SUMMARY

Variability aspects of callus formation, shoot formation, rooting and biochemical studies have been studied in the mulberry, *Morus Spp.* Moraceae, with reference to nutrient media, plant growth regulators, carbon source and anti oxidants.

1. Evaluation of suitable media

Between the two different media such as MS and B5 with 3mg/l BAP + 0.05 mg NAA tested for Morus Spp. varieties M₅, S₃₆, V₁ and Anantha, MS medium was found to be best basal medium for shoot induction when compared to the B5 media. Further, the auxiliary buds exhibited high percentage of callus formation and weight of callus in all the mulberry varieties. Hence, only MS medium was used to carry out all the *in vitro* experiments for plantlet regenerations.

2. Explant selection

In the present study different mature explants such as petiole auxiliary bud and shoot tips of Morus Spp. M₅, S₃₆, V₁ and Anantha were screened to evaluate best explant on different basal media containing BAP 3mg/l + 0.05 mg NAA. Among the various explants tested, only those of auxiliary buds and shoot tip explants showed positive morphogenetic response. In M₅, S₃₆, V₁ and Anantha species auxiliary bud explants were more effective for proliferation of shoot than shoot tip explants, whereas petiole failed to initiate shoots.
3. **Callus formation**

In our present study highest callus initiation was observed at 2mg/l 2, 4-D concentration. Mulberry varieties M₅, S₃₆, V₁ and Anantha were cultured on MS media, supplemented with 2,4-D 2 mg⁻¹. MS medium containing 2 mg/1⁻¹ 2,4-D produced maximum fresh and dry weight of callus in 20 days. Highest callus initiation was observed at the concentration of 2 mg⁻¹ of 2, 4. D. Thus the present study provides a scope of rapid callusing of M₅, V₁, S₃₆ and Anantha on MS + 2, 4. D (2,4-Dichloro phenoxy acetic acid).

4. **Shoot multiplication**

Auxiliary bud explants were cultured on basal medium (MS) supplemented with various cytokinins, auxins and antioxidants either alone or in combination. BAP, Kinetin and 2-ip were used to select best cytokine for shoot proliferation. BAP was found to be effective than 2-ip and kinetin. BAP of 2mg⁻¹ was found to be optimum concentration in four mulberry varieties. To improve further shoot multiplication rate of various combinations of three cytokinines + auxin were used. Highest shoot multiplication from the auxiliary bud explants of Morus Spp varieties were observed on MS medium with BAP 2 mg⁻¹ +NAA 1mg⁻¹.

5. **Invitro rooting and acclimatization**

*In vitro* grown shoots from nodal cultures having 2-3 nodes were excised and inoculated on half strength MS medium fortified with various auxins such as NAA, IAA and IBA in all four mulberry varieties. IBA was effective for *in vitro* rooting followed by NAA and IAA. *In vitro* plantlets with well developed roots were transferred to pots containing vermiculate and plantlets were subsequently acclimatized.
6. **Biochemical studies**

Since the building up and breaking down of protoplasm of regenerants is concerned with certain metabolites and certain enzyme activities, some biochemical studies were carried to know the plantlet regeneration and growth pattern of Mulberry varieties such as M5, S36, V1 and Anantha, during their multiplication and regeneration into shoots and plantlets. The changes in the metabolites such as starch, reducing sugars and total sugar content indicate that the accumulation of these metabolites till day '15' seems to reflect the high energy requirement of the organogenic processes, also this accumulation of starch and sugars play an important role as osmotic agents and further decrease in the levels is of much significance with associated visible manifestation of organogenesis. Increased activity of these hydrolytic enzymes such as amylases and acid and alkaline phosphatase enzymes during the present investigation indicated that degradation of different compounds proceeded in the regenerating tissues and this was concurrent with the high synthetic activity that occurs during organogenesis. Changes in the activities of soluble acid and wall bound invertase activities indicate the peak activities of the enzyme exhibits most rapid cell expansion in the regerants. The increased activities of nitrate reductase (s) and decreased activities of GDH isoforms confirm the active multiplication of explants without any sign of vitrification.

**CONCLUSION**

On the whole *in vitro* proliferated shoots were multiplied rapidly by culture of auxiliary nodal explants on MS medium. Media containing 3 mg\(^{-1}\) 2-4 D, produced maximum fresh and dry weight of callus. Highest shoot multiplication was obtained on MS media with BAP 2 mg\(^{-1}\) + NAA 1mg\(^{-1}\). Highest frequency of rooting was obtained from shoots cultured on MS medium with 0.2 mg\(^{-1}\) IBA. Biochemical studies exhibits an accumulation of total and reducing sugars, starch and total soluble proteins and hydrolytic enzymes confirm the active proliferation of explants, later regenerated into
plantlets. Increased activities of nitrate reductase (s), and decreased activities of GDH isoforms and it confirms the active multiplication of regenerants without any sign of vitrification. The invitro studies established in this study is an effective means for large scale micropropagation of commercially useful mulberry varieties. Though there was a similar trend in the entire mulberry varieties in the above said parameters, there were significant percent variations among the mulberry varieties in the same trends.