

Investigation of various factors on the germination of chia seeds sprouts (*Salvia hispanica* L.)

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Abstract. *Salvia hispanica* L. is capable to produce a large amount of green matter, which can be used as a source of biologically active substances. The purpose of this research was to select the optimal factors for the chia seed sprouts (*Salvia hispanica* L.) germination. Dark variety chia seeds (100 grains/sample) were investigated. The most significant factors for the process of sprouting were selected as the study factors, such as the water mass fraction, the temperature and the light exposure for seed germination. The output parameters of the experiment were seed germination energy, germination of seeds, speed of germination and seedling vigor. It was revealed that the mass fraction of added water had the greatest influence on the growing process of chia seed sprouts. The optimal amount of water for producing the chia seed sprouts was in the average of 4 mL/sample. As a result, it was noted that an insufficient or excessive amount of water had a negative effect on the chia seed sprouts germination. The optimum temperature for germination of chia seed sprouts was 25 °C. The optimal light factor was also determined; in particular light exposure peaks occur in the red spectrum with a wavelength of 660 nm and a blue spectrum with a wavelength of 450 nm.

Key words: chia seed sprouts, *Salvia hispanica* L., germination factors, microgreens.

INTRODUCTION

Salvia hispanica L. (Spanish sage, chia) is an annual herb that adapts to arid and semi-arid climates, particularly in the United States, Mexico, Chile, Peru, Colombia, Guatemala, Bolivia, Argentina and Australia, as well as in India, China and some countries in Europe and Africa (Ayerza, 2013; Busilacchi et al., 2013; Sreedhar et al., 2015; Zayova et al., 2016; Elshafie et al., 2018; Win et al., 2018).

This plant has received wide popularity since the chia seeds are a rich source of polyunsaturated fatty acids with a high nutritional value. The chemical composition of plant and chia seeds, in particular, the oil fraction, is critically dependent on the height

of the plant and on the temperature at which they are grown (Ahmed et al., 1994; Baginsky et al., 2016).

In addition, the plant *Salvia hispanica* L. is able to produce a large amount of leaves that can be used as a source of biologically active substances, especially in cases when the plant does not enter the reproductive phase in some regions (Zayova et al., 2016) due to its photosensitivity. In contrast to the seeds, the chemical composition, pharmacological and nutritional value of the leaves and sprouts of this Spanish sage have not been well studied (Ahmed et al., 1994; Ouzounidou et al., 2015; Zayova et al., 2016; Elshafie et al., 2018; Pająk et al., 2019).

Currently, the relevance of this study on chia seeds is to obtain microgreens for the food industry. It is promising to study the application of plant sprouts as part of functional products that can have a therapeutic effect on the state of the gastrointestinal tract and on humans, in general. According to some reports, the inclusion of sprouts in the diet can enrich it with enzymes, antioxidants, polysaccharides, etc. (Gómez-Favela et al., 2017).

In the germination process, plant resources are activated and the nutritive value of seeds is increased, including phenolic compounds and mineral composition, besides resulting in an increase in their bioavailability (Gómez-Favela et al., 2017; Pająk et al., 2019). According to studies, in the analysis of the vitamins and carotenoids content in 25 plant species, it was revealed that harvesting the microgreens contain 10 times more antioxidant compounds as compared with the traditional harvesting in the commercial ripeness phase (Ivanova et al., 2016). There is also a possible difference in chemical composition of seedlings during each day of growth due to the high intensity of bioprocesses (Pająk et al., 2019).

Chia seeds revealed a significant increase in the total content of phenolic compounds in the process of germination, including the content of γ -aminobutyric acid (GABA) (Gómez-Favela et al., 2017; Pająk et al., 2019). In the oil obtained from the leaves of *Salvia hispanica* L. about 80 compounds have been identified, most of which belong to the class of sesquiterpenes, apart from monoterpenes, phenolic compounds, and flavonoids (Ahmed et al., 1994; Amato et al., 2015; Zayova et al., 2016; Elshafie et al., 2018). It is important to note that the antioxidant activity of chia seeds is widely studied, however, data on the antioxidant effect of seedlings and of chia green part are extremely limited (Zayova et al., 2016).

Gómez-Favela et al. (2017) report that the amount of protein and insoluble dietary fibers increase in germinated chia seeds, while the content of soluble dietary fibers and the lipid component decreases. During the growth cycle of *Salvia hispanica* L., the solids content increases rapidly (from 84 to 224 g kg⁻¹ of the mass of the fresh plant) (Peiretti, 2010). The content of polyunsaturated fatty acids in plant biomass also varies depending on the growth stage. Thus, a decrease in the content of alpha-linolenic acid from 649 g kg⁻¹ at an early stage of vegetation to 499 g kg⁻¹ at the budding stage was noted, while the remaining fatty acids showed a reverse trend. The optimal composition of plants for harvesting is determined during the period of shoots before budding (Peiretti & Gai, 2009).

Germinated chia seeds are of great interest for the food industry, and the process of seed germination is of interest for agricultural production. In addition, the determination of optimal parameters could be used in the further cultivation of the plant *Salvia hispanica* L. in case of using green matter as a biologically active component.

The seed germination depends on a number of internal (dormant, genotype, maturity) and external factors, including temperature, salinity, light and moisture conditions (Jafarinia & Yazdanbakhsh, 2016; Pająk et al., 2019).

The sensitivity of various seeds to light regime depends on their species, the light factor is decisive in the process of photosynthesis. Some seeds germinate better in the dark, while in other seeds, there is also a dependence on the light spectrum (Jafarinia & Yazdanbakhsh, 2016).

Growth temperature affects both the percentage and the speed of germination, impeding water absorption and the flow of biochemical reactions (de Souza & Chaves, 2016).

The dormant dry seed must be fed with water to activate the germination process (Jafarinia & Yazdanbakhsh, 2016). In addition, it has been proven that seed germination speed decreases with decreasing external water potential, there is a critical value of water potential, below which seed germination does not occur (Hadas, 1976). One of the characteristics of chia seeds is the ability to produce a significant amount of mucilage during hydration. This gel completely envelops the seed, which also needs to be considered in the germination process (Geneve et al., 2017).

In the natural conditions, *Salvia hispanica* L. grows in tropical and subtropical regions; the minimum and maximum temperature of plant growth is 11 °C and 36 °C, respectively, with an optimal growth range from 16 °C to 26 °C (Ayerza & Coates, 2009). During the growing season, Spanish sage needs a uniform amount of rainfall and a dry climate during ripening (Win et al., 2018).

A number of studies have been conducted in the global community on the chia seed germination depending on the temperature factor and light factor (Stefanello et al., 2015b; Paiva et al., 2016; Possenti et al., 2016; Gómez-Favela et al., 2017), and the effects of salt stress (Raimondi et al., 2015; Stefanello et al., 2015a; de Souza & Chaves, 2016) and the mucilage formation factor (Geneve et al., 2017).

The optimum temperatures for germinating chia seeds are different. It is noted that the light factor does not have a significant effect on the seeds, but seedlings accumulate dry matter better in the presence of light (Stefanello et al., 2015b; Paiva et al., 2016; Possenti et al., 2016). Chia seeds are moderately tolerant to certain levels of salinity, but higher or lower values can be disastrous, especially in the early stages of seedling development (Raimondi et al., 2015; Stefanello et al., 2015a; de Souza & Chaves, 2016). Under optimal conditions, mucilage from chia seeds does not affect germination; however it slows down the germination process while increasing the survival rate of seeds under stressful environmental conditions (Geneve et al., 2017).

In this study, the experiment taking into account three significant parameters – the water mass fraction, the temperature and the light exposure was staged for the first time. These factors would determine the optimal conditions for the chia seed germination on an industrial scale.

MATERIALS AND METHODS

Materials

The chia seeds (*Salvia hispanica* L.) of dark varieties (the brand ‘Era Green’) with the following composition were used as an object of research: proteins 24%, fats 31%, carbohydrates 34%.

Conducting germination tests

Sample preparation

The research was conducted according to GOST 12038-84 'Agricultural seeds. Methods for determination of germination'. Sample preparation for germination of each experimental part was carried out by counting 100 grains of chia seeds, each sample was studied in quadruplicate.

Germination of seeds was carried out in ethanol-disinfected Petri dishes, on two layers of filter paper. Pre-prepared chia seed samples were evenly distributed in Petri dishes on paper layers and were moistened with distilled water.

Every day, Petri dishes were opened in a sterile environment for several seconds for air ventilation.

Method of counting germinated seeds

Counting germinated seeds was carried out every day for 7 days. Each Petri dish was numbered and counted accordingly, each experiment (moisture, light, temperature) was independent, and for each sample there were 4 replications. The seed germination energy of the studied samples was determined on day 3, the germination of seeds - on day 7 of the experiment.

Seeds that had formed sprouts were attributed as the 'normally germinated' seeds while the 'not germinated' seeds were hard seeds, which at the time of germination were not swollen and did not change in their appearance.

Moisture test

To assess the effect of the moisture regime on the dynamics of the chia seed germination, samples were prepared with the addition of distilled water in a different volume: sample 1 – 1 mL of water; sample 2 – 2 mL of water; sample 3 – 3 mL of water; sample 4 – 4 mL of water; sample 5 – 5 mL of water; sample 6 – 6 mL of water. The samples investigated for the effect of the moisture factor were kept at room temperature (25 °C) in a laboratory under natural daylight.

Light test

To analyze the effect of the light factor on the chia seed germination, the test samples were placed as follows: sample 1' – in the absence of light (in a dark place); sample 2' – under natural daylight; sample 3' – under artificial light at a distance of 30 cm from the lamp.

An LED luminaire with a specific wavelength was used to study sample 3'. The line emission spectrum of the installed LED lamps was the maximum value at 440 nm in the blue region of the spectrum and 660 nm in the red region. The test was carried out with the best previously detected values of moisture regime and at room temperature (25 °C).

Temperature test

A study to determine the optimal temperature for chia seed germination was carried out with given temperatures: sample 1' – 20 °C, sample 2' – 25 °C, sample 3' – 30 °C with optimum water mass fraction and values of light exposure detected in previous stages.

Evaluation methods

The arithmetic mean value (average) of the results in the above experiments for four replicates was taken as the analysis result.

The speed of germination and the seedling vigor were determined.

The speed of germination characterizes the weighted average number of days for one seed to grow. This indicator (in days) was calculated by the formula (1):

$$\text{The speed of germination} = \frac{(A1 \cdot 1) + (A2 \cdot 2) + \dots + (An \cdot n)}{(A1 + A2 + \dots + An)} \quad (1)$$

where A(n) is the number of seeds germinated in 1, 2, ... n day of germination; 1, 2, ... n is the day of seeds germination.

The seedling vigor determines the average number of seeds germinated in one day. This indicator (in grains) was calculated by the formula (2):

$$\text{The seedling vigor} = \frac{A}{N} \quad (2)$$

where A is the number of germinated seeds (in terms of 100 seeds) for the entire period of experiment (7th day) or full germination; N is the number of days of seeds germination (7 days).

Statistical analysis

The experiments were performed in quadruplicate. The data were processed by the method of mathematical statistics with using MS Excel. The value of the results is represented by the average and the standard deviation obtained using Student's criterion under the condition of 0.95 confidence intervals.

RESULTS AND DISCUSSION

In this study, for the first time, a comprehensive assessment of the influence of some of the major external factors on the process of obtaining chia seedlings (sprouts) was carried out. The optimal regimes for the production of *Salvia hispanica* L. sprouts were revealed.

The following parameters were studied as input factors of the experiment: the effect of the added water amount, the effect of light, and the effect of temperature on the process of chia seed sprouting.

At the first stage of the study, the influence of the moisture factor was evaluated. Samples under the numbers corresponding to the amount of water added, mL, were used as the test samples.

Table 1 presents the results of counting chia seeds within 7 days. According to the data obtained, all samples began to germinate on day 3. The amount of introduced moisture practically did not affect the seed germination energy of chia at the initial stage. However, the dependence on the specified factor was noted during subsequent observation, in particular, from 4 to 7 day of the experiment.

Samples 1 and 2 dried up on the fourth day due to insufficient volume of applied water, providing on the third day of observation 40 and 42% of germinated seeds, respectively. This volume, like the volume of 2 mL of water, was insufficient for the germination of 100 chia seeds. Further growth dynamics of chia seeds in these samples was not observed.

Table 1. The dynamics of chia seed germination depending on the moisture regime, %

Sample No.	Day 1a	Day 2a	Day 3a	Day 4a	Day 5a	Day 6a	Day 7a
1	0.0	0.0	40.0 ± 1.6	40.0 ± 1.6	40.0 ± 1.6	40.0 ± 1.6	40.0 ± 1.6
2	0.0	0.0	42.0 ± 1.3	42.0 ± 1.3	42.0 ± 1.3	42.0 ± 1.3	42.0 ± 1.3
3	0.0	0.0	42.0 ± 2.1	71.0 ± 1.9	85.0 ± 0.8	88.0 ± 0.6	90.0 ± 1.2
4	0.0	0.0	44.0 ± 1.4	78.0 ± 0.5	86.0 ± 1.3	88.0 ± 2.1	92.0 ± 1.0
5	0.0	0.0	40.0 ± 2.8	78.0 ± 0.8	85.0 ± 1.3	86.0 ± 1.8	90.0 ± 0.8
6	0.0	0.0	39.0 ± 0.8	70.0 ± 1.4	73.0 ± 1.4	72.0 ± 1.3	73.0 ± 1.4

^aAverage ± standart deviation ($n = 4$).

In sample 4 on the third day of the experiment, it was 44% germinated seeds (the seed germination energy), by the end of the experiment, it was 92% (the germination of seeds). Samples 3 and 5 showed similar dynamics. In sample 6, there was an excess of introduced moisture, which negatively affected the process of seed germination and, by the end of the experiment, this set showed the least amount of germinated seeds in comparison with samples 3, 4 and 5.

Fig. 1 shows the results in the most representative samples with the addition of 1, 4, 6 mL of water, which confirms the above-described results of the study.

**Figure 1.** The dynamics of chia seed germination depending on the moisture regime (from left to right 1, 4, 6 mL, respectively) on the 7th day of observation.

The seed germination energy, the germination of seeds, the speed of germination and the seedling vigor were calculated for the most viable samples of the experiment and are shown in Table 2.

Table 2. The influence of the moisture regime on the ability of chia seeds to germinate for samples 3, 4 and 5

Indicators	Sample 3 ^a	Sample 4 ^a	Sample 5 ^a
The speed of germination, days	4.0 ± 0.1	5.2 ± 0.1	3.8 ± 0.1
The seedling vigor, grains	12.9 ± 0.2	13.1 ± 0.1	12.9 ± 0.1
The seed germination energy, %	42.0 ± 2.1	44.0 ± 1.4	40.0 ± 2.8
The germination of seeds, %	90.0 ± 1.2	92.0 ± 1.0	90.0 ± 0.8

^aAverage ± standart deviation ($n = 4$).

Thus, in terms of the seed germination energy, the germination of seeds, the speed of germination and the seedling vigor, the optimal values were obtained in sample 4. It was noted that insufficiency or excess moisture had a negative effect on the chia seed germination, but does not affect the seed germination energy, which on an average for chia seeds was about 40%.

At the second stage of the experiment, the effect of light on the chia seed germination was determined. The effect of the intensity and spectral composition of light on the efficiency of photosynthesis and productivity of various plants was studied by Protasova, 1987; Protasova et al., 1990. According to the research, it was found that the most favorable condition for growing light-loving plants were light intensities within 150–220 W m⁻². The optimal composition of radiation had the following ratio of energy over the spectrum: 30% in the blue region (380–490 nm), 20% in the green (490–590 nm) and 50% in the red region (600–700 nm). In general, light of the visible radiation spectrum or photosynthetically active radiation 390–710 nm has a favorable effect on the development of the plants, in particular, in the processes of chlorophyll formation, cell division and stretching, plant growth, etc. (Massa et al., 2008). The blue spectrum with a wavelength in the range of 430–470 nm contributes to the synthesis of chlorophyll, inhibits the growth of the stem, and red with a wavelength in the range of 640–660 nm promotes flowering and growth of the stems (Massa et al., 2008; Olle & Viršile, 2013). There is also evidence that the optical spectra of the red and blue regions with wavelengths of 640–660 nm and 430–460 nm, respectively, are most effective for plant growth, in particular, the 660 + 450 nm mode (Kondratyeva et al., 2018).

Table 3 presents the results of counting chia seeds within 7 days. As at the previous stage of the study, all samples began to germinate on day 3. Obviously, light exposure did not significantly affect the number of germinated seeds, which correlates with previous data (Stefanello et al., 2015b; Paiva et al., 2016).

Table 3. The dynamics of chia seed germination depending on the light, %

Sample No.	Day 1 ^a	Day 2 ^a	Day 3 ^a	Day 4 ^a	Day 5 ^a	Day 6 ^a	Day 7 ^a
1'	0.0	0.0	46.0 ± 0.8	73.0 ± 1.6	83.0 ± 1.4	86.0 ± 1.3	91.0 ± 1.3
2'	0.0	0.0	46.0 ± 1.7	72.0 ± 1.3	83.0 ± 1.0	85.0 ± 0.5	89.0 ± 0.5
3'	0.0	0.0	47.0 ± 2.6	71.0 ± 0.5	85.0 ± 1.3	88.0 ± 0.5	91.0 ± 0.5

^aAverage ± standart deviation ($n = 4$).

However, the study results showed that the line emission spectrum of the LED lamps with peaks at 440 nm in the blue region and 660 nm in the red region of the spectrum was optimal for the process of chia seed germination.

The seed germination energy, the germination of seeds, the speed of germination and the seedling vigor were calculated by samples of the experiment and are shown in Table 4.

Table 4. The influence of the light factor on the ability of chia seeds to germinate

Indicators	Sample 1 ^a	Sample 2 ^a	Sample 3 ^a
The speed of germination, days	5.2 ± 0.2	5.2 ± 0.2	5.3 ± 0.2
The seedling vigor, grains	13.0 ± 0.2	12.7 ± 0.1	13.0 ± 0.1
The seed germination energy, %	46.0 ± 0.8	46.0 ± 1.7	47.0 ± 2.6
The germination of seeds, %	91.0 ± 1.3	89.0 ± 0.5	90.0 ± 0.5

^aAverage ± standart deviation ($n = 4$).

According to the above indicators, it could not be defined the leading sample, since all samples had relatively identical values.

Fig. 2 shows the dynamics of chia seeds germination in the samples on the 7th day of the experiment.



Figure 2. The dynamics of chia seed germination depending on the light factor (sample 1', 2', 3' from left to right, respectively) on the 7th day of observation.

Fig. 2 demonstrates the formation of various pigments in the studied samples. The germination of chia seeds in the dark ensures the formation of pale yellow etiolated cotyledons, with an elongated stem and undeveloped leaves, which indicates that chlorophyll synthesis was absent in this sample.

The germination of chia seeds in natural daylight allowed obtaining sprouts of a pale green color, which also indicated an insufficient synthesis of chlorophyll in the sample.

Only the impact of the LED lamp ensured the growth of saturated green color due to the formation of a sufficient amount of chlorophyll in the chia seeds sprouts.

Thus, the optimal conditions for the germination of chia seeds were detected with light exposure with specific wavelengths, in particular 440 nm in the blue region and 660 nm in the red region of the spectrum, since the greatest synthesis of chlorophyll was observed (green, not elongated stem and opened leaves).

The presented data confirmed the feasibility of using LED lamps to obtain a qualitative composition of chia seedlings. Another advantage of LED lamps was low heat generation, so they could be placed in the immediate vicinity of the plants without the risk of damaging them, which was used in this experiment.

At the third stage, the effect of temperature on the process of obtaining chia seeds sprouts was studied by varying the temperature factor within 20–30 °C with a step of 5 °C.

Table 5 presents the results of counting chia seeds within 7 days. According to the data obtained, all samples began to germinate on day 3, which did not contradict the studies at the previous stages. Sample 3'' dried on the day 5 of the experiment due to the high temperature and insufficient moisture.

Table 5. The dynamics of chia seed germination depending on the temperature, %

Sample No.	Day 1 ^a	Day 2 ^a	Day 3 ^a	Day 4 ^a	Day 5 ^a	Day 6 ^a	Day 7 ^a
1''	0.0	0.0	42.0 ± 0.8	75.0 ± 1.7	83.0 ± 2.5	86.0 ± 1.3	88.0 ± 0.8
2''	0.0	0.0	45.0 ± 1.3	80.0 ± 1.0	86.0 ± 1.5	92.0 ± 1.7	92.0 ± 0.6
3''	0.0	0.0	35.0 ± 1.0	61.0 ± 1.7	68.0 ± 0.8	68.0 ± 0.6	68.0 ± 0.5

^aAverage ± standart deviation ($n = 4$).

The seed germination energy, the germination of seeds, the speed of germination and the seedling vigor were calculated by samples of the experiment and are shown in Table 6.

Table 6. The influence of the temperature factor on the chia seeds ability to germinate

Indicators	Sample 1'' ^a	Sample 2'' ^a	Sample 3'' ^a
The speed of germination, days	5.2 ± 0.1	5.3 ± 0.1	5.2 ± 0.1
The seedling vigor, grains	12.6 ± 0.1	13.1 ± 0.1	9.7 ± 0.1
The seed germination energy, %	42.0 ± 0.8	45.0 ± 1.3	35.0 ± 1.0
The germination of seeds, %	88.0 ± 0.8	92.0 ± 0.6	75.0 ± 0.5

^aAverage ± standart deviation ($n = 4$).

Fig. 3 shows the dynamics of chia seeds germination depending on the temperature factor on the 7th day of the experiment.



Figure 3. The dynamics of chia seed germination depending on the temperature (from left to right 20 °C, 25 °C, 30 °C, respectively) on the 7th day of observation.

Therefore, the temperature of 25 °C was determined as the optimum for chia seed germination based on the results of the seed germination energy, the germination of seeds, the speed of germination and the seedling vigor. Previous studies confirm (Stefanello et al., 2015b; Paiva et al., 2016; Possenti et al., 2016; Gómez-Favela et al., 2017) that the influence of temperature factor was an important indicator in relation to chia seed germination. In comparative experiments, it was shown that the germination of chia seeds with low temperature (below 20 °C) and high temperature (above 30 °C) limits plant growth. The optimum range for chia seed germination is 20–25 °C, which correlates with results of this research.

Thus, according to the results of the experiment, the necessary conditions for the chia seed germination were identified for the first time; in particular, the moisture content was 4 mL of water per 100 seeds at a temperature of 25 °C with light exposure at a wavelength of 440 nm and 660 nm. These technological characteristics are recommended for utilization in obtaining chia seedlings using the soil and the hydroponic method of chia seed germination.

CONCLUSIONS

In the framework of this study, the simultaneous influence of moisture, temperature and light factors on the germination of chia seeds (*Salvia hispanica* L.) was studied for the first time. The optimal amount of water was 4 mL per 100 seeds, based on the seed

germination energy, the germination of seeds, the speed of germination and the seedling vigor. An insufficient or excessive amount of water had a negative effect on the germination of chia seeds.

It was revealed that the chia seed germination with an LED lamp with peaks at 440 nm and 660 nm promoted the accumulation of sprouts green mass and the synthesis of chlorophyll in them. The optimum temperature for germinating chia seedlings under given conditions was defined as 25 °C.

These conditions could be used for year-round cultivation of chia seedlings by the hydroponic method. However, in the further study, the established parameters would require clarification in particular the optimum experiment conditions, taking into account the realization of its multifactorial nature under hydroponic conditions.

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