CHAPTER 2

IN VITRO REGENERATION OF *Gerbera jamesonii* Bolus Ex. Hook f.

2.1 EXPERIMENTAL AIMS

Previous research has proven that *Gerbera jamesonii* could successfully be propagated *in vitro*. Different types of explants were used in order to achieve micropropagation of this plant. There are many factors that influence propagation of *G. jamesonii in vitro* such as type of explants, composition of culture media and the cultural environment. All nutrients which consist of organic and inorganic matters in the culture media must be suitable for the growth of plants *in vitro*. Generally, there are many types of culture media that suit the growth of many plant species. There is no specific medium for each species.

There are also some modifications made in the composition of culture media to meet the requirement of plant growth in the culture system. Some culture media was used specifically for the growth of woody plants, herbaceous plants, cereals, orchids and many others. To begin *in vitro* propagation, the most important aspect to be considered is the choice of explants. Explant is a small segment from a plant organ or tissue. Plant organs that can be used for this purpose are roots, petioles, leaves, young shoots, stem, peduncle, endosperm tissue, pollen and almost any other plant parts.

The selection of explants is highly dependant on the objectives of the experiments. Explants consist of young cells and tissues normally show better results

compared to the old ones. This is because, young meristematic cells divide actively. But in some cases, matured cells were also responsive. A few factors that need to be considered in selection of explants in tissue culture are source of explants (intact or aseptic plant), explants size, ontogeny, age of explants or the maturation of plant stock (Murashige, 1974a). Besides factors that are related to explant tissues, there are also other factors that play important role in the success of micropropagation. Substrates, genotypes and environmental factors influence plant regeneration *in vitro*.

In this chapter, the aim of the experiment was to study the regeneration of *Gerbera jamesonii in vitro*. The effects of culture media, plant hormones in culture media, type of explants and polarity of explants in culture media were determined and studied. The optimum growth media and conditions for regeneration were identified. At the beginning, polarity of explants in the culture media was also studied. On the culture medium, explants were placed with the adaxial surface down or the abaxial surface down. Explants were also placed horizontally and vertically in the culture media. This is to determine which polarity gives the best response when explants were cultured. The effect of polarity is very important in obtaining high rate of regeneration.

In determining the most suitable culture medium and type of explants that gave efficient regeneration of *Gerbera jamesonii*, BAP and NAA hormones were added in culture media (MS media) at various concentrations (1.0 mg/l - 3.0 mg/l). From this experiment, the optimum regeneration media and the most responsive explant in regeneration of *G. jamesonii in vitro* would be determined. As an alternative to obtain

massive regeneration of shoots from *in vitro* culture of *G. jamesonii*, the subsequent experiment would be carried out using other auxin and cytokinin besides NAA and BAP. The hormones that were used include IAA, 2, 4-D and IBA (0.5 mg/l) and Kinetin, 2iP and Zeatin (2.0mg/l).

Comparison between explants that were cultured using BAP and NAA with the other hormones was made. The best hormone combination in culture media was selected when the media produced the optimum rate of shoot formation *in vitro*. The effects of growth regulators on the root formation were also studied. Various types of hormones were supplemented to the culture media to determine which combination gave the best root formation *in vitro*. From this experiment, the most suitable culture media and growth regulators for the regeneration of adventitious shoots, multiple shoots and root could be identified. At the end of this experiment, all effects from the factors studied for the regeneration of *G. jamesonii in vitro* could be observed and recorded. The most responsive explant and optimum media for regeneration will be used for further experiments.

Liquid and solid media were used in finding the most suitable culture condition for regeneration and mass propagation of *Gerbera in vitro*. From the experiment, type of culture media that was optimum for induction of adventitious shoots was determined.

The right sucrose concentration and pH of the culture media are very essential towards the success of a regeneration process and establishment of *in vitro* plantlets. In

this experiment, various sucrose concentrations and pH level were examined to determine the optimum sucrose concentration and pH level in regeneration of *in vitro* shoots.

Coconut water was believed to have cytokinin like characters. The effect of coconut water in the culture media was also studied.

Based on all experiments, effects from all factors studied on regeneration of *Gerbera* shoots could be revealed. Most responsive explant and optimum culture media for shoot regeneration, induction of adventitious shoots and rooting induction media would be used in subsequent experiments.

2.2 MATERIALS AND METHODS

2.2.1 Source of Explants

Seeds of *Gerbera jamesonii* were imported from Horti, Singapore. These seeds were cultured and 8-week-old aseptic seedlings were used as source of explants. Organs such as leaves, petioles and roots were used throughout the micropropagation studies of *G. jamesonii*.

2.2.2 Preparation of Aseptic Seedlings

Aseptic seedlings were obtained by germinating sterilized seeds on MS basal medium (section 2.2.4). Murashige and Skoog (1962) media was used throughout this experiment since the previous researchers has proven that MS was the most suitable medium to propagate *Gerbera jamesonii in vitro* (Eduardo et. al. 1991). Seeds germinated after 6-7 days in culture. Aseptic seedlings were formed after 6-8 weeks. Leaves, petioles and roots from these aseptic seedlings were used as sources of explants.

2.2.3 Type of Explants

8-week-old aseptic seedlings were used as stock of explants. Leaves, petioles and roots excised from these aseptic seedlings were used as source of explants. All culturing

works were done with full care since all tissues used were still young, soft and fragile. The 5th or 6th leaves from shoot apex where chosen as leaf explants.

2.2.4 Sterilization of Explants

Sterilization techniques were followed carefully in order to obtain aseptic seedlings and cultures need to be free from contamination. Seeds were first washed under running tap water for minimum of 2 hours. The seeds were surface sterilized by rinsing them with sterile distilled water with 2-3 drops of Tween 20 for 3 times. Next, the seeds were soaked in 40% (v/v) sodium hypochlorite for 15 minutes and followed by 10% (v/v) alcohol for 1 minute. All sterilized seeds were finally rinsed again with sterile distilled water for 3 times for 5 minutes and ready to be cultured.

2.2.5 Preparation of Culture Media

The basic medium used in this study was Murashige and Skoog (MS). This medium was chosen based on previous studies by other researchers. The formulation for MS medium is given in Appendix I. MS medium contains macronutrients and micronutrient components, FeEDTA as iron source, vitamins, sucrose as source of carbon and myo-inositol. MS media used in this study was the ready-to-use powdered form and purchased from SIGMA Chemical Company, Sigma-aldrich, St. Louis, USA.

2.2.5.1 Preparation of MS Stock Solution

In some cases MS stock solution was used. MS stock solution consists of macronutrient, micronutrient, Fe-EDTA and vitamins stock solutions. These stock solutions were prepared separately. For the preparation of hormone stock solution, hormones were diluted with a few drops of sodium hydroxide (0.1 M) before distilled water was added. All stock solutions were kept at cool temperature. Fe-EDTA stock solution oxidized with the air easily. Thus, it needs to be kept in a dark bottle and placed in the refrigerator to avoid any chemical changes.

2.2.5.2 Preparation of Basic Medium, MS (1962)

To prepare basic MS media solution, 800 ml distilled water was poured into 1000ml beaker. Powdered MS media powder (4.4 g/l) was diluted in distilled water. 30 g/l sucrose was added to the solution followed by 8.0 g/l technical agar. Distilled water was added to the media solution until the total volume reached 1000 ml. The media solution need to be stirred constantly to dissolve all the components added into the media. The pH of the prepared media was adjusted to 5.8 with either sodium hydroxide (NaOH) or hydrochloric acid (HCL). The media was autoclaved for 21 minutes at a pressure of 104 kPa (15 Psi^2) and temperature of 121 ^oC. This procedure is to ensure the sterility of culture media. After the media has been autoclaved, it was transferred to the laminar flow cabinet and let cooled until it reached about 50 ^oC. About 10 ml of the molten culture

media was dispensed into 60 ml sterile universal container obtained from Megalab Supplies and labeled with dates, name of species, treatments, etc.

2.2.5.3 Preparation of Culture Media with Hormones

Some hormones are heat sensitive, while some are not. This means that hormones such as GA_3, Zeatin, Abscisic acid and few others need to be filtered sterilize before being added to the culture media. These hormones need to be dissolved in NaOH or alcohol for the preparation of stock. These hormones were then filtered with 0.22µm sterile membrane filters (Millipore filter). Required concentrations of sterile hormones were then added to the culture media while the media was at molten phase (50 ^{o}C).

2.2.6 Culture Conditions

All apparatus need to be cleaned and sterilized before being used. This includes forceps, scalpel, petri dishes, conical flasks and many others. These culture apparatus were wrapped in aluminium foil and autoclaved for 30 minutes. Forceps and scalpel were dipped in 70% alcohol, sterile distilled water, dried and dipped into hot bead sterilizer to ensure the sterility of culture apparatus. Hot bead sterilizer will reach a temperature of 250 ^{o}C. Forceps and scalpel need to be cooled by dipping them into sterile distilled water before being used to excise plant tissues.

All cultured explants were incubated in the culture room at the temperature of 25 ± 1 ^{o}C with 16 hours light and 8 hours dark. The intensity of light in the culture room was set at 1000 lux. This is suitable to allow cultures to respond and grow.

2.2.7 Subculture

All cultures were subcultured every 2-4 weeks. This is to provide the cultures with fresh nutrients for further growth as well as to prevent the accumulation of toxic substances in the culture media.

2.2.8 Plant Regeneration *In vitro*

2.2.8.1 Identification of the Best Polarity of Explants for Shoot Regeneration

Selected explants were cut into small segments before being cultured. Leaves explants were excised into small pieces of 10 mm x 10 mm in size. Petiole and root explants were cut into 10 mm in length. All explants were cultured in MS media supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA (shoot inducing media). For leaf explants, adaxial and abaxial surfaces of the explants were placed on the culture media. Petiole and root explants were cultured vertically and horizontally on the culture media. Cultured explants were incubated in culture room at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark with light intensity of 1000 lux.

The aim of the experiment was to identify the most suitable polarity of explants for the regeneration of shoots and the most responsive position of explants that would be used in the next experiments. Thirty replicates were used in this experiment and observations were done every week for 8 weeks.

2.2.8.2 Identification of Shoot Regeneration Media

2.2.8.2(a) Identification of Suitable BAP and NAA Combinations for Shoot Regeneration

To obtain the optimum medium for regeneration of shoots, the addition of BAP and NAA hormones in the culture media in several combinations and concentrations were examined. Leaf and petiole explants were cultured on MS media supplemented with 31 combinations of BAP and NAA at different concentrations. Thirty replicates were used in every combination. The combinations of BAP and NAA in the culture media are as below;

1. MS basal (without hormone)
2. 0.1 mg/l BAP
3. 0.5 mg/l BAP
4. 1.0 mg/l BAP
5. 1.5 mg/l BAP
6. 2.0 mg/l BAP
7. 3.0 mg/l BAP

8. 0.1 mg/l NAA
9. 0.5 mg/l NAA
10. 1.0 mg/l NAA
11. 1.5 mg/l NAA
12. 2.0 mg/l NAA
13. 0.1 mg/l BAP + 0.1 mg/l NAA
14. 0.1 mg/l BAP + 0.5 mg/l NAA
15. 0.1 mg/l BAP + 1.0 mg/l NAA
16. 0.1 mg/l BAP + 1.5 mg/l NAA
17. 0.1 mg/l BAP + 2.0 mg/l NAA
18. 0.5 mg/l BAP + 0.1 mg/l NAA
19. 0.5 mg/l BAP + 0.5 mg/l NAA
20. 0.5 mg/l BAP + 1.0 mg/l NAA
21. 0.5 mg/l BAP + 1.5 mg/l NAA
22. 0.5 mg/l BAP + 2.0 mg/l NAA
23. 1.0 mg/l BAP + 0.1 mg/l NAA
24. 1.0 mg/l BAP + 0.5 mg/l NAA
25. 1.5 mg/l BAP + 1.0 mg/l NAA
26. 1.5 mg/l BAP + 1.5 mg/l NAA
27. 1.5 mg/l BAP + 2.0 mg/l NAA
28. 2.0 mg/l BAP + 0.1 mg/l NAA
29. 2.0 mg/l BAP + 0.5 mg/l NAA
30. 2.0 mg/l BAP + 1.0 mg/l NAA

31. 2.0 mg/l BAP + 1.5 mg/l NAA

32. 2.0 mg/l BAP + 2.0 mg/l NAA

All cultures were maintained in the culture room at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark for 8 weeks. Observations were done every week and data were collected during the 8th week of culture. The formation of roots and callus on explants were also observed. From the results obtained in this experiment the most responsive explant and the most optimum medium for regeneration of shoots and formation of roots were identified.

2.2.8.2(b) Identification of Other Suitable Cytokinins and Auxins for Shoot Regeneration

Media with potentials for shoot regeneration in section 2.2.8.1 were identified. MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA gave the best response in the regeneration of shoots whereas petiole explant, was chosen as the most responsive explant compared to leaves and roots. Based on these results, the above medium was the optimum medium for regeneration of shoots and petiole explants were the most responsive explants in the regeneration of shoots *in vitro*.

Alternatively, experiments using other auxin and cytokinin hormones (besides NAA and BAP) were also done to study the effects of these hormones on the regeneration of shoots. The same concentration of the optimum medium containing NAA

and BAP (0.5 mg/l NAA + 2.0 mg/l BAP) were used to study the effect of other auxins and cytokinins in the regeneration of shoots. Thirty replicates were used in every hormone combination. Petiole explants were cultured on MS media containing these hormones;

1. 0.5 mg/l IAA + 2.0 mg/l BAP
2. 0.5 mg/l IBA + 2.0 mg/l BAP
3. 0.5 mg/l 2,4-D + 2.0 mg/l BAP
4. 0.5 mg/l NAA + 2.0 mg/l Kinetin
5. 0.5 mg/l IAA + 2.0 mg/l Kinetin
6. 0.5 mg/l IBA + 2.0 mg/l Kinetin
7. 0.5 mg/l 2,4-D + 2.0 mg/l Kinetin
8. 0.5 mg/l NAA + 2.0 mg/l Zeatin
9. 0.5 mg/l IAA + 2.0 mg/l Zeatin
10. 0.5 mg/l IBA + 2.0 mg/l Zeatin
11. 0.5 mg/l 2,4-D + 2.0 mg/l Zeatin
12. 0.5 mg/l IAA + 2.0 mg/l 2iP
13. 0.5 mg/l IBA + 2.0 mg/l 2iP
14. 0.5 mg/l 2,4-D + 2.0 mg/l 2iP

All explants cultured were incubated in the culture room at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark for 8 weeks. Observations were made every week and results were recorded during the 8th week.

2.2.8.3 Identification of Root Induction Media

Shoots of about 20mm-30mm in height were detached from shoot clumps and transferred to MS and ½ MS basal media without hormone. Shoots were also transferred to MS and ½ MS media supplemented with 1.0-2.0 mg/l NAA and 1.0-2.0 mg/l IAA. These shoots were incubated at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark. Shoots produced roots after the 4th week of culture. Thirty replicates were used in every hormone combination. The optimum medium which produced highest percentage of roots was identified.

2.2.8.4 Identification of Optimum Sucrose Concentration for Regeneration of Shoot

Petiole explant was cultured in MS media supplemented with 0.8% technical agar, 2.0 mg/l BAP and 0.5 mg/l NAA. Various concentrations of sucrose from 10-100 g/l were added in the culture media. The effect of sucrose concentrations on regeneration of *Gerbera* shoots was observed. It is important to determine the optimum level of sucrose content in the culture media especially in *Gerbera* since various plant species have specific sucrose intake for the optimum response. Furthermore, sucrose is an important energy and carbon source for the growth and development of explant in the culture medium.

2.2.8.5 Identification of Optimum pH Media on Regeneration of Shoots

The effect of different pH media on regeneration of *Gerbera* shoots was studied. The pH of MS medium supplemented with 3.0% sucrose, 0.8% agar with 2.0 mg/l BAP and 0.5 mg/l NAA was adjusted to different pH ranging from 4-8 – 6.8. Petiole explant was cultured in the culture media incubated in the culture room at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark for 8 weeks. Observation was made every day. It is important to verify the suitable pH of the culture medium in order to match the pH of growth substrates or soil where intact plants were planted.

2.2.8.6 Effect of Liquid and Solid Media on Formation of Adventitious Shoot

Media were prepared in liquid and solid forms. Shoots obtained from regeneration of petiole explant cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA (section 2.2.8.2) were used in this experiment. These new shoots were subcultured into three different types of media, prepared in both, liquid and solid forms. The media used in this study were;

1. MS basal
2. MS supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA
3. MS supplemented with 2.0 mg/l Zeatin and 0.5 mg/l IAA

Explants cultured in 50 ml liquid media were placed on rotary shaker at 100 rpm. All cultures were incubated in the culture room at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark for 8 weeks. The most suitable medium for multiplication of *Gerbera* shoots was identified based on this experiment.

2.2.8.7 Effect of Coconut Water on Shoot Regeneration

Petiole explants were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA with the addition of coconut water. Ten ml/l, 30 ml/l, 50 ml/l, 80 ml/l and 100 ml/l of coconut water, which was from green coconuts, was added in the culture media. The aim of this experiment was to examine the effect of coconut water on shoot regeneration. Cultures were incubated in the culture room at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark. Results were recorded after 8 weeks of culture.

2.2.9 Microscopic Studies (Scanning Electron Microscopy-SEM)

In this study, scanning electron microscope (SEM) was used to observe the differences between *in vivo* (intact) and *in vitro* shoots. The same developmental stages of *in vivo* and *in vitro* shoots were used. Observations and comparisons were made on the differences of leaf surfaces, number of stoma and trichomes. Standard methods and procedures for the preparation of samples for SEM process were followed (Appendix 2).

2.2.10 Data Analysis

Data obtained were analyzed using Duncan's Multiple Range Test (DMRT). Mean with different letters in the same column differ significantly at p=0.05

2.3 RESULTS

2.3.1 Identification of the Best Polarity of Explants for Shoot Regeneration

Regeneration of *Gerbera* shoots was successfully achieved when leaf and petiole explants were cultured on MS media supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA. Leaf primordia were formed on the area where the explants were cut after 3 weeks of culture. After 5 weeks, adventitious shoots developed from these leaf primordia. Peduncle and petal explants were unable to regenerate shoots *in vitro* when cultured onto the same media. Earlier experiments showed that all explants were not responsive when cultured on MS basal media. Table 2.1 showed the response of explants at different polarities cultured on MS media supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA. Comparison of regeneration rate and number of shoots per explant were made.

Leaf explant with the size of 10 mm x 10 mm produced higher shoot regeneration when abaxial part of the leaf was cultured onto the culture media (25.0 ± 1.3%) with 2.6 ± 0.5 shoots per explant compared to adaxial part (10.0 ± 0.6%) with only 1.4 ± 0.2 shoots per explant. Petiole explant produced the highest regeneration of shoots when cultured at horizontal position onto the culture media (90.0 ± 0.5%) with 8.1 ± 0.4 shoots per explant. Petiole explant showed lower regeneration of shoots when cultured at vertical positions with 55.0 ± 1.1% (3.7 ± 0.7 shoots per explant) when distal part was dipped in the culture medium and 60.0 ± 0.8% (5.3 ± 1.3 shoots per explant) when peroximal part was dipped in the culture medium.

Thus, for subsequent experiments, leaf explant with abaxial surface on the culture media and petiole explant cultured at horizontal position on the culture media were used since these two polarities of explants showed the highest regeneration ability for shoot formation.

Table 2.1: Responses of different polarity of explants cultured on MS media supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA maintained at 25 ±1 ^{o}C and 16 hours light and 8 hours dark for 8 weeks.

Source of explants	Polarity/ Source of Explant	Regeneration of Shoots (%)	No. of Shoots per explant (min ± SE)	Callus formation (%)
Leaf	Adaxial	$10.0 \pm 0.6_{d,e}$	$1.4 \pm 0.2_{d,e}$	$70.0 \pm 0.2_{c}$
	Abaxial	$25.0 \pm 1.3_{d}$	$2.6 \pm 0.5_{d}$	$81.5 \pm 0.5_{b}$
Petiole	Vertical (Distal)	$55.0 \pm 1.1_{b,c}$	$3.7 \pm 0.7_{b,c}$	0
	Vertical (Peroximal)	$60.0 \pm 0.8_{b}$	$5.3 \pm 1.3_{b}$	0
	Horizontal	$90.0 \pm 0.5_{a}$	$8.1 \pm 0.4_{a}$	0
Peduncle	Vertical (Distal)	0	0	$80.0 \pm 0.5_{b}$
	Vertical (Peroximal)	0	0	$85.0 \pm 1.0_{b}$
	Horizontal	0	0	$100 \pm 0.0_{a}$
Petal	Vertical	0	0	$10.3 \pm 0.8_{e}$
	Horizontal	0	0	$43.1 \pm 1.0_{d}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

2.3.2 Identification of Shoot Regeneration Media

Plant regeneration and organogenesis are processes for production of plant organs directly from an explant or from callus phase. Organogenesis relies on the inherent plasticity of plant tissues and is regulated by altering the components of the plant growth media. in particular, the auxin and cytokinin ratio in the culture media determines the success of plant regeneration. To propagate plant *in vitro* by using adventitious organs formation, it is important to note that the plant is capable of regenerating. The ability to regenerate is determined by the genotype, the environmental conditions (nutrient supply, growth regulators and physical conditions) and also the developmental stage of the plant.

In the present work, direct and indirect organogenesis in *Gerbera jamesonii* was successfully achieved when leaf and petiole explant were cultured onto MS media supplemented with BAP and NAA in various concentration. Explants managed to regenerate shoots directly and indirectly when cultured on culture media. Direct organogenesis occurred when leaf and petiole explants formed shoots after a few weeks being cultured on the culture media. Meanwhile, indirect organogenesis occured when *Gerbera* explants initially formed callus and subsequently the callus cells were differentiated to form shoots. With the addition of BAP and NAA in the culture media, most callus formed were mostly green in colour.

Table 2.2 showed the response of leaf and petiole explants of *Gerbera jamesonii* when cultured on MS media supplemented with BAP and NAA at various concentrations. Observations were made on the percentage of shoot regeneration and callus formation, number of shoots regenerated per explant and also percentage of rooting. Generally, all explants responded as early as 2 weeks when cultured on various culture media.

The main aim of this study was to determine which explant and hormone concentrations are the optimum in regenerating shoots. Thus, decisions were made based on which explant and hormone concentration that produced the highest percentage of shoot regeneration and number of shoots per explant in the shortest time duration.

Based on the results, for petiole explant, highest shoot regeneration (94.3 ± 2.5%) was observed when explant was cultured on MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA with 9.3 ± 0.6 shoots per explant (Figure 2.1). Multiple shoots were obtained when petiole explant was cultured on MS media supplemented with 0.1-3.0 mg/l BAP, while leaf explant produced highest shoot regeneration (25.0 ± 1.3%) through indirect organogenesis with 2.6 ± 0.5 shoots per explant. These result showed that regeneration of shoots was better achieved from petiole explant .

Regeneration potentials in petiole explant was further studied. Petiole explant was cultured on MS media supplemented with other auxin and cytokinin hormones. Auxin used were IAA, IBA and 2,4-D at 0.5 mg/l each. Meanwhile, cytokinins used were Kinetin, Zeatin and 2iP at 2.0 mg/l each. Table 2.3 showed the effect and response of

petiole explant cultured on MS media supplemented with different combinations of 0.5 mg/l auxin (2,4-D, IBA, IAA and NAA) and 2.0 mg/l cytokinin (BAP, 2iP, Kinetin and Zeatin). Thus, from this experiment optimum media for shoot regeneration from petiole explant could be identified.

Based on the result obtained in Table 2.3, 15 combinations of auxin and cytokinin were used in the culture media. Petiole explant was successfully regenerated into shoots in all the combinations used. MS media supplemented with combinations of 2.0 mg/l Zeatin and 0.5 mg/l IBA gave highest shoot regeneration (83.7 ± 1.5%) with 7.4 ± 0.9 shoots per explant (Figure 2.2).

Thus, from all the experiments, MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA was selected as the most suitable medium in regeneration of *Gerbera* shoots. This medium was used in further experiments involving regeneration of *Gerbera* shoots. Plantlets obtained were further transferred to rooting media before being acclimatized to the green house (Figure 2.5).

Table 2.2: The effect of different concentrations and combinations of BAP and NAA on leaf and petiole explants cultured on MS media at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark.

MS + Hormone (mg/l)		Explant	Observations	Callus Formation (%)	Shoot Formation (%)	No. Shoots per explant	Root Formation (%)
BAP (mg/l)	NAA (mg/l)						
0.0	0.0	Leaf	No response	-	-	-	-
		Petiole	No response	-	-	-	-
0.1		Leaf	Very few shoots formed after 2 weeks	$13.2 \pm 1.4_{e,f}$	$3.6 \pm 0.4_{f}$	$0.8 \pm 0.1_{f}$	-
		Petiole	Multiple shoots started to form after 2 weeks	-	$31.5 \pm 1.2_{d,e,f}$	$4.2 \pm 0.2_{d,e,f}$	-
0.5		Leaf	Very few shoots formed	$16.6 \pm 0.3_{e,f}$	$4.4 \pm 0.6_{f}$	$1.3 \pm 0.5_{f}$	-
		Petiole	Multiple shoots formed after 2 weeks	-	$33.2 \pm 0.4_{d,e,f}$	$4.8 \pm 1.3_{d,e,f}$	-
1.0		Leaf	Very few shoots formed	$18.7 \pm 0.2_{e,f}$	$5.1 \pm 1.2_{f}$	$1.7 \pm 0.5_{f}$	-
		Petiole	Multiple shoots formed after 2 weeks	-	$35.8 \pm 0.1_{d,e,f}$	$5.0 \pm 0.8_{d,e,f}$	-
1.5		Leaf	Very few shoots formed	$19.5 \pm 0.6_{e,f}$	$5.4 \pm 0.3_{f}$	$1.9 \pm 0.2_{f}$	-
		Petiole	Multiple shoots formed after 2 weeks	-	$37.2 \pm 0.8_{d,e,f}$	$5.4 \pm 1.2_{d,e,f}$	-
2.0		Leaf	Very few shoots formed	$20.3 \pm 0.1_{e,f}$	$5.7 \pm 0.4_{f}$	$2.3 \pm 1.4_{f}$	-
		Petiole	Multiple shoots formed after 2 weeks	-	$40.3 \pm 0.2_{c,d,e}$	$6.8 \pm 0.1_{c,d,e}$	-
3.0		Leaf	Very few shoots formed	$19.2 \pm 0.2_{e,f}$	$6.0 \pm 1.0_{f}$	$2.6 \pm 0.5_{f}$	-
		Petiole	Multiple shoots formed after 2 weeks	-	$80.4 \pm 1.6_{b,c}$	$8.8 \pm 0.9_{b,c}$	-
	0.1	Leaf	Green callus and hairy root formed	$25.0 \pm 1.0_{d,e,f}$	-	-	$10.3 \pm 0.2_{f}$
		Petiole	Green callus and hairy root formed	$36.5 \pm 0.8_{d,e}$	-	-	$23.6 \pm 1.2_{e,f}$
	0.5	Leaf	Green callus and hairy root formed	$45.2 \pm 0.3_{c,d,e}$	-	-	$70.5 \pm 1.0_{b}$
		Petiole	Green callus and hairy root formed	$45.8 \pm 0.5_{c,d,e}$	-	-	$52.1 \pm 0.7_{c}$

'Table 2.2, continued'

MS + Hormone (mg/l)		Explant	Observation	Callus Formation (%)	Shoot Formation (%)	No. Shoots per explant	Root Formation (%)
BAP (mg/l)	NAA (mg/l)						
	1.0	Leaf	Light green callus and adventitious root formed	$10.2 \pm 1.5_{e,f}$	-	-	$64.5 \pm 0.5_{b,c}$
		Petiole	Light green callus and hairy root formed	$53.6 \pm 1.0_{c,d}$	-	-	$72.3 \pm 0.8_{b}$
	1.5	Leaf	Light green callus and adventitious root formed	$20.3 \pm 1.4_{e,f}$	-	-	$45.0 \pm 0.2_{c,d}$
		Petiole	Light green callus and adventitious root formed	$53.5 \pm 1.0_{c,d}$	-	-	$30.4 \pm 0.5_{d,e}$
	2.0	Leaf	Light green callus and adventitious root formed	$10.4 \pm 2.4_{e,f}$	-	-	$70.7 \pm 2.4_{a}$
		Petiole	Light green callus and adventitious root formed	$55.2 \pm 0.5_{c,d}$	-	-	$42.2 \pm 0.1_{d}$
0.1	0.1	Leaf	Green callus and hairy roots formed.	$57.2 \pm 1.1_{c,d}$	-	-	$32.5 \pm 0.2_{d,e,f}$
		Petiole	Adventitious shoots formed	-	$42.1 \pm 1.0_{e,f}$	$3.2 \pm 0.6_{e,f}$	$37.0 \pm 0.2_{d,e}$
0.1	0.5	Leaf	Hairy roots and green callus formed	$55.0 \pm 0.5_{c,d}$	-	-	$69.2 \pm 1.2_{b,c}$
		Petiole	Green callus and adventitious with hairy roots formed	$100 \pm 0.0_{a}$	-	-	$75.0 \pm 0.1_{a,b}$
0.1	1.0	Leaf	Green callus and hairy roots formed	$53.5 \pm 1.0_{c,d}$	-	-	$70.1 \pm 1.3_{a,b}$
		Petiole	Green callus and hairy roots formed	$98.5 \pm 1.0_{b}$	-	-	$76.4 \pm 0.4_{a}$
0.1	1.5	Leaf	Green callus and hairy roots formed. A few shoots formed	$57.3 \pm 0.6_{c,d}$	-	-	$73.0 \pm 0.4_{a,b}$
		Petiole	Green callus and hairy roots formed. A few adventitious shoots formed	$78.4 \pm 1.4_{c}$	$2.3 \pm 1.0_{f}$	$1.5 \pm 0.7_{f}$	$79.1 \pm 1.5_{a}$
0.1	2.0	Leaf	Green callus and hairy roots formed	$58.9 \pm 3.1_{c,d}$	-	-	$73.7 \pm 2.3_{a,b}$
		Petiole	Green callus and hairy roots formed. A few adventitious shoots formed	$80.4 \pm 1.5_{b,c}$	$4.6 \pm 0.8_{f}$	$1.6 \pm 0.7_{f}$	$78.4 \pm 1.6_{a}$
0.5	0.1	Leaf	Yellowish green callus formed at the bottom and edges of explant	$48.0 \pm 1.1_{c,d,e}$	-	-	-
		Petiole	Yellowish green callus	$38.2 \pm 0.8_{d,e}$	-	-	-

'Table 2.2, continued'

MS + Hormone (mg/l)		Explant	Observation	Callus Formation (%)	Shoot Formation (%)	No. Shoots per explant	Root Formation (%)
BAP (mg/l)	NAA (mg/l)						
0.5	0.5	Leaf Petiole	Green callus formed Adventitious shoots and roots formed	$65.5 \pm 0.4_{c,d}$ -	- $33.4 \pm 1.2_{d,e,f}$	- $4.7 \pm 0.5_{d,e,f}$	- $39.1 \pm 1.2_{d,e}$
0.5	1.0	Leaf Petiole	Green callus formed Adventitious shoots and green callus formed	$60.2 \pm 1.4_{c,d}$ $75.0 \pm 0.1_{c}$	- $33.5 \pm 1.0_{d,e,f}$	- $2.4 \pm 0.2_{d,e,f}$	- -
0.5	1.5	Leaf Petiole	Green callus formed Green callus formed	$36.5 \pm 1.0_{d,e}$ $15.0 \pm 0.2_{d,e}$	- -	- -	- -
0.5	2.0	Leaf Petiole	Light green callus formed Adventitious roots and light green callus formed	$86.3 \pm 0.6_{b}$ $75.0 \pm 0.1_{c}$	- -	- -	$71.3 \pm 1.5_{a,b}$ $69.0 \pm 0.8_{b,c}$
1.0	0.1	Leaf Petiole	Green callus formed in the middle part of explant. Yellow callus formed at the edges Green callus formed on the surface of explant. Yellow callus formed at the edges.	$85.0 \pm 0.5_{b,c}$ $79.5 \pm 1.4_{c}$	- -	- -	- -
1.0	0.5	Leaf Petiole	White green callus formed White green callus and adventitious shoots formed	$87.2 \pm 0.3_{b}$ $40.1 \pm 1.2_{d,e}$	- $73.5 \pm 1.1_{c,d}$	- $6.5 \pm 0.7_{c,d}$	- -
1.0	1.0	Leaf Petiole	Green callus, adventitious shoots and roots formed Adventitious shoots and roots formed	$80.0 \pm 0.5_{b,c}$ -	25.0 ± 1.3e,f $90.0 \pm 0.5_{a,b}$	$2.6 \pm 0.5_{e,f}$ $8.1 \pm 0.4_{a,b}$	$42.7 \pm 1.4_{c,d}$ $56.7 \pm 1.6_{c}$
1.0	1.5	Leaf Petiole	Green callus formed Yellowish green callus formed	$11.0 \pm 1.7_{e,f}$ $24.3 \pm 0.6_{d,e,f}$	- -	- -	- -
1.0	2.0	Leaf Petiole	Green callus formed Green callus formed. Shoots and roots were also observed	$90.7 \pm 0.9_{b}$ $88.9 \pm 1.3_{b}$	- $15.4 \pm 2.7_{e,f}$	- $3.5 \pm 1.8_{e,f}$	$21.4 \pm 2.1_{e,f}$ $36.0 \pm 1.0_{d,e}$
1.5	0.1	Leaf Petiole	Yellow callus formed on surface of explant. Green callus formed in the middle Yellow callus formed at the edges of explant. Green callus formed in the middle. A few shoots formed	$75.0 \pm 0.1_{c}$ $78.1 \pm 1.5_{c}$	- $19.2 \pm 0.5_{e,f}$	- $1.6 \pm 0.3_{e,f}$	- -

'Table 2.2, continued'

MS + Hormone (mg/l)		Explant	Observation	Callus Formation (%)	Shoot Formation (%)	No. Shoots per explant	Root Formation (%)
BAP (mg/l)	NAA (mg/l)						
1.5	0.5	Leaf Petiole	Green callus formed White green callus formed. A few shoots formed	$81.2 \pm 0.4_{b,c}$ $66.0 \pm 0.4_{c,d}$	- $22.5 \pm 1.1_{e,f}$	- $2.1 \pm 0.5_{e,f}$	- -
1.5	1.0	Leaf Petiole	Green callus formed Callus, shoots and roots formed	$87.3 \pm 0.6_{b}$ $51.8 \pm 1.3_{c,d}$	- $83.1 \pm 0.5_{b,c}$	- $8.3 \pm 1.1_{b,c}$	$1.2 \pm 0.8_{f,g}$ $28.5 \pm 0.2_{e,f}$
1.5	1.5	Leaf Petiole	Green callus formed Adventitious shoots and roots formed	$17.2 \pm 0.8_{e,f}$ -	- $80.5 \pm 0.5_{b,c}$	- $6.5 \pm 1.3_{b,c}$	$6.9 \pm 1.4_{f}$ $47.6 \pm 1.1_{c,d}$
1.5	2.0	Leaf Petiole	Green callus and adventitious roots formed Green callus and adventitious roots formed	$80.2 \pm 1.5_{b,c}$ $84.2 \pm 2.1_{b,c}$	- -	- -	$31.6 \pm 0.2_{d,e}$ $40.3 \pm 1.7_{c,d,e}$
2.0	0.1	Leaf Petiole	Yellowish green callus formed Adventitious shoots and roots formed	$80.0 \pm 0.5_{b,c}$ -	- $77.5 \pm 2.0_{c}$	- $6.0 \pm 1.2_{c}$	- $23.2 \pm 0.9_{e,f}$
2.0	0.5	Leaf Petiole	Green callus formed Adventitious shoots and roots formed	$89.4 \pm 2.3_{b}$ -	- $94.3 \pm 2.5_{a}$	- 9.3 ± 0.6a	- $35.6 \pm 1.0_{d,e}$
2.0	1.0	Leaf Petiole	Green callus formed Callus, shoots and roots formed	$83.6 \pm 1.8_{b,c}$ $65.3 \pm 0.4_{c,d}$	- $52.8 \pm 1.6_{d,e}$	- $3.3 \pm 0.5_{d,e}$	- $27.9 \pm 1.4_{e,f}$
2.0	1.5	Leaf Petiole	Green callus formed Callus, shoots and roots formed	$77.1 \pm 0.5_{c}$ $43.7 \pm 1.2_{d,e}$	- $70.2 \pm 1.5_{c,d}$	- $4.1 \pm 0.2_{c,d}$	- $19.4 \pm 0.7_{e,f}$
2.0	2.0	Leaf Petiole	Green callus formed Adventitious shoots and roots formed	$80.0 \pm 0.5_{b,c}$ $32.6 \pm 0.9_{d,e}$	$17.1 \pm 0.4_{e,f}$ $53.5 \pm 1.7_{d,e}$	$1.8 \pm 0.2_{e,f}$ $6.2 \pm 0.4_{d,e}$	- $25.2 \pm 0.5_{e,f}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

Table 2.3: The effect of different combinations of auxin (2, 4-D, IBA, IAA and NAA) at the concentration of 0.5 mg/l and cytokinin (BAP,2iP, Kinetin and Zeatin) at the concentration of 2.0 mg/l on petiole explant cultured on MS media at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark.

MS + Hormone (mg/l)		Observation	Callus (%)	Shoot (%)	No. Shoot per explant	Root (%)
0.5	2.0					
IAA	BAP	Adventitious shoots formed	$45.6 \pm 1.6_{c}$	$33.6 \pm 3.4_{c}$	$3.5 \pm 2.6_{c}$	$22.0 \pm 1.1_{b}$
IBA	BAP	Adventitious shoots formed	$38.1 \pm 0.7_{c,d}$	$39.7 \pm 2.1_{c}$	$5.1 \pm 1.3_{c}$	19.2 ± 0.8
2, 4-D	BAP	Green callus formed	$89.0 \pm 0.4_{a}$	$27.6 \pm 2.0_{c,d}$	$2.2 \pm 0.9_{c,d}$	$18.5 \pm 1.0_{c}$
NAA	Kinetin	Adventitious shoots formed	$33.3 \pm 1.1_{c,d}$	$24.1 \pm 0.6_{c,d}$	$2.2 \pm 1.4_{c,d}$	$23.5 \pm 0.8_{b}$
IAA	Kinetin	Adventitious shoots formed	$37.2 \pm 0.8_{c,d}$	$14.4 \pm 1.0_{d}$	$1.8 \pm 0.2_{d}$	$21.6 \pm 1.5_{b,c}$
IBA	Kinetin	Adventitious shoots formed	$29.0 \pm 0.7_{d}$	$66.7 \pm 0.8_{b}$	$6.2 \pm 0.7_{b}$	$18.0 \pm 1.3_{c}$
2, 4- D	Kinetin	Green callus formed	$59.5 \pm 1.6_{b}$	$24.3 \pm 1.0_{c,d}$	$2.4 \pm 1.5_{c,d}$	$16.9 \pm 1.0_{c,d}$
NAA	Zeatin	Adventitious shoots formed	$46.3 \pm 0.2_{b,c}$	$54.0 \pm 1.8_{b,c}$	$4.0 \pm 0.5_{b,c}$	$21.0 \pm 0.7_{b,c}$
IAA	Zeatin	Adventitious shoots formed	$52.1 \pm 1.3_{c}$	$73.2 \pm 1.4_{a,b}$	$5.6 \pm 0.8_{a,b}$	$18.1 \pm 1.1_{c}$
IBA	Zeatin	Adventitious shoots formed	$40.5 \pm 0.9_{c}$	$83.7 \pm 1.5_{a}$	$7.4 \pm 0.9_{a}$	$32.2 \pm 0.8_{a}$
2, 4-D	Zeatin	Adventitious shoots formed	$67.3 \pm 1.3_{b}$	$22.1 \pm 1.5_{c,d}$	$2.4 \pm 1.6_{c,d}$	$27.5 \pm 0.6_{a}$
NAA	2iP	Adventitious shoots formed	$10.5 \pm 0.5_{d}$	$19.2 \pm 0.7_{c,d}$	$1.2 \pm 0.1_{c,d}$	$14.5 \pm 1.0_{d}$
IAA	2iP	Adventitous shoots formed	$17.0 \pm 1.3_{d}$	$26.3 \pm 2.0_{c,d}$	$2.4. \pm 1.0_{c,d}$	$13.8 \pm 1.0_{d}$
IBA	2iP	Adventitious shoots formed	$21.3 \pm 0.7_{d}$	$11.9 \pm 2.3_{d}$	$2.9 \pm 1.7_{d}$	$13.0 \pm 0.8_{d}$
2, 4-D	2iP	Adventitious shoots formed	$15.7 \pm 1.0_{d}$	$5.8 \pm 0.6_{d}$	$0.8 \pm 0.1_{d}$	$15.4 \pm 1.3_{c,d}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

Figure 2.1: Regeneration of shoots from petiole explants cultured on MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA

Figure 2.2: Regeneration of shoots from petiole explants cultured on MS media supplemented with 2.0 mg/l Zeatin and 0.5 mg/l IBA

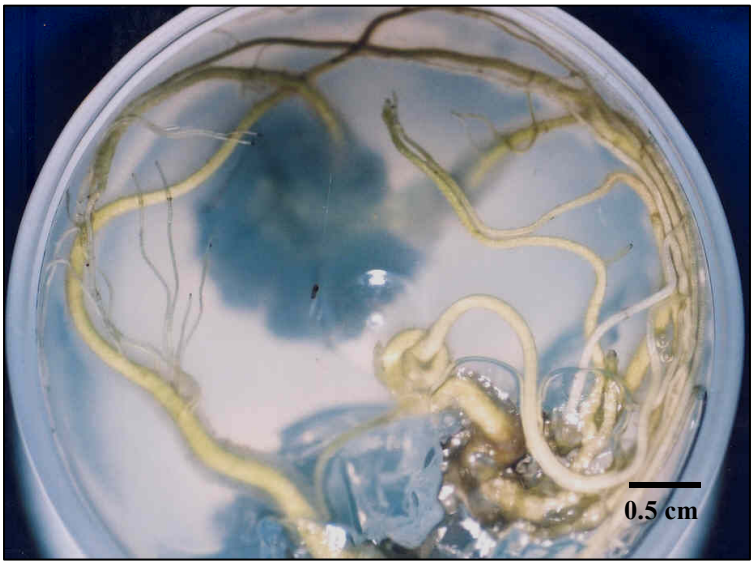

Figure 2.3: Development of roots from leaf explant cultured on MS media supplemented with 0.1 mg/l BAP and 2.0 mg/l NAA

Figure 2.4: Roots formed from leaf explants cultured on MS media fortified with 2.0 mg/l NAA.

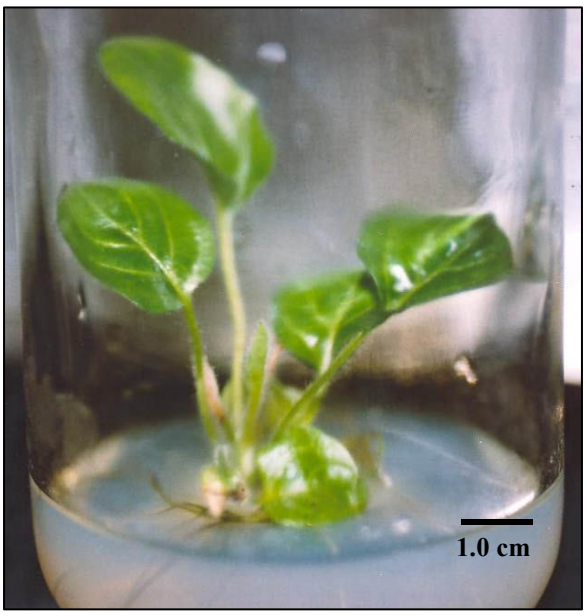

Figure 2.5: *In vitro* plantlet obtained from regeneration of petiole explant cultured on MS media supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA. Plantlet was transferred to MS basal media for root elongation.

2.3.3 Identification of Root Induction Media

Table 2.4 showed the response and development of rooting of *Gerbera* shoots when subcultured in root induction media. Shoots of about 20mm-30mm in height were detached from shoot clumps and transferred to rooting induction media. From the results, all media were able to induce rooting. However, MS basal media was identified as the optimum media for rooting of *Gerbera* shoots (90.6 ± 0.5%) with 15.5 ± 0.1 roots per explant. This is followed by MS media supplemented with 1.0 mg/l NAA with 13.6 ± 1.0 roots per explant. The lowest root production (68.7 ± 0.5%) was observed when shoots were cultured on MS media with 2.0 mg/l IAA with 5.9 ± 1.1 roots per explant. Roots produced in MS basal media were longer and thicker compared to other rooting media. Thus, MS basal media was chosen as rooting induction media for development and elongation of roots of *Gerbera* plantlets.

Table 2.4: Development of roots from *in vitro* shoots in rooting media after 4 weeks. cultures were maintained at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark.

MS Strength (with or without hormones, mg/l)	Development of Roots (%)	No. of Roots per explant (Mean ± SE)
MS	$90.6 \pm 0.5_{a}$	$15.5 \pm 0.1_{a}$
½ MS	$78.4 \pm 0.8_{b}$	$13.2 \pm 0.5_{b}$
MS + 1.0 mg/l NAA	$89.5 \pm 1.0_{b}$	$13.6 \pm 1.0_{b}$
MS + 2.0 mg/l NAA	$85.0 \pm 1.2_{c}$	$9.5 \pm 0.6_{c}$
½ MS + 1.0 mg/l NAA	$76.3 \pm 0.6_{c}$	$9.0 \pm 0.4_{c}$
½ MS + 2.0 mg/l NAA	$70.0 \pm 1.5_{c,d}$	$7.3 \pm 0.2_{c,d}$
MS + 1.0 mg/l IAA	$84.7 \pm 0.5_{d}$	$11.6 \pm 1.4_{b}$
MS + 2.0 mg/l IAA	$82.6 \pm 0.1_{b,c}$	$10.1 \pm 0.5_{b,c}$
½ MS + 1.0 mg/l IAA	$74.4 \pm 1.0_{c,d}$	$6.6 \pm 0.2_{c,d}$
½ MS + 2.0 mg/l IAA	$68.7 \pm 0.5_{c,d}$	$5.9 \pm 1.1_{c,d}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

2.3.4 Effect of Liquid and Solid Media on Formation of Adventitious Shoot

Table 2.5 showed the response of solid and liquid media in multiplication of *Gerbera* shoots. The results showed that liquid MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA produced highest shoots formation with 18.5 ± 0.8 shoots. Solid MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA produced lower number of shoots with 11.6 ± 0.5 shoots. However, shoots produced in liquid media were abnormal and smaller in size. From this experiment, solid media was selected as optimum media for regeneration of adventitious shoots since shoots regenerated developed normally.

Table 2.5: Multiplication of shoots cultured in solid and liquid media after 4 weeks. Cultures were maintained at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark.

MS + agar (g/l)	Hormone (mg/l)	No. of shoots (Mean ± SE)
0	MS basal	$5.9 \pm 1.0_{c}$
8	MS basal	$4.7 \pm 1.2_{c}$
8	2.0 mg/l BAP + 0.5 mg/l NAA	$11.6 \pm 0.5_{a}$
8	2.0 mg/l Zeatin +0.5 mg/l IBA	$9.4 \pm 1.1a_{,b}$
0	2.0 mg/l BAP + 0.5 mg/l NAA	$18.5 \pm 0.8_{a}$
0	2.0 mg/l Zeatin +0.5 mg/l IBA	$16.1 \pm 0.6_{a}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

2.3.5 Identification of Optimum Sucrose Concentration for Shoot Regeneration

Table 2.6 showed the effect of different sucrose concentration on regeneration of *Gerbera* shoots. Various sucrose concentrations from 10 g/l to 100 g/l were added in the culture media. Results showed that, the highest shoots regeneration (92.8 ± 0.4) was achieved when 30 g/l sucrose was added in the culture media with 9.1 ± 0.5 shoots per explant. Addition of 40 g/l of sucrose produced 7.1 ± 0.7 shoots per explant. Abnormal shoots were observed when 50-70 g/l sucrose was added to the culture media. Petiole explants failed to regenerate shoots when 80-100 g/l sucrose was added in the culture media. Hence, 30 g/l sucrose was chosen as the optimum sucrose concentration in the culture media for regeneration of *Gerbera* shoots. The addition of 30 g/l sucrose in the culture media was the optimum carbon source and energy level for optimal growth response of *G. jamesonii*.

Table 2.6: The effect of different sucrose concentration on regeneration of shoots. Cultures were maintained at 25 ± 1 ^{o}C with 16 hours light and 8 hours dark.

Sucrose concentration (g/l)	Regeneration of Shoot (%)	No. of Shoots per explant (Mean ± SE)
10	$35.2 \pm 2.4_{b,c}$	$4.3 \pm 0.8_{b,c}$
20	$43.7 \pm 1.2_{b,c}$	$5.5 \pm 1.1_{b,c}$
30	$92.8 \pm 0.4_{a}$	$9.1 \pm 0.5_{a}$
40	$54.4 \pm 0.9_{b}$	$7.1 \pm 0.7_{b}$
50	$49.5 \pm 1.3_{b,c}$ *	$5.4 \pm 1.3_{b,c}$
60	$13.1 \pm 1.0_{d}$ *	$3.0 \pm 0.4_{d}$
70	$9.3 \pm 2.5_{d}$ *	$1.2 \pm 1.0_{d}$
80	0	0
90	0	0
100	0	0

* formation of abnormal shoots

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

2.3.6 Identification of Optimum pH Media for Shoot Regeneration

The most suitable pH in the culture media determines the success of shoot regeneration. Table 2.7 showed the effect of different pH media in regeneration of *Gerbera* shoots from petiole explants. From the results, pH ranging from 5.6-6.0 produced high regeneration of shoots. Media with pH 5.8 resulted in the highest shoot formation (92.8 ± 0.5%) with 9.3 ± 0.6 shoots per explant. This is followed by media with pH 6.0 (90.5 ± 0.9) with 7.9 ± 0.8 shoots per explant. Media with pH lower than 5.6 and higher than 6.8 produced abnormal shoots. Therefore, pH 5.8 was chosen as the optimum pH of the culture media and used throughout all experiments. Different plant species have different adaptation to pH level in the growth substrates or soil. Some plants require acidity condition, while some other needs more alkaline environment for growth. Thus, in *G. jamesonii*, pH 5.8 was found to be optimal for shoot regeneration in the culture system. pH 5.8 was found to be similar to pH of soil for planting intact *Gerbera*.

Table 2.7: The effect of different pH in regeneration of shoots. Cultures were maintained at 25 ±1 ^{o}C with 16 hours light and 8 hours dark.

pH	**Shoot Regeneration (%)**	**No. of shoots per explant** (Mean ± SE)
4.8	$54.7 \pm 1.0_{c}$ *	$5.8 \pm 0.6_{c}$
5.2	$79.4 \pm 0.6_{b,c}$*	$5.3 \pm 1.0_{b,c}$
5.6	$88.3 \pm 2.1_{b}$	$6.6 \pm 1.3_{b}$
5.8	$92.8 \pm 0.5_{a}$	$9.3 \pm 0.6_{a}$
6.0	$90.5 \pm 0.9_{a}$	$7.9 \pm 0.8_{a}$
6.4	$87.3 \pm 1.3_{b,c}$ *	$7.3 \pm 0.4_{b,c}$
6.8	$77.2 \pm 1.1_{b,c}$ *	$6.8 \pm 0.5_{b,c}$

* formation of abnormal shoots

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

2.3.7 Effect of Coconut Water on Regeneration of Shoot

Supplementation of coconut water in the culture media exhibited rather higher number of *Gerbera* shoot regeneration from petiole explant compared to media without the addition of coconut water. Media added with 100 ml/l coconut water produced 14.1 ± 1.4 shoots per explant (Table 2.8). Culture media without the addition of coconut water (control) only produced 9.4 ± 0.5 shoots per explant. However, shoots regenerated from media fortified with coconut water were bushy and smaller in size. Therefore, regeneration of shoots from petiole explant was better achieved when explant was cultured on MS media without the addition of coconut water where the shoots produced were more uniform in size.

Table 2.8: The effect of coconut water on shoot regeneration of petiole explant. Cultures were maintained at 25 ±1 ^{o}C with 16 hours light and 8 hours dark.

Coconut water (ml)	**No. of shoots per explant**
Control	$9.4 \pm 0.5_{a}$
10	$10.1 \pm 1.2_{a}$
30	$10.7 \pm 1.0_{a}$
50	$11.1 \pm 0.8_{a,b}$
80	$12.6 \pm 1.0_{b}$
100	$14.1 \pm 1.4_{b}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

2.3.8 Microscopic Studies (Scanning Electron Microscopy-SEM)

Microscopic studies were done using scanning electron microscope (SEM). Standard procedures of sample preparation were followed (Appendix 2). Figure 2.6(a) showed abaxial surface of *in vitro* leaf, while Figure 2.6(b) showed adaxial surface of *in vitro* leaf. Figure 2.6(c) and (d) showed abaxial and adaxial surfaces of *in vivo* leaf. Numbers of stoma were higher on abaxial leaf surface compared to adaxial surface on both *in vitro* and *in vivo* leaves. Structure of stomata on *in vitro* leaf was observed in Figure 2.6(e) and Figure 2.6(f) showed structure of stomata on *in vivo* leaf. Trichomes on surfaces of *in vitro* and *in vivo* leaves were shown in Figure 2.6(g) and 2.6(h). Number of trichomes on *in vitro* leaf was found to be less compared to *in vivo* (intact) leaf. Comparisons between *in vitro* and *in vivo* (intact) leaves could be seen when samples were observed under scanning electron microscope.

2.3.9 Data Analysis

Data obtained were analyzed using Duncan's Multiple Range Test (DMRT). Mean with different letters in the same column differ significantly at p=0.05

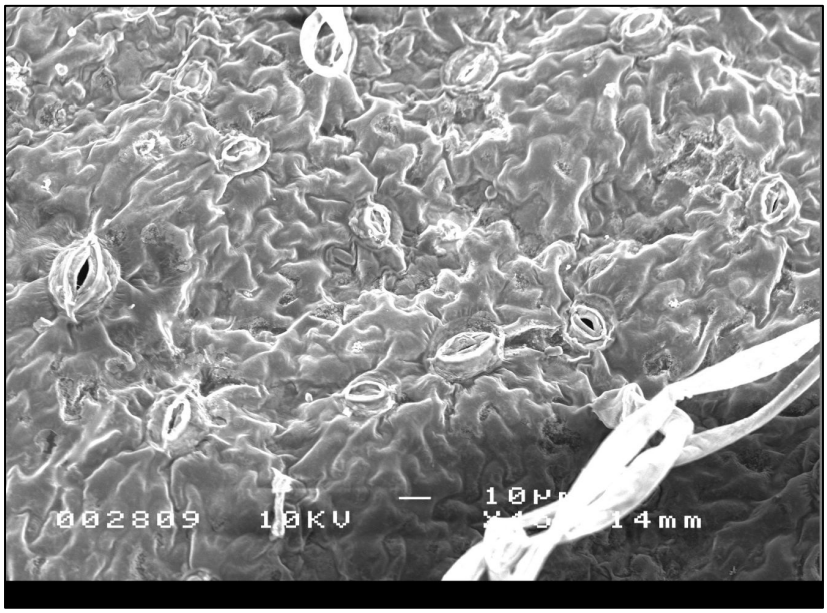

Figure 2.6 (a): SEM micrograph showing abaxial surface of *in vitro* leaf of *Gerbera jamesonii*. Stoma were clearly seen on the leaf.

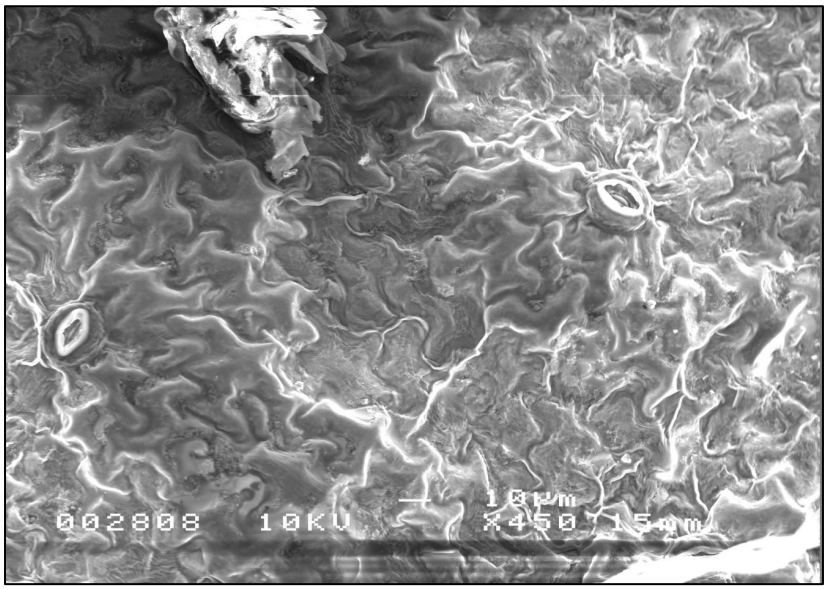

Figure 2.6 (b): SEM micrograph showing adaxial surface of *in vitro* leaf of *Gerbera jamesonii*. Very few stoma were seen on the leaf.

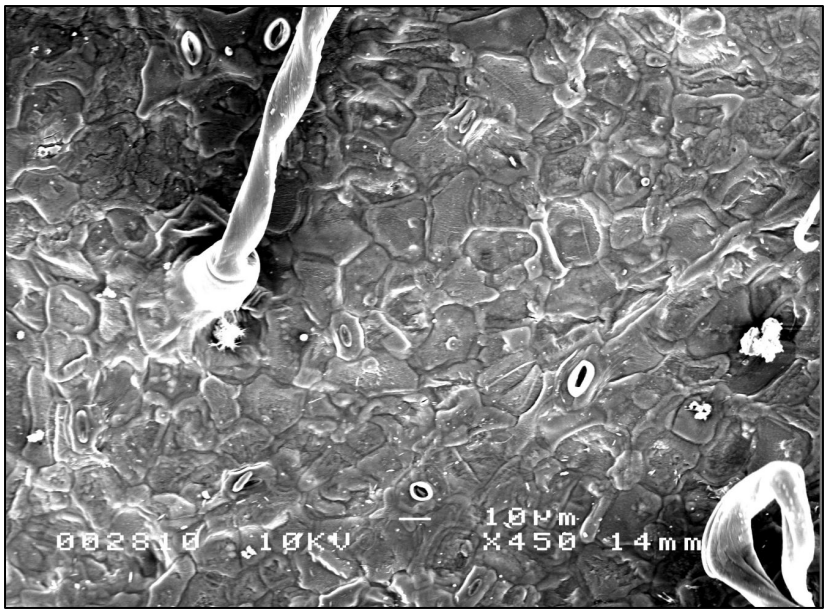

Figure 2.6 (c): SEM micrograph showing abaxial surface of *in vivo* (intact) leaf of *Gerbera jamesonii*. Stoma were seen on the leaf.

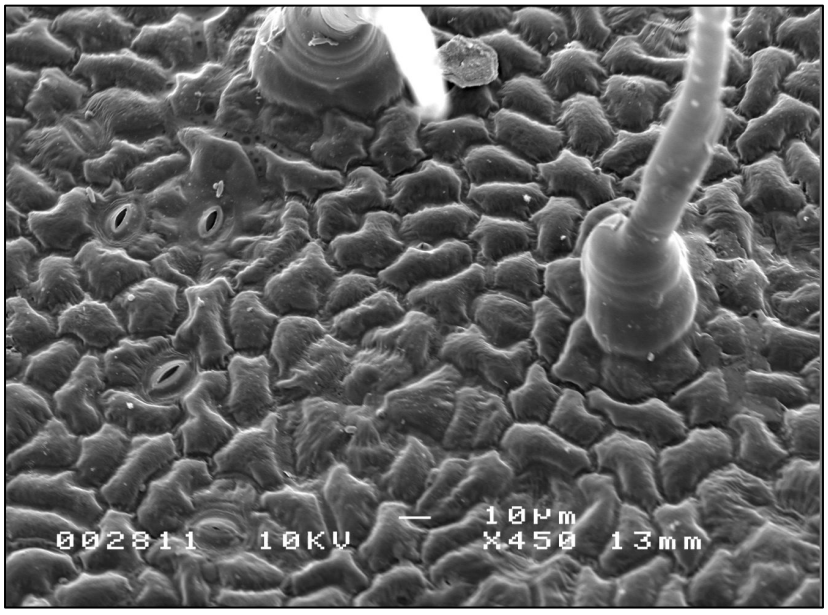

Figure 2.6 (d): SEM micrograph showing adaxial surface of *in vivo* (intact) leaf of *Gerbera jamesonii*. Very few stoma were found on the leaf.

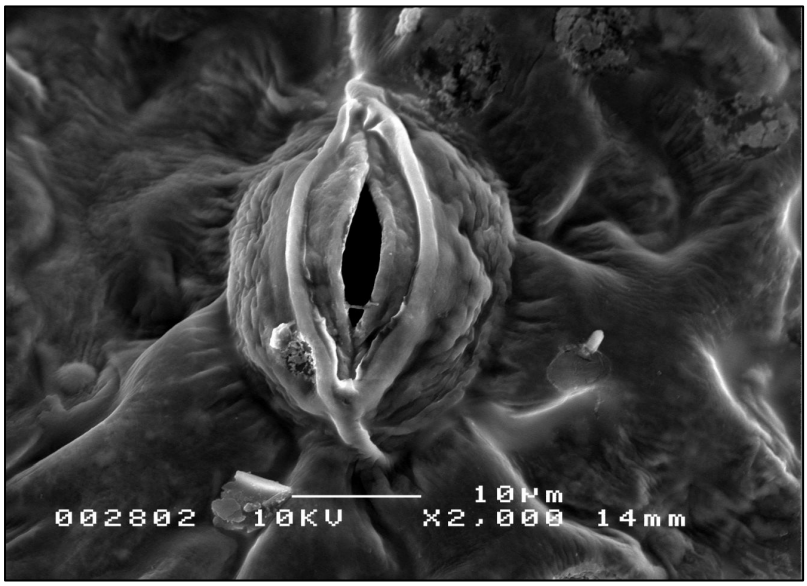

Figure 2.6 (e): SEM micrograph showing stomata on *in vitro* leaf of *Gerbera jamesonii*.

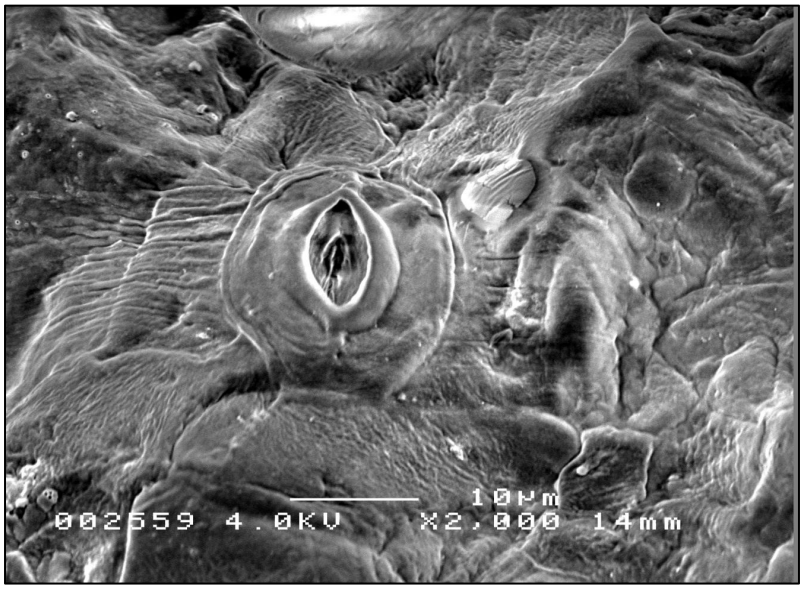

Figure 2.6 (f): SEM micrograph showing stomata on *in vivo* (intact) leaf of *Gerbera jamesonii.*

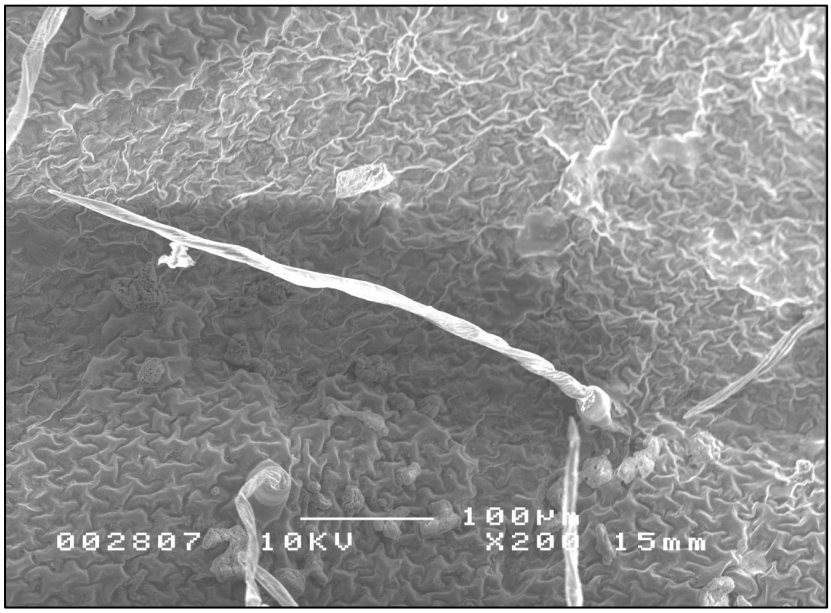

Figure 2.6 (g): SEM micrograph showing trichomes on *in vitro* leaf of *Gerbera jamesonii*

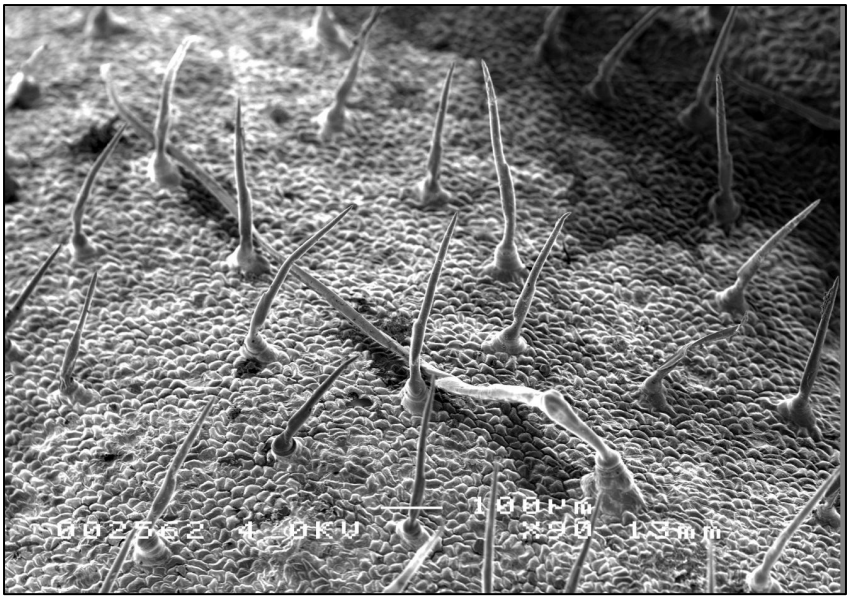

Figure 2.6 (h): SEM micrograph showing trichomes on *in vivo* (intact) leaf of *Gerbera Jamesonii*

2.4 SUMMARY OF RESULTS

1. Direct and/or indirect regeneration of shoots in *Gerbera jamesonii* were successfully achieved using petiole and leaf explants. Petiole explant was found to be the most regenerative explant.

2. Petiole explant cultured horizontally onto the culture media supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA was able to produce the highest shoot regeneration (90.0 ± 0.5%) with 8.1 ± 0.4 shoots per explant.

3. MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA was the optimum media in regeneration of shoots (94.3 ± 2.5%) with 9.3 ± 0.6 shoots per explant.

4. Petiole explants cultured on MS media supplemented with 2.0 mg/l Zeatin and 0.5 mg/l IBA produced 83.7 ± 1.5% shoots with 7.4 ± 0.9 shoots per explant.

5. MS basal media was the optimum rooting induction media, producing largest number of fibrous roots (90.6 ± 0.5%) with 15.5 ± 0.1 roots per explant.

6. Liquid MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA produced 18.5 ± 0.8 adventitious shoots in each 50 ml. However, the adventitious shoots formed were abnormal and smaller in size.

7. Solid MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA produced 11.6 ± 0.5 adventitious shoots. Adventitious shoots formed were normal in size and morphological aspects.

8. Solid MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA was chosen as optimum media for induction of adventitious shoots.

9. Addition of 30 g/l sucrose in culture media gave optimum result in regeneration of petiole explants *in vitro* with 92.8 ± 0.4% and 9.1 ± 0.5 shoots per explant.

10. Culture media with pH of 5.8 was found to be most suitable in regeneration of *Gerbera* shoots.

11. MS media added with 100 ml/l coconut water resulted in higher number of shoot regeneration with 14.1 ± 1.4 shoots per explant as compared to culture media without coconut water with only 9.4 ± 0.5 shoots per explant. However, shoots regenerated from media added with coconut water were bushy and smaller in size. Shoots regenerated from media without the addition of coconut water were more uniform and normal in size.

12. Comparisons between *in vivo* (intact) and *in vitro* leaves were made using scanning electron microscope. The number of stoma and trichome were higher on intact leaf compared to *in vitro* leaf.