Research Article **Plant Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFECT OF PLANT GROWTH REGULATORS ON *IN VITRO* PROPAGATION OF *TAGETES ERECTA*

BANANI DEKA[1] AND ANANIA ARJUNA*[2]

1Post Graduation Scholar, Faculty of Applied Medical Sciences, Lovely Professional University, Punjab, India
2Assistant Professor, Faculty of Applied Medical Sciences, Lovely Professional University, Punjab, India

ABSTRACT

Tagetes erecta is a plant of great medicinal value and is in high commercial demand due to its various uses in religious purposes, as eatables, and is used for the treatment of headache and strengthening of the heart, treating wounds and prevent them from getting infected with bacteria and toxins. Young tender leaves of *T.erecta* were collected from plants grown in pots under adequate sunlight and rainfall.. Leaves were sterilised with 70% ethanol for 5 minutes and 0.02% Mercuric chloride for 3 minutes and inoculated in MS medium supplemented with Kn, IAA, NAA and 2,4-D in different combinations (0, 0.5, 2.5, 5.0, 10 mg/L). 50% callus formation are observed in 0.5 mg/L Kn from LT, 0.5 mg/L Kn + 2.5 mg/L IAA from LM, 0.5 mg/L Kn + 10.0 mg/L IAA from LM, 2.5 mg/L Kn + 0.5 mg/L IAA from LT, 5.0 mg/L Kn + 2.5 mg/L 2,4-D from LT and 10.0 mg/L Kn + 0.5 mg/L IAA from LM. 100% callus formation occurred in the combinations of 0.5 mg/L Kn + 5.0 mg/L 2,4-D from LB and LM, 2.5 mg/L Kn from LT and 5.0 mg/L Kn + 0.5 mg/L IAA from LT. Root formation was observed in the concentration of 2.5 mg/L Kn + 0.5 mg/L IAA from LT. Root formation was observed in low concentration of Kn in combination with a low concentration of cytokinin. Callus induction resulted in high concentration of Kn and cytokinin.. It has also been observed that the explants responded differently towards different growth regulators in varying concentrations. The best callus growth was found using the Kn and IAA as growth regulators within a short time period.

ANANIA ARJUNA
Assistant Professor, Faculty of Applied Medical Sciences,
Lovely Professional University, Punjab, India

INTRODUCTION

T. erecta, also known as the African marigold, is an ancient herb with several medicinal properties and uses. It is an important ornamental crop with a great public demand. Due to low seed viability and poor germination it is difficult to fulfil the increasing demand. Tissue culture was selected as an alternative for large scale commercial propagation of this plant (Mishra and Datta, 2000). These flowers were either made with a poultice or infused into oil for application on wounds. The infused oil or cream of Marigold flowers is used extensively in aromatherapy to treat aczema, acars, cracked, skin rashes, inflammation and viral infections. The petals of flowers are used to make an eye wash. Both homeopathy and conventional medicine utilise Marigold as a healing ointment for cuts and grazes. Marigold has antiseptic, stimulant and anti- fungal properties. It is also used to heal irritating and painful mouth ulcers, stomach ulcers. It is excellent in the treatment of gastro intestinal disease as well as for gall bladder disease and indigestion and even helps to prevent stomach cancers. The flower has a number of anti oxidant components such as carotenoids, quercitin, volatile oils and saponin and mucilage (Jenkinson, 2006)

MATERIALS AND METHODS

Laboratory Requirements for Tissue Culture

General Organization

Each portion of the tissue culture procedure is localised in a specified place in the laboratory. An assembly-line arrangement of work areas (such as, media preparation, glassware washing, sterilization, microscopy, and aseptic transfers) facilitates all operations and enhances cleanliness. Media (tissue culture and nutrient agar) are available from Carolina Biological Supply Co., Burlington, NC. Laminar flow hoods are available from several suppliers (Mineo, 1990).

Glassware

Glassware that has only been used for tissue culture are used and not other experiments. Toxic metal ions absorbed on glassware can be especially troublesome. Glasswares are washed with laboratory detergent, then rinsed several times with tap water and, finally with purified water (Mineo, 1990).

High-purity Water

Only high-purity water is used in tissue culture procedures. Double glass distilled water or deionized water from an ion-exchanger are acceptable. Water should not be stored, but used immediately. Regular maintenance and monitoring of water purification equipment are necessary. Purified water for tissue culture can also be purchased (Mineo, 1990).

Plant Material

Plants used in tissue culture need to be healthy and actively growing. Stressed plants, particularly water-stressed plants, usually do not grow as tissue cultures. Insect and disease-free greenhouse plants are rendered aseptic more readily, so contamination rate is lower when these plants are used in tissue culture procedures. Seeds that can be easily surface sterilized usually produce contamination-free plants that can be grown under clean greenhouse conditions for later experimental use (Mineo, 1990).

Aseptic Technique

The essence of aseptic technique is the exclusion of invading microorganisms during experimental procedures. If sterile tissues are available, then the exclusion of microorganisms is accomplished by using sterile instruments and culture media concurrently with standard bacteriological transfer procedures to avoid extraneous contamination. Media and apparatus are rendered sterile by autoclaving at 15 lbs/inch 2 (121°C) for 15 minutes. The use of disposable sterile plastic ware reduces the need for some autoclaving. Alternative sterilization techniques such as filter sterilization must be employed for heat-labile substances like cytokinins. Aseptic transfers can be made on the laboratory bench top by using standard bacteriological techniques (i.e., flaming instruments prior to use and flaming the opening of receiving vessels prior to transfer). Aseptic transfers are more easily performed in a transfer chamber such as a

laminar flow hood, which is also preferably equipped with a bunsen burner. If experimental tissues are not aseptic, then surface sterilization procedures specific to the tissues are employed. Common sterilants are ethyl alcohol and/or chlorox with an added surfactant. Concentration of sterilants and exposure time are determined empirically (Mineo, 1990).

Procedure

Preparation of MS media

- 35 ml of stock 1 is taken in a beaker.
- To this 3.5 ml of stock 2 is added and to it 3.5 ml of stock 3 followed by 3.5 ml of stock 4 is added respectively.
- To the above mixture, 21g of sucrose is added and dissolved properly with a magnetic stirrer.
- The volume is made upto 700ml by adding distilled water using a measuring cylinder.
- Half of the medium is taken and warmed.
- 6g of agar is added to this half volume and is boiled in a micro-oven to dissolve the agar.
- After melting and dissolving the agar completely the other half is added and boiled again.
- 15ml of the medium is poured in each culture tubes followed by the addition of different growth regulators (Kn, IAA, 2,4-D) with varying concentrations (0, 0.5, 2.5, 5.0, 10.0 mg/L).
- One set of experiments consists of 50 such culture tubes with 25 different concentrations (2 culture tubes per concentration).
- The tubes were capped with plastic caps and were autoclaved along with other supplements such as forceps, scalpel, petri plates, conical flasks, filter papers, beakers etc. for 15 minutes at 15 psi.
- The autoclaved medium along with the other materials are taken to plant tissue culture room. The tubes were kept in a slanting position.
- Before inoculation the medium and the other equipments are kept in laminar air flow cabinet under UV light for 20 minutes.

Collection of explants

Fully developed as well as young tender leaves measuring about 2.5-3cm and 1.5 cm respectively in length were used as explants from the shoots of *T.erecta*, collected and grown in pots.

Sterilisation of the explants

The leaves are first washed in tape water followed by distilled water. A solution of 70% ethanol was prepared and the explants are transferred from distilled water to it and kept for 5 minutes. A fresh solution of 0.2% mercuric chloride is prepared and the leaves are transferred from ethanol and kept for 3-7 minutes.

Inoculation of explants

The leaves dipped in mercuric chloride are taken to the laminar air flow cabinet and are transferred to sterilized distilled water. The leaves were washed 3 times in separate beakers with sterilized distilled water. The explants were then cut into 3 equal portions ± tip (LT), median (LM) and basal (LB) petiolar end and are cultured with their abaxial surface on the MS medium solidified with agar with various growth regulator and different concentrations of each of them. For each treatment 2 replicates are cultured having 1 explant per culture tube. The cultures were labelled and kept in the culture room under 3000 lux light intensity and 25˚C temperatures and approximately 60-80% humidity. Light from daylight fluorescent tubes for 8 hours was given.

STOCK 1 COMPONENTS	mg\l	g\l
$MgSO_4.7H_2O$	7400	7.4
KH_2PO_4	3400	3.4
KNO_3	38000	38
NH_4NO_3	33000	33
$CaCl_2.2H_2O$	8800	8.8
STOCK 2 COMPONENTS		
H_3BO_3	1240	1.24
$MnSO_4.2H_2O$	4460	4.46
$ZnSO_4.7H_2O$	1720	1.72
$Na_2MoO_4.2H_2O$	50	0.05
$CuSO_4.5H_2O$	5	0.005
$CoCl_2.6H_2O$	5	0.005
STOCK 3 COMPONENTS		
$Na_2EDTA.2H_2O$	7460	7.46
$FeSO_4.7H_2O$	5560	5.56
STOCK 4 COMPONENTS		
Glycine	400	0.4
Thiamina HCL	100	0.1
Pyridoxine HCL	100	0.1
Nicotinic Acid	100	0.1
Myoinositol	20000	20

Table 4(a)
Composition of Stock Solutions of MS medium [Razdan, 2002]

COMPONENTS	AMOUNT(mg/l)
MACRONUTRIENT	
$MgSO_4.7H_2O$	370
KH_2PO_4	170
KNO_3	1900
NH_4NO_3	1650
$CaCl_2.2H_2O$	440
MICRONUTRIENTS	
H_3BO_3	6.2
$MnSO_4.2H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
$Na_2EDTA.2H_2O$	37.3
$FeSO_4.7H_2O$	27.8

COMPONENTS	AMOUNT(mg/l)
Sucrose	30000[mg]
ORGANIC SUPLIMENTS	
Thiamina HCL	0.5
Pyridoxine HCL	0.5
Nicotinic Acid	0.5
Myoinositol	100
Glycine	2
pH	5.8
Agar(g)	8

Table 4(b)
Basal media of MS [Razdan, 2002]

RESULTS

Differentiation of callus/shoots/roots in leaf segments of *T. erecta* was achieved using Kn, IAA and 2,4-D as growth regulators in different concentrations. A combination of IAA and Kn gave 50% callus/shoot/root formation with the concentration of 0.5 mg/L IAA + 2.5 mg/L Kn (Fig 1), 0.5 mg/L IAA + 10.0 mg/L Kn (Fig 4), 2.5 mg/L IAA + 0.5 mg/L Kn (Fig 5), 10.0 mg/L IAA + 0.5 mg/L Kn (Fig 6) and 10.0 mg/L IAA + 2.5 mg/L Kn (Fig 7). However 100% differentiation of callus/shoot/root was observed with a concentration of 0.5 mg/L IAA + 5.0 mg/L Kn (Fig 2; Fig 3). Contamination also occurred in some of the combinations. 50% contamination rate was observed in 0 mg/L IAA + 0.5 mg/L Kn, 0.5 mg/L IAA + 2.5 mg/L Kn, 0.5 mg/L IAA + 10.0 mg/L Kn, 2.5 mg/L IAA + 0.5 mg/L Kn, 10.0 mg/L IAA **+** 0.5 mg/L Kn, 10.0 mg/L IAA + 2.5 mg/L Kn, 10.0 mg/L IAA + 5.0 mg/L Kn and 10.0 mg/L IAA + 10.0 mg/L Kn. The results of these is shown in Table 4(c). Another combination of 2,4-D and Kn as growth regulator also showed callus/root/shoot formation. 50% differentiation of callus/shoot/root formation was observed in a combination of 0 mg/L 2,4-D + 0.5 mg/L Kn (Fig 8) while 100% callus/shoot/root formation resulted in 0 mg/L 2,4-D + 2.5 mg/L Kn (Fig9; Fig 10), 2.5 mg/L 2,4-D + 5.0 mg/L Kn (Fig 11; Fig 12) and 5.0 mg/L 2,4-D + 0.5 mg/L Kn (Fig 14). Contamination resulted in many of the combinations. 50% contamination occurred in 0 mg/L 2,4-D 0.5 mg/L Kn and 5.0 mg/L 2,4-D + 10.0 mg/L Kn. Most of them resulted in 100% contaminations in the concentrations of 0 mg/L 2,4-D + 5.0 mg/L Kn, 0 mg/L 2,4-D + 10.0 mg/L Kn, 0.5 mg/L 2,4-D + 0 mg/L Kn, 0.5 mg/L 2,4-D + 0.5 mg/L Kn, 0.5 mg/L 2,4-D + 2.5 mg/L Kn, 2.5 mg/L 2,4-D + 0 mg/L Kn, 2.5 mg/L 2,4-D + 0.5 mg/L Kn, 2.5 mg/L 2,4-D + 2.5 mg/L Kn, 2.5 mg/L 2,4-D + 10.0 mg/L Kn, 5.0 mg/L 2,4-D + 10.0 mg/L Kn, 5.0 mg/L 2,4-D + 2.5 mg/L Kn, 5.0 mg/L 2,4-D + 5.0 mg/L Kn, 10.0 mg/L 2,4-D + 0 mg/L Kn, 10.0 mg/L 2,4-D + 2.5 mg/L Kn, 10.0 mg/L 2,4-D + 5.0 mg/L Kn and 10.0 mg/L 2,4-D + 10.0 mg/L Kn. Contamination rate was found much higher in this combination. The results of these are shown in Table 4(d). Different types of callus with different characteristics and roots developed from different portions of young, tender leaves of *T. erecta*. The combinations that resulted in 50% callus formation are 0.5 mg/L Kn from LT, 0.5 mg/L Kn + 2.5 mg/L IAA from LM, 0.5 mg/L Kn + 10.0 mg/L IAA from LM, 2.5 mg/L Kn + 0.5 mg/L IAA from LT, 5.0 mg/L Kn + 2.5 mg/L 2,4-D from LT and 10.0 mg/L Kn + 0.5 mg/L IAA from LM. 100% callus formation occurred in the combinations of 0.5 mg/L Kn + 5.0 mg/L 2,4-D from LB and LM, 2.5 mg/L Kn from LT and 5.0 mg/L Kn + 0.5 mg/L IAA from LT. Root formation was observed in the concentration of 2.5 mg/L Kn + 0.5 mg/L IAA from LT. The results are shown in Table 4(e).

Table 4(c)
Effect of Kn and IAA on leaf explants of T.erecta

Growth regulators (mg/L) IAA	Kn	% of explants showing callus/root/shoot formation	Contamination (%)
0	0	0	0
0	0.5	0	50
0	2.5	0	100
0	5.0	0	100
0	10	0	100
0.5	0	0	0
0.5	0.5	0	0
0.5	2.5	50	50
0.5	5.0	100	0
0.5	10	50	50
2.5	0	0	100
2.5	0.5	50	50
2.5	2.5	0	0
2.5	5.0	0	0
2.5	10	0	0

5.0	0	0	0
5.0	0.5	0	100
5.0	2,5	0	100
5.0	5.0	0	100
5.0	10	0	100
10	0	0	0
10	0.5	50	50
10	2.5	50	50
10	5.0	0	50
10	10	0	50

Table 4(d)
Effect of Kn and 2,4-D on leaf explants of T.erecta.

Growth regulators (mg/L) 2,4-D	Kn	% of explants showing shoot/root/callus formation	Contamination (%)
0	0	0	
0	0.5	50	50
0	2.5	100	0
0	5.0	0	100
0	10	0	100
0.5	0	0	100
0.5	0.5	0	100
0.5	2.5	0	100
0.5	5.0	0	0
0.5	10	0	0
2.5	0	0	100
2.5	0.5	0	100
2.5	2.5	0	100
2.5	5.0	100	0
2.5	10	0	100
5.0	0	0	100
5.0	0.5	100	0
5.0	2.5	0	100
5.0	5.0	0	100
5.0	10	0	50
10	0	0	100
10	0.5	0	0
10	2.5	0	100
10	5.0	0	100
10	10	0	100

Table 4(e)
Callus and root formation on leaf explants of T.erecta.

Growth regulators (mg/L) Kn	IAA	2,4-D	% of explants showing callus formation	% of explants showing root formation	Portion of leaf showing response
0.5			50		LB
0.5	2.5		50		LM
0.5		5.0	100		LB LM
0.5	10		50		LM
2.5			100		LT
2.5	0.5		50	50	LT
5.0	0.5		100		LT
5.0		2.5	50		LT
10	0.5		50		LM

LT: leaf tip
LM: leaf median
LB: leaf base

DISCUSSION

Tender leaves were chosen as explants from *T. erecta* grown in pots under adequate sunlight because they have more potential to undergo growth and differentiation when placed in a suitable medium with growth regulators. The leaves were washed thoroughly with tape water followed by distilled water to remove the superficial dust. It is then surface sterilised with ethanol (70%) and mercuric chloride (0.02%). The concentration of ethanol was made to 70% because at this concentration the ethanol can penetrate the cell membranes of many bacteria and kills them. Mercuric chloride is a very strong sterilent. Sharma *et.al.* used a concentration of 0.1% mercuric chloride for 5 minutes during their work on *in vitro* pagation of *Bacopa moneri* in the year2010. However this concentration was found too strong for the leaf twigs of *B. moneri.* Another work performed by Nisha *et.al.* 2009, used 0.07% mercuric chloride for 3 minutes for sterilising the nodal and leaf explants of *Begonia malabarica.* Gami *et.al.,*2010, used 0.01% mercuric chloride for 5 minutes for sterilising the nodal explants of *Mimusops* elengi. Considering and looking into all the concentrations and time period, 0.02% mercuric chloride for 3 minutes was chosen to sterilise the leaf explants of *T. erecta.* The leaves were dipped for 5 minutes in ethanol and 3 minutes in mercuric chloride to kill the microbes. Long time period was avoided so as to prevent the leaves from getting damaged. After washing in mercuric chloride the leaves were again washed thoroughly in sterilized distilled water for 3 times. This was done so as to remove the mercuric chloride from the leaves whose presence in the leaves for a long time could be detrimental. This step was carried out in a laminar air flow cabinet in order to prevent the entry of microbes.

The laminar airflow principle developed by Whitfield has been widely and successfully applied in industry for the control of particulate contamination during clean assembly work. A variety of horizontal (cross flow) and vertical (down flow) laminar airflow cabinets are commercially available. The general areas of application of such units comprise (i) product protection, i.e., use of laminar airflow cabinets for operations involving manipulations of materials that must be kept sterile or free from unwanted ecological agents, and (ii) agent containment, i.e., use of laminar airflow cabinets for manipulations involving etiological agents, materials, or procedures requiring personnel protection. The item(s) in use must be confined within the working area and must not be allowed to escape from the cabinet. The horizontal laminar airflow cabinet is best suited for maximal product protection. However, when agent containment is required, the vertical laminar airflow cabinet is more applicable. Air handling-system modifications have led to the development of downflow units that have been reported to provide product protection and agent containment. In such units, a larger volume of moving air is exhausted from the cabinet than the volume of air supplied through the filter. This creates a negative pressure at the face of the cabinet, thereby drawing room air into the face or front opening of the cabinet. Theoretically, a protective air curtain is established at the cabinet face to provide both product protection and agent containment. They consist of high efficiency particulate (HEPA) filters. They are particulate filters which retain airborne particles and microorganisms (gases pass freely through). Filtration occurs by five distinct methods (primary mechanisms): 1) sedimentation 2) electrostatic attraction 3) interception 4) inertial impaction and 5) diffusion (McDADE *et.al*, 1968).

Before inoculation the media and all other equipments including forceps, sterilised blades, cotton, beakers, conical flasks, sterilised distilled water, stands, burner etc. were kept under UV light for 15 minutes in the laminar air flow cabinet to kill microbes and other bacteria that can cause contamination. The cabinet was cleaned properly with 70% ethanol. The burner was flamed and the inoculation was done near to the flame so as to prevent the entry of microbes into the culture tubes. The hot air fan was switched on throughout the experiment which is also a means of

prevention of contamination. The forceps and the scalpel were dipped in alcohol (70%), flamed each time before inoculating per explants. Hands were also cleaned with 70% ethanol before performing the experiment. Inoculation was carried out under white light in the laminar air flow cabinet.

Callus/shoot/root formation was observed in the combination of IAA and Kn. However there was contamination in many of the concentrations. This could be due to many reasons:

- The explants were not washed properly or the medium was not properly autoclaved.
- Entry of microbes during inoculation.
- The concentration of 0 mg/L IAA + 0 mg/L KN did not give any result due to the absence of growth regulators.
- Inadequate supply of growth regulators.
- Death or damaging of the tissues of the explants during washing.
- Improper placement or position of the leaf explants in the nutrient medium. Uptake of minerals and growth regulators takes place only through the dorsal surface of the leaf. Hence the explants must be placed with its dorsal surface in contact with the growth regulators.

The concentrations with 50% callus/shoot/root formation and 50% contamination (0.5 mg/L IAA + 2.5 mg/L Kn, 0.5 mg/L IAA + 10.0 mg/L Kn, 2.5 mg/L IAA + 0.5 mg/L Kn, 10.0 mg/L IAA + 0.5 mg/L Kn and 10.0 mg/L IAA + 2.5 mg/L Kn) could result into 100% callus/shoot/root differentiation if the contamination in the other halves of the tubes were avoided [Table 4(c)].

The contamination rate in the combination of 2,4-D and Kn as growth regulator is higher than that of IAA and Kn combination. Differentiation of callus/shoot/root is observed in the concentration with only Kn as the growth regulator (0 mg/L 2.4-D + 0.5 mg/L Kn and 0 mg/L 2,4-D + 2.5 mg/L Kn). This is because Kn alone has the ability to induce callus formation [Table 4(d)]. The age of the explants and the type of tissues used can also be a factor for callus and root formation. Leaf explants of *T. erecta* had a tendency to form callus which was enhanced in the presence of cytokinin (Kn), auxin (IAA and 2,4-D). Low concentration of auxin (IAA) with a low concentration of cytokinin (Kn) resulted in root formation (2.5 mg/L Kn + 0.5 mg/L IAA). With only Kn as growth regulator (0.5 mg/L and 2,5 mg/L) and with increase in concentration of auxin and cytokinin, callus formation was observed [Table4(e)]. All the three portions of the leaf (LT, LM and LB) gave rise to root and callus growth. Callus formation occurred after 10 days of culture with IAA and KN as growth regulator and after 15 days of culture with Kn and 2,4-D as growth regulator. Root formation resulted after 15 days of culture. Hence a combination of IAA and Kn is better for callus induction in *T. erecta* using leaves as explants because the time period for callus induction is less than that with the combination of 2,4-D and Kn. Comparing all the results it can be concluded that a low concentration of Kn in combination with low concentration of IAA (2.5 Kn mg/L + 0.5 IAA mg/L) lead to root formation where with increase in concentration of Kn, callus formation was observed. This shows that the response of the explants vary with varying concentration of growth regulators.

Fig1: Root formation in MS + 0.5 mg/L IAA + 2.5 mg/L Kn

Fig2: Callus formation in MS + 0.5 mg/L IAA + 5.0 mg/L Kn

Fig3: Callus formation in MS + 0.5 mg/L IAA + 5.0 mg/L Kn

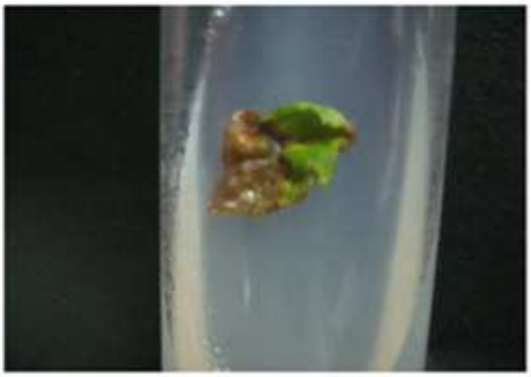

Fig4: Callus formation in MS + 0.5 mg/L IAA + 10.0 mg/L Kn

Fig5: Callus formation in MS + 2.5 mg/L IAA + 0.5 mg/L Kn

Fig6: Callus formation in MS + 10.0 mg/L IAA + 0.5 mg/L Kn

Fig7: Callus formation in MS + 10.0 mg/L IAA + 2.5 mg/L Kn

Fig8: Callus formation in MS + 0.5 mg/L Kn

Fig9: Callus formation in MS + 2.5 mg/L Kn

Fig10: Callus formation in MS + 2.5 mg/L Kn

Fig11: Callus formation in MS + 2.5 mg/L 2,4-D + 5.0 mg/L Kn

Fig12: Callus formation in MS + 2.5 mg/L 2,4-D + 5.0 mg/L Kn

Fig13: Callus formation in MS + 5.0 mg/L 2,4-D + 0.5 mg/L Kn

Fig14: Callus formation in MS + 5.0 mg/L 2,4-D + 0.5 mg/L Kn

CONCLUSION

The field of plant biotechnology is concerned with developing ways to improve the production of plants in order to supply the world's needs for food, fiber and fuel. In addition, plants provide us with many pharmaceuticals and industrial compounds. As our population grows, our needs also grow. To increase the quantity of crop production as well as to produce specific characteristics in plants, biotechnologists are using selective gene techniques. The two major methods of propagation are Plant tissue culture, Genetic engineering. Plant tissue culture comprises a set of *in vitro* techniques, methods and strategies has been exploited to create genetic variability from which crop plants can be improved (Brown and Thorpe, 1998). *Tagetes erecta* is a plant of great medicinal value and is in high commercial demand due to its various uses in religious purposes, as eatables, and is used for the treatment of headache and strengthening of the heart. It is widely used in treating wounds and prevent them from getting infected with bacteria and toxins. The cream extracted from the leaves and flowers is used extensively to treat scars, inflammation and viral infections. It has an antiseptic stimulant and anti-fungal properties (Jenkinson, 2000). Young tender leaves of *T.erecta* were collected from plants grown in pots under adequate sunlight and rainfall.. Leaves were sterilised with 70% ethanol for 5 minutes and 0.02% Mercuric chloride for 3 minutes and inoculated in MS medium supplemented with Kn, IAA, NAA and 2,4-D in different combinations (0, 0.5, 2.5, 5.0, 10 mg/L). 50% callus formation are observed in 0.5 mg/L Kn from LT, 0.5 mg/L Kn + 2.5 mg/L IAA from LM, 0.5 mg/L Kn + 10.0 mg/L IAA from LM, 2.5 mg/L Kn + 0.5 mg/L IAA from LT, 5.0 mg/L Kn + 2.5 mg/L 2,4-D from LT and 10.0 mg/L Kn + 0.5 mg/L IAA from LM. 100% callus formation occurred in the combinations of 0.5 mg/L Kn + 5.0 mg/L 2,4-D from LB and LM, 2.5 mg/L Kn from LT and 5.0 mg/L Kn + 0.5 mg/L IAA from LT. Root formation was observed in the concentration of 2.5 mg/L Kn + 0.5 mg/L IAA from LT.

Root formation was observed in low concentration of Kn in combination with low concentration of cytokinin. Callus induction resulted in high concentration of Kn and cytokinin.. It has also been observed that the explants responded differently towards different growth regulators in varying concentrations. The best callus growth was found using the Kn and IAA as growth regulators within a short time period. The callus mass when treated further by transferring it to a new medium under controlled concentration of growth regulator and other nutrients can give rise to shoots and roots which will result into small plantlets. These plantlets are then grown under glasshouse conditions to slowly adapt themselves to the changing climate and develop resistance in them. These could then

be taken out from glasshouse and can be planted in soil under natural climatic conditions. Although tissue culture has advantage of propagating a desired plant with desirable qualities in large population at any time irrespective of space and time it has many disadvantages too. The technique requires a lot of knowledge and skills in order to carry out the work. Moreover the nutrient media, the organic and inorganic compounds and the growth regulators are too expensive. The result of any work on tissue culture must be able to compensate the cost that was made during the experiment.

REFERENCES

1. Anjanasree K. Neelaandan and Kan Wang; 2012; Plant Cell Reports; Recent progress in the understanding of tissue cultured induced genome level changes in Plants and potential applications; Vol 31(4); Pg: 597-620.
2. Athar Hussain and Maida Latif; 2012; *In vitro* studies in *Tagetes erecta* (marigold) under aixins (IAA and NAA) and cytokinins (BAP, Kinetin) effect for callus formation by different explants; Vol (1&2); Pg: 41-46.
3. Basavaraj V Chivde., Karnakumar V Biradar, Rajabhau S Shiramane, Kamshetty V Manoj; 2011; International Journal of Pharma and Bio Sciences; *In vitro* antioxidant activity studies on the flowers of *Tagetes erecta* L.(Compositae); Vol 2; Pg: 223-229.
4. Brown, D.C.W. and Thorpe, T.A.; 1995; World Journal of Microbiology and Biotechnology; Crop improvement through tissue culture; Vol 11; Pg: 409-415.
5. Dixit Priyanka, Tripathi Shahilini and Verma Kumar Navneet; 2013; International Research Journal of Pharmacy; A brief study on Marigold (*Tagetes species)*: A Review; Vol 4(1); Pg: 43-48.
6. Gami, Bharat, Parabia, Minoo and Kothari, I.L.; 2010; International Journal of Pharmaceutical Sciences and Drug research; *In vitro* development of callus from node of *Mimusops elengi* – As a substitute of Natural Bark; Vol 2(4); Pg: 281-285.
7. Geeta Singh and Sudheer Shetty; 2011; Society for Applied Biotechnology; Impact of Tissue Culture on Agriculture in India; Vol 1(3); Pg: 279-288.
8. Jenkinson, Linda; 2006; Gardening Guides; Marigold – More than meets the eye; Pg: 3-12.Kuldeep Yadav, Narender Singh* and Sharuti Verma; 2012; Journal of Agricultural Technology; Plant Tissue Culture: A Biotechnological tool for solving the Problem of propagation of multipurpose endangered Medicinal Plants in India; Vol 8(1); Pg: 305-318.
9. Manoj K. Rai, N. S. Shekhawat, Harish, Amit K. Gupta, M. Phulwaria, Kheta Ram & U. Jaiswal; 2011; Plant Cell, Tissue and Organ Culture; The Role of abscissic acid in Plant Tissue Culture: a review of recent progress; Vol 106(2); Pg: 179-190.
10. McDade, J.J., Sabel, F.L., Akers, R.L. and Walker, R.J.; 1968; Applied Microbiology; Microbiological studies on the performance of a Laminar Air Flow Cabinet; Vol 16(7); Pg: 1806-1092.
11. Mineo, C.O.; 1961; Plant Tissue Culture Techniques; Pg: 152-174.
12. Mishra, Pabitra and Datta, S.K.; 2000; *In vitro* Cellular and Developmental Biology; Direct Differentiation of shoot buds in leaf segments of *T. erecta*; Vol 37(4); Pg: 466-470.
13. Nisha, M.C., Rajeshkumar, S.,Selvaral, T. and Subramanian, M.S.; 2009; Majeo International Journal of Science and Technology; A valued Indian Medicinal Plant – *Begonia malabarica* Lam.: Successful Plant Regeneration through various explants and field performance; Vol 3(02); Pg: 261-268.
14. Osman, H.A., Taha, H.S., Youssef, M.M.A., El-Gindi, A.Y., Ameen, H.H. and Lashein, A.M.S.; 2012; Australian Journal of Basic and Applied Sciences; Establishment of *T. erecta* and *T. patula* Suspension Cultures; Vol 6(6); Pg: 436-441.

15. Razdan, M.K.; 2002; Introduction to Plant Tissue culture; Pg: 23-29.Sharma, S., Kamal., B., Rathi, N., Chauhan, S., Jadon, V., Vatas, N., Gehlot, A. and Arya, S.; 2010; African Journal of Biotechnology: Vol 9(49); Pg: 8318-8322.
16. Yingchun Qi, Yaomei Ye and Manzhu Bao; 2011**;** African Journal of Biotechnology; Establishment of plant regeneration system from anther culture of *Tagetes patula*; Vol. 10(75), Pg: 17332-17338.
17. Zoltán Molnár, Emese Virág, Vince Ördög; 2011; Acta Biologica Szegediensis, Natural Substances for Tissue Culture media in Higher Plants; Vol 55(1); Pg: 123-127.