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Influence of Auxin and Cytokinin on Callus Induction of Mulberry

Dr. Sudipta Kumar Sil
Professor, Department of Botany, University of Gour Banga, Malda-732103, West Bengal, India. Email: sudiptakrsil@gmail.com

ABSTARCT

Present experimental article explained the potentialities of explants of ten mulberry varieties (*Morus* sp. var. Kajli, Kajli-OP, S1, Berhampore local, S1635, Bishnupur local, Bogura local, S2028, Tr10, BC259 and S1) on Gamborg's B5 media fortified with different combinations of Naphthalele acetic acid (NAA) and Kinetin as auxin and cytokinin respectively towards development of callus. The ratio of auxin and cytokinin ranges from 1 to 7. The combination among endogenous with exogenous auxin as well as cytokinin levels indicated pivotal role in differential induction of callus in the varieties tested. First four and the last varieties were found to be early respondents, while the others were induced for callusing at a later stage. The early respondents favoured comparatively low dose ration of auxin to cytokinin.

Key Words: Mulberry, callus development, Explants, microshoot, growth regulator, somatic embryogenesis

INTRODUCTION

Mulberry is a economically important tree as it is the main host of silkworm (*Bombyx mori*). Though mulberry can be propagated through saplings, by cutting, layering, budding and grafting, micropropagation through tissue culture has a great aspect as not only a large number of plantlets can be produced but also variation could be achieved through somaclonal variation. Germplasm identification and characterisation protocols are largely dependent upon micropropagative potentialities of mulberry.Evaluation of genetic variation is very important for breeders to study their nature. It will help to understand their conservation strategy and evolutions [1,2]. Explants like leaf disc, nodal segment, root-tip segment all have reported to have the capacity of cellular totipotency to propagate the plant in controlled physico-chemical ambience ubder laboratory conditionsOhyama used axillary buds of *M. alba* to produce a whole plant cultured in Murashighe and Skoog medium and Gamborg's B5 medium [3]. Thereafter, usefulness of node, leaf and shoot tip as explant was demonstrated by other authors as well. It was found that different culture media with different PGR'S have responses poles apart in case of different variety of mulberry [4]. As useful PGR 2,4-D, Kinetin, IAA, NAA are the most widely used either singly or sometimes mixed together to prevail better results. Use of Abscisic acid (ABA)

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also sometimes recommended to produce better results in producing callus culture [5]. There are observations to assess stress tolerance of mulberry through tissue culture technique. It was reported that assessment of salt tolerance in-vivo is an rapid, cost effective and efficient technique [6,7].

MATERIALS & METHODS

Mulberry varieties undertaken for the study were Kajli-OP, S1, Berhampore local, S1635, Bishnupur local, Bogura local, S2028, Tr10, BC259 and S1. Samples of these varieties were collected from CSRTI, Behrampore, West Bengal. Nodal part of the stem was taken as explant which was 2-3 cm long in size. Explants were rinsed with running tap water for 9-10 minutes and washed with 80% ethanol for 30 seconds. Then explants bleached with 15% commercial bleach (v/v) for 15 minutes and lastly 3-5 times with double distilled water. After sterilization dead tissues present at both ends of explants were trimmed off and cultured with culture medium.

Gamborg's B5 medium fortified with the following concentration series of NAA and Kinetin were used as media composition: NAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 µg/mL) and 0.5 µg/mL Kinetin. Media combinations were formulated by mixing different doses of NAA with fixed (0.5 µg/ml) of Kinetin. Callus development was the change-point of subculturing into microshoot and followed by rooting which was composed of MSO with IAA (0.5,1.0,1.5µg/mL).

The statistical data of the response in micropropagation through callogenesis were analysed using SPSS 15.0 software and the interactive graphical representations indicated the dose dependent efficacy of the media compositions under study.

During callogenesis and subsequent organogenesis the cultures were maintained in 25±1º C tempereture, 75% RH, 16/8 h photoperiod and 2200 lux cool fluroscent light to induce rooting initial darkness was applied on the explants, however advance stages of rooting required above physicochemical condition

RESULT

Table 1: Media compositions promotive to callus induction in ten mulberry varieties.

VARIETY NAME	OBSN.@	CM1&	CM2&	CM3&	CM4&	CM5&	CM6&	CM7&	CM8&
KAJLI	1	0.25	0.25	0.25	0.5	0.5	0.75	0.25	0

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KAJLI	2	0	0.25	0.5	0.5	0.5	0.75	0.25	0
KAJLI	3	0	0	0.25	0.5	0.5	0.75	0.25	0
KAJLI-OP	1	0.25	0.25	0.25	0.5	0.5	0.75	0.25	0
KAJLI-OP	2	0.25	0.25	0.25	0.5	0.5	0.75	0.25	0
KAJLI-OP	3	0	0.25	0.25	0.5	0.5	0.5	0.25	0
BERHAMPR	1	0.25	0.25	0.5	0.5	0.5	0.5	0	0
BERHAMPR	2	0	0.25	0.25	0.5	0.5	0.5	0	0
BERAMHPR	3	0	0	0.25	0.5	0.75	0.5	0.25	0
S1635	1	0.25	0.25	0.5	0.5	0.75	1	0.25	0
S1635	2	0	0	0.5	0.5	0.75	1	0.25	0
S1635	3	0.25	0.25	0.25	0.5	0.25	1	0	0
BISHNUPR	1	0	0	0.25	0.5	0.5	0.5	0	0
BISHNUPR	2	0	0	0.25	0.5	0.25	0.5	0	0
BISHNUPR	3	0	0	0.25	0.5	0.5	0.5	0	0
BOGURA	1	0.25	0.25	0.5	0.5	0.5	0.5	0	0
BOGURA	2	0	0	0.25	0.5	0.5	0.5	0	0
BOGURA	3	0	0.25	0.25	0.5	0.5	0.5	0	0
S2028	1	0	0.25	0.25	0.5	0.25	0.5	0	0
S2028	2	0	0	0.5	0.5	0.5	0.5	0	0
S2028	3	0	0	0.25	0.5	0.75	0.5	0	0
Tr10	1	0	0	0	0.25	0.25	0.5	0	0
Tr10	2	0	0	0	0.25	0.25	0.5	0	0
Tr10	3	0	0	0	0.25	0.25	0.5	0	0
BC259	1	0	0	0	0.25	0.25	0.5	0	0

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BC259	2	0	0	0	0	0.25	0.5	0	0
BC259	3	0	0	0	0.25	0.25	0.75	0	0
S1	1	0.25	0.25	0.25	0.5	0.25	0.75	0.25	0
S1	2	0	0.25	0.25	0.5	0.25	0.75	0.25	0
S1	3	0	0	0.25	0.75	0.75	0.75	0.25	0
VARIETIES		Kajli, Kajli OP, Berhampore local, S1635, Bishnupur local, Bogura local, S2028, Tr10, BC259, S1							
CM Callus inducing medium [Concentration of NAA and Kinetin in µg/mL] CM[&] means values mentioned in columns represent percentage (%) response per explant in callusing.		CM1 = Gamborg's B5 basal+ [concentration ratio of NAA: KINETIN=1.0], CM2 = Gamborg's B5 basal+ [concentration ratio of NAA: KINETIN=2.0], M3 = Gamborg's B5 basal+ [concentration ratio of NAA: KINETIN=3.0], CM4 = Gamborg's B5 basal+ [concentration ratio of NAA: KINETIN=4.0], CM5 = Gamborg's B5 basal+ [concentration ratio of NAA: KINETIN=5.0], CM6 = Gamborg's B5 basal+ [concentration ratio of NAA: KINETIN=6.0], CM7 = Gamborg's B5 basal+ [concentration ratio of NAA: KINETIN=7.0], CM8 = Gamborg's B5 basal medium							
OBSEN. [@] Means observation, three tubes were recorded with three repetitions.									

Table 2: Percentage of rooting from regenerated microshoot after 20 days of inoculation in different varieties: -

Sl No.	Conc. of IAA[#]	Rooting response	% of rooting$
1	0.25	No rooting	0
2	0.5	Profuse rooting	75
3	0.75	Limited rooting	50
4	1.0	No rooting	0

# Concentration of Plant Growth Regulator (PGR) in µg/mL
$ calculated from observation of 3tubes replicated thrice.

Table 3: Response of Kajli var. of Mulberry for somatic embryogenesis.

Response	Days required (Mean N=10)	% Response
Abrupt undulation on the surface of the explants (nodal segment) especially on the cut ends.	14	40 (N=10)
Globular mass of tissue appeared on the surface of the callus	8	50 (N=4)

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Cordate (Heart shaped) mass	6	75 (N=4)

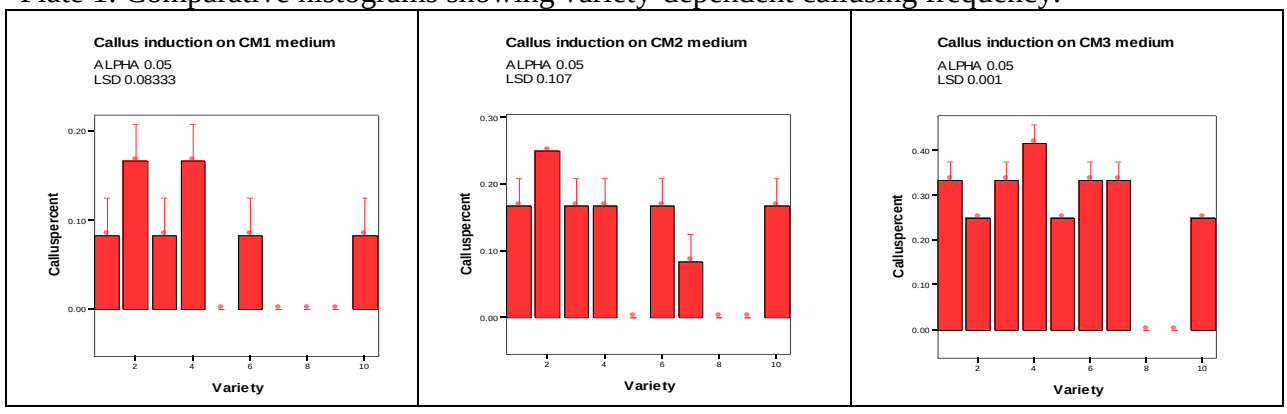
DISCUSSION

The range of proportion of plant growth regulator between Naphthelene acetic acid (NAA) and Kinetin clearly demonstrated a regime of responses among the ten varieties of Mulberry. All the varieties studied here were not homogeneous in their response towards same combination of PGR , some varieties are early-respondents, some are late whereas, few were found to be responsive to the middle doses in the combination range.

Most of the organogenetic calli were found to be of dark colour and have been noticed to be proliferated on the entire surface area of the explant, however the embryogenetic calli were comparatively light coloured and found to pass through the stages of differentiation like globular stage, heasrt stage and torpedo stage ultimate developed into bipolar meristemoids.

6-BAP was observed to be more potent in generating microshoot than Kinetin, but when Kinetin when supplemented with different combinations of 6-BAP it also induced organogenetic calli with organogenetic potential equal efficacy. Although highest percentage of microshoot developed in MS0 +1.5 mg/L 6-BAP , MS0 +2.0 mg/L 6-BAP , MS0 + 1.5 mg/L Kinetin, MS0 + 2.0 Kinetin, and also in MS0 + 1.5 mg/L 6-BAP + 1.5 mg/L Kinetin , but cumulative highest number of microshoot were obtained in the media composition of MS0 +1.0 mg/L 6-BAP and MS0 + 1.5 mg/L 6-BAP. Such condition arose due to possibility of differential activation of dedifferentiated cells and induction of variable of meristematic patches per callus. Lowest number of cumulative microshoot was recorded in media composition MS0 + 1.5 mg/L 6-BAP + 2.0 Kinetin.Rooting however induced more efficiently with IAA than other auxins. 0.5 mg/L dose of IAA was found to be potent than 1.0 mg/L dose.

Plate 1: Comparative histograms showing variety-dependent callusing frequency.



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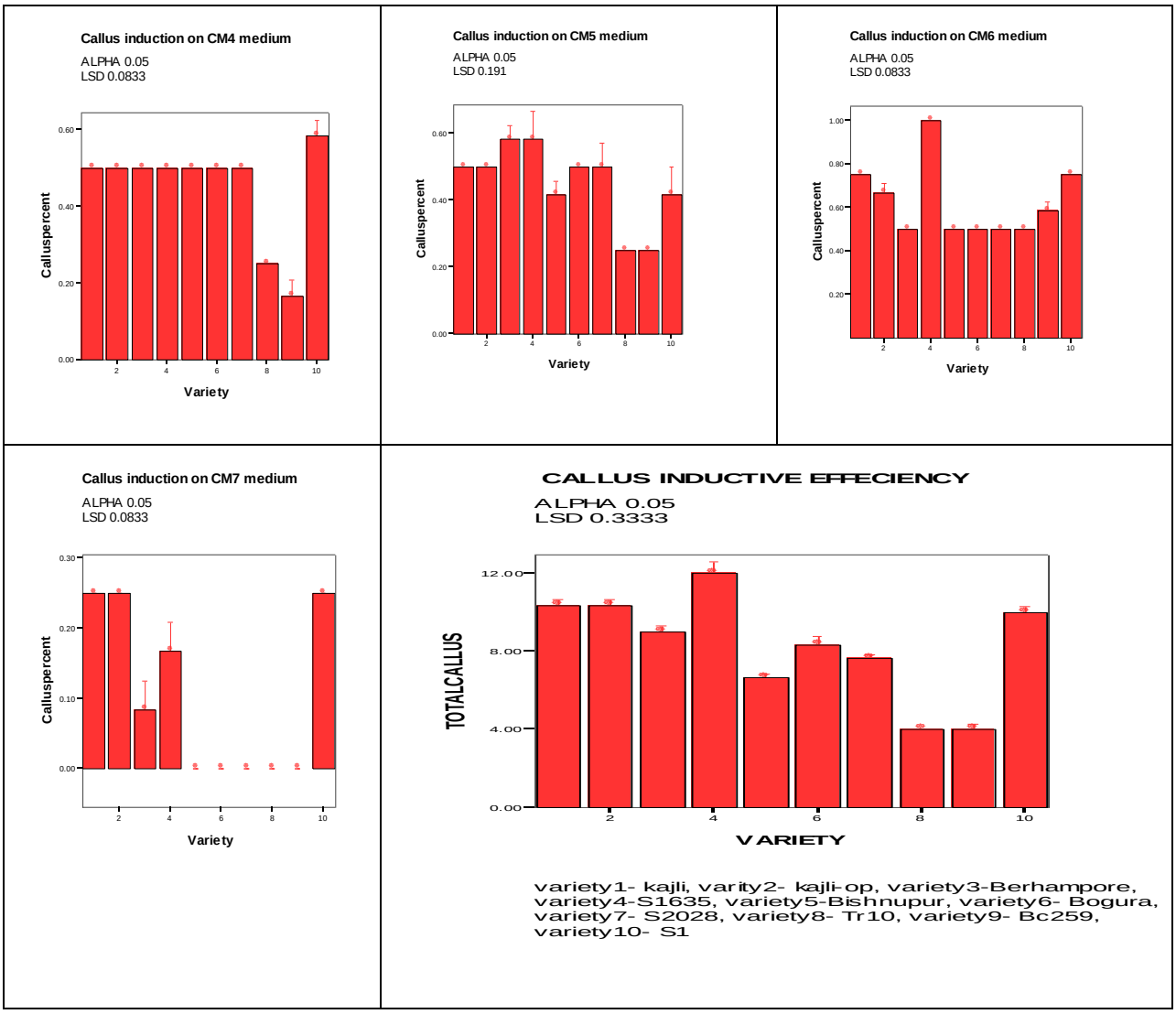
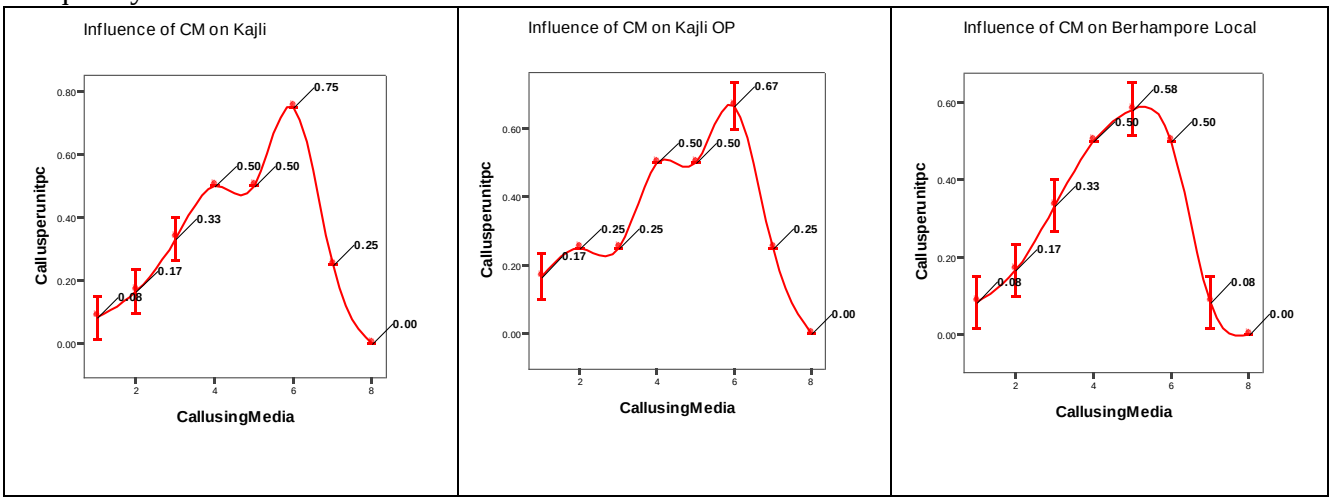


Plate 2: Comparative line-curves showing influence of callusing media composition on callusing frequency.



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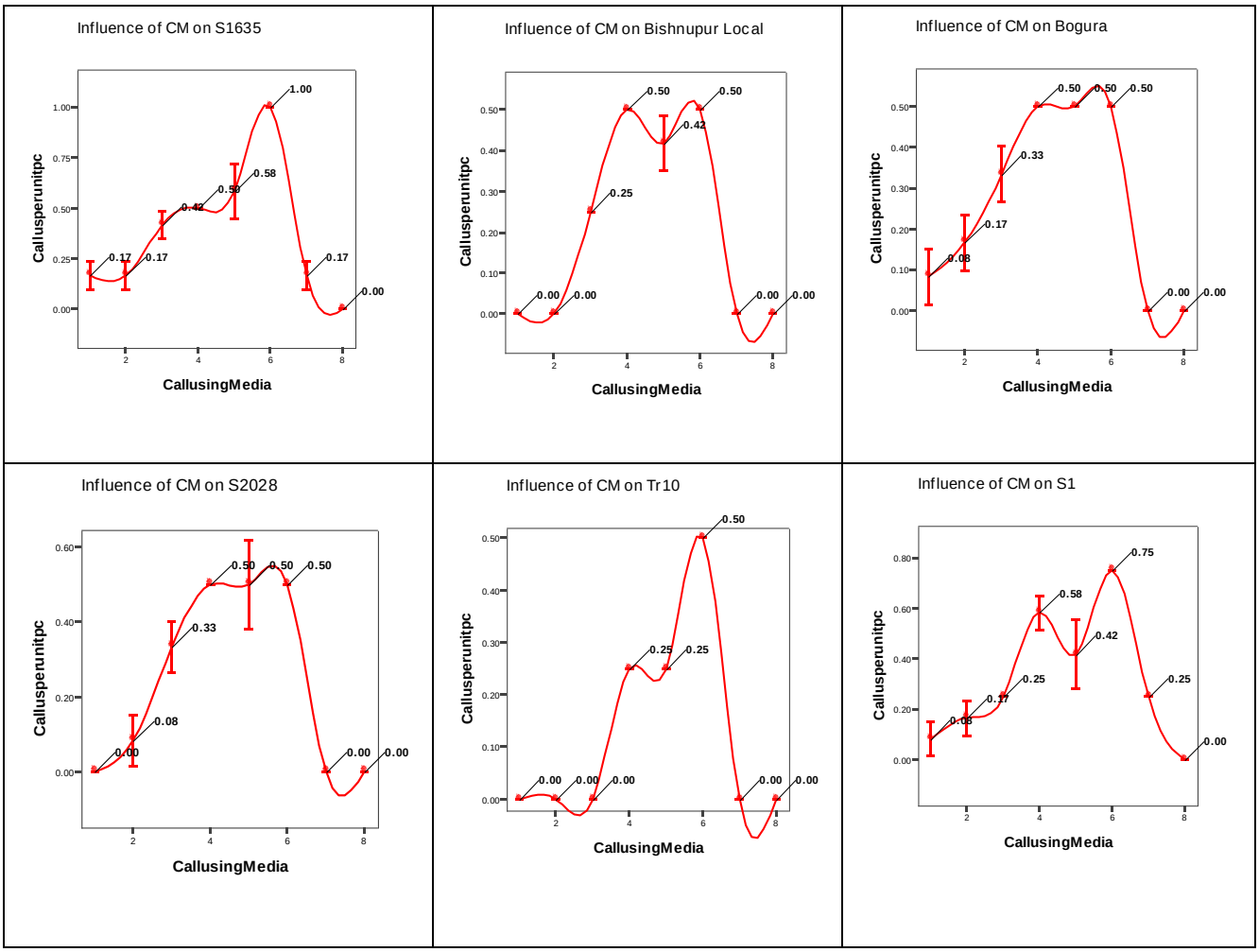
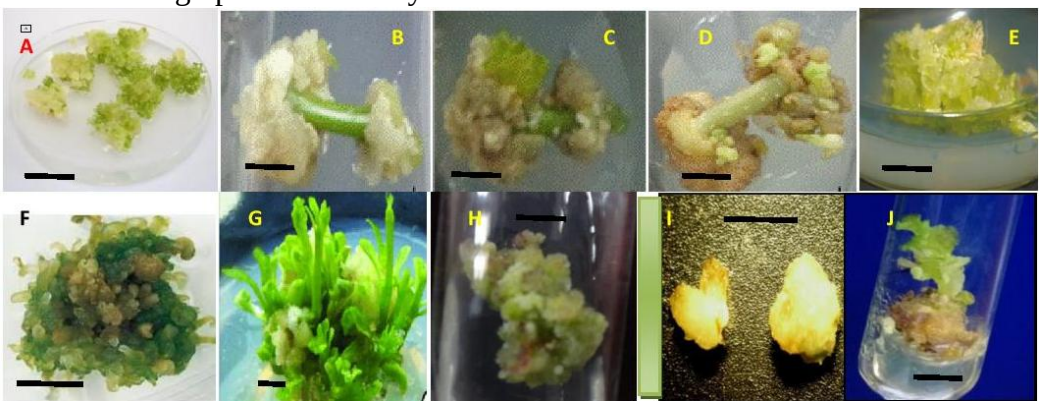


Plate 3: Photographs of Mulberry callus.



A-callus of Kajli, B, C, D - callus on both ends of explant in S1635, E- callus of S1
F- callus of Kajli-OP, G- microshoot from callus of Tr10, H- callus of Bogura,
I-Embryogenic callus of Kajli, J- leafy microshoot on callus of Berhampore local.
[scale:1.5 cm]

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The proportionate increment of auxin over a fixed dose of cytokinin has been observed among the ten varieties of mulberry. The different media compositions for callus development tested were CM1(auxin: cytokinin = 1), CM2(auxin: cytokinin = 2), CM3(auxin: cytokinin = 3), CM4(auxin: cytokinin = 4), CM5(auxin: cytokinin = 5), CM6(auxin: cytokinin = 6) and CM7(auxin: cytokinin = 7). MS basal without any plant growth regulator was used as control medium. The first four varieties were found to be early responders as evident from the histograms, such differential expressions and significant correlations were reported in other mulberry varieties and field propagation studies [8]. The effect of proportionate dose of auxin and cytokinin in callusing media on the varieties under study revealed that, callus induction increases with increase of this ratio, but not always uniform; further CM4 medium exerted nearly homogeneous effect on the genotypes, as envisaged in certain other media compositions [11]. Encapsulation of shoot bud in micropropagation was previously employed as a possible avenue of proliferation [9]; somatic embryogenesis on solid medium was exhibited by Kajli. Variation in callus induction response among the varieties under influence a specific media composition may be due to the endogenous partitioning of auxin and cytokinin levels [10, 12]. Kalji, Kajli-OP, S1625, Berhampore local and S1 varieties showed overall significantly superior potency in callus induction, while Tr10 and BC259 varieties performed with lower efficacy. The performances of S1635 in micropropagation as well as in field propagation were also found to be superior in ambient agroclimatic conditions [12]. Although mulberry is cultivated through saplings, but micropropagation augmented their utilisation in a shorter span and with greater biomass generation [13, 14].

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References

[1] Wright, S. (1978) Evolution and Genetics of populations. Vol.4. Variability within and among natural populations. University of Chicago press, Chicago.
[2] Wu, J., Krutovskii, K.V. and Strauss, S.H. (1999) Nuclear DNA diversity, population differentiation, and phylogenetic relationships in the California closed-cone pines based on RAPD and allozyme markers. *Genome*. 42, 893-908.
[3] Gamborg OL, Wetter CR (1975) Plant Tissue Culture Method. Natl Res Counc Canada, Saskatoon, Pp 1-9 Ghugale DD, Kulkarni DD, Narasimhan R (1971) Effect of auxins and

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2310 – 2318
Received 05 March 2021; Accepted 01 April 2021.

gibberellic acid on gromh and differentia tion of *Morus alba* and *Populus nigra* tissues in vitro. Exp Biol 9: 381-384.

[4] Vijayan, K., Chakraborty, S.P. and Ghosh, P.D. (2003), *In-vitro* screening of mulberry for salinity tolerance., Plant Cell Reports, 22, 350-357.

[5] Onishi. T, Yasukura. T and Tan. J (1986) Preservation of mulberry callus by the addition of both abscisic acid (ABA) and p-aminobenzoate (PABA) in culture medium, Journal of Sericulture Science, Japan, 55, 252-255.

[6] Hossain, M., Rahman, SM., Zaman, A., Joarder, OI and Islam, R., (1992), Micropropagation of M. laivegata Wall. from mature trees, Plant Cell Reports, 11, 522-524.

[7] Tewary, P.K., Sharma, A., Raghunth, M.K and Sarkar, A (2000), *In-vitro* response of promising mulberry (Morus Sp.) genotypes for tolerance of salt and osmotic stress. Plant Growth Regulation, 30, 17-21.

[8] Angrish R, Dhir KK, Nehranwalia I, Pubbi S (1986) Seasonal morphogenetic behaviour of generative buds of *Morus nigra* cultured in vitro. J Plant Physiol 122: 267-274.

[9] Bapat VA, Mhatre M, Rao PS (1987) Propagation of *Morus indica* L. (Mulberry) by encapsulated shoot buds. Plant Cell Rep 6: 393-395.

[10] Bapat VA, Rao PS (1990) In vitro growth of encapsulated axillary buds of mulberry *Morus indica* L.). Plant Cell Tissue Org Cult 20: 69-70

[11] Chattopadhyay S, Chattopadhyay S, Datta SK (1990) Quick in vitro production of mulberry (*Morus alba* L.) plantlet for commercial purposes. Ind J Exp Biol 28: 522-525.

[12] Chitra DSV, Padmaja G (2001) Seasonal influence on axillary bud sprouting and micropropagation of elite cultivars of mulberry. Sci Hort 92: 55-68

[13] Hayashi K, Oka S (1995) Formation of multiple bud bodies and plant regeneration in the mulberry, *MoruS alba*. J Seric Sci Jpn 64: 117-123.

[14] Islam R, Zaman A, Joarder N, Barman AC (1993) in vitro propagation as an aid for cloning of *Morus lavaegata* Wall. Plant Cell Tissue Org Cult 33: 339-341.