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Varietal response for *in vitro* shoot development in mulberry (*Morus* spp.)

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Abstract
A study was undertaken in the department of sericulture, with an objective to study the genotypic variations for the regeneration potential and to standardize the culture media for regenerates from nodal explant of different varieties of mulberry. The maximum bud sprouting (72.57%) was noticed in M-5 when MS medium with different concentrations of NAA and BAP. Among the different concentrations and combinations highest auxillary bud sprouting percentage of 71.50 was recorded when MS medium supplemented with 1.5 and 1.0 per cent NAA and BAP respectively in M5. In another combination of IAA and BAP, Significantly maximum response of sporting percentage (77.52%) was recorded in S13 when compared to V1, M5 and Mysore local. On the other hand among the different concentrations and combinations used the highest sprouting percentage of 81.91 was recorded when MS medium is supplemented with different concentrations of 1.5 and 1.0 mg/l IAA and BAP respectively.

Keywords: *Morus* spp., MS Media, Nodal explants, Hormones

Introduction
Mulberry belongs to the genus *Morus* and family Moraceae. Mulberry is distributed in tropical, subtropical and temperate zones. Conventional breeding is limited in mulberry due to high heterozygosity and long generation period. Many of the newly developed mulberry varieties cannot be propagated through stem cutting. Many desired cultivars do not root easily or have low rooting ability. Such difficult varieties could be multiplied by using tissue culture techniques (Priya and Padmavathi, 2017) [16]. Use of conventional method of breeding in any crop is time consuming, laborious and expensive. Therefore, applications of tissue culture techniques is found useful in solving the problems (Ram Rao *et al*., 1997) [9]. Recent advances in tissue culture and molecular biology of plants at the cellular level have provided potentially powerful new tools in the hands of biologists for generating, selecting and propagating novel and economically important plant varieties. 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) [11]. 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) [2]. Using different explants such as stem (Narayan *et al*., 1989), shoot tip and nodal segment, axillary bud (Vijayan *et al*., 2000), hypocotyl and cotyledon. *In vitro* regeneration has been attempted with various degrees of success. Since there are variations in regeneration among mulberry varieties (Bhau and Wakhlu, 2003; Rao *et al*., 2010) [1, 10]. The present investigation aim to study the genotypic variations for the regeneration potential and to standardize the culture media for regenerates from nodal explant of different varieties of mulberry. The best possible media combination and concentrations thus obtained can be further exploited for *in vitro* multiplication.

Material and Methods
Nodal explants of four mulberry genotypes of *Morus alba* L. *viz* M-5, V-1, Mysore local and S-13 maintained in germplasm bank maintained in the Department of Sericulture, UAS, GKVK Bengaluru were used for the study. Single node explants were collected from healthy greenish shoots of each genotype were washed first with water and then Labolene or tween twenty for two to three times followed by thorough washing in running tap water. Then it was then treated with 0.1 per cent Bavistin for twenty minutes followed by 0.1 per cent Streptomycin and then treated with Chlorine water for ten minutes and again wash with sterile distilled water for three to four times. The cleaned material was surface sterilized with 0.1 per cent Mercuric chloride solution for eight minutes followed by thorough wash with sterile distilled water for four to five times.

The nodal segments were cultured on MS (Murashige and Skoog 1962) medium supplemented with different concentrations and combinations (NAA + BAP and IAA + BAP) of hormones. A total of seven treatments with two combinations were tested (Vijayan *et al.*, 2011) [11].

The P^H of the medium was adjusted 5.4 before adding agar. All the culture was maintained at 26 ± 2^0 C temperature under 16 hours photo period and data were recorded periodically. Sprouting percentage was calculated after 30 days of culture, each value representing the mean (SEm±) of ten replications and repeated three times. The data collected in the study were statistically analaised by using ANOVA and factorial CRD as described by (Sunder raj *et al*., 1972).

Results and Discussion

The response of the nodal explants of the different mulberry genotypes to various optimum media combinations containing different concentration of NAA + BAP and IAA +BAP combinations were revealed that different cultivars require different hormones obtaining optimal response for sprouting. The maximum bud sprouting (72.57%) was noticed in M-5 when MS medium supplemented with different concentrations of NAA and BAP when compared to other mulberry varieties. Whereas, the minimum auxillary bud sprouting (61.23%) was recorded in Mysore local. Among the different treatment combinations and different concentrations highest auxillary bud sprouting percentage of 71.50 percentage was recorded when MS medium supplemented with 1.5 and 1.0 per cent NAA and BAP respectively in M5 mulberry variety. Whereas, the minimum sprouting percentage was recorded when MS medium is supplemented with 1.0 and 0.5 percent NAA and BAP respectively (Table 1).

These results are agreement with the findings by Hussein *et al*. (2020) [3] the *in vitro* mulberry multiplication of Yue 11, Sha 2x lun 109, Morittina, Kokuso 27 and Kantava auxiliary buds cultured on the MS medium fortified with 1.5 mg/l BAP gave the best results of the multiplication rate of 64.5 shoots/explant. However, Mhatre *et al*. (1985) [5] also opined that among all other Cytokinins, BAP was more effective in inducing auxillary bud sprouting, as it could help in breaking the apical dominance. Patel *et al*., (1983) [7] observed good shoot proliferation from auxiliary buds of *Morus indica* when MS medium supplemented with NAA in combination with either BAP or Kinetin.

In another combination of IAA and BAP, Significantly maximum response of sporting percentage (77.52%) was recorded in S13 when compared to V1, M5 and Mysore local. Whereas, the minimum sprouting percentage was recorded in V1 compared S13, M5 and Mysore local. On the other hand among the different concentrations and combinations used the highest sprouting percentage 81.91 percentage was recorded when MS medium is supplemented with different concentrations of 1.5 and 1.0 mg/l IAA and BAP respectively.

Whereas Kavyashree, 2007 opined that, when Laisemer and Skoog's Basal Medium fortified with 8.88 µ*M* BAP and 2 µ*M* TIBA was found to be the most suitable medium for apical bud multiplication, resulting into 10.6 multiple shoots per culture. Similarly, Prasad *et al.*, 2010, studied genotypic difference in shoot response to Auxin and Cytokinin concentrations and combinations as revealed by the variation in callus differentiation and shoot formation. Combination of BAP (2 mg/l) and NAA (0.1 mg/l) was found most effective in inducing higher per cent of multiple shoots. These results are disagreeing with the findings of (Singh *et al.*, 2014) [12], who reported the application of IBA higher concentration (2000 ppm) was more beneficial for over all parameters of *Morus alba* stem cuttings. However, Tewary and Subba Rao (1990) [14], studied the multiplication of apical bud on MS basal medium supplemented with different concentrations of Cytokinins (Kinetin and BAP). It clearly established the genotypic difference among the genotypes studied and also reflects the intensity of varied response in hormones. The present investigation thus indicates the marked genotypic difference in their response to BAP and Auxin concentration and combination as revealed by the variation in sprouting percentage.

Table 1: Effect of different concentrations of hormones (NAA+BAP) on auxiliary bud sprouting (%) in different mulberry varieties

Different combination of harmones MS + NAA+ BAP (mg/l)	Auxiliary bud sprouting (%) of different mulberry varieties				
	M-5	V-1	S-13	Mysore local	mean
T_1 : MS + 1.0+ 0.5	71.00(57.41)	68.00(55.55)	57.00(49.02)	56.00(48.44)	63.00(52.61)
T_2 : MS + 1.0+ 1.0	83.00(65.65)	74.00(59.34)	60.00(50.76)	59.00(50.18)	69.00(56.48)
T_3 : MS + 1.5 + 0.5	79.00(62.72)	80.00(63.43)	65.00(53.73)	62.00(51.94)	71.20(57.96)
T_4 : MS + 1.5 + 1.0	77.00(61.34)	73.00(58.69)	70.00(56.79)	66.00(54.33)	71.50(57.79)
T_5 : MS + 2.0 + 0.5	71.66(57.84)	68.00(55.55)	71.33(57.33)	70.33(57.00)	70.33(57.00)
T_6 : MS + 2.0 + 1.00	68.33(55.75)	64.66(53.53)	70.33(56.99)	62.33(52.14)	66.41(54.60)
T_7 : Basal MS (control)	58.00(49.60)	57.00(49.02)	55.00(47.86)	53.00(46.72)	55.75(48.30)
Mean	72.57(58.62)	69.23(56.44)	64.09(53.25)	61.23(51.53)	

Test of significance	F-test	SEm±	CD at 5%
Treatments	*	0.1566	0.442
Varieties	*	0.2064	0.585
Treatments X varieties	*	0.4128	1.170

***:** Significance at 5%

MS-Murashige and Skoogs medium

Note: Figures in parenthesis indicate Arc sign transformation values

Table 2: Effect of different concentrations of hormones (IAA+BAP) on auxiliary bud sprouting (%) in different mulberry varieties

Different combination of hormones MS + IAA+ BAP (mg/l)	Auxiliary bud sprouting (%) of different mulberry varieties				
	M-5	V-1	S-13	Mysore local	mean
T_1 : MS + 1.0+ 0.5	59.00(50.19)	54.00(47.29)	73.33(58.91)	70.33(57.00)	64.16(53.35)
T_2 : MS + 1.0+ 1.0	67.00(55.34)	59.00(50.18)	76.66(61.12)	74.00(59.34)	69.33(56.50)
T_3 : MS + 1.5 + 0.5	70.00(56.79)	61.66(51.74)	83.33(65.91)	79.00(62.72)	73.50(59.29)
T_4 : MS + 1.5 + 1.0	75.00(60.00)	76.33(60.90)	89.00(70.64)	87.33(69.17)	81.91(65.18)

T_5 : MS + 2.0 + 0.5	71.00(57.41)	72.66(58.48)	86.00(68.03)	84.00(66.42)	78.41(62.59)
T_6 : MS + 2.0 + 1.00	67.33(55.14)	68.00(55.55)	78.33(62.26)	78.66(62.50)	73.08(58.16)
T_7 : Basal MS (control)	54.00(47.29)	55.66(48.25)	56.00(48.44)	57.66(49.32)	55.83(48.35)
Mean	66.28(54.60)	63.90(53.20)	77.52(62.19)	75.85(60.94)	

Test of significance	F-test	SEm±	CD at 5%
Treatments	*	0.256	0.725
Varieties	*	0.338	0.960
Treatments X varieties	*	0.677	1.920

***:** Significance at 5%
MS-Murashige and Skoogs medium
Note: Figures in parenthesis indicate Arc sign transformation values

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