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Evaluation of seed germination of *Schinus terebinthifolius* Raddi from different matrices on Rio Grande do Sul state, Brazil

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ABSTRACT

Background: Native species are still poorly studied, and is the case of Schinus terebinthifolius, popularly known in Brazil as Brazilian pepper and aroeira vermelha. For some native species, the amount of seed available in natural populations is not enough to meet demand, both commercial and for research. Objective: This work aimed to study the germination of S. terebinthifolius seeds from 35 individuals from different access of Rio Grande do Sul population. In this study, before the experiment, the seeds underwent a disinfection process under aseptic conditions, which consisted of immersion for 15 minutes in solution of NaClO 3%, plus two drops of washing liquid and washing three times in distilled and autoclaved water. The plots were kept in growth chambers (BOD) with a photoperiod of 16 hours at 20°C (+/- 2 ° C). The evaluation of this experiment consisted of the first count, germination percentage and speed index of seed germination, which was performed daily for 30 days; after the first seed germination. Results: There are significant differences in the potential of seed germination, being possible perform grouping of matrices plants according to the similarity in the results of the germination percentage, first count and germination speed index of the seeds of Schinus terebinthifolius, also was observed high estimate correlation to these three variables. Conclusion: Therefore, there is importance in selecting individuals, noting features that will bring genetic and physiological representation of the species, increasing the homogeneity of the seed lot. In this way, the mother plants of the group 1 will be selected for seed collection in the production of seedlings in future reforestation programs.

INTRODUCTION

Schinus terebinthifolius Raddi, popularly known as the red pepper tree, s an arboreal species belonging to the family Anacardiaceae, native from Brazil and on most of South America and. Was introduced in Europe and in countries like the United States, being much appreciated by the beauty of its size and used for ornamental purposes (Favoreto; Gilbert, 2011). In Brazil, specifically, is found from the state of Pernambuco to Rio Grande do Sul (Fleig, 1989; Medeiros; Zanon, 1998), following the coast and also on the mainland to the west in the southern states (Fleig, 1989). Has a wide natural distribution in the states of Parana, Santa Catarina and Rio

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Grande do Sul (Nogueira, 2002). Despite being a Brazilian plant and officially listed in the Brazilian Pharmacopoeia (Jorge; Markmann, 1996), *Schinus terebinthifolius* Raddi fell into oblivion in our country.

The Brazilian pepper has many uses, especially in the food industry as a condiment, also has ornamental purposes, production of firewood and charcoal besides the traditionally used for their medicinal properties (Azevedo *et al*., 2015). In cooking the fruits, red or pink color, sweetish and aromatic, are used in the worldwide kitchen as the name Pink Pepper (Azevevo *et al.*, 2015). Of its bark and seed also it is possible to obtain essential oils for use in perfume formulation (Figueiredo, 2009). The scientific literature describes five derivatives of *Schinus terebinthifolius* Raddi for medicinal uses, from stem bark extracts, leaves and fruit until essential oils from leaves and fruits, with anti-inflammatory potential, healing, treatment of urinary system diseases, antiallergic activity, antioxidant, antiulcer, insecticide and acaricide, antifungal and antimicrobial. Among the main chemical components include simple aliphatic substances, essential oil, mono and sesqui-terpenes, triterpenoids, simple phenols and their glycosides, flavonoids, tannins and bisphenols (Favoreto; Gilbert, 2011).

The traditional use of this plant has been substantially supported by experimental and clinical trials, including the drug production. Experiments have disclosed and supported by different authors show presence of active compounds with potential anti-inflammatory, healing, treatment of urinary system diseases, ant allergic activity, antioxidant, antiulcer, insecticide and acaricide, antifungal and antimicrobial. As antimicrobial activity, *Schinus terebinthifolius* Raddi stood out due to have greater efficacy against microorganisms responsible for oral diseases when compared to other 17 medicinal plants (Mello *et al*, 2002; Favoreto; Gilbert, 2011). Moreover, polyphenols extracted from this specie have demonstrated considerable potential inhibitor against a range of carcinogenic cells, specially in cell that cause insensitive prostate cancer to androgens, wherein the extract of the leaves inhibited proliferation more than 30 times when compared to the extract gross. In the same line, the chemical composition of the oil of the fruit and leaves of *Schinus terebinthifolius* extracted from plants of Egypt, showed inhibitory activity on the viability of human cell lines of breast and brain cancer (Ibrahim *et al.,* 2004; Queires *et al*, 2006). According to Lorenzi and Matos (2002) its medicinal use includes sits baths after birth, treatment of respiratory and urinary system diseases, washing wounds and ulcers amongst others.

Several works on the main components present in *S. terebinthifolius* demonstrate that the content of these components can vary not only depending on plant maturity, but also by the location of its habitat. Study this species in the Southeast and Northeast Brazil showed genetic differentiation among existing populations in these regions (Álvares-Carvalho *et al*., 2015). Apart from the vast economic importance, the species also highlights the recovery of degraded and marginal areas, as well as reforestation programs, mainly due to its aggressive nature, a pioneer species and for have a zoochorous dispersion (Souza *et al*., 2001; Lorenzi, 1992). The Brazilian territory occupation process generated large losses from the environmental point of view and essentially forest. In this sense, one of the ways to compensate the generated degradation is to obtain seedlings from native forest seeds to be used in reforestation programs, reclamation of degraded areas, urban tree planting, preservation of native species at risk of extinction, among other activities that require the same (Vieira *et al*., 2001).

However, for some native species, the amount of seed available in populations is not enough to meet demand, both commercial and for research. This is due to the scattering of trees in small fragments of natural forests, the lack of good genetic quality seeds (Silva and Higa, 2006) and due to the delay that some species have to start producing seeds (Caldas, 2006), as well as variation of environmental factors that have intensified the reduction in the use of seeds. In the case of the species in this matter, fragmentation, predation practices and exploitation generated by fruit extraction has contributed to the reduction of seed bank, which in turn decreases the regeneration of species, causing the inbreeding phenomenon and low genetic variability of generations subsequent. Not without reason, the extraction of Brazilian pepper fruit without criteria has raised concerns on the reduction of spatial and temporal distributive potential of this species (Álvares-Carvalho *et al*., 2015).

Since, most of the native species is propagated by seed, success in the formation of seedlings depends on the knowledge of the germination process of each species and the quality of seed used (Rego *et al*, 2009). According to the Seed Analysis Rules (Brazil, 2009), there are methods defined for testing the quality of species of high commercial value for Brazil, but Brazilian forest species represent 0.2%, expressionless data on biodiversity that makes up Brazilian plant biomes (Figliolia *et al*., 2007; Piña-Rodrigues *et al*., 2004).

The evaluation of physiological quality of the seed is essential for the various segments that make up a production system, because the discovery of the effects of factors that can affect seed quality depends directly on the efficiency of the methods used to determine it (Marcos Filho *et al*., 1987b), the standard germination test is a benchmark for assessing the quality and serves as the basis of the marketing of seed. It is accomplished in ideal conditions and controlled, so as to enable standardization and reproducibility of results between laboratories (Oliveira; Pereira, 1987). However, this test has limitations for providing results that overestimate the physiological potential of seeds due to being conducted under optimal conditions. Thus, vigor tests were developed in order to provide additional data to those obtained in the germination test. According to Carvalho

(1986), the force of a seed has to be understood as the level of energy that it has to carry out the tasks of the germination process.

The first count of the germination test can be used as a strength test, since the germination rate is reduced with the advance of deterioration of the seed. Thus, samples showing higher germination values in the first count may be considered more robust. In the germination test, can determine the speed of germination index (GSI), which is calculated by the amount of seeds that germinate every day during the course of the germination test. Therefore, the higher the speed of germination index, the greater its force.

Research related to seed analysis have also received attention in the scientific community, in order to obtain information to evaluate the physiological quality of seeds (Felippi *et al*, 2012b). *Schinus terebinthifolius* is a specie reported by several authors (Carvalho *et al*, 2013b; Luz *et al*, 2015a; Luz *et al*, 2015b), however, despite the ecological importance of the great medical potential and commercial importance as a food condiment, basic research for the species in the Rio Grande do Sul state are still incipient. Thus, this work sought information about *Schinus terebinthifolius* that ensure its potential in the listed fields, studies on the germination potential are extremely important, especially for matrices occurring on this state, knowing that the content components and metabolites contained tends to vary according to the occurrence of location. Moreover, the current unsustainable exploitation of this species in regions of Brazil can derail the recovery of populations in the long term (Álvares-Carvalho *et al*., 2014), showing up for the production with high quality seedlings, either with the economic purpose or conservationist, the pressing need for morphological and silvicultural knowledge of each forest species (Felippi *et al*, 2012b) to ensure the quality of seed production in compatible scale with the usage range of species.

With this context, the objective of this study was to evaluate the germination of seeds of *S. terebinthifolius* Raddi of 35 mother plants from different populations of Rio Grande do Sul state, Brazil, making it possible to identify matrices plants with greater force to be used by nurserymen aiming the management and recovery of natural populations.

MATERIAL AND METHODS

The assays were performed in Interaction Laboratory Plant-Microorganisms belonging to the Department of Biology, Center of Natural and Exact Sciences, Federal University of Santa Maria. The botanical material used consists of seeds from the fruits of *Schinus terebinthifolius* collected from 35 individuals from five municipalities of the state of Rio Grande do Sul (Dilermando de Aguiar, Itaara, Santa Maria, São Pedro do Sul e Silveira Martins). Leafs were also collected and stored in herbarium specimens, that were incorporated to the herbarium of the Department of Biology of the Federal University of Santa Maria. Samples were collected between the months of June to August 2011 and the seeds were stored under refrigeration at 8 (+/- 2) ° C until the beginning of the experiments.

Before the installation of the experiment, the seeds have undergone a disinfection process under aseptic conditions, which consisted of immersion for 15 minutes in solution of NaClO 3%, plus two drops of liquid detergent "Girando Sol®" and three washes in distilled water and autoclaved (Wendling *et al*., 2006). The cultivation system used consisted of gerbox plastic boxes (10 x 11 x 4 cm) with a sowing paper filter as substrate for germination of seeds, moistened in the proportion of three times the volume of water relative to the weight of the paper (Mark Filho *et al*, 1987a). The plots were kept in a climate chamber of the type Biosystem Organized Development (BOD) with a 16-hour photoperiod at 20 ° C (+/- 2 ° C).

The seeds were evaluated for germination on the eighth day (first count) and on the thirtieth day. The germination percentage was determined by the number of germinated seeds relative to the number of seeds placed to germinated (Borghetti and Ferreira, 2004). The seed was considered germinated when the emergence of the radicle are viewed, as the botanical criteria proposed by Borghetti and Ferreira (2004). From the eighth day it was also held daily assessments of the number of germinated seeds for a total period of 30 days allowing the calculation of the germination speed index (GSI). The GSI is the sum of the number of germinated seeds each day, divided by the number of days elapsed from the sowing as (Nakagawa, 1994): $GSI = G1/N1 + G2/N2 + ... Gn/Nn$, being G the number of germinated seeds and N the number of days elapsed from the sowing.

The experimental design was completely randomized with four replications of 50 seeds per treatment (mother plants). Based on the first count, on the GSI and on percentage of germination, the plants were grouped by the method of k-means (Barroso & Arts, 2003; Mingoti, 2005). To validate the grouping, the data were submitted to analysis of variance (ANOVA) and compared by Tukey test at 5% probability of error. The correlation between the percentage of germination on the eighth day (first count) and on the thirtieth day and the germination speed index was determined using the Pearson correlation coefficient (ρ). Statistical calculations were performed with the Statistica software, version 7.0 (StatSoft Inc., 1984-2004, Tulsa, USA).

Results:

In the first count fulfilled on the eighth day, it was observed germination percentage ranging from 0.2 (matrix 3) to 33.2% (matrix 28) between the 35 matrix of red pepper trees, and average of 20.7%. On the thirtieth day, the germination percentage ranged from 1.7% (matrix 16) to 93.5% (matrix 35) and overall average of 40.1%. As to GSI was observed values between 0.1 (matrices 3 and 16) and 11.0 (matrix 28) and final average of 4.4 (Table 1). The mother plants 28 and 35 had the highest germination percentages, as well as the GSI, while matrices 3 and16 had the lowest percentage of germination and GSI (Table 1).

Table 1: Percentage of germination (first count) on the eighth day, germination after 30 days of cultivation and germination speed index (GSI) of 35 mother plants of *Schinus terebinthifolius.*

Matrix Plant	First Count (%)	Percentage of germination (%)	GSI
1	4.2	10.0	1.1
2	11.5	52.5	3.8
3	0.2	1.5	0.1
4	6.5	22.5	1.8
5	23.5	51.5	8.6
6	1.7	17.0	0.9
7	25.5	88.5	6.3
8	13.7	35.5	3.8
9	29.2	77.5	6.9
10	22.0	76.5	7.2
11	9.7	68.0	4.0
12	5.5	19.0	1.3
13	22.7	48.0	7.6
14	26.5	63.5	6.4
15	8.5	18.0	4.3
16	0.5	1.0	0.1
17	31.5	86.0	7.9
18	3.2	7.0	1.4
19	1.0	2.0	0.4
20	5.5	16.0	1.2
21	3.5	12.5	0.9
22	7.7	19.5	1.7
23	31.7	88.5	8.2
24	7.5	23.0	2.1
25	18.5	46.0	6.0
26	8.2	20.0	3.2
27	14.2	35.5	5.7
28	33.2	81.0	11.0
29	20.0	86.0	10.2
30	13.7	40.0	3.9
31	16.0	46.5	4.5
32	28.0	67.5	7.6
33	10.7	29.0	3.2
34	11.2	37.0	3.6
35	21.7	93.5	6.7
Average	14.3	42.5	4.4

According to the grouping performed by the method of k-means it was possible to identify the formation of five groups regarding the joint evaluation of characters percentage of germination on the eighth day (first count) and on the thirtieth day, and germination speed index (GSI) of seeds of *Schinus terebinthifolius* (Table 2).

Tabela 2: Groupings of matrices plants of *Schinus terebinthifolius* by multivariate analysis using the method of k-means based on germination percentage to 8 days (first count) and at 30 days, and germination speed index (GSI) of the seeds.

Groups	Group 1	Group 2	Group 3	Group 4	Group 5
Matrices	7; 9; 17; 23; 29; 35	2; 10; 11; 14; 28; 32	5; 8; 13; 25; 27; 30; 31; 34	4; 6; 12; 15; 20; 21; 22; 24; 26; 33	1; 3; 16; 18; 19

Through the analysis of variance was able to identify significant difference between the groups for the germination percentages of the first count and on the 30 days, and germination speed index (GSI) of the seeds of *Schinus terebinthifolius.* The average percentage of germination of the groups ranged from 4.3 to 86.67%, when comparing the average of the group, while for the first count the range was 0.90 to 28.0% and for the GSI the averages were between 4.3 to 15.8 (Table 3).

Table 3: Averages for groups of germination to 8 days (first count), at 30 days and the germination speed index (GSI) of the seeds of *Schinus terebinthifolius*.

Groups	First Count (%)	Percentage of germination (%)	GSI
1	28.0 a*	86.7 a	15.8 a
2	12.2 b	68.2 b	12.2 b
3	11.7 c	42.5 c	7.5 c
4	6.6 d	19.6 d	4.1 d
5	0.9 e	4.3 e	4.3 e

* Values followed by the same letter do not differ by Tukey test at 5% probability of error.

All variables showed a positive and significant correlation (P <0.05). The percentage of germination and GSI showed the highest value of linear correlation (0.96), and this variable also showed high correlation (0.96) with the first count. Among the GSI and the first count the estimated correlation was 0.95 (Table 4).

Table 4: Correlations of Person for germination percentage, first count and seed germination speed index of *Schinus terebinthifolius* from different access collection of Rio Grande do Sul.

	First count (%)	Germination (%)	GSI
First count (%)	1	0.96	0.95
Germination (%)	0.96	1	0.96
GSI	0.95	0.96	1

Discussion:

The results of this study showed great variation between the germination percentage of the seeds of *Schinus terebinthifolius* both the first count as the 30-day evaluation, as well as the GSI (Table 1). The overall average of germination of the seeds, on the 30-day trial, was relatively low (42.5%) compared with other native species such as cherry (*Amburana Acre* (Ducke) AC Smith), an Amazonian tree species, which showed 78% germination at 30 days (Fermino Junior *et al*., 2007), and grápia (*Apuleia leiocarpa* Vogel Macbride), which showed 82.5% of seed germination at 21 days (Lencina *et al*., 2014).

The germination test is the most widely used method for determining the quality of a seed lot and enables the evaluation of the viability under favorable conditions, and shall be taken into account that not all viable seeds will germinate. Germination is defined as a process that starts with the absorption of water, until the protrusion of the primary root through the seed coat (Borghetti and Ferreira, 2004) and may be influenced by the presence of inhibitory substances in the cell walls of the cotyledon (Bewley and Black, 1994), or the influence of environmental factors which result in the death of the embryo. According to Nakagawa (1999), lots that have higher averages in the first count can be considered more vigorous. However, what should be noted that higher percentages of germination, example of which were obtained in the best groups do not necessarily indicate lots of high force in all environmental conditions (Guedes *et al*., 2009). This is due to the fact that germination tests are conducted under favorable conditions of temperature, light, humidity and substrate, allowing the plot to express the maximum potential to produce normal seedlings. Thus, a need exists for the application of force tests complementary to germination, to separate the plots in force levels.

Measures that quantify germination, informing how long it took to occur the emergency are more suitable, giving the process a kinetic character and an evaluation of seed vigor. Such testing is important, since there may be plots or seeds that germinate faster and more slowly. Among the evaluations used highlights the germination speed index (GSI) (Borghetti and Ferreira, 2004), which is obtained based on the sum of the number of germinated seeds in each evaluation, divided by the respective time (Santana and Ranal, 2004) and is based on the assumption that more vigorous seeds germinate faster (Vieira and Carvalho, 1994), where higher germination rate of seeds, the more vigorous is the plots of seeds being analyzed (Nakagawa, 1999).

Based on the values shown in Table 1, it appears that the red-pepper tree seeds have a very different effect between the matrices that supply the seeds. Thus, the identification of matrices of *Schinus terebinthifolius* that provide more vigorous lots can be a viable alternative and contribute to the success in the production of seedlings.

The grouping carried out based on the percentage of germination of the first count at 30 days and the GSI provided the formation of 5 groups with a variable number of mother plants (5-10 plants/group). The differences found, comparing the results indicate variability of germination, assuming climate, soil and genetic conditions can influence the quality of seed, as well as the time of maximum physiological maturity. The group of best medium for germination and GSI is the group 1 consisting of 6 matrices plants. However, this number of mother plants is less than the minimum number recommended by Higa and Duque-Silva (2006). These authors suggest that seed collection is held in at least 12 unrelated plants, to be guaranteed the effective population size (Ne) greater than 48 and the appropriate genetic representativity (Higa and Duque-Silva, 2006). Thus, besides the selection of matrices based on variables of germination and seed vigor, should take into account the minimum number of mother plants.

For the definition of the best groups, ANOVA showed a difference between the average for the three variables, indicating efficiency in the clustering process and provided validation of the groups carried out by K-means. All groups showed significant differences, being group 1 (matrices 7, 9, 17, 23, 29 and 35) the best average of group for the three variables analyzed followed by the group 2 (2, 10, 11, 14, 28 and 32). These groups had equal or higher averages (Table 3) considering the average of all matrices plants (Table 1), and total 12 plants matrices corresponding to a minimum adequate number to maintain genetic variability. Differences in percentage germination are also reported between matrices of *Apuleia leiocarpa* and *Cordia trichotoma* that ranged from 13 to 96% and from 0 to 40%, respectively (Felippi *et al*, 2012a; Felippi *et al*, 2012b).

Thus, it is assumed that individuals who had the highest levels for germination (group 1) have defining characteristics, whether genetic and / or physiological, which positively influenced the seed lot. In contrast, individuals who did not have satisfactory results, which were grouped in lots of lower average germination and GSI, compromise the quality of the seed lot (Felippi *et al*., 2012a), and therefore should not be selected.

The maximum seed quality is achieved when it reaches the point of maximum germination and vigor, which is the physiological maturity point (Carvalho; Nakagawa, 1999c). After reaching physiological maturity, start to occur changes in the physical, physiological and / or biochemical nature that characterize the deterioration, and the loss of germination capacity of its final consequences (Spinola *et al*., 2000). Seed germination and seedling emergence are reflections of physiological seed quality, and in this sense, it is observed that seed lots that have similar germination may exhibit different behavior in the field and in storage. Such differences may be explained by the fact that the first changes in the biochemical processes associated with deterioration, usually occur before it is checked the decline in germination capacity (Delouche; Vieira, 1973).

The significant differences between groups, on the three parameters evaluated, did not affect the high correlation values found (Table 4). The positive and significant correlation values indicate that the higher the percentage of values in the first count, also the highest germination values at the end of evaluations and GSI. Thus, these data corroborate with Nakagawa (1999), which states that lots that have higher averages in the first count can be considered more vigorous. This variable can be the most suitable variable for germination, as it reduces the time of germination, anticipating the selection of lots more conducive to the production of seedlings both red pepper, as other tree species. The evaluation of the physiological quality of seeds for the purpose of sowing and marketing, has been fundamentally based on germination. Lots with high homogeneity are better evaluated through the germination test.

The vigor tests were developed in order to assess differences in force between seed lots, which are not possible to detect using the germination test. The force is a genetic and physiological characteristic of the seed that reflected in the responses as speed, total germination and seedling growth. Deterioration and speed are closely related to the force. At physiological maturity occur the point of maximum force and maximum germination and, also, the point at which the deterioration is minimal, and from the physiological maturation begins the process of deterioration (Harrington, 1972). However, as the seeds were collected in the same phenological stage and at the same time of year, it is assumed that the different accesses have the same maturity of seeds and the differences of the potential germination are due to genetic variability among individuals. Thus we can say that among the 35 matrices plants of *Schinus terebinthifolius* selected, the mother plants which were grouped according to the result of the germination percentage test, first count and GSI, forming Group 1, have better quality due to higher power of germination of seeds resulting from genetic character of the individual when compared to other groups formed. Thus, the results showed were consistent with the expected behavior of each matrix remained high estimate of correlation between the variables of the first count, germination percentage and germination speed index, regardless of high or low seed germination power.

These results are extremely relevant as they provide grants that can increase the production of *Schinus terebinthifolius* seeds, whose seedlings with higher quality are able to supply a market booming, mainly geared to generate raw material. In addition, the largest supply of viable seeds of *Schinus terebinthifolius* can help restore degraded areas, conserving the species in the long term through the preservation of the seed bank. The definition of methods and strategies that permit the production of quality seedlings in a short period of time and at affordable conditions, is of fundamental importance (Cunha *et al*., 2005). However, this action depends on ecological and botanical knowledge related to species (Correia *et al*., 2005), as well as the size of the genetic sample of the population (Mori, 2003) and the origin of the matrices (Piña-Rodrigues *et al*., 2007).

In addition to enabling appropriate management of this species, allowing the establishment of crops for the purpose of commercial exploitation of fruits, mainly focused on foreign markets, ensuring economical alternative to populations that depend on this activity. And finally, we suggest the continuity of research to production changes and development field, because besides the genetic character is silvicultural factors that determine the best growth for the species.

Conclusion:

There is difference of the potential germination of *Schinus terebinthifolius* seeds, collected during the same phenological stage and the same annual period, in different access of populations in the state of Rio Grande do Sul, Brazil, being possible to group matrices plants in lots of similar behavior. The germination percentage test presents high estimate correlation with the first count test and seed germination speed index of *Schinus terebinthifolius* seeds. Thus, the mother plants of group 1 will be selected for collection of seeds for seedlings in future reforestation programs.

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