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Studies of callus induction from different explants of gerbera (*Gerbera jamesonii* Bolus Ex Hook F.)

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Abstract
In present study, leaf, leaf with petiole and petiole explants of Gerbera were used as explants for callus induction. A protocol for callus induction and *in vitro* regeneration through organogenesis was established for Gerbera (*Gerbera jamesonii* Bolus ex Hook f.). Callus was induced on Murashige and Skoog (MS) basal medium supplemented with cytokinin (Kn or BA) and auxin (2,4-D/IBA/NAA) from leaf, leaf with petiole and petiole explants of this high economically valuable cut flower. Our study also presented both the approaches i.e. shoot and root multiplication. No significant callusing was observed when MS medium is fortified with 0.5 mg/l of both 2,4-D and Kn in leaf, leaf with petiole as well as petiole explants. The maximum number of callus induction was achieved from leaf explants on MS medium enriched with 2.0 mg/l BA and 1.5 mg/l IBA.

Keywords: Gerbera, explants, callus induction and growth regulators

Introduction
Gerbera is one of Asteraceae family members having high economic value as cut flower with high market demand in Indian as well as global floral industry. (Naz *et al*., 2012) [16]. It is one of the leading cut flower and ranks among the top of leading cut flowers of the world (Parathasarathy and Nagarajun, 1999) [17]. The flower has a long vase life, resistance to transportation damage and no riskiness to obtain a good market price (Chung *et al*., 2016) [5]. Though market demand to the flower increase year by year. However, development of the plants commercially is constrained by availability and sustainability the qualified planting materials. Conventionally, gerbera is generally propagated both vegetatively and generatively (Kanwar, 2008) [10]. The plant multiplication through these methods is too slow to be commercially practicable. For commercialization of this crop, however, planting material is required on large scale which requires the development of easier, quicker and economically viable methods of propagation. The vegetative method is usually carried out by rhizome divisions and cutting. Although this method maintains uniformity and genetic purity, the technique is laborious and time consuming with fewer results. While generatively, the plant is propagated by seeds. However the method produces a higher number of regenerates, the technique results in varied-regenerates and their performances (Rukmana, 1995) [22]. The technique of *in vitro* cultivation of plant cells or organs is primarily devoted to solve two basic problems. Firstly to keep the plants cells and organs free from microbes like bacteria and fungi and secondly to ensure the desired development in the cells and organs by providing suitable nutrient media and other nutrient conditions. Gerbera was propagated by direct or indirect organogenesis using various explants including stem tips floral buds, leaf and capitulum. The plants were produced from explants of capitulum in red flower Gerbera (Pierik *et al*. 1973) [19], leaves (Pierik *et al*. 1974 [18]; Barbosa *et al*. 1994 [2]), floral buds (Posada *et al*. 1999) [20], floral bracts (Maia *et al*. 1983) [13], torus (Zhang, 2002) [24] and inflorescence (Schum and Busold, 1985) [23]. Those methods cannot be used to develop the gerbera for commercial purposes and therefore clonal propagation via tissue culture works are importantly addressed in producing a large number of plants, uniform, vigorous and pathogen free in a short time (Mohammed and Ozzambak, 2014) [14]. Currently, several *in vitro* propagation protocols in different processes used for rapid and large propagation of gerbera (Roger and Tija, 1990 [21]; Erwin *et al*. 1991) [6]. Akter *et al*., (2013) [1] produced high shoots and plantlets by culturing flower buds and stalks in Murashige and Skoog (1962; MS) [15] medium supplemented with BAP and NAA for shoot initiation. Due to high economic value and market demand of Gerbera is increasing day by day. Therefore, the present investigation was undertaken to callus induction from different explants viz. leaf, leaf with petiole and petiole of *Gerbera jamesonii*.

Materials and Methods

For proper cultivation of *Gerbera jamesonii,* basal medium (BM) comprised the mineral salts and organic nutrients of the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) [15] was prepared, containing 3% sucrose and 8% agar. In our experiment MS medium were also supplemented with different type of growth regulators like auxin [kinetin (Kn) and 6-benzyladenine (BA)] cytokinin [2,4 dichlorophenoxy acetic acid (2,4-D), indole -3-butyric acid (IBA) and α-naphthalene acetic acid (NAA)] onto the basal medium with varying concentration ranges from 0.5 to 2.0 mg/l and combinations as clearly described in result section. The pH of all the media combinations was adjusted to 5.8±0.1 by using 0.1 N NaOH or 0.1 N HCl. Autoclave was done at 121ºC at 15 lbs Psi for 20 minutes and 60% relative humidity was also maintained with 16:8 hrs in light: dark photoperiod. Leaf, leaf with petiole and petiole explants collected from the healthy plants of *Gerbera jamesonii* obtained from the Division of Floriculture Block, College of Horticulture, VCSG, UUHF Bharsar, Pauri Garhwal, Uttarakhand, India. Before inoculating the explants they were washed with tap water followed by a surface sterilized by 1% (v/v) Labolene detergent then with 70-90% ethyl alcohol for 30 seconds and by 0.1 (w/v) $HgCl_2$ containing 0.2 ml Tween 80 per 100 ml solution for 1 minute. The explants were rinsed several times with sterile double distilled water (DDW) (Chakraborthy *et al*. 2010) [4]. The cut surfaces exhibiting mercuric chloride damage were aseptically trimmed with a sharp, sterile surgical blade. The medium was supplemented with various growth regulators at different concentrations as described in (Table - 2).

Result and Discussion

Induction of callus from leaf explants cultures

Callus formation from leaf explants responded well from different concentrations and combinations of BA, IBA, NAA except Kn and 2,4-D (Plate 1 ; Fig. 1 & 2; Table 1 &2). The leaf explants used for initiation of callus and were inoculated on MS medium supplemented with 2.0 mg/l BA and 1.5 mg/l IBA. The same callus was maintained on same composition of medium for six weeks of subculture (Fig. 1 & 2). MS medium containing 1.5 mg/l BA and 2.0 mg/l NAA, callus turned into browning and light greenish in long term cultures (Fig. 1). The percentage of growth response is high with BA (60%) and IBA (45%) than other auxins IAA (55%). Whereas in combination 1.0 mg/l BA + 2.0 mg/l IBA and 1.5 mg/l NAA, the percentage of growth response is (56%). Development under *in vitro* mass propagation technology was affected by several factors such as plant and explants types; medium, initiation, proliferation and multiplication of shoot; shoot rooting and acclimatization of plantlets (George, 1993) [7].

Induction of callus from leaf with petiole explants cultures

Leaf with petiole derived from *in vitro* seedling were cultured on MS medium supplemented with auxin 2,4-D, IBA and NAA and cytokinins BA (Plate 1 ; Fig. 3; Table 1 &2). In leaf with petiole explants efficient callus was initiated on MS medium fortified with lower concentration of BA 1.0 mg/l and higher concentration of NAA 2.5 mg/l achieved compact and white friable green callus (Fig. 2). Highest percentage of callus growth response was obtained by leaf with petiole explants on MS medium supplemented with 2, 4-D (5%), IBA (45%) and (55%) NAA (Table 1). The combination of Kn and 2, 4-D responded lower percentage of callus (5%) (Table 2).

Table 1: Effect of different auxins (0.5 – 2.0 mg/l^{-1}) on caulogenesis from different explants of *G. jamesonii*

Explants	2,4 –D		NAA		BA		IBA	
	% of response	Nature of response	% of response	Nature of response	% of response	Nature of response	% of response	Nature of response
Leaf	15	*	70	***	55	+++	75	***+++
Leaf with Petiole	5	*	55	++	60	+	45	*++
Petiole	10	*	60	+	65	***++	50	**++

*Callus — + Shoots buds
*** Profuse Callus — +++ Extensive
** Moderate Callus — ++ Moderate shoot bud
* Scanty Callus — + Scanty shoot bud

Table 2: Effect of various growth regulators on morphogenetic response from leaf and petiole explants of *G. jamesonii* after six weeks of cultures

Growth regulators (mg/l^{-1})	Leaf		Petiole	
	% frequency of growth response	Morphogenetic response	% frequency of growth response	Morphogenetic response
1.5 BA +1.0 IBA	70	White friable, Greenish Callus	60	Compact callus
2.0 BA+1.5 IBA	65	Brown Callus	50	Friable Callus
1.5BA + 2.0 NAA	56	Green callus	55	Green Callus
2.0 BA + 1.5 NAA	55	Callus	60	Green Callus
0.5 Kn + 1.0 2,4-D	5	No response	5	No response
2.0 Kn + 2.0 2,4-D	5	No response	5	No response

Fig-1

Fig-2

Fig-3

Fig-4

Plate 1: Callus induction from leaf, leaf with petiole and petiole explants of Gerbera jamesonii

Fig. 1: Initiation of light white from callus from leaf explants on MS + 1.0 mg/l BA + 2.0 mg/l NAA
Fig. 2: Excessive callus growth and light greenish of callus from leaf explants on MS + 1.0 mg/l BA + 2.0 mg/l IBA
Fig. 3: Browning light greening and three roots of callus from leaf with petiole explants on MS +1.0 mg/l BA + 2.5 mg/l NAA
Fig. 4: Initiation of light white from callus from petiole explants on MS + 2.0 mg/l BA + 2.0 mg/l IBA

Induction of callus from petiole explants

The petiole explants used were obtained from *in vitro* grown seedlings. The petiole induced callus on MS medium containing 1.5 mg/l BA + 2.5 mg/l NAA (Plate 1; Fig 4; Table 1). Low growth response was obtained with lower concentration of BA 0.5 mgl^{-1} and IBA higher concentration of IBA 2.5 mg/l 2, 4-D along with IAA resulted in white friable callus in long term subcultures of callus. (Plate 1; Fig. 4). A globular callus was formed on MS medium with 2.0 mg/l BA and 2.0 mg/l IBA. A granular callus was observed on same media after one month subculture. Addition of 2.5 mg/l BA and 2.0 mg/l NAA in MS medium enhanced greening of callus was obtained. The combination of various auxins and cytokinins on petiole explants evoked different morphogenetic response in *G. jamesonii* expect Kinetin and 2, 4-D combinations. Callusing response from petiole explants cultures is good. Combination of auxin (BA) and cytokinins (IBA and NAA) produced highest amount of callus in fresh and dry weight.

Discussion

The effect of explants source, growth regulators combinations and concentrations required for optimum callus growth of *Gerbera jamesonii* callus *in vitro* selection. *G. jamesonii*, callus induction was attempted by culturing leaf, leaf with petiole and petiole. The investigation so far regarding the effect of auxin and cytokinins on callus production seemed to be in agreement with these results. Callus was raised from various explants which were cultured on MS medium fortified with Kn, BA, 2,4-D, IBA, and NAA. Different combinations of growth regulators were tried BA is among the most widely used auxin for callus induction in a wide range. In our study, successful induction of potentially organogenic callus from leaf and petiole was achieved using BA. Also succeeded in the induction of shoot callus derived from petioles in presence of BA and NAA in *Cucumis metuliferus*. Similar results were reported in *Centella asiatica* by (Bisht, 2002) [3]. It was observed that of the three explants leaf, leaf with petiole and petiole maximum callus had obtained from leaf. Combination of Kn and 2, 4-D showed low response to callus formation. Combination of BA with IBA and NAA showed best results from leaf and petiole explants. Similar results were observed in teasel gourd (*Momordica dioica*). MS fortified with 1.5 mg/l BA and 2.0 mg/l NAA result found maximum percentage of callus growth, Hoque *et al.*(1995) [9]; Halder and Gadgil (1982) [8] were able to produce callus in MS media supplemented with 2.0 mg/l and 15% coconut milk.

In other way, callus induction medium, MS + BA + IBA and MS + BA + NAA were found significantly superior to other media for an early initiation of callus as well as higher fresh weight of callus. Kumar *et al.* (2003) [11] also observed identical type of callus on MS medium supplemented with varying levels of IAA and BA. About 92% of leaf explants of cucumber (*Cucumis sativus* L.) *in vitro* seedlings produced green compact nodular organogenic callus in MS medium containing 2.69 µM NAA and 4.44 µM BA. Higher concentration of 2, 4-D and NAA alone produced large amount of callus, which suppressed shoot elongation. Similar kinds of results were reported in *Trichosanthes dioica*, higher concentration of BA produced highest callus. The importance of lower levels of BA and NAA as efficient promoter of friable callus has been recognized in tomato Le *et al.* (1991) [12].

A medium containing higher level of BA with IBA and NAA were found superior in terms of production of friable and morphogenic type of calls. Growth of callus was reduced with lower concentration of BA, IBA, NAA and 2,4-D. MS medium supplemented with BA (1.0 mg/l) and NAA (2.0 mg/l) were found to induce good callusing from leaf and petiole explants. MS medium fortified with BA and IBA found most effective result from leaf explants.

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