Hormonal effect on callus induction and shoot multiplication on leaf explants in Gerbera (*Gerbera jamesonii*).

Abstract

Aims: Gerbera is very popular as a cut flower in India. Because from the spoint of view of beauty it is very attractive by different colors and it has longer vase life. It is a dwarf, stem-less, herbaceous, perennial plant that is commercially propagated by the cell culture.

Methodology: The research was conducting an investigation into In vitro method used nutrient culture media under control condition. *In-vitro* method were observed that the effect of 2,4-D +Kinetin and 2,4-D + BAP with different treatment concentrations on callus induction and shoot multiplication under in vitro condition was carried out using leaf explants.

Results: The result acquired has shown that minimum time (7.18 days) was noted under the treatment combination 2,4-D @ 2.00 mgl^{-1}+ Kinetin @1.50 mgl^{-1} whereas maximum time (35.57 Days) was found of callus with the treatment combination 2,4-D @2.00 mgl^{-1}+ BAP @2.00 mgl^{-1}under control. After the callus induction, the callus was transferred for the establishment of the shoot on different shoot multiplication media showed that time of shoot initiation 7.18 days. shoot per cent was 76.65 in these days also the number of shoots per explant 11.23 along with this, the development of shoot was completed in 11.74 days when Length of the shoot was viewed on 25 days to shoot the length was 2.17 cm, 35 days after 3.60 and 45 days after 4.56 cm. and after that count of shoot per culture was 12.62 were observed on treatment of CHU Media + BAP @ 2.50 mgl^{-1} + Kinetin @ 1.50 mgl^{-1}.

Key Words: Gerbera, *In-vitro*, PGR, Callus and Shoot

Introduction

Gerbera (*Gerbera jamesonii* Bolus ex Hook) is usually propagated by splitting of suckers or clumps resulting in production of true to type plants, however rate of multiplication is very low. Most of the cultivars are multiplied through tissue culture. There has been an increasing interest in tissue cultures as an alternative to asexual propagation of gerbera [16] reported the formation of adventitious shoots from axillary position on isolated young leaves. Plants regenerated from callus and adventitious shoots are required as a tool in mutation breeding for the production of solid mutants [6].

Multiplication of plants by meristem or non-meristem culture helps in production of large quantity of plants in shore period of time in small space [19, 22] Micro propagation is one of the viable alternatives for large-scale multiplication of gerbera [2]. Conventionally, Gerbera can be multiplied by both sexual (seed) as well as vegetative methods [3] Since last many years, a number of investigations has been carried out for development of in vitro propagation protocol of gerbera using different explants viz., shoot tip, auxillary bud, leaves, flower bud, capitulum, ovule, petal and petiole [3].

Material and Method

Collection and Identification

Preparation of CHU media

All chemicals were selected and CHU media were prepared in double distilled autoclaved water with the addition of growth regulator viz., cytokinin (BAP and Kinetin) with different treatment combination. The carbon source (sucrose 30 g/l) and gelling agent (agar powder 8 g/l).A pH of 5.8 was adjusted with 0.1 N HCl or 0.1 NaOH. The medium was immediately poured into glass tubes of 18 x 150 mm up to 20 ml in each tube. The agar is mixed in the medium and heated up

Comment [H1]: No meaning of this word

Comment [H2]: Arrange alphabetically

Comment [H3]: (Asteraceae)

Comment [H4]: italics

Comment [H5]: Provide the data, even though it is under cultivation. It is mandatory

Comment [H6]: italics

boiling for proper melting until a clear medium obtained. Then the medium was autoclaved at 121 ^{o} C at a pressure of 15 psi (pounds per square inch) for 25 minutes for sterilization. The medium was placed in growth room for one week to check any contamination before use for culture of explants.

Comment [H7]: provide the reference

Sterilization of explants

Induction of callus was carried out using leaf explants. For this, 2-4 cm leaf segments were excised from the top of the plant along with mid rib. The explants were treated with 4% sodium hypochlorite for 6-7 minutes followed by treatment of 0.1 per cent Mercuric Chloride ($HgCl_2$) for 5 minutes. After sterilization, explants were placed on a filter paper for absorption of extra water.

Comment [H8]: cite the references

Results& Discussion

1. Initiation of callus

A significant increase in average callus induction was observed from 29.76 to 35.57 days from inoculation. Minimum time duration (29.76 days) for callus induction was found in combination of 2,4-D @ 2.00 mgl^{-1}+ Kinetin @1.50 mgl^{-1} 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 mgl^{-1}+ Kinetin @1.00 mgl^{-1}, 2,4-D @2.00 mgl^{-1}+ BAP @2.50 mgl^{-1}, 2,4-D @2.00 mgl^{-1}+ Kinetin @2.00 mgl^{-1}, 2,4-D @2.00 mgl^{-1}+ BAP @1.50 mgl^{-1}, 2,4-D @2.00 mgl^{-1}+ BAP @1.00 mgl^{-1} and 2,4-D @2.00 mgl^{-1}+ Kinetin @2.50 mgl^{-1}; while the maximum time (35.57 Days) was found of callus with the used treatment combination 2,4-D @2.00 mgl^{-1}+ BAP @2.00 mgl^{-1}; The present finding similar [5,23] used leaf as explant for callus induction with the treatment combination of BAP and 2,4-D. [13,21] were also studied of callus induction in Gerbera plant.

2. Time of shoot initiation

Earliest shoot initiation was observed in medium supplemented with BAP @ 1.50 mgl^{-1} + Kinetin @ 2.00 mgl^{-1} in 7.18 days; but longest time (11.74 days) of shoot initiation was noted under BAP @1.50 mgl^{-1} + Kinetin @ 2.00 mgl^{-1}. The result is similar. [9, 25]

3. Per cent of shoot development

Maximum percentage (76.65%) of shoots regeneration was observed by application of BAP @ 2.00 mgl^{-1} + Kinetin @ 1.50 mgl^{-1} however, the minimum 51.74 per cent was noted with BAP @ 1.50 mgl^{-1} + Kinetin @ 2.00 mgl^{-1}. Observed results are akin with the findings. [11, 14]

3. Number of shoots per explants

Maximum number of shoots (11.23) were observed on treatment of BAP @ 2.50 mgl^{-1} and Kinetin @ 1.50 mgl^{-1}; whereas minimum shoots(6.17) per explants were noted in MS medium supplemented with BAP @ 1.50 mgl^{-1}+ Kinetin @ 2.00 mgl^{-1}. These results are in agree with findings of [8] reported an investigation by use of petiole explants for development of maximum number of shoots (9.3±0.6 per explant) using MS medium supplemented with 2.0 mgl^{-1} BAP and 0.5 mgl^{-1} NAA, [17]

4. Days taken for shoot development

An early development of shoots (11.74 days) were observed in BAP @ 2.00 mgl^{-1}+ Kinetin @ 1.50 mgl^{-1}; whereas maximum time duration (18.70 days) was recorded by treatment of BAP @ 1.50 mgl^{-1} + Kinetin @ 2.00 mgl^{-1}. The result is similar [20, 1].

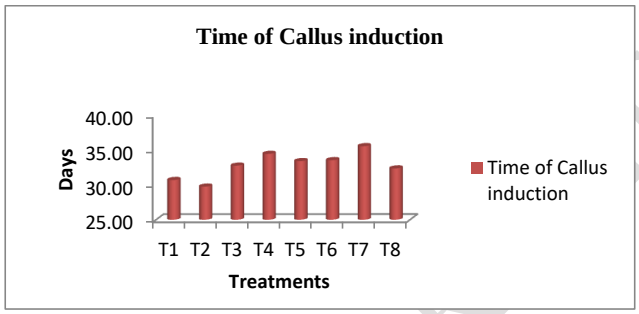
5. Length of shoot at different time interval (25, 35 and 45 days)

Highest shoot length (2.17 cm) was observed in combination of BAP @ 2.00 mgl^{-1} and Kinetin @ 1.50 mgl^{-1}, whereas, lowest (1.02 cm) was recorded in BAP @ 1.50 mgl^{-1} and Kinetin @ 2.00 mgl^{-1} supplemented MS media. Maximum shoot length (3.60 cm) was observed in treatment of BAP @ 2.00 mgl^{-1} with Kinetin @ 1.50 mgl^{-1}; while the lowest (2.05 cm) was noted in BAP @

1.50 mgl^{-1}+ and Kinetin 2.00 mgl^{-1}(35 days). Highest length of shoot (4.56 cm) was recorded in treatment of BAP @ 2.00 $mgl^{-1}$and Kinetin @1.50 mgl^{-1}; whereas the minimum 3.04 cm was noted by using BAP @ 1.50 mgl^{-1} + Kinetin @ 2.00 mgl^{-1}. The result is similar. [12, 26]

6. Number of leaves per Culture

Observation of the experiment was calculated as number of leaves per culture. Maximum number of leaves per plantlet (12.62) was observed in treatment of BAP @ 2.00 mgl^{-1}+ Kinetin @1.50 mgl^{-1}; whereas the minimum 7.50 was recorded in BAP @ 1.50 mgl^{-1} and Kinetin @ 2.00 mgl^{-1} supplemented medium. The results are similar. [27] [26] [13]



Figur-1 Effect of different combinations of IBA with Kinetin and BAP under CHU media.

Comment [H9]: Figure

Comment [H10]: Cite this in running text

T_1-2,4-D @ 2.00 mgl^{-1}+ Kinetin @1.00 mgl^{-1}, **T_2**-2,4-D @2.00 mgl^{-1}+ Kinetin @1.50 mgl^{-1}
T_3-2,4-D @2.00 mgl^{-1}+ Kinetin @2.00 mgl^{-1}, **T_4**-2,4-D @2.00 mgl^{-1}+ Kinetin @2.50 mgl^{-1}
T_5-2,4-D @2.00 mgl^{-1}+ BAP @1.00 mgl^{-1}, **T_6**-2,4-D @ 2.00 mgl^{-1}+ BAP @1.50 mgl^{-1}
T_7-2,4-D@ 2.00 mgl^{-1} + BAP @ 2.00 mgl^{-1}, **T_8**-2,4-D @ 2.00 mgl^{-1} +BAP @ 2.50 mgl^{-1}

Figure 2-Callus induction in Gerbera using leaf explants on full strength CHU media.

Comment [H11]: Cite this in running text

Treatment	Treatment detail	Time of shoot initiation	Percent of shoot development	Number of shots per explants	Days taken for shoot development	Length of shoot at different time interval			Number of leaves per
						25	35	45	

						days	**days**	**days**	**plantlet**
T1	BAP @1.00 mgl^{-1}+Kinetin @1.00 mgl^{-1}	10.64 (19.01)	62.49 (52.21)	8.48 (16.90)	15.64 (23.27)	1.69 (7.40)	2.96 (9.87)	3.44 (10.6)	11.72 (19.99)
T2	BAP @1.00 mgl^{-1}+Kinetin @1.50 mgl^{-1}	7.77 (16.16)	55.61 (48.20)	10.48 (18.85)	13.37 (21.41)	1.47 (6.90)	3.04 (10.0)	3.58 (10.8)	8.77 (17.20)
T3	BAP @1.00 mgl^{-1}+Kinetin @2.00 mgl^{-1}	9.82 (18.24)	65.34 (53.91)	6.82 (15.11)	15.28 (22.99)	1.71 (7.49)	2.29 (8.69)	3.33 (10.5)	7.84 (16.23)
T4	BAP @1.00 mgl^{-1}+Kinetin @2.50 mgl^{-1}	10.28 (18.67)	63.37 (52.73)	7.92 (16.31)	14.05 (21.97)	1.50 (6.99)	2.28 (8.67)	3.49 (10.7)	8.27 (16.68)
T5	BAP @1.50 mgl^{-1}+Kinetin @1.00 mgl^{-1}	8.77 (17.20)	61.43 (51.58)	6.83 (15.11)	16.34 (23.82)	1.69 (7.46)	2.36 (8.82)	3.26 (10.4)	9.30 (17.73)
T6	BAP @1.50 mgl^{-1}+Kinetin @1.50 mgl^{-1}	9.97 (18.38)	72.67 (58.45)	8.97 (17.40)	15.01 (22.75)	1.94 (7.99)	3.01 (9.96)	4.08 (11.6)	11.31 (19.61)
T7	BAP @1.50 mgl^{-1}+Kinetin @2.00 mgl^{-1}	11.74 (20.01)	51.20 (45.67)	6.17 (14.35)	18.70 (25.59)	1.02 (5.80)	2.05 (8.22)	3.04 (10.0)	7.50 (15.81)
T8	BAP @1.50 mgl^{-1}+Kinetin @2.50 mgl^{-1}	10.31 (18.70)	75.52 (60.32)	9.08 (17.51)	17.22 (24.50)	1.21 (6.30)	2.38 (8.86)	3.21 (10.3)	10.20 (18.61)
T9	BAP @2.00 mgl^{-1}+Kinetin @1.00 mgl^{-1}	9.21 (17.64)	74.67 (59.75)	10.98 (19.32)	15.74 (23.34)	1.34 (6.63)	2.44 (8.98)	3.39 (10.5)	9.36 (17.79)
T10	BAP @2.00 mgl^{-1}+Kinetin @1.50 mgl^{-1}	7.18 (15.50)	76.65 (61.09)	11.19 (19.52)	11.74 (20.02)	2.17 (8.46)	3.60 (10.9)	4.56 (12.3)	12.62 (20.75)
T11	BAP @2.00 mgl^{-1}+Kinetin @2.00 mgl^{-1}	10.42 (18.80)	70.05 (56.83)	10.23 (18.63)	15.22 (22.94)	1.23 (6.36)	2.66 (9.34)	3.63 (10.9)	8.71 (17.65)
T12	BAP @2.00 mgl^{-1}+Kinetin @2.50 mgl^{-1}	9.23 (17.66)	75.31 (60.19)	9.26 (17.69)	17.22 (24.50)	1.53 (7.02)	2.67 (9.36)	3.87 (11.3)	10.08 (18.85)
T13	BAP @2.50 mgl^{-1}+Kinetin @1.00 mgl^{-1}	9.35 (17.78)	65.75 (54.16)	8.38 (16.80)	16.82 (24.20)	1.88 (7.83)	3.52 (10.7)	3.81 (11.2)	8.19 (17.17)
T14	BAP @2.50 mgl^{-1}+Kinetin @1.50 mgl^{-1}	11.33 (19.65)	53.49 (46.98)	11.23 (19.18)	12.99 (21.11)	1.67 (7.36)	3.00 (9.94)	4.17 (11.7)	9.83 (18.24)
T15	BAP @2.50 mgl^{-1}+Kinetin @2.00 mgl^{-1}	9.27 (17.70)	60.64 (51.12)	10.37 (18.76)	16.72 (24.11)	2.08 (8.23)	2.75 (9.51)	3.65 (10.9)	10.27 (18.67)
T16	BAP @2.50 mgl^{-1}+Kinetin @2.50 mgl^{-1}	10.33 (18.72)	64.62 (53.48)	7.33 (15.67)	17.50 (24.70)	1.98 (8.07)	3.13 (10.1)	3.74 (11.1)	8.84 (17.26)
C.D.		1.726	1.836	1.674	1.708	1.378	1.280	1.197	1.975
SE(m)		0.597	0.635	0.579	0.590	0.673	0.442	0.413	0.682

Table 1: Effect of different combination of BAP and Kinetin on *in vitro* establishment of shoot under CHU media

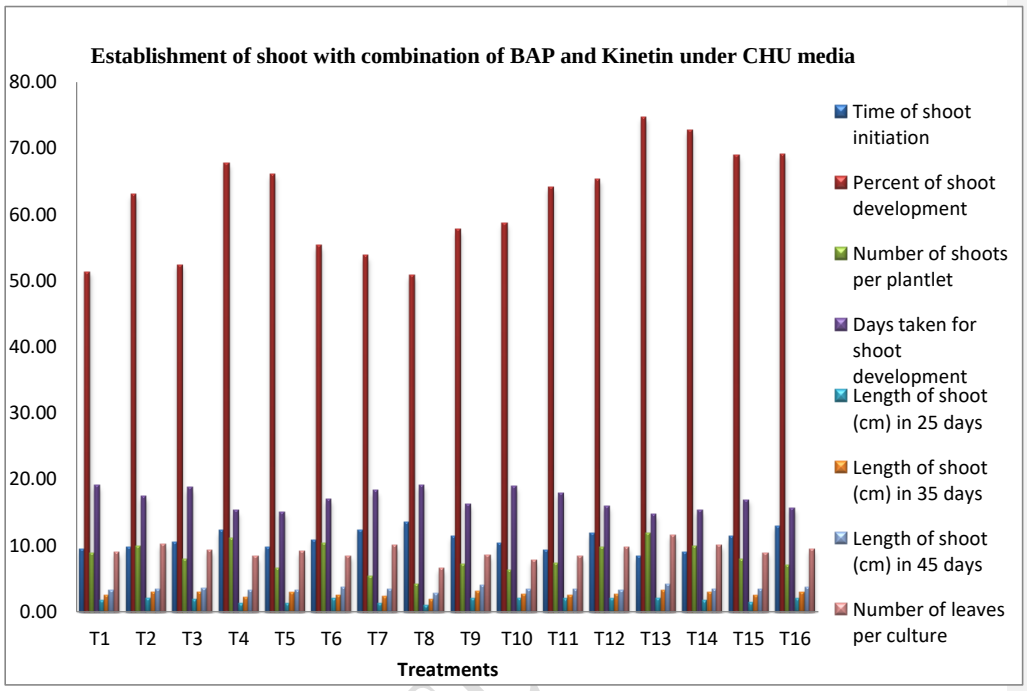


Figure-3 *In vitro* establishment of shoot with combination treatments of BAP and Kinetin under CHU media.

Comment [H12]: Cite this in running text

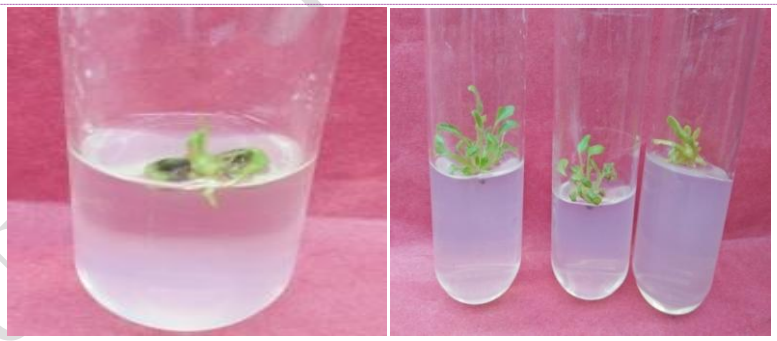


Figure-4 Combination treatment of BAP and Kinetin on *in vitro* establishment of shoot under CHU media.

Comment [H13]: Cite this in running text

Conclusion

The present investigation explained that the behavior of callus to shoot multiplication to use combination of BAP and Kinetin under CHU media. The best treatment found of this experiment

was combination BAP @1.50 mgl^{-1}+ Kinetin @ 2.00 mgl^{-1}.

References

1. Ali A, Afrasiab H, Naz S, Rauf M, Iqbal J. An efficient protocol for *in-vitro* propagation of Carnation (*Dianthus caryophyllus*). Pakistan Journal of Botany. 2008; 40(1):111-121.

2. Bhatia R, Singh KP, Singh MC. Effect of growth regulators on regeneration from leaf derived callus and shoot proliferation in gerbera. Indian Journal of Horticulture. 2008; 65:312–316.

3. Buah JN, Danso E, Taah KJ, Abole EA, Bediako EA, Asiedu J, Baidoo R.The effect of different concentrations cytokinins on the *in vitro* multiplication of plantain (*Musa* sp.). Asian Network for Scientific Information. 2010; 9 (3): 343-347.

4. Cardoso JC, Dasilva, JAT. Micropropagation of gerbera using chlorine dioxide (ClO_2) to sterilize the culture medium. *In Vitro* Cellular & Developmental Biology – Plant. 2012;48: 362-8.

5. Cardoso JC, Dasilva JAT. Gerbera micropropagation. Biotechnology Advances. 2013; 31:1344-1357.

6. Can E, Celiktas C, Hatipoglu R. Effect of auxin type and concentrations in different media on the callus induction and shoot formation of crested wheatgrass (*Agropyron cristatum*). *Biotechnol*. 2008; 22: 782-786.

7. Bhargava B, Dilta BS, Gupta YC, Dhiman SR, Modgil M. Studies on micropropagation of Gerbera (*Gerbera jamesonii* Bolus). Indian Journal of Applied Research. 2013; 3 (11): 2249-555.

8. Gaurav S, Srivastava R. Combinations and concentrations of growth regulators for somatic embryogenesis in Gerbera. Annals of Agricultural Research. 2005; 26(2): 214-217.

9. Hasbullah NA, Taha RM, Awal A. Growth optimization and organogenesis of *Gerbera jamesonii* Bolus ex. Hook *in vitro*. *Pakistan* Journal of Biological Sciences. 2008; 11 (11): 1449-1454.

10. Ikenganyia EE, Anikwe MAN, Omeje TE, Adinde JO. Plant Tissue Culture Regeneration and Aseptic Techniques. Asian Journal of Biotechnology and Bio-resource Technology. 2017; 1(3): 1-6.

11. Kumar S, Mangal M, Dhawan AK, Singh N. Callus induction and plant regeneration from leaf explants of Jojoba. Indian Journal of Biotechnology. 2013; 12: 544 -547.

12. Kadu AR. *In vitro* micropropagation of Gerbera using auxillary bud. Asian Journal of Bio Science. 2013; 8(1): 15-18.

13. Kanwar JK, Kumar S. Plant regeneration from callus and cell suspension cultures of *Gerbera jamesonii*cv. 'Diablo'. European Journal of Horticultural Science. 2005; 70(6): 265–270.

14. Mohameda SA, Ozzambakb ME. Shoot regeneration capacity of *in vitro* cultures of some Gerbera (*Gerbera jamesonii* Bolus) explants. Sudanese Journal of Agricultural Sciences. 2014; 1, 24–29.

15. Mariam A, Ali AM, Igbal A. Evaluation of micro and macro propagation techniques of Gerbera (*Gerbera Jamesonii*) under different conditions. Horticultural Research Center. Agricultural Research Corporation. 2010; P.O. Box 126, Wad Medani, Sudan.

16. Mahindrakar A, Kumar R, Aswath C. Callusing and regeneration response of *in vitro* derived leaf explants in gerbera. International Journal of Current Research. 2016; 8(11): 42039-42042.

17. Naz S, Naz F, Tariq A, Aslam F, Ali A, Athar M. Effect of different explants on *in vitro* propagation of gerbera (*Gerbera jamesonii*). African Journal Biotechnology. 2012; 11: 9048-9053.

18. Pradhan S, Yagya P, Pant B. Efficient regeneration of plants from shoot tip explants of *Dendrobium densiflorum* Lindl. a medicinal orchid. African Journal of Biotechnology. 2013; 12 (12): 1378-1383.

19. Swetha NT. Studies on Tissue Culture in Gerbera (*Gerbera jamesonii* L.). International Journal of Agriculture Sciences. 2017; 9(17) 4162-4165.

20. Prasad MP. *In-vitro* optimization of growth hormones in the micropropagation of Gerbera species. International Journal Current Biotechnology. 2014; 2(2):1-5.

21. Shabanpour K, Sharifi A, Bagheri A, Moshtaghi N. Effect of genotypes and culture medium on shoot regeneration and proliferation of *Gerbera jamesonii*. African Journal of Biotechnology. 2011; 10(57): 12211-12217.

22. Son NV, Mokashi, Hedge AN, Hedge RV, Patil VS, Lingaraju S. Response of Gerbera (*Gerbera jamesonii*Bolus) varieties to micropropagation. Karnataka Journal of Agriculture Science. 2011; 3: 354-357.

23. Sharma G, Srivastava R. Combinations and concentrations of growth regulators for somatic embryogenesis in Gerbera. Annals of Agricultural Research. 2005; 26: 214-217.

24. Shabbir K, Ahmad T, Hafiz IA, Hussain A, Abbasi NA, Ahmad J. *In vitro* regeneration of *Gerbera jamesonii*cv. African Journal of Biotechnology. 2012; 11: 9975-9984.

25. Tyagi P, Kpthari SL. Rapid *in vitro* regeneration of *Gerbera jamesonii* (H. Bolus ex Hook. f.) from different explants. Indian Journal of Biotechnology. 2004; 3(4):584-588.

26. Vijayalakshmi CL, Babu P, Bagali AN, Soregaon CD, Wadageri AH. Direct *in vitro* Regeneration of Gerbera (*Gerbera jamesonii* Bolus). International Journal of Current Microbiology and Applied Sciences. 2019; 8(1): 2610-2619.

27. Winarto B, Yufdy MP. Establishment of *In vitro p*ropagation protocol of (*Gerbera jamesonii* Bolus) explant and media selection to plantlet acclimatization. Journal of Agricultural Science. 2017; 32–40.

28. Warar MH, Kulkarni BS, Jagadeesha RC, Reddy BS. Effect of Cytokinins with Auxin on proliferation of multiple shoots in gerbera (*Gerbera jamesonii* B.) var. Sciella. Karnataka Journal of Agricultural Sciences. 2008; 21(4): 597-599.

Comment [H14]: Not cited in the text. Please check