Germination Vigour versus Delayed Luminescence of Coffee Seeds
Preliminary Series

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Abstract: Nine coffee seeds samples submitted to different post-harvest treatments were tested in terms of ultra-weak delayed luminescence and induced to germinate afterwards. Hyperbolic decay function was used to quantify the time profiles and their fitting parameters were correlated to the correspondent germination vigour (total seedlings' elongation). Good linear relation was found ($R^2 > 0.85$) for the initial value parameter as well as for the decay velocity parameter. These preliminary results point to further tests in order to validate a photonic, non-invasive, non-destructive test for coffee seed's viability analyses.

1 INTRODUCTION

THE coffee seed normally presents high germination potential just after appropriate harvest and desiccation. However, it loses its physiological quality very rapidly under common storing conditions. Therefore, it is not possible to have feasible seeds, i.e. able to germinate, for more than some months (Eira, 2006). Some techniques may improve seed's viability on long term, by improving storing conditions (Couturon, 1980; Hong & Ellis, 1992), or controlling the re-hydration process (Dussert et al., 2000) or even inducing low-temperature hibernation (Dussert et al., 2001).

Although some progress was achieved, the usual way for checking seeds' viability and vigour is to allow them to germinate, losing so the hibernation condition. In order to distinguish between feasible and not feasible seeds, and so enable an optimization of seed's storage conditions, a quick and non-destructive method is demanded, as well for other types of sensitive seeds.

The biophotonic phenomena, i.e. the ultra-weak delayed luminescence and spontaneous emission found in living organisms, with detected intensity of 10-1000 photons/cm^2.s, has been studied by many multi-disciplinary groups all over the world, in a broad variety of themes (Kobayshi and Inaba, 2000). This peculiar luminescence holds much longer than the usual bio-fluorescence, and is found far from normal thermal emission, covering the entire visible spectrum and the near IR and UV (Popp, 2000).

Correlation between the ultra-weak delayed luminescence (DL) behaviour and the germination capacity was found for barley (Yan, 2003), soya (Lanzanò, 2009; Costanzo, 2008), rice (Yong, 2010), and wheat (Wang, 2009) seeds.

A first, small trial with coffee seeds were performed by the first author ten years ago at IIB facilities (Neuss, Germany), with some indicative results of good correlation between the DL parameters and the germination capacity of tested seeds (Gallep, 2004).

Here, preliminary series of ultra-weak DL of coffee seeds are presented in relation to their germination vigor – germination rate and total seedling elongation measured in the hypocotyl root axis. Seeds submitted to different post-harvest treatments were tested for delayed luminescence, and induced to germinate afterwards.

The germination performance was established after 15 days and 30 days and correlated to DL parameters. Good correlation (R^2>0.85) was found between the germination vigor and the initial intensity and the decay velocity.

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2 MATERIAL AND METHODS

The DL of nine groups of coffee seeds (*Coffea arabica*), harvested in 2011 and treated in different conditions, were analysed in terms of hyperbolic decay in May/2013 and further induced to germinate in controlled conditions.

The different seed groups are presented on Table 1 – if mucilage is removed mechanically or by fermentation, if drying was done in drying machine, in shadow or under sun-light, and the moisture content achieved after drying (12% or 35%).

Table 1: Coffee seed groups - different post-harvest treatment.

group	post-harvest treatment	Moisture content
A7	mechanical removal of mucilage, mechanical dryer	12%
A9	mechanical removal of mucilage, dried in shadow	12%
A11	mechanical removal of mucilage, sun-dried	12%
A13	removal of mucilage by fermentation, mechanical dryer	12%
A14	removal of mucilage by fermentation, mechanical dryer	35%
A15	removal of mucilage by fermentation, dried in shadow	12%
A16	removal of mucilage by fermentation, dried in shadow	35%
A17	removal of mucilage by fermentation, sun-dried	12%
A18	removal of mucilage by fermentation, sun-dried	35%

The seeds were harvest, processed and stored in controlled conditions at Federal University of Lavras (UFLA, MG, Brazil) in June/2011. Random samples of 50 grams were taken from each seed group for the ultra-weak DL measurements and stored in dark to avoid artefacts.

The experimental setup for DL tests are shown at Figure 1; it is a dark chamber with photon-count module (photomultiplier tube + electronics) and fiber optic ring connect to external light source (halogen lamp) by fiber cable and electrical-mechanical shutter, all automatic controlled by software; it includes also temperature control for samples using fluid flow (Bertonha, 2011).

Each group of seeds was arranged in the chamber in order to complete the sample holder, which was stabilized in temperature (T = 21 ^{o}C +/- 1) to avoid seed stress. The DL measurements used photon-count mode in 100 μs time-windows for 20 thousand points (total = 2 s) and were taken after twenty-second exposure to white light (160 lux), and repeated sequentially ten times for each sample. A delay of 370 ms occurs between the end of excitation and photon-count start due technical limitation.

The 10-repetition DL data was averaged and the curve was fitted by generic hyperbolic-like decay, formulated by:

$$a + b/(1 + c.t)^d \quad (1)$$

where t is related to time, $a+b$ is the curve's initial value ($t = 0$), a is its final value ($t = \infty$), c is related to the decay velocity for small t and d is related to enhancement in velocity decay, more pronounced for great values of t.

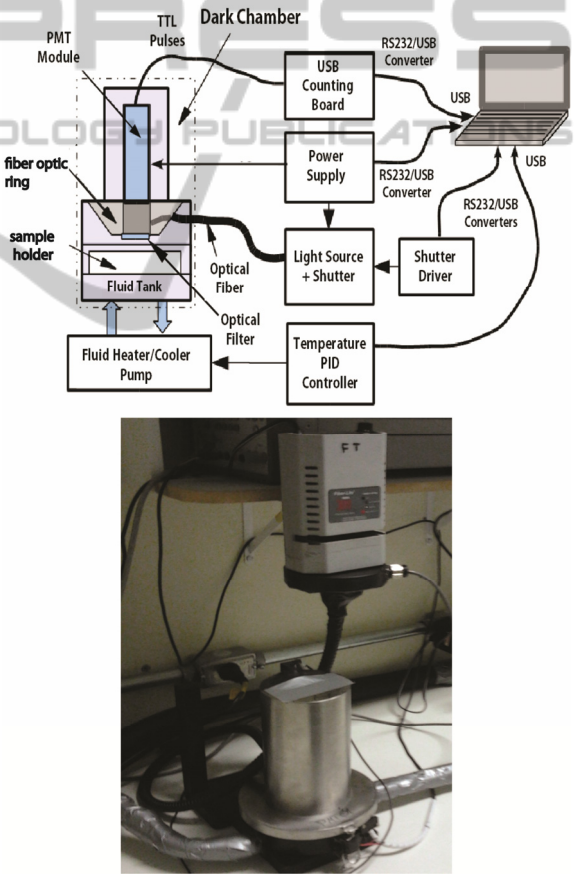

Figure 1: Setup for DL measurements in biosamples - (top) chamber, illumination and controls schematics; (bottom) picture of prototype.

After all samples were measured two hundread seeds were taken from each sample and induced to germinate in a controlled chamber (T = 30 ^{o}C +/- 1, humidity > 70%) for 30 days. For that, each group of

200 were divided in 4 x 50 seeds, and each sub-group disposed in rolls of paper towels moistened with water equivalent to 2 ½ times the dry paper substrate weght. At the 15th day after start and at the end, at the 30th day, each group was analysed in terms of hypocotyl root axis growth, measuring each seedling elongation.

3 RESULTS AND DISCUSSION

The DL time profiles of all groups are shown at Figure 2, as well as their correspondent hyperbolic decay fitting (R^2>0.995) parameters: *a, b, c* and *d*. It is clear from Figure 2 that groups A7, A11 and A13 present higher initial value than the other groups. Group A17 time profile is also distinguishable from the remaining curves, with small increase from 1 ms to 100 ms.

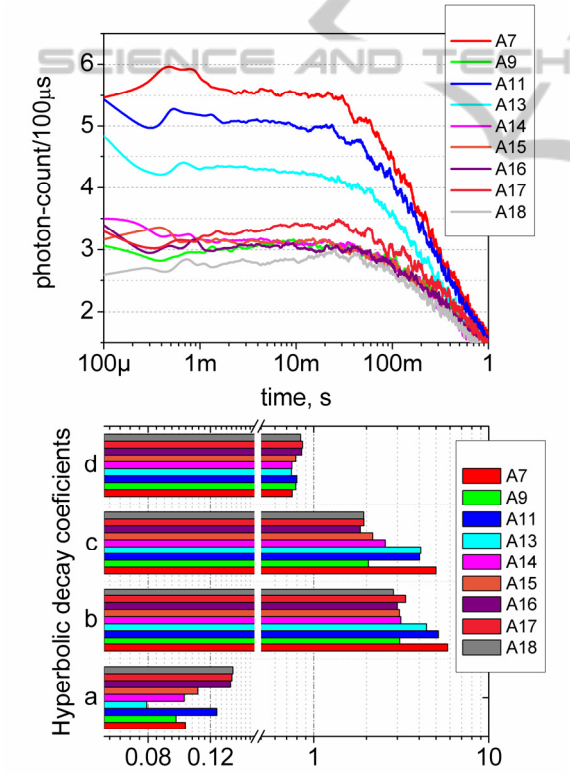

Figure 2: DL data for groups A7 to A18– (top) 10 test average time profile; (bottom) paramenters of hyperbolic decay fitting (R^2>0.995).

These facts are so reflected in the *b* parameter of their. It is also noted that the *c* parameter is also higher for the A7, A11 and A13 samples, meaning that their DL intensity decay faster that the other groups, as can be seen also in the time profiles form 10 ms to 100 ms. The A15 time profile is also noticed to have small increase at the beginning, ie. t < 1ms, as occurring for the curves of A7, A11 and A13.

The remaining groups – A9, A14, A16 and A18 – have similar time profiles, with small initial value (~3) and regular decay velocity, ie. similar *c* factor.

The germination data – seedling's elongation incidence, in a total of 200 seeds/group – at the 15th and 30th day are presented at Figure 3 for the viable groups; the ones not displayed had no seedling development at all, ie. zero seeds alive. It is noted that groups A7 and A13 present the higher development of all, followed by A11 and, with much lower development, by A15 and, much lower, A17. The A9 had few seedlings developed.

By summing all seedlings' length the total elongation is obtained for each group, for both the 15th and 30th day after imbibition. This numbers were so plotted against the correspondent *b* and *c* parameters, and these datagrams are shown at Figure 4. Good linear correlation was found between the hyperbolic fitting parameters and the total seedling elongation for both the 15th and 30th day data, with $R^2 > 0.85$ in all cases.

4 CONCLUSIONS

The ultra-weak delayed luminescence time profiles of nine groups of coffee seeds with different germination capacity were studied in terms of hyperbolic decay parameters, and good correlations were found between the DL initial value and the decay velocity versus the germinating vigour – ie., total seedling elongation at the 15th and 30th day after imbibition.

These preliminary series used old (2 years) samples, already with their vigour depreciated by the long storage time in natural condition, and so just few groups presented significant germination vigour while four of them are almost dead.

Next series of experiments would replicate this type of analysis but using fresh seed samples (< 1 year), which may present very high germination capacity, and also with artificially (thermal) stressed samples, in order to have also groups with intermediate vigour.

Although limited in number and range of seed quality, the preliminary data here presented show that the DL measurements of coffee seeds may be used as a fast, non-invasive, non-destructive test to verify sample's viability, and so help in improving post-harvest treatment, storing methods and maybe

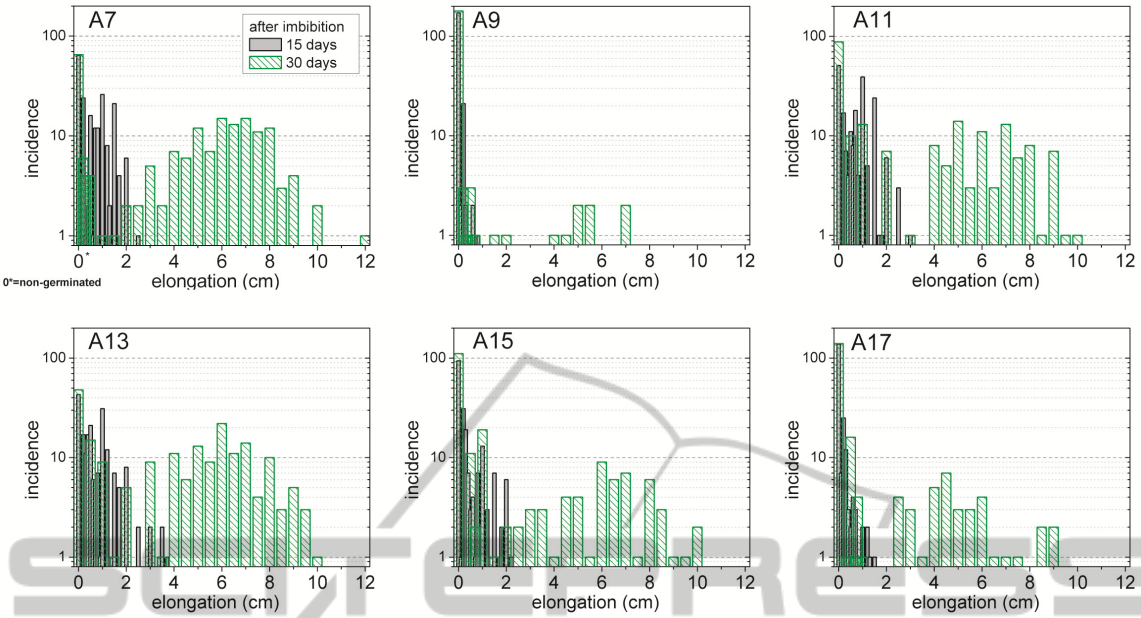

Figure 3: Seedling's length for viable groups at the 15th and 30th days after imbibition - each group has 200 seeds total.

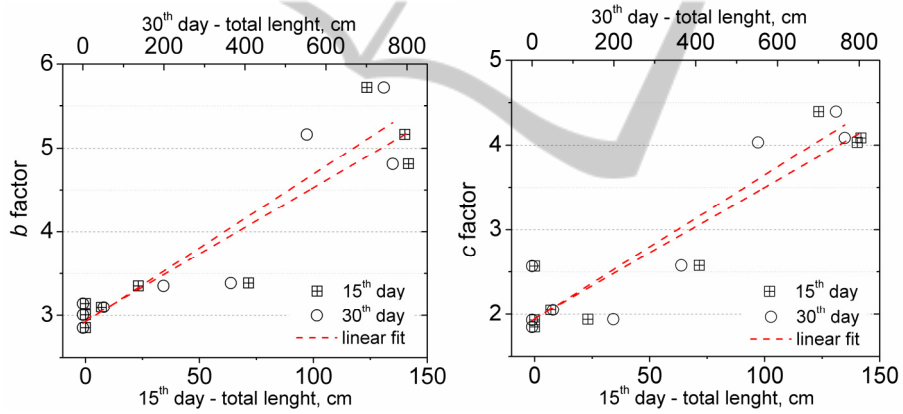

Figure 4: Seedling's total length at the 15th and 30th day *versus* the hyperbolic decay fitting parameters - (left) the *b* factor and (right) the *c* factor; dashed line correspond to linear regression (R^2>0.85).

also beverage quality.

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