Preliminary Observations on Tissue Culture of Oil Palm

Oil palm is one of the important oil seed crops which have the capability of producing high yields of oil. This crop has been introduced into our country nearly three decades ago and it has been grown successfully under irrigated conditions. The research and development on this crop has been quite recent. Propagation of this crop is by seed germination. This crop is a monocot with a single stem and it does not produce any other vegetative propagative materials such as suckers. It is not possible to clone or propagate oil palm vegetatively or by conventional methods like grafting etc. Hence, tissue culture technique can be applied to clonally propagate oil palm. At National Research Centre for Oil Palm work on tissue culture of oil palm was initiated recently. A review of the previous reports on oil palm tissue culture reveals that the pathway of regeneration is via callus and callus induction requires a mixture of media along with auxins. Tissue culture has been reported from leaf explants of seedlings (Jones, 1974: Thomas and Rao, 1995; Karun and Sajini, 1996) and also spear leaves (Schwendiman et al. 1988). In most of the studies the reported concentration of 2,4-D was high in the range of 100-200µM. Even 400 -500µM of 2,4-D has been reported for in tissue cultures of oil palm (de Touchet et al., 1991, Texeira et al., 1994). At the same time there are reports that high concentration of auxins induce variation. Hence this preliminary study was made to observe the callus induction response of leaf tissues from germinating embryos, seedlings and mature plants to low concentrations of 2,4 - D.

Leaves from mature palm (Dura palm no:117), 20 days old *in vitro* germinated seedling and 2 - 3 month old germinated seedlings from the field was collected for the experiment. The leaf explants were washed with Tween 20(20%v/v) and then rinsed with running water thoroughly. All explants were rinsed with Mercurie chloride(0.1%) and then thoroughly rinsed with sterile water several times. MS-media (Murashige and Skoog, 1962) with minor modifications were prepared along with other additives such as Myoinositol (100mg/l), Sucrose (3%), Activated charcoal (0.25%) and Agar (0.8%). 2,4-D at different concentration ranging from 2.27 to 27.18µm was added to the medium. pH was adjusted to 5.8. The explants after sterilization were cut into 10mm segments and inoculated into the media. The cultures were maintained in dark at a temperature of 25°C for eight weeks before observing for callus initiation.

In yet another experiment mature fruits were collected from dura palm (Palm No. 117) and the mesocarp was scraped out. The nuts were surface sterilized with embryos were dissected out from mature fruits of oil palm (Dura palm no:117) and washed using Tween 20(20%v/v) and then rinsed with running water thoroughly. Nuts were surface sterilized using mercuric chloride (0.1%) and then thoroughly rinsed with sterile water several times. Embryos were dissected out from the nuts and inoculated in MS-basal medium with myoinositol (100mg/l), sucrose (3%), activated charcoal (0.25%), Agar (0.8%) and BAP at different concentrations ranging from 0 to 26.6 μ M. These cultures were kept in light and the germination percentage was observed after 20 days.

The cultures after inoculation were observed for callus induction. The first response was seen in seedling and embryo leaf. There was swelling of the explants within 10 days and this response was found in MS media with 2,4-D(9.06μ M) (Fig. 1). Callus derived from seedling leaves were transferred to modified MS media with BAP (17.76μ M) and shoot regeneration was seen within a period of 4 weeks. In case of the second experiment with embryos it was observed that embryos germinated on all media but maximum germination



Fig.1 : Callus induction from seedling leaf

percentage (83%) was obtained on MS medium with BAP(17.76 μ M). Some of the embryos formed root and shoot poles simultaneously (Fig. 2).These formed complete healthy plantlets on MS basal media.



Fig.2 : Germinating zygotic embryos with root and shoot



Fig. 3 : Healthy plant from embryo

(Fig. 3). The quick response of the embryo leaf and seedling leaf for callus induction clearly indicated their meristematic nature. The preliminary observations showed that mature explants may take more time for response and also may require a modified concentration of nutrients and growth regulators for callus induction. The embryo germination under *in vitro* conditions opens up the prospects of applying embryo culture techniques for controlled crosses and also for rescuing seed embryos of pisiferas (shell less variety) which germinates poorly under *in vivo* conditions.

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