Original Research Article

Field ~~p~~Performance and ~~d~~Differential ~~r~~Response of ~~m~~Micro-propagated ~~p~~Potential F_1 ~~g~~Genotypes of *Gerbera jamesonii*

ABSTRACT

Aims: To evaluate gerbera hybrids with respect to mass propagation under *in vitro* conditions and their characterization under field conditions for floral traits for selection of promising gerbera lines for commercial utilization.

Study design: Field performance and differential response of micro-propagated gerbera were evaluated in randomized block design.

Place and Duration of Study: CSIR-IHBT, Palampur (HP), India 2012-2015.

Methodology: F_1 genotypes of gerbera were developed through controlled crossing program and subjected to *in vitro* micro-propagation to achieve quick multiplication. Mature seeds obtained from different cross combinations were used for the establishment of *in vitro* gerbera cultures. Seeds were cultured on Murashige & Skoog (MS) basal medium and the developing micro-shoots from seeds were further cultured on MS media supplemented with different doses of growth regulators to achieve shoot proliferation.

Results: Highest number of micro-shoots, number of leaves and leaf length were observed in M3 proliferation medium (MS supplemented with 1mg/l 6- Benzylaminopurine + 0.030 mg/l Indole Butyric Acid (IBA) + 0.025 mg/l Naphthalene Acetic acid) which gave best proliferation among the gerbera genotypes. The half strength MS medium supplemented with 0.4mg/l IBA was found best for *in vitro* rooting. Also, there was differential response of hybrid gerbera genotypes to micro-shoot proliferation.

Conclusion: Characterization of two hybrid F_1 genotypes (23-1 and E-3) under field conditions with respect to floral traits in comparison to parental genotypes provided information about new gerbera lines with high micro-propagation potential for commercial utilization.

Keywords: Gerbera jamesonii, in vitro proliferation, root initiation, 6- Benzylaminopurine, IBA, NAA

1. INTRODUCTION

Gerbera (*Gerbera jamesonii*) is an important commercial cut flower of the trade having extensive demand throughout the world due to its numerous colors, shapes and prolonged vase life. The plant is ideal for beds, borders, pots and rock gardens. It has a great export potential and ranks fifth among top cut flowers in the international market [1]. There is an increasing demand of quality gerbera cut flowers, planting material and new types of varieties for export as well as in domestic markets. Gerbera is out crossing in nature and the possibility of genotype fixation by clonal propagation unfolds a large potential for novel cultivar development. New cultivars of gerbera are developed by hybridization of diverse types to achieve genotypic recombination, which requires information about the genetic diversity among the parental genotypes, especially in order to create novel genotypes based on combination of desirable traits that can be easily fixed by vegetative propagation. In this context, with an aim of varietal development, selective breeding of gerbera (for desirable flower colors and complete double types) is being done under an ongoing gerbera breeding program at CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal

Pradesh, India [2,3]. The parents were selected based on prior information with respect to variations for flower color [4], shape (double, semi-double and single), size, and good performance under protected cultivation.

Although conventional vegetative propagation of gerbera, by division of suckers is comparatively easy to perform, only limited plants can be developed from one plant and it takes 10–12 months to attain the next cycle of division [5]. Floriculture industry requires about nine plants of gerbera per square meter for clonal plantation under protected cultivation and a huge number of clonal plants are required every year when gerbera cultivation is to be done on commercial scale. Under such a situation it is difficult to produce large number of clonal plants of a promising genotype within a short period of time through vegetative propagation.

In order to meet the industrial demand, we need micro-propagation for rapid and mass multiplication of elite gerbera genotypes under *in vitro* conditions. However, response of different genotypes and the genetic control of component traits constitute crucial factors for the success of mass propagation program. At least two component traits, viz., proliferation rate and *in vitro* root induction possibly influence the response of a genotype to mass propagation. Therefore, the present study was undertaken to evaluate gerbera hybrids with respect to mass propagation under *in vitro* conditions and their characterization under field conditions for floral traits in comparison to parental genotypes for selection of promising gerbera lines for commercial utilization.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

To evaluate response of F_1 gerbera genotypes to micro-propagation, seeds obtained through the crossing program were used as explants for establishing cultures for *in vitro* clonal multiplication. Twelve F_1 genotypes (23-1, 24-1, 24-2, 24-3, 24-4, A-6, B-2, D-3, E-1, E-2, E-3 and Y-1) generated in this study were evaluated for *in vitro* response and characterized for floral characteristics under poly-house conditions in the present study (Table 1).

Table 1. List of gerbera genotypes involved in the study

S.No.	Genotypes	Remarks	Shape	Colour
1.	IHBT-Gr-01 (P1)	Parent	Single	White (RHS 155 C)
2.	IHBT-Gr-02 (P2)	Parent	Semi-Double	Red-Purple (RHS 58 D)
3.	IHBT-Gr-03 (P3)	Parent	Double	Yellow-Orange(RHS158 C)
4.	IHBT-Gr-04 (P4)	Parent	Semi-Double	Orange-Red (RHS 40 A)
5.	IHBT-Gr-05 (P5)	Parent	Single	Red-Purple (RHS 66 B)
6.	IHBT-Gr-06 (P6)	Germplasm	Single	Red-Purple (RHS 58 D)
7.	IHBT-Gr-07 (P7)	Parent	Single	Red (RHS 52 C)
8.	23-1 (P2 x P3)	Hybrid F_1	Double	Yellow-Orange (RHS 16 C)
9.	24-1 (P3 x P4)	Hybrid F_1	Semi-Double	Orange

10.	24-2 (P3 x P4)	Hybrid F_1	Single	Orange-Red
11.	24-3 (P3 x P4)	Hybrid F_1	Double	Orange-Red
12.	24-4 (P3 x P4)	Hybrid F_1	Semi-Double	Yellow-Orange
13.	Y1 (P3 x P7)	Hybrid F_1*	Double	Yellow
14.	E-1 (P1 x P7)	Hybrid F_1*	Double	White
15.	E-2 (P1 x P7)	Hybrid F_1	Single	White
16.	E-3 (P1 x P7)	Hybrid F_1	Double	White (RHS 155 D)
17.	A-6 (E1 x Y1)	Hybrid F_1	Double	Yellow
18.	B-2 (P1 x P5)	Hybrid F_1	Single	Red
19.	D-3 (P4 x P2)	Hybrid F_1	Semi-Double	Orange-Red

*used as parents in breeding program

Seeds were washed with few drops of Tween-20 to remove the surface dust particles with constant shaking for 5-6 minutes and washed thoroughly with distilled water (6-7 times). The seeds were surface sterilized with 0.1% mercuric chloride solution for 2 minutes under aseptic condition in laminar air flow cabinet and rinsed 4-5 times with autoclave distilled water. The seeds were cultured aseptically on basal MS medium [6]. The pH of the medium was adjusted to 5.8 with 1N NaOH and agar (8g/l) was used for solidification of medium. The media were dispensed into tissue culture flask and each contained 100ml of culture medium. The flasks were autoclaved at 121℃ at 15 psi for 2 0 minutes and then allowed to cool at room temperature before culturing the seeds. Cultures were given a 16 h photoperiod with light intensity of 37.40 mmol-2 s-1 which was maintained by using photosynthetically active radiation (PAR) light sources and fluorescent tubes. Temperature in the culture room was maintained at 25 ± 2℃.

After germination, the apical meristems of seedlings were transferred to shoot proliferation medium supplemented with 6- Benzylaminopurine at higher levels (BAP - 1.0 mg/l) than Indole 3- butyric acid (IBA - 0.030 mg/l) and Naphthalene acetic acid (NAA - 0.025 mg/l) along with 30g/l sucrose for initiating micro-shoots in hybrid gerbera genotypes. When multiple shoots are formed in the cultures, individual shoots are separated using fine scalpel under laminar air flow and subcultures done at regular intervals of four weeks on shoot proliferation medium to attain rapid *in vitro* multiplication. To study the effect of different concentrations of BAP (0.5 (M1), 0.75 (M2), 1.0 (M3), 1.5 (M4) mg/l) and fixed concentrations of IBA (0.030 mg/l) and NAA (0.025 mg/l) an experiment was laid out employing completely randomized design with five explants per flask per treatment and replicated thrice (5 explants x 4 treatments x 3 replications = 60 explants per genotype). Data were recorded for number of micro-shoots per plant, leaf number and leaf length in proliferation medium.

To initiate rooting, shoots were transferred to rooting medium with half strength of MS salts, supplemented with varying concentration of IBA (0.1, 0.2 and 0.4 mg/l), 15 g/l sucrose and 6g/l agar were used. In case of rooting medium, days taken for root initiation were recorded. Rooted plantlets of gerbera were removed from culture flasks and washed thoroughly with

running tap water to remove agar. The plantlets were then transferred to plastic trays containing sand and covered with plastic bag to maintain humidity. After 3 weeks of acclimatization they were transplanted in sleeves for further cultivation in soil.

Data were analyzed using F-test to evaluate the response of different hybrid gerbera genotypes under proliferation medium. The data regarding different proliferation media and rooting media were analyzed using Student's t-test. All tissue culture experiments were laid out in a Randomized Block Design and the significance of differences between means was estimated at 5% level of significance.

In the field experiment conducted during 2013-14 and 2014-15, two hybrid genotypes of gerbera 23-1 and E-3 which performed well under *in vitro* conditions were evaluated along with parental genotypes IHBT-Gr1, IHBT-Gr2, IHBT-Gr3 and IHBT-Gr7. All the six genotypes were planted polyhouse in randomized block design with four replications and data were recorded for different agro-morphological and floral traits viz., leaf number per plant, plant spread (cm), number of suckers per plant, leaf length (cm), leaf width (cm), flower color, flower shape, disc color, flower number per plant, scape length (cm) and flower diameter (cm). Data were collected on five flowers of each genotype in each replication. All the data were recorded at the commercial stage of flower harvest; the stage at which the stigmata of the ray florets have been exerted [7]. Flower color of selected gerbera genotypes along with parental lines was compared using the Royal Horticultural Society's (RHS) color chart [8]. Flower shape (single - with whorls of only ray florets, semi-double - with separate whorls of ray florets and smaller-sized transition florets and complete double with ray florets covering the entire disc) [9] and disc color (light green / dark brown) were observed on visual basis. Data with respect to quantitative traits leaf number per plant, plant spread (cm), number of suckers per plant and flower number per plant were recorded on yearly basis on five random but competitive plants of each genotype while leaf length (cm), leaf width (cm), scape length (cm) and flower diameter (cm) were recorded on five mature leaves and five flowers of each genotype. The data over two years was studied separately to assess overall performance of the gerbera genotypes. Analysis of variance was conducted to work out significance of variability for different agro-morphological and floral traits.

3. RESULTS AND DISCUSSION

In the tissue culture experiments, initially the seedlings raised on MS basal medium of twelve hybrid genotypes viz. 23-1, 24-1, 24-2, 24-3, 24-4, A-6, B-2,D-3,E-1, E-2, E-3 and Y-1 when cultured on proliferation medium supplemented with BAP (1.0 mg/l) + IBA (0.030 mg/l) and NAA (0.025 mg/l) gave a differential response with respect to micro-shoot formation during first three sub-cultures. Of these, only five genotypes (23-1, 24-4, A-6, E-3 and D-3) responded well in the proliferation medium and taken forward for experimentation to evaluate their proliferation potential under varying concentrations of BAP and different concentrations of IBA for rooting (Fig.~~ure~~ 1). Since sufficient micro-shoot proliferation in other genotypes was not achieved to the same extent in the first three sub-cultures, they were excluded from the experiment so as to homogenize the experimental data set of 60 explants per genotype. The data on effect of concentrations of BAP on micro-shoot multiplication recorded after one month of culture is given in Table 2. The maximum number of micro-shoots (12.73), leaves (44.7) and leaf length (3.48 cm) were produced in the medium M3 containing 1 mg/l BAP+ 0.030 mg/l IBA+ 0.025mg/l NAA followed by M2 medium with 0.75mg/l BAP+ 0.030 mg/l IBA+ 0.025mg/l NAA.

Fig.~~ure~~ 1. Differential response of different gerbera genotypes to *in vitro* proliferation (genotypes from left to right 24-4, 23-1, E-3, D-3 and A6)

Table~~:~~ 2. Effect of BAP on number of micro-shoots, number of leaves and largest leaf length in five different genotypes of gerbera

Media	Number of micro-shoots per explant						Number of leaves per explant						Largest leaf length (cm)					
	E-3	23-1	24-4	D-3	A-6	Mean	E-3	23-1	24-4	D-3	A-6	Mean	E-3	23-1	24-4	D-3	A-6	Mean
M1	9.0	10.3	5.0	5.4	4.8	6.9	31.7	31.8	21.5	23.9	18.9	25.5	2.1	2.6	2.2	2.9	2.1	2.4
M2	10.4	10.6	5.2	5.7	5.1	7.4	32.8	33.3	23.4	25.1	21.1	27.1	2.1	2.7	2.4	3.0	2.3	2.5
M3	11.6	12.7	5.6	5.9	5.4	8.2	44.7	41.5	30.0	32.7	22.8	34.4	2.2	2.9	2.3	3.4	2.2	2.6
M4	11.2	11.5	5.1	5.6	4.9	7.6	34.2	34.4	22.4	28.9	19.5	27.8	2.2	2.8	2.2	3.2	2.2	2.5
Mean	10.5	11.3	5.2	5.6	5.0		35.8	35.2	24.3	27.6	20.5		2.2	2.8	2.3	3.1	2.2	
CD	1.86	1.70	0.41	0.31	0.42		9.53	6.84	6.14	6.34	2.78		0.12	0.20	0.14	0.41	0.14	

Currently, *in vitro* propagation is the preferred method for quick multiplication of the planting material [10, 11, 12, 13, 14]. Gerbera plants have been regenerated from calli derived from a variety of explants [15, 16, 17, 18, 19]. Adventitious shoots have also been regenerated from the flower buds of Gerbera plants grown in the greenhouse [20, 21]. However, in this study, seeds were available which were developed through crossing under an ongoing gerbera breeding program and used for *in vitro* propagation. Accordingly, standardization of culture medium with optimum concentration of growth regulators is critical for evaluating micro-propagation response of the different genotypes involved in the study.

To further understand the response of potential gerbera genotypes, data generated as per randomized block design in tissue culture experiment were analyzed and maximum micro-shoot formation was observed in 23-1 followed by E-3 (Table 2), whereas more leaves were formed in E-3 as compared to 23-1 (Fig.~~ure~~ 2). Analysis of variance of the shoot proliferation data indicates significant genotypic variations (Table 3). Overall, among the five gerbera genotypes evaluated for proliferation potential, 23-1 and E-3 have significantly better response than other genotypes under *in vitro* conditions. The present investigation suggests that number of micro-shoots leaf number and leaf size are independent traits which are affected by media conditions and contribute to overall shoot proliferation in gerberas. Considerable variations were obtained for shoot proliferation parameters indicating genotypic specificity of gerbera accessions to these component traits of *in vitro* propagation. Genotypic response to *in vitro* propagation has also been reported in other plant species such as sugarcane [22] and *Phoenix dactylifera* [23].

Fig.~~ure~~ 2. Growth of gerbera genotypes 23-1 and E-3 (left and right, respectively) after one month in proliferation medium

Table 3 ANOVA test for the differences in micro-shoot formation per explant, number of leaves per explant and leaf size among five different genotypes of gerbera

S. No.	Source of variaiton	Variance	F- value
1.	micro-shoot formation per explant	38.22	136.12*
2.	number of leaves per explant	180.27	53.06*
3.	leaf size (cm)	0.70	58.13*

*Significant at *P* = .05

Based on Student's t-test, media M3 and RM3 were significantly better from the other media for shoot proliferation and root induction in gerbera. Different combinations of cytokinin and auxins have been used to achieve multiple shoot induction in Gerbera [24, 25, 26]. In the

present studies, among the different concentrations of BAP in combination with IBA and NAA, the MS medium supplemented with 1 mg/l BAP was found to be best medium as compared to others because it results in maximum number of shoots and shoot length, whereas high concentration of BAP (1.5 mg/l) induced negative effect on plant growth (Fig.~~ure~~ 3). These results are in conformity with that of Mariam *et al*. in 2010 [27] who obtained highest shoot number in gerbera with 1mg/l BAP. Highest multiplication rates [28] were observed in gerbera with 3 mg/l BAP which are contrary to the present finding. Genotypic specificity to growth regulators may possibly be the reason for such large variations in response to concentrations of growth regulators. The gerbera genotypes which did not respond well under initial tissue culture experiment need to be evaluated for higher doses of BAP so as to ascertain their growth regulator requirements for effective micro-propagation. There are reports [29, 30] that the cultivars varied widely in their growth regulator requirements at multiplication phase. In the present study, among different genotypes evaluated for micro-propagation, genotypes 23-1 and E-3 were observed best because micro-propagation rate was found to be high in these two genotypes.

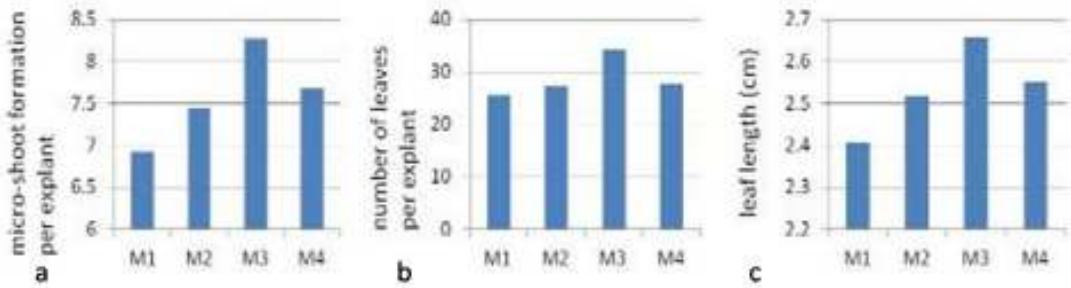

Fig.~~ure~~ 3 Comparison of proliferation media (M1-M4) for (a) micro-shoot formation per explant, (b) number of leaves per explant and (c) leaf size (cm) in gerbera

Rooting was obtained in all the root induction media, but the main difference was time taken for root initiation. Earliest response for root initiation was observed in RM3 medium (Fig.~~ure~~ 4). IBA plays important role in root induction in tissue culture plants. IBA has been reported as the best medium for root induction in Gerbera [19]. The root initiation and root development were better in MS medium with higher concentration of IBA as compared to lower concentration in all the five varieties used in the present study. 100% rooting in red yellow and white varieties was obtained the cultured on half MS containing 0.2 mg/l IBA within 15-20 days of root initiation [31]. Successful rooting was obtained from *in vitro* cultured gerbera shoots in MS medium containing 1.5mg/l IBA [27]. The results obtained in our study confirm the earlier reports. However, response to root initiation in rooting media was observed to be at par among the gerbera genotypes. A large number of rooted plantlets of gerbera were successfully hardened by removing them from culture flasks, washed thoroughly under running tap water to remove agar and then transferred to plastic trays containing sand and covered with plastic bag to maintain humidity. After 2-3 weeks of acclimatization they were transplanted in sleeves for further cultivation in soil (Fig.~~ure~~ 5). The new gerbera genotypes were characterized with respect to floral traits and showed high to moderate growth response to *in vitro* proliferation as compared to other gerbera genotypes and have good potential for commercialization (Fig.~~ure~~ 6).

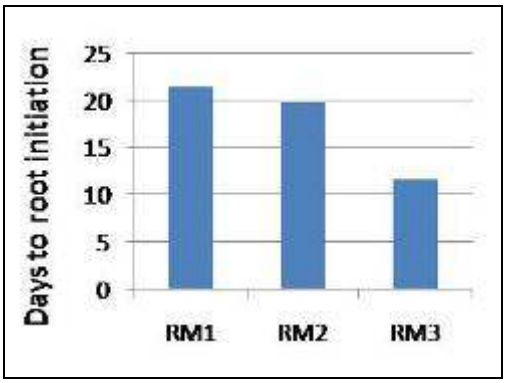

Fig.~~ure~~ 4 Mean differences of rooting media (RM1-RM3) for days to root initiation in gerbera

Fig.~~ure~~ 5. (a) Root initiation in gerbera cultures, (b) and (c) hardening of rooted plantlets in trays and (d) transfer of plantlets in sleeves in hardening chamber

Fig.~~ure~~ 6. Potential gerbera genotypes 23-1 (left) and E-3 (right) with good field performance and high micro-propagation rate

The floral description and agro-morphological performance of genotypes is mentioned in Tables 1 and 4. Significant variability was observed among the F_1 genotypes 23-1 and E-3 as compared to parental lines Gr1, Gr2, Gr3 and Gr7 for the agro-morphological and floral traits. The extent of variability based on analysis of variance estimates for different traits (Table 5) suggests that there is wide scope for improvement of agro-morphological as well as floral traits in gerbera through a breeding program. Data generated for quantitative traits

over two years were analyzed separately and not pooled because *Gerbera jamesonii* is a perennial plant species and the observations made were not repetition but continuation of the same experiment. Significant genotypic variations were recorded for the agro-morphological and floral traits although variations among the replicates were not significant which is expected considering that the plants of each genotype have clonal origin and the experiment was undertaken under controlled poly-house conditions. Based on mean performance of hybrid gerbera genotypes with the respective parents, E-3 was superior to at least one parental genotype for traits plant spread in a year, leaf length (cm) and leaf width (cm), while it was at par to parental genotypes for the other traits. In case of 23-1, the genotype was superior to at least one parental genotype for leaf width (cm), number of flowers per plant in a year and scape length (cm). Comparison of data for the two years suggests that some of the traits tend to stabilize over the years (leaf number per plant/year, leaf length, leaf width, flower number per plant/year and flower diameter), while others become more variable (plant spread, number of suckers per plant/year and scape length). As is evident from the data, characters such as plant spread, number of suckers per plant/year and scape length are associated with overall growth of the plants and may not completely express during first year of plant growth.

Table 4. Mean performance of gerbera genotypes over two years

S. No.	Source of variation	IHBT-Gr-01		IHBT-Gr-07		E-3	
		2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
1.	Leaf number per plant/year	31.25	33.15	32.05	33.05	29.3	31.85
2.	Plant spread (cm)/year	57.75	58.45	48.95	49.45	63.25*	63.8*
3.	No. of suckers / plant/year	2.45	4.45	2.7	6.25	2.75	5.7
4.	Leaf length (cm)	32.55	32.60	23.0	23.75	34.25*	33.425*
5.	Leaf width (cm)	11.55	11.3	7.17	7.075	12.87*	11.975*
6.	Flower no. per plant/year	21.15	22.95	17.6	19.55	18.8	21.8
7.	Scape length (cm)	34.6	33.55	50.05	49.05	39.3	40.75
8.	Flower diameter (cm)	8.22	8.25	7.55	7.775	8.05	8.225

*Significant at *P* = .05

S. No.	Source of variation	IHBT-Gr-02		IHBT-Gr-03		23-1	
		2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
1.	Leaf number per plant/year	20.85	23.7	22.75	24.45	25.4	28.65
2.	Plant spread (cm)/year	66.05	66.55	66.25	66.8	65.85	66.55
3.	No. of suckers / plant/year	2.3	4.5	2.45	5.15	2.8	5.75
4.	Leaf length (cm)	37.2	37.31	36.35	35.68	38	37.46

5.	Leaf width (cm)	12.17	11.67	14.67	14.6	16.2*	15.22*
6.	Flower no. per plant/year	13.95	16.35	12.75	14.75	23.5*	23.4*
7.	Scape length (cm)	32.8	31.95	32.6	31.5	45.4*	44.75*
8.	Flower diameter (cm)	9.65	9.72	9.35	9.55	10.85	10.75

*Significant at *P* = .05

Table 5. Analysis of variance for quantitative traits among new gerbera hybrid genotypes and their parental lines during 2013-14 and 2014-15 under poly-house conditions

S. No.	Source of variation	2013-14		2014-15	
		Variance	F- value	Variance	F- value
1.	Leaf no. per plant/year	85.81	367.09*	72.43	185.25*
2.	Plant spread (cm)/year	189.34	472.31*	190.21	498.80*
3.	No. of suckers/plant/year	0.16	5.66*	2.12	21.68*
4.	Leaf length (cm)	123.00	421.41*	104.66	471.24*
5.	Leaf width (cm)	38.32	388.85*	33.60	306.44*
6.	Flower no. /plant/year	67.94	323.36*	51.48	137.89*
7.	Scape length (cm)	209.45	750.73*	217.42	497.28*
8.	Flower diameter (cm)	6.04	97.88*	5.23	62.73*

*Significant at *P* = .05

4. CONCLUSION

On the basis of the present study a suitable protocol for shoot regeneration, multiplication and root induction has been identified for mass multiplication of gerbera plants. The MS medium supplemented with 1mg/l BAP + 0.030mg/l IBA + 0.025mg/l NAA produced maximum micro-shoots, leaf number and leaf length. Half strength MS medium with 0.4mg/l IBA was observed to be the best medium for early root initiation of *in vitro* raised micro-shoots of gerbera. The protocol can be utilized for mass multiplication of new gerbera genotypes on a commercial scale. Characterization of new hybrid F_1 genotypes of gerbera in comparison to parental genotypes under field conditions suggests the scope of improvement of gerbera for desirable floral traits such as double flower shape, standard size of flowers and various colours through breeding program which will result in new varietal types for cultivation in future.

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