CHAPTER 10

CONCLUSION

Gerbera jamesonii Bolus ex. Hook F. has proven to be highly regenerative in tissue culture system with 94.3 ± 2.5% of regeneration success. There are many factors that affected *in vitro* propagation of *G. jamesonii*. These factors include the type of explants used, polarity of explants in the culture medium, the effects of plant growth hormones and physical culture conditions that are somehow related to each other such as light, temperature, humidity etc. Micropropagation of *G. jamesonii* was best achieved using petiole explants cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA.

Optimum callus induction of *Gerbera* (78.8 ± 0.8%) was successfully achieved when leaf explants (10mm x 10 mm) were cultured on MS medium supplemented with 1.0 mg/l BAP and 2.0 mg/l 2, 4-D. Screening of secondary metabolites of *Gerbera* callus through thin layer chromatography (TLC) showed that, callus of *Gerbera* contain flavonoid, terpenoid and conjugated chain compounds.

Various phases of somatic embryos of *G. jamesonii* such as globular, heart, torpedo and cotyledonary phases were obtained from leaf explants through indirect somatic embryogenesis. Embryogenic callus was formed when leaf explants were cultured on MS medium supplemented with 0.01-2.0 mg/l 2, 4-D. Through double staining method, embryogenic callus can be distinguished from non-embryogenic

callus. Embryogenic callus formed were then transferred into MS cell suspension medium containing 0.1-2.0 mg/l 2, 4-D and 0.1 or 1.0 mg/l NAA. Embryogenesis was observed after 1 week in cell suspension. Cells were sieved and transferred into embryo induction medium containing 0.1-2.0 mg/l BAP and 0.1 or 1.0 mg/l NAA with the addition of 0 or 50 mM L-Proline. Embryogenic callus which has recovered from suspension medium started to develop on agar solidified medium. Optimum somatic embryos were achieved when embryogenic callus from cell clusters and aggregates from cell suspension culture were transferred to solidified MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA with the addition of 50 mM L-Proline. Somatic embryogenesis of *G. jamesonii* has many advantages. The induction of somatic embryogenesis permits the culture of large numbers of reproductive cells. Unlike shoots, somatic embryos were originated from single cells and the embryogenic cultures can be synchronized and purified so that only pure cultures of homogenous materials were obtained. The mode of somatic embryogenesis induction also allow easy scale up transfer with low labour inputs since embryos can be grown individually in liquid medium.

Synthetic seeds of *G. jamesonii* were successfully produced from micro shoots, globular phase and cotyledonary phase somatic embryo. Various factors affected the production and survival of synthetic seeds of *Gerbera* such as the concentration of sodium alginate ($NaC_6H_70_6$) and calcium chloride dehydrate ($CaCl_2.H_2O$) used in the preparation of the encapsulation matrix, the components of encapsulation matrix, content of sucrose in the encapsulation matrix, type of sowing media for germination of synthetic seeds and storage and preservation of synthetic seeds at low temperature (4 ±1 ^oC). It was

observed that 3.0 % of sodium alginate added in the encapsulation matrix and 100 mM calcium chloride dehydrate used as complexing agent was found to be optimum in the production of synthetic seeds of *G. jamesonii*. Synthetic seeds of *Gerbera* can be stored and preserved under low temperature and germinated when necessary. Production of synthetic seeds of *G. jamesonii* has become an important alternative for micropropagation of this plant species since the natural seeds of this plant is rare and difficult to obtain. In addition, the production of synthetic seeds of *G. jamesonii* allows rapid multiplication of this plant species and many other elite plant varieties. Synthetic seeds are easy to be handled while in storage and also can be transported easily from one place to another. Synthetic seeds have potential for long term storage without losing viability and the clonal nature of the resulting plants could be maintained. The production of synthetic seeds serves as a channel for new plant lines produced through biotechnological advances to be delivered directly to the green house or field.

Gerbera jamesonii was found to have irradiation response towards gamma irradiation. Various doses of gamma irradiation were exposed to *Gerbera* explants, callus and plantlets. The increase of irradiation dose has suppressed the activity of plant cells. Gamma irradiation was also found to reduce the nutrient quality of the culture medium and therefore, cells activities were also lesser. Reduction of chlorophyll and total soluble protein contents in irradiated callus were also observed as the exposure of irradiation dose increased.

Morphological characters of *G. jamesonii* plantlets were different between non-irradiated and irradiated plantlets. Variations were observed in plant height, size and texture of leaves and also the capability for *ex-vitro* flowering. In contrast to non-irradiated *in vitro* plantlets, irradiated *in vitro* plantlets failed to flower when transplanted to the green house. Somaclonal variation in regeneration system can be obtained not only when exposed to irradiation but also influenced by other factors such as the usage of various types of plant growth regulators, the type of culture medium used and also the physical environment of the culture conditions.

The present research was completed by the success of acclimatization of *in vitro* plantlets of *Gerbera* to the green house. High survival of plantlets was obtained when acclimatized in the green house. The adaptation of *in vitro* plantlets to the new environment was quite smooth. It can be concluded that, though in tropical climate country, *G. jamesonii* can be produced and cultivated successfully. Based on the morphological characters of the acclimatized *in vitro* plantlets, it was observed that plants regenerated from *in vitro* system are true-to-type i.e similar to the mother plant.

The present work on somatic embryogenesis, production of synthetic seeds and effects of irradiation on *G. jamesonii* were recent and innovative since works on these studies by previous researchers were very limited. Thus, the interesting findings on these studies could add to the present knowledge and information can be shared with others in similar field.

In future, cytological studies of *G. jamesonii* needs to be applied in order to investigate the cellular aspects of this plant species. The studies of mitotic index, ploidy level, chromosome number, cell cycle, nuclear and cell area and many more needs to be explored to look into the variation of the plant genotype, phenotype and to check for any occurrence of somaclonal variation as a result of tissue culture procedures.