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HYBRIDIZATION OF DIFFERENT GERBERA (*Gerbera jamesonii* bolus ex hooker f.) LINES FOR BREEDING NOVEL TRAITS

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ABSTRACT
Half sib mating was used where five genotypes were used as female parent, fifteen genotypes were used as male parent and the investigation was carried out at the Division of Ornamental Crops, ICAR-IIHR, Bengaluru, to study hybridization of different parents. IIHR 3 showed higher seed weight (163.73 mg), number of seeds per cross (69.60) and seed set percentage (69.86%). It can be concluded that the flowers with large flower head diameter (14.00 cm) and optimum length of flower stalk (55 cm) with visible disc florets can be taken as female parent for breeding programme. Gerbera hybrid seeds thus obtained were raised in half strength MS medium. The response of *in vitro* leaf explants to callusing and regeneration was recorded when cultured on full strength Murashige and Skoog's (1962) media supplemented with different concentrations of 2,4-D or BAP or IAA and Kinetin. Leaf explants cultured on MS medium fortified with equal concentration of BA (1 mg l^{-1}) and 2, 4-D (1 mg l^{-1}) produced green and greenish white granular callus within 25 days. But leaf explants cultured on MS medium supplemented with 2, 4-D produced yellowish callus. Plant regeneration was also found the earliest in the same media (14 days).

INTRODUCTION

The urgent need of floriculture industry especially in gerbera is the breeding of the local quality flower with biotic and abiotic resistance. The present varieties available in India are all imported by paying huge royalties, more over they are highly sensitive to biotic and abiotic stress. Hence, to meet out the international quality standards, high yielding and long lasting hybrid cultivars have to be developed which can be grown inside the polyhouse. Gerbera (*Gerbera jamesonii* Bolus ex. Hooker f.) belongs to the family Asteraceae produces very attractive flower. It is a dwarf perennial plant, used in many ways such as cut flower, pot plant and makes a good show in exhibitions on floral arrangements (Panj *et al.*, 2014)

Sorge *et al*. (2004) had collection of gerbera which had parents and four generations of progeny representing a wide range of single and crested cultivars and some spider cultivars. The pollination was done by hand and seeds were collected at maturity and stored in the refrigerator at 4ºC and later genetic analysis was done.

In recent years, most of the varieties are multiplied through tissue culture. There has been increasing interest in tissue cultures as an alternative to asexual propagation of gerbera. This method enables a million fold expansions per year of a desired plant. Axillary shoot formation from excised capitulum explants or from shoot tips (Murashige *et al.*, 1974) have been reported. Then it is possible to induce adventitious shoot formation on isolated young leaves derived from earlier developed axillary shoots. Plants regenerated from callus and adventitious shoots are required in mutation breeding as a tool for the production of solid mutants (Can *et al.*, 2008).

The purpose of the present investigation was to develop a new variety of gerbera through half sib mating and to further study the response of *in vitro* derived leaf explants to callusing and regeneration.

MATERIALS AND METHODS

Details of treatments

Twenty different lines of gerbera *viz.,* IIHR-1 to IIHR-20 were used for hybridisation. Half sib method was used, where five genotypes IIHR-1, IIHR-2, IIHR-3, IIHR-4, IIHR-5 were used as female parents, while mixed pollen of remaining 15 genotypes were used as male parent. For each cross five capitulums were used. The details of female parents used for crossing and their characters are presented in Table 1.

Hybridisation: The flower heads were pollinated in the morning between 10 am to 12 noon. The female flower (ray florets) was selected based on the receptivity of the stigma. When the stigma shows 'Y' shape exerted stigma, then the flowers were ready for pollination. The pollens were collected from different parents in a petri-dish. Using camel brush, the pollens were smeared individually on the stigma. This procedure was repeated 2-3 times on the same flower in the following day. The flowers were tagged with the details of the female parent and date of pollination.

The following observations were recorded: days taken for seed set, seed weight, number of chaffy seeds. The experiment was conducted in polyhouse with 50% shade net and the data was statistically analysed by following completely randomised design.

The seeds obtained from the individual cross were surface

sterilised, soaked in distilled water for 30 minutes with the addition of 1-2 drops of Tween-20 followed by 40% sodium chloride solution and gently agitated. Half MS medium (Murashige and Skoog, 1962) was used for inoculation of seeds. The following observations were recorded were as follows days taken for germination, days taken for first leaf emergence, days taken for rooting, number of leaves after 60 days, number of roots after 60 days, shoot length after 60 days. Individual *in vitro* plants were raised under well defined culture room conditions. Individual seeds from the cross were considered as a single line and hence basic statistical measures such as mean was applied. *In vitro* leaf explants from fourteen hybrid lines were used. The MS basal medium was used at full strength. Each basal medium was supplemented with different concentration of growth regulators in five treatments.

T_1 - MS and BA (1 mg l^{-1})

T_2 - MS and BA (1 mg l^{-1}) and IAA (1 mg l^{-1})

T_3 - MS and BA (1 mg l^{-1}) and 2,4-D (1 mg l^{-1})

T_4 - MS and Kinetin (5 mg l^{-1}) and IAA (1 mg l^{-1}) and 2,4-D (1 mg l^{-1})

T_5 - MS and BA (5 mg l^{-1}) and Kinetin (5 mg l^{-1}) and 2,4-D (1 mg l^{-1})

Completely randomized design was followed with five replications. Observations were recorded on days taken for callus initiation, callus production, days to develop plantlets from the callus, days to form shoots and roots per callus clump.

RESULTS AND DISCUSSION

The salient characters of the female parents were recorded as presented in Table 1. All the five flowers except one were double with two or three whorls of ray florets and several whorls of very short, inconspicuous transition (trans) and disc florets. Ray florets were arranged marginally with two or three rows of sleek colored florets of red group in IIHR 2, white group in IIHR 4 and IIHR 5 and orange group in IIHR 3 around the central disc florets. IIHR 1 had singe row of ray florets with color from red purple group. Trans florets were positioned between the outer ray florets and central disc florets. This paper uses self compatibility as a tool to overcome the problem which is in line with the work done by Aswath and Manjunath Rao (2006). Also, Aswath *et al*. (2003) conducted an experiment by half sib mating and the progenies were evaluated based on flower characters.

In the present study, the seed characters were recorded in Table 2. The highest numbers of chaffy seeds (32.36) were recorded in IIHR1 and the least number of chaffy seeds (9.49) were recorded in line IIHR 3. Chaffy seeds were recorded more in the flowers, where trans florets were more . The line IIHR 2 and IIHR 3 were on par (24.00 and 23.40 days, respectively) with each other for higher number of days taken for seed set. Whereas, the least number of days taken for seed set (20.00 days) was recorded for IIHR 5. The line IIHR 3 recorded maximum number of seeds per cross (69.60), seed weight (163.73 mg) and seed set percentage (69.86%). It was found that double flowers take long time for seed set compared to single flowers, and also if the ray florets are more, the seed obtained was also large. This was similar to work conducted by Barua and Bordoloi, 2012.

Least number of days for germination was observed in the line IIHR 1-1 with an average of 2.00 days. Whereas, IIHR 3-3 took the highest days (7.00) to germinate followed by IIHR 4-2 (6.00), compared to all the other lines (Table 3). The variation observed in the different genotypes might be attributed to their genetic make-up (Chobe *et al.*, 2010). The line IIHR 5-4 recorded earliest first leaf development (3.00 days) over other lines. The highest number of days taken for first leaf development (8.00) was recorded from the line IIHR 3-3. Addition of auxins together with cytokinin becomes essential for shoot induction and multiplication depending on the plant type. The right combination of auxin and cytokinin in the culture medium determined the effectiveness of micropropagation of gerbera.

Table 1: Details of female parents used for crossing and their characters

Characters	IIHR1	IIHR 2	IIHR 3	IIHR 4	IIHR 5
Diameter of flower head (cm)	12.00	11.00	14.00	11.00	12.00
Length of the flower stalk (cm)	50.00	60.00	55.00	60.00	45.00
Diameter of flower stalk (mm)	6.10	5.90	6.00	5.20	6.20
Type of flower	Single	Double	Double	Double	Double
Color of ray florets	N67C(Red purple group)	N42A(Red group)	N28A (Orange group)	NN155D (white group)	NN155C (white group)
Color of disc florets	Yellow	Yellow	Black	Black	Yellow

Table 2: Mean performance of the female parents

Lines	Number of chaffy seeds	Days taken for seed set	Seed weight (mg)	Number of seeds/cross	Seed set (%)
IIHR 1	32.36	20.00	70.54	19.50	37.60 (37.80)
IIHR 2	20.54	24.00	81.95	25.50	55.38 (48.08)
IIHR 3	9.49	23.40	163.73	69.60	88.00 (69.86)
IIHR 4	26.29	21.20	120.78	30.20	53.86 (47.20)
IIHR 5	26.30	20.40	129.70	38.60	59.46 (50.45)
Mean	22.99	21.80	113.34	36.68	50.67
S.E.m ±	2.12	0.72	9.25	3.73	1.41
C.D. at 5%	6.36	2.16	27.75	11.19	4.17

Values given in parenthesis is *Arc sine* transformed values

Table 3: Response of *in vitro* raised seeds in half MS medium

Lines	Days taken for germination	Days taken for first leaf emergence	Days taken for first rooting	Number of leaves after 60 days	Number of roots after 60 days	Shoot length at 60 days
IIHR 1-1	2.00	4.00	3.00	5.00	3.00	6.00
IIHR 1-2	3.00	4.00	3.00	4.00	3.00	6.00
IIHR 1-3	4.00	6.00	4.00	4.00	3.00	6.00
IIHR 1-4	4.00	5.00	4.00	4.00	3.00	6.00
IIHR 1-5	3.00	5.00	3.00	3.00	4.00	5.00
IIHR 2-1	3.00	4.00	4.00	3.00	3.00	6.00
IIHR 2-2	3.00	6.00	4.00	4.00	5.00	6.00
IIHR 2-3	3.00	6.00	4.00	4.00	6.00	6.00
IIHR 2-4	4.00	6.00	5.00	5.00	5.00	6.00
IIHR 2-5	4.00	6.00	5.00	4.00	5.00	5.00
IIHR 3-1	4.00	5.00	5.00	5.00	5.00	6.00
IIHR 3-2	3.00	4.00	3.00	4.00	5.00	7.00
IIHR 3-3	7.00	8.00	8.00	3.00	4.00	6.00
IIHR 3-4	4.00	6.00	5.00	4.00	5.00	7.00
IIHR 3-5	5.00	6.00	5.00	6.00	5.00	4.00
IIHR 4-1	3.00	5.00	4.00	5.00	4.00	5.00
IIHR 4-2	6.00	6.00	5.00	4.00	5.00	5.00
IIHR 4-3	3.00	6.00	4.00	6.00	5.00	6.00
IIHR 4-4	3.00	5.00	4.00	6.00	5.00	6.00
IIHR 4-5	4.00	7.00	4.00	4.00	5.00	7.00
IIHR 5-1	3.00	5.00	4.00	5.00	5.00	6.00
IIHR 5-2	4.00	6.00	4.00	5.00	4.00	6.00
IIHR 5-4	3.00	3.00	3.00	4.00	4.00	8.00
IIHR 5-5	3.00	5.00	6.00	3.00	4.00	6.00
Mean	3.52	5.16	4.12	4.16	4.20	5.72

Table 4: Effect of different MS treatments on callus induction from leaf explants at 40 days

Lines	MS + 2,4-D (1.8 mg l^{-1})	MS + 2,4-D (2 mg l^{-1}) + BA (1 mg l^{-1})
IIHR 1-1	+ +	+ +
IIHR 1-2	+ +	+ +
IIHR 1-3	+	+ + +
IIHR 1-4	+ +	+ +
IIHR 1-5	+ +	+ +
IIHR 2-1	+	+ +
IIHR 2-2	+ +	+ + +
IIHR 2-3	+ +	+ +
IIHR 2-4	+ +	+ +
IIHR 2-5	+ +	+ + +
IIHR 3-1	+	+ +
IIHR 3-2	+ +	+ + +
IIHR 3-3	+	+ + +
IIHR 3-4	+ +	+ + +
IIHR 3-5	+ +	+ + +
IIHR 4-1	-	-
IIHR 4-2	+ +	+ +
IIHR 4-3	+ +	+ +
IIHR 4-4	+ + +	+ +
IIHR 4-5	+ +	+ +
IIHR 5-1	-	-
IIHR 5-2	-	-
IIHR 5-4	+ +	+ +
IIHR 5-5	-	-

+ : Poor growth of callus + + : Moderate growth of callus; + + + : Vigorous growth of callus; No callus growth in MS medium

In Table 3 the lines IIHR 1-1, IIHR 1-2, IIHR 1-5, IIHR 3-2 and IIHR 5-4 took the least number of days (3.00 days) for rooting compared to other lines, however, IIHR 3-3 recorded maximum number of days for first rooting (8.00 days). IAA proved to be more efficient in produced maximum number of good quality, healthy and thick roots. After the seeds germinated, the plantlets were sub cultured in a medium containing BAP (2 mg l^{-1}) along with IAA (1 mg l^{-1}). The number of leaves after 60 days was found to be considerably increased in the line IIHR 3-5, IIHR 4-3 and IIHR 4-4 (6 leaves per plant) when subcultured (Table 3). In the present study it was noticed that several roots developed spontaneously from the *in vitro* grown shoots but the spontaneously developed roots were found to be inadequate for transplantation of the *in vitro* grown shoots to the soil. Therefore, separate root induction was necessary. The plants were transferred in a rooting medium containing half MS along with 1.5 mg l^{-1} IBA. Highest number of roots was obtained in the line IIHR 2-3. Aswath and Choudhary (2001) also reported maximum root induction and average number of roots per shoot when cultured on MS medium containing 1.5 mg l^{-1} IBA. Line IIHR 5-4 was significantly superior for shoot length (8.00 cm) over other lines, whereas, the line IIHR 3-5 had the lowest shoot length (4.00 cm) (Table 3).

Optimum concentration of growth regulators required varies with different cultivars every genotype had a specific range of optimum growth regulator concentration (Deepaja, 1999). The callus was initiated in all the cultures within 26-32 days of culturing, irrespective of the strength of the nutrient medium (Table 5). The growth regulator combination of 2,4-D with BAP was proved to be the best compared to other treatments in the basal nutrient medium. First callus was observed on the leaf explants cultured on MS medium supplemented with 1.0 mg l^{-1} 2, 4-D + 1 mg l^{-1} BAP (25.87 days) produced the callus among all the treatments. 2, 4-D with BAP definitely stimulated

Table 5: Effect of the treatments on callus production and regeneration

Treatments	Days to initiate callus	Days to initiate plantlet from callus	Number of shoots/ clump	Number of roots/ clump
MS and BA (1 mg l^{-1})	26.62	14.75	3.50	0.12
MS and BA (1 mg l^{-1}) and IAA (1 mg l^{-1})	29.50	14.87	1.00	2.25
MS and BA (1 mg l^{-1}) and 2, 4-D (1 mg l^{-1})	25.87	13.75	3.12	1.12
MS and Kinetin (5 mg l^{-1}) and IAA (1 mg l^{-1}) and 2, 4 D (1 mg l^{-1})	29.62	22.00	0.75	0.87
MS and BA (5 mg l^{-1}) and Kinetin (5 mg l^{-1}) and 2, 4 D (1 mg l^{-1})	32.12	22.40	0.75	0.62
C.D.(p = 0.01)	0.54	1.42	0.35	0.23

Table 6: Response of lines to callus formation and regeneration

Lines	Days to initiate callus	Days to initiate plantlet from callus	Number of shoots per clump	Number of roots per clump
IIHR 1-1	24.00	15.00	3.33	1.66
IIHR 1-2	28.00	17.00	2.33	2.00
IIHR 1-3	31.00	15.00	2.66	2.00
IIHR 2-1	32.00	14.00	2.66	2.00
IIHR 2-2	32.66	13.33	2.66	1.00
IIHR 2-5	30.00	17.00	3.00	2.00
IIHR 3-1	30.00	18.00	2.66	2.00
IIHR 3-2	24.66	20.00	2.00	1.00
IIHR 3-5	29.66	20.33	1.66	1.00
IIHR 4-1	29.33	20.00	2.00	1.66
IIHR 4-2	30.33	19.00	2.00	1.66
IIHR 4-4	29.33	20.66	2.00	1.66

callus production whereas IAA with BAP though produced callus took a long time to initiate callus. The present result is also in confirmation with Aswath *et al*. (2003) and Kumar *et al*. (2004). After 60 days of inoculation, callus produced from the explants were scored visually and analyzed (Table 4). Explants cultured on full MS medium supplemented with lower levels of 2, 4-D (2.0 mg l^{-1}) and BAP (1 mg l^{-1}) produced more amount of callus. There was no callus production on the basal medium and the leaf explants remained as it is. Growth regulator addition is a must to stimulate cell division. BAP is essentially required for the formation of callus. MS basal medium fortified with 2, 4-D and BAP produced green and nodular callus and MS medium fortified with 2, 4-D alone produced yellowish creamy callus in all the treatments irrespective of the varieties. This result is also in confirmation with Hasbullah *et al.,* 2008. The callus was observed under microscope for somatic embryogenesis validation. There was no vascular connection between the embryos and they were popping out from the clump, clearly indicated that they are somatic embryos. Naing *et al*. (2011) also reported that somatic embryos were indirectly induced from leaf derived callus. The plantlets were initiated from the callus with 13-22 days of culturing irrespective of strength of the nutritive medium (Table 5). Among the lines IIHR 2-2 took the least time of 13.33 days to form plantlet (Table 5), whereas, the combination of MS medium supplemented with BA (1 mg l^{-1}) and 2, 4-D (1 mg l^{-1}) recorded earliest days to initiate plantlet from callus (13.75 days). This is in confirmation with studies of Martin (2004) who reported that even low concentration of 2, 4-D influenced somatic embryogenesis when added to the culture medium.

The number of plantlets from the callus ranged from 1-4 (Table 5 and 6). Highest number of plants per clump (3.50) was recorded on MS and BA 1 mg l^{-1}. The line IIHR 1-1 produced 3.3 plantlets from each clump. The treatment MS and BA (1mg l^{-1}) and IAA (1mg l^{-1}) produced highest number of roots (2.25). The lines IIHR 1-2, IIHR 1-3, IIHR 2-1, IIHR 2-5 and IIHR 3-1 were recorded with highest number of roots (2.00) per callus clump. This is in confirmation with Kumar *et al.,* 2004. This variability between the lines could be attributed to genotypic difference and their response to phytohormones.

It can be concluded that half sib mating can be used as a breeding tool in gerbera. This method was significant in obtaining higher seed weight, number of seeds per cross and seed set percentage. This can be further accompanied with tissue culture for rapid propagation. Further study of histology of cultured leaves is necessary to understand the cells and factors contributing towards regeneration. The production of adventitious shoots, which will be true to type to the mother plants, can be proposed as an alternative method of propagation.

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