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Research Article

Study of Seedling Establishment and Phenological Stages of *Scrophularia striata* a Medicinal Plant in Iran

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Abstract

To study the germination traits, determine the bed culture and some of the agronomical traits of the medicinal plant (*Scrophularia striata*), a series of experiments accomplished in the Agricultural and Natural Resources Department of University of Tehran in 2016. Investigation of seed dormancy and germination was done in factorial design in three replications with three factors including gibberellic acid (at 0, 100 and 400 ppm conc.), chilling duration of 0, 1, 3, 5 and 7 days, and temperatures of 5, 10, 15, 20, 25, 30 35°C. The highest germination percentage (58.7%) occurred at 15°C and in zero and 400-ppm conc. of gibberellic acid and 7 days chilling. In the greenhouse, seven different bedding types used in a randomized complete block design with four replications to determine the establishment and emergence of seedlings. The highest percentage of seedling emergence (56.6%) found in peat moss and peat moss + perlite (1 : 1) with was no significant difference between the two treatments. In the field experiment, the effect of different dates and bedding types on phonological and agronomic traits of *Scrophularia* plant sp. performed in a split plot design with randomized complete block design at three replications. Five planting dates (June, September, October, November, and March) and three planting bed (peat moss, perlite + peat moss and coco peat + perlite + peat moss) treatments examined. The time of different phenological stages in terms of growth degree-day (GDD) estimation, showed that the June planting date (in peat moss bed) required the lowest GDD as compared to other treatments. In relation to agronomic traits of *Scrophularia* plant sp., number of main branches, dry weight of shoot, capsule weight per plant, etc. recorded the highest values of traits in the latter treatment.

Keywords: Germination; Seedling Establishment; Phenological Stages; Scrophularia striata

Introduction

According to the FAO [1], 25% of the commonly used drugs are plant sources, and 74% of the newly used herbal remedies, their therapeutic effects have been traditionally transferred from the past. On the vast plateau of Iran, due to the presence of various climates, numerous drug species found in plantations, meadows and desert areas, which are considered as scant ecological potential low-income lands. Unfortunately, owing to the abundance of herb plants in Iran, unnecessarily, the destruction of these valuable genetic resources is expanding due to the profitable and unproductive collection of medicinal plants from wild and natural habitats [2]. In this regard, many species of medicinal herbs in Iran can be introduced as new plants. *Scrophularia striata* is a native species with local name of "theshnedary" (thirsty), which belong to the Scrophulariaceae family with 200 species of flowering plants commonly famous for its medicinal uses [3]. There are five species of this valuable herb in Iran [4]. In this family, many compounds such as alkaloids, glycoside resins, iridoid and cryptophylic acid have been identified that are commonly found in different plant parts such as roots, leaves, buds, seedlings and skin [5].

A total of 34 essential oil compounds were identified in this plant, the most important of which are linalool (18.3%), 14,10,6-trimethylpentane-2-one (8.4%), dibutyl phthalate (6.9%), beta-dummies (5.9%), alfatriphenol (4.9%), and germanacrin (4.7%) [6]. The *Scrophularia* sp. is a perennial plant, with height of 90-30 cm, non-cracked branching, with alternate leaves and fruits of a capsule with numerous seeds (Ghahraman, 1986). The native *Scrophularia* sp. have a significant geographical dispersion in Iran, in the cold and mountainous areas of Zagros and in the plain slopes of the provinces such as Gorgan, Mazandaran, Gilan, East Azarbaijan and West to Zanjan, Kurdistan, Kermanshah, Ilam, Lorestan etc [4]. It has many medicinal properties, including treatment for inflammation and infection, digestive disorders, colds, skin burns, etc [7].

One of the major problems in domestication of wild medicinal species is the presence of seed germination and seed germination in crop and laboratory conditions [8]. Researchers are trying to find the causes of dormant seeds in appropriate ways to break the dormancy and increase the percentage and speed of seed germination [9]. Therefore, in the process of domestication of wild medicinal species, after proper germination, the plant should be well grown and located well so that it can produce strong and suitable seedlings for growth in the field [10]. Generally, different types of dormancy occur due to different conditions in the seed. A dormant seed is a seed that cannot germinate under normal environmental and favorable conditions [11]. The International Association for seed testing (ISTA) have proposed different methods to break dormancy and stimulate plant seed germination. The most important of these approaches can be stratification, scratching (mechanical and chemical), use of stimulating chemicals, optical rotation, temperature, etc [12].

Among various chemicals, gibberellic acid, Cytokinins, potassium nitrate and thiurea are known to eliminate certain types of seed dormancy. This substance removes light-basis dormancy and can replace cold stratification [13]. The wet cold treatment can simulate can be used for seeds that have an internal dormancy, with verbalization requirements. At cold temperatures, the amount of oxygen dissolved in water increases, as a result, seed embryos need to oxygen better provided [14]. Recognition of cardinal temperatures is also useful for evaluating germination characteristics or plant establishment potential, and it is important in the process of domestication of plants [15]. The minimum, optimal and maximum germination temperatures generally depend on the extent of the environmental adaptation of the species and the matching of germination time with favorable conditions for subsequent stages of growth, establishment, and seedling development is guaranteed [16]. Seed dormancy monitoring and recognition of suitable treatments for improving germination of *Scrophularia* sp., seedling establishment in different culture media in the greenhouse and identification of the phenological growth stages in field conditions have been the goals of this research.

Materials and Methods

Seeds in the capsule of *Scrophularia* sp. in this experiment were collected from highlands of Ilam province (Iran) in November 2015. To break the dormancy and improve seed germination, the gibberellic acid hormone (produced by Sigma) with a molecular weight of 346.3 g.mol^{-1} was used. Different planting beds including Peat moss, Cocopit and perlite were purchased from the market. Trials of dormancy break, germination and seedling establishment were performed in a laboratory and greenhouse, and study of the Phenological Stages of *Scrophularia* sp. done in the Research farm of the College of Agricultural and Natural Resources of the University of Tehran (located at Karaj city with geographical coordinates of 35° 48' North and 50° 57' East). The Lab. experiment accomplished in a completely randomized design with three replications, including the moist-chilling treatments of zero (control), 1, 3, 5 and 7 days at 3°C; acid Gibberellin application at three concentration of 0, 100 and 400 ppm and various cardinal temperature of 5, 10, 15, 20, 25, 30 and 35°C. After placing the seeds in petri dish at the desired temperatures, germinated seeds were counted daily and recorded. The germination rate of the roots was 2 millimeters or more [17]. The germinated seed counts continued for 14 days in different treatments, and the final germination percentage and rate were calculated [18].

(Formula 1) $$\mathrm{Vg} = \sum \frac{Ni}{Di}$$

Where Vg is the germination rate per seed number per day, Ni is the number of germinated seeds per day, and Di is the day's number.

In the greenhouse, wet cold-growing seeds grown in 7 different culture media (beds) in plastic pots in a randomized complete block design with four replications. Soil substrates included cocopeat, perlite, peat moss, sand, coco peat + perlite (1 : 1 ratio), peat moss + perlite ratio (1 : 1) and cocopeat + perlite + peat moss (1 : 1 : 2). In each pot, 30 wet cold-seeds sown. The pots examined on a daily basis and when the first emerged seedlings appeared the record was taken. For each culture medium, the number of days to the stages of two, four, six, eight and ten leaves was calculated.

When the seedlings reached at stage 4 to 6 leaves, transfer to field was done. The field experiment was conducted as split plot design in randomized complete block design with three replications. Planting date (main plot) was selected as mid-May, September, October, November of 2015 and March of 2016 and culture medium Peat moss, Peat moss + Perlite and cocopit + Peat moss + Perlite as a split plot. Seedlings were planted on a row at a distance of 50 and 30 cm between and within the rows, respectively. In the first year and cold winter, the plant remained in rosette. After the winter, the seedling growth started and phenological stages including first node, second node, flowering, capsule formation, seed formation and seed maturity were recorded in all treatments. Different phenological stages were calculated using the mean daily temperature and base temperature of *Scrophularia* sp. according to the growth- degree day (GDD) (Leblanc., *et al.* 2003).

(Formula 2) $GDD = \sum T_{average} - T_b$

In that case, the base temperature for *Scrophularia* sp. was considered to be 4.95°C according to previous tests.

At seed harvesting time, the plant height, number of main and sub branches, plant dry weight, dry weight of capsule, number of capsules per plant and seed number in capsule measured and recorded. Analysis of variance of data for the effect of different treatments on the final percentage of germination and rate was done using R software. To evaluate the effect of culture media on seedling emergence rate, the log-logistic model of three parameters employed using daily seedling data, and drawing of the graphs with Sigma Plot application.

(Formula 3) $f(x) = d/(1+\exp(b(\log(x)-\log(e))))$

In that case, the line gradient (b), the maximum number of established seedlings (d) and time to reach 50% of the emergence rate (e).

Results and Discussion

Response of germination percentage to the interaction of gibberellic acid with moist chilling at different temperatures

In the present experiment, the highest germination percentage of *Scrophularia* sp. (58.7%) was recorded at 15°C (Figure 1). At 5°C, almost no germination activity was observed, and germination was close to zero at 35°C. Karavani., *et al.* [19] in experiment that performed on breaking seed dormancy and germination of *Scrophularia* sp. reported the highest germination percentage (86.6%) at 25°C. In addition, at 5°C, 8% germination and at 35°C 21% germination yielded, which indicated the difference in temperature requirement of *Scrophularia* sp. for germination in these two studies, that can be attributed to differences in seed masses used in these two experiments.

In the same way, at 15°C temperature treatment, for seven days of chilling, there was no significant differences between zero and 400-ppm gibberellic acid in terms of germination percentage (Figure 1). In both cited treatments, the highest germination percentage was 58.7%. As a result, at optimum temperature for *Scrophularia* sp. seed germination instead of gibberellic acid treatment the seven days of cold chilling treatment can be used to replace. In relation to seed dormancy break, Karavani., *et al.* [19], in three treatments of 1, 5 and 7 days of chilling, with increasing temperature up to 15°C, the germination percentage increased; and with further increasing temperature ≥ 15°C, the germination percentage decreased. The most effective treatment of seed dormancy break was by applying 400 ppm of gibberellic acid through a week wet-cold, where by 92% germination was recorded.

The results of moisture cold and gibberellic acid treatments in this study have been consistent with several reports on the positive role of these treatments on seed germination of many plant species. Shariati., *et al.* [20] reported the gibberellic acid as one of the best treatments used to break seed dormancy of Yarrow seed. Rajabian., *et al.* [9] introduced the best treatment as moist-chilling for the angus seed germination. The highest squirrel seed germination percent was obtained after 30 days of cold humidification at 10°C [21].

Figure 1: Germination percentage of *Scrophularia* sp. at different gibberellic acid concentration. versus chilling time and temperature.

Seedling seedlings emergence rate in different seedbeds

The results of analysis of variance showed that the effect of planting bed type in the probability level of 1% on the percentage of emergence was significant (Table 1). The highest germination percentage (56.6%) was found in two peat moss and peat moss + perlites (1 : 1) plants, so there was no significant difference between the two treatments and the lowest 8 (35%) in the culture medium of cocopeat + perlite (1 : 1) was obtained (Figure 2).

S.O.V.	d.f.	Germination%	Planting date to two leaf stage	Planting date to four leaf stage	Planting date to six leaf stage	Planting date to eight leaf stage	Planting date to ten leaf stage
Planting bed	6	330.9**	68.1**	277.6**	1318**	2629.5**	2963.1**
Error	21	9.65	0.45	0.83	1.14	0.20	0.07
%CV.		7.15	2.91	2.54	2.74	1.22	1.67

Table 1: Analysis of variance (mean square) of planting bed effect on seedling percentage and growth characteristics of *Scrophularia* sp.

**: Indicate a significance difference at 1%probability level

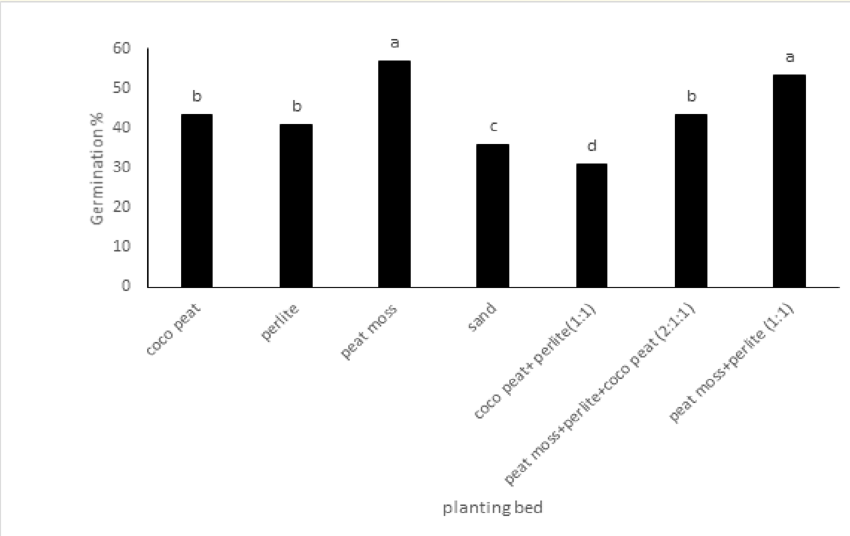

Figure 2: Comparison of the average effect of planting bed on seed germination percentage of *Scrophularia* sp.

The seed germination rate parameters including the maximum number of seedlings (d), the arrival time of 50% of emergence (e) and line gradient (b) were calculated using the three-parameter log-logistic model in different culture media (Table 2). The highest value of d parameter was found in Peat Moss (17 seedlings) and the lowest in Cocopit + perlite (9 seedlings) culture medium. In relation to parameter e, the lowest value was found in two cocopeat and sand substrate (11.2), which indicates the higher growth rate of these two bedding. In addition, faster by up to 50 percent. The highest amount of slope of the emergence rate (b), which indicates the number of seeded seeds pertaining to the past time, is related to the perlite cultivar (16.03), and the smallest amount of this parameter is related to the cocopeat culture medium (7.97) (Table 2).

Effect of different planting dates on phenological traits of *Scrophularia* sp.

The results of analysis of variance indicated the significant effect of planting date, culture media and their interaction on phenological traits of *Scrophularia* sp. at 1% probability level (Table 3). The planting date of June compared to other dates had a more favorable effect on phenological traits and yield of *Scrophularia* sp.

Delay in planting with decreasing vegetative growth and accelerating development resulted from the increased temperature, reduced the accumulation of dry matter in vegetative and reproductive organs. Two culture media of peat moss and peat moss + perlites (1 : 1) were the best substrate for all cases. The highest vegetative traits including the plant height, number of stems, shoot fresh and dry weight, root dry weight, capsule number, seed per capsule, biological yield and grain yield obtained (data not provided) in peat moss and peat moss + perlites (1 : 1). These substrates, by creating favorable plant growth conditions, have resulted in better water and nutrient absorption produced better qualitative and quantitative properties of *Scrophularia* sp. plant than other culture media in accordance with Marschner [22]. Hamidpour [23] studied the effect of zeolite, vermicompost and phosphorus on some growth parameters of common zinnia showed that the highest and lowest growth parameters related to vermicomposting by using 10% zeolite, respectively.

Culture media (plant bedding)	Slope of emerges speed(b)	Maximum number of seedlings (d)	Time to reach 50% germination rate (e)
Coco peat	7.0 ± 97.5	13.0 ± 12.2	11.0 ± 26.1
Perlite	16.1 ± 3.4	11 ± 97.2	13 ± 38.0
Peat moss	9 ± 23.4	17 ± 5.2	11 ± 63.0
sand	14.1 ± 70.4	10 ± 73.1	11 ± 28.0
Coco peat + perlite (1:1)	11.1 ± 26.2	9 ± 15.2	12 ± 8.1
Coco peat+ perlite+ peat moss (2:1:1)	8 ± 30.5	13 ± 42.2	12 ± 22.1
peat moss + Perlite (1:1)	9 ± 69.5	15 ± 82.2	11 ± 66.0

Table 2: Effect of different culture media on seedling emergence parameters of *Scrophularia* sp.

S.O.V.	d.f.	First node	Second node	Flowering	Podding	Capsulation	Maturity
Replication	2	20.5 ns	5.7 ns	205.4 ns	66.9ns	2.2 ns	350.1 ns
Planting date (A)	4	16119.6**	14861.6**	447766.3**	1395509.5**	16 827.2**	5098234.7**
Main error	8	4.9	12.5	116.8	188.2	124.4	347.5
Planting bed (B)	2	772.5**	731.1**	2770.4**	8511.4**	10475.1**	25149.2**
A*B	8	46.6**	48.7**	332.1**	1126.2**	1483.5**	3358.2**
Sub-error	20	7.2	9.04	27.5	92.0	80.0	256.8
CV%		2.5	2.5	1.4	1.4	1.2	1.2

Table 3: Analysis of variance (mean square) for the effect of planting date and planting bed on phenological traits of *Scrophularia* sp.
** & ns, respectively indicate a significance difference at 1%probability level and non-significant.

The use of different ratios of these substrates increased plant growth traits because each of them had a positive character and would not be efficient alone. For example, peat moss with large granularity and high porosity provides good ventilation, water-holding capacity and microbial activity and is used as a bedding plant for soil modification. Vermicomposting and Peat moss contain plant growth regulating agents (such as humic acid) discussed by Arancon [24]. The coco peat has been considered as one of the good growth substrates, due to its ideal acidity and electrical conductivity and other chemical properties. But then again because it has a high water holding capacity, it disconnects air-water and low aeration influences in substrate, therefore affects the release of oxygen to the roots and should be mixed with other substrates discussed by Awang [25].

Generally, the highest rate of germination of *Scrophularia* sp. (58.6%) obtained at 15°C and the effect of dormancy breaking treatments (seven days moist-chilling + 400 ppm of gibberellic acid). Cardinal temperatures including base, optimum and ceiling temperature for germination of *Scrophularia* sp. were 4.9, 17.9 and 30.5°C, respectively; indicating that cultivation of this plant in temperate regions is more feasible. The highest emergence percentage of seedlings obtained in two peat moss and peat moss + perlite (1 : 1), and the lowest germination rate (35.8%) acquired in 1 + 1 coco peat + perlite culture medium. The seedlings reached to 10 leaves stage only in two media that is perlite + peat moss (1 : 1) and coco peat + perlite + peat moss (1 : 1 : 2) and disappeared in the remaining substrates before reaching this stage. Regarding the effect of planting date and planting bed on the phenological stages of *Scrophularia* sp., it was found that in June planting and peat moss bed the plant sp. had a lower heat degree-day requirement and produced good morphological traits and shoot dry weight and seed yield compared to other treatments (data not provided) [26,27].

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