Delayed Luminescence: a non-destructive tool to evaluate watermelon seeds germination

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Abstract - The Delayed Luminescence, an ultra-weak and photo-induced emission of optical photons, is increasingly showing its potential both in scientific and commercial fields concerning agriculture and food processing. In this research, in fact, we evaluated germination performance, the principal index reflecting seed quality, of two lots of 'Mirella' F1 watermelon dried seeds in the conditions as provided by seed/breeding company. Characteristics of Delayed Luminescence emission from each single seed were correlated to the different germination levels as assessed by International Seed Testing Procedures so showing that Delayed Luminescence measurement could be a quick, cheap and non-destructive test for seed viability analysis.

INTRODUCTION

In the intensive vegetable industry, seedlings are produced in specialized nurseries, where seeds with rapid and uniform germination and growth are preferred to improve crop production and so satisfy plant commissions by grower. This last aspect is particularly important in reducing cost of grafted plants production.

In an agricultural context, seeds available for sowing come in 'lots'. The seeds of each lot have characteristics and vigour varying dramatically according to the environment in which they develop, the subsequent harvest, handling and also storage. Unfortunately, the germination performances are in general difficult to recognize by a visual observation. Conventional biochemical methods could be expensive, complex, time-consuming and destructive [1]. Techniques, able to be a sensitive indicators of the functional state of a biological sample, are desirable. Within the optical techniques, the one based on Delayed Luminescence acquisition is showing to be promising.

Delayed Luminescence (DL) is the ultra-weak photo-induced emission of optical photons by a sample at long times after the illumination source is switched off. DL applicative potential was showed in different fields such as food and agricultural crops quality [2, 3] or to evaluate the effects on seeds viability of artificial aging [4], electric fields [5], thermal treatments [6] and ion irradiation [7].

In this research [8], we used DL emission as a tool to discriminate germination performance of different seeds lots of 'Mirella' F1 watermelon, a cultivar generally grafted. Two lots, namely Lot A and Lot B, were considered, according to their different germination levels as assessed by the grower. DL emitted by each seed was measured and correlated to the germination performance of single seed.

MATHERIAL AND METHODS

Seed germination test

Two seed lots of 'Mirella' F1 watermelon (Vilmorin Italia, Italy) was used, Lot A ('Mirella' Y-HA84551F-S1K) and Lot B ('Mirella' Y-HA84551-S1KS) provided by Centro Seia srl (Ragusa, Italy). For each lot, 96 seeds were randomly selected and labelled by using the rows and columns references of a plastic multi-well plate used only to store seeds before and after DL measurements, that were completed in eight days for the two lots. Immediately after, the two multi-well plates were transferred for starting germination test. To perform the germination test, all seeds were carefully placed at the same time on top of two layers of Whatman No. 1 filter paper in 8 cm diameter Petri dishes (12 dishes per lot, 8 seeds on each dish) containing 4.5 ml of distilled water. Seeds were maintained in controlled dark condition at 28.3 °C, and starting from the beginning of sowing day (namely the day 0) germination was checked two times a day for 10 days. The seeds were considered germinated when a 2 mm root length was reached.

Delayed luminescence spectroscopy

Delayed luminescence measurements were performed by using the ARETUSA set-up developed at LNS-INFN. DL time trends decays were recorded in the time interval 10μs÷100ms by individual seeds. Spectral properties were acquired using broadband bandpass interference filters (Edmund Optics) having center wavelength λ = 450 nm, 550 nm and 650 nm (50 nm FWHM).

RESULTS

Table 1 shows the percentage of germinated seeds as a function of the day after sowing for the two lots A and B. The two lots of seeds showed very different germination performances. The percentage of seeds that did not germinate at all was 7% (Lot A) and 17% (Lot B). 76% of

Lot A seeds germinated within 2 days, 24% for Lot B's seeds.

Table 1: Germinated seeds (%)

Day	Lot A seeds	Lot B seeds
1°	50.5	9.4
2°	25.3	14.6
3°	11.6	31.3
4°	0	25
5°	5.3	3.1

For what concern DL emission, lot A and lot B showed different DL time trend decays. As example, Fig. 1 shows the DL emitted at 650nm by germinated seeds from Lot A and not germinated seeds from Lot B, respectively.

Starting from DL kinetics related to each lot and to each spectral component at centre wavelength λ (450nm, 550nm or 650nm), the experimental probability

$$p_\lambda(t) = \frac{\Delta n_\lambda(t)}{N_\lambda(t)}$$

that Δn_λ levels of the N_λ excited ones decay in radiative way, was evaluated (see ref. [8]). For each pair of wavelength λ (450nm, 550nm and 650nm), we calculated the dimensionless parameter:

$$R_p(\lambda_1/\lambda_2) = \frac{1}{t_f - t_s} \int_{t_s}^{t_f} \frac{p_{\lambda_1}(t)}{p_{\lambda_2}(t)} dt$$

where (t_s, t_f) is the time interval of the decay in which the average is evaluated. In our experimental condition: t_s, the start time of the first experimental point, was 10 µs; t_f, the final time, was assigned equal to 10 ms in order to reduce the errors connected to the low level of the signal at long times; the wavelength ratios λ_1/λ_2 were the values 450/550, 450/650 or 550/650.

Fig. 2 shows Rp(450/650), Rp(550/650) and Rp(450/550) parameters. Lot A seeds exhibit greater values than for Lot B seed ones. Furthermore, the differences are highly significant in the case of Rp(450/650) and Rp(550/650) parameters, so allowing to discriminate between the two lots of seeds that present very different germination performances.

In conclusion, the results highlights the possibility to evaluate the germination performance of different lots of watermelon seeds by using Delayed Luminescence spectroscopy. In particular, our experiments have shown that intensive parameters which describe changes in decay kinetics of DL spectral components allow to discriminate, with very high statistical significance, within different watermelon seed lots, and to characterize the lot with better performance.

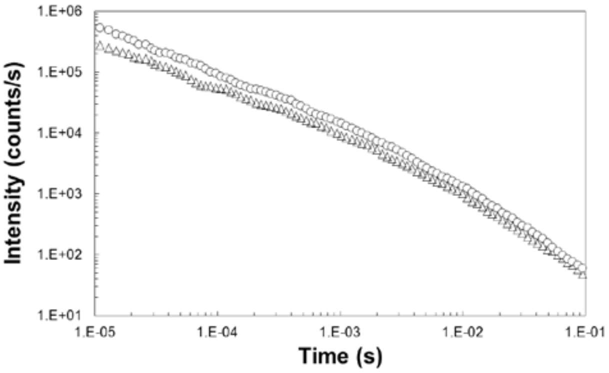

Figure 1: Delayed Luminescence time decay at 650 nm: (Δ) germinated seeds from Lot A; (○) not germinated seeds from Lot B. Markers denote average values of three independent runs performed on three different seeds. Standard errors are within markers size.

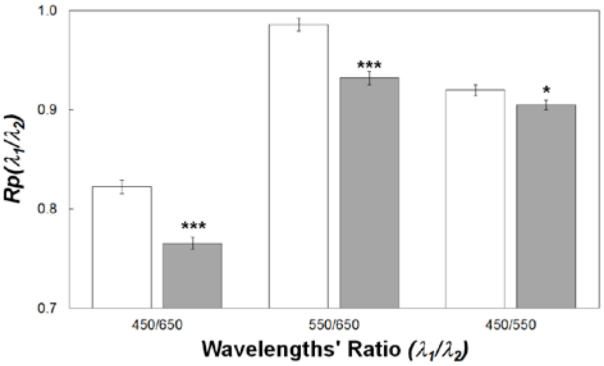

Figure 2: Comparison of the values of the R_p parameter at three different ratios of the spectral components wavelength λ_1 and λ_2: (white bar) lot A, (grey bar) lot B. Data are reported as average values of all seeds within a given lot ± SE. *p<0.05, ***p<0.001 significant differences lot B vs. lot A (ref. [8]).

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