MORPHOLOGY OF FRUITS, SEEDS AND SEEDLING AND GERMINATION OF *Hibiscus sabdariffa* L. IN DIFFERENT SUBSTRATES

Hugo Tiago Ribeiro Amaro[1] Andréia Márcia Santos de Souza David[1], Maria Aparecida Vilela de Resende[2], Cynthia Pires Guimarães[1], Miquéias de Oliveira Assis[3] Josiane Cantuária Figueiredo[4], Juliana Aparecida Santos Andrade[1]

[1]Universidade Estadual de Montes Claros (UNIMONTES). E-mail: hugo.amaro@unimontes.br; andreia.david@unimontes.br; cynthiapguimaraes@yahoo.com.br; juandrade08@yahoo.com.br
[2]Universidade do Estado de Minas Gerais (UEMG). E-mail: tida@nortecnet.com.br
[3]Universidade Federal de Viçosa (UFV). E-mail: miqueias.assis@ufv.br
[4]Universidade Federal de Pelotas (UFPEL). E-mail: josycantuaria@yahoo.com.br

ABSTRACT

The objective of this work was to characterize the morphology of fruits, seeds, and seedlings, as well as to determine the appropriate substrate for germination and initial development of *Hibiscus sabdariffa* L. seedlings. A morphological study of the fruits and the seeds conducted to germination tests on the substrates of "germitest" paper roll, between and on blotting paper and between sand was carried out to characterize seedlings and to evaluate the physiological quality of the seeds, evaluated for germination and vigor. The morphological study of *H. sabdariffa* structures is important for the understanding of the species, promoting the development of adequate management techniques. The germination is phanero-epigeal cotyledonary, starting from the second day after sowing. Germitest paper roll substrate is recommended for the germination test on *H. sabdariffa* seeds with counts at seven (first count) and 14 days (germination).

Keywords: *Hibiscus sabdariffa* L., morphological aspects, seed quality

MORFOLOGIA DE FRUTOS, SEMENTES, PLÂNTULAS E GERMINAÇÃO DE VINAGREIRA (*Hibiscus sabdariffa* L.) EM DIFERENTES SUBSTRATOS

RESUMO

O objetivo do trabalho foi caracterizar a morfologia de frutos, sementes e plântulas, assim como determinar o substrato adequado para germinação e desenvolvimento inicial de plântulas de *Hibiscus sabdariffa* L.. Foi realizado estudo morfológico dos frutos e as sementes conduzidas aos testes de germinação nos substratos rolo de papel "germitest", entre e sobre papel mata-borrão e entre areia, visando a caracterização das plântulas e a avaliação da qualidade fisiológica das sementes, sendo avaliadas quanto a germinação e vigor. O estudo morfológico das estruturas de *H. sabdariffa* é importante para o conhecimento da espécie, favorecendo o desenvolvimento de técnicas adequadas de manejo. A germinação é epígea fânero cotiledonar, iniciando a partir do segundo dia após semeadura. Recomenda-se utilizar o substrato rolo de papel germitest para o teste de germinação em sementes de vinagreira com contagens aos sete (primeira contagem) e 14 dias (germinação).

Palavras-chave: Aspectos morfológicos, *Hibiscus sabdariffa* L., qualidade de sementes

INTRODUCTION

Hibiscus sabdariffa L. (Malvaceae), known as vinegar, roselle, azedinha, quiabo-azedo, among others, is one of the species regarded as underutilized in Brazil, but with great potential to become part of the vegetable production chain at local, regional or national level (MELO, 2007). It appears as a semi-woody, biannual or perennial shrub, erect or branched depending on the conduction, able to reach 3 meters of height.

Its origin is still not clear and it is believed that *H. sabdariffa* is probably native to the Asian continent or African regions, showing good adaptation to crops in hot climates, being, in this sense, adequate for cultivation on a commercial scale in several regions in Brazil. According to Castro et al. (2004), *H. sabdariffa* has a vegetable, medicinal and ornamental uses, it is rich in vitamins A and B1 and in the following acids: citric, malic and tartaric. It is recommended as a diuretic, tranquilizer and soothing and stomachic besides being used in the production of sweets, jams, and juices, in which calyxes are the most consuming part of the plant.

Because it is a non-traditional species in cultivations with commercial purpose, it is necessary to know the morphology of fruits and seeds of *H. sabdariffa* as well as all the events that regulate its growth and development, thus providing information that can provide adequate technologies of cultivation, particularly for the quality of the seeds used in the cropping systems. According to Matheus & Lopes (2007), studies involving morphological analysis of fruits and seeds can aid to understand the germination process, vigor, storage, viability and propagation methods of the species. In addition, the morphological characterization of fruits and seeds provides subsidies to differentiate species and to characterize ecological aspects of the plant, such as dispersion, the establishment of seedlings and phase of the ecological succession.

The interest in in this crop has increased the demand for good quality seeds, however, there is no reliable information on the behavior of *H. sabdariffa* seeds during the germination process. The study of species with potential use, in seed analysis, has deserved attention in the scientific field, aiming at obtaining information, currently scarce, that evaluates the physiological quality of the seeds, both for their conservation and for the use of these plant species with the most varied objectives. However, few species regarded as underutilized are included in the Rules for Seed Analysis (BRASIL, 2009), as is the case of *H. sabdariffa* making it difficult to perform the germination test.

Several factors must be observed during the germination test, including the sowing substrate. The role of the substrate is to maintain moisture and to provide conditions for seedling germination and development. Some characteristics as seed size, water and light requirements, ease of counting and evaluation of seedlings should be considered when choosing the substrate. The substrate must maintain water availability and aeration at adequate proportions to avoid the formation of a water film around the seed, which would restrict the entry of oxygen (ALVES et al., 2002).

For *H. sabdariffa*, no studies related to the morphology of fruits, seeds, and seedlings as well as the ideal substrate for germination tests were found, indicating the need for studies. Hence, the objective of this work was to characterize the morphology of fruits, seeds, and seedlings, as well as to determine the appropriate substrate for germination and initial development of *H. sabdariffa* seedlings.

MATERIAL AND METHODS

The experiment was conducted in Horto de Plantas Medicinais (Medicine Plants Garden) and in Laboratório de Análise de Sementes in the Departamento de Ciências Agrárias, at Universidade Estadual de Montes Claros (Unimontes), *Campus* Janaúba, State of Minas Gerais, Brazil. The municipality of Janaúba is located at 15º49'51.5" S latitude and 43º16'18.2'' W, 540 m above sea level. Average rainfall in the region is approximately 870 nm and solar insolation of 2,700 annual hours. The climate in the region is classified by Köppen as "AW", tropical with dry winter.

Ripe fruits of *H. sabdariffa* were manually collected in plants cropped in the experimental area of the Garden and taken to seed laboratory for further evaluations. After that, seed extraction was carried out, and then processed for manual removal of impurities. Immediately after fruit harvest and seed extraction, the water content of the seeds was determined according to the methodology prescribed in Rules for Seed Analysis (BRASIL, 2009), and the results expressed as % of water content.

The morphological characterization of the fruits was accomplished using four repetitions of 20 fruits, observing internal and external characteristics pertaining to coloring, consistency, shape, number of seeds and dehiscence. For the description of the seeds, the coloring, shape, texture and consistency of the teguments was analyzed.

In relation to *H. sabdariffa*seedlings, it cannot be found any methodology prescribed in Rules for Seed Analysis (BRAZIL, 2009), for the performance of germination test. Thus, preliminary tests were carried out for the definition of the best temperature as well as the day for the first and last test evaluation, as recommended of Rules for Seed Analysis. Seedling characterization and evaluation of seed physiological quality were determined by means of the following tests: germination, germination speed index, seedling length, fresh and dry mass of the seedlings.

Germination: for the germination test, four replicates with 50 seeds per treatment were used by using the following substrates: Germitest paper roll (T1): the seeds were distributed over two Germitest paper sheets, moistened with water volume 2.5 times the paper weight and covered by a paper sheet, making the rolls; over the paper (T2): seeds were distributed over blotting paper sheet, moistened as previously described, in Gerbox plastic boxes; between paper (T3): seeds were distributed over a blotting paper sheet, overlapped on another paper sheet, moistened as

described above in Gerbox boxes among sand (T4): washed sand and sterilized in an oven at 200 ºC for two hours. Seeds were sown in plastic boxes at 2 cm depth keeping the substrate wet with light daily irrigation (BRAZIL, 2009).

After completion of the treatment, seeds were placed in digital germinators previously regulated at constant temperature of 30 ºC, in which visual evaluations were carried out for characterization of the germination process and seedling morphology.

The results obtained in the germination test were expressed in percentage of normal seedling accounted after pattern of normal seedlings was defined, according to recommendations of Rules for Seed Analysis (BRASIL, 2009). Seedlings were considered as normal when they presented all essential structures as primary root, secondary root, hypocotyl, cotyledon, and epicotyl.

First germination counting: the test results were obtained by the number of normal plants, determined at the first evaluation of the germination test, after establishing the days for the first and last evaluation according to recommendation of Rules for Seed Analysis (BRASIL, 2009), expressed in percentage.

Germination speed index: it was conducted together with germination test, writing down the number of seeds germinated until germination stabilization, daily. At the end of the test, the germination speed index was calculated using the formula proposed by Maguire (1962), allowing the construction of accumulated germination curves as well.

Seedling length: Length of seedlings considered normal was determined at the end of the germination test with the aid of a millimeter ruler. The results were expressed in centimeters/seedling.

Fresh and dry mass of the seedlings: normal seedlings obtained from the germination test were previously weighed for seedling fresh mass. After that, seedlings from each replicate were placed in paper bags and taken for drying in an air forced ventilation oven at constant 65 ºC for 72 hours. After that period, samples were placed for cooling in a desiccator and weighed in a 0.001-g precision scale. The results were expressed in g per seedling.

It was used a complete randomized block design (DIC), with four replicates per treatment. The results were submitted to analysis of variance and the means, whether significant, were compared by the test of Tukey at 5% of probability.

RESULTS AND DISCUSSION

The figure 1 shows the morphological patterns of fruit, seed, and seedlings of *Hibiscus sabdariffa*. It is observed that when the fruit reaches physiological maturity, it displays a very intense red coloration. The fruit of the *H. sabdariffa*is classified as capsule, oval-shaped, dehiscent, generally presenting 5 lobes contained inside the seeds.

Figure 1. General morphologic characteristics observed in *Hibiscus sabdariffa* L. A) Fruits. B) Seeds and seedlings standard.

It was verified that the set formed by the calyx and corolla is a persistent structure, constituting one of the most appreciated parts of the plant, with several culinary applications. The calyxes are fleshy, covering the oval fruits. Fruit maturation occurs heterogeneously in the plant, since it is possible to find emissions of new floral buds, fruits at the beginning of their development and mature fruits, potentializing harvests from 90 days after planting, as observed by Andrade et al., (2008). These same authors report that *H. sabdariffa*crops can produce about 8000 kg per ha fresh calyxes available for processing, thus justifying the importance of knowing the morphology and development of these fruits, since the plant is underutilized but with good potential to be part of the vegetable production chain.

It should be noted that the morphological evaluation of fruits and seeds is important since it assists in the identification of the species, as well as in the study of the factors that regulate the dispersion of the crop to new areas. *H. sabdariffa* seeds are small, bright, black colored at the end of maturation stage, and about 22 to 30 seeds per fruit are generally found. The tegument is hard

and massive (Figure 1B), which may be associated with possible mechanisms of dormancy due to the impermeability of the integument to water, as it was also verified in okra (EICHELBERGER& MORAES, 2001), which belongs to the same family of *H. sabdariffa.*

The impermeability of the tegument to the water is a common dormancy mechanism in the seeds of the families Cannaceae, Chenopodiaceae, Convallariaceae, Malvaceae, Geraminaceae, Anacardiaceae, Solanaceae, Rhamanaceae, and Fabaceae (SAMPAIO et al., 2001), being very expressive in *H. sabdariffa* seeds, which can lead to slow and irregular emergence, generating seedling unevenness in the field, contributing to increase seed expenditure. In this sense, Amaro et al. (2013) verified that pre-soaking of *H. sabdariffa*seeds for 48h was favorable for seed germination and seedling growth, which is an efficient method to overcome dormancy.

The germination test in *H. sabdariffa*seeds allowed to evaluate the general characteristics of the germination process. Germination is phanero-epigeal cotyledonary type, where the tegument is attached to cotyledons in the initial phase of seedling growth. It was observed that the radicle is small and whitish color. The hypocotyl is well developed, enclosed by well-developed green colored cotyledons.

In the initial stages of germination, little development in size and thickness of the main root was observed with the presence of absorption hair located in the final portion of the root. As seeds germinated (Figure 1B), it was possible to detect important structures that allowed to identify normal and abnormal *H. sabdariffa* seedlings, since these standards are not established in the Rules for Seed Analysis (BRASIL, 2009) for the species under study as previously reported.

According to Rules for Seed Analysis (BRAZIL, 2009), a normal seedling must have all its essential structures present, developed and healthy, as shown in Figure 1B. When this pattern does not occur, the seedlings are classified as abnormal, resulting from several factors that may act on seeds during germination, as well as factors inherent in the plant itself.

At the end of the evaluations, *H. sabdariffa*seedlings display a pair of well-developed cotyledon leaves of green color, with an average of 8.0 cm in length of aerial part and established root system, being of the pivoting type. The secondary roots are located in the upper third of the main root.

The table 1 presents the results obtained in the germination test, as a function of different substrates, except for the fresh mass of seedlings, a significant difference was found between the

substrates for the other variables analyzed. In relation to germination, it was verified that the highest percentage of normal seedlings was from the seeds sown on germitest paper (T1) and among sand (T4) reaching values of 84 and 86%, respectively, differing from the other treatments that presented lower values. The substrate directly influences the germination because, due to its water retention capacity, its structure and aeration, it interferes with the supply of water and oxygen to the seeds and serves as physical support for the development of the seedling (FIGLIOLIA et al., 1993).

Table 1. Average results of germination, (GER), germination first count (GFC), germination speed index (GSI), length of the seedlings (LS), fresh mass (FM) and dry mass of (DM) of *Hibiscus sabdariffa* L. plants in different substrates.

	Variables					
Substrates	GER (%)	GFC (%)	IVG -	LS (cm)	MF (g)	DM (g)
1*	84 A	17 A	38.7 A	22.6 A	9.10 A	2.58 B
2	57 B	11 B	30.9 B	11.9 B	8.69 A	2.79 B
3	66 B	12 B	27.1 B	14.2 B	11.46 A	3.01 B
4	86 A	13 B	21.2 C	13.1 B	10.11 A	5.72 A
Mean	73	13.2	29.4	15.4	9.84	3.52

Means followed by the same letter in the column are not different from each other by the test of Tukey, at 5% of probability. *1. Germitest paper roll; 2- between paper; 3- on paper; 4 – between sand.

It must be emphasized that in Brazil the standards for the production and commercialization of *H. sabdariffa* seeds have not been established yet. However, species of the same family as *H. sabdariffa* such as okra (*Abelmoschus esculentus*), have been commercialized with a minimum commercially required standard of 75% of germination (MAPA, 2012). Hence, in the present work, values higher than 80% were obtained in the percentage of seed germination, coming from treatment 1 and 4, being able to be considered a percentage of germination commercially acceptable when compared to other cultivated species of the Malvaceae family.

Regarding the vigor of the seeds evaluated by the test of first germination count, higher results are observed when substrate germitest paper roll was used, thus justifying better proportionate conditions in relation to the other substrates, for the establishment of the seedlings classified as normal by the first evaluation. According to the Rules for Seed Analysis (BRAZIL, 2009), within the same lot or even in different lots, the seeds that germinate first (1st evaluation), are considered the most vigorous.

The germitest paper substrate provided higher speed in the germination of *H. sabdariffa* seeds, in accordance with the results observed in the tests of first counting and germination. These results are important since they indicate greater uniformity in the germination process, thus guaranteeing better seedling development conditions in the test (Table 1). It is noteworthy that the substrate is one of the important factors that affect seed germination, consequently affecting their physiological quality.

The responses of the germination process to the substrate vary among species. In the seeds of *Parapiptadenia rigida*, a forest species of the family Fabaceae-Mimosoideae, Mondo et al. (2008) verified that the substrate between vermiculite allowed the proper development of the seedlings, particularly in relation to the physical support, unlike the treatment on vermiculite. These same authors reported that the greater contact of the seeds with the substrate facilitated tegument release, promoting the faster development of the seedling. Stockman et al., (2007) concluded that temperature and substrate interfere in the germination of *Tabebuia roseoalba* seeds, a temperature of 30°C and the germitest paper substrate are the most favorable condition for the germination test of these seeds.

Regarding length of seedlings, higher results were obtained using germitest paper (Table 1). Indeed, the higher water retention capacity of the substrate may have promoted the development of seedlings, pointing this substrate as the best for the evaluation of seed germination and development of *H. sabdariffa*seedlings. No significant difference was found between the substrates for fresh seedling mass, while for dry mass, the substrate between sand provided the best result.

The evaluation of the germination process on consecutive days allowed the preparation of the cumulative *Hibiscus sabdariffa* L. germination curves in the different substrates (Figure 2). Germination started on the second day after sowing when the germitest paper substrate was used. It is also observed that on the sixth day after sowing, 50% of the seeds had already germinated.

For the subtract between paper, this value was observed only on the seventh day; on the eighth and ninth days for substrates between sand and on paper, respectively.

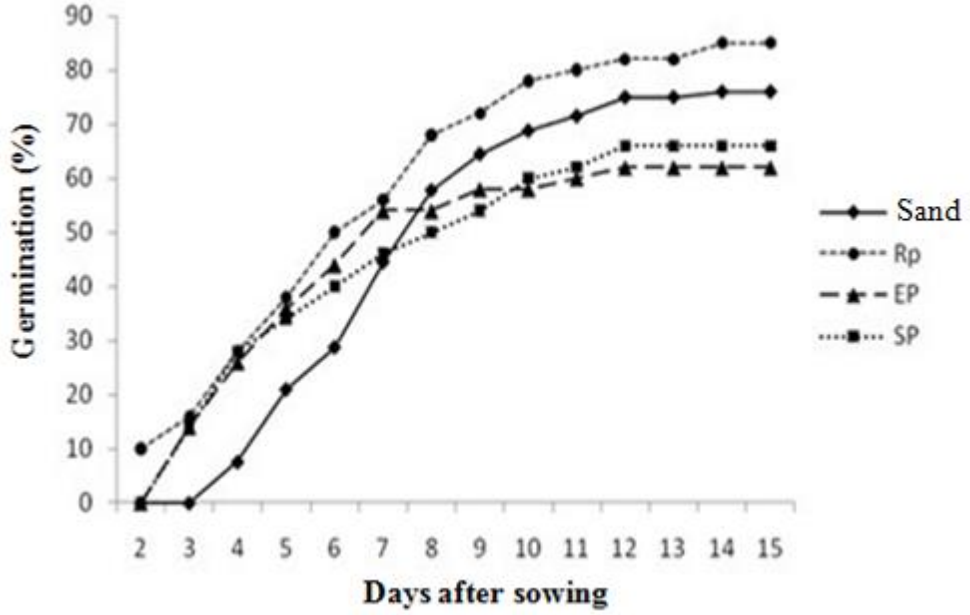

Figure 2. Cumulative germination curve (%) of *Hibiscus sabdariffa* L. seed in different substrates. Sand (between sand); Rp (paper roll); EP (between paper); SP (about paper).

For the substrates germitest paper and between sand, a similar growth was observed in the germination curve, observing a stabilization trend in the germination from day 13, with about 80 and 72%, respectively (Figure 1). For the other substrates, the curve showed variation in the germination throughout the evaluations, with stabilization in the germinative percentage from the 13th day.

Cardoso & Pereira (2009) report that although the works that evaluated the several factors acting on germination are relatively simple and easy to be performed, the analysis of the results is not always properly performed, which may compromise the interpretation of the experimental data. The most common way to present the results of a germination test is the cumulative germination curve (germination × time). According to Brown & Meyer (1988), the germination curves, with the respective adjustment models, describe seed behavior over time better than the isolated indices. The curves indicate the time required for the individual seeds of a population to germinate, thus reflecting the homogeneity or uniformity of the seed population regarding the distribution of germination times (CARDOSO & PEREIRA, 2009).

CONCLUSIONS

It was concluded that the morphological study of *H. sabdariffa* structures is important for the knowledge of the species, promoting the development of adequate management techniques.

The germination is phanero-epigeal cotyledonal, starting from the second day after sowing.

Germistest paper roll substrate is recommended for the germination standard test in *H. sabdariffa* seeds, with counts at seven (first count) and 14 days (germination) after sowing.

ACKNOWLEDGEMENTS

To Unimontes, for the technical support in this study and to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support.

REFERENCES

ALVES, E.U.; PAULA, R.C.; OLIVEIRA, A.P.; BRUNO, R.L.A.; DINIZ, A.A. 2002. Germinação de sementes e *mimosa caesalpiniaefolia* Benth. em diferentes substratos e temperaturas. **Revista Brasileira de Sementes**, Londrina, v. 24, n. 1, p. 169-178.

ANDRADE, J.A.S.; AMARO, H.T.R.; FARIA, M.A.V.R.; GUIMARÃES, C.P. 2008. Potencial produtivo de vinagreira (*Hibiscus subdariffa* L.) em Janaúba, Norte de Minas Gerais. **Horticultura brasileira**, Maringá, v. 26, n. 2 (Suplemento - CD Rom), Jul-Ago. p. 1- 4.

AMARO, H.T.R.; DAVID, A.M.S.S.; SILVA NETA, I.C.; ALVES, D.D.; SILVA, F.G. 2013. Avaliação fisiológica de sementes e crescimento de plântulas de vinagreira. **Comunicata Scientiae**, Piauí, v.4, n.1, p. 96-102.

BRASIL, 2009. Ministério da Agricultura, Pecuária e Abastecimento.. **Regras para Análise de Sementes**. Brasília: SNAD/DNDV/CLAV. 395p.

BROWN, R.F.; MAYER, D.G.1988. Representing cumulative germination.2. The use of the Weibull function and other empirically derived curves. **Annals of Botany**, Beltsville, v. 61, p. 127-138.

CARDOSO, V.J.M.; PEREIRA, F.J.M. 2009. Dependência térmica da germinação de sementes de *Drymaria cordata* (L.) Willd. ex Roem. &Schult. (Cariophyllaceae). **Acta Botânica Brasileira**, São Paulo, v. 23, n. 2. p. 305-312.

CASTRO, N.E.A.; PINTO, J.E.B.P. 2004. CARDOSO, M.G.; MORAIS, A.R.; BERTOLUCCI, S.K.V.; SILVA, F.G.; DELU FILHO, N. Planting time formaximization of yield of vinegar plant calyx (*Hibiscus sabdariffa* L.). **Revista de Ciência e Agrotecnologia**, Larvas, v. 28, n. 3, p. 542-551.

EICHELBERGER, L.; MORAES, D.M. de.2001. Preparo de sementes de quiabo (*Abelmoschus esculentus* (L.) Moench) para o teste tetrazólio. **Revista Brasileira de Sementes**, Londrina, v. 23, n. 1, p. 154-158.

FIGLIOLIA, M.B.; OLIVEIRA, E.C.; PIÑA RODRIGUES, F.C.M. 1993. **Análise de sementes**. In: AGUIAR, I.B.; PIÑA RODRIGUES, F.C.M; FIGLIOLIA, M.B. Sementes Florestais Tropicais. Brasília: ABRATES. p. 137-174.

MAGUIRE, J.A.1962. Speed of germination: aid in selection an evaluation for seedling emergence and vigor. **Crop Science,** Socienty of America**,** v.2, n.2, p.176-177.

MAPA, 2012. **Ministério da Agricultura, Pecuária e Abastecimento.** Portaria nº 111, de 4 de setembro de. Estabelece os padrões de identidade e qualidade para a produção e a comercialização de sementes de espécies olerícolas, condimentares, medicinais e aromáticas, disposto em anexo. Diário Oficial [da] República Federativa do Brasil, 5 set. 2012. Seção 1, p.3-4.

MATHEUS, M.T.; LOPES, J.C., 2007. Morfologia de frutos, sementes e plântulas e germinação de sementes de *Erythrina variegata* L. **Revista Brasileira de Sementes**, Londrina, v. 29, n. 3, p. 08-17, 2007.

MELO, A.M.T., 2007. Hortaliças subutilizadas e sua importância no contexto da agricultura familiar. **Horticultura Brasileira**, Porto Seguro, v. 25, n. 1... CD-ROM-palestras.

MONDO, V.H.V.; BRANCALION, P.H.S.; CICERO, S.M.; NOVEMBRE, A.D.L.C.; DOURADO NETO, D. 2008. Teste de germinação de sementes de *Parapiptadenia rigida* (Benth.) Brenan (Fabaceae). **Revista Brasileira de Sementes**, Londrina, v. 30, n. 2, p. 177-183.

SAMPAIO, L.S.V.; PEIXOTO, C.P.; PEIXOTO, M.F.S.P.; COSTA, J.A.; GARRIDO, M.S.; MENDES, L.N. 2001. Ácido sulfúrico na superação da dormência de sementes de sucupira-preta (*Bowdichia virgilioides* H.B.K.- Fabaceae). **Revista Brasileira de Sementes**, Londrina, v. 23, n. 1, p. 184-190.

STOCKMAN, A.L.; BRANCALION, P.H.S.; NOVEMBRE, A.D.L.C.; CHAMMA, H.M.C.P., 2007. Sementes de ipê-branco (*Tabebuia roseo-alba* (Ridl.) Sand. – Bignoniaceae): temperatura e substrato para o teste de germinação. **Revista Brasileira de Sementes**, Londrina, v. 29, n. 3, p. 139-143.

Received in: November27 2015
Accepted in: January 17, 2019