Simultaneous Biophoton Measurement of Control and Fluoride Stressed Seedlings Samples

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Abstract — **A series of 10 germinations experiments with duplicated wheat seedlings samples - one imbibed in sodium fluoride (NaF, EC50) and another in water - have their spontaneous, ultra-weak photon emission measured simultaneously for 24-h, at the 4th day of germination, in two identical measurement systems. The UPE profiles of acute stressed sprouts present initially higher increase but after 6 hours a fast decrease. These variations for the profiles local angular coefficient are so correlated to germination parameters - roots elongation and biomass gain – with clear difference between the stressed and control samples. Variations of such methodology can be developed for quick and reliable analysis of toxicity in germination tests.**

Keywords – Photon-counting, ultra-weak photon emission, biophotons.

I. INTRODUCTION

In the last decades the spontaneous ultra-weak photon emission (UPE) phenomena in plants and animals have got attention of research groups around the world, in biology, agriculture and medicine, in areas as seeds viability [1, 2, 3], water quality [4] and eco-toxicology [5].

The UPE can be found in all living beings, from near IR to UV spectrum - from 350 to 850 nm, with intensities ranging from tens to thousands photons/cm^2.s [6]. UPE can be linked back to the Russian biologist A. G. Gurwitch in 1922 [7], with primeval tests based on bio-sensors. Only in the 1950s, with the advent of the photomultiplier tube by RCA, Strehler and Arnold [8] and thereafter Colli and Facchini provided the first UPE measurements from algae and seeds, respectively [9, 10]. The very weak intensity of such experiments requires a measurement setup based on noiseless detectors - mostly used are low dark-count noise photomultiplier tubes (PMTs) cooled to minimize the inherent environmental noise constraints [11].

Recently we have shown that 24-hour UPE measurements of germination tests at the 4th day can differentiate stressed (EC50) from control samples [12]. The duplicated tests must be run simultaneously, since inherent circadian rhythms are present is such tests[13]. This paper presents new data, for NaF solution tests, where just the initial 12h were sufficient to differentiated toxic from control tests. The UPE data from stressed samples present first a high increase after imbibition, and latter (~6h) a fast decay, while the control samples mostly presents a small, constant increase during the initial 12h.

These changes in UPE local growth are further related to germination parameters measured at the end of the 4th day of germination – root elongation and net mass increase – giving good correlation in this trial.

The results indicate that procedures based on this method can be used as faster methods of germination tests in toxicology and agriculture.

II. MATERIALS AND METHODS

Nine tests of duplicated samples of 25 wheat seedlings are firstly imbibed in distilled water (3ml) plus filter paper inside 6cm petri dish, and kept inside a dark chamber for 3 days. At the beginning of the 4th day (72th hour), extra 2ml of solution was dropped in the two samples – water for control and NaF for stress - and are moved to other dark chambers to run UPE measurement for the next 24 hours. At the end of the 4th day, the sprouts are dried and measured. UPE counts are taken each 10 seconds, with dark noise of <170/10s. One extra experiment was conduct first, to measure UPE of seedlings during the entire germination period - 96 h. All tests were run under controlled temperature (22^o +/-1^{o}C), in similar photon-count chambers [14].

A screening procedure based on the Organisation for Economic Co-operation and Development (OECD) recommendation for testing chemicals [15] was firstly run to determine the concentration of the stressing solution to be used – here the effective concentration to kill 50% (EC50) for sodium fluoride (NaF) at 50 mM. The pH was adjusted to neutral (~7), and stored in proper conditions.

At the end of the 96th h the seedlings were weighed in order to calculate the biomass gain, and their roots elongation were measured individually for each seedling of each sample and afterwards integrated.

The photon count data from the two measurement systems were locally smoothed (100 points) to avoid the additive, random thermal noise. Local linear fitting was performed for two intervals - from the 73rd to the 77th hour and from the 76th to the 82th hour - and so the local angular coefficients of linear regressions were determined, respectively α_1 and α_2. With

Sponsors: Brazilian Research Council (CNPq, 552258/2009-1), FAPESP (CEPOF, FOTONICOM and 04/10146-3 + 11/10763-6).

that, the deviation in angular coefficient ($\Delta\alpha = \alpha_1 - \alpha_2$) is determined for each test. Finally, the angular coefficient deviation $\Delta\alpha$ is plotted versus the biomass gain and total sum of roots elongation of each test.

III. Results and Discussion

The UPE time profiles for the complete 4-day tests (96 h) are shown at Fig. 1. It is clear that before the 72nd hour both samples have very similar photon-count, in good synchronism, since both samples were imbibed just in water. After the second imbibition, when one sample receives stressing solution, UPE profiles strongly diverge, especially between the 73th and 82th hours. The stressed sample initially presents higher UPE, for the first six hours, and than decrease fast in the next hours. The experiment was repeated more 9 times, with UPE recorded just after the 2nd imbition, i.e. for the last 24 (72nd to 94th) hours. These curves are presented at Fig.2, with the local linear fitting for the two initial time intervals, whose angular coefficients will be further used.

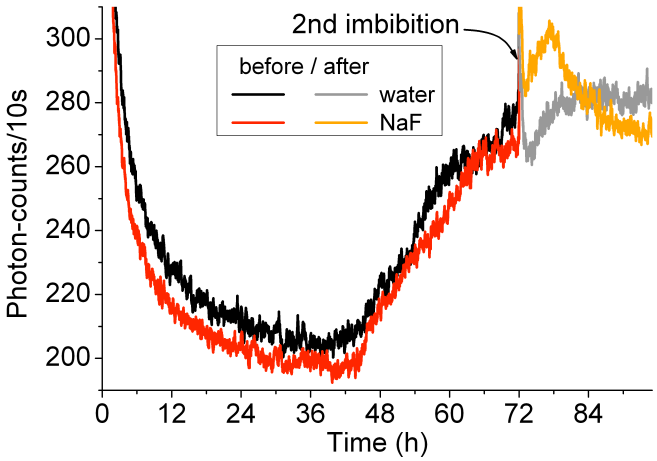

Figure 1 – UPE Temporal profiles of simultaneous germination tests; 2nd imbibition done at the 72nd hour, when one sample receives water and other the NaF (50mM) solution.

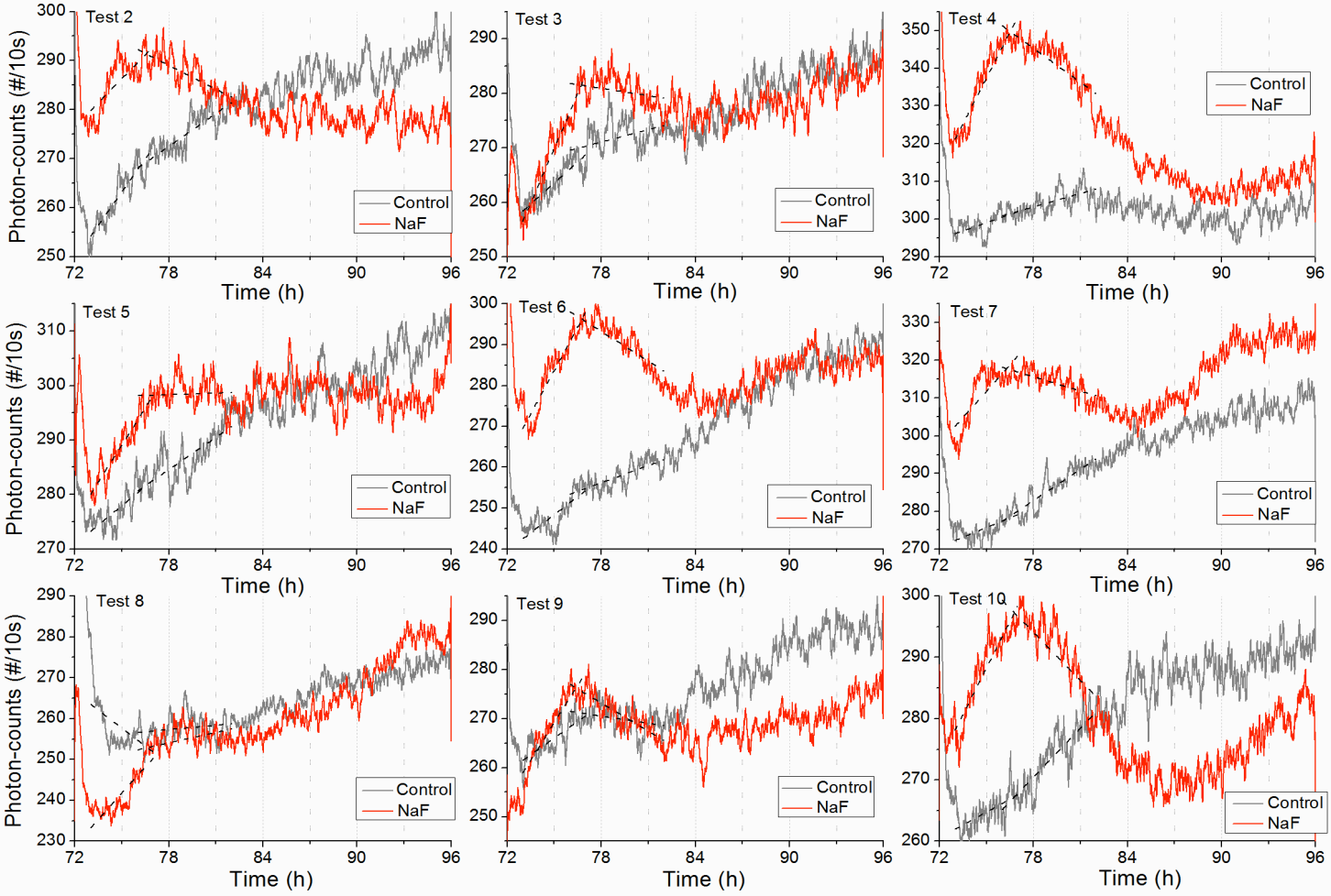

Figure 2 - UPE temporal profiles of stressed (NaF, red lines) and control (water, gray lines) wheat seedlings samples, dashed lines show local linear fitting for the 1st (73th-77th hour) and 2nd (76th-82th hour) intervals.

Similar to the final portion of Fig.1, Fig.2 subsets present the UPE curve of control samples increasing up to the experiment end, while the ones of stressed samples show faster increase in the first 6 hours, with posterior strong decrease. In resume, when calculating deviation in the local linear fitting, control tests present small $\Delta\alpha$ (<0.7) while stressed tests have mostly $\Delta\alpha > 1$. The $\Delta\alpha$ data are plotted against the corresponding biomass gain and total roots' length, as shown at Fig. 3 - stressed and control samples are so grouped very apart. As expected, control samples achieved better germination performance.

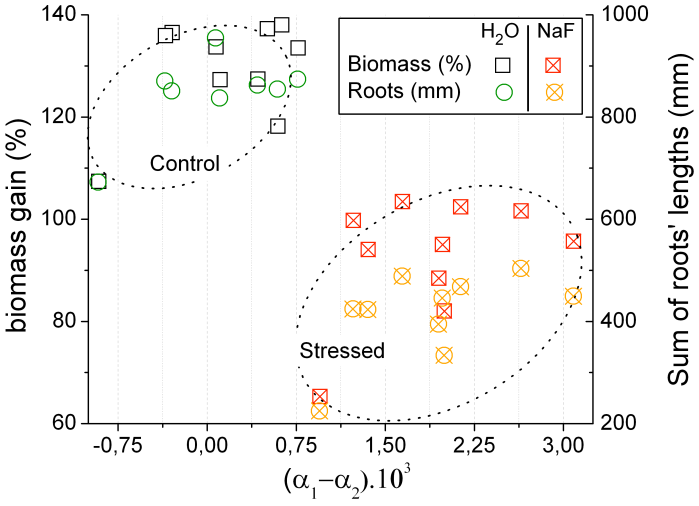

Figure 3 - Angular coefficient deviations - stressed and control seedlings - versus biomass gain and total root elongation, for all ten experiments.

By this kind of plot it is easy to see the superior performance in germination parameters - ie. the vigour - associated to the control samples in comparison to the chemically stressed samples. A hypothesis test (Student test for paired samples) were performed to establish the statistical significance of such datagram of Fig.3. The null hypothesis established is that the sample's average are equal ($\mu_0 - \mu_A = 0$), and the alternative hypothesis is that those are different ($\mu_0 - \mu_A \neq 0$), with a confidence level of 95%, or a significance level of 0.05. The results are shown in Table 1.

TABLE I. STATISTICAL ANALYSIS

Paired Samples Student Test, DF=9						
parameter	μ_0	SD_0	μ_A	SD_A	$\Delta\mu$	$t_{0.05;9}$
Value	0.1558	0.5371	1.8983	0.6450	1.7425	7.9237

The critical value of *t* for a significance level of 0.05 and degree of freedom DF=9 (n=10) is $t_{0.05;9}$=1.833, and the resulted *t* value of 7.9237 generated is much greater, assuring with confidence of 95% that the alternative hypothesis is true, ie. the difference of samples means is statistically different.

IV. CONCLUSIONS

Ultra weak photon emission from wheat seedlings samples were collected at the fourth day of germination, and it was possible to discriminate between stressed and non-stressed samples by processing the photon-counting data acquired simultaneously through two identical, simultaneous measurements. Other measurements could be performed in the future for different chemical substances, and results must be compared to map different UPE profiles that may occur.

The method may be a suitable technique for water quality assurance tests, to be applied in water treatment facilities, with advantages of speed and costs, besides being operationally simpler than traditional methods of seedling analysis.

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