

Original Research Article

Seed Germination of the Herbaceous Vine *Tropaeolum pentaphyllum* Lam. (Tropaeolaceae): A Neglected Geophyte with High Agricultural Potential

ABSTRACT

The objective of this study was to evaluate the seed germination of *Tropaeolum pentaphyllum* Lam., an endangered geophyte native of southern Brazil with agriculture potential due its edible tubers. In 2017, two experiments were carried out in natural and controlled conditions. In both experiments, germination was evaluated for two years. In the first experiment, it was evaluated the germination of 1,100 seeds, plant height, mass, and biometry of tubers. In the second experiment, it was evaluated the germination of 100 seeds. Two treatments (natural light and dark) were evaluated and each treatment contained 50 seeds. In the first experiment, in 2018 (first year), of the 1,100 seeds, 5.6% germinated and 76.5% persisted in the soil; and in 2019 (second year), 5.3% seeds germinated and no seeds remained in the soil. In natural conditions, 10.9% of seeds germinated over two years. In controlled conditions, in 2018 the dark germination was higher (48%) compared to natural light (18%); and in 2019, despite the absence of statistical differences, the dark showed a higher value of germination (12%) in relation to natural light (6%). Over two years, dark treatment showed higher germination (60%) compared to natural light (24%) and no seeds remained in the substrate. The production of tubers in plants obtained by seed germination was 98.4% and 100%, in natural and controlled conditions, respectively. The highest germination rates occurred under dark conditions indicating that the species is preferential negative photoblastic. In addition, this species forms a seed bank in the soil, in which the seeds remained for a maximum of two years. The seed germination can contribute to the genetic diversity of crops and the production of seed-tubers, decreasing the collection of tubers in situ, contributing to the conservation and agricultural use of *T. pentaphyllum*.

Keywords: edible tuber; preferential negative photoblastic; seed bank.

1. INTRODUCTION

The Tropaeolaceae family is neotropical and most species have occurrence in South America [1, 2]. This family presents annual or perennial climbing or prostrate herbs that contain rhizomes and tubers with an odor of mustard oils [1]. Tropaeolaceae occurs mainly in higher altitude areas, including two genera, *Mallagana* and *Tropaeolum*, with approximately 100 species [2, 3]. In Brazil, there are records of four native species of the genus *Tropaeolum*: *T. pentaphyllum* Lam., *T. peregrinum* L., *T. sanctae-catharinae* Sparre, and *T. warmingianum* Rohrb, distributed in the South and Southeast regions [4].

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Tropaeolum pentaphyllum Lam., in Brazil popularly known as “crem”, is an herbaceous vine species. This species is native of South America, specifically Uruguay, Northern Argentina and Southern Brazil [3, 5]. It is considered selective hygrophyte and heliophile, typical of forest edges and open areas [5]. *T. pentaphyllum* is used as an ornamental, food and medicinal plant [6]. Tuber is the more consumed part of the plant, usually as a side dish with soups and meats [7]. In addition, leaves and flowers can be also consumed [5, 8].

This species was listed as one of the plants with potential for economic use by the project “Plants for the Future – Southern Brazil” [5]. At the same time, *T. pentaphyllum* is endangered due habitat degradation and over exploitation of edible tubers by local farms [5, 9, 10]. Despite the use potential of *T. pentaphyllum*, it remains remarkably understudied [11]. Cultivation of *T. pentaphyllum* offers an opportunity for establish a sustainable commercial source of tubers and reducing the harvesting pressure in natural population.

T. pentaphyllum is a geophyte plant, in which the aerial part has an annual duration (on average 17.5 ± 4.7 weeks) and the tubers are perennial, allowing subsequent sprouting [10]. In vine species, the formation of tubers gives the ability to grow very quickly after the period in which the aerial part was inactive [12]. The propagation of this species is through of planting of “seed-tubers”, which have no standard size [13]. Another form of production of seed tubers is by stem cuttings [14]. It is also possible to propagate this species through seeds, but there are difficulties in germination [5]. Spontaneous germinations were observed in shaded places [13]. Seed dormancy, germination, and other important steps for recommendations for sexual propagation need to be studied. There are no studies in the literature involving the germination of *T. pentaphyllum*, therefore the percentage of germination of this species is unknown.

The success of the seed germination and the establishment of a normal seedling are determining features for the propagation of plant species, which are of both economic and ecologic importance. Because of its high vulnerability to injury, disease, and water/environmental stress, germination is considered to be the most critical phase in the plant life cycle [15]. Germination is a complex process during which the seed must quickly recover physically from maturation drying, resume a sustained intensity of metabolism, complete essential cellular events to allow for the embryo to emerge, and prepare for subsequent seedling growth [16]. Germination from a seed bank has the potential to be delayed so that seedling emergence coincides with a time when conditions are best for seedling establishment, and recruitment is most needed for re-establishing populations [17].

The objective of this study was to evaluate the germination and the tuber production of plants of *T. pentaphyllum* originated by seeds, seekinglooking for conservation and expansion of the cultivation of this species.

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2. MATERIAL AND METHODS

2.1 Study area

Two experiments were carried out at Instituto Federal do Rio Grande do Sul – *Campus Sertão* (28°02'42"S, 52°16'17"W and altitude of 737 m), located in the Sertão municipality, Rio Grande do Sul State (RS), Brazil. According to the Köeppen climate classification, the area has a humid subtropical climate (Cfa) presenting a well-distributed rainfall throughout the year, an annual average of 1,803.1 mm, an annual average temperature of 17.7 °C, and a photoperiod that ranges from 10.2 to 13.8 with average of 12 hours per day of sunlight during the year [18]. The region soil is classified as dystrophic red nitosol [19]. The local altitude is 735 m.

2.2 Natural Conditions Experiment

For evaluating the seed germination in natural conditions, on October 10, 2017, 1,100 seeds were sown in the soil (deep of 5 centimeters and spacing 20 centimeters) at the edge of a fragment of Mixed Rainforest and were evaluated over a period of two years under natural conditions. In this case, were evaluated the percentage of emergence of plants, the plant development over time (height in centimeters), the biometry (length, width and thickness), and mass of the tubers obtained of plants originated by seeds. At the end of December 2018 and 2019, the number of remained seeds in the soil was evaluated.

2.3 Controlled Conditions Experiment

Another experiment was implemented on October 10, 2017. Initially, it was evaluated the biometry of 146 mericarps and seeds. Of these 146 seeds, 100 were determined randomly to compose the germination experiment, which was evaluated over a period of two years. The experiment was carried out in a greenhouse with controlled environmental conditions (temperature of 25°C and daily irrigation) and the seeds were sown in seedling trays with 128 cells, containing peat as substrate. At the end of December 2018 and 2019, the number of remained seeds in the substrate was evaluated.

Two treatments (natural light and dark) were conducted in a completely casualized design, at each treatment with 5 repetitions and 10 seeds per experimental unit, totalizing 50 seeds per treatment. Were evaluated: germination rate (%); number of days from seedling until senescence of aerial stem; plant dry matter, plant height; number of leaves per plant; and biometry of the tubers obtained from plants originated by seeds.

Measurements of mass (grams) and size (length, width and thickness, in millimeters) were performed using a precision scale and a digital caliper, respectively. The height of the plants over time was measured weekly using a measuring tape (centimeters).

2.4 Data Analysis

Data were analyzed by descriptive statistics (mean, amplitude, standard deviation, and confidence interval, with $\alpha = 0.05$) using Past 4.03 software; and by analysis of variance (ANOVA) followed by Tukey's Test (at 5% probability of error) using Sisvar 5.6 software.

3. RESULTS

3.1 Biometry of Mericarps and Seeds

The length of the mericarps ranged from 4.3 to 9.4 mm ($\bar{x} = 7.3 \pm 0.9$ mm), the width ranged from 3.3 to 7.1 mm ($\bar{x} = 5.5 \pm 0.8$ mm), the thickness ranged from 2.2 to 6.9 mm ($\bar{x} = 4.9 \pm 0.9$ mm), and the mass ranged from 0.02 to 0.28 g ($\bar{x} = 0.15 \pm 0.05$ g) (Table 1).

Table 1. Biometry (length, width and thickness) and mass of mericarps and seeds of *T. pentaphyllum*

	Mericarps			Seeds		
	Mean	Amplitude	SD ¹	Mean	Amplitude	SD ¹
Length (mm)	7.3	4.3-9.4	0.9	5.2	3.8-7.5	0.5
Width (mm)	5.5	3.3-7.1	0.8	3.8	2.2-6.6	0.5
Thickness (mm)	4.9	2.2-6.9	0.9	3.5	1.5-4.8	0.5

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Mass (g)	0.15	0.02-0.28	0.05	0.06	0.01-0.14	0.02
	¹ Standard variation					

The length of the seeds ranged from 3.8 to 7.5 mm ($\bar{x} = 5.2 \pm 0.5$ mm), the width ranged from 2.2 to 6.6 mm ($\bar{x} = 3.8 \pm 0.5$ mm), the thickness ranged from 1.5 to 4.8 mm ($\bar{x} = 3.5 \pm 0.5$ mm), and the mass ranged from 0.01 to 0.14 g ($\bar{x} = 0.06 \pm 0.02$ g) (Table 1).

3.2 Natural Conditions Experiment - Germination and Tuber Formation

In 2018, of the 1,100 seeds 5.6% germinated and 76.5% remained in soil. Seed germination started in late April, the month with the highest rate (74.6%), followed by May (10.9%) and was decreasing over time until it stopped in the beginning of August.

In 2019, seed germination started at the end of April (72.41%) and ended in May (27.6%) being that 5.3% seeds germinated. Over two years, the total number of germinated seeds was 10.9% and no seed remained in the soil (Table 2).

Table 2. Germination and persistency of seeds of *T. pentaphyllum* on substrate over two years after sowing in natural condition and controlled conditions (natural light and dark)

Treatments	Percentage (%)		
	2018	2019	Total
Natural conditions			
Germinated seeds	5.6	5.3	10.9
Persistent seeds	76.5	-	-
Controlled conditions			
Germinated seeds in the natural light	18.0 b ¹	6.0 a	24.0 b
Germinated seeds in the dark	48.0 a	12 a	60.0 a
Persistent seeds	13.0	-	-
CV ² (%)	13.5	55.5	9.2

¹Means followed by different letters in the same column differ by the Tukey's test at 5% probability. ²Coefficient of variation

The total length of the aerial stem varied from 6.6 to 144.8 cm, with an average of 59.7 ± 26.5 cm. The duration of the aerial stem, from emergence to senescence, was $177,1 \pm 3.8$ days, varying from 84 to 210 days.

The results demonstrated a positive and significant linear regression between the growth of aerial stem (height) of the plant over the weeks (Figure 1). The aerial stem senescence started in the second half of July, presenting the highest percentage of senescence in the second half of October (39.7%), extending until the middle of November. None plant showed flowering in the first life cycle (emergence to senescence aerial stem system).

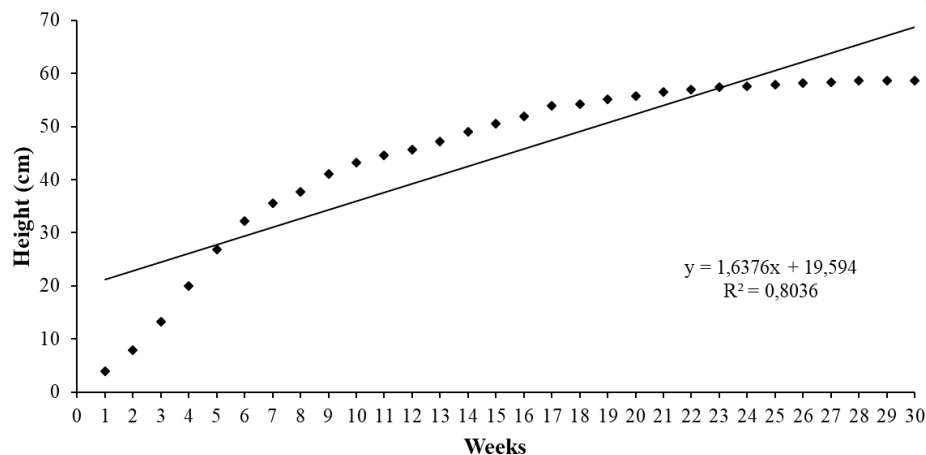


Figure 1. Height of *T. pentaphyllum* plants from seeds over time until senescence of the aerial stem in the natural conditions experiment

Of the total of 62 plants originated by seeds, 61 (98.4%) produced tubers, being a single tuber per plant. The length of the tubers ranged from 9.0 to 51.5 mm, with an average of 26.9 ± 8.0 mm; the width ranged from 4.6 to 28.7 mm, with an average of 17.4 ± 5.2 mm; and the thickness ranged from 4.2 to 21.6 mm, with an average of 15.7 ± 4.0 mm. Tuber mass ranged from 0.1 to 8.3 g, with an average of 3.7 ± 1.9 g (Table 3).

Table 3. Biometry (length, width and thickness) and mass of the tubers of *T. pentaphyllum* obtained from plants via seed germination in natural and controlled conditions experiments

Experiments	Mean	Amplitude	Standard deviation
Natural conditions			
Tuber length (mm)	26.9	9.0-51.5	8.0
Width (mm)	17.4	4.6-28.7	5.2
Thickness (mm)	15.7	4.2-21.6	4.0
Mass (g)	3.7	0.1-8.3	1.9
Controlled conditions			
Length (mm)	7.3	4.3-9.4	0.9
Width (mm)	5.5	3.3-7.1	0.8
Thickness (mm)	4.9	2.2-6.9	0.9
Mass (g)	0.15	0.02-0.28	0.05

3.3 Controlled Conditions Experiment - Germination and Tuber Formation

In 2018 in less than a year after the implementation of the experiment, the seeds in dark showed higher germination (48%) compared to natural light (18%). In 2019, although no statistical differences have been verified between the treatments, the dark showed higher germination (12%) compared to natural light (6%) (Table 2). Considering the total germination over two years, the seeds showed highest germination in dark (60%) than in natural light (24%), differing statistically between them.

The emergence of the first plant was observed in the third week of February 2018 (19 weeks after implanting experiment) and the last seed that germinated was registered in the third week of April 2019 (61 weeks after experiment implanting). Thus, some seeds germinated almost two years after the implementation of the experiment.

The germination period differed statistically between the treatments, being longer in natural light (274.8 days) when compared to dark (168.4 days) (Figure 2A). The average emergence time ranged from 145 to 528 days from sowing, with an average of 198.7 ± 97.9 (Table 2).

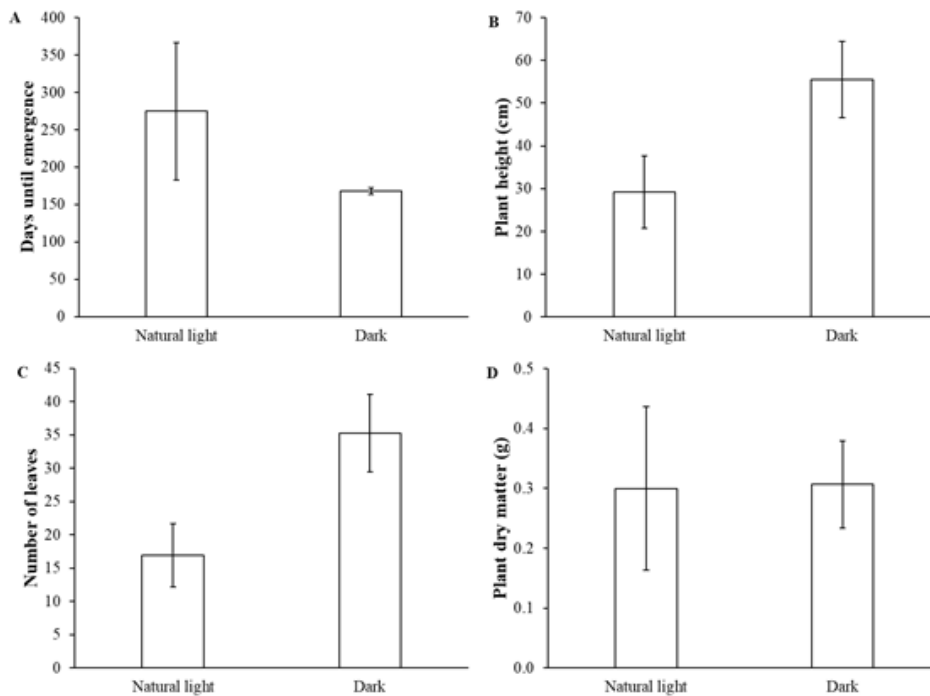


Figure 2. Days until emergence (A), plant height (B), number of leaves (C) and plant dry matter (D) of *T. pentaphyllum* plants obtained by seed germination in the controlled conditions experiment. Confidence interval ($\alpha = 0.05$)

The plant height differed statistically between treatments, being highest in dark treatment (55.5 cm) when compared to natural light germination (29.2 cm) (Figure 2B). The plant height ranged from 8.5 to 123.2 cm with an average of 48.0 ± 25.4 (Table 4).

Table 4. Days until emergence, plant dry matter (g), plant height (cm) and number of leaves of *T. pentaphyllum* plants obtained by seed germination in the controlled conditions experiment

	Mean	Amplitude	SD ¹
Days until emergence	198.7	145.0-528.0	97.9
Days plant duration	98.4	35.0-194.0	24.4
Plant height (cm)	48.0	8.5-123.2	25.4
Number of leaves	30.0	6.0-74.0	16.6

Plant dry matter (g)

0.30

0.08-0.97

0.21

¹Standard deviation

The average number of leaves was 30.0 ± 16.6 , ranged from 6 to 74 (Table 4). The number of leaves differed statistically between treatments, being highest in dark treatment (35.3 leaves) compared to natural light (16.9 leaves) (Figure 2C). None plant showed flowering in the first life cycle (emergence to senescence aerial stem system).

The aerial stem system plant dry matter did not differ statistically between treatments, being of 0.31 ± 0.1 g for dark and 0.30 ± 0.1 g for natural light (Figure 2D). The average dry matter per plant was 0.30 ± 0.21 g ranged from 0.08 to 0.97 g (Table 4).

The percentage of tuber formation was 100%, being a single tuber per plant. The dark and natural light treatments did not influence the biometric characters (length, width and thickness) and mass of the tubers obtained through seed germination (Figure 3). The length of the tubers ranged from 6.0 to 33.1 mm, with an average of 14.1 ± 5.0 mm; the width ranged from 3.6 to 13.1 mm, with an average of 8.9 ± 2.4 mm; and the thickness ranged from 3.4 to 12.9 mm, with an average of 8.3 ± 2.2 mm (Table 3). Tuber mass ranged from 0.04 to 1.2 g, with an average of 0.6 ± 0.2 g.

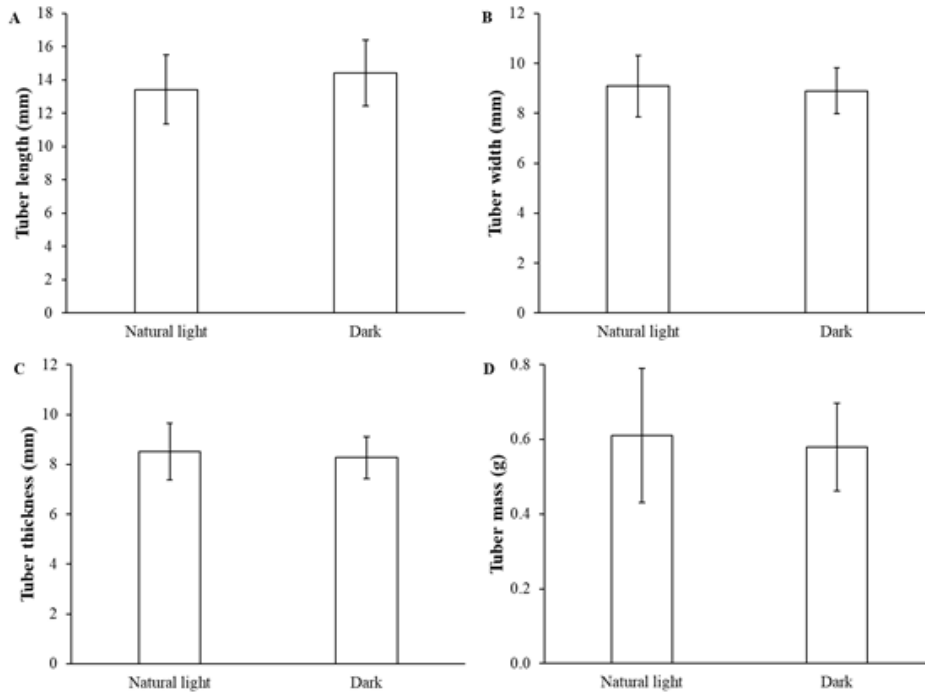


Figure 3. Tuber length (A), width (B), thickness (C) and mass (D) of *T. pentaphyllum* plants obtained by seed germination in the controlled conditions experiment. Confidence interval ($\alpha = 0.05$)

Significant correlations were found between: seed length and thickness ($r = 0.59$; $p < 0.01$); seed length and mass ($r = 0.32$; $p < 0.05$); seed length and the tuber thickness ($r = 0.31$; $p <$

0.05); seed width and thickness ($r = 0.45$; $p < 0.05$); seed thickness and days of plant duration ($r = -0.35$; $p < 0.05$); seed thickness and tuber width ($r = 0.36$; $p < 0.05$); seed thickness and tuber thickness ($r = 0.33$; $p < 0.05$); seed thickness and tuber mass ($r = 0.33$; $p < 0.05$); days until emergence and plant height ($r = -0.34$; $p < 0.05$); days until emergence and number of leaves ($r = -0.34$; $p < 0.05$); plant height and number of leaves per plant ($r = 0.80$; $p < 0.01$); tuber width and thickness ($r = 0.96$; $p < 0.01$); tuber width and mass ($r = 0.87$; $p < 0.01$); and tuber thickness and mass ($r = 0.84$; $p < 0.01$) (Table 5).

UNDER PEER REVIEW

Table 5. Simple linear correlation matrix between seed, tuber and plant parameters in the controlled conditions experiment

Characters	Seed length	Seed width	Seed thickness	Seed Mass	Days until emergence	Plant days duration	Plant dry matter	Plant height	Number of leaves	Tuber length	Tuber width	Tuber thickness
Seed width	0.20 ^{ns}											
Seed thickness	0.59**	0.45**										
Seed mass	0.32*	0.06 ^{ns}	0.07 ^{ns}									
Days until emergence	0.08 ^{ns}	0.00 ^{ns}	0.07 ^{ns}	-0.21 ^{ns}								
Plant days duration	-0.21 ^{ns}	-0.12 ^{ns}	-0.35*	0.16 ^{ns}	-0.12 ^{ns}							
Plant dry matter	-0.14 ^{ns}	-0.01 ^{ns}	0.00 ^{ns}	-0.25 ^{ns}	0.20 ^{ns}	-0.09 ^{ns}						
Plant height	0.03 ^{ns}	-0.15 ^{ns}	-0.19 ^{ns}	0.08 ^{ns}	-0.34*	0.07 ^{ns}	-0.14 ^{ns}					
Number of leaves	0.00 ^{ns}	-0.15 ^{ns}	-0.19 ^{ns}	0.06 ^{ns}	-0.34*	0.13 ^{ns}	-0.12 ^{ns}	0.80**				
Tuber length	-0.08 ^{ns}	0.07 ^{ns}	-0.17 ^{ns}	0.14 ^{ns}	0.00 ^{ns}	0.20 ^{ns}	-0.10 ^{ns}	-0.09 ^{ns}	0.04 ^{ns}			
Tuber width	0.20 ^{ns}	0.11 ^{ns}	0.36*	0.19 ^{ns}	0.09 ^{ns}	-0.12 ^{ns}	-0.10 ^{ns}	-0.05 ^{ns}	0.04 ^{ns}	0.03 ^{ns}		
Tuber thickness	0.31*	0.11 ^{ns}	0.33*	0.18 ^{ns}	0.12 ^{ns}	-0.07 ^{ns}	-0.11 ^{ns}	0.00 ^{ns}	0.10 ^{ns}	-0.01 ^{ns}	0.96**	
Tuber mass	0.14 ^{ns}	0.18 ^{ns}	0.33*	0.25 ^{ns}	0.12 ^{ns}	-0.02 ^{ns}	-0.12 ^{ns}	0.05 ^{ns}	0.00 ^{ns}	0.21 ^{ns}	0.87**	0.84**

* = significant at 5% probability; ** = significant at 1% probability; ns = non-significant at 5 % probability

4. DISCUSSION

The high remained of seeds in the soil/substrate for up to two years indicates that *T. pentaphyllum* forms seed bank but most of the germination is concentrated in the first year showing decreased viability with loss of several seeds.

Seed germination of *T. pentaphyllum* occurred mainly from April to August, which may indicate that the species require lower photoperiod and temperature for germination. Both factors also stimulate the tuber budding of *T. pentaphyllum* [10]. In congeners *T. polyphyllum* Hort. ex Loud. the cold was indicated as the main factor for overcoming seed dormancy [20] and in other species of the genus *Tropaeolum* [21]. In *T. argentinum* Buch., that also presented seeds with dormancy, a cool period following a period of warmth is necessary for germination [22].

Seed bank can be considered remained when the seeds remain viable in the soil for more than a year; and the time is determined by physiological (germination, dormancy and viability) and environmental factors (humidity, temperature, light, presence of seed predators and pathogens) [23]. The species *T. speciosum* also forms seed bank, in which the seeds remain viable for long periods. The longevity of the seed bank may be related to the complicated germination requirements, since the seed germination is difficult for this species [24].

The highest germination rate in dark related to natural light indicated that the *T. pentaphyllum* seeds may be preferential negative photoblastic. This means that the seeds require absence of light to germinate but also germinated in the presence of light [25]. Kinupp [13] had already reported the spontaneous germination of *T. pentaphyllum* seeds in shaded places.

Gibberellins (GAs) are very important for seed dormancy, responsible for shoot elongation, seed germination, and fruit and flower maturation. GAs break seed dormancy in exposure to cold or light and allow the seeds to start the process of germination [26].

The first determinant for breaking seed dormancy is the balance between the phytohormones abscisic acid and gibberellin (ABA: GA), since, while ABA inhibits germination, GAs exert a positive influence on seed germination. In this sense, environmental conditions that break dormancy act on genetic level, affecting the balance between responses to ABA and GA. Among the environmental conditions is light, where the phytochrome, responsible for the perception of red (R) and far-red (RF) wavelengths, is the first sensor to regulate germination modulated by light [27].

In this sense, research suggests the classification of seeds according to the forms of phytochrome, including the term photoblastism is replaced by forms of phytochrome that control germination, in some works. Negative photoblastic seeds, such as *T. pentaphyllum*, have phytochrome A (PhyA), and germination is controlled through a high irradiance response, or, when the level of Pfr (active form of phytochrome) is high enough to induce germination in the dark, it is controlled through low fluency responses, by phytochrome B (PhyB) [28].

Regarding the development of plants obtained from seeds, the results for the growth of *T. pentaphyllum* corroborate the classic sigmoidal curve presented by Magalhães [29]. In this curve it is possible to identify an initial phase of slow growth, where the plant is dependent on the seed reserves, after the exponential phase begins, where the leaf area index increases with growth, providing higher photosynthetic rates to the plant, and finally the

growth becomes constant and decreased, due to the lesser interception of light energy by the plant, until the process stopped. At this stage, translocation of photoassimilates to the reserve organs also takes place.

The germination rate of *T. pentaphyllum* was moderate (the maximum obtained in the experiments was 60%). However, it is important to emphasize that there are no studies in the literature involving *T. pentaphyllum* germination. Therefore, this study is the first that reported the germination of this species. In relation to other species of the genus *Tropaeolum*, in *T. tuberosum* (mashua) the germination was also low [30] but in *T. majus* (nasturtium) was of 70.9%, being considered high [31]. But it is necessary to consider that many factors are related to germination, the germination rate in *T. majus* was less than 50% in different substrates evaluated [32], while in nasturtium up to 90% of germination [33].

The results obtained in the simple linear correlation matrix allows to infer that the longer the period of days until emergence, the lower the plant development (height of plants and number of leaves) what may could be related to the short annual cycle of *T. pentaphyllum*, with peak of senescence in October [10]. Therefore, the delay in germination decreases the life cycle of plants, resulting, consequently, in lesser development of the aerial stem system and tubers.

Under natural conditions the germination of *T. pentaphyllum* seeds was low, possibly due to seed predation. In natural environments, non-synchronized germination can reduce the chances of predation and mortality. However, for crops, irregular germination is not interesting.

In this sense, further studies must be carried out in order to increase *T. pentaphyllum* germination rates, as the species may possibly have some type of dormancy associated with the seed. Seed dormancy in *T. pentaphyllum* may be related to the impermeability of the coat, to the immature or rudimentary embryo, or to the presence of inhibitory substances, or even to combinations of these factors.

Although *T. pentaphyllum* has germination rate low in natural conditions and moderate in controlled conditions, practically all plants (98.4 – 100%) originated by seed produced tubers. Thus, germination can be used aiming at the propagation of the species (production of seed-tubers), as well as to expand the genetic diversity in crops of *T. pentaphyllum*. Another propagation form that can be used to produce seed-tubers is the cutting of aerial stem with tuber formation of most 45% [14].

The tubers formed for plants obtained by seed germination in this study shows better biometrics characteristics (mass = 3,7 g; length = 26,9 mm; width = 17,4 mm and thickness = 15,7 mm) than the tubers formed by stem cutting (mass = 0,59 g; length = 10,78 mm; width = 9,47 mm and thickness = 15,7 mm) [14].

In this species small tubers are preferred for planting, which may in fact be a poor agricultural practice. By using small tubers for seed, farmers may unconsciously select less vigorous types. Seed germination can be important to maintain and/or increase the levels of genetic diversity of *T. pentaphyllum* in crops for consumption and/or commercialization, since it is traditionally done exclusively in a clonal way (planting seed-tubers). In addition, combining germination and stem cuttings for the production of seed-tubers can contribute to the conservation of *T. pentaphyllum*, as it would can decrease the tuber collection pressure *in situ*.

Compared to propagation by stem cuttings, seed germination is much more efficient from the standpoint of tuber formation, since in propagation by stem cuttings of *T. pentaphyllum*, tuber formation is only 45% [14]. In addition, seed germination contributes to the genetic diversity *in situ* and in the crops.

The non-production of flowers and fruits in the first life cycle of plants produced by seeds is possibly due to the great investment in producing of the tuber. Flowering and tuberization are distinct reproduction strategies, both of which involve the sensing of the photoperiod and generation of a signal in the leaves (a process referred to as induction), the subsequent transport of the signal (known as florigen or tuberigen), and the response in a distant organ, the vegetative meristem, or stolon tips (also called evocation) [34].

Furthermore, in perennial plants the first flowering occurs only after the accumulation of excess reserves [35]. This extra reserve allocation is an important strategic for that flowering may occur in the next cycle, in case there is a shortage of reserves, or adverse edaphoclimatic conditions. Thus, considering that the *T. pentaphyllum* tubers were in formation process (tuberization), we can indicate that the absence of extra reserves inhibited flowering in its first cycle.

As a geophyte, it is the tuber that will ensure its persistence in the next cycle. For any form of applied cultivation, *T. pentaphyllum* has a short annual cycle, but the plants obtained by seeds presented longer duration of the aerial stem system ($\bar{x} = 177,1$ days) than the plants by stem cuttings ($\bar{x} = 158$ days) [14], which could explain the larger mass and size of the tubers. Plants obtained from seeds also had a longer duration in relation to plants obtained from seed-tubers ($\bar{x} = 126$ days) [10].

Considering the great agricultural potential of *T. pentaphyllum* as food, seed germination may improve the genetic diversity of crops and decrease the collection of tubers *in situ*, contributing to their conservation. Furthermore, it may in the future be a great ally of genetic improvement programs involving the species, as it will enable the creation of different commercial varieties of *T. pentaphyllum*.

5. CONCLUSION

Seeds of *T. pentaphyllum* persist for more than one year in the soil, which indicates seed bank formation. The highest germination rates occur preferably in the month of April under dark conditions (60.0%), indicating that the species is preferential negative photoblastic. The species presented moderate germination rate. As seeds persist in the soil/substrate for up to two years it is necessary to test ways to break their dormancy and increase germination rates.

The seed germination of *T. pentaphyllum* is an excellent alternative for the propagation of the species, since the plants obtained present high formation of tubers that can be used as seed-tubers in cultivation of species and for maintaining or expand the genetic diversity of crops. Conciliate different forms of cultivation (vegetative propagation – seed-tubers and stem cutting – and sexual reproduction by seed germination) to obtain tubers can reduce the collection of tubers *in situ* contributing to the conservation of *T. pentaphyllum*.

REFERENCES

1. Bayer C, Appel O. Tropaeolaceae. In: Kubitzki K, Bayer C (eds). The families and genera of vascular plants, flowering plants, dicotyledons: Malvales, Capparales and Non-betalain Caryophyllales. Berlin: Springer; 2003.
2. Souza VC, Lorenzi H. Systematic Botany: Illustrated Guide for the Identification of Phanerogama Families Native to Brazil. Nova Odessa: Instituto Plantarum; 2008.
3. Rix M. Tropaeolum pentaphyllum Tropaeolaceae. Curtis's Botanical Magazine. 2010;27:296-300. DOI: <https://doi.org/10.1111/j.1467-8748.2010.01705.x>
4. Souza VC. Tropaeolaceae. In: Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro; 2015.
5. Kinupp VF, Lisbôa GN, Barros IBI. Plantas para o Futuro – Região Sul. In: Coradin L, Siminski A, Reis C (eds). Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial. Brasília: MMA; 2011.
6. Cruz RC, Denardi LB, Mossmann NJ, Piana M, Alves SH, Campos MMA. Antimicrobial Activity and Chromatographic Analysis of Extracts from Tropaeolum pentaphyllum Lam. Tubers. Molecules. 2016;21:1-11. DOI: <https://doi.org/10.3390/molecules21050566>
7. Bona GS, Boschetti W, Bortolin RC, Vale MGR, Moreira JCF, Rios OA, Flôres SH. Characterization of dietary constituents and antioxidant capacity of Tropaeolum pentaphyllum Lam. Journal of Food Science and Technology. 2017;54:3587-3597. DOI: <https://doi.org/10.1007/s13197-017-2817-z>
8. Kinupp VF, Lorenzi H. Unconventional food plants (PANC) In Brazil: Identification guide, nutritional aspects and illustrated recipes. São Paulo: Instituto Plantarum; 2014.
9. Pacheco TG, Silva GM, Lopes AS, Oliveira JD, Rogalski JM, Balsanelli E, Souza EM, Pedrosa FO, Rogalski M. Phylogenetic and evolutionary features of the plastome of Tropaeolum pentaphyllum Lam. (Tropaeolaceae). Planta. 2020;252:17. DOI: <https://doi.org/10.1007/s00425-020-03427-w>
10. Balestrin JT, Mattei KS, Silva D, Rogalski JM. Conservation and cultivation of crem (Tropaeolum pentaphyllum Lam.): a neglected species with high potential for use. In: Seabra G (ed). TERRA: Vulnerabilidades e Riscos Ecológicos. Ituiutaba: Barlavento; 2021.
11. Simões GD. Crem (Tropaeolum pentaphyllum Lam): chemical characterization, antioxidant and its application as a condiment in a vegetable paste. Dissertation (Master in Food Science and Technology) – UFSM; 2015.
12. Engel VL, Fonseca RCB, Oliveira RE. Liana ecology and the management of forest fragments. Série Técnica IPEF. 1998;12:43-64.
13. Kinupp VF. Unconventional food plants from metropolitan region of Porto Alegre, RS. Thesis (Doctor in Phytotechnics) - UFRGS; 2007.
14. Balestrin JT, Souza TL, Betto AS, Silva D, Rogalski JM. Propagation by Stem Cuttings of Tropaeolum pentaphyllum Lam. (Crem): An Alternative for Production of Seed Tubers. Journal of Experimental Agriculture International. 2021;43:84-90. DOI: <https://doi.org/10.9734/jeai/2021/v43i130634>
15. Rajjou L, Duval M, Gallardo K, Catusse, J, Bally J, Job C, Job D. Seed germination and vigor. Annual Review of Plant Biology. 2012;63:507-33. DOI: <https://doi.org/10.1146/annurev-arplant-042811-105550>

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16. Nonogaki H, Bassel GW, Bewley JD. Germination-still a mystery. *Plant Science*. 2010;179:574-81. DOI: <https://doi.org/10.1016/j.plantsci.2010.02.010>
17. Ooi MKJ. Seed bank persistence and climate change. *Seed Science Research*. 2012;22:S53-S60. DOI: <https://doi.org/10.1017/S0960258511000407>
18. Ramos AM, Santos LAR, Fortes LTG. *Brazilian Climate Normals 1961–1990*. Brasília: Inmet; 2009.
19. Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumberras JF, Coelho MR, Almeida JA, Araujo Filho JC, Oliveira JB, Cunha TJF. *Brazilian system of soil classification*. 5. Ed. Brasília: Embrapa; 2018.
20. Farfan J, Antonieta P, Canepa FS. Seed germination conditions and ontogeny of the *Tropaeolum polyphyllum* (Tropaeolaceae) seedling. Thesis - Universidad de Talca; 2001.
21. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). *Regras para análise de Sementes*. Brasília: MAPA; 2009.
22. Rix M. *Tropaeolum argentinum*. Tropaeolaceae. *Curtis's Botanical Magazine*. 2012. 29:355-361. DOI: <https://doi.org/10.1111/curt.12005>
23. Garwood NC. Tropical soil seed banks: a review. In: Leck MA, Parker TV, Simpson RL (eds). *Ecology of Soil Seed Banks*. New York: Academic Press; 1989.
24. Brandes U. Investigating the life cycle of *Tropaeolum speciosum* to improve future management. Thesis - University of Otago; 2007.
25. Silva EM, Pereira JC, Ferreira VM, Souza RC. Germination of *Stigmaphyllon blanchetii* Seeds in Different Temperatures and Luminosity. *Planta Daninha*. 2019;37. DOI: <https://doi.org/10.1590/s0100-83582019370100120>
26. Lymperopoulos P, Msanne J, Rabara R. Phytochrome and phytohormones: working in tandem for plant growth and development. *Frontiers in Plant Science*. 2018;9:1-14. DOI: <https://doi.org/10.3389/fpls.2018.01037>
27. Taiz L, Zeiger E, Moller I, Murphy A. *Plant physiology and development*. 6. Ed. Porto Alegre: Artmed; 2017.
28. Takaki M. New proposal of classification of seeds based on forms of phytochrome instead of photoblastism. *Revista Brasileira de Fisiologia Vegetal*. 2001;3:104-108. DOI: <https://doi.org/10.1590/S0103-31312001000100011>
29. Magalhães ACN. Quantitative growth analysis. In: Ferri MG (ed). *Plant physiology*. 2. Ed. São Paulo: Ed. Pedagógica e Universitária; 1985.
30. Grau A, Dueñas RO, Cabrera CN, Hermann, M. *Mashua (Tropaeolum tuberosum Ruiz & Pav.)*. Promoting the conservation and use of underutilized and neglected crops. Roma: International Potato Center/International Plant Genetic Resources Institute; 2003.
31. Duran CB. Evaluation of the development of nasturtium (*Tropaeolum majus* L.) cultivated in a pot with capillary irrigation in a greenhouse. Monograph. Universidade Federal do Pampa; 2017.
32. Sorgato JC, Rosa DBCJ, Moreno LB, Soares JS, Vieira MC (2014). "Capuchinha" emergence in different substrates. *Enciclopédia Biosfera*. 2014;10:954.
33. Molina R, López-Santos C, Gómez-Ramírez A. Influence of irrigation conditions in the germination of plasma treated *Nasturtium* seeds. *Scientific Reports*. 2018;8:16442. DOI: <https://doi.org/10.1038/s41598-018-34801-0>

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34. Lumsden PJ, Millar AJ. Biological Rhythms and Photoperiodism in Plants. Oxford: Bios Scientific; 1998.
35. Dafni A, Cohen D, Noy-Mier I. Life-cycle variation in geophytes. *Annals of the Missouri Botanical Garden*. 1981;68:652-660. DOI: <https://doi.org/10.2307/2398893>

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