STUDIES ON IN-VITRO SHOOT MULTIPICATION OF ROSE HYBRID " C.V. Gold Medal"

Dissertation submitted in partial fulfilment for the Master Degree of Science in biotechnology

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CERTIFICATE

This is to certify the dissertation entitled "Study on *in-vitro* shoot multiplication of hybrid tea rose cv. Gold Medal" submitted by SUNITA PRIYADARSHINI SAHOO, in partial fulfilment of the master degree of science in biotechnology, KIIT School of Biotechnology, Bhubaneswar bearing Roll no. 1661028 is a bona fide research work carried out by her under my guidance and supervision from 1st January to 15TH May, 2018.

(Research Supervisor Full Signature)
Full name and designation

DECLARATION

I SUNITA PRIYADARSHINI SAHOO hereby declare that the dissertation entitled "Studies on *in-vitro* multiplication of rose hybrid tea cv. Gold Medal" submitted by me, for the Master Degree of Science in Biotechnology to KIIT School of Biotechnology is a record of bona fide work carried by me under the supervision & guidance of "Dr. S.K. Palai, Floriculturist", Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India.

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ABSTRACT

The present investigation was carried out on an important ornamental plant Hybrid Tea Rose cv. Gold Medal of family Rosaceae. The vegetative part i.e. node was used as explants to standardize the protocol for mass multiplication through *in-vitro* techniques. The explants were excised from field grown mature plant and thereafter planted on variously supplemented Murashige Skoog's medium for multiple shoot induction. Early bud break (1-2 weeks) and maximum percentage of bud break was observed in MS medium supplemented with BAP, IAA. Maximum percentage of multiple shoots were observed in MS medium having 0.2mg/l BAP, 0.01 mg/l IAA. The maximum percentage of multiple shoots per culture were achieved when inoculated under 3000 lux light intensity and 16 h photoperiod at 25±2^{o}C. This is a rapid multiplication protocol of rose for production of healthy, disease free and high quality plant material for commercial use.

ACKNOWLEDGEMENT

At this platform, I am deeply privileged to envince a word of thanks to everyone who played a positive role in the completion of my project. I thank the almighty whose blessings have empowered me to accomplish my work successfully.

I wish to express my deepest gratitude to my guide Dr. Sashikala Beura, Director BTCC, OUAT, Bhubaneswar and Dr. S.K. Palai, Floriculturist, AICRP on Floriculture for his help, support, understanding and valuable guidance for successful completion of my research work. Without his assistance it would have been difficult for me to shape up this work. Without the help of SUPRITI mam it is not easy to work. I also want to thank all the technicians for their support in my project work.

Dt: 15th may 2018

Place: Bhubaneswar Signature

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ABBREVIATION

M.S. Media-Murashige and skoog Media(1962)

BAP-6-Benzylaminopurine

IAA- Indole-3-acetic acid

INTRODUCTION

1.INTRODUCTION

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation. The plant tissue culture refers to the In-Vitro culture of plants and plant parts on nutrient media under aseptic conditions.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant. Single cells, plant cells without cell walls (protoplast), pieces of leaves, stems or roots can often be used to regenerate a new plant on culture media given the required nutrients & plant hormones.

A rose is the most important commercial flower crop. It is a woody perennial which belongs to the Rosa species of family Rosaceae. There are over a hundred species & thousands of cultivars. They form a group of plants that can be erect shrubs, climbing or trailing with stems that are often armed with sharp prickles. Flowers vary in shapes shape & size & are usually large and showy, in colours ranging from yellows and reds. Rose plants range in size from compact, miniature roses to climbers that can reach seven meters in height.

Rose is generally propagated by vegetative methods like cutting, budding and grafting. Although propagation by vegetative means is a predominant technique in rose, yet it does not ensure healthy and disease free plants. Moreover, dependence on season and slow multiplication rates are the major limitations in conventional propagation.

The history of rose tissue culture dates back to 1945, when Nobecourt and Kofler succeeded in obtaining callus and roots on the explanted buds. In the year 1946, Lamments reported the use of embryo culture in rose breeding. The first shoot organogenesis from callus tissue culture was reported by Hill (1967) in a climbing hybrid Tea Rose “The Doctor”.

In this contest, plant tissue culture is the most efficient & reliable for rapid & mass scale production of disease free & identical plants of rose throughout the life span. The main aim of the present study was to establish a protocol for propagation of disease free & healthy planting materials through *in-vitro* culture & to ensure production of high quality planting material for commercial use of hybrid tea rose “Gold Medal”.

REVIEW OF LITERATURE

2.REVIEW OF LITERATURE

This chapter deals with the review of research work done on standardization of media & media supplements for successful *in-vitro* propagation of Rose & its related species by various scientists in India & abroad.

Rose occupies the first place among perfumery flowers. The odour of flower cannot be imitated by any known synthetic aromatic chemical or natural isolates, thus giving a unique status in the perfume world. Therefore, it has attracted the attention of researchers around the globe. Conventional breeding is not possible as seeds are not formed in rose. Therefore, researchers have tried to create variation and bring improvement in rose through *in vitro* techniques.

Schaeffer (1990) defined micro-propagation as the *in vitro* clonal propagation of plants from shoot tips or nodal explants, usually with an accelerated proliferation of shoots during sub cultures. In the last few years, *in vitro* propagation has revolutionized commercial nursery business (Pierik,1991). Micro-propagation is usually described as having the following four distinct stages (a stage "0"is added by some authors):1.stage "0":preparation of in situ donor material (fungicide and/or PGR treatments ,hedging, etiolation, etc).2:Stage "I": Initiation (including surface sterilization) of explants.3.Stage "II": shoot multiplication (optimization of proliferation media).4.Stage "III": root induction on micro-cuttings(*in vitro* or ex vitro).5.Stage "IV": acclimatization of rooted shoots (or unrooted microcuttings, to ex vitro conditions).Significant features of *In vitro* propagation procedure are its enormous multiplicative capacity in a relatively short span of time, production of healthy and disease free plants (George 1993); and its ability to generate propagates around the year(Dhawan & Bhojwani, 1986),demonstrated that using this technology upto 400,000 plants could be from a single rose on annual basis. Such a method has considerable implications for the rose breeder as it allows rapid multiplication of new varieties. Micro-propagated plants are well suited for cut flower production as they are more compacted (onesto etal,1985),branch better and sometimes yield more flowers(Reist,1985).In addition, tissue culture derived dwarf roses used for pot plant production have a faster rate of growth, early flowering , & exhibit shorter shoots & more laterals than conventionally produced (Dubois *et al.*,1988).The history of rose tissue culture dates back to 1945,when Nobecourt & Koffler succeeded in obtaining & roots on the explanted buds.

In the year, Lamments for the first time reported the use of embryo culture in rose breeding. Studies were initiated by Tulecke (1959) & Weinstein *et al.* (1962) to culture cells, cell suspension & calli with a view understand differentiation & regeneration. The first shoot organogenesis from callus tissue was reported by Hill (1967) in a climbing Hybrid Rose "The Doctor". The earliest references of rose micropropagation were those of Jacob *et al.* Elliot (1970) in R. Hybrida cv. Superstar & R. multiflora, respectively.

2.1 ESTABLISHMENT OF ASEPTIC CULTURE

Propagation of plant tissues in culture requires aseptic techniques to ensure specimens & equipment are free of contaminants such as bacteria, fungi, or algae, that may otherwise quickly overgrow the culture & destroy it. Aseptic technique combines protocols to remove contaminants from materials (sterilization, disinfection) & procedures to maintain sterility during manipulation of material. Aseptic cultures were established in Rose by Wang *et al.* (2008). The explants are sterilized the explants by soaking them in saturated laundry powder solution for 30 minutes followed by washing them 2-3 times in running tap water. Finally, the explants were sterilized by treating with 0.1 % $HgCl_2$ for 5 minutes followed by washing with sterilized distilled water for 3 times, to remove traces of HgCl2.

Li Shuyu (2009) studied on establishment of rapid micro-propagation system by taking one year old shoot, new shoots with buds, sprouting shoots as source of explants. As per his observations, the best disinfectants was 0.1% $HgCl_2$ treated for 5 minutes.

The production of phenolic compounds & necrosis of the explants maybe controlled by dipping the explants in sterilized distilled water for 2 hours followed by dipping them in a mixture of 100mg/l citric acid & ascorbic acid for 30 minutes.

2.2 *In vitro* Culture

In vitro studies are performed with micro-organisms, cells or biological molecules outside their normal biological context. Colloquially called "test-tube experiments", these studies in biology & its subdisciplines are traditionally done in labware such as test tubes, flasks, petri dishes, & microtiter plates. The epical meristem was capable of having unlimited proliferation potential to develop into shoots. Shoot buds arose from individual cells in calluses developed as a result of wound reaction from cut & injured tissues.

2.3 THE PROPAGATION OF SHOOTS

The propagation of shoots in plants is an expensive & easy way to get new plants from plants you already have. This asexual means of reproduction produces a plant that is genetically identical to its parent. There are variety of plant propagation & methods; from taking cuttings to layering to dividing & more. The technique we select will depend on the type of plant we wish to propagate & the amount of time & effort we want to put into it. The main reason is that it gives more guarantee for clonal stability. Indeed although the rate of multiplication is generally less than that which can be brought about through shoot culture. There is less likelihood associated callus development & the formation of adventitious shoots, so that stage II subculture carries very little risk of induced genetic irregularity (George & Debergh, 2008). For this reason, node culture has been increasingly recommended by research workers as the micropropagation method least likely to induce somaclonal variation (Prakash & Van Staden 2008; Ahmad & Anis 2011; *et al.* 2011).

2.4 PLANT GROWTH REGULATORS

Plant growth regulators is a natural or synthetic chemical that is sprayed or otherwise applied to a seed or plant in order to alter its characteristics. They are sometimes referred to as plant hormones. Growers can add PGRs to their crops in order to achieve a desirable goal, ranging from increasing insect & disease resistance to increasing root strength. Some PGRs also are used to stunt growth. It can be either organic (naturally derived) or synthetic. Organic sources of PGRs includes naturally sourced amendments such as sea weed 7 liquid kelp. Commercial growers, including nurseries, generally use synthetic growth regulator

2.4.1.AUXINS

The synthetic auxins 2,4 dichlorophenoxyacetic acid (2,4-D), Naphthalene Acetic Acid (NAA) & Indole 3-Butyric Acid (IBA) are commonly used in the tissue cultures. Indeed, all active auxins are weak organic acids. The relative degree of activity of individual auxins in different growth processes is very variable. It differs not only from plant to plant, but also from organ to organ, tissue to tissue, cell to cell & moreover, also with the age & physiological state of the plant tissue (Davies, 2004).

2.4.2. CYTOKININS

Among plant growth regulators, cytokinins have proven to be the most important factor affecting shoot regeneration, & their significant effects may be related to histological changes in induced tissues (Magyar Tabori *et al.*,2010). Cytokinins are N_6 substituted adenines with growth regulatory activity in plants that promote cell division & may play a role in cell differentiation (McGaw & Burch 1995). Cytokinins added to the medium are very important during culture of plants because they induce division & organogenesis (Howell *et al.,*2003) & affect other physiological & development processes (Heyl & Schmulling 2003; Ferreira & Kieber 2005; Van Staden *et al.*.2008). Considering natural cytokinins, benzyladenine (BA), or sometimes kinetin (6-furfurylaminopurine, Kn) (Barciszewski *et al.*.1999) are the most frequently used in tissue culture systems. Vengadesan *et al.* (2002) reported that BA singly was common in most of the *In vitro* micro propagation.

CYTOKININS AND AUXINS IN SYNERGY

Since the action of cytokinin and auxin has been linked from early studies, they are known to interact in several physiological and developmental processes, including apical dominance, control of cell cycle, lateral root initiation, regulation of senescence and vasculature development (Coenen and Lomex 1997; Swarup *et al.* 2002). Exogenous applications of cytokinins & auxins have been known to be important for shoot induction and elongation of many plant species *in vitro* (George1993). Various successful combinations have been reported, such as BAP+NAA for rose.

2.5 ROOTING OF *IN VITRO* REGENERATED SHOOTS

Rooting of *in vitro* regenerated shoots and transplantation of the plantlets to the fields is the most important, crucial and essential step, but difficult task in tissue culture of woody plants (Murashige 1974). Generally rooting in micropropagated shoots can be achieved by two different method, i.e. *in vitro*& ex vivo method. Root induction and elongation are complex processes that are influenced by a large number of factors, such as genotype & concentration of PGRs, & culture conditions (Bennet *et al.* 1994; Mylona Dolan 2002). The intricacies involved in adventitious rooting were reviewed by Haissig (1974), George & Sherrington (1984), Gasper *et al.* (1994) & Rout *et al.* (2000). Many researchers emphasized on ex vivo rooting because plants developed after ex vivo rooting have better root system than the plants raised after *in vitro* rooting (Borkowska 2001). In addition, ex vivo technique is comparatively less time consuming & cost effective, & requires less labour, chemicals & equipments than *in vitro* rooting because plantlets rooted ex vivo does not need any additional acclimatization prior to transplanting in the field conditions (Yan *et al.* 2010). The main advantage of ex vivo rooting is that the chance of root damage is less, rooting rate are good & root quality is better (Bellamine *et al.* 1998).

2.6 ACCLIMATIZATION

Acclimatization of *In vitro* raised plants in the greenhouse & the open field was reviewed by Preece & sutter (1991). Pierik *et al.* (1987) worked on acclimatization of *in vitro* raised plants acclimatized successfully under greenhouse conditions at low irradiance & high relative humidity for the first two weeks after transfer. Binet *et al.* (2007) took the help of VAM to increase rate of survival of *in vitro* grown plants of olive. He inoculated the micro plantlets with arbuscular mycorrhizal fungus (Glomus mosseae) during acclimatization, which significantly improved plant survived & subsequent plant development & growth.

MATERIALS AND METHODS

3.MATERIALS AND METHODS

3.1 PLANT MATERIAL

Choice of explant for initial culture is largely dictated by the method to be adopted for *in vitro* propagation. Most commonly used explant is the nodal stem segment containing axillary buds of actively field grown Hybrid Tea rose. They were cut into 3-4 cm length segments.

Different parameters influence the initial stage of micropropagation. Nodal segment taken from soft wood stem were more responsive than the woody stem. The rate of multiplication also depends upon the position of the node on the stem. The buds nearest to the base of the stem exhibited the slowest rate of development, but those from mid-stem region grewveryrapidly.

3.2 SOURCE OF EXPLANTS:

The explants were collected from the plants maintained in the garden of All India Co-ordinated Research Project on Floriculture, OUAT. The explants were then washed thoroughly in the running tap water to remove all the adhering dust or soil particles. The lateral buds were used as explants.

3.3 CULTURE MEDIA:

M.S. media (Murashige and Skoog, 1962), the most commonly used media was used as the basal media in all the experiments. The inorganic & organic constituents of the M.S. Media are listed in Table 1. Three percentage sucrose was routinely used as the carbon source. In this experiment solid media was used. The growth regulators and other additives were included in the basal media, either alone or in combinations, to test their efficiency for inducing, promoting & regulating the process of growth & differentiation**.**

TABLE 1 Composition of the stock solution for M.S. Medium (1962)

i)Major salts in 1000ml of stock solution (macronutrients):

$CaCl_2.2H_2O$	**4.4g**
KNO_3	**19.0g**
NH_4NO_3	**16.5g**
KH_2PO_4	**1.7g**
$MgSO_4.7H_2O$	**3.7g**

(Each chemical was dissolved separately, then water is mixed to make up the final volume to 1000ml).

ii)Minor salts in 100ml of stock solution:

KI	**83mg**
H_3BO_3	**620mg**
$COCl_2.6H_2O$	**2.5mg**
$Na_2MoO_42H_2O$	**25mg**
$MnSO_4.4H_2O$	**2230mg**
H_3BO_3	**620mg**
$ZnSO_4.4H_2O$	**860mg**

iii)Iron Compounds in stock solution:

Na_2EDTA	**373mg**
$FeSO_4.7H_2O$	**278mg**

iv)Myo-inositol **0.1g/l**

v)Vitamins in 100ml of stock solution:

Nicotinic acid	**50mg**
Pyridoxine-HCL	**50mg**
Thiamine-HCL	**10mg**
Glycine	**200mg**

Nicotinic acid was first dissolved in 50ml of warm distilled water; after cooling, the other two vitamins were added. The final volume was made up to 100ml. MS Medium(1000ml) was prepared by adding the stock solution in the following concentration.

a)Major salts	10ml
b)minor salts	10ml
c)$CaCl_2$	10ml
d)Na_2-FeEDTA	5ml
e)Vitamins	1ml
f)Myo-inositol	0.1gm
g)Sucrose	30gm
h)Glycine	2ml

3.4 GELLING AGENT

Agar, the widely used gelling agent was used to solidify the medium in all the experiments. 5.8gm/l of agar was added to the medium after adjusting the pH (5.7), prior to autoclaving.

3.5 pH Of THE MEDIA

After mixing all the salts and hormones the volume of the media was made up using distilled water. The pH of the media was adjusted to 5.7 in all experiments. The M.S. Medium was autoclaved at 121°C, 20 minutes for sterilization of the medium.

3.6 SOURCE OF CARBOHYDRATE AND ITS CONCENTRATION

A carbohydrate supplement to the medium was essential to maintain the osmoticum for normal growth of explants. Sucrose at a concentration of 30g/l was added in all media in addition to growth regulators.

3.7 GROWTH HORMONES

The stock solution for plant growth regulators were prepared as follows**:**

i) AUXIN

20mg of IAA/ NAA/ IBA was dissolved separately in the minimum quantity of ethanol, the volume was then made up to 80 cc by adding sterilized distilled water.

ii) CYTOKININ

20mg BAP was first dissolved in minimum quantity of 0.1N HCL, the volume was made up to 80ml with sterilized water.

The combination of hormones used in media for initiation of cultures.

TREATMENTS	BASAL MEDIA	BAP(mg/l)	IAA(Mg/l)
T1	M.S	0	0
T2	M.S	0.2	0.01
T3	M.S	0.4	0.01
T4	M.S	0.6	0.01
T5	M.S	0.8	0.01
T6	M.S	0.2	0.02
T7	M.S	0.4	0.02
T8	M.S	0.6	0.02
T9	M.S	0.8	0.02

3.8 CULTURE CONDITION

In this experiment, all the cultures were incubated under 16hours photoperiod at a light intensity of 3000 lux provided by cool, white, fluorescent lamps. The temperature and humidity of the incubation room was maintained at 25$\pm$2° C and 65-75% RH (Relative Humidity) respectively.

3.9 STERILIZATION OF EXPLANTS

Initiation of aseptic culture starts with washing the buds in running water for few minutes. The explants kept in sterilized water with few drops of Labolene

RESULTS AND DISCUSSION

for 20 minutes for removal of dust followed by the treatment in 0.2% (w/v) of Bavistin for 30 minutes. The young tender explants were surface sterilized for 5 minutes in Mercuric chloride, sodium hypochlorite, alcohol, chloroform, mercaptoethanol & ethidium bromide.

4. RESULT

4.1 Sterilization of explants

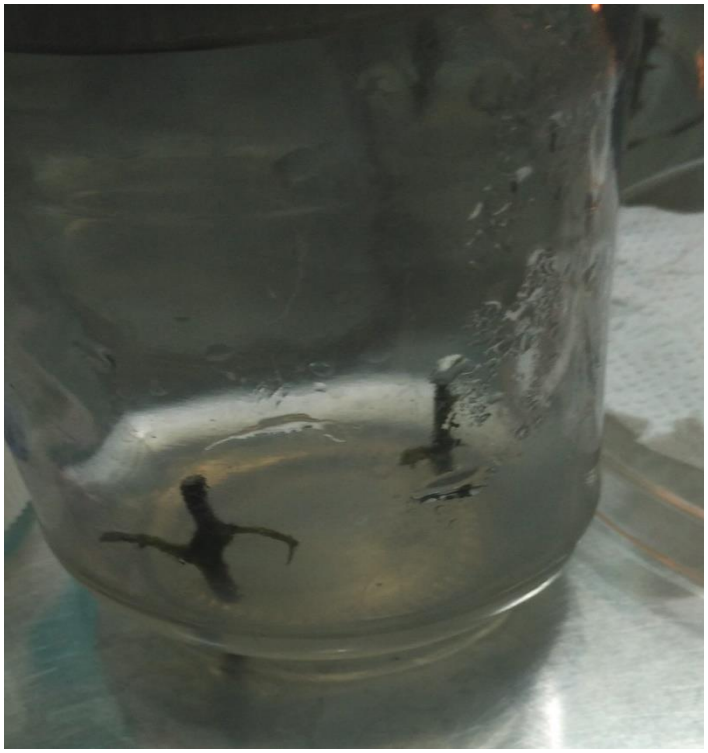
The explants were treated with different surface sterilants and the results are given below.

CHEMICALS	CONCENTRATION	OBSERVATION
Mercuric chloride	0.05%	Survived
Sodium hypochlorite	1%	Survived but infected
Ethanol	70%	Died
Chloroform	15%	Died

Among the surface sterilants used mercuric chloride at the concentration of 0.05% was found to be very effective in surface sterilization of the rose explants. The lateral shoot explants treated with mercuric chloride @0.05% resulted in infection free cultures.

4.2 ESTABLISHMENT IN MEDIUM

In this stage all explants were cultured on the M.S medium without hormones. Explants are established in the medium because in the result of oxidation of polyphenols released from the cut surface of the explants, which made the media black. After 3-4 days the explants sub-cultured to another fresh medium which is highly effective in enhancing the growth of primary explants. It was incubated in a culture room under 16 hours photoperiod with irradiance provided by proper temperature and cool white fluorescent tubes.



4.3 SHOOT MULTIPLICATION

This is the most crucial stage of micropropagation. The success rate of micropropagation to a large extent depends on the rate and mode shoot multiplication**.** For shoot multiplication the basal nutrient medium contain M.S medium combined with BAP and IAA hormone in different concentrations (in table 2). After the better hormone compositions were used in the media, the explants were sub-cultured in a fresh media in every 4 weeks. It took nearly 60 days for proper shoot development. Finally, single shoot excised from multiple shoots for root induction. Data was recorded regularly in a time interval.

4.3 EFFECTS OF GROWTH REGULATORS ON SHOOT MULTIPLICATION

Among the different concentration of growth regulators tested, the maximum percentage of multiple shoots were initiated on MS media supplemented with 0.2mg/l BAP & IAA 0.01mg/l. The cultivars did show very slow response on MS medium devoid of growth regulators. Induction of multiple shoots was better on MS medium supplemented with BAP+IAA. In 'Gold Medal' maximum percentage of multiple shoots was observed in MS medium supplemented with BAP(0.2mg/l), IAA(0.01mg/l). The average number of shoots per culture was the maximum in MS medium supplemented with BAP and IAA.

TABLE: EFFECTS OF HORMONES ON SHOOT MULTIPLICATION IN ROSE CV. Gold Medal

Sl no.	treatment	no. of shoots	no. of leaves	length of shoot (in cm)
1	T1	0	0	0
2	T2	4.2	9.6	3.24
3	T3	3.4	6.8	2.68
4	T4	2.6	4.6	2.26
5	T5	1.8	3.4	1.72
6	T6	3.0	6.0	2.30
7	T7	2.6	4.6	1.50
8	T8	1.4	2.6	0.84
9	T9	0.8	1.2	0.50

Mean of 5 cultures

5.DISCUSSION

Micro-propagation techniques are becoming important for mass cloning of many important plant species because of the uniformity and potential for planned production. Several chemical and physical factors influenced the establishment of micropropagation of plants. Shoot multiplication in Gold Medal was achieved on medium containing 0.2mg/l BAP, 0.01mg/l IAA within 8 weeks of culture.

The results indicate that the rate of multiplication of shoot declined as the concentration of BAP increased. The success of micropropagation was greatly influenced by the composition of the culture medium besides the osmoticum and the nutrient medium in the culture.

CONCLUSION,SUMMARY AND REFERENCE

6.CONCLUSION

The surface sterilization of Rose cv. 'Gold Medal' explants were successfully carried out with $HgCl_2$ at the concentration of 0.05% for 5 minutes. Maximum percentage of multiple shoots were observed in Rose on MS medium having 0.2mg/l BAP, 0.01 mg/l IAA.

7.SUMMARY

High frequency plant regeneration of Hybrid Tea Rose cv. Gold Medal through *in vitro* techniques was attempted by manipulation of nutrient media culture conditions. Nodal segment of plant were used as explant source of multiplication. Bud break was achieved on MS media without any supplemented hormone in Gold Medal. The explant release phenol from the base in 4-5 days. Shoot multiplication was observed when MS medium was supplemented with BAP (0.2mg/l), IAA (0.01 mg/l). The percentage of shoot multiplication was maximum in Gold Medal on MS medium having BAP (0.2mg/l), IAA (0.01 mg/l) than other compositions and 3% sucrose within 4 weeks of sub culture. The frequency of shoot multiplication was the highest in medium supplemented with BAP, IAA

9.REFERENCE

1. Micropropagation of roses (Rosa spp.): the effects of different media on *in vitro* rooting Metka SISKO, University of Maribor, Faculty of Agriculture and life sciences, Slovenia.

2. Barve, D.M.; Lyer, R.S.; Kendurkar, S., *et al.*. aneffective method for rapid propagation of some budded rose varieties. Indian J. Hortic. 41(1/2):1-7; 1984.

3. *In vitro* micro propagation of disease free rose (**Rosa indica** L.) Nosheen Hameed 1, Asad Shabbir 1, Aamir Ali 2 and Rukhsana Bajwa 1 Department of mycology & plant pathology, university of Punjab, Lahore, Pakistan. Punjab university seed centre, Quaid-e-azam campus, Lahore, Pakistan.

4. micropropagation of rose cultivars: Biotechnological application Floriculture Saklani kumud*, pant hem 1 and rawat vijay 2

5. department of biotechnology, Shri Guru Ram Rai (PG) College, Pathri Bagh, Dehradun, Uttarakhand.

6. Layous, L.N. (2004). Micropropagation of Jasmine *(jasminum azoricum* L.) using tissue culture techniques. king faisal university, A1-Hassa Kingdom (Saudi Arabia). College of agricultural and food science.

7. Lambardi, M., Ozudogru, E.A. and Roncasaglia, R. (2013). *In vitro* propagation of olive (*Olea europia* L.) by nodal segmentation of elongated shoots; Protocols for Micropropagation of selected Economically-Important Horticultural Plants.

8. Murashige, T. (1974). Plant propagation through tissue culture, annual review of plant physiology, 25:135-166.

9. Study of culture conditions for improved micropropagation of hybrid rose S.K. Senapti, G.R. Rout Plant Biotechnology Division, Regional Plant Resource Centre, Odisha, India

10. *In vitro* propagation of rose-a review Pratap Kumar Pati a,*, Siba Prasad Rath b, Madhu Sharma c, Anil Sood c, Paramvir Singh Ahuja ca Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar-143 005,, India, b P.G. Department of Botany, Utkal University, Vani Vihar, Bhubaneswar-751004, India c, Division of Biotechnology, Institute of Himalayan Bio-resource Technology, Palampur-176061(H.P), India

11.Micropropagation from cultured nodal explants of rose (Rosa Hybrida L. cv. 'Perfume Delight') NaphaporNak-Udom, Kantamaht Kanchanapoom & Kamnoon Kanchanapoom Centre for Genomics & Bioinformatics Research, Department of Biology Faculty of Science, Prince of Songkla University, Hat Yai , Songkhla, 90112 Thailand.

12. *In vitro* propagation of rose – A review 5 authors, including: Pratap Pati, Guru Nayak Dev University Madhu Sharma, CSIR- Institute of Himalayan Bio-resource Technology Anil Sood, CSIR-Institute of Himalayan Bio-resource Technology Paramvir S Ahuja, CSIR- Institute of Himalayan Bio-resource Technology.

13.Jasmine spp. (jasmine): Micropropagation & production of Essential oils R. Jonard.

THANK YOU