v. 3, n. 2, 2023

Journal of
Agricultural
Sciences
Research

USE OF MAGNETIC STIMULUS TO PROMOTE EFFICIENT CORN SEED (ZEA MAYS) GERMINATION EVALUATED BY BIOSPECKLE LASER USING IMAGES AND TRADITIONAL METHODS

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Abstract: The effects of the magnetic field on living organisms, especially on plants, have aroused interest of several scientists. The first research dates back to the 1930s, from this time to date, numerous works were conducted focusing on different aspects of plant development. In this research, corn seeds (Zea mays) were submitted to a magnetic field of constant intensity and values 125 mT and 250 mT for a same time interval of 1 hour. The images obtained by the Biospeckle Laser (BSL) were processed and then the results of activity levels were later compared with the data obtained by the traditional seed analysis. The dry mass of maize seedlings was measured. It was observed a close relation between the greater activity obtained by the BSL, of those seeds submitted to the field of 250 mT, with the dry mass. This work intends to use the biospeckle laser in the verification of the effects of the magnetic field on maize (Zea mays) seeds in relation to the germinative activity of the same.
Keywords: Germination, laser, dry mass.

INTRODUCTION

We are daily exposed to the terrestrial geomagnetic field which can cause several effects on living organisms, mainly vegetables and, for this reason, it has increased the interest of many researchers. First studies on effects of the magnetic field in plants using the magnetic system were performed by Savostin in 1930.

Fischer et. al. (2004) studied the alternating effects of the eletromagnetic field (variant on time) using a frequency of 54 Hz applied to sunflower seeds and to wheat seedlings, which made possible to obtain for sunflower seeds a high rate of germination, meanwhile wheat seedling had a higher plant growth and, futhermore, a higher seed mass for each plant.

Overall, the effects of the static magnetic field on the metabolism and growth of different plant species were reported by several researchers. Harichand et al. (2002) studied the effects of seed pretreatment by using magnetic fields on wheat seeds and found results with good throughput of the plant culture to the wheat species and improvement on germination and vitality of seeds.

Aladjadjiyan et al. (2003) submitted tobacco seeds to a magnetic field with the intensity of 0,15 mT[1] and verified that the magnetic field stimulates the development of the germ and leads to an increase on the energy spent for germination and of germination.

However, Fischer et al. (2004) stated that the magnetic field effects on vegetables are not restricted only to the efficiency of seedling growth. The magnetic pretreatment is widely used to increase, for example, the vitality of seeds and the throughput of seedlings, Ahmet, 2003; Penuelas, 2004.

Several studies have demonstrated that the magnetic fields can influence over a large number of cellular functions, however the exact mechanism of this interaction with live cells is not clear, Yano et al. (2004)

The main problem in using a magnetic field regards a protocol development to improve seed germination considering the temporal lack between the application of the stimulus and the validation of the effect after germination. There are some seeds in which the germination process vary from 90 to 120 days Went, (1957), depending on the temperature as in the case of coffee seeds (*Coffea arabica* L.). Thus, it is important to search alternatives aiming to reduce time for germination that, in some situations, corresponds to half time spent to produce coffee seedlings.

Therefore, it is necessary to circumvent the limits imposed by the time for germination, by using an alternative way to evaluate the effects on the germination process in early

1 mT-mili Tesla unit of magnetic field in the International System of Measurements. It is equivalent to the thousandth part of 1 Tesla.

moments, after the magnetic stimulus. Efforts for decreasing time would improve the combination of intensity for the magnetic field and time of magnetization, and will create ideal conditions to set protocols for using different seeds.

Flórez et al. (2007) exposed maize seeds to two magnetic field intensities, 125 mT and 250 mT respectively, during six intervals considering the following different times: 1 min, 10 min, 20 min, 1 hour, 24 hours and a continuous exposition until a complete development of the seedling. They found out that seedlings originated from seeds under magnetic treatments had developed more in dimensions than control seedlings. According to authors, these differences were more significant to the period of 24 hours considering both magnetic intensities at 125 and 250 mT.

Majd and Shabrangi (2009) submitted lentil seeds to magnetic fields in the intensities of: 0,06 T; 0,12 T; 0,18 T; 0,24 T; 0,30 T and 0,36 T considering the time intervals of 5, 10 and 20 minutes. They concluded that the optimum range for germination (tax of 33,7%) are the magnetic field intensity from 0,18 to 0,24 T considering time expositions ranging from 5 to 20 min.

Hozayn, Mahdy and Adbel-Rahman (2015) made experiments with onion seeds, exposing them to doses of magnetic inductions from 0,03 to 0,06 T, considering time intervals of 30, 60 and 90 minutes. Data allowed to infer that seeds treated with the magnetic field intensity of 0,06 T during 30 minutes, showed higher percentage values for germination, for example germination tax, speed of germination and growth parameters, that influenced seedling size, fresh weight, besides obtaining seedlings with more vitality. However, it must be well-known the effects caused after the magnetization of all structures of a seed in order to reduce time of germination. Therefore, additional studies must be done to elicit where and how the magnetization affects the seed.

The main hypothesis proposed for this study is that the effects of the magnetization cause early mobilization of the reserves on seed endosperm to guarantee its germination. For the alternative hypothesis, it was considered that BSL imaging is able to early identify after magnetic stimulus its effects on endosperm before the seed starts its germination. Then, this work aimed to evaluate the stimulus effects of the magnetic field on seeds aiming to speed germination, particularly trying to isolate the mobilization of the reserves in the endosperm, by using traditional techniques and BSL imaging.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

The experiment with corn seeds, Zea mays, variety/cultivar: 2B647PW, harvest: 2021/2022, Biomatrix origin without chemical treatment, were conducted under laboratory conditions, carried out at LAS - Laboratório de Sementes and at CEDIA - Centro de Desenvolvimento of Instrumentation Applied to Agriculture at ‘’Universidade Federal de Lavras” – UFLA, Minas Gerais.

The corn seeds were magnetized with two magnetic field intensities (125 mT and 250 mT) and the magnetization effects were analyzed by physiological test: measurement of dry mass and in three different regions of the corn seed: aerial, root and endosperm. The dry mass of the entire endosperm was measured and, from the germ region, the same parameter referring to the aerial and root region was measured (Figure 1).

ELECTRO-MAGNET MACHINE

A magnetic field was generated by an electromagnetic device designed by using ferromagnetic cylinders with 3 inches of diameter and forming a C shape, where an inductor was mounted using 2,000 turns of isolated wire and oppositely placed to the gap with 350 mm (Figure 2). The magnetic field generated by the device and crossing the gap was measured using a Gaussmeter (MGM MagMeter, with the resolution of 0.1 Gauss in a scale from 0 to 1,000 Gauss).

The magnetic field was generated by using a continuous electrical source of 0 to 60 V and 5 A (PS 605 D bivolt 127/220V) that excites the coil.

SEEDS PREPARATION

A belt with envelopes to receive the seeds was built using germitest paper as shown in Figure 3. Seventy seeds were placed in the individual envelopes, all the embryos to the same side, and embed using distillate water. The belt was rolled and inserted into a PVC cylinder as presented in Figure 4. Thus, the PVC cylinder with the belt rolled inside was positioned in the gap. In Figure 5, it is presented the position of each embryo directed to the north pole.

SEEDS PREPARATION

The fluxogram in Figure 6 is a summary of the methodology applied in the assays. It was selected 75 maize seeds to be part of the experiment and 8 replications were performed totaling 600 seeds. As control of the experiment, it was kept 25 seeds without magnetization and the rest of the seeds (50 seeds) were split on half to be submitted to the magnetic intensities of 125 mT and 250 mT, totalizing 25 seeds for each intensity.

The seeds were imbibed during 16 hours in the germination paper using 2.5-fold of distilled water weight in a germinator at 30 ± 2 °C.

The magnetization was performed for one hour at each intensity level (125 mT or 250 mT) individually for treatments right after the 16 hours of imbibition.

The seeds were analyzed during germination and photographed daily by Groundeye for 14 days, and on the fifteenth day the dry matter was taken.

ANALYSIS WITH THE BIOSPECKLE LASER

The illumination using the BSL were conducted just after magnetization, using a backscattering configuration as presented in Figure 7.

For the analysis with the BSL, six regions of the corn seed were chosen, two corresponding to the aerial and root regions of the embryonic axis; and two regions of the scutellum adjacent to the aerial region and two regions neighboring the root region, (Figure 8).

The images were analysed using the graphical index known as Fujii, Fujii et al., (1987), equation 1, after several tests until images reached good quality in analysis. Futhermore, numerical values were obtained when creating a histogram using data from desired region of interest (ROI).

$$Fujii(x,y) = \sum_{k=1}^{N} \left| \frac{I_k(x,y) - I_{k+1}(x,y)}{I_k(x,y) + I_{k+1}(x,y)} \right| \quad (1)$$

The Fujii method makes it possible to identify, in a biological material, areas with different levels of activity, however, there is a weighting in which the differences of subsequent images are divided by their sum, designated as weighting factor, Aizu; Asakura, (1991).

For a quantitative analysis, the Moment of Inertia MI was used, where the activity levels of the regions of interest are numerically quantified. MI is a dimensionless number and the region of greatest activity refers to the highest MI value, equation 2.

$$MI = \sum_{i,j} \frac{MOC_{i,j} \times (i-j)^2}{256} \quad (2)$$

Where the occurrence matrix (MOC)

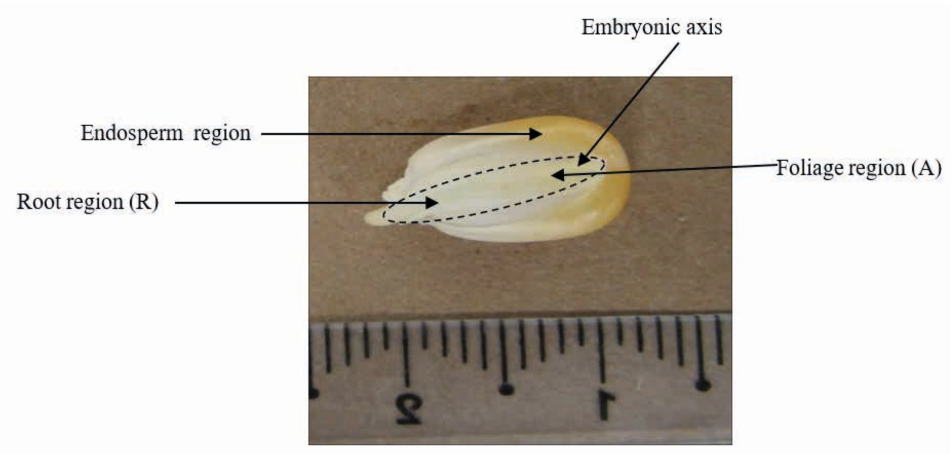

Figure 1: Regions from which the dry mass was measured.

Source: author himself.

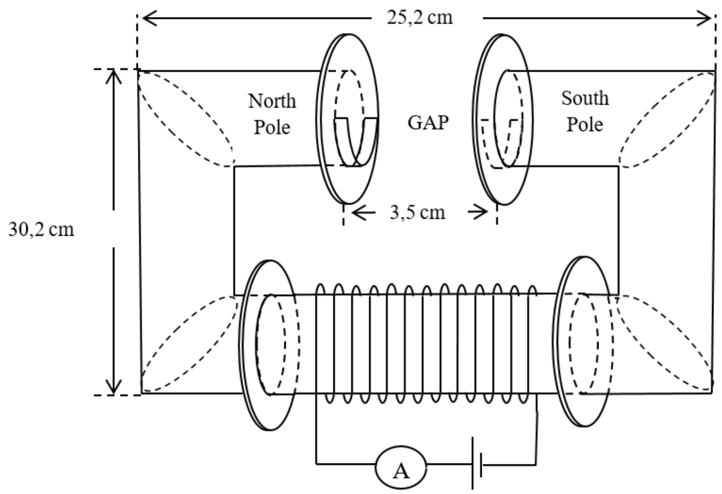

Figure 2: Magnetic system designed to receive the seeds in the gap for treatment.

Source: author himself.

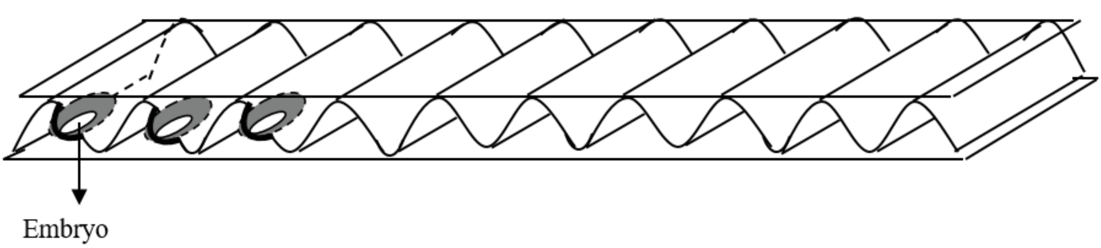

Figure 3: Seeds positioning in the belt inside individual envelopes.

Source: author himself..

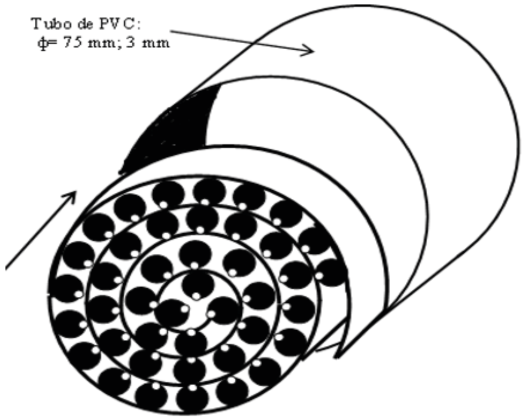

Figure 4: Positioning of the seeds in the rolled belt and inserted into a PVC cylinder.

Source: author himself.

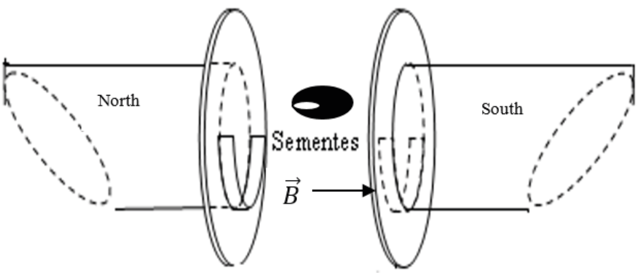

Figure 5: Direction of seeds in the electromagnetic field when exposed in the gap of the magnetic system

Source: author himself.

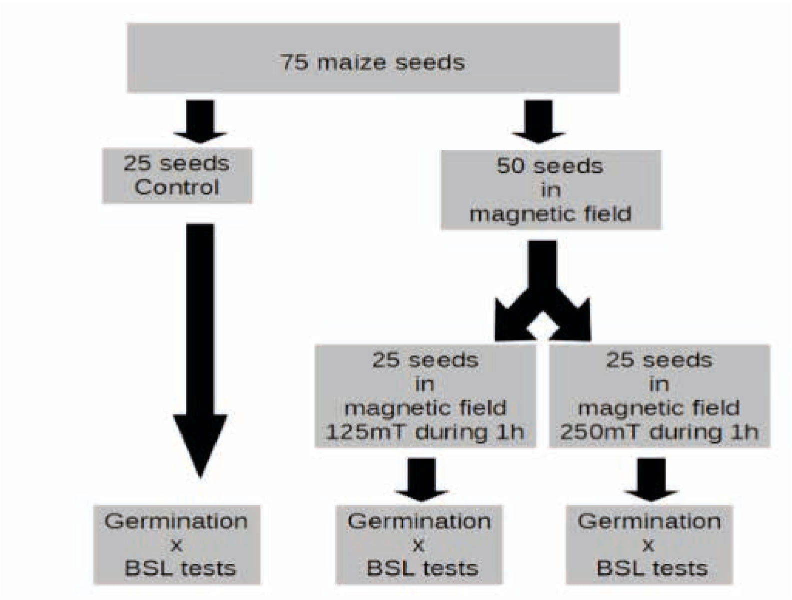

Figure 6. Flowchart of a repetition.

Source: author himself.

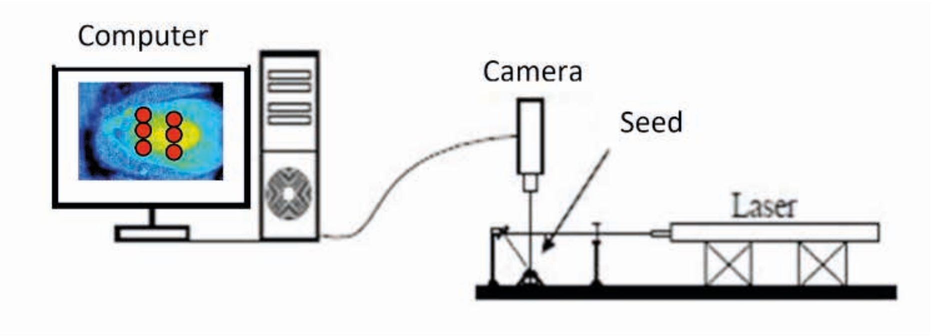

Figure 7: Backscattering configuration using a HeNe, 632 nm, laser with 10 mW, and a CCD camera capturing the images in a time rate of 80 ms.

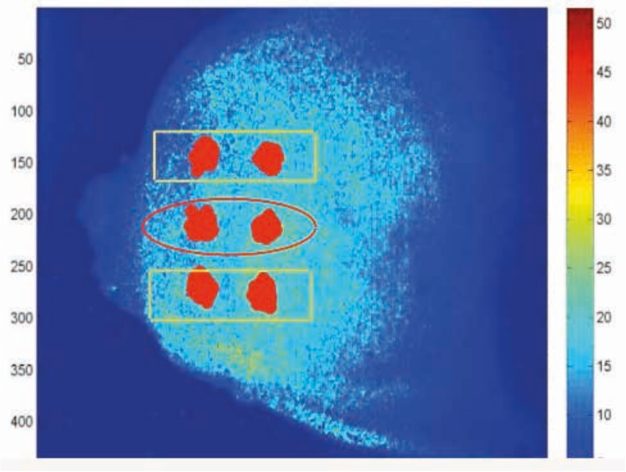

Figure 8: Regions of interest for the BSL. Red ellipse: embryonic axis with aerial and root regions. Yellow rectangles: scutellum and part of the endosperm.

Source: author himself.

Dry mass of seedlings (grams)			
	Control	125 mT	250 mT
Foliage region	0.959	1.006	1.095
Root region	2.322	2.447	2.401
Endosperm	3.179	2.483	2.323

Table 1: Dry matter mass of the foliage region for maize seedling measured in grams for the control and treatments exposed to the magnetic system

Source: Original data from research.

is defined, by Arizaga, Trivi and Rabal (1999), where the entries are the number of occurrences of an 8-bit value (0-255) of intensity i, followed by an intensity value j, equation 3.

$$MOC = [N_{i,j}] \quad (3)$$

RESULTS AND DISCUSSION

RESULTS FROM SISVAR ANALYSIS/ RESULTADOS DAS ANÁLISES DO SISVAR

It was measured the dry material mass from foliage, root regions and endosperm for maize seeds submitted to control and treatments with magnetic fields. The data obtained in analysis are described on Tables 1 and Figures 9.

Figure 10 below represents the comparison between the dry mass of the control in relation to the magnetic field intensities applied to the seed endosperm.

DISCUSSION

Analyzing Table 1 compared to figure 9, a lower dry mass of the endosperm of the magnetically treated seeds is observed in comparison with the dry mass of the endosperm of the control group, with a significant difference between these values. It is inferred that, during seed germination, there was a greater mobilization of nutrients from the endosperm destined to the germination event, being destined to the embryonic axis, as can be verified by the results of activity levels outlined by the BSL.

This occurrence is consistent with the fact that this analysis was performed in the terminal stages of seed germination, where much of the hydrolysis of reserves has already occurred and, despite this, maize seed germination is fast.

Corte et al. (2006), found something similar when they researched the mobilization of reserves during the germination of sibipiruna seeds and found that in the pre-germination period, between day zero and the fifth day, there was an exponential increase in the fresh mass of the cotyledons (region aerial) until the fifteenth day, due to the increase in the turgor of its tissues, followed by a decrease, as the reserves were depleted. Dry mass declined significantly.

There was no significant difference in terms of dry mass values between treatments performed with magnetic fields of 125 and 250 mT, which suggests that these field magnitudes cause the same effect with regard to dry mass accumulation. These results were measured after 5 days of germination including 16 hours of imbibition.

When comparing the dry mass values of the magnetically treated seeds with the dry mass of the control seeds for the root and aerial regions, it is noticed that there were no significant differences in these values.

RESULTS OBTAINED BY BIOSPECKLE ANALYSIS

Maize seeds were lightened after being embeded and magnetized. In order to study the seeds by using the Biospeckle, some relevant areas were selected in the seed for further analysis. In the maize seed shown in Figure 11, the areas of interest for the BSL were highlighted, consisting of the root (3) and aerial (4) regions and, surrounding these regions, laterally positioned regions of the scutellum (1) stand out, (2), (5) and (6); in relation to the root and aerial regions.

Once the seed is illuminated by laser light, the program makes it possible to choose the region in which the activity analysis will be carried out.

After analyses of the images, it was obtained a graphical result by applying the graphical index of Fujii Nirala, which permitted to access a qualitative result considering the

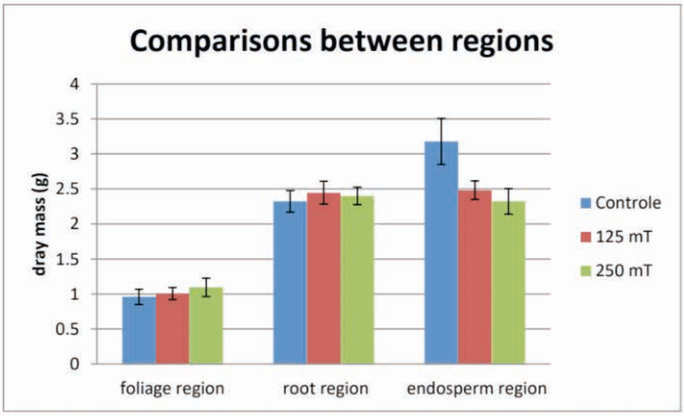

Figure 9. Dry mass of regions measured in grams for the control and treatments exposed to the magnetic system.

Source: Original data from research.

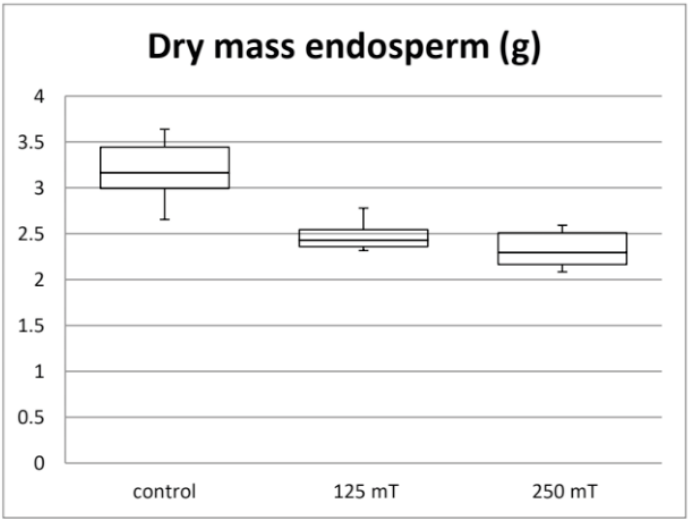

Figure 10. Dry mass of region endosperm measured in grams for the control and treatments exposed to the magnetic system.

Source: Original data from research.

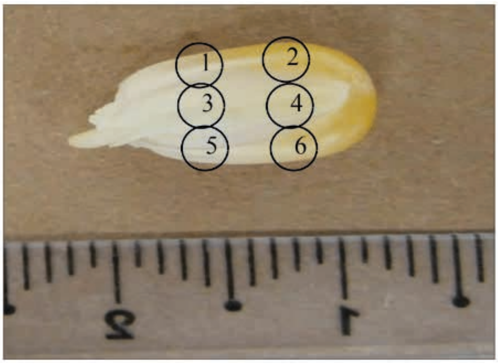

Figure 11: Regions analyzed by BSL.

Source: author himself.

regions with higher activity level.

Table 2 below lists the average activity levels for each region. Such levels refer to the Moment of Inertia of each region that were designated as of interest.

Figure 13 below represents the graphic behavior of the regions with their respective average levels of activity.

The data grouped in a box plot, the differences presented by each treatment are clearer, as shown in figure 14 below.

DISCUSSION

Analyzing graphically the data in table 2, a higher index of biological activity is observed in the regions that refer to the embryonic axis of the seed, regions 3 and 4; with region 3 referring to the root region and region 4 belonging to the aerial region.

A significant difference in biological activity is also observed when comparing the magnetic treatment of 125 mT in the root and aerial regions of the seed, suggesting a greater development of the aerial region of the seedling.

This fact corroborates the results achieved by Corte et al. (2006), when they found an increase in the dry mass of the cotyledons (a structure belonging to the aerial region) in the initial stages of germination. As the images of the seed in germination were captured shortly after 16 hours of imbibition, the Biospeckle Laser was able to detect exactly the moment when the activity of the aerial part was more pronounced due to the increase in the dry mass of that region. In addition, the BSL was precise in locating which of the regions of the embryonic axis outlined greater biological activity.

The regions surrounding the aerial (2 and 6) and root (1 and 5) regions, corresponding to the scutellum region of the seed, when compared in terms of biological activity, to the scutellum regions 2 and 6; outline greater activity and, even with the magnetic field of magnitude 125 mT, showing more promise.

The regions of the scutellum are adjacent to the root and aerial areas, if the aerial region was more active, from a biological point of view, it is to be expected that the scutellum adjacent to the aerial part has a differentiated activity, as verified by the BSL.

In seeds, not only does the endosperm form reserve tissue, the cotyledons also store nutrients. In sunflower seeds about 22 to 36% of their dry mass is constituted of lipids according to Sorokin, (1967).

These results were obtained after 16 hours of seed imbibition, that is, there is a chronological difference in the acquisition of results, both those referring to the physiology of the seed (dry mass) and those referring to the Biospeckle Laser.

CONCLUSION

1. The pre-treatment using a magnetic field of constant intensity causes physiological changes in corn seeds.

2. The Biospeckle Laser technique was efficient in detecting the regions that were physiologically altered by the application of the magnetic field, having great precision in distinguishing between two structures very close and internal to the seeds.

3. The application of the magnetic field and the use of the BSL proved, in terms of biological activity, the path taken by the nutrients at the time of nutrient mobilization.

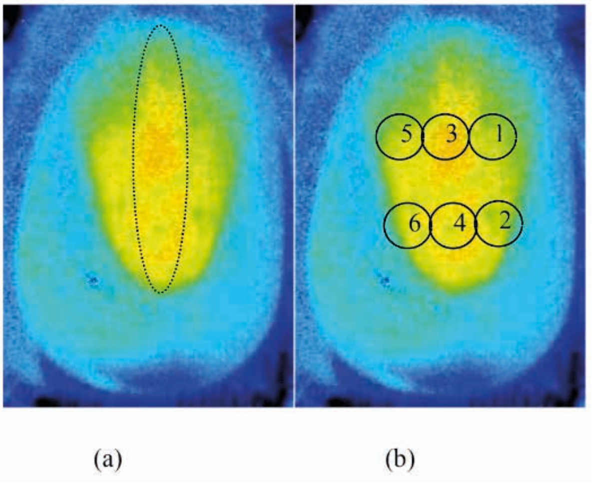

Figure 12. (a) Activity in the embryonic axis differentiated by Biospeckle analysis and by the graphical index of Fujii Nirala; (b) Relevant areas for study.

Source: author himself.

Average activity level of each region			
Region	Control	125 mT	250 mT
1	142.432	143.926	127.567
2	186.024	220.466	203.794
3	165.167	178.831	171.775
4	207.455	256.264	225.281
5	145.744	139.441	138.060
6	172.215	187.711	174.994

Table 2: Mean values of activity levels for regions (1), (2), (3), (4), (5) e (6); in seeds obtained by Biospeckle for control seeds and treatments exposed to magnetic intensity levels.

Source: Original data from research.

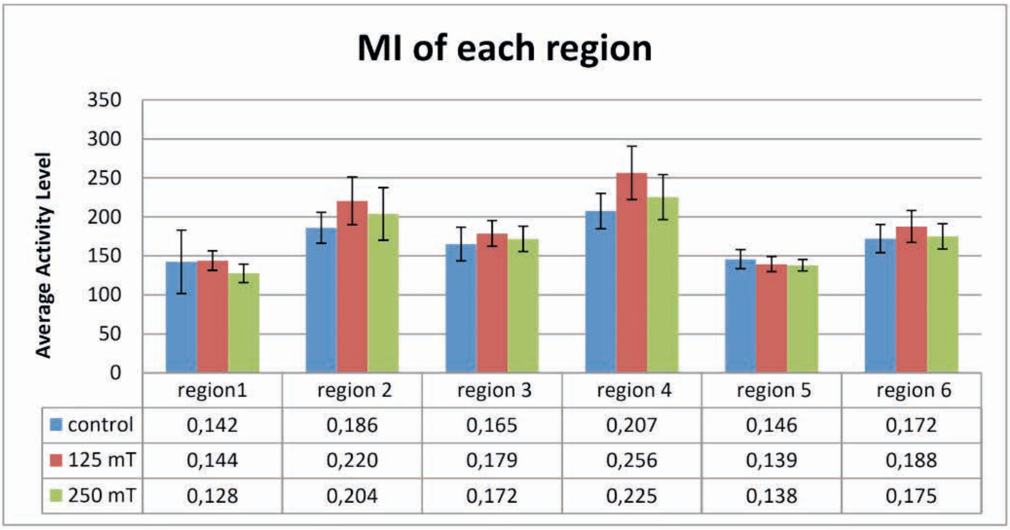

Figure 13: Mean Values for activity levels in the foliage region for control seeds and treatments exposed to magnetic intensities levels.

Source: Original data from research.

Journal of Agricultural Sciences Research ISSN 2764-0973 DOI 10.22533/at.ed.9733223100210

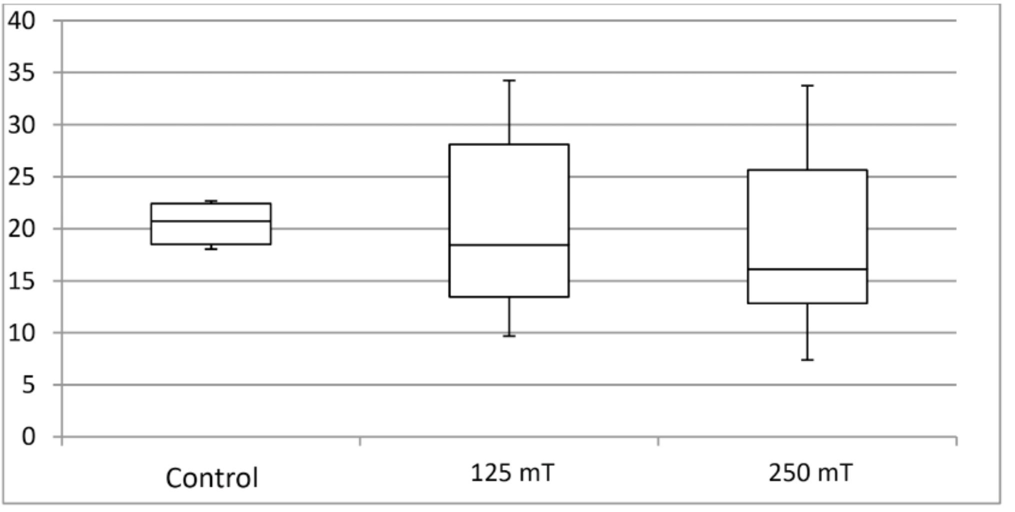

Figura 14. Activity level for each treatment.

Source: Original data from research.

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