IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 2, Issue 5 (Jul. – Aug. 2016), PP 14-20
www.iosrjournals.org

Development of a suitable plant regeneration protocol of cotton cultivar variety in comparison to non cultivar Coker

Nandini Bandyopadhyay[1], Soumitra Kr. Sen[2]
[1] *Department of Botany, Dum Dum Motijheel College,, West Bengal, India*
[2]*Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal, India*

Abstract: *We initiated our attempts with Coker-312(non cultivar) and other two cultivar varieties, Ankur-3 and JK-1. It was observed that genotype played an important role in callus induction and plant regeneration. Further, trials showed that kinetin played a major role in callus induction frequency. In case of JK-1, the MST_2 calli became dull and unable to produce embryogenic calli leading to elimination of these genotypes from further consideration. The other two genotypes, responded favorably in callus proliferation and emergence of embryogenic calli. We had used charcoal in MSG media which helped in production of root system in normal looking plantlets. Hypocotyl, cotyledon and roots have been used in the past as explants, where hypocotyls produce more friable callus than other explants. In the present study, different callus induction media with varying concentration levels of growth hormones were tested. The wide range of differences in callus initiation, proliferation and maintenance responses reflected for the influence of genotypic differences on growth pattern of cotton tissue in culture. Coker possessed the highest capacity for callus induction. Appropriate kinetin concentration level played major roles in callus induction in different genotypes. Coker is responsive to embryogenic development forming embryos on several media and the kind of explants used while Ankur-3 is fairly embryogenic. The nature of callus growth, colour, texture, friability and size, play determining roles in their ability to help regeneration via somatic embryogenesis. The embryogenic calli produced somatic embryos in MST_3 medium containing KNO_3 (played the key role in embryogenic growth) without any growth regulators. Presence of charcoal in the medium helped in formation of normal plantlets. In germinating somatic embryos, rooting is essential which has been problematic. As a result, only 5-6% of somatic embryos could be recovered to regenerate plantlets. On the contrary, we do observe that the protocol developed in this study for in vitro plant regeneration has been fairly efficient and reproducible.*
Keywords: *Callus regeneration, Charcoal, Genotypes, KNO_3, Somatic embryogenesis*

I. Introduction

Cotton is the world's most important commercial fibre crop, grown in 70 countries with about 35Mha land under cultivation worldwide. Over 180 million people are associated with the fibre industry that produces US$ 20 to 30 billion dollars worth of raw cotton. Almost 90% of the cotton value resides in the fibre (lint) that provides a source of high quality fibre for the textile industry. Cotton contributes 29.8% of the Indian agricultural gross domestic product, and nearly nine million hectares of land in India is used to produce 14.2 million bales of cotton lint (Barwale *et al*., 2004). Cotton seeds are an important source of oil and cotton seed meal is a high protein product used as livestock feed (http://apps.fao.org). Therefore, cotton production is an essential agricultural commodity to the global economy.

Somatic embryogenesis is the process by which somatic cells, under induction conditions, generate embryogenic cells, which go through a series of morphological and biochemical changes that result in the production of bipolar structure without vascular connection with the original tissue. *In vitro* development of cells and tissues depends on different factors such as genotype, type of plant, age and developmental stages of explants, physiological stage of an explants donor plant and the external environment which includes composition of media and physical culture conditions.

Several laboratories have attempted to optimize *in vitro* conditions required for plant regeneration and transformation of excised shoot tips, whereby, whole plants could be generated by organogenesis (Gould *et al*., 1991; Hemphill *et al*., 1998). Technical steps in the present study were adopted essentially based on successes as obtained earlier with Coker. The plants regenerated from shoot apices often create problem while rooting, suggesting that rooting has been highly dependent on genotype. The objective of present study is to develop a suitable *in vitro* plant regeneration protocol in cotton cultivars in comparison to non cultivar Coker.

II. Materials and methods

2.1 Plant Material

Seeds of cotton (*Gossypium hirsutum* L.), Coker-312 and two commercial varieties Ankur-3 and JK-1 were utilized in the present study.

2.2 Seed sterilization

Cotton seeds were washed with sterile water containing Tween-20 (2 drops of Tween-20 in 100ml water) for 20 min. After removing the seed coat, the seeds were placed on seed germination medium.

2.2 Seed germination

After surface disinfection, 50 seeds from each treatment lines were placed on seed germination medium in 3 replicated. The seed germination medium contained Murashige and Skoog (MS) salts (Murashige and Skoog, 1962) per liter; B5 vitamins; 3% glucose and 0.8% agar (Sigma, USA) (pH 5.8).

2.3 Induction of primary callus growth

After 6-7 days old hypocotyls developed from germinating seeds constituted the plant explants. They were cut transversely into 4-5 mm long pieces. The explants were placed on several culture media (MST1) on trial to determine the best hormonal regime that would be responsible for callus induction. MST1 medium (approx 30 ml medium/Petri plate) was supplemented with MS (Murashige and Skoog, 1962) basal salts, B5 vitamins (Gamborg et al., 1968), 3% glucose (w/v), 750 mg/l, MgCl2, 0.2% (w/v) phytagel (Sigma) and varying concentration of kinetin and 2, 4-D (growth hormones) at pH 5.8. All cultures were maintained at 28±1ºC under 16/8h (day/night) photoperiod. After 3 weeks these calli were transferred to 2nd medium MST2 (devoid of any hormone). The calli masses were then mulched and spread evenly as thin monolayer on the surface of the medium and incubated for further growth at 28±10C under 16/8h (day/night) photoperiod.

2.4 Regeneration of plants

Newly emerged calli were selectively picked up and transferred to the third medium, MST3 that contained extra nitrate source. The embryogenic calli were gently broken and spread on MST3. Thereafter, the calli were repeatedly subcultured after every 20 days on the same MST3 medium. Formation of embryos started to appear. The embryos were allowed to mature initially on MST3. When the embryos elongated, they were transferred to MSG1medium (½ MS salts, B5 vitamins, 0.1 mg/L GA3, 0.05mg/L IAA, 3%glucose (w/v), 2% active charcoal, 750mg/L MgCl2, 0.2%(w/v) phytagel and pH 5.8) in jelly-bottle for further growth for 2-3 weeks through at 28±10C under 16/8h (day/night) photoperiod. Rooting and shoot formation took place in this medium itself.

2.5 Hardening and transfer of regenerated plants in greenhouse

Regenerated plantlets 5-7 cm long shoots with 4-6 leaves with well developed roots) were placed in small cups filled with mixture of sterilized 1:1 of sand: soil in growth chamber under 60% relative humidity. The plants were then gradually hardened in growth chamber under 16/8h (day/night) photoperiod at 28±10C. After 2-3 weeks, they were transferred to large pots covered with transparent polyethylene bags which were intermittently removed to adapt the plants gradually with outdoor environment. All the regenerated plants were finally transferred to glass house.

Table 1: Composition of different media used in three genotypes of cotton

Medium	Composition		
	Genotype Coker-312	**Genotype Ankur-3**	**Genotype JK-1**
GM(Seed germination)	MS saltandB5vitamins,3%(w/v) glucose,0.8%(w/v) agar, pH-5.81	MS salt and B5vitamins,3%(w/v) glucose,0.8% (w/v) agar, pH-5.81	MS salt and B5 vitamins, 3%(w/v) glucose, 0.8% (w/v) agar, pH-5.81
MST_1(Callus induction)	MS salt and B5 vitamins,3% glucose, 0.5mg/l kinetin, 0.1mg/l 2,4-D, 750mg/lMgCl2,0.2% (w/v)phytagel, pH-5.81	MS salt and B5 vitamins,3%glucose, 0.25mg/l kinetin, 0.1mg/l 2,4-D, 750mg/lMgCl2,0.2% (w/v)phytagel, pH-5.81	MS salt and B5 vitamins,3% glucose, 0.75mg/l kinetin, 0.1mg/l 2,4-D, 750mg/lMgCl2, 0.2% (w/v)phytagel, pH-5.81
MST_2(Embryogenic calli induction)	MS salt and B5 vitamins,3%glucose, 750mg/lMgCl2, 0.2% phytagel(w/v), pH-5.81	MS salt and B5 vitamins,3%glucose, 750mg/lMgCl2, 0.2% phytagel(w/v), pH-5.81	MS salt and B5 vitamins,3%glucose, 750mg/lMgCl2, 0.2% phytagel(w/v), pH-5.81
MST_3(Regeneration)	MS salt and B5 vitamins,3%(w/v) glucose,750mg/l,$MgCl_2$,1.93gm/l KNO_3,0.2%phytagel (w/v), pH-5.81	MS salt and B5 vitamins,3%(w/v) glucose, 750mg/l $MgCl_2$,1.93gm/l KNO_3,0.2%phytagel (w/v), pH-5.81	–
MSG_1(Somatic embryogermination)	½ MS salt , B5 vitamins,3% glucose(w/v), 2gm/l active charcoal,1mg/lGA_3 750mg/l$MgCl_2$, 0.2% phytagel (w/v), pH-5.81	½ MS salt , B5 vitamins,3% glucose(w/v), 2gm/l active charcoal,1mg/lGA_3 750mg/l$MgCl_2$, 0.2% phytagel(w/v), pH-5.81	_

III. Results

3.1 Primary callus formation

Nine kinds of media formulation were tested with three different concentrations of kinetin and three different concentrations of 2, 4-D. It was observed that in case of three different varieties under study, callus induction depended on the concentrations of kinetin and 2, 4-D used. It has been found that in case of Coker, 0.5mg/l kinetin and 0.1mg/l 2, 4-D (K_2D_2) to be suitable; whereas in case of Ankur-3, 0.25mg/l kinetin and 0.1mg/l 2, 4-D (K_1D_2) have been found to be appropriate. In case of JK-1, 0.75mg/l kinetin and 0.1mg/l 2, 4-D (K_3D_2) turned out to be as suitable. The bar diagram in figure 1 shows response for callus induction of different genotypes, at varying concentrations of 2, 4-D and kinetin. After incubation for 3 weeks, whitish-yellow, friable calli masses were developed. They were then separated out from the remnants of the host tissue and subcultured in fresh medium for further growth. The genotypes showed differential effect of the composition of the medium. Critical differences have been estimated in case of the three genotypes in nine different media. In Table 2., callus induction frequency as shown in sin-x values showed that K_1D_2, K_2D_2, K_3D_2 are the most responsive media for callus induction for Ankur-3, Coker-312 and JK-1, respectively.

Table 2: Mean callus induction percentage transformed to $\sin^{-x}$ in nine treatments of two varieties

Treatment	Genotype			Mean
	Coker-312	**Ankur-3**	**JK-1**	
K_1D_1	44.36	43.24	43.84	43.81
K_1D_2	45.58	**63.52**	40.32	49.80
K_1D_3	46.16	47.9	44.42	46.16
K_2D_1	45.0	45.0	43.26	44.42
K_2D_2	**66.18**	46.74	43.84	52.25
K_2D_3	46.18	43.24	44.42	44.61
K_3D_1	45.58	45.0	46.74	45.77
K_3D_2	39.14	41.48	**61.96**	47.52
K_3D_3	39.12	37.96	45.0	40.69

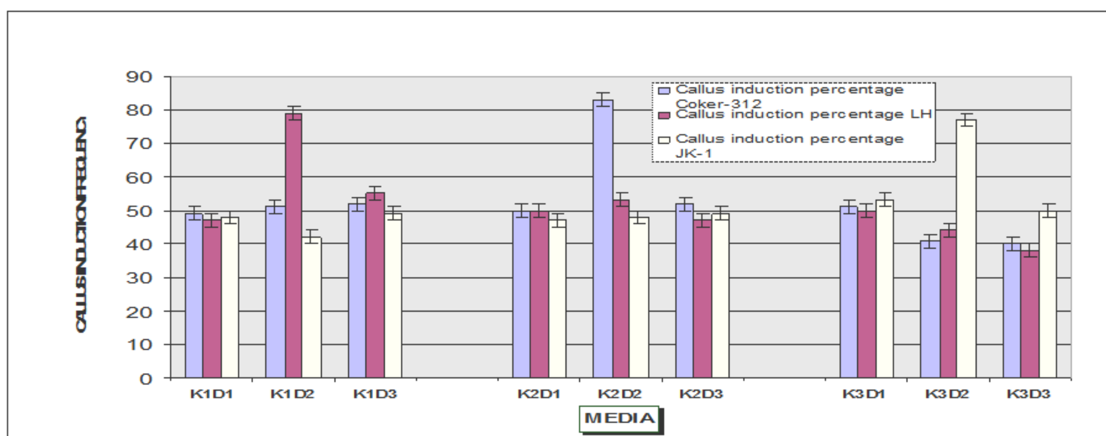

Figure 1: Callus induction frequency in varying concentration of kinetin and 2, 4-D of three genotype of cotton.

3.2 Transfer to embryogenic callus formingmedium(MST_2)

The calli mass generated in $MST_1$was further induced to proliferate for further one and half month 2-3 times in freshly prepared same medium. They were then transferred to MST_2 (MS salts + B_5vitamins + 3% filter sterilized glucose + 750 mg/l $MgCl_2$ + 0.2% phytagel, pH5.8) medium. The suitability of the developmental stage for callus growing in MST_1 medium was determined by visual observation in order to transfer them to MST_2. In case of the three different genotypes, the stages and the ages varied significantly. Four different ages viz. after two weeks, three weeks, four weeks and five weeks old calli were tried. Four weeks old calli was found to be most responsive for incubation in MST_2 medium (**Table 3**).

Table 3: Age of calli proliferating in MST_1 (primary callus induction medium) to respond to MST_2 medium (embryogenic calli formation medium) for embryogenic callus formation

Genotype	Age of callus in MST_1media(± SE)			
	Amount of callus formation in 2wks(g)[a] (Mean)	**Amount of callus formation in 3wks(g)[a] (Mean)**	**Amount of callus formation in 4wks(g)[a] (Mean)**	**Amount of callus formation in 5wks(g)[a] (Mean)**
Coker-312	2.20 ± 0.04	2.30 ± 0.37	**3.94** ± 0.17	2.62 ± 0.85

Ankur-3	2.16 ± 0.30	2.12 ± 0.05	**3.95** ± 0.10	2.42 ± 0.09
JK-1	2.34 ± 0.16	2.30 ± 0.11	**4.06** ± 0.08	2.22 ± 0.08

Values represent means of five replicates.

Table 4: Mean weight of calli in four treatments of two varieties

Treatment	Genotype (wt of calli in g)			Mean (g)
	Coker-312	**Ankur-3**	**JK-1**	
Two weeks	2.20	2.16	2.34	2.23
Three weeks	2.30	2.12	2.30	2.24
Four weeks	**3.94**	**3.95**	**4.06**	3.98
Five weeks	2.62	2.42	2.22	7.26

From the above data (**Table-4**) we found that four weeks old MST_1callus masses of all the genotypes were most responsive to proceed for embryogenic calli formation in MST_2 medium.

3.3 Embryogenic calli generation

The calli generated in MST_1were transferred to MST_2 media by spreading them into a fine lawn. They became brown after a few days. However in next 20 days, whitish or creamish (embryogenic) callus emerged from brown parent callus cells, in case of Coker-312. In case of Ankur-3, it took one and half month, whereas, in case of JK-1 no favorable response could be observed. In spite of our extensive trials to induce embryogenic calli formation in case of JK-1, we failed to make any progress. That prompted us to discontinue any further study with JK-1.The results of our attempts to encourage embryogenic calli formation in Coker-312 and Ankur-3 are represented in **Table 5** Coker took 20 days to produce embryogenic calli; whereas Ankur-3 took 40 days. The emerged embryogenic calli were transferred to MST_3 (embryo maturation) medium. The left over mulled calli were subcultured in fresh MST_2 medium for continuous embryogenic growth.

Table 5: Embryogenic growth induction in three different ages of callus (MST_2) growth

Variety	Age of calli in MST_2[a]								
	20 Days			30 Days			40 Days		
	Amt of Calli in MST_2[b]	Amt of calli[b] (±SE)	% of emb calli production	Amt of Calli in MST_2[b]	Amt of calli[b] (±SE)	% of emb calli production	Amt of Calli in MST_2[b]	Amt of calli[b] (±SE)	% of emb calli production
Coker-312	3.074	2.148 ± 0.01	**69.87**	3.014	1.476 ± 0.06	48.97	3.002	0.84 ± 0.08	27.98
Ankur-3	3.052	1.17 ± 0.01	38.34	3.133	1.722 ± 0.06	54.96	2.976	2.164 ± 0.08	**72.72**

[a-]Values represent mean of 5 replicates. [b-] Amount of callus in g.

3.4 Somatic embryo formation in MST_3 media

The embryogenic calli when transferred to nitrate rich MST_3 medium, they formed globular or heart shaped somatic embryos. The embryogenic calli took around one month to differentiate into somatic embryos. **Table 6** documents frequency of somatic embryo formation (indicated in percentage) from embryogenic calli. These embryogenic calli were repeatedly subcultured in fresh MST_3 medium to encourage renewed generation of somatic embryo formation. This can persistently be practiced for around one year, upon subculture each time in refreshed medium. After one year, these calli, lose their differentiating capacity.

Table 6: Percentage of embryogenic calli producing somatic embryos in MST_3media.

Genotype	Plantlet production from somatic embryo[a]			
	Amount ofembryogenic calli producing Embryo(g)	No. of plantlets/g of embryogenic calli	total no. of plantlet production (±SE)	% of plantlets production
Coker- 312	2.04	25	51.0 ± 1.67	49.02
Ankur-3	2.14	20	42.8 ± 0.66	46.72

[a-]Values represent mean of 5 replicates.

3.5 Optimization of regeneration frequency in cotton genotype

In case of both Coker and Ankur-3, the somatic embryos were developed from embryogenic calli in MST_3 media. Regeneration frequency of both genotypes is shown in **Table 7** Coker showed regeneration efficiency to be greater than that of Ankur-3.

Table 7: Comparison between embryo regeneration frequency of cotton genotypes

Genotype	No of observations[a]	Total no of embryognic calli	Total no. of embryogenic calli regenerated (± SE)	Regeneration frequency (%)	Mean frequency (%)
Coker-312	1	115	42 ± 0.24	36.52	**33.132**
	2	105	35 ± 0.31	33.33	
	3	95	30 ± 0.31	31.57	
	4	72	22 ± 0.26	30.55	
	5	92	31 ± 0.37	33.69	
Ankur-3	1	95	24 ± 0.37	25.26	**24.84**
	2	99	30 ± 0.37	30.30	
	3	103	25 ± 0.31	24.27	
	4	75	15 ± 0.31	20.00	
	5	90	22 ± 0.26	24.44	

[a-]Each observation is the mean of 5 plates.

3.6 Germination of somatic embryos

The somatic embryos developed from embryogenic calli in MST_3 medium were transferred to germination medium supplemented with charcoal or without charcoal. The composition of the basic medium has been as follows: MS salt, B_5 vitamins, 3% filter sterilized glucose supplemented with charcoal (MSG_1) or without charcoal (MSG_2). **Table8** shows comparative ability of two somatic embryo germination media ($MSG_1$and MSG_2) to produce normal plantlets. We found that the frequency of normal plantlet production in MSG_1 has been two to three times more than MSG_2. Thus, MSG_1 has been considered to be suitable for normal plantlet regeneration. These plantlets could adopt to greenhouse condition for further growth. It was further observed that Ankur-3 was not responsive to develop into normal plantlets in MSG_2 medium. The ill-formed plantlets that had developed were very irregular in developmental forms. They did not produce roots. But they were responsive in MSG_1 (with charcoal) while its rate of normal plantlet production had been quite low when compared to Coker-312.Thus, we could get normal plantlet formation in case of MSG_1. This prompted us to carry forward with Coker and Ankur-3.

Table 8. Comparison between normal plantlet regeneration frequency in two different germination media

Variety	Treatment[a]					
	MSG_1			MSG_2		
	No. of Total plantlets	Formation of normal plantlets	Frequency of normal plantlet production	No. of Total plantlets	Formation of normal plantlets	Frequency of normal plantlet production
Coker-312	55	14 ± 1.11	**25.45**	55	7.6 ± 0.58	13.82
Ankur-3	55	5 ± 0.224	**9.09**	55	0 ± 0.00	0.00

a-Value represents mean of five replicates.

Thus, MSG_1 was found to be a fairly suitable medium for generation of normal plantlets. **Figure 2** shows developmental stages of Cotton tissue resulting somatic embryogenesis and plantlet formation.

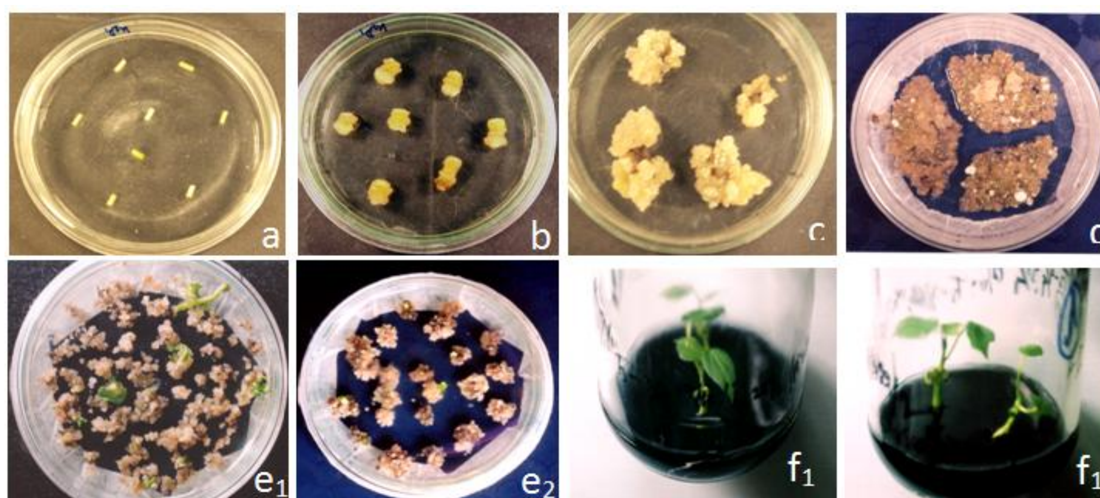

Figure 2: Different developmental stages of Cotton variety showing standardization of somatic embryogenesis. a. Cut hypocotyl of 7 days old seedling; b. Callus induction from hypocotyl in MST_1 media; c. Callus proliferation after one month which would go for MST_2; d. Emergence of embryogenic cells from MST_2 calli mass; e_1& e_2 showing somatic embryo and tiny plantlets of Coker and L-10 respectively; f_1& f_2 showing plantlets of Coker and Ankur-3 respectively in MSG_1 media.

3.7 Hardening and transfer of regenerated plants in greenhouse

Regenerated plantlets from the Coker-312 and Ankur-3 with well developed roots were hardened in growth chamber, subsequently they were transferred to greenhouse to adopt the plants gradually in outdoor environment. The plantlets grown in glass house showed 21.8% of survivability (**Table: 9**).

Table 9: Frequency of plants to survive in glass house condition

Genotype	No. of experiments[a]	No. of plantlets in MSG_1	No. of survived normal plantlets	% of survived plantlets.	Mean (%)
Coker-312	1	37	9 ± 0.31	24.3	**21.80**
	2	24	5 ± 0.22	20.8	
	3	34	8 ± 0.27	23.5	
	4	19	4 ± 0.31	21.1	
	5	26	5 ± 0.22	19.2	
Ankur-3	1	36	4 ± 0.24	11.1	**10.74**
	2	25	3 ± 0.32	12.0	
	3	39	5 ± 0.22	12.8	
	4	24	2 ± 0.21	8.3	
	5	21	2 ± 0.24	9.5	

[a-]Each observation is the mean of 5 culture bottles.

IV. Conclusion

We initiated our attempts with Coker-312 and other two cultivar varieties, Ankur-3 and JK-1. It was observed that genotype played an important role in callus induction and plant regeneration. Further, trials showed that kinetin played a major role in callus induction frequency. In case of JK-1, the MST_2 calli became dull and unable to produce embryogenic calli leading to elimination of these genotypes from further consideration. The two genotypes, Coker-312 and Ankur-3 responded favourally in callus proliferation and emergence of embryogenic calli. We had used charcoal in MSG media which helped in production of root system in normal looking plantlets. In case of Ankur-3 the frequency of normal plantlet production was very low and they did not possess the capability to revive in the green house. As a result, regeneration of normal plantlet formation failed to occur.

Different plant parts like hypocotyl, cotyledon and roots have been used in the past as explants, when it was found that hypocotyls produce more friable callus than other explants (Trolinder and Goodin 1988a, b; Chee *et al.*, 1990; and Sakhanokho *et al,* 1998). In the present study, different callus induction media with varying concentration levels of growth hormones were tested in different cotton genotypes. The wide range of differences in callus initiation, proliferation and maintenance responses reflected for the influence of genotypic differences on growth pattern of cotton tissue in culture. Genotype has been shown to play important role in callus induction and plant regeneration in several species. The nature of callus growth, colour, texture, friability and size, all play determining roles in their ability to help regeneration via somatic embryogenesis. The friable calli of MST_1 after 4 weeks were transferred to MST_2. In next 20 days, whitish / creamish embryogenic callus growth took place in case of Coker; whereas in Ankur-3 it took 40 days. In case of JK-1, no such response was observed. The embryogenic calli thereafter produced somatic embryos in MST_3 medium that contained KNO_3,without any growth regulators. It also became apparent that KNO_3 played the key role in embryogenic growth (Zhang *et al*., 2000).The embryogenic calli in case of Coker produced 49% plantlets; while in Ankur-3 46.72% plantlets were produced. The regeneration frequency was quite high. Presence of charcoal in the medium helped in formation of normal plantlets. In germinating somatic embryos, rooting is essential, unfortunately the same has received scant attention till now. As a result, only 5-6% of somatic embryos could be recovered to regenerate plantlets in Coker (Wilkins *et al*., 2000). Rooting of somatic embryos has been problematic (Gould *et al*., 1991; Hemphill *et al*., 1998). On the contrary, we do observe that the protocol developed in this study for *in vitro* plant regeneration has been fairly efficient and reproducible.

Acknowledgements

It is a great pleasure to express my deep sense of gratitude to Prof. S. K. Sen for his help and supervision during the tenure of this project. I would also like to extend my sincere thanks to all my laboratory colleages of Advanced Laboratory for Plant Genetic Engineering (ALPGE), IIT, Kharagpur for their invaluable support, help and cooperation offered to me during this research study.

References

[1]. Barwale RB, Gadwal RB, Zehr U and Zehr B (2004) Prospects for Bt cotton technology in India. *Ag Bio Forum,* 7 (1&2): 23-26. **http://www.agbioforum**.

[2]. Gould J, Banister S, Hasegawa O, Fahima M and Smith R (1991) Regeneration of *Gossypium hirsutum* and *G Barbadense* from shoot apex tissues for transformation. *Plant cell rep.* 10: 12-16.

[3]. Hemphill JK, Maier CGA and Chapman KD (1998) Rapid *in vitro* plant regeneration of cotton (*Gossypium hirsutum* L.) *Plant Cell Rep*. 17: 273-278.

[4]. Murashige T and Skoog F(1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant*. **15**: 473-497.

[5]. Gamborg O, Miller R and Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell. Res*. 50: 151-158.

[6]. Trolinder NL and Goodin JR (1988a) Somatic embryogenesis in cotton(*Gossypium*) I. Effects of source of explant and hormone regime. *Plant Cell, Tissue and Organ Culture*. 12: 31-42.

[7]. Trolinder NL and Goodin JR (1988b) Somatic embryogenesis in cotton(*Gossypium*) II. Requirement for embryo development and plant regeneration. *Plant Cell, Tissue and Organ Culture*. 12: 43-53.

[8]. Chee RP, Schultheis JR and Cantliffe DJ (1990) Plant recovery from sweet potato somatic embryos. *Hortscience*, 25:795-797.

[9]. Sakhanokho HF, Sharma GC, Zipf A, Saha S and Rajsekaran K (1998) Tissue culture potential of diverse diploid and tetraploid cotton genotypes. *Beltwide Cotton Prod. Res. Conf*. 1: 590-593.

[10]. Zhang B, Liu F and Yao C (2000) Plant regeneration via somatic embryogenesis in cotton. *Plant Cell. Tiss. Org. Cult*. 60: 89-94.

[11]. Wilkins TA, Mishra R, Trolinder NL (2004) *Agrobacterium* mediated transformation and regeneration of cotton. *Food, Agriculture & Environment*. 2(1): 179-187.