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IN-VITRO MICROPROPAGATON POTENTIALITY OF MORUS INDICA L.CULTIVAR BC-259 FROM NODAL EXPLANTS

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ABSTRACT

Callus develops and axillary shoot proliferates from nodal explants from mature trees of Morus sp var. BC-259. Explants from nodal part of var. BC-259 were collected, surface sterilized and then cultured. MS medium used as culture media either supplemented with various concentrations of BAP (N6- benzyladenine) and Kinetin singly, or in combination with each other.2, 4-D was also used in different concentrations to induce callogenesis. Microshoot generation was found more in use of 6-BAP than Kinetin. But, when Kinetin supplemented with different combinations of 6-BAP, it also induced organogenetic potential with equal efficiency. 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 MS0 + 1.5 mg / L 6-BAP + 1.5 mg / L Kinetin exhibited development of highest percentage of microshoot generation. MS0 +1.0 mg / L 6-BAP and MS0 + 1.5 mg / L 6-BAP showed highest cumulative number of microshoots. Rooting 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. Four week old micropropagatedplantlets were transferred to plastic pots, containing a mixture of soil: sand: peat moss (1:1:2) under glasshouse condition.

KEYWORDS: *BC-259, Culture Media, Explants, In-Vitro Clonally Propagation, Micro Shoots, Morussp*

INTRODUCTION

Mulberry is a perennial plant belongs to family Moraceae, which has major economic importance for Silk industry. Leaf of Mulberry plant solely used for feeding of Silkworm (Bombyx mori). It is important to improve quality and quantity of mulberry leaf for sustainability of silk industry. The prolonged juvenile period and plants perennial nature slowed the process of improvement (Thomas, 2002).Plant tissue culture is a modern technique of propagation and conservation of plant species.With a single explant, several thousand uniform plants can be multiplied in relatively short time period and space under controlled environment, irrespective of the season and weather (Akin-Idowuet al., 2009).Capacity of formation of callus tissues to develop into organs was noted by Segretain, who observed the induction of shoots in the culture of tobacco tissues; Skoog confirmed the results and reported that the callus tissues are capable of forming stem buds (Butenko, 1968). Tissue culture techniques nowdays widely used for mass propagation of cultivated varieties and forest plants(Bajaj, 1986).Plant growth regulators, commonly known as hormones like auxin, gibberellins, cytokinin play a major role in tissue culture and it depends on nature of explant used for the experiment (Ting, 1982). Explants like node, leaf, bud, inter-nodal region, shoot tip etc. are generally used for tissue culture (Narayan et al., 1989; Yadav et al., 1990; VijayaChitra and Padmaja, 1999.)The present study was performed for rapid induction, regeneration and proliferation of Mulberry variety BC-29 using nodal part as explants.

MATERIALS & METHODS

BC-259 is one of the popular varieties cultivated in West Bengal (CSRTI, Berhampore). Nodal explants (2-3 cm long) from the variety collected and sterilized by rinsing under running tap water first for 9-10 minutes, following by 80% ethanol for 30 seconds and 15 % commercial bleach (v / v) for 15 minutes and lastly washed 3–5 minutes with double distilled water. After sterilization, dead tissues present on both ends of nodal explants were trimmed and placed on desired culture mediumMSO (Murashighe& Skoog, 1962) medium used as culture medium. Different concentration of plant growth regulator (BAP and Kinetin were) used as media composition: BAP(0.25,0.5,1.0,1.5,2.0,2.53.0,3.5,4.0,4.5µg / ml) and Kinetin (0.25,0.5,1.0,1.5,2.0,2.5,3.0,3.5,4.0,4.5 µg / ml). Few culture media of combination of BAP and Kinetin were also used for the experiment. At the point of initiation of rooting, was the time for sub-culturing into rooting medium composed of MSO with IAA (0.5, 1.0, 1.5µg / ml). MSO supplemented with DIPA (0.5, 1.0, 1.5, 2.0, 2.5µg / ml) was also tested for in-vitro Callogenesis. Cultures were maintained at 25 ± 1º C temperature, 75 % RH, 16 / 8 h photoperiod and 2200 lux cool fluroscent light during callogenesis and subsequent organogenesis. Initially, darkness was applied on the explants for initiation of rooting; however advance stages require more physio-biochemical stages.

Result of micro propagation through call genesis was analyses using SPSS 16.0 software. Presented histogram represents media composition's efficacy. 35 days old microshoots were counted for their percentage induction and determination of cumulative number.

RESULTS

Organogenic response in BC-259 var. of Mulberry (35 days of inoculation), Explant -callus induced on nodal explants (Media: MS0+ different concentrations of 6-BAP, Kinetin separately). 6-BAP was found to be more potent in generating microshoot than Kinetin, but when Kinetin when supplemented with different combinations of 6-BAP it also induced organogenesis potential equally, efficiently. 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 (Table 1).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. (Table 2)

Table 1: Micro Shoot Generation % and Their Cumulative Number in Different Media Composition

Sl No.	Media Composition	Change In Callus Morphology	% of Micro Shoot[$]	Cumulative Number of Micro Shoot
1	MS0	No change	0	0
2	MS0 + 0.25 6-BAP[#]	No change	0	0
3	MS0 + 0.5 6-BAP[#]	No change	0	4
	MS0 + 1.0 6-BAP[#]	+entire surface of explants Callus green in color	33.3	17
4	MS0 + 1.5 6-BAP[#]	++ entire surface of explants Callus dark brown in color	66.6	16
5	MS0 + 2.0 6-BAP[#]	++ entire surface of explants Callus dark brown in color	83.3	15
6	MS0 + 2.5 6-BAP[#]	+ at one end Callus pale green	33.3	6

Table 1 Contd.,				
7	MS0 + 3.0 6-BAP#	No change	0	0
8	MS0 + 3.5 6-BAP#	No change	0	0
9	MS0 + 4.0 6-BAP#	No change	0	0
10	MS0 + 4.5 6-BAP#	No change	0	0
11	MS0 + 0.25 Kin#	No change	0	0
12	MS0 + 0.5 Kin#	No change	0	0
	MS0 + 1.0 Kin#	No change	0	9
13	MS0 + 1.5 Kin#	++ entire surface of explants Callus dark brown in color	75	12
14	MS0 + 2.0 Kin#	++ entire surface of explants Callus dark brown in color	50	14
15	MS0 + 2.5 Kin#	+ callus at one end Callus green in color	16.6	5
16	MS0 + 3.0 Kin#	No change	0	0
17	MS0 + 3.5 Kin#	No change	0	0
18	MS0 + 4.0 Kin#	No change	0	0
19	MS0 + 4.5 Kin#	No change	0	0
20	MS0 + 0.5 6-BAP# +0.5 Kin#	No change	0	0
21	MS0 + 1.0 6-BAP# +0.5 Kin#	No change	0	0
22	MS0 + 0.5 6-BAP# +1.0 Kin#	+entire surface of explants Callus dark brown in color	16.6	7
23	MS0 + 1.5 6-BAP# +1.5 Kin#	++ entire surface of explants Callus dark brown in color	66.6	19
24	MS0 + 1.5 6-BAP# +0.5 Kin#	+ entire surface of explants Callus dark brown in color	25	15
25	MS0 + 0.5 6-BAP# +1.5 Kin#	No change	0	0
26	MS0 + 2.0 6-BAP# +2.0 Kin#	No change	0	0
27	MS0 + 2.0 6-BAP# +0.5 Kin#	No change	0	0
28	MS0 + 0.5 6-BAP# +2.0 Kin#	No change	0	0
29	MS0 + 2.0 6-BAP# +1.5 Kin#	+entire surface of explants Callus green in color	16.6	0
30	MS0 + 1.5 6-BAP# +2.0 Kin#	+Callus at one end Callus green in color	0	7

Concentration of PGR in mg / L; $ calculated from observation of 4 tubes replicated thice.

Table 2: Root Development From Regenerated Micro Shoots After 20 Days of Inoculation (Media: 0.5 MS0 + Different Conc. of IAA)

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 PGR in mg / L; $ calculated from observation of 4 tubes replicated thrice

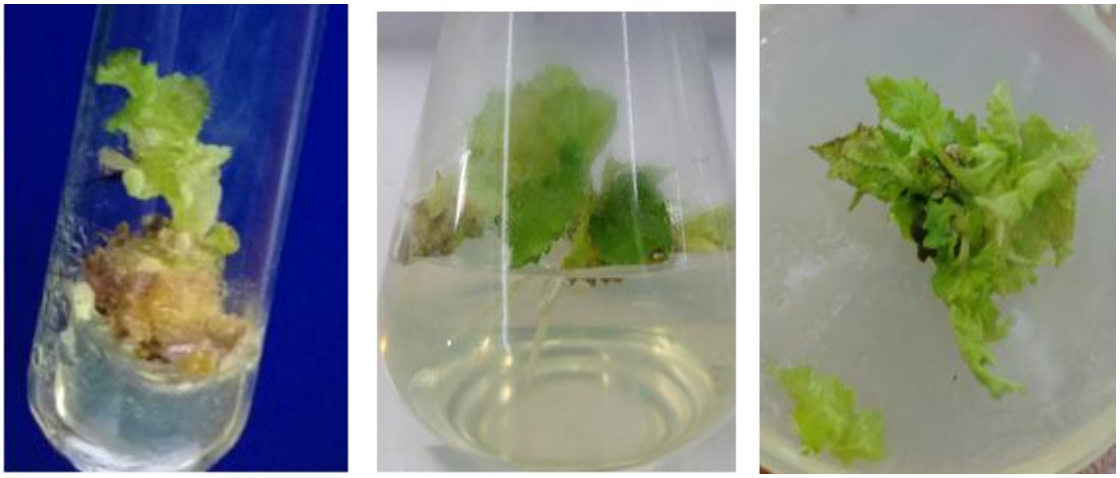

Image (Right To Left): Micro Shoot, Callus & Leafy Callus.

DISCUSSIONS AND CONCLUSIONS

Mulberry is a cross pollinated crop, and hence heterozygosity prevails. Therefore, propagation through seeds does not conserve stable genetic makeup, which limits the genetic improvement through conventional hybridization techniques. During the last three decades, micropropagation techniques have been extensively utilized as a valuable and viable tool for overcoming such constraints in mulberry.For targeted crop improvement through biotechnological approaches, attempts have been made to standardize in vitro regeneration protocols in different mulberry varieties (Sajeevan et al., 2011). Mulberry is a recalcitrant species in terms of tissue culture, and shoot regeneration is greatly dependent on the genotype, type of explant and combination of growth regulator used in the culture media (Feyissa et al., 2005). Using different explants such as stem (Narayan et al., 1989), shoot tip and nodal segment (Yadav et al., 1990; VijayaChitra and Padmaja, 1999), axillary bud (Vijayan et al., 2000), hypocotyl and cotyledon (Bhatnagar et al., 2001), leaf (Kapur et al., 2001; VijayaChitra and Padmaja, 2005). In vitro regeneration has been attempted with various degrees of success. There are variations in regeneration among mulberry varieties (Bhau and Wakhlu, 2003; Rao et al., 2010). Figure box 1 and Figure box 2 represent the histograms for exhibiting percentage microshoot generation and cumulative micropropagagative potential of mulberry BC259 variety.

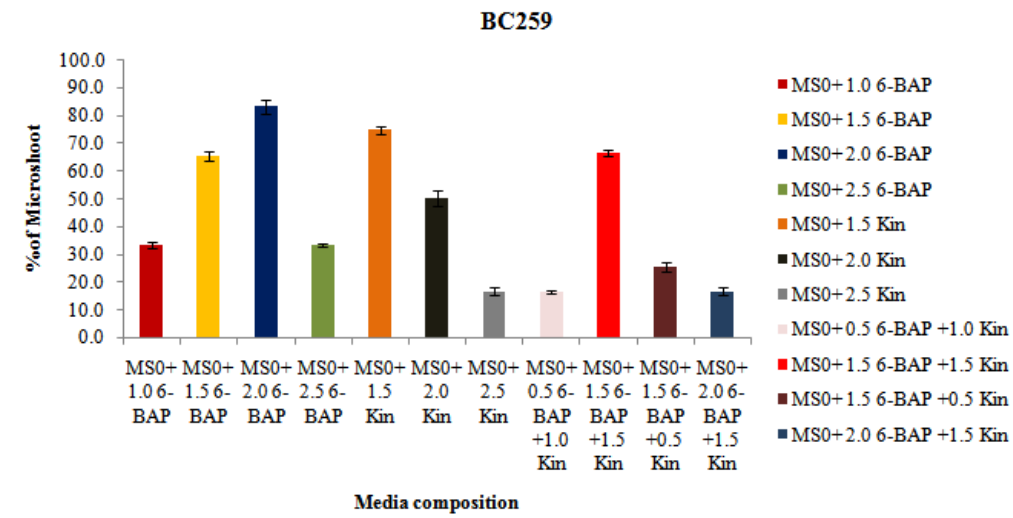

Figure 1: Percentage of Micro Shoots Generation in Different Culture Medium.

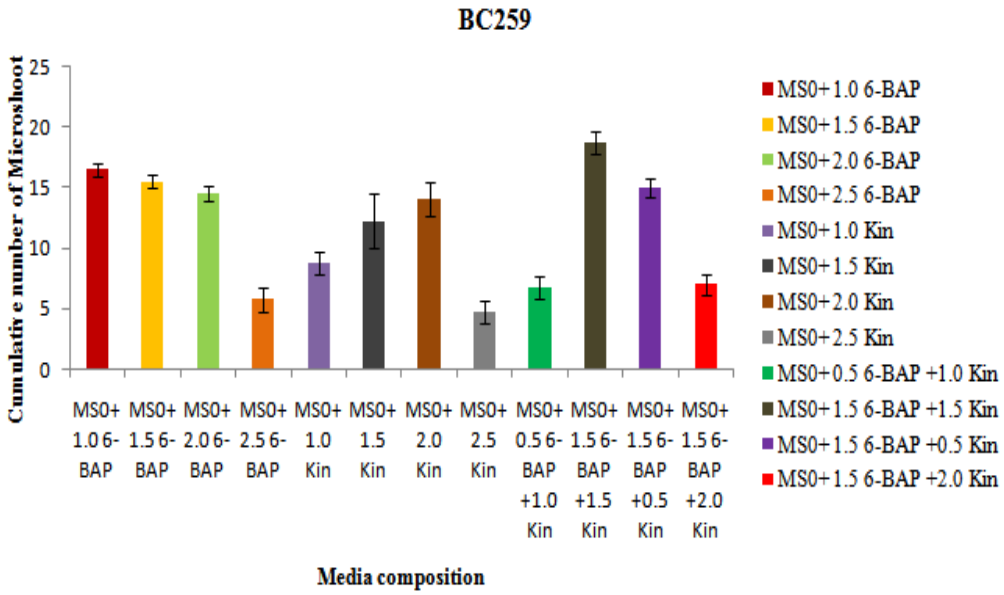

Figure 2: Cumulative Number of Micro Shoots Generation in Different Culture Medium.

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