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Effects Of Iba And Ba On Shoots Nods Internodes And Callus Of Ficus Carica Explants In Vitro

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Abstract

The study was carried out at the Ministry of Science and Technology that investigated the effects of IBA and BA on Ficus carica explants in vitro, our Data showed significant effect of plant growth concentrations on No. of shoot but the most effective concentration was (0.8mg/l.-1 of IBA) which gave highest no shoot reached (1.32) also adding BA at (8 mg/l.-1or 4 mg/l.-1) produced the highest shoots 1.867, 1.72. In general, 0.8 mg/l-1 of IBA gave highest number of shoots 1.92 for both nodes and shoot tips and there is no significant differences between them. when adding BA at concentration of 8 mg/l-1 was superior to give highest number of shoots 2.96 initiated from nodes explants. The highest callus induction% reached 82.67 % and 74.67% for the combinations of (1.5 mg/l-1 IBA + 4 mg/l-1 BA) and (1.5 mg/l-1 IBA + 8 mg/l-1 BA) respectively. IBA maximum callus induction 49.60 % at 1.5 mg/l-1 and 51.73% for 8 mg/l-1 of BA. In related to the different explants responses internodes recorded 40.48 % as compare with 31.36% and 24.80% for nodes and shoot tips respectively. For the two interactions between IBA or BA with the explants Data showed that 1.5 IBA mg/l-1 gave influenced positively on callus induction from internodes explants 75.20% and 8 BA mg/l-1 affected with percentage of 59.20% and 55.20% on nodes and shoot tips respectively.interactions among IBA , BA with the explants Data showed that highest callus induction percentage 100% for the internodes explants in media MS containing combination of 1.5 mg/l-1 IBA + 4 mg/l-1 BA or 1.5 mg/l-1 IBA + 8 mg/l-1 BA and 88.0% for nodes explants in media MS containing combination IBA + 4 mg/l-1 BA.

Key words: Ficus carica , IBA , BA, explants, callus, in vitro.

1. Introduction

Tissue culture can be defined as a science consisting of a number of different ways of growing parts of the plant, tissues or cells on artificially whose contents can be made in the laboratory and grown under controlled conditions ,And severe sterilization where Florin Stănica pointed out that the sterilization area is the heart of the laboratory of tissue culture(stănica,1999) This science has occupied many scientists and researchers in the world and has been conducted many academic researchers which in turn resulted increasing of understanding how to distinguish, detect and form organ or parts of plants separated and built in an artificial media with further invention of many modern methods in this field. These academic researches could be directed to serve the practical aspects in the development of agricultural production and overcoming many of the problems facing the production of agricultural crops of economic importance. One of the important ways to develop agricultural reality is the production of artificial seeds, which depends mainly on tissue culture.(Poon Kok Siong, etc..2012) , and according to al wasil, 2000"Tissue culture is the production of plants of cells or plant tissues in the vials in the vitro , it is one of the methods used in Asexual propagation"

Figs have a history of growth and prize as a classical fruits used by ancient civilizations. Figs are are native to western Asia spreading to Mediterranean.

Currently about 427,000 ha plantations exist worldwide yielding more than one million MT of fig fruit (FAO, 2012) producing 1.070.676 million tones. The biggest producers are Turkey, Egypt, Iran Greece and Algeria (FAO STAT, 2006).

Its production can be either a sexually pollination or through vegetative propagation (parthenocarpic. Concerning to vegetative propagation, fig trees propagated by cutting, layering and grafting. Yet, from cutting ranges only 20-30% survive (Kumar et al., 1998) demanding many planting materials and sacrificing mother plants. In addition, the possibility of infected pathogens which actually affect the yield potential (Pasqual and Ferrira, 2007.; Günver and Ertan, 1998).

2. Materials and Methods

2.1. In Vitro establishment of culture

The place in which the study was carried out is The Ministry of Science and Technology/ Directorate of Agricultural Research /Genetic engineering laboratory/ Tissue culture Unit/ Baghdad, Iraq and university of agronomic science and veterinary medicine bucharest .

2.2. In Vitro Culture sterilization and initiation of Fig (Ficus carica) experiment

2.3. Plant material and Culture conditions

Local variety of fig (Aswed Dyala) were obtained from orchard in Iraq (AlSweera) location 60 km from Baghdad. Fig explants were washed using tap water for removing the dust then, healthy shoot tips (1cm), young leaves, nodal and internodes were isolated, exposed to ethanol at 70% for 1 min washed with distilled deionizer sterilized water (D. D Water) followed by different concentration (0, 1.0 and 5.0 %) of active chlorine from commercial NaOCl Clorox active chlorine (6%) for 10 min rinsed with (D. D) Water three times 5 min each. All explants were cultured under aseptic conditions inside laminar air flow cabinet (Fig 1) on MS media free hormones previously prepared (4. 4 $g.l^{-1}$ MS+ 30 $g.l^{-1}$ sucrose, pH media was set to 5.7 before solidifying with 7 $g.l^{-1}$ of agar, heated and autoclaving for 20 min at 1.5 kg/ cm2 pressure and 121 ºC(Stănica 1999)). Two weeks later contamination percentage was recorded.

Fig 1 : sterilization of explants inside laminar air flow cabinet

2.4. Initiation stage

Shoot tips, nodal and internodes were cultured in small vial each vial contained one explant (fig 2). (Murashige and Skoog,1962) MS media providing BA at concentration of (0, 1.0, 2.0, 4 and 8 mg/l^{-1}) combined with IBA at concentration (0,0.2, 0.4, 0.8 and 1.5 mg/l^{-1}) .Culture were kept in 16 h of dark condition for one week and for the next 3 weeks in 16 h light with 8 h dark conditions. 4 weeks later, callus induction% and number of shoots were recorded (Fig 3).

It has been observed that light leads to the growth of phenolic compounds in the tissue culture of plants. Most phenols are characterized by physiological activity that inhibits the vegetative growth of different plants, as a result of blocking the elongation of new cells, which leads to stopping the elongation of plant shoots and the lack of growth. These phenolic substances work in the production of AIA-oxidase, which destroys natural auxin (AIA) in the plant. The AIA contributes to the control of several stages of growth and differentiation (Schneider and Wightman, 1974; Galston and Dalberg, 1954).

Fig (2) : Fig explants from left to right internodes, shoot tips, node and young leaves

Fig 3: different Fig explants cultured on different combination of BA and IBA after 4 weeks of culture incubation

2.4.Culture maintenance

For culture maintenance, the media fortified with (4 $g.l^{-1}$) of activated charcoal to avoid the negative effect of phenolic compound which in turn affected on explants viability (Fig 4), with a notice that cultured media is MS supplemented with (0.8 mg/Lof IBA+8mg/Lof BA) as a best combination for growth according to the results obtained .

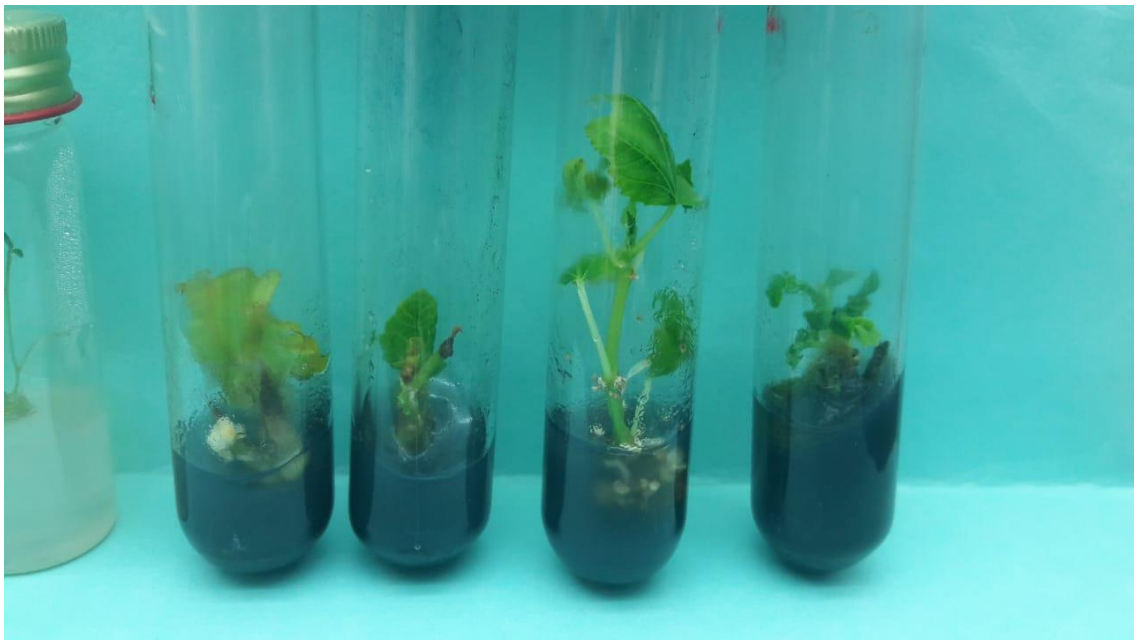

Fig 4 : Culture maintenance of Fig explants in 4 mg/l^{-1} of AC media

2.5. Data analysis

All in vitro experiment were designed according to completely randomized (C.R.D) with two interaction between fig explants (shoot tips, young leaves, nodal and internodes) and NaOCl or three interaction among , BA and fig explants , fife replicates for each treatment finally DATA were analyze used GenStat 12 ^{ed} Software .

3. Results and Discussion

3.1.Effect of different sterilization substances on contamination % of Fig explants

Based on data collected and subjected to analysis of variance at probability using GenStat 12^{ed} software data in table(1) referred that , using NaOCl at 5% gave less contamination percentage reached 14% as compared with 26%, 100% at 1% NaOCl and control treatment respectively . Concerning to explants response, highest contamination percentage reached 68.0% in leaves explants compare to lowest 37.3% in shoots explants with a notice that no significant differences were found between nods and internodes explants. For the interaction between different NaOCl concentrations and explants response, highest contamination percentage 100% for all fig explants in the absence of_NaOCl and presence of 70% ethanol as a constant treatment.

According to George (1993), the hypochlorite solutions (bleach) bactericidal action is caused by the the OCl- ion and hypochlorous acid (HOCl) and. The latter is more active improving the disinfecting chlorine significantly in slightly acid hypochlorite solutions. In earlier sterilization tests, contamination of explants were affected the explant material maturity stage and exposure time. Contamination in most cases occur in the first two weeks of in vitro culture (Mitchell et al 1995, 2003). Our research agreed

Table(1): Effect of NaOCl concentration on contamination % of Fig different explants

NaOCl %	Explants				
	Shoots	Leaves	Nodes	Internodes	Mean
0	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
1	8.0 d	68.0 b	12.0 d	16.0 d	26.00 b
5	4.0d	36.0 c	8.0 d	8.0 d	14.00 c
Mean	37.3 c	68.0 a	40.0 b	41.3 b	

Means are followed one letter show no significant differences within the single variant or there interactions at 5% level with Duncan's multiple range test

with the investigation that used of acidified bleach (pH 7) sterilization methods were examined on explant nodal and guinea hen weed leaf (Petiveria alliacia), and ackee (Bligha sapida). It produced the largest reduction in bacterial and fungal contamination (Webster et al.,2003).

3.2- Influence of IBA, BA and their interactions on number of shoot produced from different fig explants

Data in (Table 2) showed significant effect of plant growth concentrations on No. of shoot but the most effective concentration was (0.8mg/l^{-1} of IBA) which gave highest no shoot reached (1.32) also adding BA at (8 mg/l^{-1}or 4 mg/l^{-1}) producing the biggest shoot number 1.867 , 1.72. Furthermore, the interactions among (0.8 mg/l^{-1}IBA and 8 mg/l^{-1} BA) , (1.5 mg/l^{-1}IBA and 8 mg/l^{-1} BA), (0.4 mg/l^{-1}IBA and 8 mg/l^{-1} BA), (0.2 mg/l^{-1}IBA and 4 mg/l^{-1} BA), (0.8 mg/l^{-1}IBA and 4 mg/l^{-1} BA), (1.5 mg/l^{-1}IBA and 4 mg/l^{-1} BA) achieved highest number of shoots which significantly didn't differ among each other.

Table (2): Effect of BA, IBA and their interaction between on number of shoots	

IBA mg/l^{-1}	BA mg/l^{-1}					
	0	1	2	4	8	
0.0	0 j	0.333 hij	0.533 fgh	0.8 efg	1.467 cd	0.627 c
0.2	0 j	1.667 bc	1 e	2.067 ab	1.2 de	1.187 ab
0.4	0 j	0.933 ef	1.067 de	1.067 de	2.067 ab	1.027 b
0.8	0.067 ij	0.133 hij	1.733 bc	2.267 a	2.4 a	1.32 a
1.5	0.067 ij	0.467 ghi	0.2 hij	2.4 a	2.2 a	1.067 b
Mean	0.027 d	0.707 c	0.907 b	1.72 a	1.867 a	

Data in table (3) revealed various response among fig explants for number of shoots parameter. In general , 0.8 mg/l^{-1} of IBA gave highest number of shoots 1.92 for both nodes and shoot tips and there is no significant differences between them. Concerning to the effect of BA the response of different explants, data in table (4) revealed that adding BA at concentration of 8 mg/l^{-1} was superior to give highest number of shoots 2.96 initiated from nodes explants. Moreover, the response of all explants were varied, but in general nodes achieved highest number reached 1.568 which made these explants significantly superior as compared with internodes and shoot tips.

Table (3): Effect of interaction between on number of shoots initiated from Fig explants

IBA mg/l^{-1}	Explants			
	internodes	nodes	shoot tips	Mean
0.0	0.52 cd	1.36 b	0 e	0.627 c
0.2	0.8 c	1.52 b	1.24 b	1.187 ab
0.4	0.04 e	1.52 b	1.52 b	1.027 b
0.8	0.12 e	1.92 a	1.92 a	1.32 a
1.5	0.32 de	1.52 b	1.36 b	1.067 b

Table (4): Effect of interaction between BA on number of shoots initiated from Fig explants

BA mg/l^{-1}	internodes	nodes	shoot tips	Mean
0	0.04 h	0.04 h	0 h	0.027 d
1	0.56 g	0.92 ef	0.64 fg	0.707 c
2	0.32 g	1.4 d	1 e	0.907 b
4	0.44 g	2.52 b	2.2 c	1.72 a
8	0.44 g	2.96 a	2.2 c	1.867 a
Mean of Explants	0.36 c	1.568 a	1.208 b	

Table(5): Effect of three interaction among , BA, IBA and Fig Explants on number of shoots initiated

IBA mg/l^{-1}	BA mg/l^{-1}	Explants		
		Internodes	Nodes	Shoot tips
0.0	0	0 q	0 q	0 q
	1	0 q	1 hjiklmno	0 q
	2	0 q	1.6 gh	0 q
	4	1.2 hijkl	1.2 hijkl	0 q
	8	1.4 hi	3 abcd	0 q
0.2	0	0 q	0 q	0 q
	1	2.8 abcdef	1 hijklmn	1.2 hijklm
	2	0.6 ilmnopq	1.4 hij	1 hijklmn
	4	0.6 ijlmnopq	3 abcde	2.6 bdef
	8	0 q	2.2 dfg	1. 4 hi
0.4	0	0 q	0 q	0 q
	1	0 q	1.4 hijk	1.4 hi
	2	0 q	1.6 gh	1.6 gh
	4	0 q	1.6 gh	1.6 gh
	8	0.2 noq	3 abcde	3 abcde
0.8	0	0.2 nq	0 q	0 q
	1	0 q	0.2 nopq	0.2 nopq
	2	0.4 lnopq	2.4 def	2.4 def
	4	0 q	3.4 ab	3.4 ab
	8	0 q	3.6 a	3.6 a
1.5	0	0 q	0.2 nopq	0 q
	1	0 q	1 hijklmnop	0.4 lmnopq
	2	0.6 ijklmnopq	0 q	0 q
	4	0.4 lmnopq	3.4 abc	3.4 abc
	8	0.6 ijklmnopq	3 abcde	3 abcde

***Each number represent mean of 5 replicates**

**** Means next to the same letters show no significant differences in the single variant or there interactions at 5% level with Duncan's multiple range test**

The effect of three interactions among BA, IBA and Fig Explants on number of shoots in(table 5) revealed that most of media were affected positively on number of shoots initiated from different explants , for example highest response for internodes 2.8 was found at (0.2 mg/l^{-1} IBA + 1 mg/l^{-1} BA) while nodes gain positive response at (8 mg/l^{-1} BA) or (0.2 mg/l^{-1} IBA + 4 mg/l^{-1} BA) or (0.2 mg/l^{-1} IBA + 1 mg/l^{-1} BA) or (0.4 mg/l^{-1} IBA + 8 mg/l^{-1} BA) or (0.8 mg/l^{-1} IBA + 4 mg/l^{-1} BA) or (0.8 mg/l^{-1} IBA + 8 mg/l^{-1} BA) and (1.5 mg/l^{-1} IBA + 4 mg/l^{-1} BA) . Similarly , shoot tips gave similar response in media containing (0.4 mg/l^{-1} IBA + 8 mg/l^{-1} BA) or (0.8 mg/l^{-1} IBA + 4 mg/l^{-1} BA) or (0.8 mg/l^{-1} IBA + 8 mg/l^{-1} BA) and (1.5 mg/l^{-1} IBA + 4 mg/l^{-1} BA)

Our results confirmed that the testified improved regeneration on 1.0 × MS medium with NAA, IAA, and IBA plus GA3 variants on all cultivars. The shortest day period for shooting the induction on three cultivars was 4.25–5 d on 1.0 × MS medium with 0.25 mg L−1 GA3 + 1 mg L−1 NAA. Also, the longest shoots was (11.8) cm while the maximum number of nodes was 13.50. Also, the biggest number of leaves (11.00) was reported (Kumlay, 2014).

Monney et al (2016) states that shoot induction is a semi hard wood nodal segment, maintained on MS (Murashige and Skoog) different auxin and cytokinin combinations and nutrient medium. In the treatments, d 6-benzyladenine (BA) at 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 mg/L combined with 0.1 mg/L Indole 3-butyric acid (IBA) or Naphthaleneacetic acid (NAA) were used. The control subjects were hormone free MS medium. BA combined with IBA combinations proved more efficiency in the regeneration of shoot than the BA combined with NAA. Cultures on MS medium with 3 mg/L BA and 0.1 mg/L IBA produced the biggest shoot induction (100%). The same is true with the mean number of nodes per explant and mean shoot length which were (2.5) and (1.28 cm) respectively.

Benzyladenine (BA) combined with IBA is effective for shoot multiplication in for so many species of the Asclepiadaceae family (Martin, 2003). The nodes created within a period estimates the multiplication rate while shoot height is the subculture feasibility. Yet, 1.28 (cm) is the biggest mean shoot length in cultures on MS medium with 3.0 mg/L BA (in combination with 0.1 mg/L IBA). The plant growth regulator efficiency depends on its concentration (Demeke et al., 2014).

High shoot length of the control subjects indicate endogenous auxins inducing possible shoot elongation [18]. In addition, there was node significant increase by BA, which improve the capability of enhancing axillary buds in Cryptolepis sanguinolenta (Monney et al., 2016).

The IBA and BA for shooting and rooting efficiency appeared in plantlet regeneration in C. kanehirae (Chang et al., 2002). The same happened with somatic embryogenesis in Quercus semecarpifolia (Tamta et al., 2009) and BA mixed with IBA for many inductions of shoots in Arnebia euchroma (Manjkhola et al., 2005).

In different research works, the shoots multiply with a common thyme depending on the treatment used. The highest adventive shoot number on the MS media was provided with 2 or 4 mg/L BA and 0.1 mg/L IBA, employing the highest cytokinin concentrations (Karalija and Paric, 2011).

A higher auxins concentration produces more nodes (7.3) (Zaman et al., 2001). (Ghaffoor et al., 2003) (9.7) on MS medium with IBA.

3.3- Effect of IBA, BA and their interactions on callus induction different fig explants.

Data in table (6) clarified that highest callus induction% reached 82.67 % and 74.67% for the combination of (1.5 mg/l^{-1} IBA + 4 mg/l^{-1} BA) or (1.5 mg/l^{-1} IBA + 8 mg/l^{-1} BA) respectively , also plant hormones affected positively on callus induction parameter according to table (7 and 8) IBA maximum callus induction 49.60 % at 1.5 mg/l^{-1} and 51.73% for 8 mg/l^{-1} of BA . In related to the different explants responses internodes recorded 40.48 % as compare with 31.36% and 24.80% for nodes and shoot tips respectively.

For the two interactions between IBA or BA with the explants Data in table (7 and 8) showed that 1.5 IBA mg/l^{-1} gave influenced positively on callus induction from internodes explants 75.20% and 8 BA mg/l^{-1} affected with percentage of 59.20% and 55.20% on nodes and shoot tips respectively.

For the three interactions among IBA , BA with the explants Data showed that highest callus induction percentage 100% for the internodes explants in media MS containing combination of 1.5 mg/l^{-1} IBA + 4 mg/l^{-1} BA or 1.5 mg/l^{-1} IBA + 8 mg/l^{-1} BA and 88.0% for nodes explants in media MS containing combination IBA + 4 mg/l^{-1} BA while

Table (6): Interaction influence between BA and IBA on callus initiation %

IBA mg/l^{-1}	BA mg/l^{-1}				
	0	1	2	4	8
0.0	0.00 n	2.67 mn	4.00 lmn	14.67 jkl	26.67 ghi
0.2	12.00 klm	41.33 cde	29.33 fgh	42.67 cde	50.67 bc
0.4	16.0 ijk	28.00 gh	28.00 gh	25.33 ghij	40.00 cdef
0.8	25.33 ghij	36.00 defg	32.0 efgh	44.00 cd	58.67 b
1.5	29.33 fgh	21.33 hijk	40.00 cdef	74.67 a	82.67 a

Table (7): Effect of interaction between , IBA on callus percentage initiated from Fig explants

IBA mg/l^{-1}	explants			
	internodes	nodes	shoot tips	Mean
0.0	4.00 h	7.20 h	17.60 g	9.60 d
0.2	30.40 ef	44.00bc	31.20 ef	35.20 b
0.4	40.80 c	24.00 fg	17.60 g	27.47 c
0.8	52.00 b	34.40 de	31.20 ef	39.20 b
1.5	75.20 a	47.20 bc	26.40 ef	49.60 a

Table (8): Effect of interaction between , BA on callus percentage initiated from Fig

explants				
BA mg/l^{-1}	internodes	nodes	shoot tips	Mean
0	28.80 d	19.20 e	1.60 f	16.53 d
1	46.40 b	16.80 e	14.40 e	25.87 c
2	42.40 bc	19.20 e	18.40 e	26.67 c
4	44.00 b	42.40 bc	34.40 cd	40.27 b
8	40.80 bc	59.20 a	55.20 a	51.73 a
Mean of Explants	40.48 a	31.36 b	24.80 c	

Table (9): Effect of three interaction among , BA and Fig Explants on callus percentage initiated

IBA	BA	Explants		
		Internodes	Nodes	Shoot tips
0.0	0	0.00 m	0.00 m	0.00 m
	1	0.00 m	0.00 m	8.00 klm
	2	0.00 m	0.00 m	12.00 klm
	4	8.00 klm	8.00 klm	28.00 hijk
	8	12.00 klm	28.00 hijk	40.00 fghij
0.2	0	8.00 klm	24.00 ijkl	4.00 lm
	1	56.00 defg	44.00 efghi	24.00 ijkl
	2	24.00 ijkl	40.00 fghij	24.00 ijkl
	4	40.00 fghij	48.00 defgh	40.00 fghij
	8	24.00 ijkl	64.00 cde	64.00 cde
0.4	0	36.00 ghij	12.00 klm	0.00 m
	1	68.00 cd	8.00 klm	8.00 klm
	2	60.00 cdef	12.00 klm	12.00 klm
	4	20.00 jklm	28.00 hijk	28.00 hijk
	8	20.00 jklm	60.00 cdef	40.00 fghij
0.8	0	52.00 defg	20.00 jklm	4.00 lm
	1	60.00 cdef	24.00 ijkl	24.00 ijkl
	2	48.00 defgh	24.00 ijkl	24.00 ijkl
	4	52.00 defg	40.00 fghij	40.00 fghij
	8	48.00 defgh	64.00 cde	64.00 cde
1.5	0	48.00 defgh	40.00 fghij	0.00 m
	1	48.00 defgh	8.00 klm	8.00 klm
	2	80.00 bc	20.00 jklm	20.00 jklm
	4	100.00 a	88.00 ab	36.00 ghij

	8	100.00a	80.00 bc	68.00 cd

***Each number represent mean of 5 replicates**

**** Means with similar letters show no significant differences in a single variant or there interactions at 5% level with Duncan's multiple range test**

In many studies, cytokinins and auxins have an inducing role of callus formation in several plants (Darion et al., 2010; Hesar et al., 2011). They are utlised for the promotion of the callus formation in several excited and in vitro cultured explants or their body parts (Ibrahim et al., 2013) .

Data from Sharma and Nautiyal (2009) confirmed the growth regulatory requirements for callus induction variation based on the explant origion. This could be a results from the variation in morphology and biochemistry of various explant sorts, affecting cell cytokinin uptake and competence initiating callus or shoot reporting a competent and easy-to-handle protocol for organogenesis by the Cinnamomum tamala callus. Petiole which have nodal segments can be regarded the explant species source and excellent callus in Indole butyric acid (5µM) and WPM medium with Benzyladenine (2.5µM). BA combined with IBA suited the callus induction, several rooting and shooting every class of explants, different from other treatments In C. tamala.

Salim, (2016) investigated the influence of BA, 2,4-D, and IBA on white jasmine shooting and callus propagation (Jasminum azoricum L.)in vitro. Highest callus induction percent (100%) appeared in MS with 4.0 mg/L BA + 0.1 mg/L 2,4-D and 6.0 mg/L BA + 0.1 mg/L 2,4-D.

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