International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

Gibberellic Acid and Indole 3 Butyric Acid Regulation of Maturation and Accumulation of Storage Proteins (56, 34 and 26 KD) in Somatic Embryos of *Santalum album* L.

Abdul Ilah[1], Mazahirul Islam Syed[2], A. M. Reyad[3], Abdul Mujib[4]

[1]Faculty of Medical Technology, Omar Al Mukhtar University, Tobruq, Libya

[2, 3]Department Of Medical Biology, Deanship of Preparatory Year, Jazan University, Jizan, KSA

[4]Differentiation and Molecular Genetics Section, Department of Botany, Hamdard University, New Delhi-110062, India

Abstract: ***The ontogenetic path followed by somatic embryogenesis of Santalum album L. is extremely dependant on media concentrations of Gibberellic Acid (GA3), Indole 3 Butyric Acid (IBA) and 6-Benzyl Amino Purine (BAP). Initially callus was induced from nodes by using 1-Naphthyl Acetic Acid (NAA), 5.36 micro molar (µM). Aiming at embryogenesis, callus (200 mg) was incubated on Murashige and Skoog (MS) medium supplemented with NAA 5.36 µM + BAP 2.21 µM and found to produce a range of embryos types; globular, heart and premature torpedo embryos. We noted that after four weeks of culture on the same medium, premature torpedo embryos started to germinate precociously with anomalies. This problem was overcome by cultivating premature embryos on MS medium fortified with GA3 (1.44 µM) + IBA (2.46 µM) and BAP (2.21 µM). The premature embryos in low concentration of GA3, IBA and BAP supplemented medium were found to enter into short period of quiescent stage and precocious embryo germination is completely restricted and gradually gets matured and germinated. An important metabolic event we first time noted that regulation and accumulation of three storage proteins such as 56, 34 and 26 KD found in mature somatic embryos under IBA, GA3 supplemented medium. These storage proteins are essential for embryo maturation and germination. If, embryos are lacking of these proteins, embryos could not attain maturity and found to be abortive or precocious germination with anomalies.***

Keywords: Santalum album L; Somatic Embryos; Storage Proteins; Electron microscope

1. Introduction

The *Santalum album* L belongs to the family Santalaceae. This plant is known worldwide because of its unique fragrance in wood and its extraordinary medicinal and other industrial values (Lakshmisita 1986, Dikshit, Hussain 1984). The natural propagation of this plant is not easy and highly dependent on nitrogen fixing parasitic plants. It is a cross-pollinated plant, thus, seeds are extremely heterozygotic and seedlings are plagued by their variation in quality and quantity. The extensive exploitations and spike diseases has been a major threats for *Santalum album* (Coleman 1917, Verma 1969) led to the death of plant, causing huge economic loss. Thus somatic embryogenesis (SE) has been considered as important scientific method for improvement repercussion and rapid propagation. Somatic embryogenesis has been successfully applied to a large number of plant species but routine propagation of vigorous plantlets has limited the usages of this technique for large scale propagation. Plant scientists have now turned their attention to improve embryogenesis methods with evidence based high quality somatic embryos recovery and plant productions (A.Ilah et al. 2009, Sung Okimoto 1981, Sato et al. 1995, Dodeman Ducreux 1996, Alexandrova Conger 2002, Chugh Khurana 2002). Selection of high quality fully matured somatic embryos is still confusing and very few scientific reports are available. Some scientists very nicely suggested that presence of storage proteins in somatic embryos could be considered as excellent markers to assess embryos viability and maturity (Redenbaugh et al. 1986). In case somatic embryogenesis of Alfa alfa and cotton it was noticed that the storage proteins were more in later stage of embryos with greater vigor compared to other plant with lower storage proteins in somatic embryos (Redenbaugh et al. 1986, shomaker et al.1987). Comparing the storage proteins accumulation at last stage of somatic embryos with their zygotic counterpart, poor accumulations of storage proteins may be considered as poor maturations of somatic embryos (Crouch 1982). There are numerous reports on somatic embryogenesis of *Santalum album* but there is no or little evidence based recovery of mature somatic embryos for germination has been reported so far. We reported here embryo maturation marker storage proteins in late stage of embryogenesis. The protein profile of callus, heart and torpedo stages has been successfully conducted using Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis (SDS-PAGE) techniques to identify storage proteins which are utmost necessary for maturation and germination. Finally three storage proteins has been quantified (56, 34 and 26 kD) and proved to be dependable marker for harvesting fully mature somatic embryos at accurate stage of its vigor. This notable selection method of viable somatic embryos based on accumulated storage proteins would help to achieve a routine large scale matured somatic embryo recovery for artificial seed productions and germplasm conservation.

1.1 Material and Methods

Sources of explants, Culture conditions for callus induction, Plant Growth Regulators (PGRs):
Santalum album (L.) saplings were obtained from Karnataka forest department counter at Pragatimaidan New Delhi during national agricultural exhibition session. All saplings were around 6 months old. These saplings were transplanted in the herbal garden of Jamia Hamdard (Hamdard University), New Delhi, India. Plants parts were collected from those saplings and were used as experimental materials. About 5 - 7 cm shoot tips and tender nodes with dormant buds were excised from collected plants parts. All explants were soaked in 500 mL jars containing 300 mL of tap water for 40 minutes, and surface sterilized for 10 minutes with 0.1% mercuric chloride supplemented with 10 drops of Tween-20 detergent. Finally, explants were rinsed several times with sterilized distilled hwater and cultured on Murashige &Skoog (1962) basal medium (MS), supplemented with 3% sucrose, 8.0 g L−1 of agar. PGRs like 2, 4-D (2.25, 4.52, 6.78 and 9.05 µM), NAA (2.68, 5.37, 8.05 and 10.72 µM), IAA (2.85, 5.71, 8.56 and 11.42 µM) were added to the same MS medium and final pH set at 5.8, to induce callus. The cultures were maintained at 25 ± 2◦C under 16/8 h light and dark period with cool white florescent illumination (150–200 µEm−2 sec−1) and relative humidity were around 50–60%.

1.2 Estimation of callus dry biomass (dry weight)

Callus were collected (accurate 0.4g) from all treatment group of plant growth regulator and dried under oven with controlled temperature of 60^{o} C. Later on completely dried callus were weighed and their percentages calculated using formula i.e. dry wt./total fresh wt. × 100 = %. The different weight percentages were found and that helped to identify embryogenic tissue and non embryogenic tissue.

1.3 Development of somatic embryos

The callus induced from nodal or apical buds on MS medium supplemented with NAA were used as inoculums and incubated to embryogenic media fortified with NAA and BAP. The following concentrations of NAA (0.0,0.53, 5.36 µM), and BAP (0.44, 2.21, 4.42, 6.63 and 8.84 µM) were used to produce the range of somatic embryos stages such as globular , heart, torpedo and cotyledonary from the callus. The cultures were maintained at 25 ± 2◦C under 16/8 hours light and dark period with cool white florescent illumination (150–200 µEm−2 sec−1) and relative humidity were around 50–60%.

1.4 Maturation of somatic embryos

Maturation is the terminal event of embryogenesis and is characterized by the attainment of mature embryo morphology, accumulation of storage proteins, reduction in water content and often a gradual decline or loss of metabolism. Initial globular and heart embryos were incubated on MS with GA3 (0.28, 1.44µM), IBA (0.49, 2.46), and a variable concentrations of BAP (0.44, 2.21,4.42, 6.63 µM) aiming at to get developing stages of somatic embryos to be matured . The culture conditions were same as mentioned above.

1.5 Scanning Electron Microscopy (SEM)

To identify the correct morphological structure of globular somatic embryos frequently produced on NAA fortified medium we have observed under electron microscope and took photograph. For scanning Electron Microscopy, the tissues were fixed in 2 per cent glutaraldehyde adjusted to pH 6.8 in 0.1 M phosphate buffer for 24 hours at 4°C. The specimens were washed in buffer, post fixed for 2 hours in similarly buffered 1% osmium tetra oxide, and dehydrated in a graded ethanol series and finally coated with gold palladium by sputtering method by IB2 (Ion coater). The prepared samples were examined and photographed in a LEO 435 VP operating at 15-25 KV.

1.6 Protein analysis of embryogenic issues

The proteins from different collected tissues sources such as callus, heart, and torpedo embryos were isolated and used for analysis by using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

1.7 .SDS-PAGE Electrophoresis

The collected tissue was duly processed to be used for electrophoresis. Firstly 0.5 g tissue were collected in maceration plate added with 60 micro liter (µL) extraction buffer pH 6.8 (buffer contains: Tris 6.25%, mercaptoethanol 6.25% and SDS 2.5%) and with the help of maceration rod tissues were homogenized.Secondly, processed homogenized tissues were centrifuged at 10,000 rpm for 10 min at 4◦C. Then Supernatant carefully collected from the pellet and 200 µL Trichloro Acetic Acid (TCA) 10% was added and re-centrifuged at 6,000 rpm for 5 min. Finally pallet was collected and thoroughly dissolved in small volume of extraction buffer. The solution was heated at 100◦C for 5 min and after cooling kept at 4◦C until use. Just before loading into gel slab, with 1:3 ratio bromophenol blue and sample were mixed together. After complete run of the sample the *gel slab was kept for staining with Coomassie Blue R-250 solution. The stained gel slabs were washed and bands were photographed using a UV gel documentation system, Mitsubishi.*

1.8 Statistical Analysis

By using one-way analysis of variance all data were analyzed. Values are means of at least four replicates of two experiments and the mean values are separated by using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

2. Results and Discussions

2.1 Callus Induction

Juvenile apical shoot tips and nodes (1.5 – 2.0 cm) of around 6 months old plants were used as explants for better callusing response as recommended earlier by several researchers (Wernicke and Milkovits 1984, Morrish et. al. 1987, Mujib et al. 1996). Initially cultures of these explants

were marked by swelling of the explants upon incubation on auxins fortified MS medium, just after one week of incubation. Several auxin types with various concentrations were tested for better callus induction.

Table 1: Effects of 2,4-D; NAA; IAA in inductions callus of Santalum album L

PGR (µM)	Callus frequency of *S. album*	
	Explants types	
2,4.D	Apical buds	Nodes
0.45	0.0e	0.0e
2.25	70.0 a	77.4a
4.50	60.4 c	75.0b
6.75	62.5b	60.2c
9.00	35.6d	30.0 d
NAA		
0.56	0.0e	0.0e
2.68	60.2c	66.6 c
5.36	75.0a	75.7 a
8.04	70.3 b	71.1 b
10.72	38.4 d	30.9 d
IAA		
0.57	0.0e	0.0e
2.85	58.0 c	65.6c
5.70	70.7a	74.0 a
8.55	72.2b	71.0 b
11.40	24.9 d	30.6 d

Means with common letters within each column are not significantly different at $p \leq 0.05$ according to DMRT. Data were scored after 6th weeks of culture.

The NAA 5.37 µM - 8.05 µM and IAA 5.71 µM - 8.56 µM were effective by producing profuse friable callus mass slightly yellowish in colour (fig 2 a). The callusing response percentage was very satisfactory (75%) (Table1). Similarly 2.25 µM - 4.5 µM of 2,4-D fortified MS medium was also very effective (77%) for induction of whitish callus mass , while more higher concentrations of 2,4-D 6.78 µM - 9.05µM were always found to be less (35%) effective and induced callus turned brown after some times.

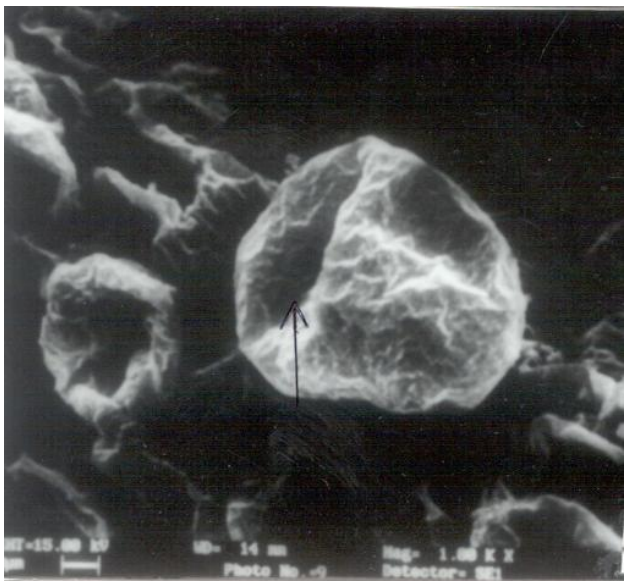

Figure 1: Scanning electron microscopic photograph: showing globular embryos (arrow) in NAA fortified MS medium

2.2 Embryo Development

We achieved best embryo development by using NAA induced embryogenic callus cell line whereas 2, 4.D induced callus cell line produces less embryos and restricted to globular stage but NAA based globular embryos (fig.1) produces more advanced stages of embryos, upon culture on embryogenic medium.

It seems that NAA based callus was metabolically more active than 2,4-D based callus . We estimated dry weight of NAA based callus cell line and found to hold heavy dry callus mass (0.035 g) 0.87 %, than 2,4-D based callus (0.31g) 0.77%. We transferred 200 mg NAA based friable callus mass in embryogenic medium which was fortified with NAA (5.36 µM) + BAP (2.21 µM -4.42 µM) and achieved profuse embryogenesis (78%) with 45 globular embryos ; 18 heart embryos and 12 under-developed torpedo embryos after 4 weeks of incubations (fig.2, b-c).

Table 2: Combined effect of NAA and BAP on somatic embryo development of Santalum album L

PGR µM		% embryo genesis/ explants		Mean number of somatic embryos/200 mg calluses/4 weeks			
				Embryo types *G – Globular; H – Heart; T* – premature Torpedo C – cotyledonary;*			
NAA	BAP	Node	Apical buds	G	H	T*	C
0	0.44	0	0.0e	0.0f	0.0c	0.0c	0.0a
0.53	2.21	11.2	12.0d	8.0e	0.0c	0.0c	0.0a
5.36	2.21	75.2c	73.0 a	35.3c	16 .0 b	10.0b	0.0a
5.36	4.42	78.2a	72.0b	45.0a	18.0 a	12.0a	0.0a
5.36	6.63	77.0 b	72.0b	38.0b	0.0c	0.0c	0.0a
5.36	8.84	70.2 d	69.0 c	30.0d	0.0c	0.0c	0.0a

Means with common letters within each column are not significantly different at $p \leq 0.05$ according to DMRT. The higher concentration of NAA (5.36 µM – 10.72 µM) + BAP (4.42 µM - 8.84 µM) found to be counterproductive (10%) and very less concentration was not effective (Table, 2). The prolong sub culturing with the same medium NAA (5.36 µM) + BAP (2.21 µM -4.42 µM) was not encouraging to reach the last steps of embryo maturations and germinations, although the proliferation of somatic embryos was continued. The progress and proliferations of embryo formation in presence of BAP and the effect of NAA on further increment of embryo frequency has been reported in many plant species (Bozena Szewczyk-T, Bozena P 2014, Mujib et al. 2005).

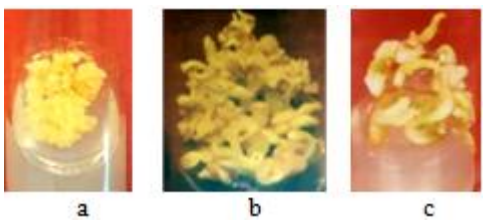

Figure 2: somatic embryogenesis and embling in *Santalum album l.*

Fig 2.a.friable callus in NAA fortified media,(b-c)Numerous somatic embryos with different stages such as globular,heart,torpedo on NAA + BAP fortified medium.(d)Fully matured torpedo embryos on BAP + IBA+ GA3 fortified media (e)Germinating cotyledonary embryos ,(f) Somatic embryo raised plantlet(embling) .

2.3 Embryo maturation and germination

Further, we found that low level treatment of GA3 (1.44 µM) + IBA (2.46 µM) and BAP (2.21 µM) supplemented MS medium was very effective (66%) and produces almost all stages embryos more regularly such as 46 globular, 25 heart , 15 torpedo and 6 Cotyledonary embryos. (Table 3).The medium with low concentration of GA3 and IBA were found to restrict new embryos formations but helped in differentiations of embryos towards higher stages of morphogenesis. The role of IBA in embryo differentiation has already been proved by Dane R et al. (1990) in interior spruce. These scientists suggested that low concentration of IBA played effective role in embryo maturation and normal cotyledon development whereas, higher level of IBA showed adverse effect with abnormal cotyledon development. The inhibitory role of GA3 for cell division and interactive role in organogenesis was reported in buckwheat tissue culture. (Veroslava S, Mirjana N 1985).We noted that, all stages of embryo development were dropped in medium with higher concentrations of BAP (4.42 µM - 6.63 µM) with GA3 (1.44µM) + IBA (2.26 µM). It is important to note that the higher concentration of BAP not only dropping all stages of embryos development but enforcing embryo irregularities which includes cotyledons.The authors reported earlier that embryo irregularity also observed on medium supplemented with BAP and NAA alone after 4 weeks of culture(Ilah A et al 2002).

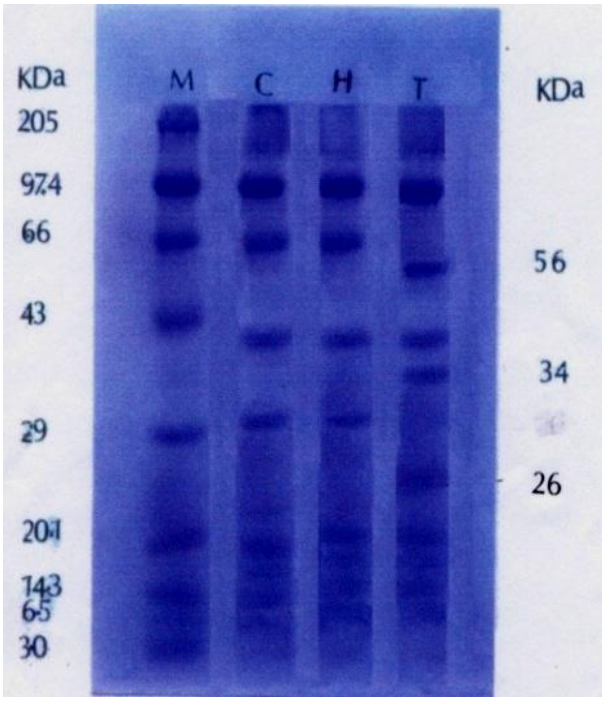

Figure 3: Stage specific proteins of various tissues during embryogenesis in *Santalum album L.* [M= Marker protein; C = Callus; H = Heart embryos; T = Torpedo embryos.]

Somatic embryo corresponding low molecular weight polypeptide chains has been characterized earlier in santalum album by some scientist (Sankara Rao K et al. 1996). They suggested that from stage 0 (callus) to stage 1 (globular) accumulated 15 and 30 kDa proteins on 2,4 –D fortified medium. But no information of proteins accumulation in torpedo stage (late stage) was reported by these scientists. Our investigation revealed that 2,4-D producing lower quality globular and abnormal heart embryos where as NAA, IAA producing high quality globular , heart embryos and premature torpedo embryos which was readily converted into torpedo embryos upon incubation on maturation and germination medium fortified with IBA+ GA3+BAP. The action of IBA and GA3 induced proteins like 56 KDa, 34 KDa, and 26 kDa that led to mature and germinate embryos without irregularities. The similar complex cellular activities produces proteins in zygotic embryo and in somatic embryogenesis in vitro conditions has been reported previously(Bian et al. 2010). We also agreed with scientists who reported that accumulations of specific proteins in last stage of somatic embryos are very much important for maturation and germination of somatic embryos. (Dane R et al. 1990; Sung and Okimoto 1981; Helleboid et al. 2000, Pasteur et al. 2001) .

Table 3: Combined effect of GA3, IBA and BAP on somatic embryo differentiations, maturation and germination of Santalum album L. on MS medium

PGR(µM)			Embryogenesis % / embryogenic callus explants	Mean number of somatic embryos/200mg calluse / 4 weeks			
				Embryo types *G – Globular; H – Heart; T – Torpedo; C – Cotyledonary*			
GA3	IBA	BAP	Node	G	H	T	C
0.28	0.49	0.44	0.0 d	0.0d	0.0d	0.0c	0.0c
1.44	2.46	2.21	66.0 a	46.0 a	25.0 a	15.0a	6.0a
1.44	2.46	4.42	65.0 b	44.0b	17.0 b	12.0b	3.5b
1.44	2.46	6.63	18.0 c	14.0 c	2.0 c	0.0c	0.0c

Means with common letters within each column are not significantly different at $p \leq 0.05$ according to DMRT. Hence the role of IBA and GA3 is very clear in embryo maturation and germination process with rare irregularities (fig.2, d-f).

2.4 Biochemical analysis and proof of embryo maturation

In support of our claims we studied biochemically three different embryo tissue types such as callus, tissue of heart embryo and torpedo embryos and were compared by analyzing their protein profile with SDS - PAGE electrophoresis. Protein profiles of all these embryo types revealed that only torpedo embryos were packed with (fig 3 three different storage proteins 56, 34 and 26 KD. The appearance of three new proteins and disappearance of one (66 KD) are the clear reflection of differential gene activity and changes in metabolic activities in embryos during maturation and embryo germination, which occurs almost universally. Many of the bands however, same and occurred at all the three different stages of development.

3. Conclusion

GA3, IBA along with BAP restricting abnormal somatic embryo development, precocious germination and promoting embryo maturations and germination by accumulating considerable amount of three main storage proteins in late torpedo stage of somatic embryos in *Santalum album* L. These storage proteins 56, 34 and 26 KD first time recorded in late torpedo stage of somatic embryos in *Santalum album* L and proved to be indicator of embryo maturations and germination with full vigor. Lacking of these storage proteins in somatic embryos did not attain maturity and become abortive. These result completely different from previously reported information that low molecular weight proteins were only recorded in early globular stage of somatic embryos (Sankara Rao K et al. 1996). Our investigation ensured viable somatic embryos production with evidence based maturation and their normal germination. These embryos could be used for artificial seed productions and germ plasm conservation.

4. Conflict of interest

The authors confirm that there is no conflict of interest for the information presented in this manuscript.

5. Acknowledgement

The first author is highly grateful to Hamdard national foundation for financial assistance for carrying out this investigation. As a team we are highly indebted to the contribution from every one for this article.

References

[1] Alexandrova K, Conger B (2002) Isolation of two somaticembryogenesis-related genes from orchard grass (*Dactylis glomerata*). Plant Sci.162: 301–307.
[2] Bian F, Zheng C, Qu F, Gong X, You C (2010) Proteomic Analysis of Somatic Embryogenesis in *Cyclamen persicum* Mill. Plant Mol. Biol. Rep. 28: 22–31.
[3] Bozena Szewczyk-T ; Bozena P (2014) Recurrent somatic embryogenesis and plant regeneration from seedlings of *Hepatica nobilis Schreb*. Plant Cell Tiss Organ Cult DOI 10.1007/s11240-014-0661-7
[4] Coleman L.C (1917) Spike disease of sandal. Department of Agriculture, Mysore. Mycology Series Bull. No 3, 1-52.
[5] Chugh A, Khurana P (2002) Gene expression during somatic embryogenesis-recent advances. Current Sci. 83(6): 715–730.
[6] Dikshit A, Hussain A (1984) Antifungal action of some essential oils against animal pathogen Fitoterapia. 55(3): 171-176.
[7] Dane R; Barry s; Flinn; David T; Webb ; Flona B; Webster; Ben CS Sutton. (1990) Abscisic acid and indole -3 nutyric acid regulation of maturation and accumulation of storage proteins in somatic embryos of *interior spruce*.Physiologia Plantarum. 78:355-360
[8] Dodeman V.L, Ducreux G (1996b) Total protein pattern expression during induction and development of carrot somatic embryos. Plant Sci. 120: 57–69.
[9] Gray D. J, Mc Colley D.W, Compton M.E (1993) High frequency somatic embryogenesis from quiescent seed cotyledons of *Cucumis melo* cultivars. J. Am. Soc. Hort. Sci. 118: 425–432.
[10] Helleboid S, Hendricks T, Bauw G, Vasseur J, Flielbart J.L (2000) Three major somatic embryogenesis related proteins in *Cichorium* identified as PR proteins. J. Exp. Bot. 51: 1189– 1200.
[11] Ilah A, Mujib A, Junaid A, Samar F, Abdin MZ (2009) Somatic embryogenesis and two embryo specific proteins (38 and 33 kD) in *Catharanthus roseus*. Biologia 64/2: 299-304
[12] Ilah A Abdin MZ, Mujib A (2002) Somatic embryogenesis irregularities in in vitro cloning of sandal (*Santalum album*). The Sandalwood Research Newsletter (SRN). Vol. 15, 2-3.
[13] Lakshmisita G (1986) Sandalwood (*Santalum album L.*) In: Bajaj YPS (Ed) Biotechnology in agriculture and forestry 1, Trees 1. Springer Verlag, Berlin, New York. 363-374.
[14] Morrish F, Vasil V, Vasil I.K (1987) Developmental morphogenesis and genetic manipulation in tissue and cell culture of the graminae . In: Scandalios G.J. (ed.). Advance in Genetics. Academic Press, New York. 431–499.

[15] Mujib A, Bandyopadhyay S, Jana B.K, Ghosh P.D (1996) Growth regulators involvements and somatic embryogenesis in *Crimum asiaticum.* Ind. J. Plant Physiol. 1(2): 84–87.
[16] Mujib A, Banerjee S, Ghosh PD (2005) Origin development and structure of somatic embryos in selected bulbous ornamentals: BAP as inducer. Somatic Embryogenesis: Plant Cell Monogr.(2) DOI 10, 1007/7089-028/published online. Springer- Verlag .
[17] Murashige T, Skoog F, (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant.15: 473–497.
[18] Noel M, Comeau D, Lenee P (1992) Embryogenese somatiquede la courgette (Cucurbita pepo L.) a partir de cotyledons, In: Comptes- rendus du ler forum jeuneschercheursde l'IAPTC-'Du gene aI'entreprise', Amiens, France. pp. 14–18.
[19] Pasteur G.M, Zappacosta D, Arena M, Curvetlo N (2001)Changes in isoperoxidase pattern during the *in vitro* rooting of Nothofogus antartica. Bulg. J. Plant Phyiol. 27: 43–53
[20] Redenbaugh K, Paasch BD, Nichol JW, Kossler ME, Viss PR, Walker KA (1986) Somatic seeds: Encapsulation of asexual plant embryos. Bio/tech. 4:797-781.
[21] Sung ZR, Okimoto R (1981) Embryogenic proteins in somatic embryo of carrot. Proc. Natl. Acad. Sci. USA. 78: 3683.
[22] Sato S, Toya T, Kawahara R, Whittler R, F Fukuda, H K omamine A (1995) Isolation of a carrot gene expressed specifically during early stage of somatic embryogenesis. Plant Mol. Biol. 28: 39–46.
[23] Sankara Rao K , Chrungoo N K , Amares Sinha (1996) Characterization of somatic embryogenesis in sandalwood (*Santalum album* L.) In Vitro Cellular & Developmental Biology - Plant. Volume 32, Issue 3, pp 123-128
[24] Shoemaker RC, Christofferson SE, Galbraith DW, (1987) Storage protein accumulation patterns in somatic embryos of Cotton (*Gossypium hirsutum* L.) Plant Cell Rep. 6:12-15
[25] Tabei Y, Kanno T, Nishio T (1991) Regulation of organogenesis and somatic embryogenesis by auxin in melon, *Cucumis melo* L. Plant Cell Rep. 10: 225–229.
[26] Vasil V,Vasil I.K(1982)Characterization of an embryogenic cell suspension culture derived from cultured inflorescences of Pennisetum americanum (Pearlmillet, Graminae). Amer.J. Bot. 699: 1441–1449.
[27] Verma A, Chenulu VV, Raychudhury SP, Nam Prakash, Rao PS (1969) Mycoplasma like bodies in tissue infected with spike and brinjal leaf. Indian Phytopath. 22: 289-291.
[28] Veroslava S; Mirjana N. (1985) Effect of gibberellic acid on organogenesis in buckwheat tissue culture. Biologia Plantarum. 27: 6, pp 432-437
[29] Wernicke W, Milkovits L(1984)Developmental gradients in wheat leaves. Responses of leaf segments in different genotypes cultured *in vitro*. J. Plant Physiol. 115: 49–58.

Author Profile

Mazahirul Islam Syed received the M.Sc degrees in Biotechnology with a specialization of plant tissue culture from Hamdard University, New Delhi, in 2001. 15 years of teaching and industrial experiences in Mumbai, Africa and French ruled countries had given great vision of understanding the subject biology. Currently teaching cell biology and human anatomy in Jazan University, KSA since 2012.