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In Vitro Flower Production in Some Species of *Hyoscyamus* (*H. pusillus* L., *H. arachnoideus* Pojark, and *H. niger* L.)

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Abstract

Various plant organ segments including segments from roots (root tip, and root collar), stems, leaves, and also seeds of several *Hyoscyamus* species (*i.e. H. pusillus*, *H. arachnoideus*, annual and biennial *H. niger* from two distinct geographical regions) were cultured on MS media with various growth regulator combinations, to assess the ability of flower production. Statistical assessments showed significant differences between various explants cultured on different media. That is, the subculture of the leaves obtained from *H. pusills* leaf culture on MS medium containing BAP (1.5 mg/L), on MS medium containing IAA (0.2 mg/L) and Kin (1.5 mg/L), led to the highest flower production. In addition, when cultured on MS medium containing 2,4-D (0.32 mg/L), the seeds of annual *H. niger* showed high flower production.

Keywords: *Hyoscyamus pusillus*; *Hyoscyamus arachnoideus*; *Hyoscyamus niger*; Flower production; *In vitro* culture

Introduction

Plant organ segments possess the ability of flower production, when cultured in certain conditions.

These flowers are named as neoformed flowers and are the same as the ordinary ones produced due to the transition of vegetative buds to reproductive parts during the development of intact plants. Ebrahimzadeh and Tarighi showed that segments from the flower stalk of *Nicotiana tabacum* cultivar Basma_5.31 upon being cultured on media without any hormones or containing IAA (5 mg/L) and Kin (0.5 mg/L), can produce flower buds [6]. Azizbekova *et al*. showed the stimulative effects of some growth regulators, such as kinetin, on increasing the number of natural flower buds in cultivated saffron [5]. Fakhrai and Evans [8] and Sarma *et al*. [9] reported high frequencies of the formation of stigma-like structures on different explants in saffron. Karamian showed that in saffron the highest production of stigma-like structures can be achieved by adding NAA (1 mg/L) to the medium [4]. The NAA to BAP ratio seems to play a crucial role in the number, shape,

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and type of stigma-like structures. Decrease of this ratio resulted in decrease of callus production while promoting the differentiation of calli. Also after two months, a neoformed flower was observed on a callus derived from a style segment cultured on MS medium containing BAP (5 mg/L). This flower possessed 3 stamens, 1 red three branched stigma, and 6 floral envelopes, and below its axis: green leaf-like organs, fine orange stigma-like structures and also contractile roots were observed [4].

Rajabian observed the formation of only one neoformed flower on style segments from cultivated saffron cultured on MS medium containing BAP (5 mg/L) and NAA (1 mg/L) [2]. Ataee showed that calli obtained using MS medium containing IAA with BA, and NAA with BA could produce embryos, and in some cases flowers were produced from these embryos [3]. Ebrahimzadeh *et al*. using cultured of segments from saffron style and floral envelope on media containing IAA and NAA and subculture of these explant segments on a medium containing high concentrations of kinetin. They observed not only the formation of numerous stigma-like organs, but also the formation of complete and incomplete floral buds [7].

The object of this study was to assess the ability of flower production on organ segments from 3 *Hyoscyamus* species.

Materials and Methods

Three species of *Hyoscyamus* genus were used for this study: *H. pusillus* L., *H. arachnoideus* Pojark, and *H. niger* L. The seeds of *H. pusillus* L. and *H. arachnoideus* Pojark, were collected from Tabriz down town (Il-Goli park, Vadi-Rahmat Rd. and Basmenj Rd.). The seeds of annual *H. niger* L. were collected from Yakhmorad way, Gachsar region, Chalus road. The seeds of biennial *H. niger* L. were collected from two distinct regions: Mandal county Damavand and Mount Oshtoran, Sefid-dasht Rd., Lorestan province. MS media with various hormones such as Kin, BAP, NAA, IBA, 2ip, and 2,4-D were used in this study [1].

Dormancy was our problem with *H. arachnoideus* and biennial *H. niger*. For *H. arachnoideus*, we solved this problem with incubation of the seeds at −4°C for two weeks. For biennial *H. niger*, dormancy could be overcome either by incubation of soaked seeds at −10°C for one week, or by gibberellin treatment (35 mg/L) for 12 h. Alcohol (70%) and calcium hypochloride solution were used for sterilization of the seeds. The seeds were also cultured on MS medium for obtaining sterile seedlings and also on MS media containing IAA (0.2 mg/L), 2,4-D (0.32 mg/L), and NAA and BAP (with various concentrations). We took the cultured seeds of *H. pusillus* to a dark place to evoke germination. Once the germination occurred, some of the samples (the ones cultured on media containing NAA and BAP) were left in the dark, while the others were incubated under 25°C with a 16/8 h photoperiod at 4000 Lux light intensity, after their germination. Germination of the remaining two species (*H. arachnoideus* and *H. niger*) took place in light. Therefore we kept all of the cultured seeds under conditions of 25°C, and light periodicity of 16/8 with light intensity of 4000 Lux, with the exception of the seeds cultured on media containing NAA with BAP which were taken to dark after their germination.

Finally, the sterilized seedlings were excised into different under-parts including roots (root-tip, root collar), stems, leaves, and flowers, and were transferred to media with various growth regulator combinations. The obtained parts (calli, leaves, stems, flowers, etc.), were then subcultured on various media.

Results

In this study, different explants from various species of *Hyoscyamus* were cultured on different media with various hormonal combinations. Flower production in *H. pusillus* stems cultured on MS medium containing IAA (0.2 mg/L), and leaves and stems cultured on MS medium containing 2,4-D (0.32 mg/L) were 30%, 20%, and 0%, respectively. Also, flower production in *H. pusillus* leaves cultured on MS media containing either Kin (1.5 mg/L) or IAA (0.2 mg/L) with BAP (1.5 mg/L) was 15%. The flower production from seed culture of *H. pusillus*, *H. arachnoideus*, and annual *H. niger* from regions 1 and 2, on MS medium containing 2,4-D (0.32 mg/L) were 10%, 15%, and 48%, respectively. When cultured on MS medium containing NAA (0.5 mg/L) and BAP (0.5 mg/L), among all of the species tested, only *H. pusillus* seeds showed 15% flower production. The phenomenon of flower production was also observed in some of the subcultures (Fig. 1).

Statistical assessments showed significant difference between various explants of different species cultured on various media (Table 1). The highest flower production was observed on the subculture of the leaves obtained from leaf culture of *H. pusillus* on MS medium containing BAP (1.5 mg/L), on MS medium containing IAA (1.5 mg/L) and Kin (0.05 mg/L) (Table 2, Fig. 2).

Further statistical assessments were performed to compare the culture of the seeds of various species on different media. The results indicated that significant difference exists between various species when cultured

on different mediums and the highest flower production was observed with annual *H. niger* seeds when cultured on MS medium containing 2,4-D.

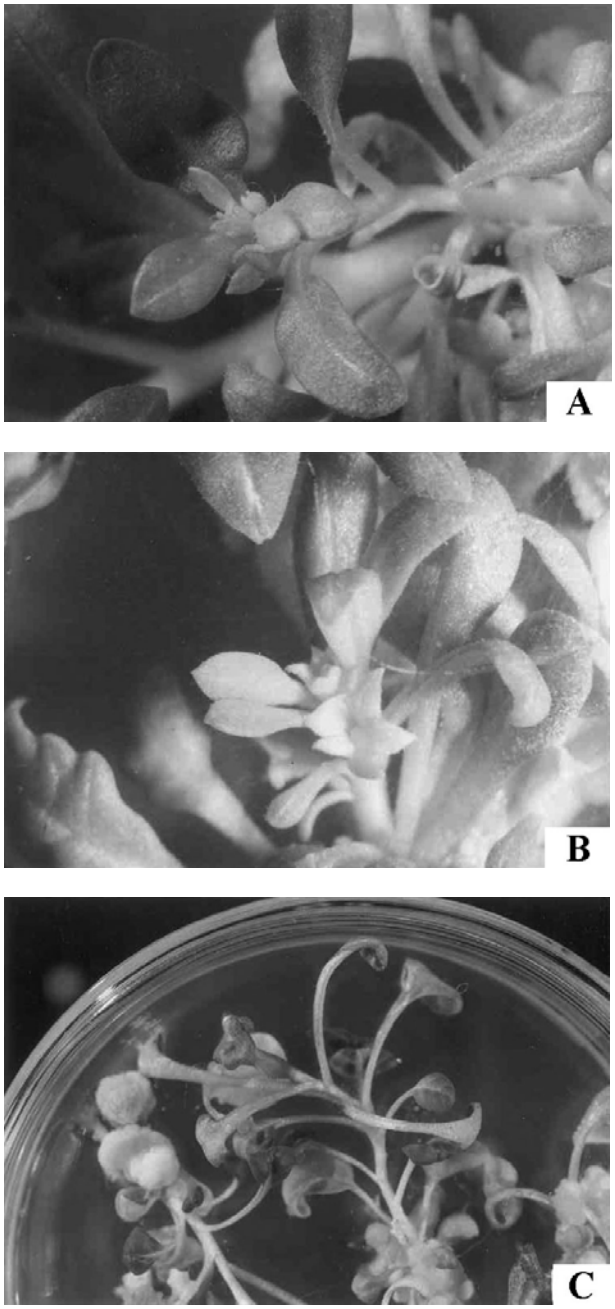

Figure 1. (A) The culture of *H. pusillus* leaves' explant segment, cultured on medium containing Kin (1.5 mg/L). (B) The group of leaves obtained from the culture of the leaves of *H. pusillus* on MS medium containing IAA (0.09 mg/L) and BAP (1.12 mg/L) subcultured on MS medium containing IAA (1.5 mg/L) and BAP (0.05 mg/L). (C) Annual *H. niger* seeds cultured on MS medium containing 2,4-D (0.32 mg/L).

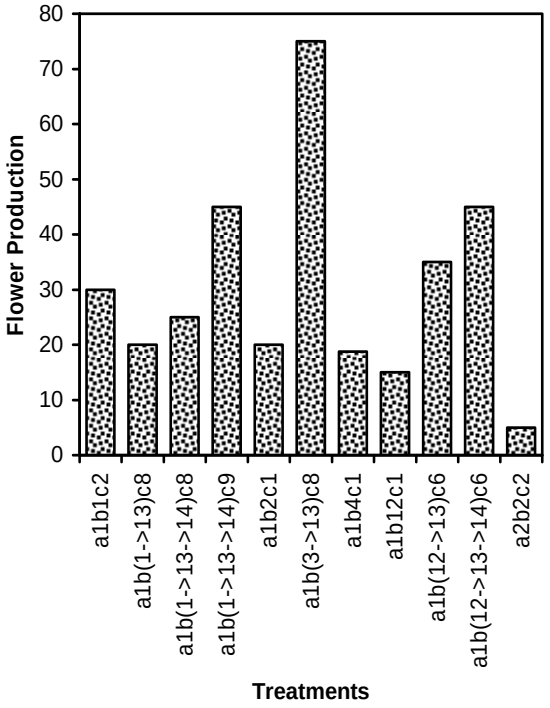

Figure 2. Flower production on various explants of different species on various media. a1b1c2: Stem of *H. pusillus* on medium No. 1 (IAA=0.2 mg/L). a1b(1->13)c8: Leaves obtained from stem culture of *H. pusillus* on medium No. 1 (IAA=0.2 mg/L) subcultured on medium No. 13 (IAA=1.5 mg/L, Kin=0.05 mg/L). a1b(1->13->14)c9: Leaves obtained from "a1b(1->13)c8" subcultured on medium No. 14 (IAA=3, Kin=1.5). a1b2c1: Leaves of *H. pusillus* on medium No. 2 (2,4-D=0.32 mg/L). a1b(3->13)c8: Leaves obtained from "stem culture of *H. pusillus* on medium No. 3 (BAP=1.5 mg/L)" subcultured on medium No. 13 (IAA=1.5, Kin=0.05 mg/L). a1b4c1: Leaves of *H. pusillus* on medium No. 4 (Kin=1.5 mg/L). a1b12c1: Leaves of *H. pusillus* on medium No. 12 (IAA=0.09, BAP=1.12 mg/L). a1b(12->13)c6: Leaves obtained from "a1b12c1" subcultured on medium No. 13 (IAA=1.5, Kin=0.05 mg/L). a1b(12->13->14)c6: Leaves obtained from stem culture of *H. pusillus* on medium No. 12 (IAA=0.09, BAP=1.12 mg/L), subcultured on medium No. 13 (IAA=1.5, Kin=0.05 mg/L), then the leaves obtained this way were subcultured again on medium No. 14 (IAA=3, Kin=1.5). a2b2c2: Stem culture of *H. arachnoideus* on medium No. 2 (2,4-D=0.32 mg/L).

Table 1. Statistical assessment of flower production percentage in various explants from different species cultured on various media

	SS	df	MS	F	Sig.
MEDIUM	18364.583	10	1836.458	12.698	.000
Error	6218.750	43	144.622		
Total	24583.333	53			

Table 2. Duncan test on flower production percentage on various media. a: the highest quantity, b: the next quantity

Treatments	N	Mean	Standard Deviation	Standard Error
a1b1c2	5	30.00 [d]	11.1803	5.0000
a1b(1->13)c8	5	20.00 [f]	11.1803	5.0000
a1b(1->13->14)c8	5	25.00 [e]	.0000	.0000
a1b(1->13->14)c9	5	45.00 [b]	11.1803	5.0000
a1b2c1	5	20.00 [f]	11.1803	5.0000
a1b(3->13)c8	5	75.00 [a]	.0000	.0000
a1b4c1	4	18.75 [f]	12.5000	6.2500
a1b12c1	5	15.00 [g]	13.6931	6.1237
a1b(12->13)c6	5	35.00 [c]	22.3607	10.0000
a1b(12->13->14)c6	5	45.00 [b]	11.1803	5.0000
a2b2c2	5	5.00 [h]	11.1803	5.0000

[*] There is no significant difference between media sharing a letter

Conclusion and Discussion

In this study, the highest flower production was observed by subculture of the leaves obtained from the leaf culture of *H. pusillus* on MS medium containing BAP (1.5 mg/L), or IAA (1.5 mg/L) and Kin (0.05 mg/L). This result is consistent with the previously obtained results [2-5] which manifested the role of auxin and cytokinin in flower production. In addition, we observed that annual *H. niger* on MS medium containing 2,4-D (0.32 mg/L) shows high flower production (after the onset of germination some calli appeared and after 10 d seedlings became visible on the collar of the calli and flowers arose on these seedlings). In this study as well as the previously performed investigations [6,7], auxin-like hormones seem to play an important role in enhancement of flower production.

Regarding the species studied in this research, flower production was observed by culture of *H. pusillus* (annual) and *H. arachnoideus* (biennial) on MS medium containing 2,4-D (0.32 mg/L) and annual *H. niger* also showed the ability of flower production. However, with the other two species of *H. niger* (which were biennial) no flower production were observed.

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