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Chloroleucon tenuiflorum (Leguminosae, Ingeae): Morphometry of Fruits, Seeds and Seedlings, Healthy and Germinability

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Abstract

To achieve a morphological and healthy characterization of fruits, seeds and seedlings of *Chloroleucon tenuiflorum* (Benth.) Barneby & J. W. Grimes, collected in northwestern Argentina, this work was performed. The mechanical scarification using sand paper and the immersion in sulfuric acid during different periods of time are the best to obtain higher levels of the % of accumulative germination. The IRP index reached 283% of increase after 48 hs; the correlation index length of fruit/ n° of seeds is optimum. The healthy seeds were the 84, 4%, while the unhealthy constituted the 15,15% (aborted, destroyed, eaten or attacked). The germinability allowed to confirm that the seeds are orthodox. Germination is epigeal, phanerocotyledonar, with photosynthesizing cotyledons, seedlings have two opposite protophylls and 1-2 alternate bipinnate eophylls. The lapse 24 - 48 hours after the moistening of pretreated seeds is optimum to obtain an effective % of germination.

Key words: *Chloroleucon tenuiflorum*, morphometry, fruits, seeds, seedlings.

Introduction

The species grow from the first slopes of the Subandean forests District (Yungas phytogeographic Province) and trough the Chaqueña phytogeographical Province (Cabrera, 1994), covering the xerophilous forests of Bolivia, north of Argentina and Paraguay, growing in monospecific woodlands owing to their radical saplings, in northeastern Argentina they are called commonly "capons" (Hoc, 1981; 2005). The trees are multipurpose because the wide applications of their wood (Tortorelli, 1956), including fine furniture (Pedersen, *com. pers.*).

The seeds of Angiosperms can be classified, owing to their morphological and physiological characters in orthodox and recalcitrant (Roberts, 1973). It has been suggested that the orthodox character should be ancestral, and it has been loosed during the evolution to the recalcitrant character (Farnsworth, 2000; Garwood, 1983). The orthodox character constitutes clearly an adaptive advantage in xeric environments or with a dry season, ought to the resistence to the scarce humidity, in the other hand, the recalcitrant character is clearly advantageous in those species that grow in humid soils or rainforests (Pammenter & Berjak, 2000). Some authors sustain that the orthodox character confers more resistence to predation (Daws *et al*., 2005).

As some authors stated during their studies in leguminous seeds (Abdala *et al.*, 2003; Araujo Neto *et al*., 2002; Parra & Maciel, 2011; Gómez da Silva *et al*., 2012; de Viana *et al*., 2014) the seed morphological analysis and their germinability are relevant in order to preserve the germplasm, specially of the native species with potential and/or in risk, having done previously a correct characterization, especially in the populations that grow in degraded areas.

The aim of this work was to achieve a morphological and healthy characterization of fruits, seeds and seedlings of *Chloroleucon tenuiflorum* (Benth.) Barneby & J. W. Grimes, collected in a neighbor of The Selvas Pedemontanas (phytogeoghraphical Province of The Yungas), (Cabrera, 1994); as well as to establish the more effective treatment in order to induce the germination of the collected germplasm and keep record the installation of the emergent seedlings.

Material and Methods

The studied material was collected circa September 2012 at the end of the fructification, that was followed by a new production of leaves and a new and profuse flowering, although a new fructification did not occured. The fruits have been harvested from five trees that grew in a population at the first slopes of the Pedemontana District (phytogeographycal Provincia de Las Yungas), (Cabrera, 1994), between Metan and Rosario de La Frontera (Salta, Argentina) besides the road n° 9/34, in the environs of the nursery-garden Vivero La Guarida (25,55698° S and 64,97031° W) placed at the south of Metan , at the Finca San José de Yatasto (25,62676º S y 64,95276º W) at 8 km of the first locality.

In the laboratory the study started on October 2012 when 100 fruits were measured and the seeds were removed. The dimensions measured in the fruits were length (using a plastic thread) following the contour of the dorsal spires and translating this to a millimeter ruler; wide measured with a millimeter ruler and thick taken with a

gauge at the middle of the fruit that had seeds. Besides, it was measured the number of spires in each fruit. All the fruits were weight with a precision scale (ε = 0, 0001 gr.).

It was registered the total number of seeds in each fruit, also it was measured the length, wide, thickness and weight of 100 seeds using a caliber and an accuracy balance, discriminating them in big and small, healthy and unhealthy, mature and immature. In each fruit it was calculated the percentage of seeds with some damage (infections, infestations, etc.).

Using all the data it were calculated the average and standard deviation, also it was calculated the correlation coefficient between the fruit dimensions and the number of seeds using the Statistics Calculator, El Imperio de los Números - Herramientas potentes de matemáticas para todos (Google+, 2016).

Taking in account the osseous consistency of the seed tegument (Hoc, 1981), it was considered the existence of physical dormancy (DF), evaluated through imbibition curves; to construct them it were used 25 seeds without any treatment and 25 seeds with mechanical scarification (using a sandpaper), all were weightened using a presition scale (ε = 0 0,1 mg). The seeds were placed in Petri dishes which contained wool covered with filter paper previously moisturized with distilled water, soon after the Petri dishes were disposed in a germination chamber at 25 ˚C. Every two hours during the first 12 hours the seeds were removed and weightened, later they were removed and weightened at 24 hours and 48 hours. After each removal and weight it was calculated the Baskin Index using the formula IRP = (final weight- initial weight) /initial weight x 100 (Baskin *et al*.., 2004); also it was performed an index of correlation (http://www.monografias.com/trabajos93/analisis-correlacion-empleando-excel-y-graph/analisis-correlacion-empleando-excel-y-graph.shtml#ixzz44Qf7UkIG). To find the optimus rank obtained by imbibitions in which the seeds should start the germination it were calculated the average and standard deviation and the "t" test of Student was performed (Statistics Calculator, El Imperio de los Números - Herramientas potentes de matemáticas para todos) (Google+, 2016).

With the experimental basis of the DF obtained, it was considerate to determine the better methods to break the latency, using the following treatments:

Treatment 1	control (without treatment)	time
Treatment 2	Scarifying with sand paper 120 mm	---
Treatment 3	Leaching with cold water	36 hs
Treatment 4	Immersion in 80˚C water, gradual cooling imbibition	36 hs
Treatment 5	Immersion in 60˚C water, gradual cooling imbibition	36 hs
Treatment 6	Immersion in sulphuric acid 98%	5 min.
Treatment 7	Immersion in sulphuric acid 98%	10 min.
Treatment 8	Immersion in sulphuric acid 98%	20 min.

After these treatments the seeds were placed in four aftershocks with 25 seeds in each one, in Petri dishes with a substratum of agar at 1% in water, then they were incubated at 25 ˚C and a photoperiod of 12/12 hs light/obscurity in a germination chamber. The number of germinated seeds was recorded daily during 30 days. The criterion used to determine if a seed started to germinate was the observation of the radical emergency. Finalized the experiment it was calculated the total proportion of germinated seeds (p) and the velocity of germination (MTG) (Pritchard & Müller, 1995). These response variables were analyzed performing an ANOVA, also, it was used the DGC test to detect the differences when the results of the ANOVA were significative. The statistical analyses were performed using the InfoStat program (Di Rienzo *et al*.., 2014).

Later, during 30 days, an experience to determine the emergengy and development of the seedlings was performed, taking into account the time of germination, the hypocotyl and epicotyl characteristics, the number and morphology of the protophylls and the later generation of the eophylls. In order to carry on this experiment, it were prepared 10 plastic pots filled with a mix of humus and perlite, it were sown five seeds in each pot, previously mechanically scarified, in order to study the seedlings development under controlled conditions. It was applied an initial and uniform irrigation, then the pots were covered with transparent plastic bags sufficiently to allow the development of seedlings. The pots were placed into a germination chamber at 25˚C and a photoperiod of 12/12 hs. It was recorded periodically the development of the seedlings, measuring all the parts that are documented here.

Results

1. Fruits and seeds morphology. Curled legumes of 29,9 (+/- 4,44) cm long. x 1,3 (+/- 0,28) cm wide. x 3,4 (+/- o,74) cm thick, with 2,5-5 whorls, 3,18 (+/- 0,805) gr weight. Thin epicarp, fleshy and fugacious mesocarp, lignified and dehiscent at maturity endocarp; with 18,14 (+/- 3,79) exalbuminated seeds with sclerotest and a "linea fisura" or pleurogram closed or not towards the micropyle, originated in anatropous ovules, sagittatus cotyledons covering incompletely the radicule. (Tab. 1; Figs. 1, 2 and 3).

Fig. 1. A , mature fruits; B, infested fruit, bar = 2 cm; C, fruits in different stages of maturation, bar = 2 cm**.**

Table 1. Dimensions of the fruits

	Fruits					**Seeds**
	Lenght (cm)	Wide (cm)	Thickness (cm)	Weight (gr)	N° whorls	N° seeds
Average	29,9	1,3	3,4	3,18	4,34	18,14
Sample size	100	100	100	100	99	100
Standard deviation	4,44	0,28	0,74	0,81	1,21	3,79

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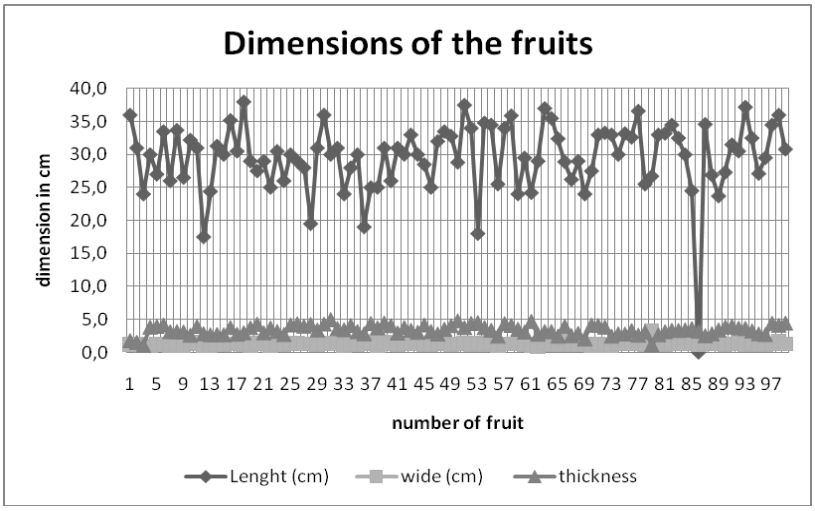

Fig. 2. Dimensions of the fruits

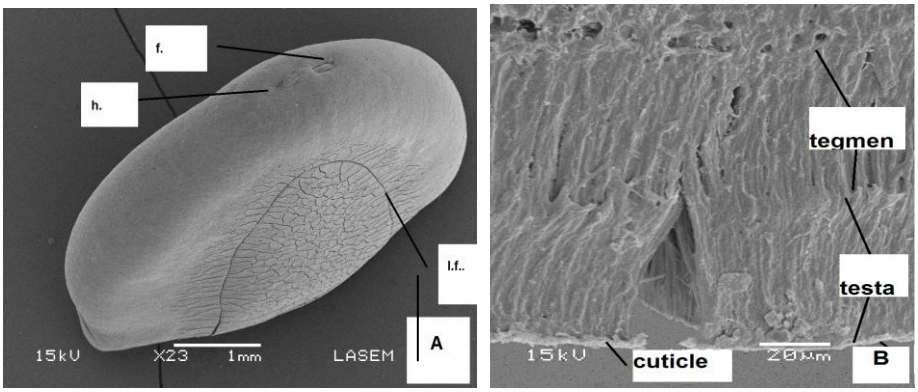

Fig. 3: Morphology of seeds. A, seed in lateral view, f = funiculum, h = hilum, l.f. = linea fisura or pleurogram; B, transversal section of a seed showing the morphology of the cells of the testa (hour glass cells) and the tegmen (irregular contour cells), the testa is externally covered by a cuticle.

2. Healthy of the fruit.: It was measured in 100 fruits, the results are synthetized in the Tab. 2. It can be seen that the 53% of them are healthful.

Table 2. Healthy of the fruits.

Characteristic	**Number and percentage**
Dehiscent,healthy	41
Indehiscent healthy	51
Perforated	12
With protuberances	7
Perforated, with protuberances, perforated predated by birds	25
TOTAL	**100**

3. Correlation index between the number of seeds per fruit and the dimensions. It has been proved that exist a positive correlation between the number of seeds and the dimensions of the fruit, standing out as perfect the relation length of the fruit/ n° of seeds. The results of this study are exposed in the Tab. 3 and the Fig. 4.

Table 3. Correlation index between the dimensions of the fruit and the number of seeds

Dimension	**Grams or cm**	**sample**	**Obtained index**	**Index from tables (*)**
Length	33,7 cm	20	**r= 1,00001**	+ perfect
Width	1,3 cm	20	**r=0,999**	+ very high
Thickness	3,97 cm	20	**r= 0,999**	+ very high
Weight	3,06 gr.	20	**r= 0,94**	+ very high

(*) http://www.monografias.com/trabajos93/analisis-correlacion-empleando-excel-y-graph/analisis-correlacion-empleando-excel-y-graph.shtml#ixzz44Qf7UkIG

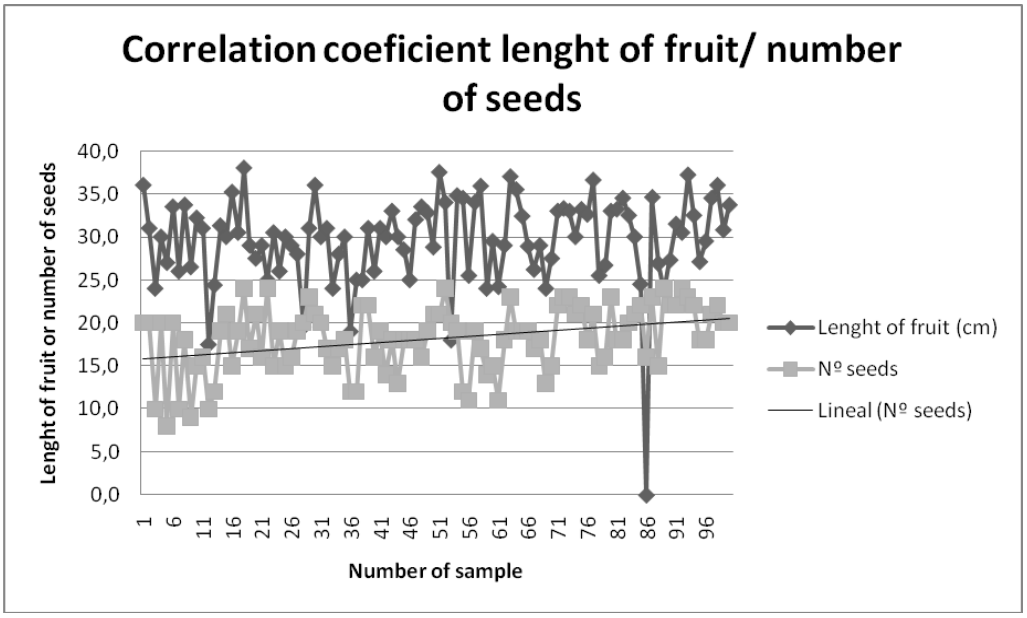

Fig. 4.: Index of correlation between the lenght of the fruit and the number of seeds.

4. Physical dormancy (DF). It has been proved its existence because of the weight of the non scarified seeds remained practically unchanged during the imbibition till 48 hs; meanwhile, the weight of the scarified seeds quadrupled at the 24 hs, more one, at the 48 hs almost all the seeds germinated.

5. Mechanical scarification using sand paper. The record of the seminal weight increase by means the of mechanical scarification is exposed in the Tab. 4.

Table 4. Increase of the seminal weight with mechanical scarification.

Initial weight	**Weight 2h**	**weight 6h**	**weight 8 h**	**weight 10h**	**weight 12 h**	**Weight 24 h**	**weight 48h**	
0,053	0,083	0,08	0,08	0,09	0,091	0,133	0,15	**Mean**
0	25	25	25	25	25	25	12	**n**
0,05	0,113	0,154	0,01	0,02	0,02	0,04	0,02	**δ**

In order to estimate the optimus time of this treatment it was performed a "t" test, which results are exposed in the Tab. 5 and Fig. 5. From this analysis it arise that the optimum time range fluctuates between the 24 and the 48 hs.

Table 5. Increase of the seminal weight using mechanical scarification, test of "t". Note: the significative differences, has been compared with t Tables, are written in bold and italics (p = 0,005).

Weight	Initial weight	Weight 2 hs	Weight 6 hs	Weight 8 hs	Weight 10 hs	Weight 12 hs	Weight 24 hs	Weight 48 hs
Initial weight	-------	1,5	1	***3***	***4***	***4***	***6,15***	***10***
Weight 2 hs	1,5	-------	0,35	0,05	0,17	0,43	2,5	***3,5***
Weight 6 hs	***1***	0,35	-----	0,13	0,37	0,33	2	2,33
Weight 8 hs	***3***	0,05	0,13	------	1,2	1,8	0,5	***11,6***
Weight 10 hs	***4***	0,17	0,37	1,2	-------	0,8	***4,7***	***8,6***
Weight 12 hs	***4***	0,43	0,33	1,8	0,8	------	***4,4***	***8,6***
Weight 24 hs	***6,15***	2,5	2	0,5	***4,7***	***4,4***	-----	***2,2***
Weight 48 hs	***10***	***3,5***	2,33	***11,6***	***8,6***	***8,6***	2,2	-------

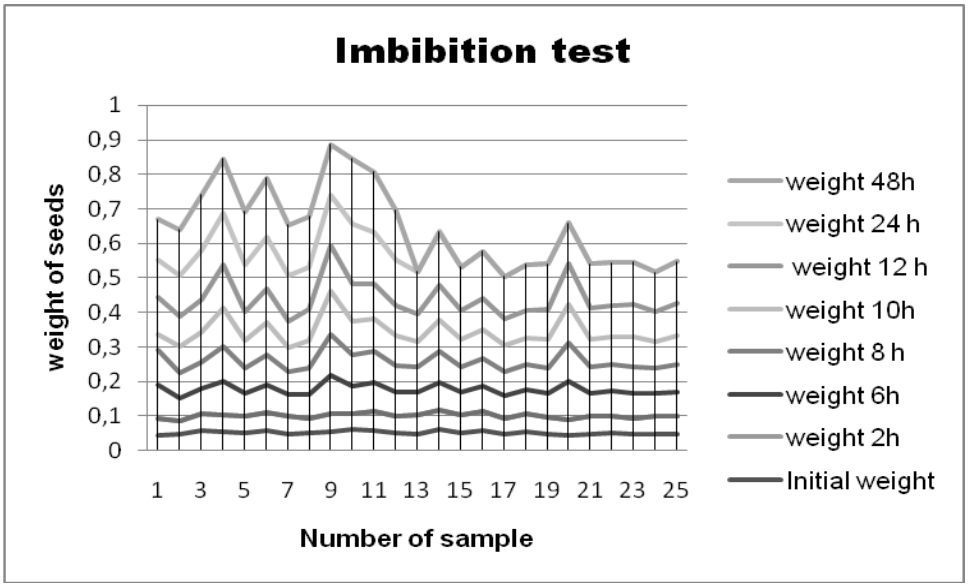

Fig. 5: Increase of the seminal weight comparing the control with the seeds scarified mechanically.

6. Index of Baskin (IRP). The index of weight increase during the imbibition after applying mechanical scarification, the results are exposed in the Tab. 6, is remarkable that the final weight is 3,5 heavier than the initial one .

Table 6. Results of IRP

Initial weight	**Weight 2h**	**Weight 6 h**	**Weight 8 h**	**Weight 10 h**	**Weight 12 h**	**Weight 24 h**	**Weight 48 h**	
0,0529	0,0825	0,0765	0,0815	0,0854	0,0929	0,1328	0,1541	**Mean**
-------	**55,95**	**44,61**	**53,87**	**61,44**	**75,61**	**149,53**	**191,11**	**IRP**
25	25	25	25	25	25	25	12	**N**

7. Seed healthy during the harvest. It has been recorded the % of healthy seeds and the % of those that had a phytosanitary problem, taking into account the % of abortion or no formation of seeds. The results show that the healthy seeds are represented by a high % in the harvested sample (85%). The results are exposed in the Tab. 7 and Fig. 6.

Table 7. Seed healthy. The discriminated % of the unhealthy seeds were calculated in base of the total of non healthy seeds. *Perhaps fungus attack. Σ = total; Mean = average; n = sample; % = percent.

N° Healthy seeds	**N° Unhealthy seeds**							
	Total	**Destroyed**	**Perforated**	**Eaten**	**Witish***	**Black & Mishappen**	**Inexistent or Aborted**	
1523	272	32	41	61	53	66	10	**Σ**
30,1584	5,8494	1,6842	1,5769	2,7727	4,8182	6,2857	5	**Mean**
102	94	19	28	23	23	1	10	**n**
84,84	**15,15**	11,8	15,1	22,4	19,5	24,3	3,7	**%**

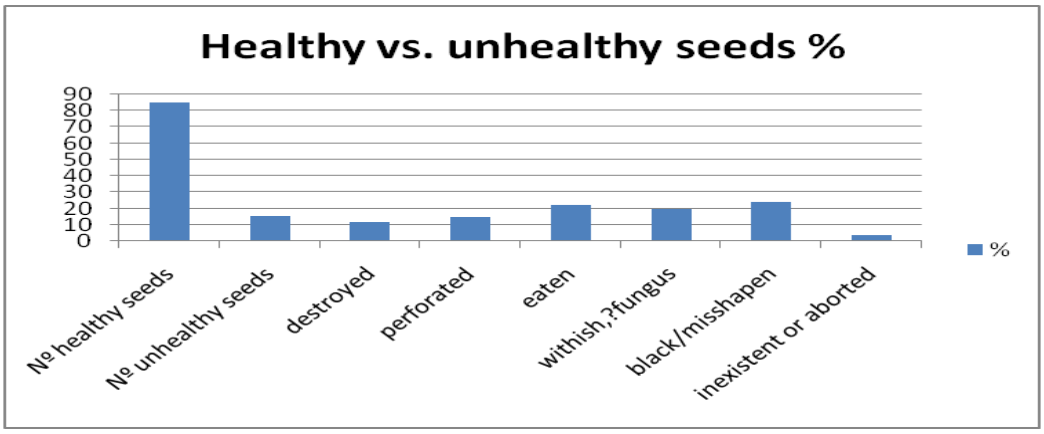

Fig.6. Gráfico de sanidad seminal.

Fig. 6. Percentage of healthy and non healthy seeds. Y axis = %; X axis = kind of seeds.

8. Proportion of germination, mean time of germination (MTG) and accumulated germination. The proportion of germinated seeds was different according to the scarification method employed (F = 47,58; P > o,oo1) (Fig. 7). The higher values were recorded in the mechanically scarified seeds and in those immersed in sulfuric acid (averages between 93 and 97%), almost the double of those submerged in water at 80 ˚C (43%). In the others methods of scarification the results were similar to the % found in the no scarified seeds (less than 10%). The velocity of germination (MTG) also fluctuated according the treatment (F = 4,86 ; P = 0,002), being lower in the seeds treated with warm water (at 60 ˚ C) and those non treated or witness (Fig. 8).

The proportion of accumulated germination recorded daily showed a differential behavior according to the imbibition and scarification method applied (Fig. 9). It were recorded the higher values (more than 87%) during the two first days in the mechanically scarified seeds or scarified with sulfuric acid, while in those treated with warm water the germination was gradual and staggered.

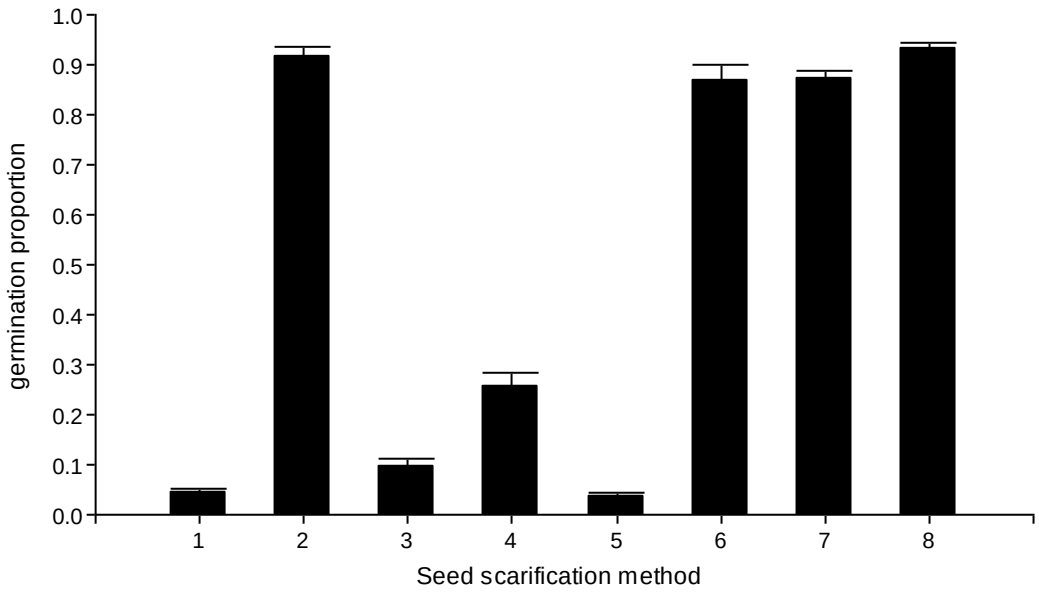

Fig. 7: Proportion of germinated seeds (average ± standard deviation) for each treatment. 1: witness, 2: mechanical scarification, 3: lixiviation 36 hs, 4: water at 80ºC, 5: water at 60ºC, 6: sulfuric acid 5 minutos, 7: sulfuric acid 10 min., 8: sulfuric acid 20 min.

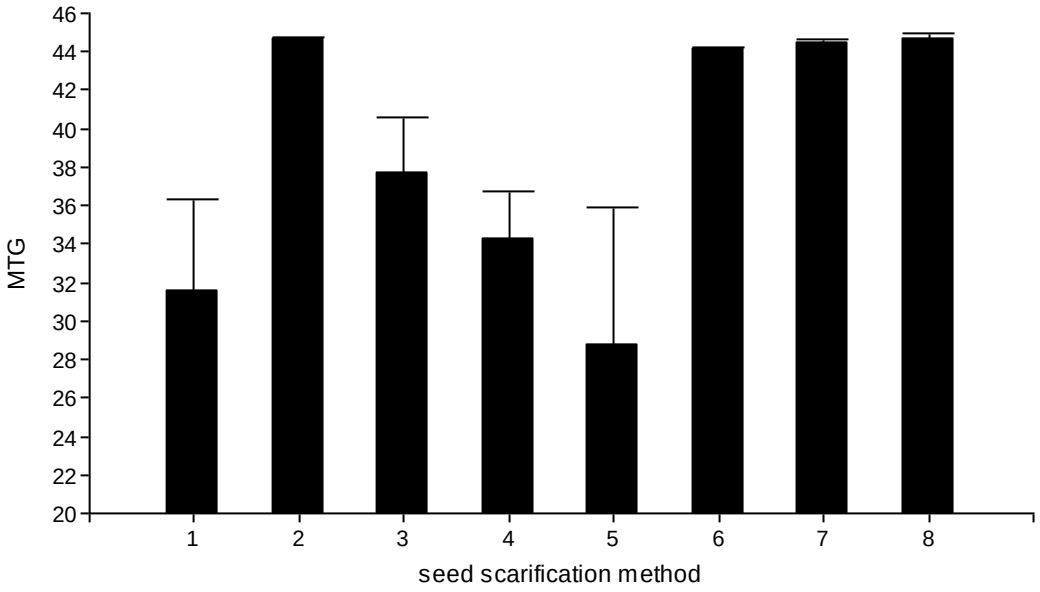

Fig. 8. Average time of germination (average ± standard deviation) for each treatment. 1: witness, 2: mechanical scarification, 3: lixiviation 36 hs, 4: water at 80ºC, 5: water at 60ºC, 6: sulfuric acid 5 minutes, 7: sulfuric acid 10 minutes, 8: sulfuric acid 20 minutes.

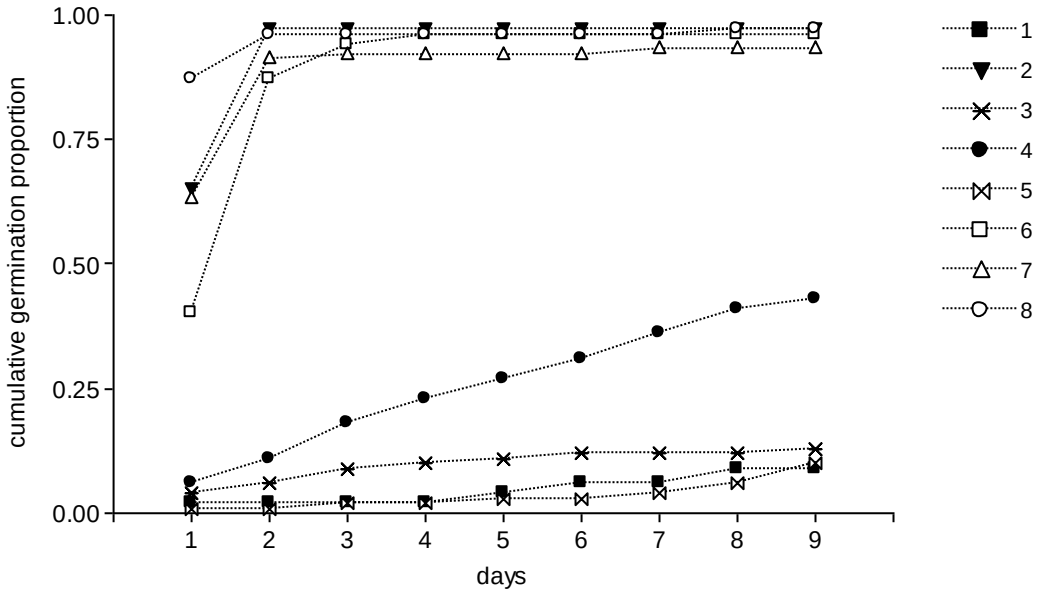

Fig. 9. Average accumulated proportion of the seeds germinated for each treatment along the days. Treatment . 1: witness, 2: mechanical scarification, 3: lixiviation 36 hs, 4: water at 80ºC, 5: water at 60ºC, 6: sulfuric acid 5 minutes, 7: sulfuric acid 10 minutes, 8: sulfuric acid 20 minutes.

With the basis on this study it can be inferred that the mechanical scarification is the more effective given that the higher proportion of germinability has been recorded at 48 hs.

9. Development of the seedlings. The experiment started on October of 2012. Since the begining of germination, and during 27 days, it was recorded the development of the seedlings, which is summarized in the Tabs. 8 and 9, and the Fig. 10.

Table 8. Development of seedlings.

Date	Root (long.) cm	Hipocotyl (long.)cm	Cotyledons (long.) cm	Epicotile	Protophylls	Morphology of Protophylls	Eophylls morphology	stem (long) cm
5days	1,6-4,9	4,2 – 6,5	7-7,5 x 5-5,5	0 - 0,2	1	pinnated	0	0
7 days	1,7-5,7	6,6-7,7	6-7,5 x4-5,5	0,3-0,4	2 opuestos	1 pinnated 1 bipinnated	0	0
20 days	6,3-8,2	8,3-9,1	Caducos	0,1-0,3	2 opuestos	1 pinnated 1 bipinnated	2 bipinnated	2,8
27 days	7-9,4	7-8	Caducos	0,2-0,5	2 opuestos	1 pinnated 1 bipinnated	3	2-4,5

Tab. 9. Morphology and dimensions of the protophylls and the eophylls.

	Protophylls	**1˚ Eophyll**	**2 ˚ Eophyll**	**3˚ Eophyll**
Phyllotaxys	Opposite (2)	Alternate	Alternate	Alternate
Division of leaf blade	1 pinnate 1 bipinnate	Bipinnate	Bipinnate	Bipinnate
N˚ leaflets	Each pinna 5 -6 pairs	Pinnae 5-6 yugae	Pinnae 5-8 yugae	Pinnae 4-yugae
Dimensions of the leaflets	1-Pinnate: 1-7 x 0,5-3 mm * 1Bipinnate: 2-8 x 1-3 mm*	3-9 x 1,5-4 mm	2-9 x -4 mm	3-6-x-1-2 mm.

Obs.: the values represent average measure. The sample is of 100 seedlings (20 pots with 5 seeds sown).

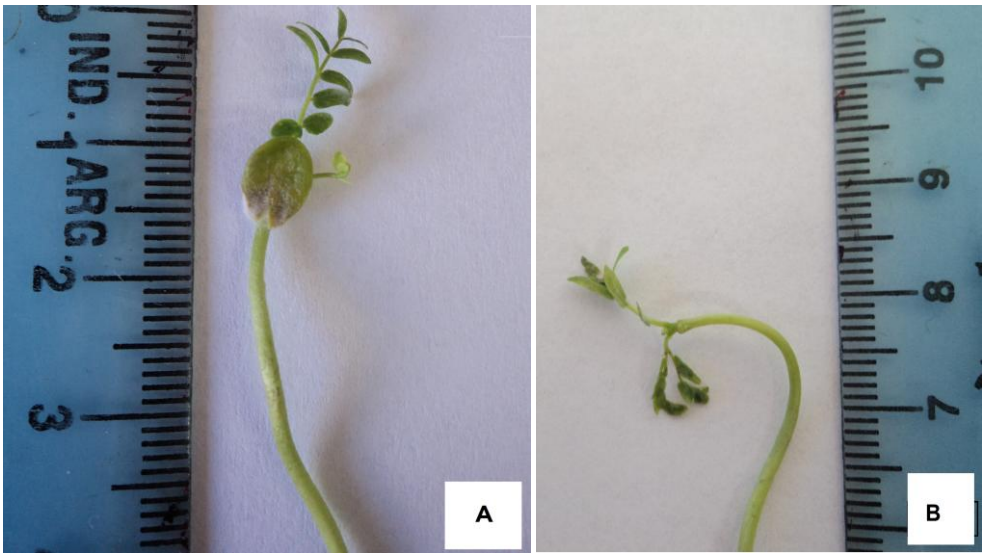

Fig. 10. Stages of development of the seedling. A, hypocotyl, cotyledon with sagitated base, protophyll developed and the other in development; B, cotyledons detached, two protophylls; C, root with nodules, protophylls opposite, two eophylls alternate, apex; D, root with nodules, cotyledons foliaceous and still persistent, two protophylls opposite and two eophylls developed, alternate with the protophylls. Scales: C, D bar = 1 cm.

Discussion and Conclusions

It has been suggested that the orthodox character should be ancestral and has been lost gradually favouring the installation of the recalcitrant seeds (Farnsworth, 2000; Garwood, 1983). The orthodox character constitutes clearly an adaptive advantage in xeric environments or in those areas with a dry season owing to the resistance to the scarce moist of the soil. Other authors points out that the orthodox character confers resistance to the predation (Daws *et al*., 2005). The results of the present study confirm the orthodox character of the studied seeds, considered by Garwood (1983) as belonging to the first group that includes the seeds that are dispersed during the dry season and persist in lethargy till the next rainy season.

Abdala *et al*. (2003) applied in seeds of *Chloroleucon tenuiflorum* mechanical scarification (doing transversal cuttings in the opposite side of the hilum) and chemical scarification (immersion in concentrated sulfuric acid and immersion in warm water), their results show that the best efficiency are obtained from the mechanical and chemical scarification, suggesting that it is needed to study the anatomy of the seeds in order to know which mechanisms are involved in the delay of the germination, probably unknowing the previous reports of Corner (1951) and Gunn (1984).

The results of this study show that the mechanical scarification using sand paper and the immersion in sulfuric acid during different periods of time are the more indicated in order to obtain the best levels of the % of accumulative germination. More one, here it was estimated the relative increase of the weight of the seeds using the IRP index recording past 48 hs a 283% of increase.

Gomes da Silva *et al.* (2012) studied the germination of *Cassia grandis* (Leguminosae, Caesalpinoideae) and conclude that the mechanical scarification and the chemical scarification (sulfuric acid) are the methods more indicated to obtain the best percentages of germination, but adopt the mechanical scarification because is less dangerous and cheaper.

de Viana *et al.* (2014) studied part of a population of *Chloroleucon tenuiflorum* that grow in the surroundings of Orán, Salta (Argentina), concluding that the weight of the fruits and seeds in each plant is smaller than the average, with hight dispersion. The results here obtained show that if the average weight of the fruit is 33 % lesser than the average obtained by de Viana *et al*. (2014), but their distribution has low dispersion. More one, the results of this work show that the correlation index length of fruit/ n° of seeds is (+) and optimum. deViana *et al.* (2014) pointed out that the average of seeds/fruit is 10,7, discriminating against the healthy seeds (69,7%) from the abortive and predated (13,7 and 16,7% respectively), in this study, however, the average of seeds /fruits was of 18,14 %; as to the health of the seeds, in this study the healthy seeds constituted the 84,8%, while the unhealthy seeds constituted the 15,15%, considering the abortions, no formation of seeds, destroying, eaten or attacked by fungus.

deViana *et al.* (2014) concluded that the seeds are probably orthodox because can tolerate the dissecation (3 -5 % of CH), with a viability of 93%. In this study it was analyzed the germinability and installation of the seedlings with and without previous treatments, therefore it can be affirmed that the seeds are orthodox.

Cajueiro Gurgel *et al.* (2012), when described seedlings of Leguminosae in order to solve taxonomical problems, followed to Ducke and Polhill (1981), distinguishing 4 categories taking into account if the germination is epigeal (phaneroepigeal, with photosynthesing cotyledons reservant or not), hypogeal (cryptohypogeal with the cotyledons reservant arising at the level of soil or below of this) placing the seedlings of Mimosoideae and Caesalpinoideae with an epigeal germination with photosynthesinzing cotyledons not reservant. The same criteria were followed by Martins da Silva (2006) who pointed out that the germination of *Copaifera* spp. is phaneroepigeal with reservant cotyledons.

The results of this work allow to describe the germination as epigeal, phanerocotyledonar, with photosynthesizing cotyledons, the seedlings have two opposite protophylls (one pinnate, the other bipinnate) and two or more eophylls bipinnate. This fact implies, as Cajueiro Gurgel *et al.* (2012) suggested, that the classification of Duke and Polhill (1981) does not cover all the variability existent in the Leguminosae, and are needed further studies.

The results obtained here allow to state that between the 24 and the 48 hours after the moistening of seeds previously treated, is optimum to obtain an effective % of germination owing to their anatomical structure, adapted to persist in the soil for a long time owing to the adaptation to the environment in which the plants grow.

This work is the first report about the index of correlation n˚ of seeds/ fruit dimensions, as well as the sanitary condition of fruits and seeds.

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