribution coefficients of the relaxation times respectively. ω (= 2πf) is angular velocity (f is frequency). X_2 and X_1 are the imaginary parts at the apex of the arcs. In the graph frequency increases from right (60 Hz) to left (8 MHz).

on a grill directly over this solution and typically took 1 week to equilibrate to a water activity of 0.7. After equilibration, the 'non-viable' seed quality sample was obtained by ageing under controlled conditions. This sample was heat-sealed in aluminium-foil plastic-laminate packets and aged at 40°C. It was found that 42 days were necessary to reduce the germination count for normal or abnormal seedling to 0%, according to a standard germination test (ISTA, 1996). The sample that was aged for this length of time will be designated as 'non-viable'. The control sample of non-aged seeds will be designated as the 'viable' seed sample.

Adjusting moisture contents of single seeds

The objective was to prepare a range of seed moisture levels from quiescence (storage at 70% relative humidity) to full imbibition. Seven target moisture contents (M_t) were used in the study: 20, 25, 30, 35, 40, 45 and 50% (fresh weight basis). The corresponding dry weight moisture equivalents were calculated (Taylor, 1997) (Table 1).

The method used to increase the MC of individual seeds to a target level employed the following steps:

- The initial percent moisture contents (M_i) of both viable and non-viable samples were determined using a standard oven-drying method in which the moisture contents were determined gravimetrically after drying at 130°C for 1 hour (ISTA, 1996).
- The initial weight of each seed was taken in grams to four decimal places (s_i), and individual seeds were placed in 15 ml glass vials (Wheaton, Millville, NJ, USA).
- The amount of additional water in µl (w_a) needed for each seed to reach M_t was calculated as follows:

$$w_a = \frac{s_i \cdot (M_t - M_i)}{100 - M_t} \cdot 1000 \qquad (4)$$

- Distilled water was added (w_a) to each seed vial. The vials were sealed with Parafilm and then with a plastic cap.
- Seed vials were kept at 25°C for 24 hours to allow complete water uptake and seed moisture equilibration.

Table 1. The target and actual seed moisture contents of seeds expressed on a fresh weight (fw) basis (% g_{H_2O}/g). The dry weight (dw) equivalents (g_{H_2O}/g_{seed}) were calculated from the target fresh weight data. SE indicates standard error

Target		Actual fw (%)			
fw (%)	dw (g_{H_2O}/g_{seed})	Viable	SE	Non-viable	SE
14	0.16	14.4	0.1	13.8	0.1
20	0.25	20.5	0.3	20.0	0.3
25	0.33	25.0	0.2	24.9	0.1
30	0.43	30.2	0.2	30.2	0.1
35	0.54	35.0	0.2	35.1	0.2
40	0.67	40.2	0.2	39.8	0.1
45	0.82	44.8	0.1	44.8	0.2
50	1.00	49.8	0.1	49.1	0.2

- The final weight in grams (s_f) of each seed was measured to calculate and verify the actual final percent moisture content (M_f):

$$M_f = 100 - \frac{s_i}{s_f} \cdot (100 - M_i). \quad (5)$$

Electrical impedance spectroscopy of seeds

The impedance spectra (IS) were measured using an impedance/gain-phase analyser (SI1260, Solartron, Farnborough, Hampshire, UK), two Ag/AgCl electrodes (RC2, WPI Inc., Sarasota, FL, USA), electrode holders and electrode gel (Signagel, Parker Laboratories, Fairfield, NJ, USA) (cf. Repo, 1994) (Fig. 2). The SI1260 analyser was controlled with a Power Mac desk computer using Labview software and a NI-488.2 general-purpose interface bus (GPIB) (National Instruments, Austin, TX, USA). The electrodes were connected with coaxial cables to the Solartron analyser. The electrodes (outer diameter 3 mm and the diameter of the contact surface 2 mm) were set in small tubes (length 15 mm, inner diameter 4 mm) filled with electrode gel. The seed was placed in a longitudinal direction in contact with the gel (Fig. 2).

The real and imaginary parts of impedance were measured between 60 Hz and 8 MHz. The frequency range was divided logarithmically into 60 frequency points. The input voltage level of the sinusoidal signal was 100 mV (rms). Short-circuit correction was made to compensate for the effect of electrode/gel interface and the gel itself in the impedance spectra of seed tissue. The gel/seed contact area (approximately 9 mm^2) at the ends of the seed maintained its position without any other support during the IS measurement. One IS sweep took approximately 50 s.

Viable and non-viable seeds were prepared at eight moisture content levels, and an impedance spectrum was taken on each seed. There were 10 seeds per treatment resulting in 10 replications, since a single seed was the experimental unit. The EIS was non-invasive as performed in this study on whole (intact) seeds. The germinability of viable seeds did not change after either electrode gel contact or an electronic scan (data not shown).

Models

Distributed models were used for all moisture contents; however, due to the change in the spectrum features with moisture content, three variations of this model were used. Seed samples with the lowest moisture content (14%) were modelled with a single DCE where the complex impedance (Z) is:

$$Z = \frac{R_1}{1 + (i \cdot \tau_1 \cdot \omega)^{\psi_1}} \quad (6)$$

It can be seen that equation (6) is the mathematical equivalent of equation (1) when R and R_2 are forced to equal zero. The features of this response, when plotted on a Wessel diagram, were characterized by a single arc where the high-frequency impedance values did not significantly differ from zero. This explicit form of equation (1) was used in a fitting program in order to ensure that its solution would not provide non-zero values for inappropriate model parameters.

The seeds with 20 and 25% MC were modelled by two distributed elements in series (double-DCE):

$$Z = \frac{R_1}{1 + (i \cdot \tau_1 \cdot \omega)^{\psi_1}} + \frac{R_2}{1 + (i \cdot \tau_2 \cdot \omega)^{\psi_2}} \quad (7)$$

This Wessel diagram is characterized by a double arc as equation (1); however, as with equation (6), the high-frequency impedance values also do not significantly differ from zero. Seeds with 30% MC and greater were modelled by the Voigt model (Fig. 1) with total impedance as shown by equation (1).

Data analysis

The equivalent circuit parameters were estimated using a complex non-linear least squares (CNLS)

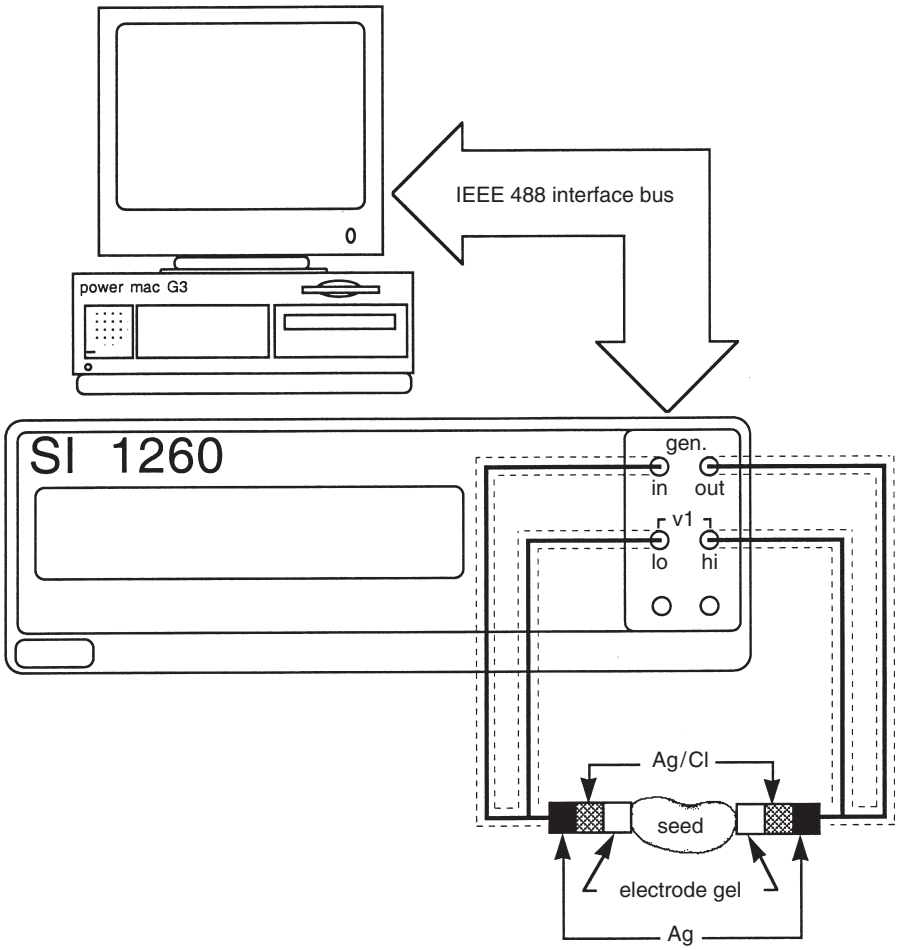

Figure 2. Custom-designed apparatus to take the impedance spectra of bean seeds.

curve-fitting program, LEVM v. 7.0 (provided by James Ross Macdonald and Solartron Instruments Ltd) and has been further developed and incorporated into a program that automates the instrument control and measurement, which expedites data collection and analysis for large numbers of samples from a single Power Mac platform. In the CNLS analysis of the 14% MC samples, the IS data of the frequency range from 1.8 kHz to 8 MHz (43 frequency points) were used. Data from 60 Hz to 8 MHz (60 frequency points) were used for samples with 20 to 35% MC. For samples with 40% MC and greater, data from 443 Hz to 8 MHz (50 frequency points) were used.

Original parameter estimates, i.e. R, R_1, R_2, τ_1, τ_2, ψ_1 and ψ_2, were obtained directly from the CNLS fit of the models. Additional parameters, R_0, R_2/R_1 ratio [log-transformed C_2 from equation (3) (i.e. $C_2 = \log(\tau_2/R_2^{1/\psi})$] and apex ratio ($X_2/X_1$), were calculated using the original parameters. The extracellular resistance (R_0) (corresponding to direct current resistance) was obtained in the case of the single-DCE model as $R_0 = R_1$, in the case of double-DCE model as $R_0 = R_1 + R_2$, and in the case of Voigt model as $R_0 = R + R_1 + R_2$ (Repo *et al.*, 1994, 2000). The apex ratio (X_2/X_1) was obtained as the ratio of the imaginary values at each apex of the IS of the low and high frequency arc (X_2 and X_1, respectively). With no literature base for EIS values of seeds, specific resistances (r_0, r_1, r_2, r) and dielectric constants (ε_{r1} and ε_{r2}) were calculated. These values and details on how these parameters were calculated are presented in the Appendix and Table 3. These parameters take into account the size of the measurement object.

The significance of the difference between treatment groups (seed quality and MC levels) was tested by a univariate general linear model (GLM) procedure (SPSS 9.0 for Windows, SPSS Inc., Chicago, IL, USA) for each of the 19 EIS parameters. For the statistical analyses, log-transformed data of original and derived parameters were used, except for ψ_1, ψ_2, R_2/R_1 and apex ratio, where the original data were

used. The data for seeds with the final MC more than 1.5% from the target MC level were omitted from the statistical analysis.

Nineteen EIS parameters (seven estimated according to the model and 12 derived) were evaluated at MC levels between 25 and 50% to determine the optimal MC range for which a specific parameter was most sensitive to the differences between viable and non-viable seeds. Principally, the higher the changes in the value of a parameter in respect to the control, the higher its sensitivity. A 'sensitivity factor' was calculated using the means of logarithms of parameter values for each treatment group. For the *i*th parameter, P_i, the absolute value of the difference of means of logarithms of non-viable ($p_{i2} = \overline{\log(P_{i,aged})}$) and viable ($p_{i1} = \overline{\log(P_{i,non\text{-}aged})}$) samples was calculated at each MC level:

$$|\Delta p_i| = |p_{i2} - p_{i1}| = \left|\overline{\log\left(P_{i,aged}\right)} - \overline{\log\left(P_{i,non-aged}\right)}\right| \quad (8)$$

where $|\Delta p_i|$ is the sensitivity factor of the parameter P_i. Standard deviation for the parameters with the highest values of $|\Delta p_i|$ was calculated as:

$$\mathrm{SD} = \pm\sqrt{\mathrm{var}\left(p_{i1}\right) - 2\,\mathrm{cov}\left(p_{i1}, p_{i2}\right) + \mathrm{var}\left(p_{i2}\right)} \quad (9)$$

where var(p_{i1}) is the variance of the log-transformed values for the parameter of viable samples at a given MC level, var(p_{i2}) is the variance of the log-transformed values for the parameter of non-viable samples at a given MC level, and cov(p_{i1},p_{i2}) is the covariance between p_{i1} and p_{i2}.

Results

Seed viability and obtaining target moisture contents

The standard germination of the viable and non-viable samples was 95 and 0%, respectively. The initial seed moisture contents (MC) after 70% relative humidity equilibration was 14% (Table 1). The target moisture contents were obtained with good reliability. Occasional deviations of more than 1.5% from the target were attributed to pipetting errors. The mean MC of retained seeds did not deviate more than 0.5% from the target, and the standard error ranged from 0.07 to 0.34% for the seven target moisture contents for viable and non-viable samples.

Impedance spectra

The magnitude of the real and imaginary part of impedance from seeds at 14% MC was high and exceeded the measurement range of the instrument at low frequencies. Thus, the lowest frequency measurements were deleted from the CNLS analysis. The remaining spectrum revealed one arc both in viable and non-viable seeds (Fig. 3A).

Two arcs were obtained in the IS with ≥ 20% MC (Fig. 3B). With increasing MC, the magnitude of real and imaginary parts decreased, and the differences between viable and non-viable seeds became more apparent. Characteristically, the apex frequencies moved to higher frequencies with increasing MC, and the proportion of the low- and high-frequency arc changed with increasing MC and loss of viability (Fig. 3A–H).

Effect of moisture content on EIS parameters

Moisture content had a strong effect on several EIS parameters of both viable and non-viable seeds. All resistance parameters decreased by several decades with increasing MC from 14 to 50% (Fig. 4; Table 2). The resistance values tended to stabilize at the highest MCs, even though the effect of MC was not absent (Fig. 4A, B; Table 2). The R_2/R_1 ratio of the viable samples increased significantly from 25 to 50% MC, whereas in non-viable seeds no significant change was found (Fig. 4C). Both relaxation times decreased significantly with MC (Table 2). The relaxation time, τ_2, was typically 100–200 times higher than τ_1. The distribution coefficients remained fairly constant with an increase in MC (Table 2). From 25 to 45% MC the coefficient ψ_1 was smaller than ψ_2, indicating that the high-frequency arc had a more depressed centre than the low-frequency arc. The apex-ratio of viable seeds increased, while the apex-ratio of non-viable seeds decreased with increasing MC (Fig. 4D). For the parameter log(C_2), the corresponding trends were opposite (Fig. 4E). There was no significant difference in the parameter C_2 or log(C_2) of either viable or non-viable seeds between MC levels from 30 to 50%.

Effect of viability on EIS parameters

Several EIS parameters differed significantly between viable and non-viable seeds. The most significant differences were measured in the MC range from 35 to 50% (Fig. 4; Table 2). In that MC range, consistently significant differences between viable and non-viable seeds appeared in the resistance parameters R_2, R_0, R_2/R_1 ratio, in the apex ratio and in the parameter log(C_2) (Fig. 4). All the resistance parameters and the apex ratio decreased with cellular decay, whereas the log(C_2) increased in non-viable seeds. Therefore, at a particular MC it may be possible to differentiate viable and non-viable seeds non-destructively by EIS.

According to the sensitivity factor (Δp, equation (8)), i.e. the difference between the means of log-

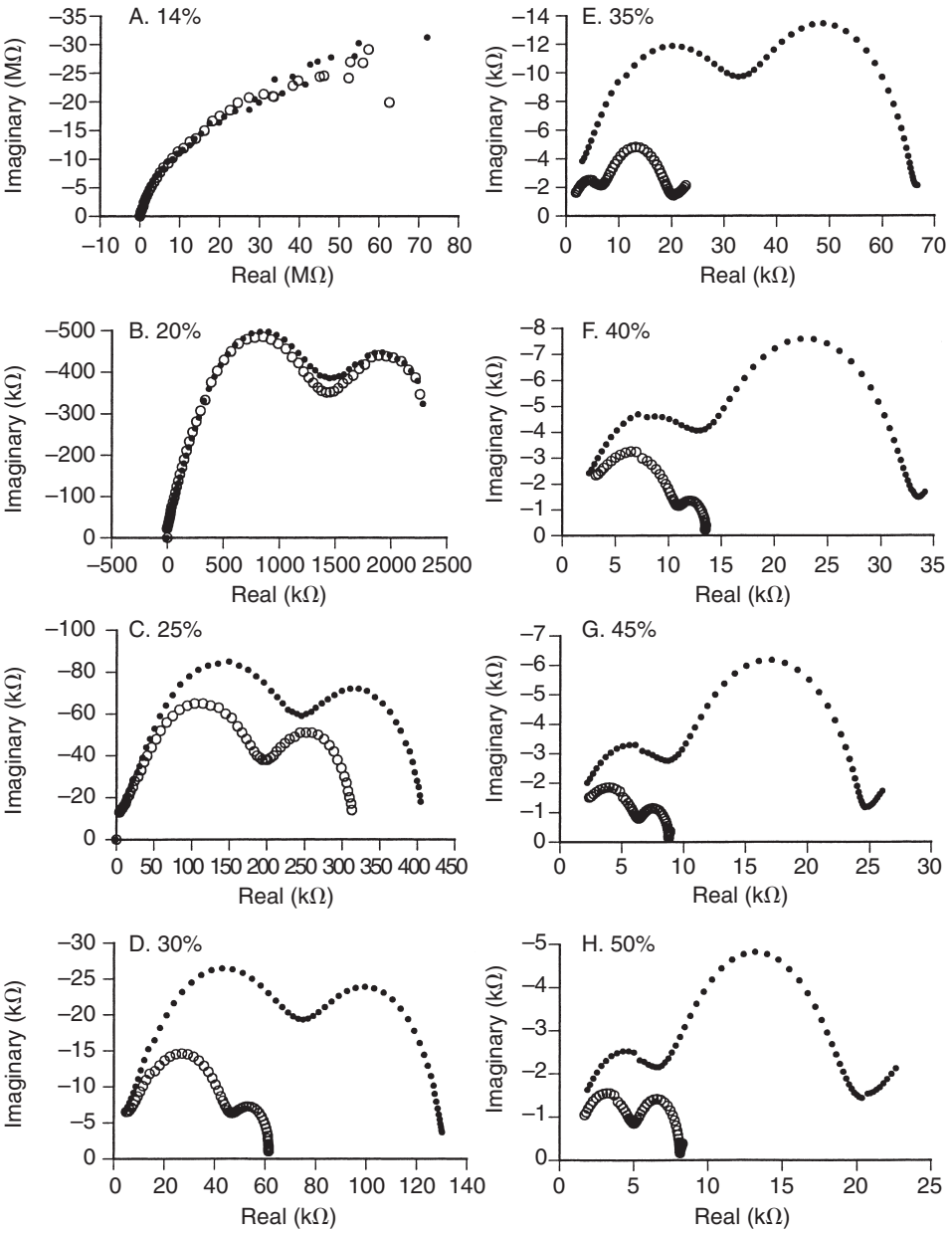

Figure 3. Mean electrical impedance spectra of viable and non-viable bean seeds at different moisture levels (fresh weight basis): 14% MC (A), 20% MC (B), 25% MC (C), 30% MC (D), 35% MC (E), 40% MC (F), 45% MC (G) and 50% MC (H). Closed symbols refer to viable seeds and open symbols to non-viable seeds. Frequency increases from right (60 Hz) to left (8 MHz).

transformed numbers, the parameters C_2, R_2, R_2/R_1 ratio and apex ratio were the most responsive at different MC levels to the effect of viability (Fig. 5). The greatest differences were typically from 40 to 45% MC (seeds partially, but not fully imbibed). Collectively, this range would be the most sensitive to detect the difference between viable and non-viable seeds. In addition, when these parameters were compared with each other, Δp-numbers and their standard deviations indicate that C_2 was the most sensitive.

Discussion

The results of this study indicate that seed viability and moisture content have a strong effect on the impedance spectra and corresponding EIS parameters

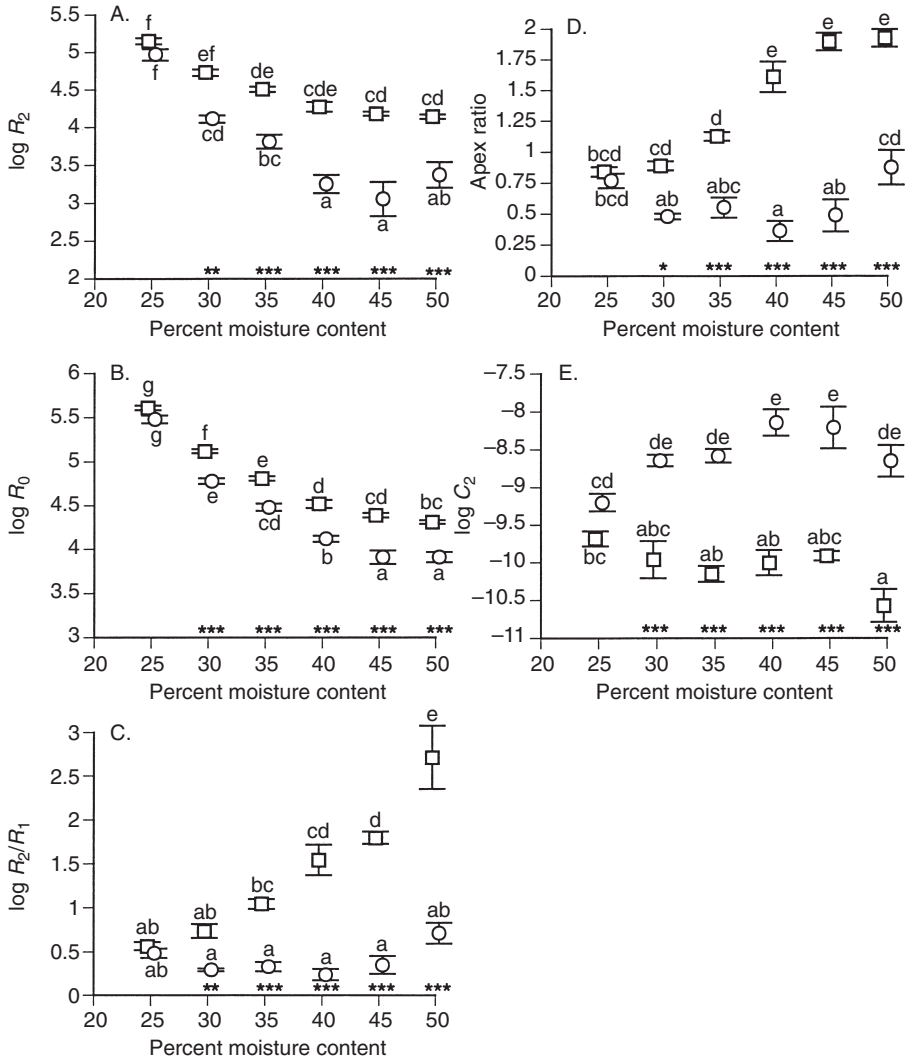

Figure 4. Relation of electrical impedance parameters of viable (□) and non-viable (○) seeds in respect to moisture level (MC). The selected parameters have the highest sensitivity to seed quality. The mean and standard error are indicated for log(R_2) (A), log(R_0) (B), R_2/R_1 ratio (C), apex ratio (D) and log(C_2) (E). Differences between treatment groups for each parameter are indicated by letters: different letters indicate significant differences at the probability level of $P < 0.05$ (Tukey test). A significant difference between viable and non-viable seeds is indicated by asterisks: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

of snap bean seeds. Furthermore, there seems to be an optimum range of moisture contents at which differences between viable and non-viable seeds can be detected. Accordingly, it may be possible to detect seed deterioration by EIS. Thus, the study represents a new field of application of EIS in the plant sciences.

Previous EIS studies of seeds are limited to one published study, which dealt with castor bean seeds during germination (Ackmann and Seitz, 1984). According to that study, seeds had one impedance arc in the complex plane instead of two. Alterations of impedance spectra were attributed to structural and biochemical changes within the endosperm cells. The characteristic frequency of castor bean seeds decreased with germination time, i.e. the apex of the arc moved towards lower frequencies. This is probably connected with an increase in seed moisture content in accordance with the results of this study (see τ_1 and τ_2 in Table 2).

Based on the results of our study, IS of hydrated *Phaseolus vulgaris* seeds had two arcs. This indicates that there are two major dispersion ranges (two relaxation times) in seeds, i.e. there may be two different cellular structures with different electrical

Table 2. The EIS parameters (mean and standard deviation) for viable and non-viable bean seeds with different moisture contents (MC) as estimated according to the distributed models (equations (6), (7) and (8)). R and R_1 are resistances. ψ_1 and ψ_2 are the distribution coefficients of the relaxation times τ_1 and τ_2, respectively. The values of parameters R_2 and R_0 are shown in Fig. 4.

		Moisture content (%)							
Parameter	Quality	14	20	25	30	35	40	45	50
R (kΩ)	Viable				2.4 ± 0.7 d	1.5 ± 0.3 bcd	1.1 ± 0.3 abc	0.9 ± 0.2 a	0.9 ± 0.2 ab
	Non-viable				1.9 ± 0.8 cd	1.4 ± 0.5 abc	1.2 ± 0.4 abc	0.8 ± 0.4 ab	1.0 ± 0.1 ab
R_1 (kΩ)	Viable	50000 ± 20000 i	1300 ± 600 h	264 ± 36 g	76 ± 8 f	31 ± 4 de	13.0 ± 2.2 c	8.5 ± 1.2 b	5.5 ± 1.0 a
	Non-viable	60000 ± 10000 i	1400 ± 500 h	212 ± 49 g	47 ± 11 e **	22 ± 5 d	9.9 ± 1.6 bc	5.8 ± 1.9 a *	4.3 ± 1.0 a
τ_2 (μs)	Viable			194 ± 41 e	62 ± 10 d	29 ± 7 c	17 ± 5 b	12 ± 2 ab	11 ± 4 a
	Non-viable			246 ± 59 e	76 ± 20 d	32 ± 7 c	25 ± 7 c *	14 ± 6 ab	11 ± 3 a
τ_1 (μs)	Viable	160 ± 60 i	11 ± 3 h	2.4 ± 0.6 g	0.8 ± 0.2 f	0.4 ± 0.1 e	0.16 ± 0.05 cd	0.10 ± 0.02 bc	0.08 ± 0.01 ab
	Non-viable	190 ± 60 i	8.7 ± 3.0 h	1.6 ± 0.4 g	0.4 ± 0.1 e ***	0.2 ± 0.1 d*	0.10 ± 0.04 bc	0.06 ± 0.03 a **	0.09 ± 0.02 ab
ψ_2	Viable			0.87 ± 0.03 bcd	0.83 ± 0.08 bc	0.81 ± 0.04 ab	0.82 ± 0.06 b	0.84 ± 0.02 bc	0.75 ± 0.08 a
	Non-viable			0.89 ± 0.04 cd	0.91 ± 0.04 d *	0.94 ± 0.05 d ***	0.92 ± 0.03 d ***	0.92 ± 0.02 d *	0.91 ± 0.01 d ***
ψ_1	Viable	0.74 ± 0.02 abcd	0.77 ± 0.08 de	0.69 ± 0.01 ab	0.74 ± 0.01 bcde	0.76 ± 0.01 cde	0.76 ± 0.05 bcde	0.77 ± 0.02 de	0.85 ± 0.05 f
	Non-viable	0.73 ± 0.03 abcd	0.72 ± 0.07 abcd	0.68 ± 0.02 a	0.70 ± 0.02 abc	0.71 ± 0.03 abcd	0.73 ± 0.04 abcd	0.74 ± 0.07 abcd	0.80 ± 0.01 ef

Statistical significance of differences between treatment groups is indicated by letters: the same letter(s) within a parameter indicates that the values are not significantly different by Tukey test ($P < 0.05$). A significant difference between viable and non-viable seeds at each MC level is indicated by asterisks: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

properties. The frequency range used in the study (60 Hz to 8 MHz) included these two dispersion ranges; thus, the parameters of the model could be accurately estimated. The only exceptions occurred with samples at 20% MC, where measurements at frequencies lower than 60 Hz would have been required for an accurate estimation of the parameters. The proportion of the two arcs and corresponding EIS parameters were strongly affected not only by MC but also viability, as both of these factors affect the cellular structure and function.

The most sensitive EIS parameters for differentiation of viable and non-viable seeds were $\log(C_2)$, R_2, R_2/R_1 ratio and the apex ratio. The first two are due to the low-frequency arc, whereas both the low- and high-frequency arcs affect the latter two. The exact interpretation of EIS parameters in relation to cellular function is not known, but we may assume that the parameters of the low-frequency arc (low-frequency dispersion) are due to cell walls. The second dispersion, i.e. the high-frequency arc, would be due to cell membranes, in particular the plasmalemma. Ageing may be directed primarily to cell membrane structure and function, but possibly also to some extent to cell walls or other constituents. As a consequence of membrane damage, intracellular ions leak out of the cells to the apoplastic space (cf. cellular injuries by frost, Palta and Weiss, 1993; Repo *et al*., 1994; Ryyppö *et al*., 1998), causing decreases of the resistance R_2 and R_0.

The physical equipment, sample fixture and techniques used to interface seed tissues were important in obtaining meaningful and reproducible data. The interfaces encountered by the alternating electric field were the electrode/gel interface (twice), gel, and gel/seed interface (twice). The seed is placed longitudinally (Fig. 2), and the current must pass the seed coat (twice), embryonic axis and two parallel cotyledons (cf. Taylor, 1997 for seed morphology). Short-circuit correction and subtraction compensated for the effect of the electrode/gel interface and the gel itself. The contact area at the gel/seed interface was kept approximately constant (9 mm^2) between seed-to-seed measurements, to eliminate variability. The effect of the seed coat in hydrated seeds was negligible on the IS and the corresponding EIS parameters (unpublished data). The embryonic axis probably had a minor effect on the IS in the whole

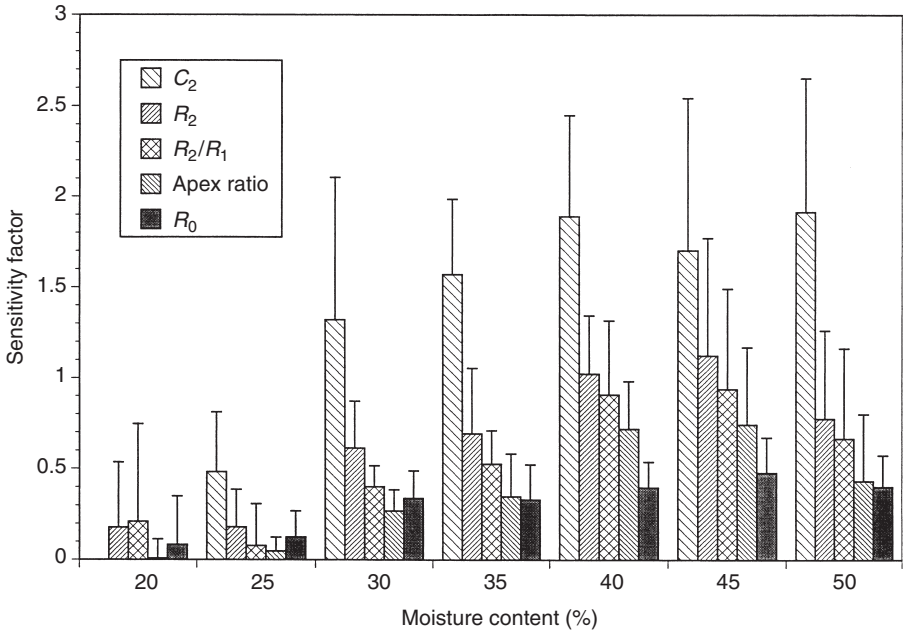

Figure 5. The sensitivity factor (Δp, see equation (8)) of selected parameters at different moisture levels. Bars indicate standard deviations.

seed measurements, since the embryonic axis is a small percentage of the total seed volume. Moreover, the axis is located on the ventral lobe of half of the seed. Collectively, most of the current passes through the cotyledons and is probably the major component affecting the EIS of bean seeds.

Several EIS parameters (especially resistances and relaxation times) of bean seeds changed with an increase in MC. The change was greatest up to 35% MC. The decisive role of MC on the impedance parameters of plant tissues has been found in earlier studies (Glerum, 1980; Pukacki, 1982; Kučera, 1986; Repo *et al*., 2000). In woody plants, the low-frequency resistance was strongly affected when the moisture content fell below the fibre saturation point, at approximately 33% MC (Tattar *et al*., 1972; Pukacki, 1982), which is in accordance with our results.

Changes in EIS with increasing seed MC may be related to ultrastructural and other changes that occur during rehydration of seeds due to the different types of water binding. In review, water covers hydrophobic sites of molecules in seeds at water potentials from –11 MPa to –3 MPa [75–97% relative humidity, i.e. Hydration III or Region III (see Vertucci and Farrant, 1995)]. A concentrated solution is achieved at Hydration IV from –3 MPa to –1.5 MPa, while a dilute solution is obtained at Hydration V > –1.5 MPa (Vertucci and Farrant, 1995). Many ultrastructural changes occur at Hydration III (Vertucci and Farrant, 1995), which might explain the transition from a single to a double arc that was observed from 14% MC to 20% MC in this study (Fig. 3A, B). Hydrolysis of storage reserves takes place in the Hydration IV, resulting in osmotically active solutes, while vacuolation and acidification occur in the embryonic axis just prior to visible germination (Obroucheva and Antipova, 1997). Moreover, water acts as a solvent for electrolytes and other constituents conducting electricity. Collectively, an increase in seed water concentration accounts for an overall decrease in impedance, while shifts in the spectra appear to coincide with ultrastructural and biochemical changes as a consequence of hydration level.

Several EIS parameters changed with the loss of seed viability. Seed ageing is accompanied by a loss of membrane integrity (Priestley, 1986), and therefore is similar to cellular injuries caused by frost or heat. Withdrawal of the plasmalemma from the cell wall, and physical breaks in the plasmalemma, leading to an accumulation of materials in the extraprotoplasmic space, are consequences of seed ageing (Smith and Berjak, 1995). Deterioration takes place in the embryonic axis, which is the primary tissue in regard to viability, but also in cotyledons. The physiological ageing in the latter tissue is the most essential for interpretation of the whole-seed EIS results obtained with the current technique. We may conclude that the variability in EIS parameters among non-viable seeds was due to the variability in cotyledon tissue viability rather than in assessment errors.

Comparing the parameters according to their sensitivity to viability, the pseudo-capacitance C_2 and

its derivative log(C_2) have the highest magnitude of change between viable and non-viable seeds. However, the variability of C_2 was typically higher than that of the other parameters that were sensitive to viability. The C_2 is an integrated number of three estimated parameters, i.e. τ_2, R_2 and ψ_2. Data showed that C_2 was especially sensitive to ψ_2, which is in the exponent of R_2. Thus, accurate CNLS estimation of the parameters corresponding to the low-frequency arc is crucial for determination of C_2.

There was some variability in the seed size of the commercial lot used in this study (data not shown). Preliminary investigations revealed that seed size (variation in length and width) had little influence on EIS parameters (unpublished data). According to the theory of electrical conduction, the resistance increases with increasing length of the sample and decreases with increasing cross-sectional area. In the case of bean seeds, the length and the cross-sectional area have a strong linear correlation. Thus, the effect of these geometrical factors on the EIS parameters may have been compensated, i.e. the longer seed has a greater circumference. Therefore, data were not normalized with respect to the seed size. However, for comparison of seeds of different species or genotypes, the specific numbers of resistances and dielectric coefficients presented in this paper may be important.

In conclusion, the EIS offers several advantages in comparison with conventional or physiological methods of assessing seed viability. The method generates a complete spectrum providing objective data to calculate a number of useful parameters. The technique is rapid and the actual measurement

Table 3. Specific resistances (r, r_2, r_1, r_0), capacitances (C_1, C_2) and dielectric coefficients (ϵ_{r1}, ϵ_{r2}) for viable and non-viable bean seeds with different moisture contents. The mean and standard deviation are indicated

		Moisture content (%)							
Parameter	Quality	14	20	25	30	35	40	45	50
r (Ωm)	Viable				9.3 ± 2.6 c	5.7 ± 1.4 bc	4.3 ± 1.2 ab	3.3 ± 0.8 a	3.6 ± 0.7 ab
	Non-viable				6.6 ± 2.7 bc	5.3 ± 1.9 ab	4.4 ± 1.4 ab	3.2 ± 1.4 ab	3.9 ± 0.6 ab
r_2 (Ωm)	Viable			560 ± 190 g	220 ± 70 efg	130 ± 40 def	80 ± 31 de	59 ± 12 cd	55 ± 17 cd
	Non-viable			380 ± 200 fg	48 ± 14 cd **	29 ± 20 bc ***	9.5 ± 9.6 a ***	9.4 ± 10.5 a ***	14 ± 8 ab ***
r_1 (Ωm)	Viable	160k ± 65k i	4.8k ± 2.1k h	990 ± 130 g	300 ± 30 f	120 ± 20 e	51 ± 12 c	33 ± 4 b	21 ± 3 a
	Non-viable	190k ± 40k i	4.9k ± 1.8k h	750 ± 180 g	160 ± 30 e ***	82 ± 22 d *	36 ± 6 bc	22 ± 7 a **	17 ± 5 a
r_0 (Ωm)	Viable	160k ± 65k i	12k ± 3k h	1.6k ± 0.3k g	520 ± 90 f	250 ± 60 e	140 ± 40 cd	95 ± 15 c	80 ± 16 bc
	Non-viable	190k ± 40k i	11k ± 7k h	1.1k ± 0.4k g	220 ± 50 de ***	120 ± 40 c ***	50 ± 15 ab ***	34 ± 18 a ***	35 ± 13 a ***
C_1 (pF)	Viable	0.006 ± 0.003 ab	0.8 ± 1.6 efgh	0.04 ± 0.01 cdef	0.21 ± 0.06 ghi	0.4 ± 0.1 hi	0.8 ± 0.5 ij	0.9 ± 0.3 ijk	3.9 ± 4.0 k
	Non-viable	0.005 ± 0.003 a	0.1 ± 0.2 bcde	0.02 ± 0.01 bcd	0.10 ± 0.05 cdefg	0.2 ± 0.1 fgh	0.5 ± 0.4 ghi	0.7 ± 0.5 ghi	2.5 ± 0.4 jk
C_2 (nF)	Viable			0.3 ± 0.1 bc	0.2 ± 0.1 abc	0.09 ± 0.07 ab	0.2 ± 0.1 ab	0.13 ± 0.06 abc	0.05 ± 0.04 a
	Non-viable			0.9 ± 0.7 cd	2.6 ± 1.7 de ***	3.1 ± 1.8 de ***	12 ± 11 e ***	24 ± 35 e ***	9.1 ± 22 de ***
ϵ_{r1}	Viable	0.2 ± 0.1 ab	21 ± 41 de	1.1 ± 0.3 cd	6.1 ± 1.8 efg	13 ± 2 efg	21 ± 16 fgh	26 ± 10 ghi	110 ± 101 i
	Non-viable	0.2 ± 0.1 a	2.9 ± 4.5 bcd	0.7 ± 0.2 bc	3.1 ± 1.6 cde	5.7 ± 3.0 ef	14 ± 12 efg	20 ± 13 efg	70 ± 10 hi
ϵ_{r2}	Viable			7.6k ± 4.2k bc	5.5k ± 3.3k ab	2.7k ± 2.0k ab	4.4k ± 4.6k ab	3.9k ± 1.7k abc	1.5k ± 1.2k a
	Non-viable			27k ± 21k cd	85k ± 54k de ***	96k ± 63k de ***	380k ± 330k e ***	710k ± 1020k e ***	290k ± 720k de ***

Statistical significance of differences between treatment groups is indicated by letters: the same letter(s) within a parameter indicates that the values are not significantly different by Tukey test ($P < 0.05$). A significant difference between viable and non-viable seeds at each MC level is indicated by asterisks: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. The abbreviation k refers to 10^3.

requires only minutes to complete. The entire procedure of preparing seeds and performing the EIS analysis can be conducted in less than 24 hours. Furthermore, biochemical techniques using staining procedures, complicated extraction protocols and the use of potentially harmful reagents are not required. The results show that the moisture content has a strong effect on EIS parameters of seeds, and that the electrical properties of seeds change upon loss of seed viability. These findings provide the basis for the application of EIS in seed-quality testing. Further studies are needed to compare the most sensitive EIS parameters with gradual loss of seed quality.

Appendix: Calculation of specific resistances and dielectric coefficients

If we assume the transversal cross-section of a seed elliptic with an area of A and the length of a seed l, for a specific resistance:

$$r_x = R_x \cdot \frac{A}{l} \quad \text{(A1)}$$

where R_x refers to an estimated resistance and r_x, to a normalized resistance. Cross-sectional area, A, of the seed was calculated according to minor (a) and major (b) diameters as:

$$A = \frac{\pi \cdot a \cdot b}{2} \quad \text{(A2)}$$

Dielectric coefficient (ϵ_{rx}) was calculated according to the estimated capacitance (C_x) as:

$$\epsilon_{rx} = \frac{C_x \cdot l}{\epsilon_0 \cdot A} \quad \text{(A3)}$$

where ϵ_0 = constant = 8.85×10^{-12} F m^{-1}. Two dielectric coefficients, ϵ_{r1} and ϵ_{r2}, were calculated using the two pseudocapacitances, C_1 and C_2, respectively, using equations (2) and (3) in the text.

The values of these parameters are given in Table 3.

Acknowledgement

The authors would like to thank Dr P. Hopman for his help with instrumentation in preliminary exploratory studies, Dr R. Schmuckler for his expertise on technical aspects of the impedance spectroscopy hardware, and Dr J. Barnard and Mr J. Heinonen for advice on statistical analysis of data. This study was supported by a grant from the American Seed Research Foundation, and from Hatch and Multi-State projects. Cornell University and the USDA-ARS provided equipment funding.

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Received 20 August 2001
accepted after revision 8 November 2001

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