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Substrates and Temperatures in the Germination of *Hibiscus sabdariffa* L. Seeds

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Abstract

Vegetable biodiversity in Brazil accounts for almost 67% of the plants on the planet, which are part of studies with focus on determinants of food security and access to adequate and healthy food, while preserving natural resources. Studies indicate that *Hibiscus sabdariffa* L. is one of the most studied unconventional food crops because of its nutraceutical quality as antioxidant and prevention of hypertension and cancer. Although relevant, substrate and temperature information for seedling production of this species are scarce. Thus, the aim of this study was to evaluate the best substrate and temperature for the vigor and germination test of *H. sabdariffa* seeds of the Talo Roxo and Rubra varieties. The experiment was laid out in a randomized complete design, in a 7 × 5 factorial arrangement, consisting of the combinations of seven substrates (paper towel, filter paper, vermiculite, between sand, on sand, plantmax® and compost) with five temperatures (20, 25, 30, 35 and 20-30 °C), with four replicates with 50 seeds each. The following variables were evaluated: germination, germination speed index, seedling length and dry mass. For germination and vigor tests of *Hibiscus sabdariffa* var. Talo Roxo seeds the paper roll substrate is recommended at temperatures of 25 or 35 °C and for var. Rubra seeds, plantmax® at 25 °C and plantmax® and vermiculite at temperatures of 25 or 20-30 °C, respectively.

Keywords: emergence, native species, physiological potential, seeds analysis, vigor

1. Introduction

Plant biodiversity of Brazil accounts for almost 67% of the plants on the planet, reinforcing the country's international role in studies on topics such as food security and access to adequate and healthy food, while preserving natural resources (Lima et al., 2016). It is estimated that 50% of the calories in the world food come from a maximum of four plant species, 90% of the food consumed comes from 103 species, but there is a potential of using at least 30 thousand plant types as food (Formagio et al., 2015). In this context, studies indicate that the use of Unconventional Food Plants (UFPs) are part of the culture, identity and agricultural practices in several regions of the world. These are represented by about 63 plant species distributed in 25 botanical families (Leal, Alves, & Hanazaki, 2018; Barreira et al., 2015).

In recent years, *Hibiscus sabdariffa* L. has been among the more studied UFPs, because of its nutraceutical quality, as an antioxidant (Djaeni et al., 2018), hypertension and cancer (Riaz & Chopra 2018) and very much discussed under food security (Guardiola & Mach, 2014). This species belongs to the family Malvaceae and produces seeds with high protein levels (28.9%), carbohydrates (25.5%) and lipids (21.4%). The main fatty acids identified are linoleic (39.31%), oleic (32.06%), palmitic (20.84%) and stearic (5.88%). Besides seeds, leaves and calyxes are widely used in cooking and traditional medicine in countries such as Mexico, India, England, China and Brazil (Nasrabadi, Zarringhalami, & Ganjloo, 2018; Singh, Khan, & Hailemariam, 2017; Nzikou et al., 2011l).

Although relevant, *H. sabdariffa* cultivation is represented by a small parcel in native environments, in Brazil it occurs in areas of the Amazon biome, such as in the State of Maranhão and does not have high production (Silva, Rêgo, & Leite, 2014). Thus, developing techniques favorable to seedling production would represent an advance in the domestication and rational exploration of *H. sabdariffa* economic potential, which is mainly obtained by extractivism and small domestic crops (Barreira et al., 2015). Information on temperature, light and substrate for nursery seedlings production is scarce (Costa et al., 2014).

The germination test is an important parameter to evaluate the seed physiological quality and allows the knowledge of seed lot physiological potential. The results of germination tests are useful to determine the rate of seedling production. However, for almost all native species, the test methodology is not yet established due to factors such as substrate and temperature (Melo et al., 2017).

For the tropical and subtropical Brazilian plant species, the optimal temperature is between 20 and 35 ºC, since these temperatures are the ones observed in the regions of origin of the plants, at the correct time for natural germination (Melo et al., 2017). In addition to substrate and temperature, germination is also affected by structure, air space, water retention capacity and other factors that may influence seed germination. The substrate represents the physical support on which the seed is placed with the function of offering and preserving the proper conditions of seed germination and seedling growth (Borges & Toorop, 2015). For *H. sabdariffa*, there was only one report on germination, initial growth and survival of seedlings under salt stress (Galal, 2017).

The choice of substrate should be based on seed requirements at sowing, since it is a factor that can facilitate the emergence of seedlings and reduce their time in the nursery. In addition, the increase in germination speed may guarantee less deterioration in seeds with high lipid content, such as *H. sabdariffa*, because oilseeds are known to have lower viability (Jeromini et al., 2018). Thus, the aim of this study was to evaluate the best substrate and temperature for the vigor and germination test of *H. sabdariffa* seeds of the Talo Roxo and Rubra varieties.

2. Material and Methods

The present study was carried out with seeds of *H. sabdariffa* var. Talo Roxo and Rubra, harvested from mature fruits of ten mother plants of each variety, grown in São Luis, state of Maranhão, northeastern Brazil. Fruits from the above-mentioned varieties were collected 30 days after the anthesis, packed in polyethylene bags and taken to the Seeds Laboratory of the Agricultural Sciences Center of the Maranhão State University, São Luís Campus. The seeds were obtained by manual processing, which consisted in the removal of the fleshy calyxes for var. Talo Roxo, drying at room temperature (25±3 °C) for 12 days and removal of the inert material by ventilation and sieves. There was no need to remove the calyxes for var. Rubra because they were dried. The seeds of each variety were homogenized for the following tests and determinations:

(1) *Initial Moisture Content*—test conducted by the greenhouse method at 105±3 °C, for 24 hours (MAPA, 2009), using four subsamples of 1 g of seeds.

(2) *Germination Test*—test carried out in germination chambers adjusted to constant 20 °C, 25 °C, 30 °C, 35 °C and alternating 20-30±3 °C temperatures and 12 h light/12 h dark. Four replicates of 50 seeds were sowed one cm deep between wet substrates of vermiculite, sand (placed between and on the substrate), compost, plantmax®, two sheets of filter paper, distributed in transparent plastic boxes (11.0 × 11.0 × 3.5 cm) and also on paper rolls. The evaluations were carried out daily by counts of normal germinated seedlings, from the 5th to the 18th day after sowing. The results were expressed in percentage.

(3) *Germination Speed Index (GSI)*—performed together with the germination test with daily evaluations from the 5th to the 18th day after sowing. The GSI was determined by the formula proposed by Maguire (1962).

(4) *Seedling Length and Dry Mass*—test carried out by washing the seedlings in water to remove the substrate residue. The seedlings were measured with a ruler graduated in centimeters, the results were expressed in cm^{-1} seedlings. Thereafter the seedling aerial parts were separated from the roots and placed in Kraft paper bags to dry in greenhouse with forced air circulation, at 65±3 °C for 48 hours. After this time, the samples were placed to cool in a desiccator and then weighed in an analytical balance with precision of 0.0001 g. The results were expressed in g. seedling^{-1} (Nakagawa, 1999).

The experiment was laid out in a randomized complete design, with the treatments distributed in a factorial scheme 7 × 5 (substrates and temperatures), with four replicates per treatment. The data were subjected to analyses of variance with the F test and the treatment means were compared by the Scott-Knott test, at 5% probability. The statistical analyses were performed in the Statistical Analysis System (SAS) software.

3. Results and Discussion

The initial water contents of *H. sabdariffa* seeds was 12.8% (data not shown in the tables). There was a significant effect ($p \leq 0.05$) of the interaction between substrates and temperatures.

The highest germination percentage of the *H. sabdariffa* var. Talo Roxo seeds was observed when paper roll was used as the substrate at temperatures of 25 °C (G = 89%) and 35 °C (G = 84%) respectively (Table 1), but these results did not differ statistically from those obtained on the treatments between sand at 20 °C, compost at 25 °C and on sand at 30 °C. For the other combinations of substrates and temperatures, the lowest germination percentage (G = 17%) was observed in compost at 20-30 °C. According to Marcos Filho (2015a), the maximum temperatures for seed germination of most plants suitable for cultivation are between 35 and 40 °C.

With regards to *H. sabdariffa* var. Rubra, the highest percentage rate (G = 90%) of germinated seedlings was found when plantmax® commercial substrate and the temperature of 25 °C were combined (Table 1). However, this result was statistically similar to those obtained on paper roll at a temperature of 20 °C, between sand at 20 °C and 35 °C and compost at 20-30 °C. There was also no statistical difference in germination of the seeds distributed in vermiculite at all temperatures evaluated, except at 20-30 °C. The lower germinative performance was observed for seeds sown on paper at 35 and 20-30 °C and between sand at alternate temperature (Table 1).

Table 1. Germination (G%) of *H. sabdariffa* L. var. Talo Roxo and var. Rubra seeds subjected to different temperatures and substrates. São Luis, state of Maranhão, northeastern Brazil, 2018

Substrates	Temperature (°C)				
	20	25	30	35	20-30
H. sabdariffa var. Talo Roxo					
Paper roll	26 dC	89 aA	61 aB	84 aA	20 Bc
On paper	22 dC	59 cA	21 cC	45 cB	20 bC
Between sand	71 aA	70 bA	45 bC	59 bB	23 bD
On sand	47 cA	46 dA	53 aA	34 dB	39 aB
Plantmax®	12 eC	66 cA	47 bB	48 cB	18 bC
Vermiculite	32 dC	76 bA	27 cC	53 bB	15 bD
Compost	62 bB	80 aA	45 bC	30 dD	17 bE
H. sabdariffa var. Rubra					
Paper roll	74 aA	65 bA	64 bA	62 bA	36 bB
On paper	44 bB	63 bA	31 dC	18 cC	21 cC
Between sand	75 aA	64 bB	62 bB	77 aA	11 cC
On sand	41 bC	68 bA	64 bA	52 bB	35 bC
Plantmax®	46 bB	90 aA	49 cB	49 bB	52 aB
Vermiculite	67 aA	80 aA	80 aA	74 aA	48 aB
Compost	50 bA	36 cB	59 bA	54 bA	52 aA
CV (%)	var. Talo Roxo 10.11			var. Rubra 10.78	

Note. Means followed by the same letter (lowercase in the column and uppercase in the row) do not differ by the Scott-Knott test at 5% probability.

Based on these results, it can be inferred that the substrate and the temperature influence in the seed germination, because, to start this process, seeds undergo metabolic reactions that involve the activation of enzymes, hydrolysis, assimilation and mobilization of the reserves, and cell division (Melo et al., 2017). Thus, in addition to the physiological activities that vary according to the initial seed quality (Mbofung et al., 2013), the substrate structure, its water retention capacity, aeration and temperature adequacy also contribute to a higher germination percentage (Silva et al., 2016).

The results obtained in this study agree with those reported by Silva et al. (2016), who also evaluated germination of oleaginous seeds, such as *Plukenetia volubilis* L., where the maximum germination percentage occurred after sowing on paper roll at 25 ºC. However, Jeromini et al. (2018) reported that the maximum germination rate of *P. volubilis* seeds, as occurred with those of *H. sabdariffa* L. var. Talo Roxo (paper roll, G =

89%) and var. Rubra (plantmax®, G = 90%) depends on the progeny, the substrate and the room temperature during the sowing period, with germination performance varying from 75 to 100%.

The best physiological performance of the *H. sabdariffa* var. Talo Roxo seeds, evaluated by the germination speed index (GSI) (Table 2), was associated to the interaction between sowing in a paper roll at 25 and 35 ºC, although it was not significantly different for the GSI evaluated at the same temperature in the vermiculite substrate. These values also did not differ from those obtained for the GSI evaluated by the combination between the temperatures of 20 and 20-30 °C, with the plantmax®, compost and sand substrates, respectively (Table 2).

With regards to *H. sabdariffa* var. Rubra, the best GSI performance was observed when seeds were submitted to 20 and 30 °C in all substrates, except plantmax® and paper roll at 20 and 30 °C, respectively (Table 2). These results did not show statistical differences of the GSI at the temperature of 25 °C in the substrates on sand, plantmax®, vermiculite and at 35 °C for the sowing done between sand and in vermiculite, as well as at 20-30 °C in all the substrates, except on paper and sand (Table 2).

Table 2. Germination speed index (GSI) of *H. sabdariffa* var. Talo Roxo and var. Rubra seeds, subjected to different temperatures and substrates. São Luis, state of Maranhão, northeastern Brazil, 2018

Substrates	Temperatures (°C)				
	20	25	30	35	20-30
H. sabdariffa var. Talo Roxo					
Paper roll	3.04 bC	8.35 aA	6.08 aB	8.97 aA	2.38 aC
On paper	2.13 bB	5.85 bA	2.02 cB	5.18 cA	3.61 aB
Between sand	4.05 aB	5.71 bA	3.79 bB	6.69 bA	2.78 aB
On sand	3.39 bA	3.90 bA	4.04 bA	2.32 dA	3.24 aA
Plantmax®	4.72 aA	5.93 bA	3.90 bB	3.91 cB	2.24 aB
Vermiculite	2.00 bC	7.16 aA	1.96 cC	5.07 cB	1.59 aC
Compost	5.03 aA	6.08 bA	4.28 bA	2.31 dB	3.11aB
H. sabdariffa var. Rubra					
Paper roll	5.76 aA	6.00 bA	6.08 aA	6.09 bA	6.08 aA
On paper	6.20 aA	6.53 bA	3.77 bB	1.59 cB	2.25 bB
Between sand	6.42 aA	6.01 bA	6.83 aA	7.58 aA	1.82 bB
On sand	6.42 aA	7.56 aA	6.25 aA	5.26 bA	6.50 aA
Plantmax®	5.76 aB	9.33 aA	6.90 aA	3.49 cB	8.29 aA
Vermiculite	8.50 aA	7.64 aA	8.96 aA	8.57 aA	6.88 aA
Compost	6.43 aA	3.73 cB	6.65 aA	6.25 bA	7.44 aA
CV (%)	var. Talo Roxo 10.55		var. Rubra 10.69		

Note. Means followed by the same letter (lowercase in the column and uppercase in the row) do not differ by the Scott-Knott test at 5% probability.

The decrease in the GSI of the seeds subjected to 35 °C can be caused by damage to their structure, because high temperatures inhibit the speed of embryonic development and cause enzymatic alterations, thus modifying the speed of metabolic reactions (Borges & Toorop, 2015). However, in addition to temperature, the humidity of the substrate where sowing is performed is one of the essential factors, since, during this process, water absorption has the function of promoting the softening of the seed coat, the increase of the embryo and the reserve tissues, favoring tegument rupture, gas diffusion and the primary root growth (Marcos Filho, 2015b).

There are substrates that are used to standardize the seed germination test and others favor seedling production, all with some advantages and disadvantages. For example, some substrates can easily dry, particularly at higher temperatures (Melo et al., 2016). Paper rolls are indicated in germination tests by the rules of the Brazilian seed analysis (MAPA, 2009), because they remain moist for a longer period of time. Probably the results obtained with this substrate in this study for G% and GSI for the var. Talo Roxo (Tables 1 and 2) are due to the interaction between the temperature and its good water retention capacity, which allowed greater contact with the seed.

Similarly, the commercial substrate plantmax® has aged manure, coconut fiber, rice husk and sawdust in its composition. These components have properties conducive to water retention and are not found in other substrates (Jeromini et al., 2018). Therefore, probably the highest percentage and germination speed rate observed for the var. Rubra (Tables 1 and 2), is not only due the proper combination between this substrate and temperature, but also to seed characteristics, such as thickness and tegument composition. While the sand does not maintain satisfactory level of humidity at high temperatures, it has a better efficiency in moderate thermal oscillations, since it presents uneven water retention and distribution, leaving the upper part dry (Melo et al., 2017).

Contrary to what was observed for *H. sabdariffa*, the most used substrate for Malvaceae seeds considered as Unconventional Food Plants (UFPs), such as *Theobroma speciosum* Willd. Ex Spreng (Varella et al., 2018) *Theobroma grandiflorum* (Willd. Ex Spreng.) K.Schum. (Moura et al., 2015) and *Theobroma subincanum* Mart. (Nascimento and Carvalho, 2012) includes sand, however, the temperature of 25 °C is the most favorable to the percentage rate and speed of germination of these plant species. It can be predicted that the knowledge of the conditions that provide a fast and uniform germination of the *H. sabdariffa* var. Talo Roxo and Rubra seeds is useful for sowing purposes, since the homogeneous seedling development reduces nursery care, therefore seedlings will develop faster, promoting a more uniform growth in the field.

Regarding the evaluation of the development of *H. sabdariffa* seedlings (Table 3), it observed that the highest seedling lengths for var. Talo Roxo occurred in the substrate vermiculite at 25 and 30 °C. These results were statistically similar to those verified for seedlings from plantmax® and compost substrates at a temperature of 20-30 °C. On the other hand, the best combinations that provided the best development for the var. Rubra seedlings length, occurred using sand, plantmax® and vermiculite and constant 35, 25°C and alternating 20-30 °C, temperatures, respectively (Table 3).

Table 3. *H. sabdariffa* var. Talo Roxo and var. Rubra seedling lenght (cm seedling^{-1}) from seeds subjected to different temperatures and substrates. São Luis, state of Maranhão, northeastern Brazil, 2018

Substrates	Temperatures (°C)				
	20	25	30	35	20-30
H. sabdariffa var. Talo Roxo					
Paper roll	4.24 cC	10.88 bA	10.71 bA	9.13 bB	11.63 bA
On paper	6.28 bB	6.53 cB	4.40 cC	5.67 cB	8.09 cA
Between sand	6.22 bB	10.05 bA	9.50 bA	7.10 cB	11.07 bA
On sand	5.77 bC	8.08 cB	5.80 cC	7.15 cB	10.50 bA
Plantmax®	8.47 aD	12.48 aB	10.25 bC	13.36 aB	14.67 aA
Vermiculite	8.66 aC	13.03 aA	13.04 aA	10.67 bB	11.90 bA
Compost	6.96 bC	11.51 bB	12.20 aA	10.41 bB	13.08 aA
H. sabdariffa var. Rubra					
Paper roll	4.69 bC	5.68 dC	6.86 bB	5.35 cC	8.14 bA
On paper	4.20 bB	3.81 eB	3.97 dB	3.09 dC	7.32 cA
Between sand	4.59 bD	6.04 dC	6.70 bB	8.30 aA	7.01 cB
On sand	4.07 bC	4.03 eC	5.40 cB	3.23 dC	8.00 bA
Plantmax®	6.41 aC	8.44 bB	7.94 aB	6.89 bC	9.55 aA
Vermiculite	6.26 aC	9.66 aA	7.56 aB	7.01 bB	8.81 aA
Compost	5.21 bC	7.22 cB	6.90 bB	8.00 aA	8.30 bA
CV (%)	var.Talo Roxo 12.54		var. Rubra 9.49		

Note. Means followed by the same letter (lowercase in the column and uppercase in the row) do not differ by the Scott-Knott test at 5% probability.

The highest *H. sabdariffa* var. Talo Roxo seedling dry mass content was observed on paper roll at 25 °C (Table 4), although this result was similar at 5% probability level by the Scott-Knott test to those obtained on sand at 20 °C, plantmax® 30 °C and vermiculite at 35 °C. With respect to *H. sabdariffa* var. Rubra, the treatment combinations that favored the seedlings dry mass accumulation were paper roll at 25 °C; between sand at 20 °C; compost at 35 °C; on sand at 25° C, 35° C and 20-30 °C; plantmax® in the thermal range between 20 °C and 30 °C and in vermiculite in all thermal conditions, with the exception of the alternating temperature of 20-30 °C.

Table 4. *H. sabdariffa* var. Talo Roxo and var. Rubra seedling dry mass (g. seedling^{-1}) from seeds subjected to different temperatures and substrates. São Luis, state of Maranhão, northeastern Brazil, 2018

Substrates	Temperatures (°C)				
	20	25	30	35	20-30
H. sabdariffa var. Talo Roxo					
Paper roll	0.276 bC	0.739 aA	0.405 cB	0.323 aC	0.030 bD
On paper	0.158 cA	0.174 cA	0.273 dA	0.214 bA	0.035 bB
Between sand	0.245 bB	0.452 bA	0.527 bA	0.306 aB	0.156 aC
On sand	0.373 aA	0.413 bA	0.172 dB	0.090 cC	0.024 bC
Plantmax®	0.057 cC	0.268 cB	0.637 aA	0.210 bB	0.177 aB
Vermiculite	0.117 cB	0.180 cB	0.222 dB	0.319 aA	0.206 aB
Compost	0.308 bA	0.215 cB	0.270 dA	0.156 bB	0.192 aB
H. sabdariffa var. Rubra					
Paper roll	0.149 bB	0.156 bB	0.349 aA	0.140 bB	0.101 cB
On paper	0.162 bA	0.150 bA	0.101 cA	0.065 bA	0.032 cA
Between sand	0.396 aA	0.280 aB	0.236 bC	0.190 aC	0.107 cD
On sand	0.172 bA	0.273 aA	0.242 bA	0.220 aA	0.300 aA
Plantmax®	0.317 Aa	0.313 aA	0.375 aA	0.164 aB	0.137 cB
Vermiculite	0.324 Aa	0.234 aA	0.283 aA	0.237 aA	0.120 cB
Compost	0.184 Ba	0.129 bA	0.227 bA	0.225 Aa	0.188 bA
CV (%)	var. Talo Roxo 9.95		var. Rubra 8.59		

Note. Means followed by the same letter (lowercase in the column and uppercase in the row) do not differ by the Scott-Knott test at 5% probability.

Lighter substrates, such as sand and vermiculite, allow greater gas exchange and better drainage, reducing the physical barrier to initial seedling development (Jeromini et al., 2018), as observed for *P. volubilis* by Silva et al. (2016). It is noteworthy that the plantmax® substrate has macro and micronutrients in its composition, as well as suitable particle quality, these led to favorable results for all *H. sabdariffa* seed evaluated variables, because just after the *H. sabdariffa* seed germination and emission of the primary roots, immediately began the process of absorption of the nutrients present in this substrate and its mobilization to the seedling structures, promoting their development and dry mass accumulation.

These results are similar to those reported by Barrozo et al. (2014) where the combination between vermiculite and the temperature of 30 °C favored the growth of *Inga laurina* (Sw.) Willd, a species cited by Barreira et al. (2015) as one of the Unconventional Food Plants (UFPs), most used as food by the communities of the Amazon biome in Brazil. The good water retention capacity of plantmax® and/or vermiculite substrates is efficient, when combined with temperatures between 20 and 30 °C, since it is possible to predict that under these conditions, according to Marcos Filho (2015b) water allows the diffusion of hormones and consequently activation of enzymatic systems, favoring the digestion, translocation and assimilation of the reserves, resulting in embryo development and seedling growth.

4. Conclusion

For germination and vigor tests of *Hibiscus sabdariffa* var. Talo Roxo seeds the paper roll substrate is recommended at temperatures of 25 or 35 °C and for var. Rubra, plantmax® substrate is recommended at 25 °C and plantmax® and vermiculite substrates at temperatures of 25 or 20-30 °C, respectively.

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