

## Chlorophyll fluorescence as a new marker for peanut seed quality evaluation

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## RESUMO

Recent advances in optical imaging technologies have contributed to rapid, accurate and non-destructive analysis in the context of seed quality. This work aimed to verify the potential of chlorophyll fluorescence images as a new marker for analyzing the physiological potential of peanut seeds. The fluorescence signals of chlorophyll a and b were detected in the excitation/emission combinations of 630/700 nm and 405/600 nm, respectively, using seeds artificially aged for 0, 16, 24 and 48 h. The images were captured using a VideometerLab4 equipment (Videometer A/S, Herlev, Denmark), and its software version 5.4.6. Data were compared with the traditional tests used to evaluate the physiological performance of peanut seeds, such as germination tests, electrical conductivity, seedling emergence and emergence speed index. The results revealed that the fluorescence intensity of chlorophyll a and b was lower in seeds with lower vigor. Therefore, the deterioration process of peanut seeds is accompanied by a breakdown of chlorophyll molecules, and consequently, changes in the fluorescent properties of seeds. From a practical point of view, chlorophyll fluorescence images can be successfully used to discriminate high-and low-vigor seed lots, quickly, accurately and non-destructively.

**Palavras-chave:** seed vigor; image analysis; non-destructive methods.

## Fluorescência de clorofila como um novo marcador para avaliação da qualidade da semente de amendoim

## ABSTRACT

Os recentes avanços nas tecnologias de imagens ópticas têm contribuído para a análise rápida, precisa e não destrutiva no contexto da qualidade de sementes. Este trabalho teve como objetivo verificar a potencialidade de imagens de fluorescência de clorofila como um novo marcador para análise do potencial fisiológico de sementes de amendoim. Os sinais de fluorescência da clorofila a e b foram detectados nas combinações de excitação/emissão de 630/700 nm e 405/600 nm, respectivamente, utilizando sementes envelhecidas artificialmente por 0, 16, 24 e 48 horas. As imagens foram capturadas com o equipamento VideometerLab4 (Videometer A/S, Herlev, Dinamarca), software versão 5.4.6. Os dados foram comparados com os testes analíticos tradicionais utilizados para avaliação do desempenho fisiológico de sementes de amendoim,

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*como testes de germinação, condutividade elétrica, emergência de plântulas e índice de emergência. Os resultados revelaram que a intensidade da fluorescência da clorofila a e b foi menor para as sementes de menor vigor. Portanto, o processo de deterioração das sementes de amendoim é acompanhado de quebra de moléculas de clorofila, e consequentemente, em alterações em propriedades fluorescentes das sementes. Do ponto de vista prático, a imagem de fluorescência de clorofila pode ser utilizada com sucesso para discriminar lotes de sementes de alto e baixo vigor, de forma rápida, precisa e não destrutiva*

**Keywords:** *vigor de sementes; análise de imagem; métodos não destrutivos.*

## La fluorescencia de la clorofila como nuevo marcador de la calidad de la semilla de maní

### RESUMEN

*Los avances recientes en las tecnologías de imágenes ópticas han contribuido a un análisis rápido, preciso y no destructivo en el contexto de la calidad de las semillas. Este trabajo tuvo como objetivo verificar el potencial de las imágenes de fluorescencia de clorofila como un nuevo marcador para analizar el potencial fisiológico de las semillas de maní. Las señales de fluorescencia de la clorofila ayb se detectaron en las combinaciones de excitación / emisión de 630/700 nm y 405/600 nm, respectivamente, utilizando semillas envejecidas artificialmente durante 0, 16, 24 y 48 horas. Las imágenes fueron capturadas con el equipo VideometerLab4 (Videometer A / S, Herlev, Dinamarca), versión de software 5.4.6. Los datos se compararon con pruebas analíticas tradicionales utilizadas para evaluar el desempeño fisiológico de las semillas de maní, tales como pruebas de germinación, conductividad eléctrica, emergencia de plántulas e índice de emergencia. Los resultados revelaron que la intensidad de fluorescencia de la clorofila ayb fue menor para las semillas menos vigorosas. Por lo tanto, el proceso de deterioro de las semillas de maní se acompaña de una descomposición de las moléculas de clorofila y, en consecuencia, cambios en las propiedades fluorescentes de las semillas. Desde un punto de vista práctico, la imagen de fluorescencia de clorofila se puede utilizar con éxito para discriminar lotes de semillas de alto y bajo vigor, de forma rápida, precisa y no destructiva.*

**Palabras clave:** *vigor de la semilla; análisis de imagen; métodos no destructivos.*

### Introduction

Peanut (*Arachis hypogaea* L.) is an oilseed of great economic importance in Brazil, in which São Paulo State has contributed with around 70-80% of the national production. Peanut seeds represent a source of energy because they are rich in oil, proteins and vitamins.

The use of high-quality seeds is of great importance as they are more resistant to stressful conditions, ensuring a uniform plant stand in the field. The physiological potential of peanut seeds is traditionally evaluated by germination and vigor tests (QUEIROGA *et al.*, 2011), which are destructive and time-consuming, and the results are subjective because they depend on the analyst's interpretation. Meanwhile, the imaging technologies are non-destructive, providing results quickly and accurately.

Chlorophyll is present in seeds during maturation, but it also plays an important role in maintaining the quality of mature seeds (GRULICHOVÁ *et al.*, 2018). Furthermore, chlorophylls are fluorescent when they are exposed to light, with wavelengths in the red range of the electromagnetic spectrum (GALLETTI *et al.*, 2020).

Therefore, the objective of this work was to assess the capacity of chlorophyll fluorescence image-based technology as a new marker for quality characterization of peanut seeds with different vigor levels..

## Material and methods

This study was conducted with peanut seeds from IAC 503 cultivar. To obtain classes of seeds with different vigor levels, the seeds were artificially aged for 0, 16, 24 and 48 h. For germination, four replications of 50 seeds were placed into rolled paper towels moistened with distilled water, kept at 25 °C. The percentage of normal seedlings was measured on the 7th and 14th days after sowing.

The electrical conductivity test was conducted with four repetitions of 50 seeds previously weighed were placed in plastic containers with 75 mL of distilled water at 25 °C for 24 h. After this period, the electrical conductivity of the imbibition solution ( $\mu\text{S cm}^{-1} \text{g}^{-1}$ ) was determined. For seedling emergence and emergence speed index, four repetitions of 50 seeds were sown in plastic trays containing sand moistened to 40% of its water holding capacity. The seedling emergence was monitored daily during 14 days to determine the emergence speed index and the final percentage of emerged seedlings.

The chlorophyll a and b fluorescence images were captured and analyzed using a Videometerlab4 equipment (Videometer A/S, Herlev, Denmark) (**Figure 1**) and its software version 5.4.6. The fluorescence was detected in the excitation/emission combinations of 630/700 nm and 405/600 nm for chlorophyll a and b, respectively. Subsequently, the images were transformed using a normalized canonical discriminant analysis (nCDA) algorithm.



**Figure 1.** VideometerLab4 equipment used to analyze fluorescence images of chlorophyll a and b in peanut seeds with different vigor levels.

The statistical analyses were performed separately for each test by analysis of variance (ANOVA) in a completely randomized design. Means were compared by Tukey's test ( $P \leq 0.05$ ).

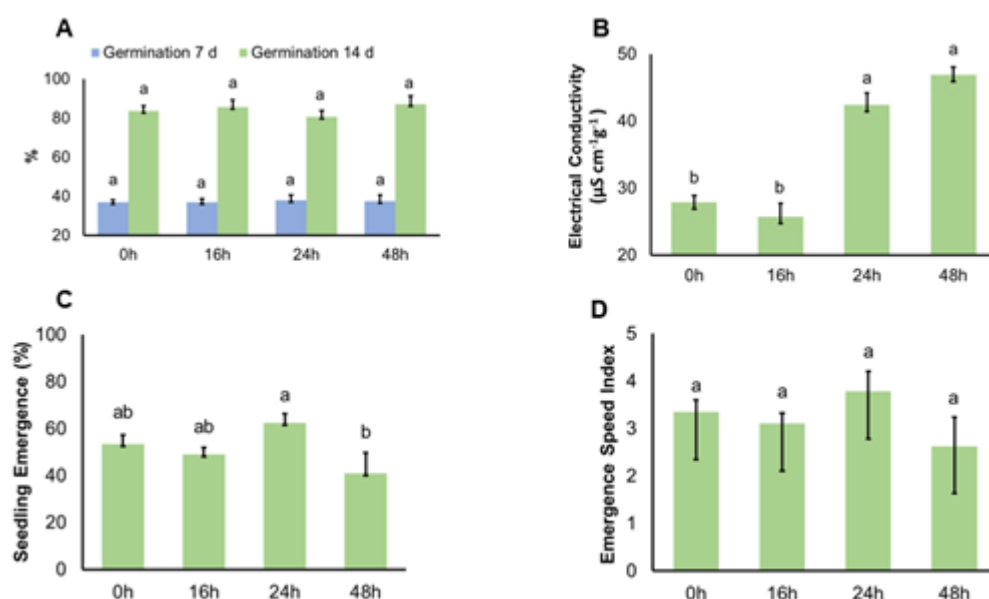
## Results and discussion

De acordo com a análise de variância, verificou-se que de modo geral, entre as variáveis analisadas, que apenas as MMSF e MMSFD ( $p < 0,05$ ) apresentaram efeitos significativos para os tratamentos. Quanto aos produtos aplicados o DHP ( $p < 0,05$ ), a MMSR ( $P < 0,01$ ), a MMSF ( $p < 0,05$ ) e a MMSFD ( $p < 0,05$ ) apresentaram diferenças significativas.

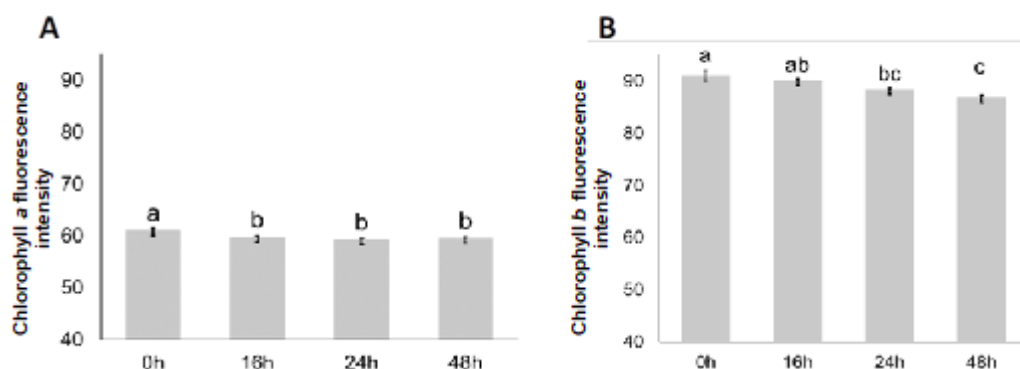
The standard germination test at 7 and 14 days did not indicate statistical differences among the classes of seeds (**Figure 2 A**). However, the electrical conductivity test showed that classes of seeds aged for 24 and 48 h had higher values than those aged for 0 and 16 h (**Figure 2 B**). Higher electrical conductivity is associated with deterioration reactions of cell membranes and, consequently, reduced seed vigor (BARBOZA DA SILVA *et al.*, 2013). Furthermore, the damages caused to the membranes resulted in lower emergence of seedlings grown from seeds aged for 48 h (**Figure 2 C**), although the emergence speed index was similar among treatments (**Figure 2 D**).

Although the standard germination test was not sensitive enough to detect differences among classes (**Figure 2 A**), the chlorophyll fluorescence

technique allowed the discrimination of seeds at different vigor levels, in which higher vigor seeds (0 h) showed higher values for chlorophyll fluorescence *a* and *b* (Figure 3 A, B).

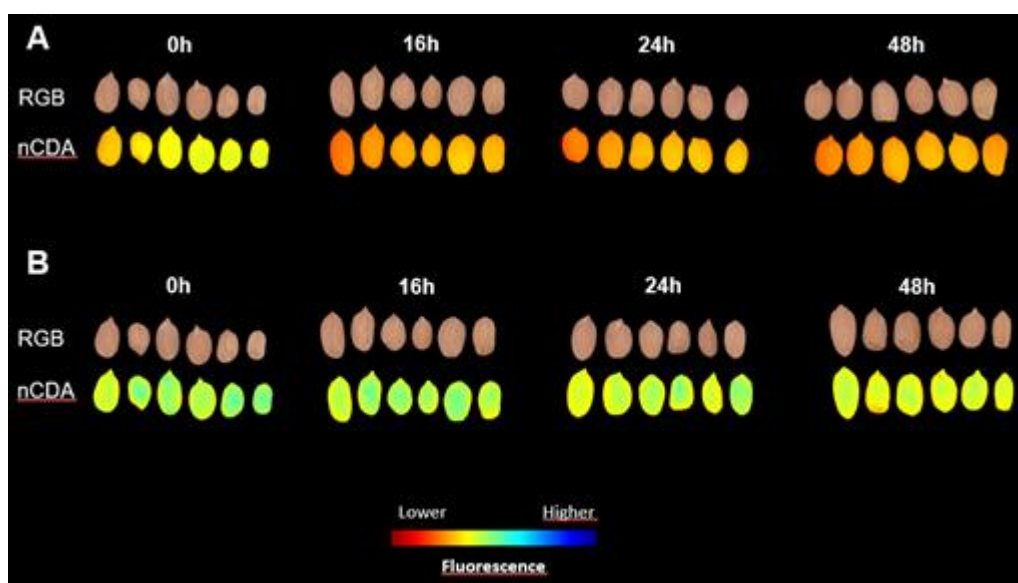


**Figure 2.** Germination and vigor tests in peanut seeds artificially aged for 0, 16, 24 and 48 h. **(A)** Germination at 7 and 14 days; **(B)** Electrical Conductivity; **(C)** Seedling Emergence; **(D)** Emergence speed index. Means with common letters do not differ significantly ( $P < 0.05$ ).



**Figure 3.** **(A)** Chlorophyll *a* fluorescence at excitation/emission combination of 630/700 nm; **(B)** Chlorophyll *b* fluorescence at excitation/emission combination of 405/600 nm. Means with common letters do not differ significantly ( $P < 0.05$ ).

Additionally, the differences in the fluorescence of chlorophyll *a* and *b* were also detected evaluating the image patterns of the seeds (Figure 4), in which the pixels of aged seed classes showed lower values (i.e., lower chlorophyll fluorescence intensity) than non-aged seeds.



**Figure 4.** RGB (Red-Green-Blue) images of peanut seeds artificially aged for 0, 16, 24, and 48 h and chlorophyll *a* fluorescence images captured at 630/700 nm excitation/emission combination (**A**), and chlorophyll *b* fluorescence images captured at 405/600 nm excitation/emission combination (**B**) after transformation by the nCDA algorithm, in which each pixel in the image is represented by a unique value that corresponds to the chlorophyll fluorescence intensity; higher pixel values indicate higher chlorophyll fluorescence intensity.

Therefore, the deterioration process in peanut seeds is accompanied by a breakdown of chlorophyll molecules, and consequently, changes in the fluorescent properties of the seeds. Similarly, Jalink et al. (1999) reported that lower chlorophyll fluorescence signals in mature tomato seeds were related to lower germination percentages, probably because chlorophylls play an important role in seed stress tolerance (NAKAJIMA *et al.*, 2012).

Hence, this pioneering study indicates that chlorophyll fluorescence imaging technique can be an innovative, practical, efficient and non-destructive method for quality classification of peanut seeds. This method can be useful and interesting for seed-analysis laboratories, breeding programs and food industries, as image analysis techniques provide results quickly and accurately. Furthermore, our findings represent a technological advance in seed biology and provide insights into the possibility of real-time diagnosis of the physiological status of peanut seeds, without relying on human vision. However, we strongly support in-depth studies with different seedlots from

different cultivars to strengthen the methodology of applying chlorophyll fluorescence imaging in the quality inspection of peanut seeds..

## **Conclusions**

The chlorophyll fluorescence imaging technique is a promising tool for rapid, accurate and non-destructive assessment of the physiological quality of peanut seeds, and it can be successfully used to discriminate high-and low-vigor seed lots in the agricultural and food industry.

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