

EVALUATION OF COCOA SEED GERMINATION BASED ON LOCATION IN THE FRUIT

AVALIAÇÃO DA GERMINAÇÃO DE SEMENTES DE CACAU EM FUNÇÃO DA LOCALIZAÇÃO NO FRUTO

EVALUACIÓN DE LA GERMINACIÓN DE SEMILLAS DE CACAO SEGÚN SU UBICACIÓN EN EL FRUTO



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ABSTRACT

Cocoa (*Theobroma cacao*) production is an agribusiness of great economic importance worldwide, as it provides the main raw material for the production of a centuries-old product: chocolate. Brazil ranks as the 6th largest cocoa producer in the world, with approximately 600,000 hectares under cultivation and 75,000 producers. *Forastero* is the most predominant variety globally, representing approximately 80% of production due to its disease resistance and high productivity. Therefore, given the growing demand for rootstocks and the importance of germination for the formation of good seedlings, this study aimed to evaluate the germination of seeds from different parts of the *Forastero* cocoa fruit, common type genotype. For the experiment, the fruits were divided into three treatments, where T1 is the base, T2 the middle, and T3 the apex. The treatments were processed, weighed, and placed to germinate in an oven at 30°C for twenty-five days. At the end of the experiment, analyses of germination, final weight, weight gain, vegetative mass, seedling emergence, among others, were performed. The results were statistically similar between the middle and apex treatments. However, the base treatment had significantly lower results than the others. It can be assumed that the low results of the base treatment may have occurred due to metabolic characteristics. Although the middle and apex treatments are considered better for seedling formation, the study highlighted the importance of further research focused on germination and its relationship with metabolic and physiological processes of *Theobroma cacao* seeds.

Keywords: Germination Test. Seed Size. Seed Position in the Fruit.

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RESUMO

A produção do cacau (*Theobroma cacao*) é um agronegócio de grande importância econômica no mundo, pois fornece a principal matéria prima para a produção de um produto secular, o chocolate. O Brasil ocupa a posição de 6º maior produtor mundial de cacau, com cerca de 600 mil hectares cultivados e 75 mil produtores. O *Forastero* é a variedade mais predominante globalmente, representando aproximadamente 80% da produção devido à sua resistência a doenças e alta produtividade. Dessa forma, visto o crescimento da demanda de porta-enxertos e a importância da germinação para a formação de boas mudas, esse trabalho teve o objetivo de avaliar a germinação de sementes de diferentes partes do fruto do cacau *Forasteiro*, genótipo tipo comum. Para o experimento, os frutos foram divididos em três tratamentos, onde T1 é a base, T2 o meio e T3 o ápice. Os tratamentos foram tratados, pesados e colocados para germinar em estufa com 30°C durante vinte e cinco dias. Ao final do experimento foram feitas análises de germinação, massa final, ganho de massa, massa vegetativa, emissão de plântulas, dentre outros. Os resultados foram estatisticamente parecidos entre o tratamento meio e ápice. Contudo, o tratamento base teve resultados significativamente inferiores aos demais. Pode-se supor que os baixos resultados da base podem ter ocorrido devido a características metabólicas. Apesar de considerar os tratamentos meio e ápice melhores para a formação de mudas, o estudo ressaltou a importância de mais pesquisas voltadas a germinação e sua relação com processos metabólicos e fisiológicos de sementes de *Theobroma cacao*.

Palavras-chave: Teste de Germinação. Tamanho de Sementes. Posição das Sementes no Fruto.

RESUMEN

La producción de cacao (*Theobroma cacao*) es un agronegocio de gran importancia económica a nivel mundial, ya que proporciona la principal materia prima para la elaboración de un producto centenario: el chocolate. Brasil se ubica como el sexto mayor productor de cacao del mundo, con aproximadamente 600.000 hectáreas cultivadas y 75.000 productores. *Forastero* es la variedad más predominante a nivel mundial, representando aproximadamente el 80% de la producción debido a su resistencia a enfermedades y alta productividad. Por lo tanto, dada la creciente demanda de portainjertos y la importancia de la germinación para la formación de buenas plántulas, este estudio tuvo como objetivo evaluar la germinación de semillas de diferentes partes del fruto del cacao *Forastero*, genotipo tipo común. Para el experimento, los frutos se dividieron en tres tratamientos, donde T1 es la base, T2 el centro y T3 el ápice. Los tratamientos se procesaron, pesaron y colocaron para germinar en un horno a 30 °C durante veinticinco días. Al final del experimento, se analizaron la germinación, el peso final, la ganancia de peso, la masa vegetativa y la emergencia de las plántulas, entre otros. Los resultados fueron estadísticamente similares entre los tratamientos de la parte media y la parte superior. Sin embargo, el tratamiento base presentó resultados significativamente inferiores a los demás. Cabe suponer que los bajos resultados del tratamiento base podrían deberse a características metabólicas. Si bien los tratamientos de la parte media y la parte superior se consideran mejores para la formación de plántulas, el estudio destacó la importancia de realizar más investigaciones centradas en la germinación y su relación con los procesos metabólicos y fisiológicos de las semillas de *Theobroma cacao*.

Palabras clave: Prueba de Germinación. Tamaño de la Semilla. Posición de la Semilla en el Fruto.

1 INTRODUCTION

The production of cocoa (*Theobroma cacao* L.) is an agribusiness of great global economic relevance, as it provides the main raw material for the manufacture of chocolate, a product of wide historical and cultural diffusion (HO; ZHAO; FLEET, 2015). In addition to industrial processing, the cocoa production chain involves agricultural and commercial stages that directly impact income, employment, and sustainability in tropical regions.

Historically, cacao was exploited by Mesoamerican peoples in humid forest areas, possibly since about 1000 BC, being used in the preparation of a strong, bitter and spicy drink in political and religious rituals by civilizations such as the Mayans and Aztecs (MODA; BOTEON; RIBEIRO, 2019). Subsequently, during the maritime expansions, cocoa was introduced to Europe in the sixteenth century, where the original drink was modified, incorporating sugar and milk and expanding its acceptance (HENDERSON et al., 2007).

From a botanical point of view, the cacao tree is a cauliflora plant, with flowers that emerge on floral cushions on the trunk and on woody branches. In the Amazon Region, a seasonal pattern is observed with two peaks of flowering associated with the rainfall regime, with emphasis on a main peak at the end of the dry season and the beginning of the rainy season (ALVIM, 1977).

The cocoa fruit can be approximately 15 to 30 cm long, with an oval shape and insertion in thicker branches. Each fruit can contain about 35 seeds, which have recalcitrant characteristics, a factor that strongly conditions their post-harvest management (VERÍSSIMO, 2012; CRUZ, 2002).

Recalcitrant seeds do not complete natural drying on the mother plant and are released with high water content. Even under controlled storage, reduction of water content below a critical, usually high, level can lead to loss of viability and embryo death (KING; ROBERTS, 1979; KING; ROBERTS, 1980a; PAMMENTER; BERJAK, 2014). This limitation is attributed, in part, to the maintenance of continuous metabolic activity, which restricts seed conservation to short periods and, frequently, until the beginning of germination (PAMMENTER; BERJAK, 2014).

In this context, germination is a central stage of the plant life cycle, involving physiological and morphological events that culminate in the formation of a structure with reproductive potential (KERBAUY, 2004; BEWLEY et al., 2013). In seed species sensitive to desiccation, such as cocoa, understanding the factors that modulate germination is decisive to ensure the formation of vigorous seedlings.

In Brazil, seed propagation has historically been predominant in the implementation of crops; However, vegetative propagation by grafting has been widely indicated aiming at

disease resistance and productivity increase. Even so, the production of rootstocks remains mostly seminal, which makes mastery of the germination process essential, due to its direct relationship with the cost and quality of seedlings (SENAR, 2018; SERRA; SODRÉ, 2021; VENIAL et al., 2017).

In view of the increase in demand for rootstocks and the importance of germination for the formation of quality seedlings, this work aims to evaluate the germination of Forasteiro cocoa seeds as a function of the location in the fruit, contributing to technical recommendations applicable to the production of more uniform and efficient seedlings.

2 OBJECTIVE

The general objective of this work is to investigate the influence of seed location in the fruit on the germination performance of cocoa seeds (*Theobroma cacao* L.) of the Forasteiro type, considering parameters of viability, germination speed and uniformity, in order to support the optimization of seedling/rootstock production in nursery.

In addition to the specific objectives: To compare the germination performance between seeds from different regions/positions of the fruit, through the percentage of germination, speed (or average time) of germination and uniformity of the process; identify the region/position of the fruit associated with the best germination performance, aiming to guide the selection of seeds for seedling production; and to generate technical recommendations applicable to the management of recalcitrant cocoa seeds in the nursery, aiming to reduce losses and increase efficiency in the formation of rootstocks.

3 METHODOLOGY

The study was conducted at the Forest Seeds Laboratory of IBEF/UFOPA, located in Santarém, Pará, with the objective of evaluating the influence of seed location in the fruit on the germination performance of cocoa seeds (*Theobroma cacao* L.) of the Forasteiro type, considering germination parameters (viability), speed and uniformity of the germination process.

Ripe fruits, intact and without signs of mechanical injury or rot were selected. After opening, the seeds were removed and classified according to their position in the fruit, establishing three treatments: basal region (close to the peduncle), median region and apical region (opposite to the peduncle). Then, the seeds were pulped (mucilage removal) and used immediately, in order to reduce viability losses associated with the recalcitrant nature of the seeds.

The experiment was carried out in a completely randomized design (DIC), with three treatments (basal, median and apical), each consisting of 4 replicates of 100 seeds, totaling 400 seeds per treatment. Sowing was carried out in a substrate composed of expandable clay and vermiculite, previously moistened and maintained with adequate moisture throughout the experimental period.

Germination was conducted under a controlled temperature of 27 °C, with follow-up for 25 days. The evaluations were performed by daily counts of the germinated seeds, adopting standardized morphological criteria for germination (e.g., radicle protrusion), applied uniformly to all treatments.

The variables evaluated were: germination percentage (G%), germination speed index (IVG) and average germination time (MGR), in addition to a measure of uniformity of the germination process, obtained by the temporal distribution of germinations throughout the evaluation period. The data were organized and submitted to statistical analysis, with prior verification of the assumptions (normality and homogeneity of variances). Next, analysis of variance (ANOVA) was performed and, when a significant effect was observed, the means were compared using Tukey's multiple comparison test, adopting a significance level of 5%.

4 DEVELOPMENT

According to Lajus (1982), the *Forastero*/common variety has fruits with a rounded shape, a rigid skin and a practically smooth surface. According to Ferreira et al. (2013), this is the true Brazilian cocoa, as it is the most cultivated in Brazil and has good resistance to pests and diseases. The summary of the analysis of variance of the characteristics evaluated as a function of the treatments applied is shown below, in Table 1.

Table 1

Initial Mass (g), Final No. of germinated seeds, Germination (%), Mortality (%), No. of seedlings emitted, Final Mass (g), Vegetative Mass (g) of the evaluated treatments

	BASE (T1)	MEDIUM (T2)	APEX (T3)
Initial Mass (g)	65.16 a	63.76 A	55.77 b
Final No. of Sprouted Seeds	185 b	189 to	191 to
Germination (%)	93.5 b	96 to	96.75 to
Mortality (%)	7.5 b	5.5 A	4.5 A
No. of Seedlings Emitted	12 b	40 to	42 to
Final Mass (g)	79.58 to	78.17 a	72.60 b
Vegetative Mass (g)	25.50 b	27.72 to	29.42 to

*Means followed in the line by the same letter are not different from each other by Tukey's test.

Source: Authors.

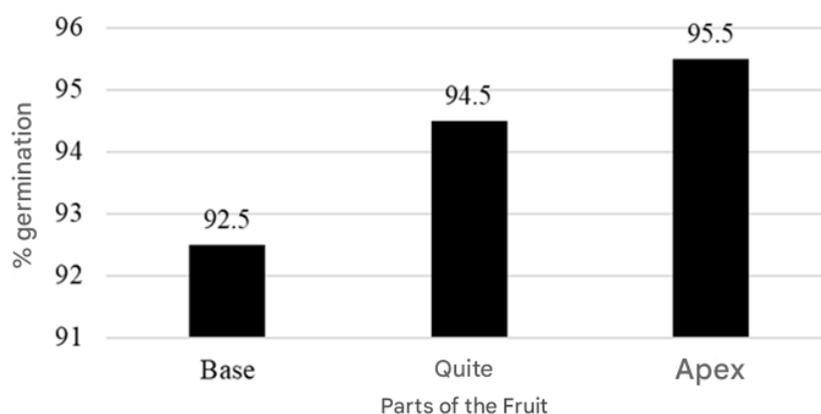
Regarding the initial mass, it was found that the means of the base (65.15g) and the middle (63.75g) were statistically similar, while the apex had a lower mean (55.77g). This distinction may have occurred due to the common variety being characterized by having a more rounded shape, however, its diameter decreases as it reaches the apex, causing the size of the seeds to become smaller (Cruz, 2012).

According to Cilas et al. (2010), genetic improvement favors seeds to have larger sizes, as seen in genotypes derived from crosses between cultivated cocoa trees. According to Carvalho and Nakagawa (2012), larger or denser seeds probably have greater vigor, due to their well-formed embryo and more reserves, but this can be influenced by many factors, such as humidity, temperature, among others. Viena et al (2017), did a study comparing eight genotypes of *Theobroma cacao*, in this one, the genotype TSH1188 which presented higher biometric values (including mass), had more tolerance to thermal stresses, however, the study did not emphasize whether only this parameter would be indicative for the formation of more vigorous plants.

In the present study, the three treatments had the same water and temperature regime (30°C). Figueiredo (1986b) did not describe the cocoa variety studied, but considered the temperature of 30 °C ideal, since it provided the maximum number of seedlings in the shortest period of time. For Freitas (2013), germination is one of the most important phases of the cultivation process. The germination percentage of the treatments was determined by the ratio of the total amount of germinated seeds at the end of the count, by the amount of seeds placed to germinate, multiplying by 100 to obtain the result in percentage. Figure 1 represents the germinated percentage of treatments at the end of the 25 days.

Figure 1

Germinated percentage of treatments at the end of the 25 days



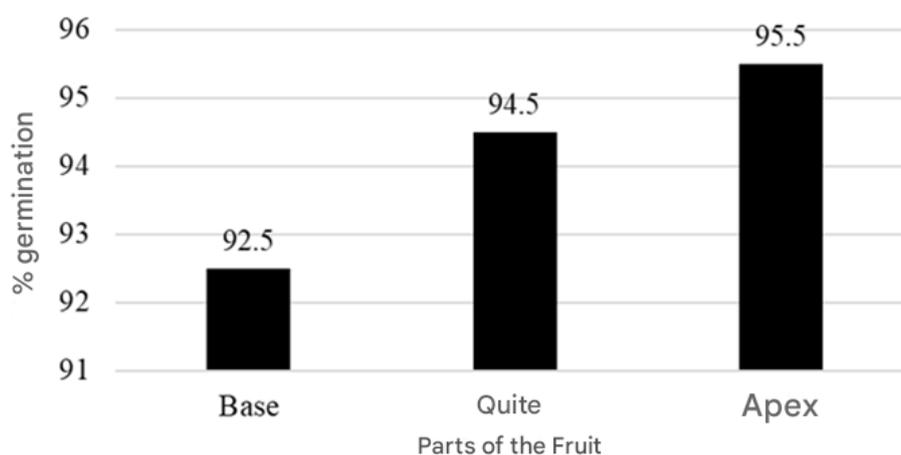
Source: Authors.

As expected, primary root protrusion was verified in 90% of the seeds of all treatments in the first analysis, in 5 days. It is possible to deduce that the properties of the cocoa tree related to seed development had an impact on this response. Given that the embryo is well developed during seed dispersal and the moisture level is high, the processing process that involves pulp removal favors rapid germination.

It is seen in figure 5 that at the end of the 25 days, the base had a lower germination percentage than the others, 92.5%, which corresponds to 185 seeds, the middle had 94.5% and 189 seeds, and the apex 95.5% and 191 seeds, the last two did not differ statistically. However, this lower germination of the base treatment, in view of the others, may have been related to its high mass gain, through water imbibition throughout the experiment (Figure 2).

Figure 2

Mass gain from the treatments at the end of the 25 days



Source: Authors.

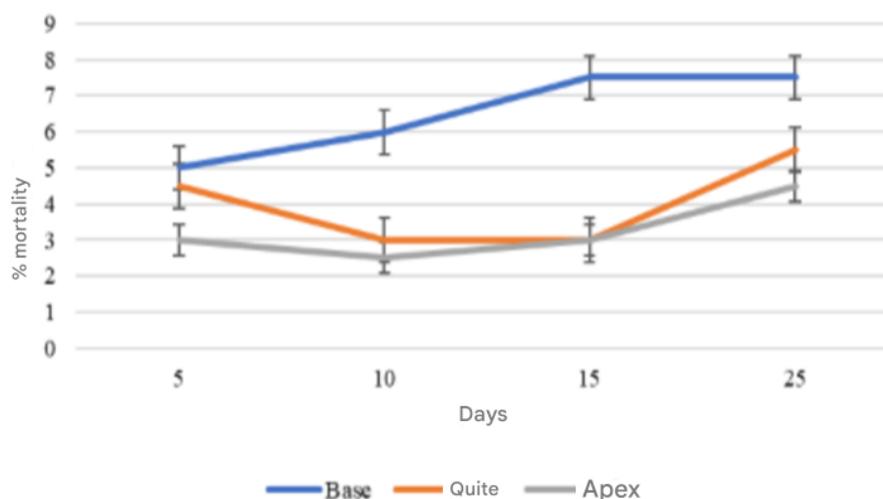
The base mass reached 79.5g at the end of the experiment, this value was considered high, since the treatment was the one that had the most seeds discarded due to mortality. The apex, which had a higher number of germinated seeds, had a lower mass than the same (72.5g), which suggests that the significant increase in base mass did not translate into benefits for survival or vegetative growth.

Schulz et al., (2014) in a study carried out with cocoa, observed that the respiration of the seeds is greater in the regions close to the fruit peduncle, where there is a higher concentration of sugars and greater enzymatic activity. Thus, the base treatment (T1) may have absorbed a significant amount of water due to its high physiological activity, but failed to transfer it for survival. As a result, it exhibited a higher weight at the end of the experiment and a higher mortality rate.

Contributing to the information cited, figure 3 expresses the mortality of the treatments, in which the base had the highest percentage of dead seeds throughout the experiment. Therefore, it is assumed that characteristics described about the different metabolic rate in the fruit explain the percentage of mortality and germination of the base if it differs from the other treatments.

Figure 3

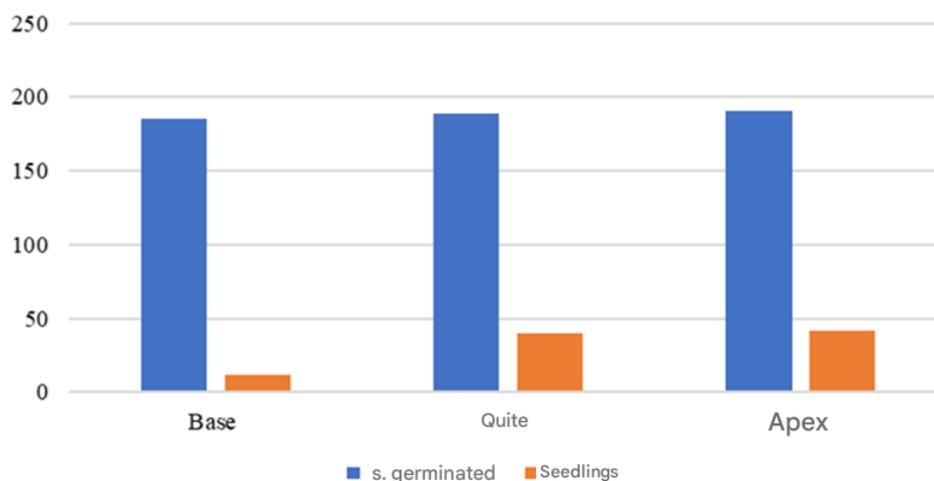
Mortality of treatments at the end of 25 days



Source: Authors.

The Mortadlity data shown in figure 6 reveal that the imbibition speed can markedly alter the germination and vigor of the seeds, and that when the recalcitrant seeds maintain this intense physiological activity, which includes increased absorption of water, therefore, from the mass, but cannot complete the germination process, the metabolism can become disorganized, leading to the consumption of reserves and the release of free radicals. Both of these activities accelerate its rapid deterioration and death (Barbedo & Marcos Filho 1998, Ferreira & Borghetti 2004, Andréo et al. 2006, Berjak & Pammenter 2008).

Seed germination is a complex physiological process that begins with the absorption of water that leads to root protrusion, with the next stage being called post-germination development, characterized by the growth and development of seedlings (Dousseau et al. 2013). To evaluate the number of seedlings, the root protrusions that developed to generate leaflets were considered. In general, seedling formation was low, in view of the number of germinated seeds, in the three treatments (Figure 4). Unlike the base, which produced only 14 seedlings, the medium developed 40 and the apex 42, where the last two did not present statistically significant differences.

Figure 4*Germinated seeds and seedlings emitted in the treatments*

Source: Authors.

Lavanhole et al., (2019) point out that this result can be understood due to the complexity of post-germination development, which is considered more complicated than germination itself. This is because seedling formation depends on a wider variety of physiological processes, such as tropisms, photomorphogenesis, root hair expression, and growth of both roots and shoots of the plant. Long et al., (2014) state that seedling malformation can also occur due to effects of cytosol viscosity, membrane fluidity and integrity, antioxidant activity, and transcription and translation rates.

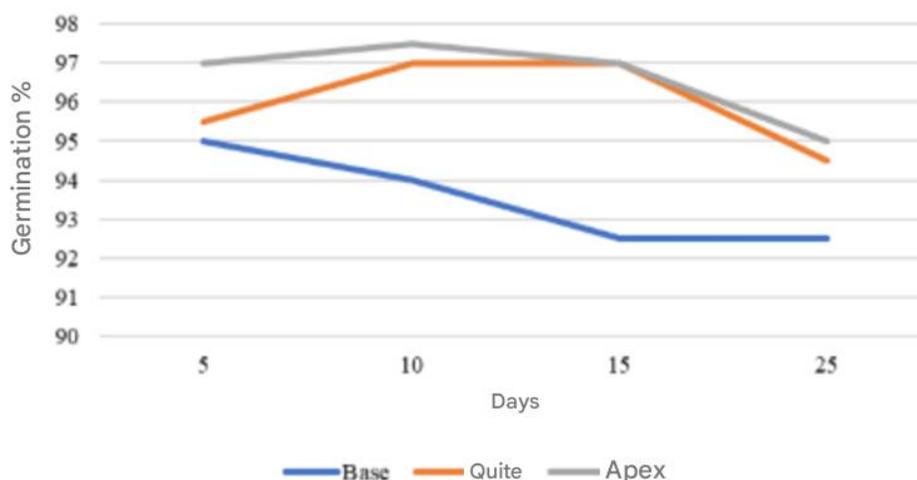
With regard to the ability of a seed to preserve its germination potential, the longevity of seeds refers to the period during which they remain alive, that is, capable of germinating (MARCOS-FILHO, 2015). However, the true longevity period of seeds could only be established if it were possible to keep them in optimal conditions, which in practice is difficult to achieve. It is possible, however, to determine viability, which represents the effective life span of the seed in a specific environmental condition (CARVALHO & NAKAGAWA, 2012). The loss of viability results in germination failures, even under favorable conditions, since it represents an irreversible degenerative change, leading to seed death.

The seeds from the middle of cocoa (T2), even with a germination result statistically equal to that of the apex, showed greater viability throughout the experiment (figure 5), demonstrating that over the days it was the treatment that had the greatest capacity to keep the seeds alive, and demonstrating its germination potential. In addition, it can be seen in the table that the seeds of the treatment were the ones that had the highest emergence rate throughout the experiment. Silveira et al., (2002), suppose that the emergence index can be

a good indicator to evaluate the vigor of the seeds of a specific matrix, as it offers more accurate predictions about how the seeds will behave when sown.

Figure 5

Feasibility of treatments at the end of the 25 days



Source: Authors.

ISTA, (2014) highlights that vigor can be defined as the set of characteristics that influence the activity and satisfactory performance of seeds in various environments. Seeds considered vigorous are those capable of performing well even in adverse environmental conditions for the species, and have great potential for a rapid and uniform germination of normal seedlings in the field (Guedes in 2009).

As for the variables evaluated, it was observed that the base treatment resulted in lower germination rate, vigor, viability and seedling formation. Therefore, it is suggested that further studies be carried out on the physiological and maturation processes of seeds located near the stalk of the cocoa fruit. These studies can help to understand the factors that led to these results, in addition to serving as a basis for future comparison and discussion.

EMBRAPA (2020) reinforces that a good seed for seedlings must have high physiological, genetic and sanitary quality, that is, it must have high germination, vigor, purity, identity and health. Seed quality directly influences the establishment, development and productivity of seedlings, as it determines the speed, uniformity and percentage of germination, resistance to pests and diseases, adaptation to the environment and expression of genetic potential.

In the treatments, the middle and apex did not present significant differences in most parameters, and were higher than the base in the variables evaluated. Thus, it can be considered that these seeds have superior quality, in ecophysiological terms. Therefore, lots

from these matrices can be more effective when used as propagation material in seedling production.

In general, the Common genotype is quite uniform, however, it has a slower development, requiring a longer period of nurseries to reach the grafting standard (Arévalo et al. 2012). Thus, the relevance of scientific research on germination is highlighted, aiming at the development of knowledge that improves the quality of plants destined for nurseries.

5 FINAL CONSIDERATIONS

Regarding the germination of the seeds as a function of different parts of the cocoa, the base was the one with the lowest performance. The middle and apex treatments showed statistically equal responses to most of the variables evaluated. In this way, the seeds of the middle and apex may have a greater chance of forming more vigorous seedlings.

There are several studies in the literature that investigate the biochemical properties of cocoa seeds. However, most of this research focuses on the modifications that occur during the drying and fermentation stages, with the main purpose of establishing a link with the quality of chocolate. (Chang et al., 2014; Febrianto et al., 2016; Gil et al., 2016). Considering that rootstock propagation is conducted mainly through seeds, studies on biochemical and physiological changes aimed at germination are extremely important, so that this knowledge can be applied in cocoa tree improvement strategies.

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