American-Eurasian J. Agric. & Environ. Sci., 4 (5): 640-645, 2008
ISSN 1818-6769

Indirect Organogenesis from Scale, Leaf Primordia and Immature Floret Explants of Hyacinth (*Hyacinthus orientalis* L.)

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Abstract: Hyacinth (*Hyacinthus orientalis*) is one of the ornamental bulbous flowers which is generally propagated through bulb and bulblet. In this research callus formation and indirect organogenesis from excised explants of hyacinth bulb through *in vitro* culture was studied. In order to obtain callus, a factorial experiment in the from of randomized complete design with 12 treatments and 3 replications was carried out. The treatments consisted of different types of explants and media. The treatments of explants in 3 levels consisted of: scale, leaf primordia and immature inflorescence, while the treatments of media in four levels consisted of Murashig and Skoog (MS) basic media containing 0.5 and/or 1.0 mg l^{-1} NAA and MS basic media containing 0.5 and/or 1.0 mg l^{-1} IBA. The results showed that in callus formation media (CFM), the media containing 1.0 mg l^{-1} NAA along with scale, during 6 weeks, the best weight of callus was achieved (1.9 g). Because of producing roots in this media, calli were obtained in media containing 0.5 or 1.0 mg l^{-1} IBA were transferred onto indirect organogenesis media (IOM). Then, this type of calli, which were yellowish, friable and nodular were cultured on 7 type of organogenesis media, as follows: 1) MS+ 0.5 mg l^{-1} BAP, 2) MS+1.0 mg l^{-1} BAP, 3) MS+3.0 mg l^{-1} BAP, 4) MS+1.0 mg l^{-1} BAP+0.1 mg l^{-1} NAA, 5) MS+3.0 mg l^{-1} NAA+0.3 mg l^{-1} NAA, 6) MS+1.0 mg l^{-1} BAP+0.1 mg l^{-1} IBA and 7) MS+3.0 mg l^{-1} BAP+0.3 mg l^{-1} IBA. The experiment was carried out as factorial in the from of randomized complete design with 21 treatments (seven types of media × produced callus from three types of explants) and 3 replications. The treatments were consisted of different types of explants and media. The results indicated that in indirect organogenesis media with 3.0 mg l^{-1} BAP+0.3 mg l^{-1} IBA, during 6 weeks, the best results of producing bulblet with the mean of 3.06 bulblet were achieved.

Key words: Hyacinth · Bulb · Indirect organogenesis · Explants

INTRODUCTION

Hyacinth (*Hyacinthus orientalis*), belonging to liliaceae family, is economically one of the most important flowers worldwide. It has attracted the attention due to variety of its colour and delightful fragrance. The genus of *Hyacinthus* consists of 30 species among which only *H. orientalis* is horticulturally important due to make long-lasting cut flowers, used as potted plants and widely grown as house plants. Hyacinth is usually propagated by separation of bulblets, but this is not easy and flowering occurs only after 4 years [1]. Also it is grown for breeding program through seed which takes 4 to 5 years. As the natural rate of vegetative propagation of bulb forming hyacinth is slow and conventional propagation techniques are labour intensive and expensive, various micropropagation procedures have been proposed for the induction of shoots and bulbs from different types of explants [2-5].

In vitro culture of bulb plants as an alternative to the conventional methods for vegetative propagation attracts much attention because of its advantages [1, 6, 7]. It increases many times the multiplication level [7-9].

The production of callus from different types of hyacinth explant via *in vitro* culture in the presence of plant growth regulators have been reported [1, 4]. Regeneration of hyacinth by tissue culture has been achieved with varying degrees of success [1, 10]. Pierik and Post [11] found that regeneration of hyacinth bulblets from scale explant could result in 240- 300 bulblets from one bulb. Hussey [3] reported that to produce whitish callus leaf, stem, ovary and basal plate of hyacinth

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explant, the explants were cultured on MS media [12] supplemented with 2.0-8.0 mg l^{-1} NAA, during 4-5 weeks. Furthermore, he cultured stem floret on MS media supplemented with different concentration of NAA which formation of callus was produced [13]. Shoot and bulb induction of hyacinth under the influence of varying light treatments was studied in *in vitro* cultures [2]. It was showed that the light as well as the types and concentrations of carbohydrates affected regeneration. *In vitro* culture of hyacinth leaf, perianth and bulb explants with varying age was investigated by Sheng Quan and Caibo [5]. They reported that age difference among the explants affected the induced bulb regeneration. Ziv and Lilien-kipnis [14] reported that the inflorescence stalk of hyacinth was a good alternative source of explants to overcome explant contamination. The absorption of BA by scale of hyacinth was investigated by Vidor *et al*. [15]. They reported that the most of the BA was absorbed during the first 36 h of cultivation and growth was greater in the part of the explant in contact with the medium. Young Byung *et al*. [16] reported that *in vitro* culture of scale segments from *H. orintalis* which treated with IBA at concentration of 1.5 and 3.0 mg l^{-1} showed higher regeneration and growth of the bulblets formed from the scale segments of bulbs than those treated with IAA at the same concentration.

The objective of the present study was to describe a method for *in vitro* propagation of hyacinth by using explants from scale, leaf primordial and immature inflorescence.

MATERIALS AND METHODS

The explants were prepared from scale, leaf primordia and immature inflorescence. Before preparing the explants, whole bulb was superficially disinfected as follows:

- The mother bulbs were immersed in benomyl (1.0 g l^{-1}) for 30 min.
- The bulbs were kept under room temperature for 1-2 h.
- The dried bulbs were placed in cold room at +4°C until the time of doing experiments.
- The dried bulbs were immersed in 70% ethanol for 1-2 min, then were soaked in benomyl (0.5 g l^{-1}) for 30 min, followed by two 5- min rinses in sterile distilled water.
- They were immersed in 20% sodium hypochlorite (NaOCl) for 15 min.
- Three changes of sterile distilled water for 5 min each.

The whole bulb was put in sterilized petri dishes and 2-3 layers of scales were discarded. The scales around the primordial leaves and immature inflorescence were mostly used. Again, for surface sterilization of explants the scale, (1.0×1.5 cm), primordial leaves (1.0×1.0 cm) and immature inflorescence were soaked in 70% ethanol for 10 second, one wash with sterilized distilled water for 3 min. Then, they were immersed in NaOCl 5% for 10 min. All the explants were rinsed with sterilized distilled water for 3-5 min, 3 times. The tissues were maintained in sterile distilled water prior to final culturing to reduce oxidation at the cut surface. The damaged ends of the explants were then trimmed and the explants placed individually in 60 ml culture jars containing 12 ml of MS medium. Cultures were incubated at 25±2°C with a 16/8 h (day/night) photoperiod and an irradiance of 1500 Lux.

Callus Formation from Explants: The sterilized explants were cultured on MS media supplemented with different concentrations of NAA or IBA as follows: 1) 0.5 mg l^{-1} NAA, 2) 1.0 mg l^{-1} NAA, 3) 0.5 mg l^{-1} IBA and 4) 1.0 mg l^{-1} IBA. The duration of cultures were 6 weeks and all the jars were kept under dark condition. After six weeks, the formed calli were weighted and then transferred to regeneration media for indirect organogenesis. The test was carried out as factorial complete design with 3 replications. Each replicate consisted of 10 jars. The mean weight of calli were compared in 5% level with Duncans multiple range test. The treatments consisted of explants in 3 levels and media in 4 levels.

Indirect Organogenesis from Callus: Due to produce roots in callus which was obtained on MS medium containing NAA therefore, the calli which were produced on MS medium containing IBA(0.5 or 1.0 mg l^{-1}) were used. This type of callus was yellowish, very friable and nodular. The calli were transferred onto organogenesis media which were basic MS supplemented with different concentrations of BAP, NAA and IBA as follows: 1) 0.5 mg l^{-1} BAP, 2) 1.0 mg l^{-1} BAP, 3) 3.0 mg l^{-1} BAP, 4) 1.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA, 5) 3.0 mg l^{-1} BAP + 0.3 mg l^{-1} NAA, 6) 1.0 mg l^{-1} BAP + 0.1 mg l^{-1} IBA and 7) 3.0 mg l^{-1} BAP + 0.3 mg l^{-1} IBA.

All the cultures were kept under light condition. After 6 or 12 weeks, the number of organs formed were noted. The test was conducted in factorial completed design with 2 treatments and 3 replications. Each replicate was 10 jars. The treatments were calli explants in 3 levels (scale, primordial leaf and immature inflorescence) and

media in 7 levels. The mean of number of organs were compared in 5% with Duncan's multiple range test.

RESULTS

Callus Formation: The results showed that after six weeks, the largest amount of callus(1.9 g) was significantly achieved from scale explant on MS medium supplemented with 1.0 mg l^{-1} NAA (P= 5%). However, calli weights which were obtained from leaf primordia and immature inflorescence explants did not significantly differ. After application of 1.0 mg l^{-1} IBA on MS medium, the produced callus weight from scale and leaf primordia explants were significantly higher than callus weight of immature inflorescence(1.32, 131 and 0.98 g, respectively). The fresh weights of calli were produced from scale, leaf primordia and immature inflorescence explants on MS media supplemented with 0.5 mg l^{-1} IBA did not significantly differ (Table 1).

Indirect Organogenesis: For the purpose of indirect organogenesis, calli obtained from different types of scale, leaf primorida and immature inflorescence explants on callus formation media which were MS basic media supplemented with 0.5 and 1.0 mg l^{-1} IBA were transferred onto 7 types of regeneration media. After 6 or 12 weeks, the number of produced bulblets were noted.

Callus Obtained on MS Medium Supplemented with 0.5 mg l^{-1} IBA after 6 Weeks: The numerous bulblets were produced with leaf primordia callus on MS medium supplemented with 3.0 mg l^{-1} BAP in combination with 0.3 mg l^{-1} IBA. These results while affecting the treatment of leaf primordia callus or scale callus on MS medium containing 3.0 mg l^{-1} BAP in combination with 0.3 mg l^{-1} NAA and also treatment of leaf primordia callus on media containing 1.0 mg l^{-1} BAP did not significantly differ (Table 2).

Table 1: The interaction different kind of explant, combination and concentration of different plant growth regulators on rate of callus (g) after 6 weeks

Explant	Growth regulators (mgL^{-1})	Callus weigth (g)
Scale	0.5 NAA	0.83 d*
	1.0 NAA	1.9 a
	0.5 IBA	1.25 bc
	1.0 IBA	1.32 b
Leaf primordia	0.5 NAA	0.89 d
	1.0 NAA	1.28 bc
	0.5 IBA	1.09 bcd
	1.0 IBA	1.31 b
Floret	0.5 NAA	1.11 bcd
	1.0 NAA	1.23 bc
	0.5 IBA	1.28 bc
	1.0 IBA	0.98 cd

* Means in each column with the similar letter are not significantly different at 5% level of probability using DMRT

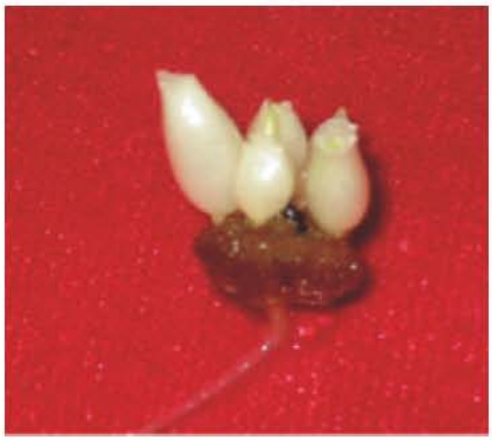

Fig. 1: Bulblets formation from leaf primordia callus on MS medium supplemented with 3.0 mg l^{-1} BAP in combination with 0.3 mg l^{-1} IBA

Table 2: The interaction different kind of callus from different explants on medium with 0.5 mg l^{-1} IBA and plant growth regulators on bulblet formation after 6 or 12 weeks

	Explant		
Growth regulators (mg l^{-1})	Scale	Leaf primordia	Floret
6 weeks			
1 BAP+0.1 NAA	1.86 bcd*	2.16 bc	0.8 efgh
3 BAP+0.3 NAA	2.43 ab	2.7 ab	0.3 fgh
1 BAP+0.1IBA	1.13 def	0.16 gh	0.0 h
3 BAP+0.3 IBA	1.46 cde	3.06 a	0.0 h
0.5BAP	1.23 de	0.83 efgh	0.96 efg
1BAP	0.7 efgh	2.7 ab	0.0 h
3BAP	0.86 efgh	1.36 cde	0.8 efgh
12 weeks			
1 BAP+0.1 NAA	3.06 b*	4.16 a	0.83 ef
3 BAP+0.3 NAA	3.4 ab	4.3 ab	1.6 de
1 BAP+0.1IBA	1.93 cd	0.33 f	0.0 h
3 BAP+0.3 IBA	2.0 bc	4.23 a	0.3 f
0.5BAP	1.8 cd	1.6 de	1.26 d
1BAP	1.9 cd	4.23 a	0.0 h
3BAP	1.83 cd	2.63 bc	0.26 f

* For each time, means in each column with the similar letter are not significantly different at 5% level of probability using DMRT

Table 3: The interaction different kind of callus from different explants on medium with 1.0 mg l^{-1} IBA and plant growth regulators on on bulblet formation after 6 or 12 weeks

	Explant		
Growth regulators (mg l^{-1})	Scale	Leaf primordia	Floret
6 weeks			
1 BAP+0.1 NAA	0.66 ghi*	1.46 b	0.2 jk
3 BAP+0.3 NAA	0.7 fgh	1.03 def	0.3 ijk
1 BAP+0.1IBA	0.8 efg	3.13 a	0.56 ghij
3 BAP+0.3 IBA	0.43 ghij	0.53 ghij	0.63ghi
0.5BAP	0.0 k	1.4 bc	0.4 hij
1BAP	0.0 k	0.56 ghij	0.23jk
3BAP	1.3 bcd	1.1 cde	0.0 k
12 weeks			
1 BAP+0.1 NAA	1.7 bcd*	2.96 a	0.86 defg
3 BAP+0.3 NAA	1.1 cdef	2.0 b	0.9 defg
1 BAP+0.1IBA	1.83 bc	3.7 a	0.83 defgh
3 BAP+0.3 IBA	1.26 bcde	1.93 bc	1.33 bcde
0.5BAP	0.53 efgh	2.1 b	0.8 efgh
1BAP	0.0 h	0.9 defg	0.23 fgh
3BAP	1.96 bc	1.93 bc	0.13 gh

* For each time, means in each column with the similar letter are not significantly different at 5% level of probability using DMRT

Callus Obtained on MS Medium Supplemented with 0.5 mg l^{-1} IBA after 12 Weeks: The numerous bulblets were produced with culture of leaf primordia callus on MS medium supplemented with 3.0 mg l^{-1} BAP in combination with 0.3 mg l^{-1} IBA and also MS medium containing 1.0 mg l^{-1} BAP (Fig. 1). These results while affecting the treatment culture of leaf primordia callus on MS medium containing 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} NAA and also culture of scale callus on MS medium supplemented with 3.0 mg l^{-1} BAP in combination with 0.3 mg l^{-1} NAA did not significantly differ. With culture of immature inflorescence callus in all treatments of plant growth regulators, the least rates of bulblets regeneration were maintained (Table 2).

Callus Obtained on MS Medium Supplemented with 1.0 mg l^{-1} IBA after 6 Weeks: The culture of leaf primordia callus on organogenesis media which was MS medium supplemented with 1.0 mg l^{-1} BAP in combination with 0.1 mg l^{-1} IBA, the number of bulblets obtained was significantly higher than all other treatments (Table 3).

After culturing of scale callus on MS medium containing 0.5 or 1.0 mg l^{-1} BAP and also culture of immature inflorescence callus on MS medium containinig 3.0 mg l^{-1} BAP, none bulblets regeneration were done.

Callus Obtained on MS Medium Supplemented with 1.0 mg l^{-1} IBA after 12 Weeks: The results of organogenesis from callus which were obtained on MS media containing 1.0 mg l^{-1} IBA, after 12 weeks, have been shown in Table 3.

After culturing of leaf primordial callus on MS medium supplemented with 1.0 mg l^{-1} BAP in combination with 0.1 mg l^{-1} IBA and also MS medium supplemented with 1.0 mg l^{-1} BAP in combination with 0.1 mg l^{-1} NAA with regard to other treatments the numerous bulblets were significantly produced (P = 5%). With culture of scale callus on MS medium containing 1.0 mg l^{-1} BAP none regeneration was done.

DISCUSSION

The results showed that the amount of callus formation of scale explant was significantly higher than leaf primordia and immature inflorescence explants. The production of callus from hyacinth scale have been reported by many people [17-19]. Hussey [3] and Sheng Quan and Cabio [5] have approved differences in callus production between different types of explants of hyacinth. It seems that one of the important reasons why the rate of produced callus in scale explant was higher than other parts of plant was because the existence of meristemoid-like cells is in basal plate of bulb that is in better situation for callus production [2]. Peak and Thorpe [1] suggested that an essential prerequisite for good regeneration of hyacinth scale explant and bulblet growth is to use the basal part of the scale. Hussey [3] reported that there was no need for plant growth regulators for regeneration of scale and basal plate of hyacinth. Young Byung *et al.* [16] reported that the normal (base- down)orientation of the scale explants of hyacinth had greater influence on the root growth than their inverted(base- up) orientation. The results also revealed the application of NAA on medium affected callus formation more than IBA. The effect of different types of auxin on tissue culture of hyacinth have been compared and the existences of differences affecting of IAA, NAA and 2/4-D [3], IBA and IAA [4], NAA and IBA [3,19] have been reported. Hussey [19] reported that application of NAA in a low concentration brought about regeneration from stem, leaf and ovary explants of hyacinth, while application of NAA in a higher concentration (2.0 and 8.0 mg l^{-1}) provoked callus formation. Although in this research, the concentration of 1.0 mg l^{-1} NAA brought about callus formation, it seems that the results of this research and previous

studies [3, 19] have shown the positive effect of NAA in callus production from hyacinth explants.

Because of application of NAA on medium, callus with roots have been formed. Therefore, in this research, IBA applied on medium in order to obtain only callus to culture onto regeneration media. It is proposed that more research should be done to find out appropriate concentration of NAA to produce callus without root formation.

The results of regeneration from calli of scale, leaf primordia and immature inflorescence explants showed the rate of regeneration from immature inflorescence callus was less than scale and leaf primordia callus. These observations support the findings of Pierik and Post [19]; Pierik and Woet [17]; Pierik and Ruibing [18] and Young Byung *et al*. [16], who showed noticeable bulblets regeneration of hyacinth from scale explant.

It seems that one of the important reasons of low level of regeneration from immature inflorescence callus into scale callus or leaf primordia callus depends on characteristics of immature inflorescence cells as well as the anatomy of the immature inflorescence explant. Raju and Mann [2] reported that the amount of callus formed seemed to be related to the internal anatomy of the leaf at the time of isolation of the leaf. The variations in regenerative characteristics among explants are sometimes attributable to difference in explant physiological age and differentiation among the constituent cells [21]. Though it was not previously compared indirect regeneration from different types of hyacinth explants, bulblet regeneration from scale explant on media which was free of plant growth regulators [3] and needed to BAP along with NAA for bulblet regeneration from hyacinth generative organs such as bud flower was reported [4]. ShengQuan and Caibo [5] showed that the cells of generative parts of hyacinth flower physiologically did not have enough potential for regeneration. Thus, attention must be paid to the existence structural differences in this connection. Peak and Thrope [1] reported explanted ovaries show a lesser morphogenetic capacity as compared with the bulb scale segments, especially with regard to bulblet formation and subsequent growth. It has been reported that one of the appropriate conditions for regeneration of bulblets from hyacinth, is the existence of basal plate section of scale in the explant [19]. The importance of basal parts of the scale along with scale in micropropagation of hyacinth have also been suggested by other researchers [22, 23]. Even in usual and natural conditions, the formation of bulblet from scale which is separated from hyacinth bulb and cultured in bedding peat, can be easily done as well [24].

The results showed that the duration of maintenance of callus on regeneration media has been affected on bulblet regeneration. Hussey [3] has also approved the importance of time in regeneration of bulblets from hyacinth explant and suggested that the appropriate period for regeneration of bulblets in 8 to 12 weeks. The results also showed that the rate of regeneration of bulblets on MS media containing BAP with low concentration of auxin, were higher than MS media containing BAP alone. Kim *et al*. [4] have reported that the regeneration of bulblets on media containing cytokinin along with auxin. Furthermore, the positive effect of auxin in bulblets regeneration of bulblets from different types of explant of hyacinth as well as the differences in type of auxin have also been reported by many other researchers [3, 16, 17, 18, 25, 26].

CONCLUSION

The scale explant was appropriated for callus formation. However, callus formation has been affected by different types of auxin. The calli of leaf primordia and scale potentially were more affected on regeneration than immature inflorescence. The cytokinin along with auxin were more affected on regeneration than cytokinin alone.

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