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Comparative efficiency of various explants for callus production in *Bunium persicum* (Boiss.) B. Fedtsch

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Abstract

Bunium persicum, family Apiaceae is a native of west Asia and has a limited distribution. During the present study an efficient in vitro protocol has been standardized viz, callus production from cotyledon, hypocotyl and nodal explants. Callus was obtained from these explants on MS medium fortified with different growth regulators both auxins (IAA, IBA, NAA, 2,4-D) and cytokinins (BAP and Kn). From cotyledon explants best callus production was achieved on MS medium supplemented with BAP (2 mg/l) +IAA (2 mg/l) within 10 days. For hypocotyls explants MS medium fortified with BAP (2 mg/l) resulted in best callus production within 24 days. MS medium supplemented with BAP (1 mg/l) +IAA (3 mg/l) proved to be most effective in callus production from nodal explants within 19 days.

Keywords: *Bunium persicum*, Apiaceae, Explants, Callus, Auxins, Cytokinins.

Introduction

Bunium persicum a Threatened plant species of the Himalayas[1] belongs to the family Apiaceae. The family consists of about 423 genera.[2] The genus Bunium contains about 166 species, including *B. persicum*, *B. carum*, *B. bulbocastenum*, *B. copticum*, *B. flexuosum*, *B. elegans*, *B. cylendricum* and *B. chaerophyllocides* that are prevalent in Central Asia, Caucasus, Crimea, and Europe.[3] In Kashmir it has been reported from Baramulla, Gurez, Harwan- Dara, Wasturwan-Tral, Chrarisharief, Khrew.[4]

The most distinctive feature of the Apiaceae is its inflorescence "umbel" (fig 1a). The other distinctive feature is its fruit, shizocarp consisting of two mericarps. The fruit are slender, dark brown in colour, and crescent shape. The plant type of "Kala Zeera" varies from dwarf (30 cm) to tall (80 cm) compact or spreading, moderately to highly branched, tuberous and perennial herb (fig 1b).[5]

The economic production of *B. persicum* is through seeds (schizocarp fruits) that are used as medicine and spices.[6] Black cumin essential oil is used in pharmaceutical, food sweetening, soft drink, food and hygiene industries.[7, 8] Ripe black cummin fruits are reported to contain an essential oil (up to 7%) rich in monoterpene aldehydes; the main components are cuminaldehyde, p-mentha-1,3-dien-7-al and p-mentha-1,4-dien-7-al (up to one third each); terpene hydrocarbons are the main components of fruits collected in the wild or harvested unripe (γ-terpinene, p-cymene, β-pinene, limonene). The latter compounds are thought to reduce the quality of the spice.[9]

The main cause of depletion of this plant has been found to be the thoughtless, improper and an unscientific commercial collection of seeds for rapid financial gains. The competition for its seeds is so severe that, instead of collecting the ripe seeds, the entire plant is removed even when the seeds are immature.[10] Thus, for the present study an efficient in vitro protocol has been developed for conservation of this important plant species.

(a) **(b)**

Figure 1: *Bunium persicum* (Boiss.) B. Fedtsch. **a)** Umbel inflorescence **b)** Whole plant

Materials and Methods

Bunium persicum was collected from Dawar-Gurez Jammu and Kashmir and transplanted at Kashmir University Botanical Garden (KUBG). Nodal explants and seeds were collected from plants grown at KUBG. Explants were first thoroughly washed under running tap water in order to remove dirt and dust followed by washing with detergent labolene and surfactant tween-20. The detergent was removed by washing the explants with double distilled water. Nodal explants were treated under laminar air flow hood with chemical sterilant (2% sodium hypochlorite) and seeds were treated with mercuric chloride 0.1% for 5-10 min. This was followed by washing with autoclaved double distilled water and finally inoculation on sterilized nutrient medium. Seeds were kept for stratification at 4ºC in dark for 80-85 days, after which seed germination took place. Cotyledon and hypocotyl explants were then excised from the germinated seeds and inoculated on MS medium.

Medium and Culture Conditions

Murashige and Skoog's (MS, 1962) medium[11], gelled with 8% agar was supplemented with different concentrations of auxins and cytokinins both individually and in combination. Auxins like 2, 4- D; IAA; NAA; IBA and cytokinins like BAP and Kn were used in concentration range of 0.1-5 mg/l. The pH of the media was adjusted to 5.8 before autoclaving at 121 °C and 15 lbs. The cultures were incubated at 22±4 ^{0}C and exposed to a regular photoperiod of 24 hours.

Results

In vitro response from nodal explants

Callus production: The nodal explants excised from plants transplants at KUBG (Kashmir University Botanical Garden) produced callus on MS medium fortified with auxins (IAA, IBA, NAA, 2,4-D) and cytokinins (BAP and Kn) individually as well as in different combinations (Table 1). Among cytokinins BAP at the concentration of 2 mg/l was effective in producing compact and greenish colored callus in 70% cultures within 11 days (fig. 2c). Among auxin, cytokinin combination BAP(2 mg/l)+IBA(1 mg/l) (fig. 2d) and BAP(1 mg/l)+IAA(3 mg/l) (fig. 2e) supplemented MS medium produced compact and greenish colored callus in 70% cultures within 21 days and compact and cream colored callus in 80% cultures within 19 days respectively. When the MS medium was fortified with Kn(2 mg/l)+ NAA(2 mg/l) (fig. 2f) compact and brown colored callus was obtained in 70% cultures within 9 days and within 8 days compact and white colored callus was obtained in 60% cultures when the MS medium was fortified with KN(3 mg/l)+IAA(1 mg/l) (fig. 2g).

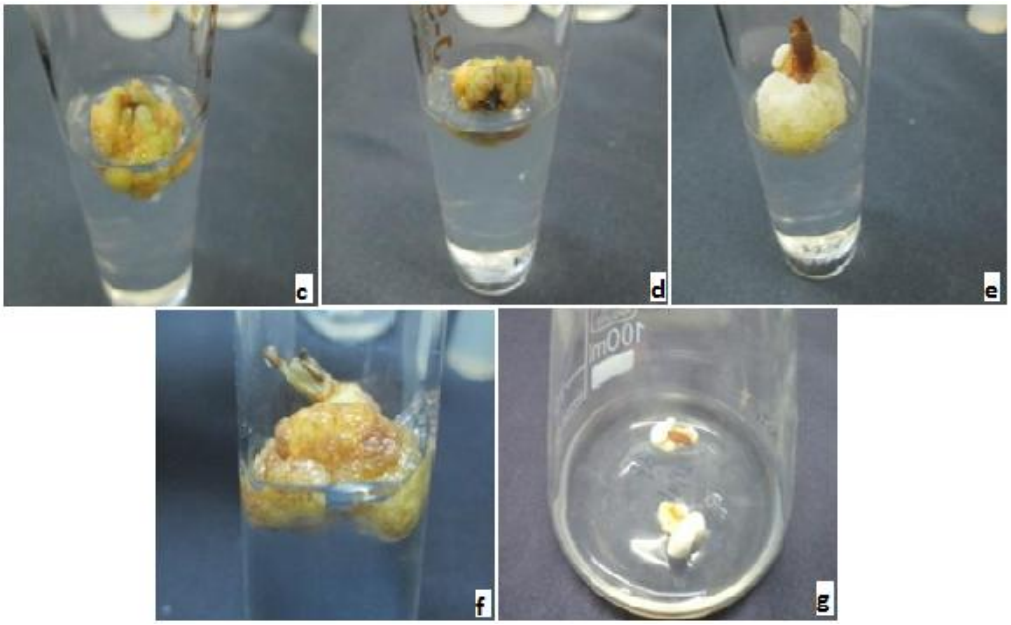

Figure 2: Callus production from nodal explant: **c)** MS+BAP(2 mg/l) **d)** MS+BAP(2 mg/l)+IBA(1 mg/l) **e)** MS+BAP(1 mg/l)+IAA(3 mg/l) **f)** MS+Kn(2 mg/l)+NAA(2 mg/l) **g)** MS+Kn(3 mg/l)+IAA(1 mg/l)

***In vitro* response from cotyledon explants**

Callus production: The cotyledon explants excised from the *in vitro* grown seedling produced callus on MS medium supplemented with different growth regulators(IAA, IBA, 2,4-D, NAA, BAP, Kn) both individually as well as different combinations (Table 2). Among cytokinins BAP at a concentration of 2 mg/l and 5 mg/l was effective in producing nodular and white colored and nodular and brown colored callus in 80% and 30% cultures within 24 and 20 days respectively (fig. 3h and 3i). Among auxins 2,4-D at the concentration of 0.5 mg/l was effective in producing friable and cream colored callus in 50% cultures within 48 days (fig. 3j). Among auxin, cytokinin combination 2,4-D(1mg/l)+ Kn(2 mg/l) supplemented MS medium produced friable and cream colored callus in 30% cultures within 34 days (fig. 3k). When MS medium was supplemented with BAP(2 mg/l)+ IAA(2 mg/l) nodular and cream colored callus was obtained in 90% cultures within 10 days (fig. 3l).

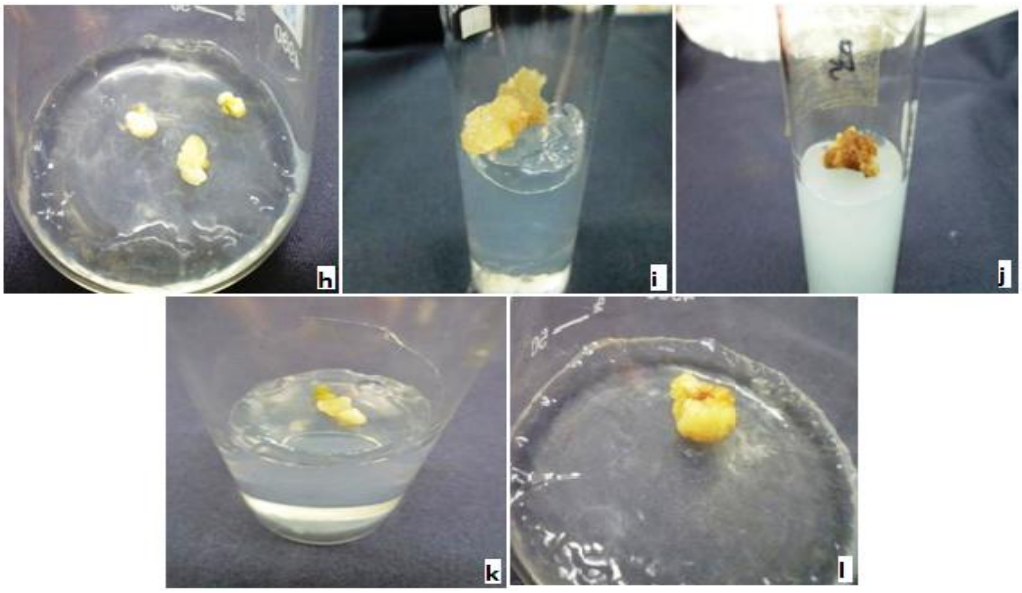

Figure 3: Callus production from cotyledon explants: **h)** MS+BAP(2 mg/l) **i)** MS+BAP(5 mg/l) **j)** MS+2,4-D(0.5 mg/l) **k)** MS+2,4-D(1 mg/l)+Kn(2 mg/l) **l)** MS+BAP(2 mg/l)+IAA(2 mg/l)

***In vitro* response from hypocotyl explants**

Callus production: Callus was obtained from hypocotyl explants when the MS medium was fortified with auxins (IAA, IBA, NAA, 2,4-D) and cytokinins (BAP and Kn) both individually as well as in combination (Table 3). Among cytokinins BAP at the concentration of 2 mg/l was effective in producing nodular and brown colored callus in 80% cultures within 24 days (fig. 4m). Among auxins 2,4-D at the concentration of 0.5mg/l was effective in producing nodular and brownish green colored callus in 60% cultures within 22 days (fig. 4n). When the MS medium was supplemented with BAP(3 mg/l)+ IAA(5 mg/l) callus differentiation was achieved in 70% cultures within 28 days (fig. 4o).

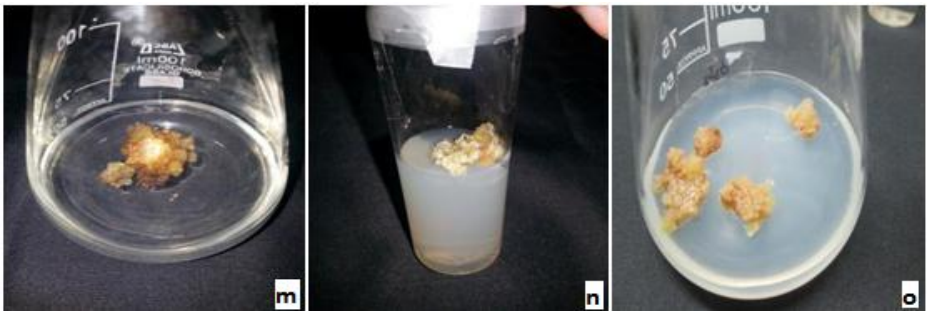

Figure 4: Callus production from hypocotyls explants: **m)** MS+BAP(2 mg/l) **n)** MS+2,4-D(0.5 mg/l) **o)** MS+BAP(3 mg/l)+IAA(5 mg/l)

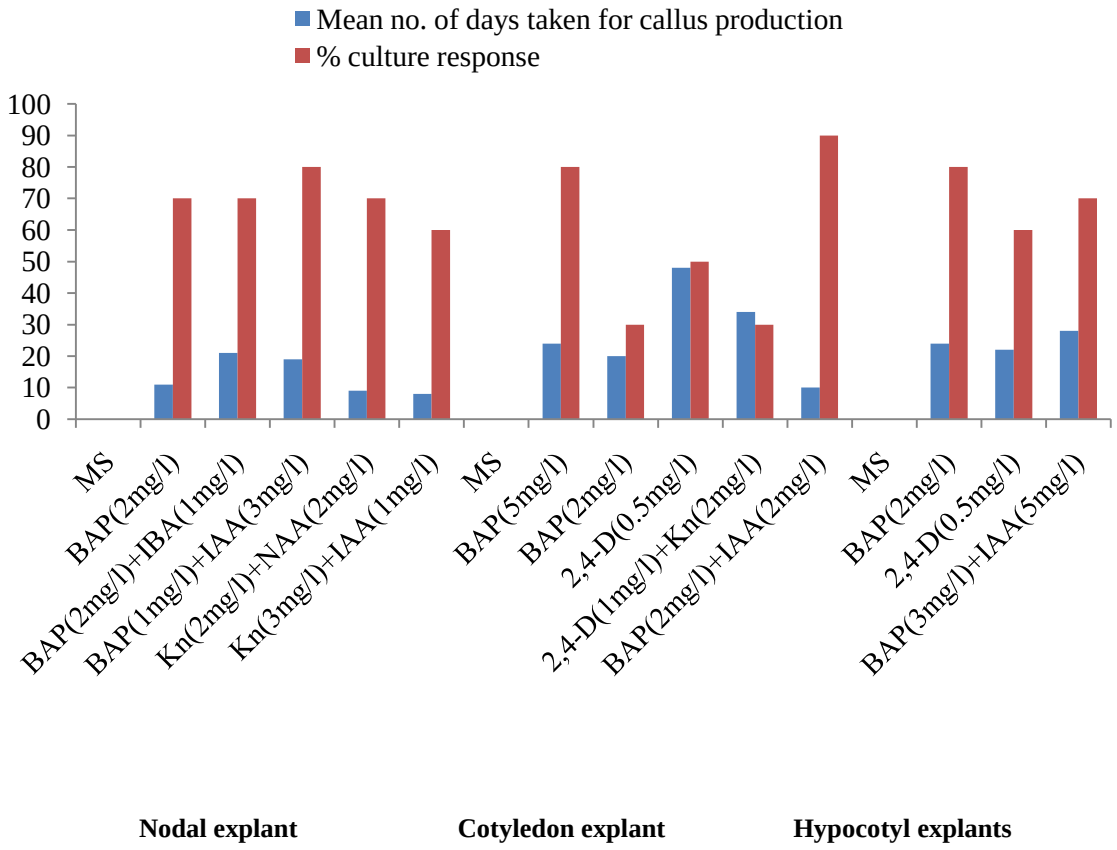

Figure 4: Comparison of Nodal, cotyledon and hypocotyl explants for callus production

Table 1: Effect of different hormones on callus production from Petiole explants

Treatments	**Mean number of days taken for callus production**	**Amount of callus produced**	**Texture and color of callus**	**Percent culture response**
MS basal	No response	-	-	-
MS+BAP(2mg/l)	11	High	Compact, Greenish coloured	70
MS+BAP(2mg/l)+IBA (1mg/l)	21	High	Compact, Greenish coloured	70
MS+BAP(1mg/l)+IAA (3mg/l)	19	High	Compact, Cream coloured	80
MS+Kn(2mg/l)+NAA (2mg/l)	9	High	Compact, Brown coloured	70
MS+Kn(3mg/l)+IAA (1mg/l)	8	Moderate	Compact,White coloured	60

(10 replicates per treatment)

Table 2: Effect of different hormones on callus production from Cotyledon explant

Treatments	Mean number of days taken for callus production	Amount of callus produced	Texture and color of callus	Percent culture response
MS basal	No response	-	-	-
MS+BAP(2mg/l)	24	Moderate	Nodular, White coloured	80
MS+BAP(5mg/l)	20	Moderate	Nodular, Brown coloured	30
MS+2,4-D (0.5mg/l)	48	High	Friable, Cream coloured	50
MS+2,4-D(1mg/l)+Kn (2mg/l)	34	Little	Friable,Cream coloured	30
MS+BAP(2mg/l)+IAA (2mg/l)	10	High	Nodular, Cream coloured	90

(10 replicates per treatment)

Table 3: Effect of different hormones on callus production from Hypocotyl explant

Treatments	Mean number of days taken for callus production	Amount of callus produced	Texture and color of callus	Percent culture response
MS basal	No response	-	-	-
MS+BAP(2mg/l)	24	High	Nodular, Brown coloured	80
MS+2,4-D (0.5mg/l)	22	Moderate	Nodular, Brownish Green coloured	60
MS+BAP(3mg/l)+IAA (5mg/l)	28	High	Friable, Brown coloured	70

(10 replicates per treatment)

Discussion

During the present study, different growth regulators both auxins and cytokinins were used both individually as well as in combination to produce callus from nodal, cotyledon and hypocotyl explants. Best callus production from nodal explants was obtained on MS medium supplemented with BAP(1 mg/l)+IAA(3 mg/l) within 19 days. Callus was also obtained when MS medium was fortified with BAP(2 mg/l); BAP(2 mg/l)+IBA(1 mg/l); Kn(2 mg/l)+NAA(2 mg/l) and Kn(3 mg/l)+IAA(1 mg/l). But the percent culture response on these concentrations was lesser. Singh *et al*., (2010) also obtained callus from nodal explants in *Centella asiatica* on MS medium augmented with Kn(3 mg/l)+IBA(3 mg/l).[12] MS medium fortified with BAP(2 mg/l)+IAA(2 mg/l) showed a 90% response from cotyledon explants within 10 days. However callus was also obtained on MS medium fortified with BAP(2 mg/l); 2,4-D(0.5 mg/l) and 2,4-D(1 mg/l)+Kn(2 mg/l),but the percent culture response was less in these cases. Callus was also obtained from hypocotyls explants with 80% response on MS medium fortified with BAP(2 mg/l) within 24 days. Valizadeh and Tabar, (2009) also obtained callus using hypocotyl explants,but they achieved best callus production on MS medium supplemenmted with 2,4-D(1 mg/l).[13] Among the three explants best explant was cotyledon explant in terms of percent culture response, however in terms of mean number of days taken for callus production nodal explants proved to be best as shown in the fig. 4.

Conclusion

An efficient *in vitro* protocol was developed for callus production using various explants of *Bunium persicum* on MS medium fortified with different growth regulators. Among all growth regulators tried, BAP was found to be more effective either alone or in combination with other growth regulators for callus production from various explants.

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