Optical analysis of the physiological quality of Triticum aestivum L. seeds with two vigor levels treated with Thiamethoxam

Análise ótica da qualidade fisiológica de sementes de Triticum aestivum L. com dois níveis de vigor tratadas com Thiamethoxam

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Ana Carla Cordeiro
ORCID: https://orcid.org/0000-0001-8718-2764
Universidade Tecnologica Federal do Paraná, Brasil.
E-mail: cordeirocarlaana@gmail.com

Fernando Augusto Henning
ORCID: https://orcid.org/0000-0002-1705-9047
Embrapa Soja – Paraná, Brasil.
Email: Fernando.henning@embrapa.br

Gustavo Henrique Couto
ORCID: https://orcid.org/0000-0001-8958-517X
Universidade Tecnologica Federal do Paraná, Brasil.
Email: gustavocouto@utfpr.com.br

Eduardo Giometti Bertogna
ORCID: https://orcid.org/0000-0002-2271-814X
Universidade Tecnologica Federal do Paraná, Brasil.
Email: ebertogna@gmail.com

Ricardo Canute Kamikawachi
ORCID: https://orcid.org/0000-0003-3079-924X
Universidade Tecnologica Federal do Paraná, Brasil.
Email: canute@utfpr.com.br

ABSTRACT

Insecticide treatment shields plants from pests. The standard tests are the ones that are most frequently used. However, some results may be unreliable or insensitive. Optical techniques such as ultra-weak photon emission and Fourier transform infrared spectroscopy can aid standard tests. In this study, two samples of wheat seeds were subjected to the accelerated aging test, resulting in two vigor levels. Every sample was divided into two groups (control and treated). The treated groups received 150 mL of Thiamethoxam for each 100 kg of seeds. The results showed that the length of the aerial part was insensitive. However, the length of the primary root showed the treated group (73% of the germination) was statistically different. The total photon counts and temporal slope profile averages associated with the ultra-weak photon emission for the treated group (73%) showed a significant difference between the control groups (73% and 94% of germination). Principal component analysis revealed differences between groups of distinct seed vigor levels. Still, it did not show differences between the groups treated and untreated. By comparing the results, optical techniques can be used to assess seed physiological quality, particularly the vigor level.

Keywords: Wheat seeds; Ultra-weak photon emissions; Fourier transform infrared spectroscopy; Thiamethoxam; Physiological Quality;
RESUMO
O tratamento com inseticida protege as plantas dos parasitas. Os testes padrão são os mais utilizados. No entanto, alguns resultados podem ser pouco confiáveis ou insensíveis. As técnicas ópticas, como a emissão de fótons ultrafracos e a espetroscopia de infravermelho com transformada de Fourier, podem colaborar. Neste estudo, duas amostras de sementes de trigo foram submetidas ao teste de envelhecimento acelerado, resultando em dois níveis de vigor. Cada amostra foi dividida em dois grupos (controle e tratado). Os grupos tratados receberam 150 mL de Tiametoxam para cada 100 kg de sementes. Os resultados do comprimento da parte aérea não foram sensíveis. No entanto, o comprimento da raiz primária do grupo tratado (73% de germinação) foi estatisticamente diferente. As contagens totais de fótons e as médias do perfil de inclinação temporal associadas à emissão de fótons ultrafracos para o grupo tratado (73%) revelaram uma diferença significativa entre os grupos controle (73% e 94% de germinação). A análise de componentes principais revelou diferenças entre os níveis de vigor. Porém, não mostrou diferenças entre os grupos tratados e não tratados. Comparando os resultados, as técnicas ópticas podem ser utilizadas para avaliar a qualidade fisiológica das sementes, principalmente o nível de vigor.

Palavras-chave: Sementes de trigo; Emissões de fótons ultrafracas; Espectroscopia de infravermelha com transformada de Fourier; Tiametoxam; Qualidade Fisiológica;

INTRODUÇÃO
Wheat cultivation is vital to the global food supply. Wheat, rice, and corn are the three most essential cereals grown worldwide due to their nutritional and economic benefits (COSTA; SOUZA; STAMFORD, 2008; DEMIR; ONDE; SEVERCAN, 2015). To achieve high crop productivity, the quality of seeds, which is found by genetic, physical, physiological, and sanitary attributes, must be ensured (FILHO, 2005; FRANCESHI et al., 2009; CONAB, 2017). Among those characteristics, the physiological one is prominent because it collects information about seed viability and vigor, which are critical factors in determining the potential for plants to establish quickly and uniformly (FILHO, 2005; FILHO, 2017).

Pathogens, insects, and harmful microorganisms, as well as chemical treatments with fungicides, bactericides, and insecticides, are all factors that can affect the physiological quality of seeds (FILHO, 2005; CARVALHO; NAKAGAWA, 2000; FILHO, 2013). Chemical treatments protect seeds and seedlings from pests and insects while increasing crop productivity (CASTRO et al., 2008; MACEDO; CASTRO, 2011). Additionally, some of them, such as hormonal control agrochemicals, specifically the bio-activator subgroup, can also interfere with the physiological quality of seeds. A systemic insecticide of the neonicotinoid chemical group with the active ingredient thiamethoxam (THX) is included in this subgroup (MACEDO; CASTRO, 2011; CASTRO, 2006).
According to Almeida et al. (2012) and Castro et al. (2009), THX treatment can boost seed vigor and contribute to the formation of deeper roots. As in Macedo and Castro (2011), standard techniques were used to investigate the physiological effects of THX on wheat plants throughout their life cycle. In that study, it was discovered that using the product in seed treatment influenced plant growth and caused a linear increase in root development (MACEDO; CASTRO, 2011). In Almeida et al., (2014), the effect of THX treated on the physiological quality of rice seeds was investigated, and the results of standard techniques revealed that insecticide had a positive impact on the percentage of germination and vigor level of treated seeds compared to the control group.

However, in Dan et al. (2012), the effect of insecticide on germination and vigor of soybean seeds was evaluated using non-optical tests; the results revealed that THX treatment did not interfere with the initial development of plants. As a result, there is no agreement on the product’s potential effects on the physiological quality of seeds, and more research is needed in this area (MACECO; CASTRO, 2011; MACEDO, 2012).

In the study performed here, the standard (non-optical) techniques used to assess the effects of THX wheat seeds treated were linked to optical techniques: ultra-weak photon emission (UWPE) and Fourier transform infrared spectroscopy (FTIR). The results found here have shown that optical techniques could be used as additional tools to analyze the dose level treatment and seeds’ vigor level. In addition, non-optical techniques can be time-consuming, labor-intensive, and produce varying results (FILHO, 2005; WIJEWARDANA; REDDY; BELLALOU, 2019). In this direction, optical techniques can provide faster results with less variability.

**MATERIALS AND METHODS**

**MATERIALS**

Materials and procedures are listed in Figure 1. The following sections describe the procedures.
TREATMENT OF THE WHEAT SEED SAMPLES

The Cruiser® 350 FS commercial product (Syngenta Proteção de Cultivos Ltda., São Paulo - SP, lot number 0070-17-11860) was used for the seed treatment. It contains 350 g of THX active ingredient per liter (SYNGENTA, 2021).

The wheat seeds were of Triticum aestivum L., cultivar ORS Madrepérola, 2021 crop, grown in Arapoti - Paraná State/Brazil (-24.26, -50.11). Two lots were used: lot number 1073 and lot number 1074. The accelerated aging test described in the Rules of Seed Analysis (BRASIL, 2009) was used in these lots separately to obtain the different vigor levels. Seeds of two lots were subjected to a temperature of 42 °C. However, the treatments were submitted in two other test groups using two different periods: 48 hours (lot 1073) and 60 hours (lot 1074) with 100% relative air humidity. This process led to the deterioration of the seeds and the appearance of abnormalities or the death of the seeds during germination. As a result, seeds with different germination percentages
were obtained: 94 percent (high vigor – lot 1073) and 73 percent (low vigor – lot 1074). Then, each group was divided into two. Those groups were: high vigor (control group of increased vigor), low vigor (control group of low vigor), high vigor – THX (treated group of high vigor), and low vigor – THX (treated group of increased vigor). Per the manufacturer's recommendation, the treated groups were 150 mL of THX for each 100 kg of seeds (SYNGENTA, 2021).

**UWPE DATA ACQUISITION**

The chemiluminescence produced by biological systems when electronically excited species are created due to oxidative metabolism or oxidative stress processes is known as ultra-weak photon emission (CIFRA; POSPÍŠIL, 2014). In the 1960s, the advancement of susceptible single-photon detectors demonstrated that all living systems emit ultra-weak photon emission. However, it was subsequently revealed that their emission intensity in the visible and near-infrared regions of the spectrum surpasses that in the ultraviolet range (VOEIKOV, 2003).

The analyses were conducted in duplicate, with samples of forty wheat seeds for each test. The seeds were placed in a Petri dish (8 cm in diameter) with five sheets of qualitative filter paper inside (both previously sterilized). Then, 6 mL of deionized water (Cloroquímica®, pH 7) was applied to the seeds. The sample was left in a germination room at 22.0 °C for 72 h. Then, another 2 mL of deionized water was added. Afterward, the samples were transferred to the photon counting chamber under low light conditions to collect UWPE data for 48 hours.

The temporal photon count profiles were obtained using custom-built equipment; more information can be found in Bertogna et al. (2016). An aluminum darkroom houses a photomultiplier (PMT) photon counting module (H6240-01 model, from Hamamatsu Corp.). The instrumentation includes a dark chamber for the Petri’s dish where the samples are placed, an electronic acquisition board for acquiring the counting pulses from the PMT module, thermal stabilizations for both the photon counting module and the seed samples, and a virtual instrument program running on a personal computer for acquiring and displaying the photon counting data.

A temperature controller kept the interior of the dark chamber, where the Petri dish was placed, at 22.0 °C. The photon count data were smoothed by the local average of one hundred adjacent counts after being transferred to the computer to reduce noise variation. The counts were integrated in 10 seconds (/10 s). Furthermore, the averages
of temporal slope profiles were calculated for each group. In addition, the total photon counts for each sample were computed.

In addition, the Scott-Knott test (5 percent probability), Dunnett test with 5 percent probability were performed in the results.

NORMAL SEEDLING AERIAL PART AND PRIMARY ROOT LENGTH MEASUREMENTS

The lengths of the aerial part (LA) and the primary root (LR) of normal wheat seedlings (classified according to Brasil (2009)) were measured. The research was conducted at the Federal University of Technology - Paraná State/Brazil.

The measurements were as follows: seed germination papers (J. Prolab®) were weighed and moistened with a volume of deionized water equal to two and a half times the mass of the dry substrate. The seeds were then placed on two paper sheets and covered with a third one. Following that, rolls were made and placed in plastic bags, and the rolls were then placed in a 75° tilted stand in a photoperiodic germination chamber (Solab®, SL 224 model), previously disinfected with 70 percent alcohol and regulated at a constant temperature of 20.0 °C under a 12-hour photoperiod. The procedure steps were adapted from Brasil (2009) and Nakagawa (1999). The experiments were set up in a completely random order.

The measurements were done in quadruplicates of twenty-five seeds for each group. The four rolls for each group were removed from the germination chamber after eight days to take LA and LR readings. The average measurement of the quadruplicate was calculated.

The Scott-Knott test with 5 percent probability and the Dunnett test with 5 percent probability were performed in the results. Moreover, the average length readings were adjusted by second-order polynomial regression.

FTIR DATA ACQUISITION

The FTIR data acquisition samples were used in the normal seedling aerial part and primary root length measurements. To do this, at the end of the measurements, all components of the seedlings (including the external and internal pericarp and teguments of the seeds), were dried individually for each sample in a thermoelectric oven (Sterilifer®) set to 80.0 °C for 24 h. Following the drying process, the samples were
macerated. After that, the samples were placed in individual glass vials previously sterilized and stored in a desiccator until the FTIR tests began.

A spectrometer (Varian®, 640 IR model) was used in those experiments. In 32 scans, a universal attenuated total reflectance (ATR) sampling accessory (Pike®, Miracle model) with a Zinc Selenide crystal with a resolution of 4 cm\(^{-1}\) and a range of 4000 to 650 cm\(^{-1}\) was used.

The fingerprint region (800 to 1500 cm\(^{-1}\)) was chosen in each range to analyze the spectra. The 16 spectra (4 for each group) were normalized by the highest absorbance value (normalization by the maximum). The dimensionality of the spectra was then reduced using the chemometric method: the principal component analysis (PCA) technique.

RESULTS AND DISCUSSIONS

UWPE DATA RESULTS

First, the dark noise of the darkroom was measured through the average of three samples, and the result was 158.0 ± 0.6 photons / 10 s.

The averages of the two temporal profiles associated with the UWPEs of the four groups: high vigor (control group of increased vigor), low vigor (control group of low vigor), high vigor – THX (treated group of high vigor), and low vigor – THX (treated group of high vigor), are shown in Figure 2.

Figure 2 - Average of temporal slope profiles associated with the UWPEs of the four groups

Source: Authors (2023)
The Scott-Knott test revealed that the low vigor - THX group differed significantly from the other groups based on the average total photon counts. Furthermore, the Dunnett test revealed a significant difference between the low vigor - THX group and the high vigor and low vigor groups, as shown by the symbols * and # in Table 1, respectively.

The Scott-Knott test revealed that the temporal slope profiles average of the high vigor and low vigor - THX groups were significantly different from the increased vigor - THX and low vigor groups. The low force and low vigor - THX groups were also quite different from the high vigor group, as indicated by the symbol *, by the Dunnett test, in Table 1. Moreover, the results from the low vigor - THX and high vigor groups were significantly different from the low vigor group, as indicated by the symbol #, by the Dunnett test, in Table 1.

**Table 1 - Total photon counts average, and Temporal slope profiles average - UWPE - Wheat seeds with two vigor levels**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total photon counts average</th>
<th>Temporal slope profile’s average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(x 10^6) (arbitrary units)</td>
<td>(x 10^-4) (arbitrary units)</td>
</tr>
<tr>
<td>High Vigor</td>
<td>3.33 ± 0.10 a</td>
<td>3.51 ± 0.42 a #</td>
</tr>
<tr>
<td>High Vigor - THX</td>
<td>3.27 ± 0.02 a</td>
<td>2.54 ± 0.51 b</td>
</tr>
<tr>
<td>Low Vigor</td>
<td>3.36 ± 0.06 a</td>
<td>2.20 ± 0.11 b *</td>
</tr>
<tr>
<td>Low Vigor - THX</td>
<td>3.12 ± 0.05 b * #</td>
<td>0.82 ± 0.46 c * #</td>
</tr>
<tr>
<td>Average</td>
<td>3.27</td>
<td>2.26</td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>2.04</td>
<td>17.95</td>
</tr>
</tbody>
</table>

Note: In the column, average followed by the same lower-case letter do not differ statistically, by Scott-Knott test, 5 percent probability; average followed by * differ statistically from the high vigor group, by Dunnett test, 5 percent probability; average followed by # differ statistically from the low vigor group, by Dunnett test, 5 percent probability.

Source: Authors (2023)

**MEASUREMENTS OF THE LENGTHS OF THE AERIAL PART AND THE PRIMARY ROOT OF NORMAL SEEDLINGS AFTER EIGHT DAYS**

The Scott-Knott test revealed no significant differences in LA between the groups. The Scott-Knott test, however, revealed that the average LR from low vigor - THX was significantly different from the other group, as seen in Table 2.
Table 2 - Length average of the aerial part (LA) and the primary root (LR) of normal seedlings

<table>
<thead>
<tr>
<th>Group</th>
<th>Length Average</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerial Part (cm)</td>
<td>Primary Root (cm)</td>
<td></td>
</tr>
<tr>
<td>High Vigor</td>
<td>8.93 ± 0.38 a</td>
<td>18.53 ± 1.02 b</td>
<td></td>
</tr>
<tr>
<td>High Vigor - THX</td>
<td>8.15 ± 0.18 a</td>
<td>19.66 ± 0.52 b</td>
<td></td>
</tr>
<tr>
<td>Low Vigor</td>
<td>8.36 ± 0.52 a</td>
<td>18.07 ± 1.53 b</td>
<td></td>
</tr>
<tr>
<td>Low Vigor - THX</td>
<td>8.84 ± 0.71 a</td>
<td>16.32 ± 1.44 c</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>8.57</td>
<td>18.15</td>
<td></td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>5.71</td>
<td>6.61</td>
<td></td>
</tr>
</tbody>
</table>

Note: In the column, the average followed by the same lower-case letter does not differ statistically, by the Scott-Knott test, 5 percent probability; the Dunnet test about the high vigor and low vigor groups were performed, but no statistical differences were found.

Source: Authors (2023)

The readings of the average length of the LA and LR and their respective standard deviations of normal seedlings on the eighth day after installing the test about the different groups were adjusted by second-order polynomial regression, according to Figure 3 and Figure 4, respectively.

Figure 3 - Second-order polynomial regression for LA averages of normal seedlings on eighth day after installing the test about seed groups

Source: Authors (2023)
**Figure 4** - Second-order polynomial regression for LR averages of normal seedlings on eighth day after installing the test about seed groups

Source: Authors (2023)

**SPECTRA ANALYSIS**

The PCA analyses of the absorption spectra for the various groups are shown in Figure 5.

**Figure 5** - PCA analyses of the absorption spectra for the various groups

Source: Authors (2023)
The first principal component accounts for 54.7 percent of the spectral variability, while the second principal component accounts for 25.4 percent of the spectral variability and 80.1 percent of the data variance. Wheat seeds with two vigor levels, whether treated or untreated, formed distinct clusters. The PCA analysis of the absorption spectra revealed two distinct clusters that identified the two existing vigor levels. This result is consistent with Larios et al. (2020a) and Larios et al. (2020b) which used the PCA technique to identify different groups of seed vigor levels. However, the PCA analysis of the spectra did not reveal any other clusters related to the insecticide treatment.

CONCLUSIONS

Traditionally, standard tests can discriminate seeds with physiological quality levels. This study used optical techniques to analyze the physiological quality of seeds. However, these procedures can supply insufficient data regarding the physiological processes that occur in wheat seeds. The UWPE technique can be used as an adjunct tool to study the physiological processes that occur in wheat seeds.

It is crucial to emphasize that there were statistically significant differences between the results of the standard tests (length of the primary root) and the outcomes obtained through the UWPEs technique (total photon counts and temporal slope profiles) for the low vigor - THX group when compared to the other groups.

Through PCA analysis, the FTIR approach classified seeds into two vigor levels. Therefore, this approach can produce faster classification results than standard procedures.

Therefore, by using the optical techniques results, it is anticipated that it may be used to analyze the effects of THX on the physiological quality of wheat seeds, assisting in decision-making and supplementing the data provided by standard testing.

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