A Protocol for Rapid Multiplication of *Gerbera jamesonii* L. –A popular Cut Flower through Organogenesis

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Abstract

A protocol for rapid multiplication of regenerated plants from callus, initiated from mature seeds of gerbera has been developed. Effect of gerbera genotypes and levels of IBA was investigated for callus formation and subsequent morphogenesis. Frequencies of callus were variable both with genotypes and IBA concentrations. 'Pale yellow' genotype and IBA 2 mgL^{-1} were identified most promising for callus differentiation. Multiple shoot regeneration was achieved on MS medium fortified with BAP 2.0 mgL^{-1} + IAA 1.0 mg L^{-1}. *In vitro* grown shoots were rooted on MS medium supplemented with IBA- 0.5 mgL^{-1}. Maximum shoot regeneration (96%), shoot number $callus^{-1}$ (9.86) and rooting (100%) was noticed for 'pale yellow' gerbera while 'white' genotype showed minimum plant regeneration (76%) and rooting (75%). Rooted plants were hardened before transplanting to soil. Survival rate of regenerated plants ranged from 57% to 89%. Maximum survival rate was found for the genotype 'pale yellow' and lowest was recorded in 'white' gerbera genotype.

Key words: Gerbera, Genotypes, Seed, Growth regulators, Callus, Organogenesis

Introduction

Gerbera (*Gerbera jamesonii* L.) is a cut flower belonging to Asteraceae family. In Bangladesh, public fascination is gradually increasing particularly in town areas about gerbera as ornamental and home decorative plants for its attractive colors and size. However, the supply of gerbera plantlets is not adequate to accomplish the local demand. It may be said here that Bangladesh has a favorable climatic condition and is capable of producing a wide range of gerberas of international standard (www. mdgbangla.org). Gerbera is also propagated through biological seeds (Murashige *et al.*, 1974). Vegetative propagation in one sense is better than sexual reproduction, because there always exist a chance of genetic segregation in the progeny if propagated by seeds and it requires longer time to produce flower. In other respect sexual reproduction has also some advantages. Development of new genotypes through hybridization followed by seed propagation is an excellent technique in gerbera breeding. Gerbera propagation through seeds may be done directly in the field or in *in vitro* condition. In *in vitro* seed culture multiple shoot formation is general phenomenon that offers huge plantlets production from single culture.

Gerbera is an exotic ornamental plant to Bangladesh with very short history of introduction. Earlier its cultivation was only confined to Godkhali of Jessore district, but now cultivation of gerbera has been spreading over a vast areas of Bangladesh due to high demand and profit. As gerbera cultivation has been expanding, the demand on propagation materials is also increasing. Still gerbera is propagated in Bangladesh through conventional method by division of clumps, but this technique cannot led to efficient solution for producing required amount of quality propagation materials. *In vitro* propagation is needed for rapid and mass

propagation of gerbera. Micropropagation techniques of gerbera have been reported in many countries, however majority of these available reports are restricted to direct regeneration through the explants of vegetative plant parts (Cardoso and Teixeira da Silva, 2013). The present study aims to develop an efficient protocol for rapid propagation of gerbera via organogenesis using seed explant to meet commercial needs.

Materials and Methods

Mature seeds of five gerbera genotypes *viz.* Yellow, Red, Pale yellow, Pink and White were used as explant in the present study. The materials were collected from the Gerbera Research Center of Agrotechnology Discipline, Khulna University. The experiment was laid out according to Completely Randomized Design (CRD).

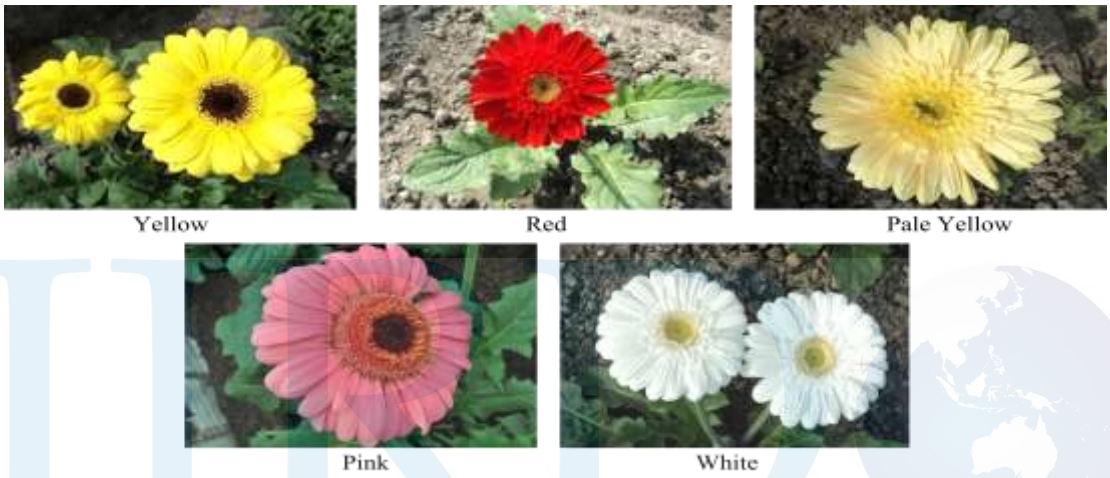

Figure 1: Gerbera genotypes used in the experiment

Gerbera seeds were surface sterilized by dipping them in 70% ethanol followed by treating with 0.2% mercuric chloride for 10 minutes then washed three times with autoclaved distilled water. Surface sterilized seeds were inoculated on MS (Murashige and Skoog, 1962) medium fortified with different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mgL^{-1}) of Indole-3-butyric acid (IBA) and the cultures were kept in the dark for four weeks at 25±1 ^{0}C temperature to induce callus. Then the calli were transferred to MS medium supplemented with 6-Benzylaminopurine (BAP) 2.0 mgL^{-1} and Indole-3 acetic acid (IAA) 1.0 mgL^{-1} for multiple shoot differentiation. The cultures were incubated at 25±1 ^{0}C under white LED light (3000 lux) maintaining 16 hours photoperiod. The cultures were subjected to two successive subcultures at four weeks interval on the same medium. Regenerated shoots were transferred onto MS medium supplemented with 0.5 mgL^{-1} IBA for root induction. In all the cases for solidifying the media 08% agar was used and the pH of the media was adjusted to 5.8.

The well rooted plantlets were transferred to small plastic glass containing sterilized soil, vermicompost, coarse sand and cocodust (2:1:1;1) and were kept in shade and moist condition for 15 days.. After that plantlets were transferred to net house. The established plants finally were transferred to the main gerbera field.

Results and Discussion

Mature botanical seeds of gerbera after surface sterilization were cultured on solidified MS medium supplemented with various levels of IBA to allow callus formation. Proliferation of callus tissues was observed within three weeks of culture initiation.

The gerbera genotype exerted a remarkable effect on callus induction from mature seeds. Frequencies of callus proliferation ranged from 78.4% to 91.8%. Among the genotypes studied 'Pale yellow' performed better in callus induction (91.8±2.86) followed by the 'White' (86.8±2.59%), while 'Pink' genotype showed lowest callus (78.4±2.30) frequency.

Table 1. Callus proliferation and shoot differentiation from plated calli in five gerbera genotypes

Genotypes	Seeds inoculated per replication	Callus induction (%)±SD	No. of calli plated	Frequency of shoot regenerated (%)±SD	No of shoot callus^{-1}±SD
Yellow	25.0	80.6±3.36	20.0	85.4±3.05	6.6±1.52
Red	25.0	84.8±1.92	21.0	91.8±1.79	8.6±1.82
Pale yellow	25.0	91.8±2.86	23.0	95.8±3.83	9.8±4.15
Pink	25.0	78.4±2.30	19.0	79.6±1.95	7.2±2.86
White	25.0	86.8±2.59	21.0	76.0±2.24	7.6±3.58

The experiment also revealed that among all concentrations, IBA- 2.0 mgL^{-1} was more suitable for callus proliferation. These results are in agreement with Chen *et al.* (1982) who observed that callus induction frequency varied with genotypes. Wu and Chen (1987) also observed that frequency of callus induction percentages differ significantly among the genotypes.

After four weeks, calli of convenient size were transferred onto solid MS medium supplemented with 2.0 mgL^{-1} BAP and 1.0 mgL^{-1} IAA. Two subsequent subcultures were done at four week interval on the same medium which resulted in organogenesis and differentiation of multiple shoots in the callus tissues.

Table 2. Effect of growth regulators on callus induction from inoculated gerbera seeds

Levels of IBA (mgL^{-1})	Seeds inoculated per replication	Frequency of callus induction (%)±SD
0.5	25.0	69.4±1.67
1.0	25.0	73.8±1.79
1.5	25.0	84.2±4.03
2.0	25.0	91.6±3.21
2.5	25.0	75.2±2.78

Calli of the genotype 'Pale yellow' performed better in shoot regeneration frequency (95.8±3.83) followed by the variety Red (91.8±1.79). The 'White' genotype showed the lowest frequency in shoot regeneration (76.0±2.24) from inoculated calli. Multiple shoot differentiation was also found genotype dependent. Responded calli of 'Pale yellow' genotype produced maximum shoots (9.8±4.15) culture^{-1} compared to other genotypes tested. 'Yellow' gerbera showed very poor (6.6±1.52) response in *in vitro* shoot formation from responded calli in regeneration media. Variations in plantlet regeneration due to genotypic differences were also reported by Islam *et al.* (2005). Naqvi *et al*. (2005) reported that regeneration frequency depends on genotype and on compositions of regeneration medium as well as concentrations of growth regulators.

Table 3. Rooting of *in vitro* grown shoots and survivability of regenerated plantles of gerbera genotypes

Genotypes	No.of shoot cultured	Frequency of shoot forming root (%)±SD	No. of roots shoot^{-1} ±SD	Root length (mm)±SD	Survivability of shoot (%)±SD
Yellow	30.0	98.6±1.52	2.4±1.14	19.8±3.70	67.4±2.97
Red	40.0	83.4±2.70	6.8±1.64	21.4±2.07	74.8±3.03
Pale yellow	45.0	99.2±0.84	7.2±2.38	18.6±2.70	89.2±3.35
Pink	35.0	80.4±5.86	5.6±1.14	23.2±1.48	71.8±2.39
White	35.0	75.2±3.03	3.6±2.19	24.4±2.41	55.8±1.30

In vitro grown shoots of gerbera were rooted on MS medium containing IBA- 0.5 mgL^{-1}. Root induction on regenerated shoots also varied with genotypes and the frequencies of root varied 75.2 % to 99.2% (Table 3). Shoots of the variety 'Pale yellow' performed better in root induction frequency (99.2±0.84) followed by the genotype 'Yellow' (98.6±1.52) while the genotype 'White' showed lower response in root induction (75.2±3.03).Variable data also recorded for root number and root length as well (Table 3).

Well rooted regenerated plantlets were transferred to small plastic pots containing soil, vermicompost, coarse sand and coco dust (2:1:1:1) and were kept under indirect sun light and high moist condition for two weeks for hardening.

Survivability of the regenerated plants was counted (Table 3). Among the genotypes, in 'Pale yellow' maximum (89.2±3.35 %) plants survived in *ex vitro* condition while minimum (55.8±1.30) rate of survivability was recorded for the plantlets of the cultivar 'White'. Gnanesh *et al.* (2012) reported genotypic differences in plantlet survivability.

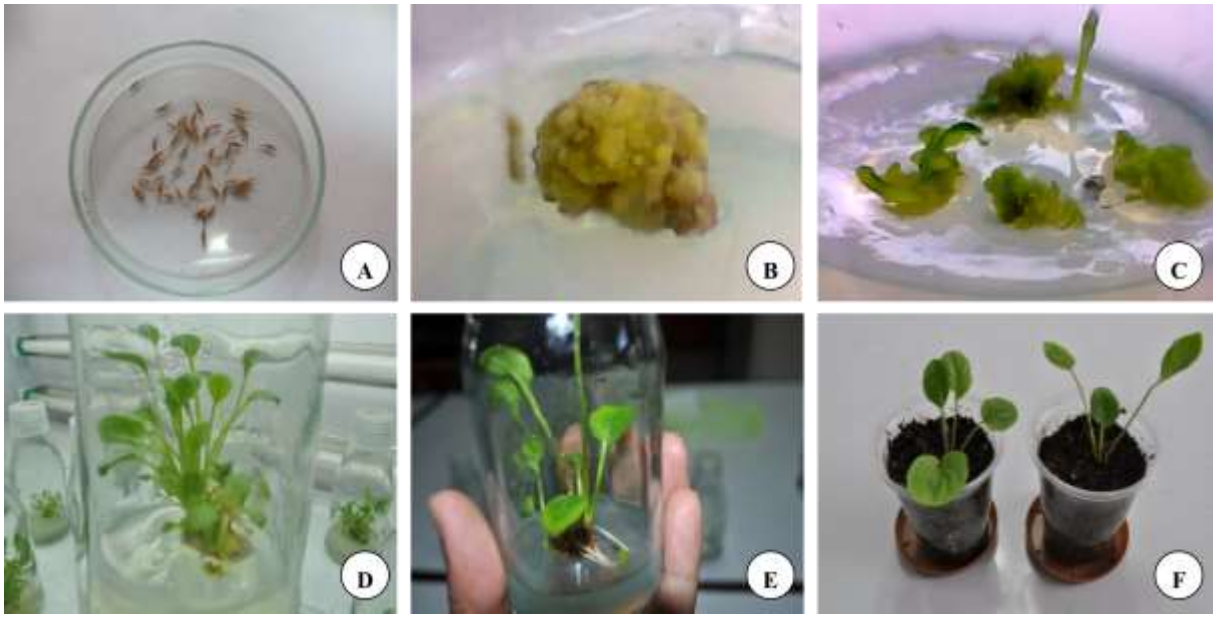

Figure 2. Different stages of *in vitro* micropropagation of gerbera genotypes on growth regulators supplemented MS. A. Mature seeds of gerbera. B. Callus produced from seed *in vitro*. C. Regeneration from seed callus. D. *In vitro* regenerated plantlets. E. Well rooted plantlets *in vitro*. F. *Ex vitro* survived plantlets.

Conclusion

All the studied genotypes responded to morphogenesis, although Pale yellow coloured gerbera genotype performed comparatively better. So this protocol of *in vitro* propagation can be recommended for mass micropropagation of gerbera.

Acknowledgement

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