Temperature Effects on Seed Germination Potential of Holy Basil (*Ocimum tenuiflorum*)

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ABSTRACT

Holy basil (*Ocimum tenuiflorum*) is an important herb used by Indians in their indigenous system of medicine, and commercially cultivated in hot and humid regions of the country. For development of quality seed standards, four parameters should be determined: optimum germination potential, optimum temperature at which maximum percent of seeds germinate, as well as first and final count days for germination and vigor testing. In this study, seed germination and two vigor indices of two holy basil varieties, CIM-Angana and CIM-Ayu, were evaluated at 20, 25, 30, 35 and 40 °C, coupled with 16 h light and 8 h dark photo periods. Maximum germination was observed at 30 °C (88%) for CIM-Angana, and at 25 °C (72%) for CIM-Ayu. Seedling vigor index I, based on seedling length and germination, was highest at 20 and 25 °C for CIM-Angana and CIM-Ayu, respectively, while seedling vigor index II, based on seedling mass and germination, was highest at 30 °C for both varieties. At 40 °C, seeds germinated but most developed into abnormal seedlings. The study revealed that holy basil seed germination should be carried out at a temperature of 30 ± 5 °C, and tests evaluated 5 to 6 d after seed sowing.

INTRODUCTION

Holy basil (*Ocimum tenuiflorum* L., syn. *O. sanctum* L.) is an important seed-propagated, essential oil-bearing medicinal herb, used for the treatment of cough, cold, asthma and general debility. It is commercially cultivated for its leaves in hot and humid regions of India. Eugenol is the major chemical constituent of the essential oil present in the leaves, with various uses in the flavoring and pharmaceutical industries.

Seed germination and vigor are the two most important seed quality parameters determining the success or failure of a crop (Hampton and TeKrony, 1995).Vigor and germination tests are essential to both commercial growers and seed production companies for ensuring crop performance (Karlovich, 1998; Oakley et al., 2004). Only those seeds that germinate rapidly and vigorously under favorable conditions in the laboratory are likely to be capable of producing vigorous seedlings in the field. Since holy basil is commercially cultivated through transplanting of nursery-raised seedlings, commercial growers often suffer from major losses due to substandard seeds. It is therefore essential to assess the germination potential and vigor in order to ensure an optimum crop stand and herb yield.

Seed germination is affected by both biotic and abiotic factors under natural conditions. Among the abiotic factors, light, moisture content, and temperature

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are the major factors affecting germination. Temperature plays an important role in seed germination capacity through its effects on seed deterioration rates, dormancy, and the germination process itself (Roberts, 1988). Temperature requirements for seed germination depend on species, variety, genotype, and sometimes on the agro-climatic condition of the cultivation area of the crop (Bewley and Black, 1994; Baskin and Baskin, 2001; Yilmaz, 2008; Verma et al., 2010; Kumar et al., 2011; Kumar, 2012). The optimum germination temperature is that resulting in the highest germination within the shortest period of time.

The aim of this research was to determine the optimal temperature regime for highest germination and seedling vigor of holy basil, so that commercial cultivation could be further diversified in various regions of India. The specific objectives were to determine (i) the highest germination percentage and seedling vigor index of variety CIM-Ayu and CIM-Angana under different temperature regimes, and (ii) optimum temperature for germination.

MATERIALS AND METHODS

Germination and seedling vigor tests

Seeds of holy basil, varieties CIM-Ayu and CIM-Angana, were collected in November 2012 from the experimental farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India. Seeds were stored at 27 ± 3°C until required for experimentation. The experiments were conducted during March–April 2013 at five constant temperature regimes, 20, 25, 30, 35 and 40 °C, coupled with a 16 h light and 8 h dark regime. Seeds were placed in Petri-dishes (16 cm diameter × 3 cm deep) on top of grade I, Qualigens single filter paper (15 cm diameter) soaked with sterile distilled water. Treatments (temperatures and varieties) were replicated four times, each Petri-dish containing 100 seeds. Data on normal seedlings were used for analyses. Germination percentage and seedling vigor index I and II were calculated according to ISTA rules (2010) and Kumar (2012). To determine seedling length, 10 normal seedlings from each replication were randomly selected at the end of the germination test, seedling length (root + shoot length) measured, and average seedling length (cm) per replication was then calculated. Seedling dry weight (g), determined using the same 10 seedlings, was obtained by placing seedlings in a paper envelop and drying under shade overnight, then transferring replicates to an oven set at 75 °C ± 5 °C for 48 h, followed by weighing to determine average seedling dry weight per replication. Seedling vigor index I (SVI-I) was calculated as:

$$\text{SVI-I} = \text{percentage germination} \times \text{average seedling length}$$

and seedling vigor index II (SVI-II) was calculated as:

$$\text{SVI-II} = \text{percentage germination} \times \text{average seedling dry weight}$$

Statistical analyses

Data were subjected to analysis of variance and mean separation using GenStat® Release 15.1. Percentage germination, SVI-I and SVI-II data were tested and verified for homogeneity of variance using Levene's statistic (PASW STATISTICS 18, Release 18.0.0; 2009). The least significant difference (LSD) test at the 5% probability level was used to compare means.

TABLE 1. Analysis of variance (mean squares) of the effect of variety and temperature regime on seed germination (%), seedling vigor index I (SVI-I), and seedling vigor index II (SVI-II), of holy basil (*Ocimum tenuiflorum*).

Source of variation	df	Germination	SVI-I	SVI-II
Replication	3	24.11	343.2	0.0001
Variety (V)	1	6815.28**	87471.8**	0.0847**
Temperature (T)	3	2537.78**	10126.6**	0.0263**
V × T	3	1902.78**	29193.9**	0.0278**
Error	18	16.31	408.6	0.0005

**Significant at $p \leq 0.01$.

RESULTS AND DISCUSSION

Seed germination of holy basil was affected by both variety and temperature. Analysis of variance indicated that variation due to these two main factors and their interaction was highly significant (Table 1). A significant variety by temperature interaction meant that the two varieties did not respond similarly across different temperatures. However, the optimum germination temperature for both varieties was between 25–30 °C (Table 2). CIM-Angana had higher germination, SVI-I and SVI-II compared to CIM-Ayu (Table 2). Overall, CIM-Angana was the better variety with respect to the three measured parameters (Table 2). Considering variety by temperature, CIM-Angana germinated best at 25 and 30 °C, followed by 20 °C. For SVI-I, 20 °C was the best temperature, followed by 30 °C and 25 °C, while for SVI-II, differences were less pronounced among temperatures. There was no significant difference in germination at 25, 30, and 35 °C of CIM-Ayu, all significantly higher than germination at 20 °C. To further explore temperature effects, when a Petri dish containing CIM-Ayu seeds exhibiting low germination (4.3%) at 20 °C was moved to 35 °C, germination increased to 85.0%, indicating that temperature played the major role in the germination process. At 40 °C, 79.8% of germinated seeds developed into abnormal seedlings and died on or after the 4th day of counting.

TABLE 2. Percentage seed germination (G), seedling vigor index I (SVI-I), and seedling vigor index II (SVI-II), under different temperature regimes, of two holy basil (*Ocimum tenuiflorum*) varieties.

	Temperature (°C)														
	20			25			30			35			Mean		
Variety	G	SVI-I	SVI-II	G	SVI-I	SVI-II	G	SVI-I	SVI-II	G	SVI-I	SVI-II	G	SVI-I	SVI-II
CIM-Ayu	4.3	12.1	0.0060	71.5	182.3	0.2400	70.5	175.8	0.2575	68.8	160.3	0.1625	53.8	132.6	0.1665
CIM-Angana	79.5	288.6	0.2775	86.0	239.1	0.2500	87.5	264.4	0.2925	78.8	156.7	0.2575	82.9	237.2	0.2694
Mean	41.9	150.3	0.1418	78.8	210.7	0.2450	79.0	220.1	0.2750	73.8	158.5	0.2100	68.3	184.9	0.2179

Variety LSD ($p \leq 0.05$): 3.6 (G), 27.8 (SVI-I), 0.0202 (SVI-II).
Temperature LSD ($p \leq 0.05$): 4.2 (G), 21.2 (SVI-I), 0.0226 (SVI-II).
Variety × temperature LSD ($p \leq 0.05$): 5.6 (G), 32.1 (SVI-I), 0.0304 (SVI-II).

Among abiotic factors, temperature is one of the critical factors in the germination of *Tagetes minuta* L. (Forsyth and Van Staden, 1983), *Cymbopogon martinii* (Roxb.) Will. Watson (Verma et al., 2010), *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Kumar et al., 2011), *Ocimum basilicum* L. (Kumar, 2012) and *Artemisia annua* L. (Kumar et al., 2013). Maximum seed germination (86–88% in CIM-Angana and 71–72% in CIM-Ayu) occurred in holy basil varieties at 25–30 °C, which seemed to be the optimum temperature for germination. In the present study, 80% of germinating seeds developed into abnormal seedlings at 40 °C. The primary reason for high temperature sensitivity in germinating seeds may be closely associated with the low rate of protein synthesis by the embryo, as reported for maize (*Zea mays* L.) (Riley, 1981). Gupta and Shahi (1998) reported 90% seed germination in *O. tenuiflorum* at 45 °C, 92% at 40 °C, 94% at 35 °C, and 95% at 30 °C, but such high percentages may be due to the inclusion of abnormal seedlings. Differential germination behavior of holy basil varieties under different temperature regimes indicated that germination capacity of the seeds was affected by temperature. Reduced temperature and critical high temperature would be expected to retard the metabolic rate to the point where pathways essential for the onset of germination would cease to operate (Roberts, 1988; Kumar et al., 2011).

Seed vigor can be influenced by genetic constitution, environmental factors, nutrition of the mother plant, maturity at harvest, seed size and mass, mechanical injury, aging, and pathogens. Uniformity would seem to be an essential component of seed vigor (Oakley et al., 2004). Rapid and uniform stand establishment is one objective for evaluating seed lots for vigor. Since mass is a better indicator of seedling growth/health than length, SVI-II (based on mass) should provide a better indication of early seedling vigor than SVI-I (based on length) (Kumar et al., 2012). In the present study, high SVI-II results at 30 °C, for both varieties, suggested that this temperature was best for raising healthy seedlings in nurseries. The decrease in SVI-I and SVI-II in both varieties at 35 °C was in agreement with results for peas (*Pisum sativum* L.) (Matthews, 1973), pigeonpea [*Cajanus cajan* (L.) Huth] (Khare and Satpute, 1999), lentils (*Lens culinaris* Medik.) (Khatun et al., 2009) and *Ocimum basilicum* (Kumar, 2012). The lowest SVI-II was recorded at 20 °C, due to lower germination and seedling dry mass. Harvesting the seed before the attainment of physiological maturity could also lead to decreased viability and vigor due to a large number of immature seeds with a relatively low degree of embryo development, along with high moisture content, as reported for lentils (Khatun et al., 2009) and peas (Matthews, 1973).

Based on results of this study, germination of holy basil seed should be carried out at a temperature of 30 ± 5 °C, and seeds evaluated 5–6 d after sowing. Growers should be advised to grow holy basil in nurseries at 30 °C, in order to obtain healthy seedlings.

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