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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* *al.,* 1997). 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 1: Germination percentage of three *Citrus* species at different temperature

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

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

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

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.

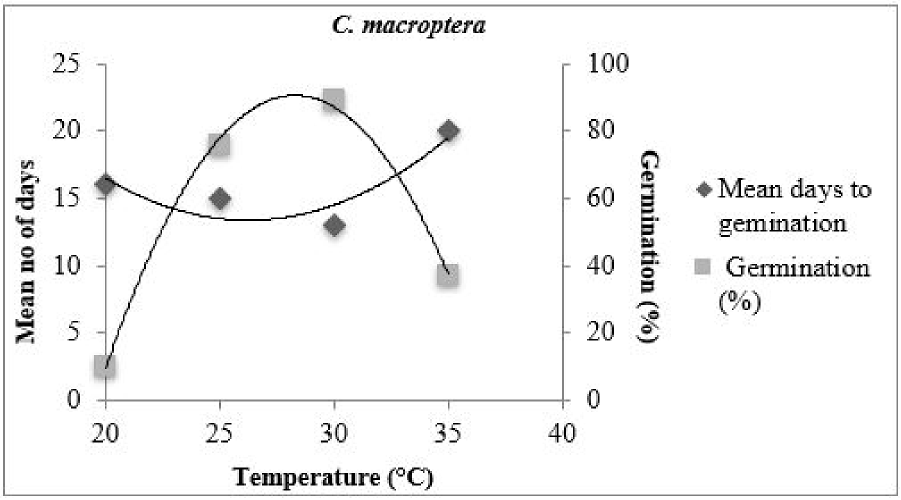

Figure 1(a): Mean days to germination and percentage germination in relation to temperature for C. macroptera

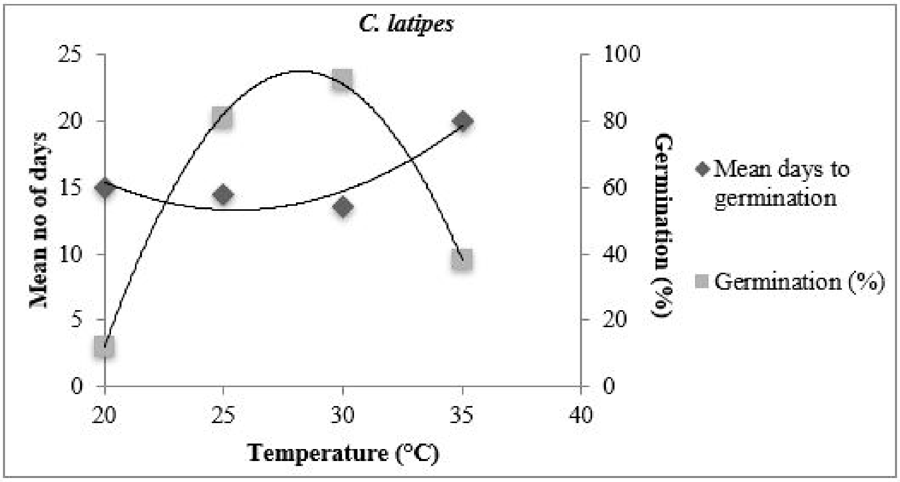

Figure 1(b): Mean days to germination and percentage germination in relation to temperature for *C. latipes*

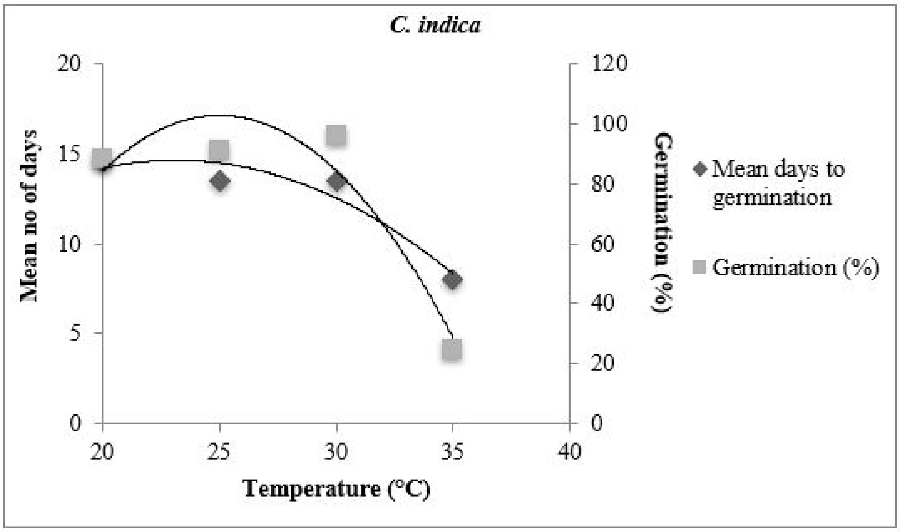

Figure 1(c): Mean days to germination and percentage germination in relation to temperature for *C. indica*.

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*.

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

Species	Optimum Temperature (°C) for Germination	Germination Temperature Range (°C)	Days to Germination Range
Citrus macroptera	28.16	20-35	7-25
Citrus latipes	28.07	20-35	7-25
Citrus indica	26.24	20-35	7-20

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.

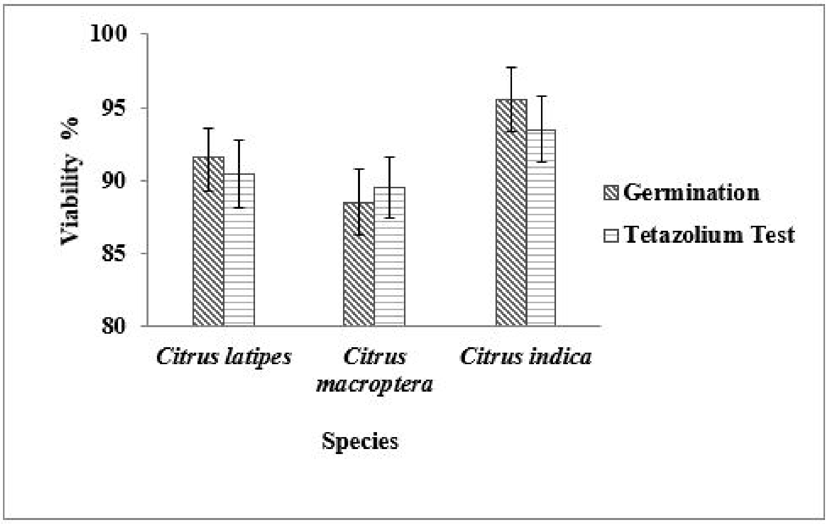

Figure 2: Percentage laboratory germination and viability of three *Citrus* species

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 sub-tropical 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.

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.

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References

Bayer, R.J., Mabberley, D.J., Morton, C., Miller, C.H., Sharma, I.K., Pfeil, B.E., Rich, S., Hitchcock, R. and Sykes, S. 2009. A molecular phylogeny of the orange subfamily (Rutaceae: Aurantioideae) using nine cpDNA sequences. *American Journal of Botany*, 96(3): 668–685.

Barton, L.V. 1943. The storage of citrus seeds. *Contributions from Boyce Thompson Institute*, 13: 47-55.

Boswell, V.R. 1961. The importance of seeds. In: A. Stefferud, (ed.), *Yearbook of Agriculture*, U.S.D.A. Washington D.C.: 1-10

Camp, A.F., Mowry, H. and Loucks., K.W. 1933. The effects of soil temperature on the germination of *Citrus* seeds. *American Journal of Botany*,20: 348–357.

Castle, W.S. 1981. A review of Citrus seed biology and its relationship to nursery practices. *Proceeding International Society of Citriculture*, 1: 113-119.

Chadha, K.L. 1995. Status report on tropical fruit species in South Asia, In: R.K. Arora and V.R. Rao, (eds.), *Proceedings expert consultation on tropical fruit specially Asia*, MARDI Serdang Kuala Lumpur, IPGRI Office for South Asia, New Delhi: 45–60.

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1985. *Handbook of Seed Technology for Genebanks*. Vol II. IBPGR, Rome, Italy: 456.

Fawusi, M.O.A. 1989. Seed germination, emergence, biochemical changes and early seedling performance in *Cleopatra mandarin* (*Citrus reticulata* Blanco) following controlled environment storage. *Biotronics*, 18: 29-35.

Heydecker, W. 1972. Vigour, In: E.H. Roberts (ed.), *Viability of Seeds*, Chapman and Hall, London: 209-252.

Kernick, M.O. 1961. *Citrus*. In: *Agricultural and Horticultural Seeds*. Food and

Agriculture Organization: 379–384.

Malik, S.K., Chaudhury, R, Dhariwal, O.P. and Kalia, R.K. 2006. Collection and characterization of *Citrus indica* Tanaka and *C. macroptera* Montr.: wild endangered species of North-eastern India. *Genetic Resource and Crop Evolution*, 53: 1485–1493.

Mobayben, R.G. 1980. Germination and emergence of *Citrus* and tomato seeds in relation to temperature. *Journal of Horticultural Science*, 55(3): 291-297.

Onwueme, I.C. (1978). *Crop Science*. Cassell, London, 16–81.

Patil, V.N. and Dadlani, M. 2009. Tetrazolium test for seed viability and vigour. In: *Handbook of Seed Testing*. 209–241.

Radosevich, S., Holt, J. and Ghersa, C. 1997. *Weed Ecology: Implications for Management.* 2nd edition. John Wiley and Sons, Inc., New York: 589.

Rouse, R.E. and Sherrod, J.B. 1996. Optimum temperature for *Citrus* seed germination. *Proceedings of Florida State Horticultural Society*, 109:132–135.

Saipari, E., Goswami, A.M. and Dadlani, M. 1998. Effect of seed drying on germination behaviour in *Citrus*. *Scientia Horticulturae*, 73:185–190.

Singh B. 1981. *Establishment of First Gene Sanctuary for* Citrus *in Garo Hills*. Concept Publication Co, New Delhi: 182.

Singh, I.P., Singh, S., Singh K. and Srivastava, R. 2001. Exploration and collection of *Citrus* germplasm from NEH region (Meghalaya) of India. *Indian Journal of Plant Genetic Resources*, 14: 70–73.

Spiegel-Roy, P. and Goldschmidt, E.E. 1996. *Biology of* Citrus. Cambridge University Press, Cambridge, New York: 230.

Soetisna, U., King, M.W. and Roberts, E.H. 1985. Germination test recommendation for estimating the viability of moist or dry seeds of lemon- *C. limon* and lime *C. aurantifolia*. *Seed Science and Technology*, 13(1): 87–110.

Upadhaya, A., Chaturvedi, S.S. and Tiwari, B.K. 2016. Utilization of wild *Citrus* by Khasi and Garo tribes of Meghalaya. *Indian Journal of Traditional Knowledge*, 15(1):121-127.

Upadhaya, A. 2013. Biodiversity and nutritive vaue of wild and semi-wild *Citrus* species of Meghalaya, Ph.D. Thesis: Department of Environmental Studies, North Eastern Hill University, Shillong Meghalaya.

Wiltbank, W.J., Rouse, R.E. and Khoi, L.N. 1995. Influence of temperature on *Citrus* rootstock seed emergence. In: *Proceedings of Florida State Horticultural Society*. 108: 137–139.