Summary And Conclusions

Genus Gloriosa has attracted the interest of cytologists, geneticits, taxonomists much more than those the phytochemists and the pharmacists. From the ancient time Gloriosa superba L. has been known as important medicinal plant and its tubers was employed in curing gout. Since the time the active principle colchicine has been extracted or characterised from G. superba L. commercial exploitation of this plant for extraction of colchicine has been of interest to the pharmaceutical companies. Moreover colchicine not only is used as a medicine but also employed in agriculture for it is the only known chemical which is able to induce polyploidy. Besides colchicine Gloriosa yields number of chemicals such as alcolchicine, gloriosin etc. which are also commercial importance. The earlier work in this laboratory developed tissue cultural methods of microprapogaton of Gloriosa (Puri, 1992). However the seedling under cultural conditions were raised successfully from the tuber eye buds and regeneration of tubers from the apical mmeristem has also been achieved in the modified MS medium. In the present investigation not only micropropagation technique has been developed and successful regeneration of apical meristem in the modified MS medium has been achieved but even callus regeration, single cell isolation and regeneration of embryoids in the suspenmsion culture have also been achieved.

The methodology employed in the present study is as per the standard technique of tissue cultura, where different solid media were prepared under aseptic conditions and the respective organs such as apical meristem, leaf tip tendril, the shoot portion below the apical meristem and the seed have been inoculated after sterilization. Culturing of all these explants has been carried out in basically three media i.e. (i) Murashige and Skoog medium (ii) Yeomans medium (iii) Whites medium, variously modified to suit the condition. The explants are inoclated in to the culture tubes under the aseptic conditions using laminar flow chamber. After securing with the cotton plug they were kept under constant temperature $25^{\circ} \pm 2^{\circ}$ C with a light regime of 2000 lux at the point of incidence. The light was supplemented with a bank of white fluroscent tubes and incadescent bulbs. The temperature was maintained with help of airconditioner. For the chromatographic detection of colchicine the method followed was of Harborne (1973).

FINDINGS

The highlights of the findings are as follows :

1 In the apical meristem of G.superba L. is transferred to MS medium supplemented with +CW, 20% + 2,4-D,4 ppm, + IAA,8 ppm, + K,5 ppm + CH,5 ppm. The shoots started growing in to seedlings while the cut end of the shoot started callusing.

2. When the explant is held in B_{MS} + CW, 20% + IBA, 5 ppm + K, 5 ppm + CH, 10 ppm it started differentiating into small protocorm like multiple tuberlets.

3. When the shoot is transferred to the medium B_{MS} + CW 20% + 2,4-D 4 ppm + IAA, 4 ppm + K, 5 ppm + CH 5 ppm callus initiation took place.

4. When the shoot is held in B_{MS} + CW, 20% + 2,4-D, 4 ppm + K, 5 ppm + CH, 10 ppm good callus growth occured.

5. The explant shoot portion below the apical meristem, leaf tip tendril and seed tried in the three different media variously modified did not give encouraging results but for remaining green in some cases, for shorter or longer period.

6. The callus mass cultured in MS modified medium subcultured in to B_{MS} + CW 20% + 2,4-D 4 ppm + BAP, 2.5 ppm + CH, 10 ppm it induces sustained growth of callus.

7. When subcultured in B_{MS} + CW, 20% + 2,4-D, 4 ppm ± BAP, 10 ppm + CH, 15 ppm good profuse callus formation which shows tendency of greening.

8. When subcultured in B_{MS} + CW, 20% + 2,4-D, 4 ppm + IAA, 8 ppm + K, 5 ppm + CH, 10 ppm or B_{MS} + CW 20% + IBA, 0.5 ppm + BAP, 5 ppm + CH, 10 ppm, tuber differentiation occurred with cluser of tubers.

When subcultured into B_{MS} + CW, 20% + 2,4-D, 4 ppm + NAA 0.1 ppm + CH, 10 ppm or B_{MS} + CW, 20% + IAA, 2 ppm + K, 10 ppm + CH, 5 ppm embryoid like structures differentiated.

9 When subcultured in to Whites medium supplemented with Cw 20% and CH 50 ppm showed numerous Embryoid like structures.

10 When subcultured into liquid broth and agitated at 60 rpm with B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm and sucrose 3% isolation of single cells occurred.

11 When subcultured into liquid broth and agitated at 60 rpm in dark condition only isolation of single cell took palce.

12 When subcultured into liquid broth and agitated at 60 rpm in continuous dark conditions in B_{MS} + CW 20% + K, 5 ppm + CH, 10 ppm cells gets seperated and differentiate embryoids.

13 When the callus mass was squashed in Acetocarmine for cytological studies both dividing and non dividing cells were noticed. Some cells with large nucleus, some with distinct chromosomes, some with unequal cytokinesis are seen. Choromosomes obtained did not exhibit any breakage or loss in number.

14 When the G.superba and G.lutea were hybridized and the former is taken as recipient and later as donar, fruit setting and seed setting occured. But in reciprocal cross failure in the fruit setting and stability has been noticed.

15 When the colchicine was extracted from the callus mass and run on the TLC plate spot developed parallel to that of standard colchicine reflecting of the callus to synthesize colchicine.