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Effect of growth hormones of callus induction activity leaf explants in *Gerbera jamesonii* (Bolus) in *in vitro* condition with SH and CHU medium

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Abstract

Present investigating was carried out on "Effect of growth hormones in induction of callus induction activity leaf explants in *Gerbera Jamesonii* (Bolus) in *in vitro* condition" at the Tissue culture Laboratory Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut during the year 2015-2019. All experiments were directed to the development of technology and motivated by practical demands. The aim of the work was to develop efficient callus inductions shoot multiplication as well as root initiation protocols in *Gerbera*. treatments showed that significantly minimum time of callus induction 29.76 days was observed in treatment 2,4-D @ 2.00 mg^l⁻¹+ Kinetin @ 1.50 mg^l⁻¹ with CHU media and showed that significantly minimum time of callus induction 31.85 days was observed in treatment 2,4-D @ 2.00 mg^l⁻¹+ Kinetin @ 1.50 mg^l⁻¹ with SH media. Significantly minimum time of callus induction 35.67 days was observed in treatment IBA @ 2.00 mg^l⁻¹+BAP @ 1.00 mg^l⁻¹ with CHU media and significantly minimum time of callus induction 37.89 days was observed in treatment IBA @ 2.00 mg^l⁻¹+BAP @ 1.00 mg^l⁻¹ with SH media.

Keywords: Growth hormones, callus induction, *Gerbera Jamesonii* and *in vitro* condition

1. Introduction

India is on the 18th rank with contributing 0.6 per cent share in global floriculture trade. During the last decade, export increased at a CAGR (Compound annual growth rate) of 4.33 per cent. The domestic Indian market is growing at the rate of 25 per cent per year in the country as a whole. About 249 thousand hectares area was under cultivation in floriculture in 2014-15 Vahoniya *et al.*, (2018) [10]. The industry is characterized by sale of most loose flowers and the export surplus from the cut-flower (Rose, Carnation, *Gerbera*, Orchid and Anthurium) industries with the growing competitiveness, floriculture units in India have been facing several constraints, so there is a need to develop optimum conditions for growing cut flowers Nirala *et al.*, (2018) [5] and it is now practiced in over an area of 249 thousand hectares area was under Cultivation in floriculture in 2015-16. Production of flowers is estimated to be 1659 thousand tons loose flowers and 484 thousand tons cut flowers in 2015-16. The country has exported 20703.46 MT of floriculture products to the world for the worth of Rs. 507.31 Crores/78.73 USD Millions in 2017-18 (The Agricultural and Processed Food Products Export Development Authority, APEDA, 2018).

In India, *Gerbera* cultivation is done in a commercially viable manner in various states like Andhra Pradesh, Orissa, Karnataka, Gujarat, Maharashtra, West Bengal, Uttar Pradesh and Uttarakhand. Its cultivation is good at 1300 to 3200 meters. It is considered as nature's one of the beautiful creations because of the excellent flowers with exquisite shape, size and vibrant colors Sil *et al.*, (2017) [8]. It is an ideal flower for cut flower, beds, borders, rock gardens and pot culture. The flowers are of attractive colour and used for a floral arrangement. Now days, *gerbera* is one of the most important cut flowers and it has tremendous demand in local as well as foreign market Chobe *et al.*, (2010) [1]. It is grown as a commercial crop in sub-tropical and temperate regions of India and it is commercially grown in Pune, Bangalore and also around Ooty as commercial cut flower.

Tissue culture technologies provide a tool to study plant development as well as applications in plant improvement, molecular biology and bio processing. Development of tissue culture protocols requires a rigorous process that begins with the knowledge of the plant/species/genotype propagation features *in vivo* and involves the optimization of the

chemical, physical and environmental factors for growth and multiplication during *in vitro* culture Lee, (2004) [4].

2. Materials and Methods

2.1 Location of experiment

The studies were carried out in the Tissue Culture Laboratory, Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut. (U.P.) from 2015 to 2019 to fulfill the objectives.

2.2 Plant Material

The gerbera plants were collected from Sheel Biotech Ltd. Gurgaon Haryana-122051 and plants were transferred in the Green house condition at Herbal Garden of Horticulture Research Centre, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut-250110 (Uttar Pradesh). The explants were collected from healthy and disease free plant Young leaves were cleaned thoroughly by repeated washing under running tap water a for period of 30 minutes, air dried and stored in the laboratory at ambient temperature. These leaves were then trimmed and used as explants for culture establishments under *in-vitro* conditions.

Table 1: Detail of different chemicals treatment tests used for the present study:

S. No.	Chemical Treatment	Percent	Treatment Time
1.	Mercuric Chloride (HgCl ₂)	0.1%	1,2,3,4 minutes
2.	Sodium Hypochlorite	4%	2,4,6,8 minutes
3.	Mercuric Chloride (HgCl ₂) + Sodium Hypochlorite	0.1+4%	1,3,5,7 minutes

Table 2: Different growth regulator concentration for Callus induction & multiplication on SH & CHU N₆ medium:

S. No.	Mediums	Auxins	Kinetin	BAP
1.	SH & CHU N ₆ medium	IBA 2.00	1.0	1.0
2.	SH & CHU N ₆ medium	IBA 2.00	1.5	1.5
3.	SH & CHU N ₆ medium	IBA 2.00	2.0	2.0
4.	SH & CHU N ₆ medium	IBA 2.00	2.5	2.5
5.	SH & CHU N ₆ medium	2,4-D 2.00	1.0	1.0
6.	SH & CHU N ₆ medium	2,4-D 2.00	1.5	1.5
7.	SH & CHU N ₆ medium	2,4-D 2.00	2.0	2.0
8.	SH & CHU N ₆ medium	2,4-D 2.00	2.5	2.5

2.3 Sterilization of culture room and Transfer facilities

The culture room was cleaned by gently washing floors and walls with a detergent. This was followed by careful wiping them with 70% ethyl alcohol and fumigation (potassium permanganate with formaldehyde) and the process of sterilization of culture room was repeated at regular intervals. The transfer area was also sterilized with UV light followed by twice a month by 70% ethyl alcohol, 30 minutes before starting the transfer work.

The explants were incubated in a culture room where the temperature was maintained at 26°C, humidity at 60 % and either under continuous dark or under a photoperiod of 16 hr. per 8 hr. light/dark at light intensity of 25μ mole s⁻² m⁻².

Table 3: Effect of different treatment combinations of 2,4-D with Kinetin and BAP in CHU media on time of callus induction inoculation (days) in (*Gerbera Jamesonii* Bolus).

Treatment	Treatment detail	Time of callus induction
T ₁	2,4-D @2.00 mg l ⁻¹ + Kinetin @1.00 mg l ⁻¹	30.71 (33.63)
T ₂	2,4-D @2.00 mg l ⁻¹ + Kinetin @1.50 mg l ⁻¹	29.76 (33.03)
T ₃	2,4-D @2.00 mg l ⁻¹ + Kinetin @2.00 mg l ⁻¹	32.77 (34.90)
T ₄	2,4-D @2.00 mg l ⁻¹ + Kinetin @2.50 mg l ⁻¹	34.47 (35.93)
T ₅	2,4-D @2.00 mg l ⁻¹ + BAP @1.00 mg l ⁻¹	33.72 (35.47)

2.4 Sterilization of Explants

Whole leaf segments were used as explants. The young leaf from the top of the plant was selected always for the explants. The cutting size of the leaf segments with mid rib were around 2-4 cm. The explants were then treated with 6-7 minutes in 4% sodium hypochlorite after 3 times rinses sterile distilled water and again use 0.1 per cent Mercuric Chloride (HgCl₂) for 5 minutes, after 3 times rinses sterile distilled water under a laminar airflow cabinet. After sterilization and cutting those explants, they were placed on a filter paper to absorb the extra water of the surface. The leaf segments were placed on the medium in both sides.

2.5 Preparation of explant Callus culture

Attempts have been made for the induction of callus using healthy and disease free plant's young leaf in CHU and SH media supplemented with Auxins (2.00 mg l⁻¹) and the different combination of Auxins (IBA & 2,4-D) with BAP and Kinetin (2.00+1.00, 2.00+1.50, 2.00+2.00, 2.00+2.50, 2.00+1.00, 2.00+1.50, 2.00+2.00, 2.00+2.50, 2.00+1.00, 2.00+1.50, 2.00+2.00, 2.00+2.50, and 2.00+1.00, 2.00+1.50, 2.00+2.00, 2.00+2.50 mg l⁻¹).

2.6 Data analysis

The data recorded were subjected to analyze as per the design. The experiment was arranged in Completely Randomized Design (CRD) each treatment was performed with three replicates. Cultures were observed routinely. The data were recorded on frequency of shoot regeneration, number of shoots per explant, shoot length in (cm), frequency of root proliferation, number of roots per shoot and root length in (cm) after weekly of explants inoculation on culture media. Interesting changes during investigation, in explants, such as proliferation, shoot initiation and root initiation were timely photographed. For statistical analysis of data generated through various experiments was qualified and the significance of difference among means were determined by ANOVA using Window stat 9.2 software.

3. Results and Discussion

Time of Callus induction with different treatments of 2,4-D + kinetin and 2,4-D + BAP treatment, it was found to be ranged from 29.76 to 35.57 day was showed in table -3 and fig. -1

In these treatments, the minimum time of callus formation was found to be 29.76 days of this treatment 2,4-D @2.00 mg l⁻¹+ Kinetin @1.50 mg l⁻¹ after inoculation followed by 30.71, 32.37, 32.77, 33.56, 33.72 and 34.47 days with the treatments of 2,4-D @2.00 mg l⁻¹+ Kinetin @1.00 mg l⁻¹, 2,4-D @2.00 mg l⁻¹+ BAP @2.50 mg l⁻¹, 2,4-D @2.00 mg l⁻¹+ Kinetin @2.00 mg l⁻¹, 2,4-D @2.00 mg l⁻¹+ BAP @1.50 mg l⁻¹, 2,4-D @2.00 mg l⁻¹+ BAP @1.00 mg l⁻¹ and 2,4-D @2.00 mg l⁻¹+ Kinetin @2.50 mg l⁻¹; while the maximum time (Days) of callus induction of this treatments, it was found (35.57 days) under 2,4-D @2.00 mg l⁻¹+ BAP @2.00 mg l⁻¹; however, it was at par 5% of Critical Difference.

T ₆	2,4-D @2.00 mg ^l - ¹ + BAP @1.50 mg ^l - ¹	33.56 (35.37)
T ₇	2,4-D @2.00 mg ^l - ¹ + BAP @2.00 mg ^l - ¹	35.57 (36.59)
T ₈	2,4-D @2.00 mg ^l - ¹ + BAP @2.50 mg ^l - ¹	32.37 (34.65)
C.D.		2.093
SE(m)		0.692

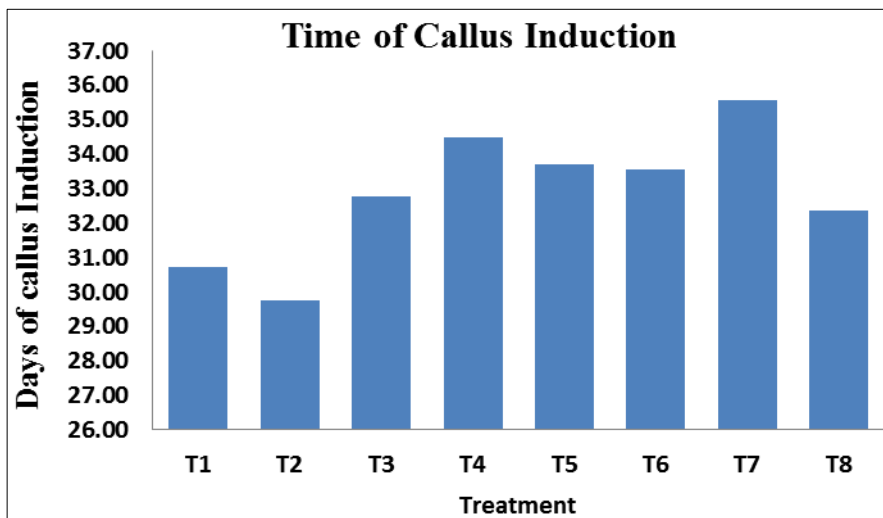


Fig 1: Time of Callus induction (2,4-D with Kinetin and BAP) in CHU media

Demonstrated that the investigation of the callus induction in days was recorded among the different combinations of 2,4-D with Kinetin and BAP. Significant increase in average callus induction in (days) sterilized explants was recorded from 31.85 to 37.32 days was showed in table -4 and fig.-2.

The treatments under study showed that significantly minimum time of callus induction 31.85 days was observed in treatment 2,4-D @ 2.00 mg^l-¹+ Kinetin @ 1.50 mg^l-¹ followed by 32.57, 33.02, 34.55, 34.99, 35.67 and 36.65 days with the

treatments of 2,4-D @ 2.00 mg^l-¹+ Kinetin @ 1.00 mg^l-¹, 2,4-D @ 2.00 mg^l-¹+ Kinetin @ 2.00 mg^l-¹, 2,4-D @ 2.00 mg^l-¹+ Kinetin @ 2.50 mg^l-¹, 2,4-D @ 2.00 mg^l-¹+ BAP @ 1.00 mg^l-¹, 2,4-D @ 2.00 mg^l-¹+ BAP @ 1.50 mg^l-¹ and 2,4-D @ 2.00 mg^l-¹+ BAP @ 2.00 mg^l-¹; while the maximum time of callus induction 37.32 days was noted under 2,4-D @ 2.00 mg^l-¹+ BAP @ 2.50 mg^l-¹; however, it was at par 5 per cent of Critical Difference.

Table 4: Effect of different treatment combinations of 2,4-D with Kinetin and BAP in SH media on time of callus induction inoculation (days) in (*Gerbera Jamesonii* Bolus).

Treatment	Treatment detail	Time of callus induction
T ₁	2,4-D @2.00 mg ^l - ¹ +Kinetin @1.00 mg ^l - ¹	32.57 (34.78)
T ₂	2,4-D @2.00 mg ^l - ¹ +Kinetin @1.50 mg ^l - ¹	31.85 (34.32)
T ₃	2,4-D @2.00 mg ^l - ¹ +Kinetin @2.00 mg ^l - ¹	33.02 (35.05)
T ₄	2,4-D @2.00 mg ^l - ¹ +Kinetin @2.50 mg ^l - ¹	34.55 (35.98)
T ₅	2,4-D @2.00 mg ^l - ¹ +BAP @1.00 mg ^l - ¹	34.99 (36.24)
T ₆	2,4-D @2.00 mg ^l - ¹ +BAP @1.50 mg ^l - ¹	35.67 (36.65)
T ₇	2,4-D @2.00 mg ^l - ¹ +BAP @2.00 mg ^l - ¹	36.65 (37.23)
T ₈	2,4-D @2.00 mg ^l - ¹ +BAP @2.50 mg ^l - ¹	37.32 (37.63)
C.D.		1.623
SE(m)		0.537

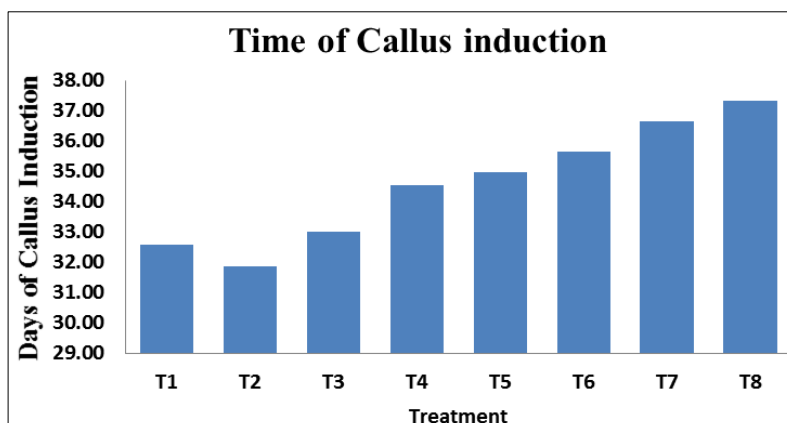


Fig 2: Time of Callus induction (2,4-D with Kinetin and BAP) in SH media

Demonstrated that the callus induction in days was recorded among the different treatment combinations of IBA with Kinetin and BAP. Significant increase in average callus induction (days) in sterilized explants was recorded from 35.67 to 41.60 days as shown in table -5 and fig.-3.

Significantly minimum time of callus induction 35.67 days was observed in treatment IBA @ 2.00 mg^l⁻¹+BAP @ 1.00 mg^l⁻¹ followed by 35.89, 36.76, 36.83, 37.68, 37.74 and 39.70

days with the treatments of IBA @ 2.00 mg^l⁻¹+Kinetin @ 1.00 mg^l⁻¹, IBA @ 2.00 mg^l⁻¹+BAP @ 1.50 mg^l⁻¹, IBA @ 2.00 mg^l⁻¹+Kinetin @ 2.00 mg^l⁻¹, IBA @ 2.00 mg^l⁻¹+Kinetin @ 2.00 mg^l⁻¹, IBA @ 2.00 mg^l⁻¹+BAP @ 2.00 mg^l⁻¹ and IBA @ 2.00 mg^l⁻¹+Kinetin @ 2.50 mg^l⁻¹; while the maximum 41.60 days was noted under IBA @ 2.00 mg^l⁻¹+BAP @ 2.50 mg^l⁻¹; however, it was at par 5% of Critical Difference.

Table 5: Effect of different treatment combinations of IBA with Kinetin and BAP in CHU media on time of callus induction inoculation (days) in *Gerbera*.

Treatment	Treatment detail	Time of callus induction
T ₁	IBA @2.00 mg ^l ⁻¹ +Kinetin @1.00 mg ^l ⁻¹	35.89 (36.78)
T ₂	IBA @2.00 mg ^l ⁻¹ +Kinetin @1.50 mg ^l ⁻¹	36.83 (37.34)
T ₃	IBA @2.00 mg ^l ⁻¹ +Kinetin @2.00 mg ^l ⁻¹	37.68 (37.85)
T ₄	IBA @2.00 mg ^l ⁻¹ +Kinetin @2.50 mg ^l ⁻¹	39.70 (39.03)
T ₅	IBA @2.00 mg ^l ⁻¹ +BAP @1.00 mg ^l ⁻¹	35.67 (36.65)
T ₆	IBA @2.00 mg ^l ⁻¹ +BAP @1.50 mg ^l ⁻¹	36.76 (37.30)
T ₇	IBA @2.00 mg ^l ⁻¹ +BAP @2.00 mg ^l ⁻¹	37.74 (37.88)
T ₈	IBA @2.00 mg ^l ⁻¹ +BAP @2.50 mg ^l ⁻¹	41.60 (40.14)
C.D.		2.066
SE(m)		0.683

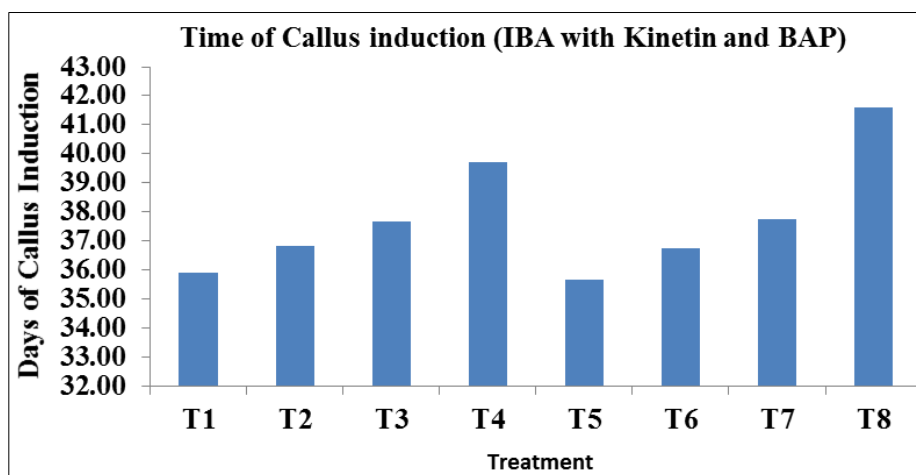


Fig 3: Time of Callus induction (IBA with Kinetin and BAP) in CHU media

Table 6 showed that significantly minimum time of callus induction 37.89 days was observed in treatment IBA @ 2.00 mg^l⁻¹+BAP @1.00 mg^l⁻¹ followed by 38.36, 39.11, 39.58, 39.75, 40.99 and 41.03 days with the treatments of IBA @ 2.00 mg^l⁻¹+Kinetin @ 2.00 mg^l⁻¹, IBA @ 2.00 mg^l⁻¹+Kinetin @ 2.50 mg^l⁻¹, IBA @ 2.00 mg^l⁻¹+BAP @ 2.00 mg^l⁻¹, IBA @

2.00 mg^l⁻¹+Kinetin @ 1.50 mg^l⁻¹, IBA @ 2.00 mg^l⁻¹+BAP @ 2.50 mg^l⁻¹ and IBA @ 2.00 mg^l⁻¹+BAP @ 1.50 mg^l⁻¹; while the minimum time of callus induction 42.39 days was noted under IBA @ 2.00 mg^l⁻¹+Kinetin @ 1.00 mg^l⁻¹; however, it was at par 5% of Critical Difference.

Table 6: Effect of different treatment combinations of IBA with Kinetin and BAP in SH media on time of callus induction inoculation (days) in (*Gerbera Jamesonii* Bolus).

Treatment	Treatment detail	Time of callus induction
T ₁	IBA @2.00 mg ^l ⁻¹ +Kinetin @1.00 mg ^l ⁻¹	42.39 (40.60)
T ₂	IBA @2.00 mg ^l ⁻¹ +Kinetin @1.50 mg ^l ⁻¹	39.75 (39.06)
T ₃	IBA @2.00 mg ^l ⁻¹ +Kinetin @2.00 mg ^l ⁻¹	38.36 (38.25)
T ₄	IBA @2.00 mg ^l ⁻¹ +Kinetin @2.50 mg ^l ⁻¹	39.11 (38.69)
T ₅	IBA @2.00 mg ^l ⁻¹ +BAP @1.00 mg ^l ⁻¹	37.89 (37.97)
T ₆	IBA @2.00 mg ^l ⁻¹ +BAP @1.50 mg ^l ⁻¹	41.03 (39.81)
T ₇	IBA @2.00 mg ^l ⁻¹ +BAP @2.00 mg ^l ⁻¹	39.58 (38.97)
T ₈	IBA @2.00 mg ^l ⁻¹ +BAP @2.50 mg ^l ⁻¹	40.99 (39.79)
C.D.		1.098
SE(m)		0.363

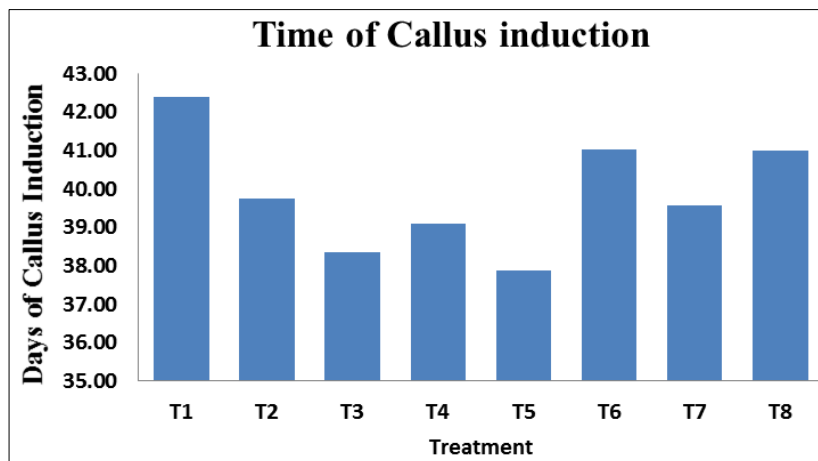
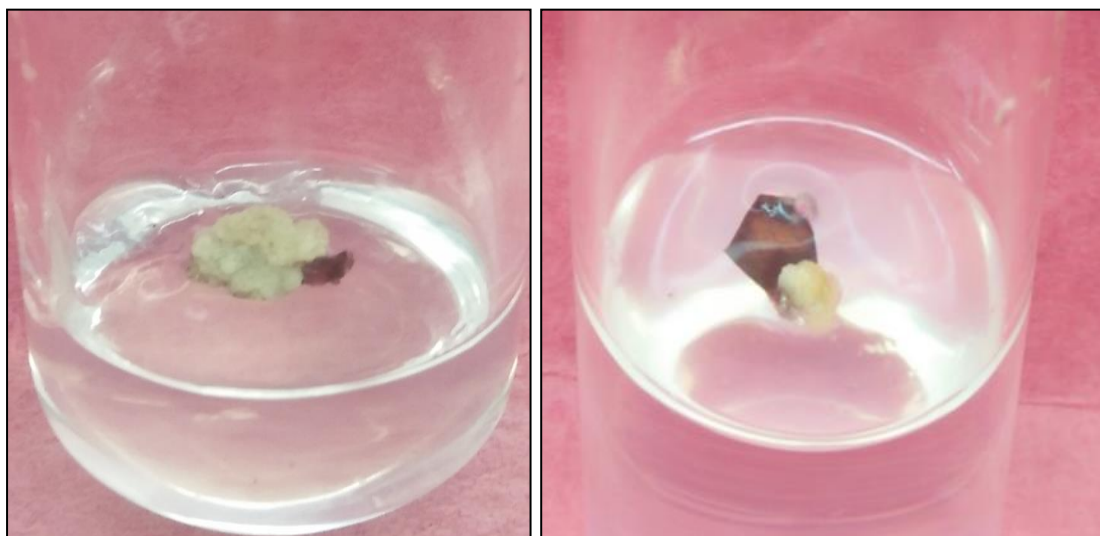


Fig 4: Time of Callus induction (IBA with Kinetin and BAP) in SH media



Initial stage of callus formation

The minimum time of callus induction (29.76 days) was noted in media supplemented with 2,4-D @ 2.00 mg l^{-1} + BAP @ 2.00 mg l^{-1} while the maximum time of callus induction (35.57 days) was observed treatment 2,4-D @ 2.00 mg l^{-1} + BAP @ 2.00 mg l^{-1} (Table-3). Similar pattern was observed in same treatment using SH media for callus induction. The minimum time callus induction (31.85 days) was noted in media containing 2,4-D @ 2.00 mg l^{-1} + Kinetin @ 1.50 mg l^{-1} ; while the maximum time of callus induction (37.32 days) was observed in treatment 2,4-D @ 2.00 mg l^{-1} + BAP @ 2.50 mg l^{-1} (Table 4). The same pattern was used for CHU media with IBA, BAP and Kinetin. The minimum time (35.67 days) was observed in treatment IBA @ 2.00 mg l^{-1} + BAP @ 1.00 mg l^{-1} While the maximum time of callus induction (41.60 days) was noted under IBA @ 2.00 mg l^{-1} + BAP @ 2.50 mg l^{-1} and however, the minimum time of callus induction (35.67 days) was observed in treatment IBA @ 2.00 mg l^{-1} + BAP @ 1.00 mg l^{-1} (Table-5). The same pattern was obtained with same treatment in SH media; whereas the minimum time of callus induction (37.89 days) was noted under IBA @ 2.00 mg l^{-1} + BAP @ 1.00 mg l^{-1} while maximum time of (42.39 days) was observed in treatment IBA @ 2.00 mg l^{-1} + Kinetin @ 1.00 mg l^{-1} (Table-6). The present finding was parsley arrangement with Sharma and Srivastva (2005) [6]; treatments were used Cytokinin with Auxin for callus induction. Gaurav and Srivastava (2005) [6]; leaf used as explant for callus induction and treatment were used the combination of BAP and 2,4-D.

Kumar and Kanwar (2005) [2]; Kumar and Kanwar (2007) [3]; Paduchuri *et al.* (2010) [9]; Shabbir *et al.* (2012) [7].

4. Conclusion

Gerbera (*Gerbera jamesonii* Bolus ex Hook) is the latest sensation to Indian Floriculture. It is commonly known as Barberton daisy, African daisy, and translates daisy and is a classic sun tracker like sunflowers. It is a dwarf stem-less herbaceous perennial herb growing in clump with solitary flower heads termed capitulum on a long slender stalk, well above the foliage. It is generally propagated by division of suckers or clumps. Propagation through seeds is not preferred as the plants exhibit heterozygosity and non-uniformity. Also, the improved semi-double and double cultivars do not set seeds. Propagation by division of suckers or clumps gives true to type plants, but the multiplication rate is very low.

1. The standardization of protocol for *in vitro* callus induction and plantlet regeneration in Gerbera was carried out by using disease free leaf as explants.
2. It exhibited that CHU media fortified with 2,4-D @ 2.00 mg l^{-1} + Kinetin @ 1.50 mg l^{-1} was superior then SH medium for *in vitro* callus induction days.
3. For earlier and higher percentage of culture establishment, the combination of BAP @ 2.00 mg l^{-1} + Kinetin @ 1.50 mg l^{-1} was proved to be best at both culture media CHU and SH medium.

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