In Vitro Flower Induction in Gerbera (*Gerbera Jamesonii* Adlam)

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ABSTRACT. *Gerbera (Gerbera Jamesonii* Adlam) *plantlets obtained from seeds grown on ¼ strength Murashige-Skoog (MS) medium supplemented with 3% sucrose were multiplied on full-strength MS medium supplemented with 0.5 mg/l BAP and 3% sucrose. The individual and combined effects of sucrose concentrations (0, 1, 3, 5 and 8%), strength of the medium (full strength and half strength MS medium), and Gibberellic Acid (GA_3) concentration (0, 1 and 5 mg/l GA_3) on in vitro flower induction of multiplied gerbera plantlets were investigated. In vitro flower induction was achieved on full-strength MS medium containing 8% (w/v) sucrose and 5 mg/l of GA_3 and 74% of the gerbera plants grown on this medium produced flowers.*

INTRODUCTION

Gerbera (*Gerbera jamesonii* Adlam), commonly known as Barbeton daisies, belongs to the family Compositae (*Asteraceae*). This is a beautiful and popular flower in the cut flower industry and it possesses characters such as different colors, high productivity, flowering precocity and long vase life. Therefore, gerbera has very high demand, which is not fulfilled, both in the local and export markets. In the local market, the price of a single flower ranges from Rs. 10.00 to 20.00 and the price of a pot ranges from Rs. 150.00 to 200.00. Therefore, to cater to this huge demand, gerbera cultivation in Sri Lanka should be expanded to areas other than the upcountry, where gerbera is predominantly cultivated. However, gerbera requires specific climatic conditions such as low temperature (24^0C) for flower production and does not produce quality flowers when these requirements are not fulfilled. Therefore, it is essential to study the requirements for flowering of gerbera. Flowering studies face difficulties associated with the control of the environmental factors such as photoperiodism, vernalization, water stress and endogenous factors that control flower initiation in a natural environment. Thus, *in vitro* flowering techniques can be used in this situation to eliminate the difficulties related to flowering in natural environment.

In vitro flowering is a novel technique in which the flowering process is induced by culturing whole plants, isolated organs or non-meristematic tissues in solid, semi-solid or liquid media under aseptic and controlled conditions. This technology helps to understand the physiological activities of the flowering process, which can never be performed under *in vivo* conditions (van Stadend and Dickens, 1991). In addition, *in vitro* flowering is a very important process in modern plant breeding techniques since it can be used for *in vitro* improvement through selective breeding methods as well as in *in vitro* seed production.

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Furthermore, this technique allows a greater control of the whole part of the plant under investigation, the whole process can be linked to micropropagation techniques, more efficient application of exogenous substances can be achieved, effect or affected sites can be isolated or eliminated, and undesirable influences such as bacterial or fungal contaminations, wounded surface and organic substances under test can be avoided.

Therefore, this study was conducted with the aim of investigating the possibility of using *in vitro* flowering techniques in identifying the factors such as C:N ratio and exogenous growth regulators, which influence on flower induction in gerbera.

MATERIALS AND METHODS

Plant materials

Gerbera (*Gerbera jamesonii* Adlam) seeds were washed with water followed by mild liquid soap (Teepol). They were then sterilized with 10% sodium hypochlorite (NaOCl) with two drops of Tween 20 for 10 min. followed by another 10 min. with a fresh 10% NaOCl solution without Tween 20. The seeds were thoroughly washed with sterilized water and established on hormone-free ¼ MS Macro nutrients (Murashige and Skoog, 1962) medium with 3% (w/v) sucrose. The seedlings obtained were multiplied on full strength MS medium supplemented with 3% sucrose and 0.5 mg/l Benzyl Aminopurine (BAP) by sub-culturing at eight-week intervals. The cultures were incubated at 16/8 h light/dark and 25^0C. After four subcultures, the plants were transferred to full-strength MS medium supplemented with 3% (w/v) sucrose without hormone and maintained for 8 weeks, to eliminate the effect of BAP, before being used for flowering studies

Effect of sucrose concentration on *in-vitro* flowering of gerbera (*Gerbera jamesonii* Adlam)

In vitro grown gerbera plantlets were established on MS medium supplemented with five different concentrations (0, 1, 3, 5 and 8% w/v) of sucrose to investigate the effect of sucrose concentration on flowering of gerbera. Each treatment was replicated four times and each replicate contained an average of 5 plants. Cultures were incubated at 25^0C and 16/8 h light/dark period and were maintained for five months by sub-culturing at four-week intervals on to the same medium.

Performance of the plants was observed at two-week intervals. Data were recorded at the end of the experiment on length of shoots and roots, and number of leaves and plants. Data were analyzed using ANOVA and CATMOD procedure with SAS computer package

Effect of the basal medium strength and gibberellic acid on *in vitro* flowering of gerbera (*Gerbera jamesonii* Adlam)

Effect of full-strength and half-strength MS medium supplemented with 3% (w/v) sucrose combined with three different GA_3 concentrations (0, 5 and 10 mg/l) on *in vitro* flowering of gerbera was investigated. Plants were sub-cultured on to the same media at four-week intervals. Performances of the plants were observed at weekly intervals and growth measurements (*i.e.* shoot and root length, number of leaves/plant, fresh and dry weight of shoots and roots) were recorded at the end of three months. Except the count data (number of leaves/plant) all the data were statistically analyzed by ANOVA (as a two factor

factorial experiment) of SAS system. Number of leaves/plant was analyzed using CATMOD procedure of SAS system.

Combined effect of strength of MS medium, GA_3 and Sucrose concentration on *in vitro* flowering of gerbera (*Gerbera jamesonii* Adlam)

Combined effect of full-strength and half-strength MS medium supplemented with two levels of GA_3 (0 and 5 mg/l) at two sucrose concentrations (3 and 8%) on *in vitro* flowering was investigated. Each treatment was replicated three times and one replicate contained an average of 15 plants. The experiment was arranged in a Complete Randomized Design. Sub-culturing of whole plant was done onto the same media every four-week. Eight weeks after initiation of the experiment, roots were trimmed in all plants and subcultured on to the same medium. Twelve weeks after initiation of the experiment, flower initiation was observed. The number of flowers produced per plant in each treatment was counted and analyzed by CATMOD procedure.

RESULTS

Effect of sucrose concentration on *in vitro* flowering

Gerbera plants did not produce any flowers at any sucrose concentration on the full strength MS medium even after five months of establishing the experiment. Although sucrose alone has no effect on flowering of gerbera, a clear increase in growth of shoots and roots with the increase in sucrose concentration was observed (Fig. 1) and this was significant at 0.1% probability level. However, response of shoot and root growth to sucrose was different. Shoots showed best performance at 3% sucrose concentration producing the highest number of leaves as well as taller shoots and thereafter gradual reduction of the shoot growth was observed. In contrast, root growth continuously increased with increase in sucrose concentration. In agreement with the results of the present study, Kristiansen *et al.,* (1999) reported reduction in number of aerial shoots and increase in root formation at high level of sucrose in *in vitro* grown Astroemeria Butterfly hybrids. Based on the results of the first experiment, it was decided to investigate the effect of nitrogen and gibberellic acid while keeping sucrose concentration at 3% (to maintain constant carbon) on *in vitro* flowering of gerbera.

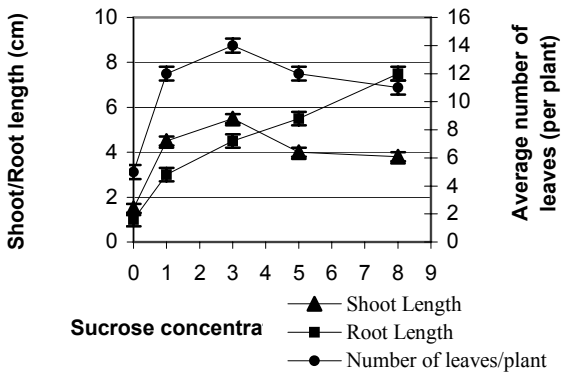

Fig. 1. Effect of sucrose on growth and development of gerbera (*Gerbera jamesonii* Adlam) plants at 5 months after initiation of the experiment.

Effect of strength of the medium and gibberellic acid on *in vitro* flowering of gerbera (*Gerbera jamesonii* Adlam)

Gerbera plants did not produce flowers at any of the tested gibberellic acid concentrations both on full or half-strength media. However, the strength of the medium as well as the gibberellic acid concentration had an effect both on shoot and root growth (Figs 2 and 3). Even though plants grown on half-strength MS medium showed better performance in relation to vegetative growth irrespective of the GA_3 concentration, only two parameters *i.e.* number of leaves per plant and root length, were significantly different at 0.5% probability level while others were not significant (Figs 2 and 3).

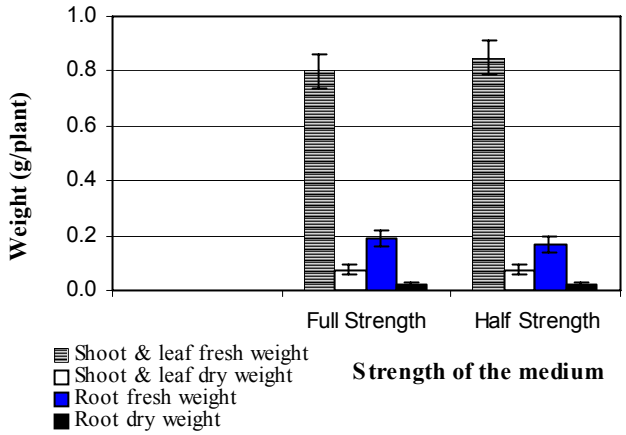

Fig. 2. Effect of full and half-strength medium on shoot and root dry weight of gerbera (*Gerbera jamesonii* Adlam) plants at 3 months after initiation of the experiment.

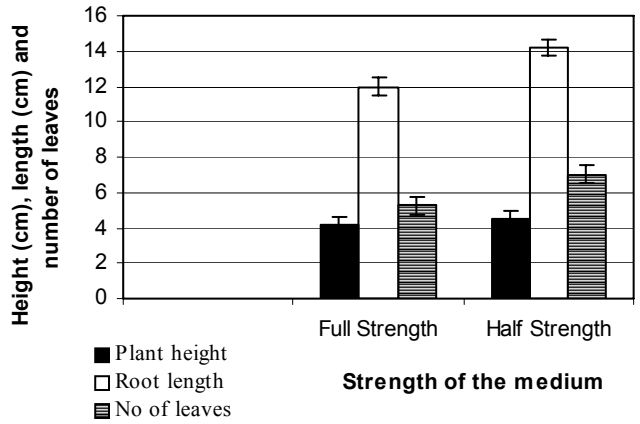

Fig. 3. Effect of full and half-strength MS medium on plant height, root length and number of leaves of gerbera (*Gerbera jamesonii*) plants at 3 months after initiation of the experiment.

Application of GA_3 showed an increase in vegetative growth compared to the plants grown on media without gibberellic acid irrespective of the strength of the medium (Figs 4 and 5). Furthermore, all the parameters measured, except root dry weight, showed better performance at 5 mg/l GA_3 concentration compared to that of 10 mg/l GA_3 and without GA_3 and this was significantly different at 5% probability level.

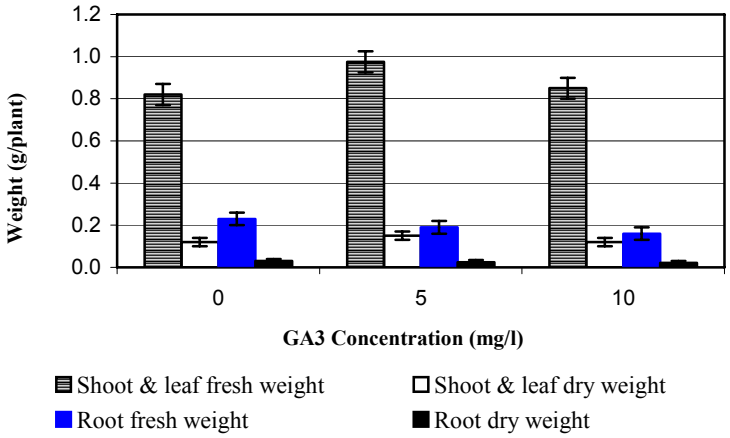

Fig. 4. Effect of GA_3 concentration on shoot and root dry weight of gerbera (*Gerbera jamesonii* Adlam) plants at 3 months after initiation of the experiment.

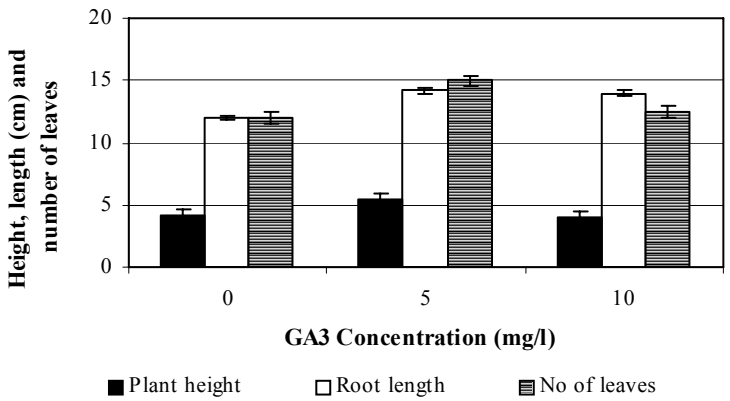

Fig. 5. Effect of GA_3 concentration on plant height, root length and number of leaves of gerbera (*Gerbera jamesonii* Adlam) plants at 3 months after initiation of the experiment.

Combined effect of strength of MS medium, GA_3 and Sucrose concentration on *In vitro* flowering of gerbera (*Gerbera jamesonii* Adlam)

Results of this experiment revealed that strength of the medium, sugar concentration and GA_3 concentration affect *in vitro* flower induction of gerbera. Flower induction was observed only in the full-strength MS medium and most of the plants (74%) grown on full-strength MS medium supplemented with 8% (w/v) sucrose and 5 mg/l of GA_3 produced flowers (Fig. 5, Plate 1). It was also observed that flower initiation occurred after the trimming of plant roots. The statistical analysis revealed that strength of the medium, sugar level and GA_3 have significant effects on flowering while full-strength MS medium supplemented with 8% (w/v) sucrose and 5 mg/l of GA_3 was found to be the best treatment combination for flower induction of gerbera under *in vitro* conditions (Fig. 6).

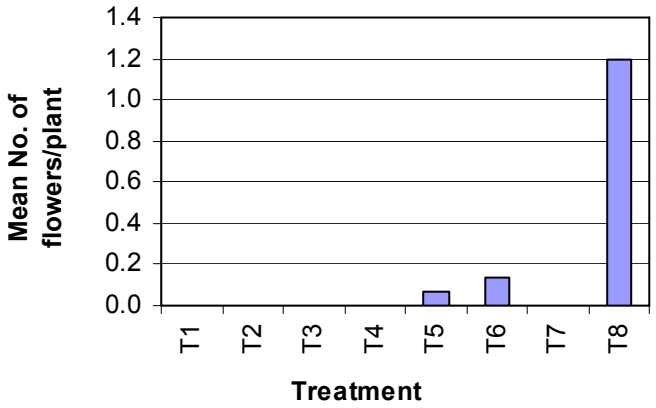

Fig. 6. The combined effect of strength of MS medium (1/2 MS and Full MS), GA_3 concentration (1 and 5 mg/l) and sucrose (3% and 8%) on *In vitro* flowering of gerbera (*Gerbera jamesonii* Adlam) at 3 months after initiation of the experiment.

Note: T1 - 0.5 MS + 0 GA3 + 3% Sugar; T2 - 0.5 MS + 0 GA3 + 8% Sugar; T3 - 0.5 MS + 5 GA3 + 3% Sugar; T4 - 0.5 MS + 5 GA3 + 8% Sugar; T5 - 1.0 MS + 0 GA3 + 3% Sugar; T6 - 1.0 MS + 0 GA3 + 8% Sugar; T7 - 1.0 MS + 5 GA3 + 3% Sugar; T8 - 1.0 MS + 5 GA3 + 8% Sugar.

Plate 1 . Different stages of in vitro flower induction of gerbra (*Gerbera jamesonii*).
a - the explant used for *in vitro* shoot multiplication
b - flower bud formation (12 weeks)
c - A flower bloomed *in vitro* (16 weeks)

DISCUSSION

Three main systems; (1) whole plant culture, (2) the culture of isolated organs or bud explants with meristematic cells and (3) non-meristematic tissues such as callus, thin epidermal layers, and pith tissues, are used for *in vitro* flowering (van Stadend and Dickens, 1991). In the present study, whole gerbera plants have been used for *in vitro* flower induction since the use of whole plant provides an opportunity to study the effects of single or a small number of compounds, while relying on the complete plant in culture to provide all the other compounds required for growth and/or flowering, in their correct concentrations.

Furthermore, the physiological state of the parent plant is critical in flowering studies. Plants which have been pre-induced or were already flowering, have often been used as the source of explants, such as in the case of *Streptocarpus* (Simmonds, 1982), *Torenia*, (Tanimoto and Harada, 1981a; 1981b), *Kalanchoe* (Margara and Piollat, 1981) and

Begonia (Ringe and Nitsch, 1968), where explants are usually taken from the inflorescence tissue. If already induced plants are used as bioassays, and flowering is brought about by the addition of some substance to the medium, it is probable that this substance simply promotes the manifestation and growth of the already induced buds or flowers. Therefore, it is desirable that the parent or donor plant used in bioassay for the flowering stimulus should be strictly vegetative at the time of culture. Therefore, in the present study, micropropagated gerbera plants, which were in vegetative stage and maintained under non-inductive conditions (16/8 hours light/dark period) were used and flower induction was attempted either by changing carbohydrate concentration, nitrogern concentration and/or GA_3 concentrations.

In most investigations of *in vitro* flowering, MS medium which has fairly high nutrient concentration, including high level of NH_4NO_3, has been used (van Stadend and Dickens, 1991; Wang *et al.,* 2002). The investigations of Tanimato and Harada (1981 a b and c) have indicated that in correct nutrient composition, flowering can be brought about in small vegetative explants of *Torenia fournier* without hormones. They found that the dilution of MS medium to one fifth of the recommended concentration enhanced adventitious bud formation and development, and in so doing it enhanced flower bud formation of *Torenia fournier*, but did not influence the ratio of flowering to vegetative buds. However, in the present study, dilution of the MS medium had no effect on flower initiation while increase in vegetative growth was observed in plants grown on ½ strength MS medium compared to that of the full strength MS medium. Thus, it may be possible that the effect of the medium strength may depend on the plant species under investigation.

In the present study, *in vitro* flower induction of gerbera was observed when plants were grown on full-strength MS medium supplemented with 5 mg/l of GA_3 and 8% (w/v) sucrose. Furthermore, it was observed that trimming of roots induced flowering of gerbera. These results can be interpreted with the support of two classical theories. Firstly, the antagonism which exists between rooting and flowering. Gaspar (1980) proposed that this antagonism was due to control mechanism where inverse variation of auxin and peroxidase enzyme are responsible for flowering and rooting. This antagonism was also found in *in vitro* grown *Kalanchoe* (van Stadend and Dickens, 1991), where reduced root mass was produced in inductive conditions and, where hormones stimulated root growth inhibited flowering.

The second classical theory that supports this result is that increase in C:N ratio can stimulate or even induce flowering. In agreement with the results of the present study, the importance of high C:N ratio for *in vitro* flowering of *Torenia fournier* (Tanimato and Harada, 1981c), and *Kalanchoe* (van Stadend and Dickens, 1991) can be shown. Furthermore, Steffen *et al.* (1986) noted that Bogenvillea floret development was promoted by fructose (83%), glucose (68%) and by sucrose (24%) revealing the role of different sugars as carbohydrate source in flowering. Therefore, in future studies, it may be possible to investigate the effect of different carbohydrate sources on *in vitro* flowering of gerbera.

Of all the plant hormones, the giberellins have been the most successful in the induction of flowering in cold requiring plants and rosette Long Day Plants (LDP) grown *in vivo* (Bernier, 1988). It has also been found that gibberellins can stimulate flower transition in some ornamental plants, which are photoperiodically neutral and do not respond to cold (Halevy, 1990). However, in short-day plants gibberellins are ineffective or have certain

limited quantitative effects on flowering (Pharis and King, 1985). In contrast, in the present study, induction of flowering of gerbera, which prefer short days for flowering, was achieved under non-inductive conditions by GA_3. In agreement with these results, promotive effects of GA_3 has also been described in *Pharbittis nil*, a model short day plant, at sub-optimal photoperiodic conditions (Galoch *et al*., 1995; Gulewska *et al*., 2000; King *et al*., 1987; Ogawa, 1981). It may be possible that gibberellic acid may be involved as a part of the signaling system operating in the plant, which trigger or prevent transition of vegetative buds to floral buds (Gulewska *et al.,* 2000). The effectiveness of this transition could be dependent on many factors such as gibberellin structure, timing, site of action and sensitivity of the organ (Gulewska *et al.,* 2000). Results of the present study clearly indicate that gibberellic acid induces flowering of gerbera at high sucrose concentrations. There is a complex relationship between carbohydrates and hormonal levels in cells and organs, which may be responsible for flowering (van Stadend and Dickens, 1991).

The results also clearly show that micropropagation techniques, which allow a degree of experimental control seldom achieved under *in vivo* studies, can be used as an ideal tool for the investigation of flowering physiology, and *in vitro* flower induction of gerbera could be achieved by manipulating the carbohydrate concentration and gibberellic acid concentration in the medium. Thus, it may be possible to regulate flowering of gerbera under *in vivo* condition by external application of GA_3 and further studies are needed to confirm these results under *in vivo* conditions.

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