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IN VITRO SHOOT REGENERATION FROM COTYLEDON AND HYPOCOTYL EXPLANTS OF DAHLIA CULTIVARS

B. Fatima, Muhammad Usman*, T. Ashraf, R. Waseem and M.A. Ali
Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad.
*Corresponding author's E-mail: muhammadusman30@yahoo.com

Different exotic dahlia cultivars namely Double Decorative and Double Giant were explored for shoot regeneration using cotyledon leaf and hypocotyl explants on MS medium supplemented with combinations of plant growth regulators (PGRs) viz. NAA and BA (A medium) and BA and IAA (B medium). Callus induction and shoot regeneration was found directly proportional while root formation was inversely proportionate to the addition of PGRs in the media. Maximum response for callus induction (60%) and shoot regeneration (13%) was found in cotyledon leaf and hypocotyl explants, respectively, on medium containing 3 mgL^{-1} each of NAA and BA. Significant genotypic variation was observed and cultivar Double Giant was found better for callus induction and organogenesis at both media types. Indirect shoot regeneration could be helpful for mass propagation of elite plant material and enhancing diversity in the ornamental plants.
Keywords: Dahlia, shoot regeneration, NAA, BA, cotyledon leaf.

INTRODUCTION

Dahlia, member of *Compositeae* family, is a beautiful gift of nature and is an important garden plant due to its diversity in colors, size, shapes, forms and profusion of flowering (De Hertegh, 1989). It is grown as both annual and perennial plant. All the dahlia cultivars are derived from one immensely variable specie *D. variables* or *D. pinnata* (Chittenden, 1981). Dahlias are also medicinally important as its tubers contain high amount of insulin and fructose and small quantities of medicinally active compounds such as phytin and benzoic acid. Many important diseases of dahlia are caused by fungal, bacterial and viral sources leading to various types of impairment (Bose and Yadav, 1989) and can be successfully eliminated using micro-propagated plant material (Sediva *et al.*, 2006).

Further, micropropagation of plants is a well known strategy for efficient production and propagation of the elite plant material. It helps in the development and rapid propagation of selected plants with desirable characters in shortest possible time and new cultivars can also be developed by protoplast fusion and genetic modifications (Pierik, 1987). A good micropropagation protocol for dahlia, therefore, could be useful for producing the low cost plant material in bulk. The regeneration of dahlia plants have been reported either directly from explants without callus formation or indirectly through callus induction and regeneration. There are a few reports available on *in vitro* propagation of Dahlia spp. However, in other flowering crops like carnation and gerbera, indirect regeneration is reported from certain explant sources like shoot meristems (Can and Koc, 1994; Poupet *et al.*, 2006), floral buds (Radice and Marconi, 1998; Posada *et al.,* 1999) and hypocotyl (Sharma *et al.*, 1999) on different media and the shoot and root regeneration occurred in the calli (Kongthong *et al.,* 1999). The present study was therefore, aimed to develop an efficient protocol for mass propagation of exotic Dahlia cultivars to enhance their production for growers and the local markets.

MATERIAL AND METHODS

Plant material and sterilization procedures

Seeds of exotic Dahlia cultivars viz. Double Decorative and Double Giant were sterilized using 70% ethanol (v/v) and 5% sodium hypochlorite (v/v) for 5 minutes followed by 3-5 times rinsing with sterilized water. Seeds were placed on MS medium (Murashige and Skoog, 1962) containing $30gL^{-1}$ sucrose and $8gL^{-1}$ for germination and plant development. These plants were multiplied using shoot tip and nodal explants on MS medium and were used as explant source for further callus induction experiments. Cotyledon leaf (CL) and hypocotyl (Hyp) explants were explored for callus induction and organogenesis on MS medium containing plant growth regulators (PGRs) NAA, BA and IAA alone and in combinations as given in table 1.

Media preparation, sterilization and culture conditions

Medium pH was adjusted at 5.8 and the media were sterilized in autoclave at temperature 121°C and 15 psi pressure for 15-20 minutes. After inoculation, cultures were placed in the growth room facilitated with 2500 lux light intensity and 25 ± 2°C temperature for proper plant growth and development.

Table 1. Media formulations for callus induction and organogenesis

Treatments	Plant Growth Regulators (PGRs) in mgL^{-1}		
	NAA	**BA**	**IAA**
A medium			
T_o	-	-	-
T_1	0.1	0.1	-
T_2	0.1	1.0	-
T_3	0.2	0.3	-
T_4	0.2	1.5	-
T_5	0.3	2.0	-
T_6	0.5	0.5	-
T_7	1.0	1.0	-
T_8	2.0	2.0	-
T_9	3.0	3.0	-
B medium			
T_o	-	-	-
T_1	-	0.05	0.05
T_2	-	0.1	0.1
T_3	-	0.2	0.2

Plant acclimatization

The regenerated plants were acclimatized following standard procedures (Khan *et al.*, 2006) and were transferred to greenhouse for plant development.

Data analysis

The experiments were laid out following completely randomized design (CRD) using three replications per treatment. Means were compared using LSD test (Steel and Torrie, 1980)

RESULTS AND DISCUSSIONS

Plant development and multiplication

Sterile plants of exotic dahlia cultivars Double Decorative and Double Giant were raised from sterilized seeds *in vitro* on MS medium (Murashige and Skoog, 1962) and germination was observed as 82% and 91% after 3 weeks of culture, respectively. Plants were multiplied using shoot tip and nodal explant to get explant (cotyledon and hypocotyl) material in bulk for further callus induction and shoot regeneration.

Callus induction and organogenesis

Different PGRs were used in combinations (NAA+BA; A medium) and (BA+IAA; B medium) to induce shoot regeneration in different explants of dahlia cultivars. However, it is worth mentioning that both the explants cotyledon leaf (CL) and hypocotyl (hyp) induced calli that subsequently regenerated into shoots when subcultured on the same medium. Significant genotypic differences among dahlia cultivars were observed for callus induction and organogenesis in CL explant whereas no variation was found for Hyp explant on both A and B media treatments (Fig. 1). However, different combinations of PGRs used significantly affected callus induction and plant regeneration.

Callus induction was increased with the increase in the levels of PGRs in A (NAA+BA) and B (BA+IAA) media for both cotyledon leaf and hypocotyl explants. However, cotyledon leaf was found significantly better for callus induction (60.50%) compared to hypocotyl (50.50%) and the callogenesis was two fold high compared to control in both the explants in A (30.33% and 24.16%, respectively) and B medium (48.00% and 38.33%, respectively). Similar trend was found for shoot induction which was also enhanced with the increasing PGR levels in both A (13.0% and 10.5%) and B (9.8 and 8.0%) media for calli derived from both hypocotyl and cotyledon leaf explants, respectively. Root formation started in the same medium and was inversely proportionate to the addition of PGRs in both A and B media. The efficiency of rooting was better in cotyledon leaf (23.50% and 21.83%) compared to hypocotyl explant (22.50% and 20.33%) in both B and A media, respectively (Table 2 and 3).

Our results showed indirect shoot regeneration from calli induced on MS medium containing BA+NAA (A medium) and BA+IAA (B medium) instead of inducing direct shoot induction from cotyledon leaf and hypocotyl explants, respectively. Several researchers have also reported similar response of the explant tissues on medium containing either of these plant growth regulators (PGRs) alone or in combinations (NAA, BA and IAA) and obtained shoot regeneration through callus formation in Gerbra (Barbosa *et al.*, 1993; 1994) Asparagus (Pontaroli and Camadro, 2005), Aster (Cammareri *et al.*, 2002), Guar (Prem *et al.*, 2005), Saussurea (Dhar and Joshi, 2005), Salvia (Ewa and Halin, 2004) and Piper (Bhat *et al.*, 1992). These findings are in agreement to our results depicting indirect organogenesis. In contrast, there are reports on response of explants on media containing these PGRs and showing direct organogenesis instead of callus formation in Snapdragon (Cui *et al.*, 2004), Dioscorea (Chen *et al.*, 2007), Perilla (Hou and Jia, 2005), Thapsia (Makunga *et al.*, 2005) and chestnut rose (Wen and Deng, 2005) and the rooting was obtained on either same media devoid PGRs (MSO) or half strength media salts and either of NAA or IAA in reduced concentration.

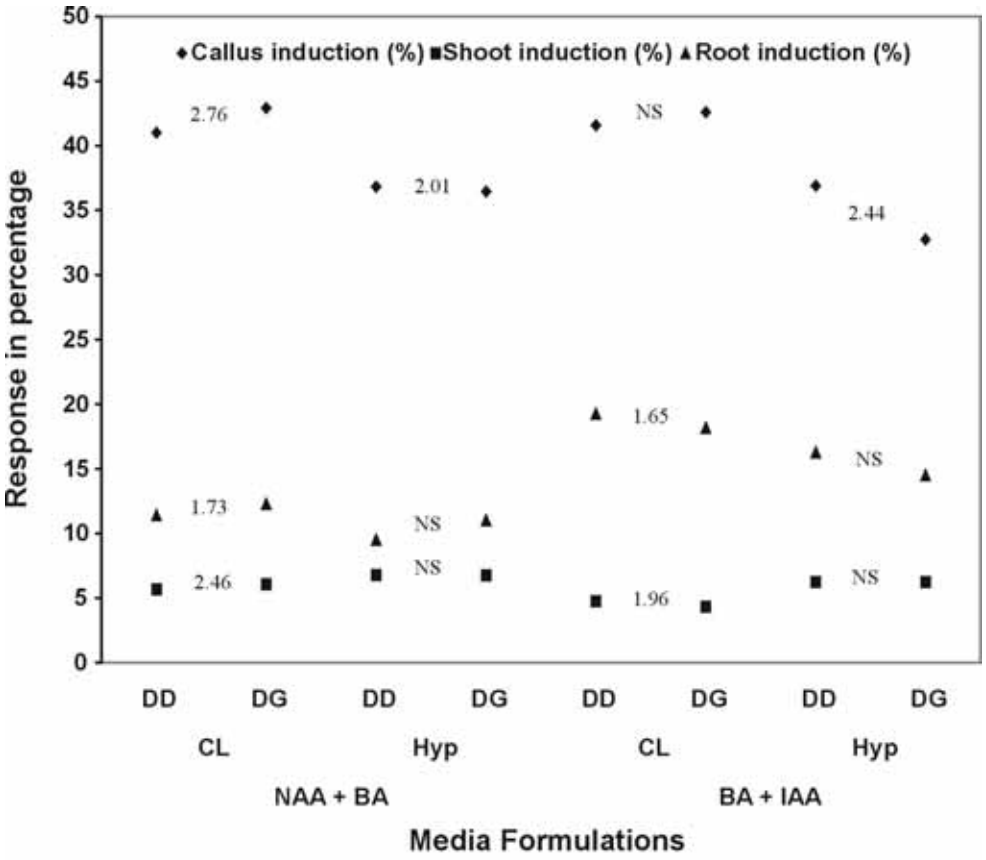

Fig. 1. Genotypic differences observed among dahlia cultivars Double Decorative (DD) and Double Giant (DG) using cotyledon (CL) and hypocotyl (Hyp) explants on MS media containing NAA+BA (A medium) and BA+IAA (B medium) for callus induction, shoot induction and root induction. Given are the LSD values at 0.05 probability.

Table 2. Callogenic and morphogenic response of explants to application of PGRs (NAA+BA) in the A medium

A medium (MS medium+NAA+BA)	Callus induction %		Shoot induction %		Root induction %	
	CL	Hyp	CL	Hyp	CL	Hyp
Control	30.33h	24.16h	1.83g	1.00gh	5.33g	6.66e
0.1 + 0.1mgL^{-1}	32.16gh	27.33g	3.16ef	1.83g	21.83a	20.33a
0.1 + 1.0 mgL^{-1}	33.16fg	30.00f	4.16e	3.50fg	14.66c	14.50b
0.2 + 0.3 mgL^{-1}	35.00f	34.66e	2.66fg	4.50f	17.66b	10.50c
0.2 + 1.5 mgL-^{1}	40.16e	35.00e	3.50ef	6.83de	13.00c	9.00d
0.3 + 2.0mgL-^{1}	41.16e	38.33d	6.33d	9.00bc	10.00de	8.00de
0.5 + 0.5mgL-^{1}	44.66b	40.83c	7.50cd	9.50bc	7.50f	7.16e
1.0 +1.0 mgL^{-1}	48.33c	41.83c	8.66bc	8.00cd	8.83ef	7.33e
2.0 + 2.0 mgL^{1}	54.00b	43.83b	8.83b	10.66b	8.50ef	8.00de
3.0 + 3.0 mgL^{1}	60.50a	50.50a	10.50a	13.00a	11.00d	11.00c
LSD values	1.935	1.421	1.224	1.167	1.744	1.389

Table 3. Callogenic and morphogenic response of explants to application of PGRs (BA+IAA) in the B medium

B medium (MS medium+BA+IAA)	Callus induction %		Shoot induction %		Root induction %	
	CL	Hyp	CL	Hyp	CL	Hyp
Control	33.83d	31.00c	1.16d	3.50c	9.00c	5.66d
0.05 + 0.05 mgL^{-1}	41.83c	33.33d	3.50c	4.16c	23.50a	22.50a
0.1 + 0.1 mgL^{-1}	44.66b	36.66a	5.50b	7.50b	23.50a	18.00b
0.2 + 0.2 mgL^{-1}	48.00a	38.33a	8.00a	9.83a	19.33b	15.33c
LSD values	1.450	1.731	1.172	1.030	1.999	2.357

Indirect organogenesis seems promisingly important in raising somaclonal variants and inducing diversity in the ornamental flowering plants like dahlia that usually lacks in the direct regeneration protocols. Further the system is efficient to be used for mass propagation of the elite cultivars in short time. However, further optimization of PGR levels and use of new explants is suggested for developing direct shoot regeneration in dahlia that could have been used for biotechnology applications.

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