African Journal of Biotechnology Vol. 11(37), pp. 9048-9053, 8 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2276
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effect of different explants on *in vitro* propagation of gerbera (*Gerbera jamesonii*)

Shagufta Naz[1], Fozia Naz[1], Amina Tariq[1], Farah Aslam[1], Aamir Ali[2] and Mohammad Athar[3]*

[1]Department of Biotechnology and microbiology, Lahore College for Women University, Lahore, Pakistan.
[2]Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan.
[3]California Department of Food and Agriculture, 3288 Meadowview Road, Sacramento, CA 95832, USA.

Accepted 26 March, 2012

This research work involves shoot induction, multiplication of induced shoot and their rooting in *Gerbera jamesonii* (Gerbera). For shoot induction, apical meristems and vegetative buds were used. Murashige and Skoog (MS) medium containing 6-benzylaminopurine (BAP) alone or in combination with indole-3-acetic acid (IAA) was tested. Best shoot induction response was obtained after 8 days of inoculation from apical meristem and after 12 days of inoculation from vegetative buds on MS medium supplemented with 10 mg/L BAP and 8.8 µM BAP + 2.87 µM IAA respectively. Apical meristems showed more pronounced effect for shoot formation than vegetative buds. Maximum number of multiple shoots was obtained on MS medium containing 10 mg/L BAP. These multiple shoots increased in their number when were given subsequent incubation period. Addition of naphthalene acetic acid (NAA) and kinetin to BAP showed good shoot multiplication response. Shoots after attaining the size of 5.0 cm were shifted for rooting. Best rooting response was obtained on MS medium containing 10 mg/L NAA. Hardening response was the best in autoclaved sand. This study provided the rapid way of multiplication of this important ornamental plant.

Key words: Gerbera, micropropagation, multiple shoot formation, growth medium.

INTRODUCTION

Gerbera is a genus of ornamental plants from the sunflower family (Asteraceae). It has approximately 30 species in the world, extending to South America, Africa, Madagascar, and tropical Asia. Gerbera species bear a large capitulum with striking, two-lipped ray florets in yellow, orange, white, pink or red colors. Gerbera species are commonly grown as potted plants or for cut flowers and it is one of the top 10 traded cut flowers in the world. *Gerbera jamesonii* is also used in the preparation of traditional Chinese medicine tu-er-feng for curing cold and also for treating rheumatism (Ye et al., 1990). The demand for tissue cultured plants is increasing more as compared to conventional production of plants. In this genus, genetic variability is relatively limited, which makes it difficult to breed for new flower colors and patterns. There is also limited genetic material that can be used to improve resistance to biotic and abiotic stress (Orlikowska et al., 1999). Gerbera plants can be propagated either sexually or asexually. Until recently, they were usually propagated by dividing clumps. However, this technique is too slow for commercial purposes (Murashige et al., 1974). Nowadays, *in vitro* propagation is the preferred method (Aswath and Choudhary, 2002; Kumar et al., 2004; Prasanth and Sekar, 2004; Reynoird et al., 1993; Xi and Shi, 2003). Gerbera plants have been regenerated from calli derived from a variety of explants (Kumar and Kanwar, 2006; Modh et al*.,* 2002; Ray et al., 2005; Tyagi and Kothari, 2004; Zheng et al., 2002). Adventitious shoots have been regenerated from the flower buds of gerbera plants grown in the greenhouse (Laliberte et al*.,* 1985; Pierik et al., 1975). Attempts at regenerating shoots using midrib explants were not successful (Pierik and Segers, 1973). In a study on the *G. jamesonii* cultivar 'Vulkan', shoots were regenerated *in vitro* using leaf blade explants (Hedtrich, 1985).

*Corresponding author. E-mail: atariq@cdfa.ca.gov.

The potential advantages of plant tissue culture technique refers to growing plant cells, tissues and organ in an artificially prepared nutrient medium static or liquid, under aseptic conditions. By using this technique, better quality and disease resistant plants can be obtained. This technique is based upon the concept of totipotency which means single cell can give rise to whole plant by cell division and involves the evaluation of large number of genotypes by using very small space in the laboratory and reduction of time between two successive generations by controlling the environmental and nutritional conditions along with reduction of differences in morphology and stages of development. In recent years, this technique has gained greater momentum on commercial application in the field of plant biotechnology and floriculture.

The most successful and most widely used discipline of plant tissue culture technique is micrpopropagation which refers to the propagation of plants by using meristem tip culture which is the transfer of apical buds and surrounding leaf primordia to sterile culture conditions (Ali et al., 2004; Ioannov, 1990; Mangal et al., 2002; Naz et 2011; Villabobos, 1986).

With the realization of foreseen advantages and unprecedented applications, this technique has received great attention all over the world including Pakistan and India. Keeping in view all the above facts, this investigation was undertaken to optimize the culture conditions for micropropagation of Gerbera (*G. jamesonii*).

MATERIALS AND METHODS

Apical meristems and vegetative buds of different sizes were used as explants. Explants were obtained from pot grown plants from greenhouse of Department of Botany, Lahore College for Women University, Lahore, Pakistan. These were washed thoroughly with tap water and then with house hold detergent to remove all the traces of dust particles. The explants were then immersed in 20% aqueous solution of sodium hypochlorite for 20 min and were thoroughly washed. Sodium hypochlorite solution was decanted and explants were rinsed three times with autoclaved distilled water to remove all the traces of sterilant. While dealing with Gerbera, it was recognized that sterilization was very tedious and the above mentioned sterilization method was not satisfactory. So to get the explants free of contamination, the following method was opted: The explants were surface sterilized with the method described above. Then, these were shifted into a mixture of MS medium (100 ml) without sugar. From this MS (100 ml) mixture, 5% mixture was removed and replaced with 5% plant preservative mixture [PPM (5-chloro-2-methyl-3(2H)-isothiazolone + 2-methyl-3(2H)-isothiazolone + inert ingredients)]. Then, the flask (containing MS mixture + explants) was placed on a shaker for 3 h at 122 rpm at 37°C for removal of all types of contamination. Inoculation was carried out in laminar air flow cabinet. It was cleaned by scrubbing with 70% ethanol solution and was irradiated with UV radiations for 25 min before use.

MS medium (Murashige and Skoog, 1962) supplemented with different concentrations of auxin and cytokinin along with 3% sucrose was used. The pH of the medium was adjusted to 5.7±0.5. Phytagel (1.5 g/L) was used for solidification of media. The media were autoclaved at 121°C for 15 min at 15 lbs/in^2 pressure.

For shoot induction and proliferation, MS media containing different concentrations and combinations of auxins and cytokinins were used as mentioned in Tables 1and 2. For rooting, MS medium was supplemented with different concentrations of NAA. All the cultures were maintained under light intensity of 2500 to 3000 lux having temperature of 22±2.0°C and photoperiod was 16 h with 8 h dark period in every 24 h cycle. Sub-culturing was carried out after every 4 weeks interval.

For acclimatization of mericlones, different types of mixtures were used that is, sand, autoclaved sand, compost and coco peat in green house. A completely randomized design with five replicates of each treatment was used for the experiment. The data for each parameter were subjected to analysis of variance (ANOVA) using the COSTAT V.63: statistical software (Cohort Software, Berkely, California). The mean values were compared with the least significant difference test following Duncans new multiple range test at 5% level.

RESULTS

Shoot Induction

For shoot induction, MS medium was supplemented with different concentrations of BAP ranging from 1 to 10 mg/L. The best shoot induction response (90%) was obtained in MS medium containing 10 mg/L BAP. At this concentration, all the explants showed shoot induction response within 8 days of inoculation from apical meristem (Table 1; Figure 1b). With the increase in the concentration of BAP, the time taken for shoot induction was decreased while the rate of shoot induction, shoot length and number of shoots per explants was increased. The other medium used for shoot induction was BAP + IAA. Best shoot induction response (80%) was obtained in MS medium containing 8.8 µM BAP + 2.87 µM IAA within 12 days of inoculation from vegetative buds.

Multiplication

For multiplication of induced shoots, MS medium was supplemented with BAP alone or in combination with NAA and KIN (Table 2). It was observed that when BAP was used alone at 10 mg/L, the response of shoot multiplication was 90% with the highest number of shoots per explant (9) and shoot length (7 cm) while at 8.8 µM, the response in all parameters was less as shown in Table 2. Among the different concentrations of BAP + NAA, the best response (60%) was obtained in 8.68 µM BAP + 2.68 µM NAA. Different concentrations of BAP + KIN + NAA in mg/L were also used for multiplication and 55% response was obtained in 1.0 mg/L BAP + 1.0 mg/L KIN + 0.5 mg/L NAA (Table 2 and Figures 1c to f).

Table 1. Effect of different concentrations of auxins and cytokinins (BAP and BAP + IAA) on shoot induction through apical meristem and vegetative buds of Gerbera.

Cytokinin + Auxin	Shoot induction time (day)	% of shoot induction	Number of shoot/explant	Shoot length (cm)
BAP (mg/L)				
1.0	23±0.527^{a}	50	5±0.441^{b}	3±0.457^{b}
2.0	22±0.601^{ab}	60	6±0.500^{c}	4±0.441^{bc}
4.0	20±0.764^{b}	60	6±0.500^{c}	3±0.457^{b}
6.0	24±0.601^{a}	70	7±0.500^{bc}	4±0.441^{bc}
8.0	19±0.601^{b}	70	7±0.500^{bc}	2±0.457^{b}
10	8±0.799^{c}	90	9±0.897^{a}	4±0.441^{bc}
BAP + IAA (µM)				
1.0+1.0	22±0.601^{ab}	40	4±0.441^{bc}	2±0.457^{b}
2.0+1.5	21±0.601^{b}	50	5±0.441^{b}	3±0.457^{b}
2.2+2.0	22±0.601^{ab}	50	5±0.441^{b}	4±0.441^{bc}
4.4+2.5	24±0.601^{a}	60	6±0.500^{c}	4±0.441^{bc}
6.6+2.75	22±0.601^{ab}	70	7±0.500^{bc}	2±0.457^{b}
8.8+2.87	12±0.764^{c}	80	8± 0.489^{a}	3±0.457^{b}

All the values are the sum of means ± SD of five parallel replicates. Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test.

Rooting

Well developed *in vitro* mico shoots after attaining the size of 5.0 cm were shifted for rooting. For *in vitro* rooting, MS medium was supplemented with NAA ranging from 1.0 to 10 mg/L. It was observed that the best response (80%) for rooting was obtained when 10 mg/L of NAA was used (Figure 1 h). By decreasing the concentration of NAA, root induction response was decreased and in 1.0 mg/L of NAA, very poor results were obtained (Table 3). Best rooting response was obtained on MS medium containing 10 mg/L NAA. For acclimatization, different media were used that is, autoclaved sand, compost and coco peat. The best response of hardening was noticed in autoclaved sand.

DISCUSSION

This study is concerned with the micropropagation of gerbera *in vitro*. Different types of explants were used for optimization of the result. For micropropagation, the best response was noticed in apical meristem followed by vegetative buds. The aspect of study was to optimize the source of explants and media for micropropagation. In this regard, different types of explants were tried. Among the different explants, the best response was noticed in apical meristem followed by vegetative buds. When the medium was supplemented with different concentration of BAP for shoot induction, the highest response was obtained in MS medium supplemented with 10 mg/L BAP. Barbosa et al. (1994) and Posada et al. (1999) also used the 0.1 to 3.0 mg/L to get the best response. When vegetative buds cultured for shoot induction in MS basal medium was supplemented with different concentrations of IAA + BAP, the best response was in 8.8 µM BAP + 2.87 µM IAA. 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. Modh et al. (2002) and Zheng et al. (2002) studied proliferated mass of shoots from mature capitula with 0.1 mg/dm^3 IAA and 10 mg/ dm^3 BA in the medium (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).

For multiplication, BAP alone or in combination with NAA, KIN in MS medium was used. At 10 mg/l BAP 90% and at 8.8 µM BAP 75% response was obtained. MS medium was supplemented with different concentrations of BAP + NAA. Highest response is obtained in MS medium supplemented with 8.68 µM BAP + 2.68 µM NAA). MS medium was supplemented with different concentrations of BAP + KIN + NAA. The highest response was obtained in MS medium supplemented with 1.0 mg/L BAP + 1.0 mg/L KIN + 0.5 mg/L NAA).

In the case of apical meristem and vegetative buds, shoot induction response ranged from 8 to 23 days, frequency of shoot induction ranged from 50 to 90% in BAP and 40 to 80% in BAP + IAA, respectively. Rapid and early shoot induction was observed in MS supplemented with 10 mg/L BAP and 8.8 µM BAP + 2.87

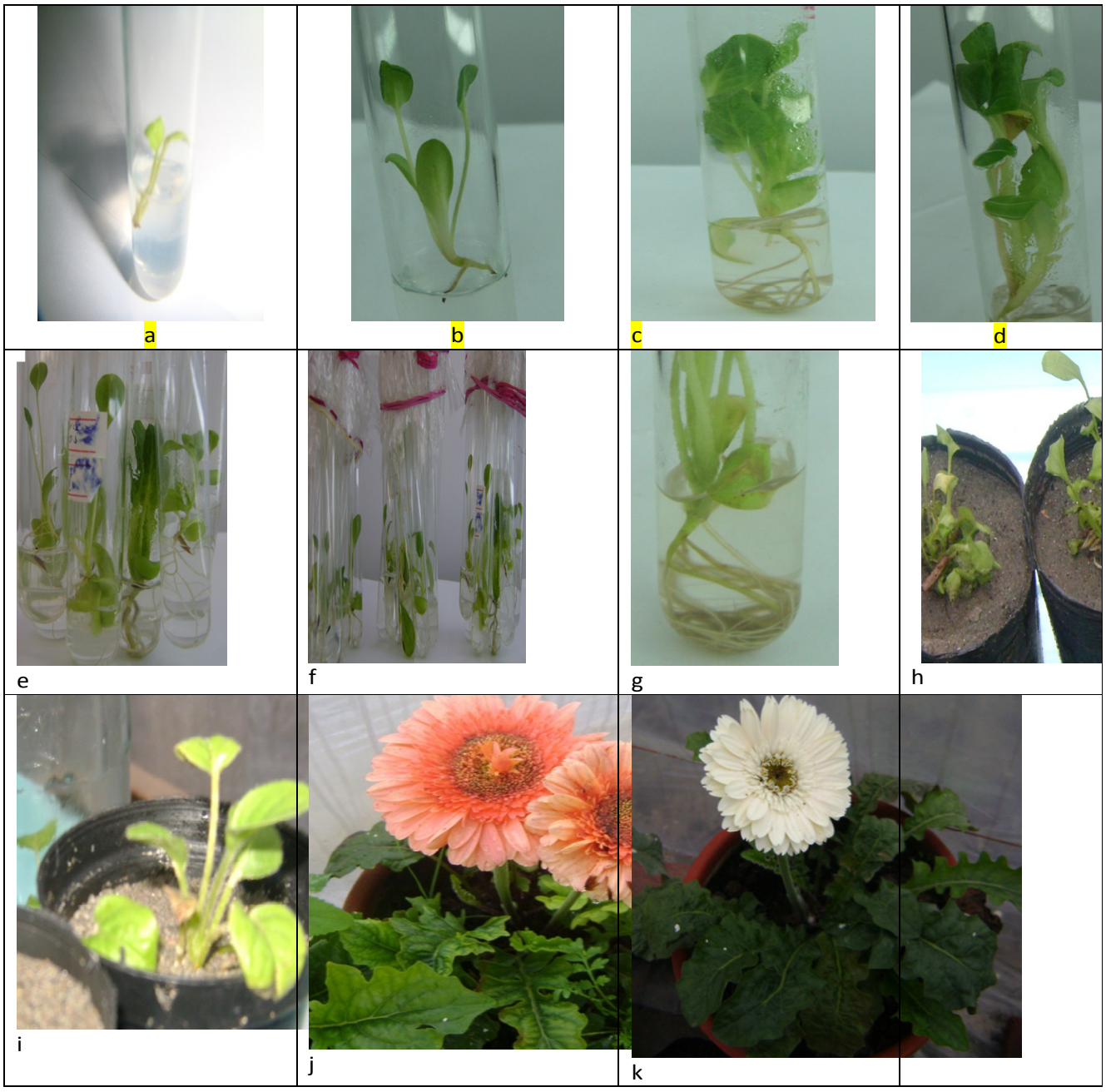

Figure 1. Different stages of micropropagation of Gerbera from different explants. a) Apical meristem as explants; b) shoot initiation on MS + 10 mg/l BAP; c to f) multiple shooting on different media; g) root initiation on NAA 10 mg/l; h and i) acclimatization in autoclaved sand; j and k) fully grown plants with beautiful flowers.

µM IAA. Gerbera shoots obtained from different combination of phytohormones were sub cultured in MS basal medium supplemented with different concentrations of BAP, BAP + NAA and BAP + KIN + NAA for further multiplication.

Gerbera shoots obtained from multiplication process were further cultured in MS basal medium supplemented with different concentrations of NAA for root induction. Rooting response ranged from 11 to 25 days of inoculation. Rapid and early rooting was observed in MS supplemented with 10 mg/L NAA. Pierik and Sprenkels (1984) cultured shoot tips of the shy-rooting gerbera cultivars Fleur and Florence in modified MS medium containing either 3 to 10 mg/dm^3 IAA or 1 or 3 mg/dm^3 NAA. The results obtained in this study could be highly significant. This efficient and reliable plant regeneration system via direct regeneration system can be exploited for improvement in quality and productivity through

Table 2. Effect of different concentrations of BAP, BAP + NAA and BAP + KIN + NAA on plant multiplication of Gerbera.

Auxin+cytokinin			% multiplication	Multiplication time (days)	Number of cultures showing multiplication	Number of shoot/explant	Shoot length (cm)
BAP	BAP+NAA	BAP+KIN+NAA					
mg/L							
1.0	-	-	55	22±0601^{ab}	3±0.457^{b}	3±0.457^{b}	12± 0.764^{c}
2.0	-	-	60	24±0.601^{a}	3±0.441^{cd}	6±0.500 ^{c}	11±0.624 ^{d}
4.0	-	-	70	23±0.527^{ab}	2±0.457^{d}	7±0.500 ^{bc}	10±0.697^{a}
6.0	-	-	75	21±0.601^{b}	4±0.441^{bc}	6±0.500 ^{c}	9±0.897 ^{ab}
8.0	-	-	86	18±0.601^{c}	5±0.441^{b}	7±0.500 ^{bc}	8±0.489 ^{a}
10	-	-	90	9±0.897^{d}	9±0.897^{a}	9±0.897 ^{ab}	7±0.500 ^{bc}
µM							
1.0	-	-	40	10±0.687^{a}	1±0.316^{c}	7±0.500 ^{bc}	11±0.624 ^{d}
2.0	-	-	45	9±0.897^{ab}	2±0.457^{bc}	4±0.441 ^{a}	10±0.697^{a}
4.0	-	-	45	7±0.500^{bc}	1±0.316^{c}	3±0.457^{b}	9±0.897 ^{ab}
6.0	-	-	50	6±0.500^{c}	2±0.457^{bc}	3±0.457 ^{b}	9±0.897 ^{ab}
8.0	-	-	65	10±0.697^{a}	3±0.457^{b}	6±0.500 ^{c}	8±0.489 ^{a}
8.8	-	-	75	5±0.441^{c}	5±0.441^{a}	7±0.500 ^{bc}	8±0.489 ^{a}
	µM						
-	1+1	-	30	17±0.578^{d}	1±0.316^{b}	5±0.441 ^{b}	11±0.624 ^{d}
-	2+1.5	-	35	18±0.601^{cd}	1±0.316^{b}	3±0.457 ^{b}	11±0.624 ^{d}
-	4+1.8	-	35	20±0.764^{bc}	1±0.316^{b}	4±0.441 ^{a}	10±0.697^{a}
-	6+2.4	-	40	21±0.601^{ab}	2±0.457^{b}	3±0.457 ^{b}	9±0.897 ^{ab}
-	8.2.5	-	40	23±0.527^{a}	2±0.457^{b}	5±0.441 ^{b}	8±0.489 ^{a}
-	8.68+2.68	-	60	13±0.441^{e}	4±0.441^{a}	6±0.500 ^{c}	7±0.500 ^{bc}
		mg/L					
-	-	0.5+0.5+0.5	25	22±0.601 ^{b}	2±0.457 ^{c}	3 ±0.457 ^{b}	12 ± 0.764 ^{c}
-	-	0.75+0.5+0.5	30	21±0.601 ^{b}	3±0.441 ^{bc}	6±0.500 ^{c}	12±0.764 ^{c}
-	-	1.0+0.5+0.5	45	28±0.897 ^{a}	4±0.441 ^{b}	5 ± 0.441 ^{b}	10±0.697^{a}
-	-	0.75+1.0+0.5	45	23±0.527 ^{b}	4±0.441 ^{b}	6 ± 0.500 ^{c}	9±0.897 ^{ab}
-	-	1.0+0.8+0.5	50	23±0.527 ^{b}	3±0.441 ^{bc}	6±0.500 ^{c}	9±0.897 ^{ab}
-	-	1.0+1.0+0.5	55	14±0.957 ^{c}	6±0.500 ^{a}	5 ± 0.441 ^{b}	7±0.500 ^{bc}

All the values are the sum of means ± SD of five parallel replicates. Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test.

Table 3. Effect of different concentrations of NAA on rooting of Gerbera.

Auxins percentage (NAA) (mg/L)	Number of shoots cultured	Duration of rooting (days)	Number of cultures showing rooting	Percentage of rooting
1.0	10	22±0.601 ^{b}	4±0.441^{bc}	40
2.0	10	22±0.601 ^{b}	5±0.441 ^{a}	50
4.0	10	25±0.667 ^{a}	5±0.441 ^{a}	50
6.0	10	20±0.764 ^{c}	6±0.500 ^{c}	60
8.0	10	19±0.601 ^{c}	7±0.500 ^{bc}	70
10	10	11±0.624^{d}	8± 0.489^{a}	80

All the values are the sum means ± SD of five parallel replicates. Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test.

genetic transformation and other cellular techniques.

REFERENCES

Ali A, Munawar A, Siddiqui FA (2004). *In Vitro* propagation of turmeric (*Curcuma longa* L.*).* Int. J. Biol. Biotechnol. 1: 511-518.

Aswath CR, Choudhary ML (2002). Rapid plant regeneration from *Gerbera jamesonii* Bolus callus cultures. Acta Bot. Croatica, 61: 125-134.

Barbosa MHP, Pinto JEBP, Pinto CABP, Innecco R (1994). *In vitro* propagation of *Gerbera jamesonii* Bolus ex Hook cv. 'Appelbloesem' using young capitulums. Revista Ceres, 41: 386-395.

Hedtrich CM (1985). Production of shoots from leaves and propagation of *Gerbera jamesonii*. Gartenbauwissenschaft. 44: 1-3.

Ioannov M (1990). Production of carnation plants by shoot tip culture *in vitro*. Tech. Bull Cyp. Agric Res. Inst. 117: 1-8.

Kumar S, Kanwar JK (2006). Regeneration ability of petiole, leaf and petal explants in gerbera cut flower cultured *in vitro*. Folia Hort. 18: 57-64.

Kumar S, Kanwar JK, Sharma DR (2004). *In vitro* regeneration of *Gerbera jamesonii* Bolus from Leaf and Petiole Explants. J. Plant Biochem. Biotechnol. 13: 73-75.

Laliberte, S, Chretien I, Veith J (1985). *In vitro* plantlet production from young capitulum explants of *Gerbera jamesonii*. Hort. Sci. 20: 137-139.

Mangal M, Bhardwaj SV, Kaur DR, Mangal AK (2002). Use of meristem tip culture to eliminate carnation latent virus from carnation plant. Ind. J. Exp. Biol. 40: 119-122.

Modh FK, Dhaduk BK, Shah RR (2002). Factors affecting micropropagation of gerbera from capitulum explants. J. Ornament. Hort. 5: 4-6.

Murashige T, Sepra M, Jones JB (1974). Clonal multiplication of gerbera through tissue culture. Hort. Sci. 9: 175-180.

Murashige I, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant 15: 473-487.

Naz S, Tabassum F , Javad S,Ilyas S, Aslam F, Munir N, Ali A (2011)micropropagation and collaboration of a recalcitrant species Richus communis. pakistan J. Bot. 43:2419-2422.

Orlikowska T, Nowak E, Marasek A, Kucharska D (1999). Effects of growth regulators and incubation period on *in vitro* regeneration of adventitious shoots from gerbera petiole. Plant Cell Tissue Organ Cult., 59: 95-102.

Pierik RLM, Sprenkels PA (1984). Improvement of the rooting of gerbera *in vitro* by NAA. Vakblad voor de Bloemisterij, 39: p. 45.

Pierik RLM, Jansen JLM, Maasdam A, Binnendijk CM (1975). Optimization of gerbera plantlet production from excised capitulum explants. Sci. Hortic. 3: 351-357.

Pierik RLM, Segers TA (1973). *In vitro* culture of midrib explants of gerbera: Adventitious root formation and callus induction. Zeit Pflanzenphysiol. 69: 204-212.

Posada M, Ballesteros N, Obando W, Angarita A (1999). Micropropagation of gerbera from floral buds. Acta Hortic. 482: 329-332.

Prasanth M, Sekar K (2004). Studies on age of explant on callus induction in gerbera cv. Mammut. Sci. Hort. 9: 207-211.

Ray T, Saha P, Roy SC (2005). *In vitro* plant regeneration from young capitulum explants of *Gerbera jamesonii*. Plant Cell Biotechnol. Mol. Biol. 6: 35-40.

Reynoird JP, Chriqui D, Noin M, Brown S, Marie D (1993). Plant propagation from *in vitro* leaf culture of several gerbera species. Plant Cell Tissue Organ Cult. 33: 203-210.

Thakur PS, Ghorade RB, Rathod TH (2004). Micropropagation studies in gerbera. Ann. Plant Physiol. 18: 133-135.

Tyagi P, Kothari SL (2004). Rapid *in vitro* regeneration of *Gerbera jamesonii* (H. Bolus ex Hook. f.) from different explants. Indian J. Biotechnol. 3: 584-588.

Villabobs AVM (1986). Obtaining virus-free carnation by *in vitro* culture of meristems and apices. Prodeecings of the tropical region. Am. Soc. Hort. 616: 227-230.

Xi M, Shi JS (2003). Tissue culture and rapid propagation of *Gerbera jamesonii*. J. Wanjing Forest. Univ. 27: 33-36.

Ye JN, Wang SQ, Cai K, Komatsu M, Mikage T, Namba T (1990). Pharmacological studies on folk medicine in Sichuan province, China .II. On Tu-er-fang derived from *Gerbera* plants. J. Pharm. Soc. Jpn. 110: 374-382.

Zheng XF, J.H. Wang and M.Y. Li. 2002. Factors affecting organogenesis in *Gerbera jamesonii* Bolus cultures *in vitro*. J. Jiangsul. Sci. Technol. 29: 29-31.