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RESEARCH ARTICLE

A SUSTAINABLE TECHNIQUE OF RAPID MULTIPLICATION OF GERBERA (*Gerbera jamisonii* L.) USING IN VITRO SEED CULTURE

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ABSTRACT

The Gerbera production continues to experience propagation problem from division of clumps. Traditional approaches of propagation through clumps have wide range of limitation. In order to address the problem of propagation technique in a system designed to develop a sustainable protocol using *in vitro* seed culture was assessed. The goal of the present research is to develop a viable protocol for rapid propagation of Gerbera. The specific objective was to select a superior genotype responding to mass *in vitro* propagation by using seed explant. Sterilized seeds of nine gerbera genotypes were germinated on MS medium supplemented with BAP 3.0 mgL^{-1}. *In vitro* grown seedlings after 30 days of seed culture were transferred onto MS medium containing BAP 2.0 mgL^{-1} + NAA 1.0 mgL^{-1} to allow multiple shoot formation. Elongated shoots were rooted on MS medium supplemented with IBA 1.0 mgL^{-1} for rooting. The regenerated plantlets were transferred to field after hardening under controlled environment. All the steps of this propagation system i.e. seed germination, multiple shoot differentiation, rooting and hardening was highly genotype dependent. Results indicate that genotype 'Kristen' showed highest germination frequency and number of leaves $plant^{-1}$, Aladin exhibited the higher shoot regeneration, Silvester as well as Wink genotype showed greater response in *in vitro* shoot elongation. Pepe genotype showed maximum root formation and root elongation. Maximum survivability rate was found with the genotype Kristen while lowest was recorded in Sonata. Kristen, Aladin and Alabanda may be the suitable gerbera plant types for *in vitro* propagation through seed explant.

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INTRODUCTION

Gerbera (*Gerbera jamesonii*) belongs to Asteraceae family is an important cut flower. It is a diploid species with somatic chromosome number 2n=50 (Sane and Gowda, 2009).The genus Gerbera consists of 30 species, which are of Asiatic and African origin (www.flower.org). Gerbera was introduced in Bangladesh from neighbouring country India about two decades ago and cultivation of this flower was concentrated only in Jessore district. At present gerbera cultivation has been extended over a vast area of the country. Local, as well as global demand on gerbera is increasing gradually and gerbera could be a major source of foreign exchange as one of the non-traditional export items in Bangladesh. It may be mentioned here that Bangladesh has a favourable climatic condition and is capable of producing a wide array of gerberas of international standard (Akter *et al.,* 2012). Instead of these admiring situations of gerbera cultivation most delimiting factor is supply of quality propagation materials. For commercial production of gerbera the farmers need continuous supply of quality plant propagules and new lucrative cultivars. So, scarcity of propagules and new cultivars are the major constraints of gerbera cultivation in Bangladesh. Gerbera is usually propagated by division of clumps. This method has some limitations; a single plant in a season can produce only 2-3 plants. Gerbera can be propagated through seeds that requires much time to flower and seeds of gerbera are very sensitive to germination in natural conditions and germination frequencies are not appreciable (Kessler, 1999), seed production and preservation techniques are also difficult. A well accepted progressive method known as plant tissue culture or *in vitro* method of propagation has been employing in many countries for propagating plantlets of commercially viable and important elite plants. Tissue culture system has already been proven as healthy and mass propagation system in many plant species. For the case of gerbera this method of propagation also known as micropropagation has been reported the best for mass commercial production of quality plant propagules (Palai *et al.,* 1998; Aswath and Choudhary, 2002 and Zhang, 2002). Many researchers have reported protocols for multiplication using explants for instance capitulum segments, leaf blade, leaf midrib, petiole, shoot tip or meristem (Kanwar and Kumar,

2008) etc. However, reports on *in vitro* propagation of gerbera using seed explants are rare. Propagation through seeds leads to produce unlike plant types because each gerbera cultivar is highly heterozygous, so segregation is common phenomenon in seed propagation. Variability and genetic segregation is highly preferred method in plant breeding for developing new cultivars. In that sense *in vitro* seed germination followed by micropropagation may offer an alternative way to successful breeding and propagation of gerbera. In this system multiple shoot formation from a single seed is very common (Jahan, 2016), whereas in traditional system one seed can produce a single plantlet. It is hypothesis that *in vitro* seed culture for sustainable multiplication technique can be used as a rapid multiplication technique that ensure quality plant propagule from selected Gerbera genotype. The goal of the present research was to develop a viable protocol for rapid propagation of Gerbera. The specific objective was to select a superior genotype responding to mass *in vitro* propagation by using seed explant.

MATERIALS AND METHODS

Mature seeds of nine gerbera genotypes *viz.* Spark, Melone-pink, Alabanda, Aladin, Kristen, Wink, Pepe, Silvester and Sonata collected from Gerbera Research Centre, Agrotechnology Discipline, Khulna University, Bangladesh. The seeds were washed with autoclaved distilled water followed by 70% ethanol treatment for 30 seconds and then immersed in 10% aqueous solution of Sodium hypochlorite for 10 minutes. Finally they were washed three times with autoclaved distilled water.

Figure 1. Gerbera genotypes used for *in vitro* seed culture

Surface sterilized seeds were cultured on solid MS (Murashige and Skoog, 1962) medium containing BAP-3.0 mgL^{-1}. The cultures were incubated in growth room maintaining a 16 hours of photoperiod with a temperature of 25 ^0C, light intensity 3000 lux. After 30 days of culture germinated plants (seedlings) were transferred to multiple shooting media (Agarified MS medium containing BAP 2.0 mgL^{-1} + NAA 1.0 mgL^{-1}), culture environment was same as previous. Sub-culture was done twice at 30 days interval on the same medium. Data on shoot number $culture^{-1}$, plant height (mm) and leaf number $plant^{-1}$ were recorded. The plantlets produced were transferred to MS medium supplemented with IBA 1.0 mgL^{-1} for rooting after 30 days of last sub-culture. Data were collected on root growth after 45 days. Regenerated plantlets were transferred to pots containing mixture of garden soil, coarse sand and vermicompost at the ratio of 1:2:1 and were kept in hardening room. Survived plants were transferred to gerbera shed house after hardening. The experiment was laid out in Completely Randomized Design (CRD), all recorded data were arranged and analysed for ANOVA in computer with the statistical package MSTAT-C. Mean data were compared by Duncan New Multiple Range Test.

RESULTS AND DISCUSSION

In vitro seed germination

Surface sterilized mature seeds of nine gerbera genotypes were cultured on solid MS medium supplemented with BAP 3.0 mgL^{-1} to allow germination. Germination frequency was found genotype dependent and was statistically significant. The frequencies varied from 70.0 to 98.33% with a LSD value of 8.056. Genotype 'Kristen' showed maximum while the genotype 'Pepe' showed minimum germination frequency.

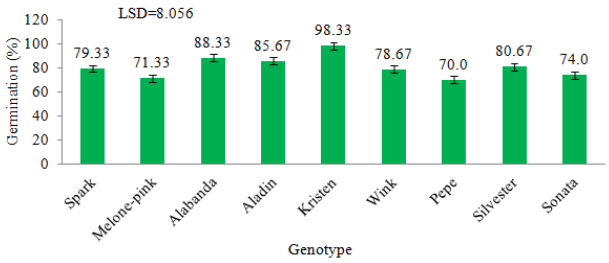

Figure 2. *In vitro* seed germination of nine gerbera genotypes

Multiple shoot differentiation

One month old *in vitro* grown seedlings were cultured on MS medium supplemented with BAP 2.0 mgL^{-1} and NAA 1.0 mgL^{-1} for shoot multiplication. Multiple shoot differentiation was achieved within 60 days of culture. As a results from this experiment testify, gerbera genotypes showed a significant variation in multiple shooting that ranged from 6.0 to 27.75 $culture^{-1}$. Genotype 'Aladin' produced highest numbers of shoot whereas the genotype 'Melone-pink' produced lowest number of shoots $culture^{-1}$ (Table 1). Variable data were also noticed for shoot length and leaf number as well and these two parameters were also identified as genotype dependent. At the end of second subculture i.e. after 60 days of seedling culture best response for shoot length exhibited by the gerbera genotype 'Wink' followed by 'Pepe' and the lowest performance was noticed for 'Spark", 'Melone-pink' and "Sonata' (Table 1). Leaf number was different with the genotype and varied from 5.5 to 8.75 $shoot^{-1}$. Genotypic dependent shoot proliferation was also observed by Jahan (2016), she reported that gerbera genotype with pink and yellow coloured flower performed best in multiple shoot formation from seed explant *in vitro*. According to many other researchers organogenesis is highly genotype dependent but they also indicated that media composition, hormonal adjustment and cultural environment significantly influences morphogenesis in gerbera (Cardoso and Teixeira da Silva, 2013; Mariam *et al.,* 2010; Modh *et al.,* 2002; Singh *et al.,* 2016; Son *et al.,* 2011).

In vitro rooting

Induction of healthy root system from the developed shoots is an essential part for successful development of plantlets. Well developed shoots were transferred to MS medium supplemented with IBA (1.0 mgL^{-1}) for root induction. Inoculated shoots started rooting within 4 weeks of culture and data on rooting were taken after 45 days of transference of shoots on rooting medium (Table 3). The highest root number found in Pepe genotype which was statistically similar with Silvester while the lowest number of root recorded in Sonata genotype which was statistically similar with Wink.

Table 1. Effect of genotypes on different shoot parameters of gerbera genotypes

Genotypes	Shoot parameters		
	No. of shoots culture^{-1}	Shoot length (mm)	No. of leaves shoot^{-1}
Spark	19.25b	25.79de	6.50bcd
Melone-pink	6.00d	24.64e	5.50d
Alabanda	20.50bc	35.67bc	7.75ab
Aladin	27.75a	30.94cde	7.25abc
Kristen	22.25b	33.48cd	8.75a
Wink	15.00c	44.15a	5.50d
Pepe	14.75c	41.65ab	6.00cd
Silvester	16.50bc	27.21de	6.50bcd
Sonata	16.00bc	23.70e	5.50d
Level of Significance	**	**	**
CV (%)	22.97	16.51	15.89

**= Significant at 1% level

Table 2. Effect of gerbera genotype on root number and root length

Genotype	Root numbers	Root length (mm)
Spark	5.00bc	8.29bc
Melone-pink	6.50abc	11.58bc
Alabanda	6.00abc	17.73b
Aladin	8.75ab	15.97bc
Kristen	7.25abc	18.10b
Wink	4.25c	13.86bc
Pepe	9.50a	33.71a
Silvester	9.25a	15.71bc
Sonata	4.00c	7.05c
Level of Significance	*	**
CV (%)	23.65	14.10

*= Significant at 5% level, **= Significant at 1% level

The maximum length of root was recorded in genotype Pepe (33.71 mm) while the genotype Sonata (7.05 mm) showed little response in root production compared to other tested genotypes. Rahman *et al.* (2014) observed regenerated shoots were rooted in MS medium with IAA or IBA and maximum frequency of rooting with highest number of roots shoot^{-1} was achieved in MS medium fortified with IBA (0.3 mgL^{-1}). Kanwar and Kumar (2006) also reported that IBA was the best medium for root induction in Gerbera. Akter *et al.* (2012) observed that of MS with IBA (0.2 mgL^{-1}) showed 95 - 100% for root induction in the red, yellow and white genotypes. Singh *et al.* (2016) obtained 100% rooting in red, yellow and white genotypes from the cultured on half MS containing IBA 0.2 mgL^{-1} within 15-20 days of root initiation. Jahan (2016) observed that Red genotype had the maximum root length Table 2.

Hardening of regenerated plants

Well rooted regenerated plantlets were transferred to plastic pot containing garden soil, coarse sand and vermicompost mixture (1:2:1). The survivability of the plantlets in *ex vitro* condition was also found genotype dependent and varied from 20.00% to 73.33% (Figure 2). Higher survival rate was noticed in gerbera genotype Kristen whereas Sonata genotype exhibited the lowest survival rate. Kaur *et al.* (1999) obtained 100% survival of regenerated plants when transferred to pots filled with a mixture of soil: sand: compost in 1:1:1 ratio. Aswath *et al*. (2003) acclimatized micro propagated plants for 3 weeks and subsequently cultured in a greenhouse using sand, FYM and red soil in 1:1:1 proportion and found maximum of 95% survivability. Gnanesh *et al.* (2012) reported genotypic differences in plantlet survivability. All the genotypes showed better performance in coarse sand and garden soil and survivability recorded to 40% to 80% (Jahan, 2016).

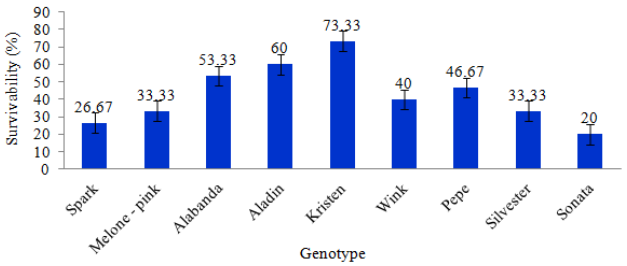

Figure 3. *Ex vitro* survivability of regenerated plants of different gerbera genotypes

The present study elucidates an easy method for direct *in vitro* shoot multiplication through seed culture in a number of gerbera genotypes. Direct regeneration is advantageous over callus culture as it saves time and cost and also maintains genetic stability. Kristen, Aladin and Alabanda genotypes appear to have the best potential for rapid sustainable propagation from *in vitro* seed culture technique. Future investigation including more genotypes and explants will be needed for better understanding of the viable multiplication technique.

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