

In Vitro Regeneration Efficiency Of Few Diploid And Triploid Mulberry (Morus Spp.) Varieties

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ABSTRACT:

The experiment was carried out to study the of micropropagation efficiency in few diploid and triploid mulberry (*Morus spp.*) varieties i.e S13, RFS135, Tr10, Vishala, M5 and G2, using axillary buds as explants. MS medium fortified with different concentrations and combinations of growth hormones were used. Alone BAP and kinetin were used in initiation media. BAP 2.0 mg/l was found to be most effective in inducing sprouting in most of the varieties. Shoots initiated from the nodal explants were transferred to the multiplication medium containing BAP (0.5 mg/l – 2.5 mg/l) + NAA (0.5 mg/l and 1.0 mg/l) and BAP (0.5 mg/l – 2.5 mg/l) + kinetin (0.5 mg/l and 1.0 mg/l) combinations of growth hormones. BAP 2.0 mg/l in combination with kinetin 1.0 mg/l was efficient in multiple shoots formation. The microshootlets were subcultured to obtain sufficient growth of the multipleshoots. The results indicated that explants inoculated in alone BAP has showed high percent of shoot initiation. For multiple shoot formation BAP in combination with kinetin was found to be effective in inducing efficient multiple shoots from the initiated shoots. Between two auxins tested for root initiation, NAA found to induced highest percentage of rooting compared to IBA.

Key words: In vitro efficiency, mulberry varieties, axillary bud explants, diploid varieties, triploid varieties.

INTRODUCTION:

Morus commonly known as mulberry is significantly associated with human civilization and spread of silk culture from Asia to Europe, Africa and Latin America. In India there are many species of which *Morus alba* and *Morus indica* are fully domesticated. The major cultivated mulberries belong to *M. alba*, *M. multicaulis*, *M. bombycis*, *M. indica* and other less important species but still being used are *M. cathyana*, *M. acidosa* and *M. nigra*. Therefore, in *Morus* as many as 150 species have been documented, of these varieties only 10-16 are generally cited and

acknowledged. Mulberry is generally propagated by conventional breeding methods such as stem cuttings, which permits perpetuation of the parental characters of the cultivars. In addition to basic propagation methods micropropagation can also be helpful in improvement of genetic features of the cultivars.

Most of the cultivated varieties are diploid in nature in addition polyploidy plants such as triploid and tetraploids having other advantageous traits, such as enhanced vigor, gigantism of plant parts, improved pest resistant and stress tolerance were also used for the propagation of mulberry through stem cuttings. The few mulberry cultivars cannot be propagated easily through conventional breeding methods, and not able to give expected production due to poor rooting, adaptability to different climatic conditions and other factors such as seasonal influence, such cultivars developed through tissue culture was found to be good and encouraging. Hence to improve and stabilize the few newly developed mulberry cultivars to the local conditions, micropropagation provide a foundation for the future work in In vitro regeneration.

In this regard an attempt has been made to evaluate the performance of few diploid and triploid mulberry varieties i.e S13, RF135, Tr10, Vishala, M5 and G2 using micropropagation technique. Among the selected cultivars S13 and RFS135 are rainfed diploid mulberry varieties, Whereas M5 and G2 were diploid irrigated mulberry varieties, Tr10 and Vishala are triploid irrigated varieties.

MATERIALS AND METHODS:

The six mulberry varieties (S13, RFS135, Tr10, Vishala, M5 and G2) used in the present study 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. 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.

Explant surface sterilization:

The explants were washed under running tap water for 15-20 minutes to remove any surface contaminants. These explants were collected in a conical flask and treated with different sterilants using tween20, bavestin and savlon for specific duration. Then the explants were treated with HgCl₂ or NaOCl. The treatment duration varied with the variety and rinsed properly before inoculation into the medium.

Inoculation:

The treated edges were carefully trimmed with the scalpel before inoculation. The MS medium containing BAP alone (0.5 mg/l to 3.0 mg/l) and Kinetin alone (0.5 mg/l – 3.0 mg/l) were used as initiation medium. The initiated shootlets were transferred to multiplication media containing BAP (0.5 mg/l – 2.5 mg/l) + NAA (0.5 mg/l and 1.0 mg/l) and BAP (0.5 mg/l – 2.5 mg/l) in combination with Kinetin (0.5 mg/l and 1.0 mg/l).

Subculturing:

The initiated shootlets were subcultured regularly into the fresh medium to get sufficient growth of multiple shoots. Prolonged culture of callus in the same media leads to slow proliferation and growth of the callus will decline, hence the shootlets were subcultured into the fresh media containing same concentration of growth hormones. Increased in length and number of shoots/shootlets was recorded after four weeks.

In vitro Rooting :

The microshootlets having 5 cm length were selected and placed in the medium containing alone NAA (0.5 mg/l – 3.0 mg/l) and IBA (0.5 mg/l – 3.0 mg/l) for rooting. Observations were made on the percentage of rooting and number of roots/shootlet after four weeks of inoculation.

RESULTS AND DISCUSSION:

Nodal explants were inoculated in the MS media supplemented with BAP alone (0.5 mg/l to 3.0 mg/l) and Kinetin alone (0.5 mg/l – 3.0 mg/l). The initiated shoots from nodal explants varied significantly among the varieties and the initiation percentage from nodal explants was presented in the table 1.

Initiation percentage from nodal explants

In BAP initiation medium maximum sprouting was observed in Tr10 79.97% at 2.0 mg/l followed by Vishala and M5 66.63% at 1.5 mg/l and 2.0 mg/l. The initiation response of the remaining varieties is G2 59.97% at 1.5 mg/l, S13 and RFS135 showed 59.97% response at 2.0 mg/l. BAP 2.5 mg/l was found to be suitable for most of the varieties.

In kinetin fortified medium, the sprouting response was very slow which varied from 19.97% to 33.30%. Highest initiation percentage was observed in S13 (33.30%) followed by M5 (26.6%) at 2.0 mg/l. The order of response of remaining varieties was RFS135 (26.6%), Tr10 (26.6%), Vishala and G2 (19.9%).

The nodal explants cultured in kinetin showed less response when compared to BAP supplemented medium. These results are corroborated with the results of Pattnaik et al (1997) and Gulabkhan rohela et al (2018) who observed that BAP at low concentration induced apical shoot bud proliferation and concluded that BAP was found to be effective in inducing apical shoot development than initiated kinetin. Among six varieties tested triploid varieties Tr10 and Vishal showed better performance than the diploid varieties, which confirms the reports of Chattopadhyaya et al., (2011).

The increase in the concentration of BAP in initiation medium produces callus formation at the base of the explants which are in agreement with the findings of Attia-o-Attia et al (2014). Triploids responded well in BAP supplemented medium whereas Kinetin enhanced initiation response in diploid mulberry varieties. Nodal explants responded well in BAP at 2.0 mg/l. These findings are corroborated with the reports of Attia-o-Attia et al., (2014) who stated that BAP at 0.5 mg/l to 2.0 mg/l was effective in inducing axillary bud proliferation and increased

concentration of BAP in initiation medium produces callus formation at the base of the explants which supports the present study.

Explants collected during April-June showed less contamination when compared to the explants collected during rainy and winter season. The initiation percentage and length of the shoots were influenced by the supplementation of exogenous hormones in the medium. This finding was in accordance with the reports of Shirin et al., (2005).

The concentration of endogenous and exogenous plant growth hormones influences the growth and multiplication of In vitro developed axillary shoots. BAP at low concentration induced maximum response in most of the varieties and found that BAP was found to be most effective compared to kinetin in inducing shoots from nodal explants. The present findings are in accordance with the reports of Patnaik and chand (1997), Yadav et al., (1990); Chitra and Padmaja (1999) who stated that BAP was superior over Kinetin in inducing shoots from nodal explants.

Multiple shoot initiation percentage from nodal explants

The shootlets developed from the initiation media were transferred to the multiplication media containing BAP (0.5 mg/l – 2.5 mg/l) + NAA (0.5 mg/l and 1.0 mg/l) and BAP (0.5 mg/l – 2.5 mg/l) + kinetin (0.5 mg/l and 1.0 mg/l) combination and observed for the percentage of multiple shoot initiation in different varieties. The percentage of multiple shoot initiation from nodal explants is presented in the table 2.

Varieties S13, Tr10, and Vishala have shown same (53.3%) response at different concentrations. In BAP + NAA combination of growth hormones, the highest multiple shoot initiation was recorded in S13 (53.3%) at BAP 1.5 mg/l + 0.5 mg/l followed by Tr10 (53.3%) at BAP 2.0 mg/l + 0.5 mg/l, Vishala (53.3%) at 2.5 mg/l + 0.5 mg/l. In RFS135 it is 46.6% at 2.0 mg/l + 0.5 mg/l, M5 39.9% at 2.5 mg/l + 0.5 mg/l and least response was observed in G2 (33.3%) at 2.0 mg/l + 0.5 mg/l. In BAP + NAA (1.0 mg/l) combination the maximum response only 33.3% was observed in S13 and Tr10. The remaining varieties showed very less percent of multiple shoots.

The addition of NAA to the medium has enhanced the shoot elongation percentage but did not influence the rate of multiple shoot formation and further reduced the frequency of bud break and also lessened the total number of shoots/explants.

In BAP + kinetin combination the multiple shoot initiation percentage varied from 39.97 to 66.63, Vishala showed maximum response of 66.63% at 2.0 mg/l + 0.5 mg/l, followed by Tr10 59.97%. In BAP + kinetin (1.0 mg/l) maximum multiple shoot initiation response was 73.30 % and least response was 53.30 %. Maximum response was observed in Vishala 73.30 % followed by Tr10 66.63 % and least response was observed in RFS135 53.30%. From the result it was observed that the multiple shoot initiation percentage was more in BAP + kinetin combination compared to BAP + NAA. These results was in contrast with the reports of Patnaik et.al., (1997) who have reported NAA in combination with BAP enhanced the frequency of multiple shoot formation and reduced the proliferation of bud break. Mohammad anis et al.,

(2003) achieved 35-80% survival percentage in shoots developed from nodal explants on MS media and further reported that MS media with 2.0 mg/l BAP + 0.2 mg/l NAA was best for multiple shoot regeneration.

Among various combinations tested BAP + Kin was found to be more suitable for increased number of shoots per shootlet. The maximum of 6 branches per shootlet was observed in Vishala and Tr 10 in BAP + Kin combination. Similar results were also noticed by most of the researches. Zaman et al., (1998) reported combined effect of BAP and Kinetin enhanced the number of shoots per plant. Balakrishna et al., (2009) noticed the increased response of nodal explants in terms of multiple shoot regeneration, when medium supplemented with BAP + kin.

In vitro root Initiation percentage

The shootlets were transferred to the rooting media consisting alone NAA 0.5 mg/l – 3.0 mg/l and IBA 0.5 mg/l – 3.0 mg/l and observed for root initiation percentage. In NAA, maximum rooting percentage was noticed in Tr10 (79.6) followed by Vishala (78.3) at NAA 1.5 mg/l. lowest rooting percentage was observed in RFS135 (40.0) at NAA 1.0 mg/l.

In IBA fortified medium the maximum rooting was observed in Tr10 (50.0%), followed by S13 (43.3%) at 1.5 mg/l. Lowest rooting response was observed in RFS135 (33.3%) and M5 (33.3%) at 1.0 mg/l IBA.

Average number of roots/shootlet

The data on average number of roots/ shootlet was presented in the table 3.

In **NAA**, the average number of roots/shootlets varied from 7-12 roots/shootlet. The maximum number of roots/ shootlet was noticed in Tr10 (12.00), followed by S13 (10.33) at 1.5 mg/l. Lowest number of roots/ shootlet was observed in M5 (7.33) at 1.5 mg/l.

In **IBA**, the maximum number of roots/ shootlet was observed in Tr10 (8.33) at 1.5 mg/l followed by M5 (7.00) at 1.0 mg/l and lowest number of roots/ shootlet was observed in S13 (6.33) at 1.5 mg/l.

Vishala showed 78.33% of rooting in In vitro conditions whereas, under field condition the rooting percentage of Vishala is 44%. The root initiation percentage was found to be more in triploid variety Tr10 and Vishala. NAA was found to be most effective in inducing rooting in most of the varieties. Increased In vitro rooting percentage in Vishala under in vitro conditions might be due to the influence of exogenous supplementation of growth hormones to the medium. There is also a significant difference in the nature of roots formed in the presence of two auxins.

The roots are strong and healthy in NAA fortified medium, where as they were weak and fragile in IBA medium. Similar results were also reported by Anuradha and Pulliah (1992), Anurag et al (1999), and Anis et al (2003), who have reported that NAA was most effective in root induction. In contrast to the present studies Chitra and Padmaja (1999) have noticed that NAA is not suitable as rooting agent.

The root initiation percentage and average number of roots/shootlets were also found to be more in triploid variety Tr10. In IBA the root initiation was slow compared to NAA.

Increased in the rooting percentage in Vishala under In vitro conditions might be due to the influence of supplementation of media with high concentration of auxin. Supplementation of auxin to the media is must for rapid growth of roots. Between two auxins tested, maximum roots initiation percentage was recorded in Tr10 and Vishala in NAA at 1.5 mg/l supplemented medium. The microshoots transferred to the medium without auxin does not showed any root initiation.

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.

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Table 1 Effect of different concentrations and combinations of growth hormones on initiation (%) from Mulberry nodal explants

Plant growth regulators (mg/l)		Initiation (%)					
		S 13	RFS 135	Tr 10	Vishala	M 5	G 2
BAP	KIN						
0.5	-	46.63±6.65	39.97±6.65	43.97±33.65	39.97±6.65	53.30±6.70	33.30±6.70
1.0	-	46.63±6.65	46.63±6.65	59.97±6.65	53.30±6.70	59.97±6.65	39.97±6.65
1.5	-	53.30±6.70	59.97±6.65	66.63±6.65	66.63±6.65	59.97±6.65	59.97±6.65
2.0	-	59.97±6.65	59.97±6.65	79.97±6.65	66.63±6.65	66.63±6.65	53.30±6.70
2.5	-	53.30±6.70	53.30±6.70	66.63±6.65	53.30±6.70	53.30±6.70	46.63±6.65
3.0	-	39.97±6.65	33.30±6.70	53.30±6.70	46.63±6.65	39.97±6.65	33.30±6.70
-	0.5	13.30±6.70	19.97±6.65	0.00±0.00	0.00±0.00	4.40±3.81	0.00±0.00
-	1.0	19.97±6.65	26.63±6.65	11.07±3.87	0.00±0.00	6.60±0.00	4.40±3.81
-	1.5	19.97±6.65	26.63±6.65	13.30±0.00	11.07±3.87	11.07±3.87	11.07±3.87
-	2.0	33.30±6.70	26.63±6.65	19.97±6.65	19.97±6.65	26.63±6.65	19.97±6.65
-	2.5	26.63±6.65	19.97±6.65	26.63±6.65	19.87±6.80	19.97±6.65	13.30±0.00
-	3.0	19.97±6.65	8.83±3.87	19.97±6.65	8.83±3.87	13.30±0.00	11.07±3.87

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

Table 2 Mutiple shoot initiation (%) from nodal explants

Plant growth regulators (mg/l)			Multiple shoots initiation (%)					
			S 13	RFS 135	Tr 10	Vishala	M 5	G 2
BAP	NAA	KIN						
0.5	0.5	-	11.07±3.87	4.40±3.81	0.00±0.00	0.00±0.00	0.00±0.00	4.40±3.81
1.0	0.5	-	33.30±6.70	26.63±6.65	26.63±6.65	8.83±3.87	11.07±3.87	11.07±3.87
1.5	0.5	-	53.30±6.70	39.97±6.65	33.30±6.70	39.97±6.65	26.63±6.65	19.97±6.65
2.0	0.5	-	46.63±6.65	46.63±6.65	53.30±6.70	46.63±6.65	33.30±6.70	33.30±6.70
2.5	0.5	-	39.97±3.81	33.30±6.70	39.97±6.65	53.30±6.70	39.97±6.65	26.63±6.65
0.5	1.0	-	4.40±3.87	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1.0	1.0	-	11.07±6.65	0.00±0.00	6.63±6.65	0.00±0.00	4.40±3.81	11.07±3.87
1.5	1.0	-	19.97±6.70	8.87±7.68	19.97±6.65	11.07±3.87	19.97±6.65	19.97±6.65
2.0	1.0	-	33.30±6.65	19.97±6.65	33.30±6.70	26.63±6.65	26.63±6.65	26.63±6.65
2.5	1.0	-	19.97±6.65	19.97±6.65	26.63±6.65	26.60±0.00	8.87±7.68	8.87±7.68
0.5	-	0.5	19.97±6.65	11.07±3.87	26.63±6.65	19.97±6.65	26.63±6.65	33.30±6.70
1.0	-	0.5	26.63±6.70	24.20±36.35	39.97±6.65	33.30±6.70	26.60±0.00	39.97±6.65
1.5	-	0.5	53.30±6.65	33.30±6.70	53.30±6.70	59.97±6.65	39.97±6.65	46.63±6.65
2.0	-	0.5	46.63±6.65	39.97±6.65	59.97±6.65	66.63±6.65	33.30±6.70	53.30±6.70
2.5	-	0.5	39.97±6.65	33.30±6.70	46.63±6.65	53.30±6.70	26.63±6.65	39.97±6.65
0.5	-	1.0	13.30±0.00	6.60±0.00	13.30±0.00	6.60±0.00	13.30±0.00	13.30±0.00
1.0	-	1.0	39.97±6.65	19.97±6.65	33.30±6.70	19.97±6.65	33.30±6.70	33.30±6.70

1.5	-	1.0	59.97±6.65	39.97±6.65	59.97±6.65	59.97±6.65	46.63±6.65	39.97±6.65
2.0	-	1.0	46.63±6.65	53.30±6.70	66.63±6.65	73.30±6.70	39.97±6.65	59.97±6.65
2.5	-	1.0	39.97±6.65	46.63±6.65	53.30±6.70	66.63±6.65	39.97±6.65	53.30±6.70

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 3 Root initiation (%) Nodal explants

Plant growth regulators (mg/l)		Root initiation (%)					
NAA	IBA	S 13	RFS 135	Tr 10	Vishala	M 5	G 2
0.5	-	33.33±5.77	30.00±10.00	40.00±10.00	30.00±10.00	30.00±10.00	40.00±10.00
1.0	-	50.00±10.00	40.00±10.00	43.33±5.77	43.33±5.77	50.00±10.00	50.00±10.00
1.5	-	50.00±10.00	36.67±5.77	79.66±4.16	78.33±10.01	43.33±5.77	33.33±5.77
2.0	-	40.00±0.00	36.67±5.77	33.33±5.77	36.67±5.77	30.00±10.00	30.00±10.00
2.5	-	33.33±5.77	23.33±5.77	30.00±10.00	30.00±10.00	16.67±5.77	16.67±5.77
3.0	-	23.33±5.77	6.67±5.77	20.00±10.00	20.00±10.00	10.00±0.00	13.33±5.77
-	0.5	23.33±5.77	20.00±10.00	30.00±0.00	13.33±5.77	20.00±10.00	16.67±5.77
-	1.0	33.33±5.77	33.33±5.77	33.33±5.77	23.33±5.77	33.33±5.77	30.00±10.00
-	1.5	43.33±5.77	30.00±10.00	50.00±10.00	36.67±5.77	20.00±10.00	40.00±10.00

-	2.0	33.33±5.77	30.00±10.00	30.00±0.00	36.67±5.77	16.67±5.77	33.33±5.77
-	2.5	23.33±5.77	30.00±0.00	26.67±5.77	23.33±5.77	16.67±5.77	20.00±10.00
-	3.0	16.67±5.77	13.33±5.77	20.00±10.00	16.67±11.55	16.67±5.77	13.33±5.77

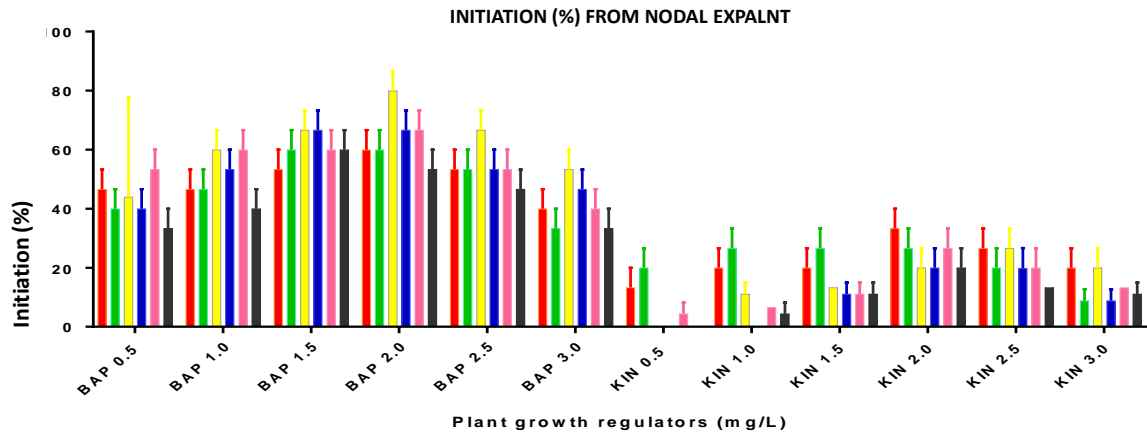
Each value represents the average 3 replications (n=3); ex plants treated with different concentrations of plant growth regulators ± indicates the standard error values

Table 4 Average number of roots

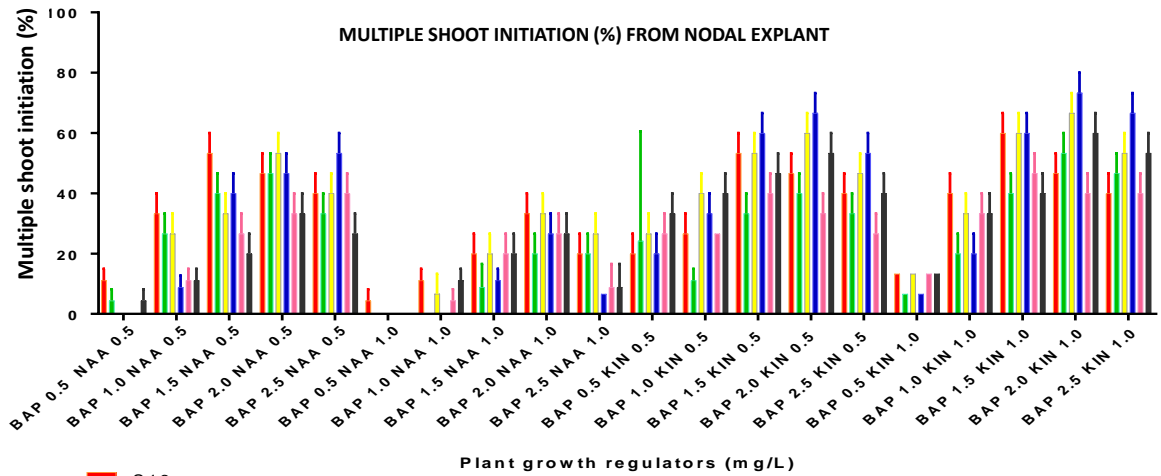
Plant growth regulators (mg/l)		Average number of roots/rootlet					
		S 13	RFS 135	Tr 10	Vishala	M 5	G 2
NAA	IBA						
0.5	-	6.00±1.00	5.33±0.58	7.00±1.00	6.00±1.00	6.00±1.00	7.00±1.00
1.0	-	7.33±2.08	6.33±0.58	7.33±0.58	7.33±0.58	9.00±1.00	9.00±1.00
1.5	-	10.33±1.53	6.67±0.58	12.00±1.00	8.33±0.58	7.33±0.58	6.33±0.58
2.0	-	7.33±0.58	8.33±0.58	6.00±1.00	7.33±0.58	5.00±1.00	6.00±1.00
2.5	-	6.33±0.58	4.44±0.58	5.33±0.58	6.33±0.58	5.00±1.00	5.33±0.58
3.0	-	5.67±1.15	3.00±2.65	5.00±1.00	5.00±1.00	4.67±0.58	5.00±1.00
-	0.5	4.67±0.58	3.00±2.65	6.00±1.00	3.33±2.89	5.33±0.58	5.33±0.58
-	1.0	6.00±1.00	5.33±0.58	6.33±0.58	5.33±0.58	7.00±1.00	6.33±0.58

-	1.5	6.33±1.15	6.67±0.58	8.33±0.58	7.00±1.00	6.33±0.58	7.33±0.58
-	2.0	6.00±1.00	6.33±0.58	6.00±1.00	6.67±0.58	5.33±0.58	6.00±1.00
-	2.5	4.67±0.58	6.00±1.00	5.00±1.00	6.00±1.00	5.00±0.00	5.00±0.00
-	3.0	4.67±0.58	5.00±1.00	5.00±1.00	5.67±0.58	4.00±1.00	3.33±2.89

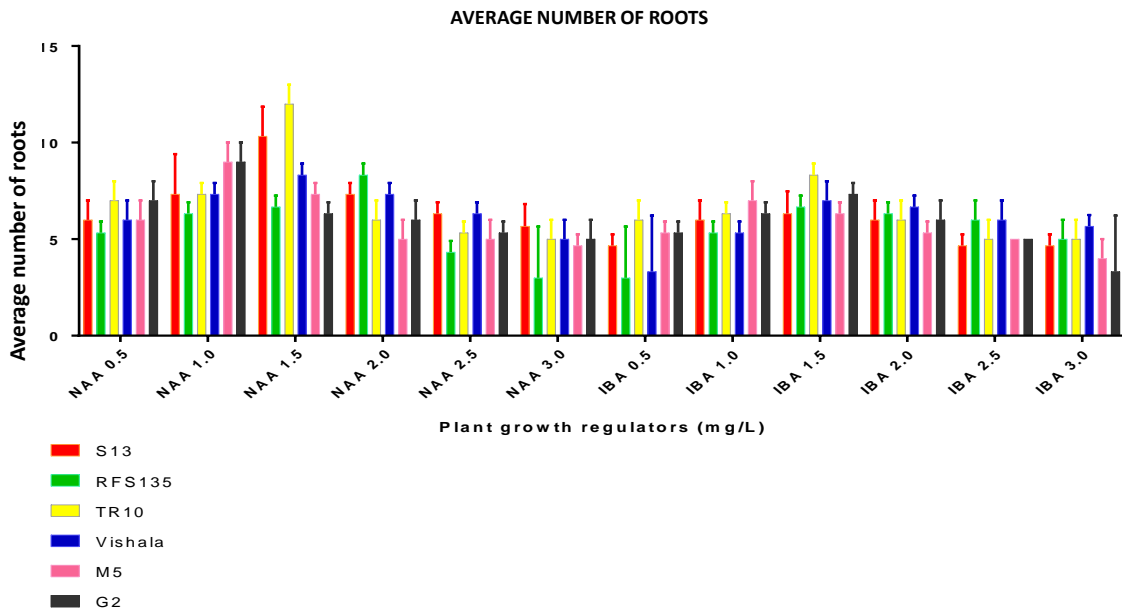
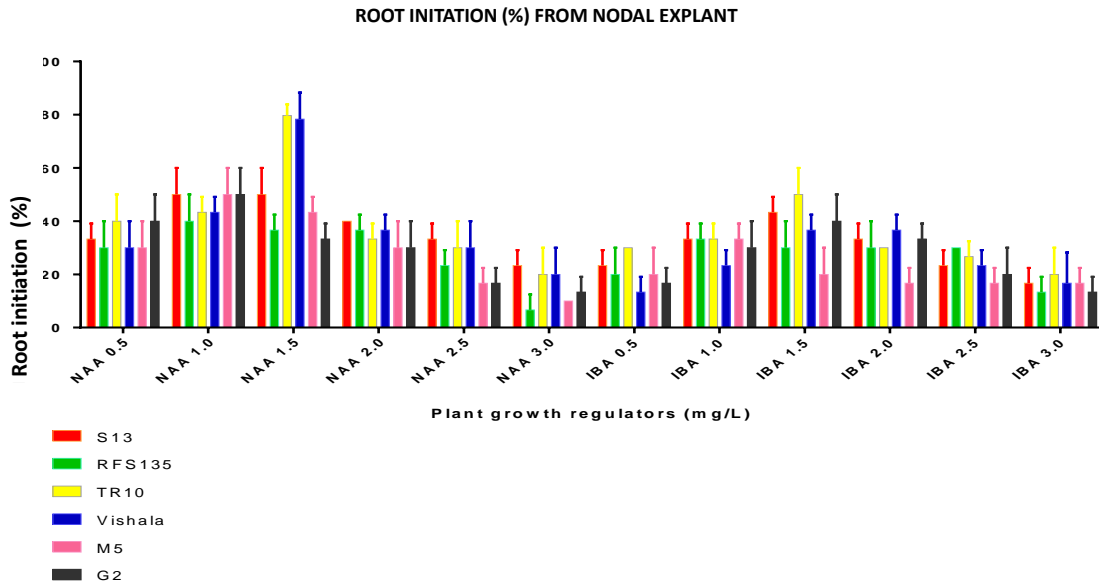
Each value represents the average of 3 replications (n=3); ex plants treated with different concentrations of plant growth regulators ± indicates the standard error values



- S13
- RFS135
- TR10
- Vishala
- M5
- G2

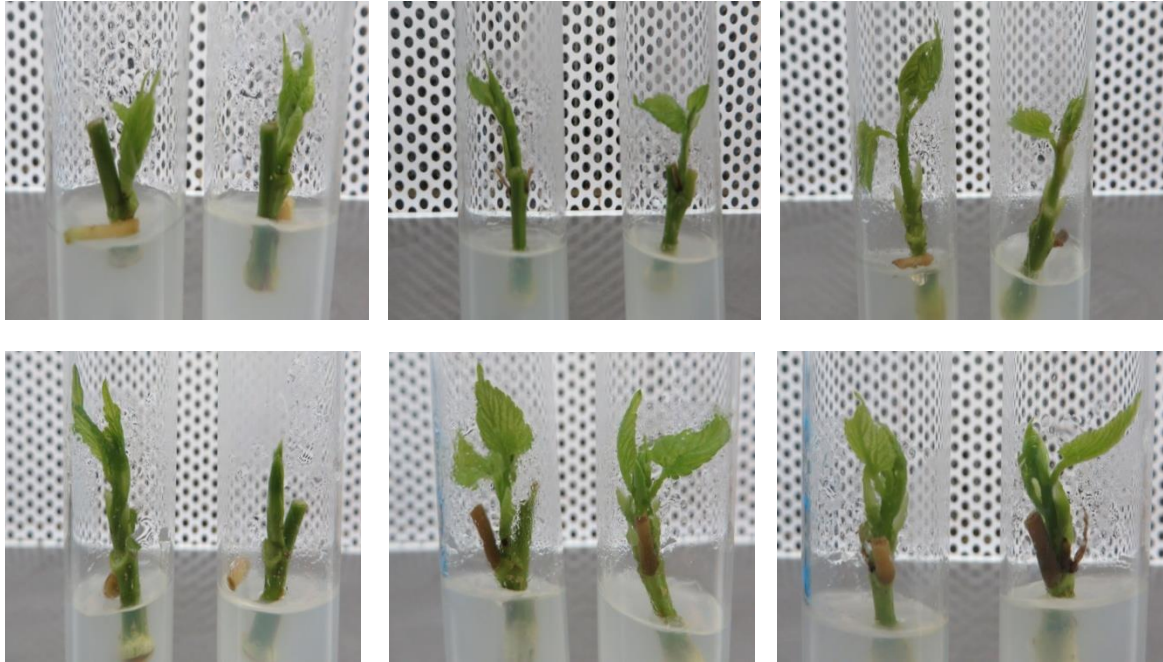


- S13
- RFS135
- TR10
- Vishala
- M5
- G2

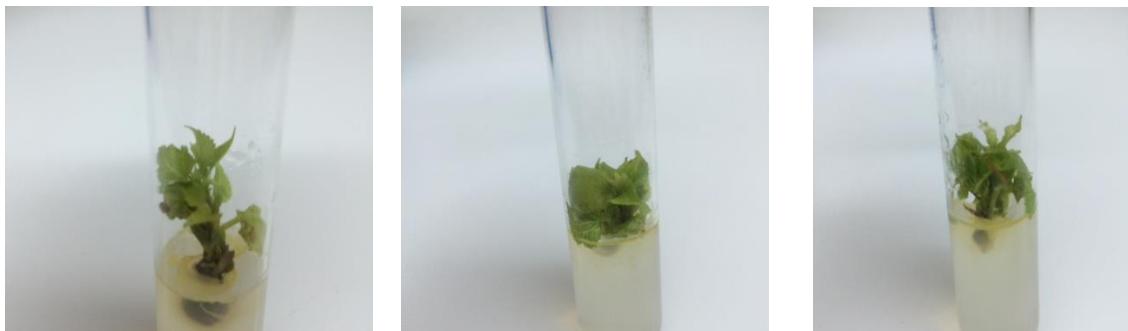


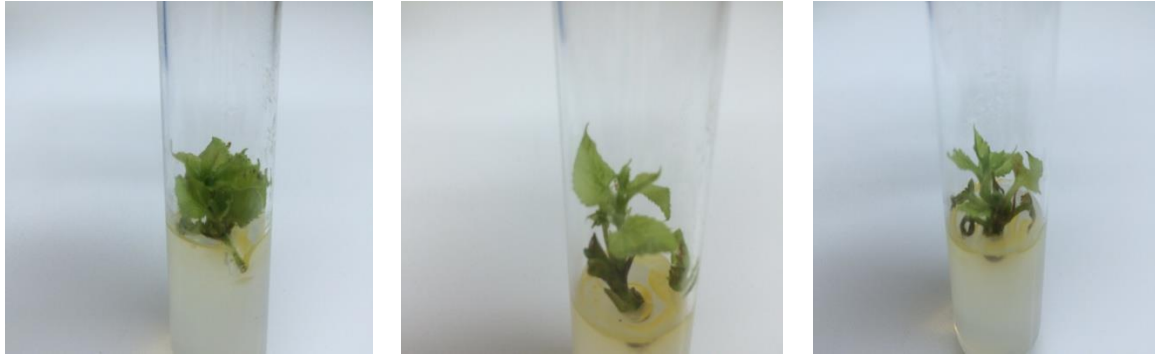
DIRECT ORGANOGENESIS

INITIATION FROM NODAL EXPLANTS OF S13, RFS 135, Tr10, VISHALA AND G2 VARIETIES

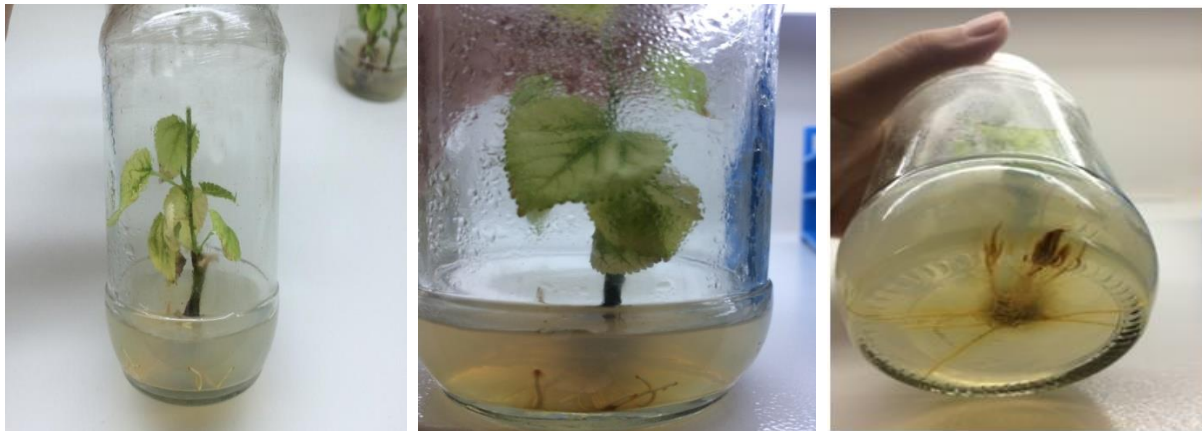


Multiple shoot initiation from Nodal explants OF S13, RFS 135, Tr10, VISHALA AND G2 VARIETIES





ROOTING FROM NODAL EXPLANTS OF S13, RFS 135, Tr10, VISHALA AND G2 VARIETIES



REFERENCES