EVALUATING THE EFFICIENCY OF THE BIOSPECKLE LASER GRAPHICAL OUTCOMES IN SEEDS

José Luís Contado
Federal University of Lavras, Food Science Department, Campus, ZIP Code 37.200-900, Lavras, MG, Brazil
Corresponding author: jcontado@ufla.br

Roberto Alves Braga Júnior
Federal University of Lavras, Agricultural Engineering Department, Campus, ZIP Code 37.200-900, Lavras, MG, Brazil

Laura Machado de Oliveira
Federal University of Lavras, Food Science Department, Campus, ZIP Code 37.200-900, Lavras, MG, Brazil

Alcebíades Fogaça de Souza Sobrinho
Federal Institute of Amapá, BR 210 KM 103, ZIP Code: 68997-000, Porto Grande, AP, Brazil.

Roney Alves da Rocha
Federal University of Lavras, Food Science Department, Campus, ZIP Code 37.200-900, Lavras, MG, Brazil

Abstract: Seeds are a fundamental input in food production programs, as well as in the restoration and conservation of ecosystems. Consequently, their physiological potential, specifically vigor, has become a key parameter for evaluating germination capacity and seedling establishment. Biospeckle Laser (BSL) technology has been increasingly used in seed vigor studies as a complement of the traditional tests. The objective of this work was to evaluate the graphical outcomes provided by the BSL in seed vigor analysis. Seeds from commercial species (maize, soybean and common bean) and native forest species (purple trumpet tree and yellow poinciana) were analyzed. Seeds were illuminated by a 632 nm laser at zero and four hours of imbibition, and the speckle images were assembled and analyzed using four graphical outcomes: the Generalized Differences (GD), the Graphical Average Value of the Differences (GraphAVD), the graphical Inertia Moment (GraphIM), and the Fujii method. The graphical outcomes represent the activity maps of the seeds, and the results presented a different response provided by the Fujii method in comparison with the other methods. The graphical analysis GD, GraphAVD, and GraphIM proved suitable for assessing seed vigor after four hours of imbibition. Among these, the GraphAVD highlighted the ability to simultaneously detect regions from low to high biological activity including the areas with medium activity. Additionally, it was possible to distinguish the viable from the non-viable purple trumpet tree seeds.

Keywords: vigor, germination, dynamic laser speckle, images.

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INTRODUCTION

Seeds are a vital role in the maintenance and perpetuation of plant species and are a fundamental input in food production programs, as well as in the restoration and conservation of ecosystems. Therefore, the physiological, physical, sanitary, and genetic quality of seeds directly influence the production of healthy and viable seedlings, ensuring the adaptability and long-term persistence of plant populations (BEWLEY et al., 2013). The germination process is the resumption of metabolic activity in the embryonic axis, leading to the protrusion of the radicle through the seed coat (BEWLEY et al., 2013). This process is considered a critical phase in the plant life cycle, as it is influenced by multiple intrinsic and extrinsic factors that interfere with physiologic processes (REED; BRADFORD; KHANDAY, 2022; POWELL, 2022)

Therefore, the physiological potential of a seed, specifically its vigor, becomes a critical parameter for successful germination and seedling establishment. Various methods are employed to assess seed viability, including traditional tests as germination, tetrazolium, accelerated aging, cold test, among others (ISTA, 2021). These analyses are typically conducted in laboratory settings under controlled conditions and require large quantities of seeds, often through destructive procedures. Results are obtained only after several days and may be influenced by the subjectivity of the analyst (KRZYZANOWSKI et al., 2020; RODRIGUES; GOMES-JUNIOR; MARCOS-FILHO, 2020; ISTA, 2021).

Optical techniques have been increasingly employed in seed analysis due to their non-invasive and objective nature, as well as their ability to provide results in near real-time. Among these, Dynamic Laser Speckle, or biospeckle laser (BSL), has emerged as alternative (SINGH et al., 2022; XING et al., 2023; BRAGA JR et al., 2025). BSL has become a valuable tool across various fields of knowledge, particularly in biology, agronomy and biomedicine. It is based on an optical interference phenomenon that occurs when a material is illuminated by coherent light, which is scattered across (BRAGA JR et al., 2025), resulting in a laser speckle pattern in a plan of observation apart from the surface. When a dynamic surface is illuminated over a period of time, continuous changes in the speckle pattern can be observed (BRAGA JR et al., 2025).

These laser speckle patterns can be analyzed through digital image processing techniques (SINGH et al., 2022; XING et al., 2023; BRAGA JR et al., 2025), allowing for the assessment of the activity of the illuminated material by tracking changes in the speckle patterns over time. A range of computational tools is available for analyzing the temporal history of speckle patterns, and the results can be presented as either graphical or numerical outcomes. In graphical analyses, image processing techniques are applied to speckle images to generate activity maps that represent the spatial variability of biological activity. In contrast, numerical analyses assign quantitative values to biological activity. Among the most commonly used computational tools for seed analysis during germination are the Generalized Differences (GD) (ARIZAGA et al., 2002), the Graphical Absolute Value Differences (GraphAVD) (BRAGA; RIVERA; MOREIRA, 2016), the Graphical Inertia Moment (GraphIM) (BRAGA; RIVERA; MOREIRA, 2016) and the Fujii method (FUJII; ASAKURA, 1975).

Specifically in the field of seed analysis, several studies have established correlations between the BSL activity and seed quality, viability, deterioration and germination potential. One of the first works was carried out *Phaseolus vulgaris* (BRAGA; SOUZA, et al., 2001; BRAGA; DAL FABBRO, et al., 2003). Digital image analysis for determining viability and vigor has already demonstrated its efficiency for various species, such as beans (GOMES-JUNIOR, 2014), maize (AZEVEDO, 2023), soybeans (BRANDAN et al., 2021), vegetables like broccoli (ABUD; CICERO; GOMES, 2017) and onions (GONÇALVES; CICERO; ABUD, 2017). One can observe the application in forest seeds, such as *Tabebuia impetiginosa* (FRACAROLLI et al., 2010) and *Anadenanthera colubrina* (CONTADO; SILVA; BRAGA JUNIOR, 2020). The application of the BSL technique in seeds and other biological material is carried out by means of a range of image processing with distinct mathematical and statistical approaches. Therefore, there is a demand for standardization of the BSL image processing in order to stabilish reliable protocols in each application.

The objective of this work was to evaluate four graphical outcomes of the BSL in seed analysis. Seeds from commercial (maize, soybean and bean) and native forest species (purple trumpet tree and yellow poinciana) were analysed.

MATERIAL AND METHODS

Seeds samples

The seeds analysed by the BSL technique were: *Zea mays* (maize), *Glycine max* (soybean) and *Phaseolus vulgaris* (common bean) representing the commercial species, *Handroanthus impetiginosus* (purple trumpet tree) and *Peltophorum dubium* (yellow poinciana) representing the native forest species. The samples (8 replicates to each variety) were

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disinfected in a 1% sodium hypochlorite solution for 1 minute, rinsed ten times with demineralized water, and then transferred to the illumination setup.

Illumination of samples

The illumination system consisted of a helium-neon (HeNe) laser light source (632 nm, 10 mW), a optical density filter and a beam-diffuser. A CCD camera (640 × 486 pixels, 1/60 s) with a macro-zoom lens (F/11) and a computer complete the backscattering configuration that was placed in an optical table with an anti-vibrational system. The seeds with intact coats were illuminated in two moments: before the imbibition (0 hour) and after four hours of imbibition (4 hours).

After disinfestation, the seeds were subjected to the imbibition process by placing four seeds per Petri dish, each containing three layers of germination paper moistened with water (2.5 times the papers weight), and incubated at 25 °C in a germination chamber. After four hours of imbibition, the seeds were illuminated again with the same image assembling process. The seeds were then returned to the BOD and remained there until the emergence of the cotyledonary or primary leaves, confirming that the seeds had developed into seedlings.

The speckle pattern images were acquired in a time-rate of 12 fps in a total of 128 frames for each seed.

Image processing

The speckle images (at 0 and 4 hours) were analyzed using four graphical outcomes.

The Generalized Differences (GD) (ARIZAGA et al., 2002) is presented in Equation 1.

$$GD = \sum_{k=1}^{N-1} \sum_{l=1}^{N-k} |I_k - I_{k+l}| \tag{1}$$

where $I(x,y)$ is the k_{th} image varying from $k=1$ to $N-1$, in a recursive way, with l varying from 1 to $N-k$, and N is the total number of images.

The *GraphAVD* is based on the Absolute Value of Differences implemented to each pixel of the image sequence (BRAGA; RIVERA; MOREIRA, 2016) (Equation 2).

$$AVD = \sum_{ij} \frac{COM(i,j)}{\sum_{lm} COM(l,m)} |i-j| \tag{2}$$

where *COM* is the cooccurrence matrix, and *i, j, l* and *m* the lines and columns of the *COM*.

The *GraphIM* is based in the Inertia Movement implemented for each pixel of the image sequence (BRAGA; RIVERA; MOREIRA, 2016) (Equation 3).

$$IM = \sum_{ij} \frac{COM(i,j)}{\sum_{m} COM(i,m)} (i-j)^2 \tag{3}$$

where *COM* is the cooccurence matrix, and *i, j, l* and *m* the lines and columns of the *COM*.

The Fujii (FUJII; ASAKURA, 1975) is presented in Equation 4.

$$FUJII = \sum_{k=1}^{N-1} \frac{|I_k - I_{k+l}|}{I_k + I_{k+l}} \tag{4}$$

where $I(x,y)$ is the k_{th} image varying from $k=1$ to $N-1$, weighted by the sum of the two images, and N is the total number of images.

RESULTS AND DISCUSSION

The BSL graphical functions in five species presented different results in the two moments of observation: 0 and 4 hours.

Results in maize seeds (*Zea mays* L.)

In the Figure 1, the four maps of activity of maize seeds at 0 and 4 hours of imbibitions are presented where the pseudo-color represents the levels of activity from low (blue) to high (red). The scale of the maps was set to each function to present the best representation of the activity level. After the setting of the scales, they were fixed comparing the two moments (0 and 4 hours).

The maps of activity provided by the functions showed, in both analysis times, the expected low activity of the seeds at 0 hour, that

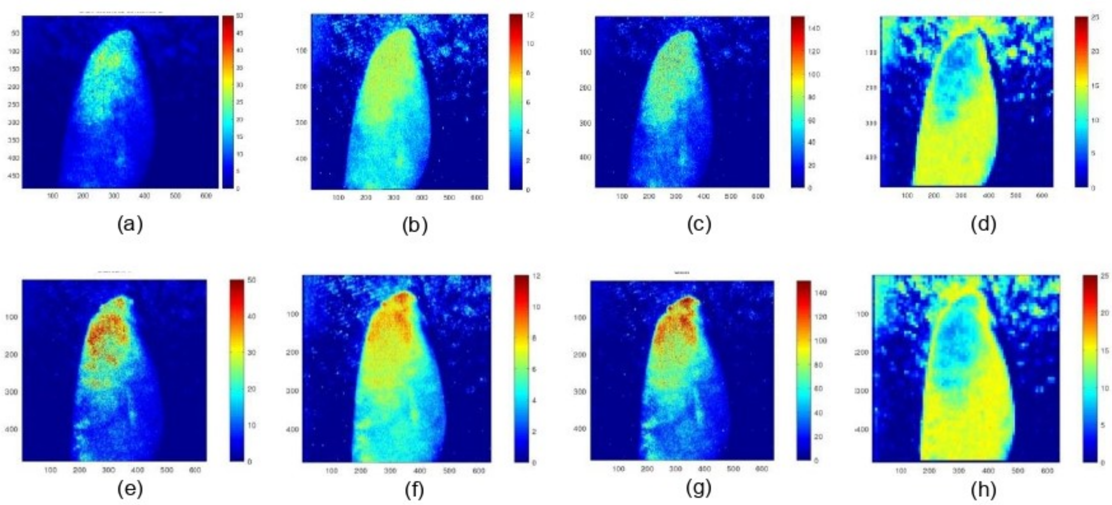

Figure 1: Graphical outcomes of the four biospeckle functions in maize seeds at 0 hour of imbibitions by: a) GD; b) GraphAVD; c) GraphIM; d) Fujii, and at 4 hours of imbibitions by: e) GD; f) GraphAVD; g) GraphIM; h) Fujii.

is due to the maize seeds being in a quiescent state.

The activity map addressed by Fujii function presented a different result if compared with the three other functions . Despite the Fujii method is well known and adopted in BSL analysis, we observed that the map of activity did not display properly the physiological areas within the seeds. The weighting process provided by the denominator is the reason for the poor outcome of Fujii in seeds. For exemple, the high activity presented by the table wasn't real, just as the locations inside the seed presented as having high biological activity by Fuji do not coincide with those presented by GD, GraphAVD and GraphIM. The ability of these three graphical outcomes to identify biological activity at the beginning of imbibition was also observed by Contado, Silva, and Braga Junior (2020).

Results in four species

The BSL graphical outcomes for soybean, common beans, purple trumpet tree and yellow poinciana seeds after 4 hours of imbibition are presented in Figure 2.

The maps at time 0 hour for all seeds were not showed since the low activity is consistent with the quiescent state and did not displayed properly the physiological areas of these species, as presented as example for the maize seed.

The same reason is related to the Fujii function, since its map of activity presented low or conflituous information about the seeds observed in Figure 1.

In the same way of the maize, the maps of activity for common bean, soybean, purple trumpet and yellow poinciana seeds presented high activity in the embryonic axis using the GraphIM and the GD functions. While the GraphAVD was able to be sensitive to the intermediary activity over the whole seed, besides the identification of the high activity in the embryonic axis. It is relevant to observe that in all cases the seeds were illuminated with their coats and without invasive adjustments as observed in *Tabebuia impetiginosa* (FRACAROLLI et al., 2010). Instead of the graphical outcomes one can observe that many works dealt with seeds vigor using numerical analysis of the BSL (SINGH et al., 2022; XING et al., 2023; BRAGA JR et al., 2025).

Dead and live Seed – purple trumpet tree Case

After the development into seedlings we could observe that the purple trumpet seeds presented different conditions, where two samples did not germinated at all.

In Figure 3, we can observe the maps of activity provided by the BSL in one viable and non-viable seeds. The graphical outcomes of the purple trumpet showed in non-viable seeds a

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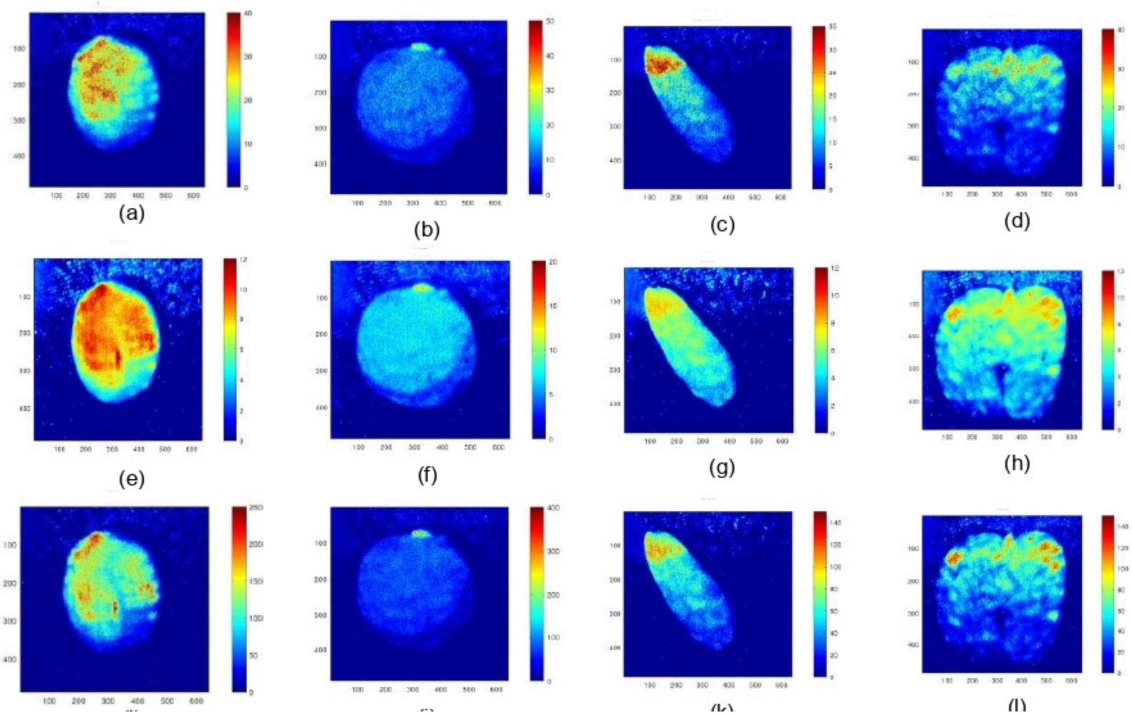

Figure 2: Graphical outcomes of the four biospeckle functions: in GD: a) soybean, b) common bean, c) purple trumpet tree, d) yellow poinciana; in GraphAVD: e) soybean, f) common bean, g) purple trumpet tree, h) yellow poinciana; in GraphIM: i) soybean, j) common bean, k) purple trumpet tree, l) yellow poinciana;n GraphAVD.

lower activity than in the case of viable seeds. Braga, Dal Fabbro, et al. (2003) reported the ability of BSL to detect dead tissue.

The visual impression can be confirmed by the numerical outcomes provided by the AVD function applied in the embryonic axis. While the biospeckle activity using the non-viable seeds presented a mean value of 5.79, the viable seeds was of 7,95. Therefore, we observe the viability of using the BSL in a graphical outcome to forecast the potential of germination just after 4 hours the imbibitions, as previously published by Contado, Silva, and Braga Junior (2020).

CONCLUSION

This work presented the ability of the graphical outcomes of biospeckle laser to identify the activity of seeds after four hours of imbibition. The four graphical outcomes evaluated presented poor results in using the Fujii method. The Generalized Differences (GD), the Graphical Average Value of the Differences (GraphAVD) and the Graphical Inertial Moment (GraphIM) methods were able to identify highest activity in the embryonic axis just after four hours of imbibition. The GraphAVD presented a map of activities varying from low to high activity including the observation of areas with medium activity. The GD, the GraphAVD and the GraphIM outcomes were able in distinguishing viable from non-viable purple trumpet tree seeds, revealing a decrease in biological activity in non-viable seeds.

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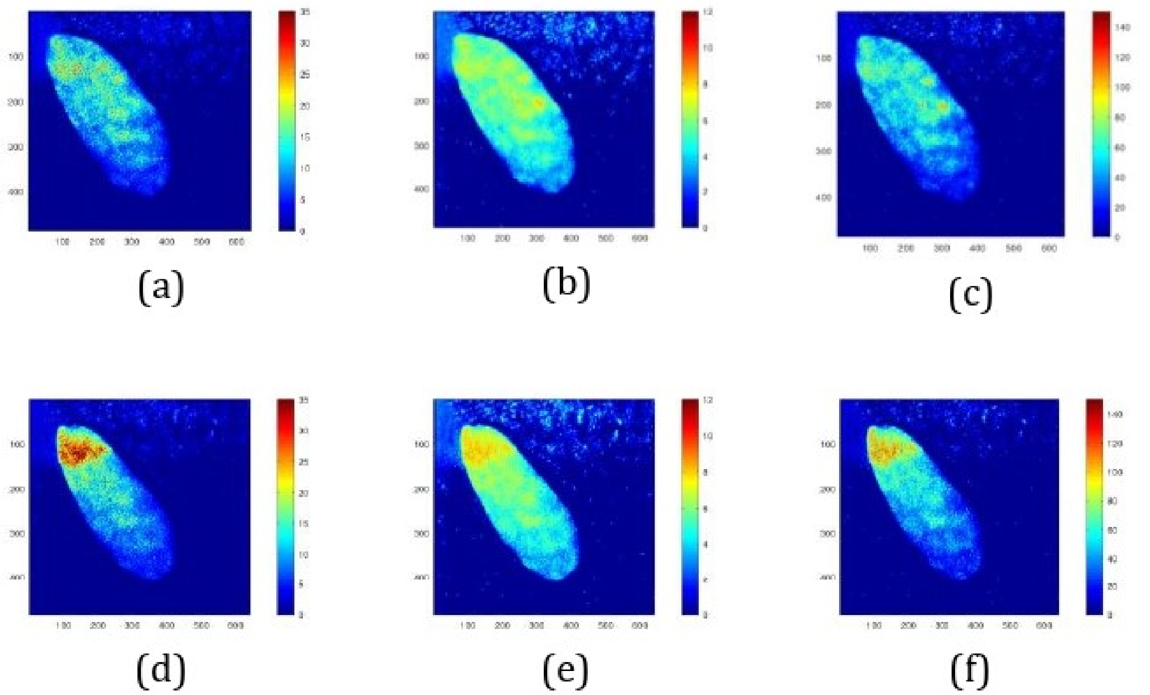

Figure 3: Graphical outcames of the 3 BSL analyses in 2 purple trumpet tree seeds at 4 hours of imbibitions: a) non-viable seed in GD, b) non-viable seed in GraphAVD, c) non-viable seed in GraphIM, d) viable seed in GD, e) viable seed in GraphAVD, f) viable seed in GraphIM.

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