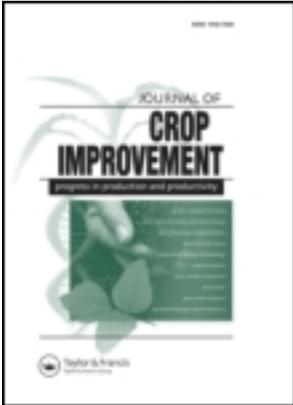


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## Temperature Relations for Seed Germination Potential and Seedling Vigor in Palmarosa (*Cymbopogon martinii*)

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*Because of non-synchronous flowering, fruiting, and dispersal of seeds, production and supply of quality seeds with well-defined germination potential parameters in Palmarosa are important issues for the growers. The objective of this study was to determine seed germination potential and seedling vigor of Palmarosa varieties under four temperature regimes at daily 16 h light/8 h dark regimes. At 25°C, germination percentage was highest for variety PRC-1 (67.8%), followed by varieties Tripta (59.3%), Trishna (57.5%), and Vaishnavi (35.5%). The mean for seedling vigor index I was highest for Trishna (277.7), followed by Tripta (259.3), PRC-1 (256.2), and Vaishnavi (140.4). However, vigor index II mean was highest for Tripta (0.2826), followed by PRC-1 (0.2615), Trishna (0.2600), and Vaishnavi (0.1388). Regardless of variety ranking by vigor index I vs. index II, the highest seedling vigor for all varieties was at 25°C using either index. However, since mass is a better indicator of seedling growth/health than length, vigor index II (based on mass) would give a better indication of early seedling vigor than index I (based on length). The low germination percentage and vigor of Vaishnavi is attributable to the genetic*

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*constitution of this self-pollinating variety. Significant decreases in germination percentage and vigor index I and II were observed at 20°C, 30°C, and 35°C. Days 2–3 and days 5–6 after sowing were the ideal times for making the first and final germination count, respectively. The study suggested that the Palmarosa nursery should have soil temperatures around 25°C with a day length of 16 h.*

**KEYWORDS** *Palmarosa, Cymbopogon martinii, temperature, germination, vigor, seed quality*

## INTRODUCTION

Palmarosa (*Cymbopogon martinii*) is an essential oil-bearing perennial crop, cultivated in India, Brazil, Paraguay, Madagascar, Guatemala, and Indonesia (Sahu, Debata, & Patnaik 2000; Singh & Kumar 2000; Verma et al. 2010). Its oil is used worldwide in the soap, perfumery, and cosmetic industries because its aroma is similar to that of rose (Sahu, Debata, & Patnaik 2000; Verma et al. 2010). India is a major producer and supplier of Palmarosa oil to the world market, and about 44 tons of Palmarosa oil were exported to about 19 countries, including the United States, France, and the Philippines during 2009–2010 (Directorate General of Commercial Intelligence and Statistics 2010).

In a laboratory test, seed germination is the emergence and development of a seedling to a stage where the aspect of its essential structures (root system, shoot axis, cotyledons, terminal buds) indicates whether it is able to develop further into a satisfactory plant under favorable soil conditions (International Seed Testing Association [ISTA] 2010). Because seeds are a basic and most important agricultural input for crop production, seedling emergence and successful establishment are critical for obtaining vigorous plants. Temperature is an important and critical factor that affects seed germination, and responses of seeds to a range of temperatures are an essential part of a germination test (Forsyth & Van Staden 1983; Roberts 1988; Bewley & Black 1994; Baskin & Baskin 2001; Kumar 2012). Germination is a complex biological process and several factors must work simultaneously for the emergence of a seedling. The percentage germination is one of the most important characteristics of the variety used for crop establishment. Only those seeds that germinate rapidly and vigorously under favorable conditions in the laboratory are likely to produce vigorous seedlings in the field. Effective stand establishment after germination is another important characteristic that gives an idea about the final population. Seed vigor is the sum total of those properties of the seed that determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. Seed vigor is an important seed quality parameter that

determines success or failure of a crop (Hampton & TeKrony 1995). High relative humidity and temperature lower seed viability and vigor. Seed vigor can be influenced by genetic constitution of the variety, environment and nutrition of the mother plant; maturity at harvest; seed size and mass; and mechanical injury, aging, and pathogen factors. Standard germination test results only provide an indication of emergence potential under favorable weather and soil conditions (Sharma 2000). The ability of seed lot to withstand inclement conditions during germination and seedling establishment is conferred by seed vigor. Thus, development of good vigor tests is essential to both commercial growers and seed production companies to ensure high crop performances (Karlovich 1998) and pre- and post-harvest processing for premium quality seed production (Oakley, Kester, & Geneve 2004). Because Palmarosa is seed propagated and commercial growers suffer major losses on account of substandard seeds, it is essential to assess the germination potential and vigor of seeds to ensure a good crop stand and high yield of oil. The present study is a continuation of our earlier study (Verma et al. 2010) to define the temperature and varietal effects and optimize the seed germination potential and seedling vigor under different temperatures for good crop establishment and high productivity.

## MATERIAL AND METHODS

### Seed

Seeds of Palmarosa varieties, viz., PRC-1, Tripta, Trishna, and Vaishnavi, were collected in November 2009 from plants growing on the experimental farm of the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. These are the improved varieties developed by CIMAP and are cultivated by growers in India. Seeds were removed and stored at 27°–39°C in paper bags until required for experimentation.

### Germination and Seedling Vigor Tests

The experiment was conducted during May–June 2010 at constant temperatures of 20°, 25°, 30°, and 35°C using daily 16 h light (180 lx)/8 h dark regimes. One hundred seeds were placed per Petri dish (16 cm diameter × 3 cm depth) lined with filter paper (TP) (15 cm diameter) that was soaked with sterile distilled water. The treatments (temperature and variety) were replicated four times. Germination was checked daily and number of normal (bearing both root and shoot) and abnormal (lacking either root or shoot, or stunted growth) seedlings recorded from the first day of counting until the day of maximum seed germination (Kumar, Verma, & Singh 2011). In Palmarosa, there are two types of abnormal seedlings, i.e., shoot without root and root without shoot, which do not exist in other crops (Figure 1).



**FIGURE 1** Shows normal seedlings (right) and abnormal seedlings (left) of the Palmarosa crop.

Data on normal seedlings were used for analyses. Germination percentage, seedling vigor index I, and seedling vigor index II were calculated as shown below (ISTA 2010; Kumar, Verma, & Singh 2011; Kumar 2012):

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds in all replicates}} \times 100$$

From each replication, five normal seedlings were selected randomly at the end of the germination test, and seedling length (root + shoot length) was measured. Mean seedling length (cm) was calculated.

The same five seedlings were placed in a paper envelope and dried under shade for 16 h. These dried seedlings were then placed in an oven at  $75^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 48 h, after which they were weighed to determine mean seedling dry mass (mg) in each replication. Seedling vigor index I and vigor index II were calculated as follows:

$$\text{Seedling vigor index I} = \text{Germination (\%)} \times \text{mean seedling length}$$

$$\text{Seedling vigor index II} = \text{Germination (\%)} \times \text{mean seedling dry mass}$$

### Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) and mean separation. Data were analyzed using GenStat Release 7.21. Least significant difference (LSD) at 5% level was computed for varieties (V) and temperatures (T) when  $V \times T$  was not significant. When  $V \times T$  was significant, an LSD to compare  $V \times T$  means was computed.

## RESULTS AND DISCUSSION

## Germination Percentage

Variety and temperature affected Palmarosa seed germination, and variations caused by these two main factors and their interaction were highly significant (Table 1). A significant variety  $\times$  temperature interaction means that all the varieties did not perform consistently across different temperatures. However, the best germination temperature for all varieties was 25°C. What varied among the varieties was the response to temperatures above and below the optimum for germination. For all varieties, except Vaishnavi, germination decreased more at a temperature above 25°C (i.e., 30° and 35°C) than it did at 20°C. In the case of Vaishnavi, maximum germination at 25°C was only 35.5%, and it was 14.0% at both 20° and 30°C and only 17.5% at 35°C. Thus for all varieties except Vaishnavi, if seeds cannot be germinated in the nursery at 25°C, then higher germination would occur at a temperature a little lower than 25°C than it would at temperatures higher than 25°C. The low germination of Vaishnavi would seem to indicate that this is a poor choice for cultivation. However, it is a self-pollinated variety and as such the quality parameters of its essential oils are stable, making it a variety of considerable interest to researchers for use in genetic improvement of Palmarosa as a crop.

The mean seed germination percentage of Palmarosa varieties across temperatures varied from 20.3% (Vaishnavi) to 50.1% (PRC-1). Across varieties, germination percentage varied from 31.4% (35°C) to 55.0% (25°C) (Table 2). Among the temperatures, 25°C recorded the highest mean germination percentage averaged across varieties (55.0%), followed by 20°, 30°, and 35°C. Averaged across temperatures, PRC-1 had the highest mean germination percentage (50.1%), followed by Tripta and Trishna (46.4% and 43.7%, respectively). Vaishnavi had the lowest mean germination percentage (20.3%). At 25°C, PRC-1 showed the highest germination percentage (67.8%), followed by Tripta (59.3%), Trishna (57.5%), and Vaishnavi (35.5%).

**TABLE 1** Analysis of variance (ANOVA) for seed germination percentage (G), seedling vigor index I (SVI-I), and seedling vigor index II (SVI-II) of four Palmarosa varieties under different temperature (T) regimes

Source	Df	Mean square		
		G	SVI-I	SVI-II
Replications	3	37.27	576.60	0.0004205
Temperatures (T)	3	1836.77**	36731.50**	0.0526267**
Varieties (V)	3	2916.10**	53758.00**	0.0359643**
T $\times$ V	9	80.65**	1817.40	0.0011100
Error	45	21.42	945.70	0.0005841

\*\*denote significance at the 1% probability level.

**TABLE 2** Mean germination percentage (G), seedling vigor index I (SVI-I), and seedling vigor index II (SVI-II) of four Palmarosa varieties (V) (PRC-1, Trishna, Tripta, and Vaishnavi) under four temperature (T) regimes

Temperature	G (%)															
	PRC-1				Tripta				Vaishnavi				Mean			
	PRC-1	Trishna	Tripta	Vaishnavi	PRC-1	Tripta	Tripta	Vaishnavi	PRC-1	Tripta	Tripta	Vaishnavi	PRC-1	Tripta	Tripta	Vaishnavi
20°C	51.0	45.0	53.0	14.0	40.8	138.1	136.1	172.4	172.4	45.3	123.0	0.1365	0.1299	0.1645	0.0562	0.1218
25°C	67.8	57.5	59.3	35.5	55.0	256.2	277.7	259.3	259.3	140.4	233.4	0.2615	0.2600	0.2826	0.1388	0.2357
30°C	43.5	34.8	40.8	14.0	33.3	238.9	201.5	239.8	239.8	74.7	188.7	0.1377	0.1429	0.1682	0.0570	0.1264
35°C	38.3	37.5	32.5	17.5	31.4	179.4	175.9	158.9	158.9	88.7	150.7	0.1196	0.1454	0.1294	0.0691	0.1159
Mean	50.1	43.7	46.4	20.3	—	203.2	197.8	207.6	21.90	150.7	—	0.1638	0.1695	0.1862	0.0803	—
LSD 5% for V means									21.90							
LSD 5% for T means									21.90							
LSD 5% for V × T means									NA							

Lowest germination percentage was recorded for Vaishnavi (14.0%) at 20° and 30°C, followed by Tripta (32.5%), Trishna (37.5%), and PRC-1 (38.3%) at 35°C (Table 2). The optimum germination in respect to all varieties was observed at 25°C and was followed by 20°C, except variety Vaishnavi; the latter recorded the lowest germination at 20° and 30°C. The discrepancy in germination behavior of Vaishnavi may be because of its deviated reproductive behavior and genotype. Decrease in germination at 30° and 35°C from 25°C was significant for all the varieties; however, from 30° to 35°C neither a significant increase (Trishna and Vaishnavi) nor a decrease (PRC-1 and Tripta) was observed. Thus, the results indicated that the temperature of 25°C was optimum for Palmarosa seed germination and that an increase in temperature beyond 25°C would lead to a decrease in germination.

### Seedling Vigor Index I & II

The variety and temperature affected both seedling vigor index I and seedling vigor index II of Palmarosa seeds, and variation attributable to these two main factors was highly significant. The mean seedling vigor index I and II of Palmarosa varieties across temperatures varied from 150.7 and 0.0803 (Vaishnavi) to 207.6 and 0.1862 (Tripta), respectively. For all varieties, the highest values for seedling vigor indices I and II were recorded at 25°C, which was the optimum temperature for germination of all the varieties. However, unlike the variety × temperature interaction for germination, there was no consistent pattern with regard to lowest seedling vigor at temperatures higher than or lower than 25°C. Growers would want to avoid the conditions that result in the lowest seedling vigor, but as can be seen in Table 2, this varies with the variety. The optimum seedling vigor index I for all varieties was observed at 25°C, followed by 20°C, except variety Vaishnavi, which had the lowest germination at 20° and 30°C. The differential response of Vaishnavi relative to seedling vigor index I may be attributed to its deviated reproductive behavior (self-pollination) and genetic background, as previously noted. Decrease in seedling vigor index I at 35°C (compared to that at 25°C) was significant for all the varieties. A significant decrease in seedling vigor index I with a temperature increase from 30° to 35°C was recorded in varieties Trishna, Tripta, and PRC-1, but not Vaishnavi (Table 2). Thus, the results indicated that 25°C was the optimum temperature for Palmarosa seedling vigor index I; temperatures beyond 25°C caused a decrease in seedling vigor index I. For seedling vigor index II, 25°C also was the optimum temperature, followed by 30°C in varieties Tripta, PRC-1, and Trishna, but not variety Vaishnavi for which 25°C followed by 35°C was optimal. Decrease in seedling vigor index II at 30° and 35°C (compared to 25°C) was significant for all the varieties. A significant decrease in vigor index II with a temperature increase from 30° to 35°C occurred in varieties Tripta

and PRC-1 but not Trishna and Vaishnavi (Table 2). Thus, the results indicated that the temperature of 25°C was optimum for Palmarosa seedling vigor index II, and temperatures beyond 25°C decreased seedling vigor index II.

Perusal of Table 3 reveals that, in respect to the studied parameters, 25°C was most conducive temperature for maximum germination percentage and high seedling vigor as indicated by index I and II. Hence, 25°C can be concluded as the optimum, but no definite pattern could be established regarding other temperatures under consideration. While 20°C was the second best temperature with respect to germination percentage with one exception of variety Vaishnavi, 30°C was the second best temperature for seedling vigor index I. The trend regarding seedling vigor index II was not decisive; the temperature of 30°C was found second best for PRC-1 and Tripta and 35°C for Trishna and Vaishnavi (Table 3).

Both seedling vigor indices are a measure of seedling growth. Vigor index I considers seedling length, which is quickly measured using a ruler in the laboratory situation. Vigor index II considers seedling mass, which requires that seedlings be weighed after they are dried in an oven. Thus, in most seed laboratories, vigor index I is used more frequently than vigor index II because it is faster. Our results showed that the same information relative to the optimum temperature conditions for seedling growth could be obtained with both indices. However, since seedling mass is probably a better indication of seedling health than seedling length (i.e., in poor light, seedlings might be etiolated), we suggest that more accurate information about the potential for early seedling growth of varieties could be obtained by using vigor index II than vigor index I.

Because the seedling vigor index is indicative of the survival potential and health of Palmarosa seedlings, information about the effects of temperature on early growth of seedlings should allow growers to manage conditions for optimal growth of each variety. For example, although 25°C is the best for germination and early seedling growth of a variety, if this temperature is not available it would be better to use 20°C for some varieties and 30°C for others (Table 3). The decision of which temperature is best would depend on the variety. Thus, if 25°C were not continuously available, it probably would be better to have a situation where the temperature is lower (20°C) during germination and then increases during the early growth of the seedlings.

The variable seedling vigor index may be attributed to the genetic constitution of individual varieties (Sharma 2000). Harvesting the seed before attainment of physiological maturity also could decrease viability and vigor potential because of a large number of immature seeds with poor embryo development and high moisture content as reported in *Lens culinaris* (Khatun, Kabir, & Bhuiyan 2009) and *Pisum sativum* (Matthews 1973).

**TABLE 3** Optimum temperatures for germination percentage and seedling vigor index I and II of four Palmarosa varieties

Variety	Trait											
	Germination (%)				Seedling Vigor Index I				Seedling Vigor Index II			
	Most optimum temperature °C	Second best temperature °C	Least desirable temperature °C	Most optimum temperature °C	Second best temperature °C	Least desirable temperature °C	Most optimum temperature °C	Second best temperature °C	Least desirable temperature °C	Most optimum temperature °C	Second best temperature °C	Least desirable temperature °C
PRC-1	25°C	20°C	35°C	25°C	30°C	20°C	25°C	30°C	20°C	25°C	30°C	35°C
Trishna	25°C	20°C	30°C	25°C	30°C	20°C	25°C	30°C	20°C	25°C	35°C	20°C
Tripta	25°C	20°C	35°C	25°C	30°C	35°C	25°C	30°C	35°C	25°C	30°C	35°C
Vaishnavi	25°C	35°C	20° & 30°C	25°C	35°C	20°C	25°C	35°C	20°C	25°C	35°C	20°C

## CONCLUSIONS

Maximum germination percentage and seedling vigor index I and II were recorded at 25°C temperature at day 2 and day 5 for first and final count day, respectively, in Palmarosa varieties. However, with regard to germination and seedling vigor there are varietal differences in how seeds respond to temperatures above and below the optimum. Knowing how seeds/seedlings of the different varieties respond to temperatures above and below the optimum (25°C) will allow growers to more effectively manage conditions for germination and early seedling growth. Further, the use of vigor index II may help identify the best variety to plant under less than optimal conditions.

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