

ISSN: 2230-9926

RESEARCH ARTICLE

Available online at http://www.journalijdr.com



International Journal of Development Research Vol. 11, Issue, 02, pp. 44135-44138, February, 2021 https://doi.org/10.37118/ijdr.21084.02.2021



OPEN ACCESS

SEED GERMINATION AND VIGOR OF INGA LAURINA (SW.) WILLD. SUBMITTED TO FRACTIONING

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ABSTRACT

Article History: Received 17th November, 2020 Received in revised form 22nd December, 2020 Accepted 05th January, 2021 Published online 24th February, 2021

Key Words: Germination, Embryo, *Inga laurina* (Sw.) Willd, Polyembryonic.

whose seeds are polyembryonic therefore have the potential to regenerate new roots and even whole plants, even if part of its mass is removed. Thus the objective was to evaluate the effects of different forms of fractioning of *I. laurina* seeds and their influence on the germination process. The experiment was conducted at the Federal University of Paraíba, in a completely randomized experimental design. The treatments consisted of the following forms of fractioning: control - TO (intact seeds), division into 1/4 of the embryo opposite the hilum (T1), fractionation in 2/4 of the embryo including the hilum (T2), division into 3/4 of the embryo including the hilum (T3), division into 3/4 of the embryo opposite the hilum (T4), fractionation in 2/4 of the embryo opposite the hilum (T5) and longitudinal splitting of the embryo (T6) . In assessing the effect of the treatments, it was determined: water content, percentage of germination, first count of germination, speed index and dry mass of seedlings. According to the data of seed germination in treatments T0, T2 and T3 the highest percentages were obtained. Regarding the first count there was the highest germination percentages in the treatment T2, for the germination speed index in the treatment T5 occurred the highest values. Thus it is concluded that the seeds Inga laurina can be separated into 3/4 of the embryo including the hilum without harming their physiological quality.

Inga laurina (SW.) Willd. It is a species of woody habit, belonging to the Fabaceae family,

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Citation: Jardel da Silva Souza, Edna Ursulino Alves, Rosemere dos Santos Silva, Flávio Ricardo da Silva Cruz and Antônio Pereira dos Anjos Neto. 2021. "Seed germination and vigor of Inga Laurina (sw.) willd. submitted to fractioning", International Journal of Development Research, 11, (2), 44135-44138.

INTRODUCTION

Inga laurina (SW.) Willd, is a species of woody habit belonging to the family Fabaceae, which has about 300 woody species distributed in 14 sections (PENNINGTON, 1997), whose seeds differ from those of the other plants of the same botanical family because it possesses in its seeds a sweet white pulp involving (RICHARDSON et al., 2001), which is known as sarcotesta and is very rich in sugars, which is the result of a differentiation of the layer of the forehead, formed by a long unicellular fiber. The name Ingá is derived from the indigenous Tupi language, meaning "that has seed in it" (RODRIGUES, 1918, apud POSSETTE, 2010), whose species is exclusively neotropical, possessing seven main phytogeographic distribution areas, among which the coast and interior of Brazil, being the main centers of diversity of the genus Southeast of Central America and the West of South America (PENNINGTON, 1997). The seeds of the genus Inga, which aggregates I. laurina are classified as recalcitrant (BILIA et al., 2003), with this they have a

low longevity and high intolerance to desiccation (BARBEDO and CICERO, 1998; FARIA et al., 2006), in other words, these seeds do not tolerate long periods of storage, and viviparity may still occur, been a phenomenon in which the seeds germinate in the fruit due to the high water content or the low concentration of inhibitory substances (FONSECA & FREIRE, 2003). With this, the seedling production in different periods of its fruiting and maturation is difficulted. In addition, I. laurina seeds have a characteristic that distinguishes them from other species within the same botanical family, such as the presence of polyembryony seeds (SCHULZ et al., 2014). This species has great importance in the reforestation of riparian forests, recovery of degraded areas, stabilization of acid soils and also as a supplier of excellent shading in coffee and cocoa plantations, whose wood can be used for firewood (BILIA et al., 2003). Its good adaptation occurs in humid regions such as river banks, where it is common to find species of this genus, besides it has importance for Brazilian fauna and flora due to the ornamental value and its fruits are source of food for wild animals (SOUZA & LORENZI, 2005). This species has great importance in the

reforestation of riparian forests, recovery of degraded areas, stabilization of acid soils and also as a supplier of excellent shading in coffee and cocoa plantations, whose wood can be used for firewood (BILIA et al., 2003). Its good adaptation occurs in humid regions such as river banks, where it is common to find species of this genus, besides it has importance for Brazilian fauna and flora due to the ornamental value and its fruits are source of food for wild animals (SOUZA & LORENZI, 2005). The form of I. laurina propagation is through seeds, in which germination occurs between 7 to 12 days after sowing. Seed fractionation is a technique that consists of cutting them into several parts or fractions, as long as the seeds have the potential to regenerate new roots and even complete plants, even if part of their mass is removed. This technique can be used successfully in the production of seedlings of *I. laurina*, since it has seeds with polyembryony, with the formation of several embryos in a single ovule, being nucellar and zygote embryos (DEGANI et al., 1993). Some authors have used the technique of seed fractionation to obtain greater seedling production, since it is possible to produce more than one seedling per seed, as verified in the studies carried out with Eugenia pyriformis Cambess. (SILVA et al., 2005) and Eugenia nvolucrata DC, Eugenia uniflora L. and E. brasiliensis Lam. (SILVA et al., 2005), in which it was observed that the seeds even fractionated in half, contain at least half of the hilum were able to matain their germination capacity and produced normal seedlings. Seeds of Eugenia species have the potential to regenerate new roots and even whole plants even when part of their mass is removed, which rarely occurs in monoembryonic seeds. In this study, we used a new technique to estimate seedlings production, which can be used to increase seedling production potential (SILVA et al., 2005). In this context, the objective of this work was to evaluate the effects of different forms of fractionation of Inga laurina seeds and their influence on the germinative process.

MATERIAL ANDMETHODS

The experiment was carried out at the Laboratory of Seed Analysis of the Department of Plant technology and Environmental Sciences of the Center of Agricultural Sciences of the Federal University of Paraíba, Areia - PB, with manually harvesting of *Inga laurina* seeds in a mother plant located in the same city. The harvest was carried out when the fruits were yellowish and transported in raffia bag to the laboratory where the procedures of seed treatment were conducted, removing all the sarcotesta that envolved the embryos, followed by the fractioning of these seeds with a blade disinfested with alcohol at 70%. **Seed fractioning:** The fractions used were: control - T0 (intact seeds), fractionation in 1/4 of the embryo on the opposite side of the hillum (T1), fractionation in 2/4 of the embryo including the hilum (T2), fractionation in 3/4 including the hilum (T3), fractionation in 3/4 of the embryo on the opposite side of the hilum (T4), fractionation in 2/4 of the embryo on the opposite side of the hilum (T5), and longitudinal fractioning of the embryo (T6).

Water content: The method used was the oven at $105 \pm 3 \circ C$ for 24 hours (BRASIL, 2009), and four replicates of 10 g of intact seeds were taken, the results of which were expressed as a percentage of water content.

Seed Germination assessment: For each treatment, 100 embryos or fragments of these were used, divided in four replicates of 25, which were distributed on two sheets of paper towel, covered with a third and organized in roll form. The paper towel was moistened with distilled water in the amount equivalent to 2.5 times its dry mass as recommended by BRASIL (2009), whose rolls were packed in transparent plastic bags, 0.04 mm thick, in order to avoid water loss by evaporation. Before distribution, the embryos and fragments were treated with Captan kg⁻¹ in the proportion of 240 g 100 kg⁻¹ of seeds to avoid the development of phytopathogenic fungal on seeds. The test was carried out in germinators type Biological Oxygen Demand (B.O.D.) regulated at 25 ° C, with photoperiod of eight hours, using daylight fluorescents (4 x 20 W). The evaluations were performed daily, from the sixth to twelfth day after the test set up. When the experiment was finished, the germinated seeds were those that emitted the primary root and the aerial part (normal seedlings) and the results were expressed as a percentage of seed germination.

First count of seed germination: The first count was performed together with the germination test, determining the percentage of normal seedlings on the sixth day after the test installation and the results were expressed as a percentage.

Germination speed index: The germination rate index (IVG) was performed in conjunction with the germination test, consisting of effective daily counts of the normal seedlings, at the same time, from six to 12 days after the test installation, and the index was obtained using the formula proposed by MAGUIRE (1962).

Length and dry mass of seedlings: After the final germination test, the normal seedlings of each treatment and repetition were measured (root and shoot), using a ruler graduated in centimeters, and the results were expressed in cm⁻¹ seedlings. In order to obtain the dry



Figure 1. Seed fractioning of *I. laurina* T1 - 1/4 of the embryo on the opposite side of the hilum, T2 - 2/4 of the embryo including the hilum, T3 - 3/4 of the embryo including the hilum, T4 - 3/4 of the embryo on the side opposite of the hilum, T5 - 2/4 of the embryo on the opposite side of the hilum and T6 - longitudinal fractionation of the embryo

conditioned in Kraft paper bags in circulation kilns and forced air renovation regulated at 80° C for 24 hours, after which period, the samples were weighed in analytical balance, and the results expressed in gram⁻¹ seedlings.

Experimental design and statistical analysis: The experimental design was completely randomized, and the data were submitted to analysis of variance by the F test and the means grouped by the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

The water content of the seeds was 51.9%, expected result for species that have recalcitrant seeds, that is, that maintains a high content of water even after reaching the physiological maturity. This moisture content did not favor that the viviparity process to occur in seeds, according to FONSECA & FREIRE (2003) when they reported that the persistence of high water content, between 62 and 52% after seed maturation and / or in seeds with low concentration of germination inhibiting substances present in the fruit and / or in the seed itself may inhibit the viviparity process. For all analyzed variables, germination percentage (G), first count (PC), germination speed index (IVG), shoot length (CPA), root length (CRA), shoot dry mass (MSPA) and root dry mass (MSRA) showed a significant effect at 1% probability (Table 1).

In other works, BONJOVANI & BARBEDO (2008) emphasized that seeds of Inga vera Willd. subsp. affinis (DC.) T. D. Penn. are recalcitrant and therefore do not undergo changes in the water content nor in the water potential of the embryos at different stages of maturation. According to KIKUTI (2002), during the process of physiological maturation of recalcitrant seeds, there is a decline in the water content of the seeds, but this is not significant when compared to the dehydration phase of orthodox seeds. In the treatments TO (intact seeds), T2 (fractionation in 2/4 of the embryo including the yarn) and T3 (fractionation in 3/4 of the embryo including the yarn), because the seeds had these characteristics mentioned above and also emphasizing that they possessed the hilum, supposedly these factors contributed to the greater production of growth promoters and lower inhibitory substances. For the data referring to the germination speed index, there was no difference between the treatments, except for the T5 treatment (fractionation in 2/4 of the embryo on the opposite side of the thread), which showed the lowest values (Table 2), also emphasizing that the same lowest percentages of germination were found. It can be assumed that this is related to the fact that the hilum is not being part of this fraction, being this region of the seed the place of the reserves and do not have embryos to give the command of germination. Because gibberellic acid acts as a promoter of germination, it can be said that in treatments T0 (Intact seeds), T1 (1/4 of the embryo on the opposite side of the hilum), T2 (2/4 of the embryo including the hilum), T3 (3/4 of the embryo including the hilum), T4 (3/4 of the embryo on the side opposite of the hilum) and

 Table 1. Mean squares referring to the first count (PC), germination percentage (G), germination speed index (IVG), shoot length (CPA) and root (CRA), shoot dry mass (MSPA) and roots (MSRA) of *I. laurina* as a function of fractionation

FV	Mean squares									
	GL	G	PC	IVG	CPA	CRA	MSPA	MSRA		
Treatment	6	5003,8095**	1171,8095**	6,4074**	16,3338**	12,1228**	0,0928**	0,2012**		
Error	21	81,5238	76,9524	1,0355	4,1649	1,9669	0,0008	0,0028		
Mean	-	63,42857	26,57143	2,37	7,870893	4,41386	0,21075	0,32543		
CV (%)	-	14,23	33,01	42,94	25,93	31,77	13,64	16,18		
(**) 1% ofprobability.										

Table 2. First count, percentage of germination and germination speed index (IVG) of I. laurina seeds submitted to fractioning

Treataments	Germination	FirstCount	IVG
	%	, D	_
T ₀	92 a	30 c	2,87 a
T_1	58 b	14 d	2,31 a
T_2	99 a	55 a	4,07 a
T_3	98 a	40 b	3,35 a
T_4	45 b	23 c	2,13 a
T_5	3 c	3 d	0,11 b
T_6	49 b	21 c	1,76 a
CV (%)	14,23	33,01	42,94

From the seed germination data, it was found that the highest percentages were obtained in treatments T0 (intact seeds), T2 (fractionation in 2/4 of the embryo including the hilum) and T3 (fractionation in 3/4 of the embryo including the hilum), while the lowest percentages occurred in the T5 treatment (fractionation in 2/4 of the embryo on the side opposite the hilum) (Table 2). In relation to the first count, the highest percentages of germination in the T2 treatment (fractionation in 2/4 of the embryo including the yarn) were observed, whereas in T1 treatments (fractionation in 1/4 of the embryo on the opposite side of the thread) and T5 (Table 2), probably due to the increase in the production of germination promoting substances, which were activated by the seed damage, which accelerated the metabolic reactions and also the germination of these seeds. Means followed by the same numbers do not differ statistically by the Scott-Knot test at 5% probability.T0 - (Intact seeds), T1 - 1/4 of the embryo on the opposite side of the hilum, T2 - 2/4 of the embryo including the hilum, T3 - 3/4 of the embryo including the hilum, T4 - 3/4 of the embryo on the side opposite of the hilum, T5 -2/4 of the embryo on the opposite side of the hilum and T6 longitudinal fractionation of the embryo. When I. laurina seeds reach maturity, they have a higher percentage of germination and the fruits are yellow-green and yellow (SCHULZ, 2014).

T6 (longitudinal fractionation of the embryo) with the exception of T5 (2/4 of the embryo on the opposite side of the hilum) the seeds damages the production of this phytormonium. With the use of plastic bags to prevent dehydration during the germination process, a large production of germination inhibiting substances such as ABA (abscisic acid) was avoided. According to FERREIRA & BORGHETTI (2004), ABA is an inhibitor of germination when in large quantities, and authors such as CARDOSO (2004) reported that ABA contributes to increase tolerance to dehydration and cold.The ABA exerts protective effects on water stress inducing the expression of genes that consequently promote the synthesis of proteins, to avoid water losses and thus restore cell damage (STACCIARINI-SERAPHIN 2004, apud MUXFELDT, 2008). The highest lengths of the primary root were verified in treatments T0 (intact seeds) and T3 (fractionation in 3/4 of the embryo including the hilum), while for the others there was no statistical difference (Table 3), resulting in a smaller root length. During the fractioning of the seed, damage to the part responsible for the development of the root could have occurred, causing the other treatments to be adversely affected, whereas for the shoot length only the T5 treatment (fractionation in 2/4 of the embryo in the side) yielded the lowest values, showing that the fractioning of the seed does not influence the growth of the aerial part, as long as it

is fractionated as close as possible to the hilum, and it can be assumed that the damage accelerated the metabolic activity of the seeds, with which they develop, but also the fact that these seeds are with a large part of the areas where the seed stores the reserves, which are used to seed germinate, emitting the photosynthetic structures. The highest values of shoot and root dry mass (Table 3) were obtained in the T3 treatment (fractionation in 3/4 of the embryo including the hilum), while the lowest dry mass accumulation occurred in the T5 treatment plants (fractionation in 2/4 of the embryo on the opposite side of the hilum). It can be assumed that the fact that this treatment did not have the hilum, nor most part of the reserve storage region of the seed, made it impossible for the seeds during the germination process to obtain sufficient energy to develop. According to SILVA (2005) the maintenance of the thread is essential for the development of the seedling.

Table 3. Length of shoot, primary root, shoot dry mass and roots of *I. laurina* seedlings originated from seeds submitted to fractioning

Treatments	Lengt	th (cm)	Drymass (g)		
	Root	Shoot	Root	Shoot	
T ₀	7,60 a	7,18 a	0,545 b	0,371 b	
T_1	2,84 b	7,03 a	0,206 c	0,090 e	
T_2	4,09 b	8,98 a	0,432 c	0,269 c	
T ₃	5,59 a	10,40 a	0,653 a	0,428 a	
T_4	4,50 b	8,31 a	0,212 d	0,184 d	
T ₅	2,50 b	4,12 b	0,017 e	0,011 f	
T ₆	3,76 b	9,06 a	0,212 d	0,121 e	
CV (%)	31,77	25,93	16,18	13,64	

Means followed by the same numbers do not differ statistically by the Scott-Knot test at 5% probability. T0 – (Intact seeds), T1 - 1/4 of the embryo on the opposite side of the hilum, T2 - 2/4 of the embryo including the hilum, T3 - 3/4 of the embryo including the hilum, T4 - 3/4 of the embryo on the side opposite of the hilum, T5 - 2/4 of the embryo on the opposite side of the hilum and T6 - longitudinal fractionation of the embryo.

ACKNOWLEDGMENT

This study was partially funded by the National Council for Scientific and Technological Development (CNPq).

CONCLUSION

The seeds of *Inga laurina* (Sw.) Willd. can be fractionated in 3/4 of the embryo including the yarn without damaging its physiological quality.

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