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Comparative Evaluation of Chia (*Salvia hispanica* L.) Plants Grown under in Vitro Culture and Natural Terrain Conditions

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Chia seeds were grown under different environmental conditions: Greenhouse Conditions (GC) and Natural Terrain Conditions (NTC). The physiological characteristics of the plants were determined by content analysis of M1 generation seeds obtained by the plant tissue culture method, including mineral matter, oil, antioxidant content, total phenolic content, and phytochemical profile. Statistical analyses revealed a significant difference in germination rate and length of the seeds. In vitro culture of cells, tissues, organs, and components defined as sterile by others under controlled environmental conditions (temperature, humidity, and lighting). The seeds were germinated in vitro in Murashige and Skoog basal medium (MS) containing 3% sucrose, 7% agar, 0.4 mg l^{-1} gibberellic acid (GA_3), and 10 mg l^{-1} ascorbic acid (AA). 16 hours of light and 8 hours of darkness. In the rooting experiment, different concentrations of IBA (0, 1, 2) mg L^{-1} were used, and measurements were taken after one month.

Keywords: *In Vitro* Culture, Natural Terrain Conditions, Chia (*Salvia hispanica* L.)

INTRODUCTION

Chia (*Salvia hispanica* L.), a member of the Lamiaceae family, is an annual herbaceous plant, the most commonly used plant in this family. (Ghafoor et al. 2018, Pelaez et al. 2019, Arnak, 2020). The most widely cultivated variety of *S. hispanica* worldwide is known as Spotted (Pelaez et al. 2019). Chia is derived from the Spanish words chian and chien, meaning oily (Yurt & Gezer, 2018). Its origins lie in Guatemala and Mexico (Güler Çelik, 2017, Coşkun & Bahar, 2020). *Salvia hispanica* L. is an annual herbaceous plant belonging to the Lamiaceae family, originating from southern Mexico and northern Guatemala. The genus Salvia consists of approximately 900 species in the Lamiaceae family (Lu & Foo, 2002). This genus; Numerous chemical studies have been conducted describing the isolation of diterpenoids, tanshinones, and polyphenols from different parts of plants (Dorman et al. 2003). *Salvia hispanica* L. is commonly known as "Chia" (Ayerza 1995). The chia plant has purple flowers and small black-and-white seeds (Ali et al. 2012). Chia is a fast-germinating plant with slow growth and development. Germination takes 3-7 days, and the development process is completed approximately 9-10 months after germination. Sowing takes place in February-March, and harvesting occurs in November-December. Although the chia plant does not have a natural distribution area in our country, its cultivation is also difficult here. Seed maturation occurs in late autumn and early winter, and it is greatly affected by changing weather conditions. Chia is a plant that grows naturally in tropical and subtropical climate zones and is cultivated in regions with these types of climate characteristics (Orozco et al. 2014). Some morphological characteristics, such as plant height, leaf size, and number of branches and leaves per plant, were affected by the propagation method. No differences were found in free radical scavenging activity, flavonoid, and lipid composition among extracts of plants developed in vitro, ex vitro, and in vivo. This is the first comparative study of micropropagated and in vivo seed-derived chia plants. The study also presents, for the first time, data on the antioxidant potential and lipid components of *S. hispanica* leaves. It is commercially cultivated in Argentina, Colombia, Ecuador, Peru, Bolivia, Paraguay, and Australia (Busilacchi et al. 2013). The chia plant is widespread in South America and is commercially cultivated there (Ayerza & Coates, 2001). Because chia seeds mature in late autumn, cultivation is difficult in our country. Since chia seeds ripen in late autumn, cultivating them in our country is difficult. For this reason, the plant tissue culture method has been used to propagate and produce the plant. This study aims to compare

comparison between tissue culture-derived plants and those grown under conventional field conditions. the physiological, morphological, and anatomical characteristics of Chia plants grown under different environmental conditions using the plant tissue culture technique, greenhouse (GC), and M1 generations grown under normal conditions. (NTC).

MATERIALS AND METHODS

Plant Material

Chia (*Salvia hispanica* L.) seeds of Bolivian origin, obtained from Genartek Biotechnology Company, were grown in greenhouse conditions. Chia seeds were sown in March and completed their plant development in 10 months. M1 seed generation was obtained from greenhouse-grown plants. Using chia seeds of Bolivian origin, tissue culture has made in the Pamukkale University greenhouse. Seeds, basal medium (MS) 3% sucrose, 7% agar with 0,4 mg l^{-1} gibberellic acid (GA_3), and 10 mg l^{-1} were grown in vitro in the medium supplemented with ascorbic acid (AA). About 4 weeks after germination, the flowerpot was transferred in greenhouse (Fig. 1).

Figure 1: Germination of chia (*Salvia hispanica l.*) seeds on MS medium.

Chia is a slow-growing plant that germinates quickly. The chia plant has completed its development in about ten months (Fig. 2,3). Chia seed germination was within one week. The chia seedling completed its development process in about eleven months.

Figure 2: Transplanting and adapting chia (*Salvia hispanica l.*) seedlings into pots.

Figure 3: Flowers and seeds of the chia (*Salvia hispanica l.*) plant.

Figure 4: Seeds obtained from chia (*Salvia hispanica l.*) plants grown in natural terrain conditions (a) and greenhouse (b)

Statistical Calculations

Statistical calculations were performed using the Mann-Whitney U test in the IBM SPSS Statistics 23 program to correctly interpret the data.

RESULTS AND DISCUSSION

Chia seeds were germinated in tissue culture medium and then germinated in a greenhouse and in the natural field. Different growing conditions affected some physiological states of the chia seeds (Table 1).

The germination rate of chia seeds varied greatly in different environments. While 50% of the seeds sown in natural field conditions germinated, 95% of the seeds sown in the greenhouse germinated (Fig. 4). The ecological environment greatly affected the physiology of the chia plant's height. Chia plants grown in the greenhouse varied between 1.50-2 m, while chia plants grown in the natural field varied between 0.8-1.5 m, with an average height of 1.40 m (Fig. 5 a-b). Morphological differences are observed in the leaves of chia plants grown under different environmental conditions. Chia plants grown in greenhouses have large leaves with thick leaf blades. Chia plants grown in natural field conditions, on the other hand, have small leaves with thick leaf blades (Fig. 6 a-b).

Table 1: Morphological and anatomical characteristics of chia (*Salvia hispanica* L.) plants grown under different conditions.

Chia (*Salvia hispanica* L.)	Natural Terrain Conditions	Greenhouse Conditions
Germination time (day)	7 day	3-5day
Germination rate (%)	50	95
Plant height (average) (m)	1,50	1,95
Leaf	Laminate is thick and large	Laminate is thick and small
Flowering	+	+
Seed yield	+	+

*NTC: Natural Terrain Conditions, GC: Greenhouse Conditions

[A] [B]

Figure 5 a-b: Flowering and seed production morphologies of chia plants grown under greenhouse and natural field conditions.

[A] [B]

Figure 6 (A and B): Flowering status of chia plants grown in greenhouse and natural field conditions.

All these and similar studies conducted have influenced the determination of our study topic, based on the idea that the production and consumption of the Chia plant can be widespread in our country. Chia (*Salvia hispanica* L.) seeds, which have a rich nutritional content, were germinated and grown under different environmental conditions, and new generation seeds were obtained. The extracts of the obtained new generation seeds were analyzed, and their phenolic, antioxidant, mineral, and omega fatty acid content were tested. In one study, medicinal chia plants were propagated using plant tissue culture techniques. It was observed through the obtained results that there were significant differences between the different concentrations of growth regulators used in the experiment in the multiplication and rooting of the branches (AL-Alwani and Mohammed, 2023) Chia plants grown under various environmental conditions were compared with the type grown in another environment in terms of germination rate and duration, physiological characteristics of the plant, and development status. Morphological, anatomical, and physiological differences were determined. The effect of chia gel, a sports drink containing chia seeds (*Salvia hispanica* L.), on the VO2max capacity of football and futsal players was investigated (Mardiana et al. 2022). According to a study, a tissue culture technique was developed for the micropropagation of Chia (*Salvia hispanica* L.) plants. After a one-week culture, a high seed germination rate (100%) was recorded in Murashige and Skoog medium enriched with 0.4 mg l^{-1} gibberellic acid and 10 mg l^{-1} ascorbic acid. After a five-week culture, the maximum number of shoots per explant was obtained in MS medium containing 2 mg l^{-1} 6-benzylaminopurine (Salih 2025). The best plant rooting has achieved after a four-week culture in semi-strength MS medium containing 0.1 mg l^{-1} indole-3-butyric acid. Multiple plants were successfully adapted ex vitro with a 95% survival rate. After six months, the key morphometric characteristics of the plants were evaluated ex vitro and in vivo under field conditions.

Germination occurs in a short period of 3-5 days under greenhouse conditions, while in natural field conditions (NTC), this period extends to approximately 7 days. This difference can be explained by the more balanced and optimal temperature, humidity, and light conditions in the greenhouse environment.

Furthermore, the germination rate reaches a very high value of 95% under greenhouse conditions, while it is around 50% under natural conditions. This finding emphasizes the positive effect of controlled environmental conditions on seed viability and emergence success.

Plant Height

Plant height was determined to be an average of 1.95 m in the greenhouse environment and 1.50 m under natural conditions. In other words, greenhouse conditions provide approximately a 30% increase in plant height. This increase in plant height in controlled environments may be related to optimal temperature, regular irrigation, and sufficient nutrient uptake.

Leaf Characteristics

In both growing conditions, the leaf laminae are thick; however, leaves were described as smaller in the greenhouse environment and larger under natural conditions. This situation may be an indicator of the plant's light adaptation depending on different light intensities and relative humidity values. While leaf width increases to increase the photosynthetic surface area under high light intensity in natural conditions, compact leaf development may have been observed under more balanced light in greenhouse conditions.

Flowering and Seed Yield

Flowering and seed yield were indicated as (+) in both conditions. This shows that the chia plant can adapt to both natural and controlled conditions, i.e., it has a wide adaptation ability in terms of reproduction. However, considering the differences in germination and plant development, it can be said that greenhouse conditions offer a higher and more efficient production potential.

CONCLUSIONS

Chia seeds were grown under different environmental conditions, namely Greenhouse Conditions (GC) and Natural Field Conditions (NTC). The physiological characteristics of the plants, the bioactive content of M1 generation seeds obtained through plant tissue culture, and their phytochemical profile were analyzed. Statistical analyses revealed a significant difference in germination rate and seed length. In vitro culture of cells, tissues,

organs, and components was determined under specific chemical and physical conditions, within controlled environmental conditions (temperature, humidity, and lighting). The results obtained in the study show that chia plants grown under greenhouse conditions are more advantageous in terms of morphology. In particular, significant increases were observed in germination success, plant height and overall growth rate. Under natural conditions, the plants germinate later and their development is slower. These differences reveal that optimum environmental control (temperature, humidity, irrigation, light) is critical for tropical plants such as chia. It can be concluded that chia plants grown under suitable greenhouse conditions have the potential for high yield and quality seed production.

Supplementary materials
Not applicable

Author contributions
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All data are included within the article and in the hands of the first author if needed

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Conflict of interest
The authors declared that the present study was performed in the absence of any conflict of interest.

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