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ORGANOGENIC RESPONSE THROUGH CALLUS PHASE IN FEW MULBERRY (*Morus* spp.) VARIETIES

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ABSTRACT:

The experiment was conducted to know the of micropropagation efficiency of Tr10, Vishala, M5 and G2 mulberry (*Morus* spp.) varieties using leaf and nodal explants. Regeneration and multiple shoot formation were achieved from the callus phase from four mulberry varieties via Tr10, Vishala, M5 and G2. MS medium fortified with different concentrations and combinations of growth hormones were used, such as alone 2, 4 D (0.5 mg/l - 2.5 mg/l) and in combination with BAP (0.5 mg/l and 1.0 mg/l) for callus initiation from leaf and nodal explants. Shoot buds developed from callus were transferred to the multiplication medium containing BAP (0.5 mg/l – 3.0 mg/l) and NAA (0.1 mg/l) combination of growth hormones. The micro shootlets were subcultured to obtain sufficient growth of the shoots. Then shootlets were transferred to the rooting media containing NAA and IBA (0.5 mg/l – 3.0 mg/l). The results indicate that explants inoculated in alone 2, 4 D has enhanced the callus formation percentage. The best callusing was observed from nodal explants in alone 2, 4 D at 2.0 mg/l concentration in most of the varieties. Whereas the leaf explants did not show any further regeneration and proliferation was ceased in callus phase.

Key words: In vitro efficiency, mulberry varieties, leaf explants, nodal explants.

INTRODUCTION:

Mulberry silk is one of the most renowed and popular forms of silk, particularly when it comes to textiles. The mulberry is the sole source of food for silkworms (*Bombyx* mor L.). The role of mulberry in silk industry is very significant and most of the mulberry varieties in India belong to the *Morus* indica. Mulberry is a fast growing deciduous trees belong to the genus *Morus*. Mulberry cultivation is practiced under various climates. Conventional breeding methods are time consuming and slow, contributing to the high cost of the plants. Perpetuation of some mulberry varieties through cuttings faced difficulties due to adaptability to the new localities and poor rooting ability. Thus release of a new cultivar may require many years of production before enough plants are obtained to supply. As a result many attempts have been made to microprpoagate the plants using different explants with varying success.

The present experimental work aims to study the regenerative efficiency of leaf and nodal explants using four mulberry cultivars i.e. Tr10, Vishala, M5, and G2. Change in the composition of the media and type of the explants used are still among the key factors for successful regeneration of plants from different cultivars. It is generally said that higher the efficiency of callus induction higher is the regeneration from callus. As an objective many

factors involved in the regeneration procedure which includes sterilization of the explants, type of the explants used and growth hormone combination etc.

MATERIALS AND METHODS

The four mulberry varieties (Tr10, Vishala, M5 and G2) used in the present study includes were collected in the form of cuttings and raised in the department of Biosciences and Sericulture, Sri Padmavati Mahila Visvavidyalayam, Tirupati by adopting recommended package of practices. Leaf explants were excised from actively growing pruned branches and were cut into bits of around 2cms size. The nodal explants were excised from young actively growing shoots, kept in conical flask containing water and brought to the laboratory. The leaves were removed and nodal region measuring about 2-3 cm each containing an axillary bud was excised and used as explants for the present study.

Sterilization of leaf and nodal explants

The sterilization and the treatment of duration varied for leaf and nodal explants and also for variety to variety. The explants were washed several times with tap water for 15-20 min and then with double distilled water for 5 min. The explants were surface sterilized using different sterilants such as tween 20, savlon and bavistin for specific period. Explants were raised properly with double distilled water after every treatment with sterilants.

Sterilization prior to Inoculation

The sterilized leaf and nodal explants were taken into the laminar air flow cabinet and again sterilized with 70% ethanol for 15 sec and $HgCl_2$ for 45 sec and washed with sterilized double distilled water for 3 times to remove the traces of sterilents. The sterilized edges of leaf explants were cut into small bits of $1cm^2$ and used as explants. Nodal explants were sterilized 70% ethanol for 1 min and $HgCl_2$ for 3 min. The nodal explants were properly rinsed with double distilled water thrice after every treatment to remove the traces of sterilents.

Inoculation

The treatment edges or leaf and nodal explants were carefully trimmed with scalpel before inoculation of explants into the MS medium and inoculation was done with the help of sterilized forceps. After every inoculation the instruments were dipped in alcohol and flamed in order to avoid contamination. The explants were inoculated in MS media supplemented with 2, 4 D (0.5 mg/l to 2.5 mg/l) alone and in combination with BAP (0.5 mg/l and 1.0 mg/l) for inducing callus. Observations were made daily to record about percentage of callus initiation, nature of callus (compact, loose, friable) and color (green, white, brown and yellow).

Subculturing

Prolonged culture of callus in the same media leads to slow prolifiration and growth of the callus will decline, hence the callus was subcultured into the fresh media containing same concentration of growth hormones to obtain rapid and sufficient growth. Increased in the mass of callus was recorded after four weeks.

Initiation of multiple shoots

Callus initiated from the leaf and the nodal explants was used for further studies for initiation of shoots. The callus was inoculated into the MS medium containing BAP (0.5 mg/l – 3.0 mg/l) alone and in combination with NAA 0.1 mg/l.

In vitro rooting

Microshootlets developed from callus in multiplication media was transferred to the rooting media containing NAA (0.5 mg/l – 2.5 mg/l) and IBA (0.5 mg/l – 2.5 mg/l). Observations on rooting percentage were recorded after 30-40 days of inoculation in the rooting media. The rooted microshootlets were taken out from the media and washed properly to remove the traces of agar and planted in a plastic cup containing autoclaved vermiculite and sand in 1:1 ratio. Then after 10 days the plantlets were transferred to polybags containing soil and organic manures in 2:1 ratio. Plants were watered for every four days for 2 weeks and transplanted to the main field.

RESULTS AND DISCUSSION

Leaf and nodal explants were inoculated in the MS media supplemented with auxin 2,4 D (0.5 mg/l - 2.5 mg/l) alone and in combination with BAP (0.5 mg/l and 1.0 mg/l). The callus initiated from leaf and nodal explants varied significantly among the varieties and the reports are presented in the table I.

Callus initiation from leaf explants

In the present study, alone 2, 4 D induced maximum callusing percentage of 26.6 in Tr10. where as 2, 4 D in combination with BAP showed less callusing response from leaf explants.

Among all the concentrations and combinations tested, 2, 4 D alone showed best response. The response of the callus initiation varied with the concentration of the growth hormones used. This clearly indicated that the requirement of growth hormones for each variety is not same. Maximum initiation percentage was observed in Tr10 (26.60) at 1.5 mg/l 2,4 D and least response was observed in Vishala (20.0) at 2.5 mg/l 2,4 D. whereas G2 and M5 showed 24.4, 22.2 respectively at 2.0 mg/l.

In 2,4 D + BAP (0.5 mg/l) combination maximum initiation percentage (22.2) was observed in Tr10 and G2 at 2,4 D 1.5 mg/l + 0.5 mg/l and 2,4 D 2.0 mg/l + BAP 0.5 mg/l. The initiation of callus took too long in 2,4 D+ BAP combination compared to alone 2,4 D. The callus initiation percentage was very less in 2,4 D + BAP 1.0 mg/l compared to alone 2,4 D, 2,4 D + BAP 0.5 mg/l, showing only 13.3 percent of callus in Tr10 and M5. In contrast Vijayan *et al* (2000) achieved rapid response in BAP initiated medium and reported that BAP was found to be the major factor responsible for callus initiation capacity in mulberry leaves.

Increase in the concentration of 2,4 D up to 2.5 mg/l increased the frequency of callus initiation in some varieties up to certain level, later the response was decreased gradually and ceased. The percentage of callus initiation was drastically reduced in leaf explants. The present study reports are not in conformity with the findings of Bhau and Wakhlu (2001) who stated that leaf explants are suitable for best callusing. The initiated callus when transferred to the fresh media failed to

proliferate and dried. No organogenesis was noticed in both the combinations of growth hormones after subculturing.

Callus initiation from nodal explants

In 2,4 D alone, maximum response of callus initiation was achieved in Vishala (73.3%) followed by Tr10 (66.6%) and G2 (66.6%), least response was observed in M5 (53.3%). 2,4 D at 2.0 mg/l showed best response in all the varieties except M5 which showed only 53.3% response at 1.0 mg/l. when the concentration of 2,4 D was increased above 2.5 mg/l callus initiation percentage was decreased slowly and ceased. Mandoji Mansoor Khan and Shankar Naik (2016) have reported that higher concentration above 2.0 mg/l of 2,4 D showed reduced sprouting.

In 2,4 D and BAP 0.5 mg/l combination maximum callusing percentage was observed in Vishala (66.3) and least response was noticed in M5 and G2 (46.6). In 2,4 D and BAP 1.0 mg/l maximum callusing was observed in M5 (53.0%) and less callusing was observed in G2 (33.3%). There was a significant variation among the varieties depending on the concentration of the growth hormones supplemented in MS medium. Among the growth hormones, alone 2,4 D 2.0 mg/l was found to be most suitable for callus initiation for all the varieties.

Nodal explants were more responsive than the leaf explants in callus inducing capacity. In 2,4 D alone and combination of 2,4 D and BAP, high percentage of callus was noticed in alone 2,4D and from the result it can be concluded that 2,4 D plays an effective role in inducing callus, which confirms the reports of Prasad Rao *et al* (2010) who have reported that 2,4 D 2.0 mg/l along with low concentration of BAP showed best response.

The percentage of callus formation varies with the explants used for the study, nature of the explants, and also the combination of the growth regulators used in MS medium, which confirms the studies of Kathiravan *et al* (1995). The regeneration efficiency depends on the various factors such as genotype, type of the explants, concentration and combination of the growth hormones provided in the medium. These results are in conformity with reports of Gonzalez *et al* (2007) and Melur Kodandaram *et al* (2013). The callus initiation percentage was found to be more in nodal explants compared to the leaf explants. These findings are in contrast with the reports of Vijaya chitra and padmaja (2005) who stated that enhanced organogenesis with highest proliferation from the leaf explants of mulberry in BAP supplemented medium.

Multiple shoots initiation percentage from nodal explants

For multiple shoot initiation alone BAP 0.5 mg/l – 3.0 mg/l and in combination with NAA (0.1 mg/l) were used. The percentage of multiple shoot formation from nodal callus is presented in the table II.

In alone BAP maximum response was observed in Vishala (53.3) at 2.0 mg/l, followed by Tr10 (46.6). Least response was found in M5 (39.9) at 2.0 mg/l. Increase in the concentration of BAP above 2.5 mg/l decreased the shooting ability from callus. These findings were supported by the reports of Narayan *et al* (1989) who have reported that increased BAP concentration decreased the shoot formation from callus. All the varieties showed best response at 2.0 mg/l of BAP.

Combination of BAP+NAA showed best response for organogenesis compared to alone BAP. Maximum organogenic percentage was noticed in Vishala (73.3) at BAP 2.0 mg/l + NAA 0.1 mg/l. The initiation of multiple shoot from the nodal callus of different varieties, have reported positively in the presence of BAP+NAA. The presence of BAP in MS medium is responsible for the development of multiple shoots from the callus. The present results are in conformity with the results of following researches, Narayan *et al* (1989); katiravan *et al* (1995), (1997); Sahoo *et al* (1997); Vijayan *et al* (2000); Vijayan *et al* (1998) and Prasad Rao *et al* (2010) who have stated that callus when transferred to BAP+NAA media has enhanced callusing percentage with development of increased number of shoots/plants.

In vitro Rooting

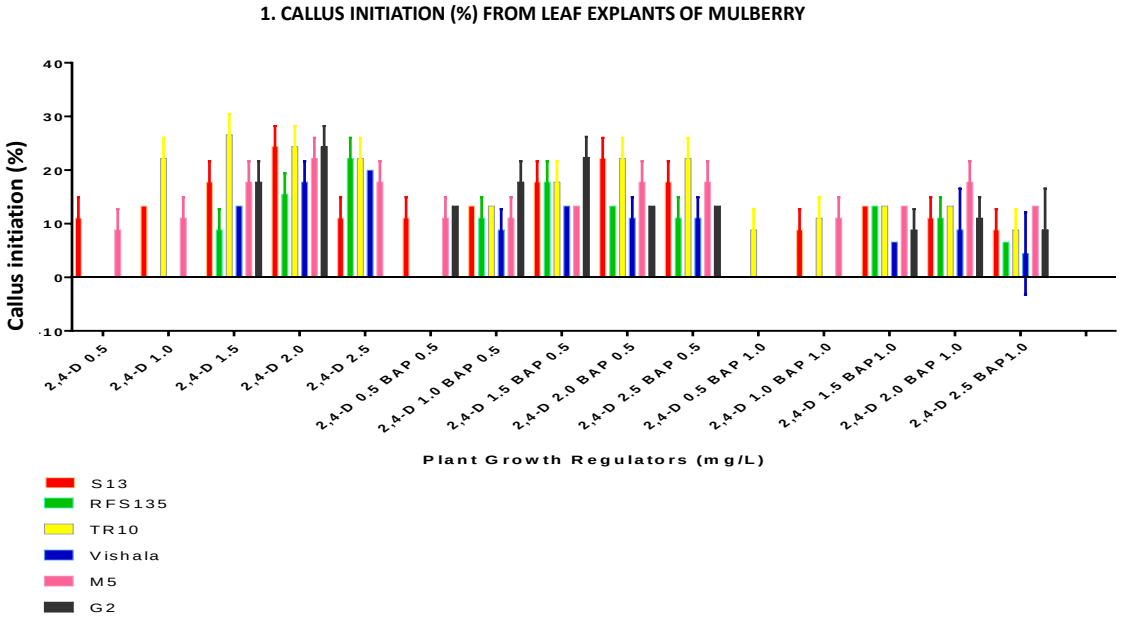
The microshootlets were transferred to the rooting medium containing alone NAA 0.5 mg/l- 3.0 mg/l and alone IBA 0.5 mg/l- 3.0 mg/l and the percentage of rooting observed in different varieties is presented in the table III.

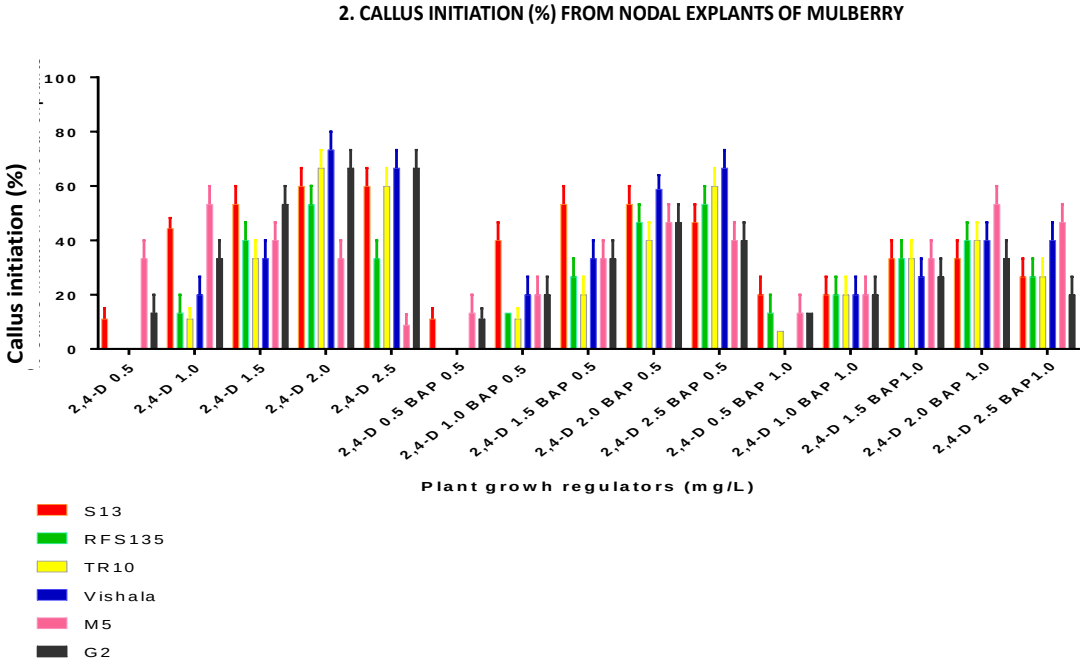
NAA induced highest root initiation in all the varieties tested. Maximum rooting was observed in Tr10 followed by Vishala (73.6 and 70.3) respectively. Successful rooting is one of the important criteria for micropropagation. The whole plant developed from the callus was successfully established from nodal explants. Profuse rooting was noticed from the base of the shootlets in all the varieties. The percentage efficiency of root formation was decreased with the increased concentration of NAA. Rapid rooting response was observed in NAA at 1.0 mg/l and 1.5 mg/l in all the varieties which was in accordance with the reports of Narayan *et al* (1989) and Prasad Rao *et al* (2010) who have achieved successful 95% rooting in NAA supplemented medium.

Acclimatization

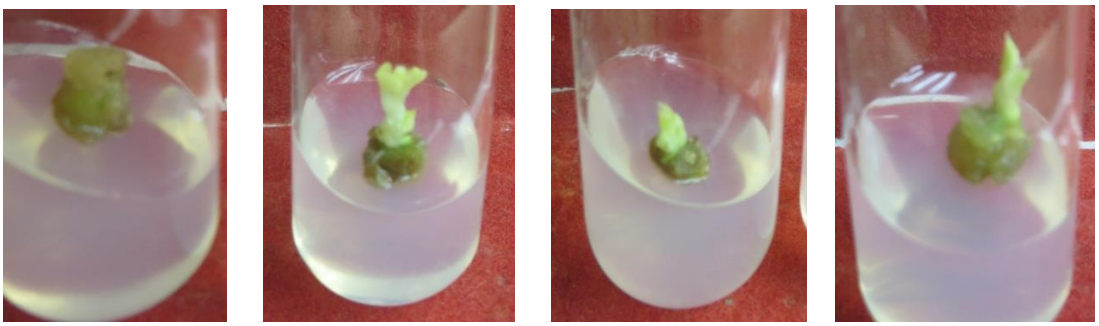
The microshootlets with well established root system were taken from the culture media and washed gently and kept in plastic cups containing sand and vermiculite in 1:1 ratio and covered with polythene sheet to maintain humidity. These plants were transferred to the green house and kept in poly bags containing soil and organic matter. The plants were watered regularly and transferred to the main field with 85% survivability.

The percentage of callus initiation from Leaf and Nodal explants of
mulberry varieties (*Morus* spp.)



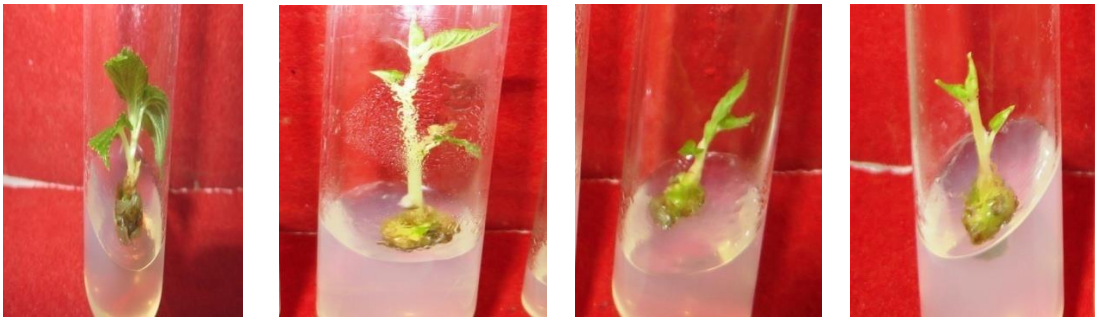


CALLUS INITIATION FROM MULBERRY NODAL EXPLANTS



Tr10 **Vishala** **M5** **G2**

MULTIPLE SHOOT INITIATION FROM NODAL EXPLANTS



Tr10 **Vishala** **M5** **G2**

ROOT INITIATION FROM NODAL CALLUS

Tr10 **Vishala** **M5** **G2**

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Table I Effect of different concentrations and combinations of growth hormones on Callus initiation from Mulberry leaf and nodal explants

Plant growth regulators (mg/l)		CALLUS INITIATION PERCENTAGE (%)							
		Leaf Explants				Nodal Explants			
2,4-D	BAP	Tr 10	Vishala	M 5	G 2	Tr 10	Vishala	M 5	G 2
0.5	**-**	0.00±0.00	0.00±0.00	8.83±3.87	0.00±0.00	0.00±0.00	0.00±0.00	33.30±6.70	13.30±6.70
1.0	**-**	22.20±3.81	0.00±0.00	11.07±3.87	0.00±0.00	11.07±3.87	19.97±6.65	53.30±6.70	33.30±6.70
1.5	**-**	26.60±3.81	13.30±0.00	17.77±3.87	17.77±3.87	33.30±6.70	33.30±6.70	39.97±6.65	53.30±6.70
2.0	**-**	24.40±3.81	17.77±3.87	22.20±3.81	24.40±3.81	66.63±6.65	73.30±6.70	33.30±6.70	66.63±6.65
2.5	**-**	22.20±3.81	20.00±0.00	17.77±3.87	0.00±0.00	59.97±6.65	66.63±6.65	8.83±3.87	66.63±6.65
0.5	**0.5**	0.00±0.00	0.00±0.00	11.07±3.87	13.30±0.00	0.00±0.00	0.00±0.00	13.30±6.70	11.07±3.87
1.0	**0.5**	13.30±0.00	8.83±3.87	11.07±3.87	17.77±3.87	11.07±3.87	19.97±6.65	19.97±6.65	19.97±6.65
1.5	**0.5**	17.77±3.87	13.30±0.00	13.30±0.00	22.40±3.81	19.97±6.65	33.30±6.70	33.30±6.70	33.30±6.70
2.0	**0.5**	22.20±3.81	11.07±3.87	17.77±3.87	13.30±0.00	39.97±6.65	58.87±5.10	46.63±6.65	46.63±6.65
2.5	**0.5**	22.20±3.81	11.07±3.87	17.77±3.87	13.30±0.00	59.97±6.65	66.63±6.65	39.97±6.65	39.97±6.65
0.5	**1.0**	8.83±3.87	0.00±0.00	0.00±0.00	0.00±0.00	6.60±0.00	0.00±0.00	13.30±6.70	13.30±0.00
1.0	**1.0**	11.07±3.87	0.00±0.00	11.07±3.87	0.00±0.00	19.97±6.65	19.97±6.65	19.97±6.65	19.97±6.65
1.5	**1.0**	13.30±0.00	6.60±0.00	13.30±0.00	8.83±3.87	33.30±6.70	26.63±6.65	33.30±6.70	26.63±6.65
2.0	**1.0**	13.30±0.00	8.87±7.68	17.77±3.87	11.07±3.87	39.97±6.65	39.97±6.65	53.30±6.70	33.30±6.70
2.5	**1.0**	8.83±3.87	4.43±7.68	13.30±0.00	8.87±7.68	26.63±6.65	39.97±6.65	46.63±6.65	19.97±6.65

Each value represents the average of 3 replications (n=3); explants treated with different concentrations of plant growth regulators± indicates the standard error values

Table II Multiple shoot initiation (%) from nodal callus

Plant growth regulators (mg/l)		**Multiple shoot initiation (%)**			
		Tr 10	**Vishala**	**M 5**	**G 2**
BAP	**NAA**				
0.5	**-**	11.07±3.87	0.00±0.00	11.07±3.87	11.07±3.87
1.0	**-**	13.30±0.00	0.00±0.00	19.97±6.65	13.30±0.00
1.5	**-**	19.97±6.65	8.83±3.87	20.00±0.00	33.30±6.70
2.0	**-**	46.63±6.65	53.30±6.70	39.97±6.65	46.63±6.65
2.5	**-**	39.97±6.65	53.30±0.00	33.30±6.70	39.97±6.65
3.0	**-**	19.97±6.65	46.63±6.65	26.63±6.65	0.00±0.00
0.5	**0.1**	11.07±3.87	11.07±3.87	13.30±0.00	33.30±6.70
1.0	**0.1**	19.97±6.65	13.30±0.00	33.30±6.70	39.97±6.65
1.5	**0.1**	20.00±0.00	33.30±6.70	39.97±6.65	39.97±6.65
2.0	**0.1**	66.63±6.65	73.30±6.70	46.63±6.65	53.30±6.70
2.5	**0.1**	53.30±6.70	59.97±6.65	39.97±6.65	46.63±6.65
3.0	**0.1**	46.63±6.65	53.30±6.70	33.10±7.00	13.30±0.00

Each value represents the average of 3 replications (n=3); explants treated with different concentrations of plant growth regulators ± indicates the standard error values

Table III Root initiation (%) from nodal callus

Plant growth regulators (mg/l)		**Root Initation percentage**			
NAA	**IBA**	**Tr 10**	**Vishala**	**M 5**	**G 2**
0.5	**-**	40.00±10.00	30.00±10.00	30.00±10.00	40.00±10.00
1.0	**-**	43.33±5.77	43.33±5.77	63.33±5.77	50.00±10.00
1.5	**-**	73.66±4.04	70.33±4.72	43.33±5.77	43.33±5.77
2.0	**-**	43.33±5.77	36.67±5.77	30.00±10.00	33.33±5.7
2.5	**-**	30.00±10.00	30.00±10.00	16.67±5.77	30.00±10.00
3.0	**-**	20.00±10.00	30.00±0.00	10.00±0.00	20.00±10.00
-	**0.5**	30.00±0.00	20.00±10.00	20.00±10.00	16.67±5.77
-	**1.0**	33.33±5.77	23.33±5.77	40.00±10.00	30.00±10.00
-	**1.5**	50.00±10.00	30.00±10.00	33.33±5.77	40.00±10.00
-	**2.0**	30.00±0.00	40.00±10.00	23.33±5.77	33.33±5.77
-	**2.5**	20.00±10.00	23.33±5.77	20.00±10.00	20.00±10.00
-	**3.0**	16.67±5.77	23.33±5.77	6.67±5.77	13.33±5.77

Each value represents the average of 3 replications (n=3); ex plants treated with different concentrations of plant growth regulators ± indicates the standard error values