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Cardinal Germination Temperatures of Some Medicinal Plant Species

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ABSTRACT

The germination behavior of 11 medicinal plant species under different temperatures was studied. Seeds of all species were exposed to eight constant temperatures ranging from 5–40 °C. A linear model was applied to describe the germination rate-temperature relationship. Tested species showed clear differences in temperature requirements for seed germination. The highest germination percentage was observed within the ranges of 15–25 °C for *Marrubium vulgare* L., 15–30 °C for *Origanum majorana* L., 10–30 °C for *Hyssopus officinalis* L. and *Silybum marianum* L., 10–25 °C for *Thymus daenensis* Celak subsp. *daenensis* and *Ocimum basilicum* L. Germination of most species ceased or highly declined at temperatures of 35 °C. The highest germination rate was observed within temperature ranges of 15–30 °C for *Nepeta racemosa* Lam., *Oenothera biennis* L. and *H. officinalis*, 10–25 °C for *O. basilicum* and *Salvia nemorosa* L., 20–25 °C for *O. majorana* L. and *T. daenensis* and 10–30 °C for *S. marianum*. The lowest base temperature was observed for *Salvia sclarea* L., *S. nemorosa, H. officinalis* and *Centaurea benedicta* (L.) L. All medicinal species studied in this research had a low, below 5 °C, base temperature. Optimum temperature ranged from 13–24 °C, and the lowest optimum temperature belonged to *C. benedicta*. These germination requirements are insufficient to make a general conclusion for introducing the species into cultivation, however, these data provide basic temperature requirements for further research.

INTRODUCTION

The global demand for herbal medicine is not only large, but growing (Srivastava, 2000). The world market for herbal remedies in 1999 was calculated to be worth 19.4 billion USD, with Europe in the lead (6.7 billion USD), followed by Asia (5.1 billion USD), North America (4.0 billion USD), Japan (2.2 billion USD), and the rest of the world (1.4 billion USD) (Larid and Pierce, 2002). Still most species of medicinal plants are collected from the wild and the total number of species of medicinal plants cultivated on any scale is small. Only 130–140 of the 1200–1300 species that are traded in and native to Europe are derived predominantly from cultivation (Lange, 1998). The wide array of medicinal plants requires many different techniques for production, harvesting and storage, yet the requirements of these species are poorly documented and often unsuccessful (Uniyal et al., 2000).

Germination is one of the most important factors in the success of an annual crop and hence plays a key role in crop production (Khajeh-Hosseini et al.,

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2003; Soltani et al., 2006). Germination requirements are species specific and the extent and rate at which the process occurs in non-dormant seeds is affected by environmental factors such as light, oxygen, water and temperature (De Villiers et al., 2002). Temperature affects the onset, potential and rate of germination (Roberts, 1988; Flores and Briones, 2001), and is thereby always the most critical factor determining success or failure of plant establishment (Kader and Jutzi, 2004). Generally, temperatures below the optimum result in progressively poorer germination (Nykiforuk and Flanagan, 1994). Germination rate usually increases linearly with temperature, at least within a well-defined range, and declines sharply at higher temperatures (Mwale et al., 1994; Kamkar et al., 2006). Each plant species in each given phase of its life cycle, and under a given series of conditions, has a base temperature (T_b) below which it will not grow, an optimum temperature (T_o) at which it grows best, and a ceiling temperature (T_c) above which growth ceases completely (Jami Al-Ahmadi and Kafi, 2007). To improve establishment success rates and reduce the cost of restoration operations, it is essential to have a good understanding of seed germination requirements (Sy et al., 2001). The objective of this study was to investigate the germination behavior of some medicinal plant species under different temperatures in order to facilitate their cultivation practices.

MATERIALS AND METHODS

Mature seeds of eleven medicinal plant species (*Salvia sclarea* L., *Salvia nemorosa* L., *Marrubium vulgare* L., *Hyssopus officinalis* L., *Origanum majorana* L., *Ocimum basilicum* L., *Nepeta racemosa* Lam., *Thymus daenensis* Celak subsp. *daenensis*, *Oenothera biennis* L., *Silybum marianum* L., and *Centaurea benedicta* (L.) L. (=*Cnicus benedictus*) were collected randomly in 2008 from the herbal garden of Medicinal Plants and Drugs Research Institute of Shahid Beheshti University, Tehran, Iran. After collection, immature and insect-damaged seeds were removed. Seeds were surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 5 min and then thoroughly rinsed with sterilized water. All germination experiments were conducted using three replications of 25 seeds for each treatment. Seeds were placed on double-layered Whatman no. 1 filter paper moistened with 5 ml distilled water in sterilized petri dishes. The effects of eight constant temperatures (5, 10, 15, 20, 25, 30, 35, 40 °C) were studied using dark germinators with 70–75% relative humidity for 21 days. Species that maintained a high germination rate at 5 °C were also exposed to 3 °C. Germinated seedlings were counted and removed every 24 h. A seed was considered germinated when the tip of the radicle had grown free of the seed coat (Wiese and Binning, 1987; Auld et al., 1988). The germination rate (GR) was calculated according to McKenzie et al (1980) as follows:

$$GR = \sum_{n=0}^{t-1} X_n (t-n)/t$$

where X_n is the germination percentage on the nth count, n is the nth day of counting germination-1, and t is total days of counting germination. After arcsine transformation, the percentage of germination and germination rate were subjected to an analysis of variance. For each species at different temperatures, the data were analyzed as a completely randomized design with three replications, and means separated using Duncan's new multiple range test (LSR, $p \leq 0.05$). The following linear model (Summerfield et al., 1991) was used to calculate the cardinal temperatures:

$$f = B\,(T - T_b) \text{ if } T < T_o$$
$$f = C\,(T_c - T) \text{ if } T > T_o$$

where f is the daily development rate, T is the temperature in °C ($T_b < T_c$), T_b is the base temperature below which f = 0, T_c is the ceiling temperature above which f = 0, T_o is optimum temperature, and B and C are model parameters. From the above analysis, the optimum germination temperature was determined for each species. The model was evaluated using the linear regression procedure of Sigmaplot (1993).

RESULTS AND DISCUSSION

Results showed that different species responded differently to constant temperatures, and significant ($p \leq 0.05$) temperature differences for germination percentage and rate were observed for each species (Tables 1 and 2). The highest

TABLE 1. Temperature effects on seed germination percentage of 11 medicinal plants.

	Temperature (°C)								
Species	**3**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
Centaurea benedicta	36.3^{c*}	71.9^{ab}	65.9^{ab}	77.9^{a}	56.9^{bc}	40.9^{c}	11.9^{d}	0.0^{d}	—
Hyssopus officinalis	0.0^{e}	56.9^{bc}	65.0^{abc}	67.7^{a}	55.8^{c}	71.9^{a}	66.2^{ab}	29.5^{d}	0.0^{e}
Marrubium vulgare	—	17.4^{c}	21.6^{c}	68.6^{a}	62.4^{ab}	77.7^{a}	46.9^{b}	26.4^{c}	0.0^{d}
Nepeta racemosa	—	0.0^{d}	43.0^{bc}	54.7^{ab}	43.0^{bc}	57.6^{a}	51.7^{ab}	38.1^{c}	0.0^{d}
Oenothera biennis	—	0.0^{c}	48.8^{b}	61.1^{ab}	52.4^{b}	51.7^{b}	70.1^{a}	49.2^{b}	0.0^{c}
Ocimum basilicum	—	0.0^{d}	40.1^{c}	55.9^{b}	66.1^{ab}	71.3^{a}	58.5^{ab}	31.6^{c}	0.0^{d}
Origanum majorana	—	29.3^{c}	36.9^{bc}	49.8^{ab}	55.0^{a}	45.9^{ab}	41.1^{bc}	8.6^{d}	0.0^{d}
Salvia sclarea	0.0^{d}	47.8^{b}	51.1^{b}	44.9^{b}	71.9^{a}	52.1^{b}	51.7^{b}	15.6^{c}	0.0^{d}
Salvia nemorosa	0.0^{c}	46.1^{ab}	42.9^{ab}	58.9^{a}	58.7^{a}	59.0^{a}	41.1^{ab}	28.6^{b}	0.0^{c}
Silybum marianum	0.0^{b}	5.4^{b}	78.1^{a}	67.4^{a}	78.1^{a}	76.8^{a}	71.3^{a}	4.3^{b}	0.0^{b}
Thymus daenensis subsp. *daenensis*	—	4.3^{de}	16.5^{d}	36.0^{c}	65.0^{a}	52.7^{ab}	50.4^{abc}	43.8^{bc}	0.0^{e}

*Means followed by the same letter in each row are not significantly different according to Duncan's new multiple range test ($p \leq 0.05$).

germination percentage was observed within the range of 15–25 °C for *M. vulgare* and *O. majorana*, 10–30 °C for *H. officinalis* and *S. marianum*, 20–30 °C for *T. daenensis* and *O. basilicum*, and 5–30 °C for *S. nemorosa*. With the exception of *S. sclarea* (20 °C), *O. biennis* (30 °C) and *C. benedicta* (15 °C), most species maintained maximum germination percentage over a wide temperature range (Table 1). Germination of most species ceased or greatly declined at temperatures of 35 °C and higher, but germination percentage of *S. sclarea* and *C. benedicta* declined at 25 and 20 °C, respectively (Table 1). Bannayan et al. (2006) reported that most medicinal plant species they studied also maintained high germination percentage over a wide temperature range, ceasing or declining at 35 °C. Similar to germination percentage, most species maintained the highest germination rate over a wide temperature range, indicating the ability for fast germination and establishment under wide thermal conditions.

The highest germination rate was observed at temperatures ranging from 15–30 °C for *N. racemosa* and *H. officinalis*, 20–30 °C for *O. basilicum*, 10–25 °C for *S. nemorosa*, 15–20°C for *O. majorana*, 20–25°C for *T. daenensis*, and 10–30°C for *S. marianum* (Table 2). The highest germination rates for *S. sclarea* and *C. benedicta* were observed at temperatures of 20 and 15 °C, respectively (Table 2).

Table 2. Temperature effects on seed germination rate (germination percentage per day) of 11 medicinal plants.

	Temperature (°C)								
Species	**3**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
Centaurea benedicta	19.6^{c*}	46.0^{b}	44.5^{b}	61.3^{a}	44.7^{b}	42.1^{b}	13.8^{cd}	0.0^{d}	—
Hyssopus officinalis	0.0^{e}	32.0^{d}	43.0^{c}	54.5^{ab}	49.8^{b}	59.2^{a}	59.0^{a}	26.3^{d}	0.0^{e}
Marrubium vulgare	—	7.1^{de}	16.2^{dc}	47.3^{a}	43.2^{a}	43.8^{a}	31.7^{b}	20.1^{c}	0.0^{e}
Nepeta racemosa	—	0.0^{d}	31.0^{c}	40.4^{abc}	37.3^{abc}	46.6^{a}	42.8^{ab}	32.8^{bc}	0.0^{d}
Oenothera biennis	—	0.0^{d}	27.2^{c}	48.1^{b}	51.7^{b}	49.0^{b}	55.8^{a}	32.3^{b}	0.0^{d}
Ocimum basilicum	—	0.0^{d}	30.8^{c}	54.3^{b}	60.7^{ab}	58.6^{a}	46.9^{ab}	26.4^{c}	0.0^{d}
Origanum majorana	—	7.4^{e}	29.7^{d}	32.1^{ab}	43.0^{a}	38.6^{bc}	23.5^{cd}	1.6^{ef}	0.0^{f}
Salvia sclarea	0.0^{f}	27.8^{d}	39.0^{bc}	37.1^{c}	65.0^{a}	46.2^{bc}	47.7^{b}	14.5^{e}	0.0^{f}
Salvia nemorosa	0.0^{d}	35.3^{b}	48.9^{a}	54.3^{a}	55.2^{a}	55.0^{a}	31.4^{b}	19.4^{c}	0.0^{d}
Silybum marianum	0.0^{b}	4.0^{b}	56.0^{a}	54.5^{a}	63.7^{a}	61.5^{a}	55.0^{a}	1.9^{b}	0.0^{b}
Thymus daenensis subsp. *daenensis*	—	1.9^{d}	30.7^{c}	28.4^{c}	54.1^{a}	48.6^{ab}	42.9^{b}	33.0^{c}	0.0^{d}

*Means followed by the same letter in each row are not significantly different according to Duncan's new multiple range test ($p \leq 0.05$).

Germination behavior is highly related to ecological conditions of species' natural habitat. A study of germination behavior under different temperatures aids in determining optimum planting time and suitable regions with optimum temperature ranges for cultivation (Nadjafi, 2006). Most of the species in this study naturally found in Mediterranean and arid environments, germinated well at temperatures ranging from 15 to 30 °C, indicating a preference for moderate temperatures for germination. Estrelles et al. (1999) also showed that typical Mediterranean optimal temperatures between 15 and 25 °C led to maximum germination in species characteristic of arid environments like *Lavandula dentata* L., *Teucrium gnaphalodes* L'Hér., *Thymbra capitata* (L.) Cav. and *Thymus hyemalis* L. and that the upper temperature was a significant limiting factor for these species. The highest germination percentage and rate for *C. benedicta* were observed at temperatures of 15 and 20 °C, respectively, indicating an adaptation to cooler regions (Tables 1 and 2). Late planting of this species can therefore reduce its germination and subsequent field establishment. Similar results have been reported for *Mentha cervina* L. and *Sideritis spinulosa* Cav. (Estrelles et al, 1999), found in habitats with relatively low average temperatures. In contrast, *O. biennis* had the highest germination percentage and rate at 30 °C, indicating an adaptation to warmer conditions.

Our results have shown that *O. majorana* seeds germinate best under moderate temperatures (Tables 1 and 2). Other close relatives of this species exhibited similar germination response to temperatures. *Origanum dictamnus* L. seeds showed optimal germination at relatively cool Mediterranean temperatures ranging between 10 and 20 °C; at sub-optimal temperatures, germination was simply inhibited (Thanos and Doussi, 1995). The highest germination percentage of *O. majorana* was obtained at a range of 15–20°C (Thanos, 2000), but germination declined at 25 °C. The highest germination percentage of *Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw. was obtained between 15 and 20 °C, while the highest germination rate was observed at 20 °C (Thanos et al., 1995). Comparable to our results, the optimum temperature for seeds of *Ocimum gratissimum* L. subjected to combination of light and temperature treatment was 25 °C (Obeme and Agboola, 2008). Based on those results, it seems that different species of a genus originating from similar environmental conditions (such as the Mediterranean) have similar germination responses to temperature.

Calculated cardinal temperatures (Table 3) showed that GR was strongly correlated with temperature. Similar linear relationships between GR and temperature have been reported by Bannayan et al. (2006), Kamkar et al. (2006), Zehtab-Salmasi (2006), Jami Al-Ahmadi and Kafi (2007), and Ghaderi et al. (2008). The temperature at which seed germination could be initiated, T_b, was quite similar and low (below 5 °C) for all plant species, ranging from 0–4 °C, with zero base temperatures observed for *S. sclarea*, *S. nemorosa*, *H. officinalis* and *C. benedicta* (Table 3). Optimum calculated temperatures ranged from 13–24 °C (Table 3), the lowest for *C. benedicta*. The calculated base and optimum temperatures of this species confirm its adaptation to low temperatures, similar to a conclusion reached by Bannayan et al. (2006) for *Rubia tinctorum*

TABLE 3. Calculated cardinal temperatures (°C), based on a linear model of germination rate-temperature of 11 medicinal plants.

Species	T_b	T_o	T_c	R^2
Centaurea benedicta	0	13.0	35.5	0.83**
Hyssopus officinalis	0	17.8	44.6	0.72*
Marrubium vulgare	4.0	17.8	42.6	0.91**
Nepeta racemosa	1.3	24.1	42.6	0.78*
Oenothera biennis	3.4	22.5	42.5	0.85**
Ocimum basilicum	4.0	23.2	41.3	0.95**
Origanum majorana	1.0	18.4	40.1	0.95**
Salvia sclarea	0	21.0	40.3	0.81**
Salvia nemorosa	0	17.0	41.0	0.85**
Silybum marianum	2.7	18.0	40.3	0.84**
Thymus daenensissub subsp. *daenensis*	3.1	22.7	42.4	0.86**

T_b: base temperature; T_o: optimum temperature; T_c: ceiling temperature.
*Significant at $p \leq 0.1$;
**Significant at $p \leq 0.05$.

L. and *Zataria multiflora* Boiss. All medicinal plant species of this study germinate in a wide range of temperatures from well below 5 °C up to 40 °C (Table 3). This indicates their ability and potential for germination and establishment in areas with different thermal conditions. Similarly, Jami Al-Ahmadi and Kafi (2007) reported a wide thermal range of germination for kochia (*Kochia scoparia* (L.) Schrad.), from 3.5 °C (T_b) to a maximum of 50 °C, and related this ability to potential successful establishment in a wide array of areas.

This study provides data for optimum and base temperatures for seed germination of 11 medicinal plants. Although this information is insufficient to make definite conclusions for introducing the species into cultivation, the germination data provides basic temperature requirements that can be used in further research.

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