FACTORS AFFECTING GERMINATION AND SEEDLING GROWTH OF AN ENDANGERED FOREST TREE WRIGHTIA TOMENTOSA (ROXB.) ROEM. & SCHULT. THROUGH IN VITRO ZYGOTIC EMBRYO CULTURE

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Abstract: The species Wrightia tomentosa has medicinal value and importance in toy making industry. Due to its over exploitation the species has become an endangered. The present investigation reports on a protocol for conservation of W. tomentosa through zygotic embryo culture. The zygotic embryos were cultured on MS (Murashige & Skoog), WPM (Woody Plant Medium) and B5 media. The germination percentage was found more on MS medium compared to WPM and B5 media. When the zygotic embryos cultured on full and half strength MS media containing 15-30 g/L sucrose it showed less percentage of germination and more days for germination. Maximum percentage (96%) of germination was obtained by culturing zygotic embryos on ¼ strength MS medium containing 7.5gm/L sucrose in comparison to the other media used. Germination was also affected by the orientation of zygotic embryos in culture. The zygotic embryos placed vertically on the medium were germinated early with more percentage and with healthy seedlings. The in vitro germinated seedlings were acclimatized in the culture room, transferred to research field and maintained under shady conditions. The survival percentage of plantlets was found to be 90% and the plants were morphologically normal, healthy and similar to the mother plant. This protocol can be useful for overcoming seed dormancy and also for rapid multiplication and conservation of an endangered forest tree W. tomentosa using zygotic embryo culture.

Key words: Wrightia tomentosa, Zygotic embryo culture

INTRODUCTION

India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world [1]. The pharmacological or biological tests of 20 % of the plants found in the world have been submitted [2]. In vitro propagation of many medicinal and/or endangered plant species was reported [3,4]. The protocols on in vitro micropropagation of many threatened medicinal species have been developed [5-7]. Wrightia tomentosa (Roxb.) Roem. & Schult. of family Apocynaceae is an endangered medicinally important tree species. It is commonly called as “Tella pala”(Vern.) [8]. It is a deciduous tree upto 20m tall, abounding in yellow milky juice; bark smooth yellowish grey, branchlet pubescent; young parts densely tomentose. Flowers are yellow in terminal corymbose cymes, fruit of follicles with a deep groove on each side, and seeds are with white silky coma [9,10].
The species is medicinally useful for stomach ache, tooth ache, snake bite, fever, colic, dysentery, hemorrhage, hemostat, renitis and tumour. The cytotoxic activities of Wrightiadione isolated from *W. tomentosa* was studied on the murine P388 lymphocyte leukemia cell line [11, 12]. The species is not only medicinally useful but also the wood of this species plays an important role in toy making industry. Due to regular cutting and over exploitation, the species has become an endangered and the plant is placed as category I of Red Data Book [13].

Although the species has medicinal value and importance in toy making industry, there is no report on *in vitro* zygotic embryo culture in *W. tomentosa* an endangered forest tree. Hence the present investigation reports on the role of different types of media and sucrose concentration on germination and plantlet establishment through *in vitro* zygotic embryo culture in *W. tomentosa*.

**MATERIALS AND METHODS**

**Plant Material:** The germplasm has been collected from the trees growing in the Kakatiya Arboretum, Forest Research & Development, Warangal. Fruits were washed thoroughly under running tap water followed by tween-20 (5%-v/v). These were surface sterilized with 0.1% (w/v) HgCl₂ for 10 min. After surface sterilization the fruits were opened with the help of sterilized scalpel. The fresh seeds were dissected, isolated the zygotic embryos in the laminar-air-flow chamber aseptically and were placed vertically on various culture media.

**Culture Media and Culture Conditions:** The isolated zygotic embryos were inoculated on different types of media, such as full strength MS [20], WPM [21] and B₅ (Gamborg’s medium) [8]. ½ strength, ¼ strength MS media containing different concentrations of sucrose (30 g/L, 15 g/L and 7.5 g/L) (Fig.1a-c). Zygotic embryos were also cultured on ¼ strength MS medium containing 7.5 g/L sucrose with different orientations viz., vertically placed, half dipped and horizontally placed. The zygotic embryo explants were cultured in flasks (100 ml & 150 ml), culture bottles (300 ml & 400 ml) and closed with polypropylene lid. All the cultures were kept in controlled culture conditions of temperature (25 ± 2°C), light (45 µmol m⁻² s⁻¹ for 16 h/d provided by fluorescent tubes) and 60-70% relative humidity (RH).

**Plantlet Establishment:** The *in vitro* germinated seedlings were taken out from the culture vessels and washed with sterile distilled water to remove remains of agar. Later shifted to plastic pots containing potting mix and kept in culture room for acclimatization. These were covered with plastic bag to maintain the RH (80-90%). After the acclimatization these were shifted to green house.

**RESULTS AND DISCUSSION**

The zygotic embryos of *W. tomentosa* were cultured on different types of media viz. MS, WPM and B₅ containing 30 g/L sucrose. The percentage of germination was found more on MS basal medium compared to WPM and B₅ media (Table-1).

The zygotic embryos were also cultured on full strength MS medium containing different concentrations of sucrose (Table-2). Maximum percentage of germination (74%) was observed at 7.5 g/L sucrose than the other concentrations of sucrose used. These zygotic embryos were also

\[\text{Table 1: Effect of different types of media on } \textit{in vitro} \text{ zygotic embryo culture of } W. \textit{tomentosa}. \textsuperscript{a} \text{Mean ± Standard Error} \]

<table>
<thead>
<tr>
<th>Type of medium</th>
<th>% of germination</th>
<th>Days for germination</th>
<th>Average length (cms) of seedlings (± E)\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>58</td>
<td>8</td>
<td>3.2 ± 0.18</td>
</tr>
<tr>
<td>WPM</td>
<td>24</td>
<td>12</td>
<td>3.4 ± 0.22</td>
</tr>
<tr>
<td>B₅</td>
<td>20</td>
<td>12</td>
<td>3.6 ± 0.20</td>
</tr>
</tbody>
</table>

\[\text{Table 2: Effect of MS medium containing different concentrations of sucrose on } \textit{in vitro} \text{ zygotic embryo culture of } W. \textit{tomentosa}. \textsuperscript{a} \text{Mean ± Standard Error} \]

<table>
<thead>
<tr>
<th>Type of medium + Conc. of sucrose</th>
<th>% of germination</th>
<th>Days for germination</th>
<th>Average length (cms) of seedlings (± SE)\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + 30 g/L sucrose</td>
<td>58</td>
<td>8</td>
<td>3.2 ± 0.18</td>
</tr>
<tr>
<td>MS + 15 g/L sucrose</td>
<td>72</td>
<td>6</td>
<td>3.4 ± 0.21</td>
</tr>
<tr>
<td>MS + 7.5 g/L sucrose</td>
<td>74</td>
<td>6</td>
<td>4.4 ± 0.21</td>
</tr>
</tbody>
</table>
Fig. 1 (a-g): Zygotic embryo culture and plantlet establishment in *Wrightia tomentos*: a) Fruit b) Dehisced fruit c) Seeds d) Zygotic embryo culture on ¼ strength MS + 7.5g/L sucrose e) Showing different stages of embryo germination f) Acclimatization g) Growing in the greenhouse.
cultured on ½ strength MS medium with different concentrations of sucrose (Table-3). ½ strength MS medium containing 7.5 g/L sucrose gave maximum percentage of germination (84%). Zygotic embryos were also found to be germinated early on the same medium.

The zygotic embryos were cultured on ¼ strength MS medium containing different concentrations of sucrose (Fig.1). Maximum percentage of germination (96%) and early germination was achieved (Table-4) in comparison to all other media with different concentrations of sucrose used. In higher concentration of MS medium and sucrose levels the germination percentage was decreased. When, the strength of MS medium and sucrose levels decreased showed the gradual increase in the percentage of germination. These results indicate that sucrose plays an important role in embryo germination of W. tomentosa. Unlike the MS medium, embryos germinated poorly on WPM and B5 media in the present investigations. Apart from germination, less number of days taken for germination, seedlings also grew better and in terms of plant length with number of roots on ¼ MS medium containing 7.5 g/L sucrose (Table-4; Fig.1d-g). When the zygotic embryos cultured on ¼ strength MS medium containing 7.5 g/L sucrose with different orientations, showed maximum percentage (96) of germination from vertically placed zygotic embryos in comparison to half dipped (53%) and horizontally placed (48%).

**Plantlet establishment:** The plantlets were taken out from the culture vessels and washed with sterile distilled water to remove the remains of agar. Later shifted to plastic cups containing potting mix and grown under laboratory conditions. The survival percentage was found to be 90%, when plantlets were subjected to *in vitro* hardening before being transferred to green house. During *in vitro* hardening, shoots elongated, leaves turned greener and expanded and root system became extensive (Fig.1f-g)

**DISCUSSION**

During the present investigation the best zygotic embryo germination was achieved on ¼ strength MS medium containing 7.5 g/L sucrose. Samuel et al. [14] developed a protocol to overcome seed dormancy by culturing zygotic embryos. Best germination frequency (78.3%) was achieved from mature zygotic embryo axes isolated from fresh seeds when cultured on MS medium (half – strength major salts) with 28.9 µM GA3.

Joshi et al.[15] have studied the role of gelling agents, carbon source, type of vessel and liquid culture system on *in vitro* micropropagation of W.tomentosa. Effect of different concentrations of sucrose-sources (Sucrose AR, Sugar cubes, commercial sugar and Jagger) was also investigated on *in vitro* multiplication of W.tomentosa. Sugar cubes containing medium was obtained a higher multiplication rate than the other three sucrose-sources. The other three sucrose-sources showed the reduced rate of shoot multiplication.

Similarly Ghorpade et al. [16] have established an efficient protocol for development of seedlings of an endangered medicinally important forest tree *Boswellia serrata*, the excised green zygotic embryos were cultured on B5, WPM and SH media

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**Table 3:** Effect of ½ strength MS medium with different concentrations of sucrose on *in vitro* zygotic embryo culture of *W.* tomentosa. *Mean ± Standard Error*

<table>
<thead>
<tr>
<th>Concentration of sucrose</th>
<th>% of germination</th>
<th>Days for germination</th>
<th>Average length(cms) of seedlings(±S£)</th>
</tr>
</thead>
<tbody>
<tr>
<td>½ strength MS + 30 g/L sucrose</td>
<td>75</td>
<td>8</td>
<td>3.6±0.22</td>
</tr>
<tr>
<td>½ strength MS + 15 g/L sucrose</td>
<td>80</td>
<td>6</td>
<td>4.2±0.46</td>
</tr>
<tr>
<td>½ strength MS + 7.5 g/L sucrose</td>
<td>84</td>
<td>5</td>
<td>6.2±0.59</td>
</tr>
</tbody>
</table>

**Table 4:** Effect of ¼ strength MS medium with different concentrations of sucrose on *in vitro* zygotic embryo culture of *W.* tomentosa. *Mean ± Standard Error*

<table>
<thead>
<tr>
<th>Concentration of sucrose</th>
<th>% of germination</th>
<th>Days for germination</th>
<th>Average length(cms) of seedlings(±S£)</th>
</tr>
</thead>
<tbody>
<tr>
<td>¼ strength MS + 30 g/L sucrose</td>
<td>75</td>
<td>7</td>
<td>5.0±0.42</td>
</tr>
<tr>
<td>¼ strength MS + 15 g/L sucrose</td>
<td>82</td>
<td>5</td>
<td>6.4±0.58</td>
</tr>
<tr>
<td>¼ strength MS + 7.5 g/L sucrose</td>
<td>96</td>
<td>4</td>
<td>8.2±0.87</td>
</tr>
</tbody>
</table>
fortified with different concentrations of sucrose and MS medium containing 3% sucrose, polyvinylpyrrolidone (PVP) (0 – 300 mg L\(^{-1}\)) and also fortified with GA\(_3\), IAA, NAA, IBA or 2, 4-D and BAP (or) Kinetin individually. The highest frequency of zygotic embryo germination (96%) and conversion into seedling was obtained on MS medium containing 3% sucrose together with 200 mg L\(^{-1}\) PVP; other media were either inferior or induced abnormalities in the seedlings inducing callus formation from the zygotic embryos.

The strength of basal medium, sucrose concentration, and percentage of agar affected \textit{in vitro} rooting percentage [17]. The beneficial effect of reduced salts and sucrose concentrations during rooting phase was reported in \textit{Garcinia indica} by Chabukswar et al. [18]. Low sucrose concentration enhanced the percentage of zygotic embryo germination in several species of \textit{Garcinia indica} as observed in the present investigations. Whereas Rambabu et al. [19] reported that the zygotic embryos grown on half-strength MS medium containing different concentrations of sucrose showed lower percentage of germination (15-40%) compared to those germinated (100%) on full-strength MS medium (30 gl\(^{-1}\)) in \textit{Givotia rottleri-formis}. Lower germination was observed at lower concentrations of sucrose (7.5-15gL\(^{-1}\)) and at high concentration (40gL\(^{-1}\)) promoted callus formation in \textit{G. rottleri-formis}. These observations are in contrary to the present investigations on \textit{W. tomentosa}.

**Data Analysis:** The data were recorded on percentage of germination, days for germination and average length of seedlings after 4 weeks of incubation. For each experiment, a minimum of 20 replicates were maintained and each experiment was repeated at least thrice. Observations were recorded for every 3 weeks.

**CONCLUSION**

In conclusion, it is reported that the germination percentage and seedling growth were successfully enhanced with low concentration of MS medium and sucrose concentration levels in \textit{W. tomentosa}. The vertically placed zygotic embryos showed the best results in comparison to half-dipped and horizontally placed. The efficient protocol developed during the present investigation is useful to overcome the seed dormancy and also conservation of \textit{W. tomentosa}. The present study has also demonstrated that the cost of micro propagation of \textit{W. tomentosa} could be reduced significantly by the low amount of MS medium and sucrose concentrations.

**Abbreviations used:** MS – Murashige & Skoog, WPM – Woody plant medium, \(B_3\) - Gamborg’s medium

**ACKNOWLEDGEMENTS**

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**REFERENCES**