

***In vitro* Seed Germination of *Citrus macroptera* Mont. – An Endangered Species of Meghalaya**

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Abstract *Citrus macroptera* Mont. locally known as Chambil is a fruit tree growing in semi-wild conditions of West Garo Hills and South Garo Hills districts of Meghalaya. *Citrus macroptera* is listed as rare, semi-wild and endangered species by the National Research Center for Citrus, Nagpur. The embryos of this species are mostly under developed, thus resulting in poor natural germination. The seeds being recalcitrant in nature cannot be preserved for long. As an alternative strategy for conservation of this endangered species an experiment was conducted to standardise the medium and culture environment for *in vitro* seed germination. Among the different media evaluated, full strength MS medium with pH of 5.8, temperature of 25°C, photoperiod of 10/14 h (light/dark) under 1000 lux light intensity showed early germination (7-9 days) and exhibited the highest percentage of seed germination (63-73%) with highest shoot length (2.09-2.64 cm) and root length (1.52-1.99 cm).

Keywords *Citrus macroptera*, Endangered, *In vitro* seed germination, Meghalaya.

Introduction

Citrus macroptera Mont. locally known as Chambil grows in semi wild conditions of West Garo Hills and South Garo Hills districts of Meghalaya. It is also found in the vicinity of Shella and Dawki areas near Cherrapunji (NRC 2013). The thick rind as well as juice of the fruit are used in preparation of appetizing Garo cuisines and also for making pickles. The fruits are also used for treatment of cough, cold and high blood pressure by the locals. Besides, it has great potential for use as commercial refreshing drink. The National Research Center (2013) for Citrus at Nagpur has listed *Citrus macroptera* as rare, wild, semi-wild and endangered (NRC 2013). *Citrus macroptera* is in need of special and immediate attention for conservation due to its endemism and high degree of threat perception (Malik et al. 2009). The slow regeneration of this species and increasing human intervention by clearing of forests for jhuming and human habitation, have become a severe threat to this species. The recalcitrant nature of seeds makes it difficult to store for longer duration. Natural seed germination is a rare phenomenon owing to rudimentary embryos. *In-vitro* seed germination, mass propagation and regeneration of this species under *in-situ* and *ex-situ* conditions require urgent attention to facilitate effective conservation and utilization. The objective of the study was to standardise the medium and culture environment for *in-vitro* seed germination of *Citrus macroptera*.

Materials and Methods

Fruits of *Citrus macroptera* were collected from

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nearby villages of Tura in West Garo Hills district. The seeds were separated from fruits and washed with Teepol and rinsed with tap water by keeping under running water for about half an hour. Seed were then washed with double distilled water five times. Healthy seeds were isolated from the lot and surface sterilized with 70% absolute alcohol for 2 minutes. Seeds were subsequently rinsed with double distilled water six times. Later seeds were surface sterilized under laminar flow chamber with 10% sodium hypochlorite for 15 minutes followed by 0.1% mercuric chloride for 5 minutes. Seeds were then washed thoroughly with double distilled water six times.

Sterilized seeds were given longitudinal incision on both sides of the seed coat and then inoculated on four different basal media viz. MS media (Murashige and Skoog 1962), LS media (Linsmaier and Skoog 1965), WPM (Woody Plant Medium) (McCowan and Lloyd 1981) and White media (White 1963) containing 3% sucrose with no growth regulators at pH 5.8 under temperature of $25 \pm 2^\circ\text{C}$ for 8 h photoperiod and 80% relative humidity. The number of days required for germination was recorded. Percentage of germination, length of shoot and root were recorded at eight weeks after culturing. Out of the four media, the medium showing best results was then used for seed inoculation at three different strengths (viz., full strength, half strength and one fourth strength with 3% sucrose) with pH 5.8, temperature $25 \pm 2^\circ\text{C}$, photoperiod 8 h, light intensity 1500 lux and relative humidity 80%. The number of days required for germination percentage of germination, length of root and shoot were recorded. The strength of the medium which showed best results was further used for seed germination with varying pH levels (4.5,

5.8, 6.5), temperatures ($20 \pm 2^\circ\text{C}$, $25 \pm 2^\circ\text{C}$ and $30 \pm 2^\circ\text{C}$), photoperiods (8/16 h, 10/14 h, 12/12 h light/dark) and light intensities (500 lux, 100 lux and 1500 lux) to standardize the culture environment and similar observations were recorded as mentioned above.

Ten replicates per treatment were taken and the experiment was repeated three times. The experiment was laid out in completely randomized design. The data were subjected to statistical analysis using Fischer's one-way analysis of variance. The level of significance used in F test was $p < 0.05$. Critical difference was calculated for comparison wherever the F test was found significant (Panse and Sukhatme 1989).

Results and Discussion

Among the four media (MS media, LS media, WPM and White media containing 3% sucrose with no growth regulators) tested for *in vitro* seed germination of *Citrus macroptera*, MS medium showed early germination (8.3 ± 0.33 days), highest germination percentage ($60 \pm 0.57\%$), highest shoot length (2.64 ± 0.64 cm) and root length (1.86 ± 0.46 cm). The least percentage of seed germination was observed in White medium (Table 1).

Further, three different strengths of MS basal medium (with 3% sucrose and no growth regulators) viz. full strength, half strength and one fourth strength were used for *in vitro* seed germination. It was observed that full strength MS medium showed earlier (8.3 ± 0.88 days) and higher percentage of germination (63%) with maximum length of shoot (2.44 ± 0.60

Table 1. Effect of different media on *in vitro* seed germination of *Citrus macroptera*.

Media	Days for germination Mean \pm SE	% of germination Mean \pm SE	Length of shoot (cm) Mean \pm SE	Length of root (cm) Mean \pm SE
MS	8.3 ± 0.33	60 ± 0.57	2.64 ± 0.64	1.86 ± 0.46
LS	14 ± 0.57	43.3 ± 0.33	1.69 ± 0.62	0.99 ± 0.37
WPM	14.6 ± 0.33	36.6 ± 0.33	1.21 ± 0.53	0.81 ± 0.34
White	19 ± 0.57	23.3 ± 0.88	0.476 ± 0.25	0.35 ± 0.16
CD 0.05	0.89	1.09	1.53	1.01

Table 2. Effect of different strength of MS medium on *in vitro* seed germination of *Citrus macroptera*.

Strength of MS medium	Days for germination Mean \pm SE	% of germination Mean \pm SE	Length of shoot (cm) Mean \pm SE	Length of root (cm) Mean \pm SE
Full strength	8.3 \pm 0.88	63.3 \pm 0.88	2.44 \pm 0.60	1.85 \pm 0.43
Half strength	11 \pm 0.57	43.3 \pm 1.33	0.96 \pm 0.32	1.12 \pm 0.37
One fourth strength	15 \pm 0.57	33.3 \pm 0.88	0.74 \pm 0.28	0.45 \pm 0.15
CD 0.05	4.15	1.99	1.25	0.98

Table 3. Effect of different pH on *in vitro* seed germination of *Citrus macroptera*.

pH of medium	Days for germination Mean \pm SE	% of germination Mean \pm SE	Length of shoot (cm) Mean \pm SE	Length of root (cm) Mean \pm SE
4.5	15 \pm 0.57	33.3 \pm 0.88	0.60 \pm 0.26	0.41 \pm 0.14
5.8	9 \pm 0.57	66.6 \pm 0.88	2.60 \pm 0.56	1.99 \pm 0.42
6.5	10 \pm 0.57	43.3 \pm 0.33	0.94 \pm 0.35	0.54 \pm 0.25
CD 0.05	1.09	1.41	1.20	0.85

cm) and root (1.85 \pm 0.43 cm) (Table 2).

Further evaluation of seed germination was done with full strength MS basal medium (with 3% sucrose and no growth regulators) with varying pH, temperature, photoperiod and light intensity to standardize the culture environment. Seed inoculation was carried out in full strength MS medium at different pH levels viz., 4.5, 5.8 and 6.5, with temperature maintained at 25 \pm 2°C, photoperiod 8 h, light intensity 1500 lux and relative humidity of 80%. Observations revealed that pH of 5.8 showed early germination (9 \pm 0.57 days), maximum percentage of germination (66.6 \pm 0.88%), maximum shoot (2.60 \pm 0.56 cm) and root (1.99 \pm 0.42 cm) length. A significant difference was recorded in all the growth parameters when compared between pH 5.8, 4.5 and 6.5 (Table 3).

Seed inoculation was done in full strength MS medium with varying temperatures viz., 20 \pm 2°C, 25 \pm

2°C and 30 \pm 2°C, at 5.8 pH, maintaining a photoperiod of 8 h, with 1500 lux light intensity and relative humidity of 80%. Best results were obtained at 25 \pm 2°C temperature with earlier germination (9 \pm 0.57 days), higher percentage of germination (66.6 \pm 0.66%), highest shoot length (2.41 \pm 0.55 cm) and root length (1.52 \pm 0.42 cm) (Table 4).

Table 5 reveals that photoperiod of 10/14 h (light/dark) showed earlier germination (7 \pm 0.57 days), highest percentage of germination (73.3 \pm 1.20%), highest shoot length (2.56 \pm 0.42 cm) and highest root length (1.65 \pm 0.33 cm).

Different light intensities viz., 500 lux, 1000 lux and 1500 lux were evaluated for *in vitro* seed germination of *Citrus macroptera* in full strength MS medium. It was noted that 1000 lux light intensity showed early germination (9 \pm 0.57 days), higher per-

Table 4. Effect of different temperatures on *in vitro* seed germination of *Citrus macroptera*.

Temperatures	Days for germination Mean \pm SE	% of germination Mean \pm SE	Length of shoot (cm) Mean \pm SE	Length of root (cm) Mean \pm SE
20°C	15 \pm 0.57	40 \pm 0.57	1.12 \pm 0.42	0.834 \pm 0.29
25°C	9 \pm 0.57	66.6 \pm 0.66	2.41 \pm 0.55	1.52 \pm 0.42
30°C	16 \pm 1.15	43.3 \pm 0.33	0.74 \pm 0.27	0.45 \pm 0.18
CD 0.05	0.97	1.03	0.76	0.91

Table 5. Effect of different photoperiods on *in vitro* seed germination of *Citrus macroptera*.

Photoperiod (h) (light/dark)	Days for germination Mean \pm SE	% of germination Mean \pm SE	Length of shoot (cm) Mean \pm SE	Length of root (cm) Mean \pm SE
8/16	10.6 \pm 0.66	53.3 \pm 0.33	1.10 \pm 0.32	0.49 \pm 0.15
16/14	7 \pm 0.57	73.3 \pm 1.20	2.56 \pm 0.42	1.65 \pm 0.33
12/12	10 \pm 1.15	46.6 \pm 1.20	1.47 \pm 0.42	1.09 \pm 0.32
CD 0.05	1.59	1.89	1.25	0.82

Table 6. Effect of different light intensities on *in vitro* seed germination of *Citrus macroptera*.

Light intensity (lux)	Days for germination Mean \pm SE	% of germination Mean \pm SE	Length of shoot (cm) Mean \pm SE	Length of root (cm) Mean \pm SE
500 lux	13 \pm 0.57	33.3 \pm 0.33	1.01 \pm 0.37	0.52 \pm 0.36
1000 lux	9 \pm 0.57	63.3 \pm 0.88	2.09 \pm 0.46	1.92 \pm 0.20
1500 lux	10 \pm 0.88	40 \pm 0.57	0.73 \pm 0.32	0.65 \pm 0.32
CD 0.05	1.31	1.20	1.14	0.88

centage of germination (63.3 \pm 0.88%), highest shoot length (2.09 \pm 0.46 cm) and root length (1.92 \pm 0.20 cm) (Table 6).

Out of the four basal media (viz. MS, LS, WPM and White media) tested for *in vitro* seed germination of *Citrus macroptera* in the present study. MS medium showed the best result with early and highest percentage of germination, highest shoot and root length. Similar trials were conducted on *in vitro* seed germination of *Cymbidium aloifolium* (Bhowmik and Rahman 2016) *Psoralea corylifolia* (Pandey et al. 2014) *Malaxix khasiana* (Deb and Temjensangba 2006), *Cleisostoma racemiferum* (Deb and Temjensangba 2007) and *Coelogyne suaveolens* (Sungkumlong and Deb 2008) where MS medium was found to be the best over other nutrient media. Among the three different strengths of MS basal medium (viz. full, half and one fourth) used for seed germination of *Citrus macroptera*, full strength MS medium showed earlier and higher percentage of seed germination, highest length of shoot and root with overall healthy seedlings with dark green and broad leaves. Effect of different basal media (Viz., MS, KC, PM and VW) in full and half strength were evaluated for *in vitro* seed germination of *Cymbidium aloifolium* and noted that full strength MS medium showed best results (Bhowmik and Rahman 2016). Full strength MS medium was found to be more effective for *in vitro* seed germination of *Psoralea corylifolia* compared to other

media viz., B5 and White medium (Pandey et al. 2014).

Among the different levels of pH (4.5, 5.8 and 6.5), temperature (20 \pm 2°C, 25 \pm 2°C and 30 \pm 2°C), photoperiod (8/16 h, 10/14 h, 12/12 h light/dark) and light intensity (500 lux, 1000 lux and 1500 lux) evaluated for *in vitro* seed germination of *Citrus macroptera* on full strength MS basal medium, it was observed that pH of 5.8, temperature of 25 \pm 2°C, photoperiod of 10/14 h (light/dark) and light intensity of 1000 lux showed best results in terms of days required for initiation of germination, germination percentage, root length and shoot length. Similar experiments were conducted by researchers on other rare and endangered species to study the effect of different *in vitro* culture conditions. The effects of photoperiod and temperature on *in vitro* seed germination of *Centaurea zeybekii* were investigated and observed that highest germination was obtained at 24 \pm 2°C (Kurt and Erdag 2009). The optimum temperature for *in vitro* seed germination of *Hostayingeri* was noted to be 30 \pm 2°C (Kim et al. 2016). A temperature of 25°C produced highest *in vitro* seed germination in *Bupleurum latissimum* (Kee Hwa Bae 2015). Effect of light intensity and pH on *in vitro* seed germination of *Galaxur ceolata*, an evergreen perennial monocot species, was studied and no significant difference was observed in germination between the two light levels and the three pH levels studied (Yang et al. 2013). Effect of temperature on *in vitro* seed germi-

nation *Citrus reticulata* was evaluated and observed that 25°C produced highest germination percentage (Hassanein and Azooz 2003).

Conclusion

Among the four basal media evaluated for *in vitro* seed germination of *Citrus macroptera*, MS medium proved to be the best. Full strength MS medium with pH of 5.8, temperature of 25°C, photoperiod of 10/14 h (light/dark) and 1000 lux light intensity showed early germination (7-9 days), highest seed germination percentage (63-73%), highest root length (1.52-1.99 cm) and highest shoot length (2.09-2.64 cm).

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