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Effect of Media and Culture Environment on *In vitro* Seed Germination of *Citrus indica* Tanaka

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Abstract *Citrus indica* Tanaka commonly known as Wild Indian Orange is believed to be the progenitor of cultivated Citrus. It grows wild in the buffer zone of Nokrek Biosphere Reserve of Meghalaya. The slow regeneration of this species in its natural habitat and increasing human intervention around the Biosphere Reserve have led to rapid decline in numbers of this species during the past few years. In order to protect this species from extinction, efforts should be made for multiplication under *in situ* and *ex situ* conditions. An experiment was conducted to study the effect of different media, strength of medium and culture environment on *in vitro* seed germination of *Citrus indica*. Half strength MS medium with pH 5.8 temperature of 25°C, photoperiod of 10/14h (light/dark) under 1000 lux light intensity showed early germination (6.6-9 days), highest germination (70—83.3%), highest shoot length (2.14—3.88 cm) and root length (1.66—3.61 cm).

Keywords *Citrus indica, In vitro* seed germination, Culture environment, Media, Nokrek Biosphere Reserve.

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Introduction

Citrus indica Tanaka commonly known as Wild Indian Orange is believed to be the most primitive and perhaps the progenitor of all cultivated Citrus. Malik et al. (2006) reported that *Citrus indica* was found distributed in the buffer zone of Nokrek Biosphere Reserve spreading in the East, West and South Garo Hills Meghalaya. *Citrus indica* is typically a dwarf shrub bearing small fruits which are usually bright orange when fully ripe. The fruit is locally called 'Memang Narang' and is used to cure jaundice, small pox, stomach ailments of humans and domestic animals and is also used in traditional rituals (Malik et al. 2006). The fruits are also reported to cure hypertension and used as an antidote for food poisoning and snake bite. The juice is used as energy drink for relief from fatigue and dehydration. The trees are preferred in home gardens in villages of lower elevation of Khasi and Jaintia Hills for its aesthetic and ornamental value (Upadhyay et al. 2016). Dur-

ing the last few years, the numbers of this species has been declining rapidly. The slow regeneration of this species in its natural habitat and increasing human intervention around the buffer zone byjhum cultivation and large scale deforestation has become a matter of serious concern. Efforts should be directed towards regeneration of *Citrus indica* under *in situ* and *ex situ* conditions to facilitate effective conservation and utilization. *In vitro* seed germination has been successfully carried out as an alternative for natural germination, in a number of rare and endangered species. Keeping in view the above facts, an experiment was conducted to study the effect of different media, strength of medium and culture environment on *in vitro* seed germination of *Citrus indica.*

Materials and Methods

Fruits of *Citrus indica* were collected from Nokrek Biosphere Reserve. The seeds were separated from fruits and washed with Teepol and rinsed with tap water by keeping under running water for half an hour. Seeds were then washed with doubled distilled water four to five times. Healthy seeds were isolated from the lot and surface sterilized with 70% absolute alcohol for 2 minutes and subsequently rinsed with double distilled water six times. Later seeds were surface sterilized under laminar flow chamber with 10% sodium hypochlorite for 15 minutes and then washed thoroughly with doubled distilled water six times.

Sterilized seeds were then inoculated on four basal media viz., MS medium (Murashige and Skoog 1962), LS medium (Linsmaier and Skoog 1965), Woody Plant medium (McCowan and Lloyd 1981) and White medium (White 1963) containing 3% sucrose and no growth regulators at pH 5.8 under temperature of 25 ± 2°C for 8 hours photo period at 80% relative humidity. The number of days required for germination was recorded. Percentage of germination was calculated and length of shoot and root were recorded after eight weeks of culturing. Out of the four media, the medium showing best results was 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 ± 2°C, photoperiod 8 hours, 1500 lux light intersity and relative humidity of 80%. The number of days required for germination, percentage of germination, length of shoot and root 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 ± 2°C, 25 ± 2°C and 30 ± 2°C), photo periods (8/16h, 10/14h, 12/12h light/dark) and light intensities (500 lux, 1000 lux and 1500 lux) to standardize the culture environment. Similar observations as mentioned above were recorded.

Ten replicates were taken per treatment 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 $p \leq 0.05$. 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 with 3% sucrose and no growth regulators evaluated for *in vitro* seed germination of citrus indica, MS medium showed early germination (7.6 ± 0.33), highest germination percentage (73.3 ± 0.88%), highest shoot length (2.27 ± 0.37 cm) and root length (2.78 ± 0.45cm) (Table 1). Three different strengths (full strength, half strength and one fourth strength) of MS medium (with 3% sucrose and no growth regulators) were then tried for seed germination and observed that half strength MS medium showed earlier (6.6 ± 0.33 days) and

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

Media	Days for germination	% of germination	Length of shoot (cm)	Length of root (cm)
MS	7.60	73.30	2.27	2.78
LS	9.30	33.30	0.63	0.46
WPM	9.00	35.30	1.60	0.98
White	17.60	16.60	0.29	0.23
CD (0.05)	NS	0.94	0.99	NS

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

Strength of MS medium	Days for germination	% of germination	Length of shoot (cm)	Length of root (cm)
Full strength	6.60	70.00	2.89	2.14
Half strength	6.60	83.30	3.88	3.61
One fourth strength	11.60	53.30	1.93	1.92
CD(0.05)	1.67	1.03	NS	NS

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

pH of medium	Days for germination	% of germination	Length of shoot (cm)	Length of root (cm)
4.5	13.30	36.60	0.80	0.76
5.8	7.60	80.00	2.73	3.20
6.5	10.60	53.30	1.27	1.03
CD(0.05)	2.36	1.36	Ns	0.69

higher percentage of germination (83.3 ± 0.33%) and highest length of shoot (3.88 ±0.53 cm) and root (3.61 ±0.49 cm) (Table 2).

Further assessment of seed germination was done with half strength MS medium (with 3% sucrose and no growth regulators) with varying pH, temperature, photoperiod and light intensity to standardize the culture environment. Seed inoculation was done in half strength MS medium at different pH levels viz., 4.5, 5.8 and 6.5 with temperature maintained at 25 ±2°C, photoperiod 8 hours, 1500 lux light intensity and relative humidity of 80%. It was noted that pH of 5.8 showed early germination (7.6 ± 0.88 days), highest percentage of germination (80 ± 1.15%) and highest shoot (2.72 ± 0.30 cm) and root (3.2 ± 0.32 cm) length (Table 3). Seed inoculation was done in half strength MS medium with varying temperatures viz., 20 ± 2°C, 25 ± 2°C and 30 ± 2°C, maintaining a photoperiod of 8 hours, 1500 lux light intensity and relative humidity of 80%. Early germination (9 ± 0.57 days), higher percentage of germination (73.3 ± 0.66%), highest shoot length (2.54 ± 0.47 cm) and root length (2.65 ± 0.46 cm) were recorded at 25 ± 2°C temperature (Table 4). Different photoperiods (viz.,8/16h, 10/14 h, 12/12h light/dark) were tried and noted that photoperiod of 10/14 h (light/ dark) resulted in early germination (8 ±0.57 days), highest percentage of germination (73.3 ±0.66%), highest shoot length (2.29 ± 0.44 cm) and highest root length (2.12 ± 0.41 cm) (Table 5). Different light intensities viz. 500 lux, 1000 lux and 1500 lux were tried for *in vitro* seed germination of *Citrus indica* in half strength MS medium and observed that 1000 lux light intensity produced early germination (7.6 ± 0.33 days), higher percentage of germination (70 ± 0.33%) and highest shoot length (2.14 ± 0.42 cm) and root length (1.66 ± 0.33 cm) (Table 6).

Out of the four basal media evaluated for *in vitro* seed germination of *Citrus indica*, MS medium showed the best result. MS medium proved to be the best in similar trials carried out on *in vitro* seed germination of *Cymbidium aloifolium* (Bhowmik and Rahman 2016), *Malaxix khasiana* (Deb/and Temjensangba 2006), *Cleisostomara cemiferum* (Deb and Temjensangba 2007), *Coelogynesu aveolens* (Sukumdong and Deb 2008) and *Psoralea corylifolia* (Pandey et al. 2014). Among the three

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

Temperature	Days for germination	% of germination	Length of shoot (cm)	Length of root (cm)
20°C	8.00	60.00	1.55	1.03
25°C	9.00	73.30	2.54	2.65
30°C	11.00	23.30	0.69	0.45
CD(0.05)	1.09	1.03	1.02	0.99

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

photoperiod (h) (light/dark)	Days for germination	% of germination	Length of shoot (cm)	Length of root (cm)
8/16	8.00	60.00	1.07	1.03
10/14	9.00	73.30	2.29	2.12
12/12	11.00	23.30	1.15	1.08
CD(0.05)	1.09	1.03	1.05	0.99

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

Light intensity (lux)	Days for germination	% of germination	Length of shoot (cm)	Length of root (cm)
500 lux	9.60	43.36	0.79	0.55
1000 lux	7.60	70.00	2.14	1.66
1500 lux	9.00	50.00	1.01	1.04
CD (0.05)	NS	0.76	1.00	0.85

strengths of MS medium (full strength, half strength and one fourth strength) assessed for *in vitro* seed germination, it was observed that half strength of MS medium showed earlier germination, higher percentage of seed germination, highest length of shoot and root. Kone et al. (2015) studied the effect of different strengths (one fourth, half and full) of MS medium on *in vitro* seed germination of Bambara Groundnut and observed that half strength MS medium showed optimal growth performances.

Among the different levels of pH (4.5, 5.8 and 6.5), temperatures (20 ±2°C, 25 ±2°C and 30 ±2°C), photoperiods (8/16 h, 10/14h, 12/12h light/dark) and light intensities (500 lux, 1000 lux and 1500 lux) evaluated for *in vitro* seed germination of *Citrus indica* on half strength MS medium, it was noted that pH of 5.8, temperature of 25 ±2°C, photoperiod of 10/14h (light/ dark) and light intensity of 1000 lux produced best results in terms of days required for germination, germination percentage, root length and shoot length. Similar trials were carried out by researchers on other rare and endangered species to standardize the culture environment for *in vitro* seed germination. Kurt and Erdag (2009) observed highest *in vitro* seed germination at 24 ± 2°C in *Centaurea zeybekii* an endangered endemic species of Western Turkey. The effect of illumination was also examined and noted that seeds of *Centaurea zeybekii* can germinate well both in light and dark condition. A temperature of 25°C produced highest *in vitro* seed germination in *Bupleurum latissimum* a threatened to extinct species of South Korea (Kee Hwa 2015). Hassanein and Azooz (2003) observed highest *in vitro* seed germination in *Citrus reticulata* at a temperature of 25 °C.

Conclusion

Among the different media evaluated for *in vitro* seed germination of *Citrus indica*, MS medium exhibited the best results. Among the different strengths of MS medium evaluated, half strength MS medium with pH 5.8, temperature 25°C, photoperiod 10/14 h (light/dark) and 1000 lux light intensity at 80% humidity proved to be the best in terms of early germination, percentage of seed germination, root length and shoot length.

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