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THE EFFECTS OF (Z)-7-METHOXY-1, 5-DIHYDROBENZO [C] OXEPINE ON *IN VITRO* CULTURE OF *CURCUMA LONGA* L

ARGHYA GHOSH

U.G. & P.G. Department of Botany
Darjeeling Government College, Darjeeling
Email: agbotphd@rediffmail.com
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The plant *Curcuma longa* L. is a pharmaceutically important one and almost all the parts of the plant especially rhizomes were reported to contain different types of terpenoid. Rhizomatous explants from three months old buds were cultured on Murashige and Skoog's (MS) medium supplemented with different concentrations of cytokinins and auxins. When the culture was performed in MS medium supplemented with 3 mg/L of 6-benzylamino purine, it yields 7.12 ± 0.34^{bc} numbers of shoots with an average shoot length of 8.32 ± 0.47^{abd} cm. The elongated shoots produced 6.12 ± 0.73^{def} roots with an average length of 4.93 ± 0.30^{abc} on MS medium supplemented with 2.5 mg/L of Indole 3 butyric acid (IBA) and showed 82% survivability after hardening. When the culture on 3 mg/L of 6-benzylamino purine was supplemented with 12 mg/L of the (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine isolated from *Curcuma caesia* Roxb., multiple numbers of shoots having an average shoot length of 3.29 ± 0.17^{e} cm were produced. The elongated shoots produced roots with an average length of 7.91 ± 0.93^{ijk} on MS medium supplemented with 2.5 mg/L of Indole 3 butyric acid (IBA) and 14 mg/L of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine. Hence, it can be concluded that although this terpenoid is a shoot growth retardant yet acts as elicitor for rooting.

Keywords: *Curcuma longa* L., *Curcuma caesia* Roxb., (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine (MDHBO), Plant growth regulator, 6-Benzylamino purine, Indole 3 butyric acid.

In plant tissue culture elicitation is a process of induction of morphogenesis by applying some plant growth regulators or some active principles to the medium. Nowadays application of elicitors during tissue culture is currently the focus of research. This method has been considered as one of the most effective protocols to improve the biogenesis of different organs as well as some metabolites in medicinal plants. Plant metabolites can be used as unique sources for pharmaceuticals, food additives, flavours, industrial ingredients etc. Commonly used phyto-metabolic elicitors are salicylate, methyl salicylate, benzoic acid, chitosan and so forth (Patel *et al*. 2013). In this research work terpenoids are taken as elicitor molecules for smooth and rapid organogensis of the rhizomatous bud taken from the plant *Curcuma longa* L. There are various kinds of synthetic plant growth regulators having morphogenetic effect but they also possess some abnormalities to the dividing cells which leads to production of non *true to type* plants during *in vitro* culture. There are several reports of uses of conjugated terpenoid ketones as a new group of plant growth regulators which can be used solely or in combination with some synthetic plant growth regulators (Kalsi *et al*. 1978). (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine (MDHBO) is the novel terpenoid (isolated from *Curcuma caesia* Roxb.) extracted, purified and physiochemically characterised using UV spectroscopy, FT- IR spectroscopy, Mass spectroscopy and different NMR spectroscopy (Ghosh *et al*. 2013, Figure 1). This is a diterpenoid derivative having molecular formula of $C_{12}H_{12}O_2$ and reported for first time in *Curcuma caesia* Roxb. Presence of this diterpenoid derivative is also not reported till date, from other plant species also. This novel terpenoid, (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine also have the morphogenetic effect like synthetic plant growth regulators during *in vitro* culture. In addition to that (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine also have some antibacterial and antifungal potentialities as per earlier reports (Ghosh *et al*. 2013a). There are several reports on activities

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of terpenoids as plant growth regulators. In mung bean, *Cyperus scariousus, Phaseoulus aureus* etc. it is experimentally proved that morphogenesis of this plants is triggered by application of some terpenoids in *in vitro* condition (Kalsi *et al*. 1978). As a model plant of Zingiberaceae, *Curcuma longa* L. is chosen as host to screen the effect of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine as plant growth regulators. As (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine is a natural product, one can expect that application of this metabolite as growth regulator might not affect the genetic fidelity of *in vitro* raised plants and enhances the probability of producing *true to type* plantlets. Keeping all these notes in mind, sole and combinatorial effects of synthetic plant growth regulator as well as extracted terpenoid of *Curcuma caesia* Roxb. were studied. In this experiment two sets were prepared first. Set one containing only the synthetic plant growth regulators and set two containing only the said terpenoid. In set one, there are some positive effect regarding organogenesis and in set two the effect if negative. Most interesting result found when both the chemicals were used simultaneously. During the studying of combinatorial effects, presence of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine elicits the *in vitro* regeneration process over the solely used synthetic plant growth regulators containing experimental set (i.e. set one). From this result it can be concluded that (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine may have some synergistic effect than the synthetic plant growth regulators used here in.

MATERIALS AND METHODS

Collection of Plant Material and MDHBO

Collection of Plant Material : The Rhizomatous buds of *Curcuma longa* L. was collected in the month of August 2019 from experimental garden of Department of Botany, Darjeeling Govt College, which is located at 27.0533^0 N latitude, 88.2601^0 E longitude with an average altitude of 2134 metres above mean sea level. The plant was identified in the Taxonomy and Ecology laboratory of department of Botany, Darjeeling Government College, Darjeeling.

Collection of MDHBO: 2.5 kg shade dried rhizomes of black turmeric plant was powdered of approximately and extracted three times with 1 liter of 95% EtOH at room temperature to give an extract of 479 gms. The extract was evaporated under reduced pressure and a solid residual mass was obtained. The above obtained residual sample was subjected to repeated preparative thin layer chromatography using different solvent systems, e.g solvent system 1. Methanol (5%): benzene (95%) and solvent system 2. Chloroform (60%): benzene (30%): acetic acid (10%). Three homogeneous spots were collected in solvent system 2, having Rf values of 0.87, 0.79 and 0.75 respectively. The sample with Rf value 0.75 was taken up for further study. This sample was positive in Liebermann's Burchard test and gave purple colour indicating its terpenoid nature. The compound (MDHBO) had melting point of 57^0C. The sample was then further analysed through various spectroscopic techniques like UV spectroscopy (UV- 1601PC, UV-Visible Spectrophotometer, Shimadzu), FT-IR spectroscopy (Perkin Elmer Spectrum- 1 Spectrophotometer), High Resolution Mass spectroscopy (JEOL- JMS 600 Instrument) and Nuclear Magnetic Resonance spectroscopy, ^{1}H & ^{13}C (Bruker Avance-400 Spectrometer) for its proper physicochemical characterization.

Explant Preparation: Young disease free rhizomatous buds of two to three cm were collected from three months old plant. Explants were washed thoroughly under running tap water and, treated with 5% (m/v) Teepol (Qualigen, Mumbai, India) for 20 min, followed by rinsing three to five times in sterile double distilled water. Another round of dis-infestation was done with 70% alcohol for 5

min followed by immersion in 0.1% (m/v) aqueous mercuric chloride ($HgCl_2$) solution for 5 - 6 min and finally rinsed with sterile double distilled water (five to six times) in a flow chamber. The surface sterilized explants were trimmed at cut ends and about one to two cm prior to inoculation on culture media (Gopal *et al*. 1998, Ghosh *et al*. 2013b).

Media and Culture Conditions: Surface sterilized rhizomatous segments (1 - 2 cm) were cultured on MS basal medium (Murashige *et al*. 1962) containing 3% (w/v) sucrose (Himedia, Mumbai, India) for culture initiation and served as explant sources for subsequent experiments. The pH of the medium was adjusted to 5.8 before gelling with 0.8% (w/v) agar (Himedia, Mumbai, India). The explants initially were implanted vertically on the culture medium in test tube (150 × 25 nm) and plugged tightly with non absorbent cotton. All the cultures were kept under cool fluorescent light (16 h photo period 40 µmol· m–2 s–1, Philips, India at 25°C ± 2°C) and 60% - 70 % relative humidity (RH) (Ghosh *et al*. 2013b).

Shooting: For initial multiple shoot induction, the explants were cultured on MS medium (Murashige *et al*., 1962) supplemented with various concentrations of BA (0.5 - 5.0 mg/L) solely and also in combination with (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine (2 - 20 mg/L). The induced shoots were allowed to grow for 15 days (Ghosh *et al*. 2013b).

Rooting: Small micro shoots grown on subculture medium were transferred to full strength MS media (Murashige *et al*., 1962), supplemented with various concentrations of IBA (0.5 – 5 mg/L) solely and also in combination with (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine (2 - 20 mg/L) for root development. IBA was filter sterilized and added to the medium after autoclaving under the sterilized environment of laminar air flow cabinet (Ghosh *et al*. 2013b).

Hardening: The completely rooted plantlets with 5 - 7 fully expanded leaves were removed from the culture medium and the roots were washed gently under running tap water to remove agar. The plantlets were transferred to plastic pots (5 cm diameter) containing a mixture of sterilized garden soil and vermiculite in the ratio of 2:1 and covered with transparent plastic bags to ensure high humidity. Each was irrigated with 1/6 MS basal salt solution devoid of sucrose and inositol every 4 days for 2 weeks. The growth chamber was maintained at 26°C ± 1°C, 80% - 85% relative humidity with light intensity of 50 µmol· m–2· s–1 on a 16 h photoperiod inside the culture room conditions. The relative humidity was reduced gradually and after 30 days the plantlets were transferred to pots (25 cm diameter) containing garden soil and kept under green house for another 2 weeks. There are no changes in respect to morphology, growth characteristics and floral features etc in between tissue culture regenerated plants and naturally grown field plants (Ghosh *et al*. 2013b).

Statistical Analysis: Experiments were set up in completely randomized block design. Each experiment was repeated three times with 10 - 12 replicates. Data were analyzed by one way analysis of variance (ANOVA) and the difference between means were scored using Duncan's Multiple Range Test P = 0.05 (Duncan D.B., 1955) on the statistical package of SPSS (Version 10).

RESULTS AND DISCUSSION

Establishment of Explants: To overcome contamination problem, surface sterilization of explants was done with 0.1% aqueous solution of Mercuric chloride ($HgCl_2$) for 1, 2, 3, 4 and 5 minutes. Mercuric chloride ($HgCl_2$) is a very strong sterilant (Gopal *et al*., 1998). When the explants sterilization was done with 0.1% aqueous solution of $HgCl_2$ for 3 minutes, 83 % of the explants get survived even after nine days of inoculation. Whereas, exposure of

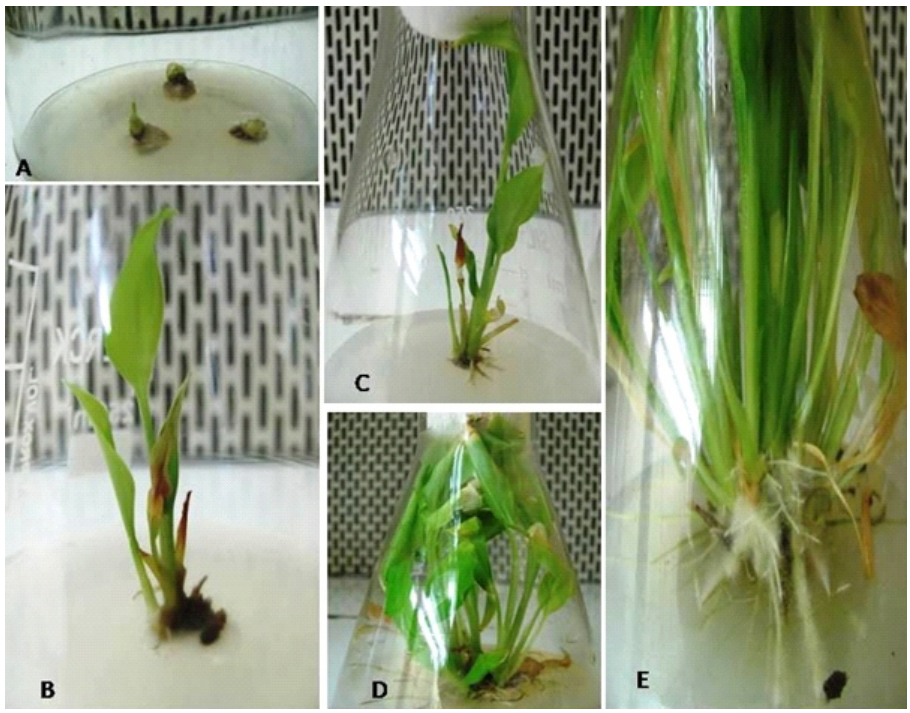

Plate 1 (A-E): A- Establishment of explants and induction of shoot on MS medium supplemented with PGR. **B-** Shoot development in the MS medium without MDHBO supplementation. **C-** Root induction in the MS medium with MDHBO supplementation. **D-** Less root and more shoot development in the MS medium without MDHBO supplementation**. E-** Faster maturation of roots in the MS medium with MDHBO supplementation.

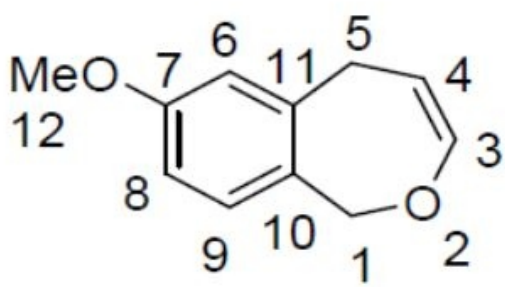

Figure 1- Structure of MDHBO

0.1% aqueous solution of $HgCl_2$ above and below 3 minutes prove to allow death or contamination of explant respectively (Table 1,Plate 1A) (Ghosh *et al*. 2013b).

Shoot Development: The Explants which were cultured on basal MS medium (Murashige *et al*. 1962) failed to produce shoots even after 4 weeks of inoculation. MS medium supplemented with different concentrations of cytokinins (BA) and (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine showed variation in the regeneration percentage and number of shoots formed. To establish the effect of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine as elicitor molecule two experimental sets were performed. In one experiment the MS medium (Murashige *et al*., 1962) is only supplemented with 0.5 -5 mg/L of BA and in another BA for 0.5 -5 mg/L as well as 2-20 mg/L of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine. Among the different concentrations of cytokinin tested, the best response was obtained in the presence of 3 mg/L BA (Ahuja *et al*. 1982, Sen *et al*. 1991, Vincent *et al*. 1992, Sahoo *et al*. 1997, Guru *et al*. 1999, Begum *et al*. 2002; Hiregoudar *et al*. 2006, Saha *et al*. 2010). When that 3 mg/L of BA is supplemented with different concentrations of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine, the effect of terpenoid looks like a shoot retardant. The average length of shoot in the medium supplemented with 3 mg/L of BA was

Table 1: Standardization of 0.1% $HgCl_2$ for surface sterilization

Sl No.	Treatment duration in minute with 0.1% $HgCl_2$	No. of explants inoculated	Rate of contamination after following day(s) of treatment						Percentage of contamination free living explants after 9 days
			1	2	3	5	7	9	
1	1	6	5	6	6	6	6	6	0
2	2	6	4	4	3	3	2	2	33
3	**3**	**6**	**0**	**0**	**1**	**1**	**1**	**1**	**83 (Taken for further programme)**
4	4	6	0	0	0	0	0	0	100 (Turns black and died)
5	5	6	0	0	0	0	0	0	100 (Turns black and died)

Table 2: Results of shoot and root growth on MS medium supplemented with different concentrations as well as combinations of said plant growth regulators and MDHBO

Shooting MS medium supplemented with different concentrations of BA (mg/L)	Number per explants	Average length in centimetre
MS basal medium (Control)	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}
0.5	7.53 ± 0.36^{cd}	6.15 ± 0.45^{de}
01	7.26 ± 0.61^{b}	5.73 ± 0.89^{d}
1.5	7.89 ± 0.50^{a}	5.29 ± 0.73^{bcd}
02	6.37 ± 0.23^{abc}	4.31 ± 0.41^{e}
2.5	6.23 ± 0.79^{b}	4.75 ± 0.39^{a}
03	$\mathbf{7.12\pm0.34^{bc}}$	$\mathbf{8.32\pm0.47^{abd}}$
3.5	5.89 ± 0.12^{a}	5.17 ± 0.91^{b}
04	6.75 ± 0.95^{b}	4.89 ± 0.65^{e}
4.5	5.32 ± 0.72^{de}	4.12 ± 0.23^{de}
05	4.39 ± 0.68^{e}	3.95 ± 0.33^{a}
MS medium supplemented with different concentrations of BA (mg/L) & MDHBO (mg/L)		
03 (mg/L) & MDHBO 2 (mg/L)	7.31 ± 0.56^{b}	6.10 ± 0.79^{ac}
03 (mg/L) & MDHBO 4 (mg/L)	6.75 ± 0.43^{abc}	5.21 ± 0.31^{cd}
03 (mg/L) & MDHBO 6 (mg/L)	6.12 ± 0.89^{cd}	4.29 ± 0.93^{d}
03 (mg/L) & MDHBO 8 (mg/L)	5.97 ± 0.67^{e}	4.12 ± 0.38^{bc}
03 (mg/L) & MDHBO 10 (mg/L)	5.12 ± 0.93^{de}	3.75 ± 0.29^{e}
03 (mg/L) & MDHBO 12 (mg/L)	$\mathbf{4.39\pm0.32^{a}}$	$\mathbf{3.29\pm0.17^{e}}$
03 (mg/L) & MDHBO 14 (mg/L)	4.12 ± 0.95^{cd}	3.03 ± 0.79^{abd}
03 (mg/L) & MDHBO 16 (mg/L)	3.75 ± 0.69^{b}	2.72 ± 0.47^{cd}
03 (mg/L) & MDHBO 18 (mg/L)	3.41 ± 0.37^{ab}	2.59 ± 0.62^{cd}
03 (mg/L) & MDHBO 20 (mg/L)	3.29 ± 0.59^{e}	2.12 ± 0.38^{d}
Rooting **MS medium supplemented with different concentrations of IBA (mg/L)**		
MS basal medium (Control)	0.00 ± 0.00^{i}	0.00 ± 0.00^{j}
0.5	2.34 ± 0.42^{f}	5.18 ± 0.59^{j}
01	2.75 ± 0.79^{i}	4.70 ± 0.37^{df}
1.5	4.89 ± 0.63^{abc}	5.41 ± 0.18^{ef}
02	4.92 ± 0.80^{ef}	5.11 ± 0.74^{cd}
2.5	$\mathbf{6.12\pm0.73^{def}}$	$\mathbf{4.93\pm0.30^{abc}}$
03	5.83 ± 0.40^{ghi}	4.79 ± 0.81^{ijk}
3.5	4.77 ± 0.29^{abc}	4.12 ± 0.69^{ad}
04	3.89 ± 0.28^{bc}	3.15 ± 0.35^{cd}
4.5	3.72 ± 0.93^{de}	3.20 ± 0.82^{abcd}
05	4.15 ± 0.59^{fg}	3.89 ± 0.60^{bd}
MS medium supplemented with different concentrations of IBA (mg/L) & MDHBO (mg/L)		
2.5 (mg/L) & MDHBO 2 (mg/L)	2.75 ± 0.47^{gh}	5.23 ± 0.69^{ij}
2.5 (mg/L) & MDHBO 4 (mg/L)	2.89 ± 0.28^{jk}	4.92 ± 0.57^{bc}
2.5 (mg/L) & MDHBO 6 (mg/L)	4.90 ± 0.18^{ef}	5.76 ± 0.84^{gh}
2.5 (mg/L) & MDHBO 8 (mg/L)	4.98 ± 0.79^{cd}	5.95 ± 0.37^{h}
2.5 (mg/L) & MDHBO 10 (mg/L)	6.73 ± 0.36^{ef}	6.89 ± 0.29^{def}
2.5 (mg/L) & MDHBO 12 (mg/L)	6.89 ± 0.58^{ghi}	7.03 ± 0.80^{cd}
2.5 (mg/L) & MDHBO 14 (mg/L)	$\mathbf{7.21\pm0.82^{ij}}$	$\mathbf{7.91\pm0.93^{ijk}}$
2.5 (mg/L) & MDHBO 16 (mg/L)	6.95 ± 0.80^{gh}	7.29 ± 0.25^{abc}
2.5 (mg/L) & MDHBO 18 (mg/L)	6.83 ± 0.69^{de}	6.83 ± 0.49^{gh}
2.5 (mg/L) & MDHBO 20 (mg/L)	6.02 ± 0.72^{b}	6.55 ± 0.17^{a}

**Values are means ± SE. n = 10 - 12 (in triplicate); Means followed by same does not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$).

8.32 ± 0.47^{abd}. The average length of shoot in the medium supplemented with 3 mg/L of BA and 12 mg/L of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine was 3.29 ± 0.17^{c}. However, increasing or decreasing concentration of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine exhibits no such positive effect on shoot development. This is probably due to higher concentration of nitrogen and potassium. The stimulating activity of BA on multiple shoot formation has been reported earlier for several medicinal plants (Table 2, Plate 1) (Ghosh *et al*. 2013b).

Root Development: In the preliminary experiments conducted, no rooting was observed when the shoots were culture on basal (Control) MS medium. For root development also two sets of culture were prepared. In one set healthy shoots were excised and placed MS basal medium (Murashige *et al*., 1962) supplemented with different concentrations of auxin (IBA) at the range of 0.5 - 5.0 mg /l (Chawdhury *et al*., 1993; Gopal *et al*., 1998; Andrade *et al*., 1999; Juliani *et al*., 1999; Ghosh *et al*., 2013b). In another set the shoots were placed in MS medium supplemented with auxin (IBA) at the range of 0.5 - 5.0 mg /l and (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine at a concentration of 2 – 20 mg/L. Best response for rooting were recorded at 2.5 mg/L of IBA in first set. Where as in another set where the MS medium is supplemented with 2.5 mg/L of IBA and 14 mg/L of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine the response regarding rooting becomes more positive over the first experimental set. After 25 days of repeated subculture the medium containing only 2.5 mg/L of IBA gives rise to 6.12 ± 0.73^{def} number of roots with an average length of 4.93 ± 0.30^{abc} centimetre. In another set when that same medium was supplemented with different concentrations of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine, at 2.5 mg/L of IBA and 14 mg/L of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine, response was more positive. In 2.5 mg/L of IBA and 14 mg/L of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine the average response was 7.21 ± 0.82^{ij} with an average length of 7.91 ± 0.93^{ijk} centimetre (Table 2, Figure C, D, E). When MS medium was supplemented with only (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine (@ concentrations of 2 – 20 mg/ L), no such effect was observed except initiation of formation of hairy root structure in a very less number.

Hardening : Matured rooted plantlets were taken out gently from the test tubes and thoroughly washed with sterile water to remove adhered agar and traces of the medium to avoid contamination. The *in vitro* generated plantlets were transferred to plastic pots containing potting a mixture of (2:1) soil and vermiculite in green house. Finally the acclimated plants were, shifted to the field conditions showing 82% of survival rate. The growth characteristics of *in vitro* raised plants were identical morphologically with naturally occurring field plants (Ghosh *et al*., 2013b).

Unsaturated terpenoid ketones have a root inducing property on hypocotyls cuttings of *Phaseoulus aureus*. Significantly isopatchoulenone is distinctly more active in root development over auxins. Terpenoids with a cross conjugated ketone moiety have been shown to cause adventitious root formation in the hypocotyl of mung bean cuttings. The essential oils from the tubers of *Cyperus scariousus* significantly induce the formation of roots in the stem cuttings of *Phaseoulus aureus.* Such kind of effects of terpenoids could be evaluated by knowing the structure - activity relationship of these biomolecules and their biological activities. Presence of different functional group like conjugative α, β, unsaturated ketone moiety, exomethylene moiety, conjugative lactone carbonyl moiety, and conjugative enone chromophore moiety might cause some changes in signalling pathway in some way so that developmental stages could be influenced (Nerali *et al*., 1965; Shibaoka *et al*., 1967; Kalsi *et al*., 1977; Kalsi *et al*., 1978).

There is a synergistic effect on

development root when the MS medium supplemented with IBA and MDHBO. The molecular formula of IBA is $C_{12}H_{13}NO_2$. Whereas, the molecular formula of MDHBO is $C_{12}H_{12}O_2$. MDHBO contains a 7 membered unsaturated oxocyclic dihydro oxypine ring which resembles the pyrene ring of auxins or IBA. The molecular formula of BA is $C_{12}H_{11}N_5$ which does not contain any pyrene ring like auxinwhen s. Probably this may be the cause of synergistic auxin's effect (positive effect on root growth) when MS medium is supplemented with IBA and MDHBO. This may also the reason why there is an antagonistic effect on shoot development when MS medium supplemented with BA and MDHBO, as more auxin : cytokinin ratio prevents shoot development.

CONCLUSION

In this experiment it is found that the said metabolites have some morphogenetic effect over synthetic plant growth regulators. When the plant growth regulators are only used, the effect and pattern of morphogenesis differs with the experimental set containing plant growth regulators and said metabolite. The figures placed here are looking similar with each other as the chemical or artificial plant growth regulator and the natural metabolite both have null effect on development of any morphological anomalies on the experimental plant. The difference in lengths and numbers of shoot and root production from the explants were seen properly.

The results showed that although this terpenoid molecule (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine have negative effect on development of shoot yet also possess some positive effect during rooting. Hence, from above findings it could be reported that (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine can be used as shoot retardant but root elicitor. Furthermore, as this terpenoid is a natural one so it also can be hypothesised that using of this metabolite only will not influence the genetic blueprint of the *in vitro* raised plants which is a major drawback of using of chemical plant growth regulators. So this metabolite could be used in ecofriendly manner as shoot retardant and root elicitor molecule in *in vitro* culture to propagate *true to type* plants.

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