Results And Discussions

A) RESULTS

In the present investigation greater emphasis has been given on successful culturing of Gloriosa superba, whose commercial importance has been discussed time to time the efforts have also been made to culture rather less seriously a temperate species Gloriosa lutea and which has been under cultivation from last more than five years in the botanical garden and has aclamatiazed as already discussed it is well known that these Liliaceous member are able to propagate by two means (i) predominentaly undergound tuber which posses eye buds, (ii) through seeds. Successful propogation of Gloriosa superba by tissue clture means througn adventitious bud i.e.eye bud has been achieved by earlier workers (Samarajeewa, P.K. ,1993, Puri, 1992). The formers not only raised the seedlings in vitro condition but even could achieve hardening beyond the laboratory condition and successfully transfered to the field. However they did not succeded in rasing the seedling through meristem culture, not did they reported to have tried culturing of various organs such as leaf tendril, seed and so on. Nonethe less (Puri 1992) did try to culture leaf tendrial, shoot merisstem besides tuber eye bud. Except tuber eye bud which could be sussfully cultured rest met with failure. In the present investigation therefore the effortrs have been made to culture followng explants.

i) Meristem cutlure

a) Shoot apical meristem

b) Leaf tip tendrill

ii) Shoot region below the apical dome

iii) Seed culture

iv) Callus culture

The different media tried are :

i) Murashige and Skoog medium (1962)

ii) Yeomans medium (1982)

iii) Whites medium (1963)

It is necesary to mention here that these media were modified to suit the situaiton

I) Murasshinge and Skoogs medium :

Composition of Basic medium and (B_{ms} (Reinert and Bajaj(1977)

Constituents	Mg/1 mg/L
$(NH_4)NO_3$	1,650
KNO_3	1,900
$CaCl_2{\cdot}2H_2O$	440
$MgSO_4{\cdot}7H_2O$	370
KH_2PO_4	170
$FeSO_4{\cdot}7H_2O$	27.8
Na_2EDTA	37.25
$MnSO_4{\cdot}4H_2O$	22.3
$ZnSO_4{\cdot}7H_2O$	8.6
H_3BO_3	6.2
KI	0.83

Constituents	Mg/1 mg/L
$Na_2MoO_4{\cdot}2H_2O$	0.025
$CuSO_4{\cdot}5H_2O$	0.025
$CoCl_2{\cdot}2H_2O$	0.025
Myo-inositol	100.0
nicotinic acid	0.5
Prydixine HCl	0.5
thiamine HCl	0.1
glycine	2.0

The medium was supplemted with :

Agar	-	6 gm/1,8 g /1
Sucrose	-	3%,5%
Coconut water	-	10%,.15%,20%
2,4-D	-	4 ppm
IAA	-	4 ppm,, 8 ppm
IBA	-	5 ppm
NAA	-	0.1 ppm
Kinetin	-	2-5 ppm
Casein hyrdolysate	-	5 ppm, 10 ppm

This suplimented medium was ued to culture various explants listed below.

Experiment No.1

Apical shoot meristem as an explant

The shoot tip was surface sterilized and inoculated in MS medium supplemented with :

Agar	:	8 gm/1
Sucrose	:	3%
Coconut water	:	10%,20%
IAA	:	4 ppm, 8 ppm
IBA	:	5 ppm
Kinetin	:	5 ppm
Casein hydrolysate	:	210 ppm
2,4 D	:	4 ppm
BAP	:	4 ppm

Table-I

	Values expressed in ppm							
C.W.	2,4-D	IAA	IBA	NAA	K	BAP	CH	Observations
10%	4	-	-	-	-	4	-	Yellowing explant
20%	4	-	-	-	5	-	-	Initiation of callus but fails develop further
20%	4	4	-	-	5	-	5	Callus initiation, growth rate very slow
20%	4	-	-	-	3	-	5	Better callus growth
20%	4	-	-	-	5	-	10	Very good callus growth
20%	-	8	-	-	5	-	5	Callus initiation at the base and shoot grow into seedling.
20%	-	-	5	-	5	-	10	Callus differentiates into many small protocorn type tubers

Table I gives the range of variations made in the supplements and the observations recorded. The major variation made in this experiment is with respect to the concentration of coconut water. The results obtained in the varying combinations of CW ,2,4-D,IAA,IBA, Kinetin and CH has been listed in the column of the table. It becomes clear that the explant failed to even remain green or show any tendency of growth in the combination B_{MS} + 10% CW + 2,4-D 4 ppm + BAP 4 ppm .Very interesting results have been obtained with these varying concentrations of supplements. The combination B + CW + 2,4-D 4 ppm + K 5 ppm initiates callusing of the explant esppecially from thecut end of the lateral leaf petriole. However, it failed to develop further with the combination B + Cw 20% + 2,4-D 4 pppm + IAA 44 ppm + K 5 ppm + CH 5 ppm. Here callus initiation did take place but growth was very slow. While from the above combination when IAA was removed and the concentration of kinetin was reduced to 3 ppm callus grow better indicating there by possible suppressive action of IAA on callus growth. Interesting took place but even explant started growing and differentiaed into seedlings: B_{MS} + CW 20% + IAA 8 ppm + K 5 ppm + CH 5 pm (Plnate III) . However, in the combination B + CW 20% + IBA 5 ppm + K 5 ppm + CH + 10 ppm instead of shoot growth callus differentiated into many small tbuer like structures which failed turn green. (Plate II, 5)It is notworthy to point out here that in the above set of experiemnt the concentration and combination

.f the supplement CW 2,4-D K and CH are same as that of the third combination listed in the table I except IAA which is double. In the former the callus initiation occurd and callus ketp growting at a slow rate. But in the later both callus growth as well as the shoot growth have been stimulated (Plate III).

Experiment No.2

Leaf tip tendril as an explant

The young leaves wsere first surface sterilized and with the help of sterile scissor leaf tip tendrils of 5-8 mm were excised and were inoculated in the MS medium supplimented with :

Agar	-	8 gm/1
Sucrose	-	3%
Coconut water	-	10%, 15%, 20%
IAA	-	4 ppm
Kinetin	-	2-5 ppm
Casein hydrolysate	-	5 ppm, 10 ppm
2,4-D	-	4 ppm

Table-II

	Values expressed in ppm							
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	Observations
10%	4	-	-	--	2	-	-	No significant result
10%	2	-	-	-	3	-	-	-do-
15%	4	-	-	-	3	-	-	-do-
15%	4	-	-	-	5	-	-	Explant remains green and afterward turns yellow
20%	4	-	-	-	5	-	-	Explant remained green for long time but failed to differentiate in callus
20%	4	4	-	-	5	-	5	-do-
20%	4	-	-	5	5	-	10	-do-

Since the leaf tip is modified in to tendril and the tendril keeps on growin simlar to that of a cucurbit members it is imperative that it possesses meristematic tissue. Therefore this part has also been taken for culturing to see whether it has any regeneration potential. The results of this experiment are tabulated in Table II in following three combination i) B_{MS} + CW 10% + 2,4-D 4ppm + K 2 ppm (ii) B_{MS} + CW 10% + 2,4-D 2 ppm + K 3 ppm and (iii) B_{MS} + CW 15% + 2,4-D 4 ppm + K 3 ppm no significant development has been noticed. However, in combination B_{MS} + CW 15% + 2,4-D 4 ppm + K 5 ppm explant remain green for some period later on it showed signs of death. Whereas, it with the same combination but concentration of CW increased to 20% made explant remain green for very long time. However, they did show any signs of differentiation. Similar is the situation even if the combination is changed to addition of IAA or NAA or CH (7th and 8th set in Table II).

Experiment No.3

shoot portion below apical region as an explant

Surface sterrilized pieces of about half centimetre shoot portion bvelow apical dome were incoculated on MS media supplemented with :

Agar	-	8 g/1
Sucorose	-	3%
Coconut water	-	10%,20%
IAA	-	4 ppm, 8 ppm
2,4-D	-	4 ppm
Kinetin	-	2-5 ppm
Cesein hydrolysate	-	5 ppm. 10 ppm
	-	

Table -III

	Value expressed in ppm							
C.W	2,4-D	IAA	IBA	NAA	K	BAP	CH	Observations
10%	4	-	-	-	2	-	-	No significant results
20%	4	-	-	-	5	-	-	-do-
20%	4	4	-	-	5	-	-	Explant remained green for long time
20%	4	-	-	-	3	-	-	Explant soon turned yellow
20%	4	8	-	-	5	-	5	Explant remained green for long time
20%	4	-	-	-	5	-	10	Explant remained green for long time and swelled butt failed to calluse
20%	-	-	5	-	5	-	10	No significant result

In the third experiment shoot portion just below the apical region has been tried for culturing . This is because of the reason known that in this plant branching is rare and even if branches possibly not the axillary bud develops into branches but sprouts from apical meristem itself. The observations are recorded in Table III. It is clear from the table that with the variation in the combination and concentration of supplement responses varied. Incombination B_{MS} + CW 20 % + 2,4-D 4 ppm + K 5 ppm no responses has been recorded whereas in the latter combination with only addition of IAA the explant remained green for long period when the IAA is not added and the concentration of kinetin is reduced to 3 ppm the explant started turning yellow (4th set in Table III). In combination B_{MS} + CW 20% + 2,4-D 4 ppm + IAA 8 ppm + K 5 ppm + CH 5 ppm also the explant remain green for a long time but failed to differentiate. But B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm the green explant showed the sign of swelling but did not burst in to callus (Plate II ,4) No change is seen in combination B_{MS} + CW 20 % + IBA 5 ppm + K 5 ppm + CH 10 ppm.

Experiment No.4
Seed culture

the seeds of G.Superba were soaked in running water for four days and were then surface sterilized and inoculated in MS media supplemented with :

Agar	-	8 g/1
Sucropse	-	3%
Coconut water	-	10%, 20%
2,4-D	-	4 ppm
IAA	-	4 ppm
IBA	-	5
Kinetin	-	5

Table-IV

	Values expressed in ppm							
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	Observations
10%	-	-	-	-	5	-	-	No significant result
10%	4	4	-	-	5	-	-	-do-
20%	4	-	-	-	5	-	-	-do-
20%	4	-	-	-	5	-	5	-do-
20%	4	-	-	-	5	-	10	-do-
20-%	4	-	5	-	5	-	10	-do-

In Glorisa there is poor germination of seeds. Possibly it has prolonged dormancy or the inhibitory substances present in seeds prevent germination. Therefore, in the present experiment seeds harvested in earlier period which were dried and stored in the laboratory were soaked in running water for four days to ensure removal of inhibitiors and then it was taken for culturing. The results have been tabulated in Table IV . It can be seen seen from the results that all the combinations attempted in the earlier experiments almost tried keeping only one factor constant i.e. Kinetin. However, the seeds did not show any signs of germinations.

II) Yeomans medium :

Basic Medium

Constituents	mg/1
$MgSO_4 \cdot 7H_2O$	480
KCl	29.8
$CaCl_2.2H_2O$	88.8
KH_2PO_4	81.6
$FeSO_4.7H_2O$	2.0
$MnSO_4.4H_2O$	2.0
$ZnSO_4.7H_2O$	0.4
$CuSO_4.5H_2O$	0.04
KI	0.04
H_3BO_3	0.04
$NH_4MoO_2.2H_2O$	0.04
Casein hydroilysate	500
Nicotinic acid	0.1
Pyridoxin HCl	0.2
Thiamine HCl	0.2

The Yemoans medium was suppmented with :

Agar	-	6 g/1
Sucrose	-	3%
Coconut water	-	10%, 15%,20%
2,4-D	-	4 ppm
IAA	-	4 ppm,8 ppm
Kinetin	-	1-5 ppm
Casein hydrolysate	-	1500 ppm

Experiment No.5

Apical shoot meristem as an explant

Surface sterilized apical shoot tips were inoculated in Yeomans medium supplemented with :

Agar	-	6 g/1
Sucrose	-	3%
Coconut water	-	10%,15%,20%
2,4-D	-	4 ppm
Kinetin	-	1-4 ppm
IAA	-	4 ppm, 8 ppm
Casein hydrolysate	-	1500 ppm

Table-V

Values expressed in ppm								Observations
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	
10%	-	-	-	-	-	-	-	No significant result
10%	-	-	-	-	1	-	-	-do-
15%	-	-	-	-	2	-	-	Explant turned yellow after some days
20%	-	-	-	-	4	-	-	explant remained green for long time but to differentiate
20%	4	4	-	-	4	-	-	Explant remained green for long time and after 4 weeks showed emergence of a shoot tip
20%	4	8	-	-	4	-	-	Explant remained green for long time

The standard basic medium(B_Y) has been supplemented and the list of supplements are given in the text. Simialr to that of culturing different expalts in MS medium here also four differenr organs have been tried

Experiment No.5 gives the account of apical shoot meristem culture. Results obtained are given in Table V. In combination B_Y + CW 10% and B_Y + CW 10% + K 5 ppm no change in the explant has been noticed on the other hand in B_Y + CW 20% + K 2 ppm explant show the sign of deterioation Where as in combination B_Y + CW 20% +, K 4 ppm explant remain green for long time but failed to differentiate (Plate IV,8). Similarly in the B_Y +CW 20% + 2,4-D 4 ppm + IAA 8 ppm + K 4 ppm also the explant remain green.The interesting result is in B + CW 20% + 2,4-D 4 ppm + IAA 4 ppm + K 4 ppm explant remained green for long time for four weeks and showed slight sign of growth but failed to keep up (plate IV,9).

Experiment No.6

Leaftip tendril culture

Surface sterilized leaftips were inoculated in Yeomans medium supplemented with :

Agar	-	6 g/1
Sucrose	-	3%
Coconut water	-	10%,20%
2,4-D	-	4 ppm
IAA	-	4 ppm
Kinetin	-	1-4 ppm
Casein Hydrolysate	-	1500 ppm

Table-VI

	Values expressed in ppm							
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	Observations
10%	-	-	-	-	-	-	-	Fail to survive
10%	-	-	-	-	1	-	-	-do-
20%	-	-	-	-	2	-	-	Explant remained green for some days
20%	4	4	-	-	4	-	-	Explant remained green for long time

In this experiment similar to that described earlier the leaf tip tendril has been inoculated in the supplemted Yeomans medium. The list of supplements is given in the text. The results are tabulated in table VI. In this experiment again no encouraging results have been obtained except in B_Y + CW 20% (2) K 8 ppm and B_Y + CW 20% + 2,4-D 4 ppm + K 4 ppm and (3) B_Y + CW 20% + 2,4-D 4ppm + K 4 ppm +

In this experiment similar to that described earlier the leaf tip tendril has been inoculated in the supplemented Yeomans medium. The list of supplements is given in the text. The results are tabulated in Table VI . In this experiment again no encouraging results have been obtained except in combination B_Y + CW 20% + K 2 ppm and B +CW 20% + 2,4-D 4 ppm + K 4 ppm and B +CW 20% + 2,4-D 4 ppm ± K 4 ppm + IAA 4 ppm where some sing of survival is seen by the fact that they remained green for shorter or logner periods.

Experiment No.7

Shoot Portion below the apical region

Small portions of shoot region below the apical region of about 0.5 cm size were surface sterilized and inoculated on Yeomans medium supplemented with :

Agar	-	6 g/1
Sucrose	-	3%
Coconut water	-	10%,15%,20%
2,4-D	-	4 ppm
Kinetin	-	1-4 ppm
IAA	-	4 ppm ,8 ppm
Casein hydrolysate	-	1500 ppm

Table-VII

Values expressed in ppm								Observations
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	
10%	-	-	-	-	-	-	-	fail to survive
10%	-	-	-	-	1	-	-	-do-
15%	-	-	-	-	2	-	-	-do-
20%	-	-	-	-	4	-	-	Explant remained green for long time
20%	4	4	-	-	4	-	-	-do-
20%	4	8	-	-	-	-	-	Explant which was green for a long time gradually turned yellow

The culturing of shoot portion below apical meristem region has been carried out and the supplements added to B_Y is listed in Text, the various combinations were tried and given in Table VII. In the combination B_Y + CW 10% and B + CW 10% + K 1 ppm and B_Y + CW 10% + K 2ppm no change have been noticed. But in combination B + CW 20% + K 4 ppm and B_Y + CW 20% + 2,4-D 4 ppm + IAA 4 ppm + K 4 ppm there are signs of survival by the fact that it remain green but failed to differentiate. But incombination B_Y + CW 20% + 2,4-D 4 ppm + IAA 8 ppm the explant remained green for long time but gradually started drying.

Experimental No. 8

seed culture

Pre soaked seeds were surface sterilized and inoculated in Yeomans media supplemented with :

Agar	-	6 g/1
Sucrose	-	3%
Coconut water	-	10%,20%
2,4-D	-	4 ppm
Kinetin	-	1,4 ppm
IAA	-	4 ppm
Casein hydrolysate	-	1500 ppm

Table -VIII

	Values expressed in ppm							
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	Observations
10%	-	-	-	-	-	-	-	No development
10%	-	-	-	-	1	-	-	-do-
20%	-	-	-	-	2	-	-	-do-
20%	4	-	-	-	4	-	-	-do-
20%	4	4	-	-	4	-	-	-do-

This experiment gives the account of seed culture in Yeomans medium. The suppliment list is given in the text the combination and the results have been recorded in Table VIII. In this experment non of the combinations tried showed any development In other words it failed to develop.

(II) White Medium:

Basic Medium

Constituents	mg/1
$Ca(NO_3)_2 .4H_2O$	300.00
KCl	65.00
KNO_3	80.0
$ZnSO_4.7H_2O$	3.0
H_3BO_3	1.5
Na_2SO_4	200.0
$NaH_2PO_4.2H_2O$	16.5
$MnSO_4.4H_2O$	7.0
KI	0.75
$MgSO_4.7H_2O$	720.0
Thimine HCl	0.1
Pyridoxin HCl	0.1
nicotinic acid	0.5
Glycine	3.0
Sucrose	20,000.0
pH	5.5

The whites Medium was supplemented with :

Agar	-	8 g/1
Sucrose	-	2%
Coconut water	-	10%,20%
2,4-D	-	4 ppm
IAA	-	4 ppm
Kinetin	-	2-5 ppm
Casenin hydrolysate	-	5 ppm,10 ppm,50 ppm
Ferric citrate	-	5 ppm
$CaCl_2.2H_2O$	-	0.33 ppm
Na_2 MoC $4H_2O$	-	0.28 ppm

Experiment No.9

Apical shoot tip culture

Apical shoot tips were surface sterilized and were inoculated in to Whites medium supplemented with :

Agar	-	8 g/1
Sucrose	-	2%
Coconut water	-	10%,20%
2-4-D	-	4 ppm
IAA	-	2 ppm
Kinetin	-	2-5 ppm
Casein hydrolysate	-	5 ppm,10 ppm,50 ppm

Table No.IX

Values expressed in ppm								Observations
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	
10%	-	-	-	-	-	-	-	No development
10%	-	-	-	-	2	-	-	-do-
20%	4	2	-	-	2	-	-	Remained green for a short period
20%	4	-	-	-	3	-	5	Remained green for long time medium turned brown
20%	4	-	-	-	4	-	10	Remained green for long time and get swollen but soon turned brown

The basic constituents of white medium and supplements added to that in present experiments are given in the text. Even in this medium culturing explants of shoot meristem, shoot portion below the apical meristem, leaf tip tendril and seeds have been tried.

In experiments No.9 the efforts have been made to culture apical meristem in supplemented whites medium. The observations are recorded in table No.IX. In this experiments various combinations of basic medium + variation in the concentration of coconut water 10 to 20% and 2,4-D IAA, K and CH have been tried. In B_W + CW 10% or B_W + CW 10% + K 2 ppm have been tried. In B_Y + CW 10% and B_Y + CW 10% + K 2 ppm no development could be seen. But in copmbination B_Y + CW 20% + 2,4-D 4 ppm + IAA 2 ppm + K 2 ppm the expant remained green but evantually failed to develop (Plate V,10). But incombiantion where CW and 2,4-D have been kept constant, K and CH varied respectively3,4,5 ppm and 5,4,50 ppm explant remained green for long time but still faied to develop.

Experiment No.10

Leaftip tendril culture

Leaftip tendril from young leaves were surface sterilized and were inoculated in whites medium supplemented with :

Agar	-	8 g/1
Sucrose	-	2%
Coconut water	-	10%,20%
2,4-D	-	4 ppm
IAA	-	2 ppm
Kinetin	-	2-5 ppm
Casein hydrolysate	-	5 ppm, 10 ppm

Table-X

Values expressed in ppm								Observations
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	
10%	-	-	-	-	-	-	-	No significant development
10%	-	-	-	-	2	-	-	-do-
20%	4	2	-	-	2	-	-	-do-
20%	4	-	-	-	3	-	-	Remained green for long time but failed to Calluse
20%	4	-	-	-	4	-	10	-do-
20%	4	-	-	-	5	-	50	-do-

In this experiments the combination tried and observations recorded are given in The Table No.X. No significant achievements have been made in supplemented whites medium but for remaining explant green for long time. In combination where CW and 2,4-D were kept copnstant at 20% and 4 ppm respective and K and CH varied the explant died.

Experiment No.11

Shoot region below apical merisitem

Young explant surface sterilized and were incoculated in whites medium supplemented with :

Agar	-	8 g/1
Sucrose	-	2%
Coconut water	-	10%, 20%
2,4-D	-	4 ppm
IAA	-	2 ppm
Kinetin	-	5, 10 ppm
Casein hydrolysate	-	5 ppm. 10 ppm, 50 ppm

Table-XI

Values expressed in ppm								Observations
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	
10%	-	-	-	-	-	-	-	No development
10%	-	-	-	-	2	-	-	-do-
20%	4	2	-	-	2	-	-	Remained green for a short period
20%	4	-	-	-	3	-	5	Remained green for long time, medium turned brown
20%	4	-	-	-	4	-	10	Remained green for long time and get swollen but soon turned brown

In this experiments the shoot region below apical meristem has been tried in the supplemented whites medium. In Table No.XI varation in the combination the combination B_W+ CW 20% and B_W+ CW 10% + K 2 ppm + no development occured but in combination B_W+ CW 20%+ 2,4-D 4 ppm + IAA 2 ppm + IAA 2 ppm + K 2 ppm it remained green for short period. But in combination B_W + CW 20% + 2,4-D 4 ppm + CH 5 ppm explant remained green for a long time but elicited some substances which coloured the cultured the culture medium brown. In combination B_W + CW 20% + 2,4-D 4 ppm + CH 10 ppm + K 4 ppm the explant showed the sign of swelling but failed to differentiate and soon turns brown.(Plate V,11)

Experiment No.12

Seed Culture

Presoaked seeds were surface sterilized and were inoculated in whites media supplemented with :

Agar	-	8 g/1
Sucrose	-	2%
Coconut water	-	10%, 20%
Kinetin	-	2 ppm ,3 ppm
Casein hydrolysate		5 ppm,10 ppm, 50 ppm

Table-XII

Values expressed in ppm								Observations
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	
10%	-	-	-	-	-	-	-	No significant result but browing of medium occurs
10%	-	-	-	-	2	-	-	-do-
20%	-	-	-	-	2	-	-	-do-
20%	-	-	-	-	3	-	5	No significant development
20%	-	-	-	-	-	-	10	-do-
20%	-	-	-	-	-	-	50	-do-

Culturing of the seeds in supplmented whites medium has been tired and results are given in Table XII. Even in the whites medium the different combinations of CW,K and CH have been tired which were of no avail and seeds failed to sprout.

Experiment No.13

Callus culture

Values are expressed in ppm									Observations
CW	2,4-D	IAA	IBA	NAA	K	BAP	CH	GA_3	
20%	-	2	-	-	10	-	5	-	Embryoid like small structures were formed
20%	-	-	1	-	-	5	10	-	Callus was showing small green patches
20%	-	-	-	-	-	5	10	4	Cellus turned yellowish brown very slow growth
20%	4	-	-	-	-	10	15	-	Good profuse callus development, greenish yellow colour but differentiation of shoot buds
20%	4	-	-	-	-	50	10	-	Good callus growth showing initiation of shoot buds in 1-2 test tubes but failed to develop
20%	4	-	-	-	-	2.5	10	-	Good callus growth
20%	4	-	-	0.1	-	-	10	-	Callus becomes whitish yellow giving rise to small embryoid like structures
20%	4	8	-	-	5	-	10	0	Small tuber like structures developed which further gave well organised clusters of tubers
20%	-	-	0.5	-	-	5	10	-	Small tuber like structure developed

Successful initiation of callus growth has been achieved in MS medium supplemented variously with CW ,2,4-D ,IAA,IBA, NAA, K ,BAP and CH. The observations are recorded in Table XIII. In the supplemented Ms medium very good callus growth could be achieved with the combination B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm. Prepatual growth of callus even by subculture could be seen which in it self is an achievement. To see whether there is any responses of callus to different permutations and combinations of supplement this experiment has been designed. The various combinations tried and the obsevations recorded are given in the TAble XIII It could be seen that when the callus is transferred to a medium with a combination B_{MS} + CW 20% + IAA 2 ppm + K 10 ppm + CH 5 ppm differentiation or development of embryoid like small structures were noticed . When transferred to a combination B_{MS} + CW 20% + IAA 1 ppm + BAP 5 ppm callus showed tendency of greening but the same callus when transferred to medium devoide of IBA but supplemented with GA 4 ppm callus turned yellowish brown and the growth is retarded.

In combination B_{MS} + CW 20%+2,4-D 4 ppm + BAP 10 ppm + CH 50 ppm good profused callus with tendency of growing is seen but failed to differentiate in to shoot.But in combination where concentration of all other supplements are same as above but BAP concentration has raised to 50 ppm callus showed tendency of growth and initiation of shoot buds but failed to develop. When transferred to the combination B_{MS} + CW 20%+2,4-D 4 ppm + NAA 0.1 ppm + CH 10 ppm callus become withish yellow but

but gave rise to small embryoid like structures. But when it was transferred to a medium B_{MS}+ CW 20% + 2,4-D 4 ppm + IAA 3 ppm + K 5 ppm + CH 10 ppm tendency of tuber development was seen which further differentiated into organised cluster of tubers.

Experiment No.14

In the preceeding experiment (i.e. EXpt. 13) the callus that has been successfully raised from shoot meristem in supplemented MS medium was subcultured. This experiment has been designed to see wheather the small tuber like structures differentaiated from the callus recorded in the 8th combination listed in the table XIII of the experiment 13 is able to regrate or differentiate in to shoot. Therefore in this experiment tuber like structures differentiated in the combination B_{MS} + CW 20% + IBA 0.5 ppm + BAP 5 ppm + CH 10 ppm were transferred or subcultured in to a medium B_{MS} + BAP 5 ppm + GA 4 ppm. As recorded in the table XIV it has been noticed that the clusters of tubers at their junction started turning green. However, the tuberlet tips remained colourless.

In the second half of the experiment the callus was transferred from B_{MS} + CW 20% + 2,4-D 4 ppm + BAP 5 ppm + CH 10 ppm to medium with a combination B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm. Within a few days of transfer the callus started differentiating into a small embryoid like structure whitish in nature preipheraly. However, repeated subculturing of the callus in B_{MS} + CW 20% + 2,4-D 4 ppm ± K 5 ppm + CH 10 ppm no differentiation occurred but callus kepts on growing with a slimy coating on the surface of it .When the callus was transferred from B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm of the experimewnt No.1. to the whites medium supplemented wth Cw 20% and CH 50 ppm it showed numerous embryoid like structures whitesh in nature(Plate VI)

Experiment No.15

Suspension culture

Various tenets of tissue culture are available.Having achieved the callus differentation the main objectives of the present investigation remains to be looked for is to see whether the callus cells still possess the ability to synthesize colchicine and also to make observations how the callus divides. Therefore the callus was variously subcultured and also transferred to suspension liquid broth so that the cells would be seperated. As given in the earlier experiments the media that gives a callus growth is B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm. The same medium was followed for the suspension culture but in the liquid form with a varied combination of light and temprature regims. The basic medium was supplemented With ingredients mentioned as above except sucrose whose concentration was varied in 3% and 5%. The conical flasks carrying the suspension of callus mass in to the liquid broth were clamped on horizontal roratory shaker and agitated at 60 rpm under the light with a constnat temperature of 25° C ± 2° C. They were periodicaly sampled to examine the seperation of cells. The results are given to in the Table. In them medium B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm and sucrose 3% cells got seperated and cells of various shapes and sizes were seen, both under continuous light and continuous dark

conditions. In a medium B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm and sucrose 5% the same result as in the previous combination have been obtained. When it was tried in the medium B_{MS} + CW 20% + K 5 ppm + CH 10 ppm + sucrose 3% i.e. devoid of 2,4-D or auxin and kept under agitation under continous light cells got seperated and they were of various shapes and size (Plate VII). But paralle to this the callus mass in the same cobination but in dark cells get seperated immediately on third day of inoculation and out of these cells some slowly differentiated in to embryoids in about 15 days time (Plate No. VIII).

Media composition	Results
i) B + CW 20% + 2,4-D 4 ppm + K 5 ppm, + CH 10 ppm (Sucrorse-3%)	Cells gets seperated and showed various shapes size in both light ,dark conditions
ii) B + CW 20%+2,4-D 4 ppm + K 5 ppm + CH 10 ppm, (Sucrose-5%)	Merely same results as above embryoid formation But some of the cells have shown correct shape both in dark and light conditions
iii) B + CW 20% + K 5 ppm+ CH 10 ppm (Sucrose 3%)	In light condition,cells get seperated showing varied shapes In Dark condition cells get seperated immediately on 3rd day of inoculation while embryoid have been reported on 15th day.

Experiment No-16

Chromatographic seperation of colchicine

In order to see whether the callus growing under culture condition is able to synthesize colchicine, the callus sample was taken and the alkaloid was extracted. After condensing the extract it was run on the TLC plates. Parallely

standard colchicine was also run in the solvent prepared by mixing Methanol and Ammonium hydroxide in the proportion 200:3 respectively. After running the plate 6 h were dried and Dragendroffs reagent was sprooid to detect colchicine. On the phtographicplate it can be clearly seen that there is faint development of spot parallel to that of standard colchicine indicating there by the ability of callus to synthesize colchicine.(Plate IX).

The same extract of callus was run even of the Whatman filter paper strips. However, the spots failed to develop.

Experiment No.17

Cytological studies in callus

When the explant shows tthe sign of growth in the suitable medium first thing that is noticed is meristem starts callusing and if the medium is accomplished for undifferentiated growth of the explant callus continues to grow in to a mass by repeated divisions. Often such an undifferentiated growth of cells also sho[illegible] variation in cell division. In order to study how the cells divide the callus has been put in to the suspension culture for seperation and tne cells that seperated have been studied for further cytology. The cells having different stages of cell division have been obtained in suspension culture and they have been microphotographic (Plate X)

The cells of different size and shape seperated from the callus mass could be seen. Moreover normal large turgid cells with prominant nucleus which appear to have potential for division have also seen (Plate X) Such cells

whenever division chromosomal differentiation started and the cells started dividing. From plate it can be seen that dividing cells are more or less same in size and shape with equal distribution of chromosomal mass. Occasionally cytokinesis did not appear to be equal though the chromosomal distribution appeared to be equal resulting into two cells of unequal size and shape.

Experiment No.18

Hybridization

Hybridization between Gloriosa superba and Gloriosa lutea has been carried out in the mansoon season of 1993. Twelve to fifteen crosses were made between them. Following observations have been recorded.

When G. superba is used as a fema le and G.lutea as a pollen donar fruit and seed development took place (Plate No XI,33) Where as in the reciprocal crossed i.e. when G.lutea was chosen as a female plant and G.Superba as a pollen donor no fruit setting occurred.

B) DISCUSSION

India being a tropical country it is a treasure of diversified plants. Moreover a vast stretch of land mass with its mountaious terains rivers, the varied ecogeographical conditions covering both tropical and pantropical climate is able to sustain both the tropical and semitemperate to temperate plants, growing in extreme drought condtion to plants exposed to torential rains thaugh out the year This ecogeographical condition being unique inflict upon vegetation diversity. For instance in the genus Gloriosa it self out of all species, 7 species are found in India. Especially among the monocots the family Liliaceae is considered to be the largest one and maximum number of species are found in India. With its mode of propagation both through underground organ as well as seeds it is able to withstand and overcome unforseen vegaries of nature. But it is a treasure of diversified chemical principles of great importance medicinal values. For instance Gloriosa has been known in ancient time both in East as well as in West that it cures gout. Therefore the entire pharamaceutical industries of the world is concentrating its eye on these treasure and hence the only way of conserving and perpatually harness the vast chemical potential is the rapid cultivation and culture and develop method of prapagation and there-by to divertify the exploitation of natural sources to the four walls of the laboratory where the

modern technology can be employed to make the tissues to grow as well as perpatually yield the chemical principle.

1 MS Medium

Efforts have been made earlier to propogate Gloriosa through tissue culture means where different organs have been used (Puri, 1992, Samarajeewa et al, 1993). Puri (1992) tried micropropagation method through various organs such a tuber eye bud, the apical shoot meristem, seeds, leaf tip tendril and so on. Out of them she was able to raise the seedlings successfully from the tuber eye buds under laboratory conditions. While Samarjeewa et al (1993) tried to raise the seedlings of Gloriosa superba the shoot meristem successfully. However, the different tanets of tissue culture as well as recipies have not been tried with reference to G. superba. In the present investigation culturing of shoot meristem, induction of callus, suspension culture, single cell isolation and cytological studies tabulated in the tables give the achievements.

Although the greater emphasis has been given to the callus initiation in the shoot meristem using the modified MS medium, the other observations have also been recorded. As indicated in the table No.I B + CW, 20% + 2,4-D, 4 ppm with varying concentrations of K(3-5) and CH (5-10 ppm) very good callus growth could be achieved. The same combination both K and CH 5 ppm but addition of IAA 8 ppm led to the sprouting of the shoot meristem in to a seedling, although

basal cut end starts callusing (Plate No. II)Puri (1992) tried to culture shoot meristem in the modified MS medium where she tried with various combinations of CW,IBA,2,4-D,NAA,IAA and K it was of no avail. However with a modified MS medium having combination CW 15% + IBA 0.025-16 ppm + K 0.025 -8 ppm the explant burst in to a mass of cells and gave rise to tiny root like growth which later on turned in to which brown tuber like organ. Besides MS, she also tried several other media, none gave the successful results. Where as the same modified MS medium suplemented with CW 20% + 2,4-D 4 ppm + IAA 8 ppm + K 5 ppm + CH 5 ppm in the present investigation induced the shoot initiation. Samarjeewa et al (1993) successfully could induce shoot initiation where first they inoculated the explant shoot meristem to B_5 i.e. Gamborgs medium containing BAP or K 0.1 to 5 mg^{-1} IAA, IBA,NAA or 2,4-D 0.01 to 5 mgl^{-1} the shoot meristem did not show any sign of growth till they transfered 3 week after to a liquid MS medium with BAP 0.01 -10 mgl^{-1}. IBA 0.01 -5 mg^{-1} the multiple shoots that developed in to this liquid broth after agitation were transferred to solid medium containing IAA,IBA,NAA or 2,4-D with a concentration varying from 0.05 to 5 mg^{-1} for root induction . Three months after holding the shoot in the above liquid recipy. It was transferred to solid B_5 medium for root initiation. In other words the circutus way and different recipies employed and need to transfer periodically from one to another, plus the time required does not provide the easy way of micropropagation in Gloriosa. Where as in the present investigation only the MS modified medium is used as mentioned in the discussion

end given in the table No.I. And the same MS medium but with IBA led to the root initiation, is in itself reinforces the easy methodology of micropropagation. The only thing is they have not been tried for hardening. The seedling were robust and healthy with multiple shoots (Plate No.III) Secondly the time required for raising the seedling by this micropropagation method in only 9 weeks that is within 2-3 months we have well developed seedling. The combination that B_{MS}+ CW 20% + 2,4-D 4 ppm + IAA 8 ppm + K 5 ppm + CH 5 ppm that initiates the shoot growth as well as initiates the callus growth at the cut end has a duel purpose. (i) Leading to the micropropagation and (ii) to the callus biomass.

It is well documented in the literature that the auxin and cytokinin are the two important growth regulators responsible for differentiation . The ratio of Cytokinin to auxin is important during the process of organogenesis (Skoog and Miller ,1957, Reinert, 1973, Fonnesbech, 1974). According to them it does not follow that the exogenous auxin is obligatory. The auxin stimulates shoot cell elongation while the cytokinin promotes the cell division in plant tissue and regulate growth and development in the same manner as Kinetin (6-furfurylaminopurie). Cytokinins are mainly N-6 substituted aminopurine derivatives (Doods and Roberts, 1985). However Benzyl aminopurine (BAP) also

falls in to this category or genus. In brief cytokinin suplements are instrumental in regulatiion of cell division, cell elongation, cell differentiation and organ formation. The most frequently employed auxins are IAA, IBA, NAA and 2,4-D amongst which the first is of natural occurence. Among the many auxins available IAA, the naturally occuring auxin is least active and is readily broken down by plant tissues. The most stable analogues are IBA and NAA which are generally more effective. The most active auxin is 2,4-D which is known to induce rapid callus formation and supress organogenesis strongly in many plant species (Hussey, 1986). As a matter of fact 2,4-D is regarded as antiauxin rather than auxin for it is notrious in desturbing the polarity but is taken as a 'prodigy' in callus growth. The fact the combination 2,4-D and IAA in ratio of 1:2 maintaining the concentration of K and CH same favours the shoot growth while 1:1 combination with a same the concentration of K and CH (Table-I) only induces callus initiation is reflective of the fact that IAA surmounts the effect of 2,4-D allowing it to function only at cut end region stimulating callus initiation.

Since the time micropropagation of plants through shoot meristem has been developed and the technique has been mastered, various media where developed. Most basic media were modified to suit the condition. Among the exaustive

list of such successful cultures published by Hu and Wang (1983) indicates that in maximum number of cases MS media has been used as a basic medium with additions of either IAA or IBA or NAA possibly because versatality of MS medium is grant besides less combursum. However, though Samarajeewa et al (1993) to initiate the shoot growth used B_5 medium supplemented with one of the auxins. They had to transfer to eventually MS medium for rooting. Micropropagation of Teccoma stans carried out by Baburaj and Gunasekaran (1993) also involved MS medium supplemented with BAP and IAA in the same way as Samarjeewa et al (1993). The fact that Gloriosa is able to regenerate and exhibit shoot growth in different media in itself is a testimony for the extreme elasticity of this plant.

Experiment No.2,3, and 4 have been made to culture leaf tip tendril, shoot portion below the apical region and seed in the same modified MS medium. It is noteworthy to point out that both the leaf tip trendril and shoot portion below the apical region more or less exhibited the same tendency. But for the explant remaining green for a long time no regeneration was obtained least is the achievement insofar as seed culture is concerned. Puri (1992) also tried to grow leaftip tendril under culture condition in MS medium with a negative result. Although the tendril taken as a explant to regenerate the seeding has not been reported by

Hu and Wang (1983), the successful tendril culture has been achieved by Batta (1972).

2 **Yeomans Medium**

Although successful culturing of shoot meristem in modified MS medium has been achieved, it was felt interesting to try even other media. The Yeomans medium as another important medium which differs from that of Murashinge and Skoog by lacking myo-inositol, EDTA and Glycine. However, it contains casein hydrolysate which provides almost all amino acids. Supplements added to basic medium has been given in the text.

When the explant of shoot meristem was transfered to this medium maintaining the same light and temperature regime as in earlier experiment no significant achievement but for explant showing the tendecy of growth by way of shoot tip emergence four weeks after the transfer in the combination B_Y + CW 20% + 2,4-D 4 ppm + K 4 ppm; even the explant didnot show the sign of callusing at the cut end. The tendency of growth exhibited by this which eventually failed may be due to auxin and Kinetin. When these thing are lacking tissue showed the sign of death. In the subsequent experiments in the Yeomans medium leaf tip tendril, shoot portion below apical region and seeds were tried in various supplemented media of Yeoman. None gave encouraging results. The possible reason may be, in

tne present course of study Kinetin has not been substituted by BAP and since the casein hydrolysate is a basic ingrodient it has been supplemented to very high concentration and this medium is lacking Myo-inositol, it is not able to effect or stimulate the growth. Nontheless it is evident from the literature that Kinetin is vis-a-vis BAP though, at time BAP is prefered (Dodds and Roberts, 1985).

3 Whites Medium

Whites medium is an another medium which lacks myo-inositol, casein hydrolysate, EDTA and Iron but contains sucrose in very high concentration (2%). May be that it provides a lattitude to vary the concentrations of some of the ingradients. However, the supplemented medium does contain Iron Calcium, Molybedanum amongst the inorganic and casein hydrolysate as organic supplement. Although most often this medium is used for initiation of embryo there are examples in the literature where organogenesis and callus development have also been achieved. It need not mentioned here that each plant is unique in its nutritional requirement for the growth which need to be tried by varying these parameters. So far as apical shoot tip is concerned no encouraging results nave been obtained evenwith various combinations of coconut water 2,4-D IAA Kinetin or

casein hydrolysate. Similar is the situation with the shoot tip below the apical meristem, Leaf tip tendril, where tissue remains green for shorter or longer period no reportable changes. So far as seed is concerned it remained recalcitrant.

It is noteworthy to mention here that although the seeds with a vigerous method of scarification by putting in to the runing water to washout inhibitors and transfering to different media variously supplemented with different auxins and cytokinins and so on, did not show any sign of germination. But the same seed when dissected and embryo was salvaged and put into whites medium gave most encouraging results by way of growing. However, the same seed when was put in to the soil started sprouting. These results make further problems of investigation.

4 Callus culture

We come to the aspect of great amusement and enchantment of successful initiation of callus growth in the MS medium (Table -XIII) variously supplemented with the coconut water ,2,4-D,Kinetin, IAA,IBA,BAP,NAA and CH. The fact that the very good callus growth could be achieved with a combination B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm provided the vast opportunity for many series of experiments and raise the callus mass (Tables 13 and 14). The explants mainly responded to

culture by way of callusing is in the cut end of the shoot meristem. However, other organs such as leaf tip tendril, seeds or regions below the apical shoot did not show any sign of growth or regeneration and callus formation. Samarajeewa et al (1993) reported that B_5, medium supplemented with BAP and IAA, IBA or NAA initiated primary growth and IAA,IBA and NAA at a high concentration of 1 mg^{-1}, and 2,4-D 0.1 mg^{-1} induced callus formation suppressing shoot growth. Where as in the present investigation not only a profuse callus development occured when the MS medium was supplemented with CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm without any of the other auxins IAA or IBA but entire explant absolved into callus mass with a rapid sustained growth of it when it was subcultured (Table XIII). They reported that 2,4-D suppresses the shoot growth in Gloriosa may be that in the present experiment, Even with the concentration of BAP 10 ppm, 2,4-D supresses the differentiation. The effect of 2,4-D could be subverted when the concentration of BAP is increased four fold which can be evidenced by the fact that callus mass show the sign of shoot initiation(Table Plate) .But when 2,4-D is totally eliminated and IAA K and CH is taken, embroyid like small structures started differentiating. Similar was the situation with 2,4-D 4 ppm + NAA 0.1 ppm and CH 10 ppm. The tendency of embryoid formation is seen but the autotrophic tendency has been throttled when BAP is removed from the culture.

On the other hand with a combination 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 + K 5 ppm + CH 10 ppm induced tuber formation from the callusing end of the shoot is very interesting aspect which has never been observed by Samarajeewa et al (1993). However, Puri (1992) observed tuber differentiation in the medium B_{MS} + CW 15% + IBA 16 ppm + K 2 ppm . In other words if the 2,4-D replaces any of the other auxin IAA, IBA or 2,4-D is replaced by IBA it does not make greater difference for all the group of auxins possibly work more or less in the same way by inducing the tuber formation. She also automatically varified these tuber to ensure that they are not mistaken for roots. No such observation hither to have been reported. But this opens an opportunity for rapid multiplication of Gloriosa by isolating these tubers and subsequently transferring them to soil conditions so that they develop into the seedlings.

The efforts were made to subculture the callus in the Whites medium as given in experiment 15. The combination B_{MS} + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm even under light conditions show embryoid like structural differentiating along the priphry of the callus mass. With an increased concentration of CH to 50 ppm

no change is seen. In other words this leads to hopefully looked forward for another means of propagating Gloriosa i.e. by way of successfully isolating hundreds and thousands of embryoids and subsequently the seedlings. This efforts has not been made by earlier workers in Gloriosa.

5 Suspension culture

When the callus mass was transferred to the liquid medium of MS supplemented with Sucrose, Coconut water, Kinetin, Casein hydrolysate and 2,4-D under varied combination of light and temperature regime and agitated at 60 rpm it led to the cell seperation especially in the medium MS + CW 20% + 2,4-D 4 ppm + K 5 ppm + CH 10 ppm and sucrose 3%. If the entire broth is kept under dark they differentiated in to embryoids. This enables to look for single cells having potentiality of synthesizing secondary metabolites under culture condition. Single cell if showing any genetic variation and evetntually differentiating in to embryoids enables better selection.

There are many examples where from the callus mass single cell isolation and eventual differentiation of them into embryoid have been accomplished. One of the recent studies of Ohashi Shin-Ichi et al (1993) developed a novel embryo forming system in which primary explant of carrot hypocotyl exposed to 2,4-D containing MS solid

or liquid medium for short time and released single cells and these single cells develop in to callus or embryogenic culture. And they found somatic embryos in absence of 2,4-D. In the present investigation successful isolation of single cells from the callus mass and eventual development of these cells into embryoids in the MS medium devoid of 2,4-D but supplemented with Kinetin and Casein hydrolysate and sucrose has been accomplished. It is surmised that if these embryoids are transferred to MS medium supplemtnted with 2,4-D it may lead to the dedifferentaitation of embryoid into callus mass which will be tne further plan of work.

6 **Cytological Studies**

The experiment No.17 is concerned with the cytological studies of the callus. Obviously when the callus is growing rapidly the cell division has to take place. Most often it is at tnis stage somaclonal variants are obtained. Therefore, the callus was squshed in acetocarmine and the chromosomes have been prepared, (Plate No. ×) gives the complete picture of the cytological structures of the callus cells. another important point to be emphesize here is callus is nothing but undifferentiated mass of cells and hence variing in shape and size are obtained which can be seen from the Plat No. × .Some of the preparations

that were microphotographed exhibited chromosomes in the process of condensation (Plate No. X). Yet some unequal distribution of cytoplasm leads to the cells of different sizes and shape. However, no chromosomal variations could be seen in the callus and hence it is difficult to conclude about somaclonal variation in ât this stage. Moreover somaclonal variation no are rare in diploids.

7 **Hybridization**

The results of hybridization between G.superba and G. lutea are given in he experiment No.18. The intersting aspect of this is when G. superba is taken as a female parent and G.lutea as male, fruit as well as seed setting occurred. But when the reciprocal crosses were made taking G. lutea as a female fruitbearing did occur but wither away soon. This only leads to argue that G. superba has a good combining ability while G. lutea dosenot have. this primiminary study challanges further investigation as to what exactly happens. When the gloriosa flower is pollinated by G. superba pollens as such max fail to germinate the study of pollen germination in G.superba was carried out and shown that pollens radily germinated and they do not have sterility (Lugade, 1987).

8 **Chromatographic seperation of colchicine**

Since the main bojective of callus growth was to test whether call using ofgan of G. superba maintains the ability to synthesize colchicine the callus mass was teste

by extracting this alkaloid and preparing the chromatogram in experiment No.16. Although no bright spot could be seen it did possess the ability to synthesize colchicine. Modification of medium by adding some of the stress causing chemicals may probably stimulate the synthesis of colchicine which will be further challange in the field.