

**INDUCTION OF SOMATIC EMBRYOGENESIS IN
PINUS KESIYA ROYLE EX. GORD**



By

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CERTIFICATE

I, Chitta Ranjan Deb, hereby, declare that the subject matter of the thesis entitled "Induction of somatic embryogenesis in *Pinus kesiya* Royle ex. Gord." is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Botany.

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Summary

Pinus kesiya seeds collected during late January to late February were ideal for initiation of embryogenic cultures, whereas the seeds collected during March exhibited poor embryogenic response. The optimum embryogenic response was recorded in the secondary needles collected during April and May where whole needle surface was full of white, soft and translucent callus. About ~90.0% embryogenic response was recorded in apical domes (collected during May to July) before emergence of the needles. This response declined considerably in apical domes showing emergence of needles.

Incorporating PVP and citric acid in the culture media prevented the browning of both explants and media. 200 mg/l of PVP was found to be very effective for zygotic embryos and secondary needles whereas, a combination of 200 mg/l PVP and 100 mg/l citric acid for apical dome sections.

The optimum embryogenic cultures (79.6%) from zygotic embryos (from seeds stratified at 4°C for 24 h) resulted within 3 weeks in mMS medium containing 2,4-D, NAA (5 mg/l each), BAP (2.5 mg/l), sucrose (2.0%), CH and myo-inositol (1000 mg/l each) and L-glutamine (500 mg/l). The embryogenic cultures were white, soft, translucent and gelatinous whereas, the non-embryogenic cultures were hard and green. The embryogenic response in ½mMS (57.4%), DCR (57.4%), ½DCR (55.5%), LP (51.8%) and ½LP (24.2%) media were recorded in decreasing order. In case of secondary needles, the use of MS medium containing 2,4-D, NAA (3.0 mg/l each), BAP (1.0 mg/l), sucrose (2.0%), CH and myo-inositol (1000 mg/l each) and L-glutamine (500 mg/l) was optimum for initiation of embryogenic cultures (88.6% response) followed by mMS (76.4%), ½MS (59.3%), DCR (50.7%) and ½ mMS (43.6%) media. Pre-culture of the apical dome sections on ½DCR medium containing activated charcoal (0.4%) at 4°C for 72 h was found suitable for initiation of embryogenic cultures. The culture of these sections in ½DCR medium containing 2,4-D, NAA (5.0 mg/l each), BAP (2.5 mg/l), sucrose (2.0%), CH and myo-inositol (1000 mg/l each), L-glutamine (500 mg/l) resulted in optimum initiation of embryogenic cultures (92.6%)

followed by DCR (64.8%), $\frac{1}{2}$ mMS (59.3%), mMS (38.9%) and LP (37.0%) media. For all the explants, cytokinins alone were found to be inhibitory for initiation of embryogenic cultures. The embryogenic cultures from all the explants were obtained in the dark at $25 \pm 2^\circ\text{C}$. Light was inhibitory for initiation of embryogenic cultures particularly for apical dome sections. The cultures turned hard and green in the light.

The embryogenic cultures obtained from different explants proliferated well on respective initiation medium when cultured at 2 weeks interval for 2-3 passages. Softer embryogenic cultures were obtained when embryogenic cultures from apical dome sections were cold-treated at 4°C for 24 h after two subcultures on initiation medium. PEMs and proembryos were formed on maintenance medium (respective basal medium) containing $1/10^{\text{th}}$ growth regulators of initiation medium along with other adjuvants. The proembryos, developed from zygotic embryos and secondary needles, converted into cotyledonary embryos within 2 passages on respective basal medium devoid of auxin and cytokinin but containing ABA (4.0 mg/l) and sucrose (4.0%). It was not possible to convert pro- and globular embryos into cotyledonary embryos in case of apical dome sections.

The cotyledonary embryos, obtained from zygotic embryos, elongated on mMS medium without any growth regulators, CH, L-glutamine but containing myo-inositol (100 mg/l), sucrose (3.0%) and activated charcoal (0.2%) at 12 h photoperiod (1900 lux light). These somatic embryos germinated in 3-4 weeks time on mMS medium containing sucrose (3.0%), kinetin (5.0 mg/l) and NAA (1.0 mg/l) at 12 h photoperiod. A 40% conversion frequency of somatic embryos to emblings was recorded.

The cotyledonary somatic embryos, obtained from culture of secondary needles, elongated on MS medium free of all growth regulators, CH, L-glutamine and contained myo-inositol (100 mg/l), sucrose (2.0%) and activated charcoal (0.4%) under 12 h photoperiod (1900 lux light). However, it was not possible to convert cotyledonary embryos into emblings.

About 150-200 mg inoculum per 15 ml of medium was found suitable for initiation of suspension cultures. The elongated single cells formed in the culture were proliferated by subculturing at 6-7 days interval for 3-4 passages. The cultures were diluted at 1:4 ratio at every subculture for zygotic embryos and secondary needles and for apical dome sections at 1:5.

In 3-4 passages, the ESMs formed from single cells through cleavage in respective basal medium containing $1/10^{\text{th}}$ growth regulators of initiation medium. The suspensor cell masses started accumulating and formed proembryonal head in growth regulators free medium containing sucrose (2.0% for zygotic embryos, secondary needles and 3.0% for apical dome sections) at 100 rpm.

It was observed that the specific activity of peroxidase increased after 10 days of culture on initiation medium. It was maximum at 10^{th} day of first subculture and followed a declining trend during the second subculture. Increase in total protein content was recorded upto 10^{th} day of first subculture after which it declined considerably which followed a similar pattern as that of peroxidase activity.