Effect of temperature on germination of *Citrus macroptera*, *Citrus latipes* and *Citrus indica* seeds

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**Abstract**

Seeds are an important means of propagation of Citrus species. Seeds of three wild Citrus namely; *Citrus macroptera* Montrouz., *Citrus latipes* (Swingle) Tanaka and *Citrus indica* Tanaka were germinated at 20°C, 25°C, 30°C and 35°C temperature to observe the effect of temperature on germination. Mean germination time and percentage seed germinated were recorded and used to determine optimum temperature for germination. Viability of seeds determined using chemical and germination tests yielded similar results. Optimum temperature for germination was found to be 28°C for *C. macroptera* and *C. latipes* and 26°C for *C. indica*.

**Keywords:** Germination, wild, *C. macroptera*, *C. latipes*, *C. indica*, Meghalaya

**Introduction**

*Citrus* has been domesticated since ancient times, and where ‘natural’ populations are located, it is often difficult to determine whether they represent wild ancestors or are derived from naturalized forms of introduced varieties. Though relatively rare in wild, *Citrus* are mostly found as scattered trees in primary forests in remote areas rather than as pure stands. In India, a vast reservoir of *Citrus* diversity exists both in wild and in cultivated forms. North-eastern India is considered as natural home of many *Citrus* species with wide occurrence of indigenous species like *C. macroptera*, *C. latipes* and *C. indica* (Malik et al., 2006). Though cultivated worldwide, some species of *Citrus* like *C. macroptera*, *C. latipes*, *C. indica*, *C. ichangensis* and *C. assamensis*, are still found in wild (Singh, 1981). Researchers have described this region as hot spot for *Citrus* biodiversity and have highlighted that erosion of these genetic resources is a cause of concern (Chadha, 1995; Singh et al., 2001). *C. macroptera* and *C. latipes* are used by many traditional societies for their medicinal and culinary properties (Upadhaya, 2013; Upadhaya et al., 2016). However, lack of cultivation of these species and loss of natural forest cover has underlined need to adopt complementary conservation strategies to ensure continued existence of these species in future (Malik et al., 2006).

Seeds offer a convenient way to store germplasm over time and to transport it over long distances even across international boundaries (Boswell, 1961; Heydecker, 1972) and adequate seed germination is the key to successful tree establishment (Radosevich et
Seeds are particularly valuable in *Citrus* culture because of their importance in the establishment of nursery stocks and because seeds are known to be relatively free from the common *Citrus* virus and fungal diseases (Kernick, 1961; Onwueme, 1978; Fawusi, 1989). Moreover, *Citrus* seeds are easily obtainable, relatively inexpensive, plentiful, and grow true-to-type (Castle, 1981; Rouse and Sherrod, 1996).

The effect of temperature on the germination of various commercially important species of *Citrus* has been studied by a number of researchers (Wiltbank et al., 1995; Saipari et al., 1998). However, such information is lacking on the fast diminishing wild *Citrus* species that have been used by tribal communities since ages. This study was conducted to determine the viability of the seed and its optimum temperature for germination.

**Materials and Methods**

Khasi Papeda (*Citrus latipes* (Swingle) Tanaka), Melanesian Papeda (*Citrus macroptera* Montr.) and Indian wild orange (*Citrus indica* Tanaka) are the three wild *Citrus* found in Meghalaya. They are culturally important species and are used in traditional healing system. The three species belongs to two subgenera, *Citrus* and *Papeda* (Spiegel-Roy and Goldschmidt, 1996), distinguished by leaf, flower and fruit characteristics. Seeds of these fruits were extracted from ripe fruits, rinsed thoroughly and soaked for 24 hrs in distilled water. These were then treated with sodium hypochlorite solution (0.1 %) and rinsed. As a general rule the fresh seeds as soon as they were removed from the fruit were used for the tests.

Seed viability test for these species was done using TTZ test given by Patil and Dadlani (2009) and compared to result of germination. Germination under different temperature was tested in the controlled condition in a Seed Germinator. Ten replicates of 10 seeds each in the wet filter paper was placed in Petri-dish and kept at 20°C, 25°C, 30°C and 35°C under light conditions of 16 hr light and 8 hr dark following Barton (1943). Daily records were made to observe the germination and the percentage of germination (100 X number of seedlings emerged ÷ number of seeds planted) was calculated for each temperature.

**Results**

Seed viability test of the three species of *Citrus* using TTZ test showed an average of 90 % viability in *C. macroptera*, 91 % in *C. latipes* and 94 % in *C. indica*. The percentage seed germination of the three species of *Citrus* under different temperature is given in the Table 1. Germination in all three species increased with increase in temperature up to 30°C and decreased at 35°C. For *C. macroptera* and *C. latipes*, gradual increase in temperature enhances seed germination rate and decreases the number of days required for germination, whereas at 35°C temperature, the number of days required for germination also increased. While for *Citrus indica* unlike other two species, the number of days required for germination decreased at even 35°C.
ANOVA was performed to determine the effect of temperature on the percentage of germination and mean number of days required for the germination for all the three species. Variation was found to be statistically significant (Table 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature (°C)</th>
<th>No of days</th>
<th>Percentage germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>mean number of days</td>
<td></td>
</tr>
<tr>
<td>Citrus latipes</td>
<td>20 10-20</td>
<td>15</td>
<td>12***</td>
</tr>
<tr>
<td></td>
<td>25 9-20</td>
<td>14</td>
<td>81***</td>
</tr>
<tr>
<td></td>
<td>30 7-20</td>
<td>13</td>
<td>92***</td>
</tr>
<tr>
<td></td>
<td>35 15-25</td>
<td>20</td>
<td>38***</td>
</tr>
<tr>
<td>Citrus macroptera</td>
<td>20 12-20</td>
<td>16</td>
<td>10***</td>
</tr>
<tr>
<td></td>
<td>25 10-20</td>
<td>15</td>
<td>76***</td>
</tr>
<tr>
<td></td>
<td>30 7-20</td>
<td>13</td>
<td>89***</td>
</tr>
<tr>
<td></td>
<td>35 15-25</td>
<td>20</td>
<td>37***</td>
</tr>
<tr>
<td>Citrus indica</td>
<td>20 9-20</td>
<td>14</td>
<td>88***</td>
</tr>
<tr>
<td></td>
<td>25 7-20</td>
<td>13</td>
<td>91***</td>
</tr>
<tr>
<td></td>
<td>30 7-10</td>
<td>13</td>
<td>96***</td>
</tr>
<tr>
<td></td>
<td>35 7-9</td>
<td>8</td>
<td>24.7***</td>
</tr>
</tbody>
</table>

LSD: the mean difference is significant at the 0.05 level (P < 0.001)

Further, correlation analysis was also performed to obtain the optimum temperature required by the Citrus seeds to germinate. The regression equation derived from the second degree polynomial (parabolic curve) best describes the correlation of the effect of temperature on time for Citrus seed germination (Wiltbank et al., 1995). The optimum temperature for germination was calculated from the derivative given by Rouse and Sherrod (1996).

\[
\frac{dy_{diff}}{dx} = 2ax+b
\]

\[
0 = 2ax+b
\]

\[
x = \text{Max/Min or optimum temperature}
\]

The equation was derived by subtracting the polynomial regression equation of the mean days to germination from percentage equation for each species.
Table 2 and Figure 1 describe the polynomial regression equation of mean days to germination and percentage germination by temperature for all three species. Germination percentage and number of days required to germinate was positively correlated to the temperature. The germination increases with the increase in temperature from 20b°C to 30 °C whereas with further increase to 35 °C, the germination percentage decreases.

Table 2: Regression equations, correlation coefficient of temperature on days to germination and percentage germination of seeds of three Citrus species with significance level

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable</th>
<th>Regression equation</th>
<th>$R^2$</th>
<th>Significance P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus macroptera</td>
<td>Mean days to germination</td>
<td>$0.075x^2 - 3.915x + 64.725$</td>
<td>0.844</td>
<td>0.000 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Percentage germination</td>
<td>$-1.180x^2 + 66.780x - 854.2$</td>
<td>0.8</td>
<td>0.000 (0.05)</td>
</tr>
<tr>
<td>Citrus latipes</td>
<td>Mean days to germination</td>
<td>$0.070x^2 - 3.570x + 58.5$</td>
<td>0.873</td>
<td>0.000 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Percentage germination</td>
<td>$-1.230x^2 + 69.430x - 884.950$</td>
<td>0.946</td>
<td>0.000 (0.05)</td>
</tr>
<tr>
<td>Citrus indica</td>
<td>Mean days to germination</td>
<td>$0.005x^2 - 0.765x + 28.255$</td>
<td>0.893</td>
<td>0.429 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Percentage germination</td>
<td>$-0.910x^2 + 47.270x - 512.550$</td>
<td>0.698</td>
<td>0.000 (0.05)</td>
</tr>
</tbody>
</table>

Table 2 and Figure 1(a) describe the polynomial regression equation of mean days to germination and percentage germination by temperature for all three species. Germination percentage and number of days required to germinate was positively correlated to the temperature. The germination increases with the increase in temperature from 20b°C to 30 °C whereas with further increase to 35 °C, the germination percentage decreases.

**Figure 1(a):** Mean days to germination and percentage germination in relation to temperature for C. macroptera
The number of days required for germination decreased with the increase in temperature from 20 °C to 30 °C, but further increase in temperature to 35 °C, led to the reversing of effect leading to increase in the number of days required for germination except in *Citrus indica*. In *Citrus indica*, even after increase in temperature from 30 °C to 35 °C, time required for germination did not increase. The equation was positively correlated to temperature with the coefficient value of more than 0.8 in all cases except for *C. indica*.
Thus the relationship between the temperature and germination and time required based on regression analysis indicated that temperature enhances percentage germination and also time up to the optimum temperature beyond which it could be detrimental. Table 3 shows the calculated optimum temperature for germination for all three species. The graphical representation of the result also conforms to the obtained data. The optimum temperature for germination was near to 30°C. This point of optimum germination, defined as the temperature at which the greatest number of seeds germinated in the least number of days and also the overlap of the two curves are at the furthest distance from each other (Figure 1). Table 3 shows the optimum temperature for germination with the range of temperatures in which seed germination was carried out and range of days required for germination.

Table 3: Optimum Temperature, Germination Temperature Range and Days to Germination Range for the three Citrus species

<table>
<thead>
<tr>
<th>Species</th>
<th>Optimum Temperature (°C) for Germination</th>
<th>Germination Temperature Range (°C)</th>
<th>Days to Germination Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus macroptera</td>
<td>28.16</td>
<td>20-35</td>
<td>7-25</td>
</tr>
<tr>
<td>Citrus latipes</td>
<td>28.07</td>
<td>20-35</td>
<td>7-25</td>
</tr>
<tr>
<td>Citrus indica</td>
<td>26.24</td>
<td>20-35</td>
<td>7-20</td>
</tr>
</tbody>
</table>

Figure 2: Percentage laboratory germination and viability of three Citrus species

Seed viability test of the three species of Citrus using TTZ test showed an average of 90% viability in C. macroptera, 91% in C. latipes and 94% in C. indica. Seed viability tested using germination at the optimum temperature of 30°C for the three species showed the seed viability of 89%, 92% and 96% respectively. Seed viability measured by tetrazolium staining, paralleled the germination percentage tested by seed
germination in the germinator at 30°C, the optimum temperature (Figure 2). ANOVA was used to test whether the two methods showed different results. It was found that the two tests did not differ significantly.

Discussion

Citrus seeds have been reported to germinate at a wide range of temperatures with the minimum temperature of about 6 °C and a maximum of about 39 °C and the optimum temperature for several varieties range from 26 °C to 30 °C (Camp 1933; Mobayben 1980; Soetisna et al., 1985). In the present study, the optimum temperature for germination for the three species of Citrus, namely, C. macroptera, C. latipes and C. indica ranged between 26-28 °C. This result is in conformity with that of Ellis et al. (1985) and Rouse and Sherrod (1996), who also found similar temperature optima for Citrus seed germination.

Percentage germination increased with increase in temperature up to the optimum and subsequently it decreased with further increase in temperature above optimum, which is similar to the findings of Rouse and Sherrod (1996) in case of 17 varieties of Citrus. Detrimental effect of temperature higher than the optimum on germination of Citrus seeds has also been reported by Wiltbank et al. (1995). Mean days required for germination of seed of the three species of Citrus decreased as temperature increased up to the optimum. However, continued increase in the temperature above the optimum resulted in the increase in mean days required for germination of seeds of C. macroptera and C. latipes. Rouse and Sherrod (1996) and Ellis et al. (1985) also reported similar effect of temperature on germination of seeds of Citrus species. While in case of C. indica, with the increase in temperature from 30 to 35°C, the germination time decreased sharply but this resulted into very less total percentage germination as a large number of seeds were damaged at this temperature.

All the three species viz., C. macroptera, C. latipes and C. indica grow in subtropical climate mostly on the hills at an elevation ranging from 500 m to 1900 m above msl. Temperature range suitable for seed germination of these species recorded in experiment are closer to ambient temperature of their places of occurrence in nature suggesting that probably temperature is one of the important ecological factor regulating the distribution of the Citrus species. The study has shown that sensitivity of three species to temperature varies. C. indica showed a high germination of 88% at a temperature as low as 20 °C, while in case of the other two species the seed germination at 20 °C was about 10%. The effect of temperature on seed germination of C. latipes and C. macroptera was found to be similar, whereas C. indica behaved in a different way. Bayer et al. (2009) based on their findings from cpDNA sequence study showed that C. macroptera and C. latipes are closely related while C. indica is genetically away from these two. Spiegel-Roy and Goldschmidt (1996) also classified C. macroptera and C. latipes in subgenera “Papeda” and C. indica in subgenera “Citrus”. These genetic variability may be the reason for the variation in their sensitivity to temperature.
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Seed viability measured by tetrazolium staining, paralleled the germination percentage tested by seed germination in the germinator at 30 °C, the optimum temperature. This indicates that almost all viable seeds of the *Citrus* species studied germinated at optimum temperature. Similar findings were reported by Malik *et al.* (2006) on the viability of seeds of *C. macroptera* and *C. indica*. The findings of this study is also in close conformity with that of Saipari *et al.* (1998) who worked on the viability of seeds of *C. karna, C. jambhiri* and *C. grandis*. It was found that the optimum temperature for seed germination for the three species varied between 26 – 28 °C.

Acknowledgement

The authors are thankful to the University Grants Commission, New Delhi for the financial assistance to A. Upadhaya in the form a Junior Research Fellowship.

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