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Full Length Research

BIOSPECKLE AS TOOL AUXILIARY IN EVALUATION DORMANCY OVERCOMING *ARAÇÁ-BOI* SEEDS

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The araçá-boi is a native fruit of the Amazon known for its organoleptic properties of fruit, however it presents the seed dormancy. The use of coherent light in the evaluation of biological activity has great potential for allowing identify damage in seeds. This technique is based on the phenomenon known as dynamic speckle or biospeckle. So we proposed the use of biospeckle technique in determining the viability and vigor correlating with the germination behavior and seedling development in the field. The seeds used in Experiment 1 were grouped according to their dimensions in large, medium and small. Then were subjected to the following treatments to break dormancy: (A) control, (B) removal of seed coat with sandpaper aid and (C) removal of seed coat with scalpel. In the osmoconditioning experiment similar seed size were used. To break dormancy were placed in germination boxes, previously lined with filter paper soaked in potassium nitrate solution for 24 h at 25°C. Then the images were obtained by biospeckle technique of seed groups submitted to these two different treatments. The use of biospeckle technique was efficient in determining the viability and vigor of the seeds of araçá-boi. Large seeds and medium were more active. The speed emergence of large and medium sized seeds and seedling development observed in the experiment corroborate with the data of the activity observed by biospeckle technique. Similarly seeds osmoconditioned in KNO_3 for 24 h had higher emergence rate and activity observed by biospeckle analysis.

Terms for indexing: dynamic speckle, Eugenia stipitata, germination, laser.

INTRODUCTION

The *Eugenia stiptata*, Araçá-boi, is a fruit species from the Amazon showing great potential agro-industrial presenting fruit of interest physical and chemical characteristics and sensory attributes of high acceptability (Rogez et al, 2004).

The major difficulty in the cultivation of this fruit is the long period for seed germination and seedling emergence which relates to the seed dormancy. This may be related to dormancy hardness and impermeability to water and gases integument, the presence of immature embryo and germination inhibitors (Sert e Bonato, 2009).

Another important factor is the size of the seed, since this characteristic influence on the germination, emergence and also in the germination speed of the same (Costa et al, 2006).

The research for methods of assessing the viability of seeds non-destructive and effectively makes promising new techniques are developed. The biospeckle or dynamic speckle is a known phenomenon by using coherent light in the evaluation of the biological activity of living tissue or to study the dynamics of particulate systems.

This phenomenon can be seen when a beam of coherent light is dispersed to cover a surface that displays some kind of dynamic activity (Rodrigues, 2007).

The biospeckle is an interference pattern formed by

The diffuse reflection of coherent light scattered by interacting with an object that has some type of activity biological or not. The interference pattern changes over time due to structures that scatter the light (Oliveira et al, 2009).

Rodrigues et al. (2007) have postulated that the diffuser object modifies the individual grains of the speckle pattern also change.This suggests that the biospeckle patterns contain information about the subject's movement.

In biological material activity analysis level which has been proposed with the use of laser is the analysis of information derived from the autocorrelation function of the Temporal and Spatial Pattern of Speckle (Spatial Temporal Speckle-STS) as presented by (Rabelo, 2000), and (Romero, 1999). This tool also already been used for (Bergkvist, 1997), (Oulamara et al, 1999) and (Xu et al, 1995).

Another STS analysis of the properties is the calculation of the scattering intensities module also termed by some authors as the moment of inertia (mi) which is obtained by Occurrence Matrix (MOC) proposed by (Arizaga et al, 1999).

Due to the complexity of biospeckle would be very useful to have a model to describe at least the main features of the phenomenon (Braga Jr, 2000). The pattern is generated by the dynamic and random biospeckleand should be analyzed with standard processing techniques and statistical processing since the visual inspection only permits the identification of the existence of the phenomenon but does not quantify it (Rabal et al,. 2000).

The biospeckle appears as a valuable tool in the studies of different areas of knowledge especially agronomy and can be used to assess the potential germination of seeds (Seitz, 1979).

Braga Jr (2001) conducted the analysis of seeds with the technique of biospeckle and demonstrated that the use of laser has great potential since it identifies damage in seeds for purposes of evaluating the feasibility.

In studies conducted in bean seed (*Phaseolus vulgaris L*), Rodrigues et al. (2007) separated living anddead tissue using the biospeckle analysis. Bergkvist (1997), emphasize that the incidence of the laser does not significantly affect the activity sheets confirming Seitz (1979) and Rabelo (2000), what evaluated the influence of laser in oranges metabolism showing that the tissue subjected to a low-power laser did not alter the results biospeckle in the time.

The objective of this work was to study the use of biospeckle technique in determining the viability and vigor correlating with the germination behavior and seedling development in the field. For this two experiments were conducted in order to evaluate the possibility of biospeckle technique use as a tool in reducing the time to evaluate treatment of overcome dormancy in *E. stipitata* seeds.

MATERIAL AND METHODS

Material collection

The seeds used in this study were derived from fruits of different populations of *E. stipitata* collected in the city of Manaus in the period 7-9 November 2013. The seed extraction was performed manually followed by washing under running water with the aid of sieve for complete removal of mucilage. Then were placed to dry in the air for 2 hin average temperature of 25º C.

Experiment 1

Choice of seed

The seeds were quantified and separated according to their size into three groups: (1) Large, seeds weighing more than 0.78 g; (2) Medium, seeds with mass between 0.77 g and 0.19 g and (3) Small, seeds mass less than 0.18g.

Treatments to break dormancy

We selected 96 seeds from each group (Small, Medium and Large) and sequentially numbered from 01 to 288. The seeds were subjected to the following treatments to break dormancy: a) intact seed (control), b) removal of seed coat with grip tape aid, c) with a scalpel and were then submerged in water for 12 hours. After this period the seeds were taken to the Plasma Laboratory of Atomic Spectroscopy (LaPEA) of the Federal University of Roraima in order to employ the biospeckle technique.

Experiment 2

Choice of seed

In this experiment all the seeds were selected having the same size obtaining as uniform as possible.

Treatments to break dormancy

80 seeds evenly selected and 40 was separated from the seeds used as control samples then were immersed in a solution of NaClO (2%) in active chlorine for 60 minutes for disinfection. Then washed in distilled water and placed in pre-germination boxes lined with filter paper impregnated in solution of potassium nitrate (KNO_3 -1Mpa osmotic potential and its concentration was based on the van't Hoff equation (Hillel 1971) for 24 hours for osmopriming. The osmopriming was carried

out in BOD (Biochemical Oxygen Demand) with temperature of the 10º C. The pre-germination boxes have been involved with plastic bags to prevent evaporation of the solution. After treatment seeds were rinsed in distilled water and were then submerged in water for 12 hours. After this period the seeds were taken to the Plasma Laboratory of Atomic Spectroscopy (LaPEA) of the Federal University of Roraima in order to employ the biospeckle technique.

Obtain images in the experiments

Seeds were removed from the water and after the 5 minutes was carried out by the reading laser. The time of image acquisition was around 12 seconds where each treatment consisting of 32 seeds. 288 videos were generated at the end of all the readings of the samples.

The experimental apparatus consisted of He-Ne laser whose wavelength was 632 nm, CCD camera and biconvex lens to the laser divergence in order to ensure that all seed would be illuminated and a microcomputer coupled. The figure 1 shows the layout of the experimental apparatus used.

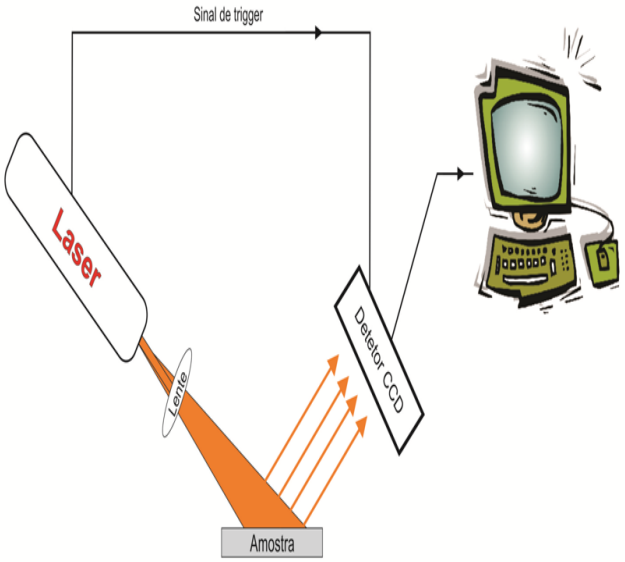

Figure 1: Scheme of the experimental apparatus used in LaPEA

Images of interpretation

Each 12-second video was converted into 94 pictures. After obtained these images they were edited by Gimp. The images were compared two by two superimposed so that at the end obtains only the difference between them. After editing these images we obtained 15 patterns of differences for each seed and through these differences we determined the changes occurred through three tracks of pixels of the images chosen randomly.

To interpret the results it was established an area with different activity in the image and given the value of 1 (white color) and the region that showed no difference that is lack of activity received 0 (black color).

Sowing

After the generated images of the experiment 1 the seeds were sown in bed containing sand + sawdust (1:1), evaluated the percentage of emergency and the emergency rate (index), shoot length with ruler aid and number of leaves. At the end of 100 days we assessed the length of the root system, dry weight of shoot and root system using a digital balance with precision 0,001 g.

The experimental design was completely randomized in a factorial 3 x 3 wherein the three seed sizes and three treatments to break dormancy with 9 treatments and four replicates of 8 seeds per replication totaling 288 seeds.

In experiment 2 the seeds were sown in bed containing sand + sawdust (1: 1) being evaluated the percentage of emergency and the emergency rate (index). The design was completely randomized with two treatments to break dormancy and four replicates of 10 seeds per replication totaling 80 seeds.

The analyses were done using the software System for Analysis of Variance - SISVAR (Ferreira, 2011). Qualitative data were submitted to Turkey test at 1% probability (Gomes, 2000).

Comparison with the data obtained in the field

The characteristics evaluated after sowing were then compared to images obtained by biospeckle to verify if the activities observed with the technical corroborate those obtained in the field.

RESULTS

From Figure 2 it can be seen the difference of activity in large and small seed of *E. stipitata.*

Most sequence of observed activity in the images generated by biospeckle technique in experiment 1 was obtained by large and medium seeds of *E. stipitata*, while small seeds had low activity. The treatments used to speed up the germination process did not influence the activities presented (Figures 3).

We can see that the small seeds showed low activity and consequently had low germination rates. This result is related to the behavior of seeds in the field where it was observed that the seeds sizes were significant in germination percentage and the treatments used to

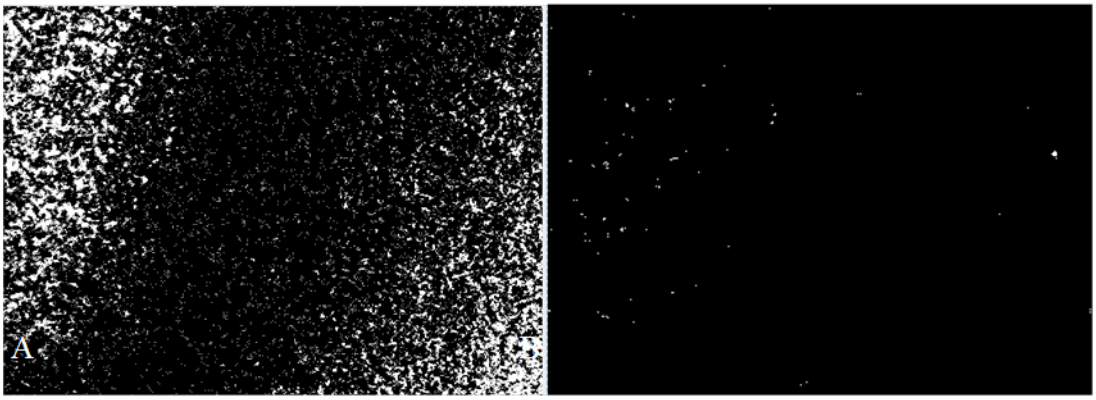

Figure 2: Activity of images (MOC standard) obtained by biospeckle technique used to evaluate the feasibility of large (A) and small (B) seeds of ***E. stipitata***.

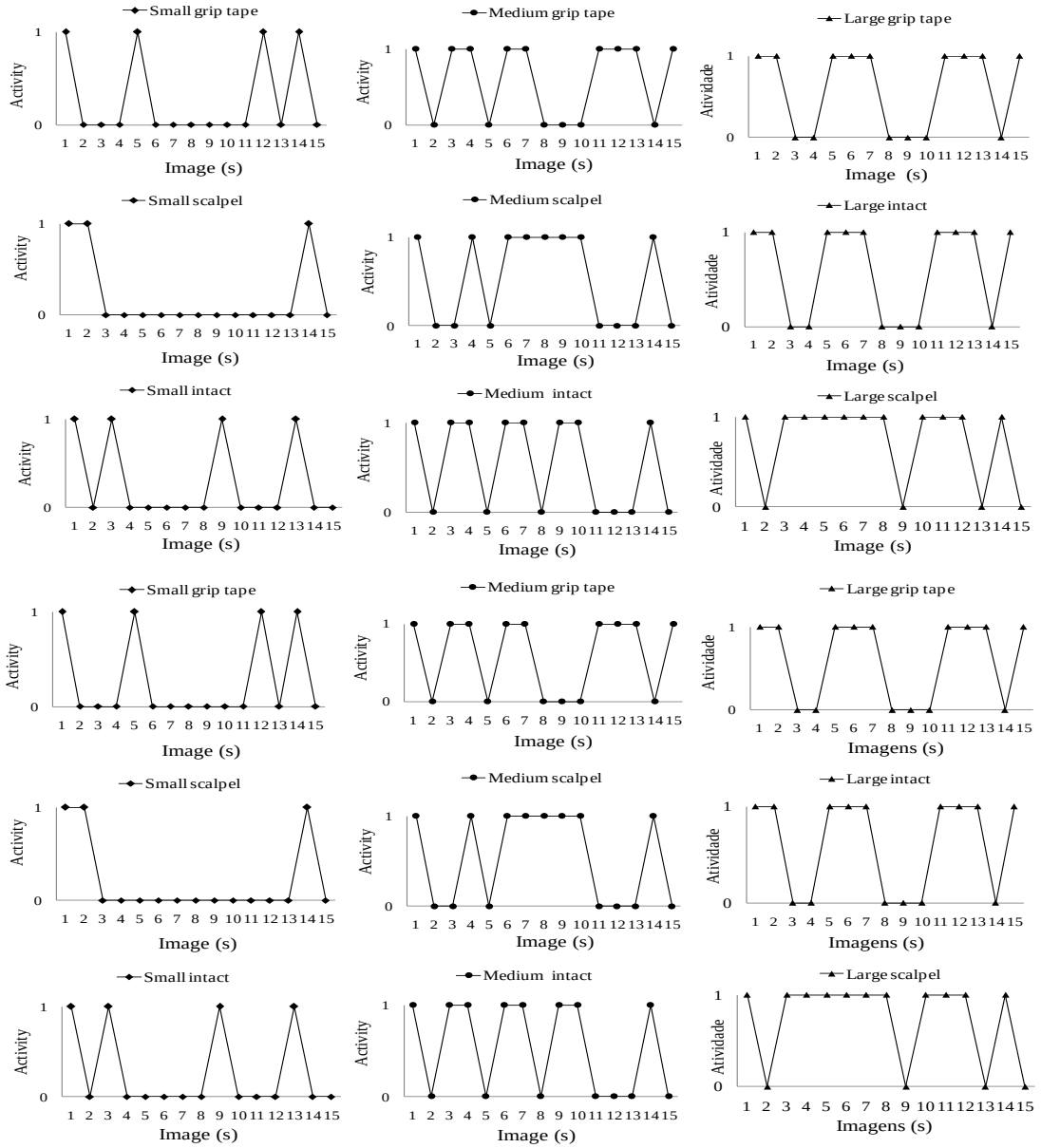

Figure 3: Activities made by smaller seeds, medium and large after treatments for acceleration of germination process recorded by the technique of biospeckle, Boa Vista, Roraima.

Table 1: Values of mean squares and their significance for the emergence data of large, medium and small *E. stipitata* seed after treatments to break dormancy, Boa Vista, RR, 2014

Variation sources	Degrees of release	Square mean
Treatments	2	506,77$^{n.s}$
Seeds size	2	19272,44**
Treatments x Seeds size	4	89,65$^{n.s}$
Error	27	172,34
Total	35	
C.V. (%)		17

$^{ns, **}$not significant and significant in 1% in Tukey test, respectively. C.V.%= percent coefficient of variation.

Table 2: Emergency Percentage of large, medium and small ***E. stipitata*** seeds sowed in substrate sand + sawdust over 140 days of cultivation, Boa Vista, RR, 2014

Seed size	Emergency Percentage	
Large	77	a
Medium	70	a
Small	3	b

Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

break dormancy also did not influence the field germination (Table 1).

Large and medium seeds of *E. stipitata* showed high percentages of germination in the field highlighting the activities observed by biospeckle technique. Smaller seeds, which showed low activity, had the lowest percentage of seeds germinated over 140 days of cultivation (Table 2).

The percentage of emergence of large and medium seeds did not differ statistically at the end of 140 days showing the positive correlation with the images seen by the technique of biospeckle which showed no difference between the activities presented by large and medium sized seeds.

Large and medium seeds also had higher emergency velocity showing good development in the field (Figure 4).

Large and medium seeds presented emergency rate similar to the 60 days of cultivation about and from this date there was a greater emergence speed index of large seeds. This result did not influence the emergence percentages of large and medium sized seeds which did not differ statistically at the end of 140 days of cultivation (Table 2).

The characteristics evaluated up to 140 days of cultivation proved that large and medium seeds besides larger germination percentage also showed better seedling development (Table 3).

Figure 5 shows that most sequence of the activity observed in the images generated by biospeckle technique in experiment 2 was obtained by the seeds osmoconditioned in KNO_3 for 24 hours while the seeds witnesses showed low activity.

Table 4 it is observed that there was significant effect of treatment used in breaking dormancy of seeds.

Note that the osmoconditioned seeds in KNO_3 for 24 hours showed high activity and therefore had high germination rates. There was a statistical difference between the seeds of *E. stipitata* osmoconditioned in KNO_3 for 24 hours and the witnesses (Table 5).

The percentage of emergence of osmoconditioned seeds differ statistically witnesses reaching 100% of emergency at the end of 63 days showing a positive relationship with the images seen by biospeckle technique that showed differences between the activities provided by the osmoconditioned seeds and witnesses.

In the osmoconditioned processthe KNO_3 moleculesallows better aeration of the seedand ionssalts influx within the cells of the seeds directly influencing the cellular metabolism.

The speed of emergence of osmoconditioned seed was proportional to emergence percentages (Table 5).

The osmoconditioned seeds also had higher emergency velocity demonstrating the positive relationship between images acquired by biospeckle technique and the behavior of seeds in the field.

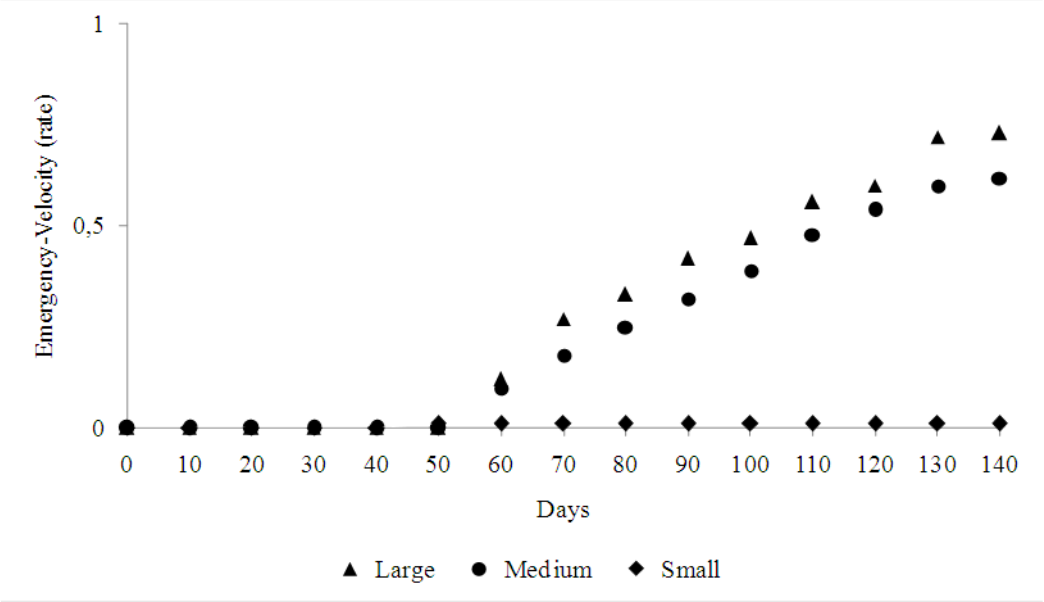

Figure 4: Emergency-velocity (rate) of cumulative seedlings obtained from large, medium and small seeds *E. stipitata* to 140 days.

Table 3: Shoot length (SL), length of the root system (LRS), dry weight of shoot (DWS), dry root weight (DRW) and number of leaves (NL) in leaves of *E. stipitata* emerged to os140 days of cultivation in substrate sand + sawdust after feasibility analysis by biospeckle, Boa Vista, RR, 2014

Seed size	SL (cm)		LRS (cm)		DWS (g)		DRW (g)		NL	
Large	15,61	a	12,85	a	0,210	a	0,110	a	20	a
Medium	11,91	b	11,17	a	0,120	b	0,060	b	18	a
Small	0,92	c	1,25	b	0,003	c	0,002	c	3	b
C.V (%)	3,89		4,01		3,32		2,09		2,28	

Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

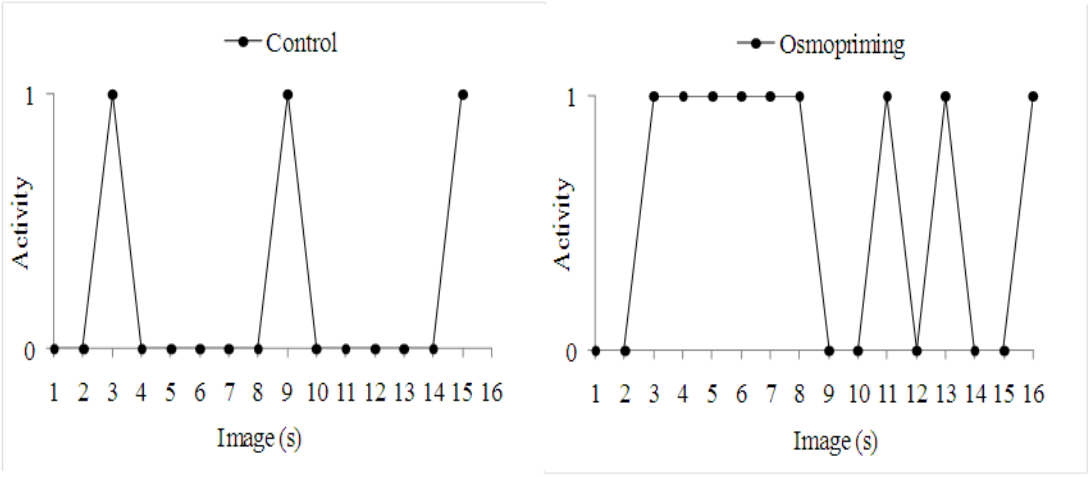

Figure 5: Activities presented by the osmoconditioned seeds in KNO_3 for 24 hours and seeds used as witnesses recorded by the technique of biospeckle, Boa Vista, Roraima.

Table 4: Values of mean squares and significance for emergency osmoconditioned seeds in KNO_3 for 24 hours, Boa Vista, RR, 2014.

Variation sources	Degrees of release	Square mean
Treatments	1	19012,5**
Error	6	12,5$^{n.s}$
Total	7	
C.V (%)	7	

$^{ns, **}$not significant and significant in 1% in Tukey test, respectively. C.V.%= percent coefficient of variation.

Table 5: Seed emergency percentage of *E. stipitata* osmoconditioned in KNO_3 for 24 hours sown in substrate sand + sawdust over 63 days of cultivation, Boa Vista, RR, 2014

Treatments	Emergency Percentage	
Osmopriming	100	a
Control	2,5	b

Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

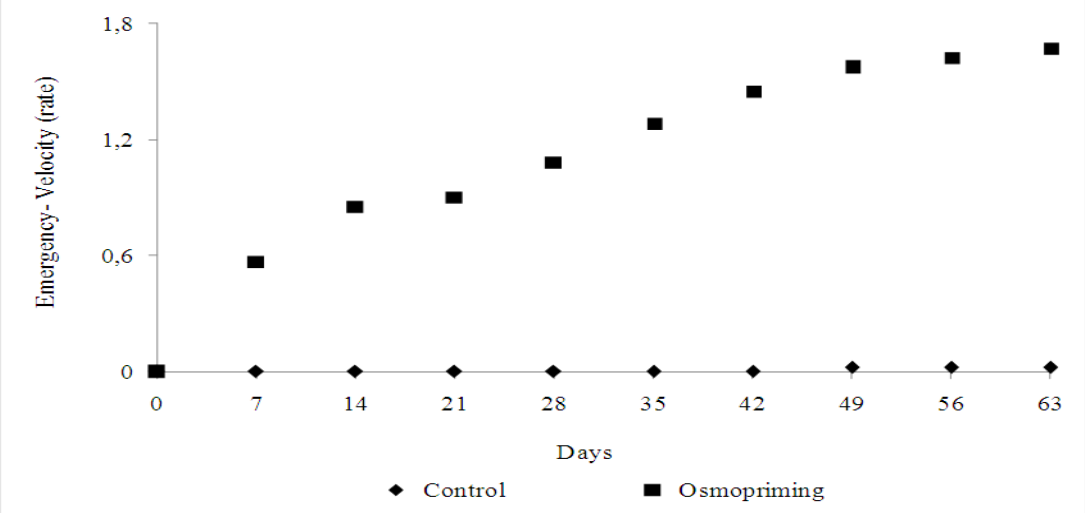

Figura 6: Emergency-Velocity (rate) of cumulative seedlings obtained from osmoconditioned seeds *E. stipitata* to in KNO_3 for 24 hours up to 63 days.

DISCUSSION

The images obtained through this technique indicate the index of the biological activity of seeds can be used to evaluate the germination potential (Silva, 2007).

According to (Braga Jr, 2001) the use of the laser offers great potential for identify damage to seeds in the evaluation of viability.This information corroborates studies in bean (*Phaseolus vulgaris* L), by (Rodrigues et al, 2007). The authors managed to separate living tissues of dead using biospeckle technique.

In studies on the influence of seed size in the germination of early bacuripari (*Rheedia gardneriana*), (Nascimento, 2004) observed higher speeds and germination percentages for larger seeds. Further emergency rate were also observed by large and medium seeds of jambo-vermelho (*Sysygium malaccense*) (Carvalho, 2012).

The seedlings emerged of the large seeds had higher shoot lengths and greater dry mass of shoot and root. However, in relation to the length of the root system and the number of leaves, medium and large seeds

seedlings did not differ statistically, presenting this way, uniformity.

These results showed the efficiency of the use of biospeckle technique in determining the viability of the seeds of *E. stipitata* and in its place demonstrating that large and medium seeds presented more viable and more germination percentage.

According to (Carvalho, 2012) the larger seeds generally were better nourished during its development having well-formed embryos and greater amount of reserve substances and consequently the most vigorous.

Popinigis (1997), stated that the size of the seed in many species is indicative of their physiological quality. Thus, within the same batch the small seeds have a lower germination and vigor of the seeds of medium and large size.

This result is related to the behavior of seeds in the field where it was observed that osmoconditioned seeds showed higher germination percentage. According to (Silva, 2007), the images obtained by the technique biospeckle indicate the biological activity index of the seeds which can be used to evaluate the germination potential of the same.

The osmoconditioned is the controlled hydration of the seeds in an osmotic solution stimulating your metabolism without, however, allow the emission of the primary root (Nascimento, 2004). This conditioning active degradation, uptake and translocation of reserves allowing the seeds to achieve relatively uniform metabolic state by interrupting the water supply.

CONCLUSIONS

The use of the technique biospeckle is efficient to determine the viability and vigor of the seeds of *E. stipitata*. Large and medium seeds have higher activities.

The emergence of large and medium seeds, emergency speed and seedling development relate to the activity observed by biospeckle technique.

The seeds osmoconditioned in KNO_3 for 24 hours had higher activities.

The emergence of seeds osmoconditioned in KNO_3 for 24 hours and the emergence speed relate to the activity observed by biospeckle technique.

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Conflict of interest

None.

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