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Plant Biotechnology

[B.Sc. Biotech Part-III]

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Note: **A feedback form is enclosed along with think tank. Kindly fill the feedback form and submit it at the time of submitting to books of library, else NOC from Library will not be given.**

Ritu Dhingra

Syllabus

Section A

1. Tools of plant biotechnology: Inducible gene expression systems as tools for plant functional genomics, Somatic embryogenesis system and artificial seed production.
2. Plant genetic transformation: Methods and emerging trends. Screening procedures. Micropropagation- Seed versus soma, use of micropropagation in multiplicaotn of specific genotypes, hardening of micropropagated plants & their transfer to soil.

Section B

3. Micropropagation methods for the following category of plants(one example for each category) – (a) Floriculture, (b) Horticulture, (c) Medicinal and ornamental plants, (d) Cereal, pulse, oilseed and fiver crops, (e) Forest trees, fruit trees-Problems in propagating trees namely systemic contaminates, phenolic leaching, seasonal variation in response to genotypic recalcitrance. Designing new plant genotypes with enhance res9istance to salinity.

Section C

4. Micropropagation and variation among species– The engineering of recombinant plastids in higher plants.
5. Isolated Microspore Embryogenesis in cereals: Aspects and Prospects.
6. In vitro propagation of tropicl and sub-tropical fruit crops.
7. Application of biotechnology: in Indian ginseng (Ashwagandha).
8. Ornamental foliage plants improvement through biotechnology.
9. Wild species of Oryza as an important reservoir of useful alleles.
10. In vitro propagation of tropical and sub-tropical fruit crops. Genetic transformations of foodgrains, legumes, fruits and horticultural crops.
11. somaclonal variation: Application and limitation, Exploitation for selecting superior paonetype disease resistant, stress tolerant, high secondary metabolite producing.

12. Disease elimination. Morphogenesis, regeneration of plantloes, multiplication of plantlets, rooting, .
13. commercial production of tissue cultured plants-(i) Technology transfer, equipment and procedures, (ii) Aseptic techniques and control of contamination in a commercial laboratory, quarantine, pathological indexing, packaging, cost analysis,
    marketing.

Section D

14. GM crops and protoplast cufure – Genetic improvement of plants through tissue culture-comparison with classical methods(a) Transgenic Plants, antisense RNAs, tissue specific sequences, elimination of plant viruses, Homozygous plant production through anther, ovule, pollen cultures.
15. *In vitro* pollination and fertilization, embryo rescue, endosperm culture and production of seedless plants.
16. Protoplast culture and its genetic improvement: (i) Somatic hybridization, cybrids: limitations, (ii) Micromanipulaitn of genes using protoplasts,
17. Genetic engineering in plant: Identity sreservation in Genetically Modified Crops.

Contents

Section –A

Tools of Plant Biotechnology & Plant genetic transformation

Multiple Choice Questions

Q.1 The method of producing thousands of plants through tissue culture is called
A) Micropropagation
B) Somatic hybridization
C) Biofortification
D) Biomagnification
Ans. A) Micropropagation

Q.2 Which one of the following is commonly used in transfer of foreign DNA into crop plants?
A) Penicillium expansum
B) *Trichoderma harzianum*
C) *Meloidogyne incognita*
D) *Agrobacterium tumefaciens*
Ans. D) *Agrobacterium tumefaciens*

Q.3 What is/are the benefit(s) of micropropagation or clonal propagation?
A.Rapid multiplication of superior clones
B.Multiplication of disease free plants
C.Multiplication of sexually derived sterile hybrids
D.All of the above
Ans. D) All of the above

Q.4 Cellular totipotency is the property of
A.plants
B.animals
C.bacteria
D.all of these
Ans. A) Plants

Q.5 Agrobacterium based gene transfer is efficient
A.only with dicots
B.only with monocots
C.with both monocots and dicots
D.with majority monocots and few dicots

Ans. A) only with dicots

Q.6 Which one of the following is a case of **wrong** matching?
A Micropropagation-In vitro production of plants in large numbers
B Callus - Unorganized mass of cells produced in tissue culture
C Somatic hybridization - Fusion of two diverse cells
D Vector DNA - Site for t-RNA synthesis

Ans. D) Vector DNA - Site for t-RNA synthes

Q.7 Which part would be most suitable for raising virus-free plants for micropropagation?
A Meristem
B Node
C Bark
D Vascular tissue

Ans. A) Meristem

Q.8 For transformation, micro-particles coated with DNA to be bombarded with gene gun are made up of
A Silicon or Platinum
B Gold or Tungsten
C Silver or Platinum
D Platinum or Zinc

Ans. B) Gold or Tungsten

Q.9 In plant biotechnology, PEG is used in
A Protoplast fusion
B Cell culture preparation
C Protoplast isolation
D Hardening

Ans. A) Protoplast fusion

Q.10 Virulence trait of Agrobacterium tumefaciens is borne on

A.chromosomal DNA
B.tumour inducing plasmid DNA
C.both chromosomal and plasmid DNA
D.cryptic plasmid DNA

Ans. B) tumour inducing plasmid DNA

Q.11 The size of the virulent plasmid of Agrobacterium tumefaciens is
A.40-80 kb
B.80-120 kb
C.140-235 kb
D.>235 kb

Ans. C) 140-235 kb

Q.12 Co-integrating transformation vectors must include a region of homology in
A.the vector plasmid
B.the Ti-plasmid
C.between vector plasmid and Ti-plasmid
D.none of these

Ans. C) between vector plasmid and Ti-plasmid

Q. 13 Opines are
A.amino acid derivatives found in tumor tissues
B.amino acid derivatives found in normal tissues
C.amino acid derivatives found in both normal as well as tumor tissues
D.none of the above

Ans. A) amino acid derivatives found in tumor tissues

Q.14 In the liposome mediated gene transfer in plants, nucleic acids are
A.protected from nuclease digestion
B.stable in liposomes
C.both (a) and (b)
D.not stable in liposomes

Ans. C) both (a) and (b)

Q.15 Advantage of microprojectile method over microinjection method for gene transfer in plants include
A.intact cells are used
B. method is universal in its application irrespective of all shape, size, type and presence or absence of cell wall
C.gene can be transferred to many cells simultaneously
D.all of the above

Ans. D) all of the above

Q.1. Write short notes on :

(a) Somatic embryogenesis;

(c) Inducible gene expression systems as tools for plant functional genomics..

Ans 1. (a) Plant cells are tati potent and can produce whole new plants under favorable conditions of nutrients and plant growth regulators.

These somatic embryos were similar to zygotic embryos in development and structure. The origin of somatic embryos morphologically developed through the 3 stages:-

1. Globular
2. Heart
3. Torpedo

Embryogenesis is a two step process:-

Induction of embryogenesis Development of embryo ultimately leading to germination.

It is of 2 Types:-

1. Direct embryogenesis
2. Indirect embryogenesis

Indirect embryogenesis:- When explants produce callus and the callus forms then its known as indirect embryo. Direct embryo:- Eves is when embryo occur direct on the explants without production of callus it known as direct embryo.

- ❖ When exogenously supplied Quxin is present in appropriate concentration for the indi somatic embryogenesis from callus or explants.
- ❖ In direct embryogenesis cells of explant are already determined for embryonic and termed as per-embryogecsnic determic (PEDC's).
- ❖ In direct embryogenesis cells require redetermination through a period and termed as induced embryogenic developing (IEDC's)
- ❖ Embryogenesis occurs form a single cell or from a group of cells.
- ❖ Embryogenic cells are small cellor isodiametric shape, filled with dense cytoplasm and conspicuous nucleus.

- When somatic embryos, early stage or developed are transferred on induction medium they give rise to somatic embryos. This method of obtaining embryos recurrently is known as repetitive or cyclic embryogenesis. This method is

useful for continuously obtaining embryos in large no eg in Jatropa balladona, Ranemculus.

- During somatic embryogenesis in cell suspension embryos of different sizes are produced, this can be achieved by sieving or fractionation of suspeusic…. With appropriate sieve size. Such cultures may be fully synchronized for their growth.

Factor's Affecting Somatic Embryogenesis

(a) **Composition of medium:-** Levels of sucrose and nitrogen is to be monitored Reduced nitrogen is not require for the induction and nitrogen alone in high amount is sufficient for induction of somatic embryos however reduced nitrogen in the embryo development medium supports embryo development increased osmotic concentrations by sucrose affect the embryos.

(b) **Auxins**: -2,4 D appear to be required for embryo induction but adversely affect embryo development.

(c) **Cytokinins**:- Except zcatin suppress embryo genes.

(d) **Ethylene**:- Suppresses embryogenesis.

(e) **Abscisic acid** :-

- Suppress abnormal development embryos.
- Imparts dormancy and help the formation of cotylidonary stage somatic embryo.

Applications: -

(i) It provides potential planfect in the form .. somatic seeds. It can be used for the production of synthetic seeds.

(ii) Somatic embryos provides organized culture system such cultures produce organ specific or differentiation related compounds in high Amount compared to cell culture of that sps..

Advantages Of Somatic Embryogenesis

Rapid multiplication through cell culture and use of bioreactors.Presence of bipolar structure avoids the rooting steps required in organogenesis. Possible to induce dormancy and store the culture for long duration.Possibilities of encapsulation and other methods of packing and direct delivery system can be employed.

Ans. (b) Tet-On Advanced Inducible Gene Expression System

- **Virtually eliminate background expression.**
- **Doxycycline sensitivity is increased tenfold.**
- **Well-suited for mammalian cells and transgenic animals.**

The Tet-On Advanced rtTA protein (Urlinger, S., et al. 2000) contains mutations that both enhance its sensitivity to doxycycline (Dox) tenfold and diminish residual binding to tetracycline reponse elements (TREs) in the absence of Dox Moreover, human codon optimization, combined with the use of 3 minimal VP16 transcription activation domains, enable signifi cantly elevated expression levels of the rtTA-Advanced protein.

Express 2 genes with Bidirectional Tet Expression Vectors

Each pTRE-Tight-BI vector contains a bidirectional promoter, composed of a modifi ed TRE, flanked by minimal CMV promoters. This configuration allows gene expression studies based on coregulation of two independent genes (pTRE-Tight- BI) or co expression of your gene of interest with a fl uorescent reporter (AcGFP1, ZsGreen1, DsRed-Express, or DsRed2),

Improved TRE Response Plasmid

pTRE-Tight contains a modifi ed TRE that reduces basal expression and binds tightly to rtTA-Advanced. In the presence of Dox, rtTA-Advanced binds to the TRE-Tight promoter and activates transcription of your downstream gene.

n Expressi Syst

Q.2 Explain micropropagation technique. How is it useful in propagation of rare and/or endangered variety?

or

Write short notes on : Medicinal and rare plants

or

Application of biotechnology in Indian ginseng

Ans: *Withania somnifera* Dunal, a member of the Solanaceae, is a widely used medicinal species useful in the treatment of inflammatory conditions, tuberculosis, rheumatism, as a tonic, or as an anti-tumour agent (Chopra et al., 1958; Suffness and Douros, 1982). This species also contains tropane alkaloids and withanolides (Tyler et al., 1981). Propagation is mainly by seed, but seed viability is limited to one year. Roja et al. (1991) reported callus formation from axillary meristem explants in MS medium with 2,4-D (2 mg /l) whilst Baburaj and Gunasekaran (1995) have also reported callus induction from leaf explants of *W. somnifera* usingMS medium supplemented with NAA (2 mg /1) and KN (0.5 mg /l).

Materials and methods

Plants of *W. somnifera* grown in the glasshouse can be used as experimental material. Plants were grown in the beds. The temperature in the glasshouse varied from 28 to 32 C. No artificial light was provided. Different explants were used.

Axillary leaves and nodal segments were taken from 1 year-old plants while hypocotyl and root segment explants were removed from 10–15 day-old seedlings.

Axillary leaves

Axillary leaves (0.5–0.8 cm) taken from 1st to 3rd node from the apical region of lateral branches were surface-sterilized with 0.01% (w/v) mercuric chloride for 2–3 min, washed 3–4 times with sterile double distilled water and inoculated on agar-solidified MS medium supplemented with different concentrations of 2,4-D and KN, either alone or in combination. The pH of the medium was adjusted to 5.6 before sterilization. Cultures were maintained at 25C with a 16-h photoperiod and white fluorescent 24 tubes. Callus was subcultured after 25 days on the original callus-inducing medium.

Axillary shoots

Axillary shoots arising from the nodes of nodal segments were used for establishing callus. Nodal segments (1–2 cm) were taken from lateral branches and surface-sterilized as for leaves with 0.1% mercuric chloride and cultured on agar-solidified MS medium supplemented with different concentrations of BA and KN.

Hypocotyls and root segments

Seeds normally collected from sibbed plants and stored at 4 _C for about 6 months were used. These were soaked in water for 24 h, washed with 5% (v/v) teepol solution (5 min), surface-sterilized with 0.1%mercuric chloride (12–13 min), washed 3–4 times with sterile double-distilled water, and inoculated on half strength MS medium with 1% (w/v) sucrose without any growth regulators (Sen and Sharma, 1991). Seeds were incubated at 25 _C (dark) for 1 week and then transferred to white fluorescent lights with a 16-h day length. Hypocotyls and root segments of 10–15 day old seedlings were cultured on the MS-based agar solidified medium supplemented with 2,4-D (2 mg/l) and KN (0.2mg/l) for callus formation. Tubes inoculated with root segments (0.8–1.0 cm) were incubated both in light and dark at 25 _C. Callus obtained from various explants was used for regeneration assessments on MS medium supplemented with 2,4-D, KN or BA, either alone or incombination. For each treatment, 25 tubes were inoculated with desired explants, i.e. axillary leaves, axillary shoots, hypocotyls and root segments for callus induction and incubated under optimal conditions as defined above. The experiment was terminated after an interval of 30 days.the callus obtained from each explant was inoculated into the tubes containing MS medium supplemented with 2,4-D, kinetin or BA, either alone or in combination

.

Results and discussion

(a) Callus initiation

Callus initiation appeared, for axillary leaves, after 7–8 days, and callus was creamish-white and friable. Callusing was best (92%) on medium supplemented with 2,4-D (2 mg/l) and KN(0.2mg/l). Copious callus was found at the base of axillary shoots emerging from the nodes on MS medium with BA (2 mg/l). Hypocotyl callusing (90%) on MS medium was noticed between 15 and 20 days. Callus was also creamish and friable when the medium contained 2,4-D (2 mg/l) and KN (0.2 mg/l). Root segments of seedling explants showed a 100% callusing response (30 days) both in the light and dark.

(b) Shoot regeneration

Shoots were initiated, from axillary leaf-derived callus,only on medium supplemented with 2,4-D (2 mg/l) and KN (0.2 mg/l). Callus obtained from the axillary shoot base showed multiple shoot formation with BA (2 mg/l) alone. Shoot buds were initiated from hypocotyl-derived callus on medium supplemented with 2,4-D (2 mg/l) and KN(0.2 mg/l). Shoots were excised and transferred to medium supplemented with BA (2 mg/l) alone to faster multiplication. Callus derived from root segments did not regenerate To increase rooting, shoots derived from hypocotyls or axillary shoot base callus were excised and cultured on MS medium with different concentrations of auxins. After 12–15 days post-transfer to rooting medium, roots appeared and, by day 30, many were found to be 4.5–5.5 cm long. Regenerated shoots obtained from the axillary shoot base callus were rooted best on MS medium containing IBA (2 and 4 mg/l), or IBA (2mg/l) with IAA(2 mg/l) .Plantlets were transferred to pots containing a sand/soil mixture (1:1) initially covered with beakers, and kept at 25C and transferred (25– 30 days) to the glasshouse.

(a) The survival rates were as high as 83%. Callus from root segments with 2, 4-D (2 mg/l) and KN (0.2mg/l).

(*b*) Multiple shoots obtained from axillary shoot base callus.

(*c*) Transplanted plant in pots. Callus induction and shoot regeneration for axillary leaves, axillary shoots,

Hypocotyls and root segments of *W. somnifera* on MS medium supplemented with various growth regulators after 30 days of initial culture. The experiments were repeated twice, each experiment consisting of 25 replicates.

The comparison of callusing and shoot regeneration potential of different explants showed that axillary shoot explant is the best one, as its callusing capacity was 83% and the number of shoot averagewas 8 per callus. Moreover, the height attained by the shoots within 30 days was approximately twice that of hypocotyls and 6-fold that of axillary leaves. Though root segment was best in callusing potential, no shoots could be regenerated from this source of callus even by using different combinations of growth regulators.

Withania somnifera is normally propagated through seeds and, being an open pollinated plant, the inherent variability attributable to recombination is expected. It is, therefore, not unusual that chemotypes on the basis of withanolides have been recognized (Kirson et al., 1971; Glotter et al., 1973). This species is of economic interest for its wide ranging pharmacological activity and one of the major constraints in utilizing natural populations is the existence of plant to plant chemovariability.A standard protocol to induce multiple shoots in culture may provide a more homogeneous source of plants.

Q,3 Explain micropropagation technique. How is it useful in propagation of specific genotypes, endangered varieties?

Ans. In *Dianthus superbus* ssp. *superbus* which is critically endangered, the possibility of multiplying was tested with help of tissue culture. *In vitro* the culture was derived by sterile sowing of seeds. Species *Diantus superbus* ssp. *superbus* was described by many authors taxonomically (Dostál 1989, Kovanda 1990, Podpìra 1906). In Moravia it was signed as largest from genus *Dianthus* (Podpìra1906, Èelakovský 1879). In present it belongs among critically endangered species **(**Holub, Procházka et. Èeøovský 1979, Rybka 1995). One of possible methods of protection of endangered taxon is multiplying and conservation of plants with help *in vitro* cultures. This multiplying of plants with help of explant at culture is called as a micropropagation.

The reason is that parts of plants used for multiplying are very small in comparison with classic vegetative multiplying (0,5.1,0 mm). Micropropagation has a lot of advantages. For example: high coefficient of multiplying, small demands on number of initial plants, small demands on space and multiplying of plants regardless seasons of the year (Kováè 1995). On the other side there are some disadvantages, which essentially restrict using tissue cultures in multiplying of endangered species and so they represent one of the last solutions of this problem. Disadvantages are e. g. economic (expensive equipment, high elaborateness) and multiplying of high number of genetic identical plants has no sense from protection stand point of view. There is a possibility of occurrence of somaclonal variability, which can be successfully eliminated by suitable methods of micropropagation (Novák 1990, George 1993). Some authors think over utilization of somaclonal variability to intensify genetic variability of extremely reduced populations of endangered species. Nevertheless it is important to evaluate variability of regenerated plants. For that we rise morfological and cariological criteria, identificate isozyme marker or watch variability on level DNA with help of methods of molecular biology e. g. PCR, RFLP (Schaal et al. 1991, Potter et Jones 1991). Multiplying of endangered species should be used in these species, which have very strongly reduced populations, in species with low production and germinative activity of seeds and in species which cannot be sexually reproduced.

Material and methods

(a) Establishing of plant tissue culture and setting conditions *in vitro*.

As collected initial seeds material was used *Dianthus superbus* ssp. *superbus* .From the seedlings explant at culture was derived by sterile sowing. Seeds were wrapped into filter paper and they were shaken for 15 minutes in 15% solution of Savo super with three drops of Jar. Then they were washing three minutes three times in sterile water. Then the seeds were placed into test tubes in aseptic condition, contained 10 ml of modified MS media. Test . tubes with seeds were put into cultivation room, where germination passed at the temperature of 25 °C, photoperiod 16 hours, illumination 2000 lux. Shoots forth plants of the test growing clone were cut into pieces and after multiplying on initiatory medium were tested on 6 types of medium with various contents of growing regulators . Nodal segments were cut off leaves and then they were into cultivation glass with the set medium. Three nodal segments were put into each cultivation test . tube, and on each type of medium 80 segments were placed. The testing was carried out twice. Cultures were cultivated in the same conditions as initial culture. For assessment of growth of regulators the coefficient of increasing was evaluated (the number of shoots arising from one explantat), the length of shoot in mm, the number of nodes arising from one axillar shoot, the percentage of rooting plants and appropriate formation of callus. The growth was evaluated after 30 days.

(b) Transfer of plants into unsterile conditions

After cultivations *in vitro* the plants were taken out of cultivation test tube and rinsed to be protected against contamination on the rest of media. Then they were put into four plastic boxes and were planted out into clammy perlit. Planted boxes were covered with transparent plastic lid and placed into cultivating room at the temperature 25±1 °C , illumination 2000 lux and photoperiod 16 hours. Plants were cultivated in boxes for 30 days. Then the boxes were brought into a greenhouse, where the aclimatization of plants passed. Ventilation in boxes was carried out twice a day during the period of 30 days. After aclimatization of plants they were put into flowerpots contained mixture of horticultural substrate and perlit in ratio 3 : 1. After next 30 days the plants in size about 10 cm were placed into experimental area, where the control of their growth was assessed. The percentage of successfully transferred plants were evaluated on every stage of their transfer into field condition.

Results

The establisment of tissue culture and setting of suitable condition of cultivation *in vitro*. The evaluation of growth of explantat was carried out after 30 days . The coeficient of increasing on particular types of media was not significantly different. The highest value was reached on medium H2 (BAP mg×l.1) where it was 1,5±0,35, the smallest on medium H1 with content (BAP 2,25 mg l.1 and IBA 0,1ml×l.1) and it

was 1,1±0,29. longest axial shoots were created on medium H3 without growth regulators (45,32±15,87). The shortest shoot grew on H1 medium (2,25 mg×l.1 and IBA 0,1 mg×l.1) and it was 9,16±3,42. The greatest number of nodes on first shoot was created on medium H3 and it was 4,4±0,93. The greatest percentage 100% of rooting shoot was created on medium H5 with IBA (0,1 mg×l.1). On medium H6 (without BAP, IBA) rooting percentage 97,5% of shoot and on medium H3 rooting percentage of shoot 86,6%. Calus was not created on any medium, only H1 medium 0,64% and H4 0,68% of explantats. Transfer of plants into unsterile conditions, rooting and acclimatization Transfer of plants derived *in vitro* carried out in several stages. In the first part of transfer 4,5% of plants died (tab. 4). The greatest percentage of dying represents the beginning of indurating of plants in a greenhouse. Dying was 26,75%. In the next steps of transfer into unsterilized condition the dying was not so high. During setting of plants into flowerpots the loss was 4,5% and after setting on experimental area the loss was 2,75%. The percentage of successfully transferred plants was 61,5% from the whole number of 100 plants during two repetitions.

Discussion

The creation of axilry shoots was the greatest on medium H2. The meaning full influence of cytokinins, which stimulate proliferation of axilry shoot from lateral buds and partial creation of callus was not so significant after one month of cultivation as in species *Dianthus arenarius* (Kováè1992). The lowest coefficient of increasing was noticed on medium H1. The inhibitor influence of auxin appeared on media H5,H6 and it regenerated one long axillar shoot. Plants on media H3 without growth regulators reacted similarly. On the other hand cytokinines inhibit the effect of apical peak on side buds and stimulate proliferation of axilar shoots from these buds. This effect is usual *in vitro* cultures (Pierik 1987) and was also described in various species *Dianthus* **(**Crouch et al. 1993, Kováè 1995, Kozai et al.1998) and was described even in species *Dianthus arenarius* ssp. *bohemicus* (Kováè 1995). The influence of cytokinins can cause the creation of adventitious shoots and possible fyziological and genetic changes (somaclonal variability) (Kováè,1995). Creation of roots was inhibited by content of BAP, on the other hand auxin had a stimulating influence on creation of roots. There was great precentage of rooting shoots on both medium H5 and H6. On medium H6 shoots rooted less what can confirm that too high concentration of auxins can inhibit the growth of roots (Kováè1992, Procházka 1998). On medium H3 there was great percentage of root shoots even without the influence of auxins. The longest shoot with the greatest number of nodes were created on medium H3 without growth regulators. These shoots gave during cutting into one nodal segments high number of explantlets. Partial creation of calus, which appeared on media containing cytokinins increase the risk of rise origin of somaclonal variability. Regeneration of unaltered genetically stable plants can be reach if only the culture *in vitro* without the stage of dedifferentiate and calus proliferation is developing (Novák 1990). From that reason it is more suitable for micropropagation of *Dianthus superbus* ssp. *superbus* to choose cultivating media, on which the creation of calus is not remarkable. From these media the best is medium H3 without

growth regulators. From the economical point of view this medium is suitable for increasing of plants species *Dianthus superbus* ssp. *superbus*. The whole successness of transfer of plants *Dianthus superbus* ssp. *superbus* from conditions in vitro into field conditions was 61,50% . The most critical part of transport was aclimatization of plants into greenhouse conditions (26,75%). At plants grown by that time *in vitro* there are great changes e. g. transfer into autotrophic nutrition, vitrification (rise stomata and cuticula) (Dvoøák 1991, Pierik 1987). Conditions in greenhouse influenced the transfer of plants. The transfer was held in June, the temperature about 26°C was not ideal. Maybe therefore the successness of transfer of *Dianthus superbus* ssp. *superbus* was only 61,50%. Shoots of plants derived *in vitro* have decumbent character, gradually become ligneous and give rise to axilar shoots, which are already erected and differ in colour and look of leaves. This phenomenon is usual in plants derived *in vitro.*

Propagation of the rare *Trillium persistens in vitro*

Trillium persistens Duncan (Trilliaceae), or persistent trillium, is a rare native woodland species which has been listed as nationally endangered (U.S. Dept. of the Interior, Fish and Wildlife Service, 1978). First described in 1971 (Duncan, *et al.*, 1971), it is known from only 4 populations in Georgia and South Carolina. The species is threatened by habitat loss to development and logging and is also of interest to wildflower collectors .

Material and Methods

Two dormant rhizomes of *T. persistens* were collected .Buds were dissected from the rhizome and surface sterilized in a 10% (v/v) dilution of commercial sodium hypochlorite for 10 minutes, followed by two rinses of sterile distilled water. Later re sterilization was done using a 5% (v/v) dilution of sodium hypochlorite. The buds were then cut into 4-5 small pieces and cultured on ½MS + 1 mg/l naphthalene actic acid (NAA), 1 mg/l benzyl aminopurine (BAP), 3% sucrose, and 0.8% agar in 60 mm x 15 mm sterile disposable culture dishes, approximately 15 ml/dish. Cultures were maintained in a growth room at 26°C with 16h light, under fluorescent cool white lights.

After 12 days, all pieces were transferred to fresh medium. Because of a light bacterial contamination, all pieces were resterilized at 19 days and again transferred to fresh medium. Once MRs were established, the tissues were subcultured onto ½MS medium without growth regulators. *In vitro* produced shoots were excised and placed onto the same medium in 25 mm x 150 mm culture tubes, 25 ml/tube, for root initiation. Rooted shoots were transplanted to potting soil. The plants were placed in a greenhouse either under a misting system, or under plastic bags. When bags were used, holes were punched in the bags to gradually acclimate the plants.

Results and Discussion

With rare species there is often not a sufficient amount of tissue available for experimentation. In this case, a growth regulator combination which was successful with a related species, *T. erectum*, was selected for use. In addition, half-strength salts were used, which had been found to be beneficial to *T. grandiflorum*, and not detrimental to other *Trillium* spp. which have been tested (Pence & Soukup, 1993).

With this medium, two of the nine resterilized bud pieces gave rise to swollen, smooth-surfaced, cream-coloured tissues resembling rhizome tissue, designated mini-rhizome (MR) tissues. Each MR gave rise to a single shoot and, in addition, produced new MRs which gave rise to new shoots. Once the MR tissue was initiated from the original explant on the hormone-containing medium, further growth was autonomous and continued in the absence of growth regulators. Shoots were rooted in tubes on medium lacking growth regulators, and once roots had formed these were transferred to pots.

The production of MRs by *T. persistens in vitro* is similar to the response observed with bud, leaf, and stem explants from other *Trillium* spp. in the presence of auxin and cytokinin (Pence & Soukup, 1986; 1993). The direct formation of MR tissue is also similar to the direct formation of bulblets in other liliaceous species.

Compared with other tissue culture systems, *Trillium* grows slowly *in vitro*. It may take 4-8 months for a piece of MR tissue to produce new MRs and for those to develop shoots which can be isolated for rooting. Rooting may then take another 6-8 months. However, young *Trillium* plants can be produced in tissue culture in 2-3 years less than from seed. In addition, once MR cultures are initiated, many plants may be propagated from a single source. During the course of these studies, more than 230 plants were obtained from two original source plants. This is the approximate equivalent of one-sixth of all the plants remaining in the wild.

Section –B

Micropropagation

Multiple Choice Questions

Q.1 The part of cotton producing pure cellulose is:
(a) Root hair
(b) Leaf hair
(c) Seed hair
(d) Stem hair
Ans. (c) Seed hair

Q.2 The first anther culture in Datura was carried out by
a. Beadle and Tatum b. Swaminathan c. Guha and Maheswari
d. Linsmaier and Skoog e. none
Ans. (C) Guha and Maheswari

Q.3 The bread wheat, *Triticum aestivum*, that is commonly used all over the world, is
A. hexaploid
B. tetraploids
C. Triploid
D. Diploid
Ans. A) hexaploid

Q.4 Which tropical fruit crop has been successfully engineered to be protected against a lethal virus?
A.Passion fruit
B.Papaya
C.Mango
D.Lychee
Ans. B) Papaya

Q.5 Transgenic plants
A.contain foreign genes in their cells
B.are used to produce human antibodies
C.both (a) and (b)
D.are plants that differ in geographical locations
Ans. C) both (a) and (b)

Q.6 A carrot is an example of a _____.
A) flower
B) fruit
C) taproot
D) cone
Ans. C) taproot

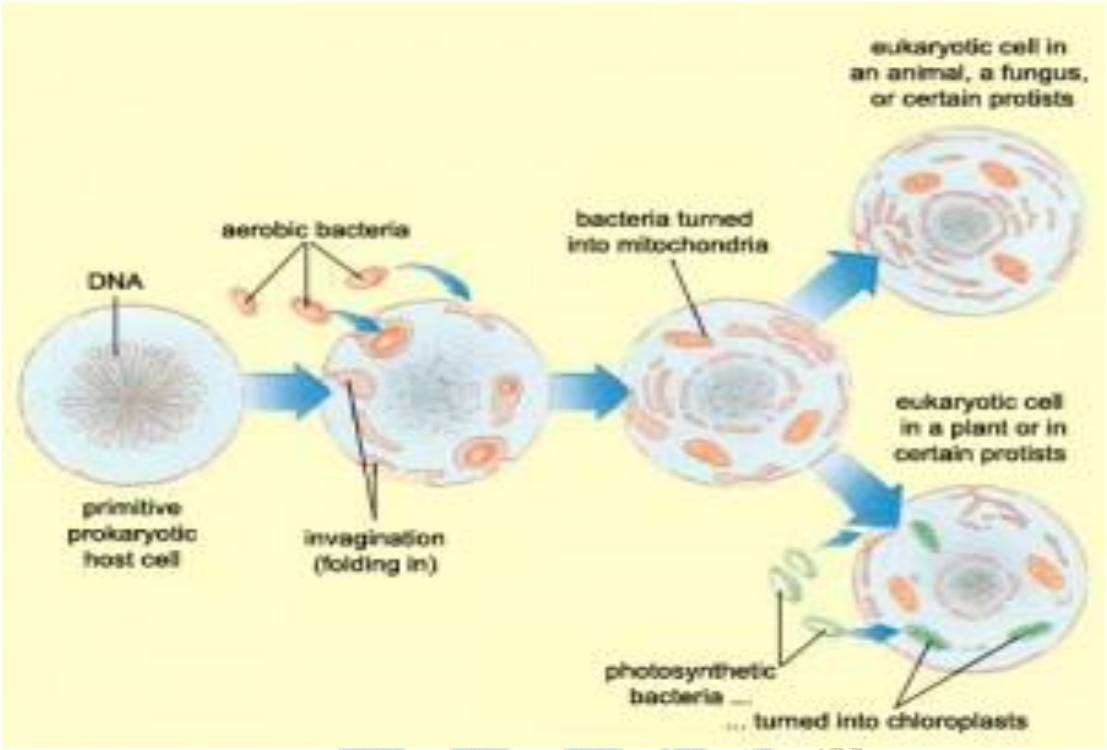
Q.7 Which term BEST describes the fusion of male and female gametes in flowering plants?
A) self-pollination
B) double fertilization
C) cross-pollination
D) pollination
Ans. B) double fertilization

Q.8 Which of the following is NOT an example of a fruit? (Points :1)
A) tomato
B) watermelon
C) strawberry
D) potato
Ans. D) potato

Q.1 Write short note on :
The engineering of recombinant plastids in higher plants.

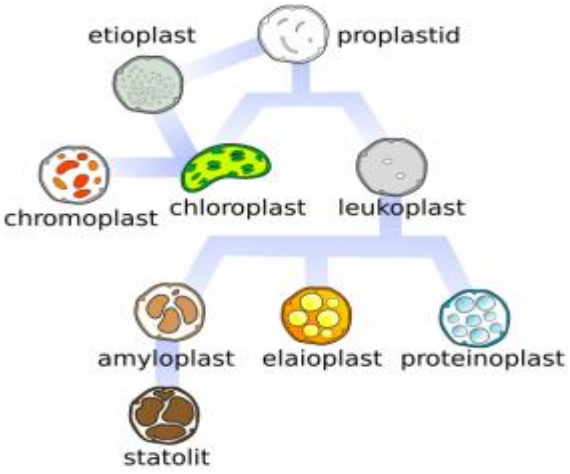
Ans. **(Dr. Pal Maliga)**

In traditional plant genetic engineering, a foreign gene (transgene) is inserted into the nuclear genome. A current controversy regarding plant genetic engineering is the possibility of transgene escape to wild relatives through cross-pollination. Chloroplast transformation is emerging as an alternative to nuclear transformation, and may address some of these concerns. In addition to gene containment, other advantages of chloroplast transformation may include the feasibility of obtaining high levels of protein production and the possibility of producing multiple proteins using polycistronic mRNAs.

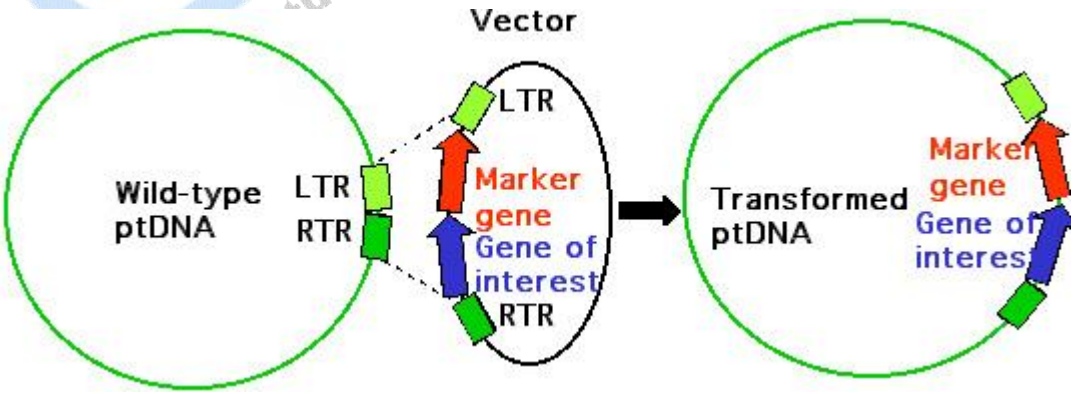


In nuclear transformation, transgenes are integrated into the genome at random positions. The context of transgene insertion may influence its level of expression, a phenomenon known as "position effect". In chloroplast transformation, on the other hand, transgenes are integrated by homologous recombination, allowing targeted insertion. Each plant cell has up to 10,000 identical copies of each plastid gene. Therefore, the expression of the transgene in transplastomics is many-fold and, once wild-type plastid genome copies are eliminated through repeated rounds of selection, a genetically stable population can be generated. several vector and selection systems used for plastid transformation. Vectors used for plastid transformation utilize left (LTR) and right (RTR) targeting regions to direct insertion of the transgene into plastid intergenic regions. Two recombination events targeted by the homologous regions direct insertion of the marker gene and the transgene into the LTR and RTR region of the plastid. The author describes several commonly used plasmid transformation vectors, such as the plasmid repeat vector (pPRV) and vectors pRB94 and pRB95, in some detail. In some of these systems, read-through transcription facilitates expression of ribosome binding site regions inserted at intergenic regions, allowing production of the protein(s) of interest from polycistronic mRNA transcripts. Selectable markers currently used in plastid transformation include spectinomycin-streptomycin resistance (conferred by the bacterial *aadA* gene) and kanamycin resistance (conferred by the *neo* gene). A positive selection marker is betaine aldehyde hydrogenase, which confers resistance to betaine aldehyde. The bacterial enzyme cytosine deaminase has been used as a negative selection marker system. Cells that express cytosine deaminase convert 5-fluorocytosine to the toxic compound 5-fluorouracil, and transformed seedlings can thus be identified using

medium containing 5-fluorocytosine. The reporter genes beta-glucuronidase (GUS) and green fluorescent protein (GFP) have made it relatively easy to detect transient expression of the transgene as well as stable transformation events in chloroplasts. Enzymatic activity of GUS is visualized by histochemical staining, while GFP can be visualized by direct imaging under UV.



Regulatory regions in plastid expression cassettes include a 5' regulatory region (PL cassette) and 3' regulatory region (T cassette). Plastid genome promoters are recognized by plastid-encoded RNA polymerase (PEP) or nucleus-encoded plastid RNA polymerases (NEP). Most plastid transformation systems have used derivatives of the strong sigma-70-type PEP promoter of the rRNA operon promoter (Prrn). The mRNA 3'UTR is encoded by the T cassette, and typically includes an RNA stem-loop structure, which functions as an inefficient transcription terminator. Most T cassettes are derived from plastid *pbsA*, *rbcL* and *rps16* genes, and differ in degree of stability that they confer to the transgene mRNA. In general, the expression cassettes are designed to optimize transcript stability and translation efficiency.



the 5’ UTR structure of m-RNA , plays an important role in translation efficiency and thus affects the yield markedly. The translation efficiency of chloroplast ribosomes is

considerably influenced by the sequence around the AUG initiation codon. The codon usage differs between chloroplast and nuclear genes, necessitating careful optimization of transgene codons. However, despite the prokaryotic nature of the plastid expression system, chloroplast codon use preferences are also distinct from codon usage in *E. coli*. This was illustrated by experiments using a synthetic, codon-optimized version of CP4 5-enolpyruvylshikimate-3-phosphate synthase (ESPS) containing 77% plastid-preferred codons versus a non-optimized, bacterial CP4 ESPS version. The synthetic version of CP4 ESPS resulted in just 1.5 to 2-fold higher protein levels than the bacterial version. Other such examples indicate that care must be taken when incorporating heterologous coding regions in chloroplast transformation studies.

Plastid transformation has been successfully performed in several solonaceous species, such as potato and tomato, although plastid transformation in other systems remains problematic. Tomato plastid transformation has resulted in relatively high levels of protein production in tomato fruits, and holds promise for production of oral vaccines. Plastid transformation in *Arabidopsis* is presently feasible but inefficient, and although the plastid transformation in rice is feasible, regeneration of plants from cultured cells remains difficult.

In conclusion, with the advantages of plastid transformation, including gene containment, expression of multiple genes, lack of position effects, high expression levels, and the possibility of expressing unmodified human and bacterial cDNAs.

Q.2 What are the different methods of propagating arid and semi arid tree?

Ans.

In vitro* multiplication of the semi-arid forest tree, *Balanites aegyptiaca

Balanites aegyptiaca an evergreen tree is a multi purpose plant known for its many uses as fodder, charcoal, timber, fuelwood, antifeed etc (Von Maydell, 1984).
The tree is drought and fire resistant, and withstands up to 2 months flooding in areas near a river but it can not tolerate prolonged water logging .
Micropropagation of tree species offers a rapid means of producing clonal planting stock for afforestation, biomass, and conservation of elite germplasm (Bonga and Durzan, 1982). The tissue culture of forest trees has shown promise in obtaining regenerants and clonal multiplication for domestication of wild populations, afforestation and improvement of economically important trees that have been cultivated for generations. Woody taxa are generally difficult to regenerate by *in vitro* techniques, but some success has been achieved in a few leguminous tree species (Dhawan,1987). This paper describes preliminary observations on clonal multiplication of axillary buds of mature *B. aegyptiaca* tree, popularly known as "desert date".

Materials and methods

Vegetative shoots were collected from ten year old tree of *Balanites aegyptiaca* located in Hann forestry Park .Nodes (1.5 to 3 cm) were used as source of explant. Nodes segments bearing axillary buds were surface sterilized by quick dip in 90% ethanol followed by first, sodium hypochlorite solution for 5 min, and rinsed 5 times in sterilized distilled water, then in 0.1% HgCl2 containing tween 20, finally 5 rinsed times in sterilized distilled water.

Culture medium and condition

The explants were placed on solid basal MS (Murashige and Skoog, 1962) medium supplemented with different concentrations and combinations of BAP: 0, 1, 2.5, 5 mg/l; kin: 0, 1, 2 and IAA 0, 0.1 mg/l for shoot proliferation and multiplication. The pH was adjusted to 5.6 using 0.1 N NaOH or 0.1 N HCl before autoclaving (110°C, 20 min). 20 ml of medium were dispensed into culture tubes . The cultures were maintained at 25±2°C either under 16 h/day photoperiod from cool white fluorescent lamps (3000 lux). The shoots were maintained by regular subcultures at 4- week intervals on fresh medium with the same compositions.

Root induction and acclimatization

For root induction, excised micro shoots (1-2 cm length) were transferred in liquid or solidified MS basal medium supplemented with different concentrations of NAA or IBA (5 and 20 mg/l). One excised shoot was placed in each tube (25 x 150 mm) containing 15 ml of the culture media. All the cultures were incubated at 25±20C under 14 h photoperiod with cool, white fluorescent lamps.Rooted explants were planted in pots containing a sterile soil and
kept in the greenhouse for acclimatization.

Observation of cultures and presentation of results

24 explants were used per treatment. The data pertaining to mean percentage of cultures regeneration, number and length of shoots/explant were statistically analysed by the Fisher's test.

Results and discussion

Among all the used media, the explants in MS medium were healthy and grew vigorously. 100% of segment explants produced shoots within 2 weeks and explants possessed 2.25 shoots having broad leaves. B5 (Gamborg et al., 1968) and SH media (Schenk andHildebrandt, 1972) induce vitrifaction with an average of 97% of new shoots produced. B5 medium produced rosette plants. Cultured axillary buds started to grow within 5 to 10 days. Excised explants cultured on MS medium formed callus at the cut and along the margin which remained unorganised.

Addition of BAP (1 – 5 mg/l) increased the number of shoots. Medium supplemented with 5 mg/l BAP induced a mean of 4.30 shoots per node explant (Table 1), while medium supplemented with Kin resulted in a reduced number of shoots although with longer internodes. Shoot multiplication required BAP, and Kin

was found to be less effective than BAP. 2.5 mg/l BAP in combination with 0.1mg/l NAA was the most effective combination for axillary bud multiplication, resulting in 3.13 shoots per node explant.

Individual shoots were excised from 4 – 6 week old cultures on multiplication medium and placed on the rooting medium. Root on induced shoots has been observed 10 days after rooting induction. Rooting ofshoots was best achieved using high concentrations (10 to 20 mg/l) of auxin .Shoots produced *in vitro* rooted well when treated with 20 mg/l of IBA or NAA. The number of roots per shoot is rather variable with NAA N doye et al.

Means within a column followed by a same letter was not significantly different (P= 0.05) according to Fisher's test. treatment. Higher concentrations of NAA caused formation of callus. For the control (without auxin), the rooting was 0% after 30 days. According to El Nour et al.(1991), the IBA hormone did not improve rooting significantly, which is contrary to our results. After 4 weeks, plantlets were transfered to pots and later established in soil. 48% of transferred plantlets survived with subsequent establishment. The results of this study show that axillary bud explants of *B. aegyptiaca* from mature mother plants can be readily established in MS medium containing 2,5 mg/l BAP to produce shoot multiplication. Multiple shoot regeneration has also been reported in *Ziziphus mauritiana* L.. However, there are no published reports on the micropropagation of *B. aegyptiaca*. MS media without any growth hormones enhanced shoot growth and elongation,while media with BAP enhanced axillary branching explants furthering multiple shoot development of nods.

Q3. Write short note on micropropagation of woody or forest trees in vitro rooting of teak (*Tectona grandis* L.)

Ans.

Teak (*Tectona grandis* L.), a leafy tree species, native to India, Myanmar, Laos and Thailand, is one of the world's premier hardwood timbers, attractive for its yellow colour, fine grain and durability. It is highly sought after for shipbuilding as well as interior and exterior luxury furnishings.

(EDP Sciences) However, in vitro rooting is often preferred because the plants perform much better in terms of plant quality due to the advantage of possessing already roots during the acclimatization phase.

2. MATERIALS AND METHODS

Explant source and sterilization

Sixty shoot tips and single nodes of young twigs from a single sexually mature teak *plus* plant were cut off from the explant source and were surface sterilized with ethanol (70% v/v) for 60 s and then dipped in a 0.05% mercuric chloride solution for 5−10 min. After rinsing three times with sterile distilled water, explants were dipped in 5−10% commercial bleaching solution containing 5% sodium hypochloride and a few drops of Tween-80 for 5−10 min, followed by three rinses in sterile distilled water.

Culture establishment

The nodal explants were then singly placed in test tubes with basal MS medium supplemented with 6-benzyl amino purine (1.0 mg L−1) and adenine sulphate (20 mg L−1), sucrose (3%) and solidified with 0.6% agar to induce bud sprout and to select sterile shoots. Activated charcoal 3 g L−1, was added to the medium to reduce browning. Medium pH was adjusted to 5.8 before autoclaving. After 30 days, the aseptic axillary shoots were transferred to a fresh medium of the same composition for 3 subcultures to produce a large number of shoots; then they were transferred to MS medium (free of PGR's) for 2 weeks in an attempt to eliminate any residual prior to placing the explants on several proliferation media. The aseptic shoots were cut into single nodes with their respective 2 leaves and placed, randomly distributed, into the different proliferation media. In general, from each shoot 4−5 nodes were obtained.

Shoot multiplication

The culture media were: MS medium and modified MS medium, the latter containing half the concentration of NH4NO3; both media contained 3% sucrose, 0.6% (w/v) agar 0.8% (w/v) pectin as anti-hyper hydricity agent, MS vitamins and PGR's.The pH was adjusted to 5.8 before autoclaving. In some treatments, the explants (nodal segments) were dipped in pre-filtered aqueous solution of GA3, for 10 s at the concentration of 100 mg L−1, prior to being placed into the jars +GA3.

The cultures were covered with a polyethylene film and incubated.

Rooting

Elongated shoots derived from nodal explants (grown on MSA+ GA3), were excised and their response to a range of rooting treatments was determined. The rooting medium consisted of mMS plus MS vitamins and 3% sucrose and solidified with 0.6% of agar. Indole- 3-butyric acid (IBA) was tested either alone (at 0, 0.5, 1, 1.5 mg L−1) or in combination with putrescine (160 mg L−1). The cultures were initially maintained for 6 days under dark and then exposed to light and temperature as mentioned above. Eight shoots were inserted with their basal ends into 100 mL of medium in a 580 mL glass jar. Each treatment consisted of four jars, every one containing eight explants. All experiments were repeated two times. The percentage of rooted shoots, the total number of roots and root length for each rooted microcutting were evaluated after 4 weeks of culture on the rooting medium.

The plantlets were transplanted to jiffy pots containing a soil: vermiculite (1:1) mixture. Initially plantlets were covered with a polyethylene film, which was gradually eliminated in two weeks time. Plants were transferred to pots and placed in a greenhouse for completing their acclimatization .

3. RESULTS AND DISCUSSION

Explant responses

Approximately 80% of the node explants remained aseptic. Three to four days after incubation on establishment medium, the axillary buds started to burst. At least, one axillary shoot developed per node, rarely two, however only one was able to elongate. The process of teak culture establishment in this experiment coincided with Devi et al. who reported that axillary shoots developed after 7−10 days and with Tiwari et al. who observed axillary shoots after 6 weeks on several nodes.

Shoot proliferation

Among the media tested, modified MS, without adenine sulphate, was superior to MS in terms of average number of shoots per explant resulting in a statistically significant difference with 3.8 axillary shoots per explant of MS-A+GA3 medium versus 2.7 shoots in MS-A medium supplemented with adenine sulphate GA3, both in the medium or used by soaking the explants, was effective in full MS medium, where it slightly increased number of nodes and drastically increased shoot length. BAP has been the most commonly used in micropropagation of teak alone or with kinetin. High concentration of BAP (5 mg L−1) was applied for a few hours .However, high concentrations of BAP can be inhibitory to the growth of axillary bud sprouts and can present some risks of unexpected abnormalities of new shoots after this treatment such as vitrification .and decreasing the cytokinin concentration generally reduces the multiplication rate. Ammonium is another compound involved in vitrification . We took steps to overcome vitrification problems by modifying the MS medium (reducing the ammonium quantity) and by adding pectin (from grape must) to reduce the water content in the medium and by adding GA3 since this was beneficial in olive. According to general observations, these changes probably contributed in the improvement of the culture quality reducing visual vitrification symptoms.

Rooting

After 4 weeks of culture, on the rooting medium, rooting took place only in the presence of auxin or putrescine. IBA alone induced 30−50% rooting depending on its concentration, producing swollen and brittle roots. Putrescine alone (160 mg L−1) promoted about 70% of rooting. Rooting reached 100% when putrescine was combined with 0.5mg L−1 IBA .Callus formation at the base of shoots increased with increasing concentrations of IBA. The number of roots per explant was low with the lowest IBA concentration, but increased when IBA was combined with putrescine . The use of a low concentration of IBA is advisable since it does not interfere with shoot growth, root elongation and keeps the basal callus small, however, it induces only a few thin roots without lateral ramifications. The combination of IBA with putrescine resulted in vigorous roots with multilateral ramifications. Roots emerged at the base of shoots after 2 weeks. An additional 2 weeks on this medium increased the number of roots and promoted shoot elongation.

4. CONCLUSIONS

This study provides an efficient in vitro propagation method which could be commercially feasible for teak, by providing a protocol for producing genetically uniform plants from selected genotypes. This work showed the highest number of micropropagated shoots reported for teak, up to now, in the literature and in a relative short period of time, producing about 4 shoots with 4 nodes within 4 weeks. Particular emphasis should be done to the use of putrescine, which in combination with low concentration of auxin, in rooting medium, was essential in stimulating high rooting percentage with high quality of roots, resulting in fast growing plantlets during acclimatization phase, reaching 100% of plant survival. Some steps were important for improving quality of shoots both during proliferation and rooting phases, aimed at reducing vitrification problems and aimed at improving shoot length and rooting percentage, by modifying the medium composition in terms of salt balance, type of gelling agent, by adding pectin of must wine, hormones and method of their administration, resulting in a reduction of the process time from the usual 4−5 months reported in the literature to less than 3 months including the greenhouse acclimatization period.

Section –C

Micro propagation and Variation Among Species

Multiple Choice Questions

Q.1 Plants having similar genotypes produced by plant breeding are called
A) Autopolyploid
B) Haploid
C) Clone
D) Genome
Ans. C) Clone

Q.2 Variations observed during tissue culture of some plants are known as
(A) Clonal variations
(B) Somatic variations
(C) Somaclonal variations
(D) Tissue culture variations
Ans. (C) Somaclonal variations

Q.3 Virus free plants can be obtained through
(A) Anitibiotic treatment
(B) Bordeaux micture
(C) Root tip culture
(D) Shoot tip culture
Ans. (D) Shoot tip culture

Q.4 In plant tissue culture one of the following is used as growth factor
a. GA3 b. Zeatin c. ABA d. urea e. all these
Ans. e. all these

Q.5 The laminar air flow apparatus is used in
a. silk reeling b. honey extraction c. plant tissue culture d. genetic engineering e. plant breeding
Ans. c. plant tissue culture

Q.6 Which one is used to sterilize the plant material before culturing
a. ethanol b.methnol c. sodium hypochlorate d. calcium hypochlorate
e. either c or d
Ans. e. either c or d

Q.7 The White's medium was once used to culture
a. SP algae b. bacteria c. fungi d. plant tissues e. none
Ans. d. plant tissues

Q.8 One of the best combination of growth regulators in tissue culture
a. salt and sucrose b. glucose and sucrose c. glucose and salt
d. sucrose and gelatin e. sucrose and minerals
Ans. a. salt and sucrose

Q.9 The advantage of somatic embryoculture is that a complete plant with tap root system can be produced. Which of the plant was produced through this method
a. carrot b. celery c. alfalfa d. all these e. none
Ans. d. all these

Q.1 Write short note on:

(a) Wild species of Oryza as an important reservoir of useful alleles.
(b) Fiber crops
(c) Genetic transformation of food grains and legumes

Ans.

Micropropagation of rice (*oryza sative* l. Cv swat-ii) through somatic embryogenesis
BY :- Ihsanilahi , Shazia Bano, Musarrat Jabeen and Rahim

Callus was induced on mature caryopsis of a local variety of rice (*Oryza sativa* L. cv. Swat-II). The frequency of callus induction was studied on modified MS medium using a variety of combinations of 2,4-D and Kn. Addition of tryptophan to different combinations of auxins and cytokinins increased the embryogenic callus mass. Calli have been successfully proliferated on MS supplemented with 1.0 mg/l of Kn and 0.5 mg/l of NAA. For regeneration the embryogenic callus was cultured on MS containing different concentrations of NAA and Kn and BAP and IAA. The somatic embryos developed into complete plantlets on regenerative media. The plantlets were then transferred to natural conditions for acclimatization.

Introduction

Rice is a cereal crop of tremendous economic importance. It is a staple food for a large human population. It is feared that world population would be around 10 billion by 2050. Thus more food will be required to feed the human population. This will be in the backdrop of diminishing cultivated land. Studies are underway to increase yield as well as quality of rice. Introduction of golden rice is a tremendous achievement in this direction. The available cultivable land is being utilized for non-agricultural purposes (Shazia, 1996). Similarly, salinity is making a great chunk of land uncultivable. Attacks by pests and insects are responsible for decrease in production. Thus there is a constant need to improve crops to overcome all these hazards. Resistant varieties/cultivers have to be developed to cope up with the situation. Induction of tolerance requires an efficient breeding system. uction of tolerance.

Somatic embryogenesis in rice have been reported from culture of leaf tissue (Wernicke *et al.,* 1981), root tissue (Abe & Futsuhara, 1985), inflorescence (Chen *et al*., 1985) and protoplast (Yamada *et al*., 1986). Rueb *et al*., (1994) developed a reproducible and efficient procedure for regeneration of rice plants from callus through somatic embryogenesis. Organogenic capacity of callus tissues depends upon the plant species, type of explant from which the callus was derived, age of callus tissue and composition of the nutritional medium. Another important factor is nature and level of various growth regulators.

Materials and Methods

Callus cultures of rice were initiated on mature caryopsis on a modified Murashige & Skoog's (1962) medium supplemented with 4% sucrose. In addition this medium contained various growth hormones. After dehiscing, the seeds were washed with tap water to remove dust and other particles. The cleaned explants were placed in 70 % alcohol for five minutes and surface sterilized with 1% HgCl2 solution for three minutes. The seeds were then rinsed three times with sterile distilled water to remove all the traces

of mercuric chloride. The explants were afterwards inoculated onto the medium. The pH of the medium was adjusted to 5.6 and then solidified with 0.9% agar. The phytohormones were added to the medium before pH adjustment. The medium was then autoclaved at 15 psi for 15 minutes at 1200C. Cultures were kept in 16 hours cycled fluorescent light cooled incubators with temperature regulated at 25±10C. Phytohormones used and their respective concentrations are mentioned in the results section.

Results

To achieve embryogenesis and plantlet regeneration the following procedures were adopted.

a. Callus formation:

Sterilized caryopsis of rice (Oryza sativa L. Swat-II) were inoculated on MS medium supplemented with 4% sucrose as the carbon source. Various growth regulators at 1.0mg/l 2,4-D + 0.5 mg/l Kn + 50 mg/l TPN, 1.0 mg/l 2,4-D + 1.0 mg/l Kn + 50 mg/l TPN, 1.0 mg/l 2,4-D + 0.5 mg/l BAP + 50 mg/l TPN, 1.0 mg/l 2,4-D + 0.3 mg/l Kn were added to the basal medium. Before the actual experimentation, the seeds were inoculated on plain basal medium (i.e. MS). After about three days the caryopsis germinated on MS containing 4% of sucrose. Moreover, a little callus formation was observed at the base of the coleoptile after another 10 days. This callus was isolated and inoculated onto the same fresh medium. However, it did not exhibit a reasonable growth. The callus so formed was then inoculated onto MS containing 2,4-D (1.0 mg/l) and Kn (0.3 mg/l). A reasonable callus formed after about four weeks of culture. This callus was soft, friable, brown coloured and non-embryogenic . Further proliferation was observed when this callus was subcultured on the same medium..

To induce embryogenic callus, when the caryopsis were inoculated onto MS containing 2,4-D (1.0 mg/l) + Kn (0.5 mg/l) + TPN (50 mg/l), a yellowish white, compact and somewhat embryogenic callus got induced after about six weeks Nonetheless, a soft, friable and non-embryogenic callus resulted on MS containing 1.0 mg/l each of 2,4-D and Kn and 50 mg/l 0f TPN. However, a copious, compact and embryogenic callus also got induced on MS containing 2,4-D (1.0 mg/l) +BAP (0.5 mg/l) +TPN (50 mg/l). All these calli when subcultured on the same fresh media, which subsequently exhibited reasonable proliferation and maintained their embryogenic or non-embryogenic nature for a long duration.

b. Callus proliferation:

The callus obtained on MS + 2,4-D (1.0 mg/l)+Kn (0.3 mg/l) was inoculated onto MS with various growth hormones. When the callus was inoculated onto MS + IAA (0.5 mg/l) + BAP (1.5 mg/l), a slight callus proliferation was observed Green columnar projections also developed in this callus which later on developed into plants. Probably the structures developed were shoot primordia.

The callus obtained on MS + 2,4-D (1.0 mg/l) + Kn (0.3 mg/l) was sub-cultured onto MS containing various concentrations of Kn and NAA. When the callus was inoculated on 0.5 mg/l each of Kn and NAA, callus proliferation alongwith shoot buds (meristemoids) formation was observed. These meristemoids afterward developed into plantlets. Meristemoid formation increased when the concentration of Kn was raised from 0.5 mg/l to either 1.0 or 1.5 mg/l . The callus, which formed on MS + 2,4-D (1.0 mg/l) + Kn (0.5 mg/l), was subcultured on MS containing various concentrations of different growth hormones . As evident from the result, bud like structures (meristemoids) formed in this callus which exhibited reasonable regenerating capability.

c. Somatic embryogenesis: Embryogenic calli obtained on mature caryopsis were further transferred to different regeneration media i.e., Kn 0.5 mg/l + NAA 0.2 mg/l, Kn 1.5 mg/l

+ NAA 0.5 mg/l, Kn 2.0 mg/l + NAA 0.5 mg/l and BAP 1.5 mg/l + IAA 0.5 mg/l etc. When the embryogenic callus was sub-cultured on MS medium containing 0.5 mg/l Kn and 0.2 mg/l of NAA, excellent callus growth with moderate regeneration was observed within a culture period of three weeks. This callus was friable, loose textured and creamy- yellow with slight tinge of green colour. This greening was due to the globular bably the embryoids, which later on gave rise to complete plantlets within four weeks. Similarly, luxuriant callus proliferation along with abundant embryoid formation was also observed on MS medium supplemented with 1.5 mg/l Kn and 0.5 mg/l NAA. These embryoids gave rise to plantlets after about four weeks . These plantlets were healthy and could be easily transferred to field conditions.

Discussion

The present studies were undertaken to induce reproducible embryogenic callus and produce plants through somatic embryogenesis. As evident from the results a reasonable callus was induced when MS was supplemented with 2,4-D and Kn. (Shazia (1996). This combination was excellent for callus proliferation and regeneration. Bud like structures and subsequent plantlets formation has also been reported in wheat (Ilahi, 2000). Further a soft, friable and non-organogenic callus formation occurred in rice caryopsis when the MS medium contained 1.0 mg/l 2,4-D and 0.3 mg/l Kn. The addition of tryptophan to increase the formation of green and compact regions in the callus also helped in an increase in embryogenic callus mass. Similarly excellent embryogenic callus growth was observed with 1.0 mg/l 2,4-D + 0.5 mg/l Kn + 50 mg/l TPN. These findings are in line with those where TPN was found to promote the growth of embryogenic callus in rice culture (Siriwardana & Nabors, 1983). In rice somatic embryos got developed on the embryogenic callus under the influence of appropriate balance of exogenous auxins and cytokinins.However, tryptophan is also required for embryogenic callus induction.

Ans. (b)

Cotton Shoots

(By Bushra Rashid*, Tayyab Husnain and S. Riazuddin)

An efficient *in vitro* rooting technique was developed for recovery of transgenic cotton plants. The loss of transgenic shoots due to failure to form roots is genotype dependant and represents a significant limiting factor in the overall recovery of transgenic plants from cultures. Transgenic shoots following selection on antibiotic medium were efficiently rooted on MS containing different combinations of kinetin, IBA and IAA. Healthy and efficient rooting was achieved when the shoots with blackish and dead root portion were treated with 1.0 mg/ml IBA and cultured on MS medium containing 2% sucrose. This method for *in vitro* rooting of cotton shoots proved to be a simple and reliable allowing 98% recovery of non-rooting shoots from culture. All the rooted plants normally survived in soil and flowered.

Cotton crop has been difficult to manipulate with high efficiency since the tissue culture methods used for regenerating transgenic plants by indirect transformation via callus. So, *in vitro* regeneration by somatic embryogenesis is limited to a few

Rooting in transgenic cotton plants is a major limiting factor, so whatever be the transformation system used, all methods ultimately depend on root formation for the recovery of plants from culture (Luo and Gould 1999). Genotype, position of explants, components of medium and proportion of phytohormone will influence plant regeneration. In the present findings, the root portion in transgenic shoots after selection on antibiotic medium was getting black and ultimately shoots died. In this report a more efficient and simple procedure for rooting of transgenic cotton plants on the tissue culture media .

Different concentrations i.e. 1, 1.5, 2, 2.5 and 3% of sucrose were added to MS medium. Plant growth regulators were added to the medium in combinations or separately as Kn (1 mg/l), IAA (1 mg/l), and IBA (1 mg/l). Then pH was adjusted to 5.7 - 5.8 and medium was sterilized at 121ºC and 15 lbs psi for 20 min. Transformed shoots of cotton variety CIM-482 with black and dead root portion were cultured on this modified medium. Another modification was made that the dead and black root portion was cut with a surgical blade and the base of the shoot was just dipped in solution of IBA (1 mg/l) and then cultured on simple MS. The cultures were kept at 27 ± 2°C with a photoperiod of 16 h under the light regime, 100 - 120 µm m^2/s.

Rooted shoots were taken out of the culture vessel and the medium was removed by washing roots with water. The root portion was dipped into solution of IBA (1 mg/l) and planted into the soil pots (Rashid et al. 2004). Non-transformed plantlets were shifted to soil without IBA treatment. Pots were covered with polythene bags after adding 20 - 25 ml of nutrient solution and kept at 30 ± 2°C for 16 hr photoperiod in light intensity of 250 - 300 µmol/m^2/s and removed completely after acclimation of plants.

Table 1. Effect of growth hormones on rooting of transgenic shoots. (mg/l)	Rooting (%)
MS + Kn (1.00) + (IAA 1.00)	45
MS + Kn (1.00) + IBA (1.00)	77
MS + IAA (1.00)	52
MS + IBA (1.00)	80
IBA 1.00 (Black root cut and treated with IBA)	98
MS (Transgenic)	0
MS (Non-transgenic)	92

Ans (c) (*Department of Field Crops, Faculty of Agriculture, Ankara University*),

Cowpea (*Vigna unguiculata* L. Walp) is an important legume grown all over the world as grain crop, animal fodder, cover crop, gren manure and vegetable. Cowpea (*Vigna unguiculata* L. Walp.) is a summer annual herbaceous, drought tolerant legume, widely grown in Africa and many parts of the world as grain crop and animal fodder. Sometimes grown for cover crop, green manure but is more generally used as vegetable. Cowpea seed is a nutritious component in the human diet, as well as a livestock feed and is consumed by more than 200 million people on daily basis in many parts of Africa (Popelka *et al*., 2006).

Shoot tip multiplication is generally used for producing virus free material and maintining germplasm via cryopreservation (Nehra and Kartha, 1994).
Agar has been used as gelling agent in most of the plant regeneration studies on cowpea with the exception of Ramakrishna *et al*. (2005) and Mao *et al*. (2006); who used phytagel. However, no report describes comparative effect of gelling agents on plant regeneration in cowpea.The paper compares effects of agar and gelrite on micropropagation from shoot tip of two Turkish cowpea cultivars with aim to investigate the factors responsible for plant regeneration and possible use of the results in breeding and genetic transformation experiments.

Materials and methods

Seeds of two cowpea cultivars were obtained from the Department of Field Crops, Faculty of Agriculture, Ege University, Izmir, Turkey. Surface sterilized seeds were germinated in growth chamber at 24 ± 2 oC in 16 h light photoperiod on MS basal medium containing 0.7% agar (Duchefa, Germany) and 3.0 % sucrose. Shoot tip explants were obtained from 3-4 days old *in vitro* grown seedlings.
They were cultured in upright position on MS medium (Murashige and Skoog, 1962) with 3.0 % sucrose gelled with 0.65% agar, and 0.21% Gelrite (Sigma) containing 0.15, 0.25 and 0.35 mg/l Thidiazuron-TDZ, 2 mg/l yeast extract with and without 1.25 mg/l PVP (PolyVinyl Proline) as an anti oxidant and 3 g/l active charcoal. The pH of all cultures was adjusted at 5.6-5.8 before autoclaving. Initial experiments showed that the explants were infested with high endogenic bacterial contaminations; therefore, to overcome the problem, all cultures contained 500 mg/l of an anti biotic-Augmentin (Glaxo-Smith Kline, England). The explants were incubated in growth chamber at 24± 2 oC temprature with 16 h light photoperiod.
After 8 weeks of culture, the explants were transferred to MS medium to reduce stress of plant growth regulators on shoot regeneration and elongation. Thereafter, two weeks these shoots were excised from explants and transferred to MS medium containing 0.5 mg/l IBA for rootng.
After two weeks on rooting media, the rooted shoots (plantlets) were taken out from the Magenta vessels® and submerged in water for 30 minutes before transfer to pots containing sterilized peat moss. Pots were covered with transparent polythene bags and placed in growth chamber at room temprature. After seven days, the bags were

removed and the plants were allowed to grow at room temprature with 50 % relative humidity.

Results

After two to three days on culture media, the explants started bleeding reddish pink coloured phenolic compounds which were more visible in gelrite compared to agar gelled medium in cultures that did not contain PVP and active charcoal, irrespective of the TDZconcentration. The explants swelled after 9-10 days and showed development of variable number of shoot meristems but the phenolic compounds inhibited the growth of these shoot meristems in to full shoots (numerical results not shown). Therefore, these experiments were discarded at initial stages.

Q.2 Explain the micropropagation technique of ornamental plant *in vitro* mass multiplication of orchid (*Oncidium* sp.).

Ans. Orchid constitutes an order of royalty in the world of ornamental plants. They are of immense horticultural importance and also play a very useful role to balance the forest ecosystems (Kaushik, 1983). They are one of the most pampered plants and occupy top position among all flowering plants valued for cut flower production as potted plants, which fetch a very high price in the international market. In India, orchids form nine percent of flora and about 1300 species are found in Himalayas with others scattered in Eastern and Western Ghats (Jain, 1980).The horticultural trade depends on wild orchid population as a source of stock plants, but most are not propagated commercially. In the present study, an attempt was made to have a mass clonal propagation of an orchid within a short span of time. *Oncidium* sp., one of the commercially important orchids, was selected for this study because it produces a brightly attractive flowers and it is highly adaptable to culture under a wide range of climatic conditions.

MATERIALS AND METHODS

(a)Explant source

The green pods still containing the dry petals at their tips were collected from our nursery and used for embryo culture without delay. First the dry petals attached to the green pods were removed, then the pods were washed thoroughly using running tap water. The pods were surface decontaminated by immersion in 5% sterlique (Sodium hypochloride solution) for 20-30 min followed by thorough wash in sterile double distilled water. Then the pods were dipped quickly in 70% alcohol and flamed over a spirit lamp. Each
pod was then transferred to a sterile Petri dish.

(b) Inoculation of seeds

The pods were cut longitudinally into 2 halves using a surgical knife, and the seeds together with cottony fibers in between were scooped out into the thin water film. After careful separation of the seeds from the fibres, the seeds suspension was sucked into sterile wide mouthed Pasteur pipette and transferred in MS medium (Murashige and Skoog, 1962) in 100 ml conical flasks. The pH of all the media were adjusted to 5.6 prior to adding 0.8% agar was used as gelling agent.

(c) Maintenance of culture

Culture bottles were autoclaved for 20 min at 121ºC. All the cultures were maintained at 25ºC continuous light (3000 lux) with a photoperiod of 12 h daily and 60 – 70% relative humidity.

Seedling development

The MS medium was supplemented with two plant growth regulators, BAP and NAA, seedlings were developed by various concentration of growth regulators. NAA ranging from 0.5 – 0.4 mg l-1 and 0.5 – 2.5 mg l-1, respectively,were prepared and their individual and combined effects were analyzed through the parameters such as production of protocorm like bodies, number of shoots and number of shoots with roots.

Subculture and multiplication of protocorms

Protocorms development from the embryos in 2-month time were collected over a sterile filter paper disc, blotted and then transferred in aliquots (50-70 protocorms per bottles) on to agar nutrient medium in bottles as the case may be. The cultures were incubated again under the same conditions for the multiplication of protocorms.

Hardening

The well-developed seedling 3-4 cm height were removed from culture vessels and thoroughly washed with tap water to remove adhering medium completely without causing damage to the roots.

Then the plantlets were treated with the fungicide solution (Bavestin) at 5% concentration and transferred to perforated plastic pots. Plastic pots were filled with a mixture of uniform, small charcoal pieces and brick pieces (1:1). After a thorough wash of the pots and the potting media in water and treatment with 0.2% diethane fungicide, the seedling were transplanted. The roots of the seedlings were closely touching on the surface of the charcoal pieces. Care was also taken so that 1-2 roots of the seedlings passed through the space in between charcoal pieces. The potted plants were kept under a green house (25% light) andmmist irrigated. After two weeks both misting and foliar application of

NPK mixture were followed. The application of the later was done twice in a week. Observations on the establishment of the seedlings were recorded by weekly intervals.

RESULTS AND DISCUSSION

Tissue culture production of ornamental plants in general and orchids in particular forms the basis for the entire horticultural industry. Among the various explants, the shoot tip and axillary buds are most commonly used. Possibility may arise like intervarietal, interspecific or intergeneric hybrids during the embryo /seed culture when they are taken as a source of explant. Germination of orchid seeds is different from other seeds. Orchid seeds are produced in large number within a capsule or pod. The seeds are very minute, contain undifferentiated embryo and lack endosperm. In certain orchids, self-pollination is not possible and even if possible as in the case of Vanda, one has to wait for 4-6 months for pod development (Fitch, 1981). Green pod cultures as against mature/dehisced pod culture is desirable to save time and to avoid contamination. The seeds taken from the green pods were sown on the MS medium containing various concentrations of two plant growth regulators, namely BAP and NAA. Invariably all the embryos transferred to the MS medium germinated within two weeks. Swelling and glistering of the embryos were first noticed within 10 days. The swelling of the embryo was followed by pigment synthesis. The embryos turned from yellow to yellowish green and finally becoming green as they grew. Due to the non-endospermic nature of the seed, the germination in nature is a unique phenomenon and requires fungal infection. Germination is much more successful in *in vitro.*;

. In the present investigation also same sequence of seedling development was observed when the selected orchid, *Oncidium* spp was grown on the medium. As the embryos developed into globose protocorms, seed coat (testa) got ruptured and rhizoids and shoot initials were getting formed. After 90 days, well-developed protocorms obtained from MS medium were subcultured on the same
medium along with five different concentrations of BAP (0.5, 1.0, 2.0, 3.0 and 4.0 mg l-1) and NAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg l-1). Both individual and combined effects of BAP and BAP + NAA were analyzed During the first subculture, the protocorms further increased in size divided and formed clusters. Rhizoids differentiation was prominent in certain clusters. Some times they could be easily mistaken as roots. The quantity of protocorms produced by the seeds of the green pods were categorized into three groups, namely,

i) very less number of protocorm like bodies formation (+),
ii) less number of protocorm like bodies formation(++) and
iii) more number of protocorm like bodies formation (+++).

For subsequent development (of *in vitro* orchid seeds into seedlings), several modifications were made in the media by changing the ingredient and their quality and quantity. The most important development in culture media was the incorporation of growth regulators like auxins and cytokinins. The discovery of various plant hormones let to their utilization in attempts to promote orchid and seed germination on seedling growth. Growth hormones inhibit as well as promote orchid seed

germination in orchids, depending on the type. Several workers have tried various growth regulators and various concentrations of growth hormones used in an attempt to promote seed germination and seedling growth (Arditti, 1979).

In relation to germination behavior, it was observ-ed that epiphytic orchids germinated more quickly. The same observation was also made in the present study. Auxin was the first plant growth hormone added to the seed culture. In majority of the cases auxins (mostly NAA, IAA and IBA) enhanced the germination and seedling growth (Arditti, 1979). In the present study BAP and NAA stimulated shoot and root growth in *Oncidium* sp. as also reported in *Epidendrum nocturunum* (Yates and Curtis, 1949) and growth of cymbidium seedlings. More protocorm like bodies was produced on the medium which containing 2 mg l-1 BAP. It was followed by 1.0 mg l-1 BAP and 1.5 mg l-1 NAA in the present MS medium.

Only a small number of protocorm like bodies was produced on the medium contained 4 mg l-1 BAP. The same principle was followed to quantify the combined effects of BAP and NAA. More protocorm like bodies was produced by the medium which contained 2.0 mg l-1 BAP + 1.5 mg l-1 NAA. Less number was produced by the medium containing 3.0 mg l-1 BAP + 2.0 mg l-1 NAA. Very poor result was observed in the medium containing 4.0 mg l-1 BAP + 2.5 mgl-1 NAA.Cytokinins usually have no beneficial effect on orchid seed germination. In *Coeloglossum viride* and *Platanthera bifolia*, kinetin (1-10 mg l-1) retarded the seed germination and seedling growth but increased the growth rate of protocorms. On the contrary, in the present investigation, the seedling development of *Oncidium* sp. was best on the MS medium supplemented with 2 mg l-1 BAP. These findings are in agreement with the reports of Sharma (1996). A well-developed cluster were selected and transferred to second subculture for root induction (Figure I). These subcultures were grown using the same concentrations and combinations of the same plant growth regulators.

These cultures media were used to study their stimulatory effect of the number of shoots and roots per shoot. The number of shoots and roots were counted. The initiation of 100% of root was observed both in2 mg l-1 BAP and 2.0 mg l-1 BAP + 1.5 mg l-1 NAA. The present investigation revealed that the MS medium supplemented with certain concentrations of plant growth regulators influenced on seed germination, production of protocorm like bodies, shoot multiplication and root initiation. The *in*
vitro raised seedlings were successfully established in the potting medium. Further

Section-D

Gas Crops and Protoplast Culture

Multiple Choice Questions

Q.1 Protoplasts are the cells devoid of
A.cell membrane
B.cell wall
C.both cell wall and cell membrane
D.none of these

Ans.B.cell wall

Q.2 Desired improved varieties of economically useful crops are raised by:
(a) Migration
(b) Biofertilizer
(c) Hybridization
(d) Natural selection
Ans. (c) Hybridization

Q.3 Emasculation is concerned with:
(a) Hybridization
(b) Clonal selection
(c) Mass selection
(d) Pure line selection
Ans. (a) Hybridization

Q.4 To obtain naked protoplasts during somatic hybridization the enzymes needed are:
(a) Cellulase and protease
(b) Cellulase and amylase
(c) Cellulase and pectinase
(d) Cellulase and lipase
Ans. (c) Cellulase and pectinase

Q.5 Transgenic plants are developed by
A Introducing foreign genes
B Introducing gene mutations
C Deleting certain chromosomes parts
D Stopping spindle formation
Ans. A Introducing foreign genes

Q.6 Cry genes or Bt genes are obtained from
A DNAase
B Amylase
C Lipase
D Restriction endonuclease
A Cotton pest
B Tobacco plant
C Bacillus thuringiensis
D E - Coli

Ans. C) Bacillus thuringiensis

Q.7 Which enzyme is useful in genetic engineering?

Ans. D) Restriction endonuclease

Q.8 Which is related to genetic engineering?
A Plastid
B Plasmid
C Heterosis
D Mutation

Ans. B) Plasmid

Q.1 What is protoplast? Write down different methods at protoplast isolation and culture?

Or

Micromanipulations of gene? Write short note on micro somatic hybridization and its application?

Ans. "Protoplast" is known as "Naked plant cell" referred to all the components at a plant cell excluding the cell wall
Protoplast are isolated by (i) mechanical method (ii) enzymatic method.

Mechanical Method:-
Highly vacuolated cells of storage tissue (Ohiou Dulb) radish root could be used for isolation. Mechanically protoplasts have been isolated by gently teasing the new callus froev expanded leaves. In this method yield of protoplast is generally very low and is very laborious and tedious method. Liability of protoplast is low.

Enzymatic method: - Cocking used concentrated solution of cellulose to degrade the cell walls.Protoplasts are isolated by treating tissue with a mixture of cell wall degrading enzymes in solution, which contain osmotic stabilizers:

Enzymes generally used are (pastiness) macerozyme driseleose helicase, colonase, zymolyase etc.
The activity of Enzymes is pH dependent and is generally indicated by the Manufacturer.
Purification at protoplast is necessary because if contains sub-cellular debris, undigested, cells and broken protoplast. This can be purified by a combination of filtration, centrifugation and washing. Enzyme solution containing protoplast is tilitered through nylon mesh
Viability of protoplast can be checked by using different stain like fluoresce in dictate(FDA) phenosal ravine, calcofluor white.

Culture techniques:-Lecturer in Biotechnology.Isolated protoplasts are usually cultured in either liquid or semisolid agar media plates. Protoplast are some times allowed to regenerate cell wall in liquid culture before they are transferred to agar media.
Agar culture: - Agarose is most frequently used to solidify the culture media. Bergmann cell plating is used for plating of protoplasts. Protoplast suspension at double strength of agar is required the Concentration of agar should be chosen to give a soft agar gel when mixed with the protoplast suspension. Petri dishes are then sealed with Para film and incubated upside down.

Liquid Culture: -It has been generally preferred in earlier stages at culture become.

(i) It allows easy dilution and transfer.
(ii) Protoplast at souse species do not divide in agarified media.
(iii) asmotic pressure of the medium can be effectively reduced.
(iv) Various modification of these culture methods are:-

Liquid droplet method:- It involves suspending protoplast in culture media and pipe ting doublets into plastic petui dishes. Five to seven drops can be cultured plat. The plates are then sealed and incubated. This method is convenient for microscopic examination and fresh me dig can be added to the developing suspensions at 5-7 days in fervals.

Hanging droplets method:- Small drops of protoplast suspension are placed on the inner side of the lid at dish. When the lid is applied to dish culture drops are hanging or suspended from lid.

Co-culturing:- It is the culturing of two types of protoplast viz. slow growing and fast growing. Reliable fast growing protoplast preparation is mixed with protoplast of a slow growing recalcipant species. Fast growing protoplast provide they other species with growth factor and un defined diffusible chemicals.

Culture medium :- Protoplast have nutritional requirements similar to other plant cells culture medium should be devoid of ammonium as it is detrimental to its survival end quantity of iron of Zinc should be reduced.
Calcium concentration should be increased 2-4 times over. The concentration normally used for cell culture i.e. important for important for membrane stability.

For osmolarity Manitol/and sorbitod/ are most frequently used compounds auxin and cytokinin stumaltat protoplast division and growth.
Light is necessary for initiating protoplast division pH should be 5.5-5.9.

Protoplast development

Cell wall fore nation:-_cultured protoplast generally starts to regenerate a cell wall within a few hours after isolation.

Growth and plant regeneration:-Cell wall formation is required before cytokines is occur. After regenerate of cell wall they show increase in cell size. The first cell division occurs within 2-7 days. Second division occurs within a week and by the and at the second week in culture small aggregates of cells are present.
Once small colonies have formed their further growth is inhibited if they are allowed to be main or high osmotic indium. First protoplast regeneration was observed in picotiana tobauum.

Somatic hybridization:-Somatic cell fusion bas been suggested a inethod to overuse the species barrier to sexual hybridization. The technique of hybrid production through the fission of somatic body under in vitro conditions to a hybrid plant is known as somatic hybridization in this nucleus and cytoplasm of both parents are fused it the hybrid cell.

Protoplast fusion:- It consist of mixing of protoplast of two different genowes.

Spontaneous fusion method:- During isolation of protoplast cell wall degrades and this led people to believe that there would be spontaneous fusion that leading to the fore nation of homokaryom spontaneous fusion at protoplast is observed.

Induced fusion method:- When protoplast at different origins required then there is a suitable agent(fusogen) is added to fare the plant protoplast of different origin. Some of the methods are:-

(a)**Treatment with sodium nitrate:-** Isolated protoplast are suspended, in a mixture at 5.5% Nano in 10% sucrose solution and then incubated at 35^0C for 5 min and they centric taged for 5 min. after that most of the supernatant is decanted and pellets are

transferred to the water both for 30 min. at this time most of the protoplasts undergo cell fusions.

(b) **Ca^{+2} ion at high pH:-** Isolated protoplasts are centrifuged in a 0.5 m maunitol with 0.05 M CaCl2 at pH 10.5 centrifuged tubes then incubated in a mater both at 37^0C for 40-50 min.

PEC method:-Isolated protoplast suspended in a solution containing PEC which enhances agglutinating and fusion at several species. This tube is then shaken for 5 second and allowed to settle for 10 min. the protoplasts then washed by the addition at protoplast culture medium to remove PEG.

Electro fusion :- A potential difference is applied to container with protoplast then a shoal shock is applied as a result protoplasts from different origin get fuse.

A two step procedure is fallowed with application at an alternation current (AC) and direct current(DC) with pulse of high intensity. This technique is simple quick and efficient. S different steps in fusion are:-

(i) Agglutination
(ii) Plasma membrane fusion at localized sites.
(iii) formation of hetero korgan.

After fusion at different protoplast. Hetero karyon formed and this can be identify and selected by different methods.

Chromosome number in somatic hybrids. Jute specific and intergeneric somatic hybrids are mostly polyploidy. Chromosome no of these hybrids indicates that only few hybrids have the sum total chromosome no of both the parents as expected in on amphipod. There is an indication that loosely related species would yield true amphidiploids through somatic hybridization.

Variability in chromosome no of hybrids could be one to following reason:

(i) Multiple fusion give a higher chromosome number.
(ii) Fusion of more than two protoplasts with subsequent mitotic irregularities.
(iii) Asymmetric hybrids results from fusion at protoplasts isolated from actively dividing tissue of one parent and quiescent tissue of other.

Micromanipulation:-Asymmetric hybrid:- Jute specific somatic hybrids contras many undesirable traits besides desired ones & remove unwanted genes if requires backcross. Hybrid callus is strife, incapable of regenerate plants are narcotic and are before maturity, somatic hybrids have lose their chromosome of one parent and phenotypic ally close to another parent called asymmetric hybrid.

Researchers produced asymmetric hybrid by fusion with B. oleracea with X-irradiated B. Compestris and morphology isozymes and chromosome no.

Symmetric hybrid:- In this fusion of protoplast of distantly related sps. Inters pacific inter generic inter bible or even interfamily have been produced.
In brassiere family B. nopus synthesized by fusion between B. oleracia (2n=18) X B. Compesbis (2n=20) pomato developed by Potato+Tomato were sterile because of protoplast derived from oneupcoid callus cells.
Several inter specific & inter generic hybrids of citrus with sexually compatible incompatible partner have been developed.

Cybrid:- In somatic hybrids the cytoplasm is derried from both the parents. However somatic hybrids can be obtain where nucleons is derived from are parent and cytoplasm from both thus producing cytoplasmic hybrid (cybuid). Pre segregation of noclei in a fused product can be stimulated and cytoplasm while the other contributes the nucleus alone or both nucleus and cytoplasm there are different ways of inactivating the

Nucleus of one protoplast
Different methods of achieving this type of hybrids are:-
1. By Application of lethal dosage of X-ray gamma irradiation foone parental protoplasts.
2. By beatment with isdoacetat to metabolically inactivate the protoplasts.
3. Fusion of normal protoplast with enucleated protoplasts that can be obtained by high speed centrifugation.
4. Fusion of normal protoplast with another in which nuclear civision is suppressed.

Application of somatic hybridization:-
1. Production of hovel varieties.
2. Production of disease resistance A biotic, biotic, stress resistance plant and plasmic male sterility can be obtained.
3. Production of auto tetraploid.
4. Protoplast of sexually sterile plants can be fused to produce vertile discoid.
5. Production of homozygous line.
6. production of unique nuclear cyto plasmic combinations Production of

Q.2 What are GM crops? Write different concerns about G M crops?

Ans. A large area is planted with GM Crops However just two baits. Herbicide resistance and insect resistance and four Crops grown dominantly in America.
The agro biotech multinational like Monsanto and syngento drove the development of these crops. The main beneficiary other than agro biotech multinational appears to have been the farmer who has generally enjoed increased yields and from growing GM Crops.
There does seem to be a real reduction in the use of herbicides or pesticides associated with growing GN crops. The introduction of herbicide. To/ errant cotton

resulted I increased yield but no decrease in herbicide usage, whereas the adoption of herbicide resistant soybeans led to significant reduction in herbicide use and only small increases in yield. The herbicides used an gas crops are considered to be more environ mentally friendly than are some of the alternatives. Other benefits from growing GM crops might also be apparent dg. More environmental friendly cultivation method can be used that reduced soil erosion bio diversity may also be increased as weeds can be allowed to grow post emergence if herbicide resistant crops are being growing / tjos development at commercial level has implications for the future of G M crops. The main perception is that G M crops have failed to live upto the considerable claims that were made for them. A pioneered example of G M crop is Golden rice like many of the new developments in G M crops if was primarily developed in academia with backing from public funding. However until the benefits of these "new generation" G M crops because apparent. The tech. faces some considerable hurdles to wide spread acceptance.

Plant transformation is a very low- frequency event and some means of selecting the transformed cells from the untransformed cells is required.

The use of antibiotics as selective agents in plant biotechnology was logical. However the me of antibiotic marker genes has proved to be one of the hurdles to the widespread, acceptance of G N crops except in maize the amphibolites resistance generation of G M crops that have so much ethical hurdle.

Amperilin is an antibiotic of the penicillin family that is widely used to treat a variety of infections in human.

1. The antibiotic resistance genes used in creating G M crops were isolated from bacteria. So the development of antibiotic resistance strain is a real problem in hospitals where antibiotics are used routinely emd create a selective pressure for the development of resistance.Transfer of intact antibiotic resistance genes to gut flora ingested material of plant is unlikely.
2. Many of the out biotic resistance genes ealumanly found if G M crops confer resistance to antibiotics that are not used to beat disease in human.

Herbicide resistance and super weeds:-

One problem with the use of herbicide resistance markers is the potential for create of super weeds Transfer of herbicide resistance genes t weedy relatives of G M crops could creates such super weeds. We plant biotech no logist can decrease the use of herbicide resistance genes as selectable marker by again employing alternatives or by using clear gene technology.

Gene containment:-

Preventing the transfer of foreign gene from G M crops to other plants is a wider environmental issue a concern that does not only apply to herbicide resistance genes.

A great variety of foreign once are being introduced into G M crops but the environmental impact all these genes is currently difficult to predict.

Gene transfer usually occurs through pollen although G M crops if a valid relative pollinated them could also serve as a female parent for hybrid speeds.
The dispersal of seeds from G M crop if amongst weedy relatives could also produce mixed populations with inbo egression of a herbicide resistance genes resulting in herbicide resistance weeds.
The potential for foreign genes from G M crops to be transferred to weedy relatives depends on a great many variables.

Techniques for gene containment:-
Elide variety of techniques are capable of preventing reducing transfer.
A variety of techniques are capable of preventing gene transfer.
Terminator gone technology has been the because of apposition to G M crops as seed can not be collected in subsequent years.
The threat of terminator technology being introduced led to protests in many parts of the world and was seen as yet another example of big business imposing its wishes on farmers and consumer alike.
Terminator technology has the potential to make G M crops safe by reducing gene transfer to weeds relatives.
This technique had already been introduced led to G M Colton crops being burnt in Indio. It has become a problem for farmers in developing countries.

Regulations of G M crops and products:-
The future of G M crops is determined by the regulatory framework that is applich to their growth and processing.
Regulations are design to ensure that the public is reassured and confident that any G M crops or products are safe and that they are not being consumed without their knowledge.
There should be proper labelling of the G M food so that consumers can make informed choices about eating G M food or not.
One point warth noting is that foods derived from MOs can be regulated by a simplified procedure.
Concerns about G M food safety:-
It is important to remember there is no evidence that G M foods are any less safe than non- G M crops. However become much broader.

Q.3 Write short note on:-

(i) In vitro pollination.

(ii) In vitro fertilization and its applications.

Ans

Kanta et al. (1962) have described the technique of seed development through *INVITRO POLLINATION* of exposed ovules as test tube fertilization seed development following stigmatic pollination of cultured whole pistils has been referred to as "IN VITRO POLLINATION".

However in either case fertilization of the egg occurs inside the ovules by sperms delivered by the pollen tube almost in a natural fusion.

The term test take fertilization refer to in vitro fusion of excised egg and sperm cells. In vitro application of pollen to excised ovules is referred to as in vitro ovular pollination to the ovules attached to the placenta as in vitro placental pollination and to the stinging of intouh as inlitro stigmatic pollination under the term of in vitro pollination.

In Vitro Fertilisaiton:-

Initial steps for IN VITRO POLLINATION and INTRA OVARIAN POJJIMATION are the save eg me termination at the time of an thesis dehiscence of anthers. Pollination entry of pollen tube into the ovules and fertilization

(c) Emasculation and bagging of flower buds and (c) Collection of pollen graries.

(d) For in vitro pollination a suitable nutrient medium is required that will favour the germination of pollen grains and the development of fertilized ovules into mature seeds.

main requirement for in vitro pollination is the maintenance of reasonable sterility during pollen and ovule collection.

To prevent chance pollination the buds are emasculated (tamales partner) before an thesis and bagged. After an thesis the buds are aseptic culture.

After that rinsing at pistil with pedicel is done with 70% alcohol and suitable agent. Stigma and style are remove and the ovary wall is beeled to expose the ovules. Whaled placenta is used for placental pollination.

For collecting the pollen under aseptic conditions undehisced emthers are removed from buds and kept in sterile Petri plates until they dehisce.

The discharged pollen is aseptically deposited an the cultured ovules, placenta or stigma as the case may be.

Factors affecting in vitro pollination

(i) Explant:-in petunja in vitro pollinated excised ovules aid not form viable seeds.

Because the pollen grain germinated normally but the pollen tubes fasted enter the ovules but when intact placenta with undisturbed ovules were pollinated

normally event's from pollen germination to the development of viable seeds accrued.

In maize the ovaries attaché a to cab tissue give better results then single ovaries. Reducing the no of ovaries ares not affect fertilization but has delectations.Effect an kernel development lob piece with one ovary did not form fully developed kernel. Whereas with loeverice blocks had one two fully developed kernels.

Time of excising the ovules from pistils. Las a definite influence on in vitro pollination. The incidence of seed set is higher when ovules are excised I-L days after enthuses than on the day of an thesis. Optical stage for maize Spike for in vitro pollination is 3-4 days after silking.

(2) Culture medium:-the technique of in vitro pollination involves two major processes:-

(a) Germination of pollen grains and pollen tube growth leading to fertilization.

(b) Development of fertilized ovules into mature seeds with a viable embryo.

Composition of culture medium support both of the processes. Where pollen grains fail to germinate on the surface of ovules they may be separately grown on a suitable medium and pollen tubes applied to the ovules. A modified technique was developed to obtain germinable seeds in B. *oleracea.* i.e. dose by dipping isolated ovules in a 1% $Cacl_2$ solution planted them on a slide procoated with 10% gelatin solution.

The most imp. Sole of culture medium is in supporting normal development of fertilized ovules.

(3) CO_2 – in some plants elevated level of CO_2(N.) for 24h after in vitro pollination increased the yield of selfed seeds.

(4) Storage of cultures: - Cultures are usually stored in darkness or heen darken. In some cases seed set may be influenced by temperature.

(5) Genotype: - Some evidence are there of genotypic variation in response of in vitro pollinated ovaries of maize.

In vitro fertilization:-

In vitro fertilization can be done by fusion of male and female gamete in vitro. Regeneration is done via embryogenesis fully fertile hybrid plant from the fusion product is a major peak through in the field of plant biotechnology. In this process firstly isolation of male and female gamete is done by different method. After that isolated egg is fused with sperm by electro fusion method fertilized egg divided and formed mini callus with a higher frequency after being injected with plasmid DNA.

- Egg cells were isolated by micro dissection from the ovules incubated for up-down in du enzyme solution containing pectinase, pectolyase, hemiallulase with maunitol. pH set as 5.
- Sperms are isolated from pollen grains by osmotic shock in nannitol solution.
- To fuse isolated gametes single sperms and egg cell were selected and carefully transferred to a fusion solution in the form of droplets. The fusion droplets were covered with this layer of mineral oil.
- Fusion of gametes was performed with a pair of adjustable electrodes mounted on the microscope.
- Alignment of the gametes in a manner that the egg was in costal with the electrodes fewoureh better fusion.
- In vitro fertilization eggs were cultureh on a semi permeable transparent membrane with 0.1 ml. of nutrient solution.
- This dish was inserted into the middle of Petri plates filled with nutrient medium containing feeder layer derived from bred embryogenesis suspenslon culture of omothe line.
- These cultures were maintaiueh under light/dark.
- Karyogamy occurred within the of fusion.
- 90% of fusion products produced imni colonies and 41% fo cells developed into globular embryos.
- Full plants could be regenerated by transferring there organized structures to semisolid medium of an altered composition.

Application:-

Several potential application of in vitro pollination in genetic manipulation of plants have been suggested.When this new technique becomes applicable to save other species. Fusion of genetically modified gametes may become omother approach to genetic engineering of crop plats. In vitro ovular and placental pollination are very useful in inbreeding and hybridization programs when the zone of incompatibility lies in the stigma style or ovary. In vitro pollination has also helped is obtaining haploids.

(i)Inbreeding: -

In self pollinated the pollen germination is good but the pollen tube does not enter the ovary. So self incompatibility can be over come by it vivo bud pollination. Production of homozygosis plants via androgen sis lgynogenesiss last not been successful so far. In the legumes with gametophilic incompatibility to achieve homozygosity not applicable. Researchers have reported the frequency of seed set following in vitro self pollinations was 5-10 times greater them in vitro.

(2) Hybridization: -

the most important application of invibo placental pollination is is raising hybrids produced fertile hybrids with ac chromosomes. The sexually incompatible cross has also been made through protoplast fusion but all the somatic hybrids were encnploid. When ovules were pollinated with pollen the hybrid embryos developed up to the globular stage and well developed endosperm was formed. Embryo exhibit budding but did not developed further normally.

(3) Haploid production: -

Researchers raised haploids of a plant by pollination their exposed ovules with the pollen. These haploids developed part hero genetically.

Pat hero genetically development of haploid plant in the cultures of unfertilized ovules and ovaries is well known.

(4) Production of stress tolerant plant: - In vitro pollination at higher temperature resulted in the production of heat- stress tolerant plants of maize.

At the elevated temperature only heat stress tolerant pollen gains were able to effect fertilization and the resulted sporophytes expressed the gametophyte trail. These plant exhibited better agronomic performance of high temperature compened to the plants produced through in vitro pollination of normal temperature.

Q.4 What are haploids? Give a brief description of another and pollin culture.

Ans.

- Haploids are plants which has game tic chromosome (n.) in their sporophyties.
- Haploids may be grouped into two broad categories:-

(a)Monoploids – Which possess half the no of chromosomes from a diploid species.

(b)Polyhaploids – It possess half the number of chromosomes from a polyploidy species.

Haploid production through anther culture has been referred to as androgenesis while gynogenesis is the production of haploid plants from ovary to ovule culture where the female gamete or gametophyte is triggered to sporophytic development.

Androgenic Methods:-

It is a method of haploid production which is done from the male gametophyte of an emaiosorm plant ie microspore principle is to stop the development of pollen cell whose fate is normally to become a gamete (Sexual cell) and force its development directly into a Haploid can be obtained by the culture excised anthers and pollen.

Anther Culture:-

Young flower buds with immature anther which have the microspores are surface sterlized and rinsed with sterile water. One of the anthers is crushed in acetocarmine to know the stage of polles development. Anthers of appropriate stage are inoculated in the nutrient media.

The anthers in later stage gradually turn brown and within 3-8 weeks thes burst open due to the pressure exerte by the growing pollen callus or pollen plants.

They attains a height of about 3-5 cm the individual plants or shoots emerging from the callus are separated and transferred to a medium that would support further development.

Microspore Culture

- Haploid plants can be produced through in vitro culture of male gametopytic cells i.e. microspores or immature pollen.
- General procedure of culture is:-

  Anthers are collected from sterlized flower buds in a small beaker containing basal media. Microspores are then squeezed out of the anthers by pressing them against the side of beaker with a glass rod. Anther tissue debris is removed by tilt ring the suspension through a nylon sieve.

This wuwpension is the centrifuged at low speed.

The supernatant containing fine debris is discarded and the pellet of pollet is resuspended in fresh media.

The microspores obtained are then mixed with an appropriate culture medium.

Final suspension in then pipetted into small Petri dishes. (For creation, thin layer of liquid is made)

Each dish is then sealed with Para film to avoid dehydration and is incubated. The

various factors affecting the androgensis are:-

Genotype: - For successful culture, the genotype of anther is predominant.

Physiological status of the donor plant:- The physiological status of the plant at the time of excision of anther influences the sporophytic efficiency of microspores.

Stage of pollen: - Selection of anthers at an appropriate stage of pollen development. Anthers with microspores ranging from tetrad to the binudeate stage are responsive.

Pretreatment of anthers: - As the androgenesis is the duration from the mormal development so for the induction certain treatments are given:

(a) **Cold treatment:** - It is given between 3^0 to 6^0C for 3 to 15 days. As a result weak and non- viable anthers and microspores are killed and the material gets enriched.

This treatment retards aging of the anther wall.

(b) **Hot treatment** - Explants are subjected 300C for 24 hrs or 400C for 1 hr stimuli embryogenesis.

(c) **Chemical treatment**-Chemicals induce par example- 2 chloroethylphosphonic acid.

5. **Culture Media:** - Presence of sucrose nitrati… ammonium salts and amino acids are essential components to be present in a culture medium. Activated charcoal also the percentage of androgenic anthers in x pollen embryogenesis can be induced on mineral sucrose medium.

Process of Androgen sis:-

Haploid plantlets are formedin t ways:-

(a) **Direct embryogenesis:** Embryos originate directly from the microsopor anthers without callusing.

(b) **Indirect embryogenesis:** It is also know organogenesis pathway in this microspores undergo prolifera to form callus which can induced to differentiate into plants. **Process of androgensesis** shows microspores under go division which continues until a 40-50 celled prembryo is formed. The embryos undergo various stage of development stimulating those of normal zygotic embryo formation. However when the microspore take organ genetic pathway, when these all increase in size exerting pressure and the contents are released in the form of callus.These calluses differentiate into plantlets. The plants with developed shoots and roots are then transferred to pots.

The physical environmental conditions in which the cultures are to placed can enhance differentiation. These are:-

(a) Incubation at 24-28^0C

(b) Hight intensity of 500 Lux.

(c) After induction leapt at 14 hr day light at 2000-4000 Lux.

For obtaining homozygous lines the plants derived from their atuher culture are analyzed for their polity status. Some of these methods are:-

1. Counting of plastids in the stomata:- count number of plastids in the stomato leaf.

2. Chromosome number:- It can be con from pollen mother cells of buds which can be collected form regenerated plan acetocarmine is used for staining of chromosome.

3. Number of nucleoli:- Haploids contain of nucleoli where as diploids have 2 nucleoli

4. Flow cytometric analysis:- Nuclear DNA content reflects the ploidy state of the donor which is determined by flow cytometery.

Diploidization

Haploids can be diploidized to produce homozygous plants by following methed
Colchicines treatment.
Endomitosis (Chromosome duplication without nuclear division)

Significance and uses of Haploids

(a) Development of pure homozygous lines.
(b) Hybrid development.
(c) Induction of mutation.
(d) Induction of genetic variability.
(e) Gem ration of exclusively male plants.
(f) Cytogenetic research.
(g) Significance in the early release of varieties.
(h) Hybrid sorting in plant breeding.
(i) Disease resistance.
(j) Insect resistance.
(k) Salt tolerance.

Gynogenic Haploids

- Recent advances has lead to the induction of haploid from ovar ovule culture.
- The megaspores or female game top of angiosperms can be triggered into sporophytic development.
- In vitro culture of unpollinated ou and ovules represent an alternative the production of haploid plants in for which anther culture has gi msatisfactory results.
- Ovaries can be cultured as polling empollinated

Procedure Ovaries are removed and seew sterilized Before culturing the lip of dis part of the pedicel is cut otf the ovary is implanted wit cut and inserted in the nutria

When liquid medium is to be employed the ovaries can be placed on a filter paper and inserted into the medium.

Factor affecting cuture:

- Genotype
- Growth condition of the donar plant.
- Stage of harvest of ovule
- Embryo soc stage.
- Culture conditions
- Seasonal effects
- Physical factors.

Key Terms

ABIOTIC STRESS - The stress caused to plants due to herbicides, water deficiency, ozone, intense light etc.

ADHERENT CELLS - The cells which grow adhering to cell culture vessel and are adherent dependent are called adherent cells

AEROBE - A microorganism dependent on oxygen for it's growth.

AGAROSE GEL ELECTROPHORESIS - Electrophoresis carried out on agarose gel to separate DNA fragments.

Agrobacterium tumefaciens – A rod shaped bacterium that causes crown gall disease by inserting it's DNA into plant cells.

AMINO ACIDS - The building blocks or monomeric units of protein.

Anaerobe- A microorganism that can grow in the absence of O2

Androgenesis- Development of plants from male gametophytes

Aneuploidy - An abnormal condition of chromosomes, differing from the usual diploid constitution. This may be due to a loss or gain of chromosomes.

Annealing - The pairing of complimentary single strands of DNA to form a double helix.

Apoptosis - Programmed cell death

ARS - Autonomously Replicating Sequence

ATCC - American Type Culture Collection

ATP - Adenosine Triphosphate

Autoradiography - The process of detection of radioactively labeled molecules by exposure of an X-ray sensitive film.

Auxins - A group of plant growth regulators which are involved in cell elongation, root initiation etc. e.g. indole acetic acid.

BATCH CULTURE - Batch culture is a closed culture system containing limited amount of nutrients

Bergmann's plating technique - The most widely used method for culture of isolated

single plant cells.

Bioaccumulation - Concentration of a chemical agent (e.g. DDT) in the increasing amount in the organisms of a food chain.

Bioaugmentation - The addition of microorganisms to waste sites so that the hazardous wastes are rendered harmless.

Biochemical oxygen demand (BOD) - The oxygen required to meet the metabolic needs of aerobic organisms in water containing organic compounds.

Biodegradation - Biological transformation of organic compounds by living organisms, particularly the microorganisms.

Biofertilizer - Commercial preparation of microorganisms by using which the nitrogen nd phosphorus level and growth of plants increase.

Biofilteration - The process of removing complex wastes from domestic and industrial sources by using microorganisms.

Biohazards - The accidents or risks associated with biological materials.

Biotic stress - The stress caused to plants by insects, pathogens (viruses, fungi, bacteria), wounds etc.

Biotransformation - The use of biological systems for the conversion of biomolecules.

Biodiversity - The variability among the living organism from all sources, soil, water, air, extreme habitat or associated with organisms.

BIOTIN - A non-radioactive label used for labeling probes, detected through a cyto-chemical reaction

Bt plants - The plants carrying the toxin producing gene from Bacillus thuringiensis, and capable of protecting themselves from insect attack.BLAST- Basic Local Alignment Search Tool

Broth - Any fluid medium supporting the growth of microorganisms.

Callus - A mass of undifferentiated plant tissues formed from plant cells or tissue cuttings when grown in culture.

Cell culture - The culture of dispersed (or disaggregated) cells obtained from the original tissue, or from a cell line.
Cell lines - animal or plant cells that can be cultivated under laboratory conditions.

Cell - mediated immune response- The activation of the T-lymphocytes of the immune system in response to a foreign antigen.

CLONE - All the individuals derived by asexual reproduction from a single original individual. In molecular biology, a strain of organisms that carries a particular DNA sequence.

Cloning vector - A plasmid or a phage that carries an inserted foreign DNA to be introduced into a host cell.

Codon - A triplet nucleotide sequence of mRNA coding for an amino acid in a polypeptide.

Colony hybridization - A technique that employs nucleic acid probe to identify a bacterial colony with a vector carrying specific gene (s).

COMPETENCE - Ability of a bacterial cell to take in DNA

CONTINUOUS CELL LINES - The cell lines that get transformed and under in vitro conditions grow continuously are called Continuous cell lines. These cells show no contact inhibition and no anchorage dependence.

Cross-pollination: Fertilization of a plant with pollen from another plant. Pollen may be transferred by wind, insects, other organisms, or humans.

Culture - A population of plant or animal cells/microorganisms that are grown under controlled conditions.

Culture medium - The nutrients prepared in the form of a fluid (broth) or solid for the growth of cells/tissues in the laboratory.

CYBRIDS - The cytoplasmic hybrids obtained by the fusion of enucleated and nucleated protoplasts are called Cybrids

Cybridization - The process of formation of cybrids.

CYTOKINES - Various chemicals produced in the body which mediate immunological responses

Cytotoxicity - The toxic effects on cells that result in metabolic alterations including the death of cells.

Diazotrophs - The microorganisms involved in diazotrophy.

DMSO - Dimethyl sulfoxide

DNA - Deoxyribonucleic acid

DNA hybridization - The pairing of two DNA molecules used to detect the specific sequence in the sample DNA.

DNA marker- A DNA sequence that exists in two or more readily identifiable forms (polymorphic forms) which can be used to mark a mal position on a genome map.

DNA repair - The biochemical processes that correct mutations occurring due to replication errors or as a consequence of mutagenic agents.

DNAse - Deoxyribonuclease

Electrophoresis - An analytical technique that separates charged molecules in an electrical field.

Electroporation - The technique of introducing DNA into cells by inducing transient pores by electric pulse.

Embryonic stem cells (ES cells) - The cells of an early embryo that can give rise to all differentiated cells, including germ cells.

Embryo rescue - The culture of immature embryos to rescue them from unripe or hybrid seeds which fail to germinate.

Embryo transfer - The process of implantation of embryos from a donor animal, or developed by in vitro fertilization into the uterus of a recipient animal.

Enzyme - linked immunosorbent assay (ELISA)- A technique for the detection of small quantities of proteins by utilizing antibodies linked to enzymes, which in turn catalyse the formation of coloured products.

EXPLANT - The whole plants can be regenerated virtually from any plant referred to as explant.

Exponential phase - This refers to a phase in culture in which the cells divide at a maximum rate.

FED-BATCH CULTURE - In a Fed Batch culture, the culture is continuously or sequentially fed with fresh medium with out removing the growing culture

Fermentation - The growth of cells or microorganisms in bioreactors (fermenters) to synthesize special products. Fermentation in biochemistry refers to the biodegradation of

carbon compounds by cells or organisms under anaerobic (lack of oxygen) conditions.

FINITE CELL LINES - Finite cell lines are those which have a limited life span and they grow through a limited number of cell generations

Fusogen - An agent that induces fusion of protoplasts in somatic hybridization.

Fusion protein - A protein that is formed by fusion of two polypeptides, normally coded by separate genes

Gametoclonal variations - The variations observed in the regenerated plants from gametic cells (e.g. anther culture).

Gene bank - A library of genes or clones of an entire genome of a species.

Gene Cloning - It basically involves the insertion of a gene (a fragment of DNA) or recombinant DNA into a cloning vector, and propagation of the DNA molecule in a host organism.

GENOMIC DNA -The DNA of an organism containing the essential genes of the organism

GENETIC MAPS - Maps giving relative distance and position of one gene with respect to the other, the distances are based on recombination values

Gene therapy - Treatment of diseases by use of genes or DNA sequences.

Genetically engineered microorganisms (GEMs) - The microorganisms with genetic modifications are collectively referred to as GEMs.

Genetically modified (GM) foods - The entry of transgenic plants and animals into the food chain represents GM foods.

Genetically modified organisms (GMOs) - A term used to represent an organisms that are genetically engineered. It usually describes the transgenic plants and transgenic animals.

Genetic engineering - Broadly involves all the in vitro genetic manipulations.

Genome - The total content of DNA represented by the genes contained in a cell.

Genomics- The study of the structure and function of genomes.

Genetic library- A collection of clones representing the entire genome of an organism.

Germ line- Reproductive cells that produce gametes which in turn give rise to sperms and

eggs.

GERMPLASM - Germplasm refers to the sum total of all genes preset in a crop and its related species G.M. or Genetically modified crops etc which have a higher productivity

Golden rice - The genetically engineered rice with provitamin A (beta-carotene) enrichment.

Green biotechnology is biotechnology applied to agriculture processes. An example would be the selection and domestication of plants via micropropagation. Another example is the designing of Transgenic plants to grow under specific environments in the presence (or absence) of chemicals. Using geentic engineering, plants have been created that can express a pesiticide, thereby ending the need of external application of pesticides. eg Bt corn. Whether or not green biotechnology products such as this are ultimately more environmentally friendly is a topic of considerable debate.

Heterologous - These are gene sequences that are not identical, but show variable degrees of similarity.

HIGH THROUGH PUT- Fast rate of sequencing

Histotypic cultures - The growth and propagation of cells in three dimensional matrix to high cell density.

HPLC - High Performance/Pressure Liquid Chromatography

HYBRID DNA -DNA composed of sequences from two different organisms, also called as Recombinant DNA.

HYBRIDIZATION - The annealing of a single stranded DNA to its complimentary region on another single stranded DNA

Karyotyping - The method of photographing the complete set of chromosomes for a particular cell type and organizing them into pairs based on size and shape.

LAF - Laminar Air Flow Hood

Meristem - A localized region of actively dividing cells in plants i.e. tips of stems and roots.

MICROARRAY - Large number of DNA spots present on a glass slide representative of the total mRNA of a cell, used for detecting expression patterns

MICROPROPAGATION - This method of tissue culture utilizes the culture of apical shoots, auxillary buds, meristems.

Microinjection - The delivery of DNA or other compounds in to eukaryotic cells using fine microscopic needle.

MOLECULAR PHARMING - Use of Transgenic animals to obtain products of medicinal commercial purposes through recombinant DNA technology

MOLECULAR BREEDING - Breeding assisted by molecular (nucleic acid) markers, is known as molecular breeding.

Morphogenesis - The growth and development of an undifferentiated structure to a differentiated structure or form.

MTCC - Microbial Type Culture Collection

Multicellular tumour spheroids (MCTs) - In vitro cellular three dimensional proliferating models for the study of tumour cells.

Nitrogen fixation - The process of conversion of atmospheric nitrogen to ammonia. Biological nitrogen fixation occurs in prokaryotes and is catalysed by the enzyme nitrogenase.

Northern blotting - The transfer of RNA from an electrophoresis gel to a membrane to perform Northern hybridization

Northern hybridization - The technique used for the detection of specific RNA molecule through Northern blotting

Oligonucleotide - directed mutagenesis- A technique to alter one or more specific nucleotides in a gene (DNA sequence) so that a protein with specific amino acid change is produced

Organ Culture - The in vitro culture of an organ so as to achieve the development and/or preservation of the original organ.

Organogenesis - The process of morphogenesis that finally results in the formation of organs e.g. shoots, roots.

Patent - A government issued document that provides the holder the exclusive rights to manufacture, use or sell an invention for a defined period usually 20 years.

Phosphinothricin (glufosinate) - A broad spectrum herbicide

Phytoalexins - The secondary metabolites produced in plants in response to infection

Plantlet - Small rooted shoot or germinated embryo

Plating efficiency - The percentage of cells plated which produce cell colonies.

Plasmid- An autonomous, circular, self replicating extrachromosomal DNA, found in bacteria and some other cells.

PAM - Point Accepted Mutation

pI - Isoelectric point

Polymorphism - The allelic variations in the genomes that results in different phenotypes

PEG - Polyethylene glycol

PGDF - Platelet derived growth Factor

Primary Cells - The eukaryotic cells taken directly from an animal for culture purpose.

Primary Cell Culture - The culture produced by the freshly isolated cells or tissues taken from an organism.

Probe - A labeled molecule used in hybridization technique.

Protein Engineering - Generation of proteins with subtly modified structures conferring improved properties e.g. higher catalytic function, thermostability etc.

Protein Targeting - The process of transport of proteins from one compartment to other with in a cell. Also called as protein sorting.

PROTEOME - The complete protein complement of cells, tissues and organisms is referred to as its proteome.

PROTEOMICS - Large scale characterization of the entire protein complement of cells, tissues, and organisms is called proteomics.

PROTEIN ENGINEERING - Production and modification of proteins for medicinal, Industrial, and research purposes

PRIMARY CELL CULTURE - The maintenance of growth of cells dissociated from the parental tissue in culture medium is known as primary cell culture

RNA - Ribonucleic acid

RNAse – Ribonuclease

R- HuEPO - Recombinant human erythropoietin

Recombinant DNA (rDNA) technology - The techniques involved in the construction, and use of recombinant DNA molecules.

Recombinant protein - A protein that is produced by the expression of a cloned gene of a recombinant DNA molecules

Restriction endonuclease - An enzyme that specifically cuts DNA molecule at specific nucleotide sequences.

Retro virus - A virus with RNA as genetic material

Reverse transcription - The process of synthesis of DNA from RNA

RNA vaccines - RNA molecules which can synthesize antigenic proteins and offer immunity

Satellite DNA - Repetitive DNA that forms a satellite band in a density gradient

Scale up - The expansion of laboratory experiments to full-sized industrial processes.

Secondary metabolite - A metabolite that is not required for the growth and maintenance of cellular functions.

Septic tanks - Anaerobic digesters of solids of the sewage settled at the bottom of tanks.

Sewage - The liquid waste arising mainly from domestic and industrial sources.

Shot gun approach - A technique for sequencing of genome in which the molecules to be sequenced are randomly broken down into fragments, which are then individually sequenced.

Shuttle vectors - The plasmid vectors that are designed to replicate in two different hosts e.g. E. coli and Streptomyces sp

SNPs - Single Nucleotide Polymorphisms

Single cell protein (SCP) - Cells or protein extracts of microorganisms produced in large quanities for use as human or animal protein supplement.

Signal peptide - A short sequence of amino acids at the N terminal end of some proteins that facilitates the protein to cross membrane

Sludge - The semi solid mass produced during the course of sewage/waste water treatment processes.

Somaclonal variation - The genetic variations found in the cultured plant cells when compared to a pure breeding strain.

Somatic cell - Any body cell as opposed to germ cell. Somatic cell is non-reproductive and divides by mitosis.

Somatic cell gene therapy - The delivery of gene(s) to somatic cells to correct genetic defects.

Somatic embryogenesis - Formation of embryos from asexual cells

Southern hybridization - A technique used for the detection of specific DNA sequences (restriction fragments)

Sparger - A device that introduces air into a bioreactor in the form of a fine stream

SDS-PAGE Sodium Dodecyl Sulphate - Polyacrylamide gel Electrophoresis

Stem cells - A progenitor cell that is capable of dividing continuously through out the life of an organism.

SUB CULTURING - Subculturing involves removing the growth media, washing the plate, disassociating the adherent cells, usually enzymatically/or removing by using pipette, and diluting the cell suspension into fresh media.

SUSPENSION CULTURES - Cells which do not attach to the surface of the culture vessel and grow in a suspended manner in the culture medium are called suspension cultures

T-DNA - The part of the Ti plasmid that is transferred to the plant DNA

T-lymphocytes (T cells) - The lymphocytes that are dependent on the thymus for their differentiation, and are involved in cell-mediated immune response.

Thaumatin - A protein extracted from berries which is about 3000 times sweeter than sucrose

Tm - Melting temperature

Ti plasmid - The large-sized tumour inducing plasmid found in Agrobacterium tumefaciens. It directs crown gall formation in certain plant species.

Tissue culture - A process where individual cells, or tissues of plants or animals are grown artificially.

Tissue engineering - The application of the principles of engineering to cell culture for the construction of functional anatomical units.

TRANSFORMATION - Uptake of naked DNA by bacterial cells

Totipotent - A term used to describe a cell that is not committed to a single developmental pathway, and thus it is capable of forming all types of differential cells.

Traditional (old) biotechnology - The age old practices for the preparation of foods and beverages, based on the natural capabilities of microorganisms.

Transgenic - An organism that carries a foreign DNA (transgene).

VECTOR - A vehicle for carrying cloned DNA

Vegetative propagation - The asexual propagation of plants from the detached parts of the plants.

Zygote- The fertilized egg formed by the fusion of two gametes.

B.Sc./M.Sc. (Part III) Examination, 2011

(FACULTY OF SCIENCE)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

PAPER BT-702

PLANT BIOTECHNOLOGY

Year-2011

Time.: 3 Hours ***Max. Marka : 50***

Attempt FIVE questions in all, selecting ONE question from each Section. Question No. 1 is compulsory. Each question carries 10 marks.

1. Give short answers:
   - (i) Define Cytodifferentiation.
   - (ii) What are DNA microarrays?
   - (iii) What do you mean by Phenolic leaching?
   - (iv) Explain Genotypic recalcitrance.
   - (v) What do you mean by Embryo rescue?
   - (vi) Define chimeric DNA
   - (vii) What are cisgenic plants?
   - (viii) Explain biolistics.
   - (ix) What are sphaeoplasts?
   - (x) What do you mean by Golden rice? 1 x 10 = 10

Section-A

2. Define functional genomics. What approaches are used to determine the functions of unknown genes? 4+6=10

3. Write notes on:
   (i) Use of micro-propagation in Agriculture
   (ii) Hardening of regenerated plants and transfer to soil.
   (iii)Microinjection. 4+3+3

Section-B

4. Describe micro propagation and morphogenetic pattern of cereal plants with a suitable example. 10

5. Write short notes on:
   (i) Micro propagation of legumes
   (ii) Seasonal variation and geotypic dependent regeneration system. 5+5

Section-C

6. Describe the following: 5+5
   (i) The engineering of recombinant plastids in higher plants.
   (ii) Genetic transformation of food grain corps.

7. Write notes on :
   (i) Disease elimination through tissue culture.
   (ii) Aseptic techniques and control of contamination in a commercial laboratory.
   (iii) Quarantine 4+3+3

Section-D

8. Describe androgenesis and production of homozygous plants and their use in agriculture. 8+2

9. Write notes on
   (i) Somatic hybridization
   (ii) Production of seedless plants.
   (iii) Tissue specific sequences 4+3+3

B.Sc./M.Sc. (Part III) Examination, 2009

(FACULTY OF SCIENCE)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

PAPER BT-702

PLANT BIOTECHNOLOGY

Year-2009

Time.: 3 Hours ***Max. Marka : 50***

Attempt FIVE questions in all, selecting ONE question from each Section. Question No. 1 is compulsory. Each question carries 10 marks.

1. Give short answers:
   (a) What is Floriculture?
   (b) What do your mean by phenolic leaching?
   (c) What do you mean recalcitrance?
   (d) What do you mean by quarantine
   (e) What is the main difference between somatic hybrid and cybrid?
   (f) What is a GMO? Give an example.
   (g) What is pathological indexing?
   (h) What do mean by hardening?
   (i) What are recombinant plastids?
   (j) Give two examples of endangered species whose multiplication through tissue culture is desirable. 1 x 10 = 10

Section A

2. Explain micropropagation technique. How is it useful in propagation of specific genotypes, rare and/or improved varieties? 5+5

3. Write short notes on :
   (a) Somatic embryogenesis;
   (b) Screening procedures in genetic transformation;

(c) Inducible gene expression systems as tools for plant functional genomics.
4+3+3

Section B

4. Explain in detail the problems in propagating trees.

5. Write short notes on :
   (a) Write a protocol for micropropagation of a medicinal and ornamental plant with example.
   (b) Design one micropropagation experiment to obtain new plant genotypes with enhanced resistance to salinity 5+5

Section C

6. Write short notes on any three :
   (a) Application of biotechnology in Indian ginseng;
   (b) The engineering of recombinant plastids in higher plants.
   (c) Aseptic techniques and control of contamination in a commercial laboratory.
   (d) Wild species of Oryza as an important reservoir of useful alleles.
   (e) Genetic transformation of food grains and legumes., 3+4

7. Explain the technique of somaclonal variation, its application and limitation 5+5

Section D

8. Explain stepwise the protoplast culture technique and how icromanipulation of genes is done using protoplast. 6+4

9. Write short notes on :
   (a) Embryo rescue technique;
   (b) Transgenic plants;
   (c) Anther

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