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Single seed viability checked by delayed luminescence

Evelina Costanzo · Marisa Gulino · Luca Lanzanò · Francesco Musumeci · Agata Scordino · Salvatore Tudisco · Li Sui

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Abstract Time resolved spectral components of delayed luminescence (DL) from single dry soybean seeds were measured using a device with single photon sensitivity. The seeds were aged by a thermal treatment to change their viability. A correlation was observed between the seeds viability and some DL parameters, i.e. the total number of photons emitted and the relative decay probability of excited states. This relevant result confirms the close connection between the state of biological systems and their DL, and it can allow the development of a quick selection technique for single dry seeds, a goal impossible up today.

Keywords Laser induced luminescence detection · Aging effects · Seed quality determination · Non invasive diagnostic

E. Costanzo
Dipartimento di Fisica e Astronomia,
University of Catania, Catania, Italy

E. Costanzo
I.N.F.N. Sezione di Catania, Catania, Italy

M. Gulino (✉) · L. Lanzanò · F. Musumeci · A. Scordino · S. Tudisco
Dipartimento di Metodologie Fisiche e Chimiche per l'Ingegneria, University of Catania, Catania, Italy
e-mail: gulino@lns.infn.it

M. Gulino · L. Lanzanò · F. Musumeci · A. Scordino · S. Tudisco · L. Sui
Laboratori Nazionali del Sud, I.N.F.N,
Catania, Italy

L. Sui
Department of Nuclear Physics,
China Institute of Atomic Energy,
Beijing, China

Introduction

In the last few years, thanks to the increased capability to measure very weak light signals (Ntziachristos et al. 2005; Fujimoto et al. 2000; Watanabe et al. 2007), several non-invasive techniques to explore the state of biological systems have been developed. Indeed photons can be regarded as the information carriers of matter due to their interactions at atomic and molecular level, so giving information about the chemical components and the complex structure of such systems, even if in an encoded way.

Even in agricultural sciences several attempts to use light to extract information from biological systems have been made. In particular, with special reference to the argument of this paper, the viability of several kinds of seeds (Veselova et al. 1985; Lanteri et al. 1998; Yan et al. 2003) has been successfully correlated to the DL.

The DL is present in both solid state and biological systems. In the last years several papers have demonstrated that it is a sensitive indicator of the physiological state of biological systems, opening new application perspectives in medicine, environmental pollution and food quality control (Scordino et al. 1996; Chung et al. 2004; Chen et al. 2005a, b; Musumeci et al. 2005; Eschrigl et al. 2007).

The crucial problem of the DL measurements of small systems, including single seed, is that the signal is often too low to be measured with current equipments. For this reason, up to now, the measurements were performed on samples of tenths up to hundreds seeds, giving only statistical information on the total number of emitted photons, without any spectral or time characterisation.

Nevertheless the possibility to evaluate the vegetative performances of a single seed appears of great interest, because it could allow the development of a fast, cheap and non-invasive selection technique. To demonstrate such

possibility a new measurement set-up has been developed to detect the DL of a single seed. This paper shows the obtained results on artificially aged Soybean seeds and suggests further possible improvements.

Materials and methods

Soybean seeds (*Glycine max*) have been selected in order to have about similar size (5.0 ± 0.1 × 3.0 ± 0.1 mm). Their average weight was 70 ± 6 mg. Their germination percentage was about 98% and they grow, in the first six days after the germination, at an average speed of 1.2 ± 0.2 mm/h. To change these performances six groups, each of 14 seeds, have been artificially aged by a thermal treatment in a stove at temperature of 75 ± 2°C and a relative humidity less than 10%. The six groups were treated for 2, 3, 18, 27, 48 and 72 h, respectively. A seventh group was used as native (untreated) sample.

After the thermal treatment the final weight and the DL of each seed were measured. The seeds were then placed inside a constant climate chamber (temperature 25.0 ± 0.5°C, relative humidity 60 ± 5%) to measure their vegetative performances. As shown in Fig. 1a each seed has been placed within a single cell of alveolar plates, closed on the bottom with filter paper, and dipped into distilled water. For six days, every 24 h, the plates were photographed and the length of every seedling was calculated from the photo. The viability has been quantified by the parameter:

$$G = L/L_{AV} \tag{1}$$

where L is the average length of a single seedling in the six days and L_{AV} is the average value of L calculated for the group of native seedlings. The G parameter relative standard deviation for the group of native seedlings was 14%.

The DL has been measured using the set-up shown in Fig. 1b (Tudisco et al. 2003). The excitation source was a pulsed Nitrogen laser (λ = 337 nm, pulse width 5 ns, pulse energy 0.1 mJ). A bifurcated fibre bundle (Lot-Oriel LLB321) was used to send the laser pulse to a single seed putted in a dark holder and to collect the emitted light (see Fig. 1b). Spectral components of DL were obtained by using optical broadband (80 nm FH) Lot-Oriel filters and detected by a single-photon count photomultiplier (PMT) (Hamamatsu R-7206-1, spectral response 300–850 nm) cooled down to –20°C. An electronic gate (Tudisco et al. 2003) enables the PMT to start the photon counting few microseconds after the end of the laser pulse.

Results and discussion

The measured emission spectra of untreated Soybean seeds shows a quite broad spectrum having two different regions:

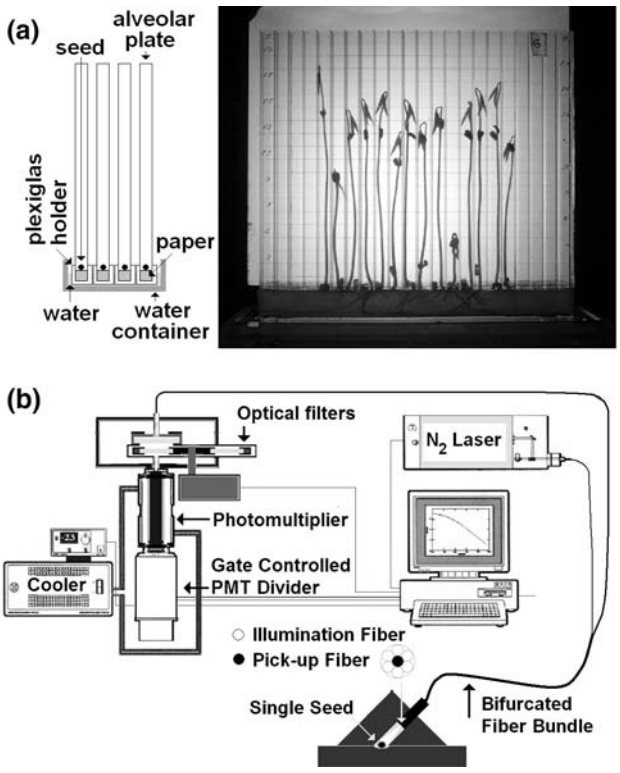

Fig. 1 **a** Photography of the polycarbonate alveolar plate in which the seeds have been grown and picture of the plates arrangement inside the thermostatic chamber. **b** Synthetic scheme of the DL measurement system

a weak local maximum at around 600 nm and a further increase up to 800 nm. At longer wavelengths, due to the low quantum efficiency of the used PMT, no significant results can be obtained.

Differently from other vegetable systems (Scordino et al. 2000) in our case the time trend of the DL spectral components depends on the wavelength λ. In particular there are two different decay slopes in the two regions of the emission spectra at $\lambda < 600$ and $\lambda > 600$ nm, respectively. For this reason only two spectral components characterizing the two region (460 and 645 nm) have been measured for all the seeds.

The Fig. 2 reports the behaviour of the two spectral components for two different seeds, one untreated (black markers) and one treated for 72 h (open markers). In order to avoid overlaps the values of the treated seed have been multiplied by ten. The differences in the DL intensities and in time trends could be used to discriminate between the different vegetative performances.

The time trends of the shown experimental data can be well described by the sum of two power laws (Frauenfelder et al. 1999):

$$I(t) = I_1/(1 + t/t_{0_1})^{m_1} + I_2/(1 + t/t_{0_2})^{m_2} \tag{2}$$

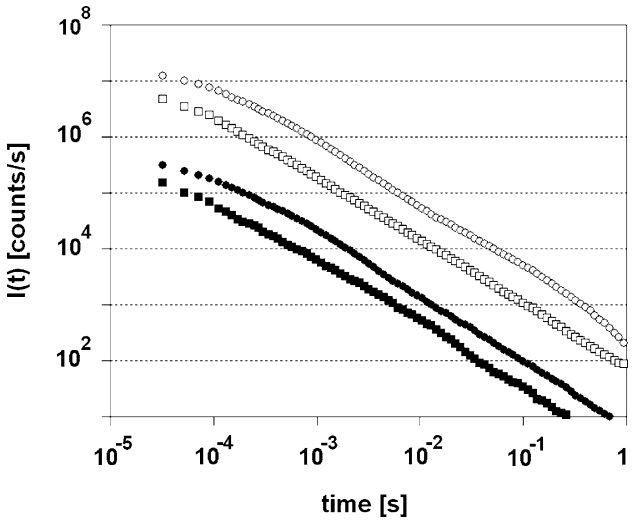

Fig. 2 Behaviour of the two DL spectral components for two different seeds: untreated seed at emission wavelength 460 nm (*filled circle*) and 645 nm (*filled square*), thermal treated for 72 h seed at emission wavelength 460 nm (*open circle*) and 645 nm (*open square*). In order to avoid overlaps the values related to the treated seed have been multiplied by ten

Such approximation is consistent with the existence of a distribution of relaxation kinetics (Palazzo et al. 2002). In fact DL has been described as the photo-induced emission from collective excited states associated with the ordered structures inside the biological systems (Brizhik et al. 2001). In other words, as concerns the DL, biological systems behave as solid-state dielectric systems (Scordino et al. 2000).

In this frame the parameters I_i, t_{0i} and m_i in Eq. (2) do not have any simple physical meaning. Alternatively, the decay process can be characterised by the total number N of excited levels which decay radiatively and the probability $p(t)$ that Δn levels of the n excited ones decay radiatively. So for each spectral component λ we can evaluate:

$$N_\lambda = \int_{t_s}^{\infty} I_\lambda(t)\,\mathrm{d}t, \quad p_\lambda(t) = \frac{\Delta n_\lambda}{n_\lambda} = \frac{\int_t^{t+\Delta t} I_\lambda(t')\mathrm{d}t'}{\int_t^{\infty} I_\lambda(t')\,\mathrm{d}t'} \tag{3}$$

where t_s = 32 μs is the start time of DL recording.

Numerical evaluation of Eq. (2) was carried out extrapolating to longer (infinity) time the fit of the last part of time trend.

More precisely for each seed we used as DL parameters:

$$R_N = 0.5(N_{460}/{<}N_{460}{>}_{\text{Native}} + N_{645}/{<}N_{645}{>}_{\text{Native}}) \tag{4}$$

the average value of the ratios between the photons total number at each λ and the corresponding average native one, and:

$$R_p = \frac{1}{t_f - t_s}\int_{t_s}^{t_f} \frac{p_{460}(t)}{p_{645}(t)}dt \tag{5}$$

the average in the measurement time interval of the ratio between the two spectral decay probabilities. Here t_f = 0. 2 s is the time at which signal-to-noise ratio of DL signals becomes lower than ten.

To test the reproducibility the same seed has been measured several times, moving and replacing it in the seed holder. The measured R_N value has a standard deviation of 15% while R_p is affected by a standard deviation of 8%. This error is essentially due to the uncertainty of the seed's position in the holder. Indeed when the seed is left in the same position the standard deviation of both R_N and R_p decreases to only a few percent.

The thermal treatment produces a decrease of the seeds weight, due to dehydration, marked at the beginning of the treatment (0.9%/h) and less pronounced afterwards (0.02%/h at 72 h). In Fig. 3 for each group of treated seeds the average values of G, R_N and R_p are plotted as a function of the weight decrement. It is possible to note that the three parameters smoothly changes for weight decrements up to 8% while they quickly change in the last part, where, a further weight decrement of about 0.5% produces a sudden increase in DL parameters and decrease in seeds germination. Similar correlation between the water content and the DL parameters were previously found for other biological systems (Gulino et al. 2005), supporting the idea that DL parameters depend on the system structure (Scordino et al. 2000). So this sudden variation can be explained by an irreversible change of the seed structure.

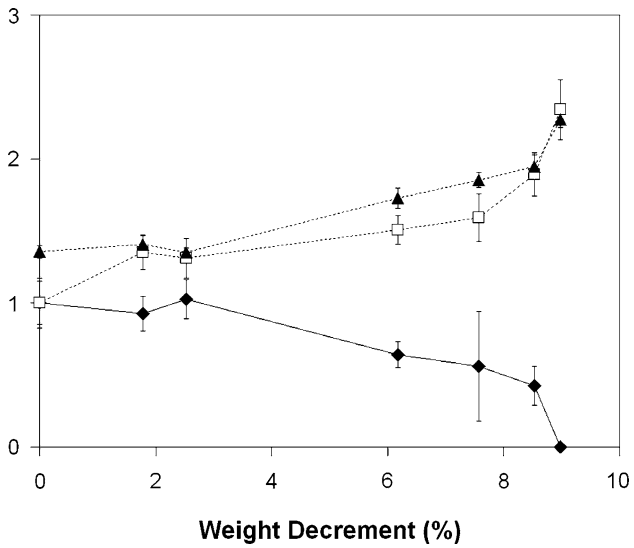

Fig. 3 Plot of average values of the parameters Eqs. (1), (4) and (5) G (*filled diamond*), R_N (*open square*) and R_p (*filled triangle*) as a function of the average weight decrement of seeds

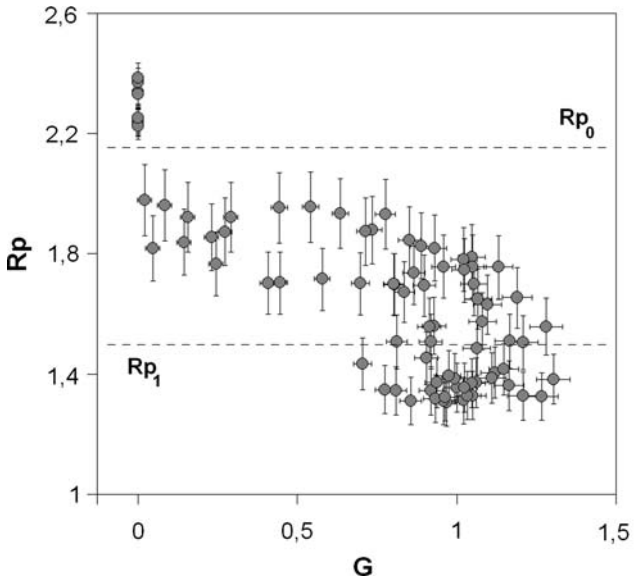

Fig. 4 Correlation between the parameters Eqs. (1) and (5) R_p and G measured for each studied seed

As a first approximation a significant linear correlation between R_N and G and between R_p and G can be considered ($r = -0.75$ with $P_N(|r| > 0.75) < 0.001$ and $r = -0.84$ with $P_N(|r| > 0.84) < 0.001$, respectively). This is a new and important result because for the first time the viability of a single seed is correlated with DL parameters. It must be stressed that the growth parameter G shows a better correlation with R_p, which is connected to the time trend of DL here measured with a good reproducibility. However, due to the errors affecting both the parameters evaluated on the single seeds, it is not possible to establish an unambiguous analytical relation R_p versus G as shown in Fig. 4.

Nevertheless an effective prediction of vegetative performances of single seed appears to be possible. In fact it is possible to divide the seeds in three groups: seeds without any germination performance (average value $R_p = 2.27 \pm 0.06$ SD), partially damaged seeds (average value $R_p = 1.81 \pm 0.17$ SD) and untreated seeds (average value $R_p = 1.42 \pm 0.09$ SD). The statistical analysis of the data reported in Fig. 4 shows that it is possible to consider two values $R_{po} = 2.15$ and $R_{p1} = 1.5$ such that the seeds with $R_p > R_{po}$ have a probability bigger than 96% to be devitalized and the seeds with $R_p < R_{p1}$ have a probability greater than 92% to exhibit a normal vegetative performance. This fact suggests the way to achieve a method to discriminate between devitalized and living seeds in a fast and not destructive way.

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