OPTIMIZATION OF TISSUE CULTURE PARAMETERS FOR COMMERCIAL PRODUCTION OF PLANT MATERIAL OF *Gerbera jamesonii*

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CERTIFICATE

This is to certify that the work entitled, "Optimization of tissue culture parameters for commercial production of plant material of *Gerbera jamesonii*" submitted by Taru Prasad and Zain Taha in partial fulfillment for the award of degree of Bachelors of Technology in Biotechnology of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Dr. Hemant Sood
Date: 22/5/10

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Date:

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LIST OF ABBREVIATIONS

KN	Kinetin
IBA	Indole-3-butyric acid
NAA	1-naphthalene acetic acid
BA	Benzyl adenine
IAA	Indole-3-acetic acid
PAA	Phenylacetic acid
BAP	Benzyl amino purine
2,4-D	2,4-Dichlorophenoxyacetic acid
MS	Murashige & Skoog
LS	Linsmaier & Skoog
dm^3	Decimeter cube
µM	Micro molar
TDZ	Thidiazuron
$HgCl_2$	Mercuric Chloride
psi	Pound per square inch
UV	Ultra Violet
g	Gram
mg	Milligram
l	Liter
cm	Centimeter
°C	Degree Celsius

ABSTRACT

Gerbera has gained popularity in the past few years in many countries of the world and it is in great demand in the floral industry as cut flower as well as potted plant due to its beauty, color, long vase life, and ability to rehydrate after long transportation. The most commercial cultivars are propagated through vegetative means by multiplication through divisions of clumps. However, the multiplication by this method is too slow to be commercially viable. To commercialize this crop and to meet the growing demand for planting material, tissue and organ culture techniques are being used as alternative methods for propagation in many countries. Attention should be paid to improve the technology to achieve 100% success in all species/cultivars to meet growing demands of the growers globally. From the literature, it is evident that gerberas are highly amenable to *in vitro* studies, as various explants were found to favorably respond to different culture media with different types and concentrations of growth regulators. Hence, the present investigation aims at *in vitro* establishment and long-term maintenance of callus and regeneration of *Gerbera jamesonii* for eventual commercial purposes.

In vitro propagation of *Gerbera jamesonii* was carried out by taking shoot apices from the field grown plants and rapid micropropagation technique was developed by optimizing cost effective medium (MS + KN 3 mg/l + BA 2mg/l + sucrose 30 gm/l + agar-agar 9g/l) for maximum shoot proliferation and *in vitro* root induction was carried out by optimizing medium with different concentrations and combinations of auxins (MS + NAA 2mg/l + IBA 2mg/l + sucrose 30 g/l + agar-agar 9g/l). For the development of somaclonal variants for different flower color, callus induction and regeneration into shoots was carried out. Those regenerated shoots were further kept on rooting medium to develop into complete plantlets and were finally transferred to pots in glasshouse conditions and are expected to show flowering with a wide range of colors.

CHAPTER 1 INTRODUCTION

Gerbera jamesonii is an important commercial cut flower crop. It is one of the top ten traded cut flowers in the world (Parthasarathy, Nagaraju 1999). It is a perennial herb with deeply lobed leaves covered with silky hairs arising from a crown. The striking inflorescence is borne on a long stalk and the outermost petals (ray florets) may be cream, red, orange or pink, while the central flowers (disc florets) are cream.

The genus name *Gerbera* is in honor of the German naturalist Traugott Gerber, and the species was named after Robert Jameson who collected live specimens while on a prospecting expedition to the Barberton district of Mpumalanga Province, South Africa in 1884, even though the species had been collected on three earlier occasions by other people. The flower is also known as Barberton Daisy deriving its name from where it was first collected.

Like any ornamental plant, gerberas and so *Gerbera jamesonii* too is produced exclusively for its aesthetic values. Thus, the improvement for quality attributes such as flower color, longevity and form, plant shape and the creation of novel variants are important economic goals. However, the prime target at first is to increase its propagation to meet the demands of the commercial ornamental industry.

Its vegetative propagation is slow and other conventional methods of propagation are inadequate for the production of large number of uniform propagules for commercial cultivation. Over the years many workers have attempted tissue culture propagation of gerbera from different explants such as shoot tips, inflorescence, ovule and used callus regeneration and finally acclimatized it to the *ex vitro* environment.
(J. K. Kanwar, S. Kumar 2008).

From the literature, it is evident that gerberas are highly amenable to *in vitro* studies, as various explants were found to favorably respond to different culture media with different combinations and concentrations of growth regulators.

Therefore the present study was carried out with following objectives

1. Establishment of *in vitro* shoot cultures of *Gerbera jamesonii.*

2. Callus induction and regeneration of Gerbera plantlets.

3. Hardening of *in vitro* grown plantlets.

CHAPTER 2 REVIEW OF LITERATURE

Review is discussed in the light of available literature relevant to the research on *Gerbera jamesonii*.

2.1 General Micropropagation

Out of the recorded species, only one species *Gerbera jamesonii* is under cultivation. *Gerbera* belongs to the family *Asteraceae* and can be propagated by both sexual and asexual methods. Most of the commercially grown cultivars are propagated through vegetative means, to maintain uniformity and genetic purity (Peper *et al.* 1971). Among the vegetative means, multiplication through divisions of clumps is the most common method used for several decades. Gerbera can also be propagated through cuttings (Schiva 1975). The plant multiplication by these methods is too slow to be commercially practicable. Hence, there was a need to develop an easier, quicker and economically viable method of propagation. A tissue culture procedure has been proven to be commercially practical in gerbera propagation. This method enables a million fold expansion per year of a desired plant (Murashige *et al.* 1974; Aswath *et a.l* 2002,2003).

2.2 Types of explants

Gerbera was propagated by direct or indirect organogenesis using various explants including stem tips, floral buds, leaf, capitulum etc. The plants were produced from explants of capitulum in red flower gerbera (Pierik *et al.* 1973,1975), leaves (Hedtrich 1979; Barbosa *et al.* 1994), floral buds (Posada *et al.* 1999), floral bracts (Maia *et al.* 1983), torus (Zhang 2002) and inflorescence (Schum, Busold 1985).

2.3 Media

The nutrient medium is important for successful tissue culture. Information is still scanty on the various media used for gerbera explants. MS medium (Murashige, Skoog 1962) was successfully used by many workers for callus formation as well as shoot regeneration (Pierik *et al.* 1982; Le *et al.*1999; Modh *et al.* 2002; Aswath, Wazneen 2004; Kumar *et al* 2005, 2006). Parthasarathy *et al.*1996; Verma and Anand 2006; Chu 1978; Gamborg *et al.* 1968 used N6 and B5 media for culture establishment in gerbera. Mandal and Datta 2002; Chen *et al.* 2006 used LS (Linsmaier, Skoog 1965) medium for establishing organogenic callus cultures from immature flower buds.

The basal medium used for regeneration studies of *Gerbera jamesonii* has been varied many times in different studies. Murashige *et al* used MS medium with modification while Laliberte *et al* used Heller's mineral salt along with MS minerals. Mandal and Datta also used MS and Heller's salts to induce shoot buds from capitular sections. The Authors have also used combinations of MS Macro and Heller's micro salts.

2.4 *In vitro* Shoot Multiplication

Purnima Tyagi and S L Kothari (2003) reported a multiplication rate of up to 8 to 15 shoots from one *in vitro* shoot every 4 weeks on MS medium with 4mg/l KN and 0.1, 0.5 mg/l IAA. KN was better for shoot multiplication than BAP. The authors observed that apart from IAA, PAA also supports shoot initiation. Thus its effect on maintenance of cultures was checked and BAP or KN (2-4 mg/l) was combined with PAA (0.05-2.5 mg/l) where KN was more efficient with PAA than BAP. On 2 mg/l KN + 0.5 mg/l PAA, the cultures multiplied rapidly and 20-25 shoot buds developed. Effects of PAA in improvement of elongation and normal shoot recovery has been well established in other plants systems such as *Phaseolus vulgaris, Capsicum annuum* and *Helianthus annuus.*

Sahavacharin (1985) used young capitulum of gerbera hybrids for rapid multiplication of shoots on MS medium supplemented with 0–1 mg/dm^3 IAA and 0–12 mg/dm^3 KN.

Topoonyanont and Dillen (1988) cultured capitulum explants of orange,yellow, red and pink cultivars on half-strength MS basal medium supplemented with 5–15 mg/dm^3 BA. The orange cultivar produced eight shoots per explant with 5.0–7.7 mg/dm^3 BA. No shoot induction from pink cultivar was observed.

Barbosa *et al* (1994) obtained the best capitulum establishment with 3 or 9 mg/dm^3 BA and best propagation with half-strength MS supplemented with 2.27 mg/dm^3 BA.

Ray *et al* (2005) developed an efficient protocol for large scale propagation using young capitulum as explants on a medium containing 7 mg/dm^3 BA and 0.1 mg/dm^3 IAA, which initiate multiple shoots (10 shoots per explant).

Modh *et al.* and Zheng *et al.* (2002) reported proliferated mass of shoots from mature capitula with 0.1 mg/dm^3 IAA and 10 mg/ dm^3 BA in the medium.

Pierik *et al.* (1974) reported a rapid method for the development of shoots from the dormant buds situated in the axils of the bracts surrounding the receptacle of the flower capitulum.

Huang *et al.* (1987) reported a scheme for commercial multiplication through shoot tips, cultured on a medium containing 5% MS nutrients, 5 mg/dm^3 BA, 0.1 mg/dm^3 IAA and 1% agar, pH 5.6.

Aswath and Choudhary (2001,2002a,b) and Aswath *et al.* (2003) cultured shoot tips for establishing and proliferation of multiplication in *Gerbera jamesonii.*

2.5 *In vitro* Root induction

The nutrient medium of Murashige *et al.* (1974) gave good results and the incorporation of cytokinin (especially zeatin) favored the formation of new buds (Gregorini *et al.* 1976), which quickly formed roots on transfer to nutrient medium containing NAA.

2.6 Callus induction and regeneration

Napaskamon (1991) studied the effect of KN and NAA on induction and growth of callus from mature capitulum of local gerberas. The plantlets showed enhanced callus formation and growth with NAA whereas KN had no effect.

Mandal and Datta (2002) established organogenic callus cultures from immature flower buds on modified MS medium with IAA and BA.

Kumar and Kanwar (2006) observed maximum callus induction and growth in petal explants with 1, 1.5 and 2 mg/dm^3 2,4-D.About 53% of the calli developed five shoots per callus when transferred to regeneration medium containing 2 mg/dm^3 BA and 0.5 mg/dm^3 IAA.

Prasanth and Sekar (2004) used leaf bits excised from cv. Mammut and cultured them on medium containing 0.1 mg/dm^3 BA and 0–2 or 3 mg/dm^3 NAA. The combination of BA and NAA was more effective in enhancing callus formation than either NAA or BA, and this effect increased with increasing concentrations of both growth regulators.

Parthasarathy *et al.* (1996) reported callus induction and subsequent plant regeneration from leaf explants of *G. jamesonii* on MS medium supplemented with NAA, BA, KN and IBA in different concentrations. Callus growth was greatest with 1 mg/dm^3 of NAA, BA and IBA each.

Orlikowska *et al.* (1999) produced callus from petiole explant of the youngest 3–4 leaves detached from axillary shoots produced *in vitro* with 2.3 μM TDZ and 0.53μM NAA.

2.7 Hardening and acclimatization of *in vitro* rooted plants

Reynoird *et al* (1993) rooted *in vitro* shoots with half-strength MS containing 0.25μM NAA and acclimatized the regenerated plants in greenhouse under a plastic tunnel in trays containing peat:perlite (1:1) medium and achieved 100% success.

Petru and Matouš (1984) successfully transferred the plantlets into sterilized peat:perlite (1:1) substrate and then into a standard horticultural substrate.

Parthasarathy and Nagaraju (1999) achieved a 90–100% success in polythene bags containing equal amount of soil:sand:farmyard manure.

Kumar *et al.* (2004) reported 60–70% success in pots containing a mixture of sand:farmyard manure mixed in the ratio 1:1.

Kaur *et al.* (1999) obtained 100% survival rate of *in vitro* shoots when transferred to pots filled with a mixture of soil:sand:compost in 1:1:1 ratio.

Olivera *et al.* (2000) studied the effect of acclimatization on growth and plant development of gerbera under greenhouse conditions with 82.4% survival of plantlets.

Xi and Shi (2003) observed that the rooted plantlets could survive for 15–25 days when transferred to peat.

CHAPTER 3 MATERIALS & METHODS

The present experiments of Establishment of *in vitro* shoot cultures of *Gerbera jamesonii*, callus induction and regeneration of Gerbera plantlets & Hardening of *in vitro* grown plantlets were carried out in the Department of Biotechnology, Jaypee University of Information Technology, Waknaghat, during 2009-2010.

The Experiments and methodology followed to carry out the above-mentioned experiments have been described under the following headlines.

3.1 Micropropagation of *Gerbera jamesonii*

3.1.1 Collection of Plant material

3.1.2 Preparation of explants

3.1.3 Surface Sterilization

3.1.4 Media Preparation

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3.2 Callus induction and regeneration of *Gerbera jamesonii*

3.2.1 Preparation of explants

3.2.2 Media preparation

3.2.3 Induction of callus

3.2.4 Callus Regeneration

3.3 Hardening and acclimatization of *in vitro* rooted plantlets in glass house

3.1.1 Collection of Plant material

The field grown plant of *Gerbera jamesonii* was procured from Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Solan, H.P. and was brought to the laboratory of the Department of Biotechnology of this University for further studies.

3.1.2 Preparation of Explant

To prepare the explants, the plants were washed under running water so as to remove all soil debris. Then all the shoots and leaves were removed and the shoot apex was excised with the help of a scalpel blade and collected in water in a beaker.

3.1.3 Surface Sterilization

Surface Sterilization of the shoot apices was carried out by using different surface sterilants for different time intervals. The different sterilants used are as follows

i) Bavistin - 0.5 %

ii) Mercuric chloride - 0.1 %

The shoot apices collected in beaker were surface sterilized first by washing 3-5 times with tap water, later treated with Labolene (2-3 drops/100 ml water) for 2-3 minutes and then washed thoroughly with distilled water. Henceforth, the surface sterilization was carried out in Laminar air hood.

i) The washed shoot apices were then treated with Bavistin (0.5 %) for 2-3 minutes. Subsequently, the shoot apices were washed with distilled water 1-2 times.

ii) After being treated with Bavistin, the shoot apices were treated with $HgCl_2$ (0.1%) for 0.5-1 minute. Finally, the shoot apices were washed with distilled water 3-4 times.

3.1.4 Media Preparation

The composition of Media was based on Murashige & Skoog (1962) medium divided into different stocks (Annexure I).The Stock Solutions were prepared and kept in the refrigerator. Each salt of the stock solution was dissolved separately in distilled water so as to avoid precipitation. The Chemicals used were of reliable grade and were obtained from reliable firms like Sigma Aldrich. Each Stock solution of MS Media was added one by one to prepare the desired medium. After addition of sucrose (30g/l), growth hormones, desired volume was made with distilled water and the pH of the medium was adjusted to 5.6-5.7 by using pH meter. Then, agar-agar (9g/l) was added and dissolved by boiling the medium till it became homogenous. The medium was finally dispensed in 250 ml culture jars for culturing. These were closed with the jar caps and autoclaved at a pressure of 15 psi, 121 °C for 20 minutes for proper sterilization of culture medium. This medium was allowed to solidify and was used after 2 days of preparation. In all 10 different combinations of media was prepared using different concentrations of appropriate growth hormones like KN, BA, IBA, NAA, 2,4-D for each of the experimental setups.

3.1.5 Preparation of Modified MS Media

Basal Media composition was same as based on Murashige & Skoog (1962) medium and manipulations were carried out for cost effectiveness with respect to 1 component for the preparation of modified MS media. Here, distilled water was replaced with Tap water. Upon observing inoculation results in the first month, the standard and modified media showed no differences and therefore modified media was used for the rest of the project because it proved to be cost effective than the standard media.

3.1.6 Cleaning of Glassware

All the glassware which were not used before were cleaned in a dilute solution of Labolene with test tube brush and rinsed thoroughly in tap water. The Glassware was then kept for drying in hot dry air oven at 80-100 °C for 2-3 hours.

The used glassware with contaminated cultures were first autoclaved and molten media was disposed off and the culture jars were cleaned.

3.1.7 Culture Conditions

All the operations were carried out aseptically in a Laminar Air Hood filled with UV Light. The Laminar Flow Chamber was thoroughly wiped with rectified spirit prior to use and the UV Light was kept on for 15-20 minutes in Laminar Flow Chamber. Thereafter, the airflow was allowed to run and the UV Light was switched off. Before starting with aseptic manipulations, hands and arms were washed with soap and water. Hands were frequently wiped during manipulations with rectified spirit which was allowed to evaporate. All the metallic tools like scalpel blade, forceps and needles were first autoclaved and wiped with rectified spirit and flame sterilized at the time of use. The rims of tubes and jars were quickly flame sterilized before and after inoculations. Subsequently, the jars were kept in trolleys in culture room at 25 ± 2 °C under 16 hours photoperiod and 3000 flux light intensity.

3.1.8 Inoculation of prepared ex plant

For the establishment of the cultures, the surface sterilized explants were inoculated onto the MS Medium supplemented with various combinations of growth hormones. The cultures were then incubated for the proliferation of shoot apex. The proliferation of shoot apex was indicated by the emergence of micro shoots and leaflets.

Observations

Average number of micro shoots proliferated per shoot apex on prepared MS media after 15 days of inoculation was recorded.

3.1.9 *In vitro* shoot multiplication on prepared MS media

The shoots obtained from the shoot apex were subcultured on prepared MS media containing various combinations of growth hormones including BA, IBA & KN. Subculturing for multiplication was carried out at 3 weeks interval up to 5 subcultures.

Observations

Average number of shoots, shoot length after 3 weeks of sub culturing was recorded.

3.1.10 *In vitro* rooting on prepared MS media

Rootable shoots were excised from lavishly multiplying shoot cultures having at least 2 cm length and were transferred singly to separate culture jars containing MS Media enriched with rooting growth hormones NAA and IBA of different concentrations.

Observations

Average number of roots and root length after 3 weeks was recorded.

3.2.1 Preparation of explants for callus induction

There were three types of explants prepared for callus induction. The leaves, petioles and rootlets of *Gerbera jamesonii* were taken from *in vitro shoots* and cut into small sized explants; five to six millimeters in size under sterile conditions.

3.2.2 Callus Induction

The prepared explants were cultured on MS medium (Murashige and Skoog, 1962) with the addition of growth hormones 2,4-D and NAA of varying concentrations. The explants were incubated in a culture room at 25 °C ± 2 °C to encourage the formation and growth of callus. After 4 weeks, the incised explants were sub cultured in newly prepared MS medium enriched with the calli regeneration growth hormones.

3.2.3 Regeneration of shoots from calli

After 4 weeks, the developed calli were cultured on regeneration media containing different concentrations and combinations of KN, IBA and BA for initiation and proliferation of shoots. Shoot cultures were raised in culture jars as regenerants and were multiplied further to get their large numbers.

3.3 Hardening and acclimatization of *in vitro* rooted plantlets in glass house

Transplantation and hardening of *in vitro* regenerated plants was carried out in plastic pots. After growth of 5 weeks on the rooting medium, roots were well developed and the plantlets from all rooting media were taken out of culture jars. Precaution was taken to avoid any possible damage to delicate root system. The agar medium from roots was washed gently under running tap water. After removal of the adhering medium, the plantlets were kept in Bavistin solution (0.5%) for 15-20 minutes.

Plantlets were transferred to plastic pots containing pre-autoclaved mixture of sand, soil and perlite, vermiculite and coco-peat in combinations. The root portion of plantlets was then gently placed in the pots containing the various rooting mixtures and sand at the bottom. The plantlets were watered and covered with glass jars to maintain high humidity.

After a week, when the plantlets showed initial signs of establishment in pots, with the appearance of new leaves, the pots were initially uncovered for overnight. Then after 2-3 weeks of transfer to pots, the plantlets were totally uncovered.

Observations

Percentage of survived plants in different potting mixtures was recorded.

CHAPTER 4 EXPERIMENTAL RESULTS

4.1 Proliferation of shoot apex on prepared MS media

4.2 *In vitro* shoot multiplication on prepared MS media

4.3 *In vitro* rooting on prepared MS media

4.4 Callus induction and regeneration

4.5 Hardening and acclimatization of *in vitro* rooted plantlets in glass house.

4.1 Proliferation of shoot apex on prepared MS media

After 3 weeks of inoculation of shoot apex, we observed 3 to 8 micro shoots emerging from each inoculated explants (Figure 1).

4.2 *In vitro* shoot multiplication on prepared MS media.

After 3-4 weeks of growth, the shoots thus proliferating from the shoot apex were excised under aseptic conditions and subcultured on the prepared MS medium enriched with different concentrations of BA, KN & IBA growth hormones (Table 1) The maximum shoot multiplication was seen in MS Media containing (MS + IBA 2mg/l + KN 3mg/l + Sucrose 30g/l + agar-agar 9g/l). (Figure 2)

TABLE 1: *In vitro* shoot multiplication on different combinations and concentrations of cytokinins and auxins.

MS + Growth regulators (mg/l)			Parameters of shoot multiplication		
IBA	BA	KN	Days to multiple shoot formation	Avg No. of Shoots per explant	Avg shoot length per explant (cm)
0	0	0	18-20	6.3	12.4
0	0.5	0	9-10	12.3	14.8
1	0.5	0	9-10	13.6	13.2
1	1	0	8-9	18.2	16.5
1	2	0	8-9	18.3	20.6
0	2	1	6-8	21.3	21.1
0	3	2	6-8	20.6	21.6
0	2	3	5-6	22	20.2
0	2	4	5-6	21.6	25.4
1	0	3	6-7	20.2	27.5
2	**0**	**3**	**6-7**	**25**	**29.1**
2	3	2	5-7	22.2	28.8
1	2	3	5-7	20.2	26.4
1	3	4	5-6	23.3	30
2	3	4	5-6	23.6	29.4

4.3 *In vitro* rooting on prepared MS media

The Rootable shoots which were excised and put in separate culture jars containing MS Media enriched with rooting growth hormones NAA, IBA & KN of different concentrations (Table 2) were observed after 1-2 week(s) and the best results for number of roots and root length was seen in media containing (MS + NAA 2mg/l + IBA 2mg/l + Sucrose 30 g/l + agar-agar 9 g/l). (Figure 3)

TABLE 2: *In vitro* root induction on different combinations and concentrations of auxins.

MS + Growth Hormones (mg/l)		Days to root formation	Avg No. of Roots per explant	Avg root length per explant (cm)
NAA	IBA			
0	0	17-19	2.3	0.3
0	0.5	9-10	2.7	0.4
1	0.5	9-10	3.2	0.45
1	1	7-8	3.9	1.2
1	2	7-8	3.4	1.4
0	2	6-8	5.3	1.9
0	3	5.6	4.4	2.3
0	2	5-6	4.9	2.3
0	2	5-6	5.3	2.7
1	0	6-7	5.1	3.3
2	**2**	**4-5**	**6.6**	**4.5**
2	3	5-6	6.1	4.4
1	2	5-7	5.9	4.5
1	3	5-7	6.5	4.23
2	3	5-6	6.6	4.6

4.4 Callus induction and regeneration

Callus formation initiated in all explants within 10-15 days at the cut surfaces in all media concentrations. But leaf discs were transformed into complete callus mass within 4 weeks of culture as leaf discs responded with highest frequency for callusing (Figure 4). Overall, MS medium supplemented with 2,4-D (2 mg/l) + IBA (2mg/l) + sucrose (30 g/l) + agar-agar (9 g/l) (Table 3) was found to be the best for callus induction. Callus cultures were subcultured after 6-8 weeks so as to proliferate on suitable callus induction medium. During subculturing, some of the calli turned brown and eventually their proliferation stopped. Those calli were not carried further for regeneration. Calli with a creamy appearance were taken for regeneration.

Proliferating callus cultures or parts thereof were subcultured onto optimized regeneration media containing different concentration and combination of cytokinins & auxins. Medium having MS + BA(2 mg/l) + KN(3 mg/l) + IBA(0.5 mg/l) was

found to be the best for differentiating calli into shoot primordia (Table 4). These somaclonal variants were further subcultured on same regeneration medium to get large number of regenerants, which were further cultured on root induction medium as mentioned earlier to develop into complete plantlets. (Figure 5)

Table 3: Effects of various growth hormones on callus induction in leaf as explants

MS+Auxins (mg/l)		Percent explants forming callus
2,4-D	NAA	Leaf discs
0	0	0
0.5	0	16.3
1	0.5	33.3
1.5	0.5	46.4
2	**0**	**50.2**

Table 4: Effect of various growth hormones on shoot regeneration in calli- derived from leaf explant.

MS+ Growth hormones (mg/l)			No. of Shoots per callus (Mean)
BA	KN	IBA	Leaflets
0	0	0	0
0	1	0.5	0
0	1.5	1	0
0	2	1.5	2.3
0	2	2	3.5
0	3	2	4.2
0.5	0	1.5	4.2
1	0	2	5.3
1.5	0	2	5.6
2	0	2	5.6
1	2	0	6.9
1.5	2.5	0	6.4
2	**3**	**0.5**	**7.6**
1	2	1	7.4
2	3	1.5	7.3

4.5 Hardening of *in vitro* rooted plantlets in glass house

After 5 weeks of growth on rooting medium, roots were well developed and the plantlets from all rooting media was taken out from all culture jars and after the treatment already mentioned, were potted in potting plastic pots containing different potting mixtures (Table 5). After a week, when the plants showed initial signs of establishment in pots, with the appearance of new leaves, the pots were initially uncovered for overnight. After 2 weeks of transfer to pots, and when the plantlets were totally uncovered, we observed that the potting mixture of sand, soil with perlite, vermicultite, coco peat in ratio 1:1:1 gave us the best results in terms of plant survival and growth (Figure 6 & 7).

Table 5: Growth and Survival of hardened plants in different potting mixtures under glass house conditions.

Potting Mixture (Sand+Soil)	Ratio	No. of Leaves	Percentage Survival of plants
Vermiculite	--	3	22.25
Perlite	--	3	22
Coco-peat	--	5	25
Perlite+Vermicultie	1:1	5	20.90
Perlite+coco-peat	1:1	8	32.45
Vermiculite+coco-peat	1:1	7	32.20
Perlite+Vermiculite +coco-peat	**1:1:1**	**14**	**50.23**

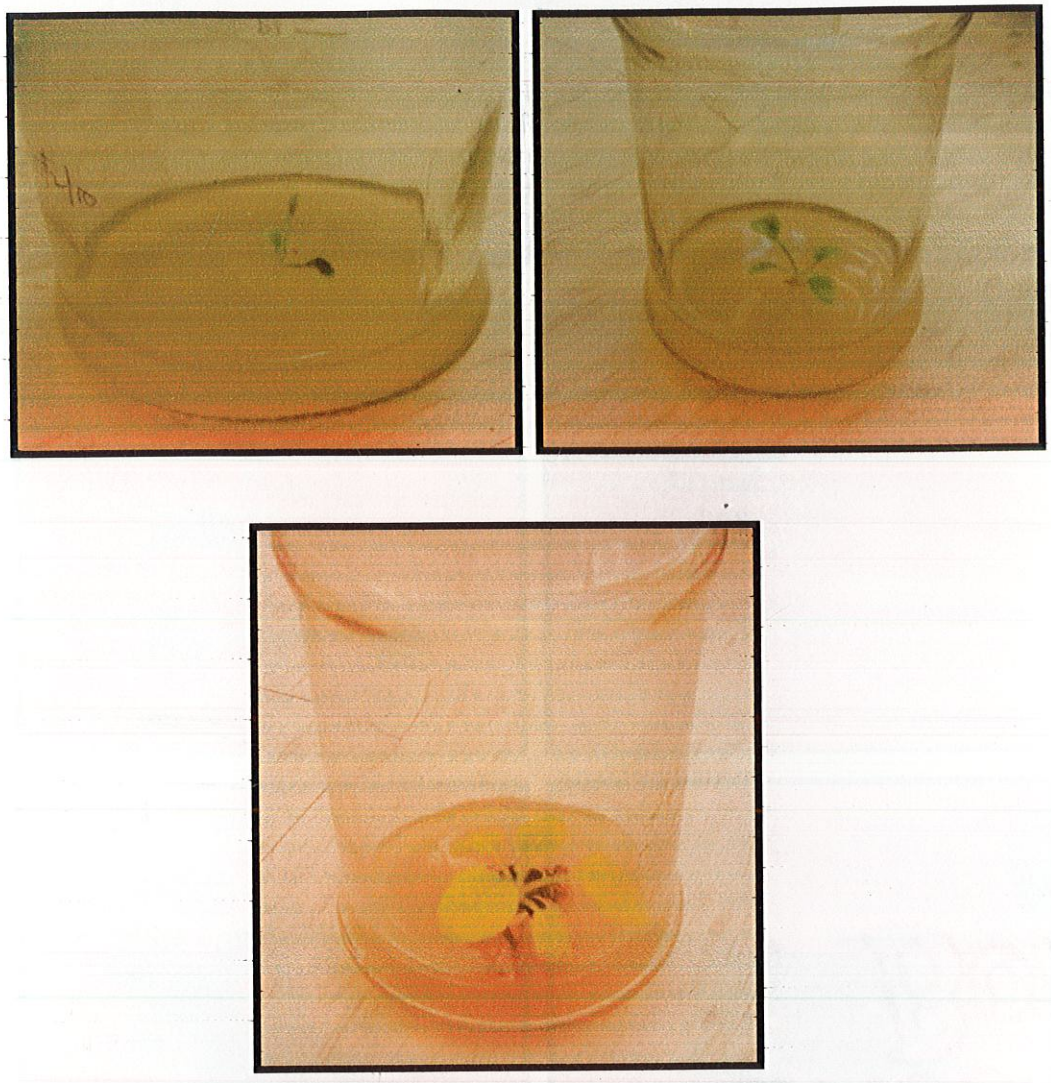


Figure 1: Proliferation of shoot apex after 15 days of culturing

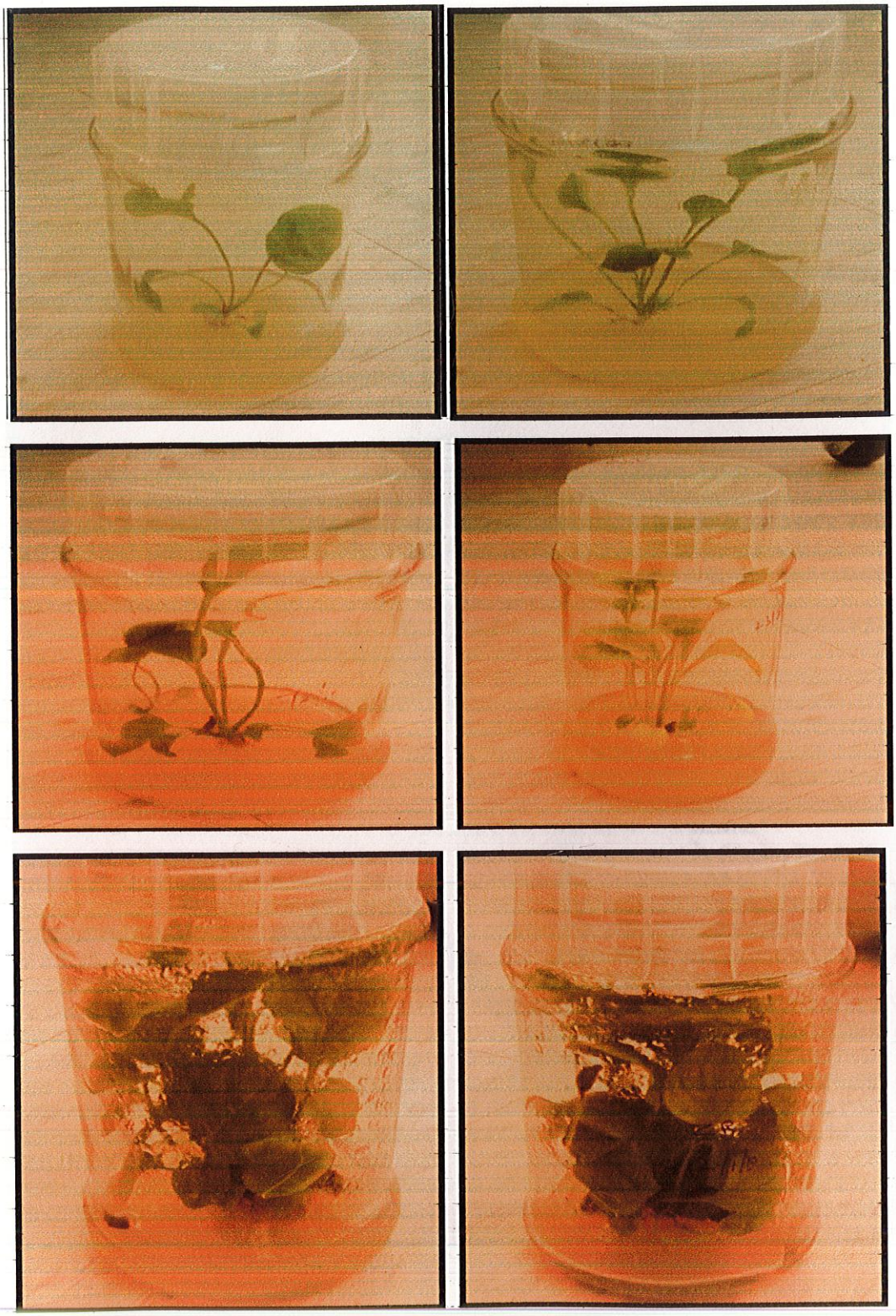


Figure 2: Pattern of shoot multiplication from 1st to 5th week under *in vitro* conditions

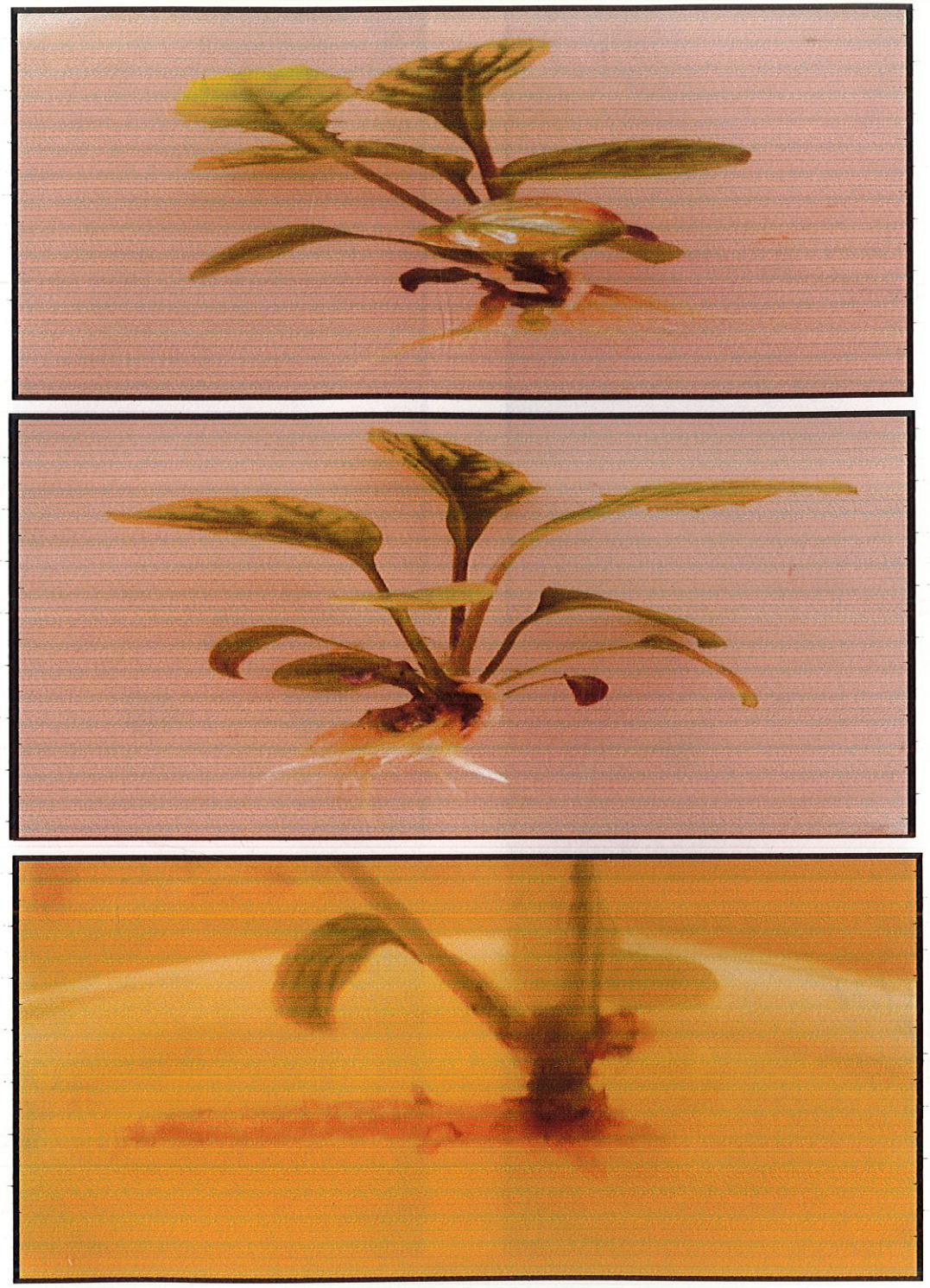


Figure 3: Root induction in *in vitro* grown shoots

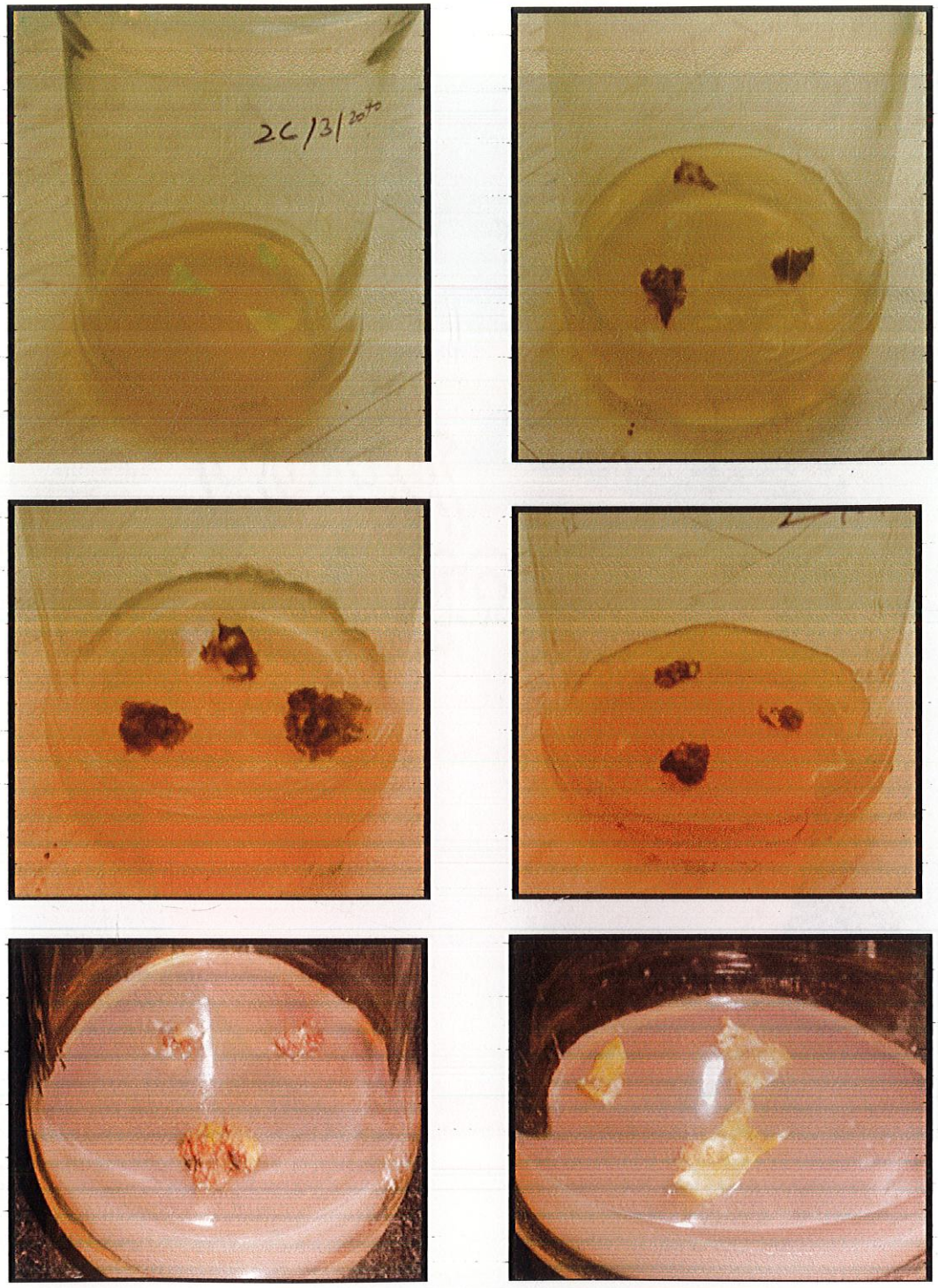
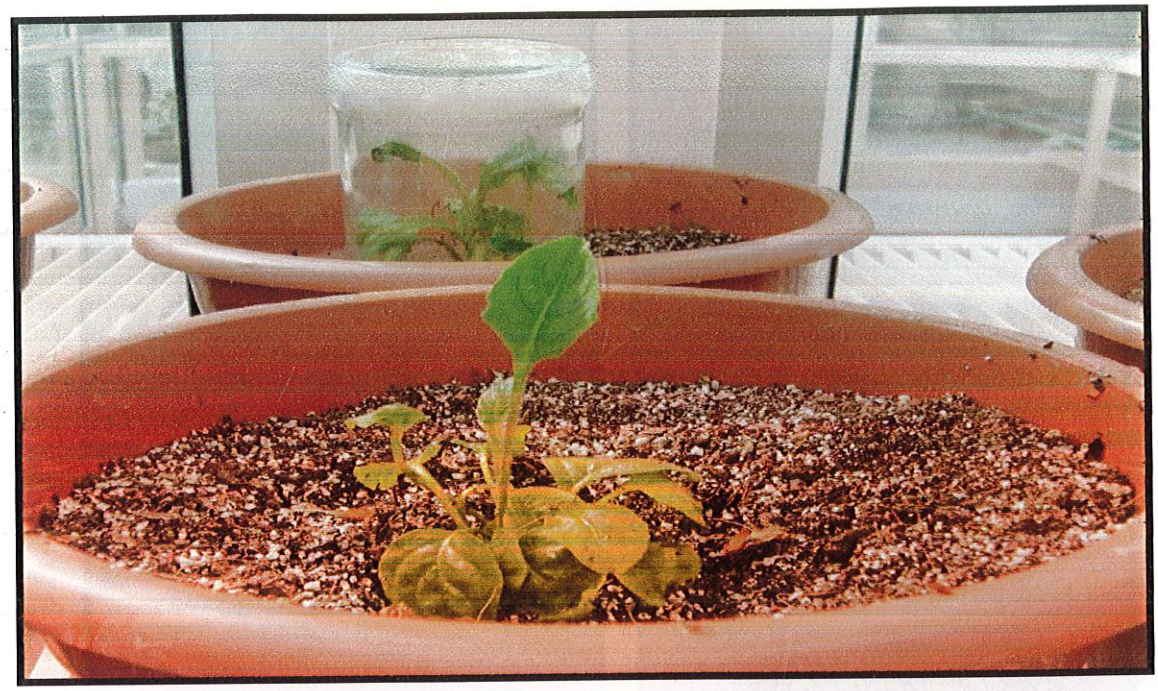


Figure 4: Callus induction and growth within 3 weeks of culturing

Figure 5: Shoot regeneration from callus after 6-7 weeks of culturing



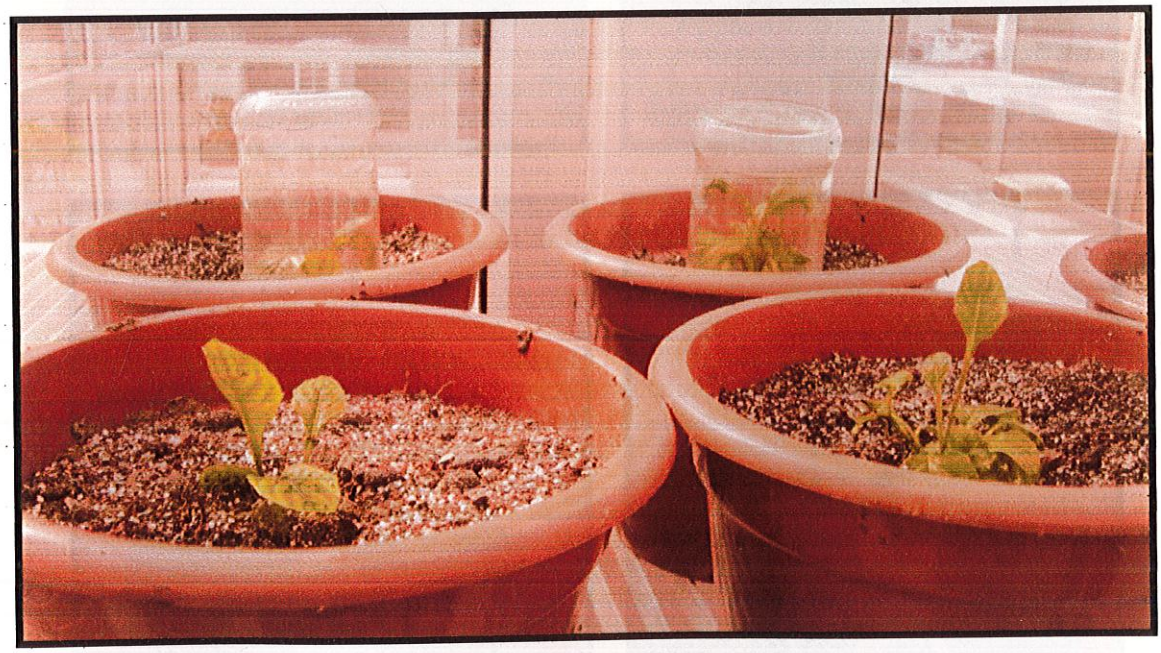


Figure 6: Transplantation and hardening of *in vitro* grown plantlets in glass house

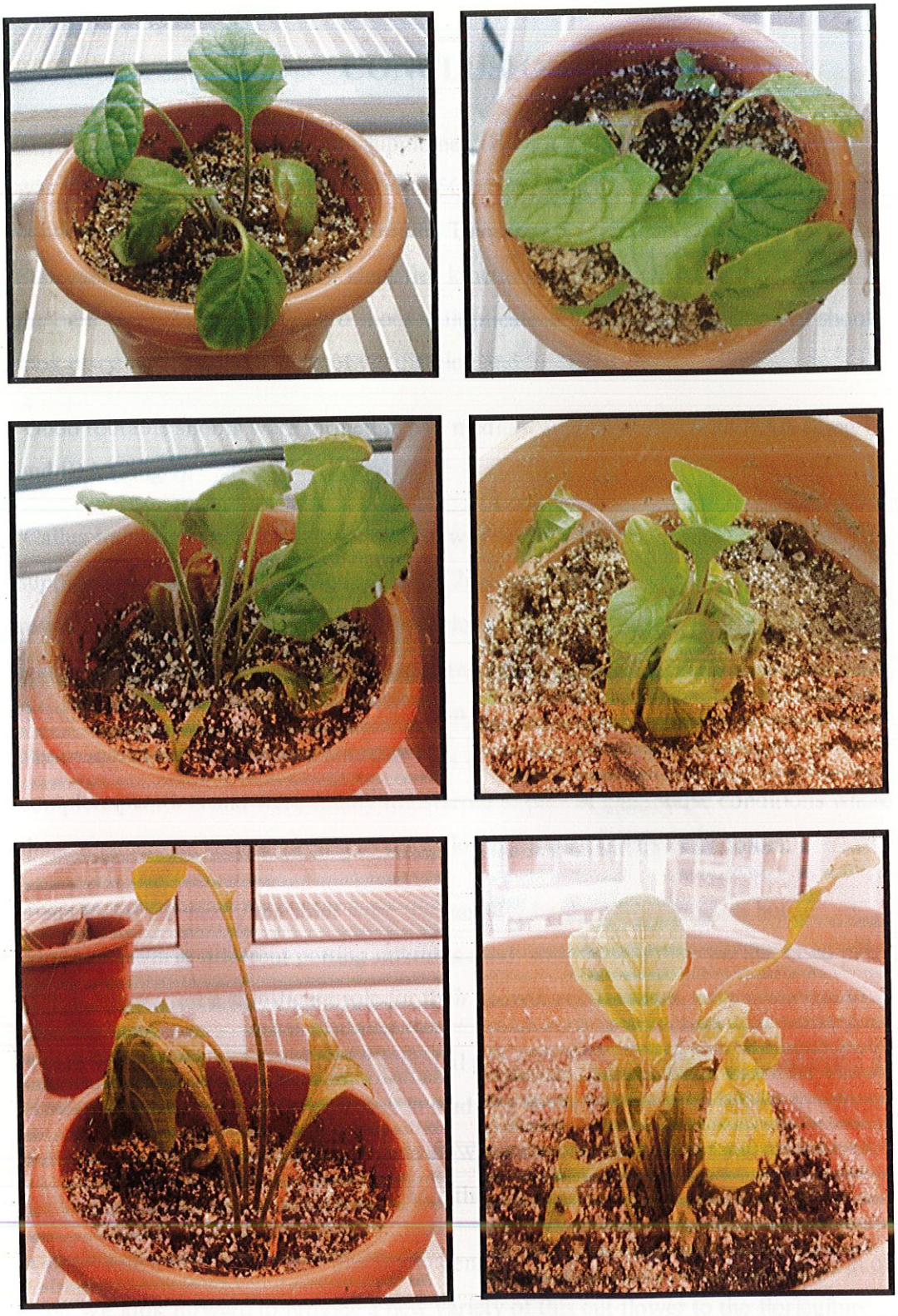


Figure 7: Hardened plants of *G.jamesonii* after 4-5 weeks of transfer to glass house

CONCLUSION

The identification of MS medium supplemented with KN (3 mg /l) + IBA (2 mg/l) was found best for shoot multiplication of Gerbera, which was reported in the studies conducted by Sood *et al.* (2009), Purnima Tyagi and S L Kothari (2003), Sahavacharin (1985), who have identified kinetin with auxin (IBA) as the best medium combination for *in vitro* shoot multiplication. Rapid multiplication of shoots took place with no occurrence of vitrification and fasciated shoots.

Good rootable shoots were formed in MS medium supplemented with NAA (2mg/l) and IBA (2mg/l).

Callus Induction and Callus regeneration was successfully done on MS medium supplemented with 2,4-D (2mg/l) and BA(2mg/l) +KN(3mg/l) +IBA (0.5mg/l) respectively for the development of somaclonal variants. As there was less diversity and variation with respect to flower color reported by Kanwar & Kumar *et al* in 2008, therefore, induction of somaclonal variation was added as another flavor in this study. Those regenerated shoots were further kept on rooting medium to develop into complete plantlets and were finally transferred to pots in glasshouse conditions where those regenerants were expected to show flowering with diversity of colors.

Plantlets of *Gerbera jamesonii* were successfully hardened under glass house conditions where different potting mixtures sand, soil, vermiculite, perlite, coco peat (1:1:1:1:1) played a significant role for their growth and survival.

As we have optimized virtually all essential parameters for commercial production of *Gerbera jamesonii*, we are interested in studying the somaclonal variations in which regenerated plantlets would mature and flower into different desired and commercially demanded colors to capture the major section of flower market.

A good research is one which ultimately benefits mankind, no matter in what form or way. We look forward to provide a new variety of this cut flower to the floriculture industry.

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ANNEXURE – I

Composition of used Murashige and Skoog (1962) basal medium (MS Medium)

STOCKS	CHEMICALS	ORIGINAL STRENGTH (mg/l)	STOCK (g/l)	FINAL VOLUME
A - 10X	KNO_3	1900	19	
	$MgSO_4.7H_2O$	370	3.7	100ml/l
	KH_2PO_4	170	1.70	
B - 20X	NH_4NO_3	1650	33	50ml/l
C - 100X	$CaCl_2.2H_2O$	440	44	10 ml/l
D - 100X	Na_2EDTA	37.26	3.72	10 ml/l
	$FeSO_4.7H_2O$	27.85	2.28	
E - 100X	KI	0.83	0.083	100 ml/l
F - 100X	H_3BO_3	6.2	0.62	
	$CoCl_2.6H_2O$	0.025	0.0025	
	$ZnSO_4.7H_2O$	8.6	0.86	
	$CuSO_4.5H_2O$	0.025	0.0025	10 ml/l
	$MnSO_4.4H_2O$	22.3	2.23	
	$Na_2MbO_4.2H_2O$	0.25	0.025	
G - 100X	m-INOSITOL	100	10	10ml/l
	GLYCINE	2	0.2	
H -100X	PYRIDOXINE-HCl	0.5	0.05	
	NICOTINE ACID	0.5	0.05	10 ml/l
	THIAMINE	0.1	0.01	