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Callus Induction in Ailanthus Excelsa Roxb. –A Multipurpose Tree

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ABSTRACT:

Ailanthus *excels RoxB* is well known for its ayurvedic, pharmaceutical and pharmacological importance. It is the member of *Simaroubaceae* Family. The anti-cancerous, antiviral, anti-malarial, anti-leukemic, anti-feedent, hepato protective and anti-anesthetic properties of this plant earned its name as "Tree of Heaven". It is fast growing tree. However, short seed viability, poor competitiveness and lake of proper cloning techniques it is difficult to cope up with the demand. Therefore micro propagation becomes prime necessity. An attempt was made to established callus culture from various explants in two different medium named as Murashige-Skoog Medium (MS medium) and Woody plant medium (WPM) supplemented with growth regulators. MS medium with half strength of mineral salts, MS medium with full strength of mineral salts with silver nitrate as well as ascorbic acid and citric acid and WPM without silver nitrate, along with different auxin and/or cytokinin concentration were checked for callus induction. Among the different explants used, rachis with base of petiole and internodes responded well in the medium supplemented with picloram (1mg/L) and 6-benzyle amino purine –BAP- (1 mg/L) as well as naphtalene acetic acid -NAA- (1 mg/L). The browning of callus could be prevented by supplementing medium with silver nitrate. Further maintenance required frequent subculturing. Ascorbic acid and citric acid was also used as alternative of silver nitrate. Media supplemented with ascorbic acid and citric acid at concentration of 1mg/L and 1.5mg/L respectively, found effective to keep the callus viable even after a month.

KEY WORDS: Ailanthus *excelsa*RoxB.,Antioxidant, Browning, Callus, Sub culturing.

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INTRODUCTION:

"The tree of Heaven"- scientifically called as *Ailanthusexcelsa*RoxB is well known for medicinal importance since very long time[1,2]. The Softwood of the tree is used for making toys and bark is used as painkiller. This large deciduous tree is known as "Arduso" in Gujarati , "Araluka", "Aralu" and Araluvrksa" in Sanskrit "Maharukh" in Hindi, "Marukh" and "Mahanimb" in Marathi, "Mattipongilyam" in Malayalam "Agal" "Perumaruntu" and "Perumaram" in Tamil. It predominantly found in well cultivated areas of Gujarat, Maharastra, Karnataka Tamil Nadu, Madhya Pradesh, Andhra Pradesh, Chhattisgarh, Orissa and Rajasthan. It less found in forest area.

It belongs to the "*Simaroubaceae"* family. It is fast growing softwood tree in wet areas but its competitiveness is very poor. Because of its medicinal, antimicrobial and pharmacological applications, their demand is high. Commercially it is used forculture of silk warm as well as material for fodder, matchbox, puppets, and sward case etc[3]. Its analgesic[1], antiparasitic[2], anti-inflammatory[4], anti-diarrheal[5], antibacterial and anti-cancerous[6] properties were extensively reviewed.

Table-1 Applications of different parts of *Ailanthus excelsa*RoxB.

Purpose	Part Used	Properties	References
Against stomach worm	Leaf	Anti parasitic	2
Tetanus and Joint Pain	Bark	Pain relief	1
Protection against Myocardial Infarction and Transplantation Complications	Bark	Anti-inflammatory	4
Inhibitory activity against diarrhea and reduction in gastrointestinal motility	Bark	Anti-diarrhoeal and Anti-inflammatory	5
Silver nanoparticle synthesis	Leaf	Anti-bacterial and anti-cancerous	6
Reduced total Cholesterol	Leaf	Hypolipidemic	7

Though it is fast growing tree, poor competitiveness and lack of suitable clonal techniques[3], short seed lifespan[8] and fungal contamination in growing branches[9] are the major problem seen with *Ailanthus excelsa*RoxB. Microporpagation may cope up with demand to provide healthy plants. Therefore main objective of the present study focused on induction of callus in*Ailanthus excels* RoxB from various explants.

EXPERIMENTAL SECTION

Plant material

Leaf, rachis, petioles, apicalnodes and internodes were used as explants. Explants were collected from farm near Post Graduate Department of Biosciences (22.55^{o}N; 72.92^{o}E), Sardar Patel University and Vadodara Railway station (22.30^{o}N 73.20^{o}E) near platform number seven.

Sterilization of Explants

Explants were collected early in the morning (before 9:00 am) and washed under running tap water for 30min to remove dirt followed by wash with mild soap solution (Tween-20) for five min. Trace of soap solution was removed by washing under tap water. After that explants were treated with 0.2% Bavistin for 20 min under sterilized laminar air hood followed by sterile distilled water wash. Explants were washed with 70% ethanol and 0.1% $HgCl_2$ for two and one min respectively by intermittent sterile distilled water washes. Trace of surface sterilizing agents were removed by washing the explants three times with sterile distilled water. Explants were cut in appropriate size with sterile surgical blade (No-24) under laminar air hood.

Medium composition

Explants were inoculated in woody plant medium and Murashige-Skoog Medium with minor variation. WPM was used without any antioxidant. MS medium was used with two types of anti-oxidants (a) 1.7 mg/l silver nitrate and (b) 1.0mg/l ascorbic acid along with 1.5 mg/l citric acid. MS medium composition was also varied by half strength as well as full strength of mineral salt composition. Growth hormones used in media were auxin namely naphthalene acetic acid (NAA), 2-4-dichlorophenoxy acetic acid (2, 4-D),Indol acetic acid (IAA), Indol butyric acid (IBA) and Picloram. Cytokinins used were 6-Benzylaminopurin (BAP), Kinetin (Kn) and thidiazuron (TDZ).

Composition of media,

1. MS Medium with Full Strength of Mineral Salts + 1.7mg/l $AgNO_3$+ Growth Hormones (NAA and Kn; BAP and IAA; BAP and NAA; 2,4-D and BAP; NAA and TDZ)
2. Woody Plant Medium + 1.7mg/l $AgNO_3$+ Growth Hormones (NAA and Kn)
3. MS Medium with Half Strength of Mineral Salts + 1.7mg/l $AgNO_3$+ Growth Hormones (2,4-D and BAP; NAA and TDZ)
4. MS Medium with Full Strength of Mineral Salts + 1.7mg/l $AgNO_3$+ Auxin (IAA, IBA, NAA, 2, 4-D and Picloram)
5. MS Medium with Full Strength of Mineral Salts + 0.1mg/l Ascorbic acid + 0.15mg/l Citric Acid + Growth Hormones (BAP and NAA)

Duration of sub-culturing of explants was also optimized by transferring them to fresh medium at 20th, 10th and 5th day.

Statistical analysis

Analysis of variance of data was done by one-way ANOVA using MicrosoftOffice Excel 2007 program (p = 0.05).

RESULTS AND DISCUSSION

Response of callus induction was checked between WPM without antioxidant and MS medium with 1.7 mg/l of $AgNO_3$ along with NAA (0.5, 1, 2, 3 and 5 mg/l) or (0.54, 10.74, 16.11 and 26.85 µM) and Kinetin (1, 2 and 3 mg/l) or (0.46, 0.93 and 1.39 µM) as growth regulator. 1mg/l kinetin (0.46 µM) with 1mg/l NAA (10.74µM) gave 80% of response when supplemented in MS medium (Figure-1). So MS medium was preferred over woody plant medium. $AgNO_3$ was reported to protect the explants from browning by ethylene inhibition[10,11].

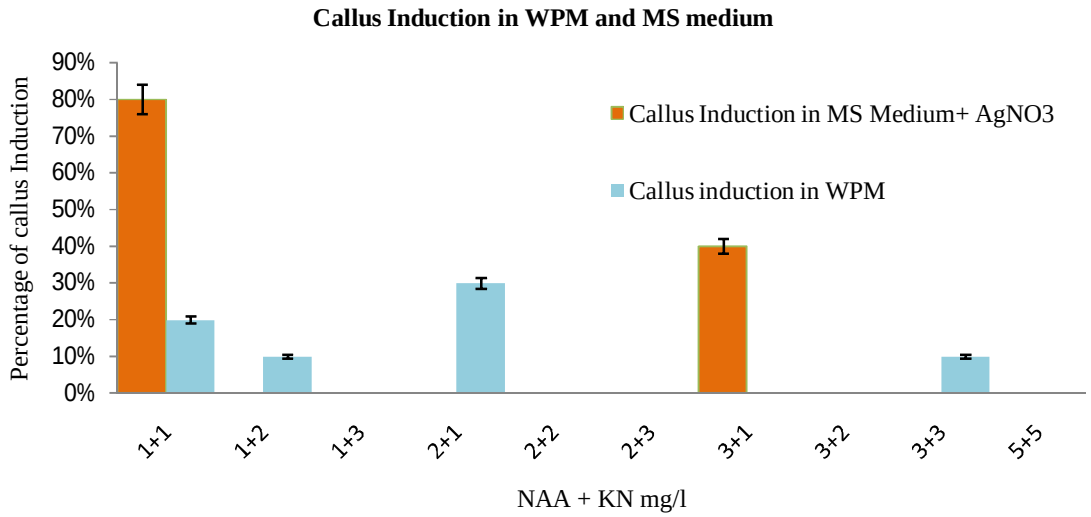


Figure- 1 Response of leaf for callus formation in MS media + Antioxidant and WPM without antioxidant

Much changes were not noticed when half strength of micro-elements of MS medium was used. Among the tested concentration, 40% of plants were responsive for 6mg/l NAA in the combination with 3 mg//l TDZ (Figure-2a and b). Leaf turned brown after 10 days of incubation (Figure-3). Callus formation startedatcutedge of leaf. NAA and TDA induced callus in *Primula vulgaris*at concentration of 0.5 mg/l and 3 mg/l respectively [3]. Callus induction rate was 100% in *Primula vulgaris* contradictory to our results which showed only 40% of response.

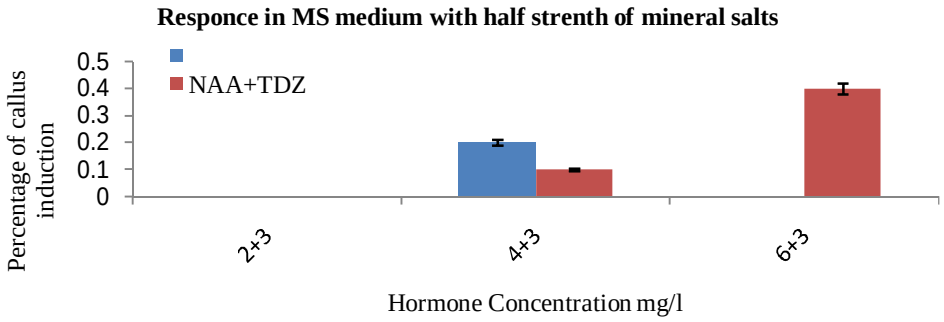


Figure 2(a) Response of leaf for callus formation in MS media with half strength of mineral salts + Antioxidant +

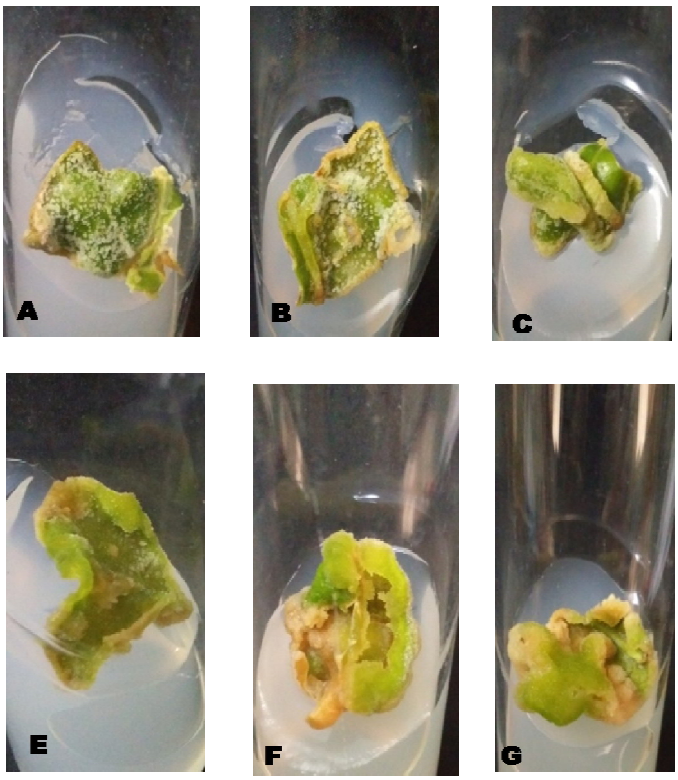


Figure 2(b) – MS medium with half strength of mineral salt medium + 1.7 mg/l $AgNO_3$

Growth hormones

A: 2mg/l 2,4-D + 3 mg/l BAP
B: 4mg/l 2,4-D + 3 mg/l BAP
C: 6mg/l 2,4-D + 3 mg/l BAP
E: 2mg/l NAA + 3 mg/l TDZ
F: 4mg/l NAA + 3 mg/l TDZ
G: 6mg/l NAA + 3 mg/l TDZ

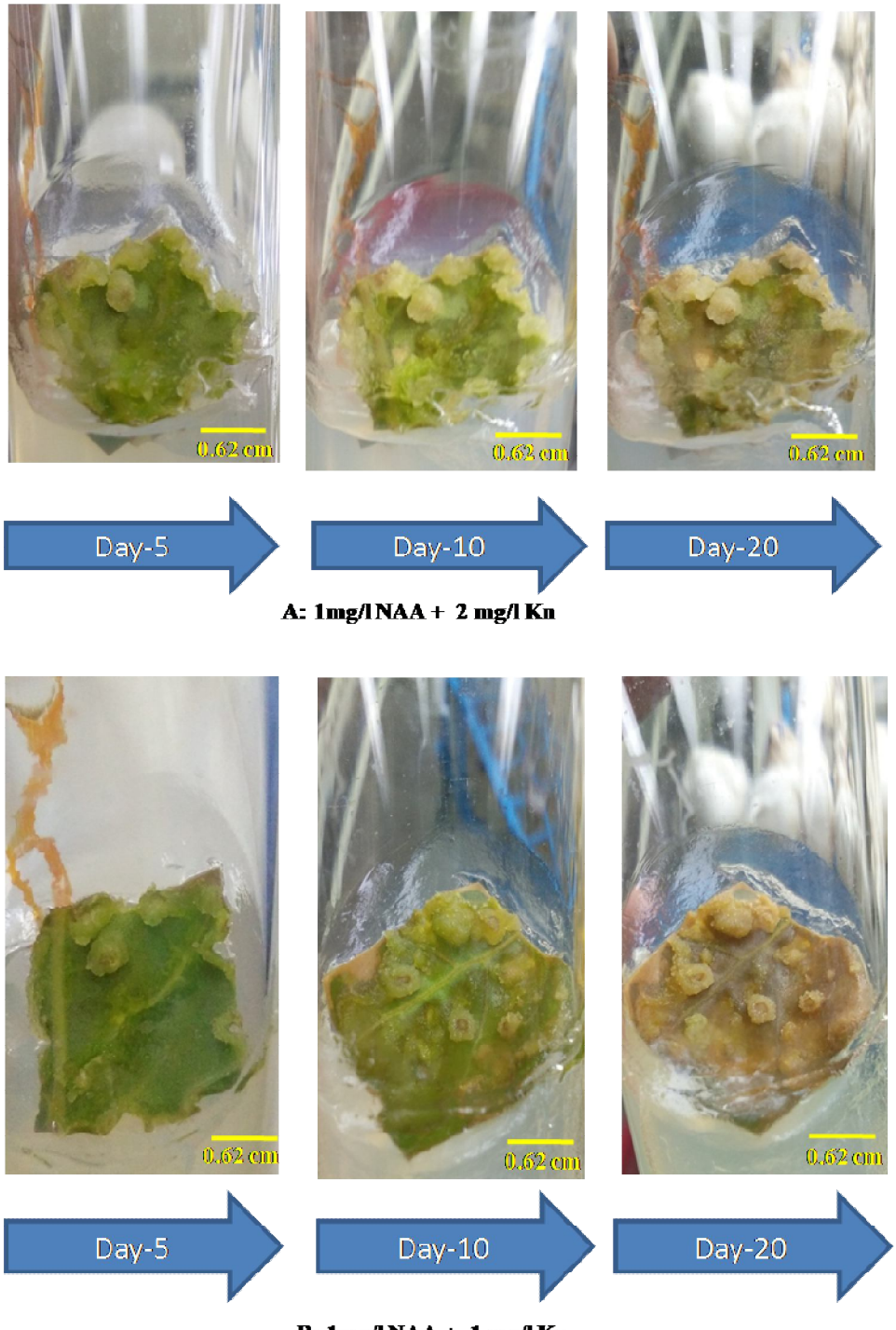


Figure 3 Browning of leaf explant over incubation period of 20 days.

Responses of rachis and petioles towards the different auxin concentrations were checked. Full strength of MS medium was used along with silver nitrate as anti-oxidant. Among the tested

auxins, picloram and NAA induced better response for callus induction in petioles and rachis. Other auxins *viz* IAA and IBA were not induceing callus in neither rachis nor in petioles under dark condition (Figure-4 a and b). Incubation in dark condition reduces browning by inhibiting phenol synthesis[12]. Picloram showed highest callus induction at 1mg/l (4.14 µM)concentration in rachis. Similarly 3 mg/l and 4 mg/l (16.11 µM and 21.48 µM) NAA induced noticeable callus in petioles (Figure-5). The results were similar as reported in *Eurycomalongifolia*, a plant belongs to *Simaroubaceae*[13]. Picloram, NAA and IAA induced callus in petioles of*Eurycomalongifolia*at 4 mg/l, 3 mg/l and 3mg/l concentration respectively[13]. Contradictorily IAA provedto be ineffective in inducing callus in petiole as well as rachis of*Ailanthus excelsa*. One way ANOVA for picloramand NAA for callus development from rachis were showed Table-1(a-d) which showed that p values are 0.0047 and 0.002 respectively (less than 0.05). Similarly for one way ANOVA for callus induction in petioles using picloram and NAA, p values were observed 0.005 and 0.002 (less than and equal to 0.005). Statistical analysis indicates that picloram and NAA significantly influence the callus induction in MS medium with rachis as well as petioles. Callus growth was not sustained and got brown even after subculturing at tenth day. This indicates incubation in dark and silver nitratesupplementation were less efficient to prevent browning.

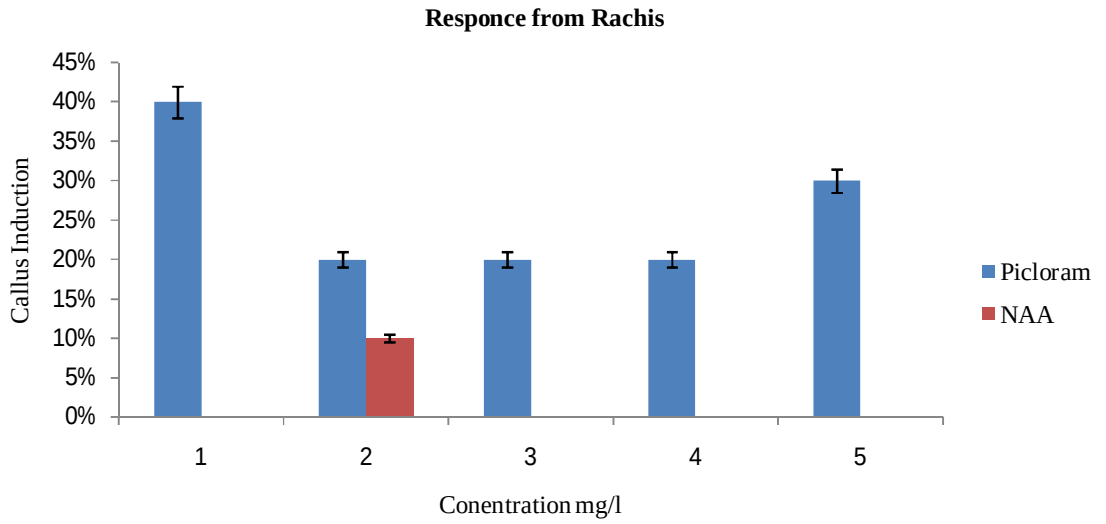


Figure4 (a) Response of rachis for callus induction in MS medium with Full strength of mineral salts + Auxin

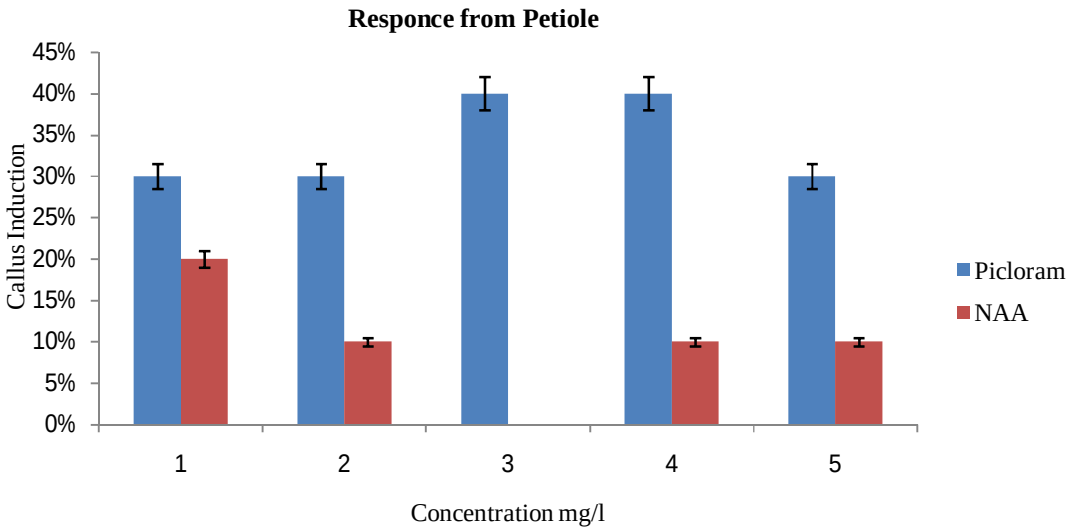


Figure 4 (b) Response of Petioles for callus inductions in MS medium with Full strength of mineral salts + Auxin

The browning could be preventedby replacing silver nitrate with ascorbic acid and citric acid as anti-oxidants..Internodes were inoculated in MS media containing 1.0 mg/l of Ascorbic acid and 1.5mg/l citric acid as antioxidant with BAP and NAA. Highest rate of callus induction was observed in 1mg/l of NAA (5.37 µM) and BAP (4.44 µM), followed by 51.85% in 3mg/l NAA (16.11µM) and 1mg/l BAP (4.44 µM), 48.14% in 3mg/l of NAA (16.11µM) and BAP (13.32µM) and 40.74 % in 1mg/l NAA (5.37 µM) and 3mg/l BAP (13.32µM). Callus induction from apical node reported to be 14.28% with 1mg/l NAA+ 1mg/l BAP , 9.52% in 3 mg/l NAA+ 1 mg/l BAP and 3.70% in 1 mg/l NAA+ 3 mg/l BAP[Figure6a & b]. Callus derived in this way remained green and viable till one month. It indicated ascorbic acid and citric acid play better role in preventing effect of secondary metabolites than silver nitrate. NAA and BAP induced shoot in *Zingiberofficinale*at 0.05 mg/l and 4 mg/l of concentration respectively. 2.0 mg/l BAP with 0.5mg/l NAA in MS medium induced shoot in *Zingier officinale*Rosc[14]. NAA (1.25 mg/l) with kinetin (1mg/l) optimum for callus biomass in *Eurycomalongifolia*[15]. Reports are contradictory with our results that instead of shoot induction callus was obtained with BAP and NAA in combination.

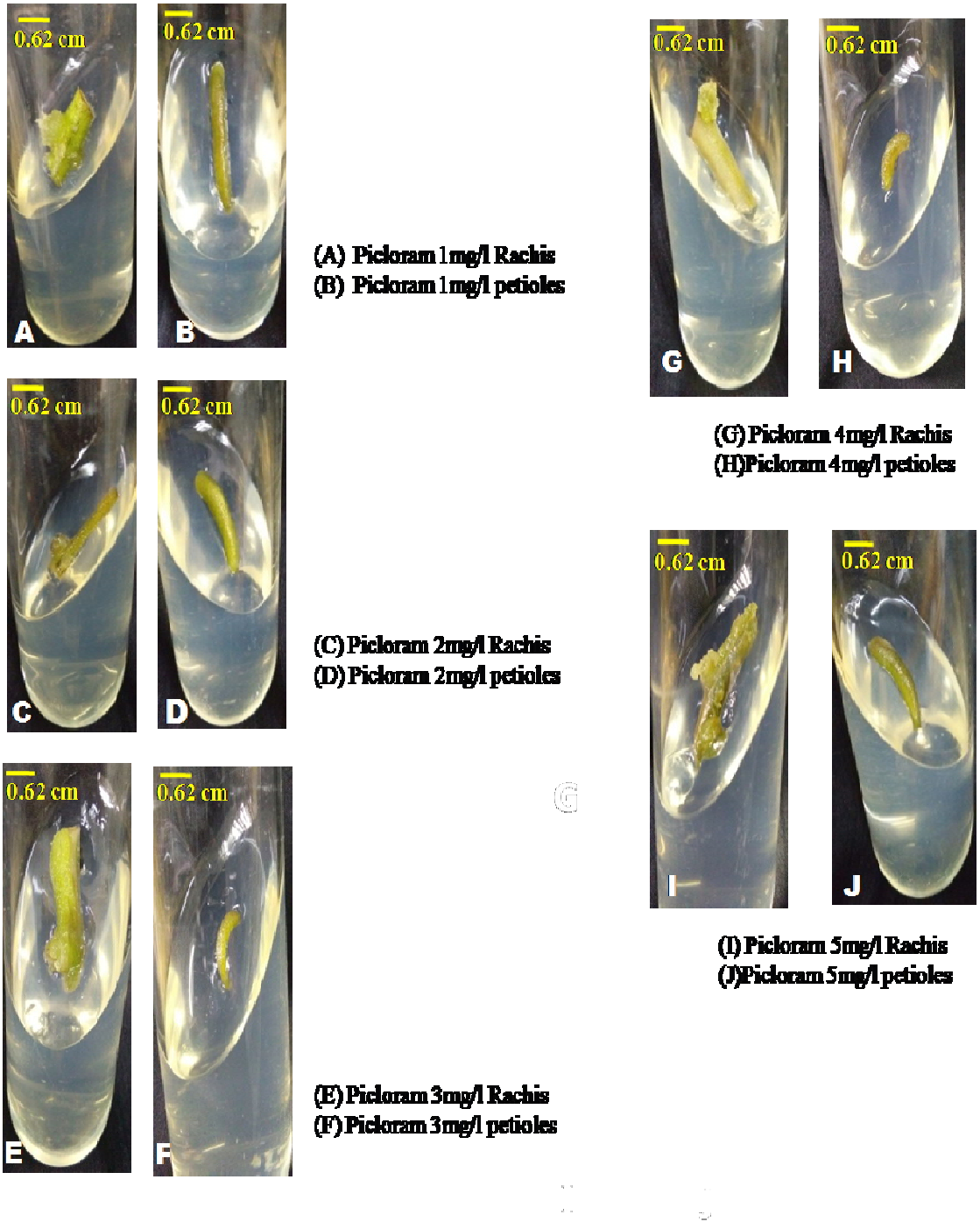


Figure 5: Callus induction from rachis and petioles in media supplemented with picloram on day 5.

Table 1(a) One Way ANOVA for various concentration of picloram to induced callus from rachis (p=0.05)

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Concentration	5	15	3	2.5		
picloram	5	1.3	0.26	0.008		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18.77	1	18.769	14.9673	0.00475	5.317655
Within Groups	10.03	8	1.254			
Total	28.801	9				

Table 1(b) One Way ANOVA for various concentration of NAA to induced callus from rachis (p=0.05)

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
NAA	5	0.1	0.02	0.002		
Concentration	5	15	3	2.5		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	22.201	1	22.201	17.7466	0.002945	5.317655
Within Groups	10.008	8	1.251			
Total	32.209	9				

Table 1(c) One Way ANOVA for various concentration of Picloram to induced callus from petioles (p=0.05)

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Concentration	5	15	3	2.5		
Picloram	5	1.7	0.34	0.003		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17.689	1	17.689	14.13424	0.005548	5.317655
Within Groups	10.012	8	1.2515			
Total	27.701	9				

Table 1(d) One Way ANOVA for various concentration of NAA to induced callus from petioles (p=0.05)

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
NAA	5	0.5	0.1	0.005		
Concentration	5	15	3	2.5		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	21.025	1	21.025	16.78643	0.003451	5.317655
Within Groups	10.02	8	1.2525			
Total	31.045	9				

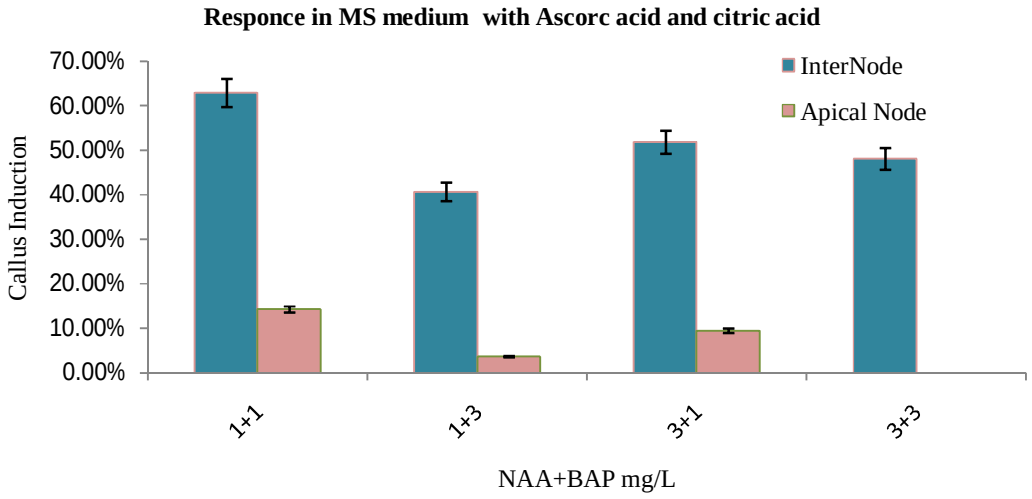
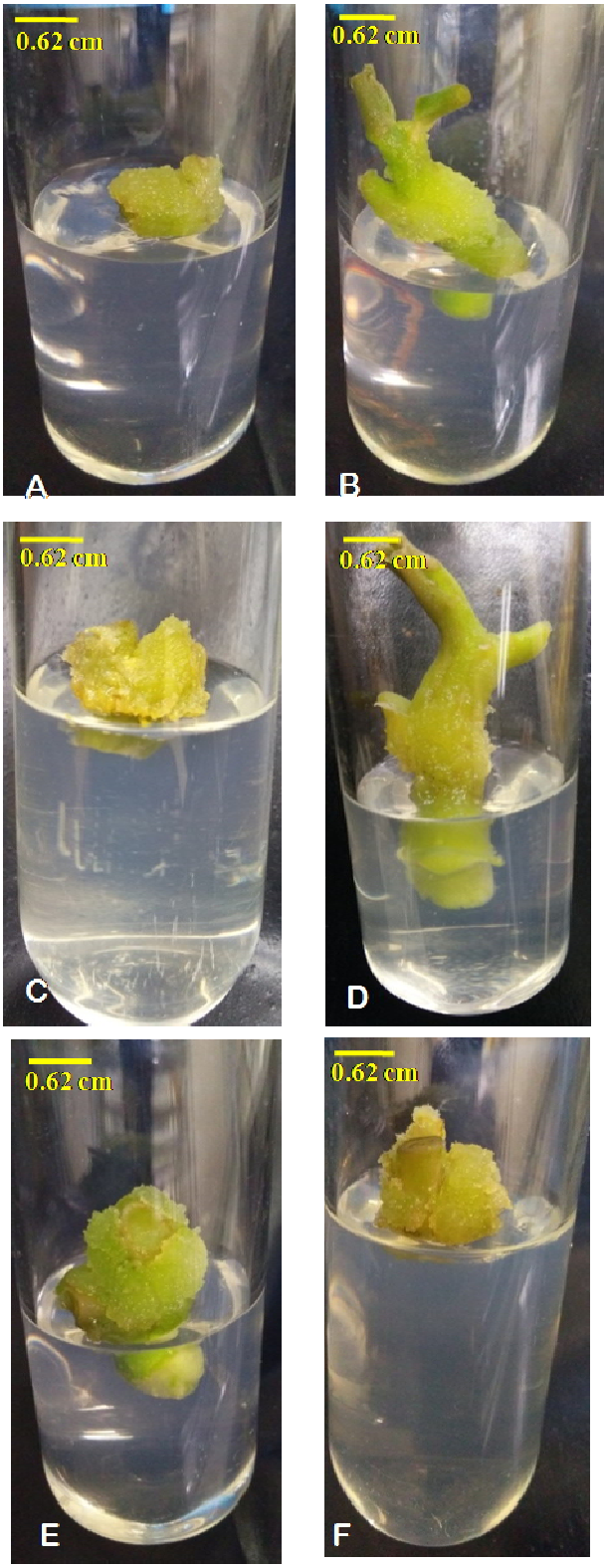


Figure 6 (a) Response of apical and internodes for callus induction in MS medium with Full strength of mineral salts + Growth Hormones



(A) BAP 1 mg/l + NAA 1mg/l Internode
(B) BAP 1 mg/l + NAA 1mg/l Apical Node

(C) BAP 1 mg/l + NAA 3mg/l Internode
(D) BAP 1 mg/l + NAA 3mg/l Apical Node

(E) BAP 3 mg/l + NAA 1mg/l Internode
(F) BAP 3 mg/l + NAA 3mg/l Internode

Figure 6 (b): Callus induction from internodes and/or apical nodes in MS media full strength of mineral salts supplemented with BAP and NAA on day 10.

CONCLUSION

MS medium found suitable medium for callus induction with leaf explants. Kinetin (80% response) was betterthanthidiazuron (40% response). Explants were prevented from browning by subculturing before 10 days. Internodes (explants) gave considerable callus induction with NAA (1mg/L) in combination with BAP (1mg/L) under MS medium supplemented with antioxidant (1mg/L Ascorbic acid and 1.5 mg/L of citric acid).

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