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The germination of bush mint (*Hyptis marrubioides* EPL.) seeds as a function of harvest stage, light, temperature and duration of storage

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ABSTRACT. This study evaluated the effect of light, temperature, physiologic stage at harvest and length of time on the germination of *Hyptis marrubioides* seeds. Two trials were conducted. The first experiment was performed immediately after the seed harvest and consisted of a 2 x 3 x 3 factorial design with 2 environmental conditions (light and dark), 3 temperatures (20, 30 and 20/30ºC) and 3 seed harvest times (green colored seeds, light brown seeds and dark brown seeds). The second experiment was conducted in the presence of light at a temperature of 30ºC and consisted of a 4 x 3 factorial design, with 4 storage times (0, 6, 12 and 18 months) and the 3 three harvest physiologic stages used in the previous experiment. Both of the experiments were conducted in randomized blocks, with 4 replications of 100 seeds. Light did not affect germination. By contrast, a temperature of 20ºC retarded the germination process, although the percentage of germinating seeds was not affected. Seed storage and the different harvest physiologic stages affected the Speed of germination index (SGI) and the germination percentage. Seeds that were harvested at the more mature stage (dark brown color) could be stored for up to 18 months.

Keywords: medicinal plant, viability, Lamiaceae.

RESUMO. Germinação de sementes de hortelã-do-campo (*Hyptis marrubioides* EPL.) em função da época de colheita, da luz, temperatura e armazenamento. O presente trabalho teve como objetivos avaliar a influência da luz, da temperatura, do estádio fisiológico na colheita e do armazenamento na germinação das sementes de *Hyptis marrubioides*. Foram realizados dois experimentos, sendo o primeiro implantado logo após a colheita das sementes, constituído por um fatorial 2 x 3 x 3, com 2 ambientes (luz e escuro) x 3 temperaturas (20, 30 e 20/30ºC) x 3 épocas de colheita das sementes (sementes com coloração verde, coloração marrom claro e coloração marrom escuro). O segundo experimento, em presença de luz e à temperatura de 30ºC, foi constituído por um esquema fatorial 4 x 3, sendo 4 tempos de armazenamento (0, 6, 12 e 18 meses) e 3 três estádios fisiológicos de coleta mencionados no experimento anterior. Ambos os experimentos foram implantados no delineamento em blocos ao acaso, com quatro repetições de 100 sementes. Foi verificado que o fator luz não influenciou a germinação. Por outro lado, a temperatura de 20ºC retardou a germinação, apesar de não influenciar a sua porcentagem. O armazenamento e os diferentes estádios fisiológicos de colheita das sementes influenciaram o IVG e a porcentagem de germinação. Sementes coletadas no estádio mais maduro (coloração marron escuro) demonstraram ser apropriadas para o armazenamento por até 18 meses.

Palavras-chave: planta medicinal, viabilidade, Lamiaceae.

Introduction

The genus *Hyptis* (Lamiaceae) is composed of more than 300 species and displays the greatest morphological diversity in the Brazilian savannah (WILLIS; SHAW, 1973). Species in the genus *Hyptis* are quite aromatic and are frequently used in the treatment of enteric infections, cramps, pain and skin infections (CORRÊA, 1931).

The indiscriminate use of medicinal species by individuals practicing folk medicine and by the pharmaceutical industry has caused a considerable decrease in population density of some of these plants in their natural areas. Given the potential negative impact to wild population of medicinal species, the development of effective protocols that allow for the commercial propagation of these species is of utmost importance (NADEEM et al., 2000). The propagation of bush mint can be achieved through cuttings as well as by planting the seeds. However, one of the first steps toward successful commercial propagation of a species is to understand the seed production system in terms of viability, quality retention and storage.

Seed germination is an ordered process of metabolic activities that occur in phases. The result of these processes is the formation of a seedling. Germination is a critical step in the vegetative biocycle due to its association with several extrinsic (physical environment) and intrinsic (physio-metabolic processes) factors (BEWLEY; BLACK, 1994; POPINIGIS, 1985).

The most adequate moment for seed harvest occurs as close as possible to the point of physiological maturity. Upon reaching the physiologic maturity, the seeds contain a maximum amount of dry matter, which is an important indicator of independence from the mother plant. A delay in ripe seed harvest contributes to deterioration as the process of field “storage” begins. During field storage, the seed is subjected to highly unfavorable conditions, such as rain, insect damage and microorganism attack. Seed harvest should therefore be conducted at the appropriate moment, in order to reduce the eventual qualitative and quantitative losses (VON PINHO, 1997).

Harvest anticipation becomes relevant to maintaining good physiologic quality, avoiding the rapid deterioration of the seed in the field and, subsequently, during the storage period (CARVALHO; NAKAGAWA, 2000).

Seed deterioration is a continuous degenerative process that begins after physiologic maturity and continues until the loss of seed viability and eventual seed death. Depending on the environmental conditions and management, the reduction of the physiological quality of the seed can occur through the intensification of the deterioration phenomena (MARCOS FILHO, 2005).

Storage conditions determine the maintenance of seed quality. Seeds present the best quality at the initial stage of physiologic maturity. From that point onward, the germination power and the vigor decline at a variable intensity that depends on the conditions to which the seeds are subjected (PELEGRINI, 1982). A variety of factors affect the stored seed quality, such as seed longevity, initial quality, maturation stage, moisture contents, the physical conditions of the seed, the conditions of the storage environment, phytosanitary treatment and type of packaging. Management strategies that halt or block the mechanisms of aging are therefore required (CARVALHO; NAKAGAWA, 2000; PELEGRINI, 1982).

Light is one of the most important environmental factors that is necessary for releasing seed dormancy in many species. The activation of seed germination by light is linked to a pigment system called phytochrome. The phytochrome pigment is found in every higher plant and acts as a mediator of light responses. By absorbing light at a given wavelength, phytochrome changes its biochemical structure and allows, or restricts, a photomorphogenetic response (BORGES; RENA, 1993). Phytochrome is always associated with the action of biological membranes, and it likely regulates their permeability and controls the flow of many substances within and among the cells (TAIZ; ZEIGER, 1991).

Temperature is an ecological factor that is of great importance in the seed germination process. Temperature determines and limits and rate of occurrence of germination, acting on dormancy and subsequently inducing or halting the process (BEWLEY; BLACK, 1994). Arnold et al. (1990) state that it is essential to quantify the effect of alternate temperatures on seed populations with different dormancy levels in order to understand the timing of dormancy breaks in the field. Some of the interactions of temperature with light can be relevant to the performance of seeds in their natural environment (MAYER; POLJAKOFF-MAYBER, 1989). A large number of species display positive germination reactions at alternating temperatures, paralleling the trends seen in natural environments where day temperatures are higher than night temperatures (CARVALHO; NAKAGAWA, 2000; POPINIGIS, 1985).

This study evaluated the effects of light and temperature on germination, as well as on the physiologic stage at harvest and storage time on bush mint (*Hyptis marrubioides)* seed quality retention.

Material and methods

Bush mint seeds were harvested from native plants growing in Fazenda Três Barras, Minas Gerais State, Brazil. Herbarium material was identified from these individuals and was deposited at the herbarium of the Universidade Federal de Lavras under the accession number “ESAL 13955”. One hundred bush mint seeds weighed 0.0174 g. Three harvest times were used: stage 1 – seeds completely formed and with a green color; stage 2 – seeds collected 2 weeks after the first harvest, with light brown color; and stage 3 – seed that were collected 10 days after the second harvest, at the beginning of dispersion and having a dark brown color. The harvested plants were dried in the shade, and the seeds were thrashed manually. The impurities were separated using a blower (Model: South Dakota).

The germination experiments were conducted at the Laboratory of Seed Analysis and at the Laboratory of Tissue Culture and Medicinal Plants at the Department of Agriculture at the Universidade Federal de Lavras. The seeds were sown in a "gerbox" containing moistened paper towels with added water equivalent to 2.5 times the weight. In the first trial, the seeds were placed in BOD (Biochemical Oxygen Demand) chambers at 20°C, 30°C or at an alternating temperature cycle (20/30°C) with 12 hours of light daily. Complete darkness was simulated by wrapping the gerboxes with aluminum foil. The evaluations were done daily, counting the number of seeds that had a protruded radicle to determine the Speed of germination index (SGI), which was computed according to Maguirre's formula (MAGUIRRE, 1962).

The germination percentage was obtained 10 days after sowing, when it was stable, by computing the number of normal plants, according to the Rules for Seed Analysis (BRASIL, 1992).

In the second trial, the seeds were stored in paper bags in a cold chamber at 10ºC and 50% RH, for up to 18 months. The quality of the seeds was evaluated every 6 months. From the previous trial, a temperature of 30ºC under light conditions was determined to be optimal. The second trial was carried out under these conditions and according to the previously mentioned methodology.

Both trials were conducted as factorials. The first experiment consisted of a 2 x 3 x 3 factorial design, using the variables of light and darkness at 3 temperatures and 3 harvest times. The second experiment was a 4 x 3 factorial design, with 4 storage times and 3 harvest times. The experimental design was set-up as a series of randomized blocks, with 100 seeds/gerbox in 4 replications.

Results and discussion

Trial 1

The seeds of *Hyptis marrubioides* were light insensitive, germinating both in the presence and in the absence of light. A greater germination speed was observed at the highest temperature tested (30ºC) and in the presence of light. No differences were observed with the alternating temperature, (20-30ºC), except for those harvested at harvest time 2 (Table 1). These data are consistent with results found for scarlet sage (*Salvia splendens* Sellow), Lamiaceae, by Vazquez-Yanes and Orosco-Segovia (1993), and for common mallow (*Malva sylvestris* L.), by Malvaceae, by Figueiredo and Popinigis (1980).

In general, greater germination velocities were observed at 30ºC and under alternating temperatures (20-30ºC) (Table 1) than at 20ºC, both in the presence and in the absence of light (CARVALHO; NAKAGAWA, 2000; HENDRICKS; TAYLORSON, 1976).

The germination of coat button (*T. procumbens* L.*)*, Asteraceae, seeds was greater at 25, 30 and 35ºC, reaching more than 90% germination. The process was faster at 30ºC and more evenly distributed at 35ºC. Germination was less than 6% at 15, 20 and 40ºC. At 40ºC, coat button seeds lost up to 80% of their viability (GUIMARÃES et al., 2000).

There were differences detected in seed quality at 20ºC using the SGI results (Table 1). These significant changes were seen among all of the harvest physiologic stages, both in the presence and in the absence of light, presenting the lowest speed of germination indices.

Table 1. Speed of germination index (SGI) of *Hyptis marrubioides* seeds, harvested at different physiologic stages, under different temperatures, in the presence or absence of light.

	SGI					
T°C	With light			No light		
	Stage 1	Stage 2	Stage3	Stage 1	Stage 2	Stage 3
20	12.87 B a	09.92 C a	12.35 B a	13.60 B a	16.27 B a	15.03 B a
30	23.86 A a	22.45 A a	24.82 A a	24.34 A a	24.04 A a	23.87 A a
20-30	21.57 Aab	17.94 B b	22.13 A a	22.66 A a	21.80 A a	22.21 A a

Averages followed by the same capital letter in the columns, and a small cap in the lines for each light condition, are not significantly different according to Tukey's test at 5% probability.

A high germination percentage was observed, independent of the light condition, the physiologic stage or the temperature. However, differences were observed among the factors analyzed (Table 2), except for the comparisons between the seeds harvested at maturation stage 2 that were germinated under light at 20ºC, and those seeds harvested at stage 1 that were germinated in the dark at 20ºC.

Table 2. *Hyptis marrubioides* seed germination (%), harvested at different physiological stages, under different temperatures, in the presence or absence of light.

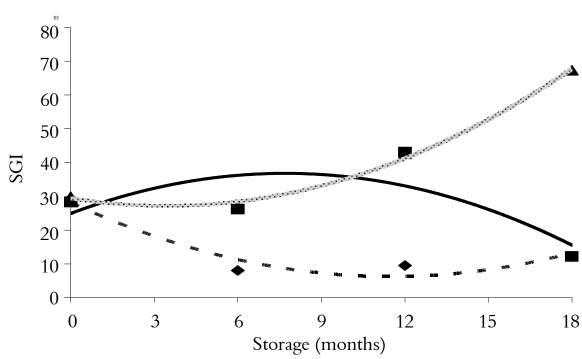
	Germination (%)					
T°C	With light			No light		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
20	86.25 Aa	78.75 Ba	87.50 Aa	75.50 Ba	90.00 Aa	90.50 Aa
30	97.50 Aa	98.50 Aa	100.0 Aa	98.50 Aa	99.25 Aa	96.75 Aa
20-30	98.25 Aa	97.75 Aa	99.75 Aa	97.25 Aa	98.75 Aa	99.25 Aa

Averages followed by the same capital letter in the columns, and a small cap in the lines for each light condition, are not significantly different according to Tukey's test at 5% probability.

The stage 1 seeds presented the lowest germination percentages, highlighting that immature seeds are more sensitive to lower temperatures. For instance, in sage (*Salvia splendens* Sellow), Lamiaceae, it has been observed that temperatures of 15, 20 and 25°C affect the seed germination velocity, and that the germination process was retarded at 15°C, due to the fact that lower temperatures reduced the seed metabolic rate (MENEZES et al., 2004).

Trial 2

Significant differences were observed in seed quality during storage. These differences were demonstrated with the SGI of the *Hyptis marrubioides* seeds harvested at different physiologic stages and stored for different periods of time (Figure 1). Seed stage did not significantly alter the speed of germination of unstored seeds when compared using the SGI. However, there was a pronounced reduction in SGI for stage 1 seeds after the first months of storage, demonstrating that seeds harvested at this stage were likely not mature and subsequently susceptible to suffering deterioration during storage. In contrast, the seeds harvested at the light brown stage (stage 2) had a small increase in SGI in the first months of storage. After 7.7 months of storage, a decrease in the SGI was observed.



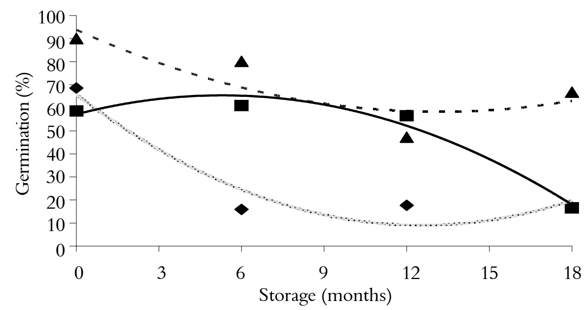
Dark brown seeds $\hat{Y} = 28.1624 - 3.8036x + 0.1653x^2$ $R^2 = 92.01\%$*
Light brown seeds $\hat{Y} = 24.9446 + 3.0816x - 0.2002x^2$ $R^2 = 53.59\%$*
Green colored seeds $\hat{Y} = 29.4384 - 1.3084x + 0.1910x^2$ $R^2 = 99.60\%$*

Figura 1. Speed of germination index (SGI) of *Hyptis marrubioides* seeds as a function of harvest stage and storage period.
*Significant at 5% probability by the F test. Data transformed by Arcsine $\sqrt{X}$.

Guimarães et al. (2004) observed that coat button seeds (*Tridax procumbens* L.), Asteraceae, that were subjected to variations in temperature, storage environment and air moisture during storage displayed a reduction in viability within the first year.

In contrast, the seeds harvested at the dark brown stage (stage 3) had an increased SGI throughout the storage period. This effect was probably due to dormancy release, demonstrating that mature seeds can be stored without having their germination time reduced.

The germination percentage results were similar to those of SGI (Figure 2). Seeds harvested with a green color (stage 1) presented pronounced reduction in germination after the first months of storage, while seeds harvested in the second stage maintained germination until almost six months, with a subsequent reduction thereafter. In contrast, mature seeds (stage 3) presented the greatest germination values both at the beginning and throughout storage, maintaining approximately 70% germination after 18 months. This response is likely due to the seed at this stage being better structured and containing larger amounts of food reserves.



Dark brown seeds $\hat{Y} = 93.7794 - 5.928x + 0.2047x^2$ $R^2 = 72.21\%$*
Light brown seeds $\hat{Y} = 57.1576 + 3.1094x - 0.2935x^2$ $R^2 = 96.95\%$*
Green colored seeds $\hat{Y} = 65.7305 - 9.0330x + 0.3599x^2$ $R^2 = 91.93\%$*

Figure 2. Germination percentage of *Hyptis marrubioides* seeds as a function of harvest stage and storage period.
*Significant at 5% probability by the F test. Data transformed by Arcsine $\sqrt{X}$.

Cabral et al. (2003) did not find significant differences associated with storage in the germination percentage of caraibeira (*Tabebuia aurea* Manso D.C.), Bignoniaceae (60, 90 and 120 days). Variability was maintained during this period, with the germination percentage varying from 88 to 97%.

Conclusion

Light did not affect the percentage or the speed of germination in recently harvested *Hyptis marrubioides* seeds; temperatures of 30ºC and alternating temperatures between 20-30ºC were favorable for *Hyptis marrubioides* seed germination; dark brown colored *Hyptis marrubioides* seeds maintained quality over a longer storage period.

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