6. SUMMARY AND CONCLUSION

6.1. Micropropagation

1. MS medium proved to be the best for micropropagation of all the explants among the various media tested.

2. The explants failed to grow in growth regulator- free medium.

3. Among cytokinin treatments the synergetic effect of BA (2.0 mg/l) and KN (1.0 mg/l) proved to be the best when compared with individual treatments.

4. Increased shoot formation was obtained by the addition of auxins IAA/NAA to optimal cytokinin concentration BA (2.0 mg/l) + KN (1.0 mg/l).

5. Indole acetic acid (0.5 mg/l) and NAA (0.75 mg/l) proved to be effective for shoot proliferation when treated with BA (2.0 mg/l) + KN (1.0 mg/l).

6. Adenine sulphate proved to be inhibitory for both shoot induction and number of shoots formed when treated with optimum concentration of auxin and cytokinin.

7. Gibberellic acid significantly increased the shoot induction response, number of shoots formed and shoot length when treated with BA (2.0 mg/l) + KN (1.0 mg/l) + IAA (0.5 mg/l) / BA (2.0 mg/l) + KN (1.0 mg/l) + NAA (0.5 mg/l).

8. Phenolic exudation, which was inhibitory of shoot induction, was considerably reduced by the addition of antioxidants.

9. Ascorbic acid at 100mg/l proved effective in reducing phenolic exudation and thereby considerably increased shoot number followed by PVP.
10. Charcoal at higher concentration was found to be totally inhibitory to shoot bud induction response.

11. Among the various explants tested, CN showed maximum response with 100% bud break and 36 shoots/explant on MS medium fortified with BA (2.0 mg/l) + KN (1.0 mg/l) + IAA (0.5 mg/l) + GA3 (0.1 mg/l) + Ascorbic acid (100 mg/l).

12. Young axillary buds and shoot tip explants responded better than mature axillary buds and shoot tip explants proved that the age of explants was an important determinant for micropropagation.

13. Half-strength MS + IBA (0.5 mg/l) was essential for rooting shoots, since the shoots failed to form shoots in medium devoid of growth regulators.

14. Average shoot length obtained was 5.5-7.0 cm in all explants.

15. NAA 0.5 mg/l also produced rooting with highest root length of 4.6 cm, with increase in the concentration of auxins there was increase in the frequency of callus formation.

6.2. Organogenesis

1. MS media containing different concentrations of auxins was used for callus initiation.

2. Morphology of callus produced depended entirely on the type of auxin used.

3. MS+IAA at 2.0 mg/l and MS+NAA 3.0 mg/l concentration were proved to be the best for callus initiation. The callus was whitish yellow and nodular.

4. Stem and petiole explants showed high phenolic exudation and low callus formation in all the treatments.

5. The calli turned brown with increasing auxin concentration and number of days of inoculation.

6. Proliferation of callus was achieved on the same medium. Addition of cytokinins suppressed callus initiation.
7. For shoot bud regeneration combined effect of auxins and cytokinins were found essential and the callus failed to produce shoot buds both in the medium devoid of growth regulators and individual treatments of auxin and cytokinin.

8. Modified MS medium (MS+B_5) was found suitable rather than MS medium for shoot bud regeneration in addition with BA (2.0 mg/l) + KN (1.0 mg/l) + IAA (0.5 mg/l).


10. The rate of shoot multiplication was high in the initial subcultures on the fresh medium and latter declined.

6.3. Direct organogenesis

1. Direct regeneration was obtained from cotyledon and young leaf explant when cultured on mMS (MS+B_5) medium.

2. Shoot buds regenerated only from mid rib region of both cotyledon and young leaves and not from any other areas. Cotyledon explants gave better response than young leaf explants.

3. An average of 20 shoots / explant was produced from cotyledon explant when cultured on BA (2.0 mg/l) + KN (1.0 mg/l) + Ascorbic acid (100 mg/l) + IAA (0.5 mg/l) + GA_3 (0.1 mg/l).

4. An increase or decrease in the optimum concentration of auxin and cytokinin drastically reduced the percentage response and number of shoot buds produced.

5. Shoot elongation occurred on the same medium. Highest shoot length obtained was 8.3 cm in cotyledon and 8.0 cm in leaf explant at GA_3 0.5 mg/l concentration.
6. Rooting of shoots for both direct and indirect organogenesis was attained on half-strength MS + IBA 0.5 mg/l and half-strength MS+NAA 0.5 mg/l with cotyledon explants showing an average root length of 5.0 cm.

7. There was 75-80% survival of the plants on transfer from in vitro to in vivo condition.

6.4. Somatic embryogenesis

6.4.1. Somatic embryogenesis on solid medium

1. Cotyledon and young leaf explants produced white friable callus on 2, 4-D treated medium within 15 days of inoculation.

2. Optimal concentration of 2.0mg/l of 2, 4-D was needed for both callus initiation and proliferation.

3. Cotyledon explants showed highest response (95.4%) followed by leaf explants (93.1%), whereas petiole and internodal explants failed to produce embryogenic callus.

4. Somatic embryos formed from the callus when cultured on MS medium containing 2, 4-D (2.0 mg/l) and BA (1.0 mg/l), high ratio of 2, 4-D to low ratio of BA triggered somatic embryo formation.

5. Average number of 56 somatic embryos formed in cotyledon explant, whereas leaf produced 48 embryos/explant.

6. Development and maturation of somatic embryos was achieved on 2, 4-D free medium as 2, 4-D as found to be totally inhibitory of somatic embryo maturation.

7. Eighty six percent of globular embryos from cotyledon explant and 75.8% from leaf explants matured on MS medium containing glutamine10mg/l.
6.4.2. Somatic embryogenesis in liquid medium

1. Somatic embryos occurred only in the medium containing 2, 4-D. Highest frequency of somatic embryo induction occurred in MS medium containing BA (1.0 mg/l) + glutamine (10 mg/l) + 2, 4-D (2.0 mg/l).

2. Cotyledon callus cultures showed 93.3% embryo induction and 86.0% in leaf explants.

3. Frequency of somatic embryos formed in the liquid medium is higher than that in solid medium.

4. The number of somatic embryos obtained from cotyledon explant was 87 embryos and from leaf explant was 78.9 embryos.

5. Gibberellic acid at 0.1 mg/l produced 80% maturation in cotyledon derived embryos and 73.6% in leaf explants.

6. The matured embryos failed to develop into plantlets both in solid and liquid medium; only shootless roots developed in 40% of the embryos.

6.5. Determination of Berberine

1. NAA 3.0mg/l produced high amount of callus 15.1mg/l DCW followed by IAA 12.1 mg/l.

2. The highest biomass accumulation in terms of percent increase in callus biomass was at 25 to 35 days after inoculation.

3. Berberine was identified only in regenerative callus cultured on mMS medium containing BA (2.0mg/l) + KN (1.0mg/l) + IAA (0.5mg/l) and in field grown plant material.

4. The results showed that there was no correlation between biomass production and accumulation of berberine; rather it was growth hormone dependant.

5. The amount of berberine in field grown plant material was found to be higher than in callus cultures.
6. The amount of berberine present in field grown plant material was 2.796 mg/g and in callus it was 0.886 mg/g.

7. The presence of berberine in callus cultures has generated a great interest in tissue culture system of this plant, which may provide new avenues for large scale production of berberine in vitro. However, further studies are in need to improve the berberine production in vitro.