In vitro propagation of *Gerbera* – A Review

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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, colour, 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. Most of the work has been carried on plant regeneration by adventitious organogenesis from capitulum, shoot tip, leaf, petiole and other parts of the plant. 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 favourably respond to different culture media with different types and concentrations of growth regulators.

Keywords: micropropagation; explants; *Gerbera;* growth regulators

The Barberton/Transvaal daisy, or gerbera, is a flower with increasing commercial significance. *Gerbera* is one of the leading cut flowers and ranks among the top ten cut flowers of the world (Parthasarathy, Nagaraju 1999). The production of gerbera was approximately US$ 220 million in 2001 representing 70 million stems sold in US alone (Broek et al. 2004). It has a wide applicability in the floral industry as cut flower and potted plant. The flowers are hardy and stand the rigors of transportation and a long keeping quality fetches a good market price. The species, a perennial herb, is native to South Africa and Asia. It is an important commercial flower grown throughout the world in a wide range of climatic conditions. Gerberas mostly inhabit temperate and mountainous regions. In India they are distributed in the temperate Himalayas from Kashmir to Nepal at altitudes from 1,300 to 3,200 m. The genus *Gerbera* was named in honour of a German naturalist Traugott Gerber, who traveled in Russia in 1743. The genus consists of about forty species (Das, Singh 1989). Out of the recorded species, only one species *Gerbera jamesonii* is under cultivation. Few important cultivars of gerbera are Cream Clementine, Maron Clementine, Delphi, Vesta, Uranus, Terraqueen, Dusty, Valentine, Diablo, Mariso and Pascal. Based on the flower heads, they may be grouped into single, semi-double and double cultivars (Loeser 1986).

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. For commercialization of this crop, however, planting material is required on large scale, which requires the development of 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 al. 2002, 2003). Micropropagation of gerbera is being used in many countries from a range of explants. In tissue culture studied so far, plant regeneration was uniformly achieved with different explants as the source material (Murashige 1977). Bud regeneration in gerbera may represent an effective alternative to the current methods of micropropagation via axillary branching, provided that the phenotype and flower production of the regenerants are maintained (Reynoird et al. 1993).

Like any ornamental plant gerberas are produced exclusively for their aesthetic values. Thus the improvement for quality attributes such as flower colour, longevity and form, plant shape and the creation of novel variants are important economic goals. Conventional breeding of gerbera by crossing and selection has generated several clonally propagated elite genotypes that have desirable traits such as colour, shape, vase life and resistance against pests and diseases. However, one of the disadvantages of traditional breeding is the limited genepool of any single species. An important application of genetic engineering to the ornamental plant is the possibility of changing flower colour. Flavonoids, which are common flower pigments, are synthesized via phenylpropanoid pathway. Cloning and transferring into plants, the genes affecting flavonoid synthesis, has led to changes in flower colour or pattern. Elooma et al. (1993) used *Agrobacterium tumefaciens*-mediated transformation to introduce an antisense gene for chalcone synthase into Terra Regina. *Gus* and *nptII* marker genes have been introduced into five cultivars of gerbera (Nowak et al. 1997). Nagaraju et al. (1998) reported *Agrobacterium*-mediated genetic transformation protocol for *Gerbera hybrida* using petiole, leaf and shoot tip explants. Reynoird et al. (2000) revealed differences in bud regeneration capacity from *G. hybrida* leaf explants and concluded that genetic transformation depends upon regeneration frequency of the explant. Orlikowska and Nowak (1997) found that young leaf petiole was not efficient enough to enable successful transformation in gerbera.

The objective of this article is not to exhaustively review the literature but to overview the work done in tissue and organ culture and bring forth some points that we consider as the major thrust of contemporary and future research.

Establishment and regeneration of cultures

The technique of *in vitro* cultivation of plant cells or organs is primarily devoted to solve two basic problems. Firstly, to keep the plant cells and organs free from microbes like bacteria and fungi, and secondly, to ensure the desired development in the cells and organs by providing suitable nutrient media and other environmental conditions. Using modern equipment and careful handling during various operations can eliminate the first problem. The second problem remains the area of active research and is likely to do so for some times in the future. At present, it relies mainly on the manipulation of culture medium especially growth regulators, and to a lesser extent on other factors, including environmental conditions.

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).

Virtually all tissue culture media are synthetic or chemically defined, only a few of them use complex organics as their normal constituents. A variety of methods were developed, but none of them is suitable for either all plant species or for every purpose. 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, Kanwar 2005, 2006). Parthasarathy et al. (1996) and Verma and Anand (2006) used N_6 (Chu 1978) and B_5 (Gamborg et al. 1968) media for culture establishment in gerbera. Mandal and Datta (2002) used LS (Linsmaier, Skoog 1965) medium for establishing organogenic callus cultures from immature flower buds. Chen et al. (2006) used DKW (Driver, Kuniyuki 1984) medium for tissue culture studies of gerbera stem nodes with buds. *Gerbera* was regenerated *in vitro* through various explants as given below.

Capitulum

The capitulum explants for propagation of gerbera were used by a number of workers (Pierik et al. 1982; Modh et al. 2002; Tyagi, Kothari 2004; Ray et al. 2005). The advantages of the capitulum method over shoot tip are the easier sterile isolation *in vitro* and it is also non-destructive, only inflorescences are used and no shoots are lost from the plant (Pierik et al. 1982). The results of clonal propagation *in vitro* using either capitulum explants or subcultured shoots depended on the cultivar and on the cytokinin level in the medium. With capitulum explants shoot formation of some cultivars was very low and almost independent of the BA (Pierik et al. 1982). 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 kinetin. 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. Huang et al. (1987) also reported propagation using capitulum explant on half-strength MS medium containing 0.03 mg/dm^3 NAA. The cultivars Pastourelle and Mardi Gras were propagated from fragments of capitulum (0.5–0.7 cm diameter). The medium containing 0.1 mg/dm^3 IAA and 1 or 2 mg/dm^3 BA gave the best shoot production (Laliberte et al. 1985). 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 in Appel Bloesem. Ray et al. (2005) developed an efficient protocol for large scale propagation using young capitulum as explant on a medium containing 7 mg/dm^3 BA and 0.1 mg/dm^3 IAA, which initiate multiple shoots (10 shoots per explant). Tyagi and Kothari (2004) used capitular sections for rapid *in vitro* multiplication of shoots with 4 mg/dm^3 kinetin and 0.5 mg/dm^3 IAA. About 20–25 shoot buds developed from the callus. Kuan et al. (2002) conducted an experiment to study the effect of carbon source and concentrations on the *in vitro* growth and maintenance of plantlets produced from young capitulum explants. The addition of lactose at 1% was effective in lowering the growth rate of explants up to five months and the growth vigour could be resumed after transferring the plantlets to culture medium with 1% sucrose.

Proliferated mass of shoots was obtained from mature capitula with 0.1 mg/dm^3 IAA and 10 mg/ dm^3 BA in the medium (Modh et al. 2002; Zheng et al. 2002). Capitulum explants of the varieties Appel Bloesem, Marleen, Clementine and Pimpernel were cultured on modified MS media supplemented with 2 mg/dm^3 BA and 0.5 mg/dm^3 IAA, the only treatment to produce a large translucent callus in Appel Bloesem and Marleen (Arello et al. 1991). Napaskamon (1991) studied the effect of kinetin 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 kinetin had no effect.

Several other floral parts such as inflorescence buds, peduncle and floral bracts were used for *in vitro* propagation, although to a lesser extent. *In vitro* shoot production of 17 *G. jamesonii* cultivars from 1 to 2 cm long flower bud explants was higher and quicker than from explants of pedicels of flowering plants. Shoot development in the axils of involucral bracts was the most common in explants from buds, while shoots from undifferentiated callus occurred more frequently in explants from fully developed inflorescence (Schum, Busold 1985). Maia et al. (1983) gave details of a newly developed method using floral bracts as explant and described the choice of mother plants, culture media, propagation rate and conditions of *in vitro* culture. Pawlowska (1977) studied the capacity for shoot formation from inflorescence buds. Explants were excised when the peduncle reached 12–20 cm in length and the ligulate flowers were yet visible or when the young buds reached 1.0–1.5 cm in length. Pierik's medium enriched with IAA and kinetin or BA was used for the induction of shoots when the young buds reached 1.0–1.5 cm in length. 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 was described by Pierik et al. (1974). Wang and Yu (2001) studied the tissue culture of floral receptacle on MS medium supplemented with 8 mg/dm^3 BA and 0.1 mg/dm^3 NAA. Mandal and Datta (2002) established organogenic callus cultures from immature flower buds on modified MS medium with IAA and BA.

Tubular florets and anthers of 126 genotypes were grown on a simplified Linsmaier and Skoog (1965) medium containing 0.5 mg/dm^3 IAA, 0.1 mg/dm^3 2,4-D and 2 mg/dm^3 kinetin. After 1-day incubation most of the pollen mother cells reached the tetrad stage of development and 4 days later some anthers contained cells with 2, 4 and more nuclei. Embryoids were formed on callus from seven tubular florets and seven anther genotypes (Backer-Zens et al. 1983). *Gerbera* plants Tilusion, Jamilla and Mariola were regenerated and propagated *in vitro* from floral peduncle explants. *De novo* regeneration of adventitious buds was made possible by culturing explants on medium with 0.1 mg/dm^3 TDZ alone or in combination with 0.25 mg/dm^3 NAA. Transfer of the shoots to the medium with 1mg/dm^3 BA led to the production of axillary buds at the rate of 10 new-formed-shoots per initial explant (Le et al. 1999). Posada et al. (1999) reported micropropagation of gerbera using floral bud explants with 0.1 mg/dm^3 IAA and 1 mg/dm^3 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.

Shoot meristem and shoot tip

The inconsistent behaviour of callus cultures with respect to organ regeneration necessitated the use of alternative tissue and methods for the multiplication of gerbera. It involves an increase in the number of miniature divisions *in vitro*. Murashige et al. (1974) developed a tissue culture method for rapid clonal multiplication of gerbera. Shoot tips were used as initial explants and the miniature divisions, which arise from them, were repeatedly separated and recultured. 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. *In vitro* culture of vegetative apices proved a valid method for the clonal multiplication of gerberas. The nutrient medium of Murashige et al. (1974) gave good results and the incorporation of cytokinin (especially zeatin) favoured the formation of new buds (Gregorini et al. 1976), which quickly formed roots on transfer to nutrient medium containing NAA. Pierik and Sprenkels (1984) cultured shoot tips of the shy-rooting gerbera cultivars Fleur and Florence in modified MS medium containing either 3–10 mg/dm^3 IAA or 1 or 3 mg/dm^3 NAA. Aswath and Choudhary (2001, 2002a,b) and Aswath et al. (2003) cultured shoot tips for establishing and proliferation of multiplication in *G. jamesonii* AV101, GJ-1, GJ-2 and GJ-3 on MS medium supplemented with different concentrations of growth regulators. Thakur et al. (2004) developed a protocol for *in vitro* culture of gerbera Kazak and Gold Disk using shoot tip as explant. The maximum establishment percentage of Gold Disk and Kazak was observed with 2.5 and 2 mg/dm^3 BA, and maximum number of shoots with 1.5 and 1 mg/dm^3 BA, respectively. Aswath and Wazneen (2004) studied the effect of growth regulators and media on *in vitro* shoot regeneration and proliferation using shoot tips obtained from *ex vivo* plants of TIHR selection GJ-23 and showed the effectiveness of this system for commercial multiplication of gerbera. Giles et al. (1986) initiated cultures from shoot meristem and intended to identify the metabolites involved in multiplication of gerbera. Constantinovici and Sandu (1995) established a method for regenerating gerbera plants *in vitro* using flower meristem and vegetative apices on a medium containing 5 mg/dm^3 BA and NAA for regeneration phase of gerbera plantlets.

Leaf and petiole

The first step in the regeneration of gerbera plants *in vitro* is axillary shoot formation from excised capitulum explants (Pierik et al. 1982) or from isolated shoot tips (Murashige et al. 1974). Then it was possible to induce adventitious shoot formation on isolated young leaves from earlier developed axillary shoots. Adventitious shoots are needed in mutation breeding as a tool for the production of solid mutants, as the chances of solid mutants arising with adventitious shoot technique are far higher than with axillary shoot techniques. Hedtrich (1979) observed the regeneration of adventitious shoots from leaf blades during *in vitro* propagation of Vulkun on modified MS medium supplemented with 1 mg/dm^3 BA and 0.1 mg/dm^3 GA_3. Reynoird et al. (1993) gave modified MS medium supplemented with 10μM BA and 2.5μM NAA for plant regeneration from *in vitro* leaf culture of several gerbera species. The morphogenetic potential varied with the developmental stage of the leaves and up to 90% of excised developing leaves formed 3–5 shoots per explant. Jerzy and Lubomski (1991) examined the effects of preparation of leaf explants, composition of culture medium, the kind of *ex vitro* stock plant and low temperature pre-treatment on the formation of adventitious shoots in cv. Sardis. Adventitious shoot formation occurred at the base of leaf petiole with 0.5 mg/dm^3 IAA.

Palai et al. (1998) developed an efficient protocol for mass cloning of gerbera cv. Fredaisy *in vitro* by manipulating growth regulators and culture conditions. Leaves were used as the explant source for callus culture. The cultures inoculated in the light exhibited a higher rate of shoot bud differentiation than those inoculated in the dark. *In vitro* propagation of gerbera was studied by culturing 3-leaf-bud explants on a medium containing 0–4 mg/dm^3 BA and 0–1 mg/dm^3 IAA. Highest multiplication rates (9.5–11.2) were obtained on 1 mg/dm^3 BA, irrespective of the IAA concentration used (Barbosa et al. 1994). A high frequency of shoot organogenesis and plant establishment protocol was developed for *ex vitro* leaf derived callus with 0.4 mg/dm^3 BA and 4 mg/dm^3 NAA (Aswath, Choudhary 2002a). Xi and Shi (2003) micropropagated four gerbera cultivars using 0.5–1 cm long young leaves with 1 mg/dm^3 BA and 0.1 mg/dm^3 NAA. 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.

Kumar and Kanwar (2006) observed callus induction on leaf and petal explants on MS medium

supplemented with 1, 1.5 and 2 mg/dm^3 2,4-D. The calli derived from leaf explant differentiated into roots with NAA. BA and kinetin failed to induce callus on leaf and petal explants in cut flower gerbera. Ruffoni and Massabo (1991) indicated that *in vitro* plant, leaf petiole and shoot apices were able to regenerate some buds, whereas leaf laminas were only able to regenerate callus and roots. Barbosa et al. (1994) cultured leaf bud explants of Appel Bloesem on MS medium supplemented with 3% sucrose, 80 mg/dm^3 adenine sulphate, 100 mg/dm^3 tyrosine, 7 g/dm^3 difcobacto agar, 0.01, 2 and 4 mg/dm^3 BA and 0, 0.25 and 0.5 and 1 mg/dm^3 IAA. Highest multiplication rate was obtained on 1 mg/dm^3 BA, irrespective of the IAA concentrations used. Parthasarathy et al. (1996) reported callus induction and subsequent plant regeneration from leaf explant of *G. jamesonii* on MS medium supplemented with NAA, BA, kinetin and IBA in different concentrations. Callus growth was greatest with 1 mg/dm^3 of NAA, BA and IBA. Adventitious shoots were observed on MS medium supplemented with 1 mg/dm^3 NAA and 0.75 mg/dm^3 IBA. Orlikowska et al. (1999) produced callus from petiole explant of the youngest 3–4 leaves detached from axillary shoots produced *in vitro* with 2.3μM TDZ and 0.53μM NAA in Bal, Mariola and Rebecca. Direct shoot regeneration occurred during the first four weeks and the effectiveness of shoot regeneration depended on the cultivar, the sequence of passage on regeneration medium, growth regulators and duration of the induction period. Xu et al. (2002) and Kumar et al. (2004) regenerated adventitious shoots from petiole and leaf pieces of *G. jamesonii* in a medium supplemented with different concentrations of auxins and cytokinins.

The shoots obtained from capitulum method can be used as sterile starting material for axillary branching experiments and the shoot formation in most of the cultivars strongly depended on the level of BA in culture medium. The initiation of callus and subsequent establishment of cultures is not very specific with respect to the tissue used or the type and concentration of the growth regulators. Shoot apices and/or shoot tips are alternative methods for regeneration and clonal multiplication of gerbera for commercial use whereas leaf explant and petiole could be used for the production of adventitious shoots, which are needed in mutation breeding to induce genetic variability.

Haploid production

An important aspect of plant breeding is the induction of maximum genetic variability of germplasm sources to secure a wider scope for selection and introduction of better trait qualities in existing crop species. Plant breeders have worked intensively to obtain haploids either *in vitro* or *in vivo*. Guha and Maheshwari (1964, 1966) published the first report showing that the isolated anthers of *Datura innoxia* were able to form haploid embryos *in vitro*. During the last few decades much progress has been made in anther culture of rice, wheat, maize, brassica, pepper and many other crop species. These advances attracted the attention of geneticists to survey the advantages offered by haploid plants over their relative diploids. Pavlova (1986) reported that the cells of female gametophytes are considered as possible alternative sources of haploid cells for plant breeding work and production of homozygous haploid plants. Tosca et al. (1999) studied the effect of genotyping and season on gynogenesis efficiency in four genotypes of gerbera. Naked unfertilized ovules were collected between April and October, cultured on MS medium supplemented with 0.88μM BA and 0.57μM IAA. Both the genotypes and the season significantly interacted in the recovery of haploid calli depending upon the genotype and season. The ability to produce haploid callus was found not to be predictive for efficient shoot regeneration. Miyoshi and Asakura (1996) cultured unpollinated ovules of several genotypes of pot gerbera in Ahmin-Vieth medium for callus. The highest frequency of callus formation was 17.5%. Shoot formation was 19.6% and 80.4% of plants were haploid, 15.2% were diploid and 4.3% were mixoploid. Cappadocia et al. (1988) emphasized the use of unpollinated ovules and ovaries for the production of haploid in those species where anther culture is not effective and obtained about 76% haploid plants. Ahmin and Vieth (1986) studied the influence of sucrose and various growth regulator combinations on *in vitro* cultivation of gerbera from unfertilized ovules cloned from cv. Super Gerbera. The successful regeneration of haploid plants was reported with 0.1 mg/dm^3 IAA and 0.2 mg/dm^3 BA. Meynet and Sibi (1984) reported that *in vitro* culture of 2,500 unfertilized naked Fresultane ovules gave rise to more than 100 plants, of which most were haploid (n = 25). Sitbon (1981) cultured unfertilized ovules near maturity from flowers of Appel Bloesem, Clementine, Lambda and Pimpernel in 12 different media with various levels and combinations of growth substances in light and dark. Chromosome number was determined for 16 clones obtained by callus culture. Two clones were diploid, 14 were haploid, different in size and leaf shape from their diploid parent. Callus of haploid clones probably originated from one or more gametophytic cells.

It is evident that *in vitro* techniques have been employed to induce haploids in gerbera using unfertilized ovules. Contrary to *in vivo* methods, the success achieved using *in vitro* methods has been spectacular. *In vitro* production of haploids can solve some problems in genetic studies since gene actions are readily manifested due to a single allelic dose present in chromosomes of an entire genome. Though a rapid achievement of homozygous traits in double-haploids, pollen derived haploid plants have been used in breeding and improvement of crop species. The important achievement of haploid production is in selection programs to create parents with new gene combinations, and to study the conformity of ovule-derived plants from a dihaploid strain as a possible variability source.

Cell suspension and somatic embryogenesis

Cell suspension cultures and regenerative callus have found a vast applicability in biotechnology, i.e. *in vitro* selection, protoplast fusion and genetic transformation. Suspension cultures are generally initiated from friable callus and during the regeneration phase somatic embryogenesis is easily observed. Somatic embryogenesis is a process of single cell or a group of cells initiating the developmental pathway that leads to reproducible regeneration of non-zygotic embryos capable of germinating to form complete plants. In tissue cultures somatic embryogenesis occurs most frequently as an alternative to organogenesis for regeneration of whole plants. Adherence to this pattern of morphogenesis depends on coordinated behaviour of a cell or cells to establish polarity as a unit and thereby initiate gene action sequentially specific to emerging tissue region. Ruffoni and Massabo (1991) reported the callus and suspension cultures of *G. jamesonii* hybrida by using fragments of apices, petioles and leaves as explant sources from its three cultivars and 11 other selected clones on MS basal medium along with 27 combinations of several hormones. They reported the best proportions between callus, shoot and root regeneration in apex explant and best suspension culture with 90% viability on MS basal medium supplemented with 0.2 mg/dm^3 pCPA and 0.2 mg/dm^3 BA. They studied only the cellular features of the liquid cultures. Kumar and Kanwar (2005) achieved plant regeneration from callus and cell suspension cultures derived from disc floret, ray floret and petal explants of Diablo.

Sharma and Srivastava (2005) achieved somatic embryogenesis from callus cultures derived from leaf disc explants of Alsmeera. Maximum percentage response (64.56%) for somatic embryo induction from callus clumps was obtained with 0.5 mg/dm^3 BA and 1 mg/dm^3 NAA. The histological studies confirmed the presence of somatic embryos in different developmental stages.

Embryogenic cell suspension offers the possibility for large-scale clonal propagation and provides an excellent tool for both theoretical and practical applications. Selection scheme with embryogenic cultures gives rise to variants for various abiotic and biotic stresses. Only a few attempts have been made to regenerate gerbera through embryo-like structures produced through somatic embryogenesis. These cultures could be used in future to obtain transgenic plants for breeding and cytological studies.

Greenhouse acclimatization

Attempts have been made for hardening and acclimatizing gerbera plantlets, one of the most important aspects of *in vitro*-raised tissue cultured plants. A few reports are available where different soil media were used and standardized with 50–100% success depending upon the soil mixture. 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. Regenerants never displayed phenotypic variations during subsequent vegetative and floral development. Petru and Matouš (1984) successfully transferred the plantlets into sterilized peat:perlite (1:1) substrate and then into a standard horticultural substrate. Laliberte et al. (1985) transferred rooted plantlets to Jiffy-7 peat pellets, in glass covered acclimatization module and later to a mixture of 1 perlite:1 sphagnum moss in greenhouse with 95% success. The rooted plantlets exhibited 100% survival in plastic pots filled with coco peat, red soil and sand at a ratio 3:1:1 in *G. jamesonii* cv. AV 101 (Aswath, Choudhary 2002b). Parthasarathy and Nagaraju (1999) achieved a 90–100% success in polythene bags containing equal amount of soil:sand:farmyard manure. Woltering (1990) studied the gaseous composition of atmosphere in polypropylene (PP), polyvinylchloride (PVC) and glass containers using *Gerbera jamesonii* plantlets. Ethane, ethylene and propane were present in all types of containers. Propylene and butane were found only in PP and PVC containers. An unknown phytotoxic component was found under normal cultural conditions only in PP containers; it caused severe loss of quality in plants grown in PP contain-

ers. 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. Kumar and Kanwar (2005) achieved 50–60% success in cv. Diablo in a mixture of sand:farmyard manure (1:1). Ray et al. (2005) reported that the plantlets micropropagated in garden soil were uniform and identical to the mother plant with respect to growth characteristics and morphology. Posada et al. (1999) observed 50% survival rate on the media with buried rice hull and coke scoria under high humidity.

Micropropagation on large scale can be successful only when plants after the transfer from culture to the soil show high survival rates and the cost involved in the process is low. Tissue culture plants generally show some structural and physiological abnormalities. A heterotrophic mode of nutrition and poor mechanism for water loss control further renders micropropagated plants vulnerable to transplantation shocks. Therefore, transfer of individual plantlets to a potting mixtures and their acclimatization under greenhouse conditions require the application of various methods to harden the plants for transplantation. Although different workers using different soil/potting mixtures achieved 50–100% success in gerbera, still the technology needs improvement to achieve 100% success in all species/cultivars to meet the growing demands of growers globally.

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Množení gerber v *in vitro* podmínkách – Přehledová studie

ABSTRAKT: V posledních několika letech se *Gerbera* stala populární v mnoha zemích světa. Na trhu je vyhledávaná nejen jako květina k řezu, ale také jako hrnková květina, a to díky své kráse, barvě, dlouhé době trvanlivosti ve váze a možnosti transportu nasucho. Většina komerčně pěstovaných kultivarů se rozmnožuje vegetativním způsobem pomocí dělení trsů. Tato metoda množení je však příliš pomalá. V mnoha zemích se pro uplatnění gerber na trhu a pro uspokojení rostoucí poptávky po rostlinném materiálu používají techniky explantátových a orgánových kultur jako alternativní metoda množení. Ve většině prací se pro regeneraci rostlin cestou adventivní organogeneze používá úbor, vrchol, list, řapík a další části rostlin. Pozornost by měla být zaměřena na zlepšení technologie množení, aby se dosáhlo 100% úspěchu při množení u všech skupin a kultivarů gerber a tím k celkovému uspokojení poptávky pěstitelů. Z literatury je zřejmé, že existuje mnoho *in vitro* studií provedených na gerberách, kde byla zjištěna dobrá odezva různých typů explantátů na odlišná kultivační média s rozdílnými typy a koncentracemi růstových regulátorů.

Klíčová slova: mikropropagace; explantáty; *Gerbera*; růstové regulátory

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