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Optical properties of maize seeds

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A b s t r a c t. The aim of this study was to determine the optical properties of raw and dyed maize seeds using photoacoustic spectroscopy. The experimental photoacoustic spectroscopy results are verified by using transmission photometry and the Beer law. The obtained values of the optical absorption coefficient for raw and dyed seeds only change at superficial level and also depend on the incident wavelength of light. The experimental results show an increment of the superficial optical absorption coefficient values in the dyed seeds, when compared to the raw seeds, of 45, 37, and 8.9% at 633, 635 and 650 nm, respectively. The results show that the photoacoustic spectroscopy technique can be useful in the optical characterization of seeds in the agricultural sector.

K e y w o r d s: maize, optical absorption coefficient, photoacoustic spectroscopy

INTRODUCTION

Phytochromes are photoreceptive signaling proteins responsible for mediating many light sensitive processes in plants, including seed germination seedling de-etiolation and shade avoidance (Levskaya *et al*., 2009). Phytochrome is located in the embryo (Hernandez *et al*., 2010) and is the basis of the mechanisms of laser biostimulation with low power (Samuilov and Garifullina, 2007). Seeds can be pigmented, which alters the spectral composition of the light in the seed. Hence, when interpreting light responses of seeds, the optical properties of them must be taken into account.

Lasers have been used in different phenological stages of plants to carry out processes of biostimulation. Different lasers have been applied within the range of visible and ultraviolet light as the nitrogen laser 337.1 nm (Govil *et al*., 1985), a combination of nitrogen laser (337.1 nm) and argon (514.5 nm) (Govil *et al*., 1991), He-Ne laser (632.8 nm) (Osman *et al*., 2009) and in recent years diode lasers at 650 and 660 nm (Benavides *et al*., 2003; Hernandez *et al*., 2004; 2006; 2008a; 2009; 2010).

The beneficial effect of biostimulation of seeds on the stages of germination, seedling development and production has been proven by numerous studies (Jun Lin *et al*., 2007; Gładyszewska, 2006; Podleśny, 2002; 2007). Effects related to low-intensity laser light punctuate the importance of the characteristics of the irradiation parameters: wavelength (λ, nm), irradiation time (t, s) and surface density of energy (DSE, J cm^{-2}) to produce favorable effects of biostimulation (Hernandez *et al*., 2006) in the establishment of plants.

The laser most reported in the literature for biostimulation processes is the He-Ne laser. To carry out the seed biostimulation, these are irradiated with low-power laser light before sowing, alone or in combination with photosensitizers. There is an optimal intensity of the laser beam to obtained positive results, which is different for seed without dye and seed with dye (Hernandez *et al*., 2006; 2007; Ouf and Abdel-Hady, 1999). It is necessary to understand the cause of this difference and quantify the optical energy absorbed at various depths of the seed.

Photoacoustic spectroscopy (PAS) is a technique that has been widely used in different areas and applications, from semiconductor to biological materials (Fesquet *et al.*, 1984; Ramon *et al.*, 2001). Obtaining the optical absorption spectra of different samples by this technique has advantages over optical conventional measurements by two fundamental features: first, the scattered light does not alter the

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measurements and second, the sample does not need to be prepared to have a surface with a good optical quality (Lomeli *et al*., 2005); so that seeds can be directly investigated (Hernandez *et al*., 2008b), then PAS appears to be a potential method to study optical properties of seeds (Hernandez *et al*., 2008a; 2009).

The aim of this study was to determine the optical properties of the seeds of a maize genotype for two conditions, raw and dyed seeds (dyed with methyl red), by using photoacoustic spectroscopy and optical transmission measurements.

MATERIALS AND METHODS

Seeds of single-cross hybrid CL-12 x CL-11 were provided by the Mexican Institute of Genetic for the Seed Quality Control (IREGEP) in the present study. The seeds were homogenized by size, shape and colour using the UTHSCSA Image Tool program, version 3.1 (2002), University of Texas. The average mass of the selected seeds was 240 g in 1 000 seeds, measured in four samples of one thousand seeds each one.

The seeds were photosensitized by soaking in a dilution, 1 g of methyl red per liter of water, during 25 min, then these seeds were dried and three seeds were randomly selected from each condition, raw and dyed. These seeds were used to obtain their optical absorption spectra by PAS technique and optical transmission measurement by transversal photometry (making nine measurements per each seed, in each condition and per each technique, having a total of 108 measurements).

The measurements were carried out as a function of the seed thickness, pulled off consecutive layers, to obtain their optical absorption spectrum by PAS and the optical transmission measurement of the consecutive seed thicknesses. From the PAS optical absorption spectra is possible to obtain, by applying the Rosencwaig and Gersho model, the seed optical absorption coefficient (β) at different wavelengths (Hernandez *et al*., 2008b; 2009). Additionally, from β values obtained in different thickness at 632 nm (a He-Ne laser was used in transmission measurements), and applying the Beer law for optical absorption, the results obtained by both methods (PAS and transmission) were compared at this wavelength. The seed optical absorption spectra were obtained in the range of 620-700 nm by using PAS technique. The experimental set-up consisted of a xenon lamp (Oriel) whose beam is focused to pass through a monochromator (Oriel) and the emergent monochromatic light, which is periodically modulated by a mechanical chopper (Stanford Research) at 17 Hz of frequency. The modulated light beam is directed, through an optical fiber, to the window of the photoacoustic cell, so that the light insides on the analyzed sample. The generated signal in the photoacoustic cell is detected by a lock-in amplifier (SR-850) which is interfaced to a personal computer, to record the photoacoustic (PA) signal amplitude and phase as a function of the incident light wavelength. The PAS spectra were always normalized to the signal obtained from charcoal powder. Starting from the depth 0, which means the whole seed with 3 mm thickness was reduced; that is the thickness was reduced eight times and each reduction was 0.3 mm (Fig. 1); these processes of seeds thickness reduction were conducted for both raw and dyed seeds. Then, each seed for each group (raw and dyed seeds) was placed in the photoacoustic cell and the optical absorption spectra of the samples were obtained. These spectra were carried out at the different thickness of the samples, to obtain their optical absorption spectrum in each layer (a total of nine layers of each seed).

The optical absorption coefficient, β, for typical wavelengths of diode lasers (632, 635, 650 and 660 nm), was determined from the PA signal amplitude by using the method proposed by Fesquet *et al.* (1984) and used by Hernandez *et al*. (2008b). The total optical absorption coefficient for thermally thick samples $a_s\ l_s$>1, where a_s is the thermal diffusion coefficient, defined in Eq. (2), and l_s the sample thickness) was obtained from the normalized photoacoustic signal amplitude:

$$\beta = \frac{(a_s)\left\{q^2 + q(2-q^2)^{1/2}\right\}}{(1-q^2)}, \qquad (1)$$

$$a_s = (\pi f / \alpha)^{1/2}. \qquad (2)$$

To obtain a profile of light intensity inside of the seed and therefore the β value from each layer, then q, is obtained by PAS technique as described above, and compared thorough the Beer law (Eq. (3)), with the ratio of the measured transmitted laser intensity (I/I_0) at 632 nm (Atkins, 2001).

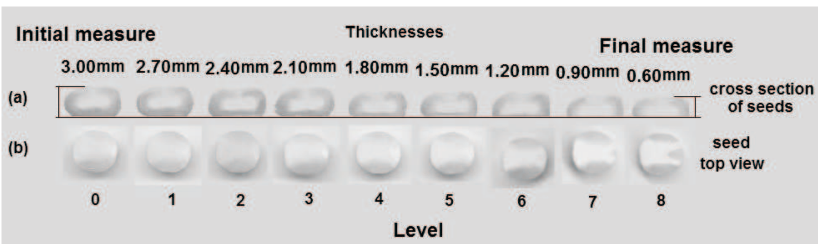

Fig. 1. Thicknesses of the samples (raw and dyed) for the optical measurement sequence, a – cross section of seeds, b – seed top view.

$$I = I_0 e^{-\beta x}, \quad (3)$$

$$I / I_0 = e^{-\beta x}, \quad (4)$$

where: I, I_0 – the light intensity absorbed at the depth x and on the surface of sample.

Using a He-Ne laser, the optical transmission measurements were performed to obtain the ratio of the transmitted laser intensity to the incident intensity (I/I_0). These intensity ratios were obtained, as well as the PAS measurements, at the same depths of each seed. In the experimental set-up of transversal photometry for the light transmission measurements, the light emitted by the He-Ne laser (Uniphase), 0.5 mW of power, is passed through a beam stretcher, which contains two optical lenses. The beam diameter was increased to 5 mm and then impinges on the seed. A certain amount of this light passes through the seed and is detected by an optical power meter (model 45-545, Metrologic Instruments Inc.).

The analysis of variance with PROC GLM procedure was used and the variables that had a significant effect between conditions were applied; also it was performed the Tukey test of multiple comparisons of means with the procedures of SAS (1999).

RESULTS AND DISCUSSION

The average values of the optical absorption coefficient (β), obtained by PAS and by using Eq. (1), are shown in Figs 2 and 3 for raw and dyed seed conditions respectively. In both figures, each curve represents the value, as a function of wavelength, by decreasing the thickness of the seed in 0.3 mm by 8 times, being the level 0 the whole seed. It is possible to observe that these curves of β are similar from layers 1 to 8 for both seed conditions. The optical properties only change at the surface (layer 0- whole seed).

Since only in the surface layer were observed differences in the optical absorption coefficient, we will focus the statistical analysis in this layer. Tables 1 and 2 show the results of analysis of variance and comparison of means for β on the surfaces of seeds (layer 0) studied in conditions raw and dyed, for the case of the wavelengths used in applications of agricultural seed biostimulation process *ie* at 632, 635, 650 and 660 nm. Table 1 shows that there were significant differences in ($p \leq 0.01$) for seed conditions raw and dyed at 632, 635 and 650 nm wavelengths. At 660 nm wavelength there was not statistically significant differences. β for dyed seeds condition, increased significantly, at 632, 635 and 650 nm, when compared with the raw seed condition, increasing the β value for these wavelengths in 45, 37, and 8.9%, respectively. On the other hand, at 660 nm no significant difference was found. In order to validate the use of the PAS technique for optical characterization of agricultural seed, this research compares the results obtained by PAS with the transmission measurements through Beer law, which relates the intensity of transmitted light with the obtained β as described in Eq. (4).

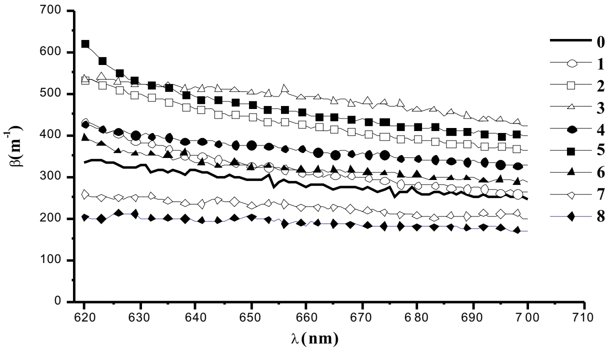

Fig. 2. Optical absorption coefficient (β) as a function of wavelength (λ), raw seeds, 8 different layers (layer 0-whole seed).

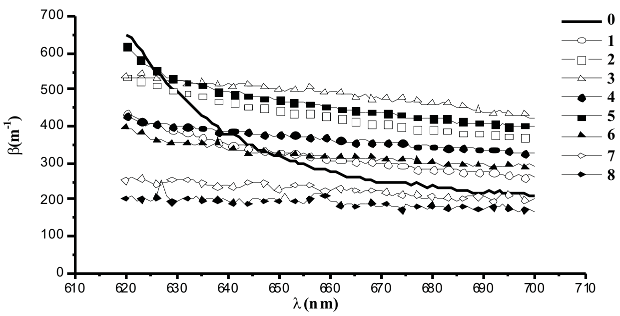

Fig. 3. Optical absorption coefficient as a function of wavelength, dyed seeds, 8 different layers (layer 0-whole seed).

In this way, Fig. 4 shows in continuous (raw seeds) and dashed (dyed seeds) lines the $e^{-\beta x}$ values (right side of Eq. (4)), from β value obtained by the PA measurements, at 632 nm, for different x thicknesses of seeds (where the minimum thickness of seed corresponds to level 8 and the maximum thickness corresponds to zero level according, Fig. 1). The wavelength at which the β value was taken (632 nm), corresponds to the He-Ne laser wavelength, used in the optical transmission measurements (I/I_0, left side of Eq. (4)), these transmission measurements, performed at same thicknesses of seeds to obtain $e^{-\beta x}$ values, are shown in Fig. 4.

Figure 4 shows a good agreement between the $e^{-\beta x}$ results and I/I_0 measurements in the range of the smaller thicknesses of the seeds for both seed conditions (raw and dyed). In the case of the bigger thicknesses of the seeds some differences between the $e^{-\beta x}$ results and I/I_0 measurements are evident. These differences can be due mainly to the fact that the samples become more inhomogeneous in the case of the bigger thicknesses; then there is a higher light dispersion in the seed and also more reflection could appear.

T a b l e 1. Analysis of variance of for typical wavelengths of laser light applied as presowing treatment

Source of variation	D.F.	β at λ = 633 nm	β at λ = 635 nm	β at λ = 650 nm	β at λ = 660 nm
Repetitions	2	738.1	697.69352	103.45	27.75
Seed condition	1	28868.8**	20037.1 **	1019.81**	1.55
Error	2	68.02	20.9	0.676868	8.68
R^2		0.99	0.99	0.99	0.766678
CV (%)		2.14	1.23	0.267853	1.065884
Mean		385.1	371.46	307.1539	276.4329

R^2 – coefficient of determination, CV – coefficient of variation, D.F. – degree of freedom, β – optical absorption coefficient of maize seeds, **significant differences at the 1% probability of error.

T a b l e 2. Comparison of the mean of the variables obtained by spectroscopy photoacoustic (PAS)

Seed condition	β (λ = 633)	β (λ = 635)	β (λ = 650)	β (λ = 660)
Dye seed	454.383a	429.246a	320.1911a	276.942a
Raw seed	315.654b	313.669b	294.1167b	275.924a
HSD	28.973	16.062	2.89	10.351

Mean values with the same letters are statistically equal (Tukey, α = 0.01), β (m^{-1}) - optical absorption coefficient of maize seeds, λ(nm) – wavelength, HSD – honestly significant difference.

These results are important in the laser light applied in different phenological stages of plants to carry out processes of biostimulation, for example the laser light is applied in combination with dye to inhibit the mycoflora associated with the seed; this can be external or internal. Thus, the optimal doses to eliminate pathogens vary if using a dye (methyl red) or not because there is greater absorption of light in the first layer of the seed with dye, in agreement with the results of the present research; in this way, optimum levels of irradiation of seeds vary. Ouf and Abdel-Hady (1999) noted that the effects of laser irradiation increased when the seeds was dyed, pre-irradiation, in the germination and mycoflora tests when compared with seeds without a photosensitizer.

Biostimulation implies the irradiation of seeds by using low-power laser. The seeds can be in different conditions; for example photosensitized with red methyl or other photosensitizers. Hernandez *et al*. (2006) found that the optimal parameters of irradiation to improve the vigor of maize seed, with and without photosensitizer, are similar in irradiation time and wavelength, and only the optimum laser intensity level of laser light was different. Also, studies about pre-treatment laser light on maize seed found that seeds with methyl red dye required, to improve their seed vigor, a laser intensity of 20 mW cm^{-2} and seeds without dye required 10 mW cm^{-2} of laser intensity (Hernandez *et al*., 2007), once again the superficial light absorption due to a photosensitizer becomes important.

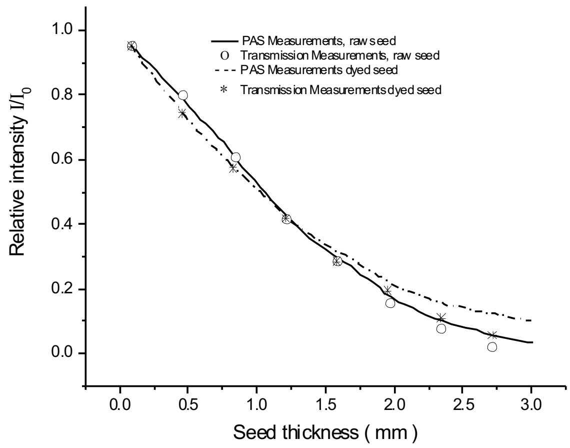

Fig. 4. $e^{-\beta x}$ obtained values in continuous (raw seeds) and dashed (dyed seeds) lines. The optical transmission measurements (I/I_0) are shown by open circles (raw seeds) and asterisks (dyed seeds).

In this research can be seen that is important to determine the optical absorption coefficient. It has been found that the β value of the seeds only change at superficial level, when compared both conditions of seeds used in the present study (raw and dyed). It was showed that photoacoustic spectroscopy can be considered a suitable technique for optical characterization of maize seed.

CONCLUSIONS

1. Photoacoustic spectroscopy is a suitable method to measure in maize seeds.

2. The results of this study indicated that dyed seeds increased their optical absorption coefficient only at the surface layer comparing to raw seeds.

3. Using Beer law it was found a good agreement, at the smaller thicknesses of the seeds, between the $e^{-\beta x}$ results, obtained from PAS method, and I/I_0, obtained from direct optical transmission measurements.

4. Some differences appear in the case of the bigger thicknesses of the seeds due mainly to the fact that these samples are more inhomogeneous and then increase the scatter of light and also more reflection could appear.

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