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Effective *in Vitro* Propagation by Bulb Scale Segments Culture of *Muscari comosum* var. *plumosum*

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Abstract - A rapid and mass propagation method for multiple shoots and plant regeneration using bulb scales of *Muscari comosum* var. *plumosum* were developed. *In vitro* different parts of bulb scale as explants were cultured on 11 kinds of MS (1962) media supplemented with various plant growth regulators to induce shoot and callus. A combination of 2.0 mg/L 6-BA and 2.0 mg/L IBA on MS medium was the most favorable and induced the highest production (80%) of shoot formation after 30 days. We also found that the middle part of bulb scale was the best for mass propagation of *Muscari comosum* var. *plumosum* of which production could reach 64.4%.

Key words - Bulb scale, Direct shoot, Muscari, Propagation, Rapid

Introduction

Muscari, characterized by itsclusters of small, bell-shaped, cobalt-blue flowers which look like clusters of upside-down grapes, is one kind of bulbous plant. It belongs to the family *Liliaceae* and is divided into three subgenus*Muscari*, *Leopoldia* and *Botryanthus*. Commonly it is named grape hyacinth and cultivated for pot or garden use. Muscari grows throughout the temperate regions of the world from sea level to 2000 m high, and conventionally propagate by scaling from mother bulbs. Only 2~5 bulblets can be produced by this method, depending on species, cultivar and bulb size. It takes 3~4 years from bulblet to blossom. So it is difficult to obtain large numbers of bulbs from scale propagation in a short period of time. Therefore, rapid and mass propagation *in vitro* is essential to produce large numbers of bulblets of Muscari.

Previous studies on the plantlet regeneration on Muscari utilized protoplasts (Nakano *et al.*, 2005). The effect of leaf cutting and leaf part on bulblet formation of Muscari was investigated, and the survival rate of cutting and forming capacity of bulblets were higher in autumn cutting than those of spring (Choi *et al.*, 2000). The effect of leaf order and position on bulblet formation of Muscari was also reported. The forming capacity of bulblet was higher in middle leaf than inner or outer leaf (Park *et al.*, 2000). Studies showed the effect of scaling time and scale position on the capacity of bulblet formation, and the capacity of bulblet formation was higher in outer scale than inner scale (Bae *et al.*, 2000). Peck and Cumming (1986) reported that the production of bulblets of Muscari through tissue culture was enhanced when 1g/L activated charcoal was added to a modified MS medium. Bulbous plants are always as the object for plant regeneration and propagation from bulb scale like lilies (Li *et al.*, 2004; Wang *et al.*, 2005) and lycorises (Wang *et al.*, 2005; Zhu *et al.*, 2002). In our experiment we also used different sections of bulb scales, and aimed to develop a rapid method for inducing shoot clumps of *Muscari comosum* var. *plumosum*.

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Materials and Methods

Plant material

Healthy bulb of *Muscari comosum* var. *plumosum* was chosen from pot, and discarded the brown damaged outer scale, then cut the stalk and roots off to make the bulb left. Used running water to rinse the bulb for 1~2hours and blotted dry. The bulb was sterilized by dipping 70% ETOH for 1 minute , following by soaking 7% calcium hypochlorite added 1 or 2 drops Tween-20 for 30 minutes. After rinsed five times by distilled water, separated the bulb into three parts from basal disc to top on average, and marked them as upper, middle and bottom part.

Culture condition

Small pieces of scales from three different parts (upper, middle and bottom) as explantswere cultured continuously either on MS medium containing 0.1 mg/L to 5.0 mg/L IBA and BA, or NAA, 2,4-D and BA in 9cm petri dishes (6~8 explants per dish). The media were adjusted to pH 5.8 before autoclaving at 121→ for 15 min. All cultures were incubated at 25±2→, 50mol·m-2·s-1 , 16/8h photoperiod per day in a growth chamber.

Results

Effect of IBA and BA on direct shoots and bulbs formation of Muscari

After two weeks of culture, some small shoots emerged from the surface or cut edge of the bulb scale (Fig. 3A-3B), and grew up persistently. Thirty days later, we found that shoots could be induced on all of the different media. MS medium supplemented with 2.0 mg/L BA and 2.0 mg/L IBA, was the most responsive for direct shoot development, on which the earliest shoots emerged (Fig. 3C). The production of shoot formation could achieve up to 80%. Root produced at the cut edge of bulb scale on MS medium containing 2.0 mg/L BA and 2.0 mg/L IBA or 3.0 mg/L BA and 3.0 mg/L IBA (Fig. 1). On MS media supplemented with 5.0 mg/L BA and 5.0 mg/L IBA, a lot of regenerated plants were induced (Fig. 3F, G, H). However, MS medium containing IBA from 0.1mg/L to 1.0mg/L could make the shoots emerged but only 1 or 2 per scale, and much smaller. The number of shoots per explants was obviously higher on MS medium with 2.0 mg/L to 5.0 mg/L BA. More than 50 shoots could be produced on one scale, and the average were 20~40. On MS medium without auxin and cytokinin, there was no response, no shoot, callus and root.

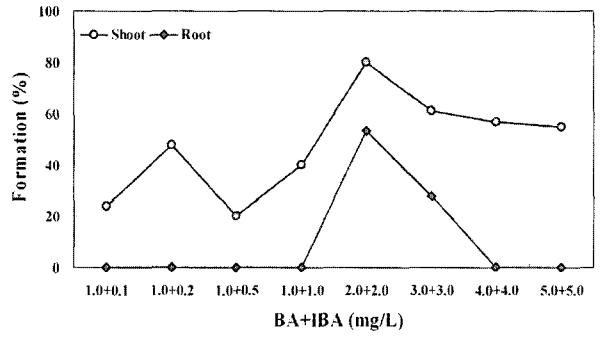

Fig 1. Effect of IBA and BA on shoot and root production in bulb segment culture of Muscari after 30 days of culture.

Effect of 2,4-D , NAA and BA on callus and multishoot induction

MS medium supplemented with 1.0 mg/L BA and 0.1 mg/L 2,4-D or 1.0 mg/L BA and 0.5 mg/L NAA was good for shoot and callus formation. Organogeneticcalli from bulb scale vigorously produced on these two media. The compact and yellowish calli changed to green spot and shoot differentiation. (Fig. 3E)

Effect of different parts of bulb scale on multishoot formation

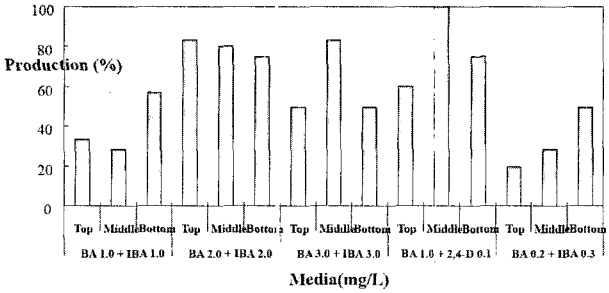

Fig. 2. Production of shoot and bulblets from different parts of bulb scale on five media after 30 days of culture.

TTable 1. Effect of different parts of bulb scale on shoot producing

Explant parts	No. of cultured explants	No. of formed shoots	% of producing
Top	61	16	26.2
Middle	73	47	64.4
Bottom	73	34	46.6

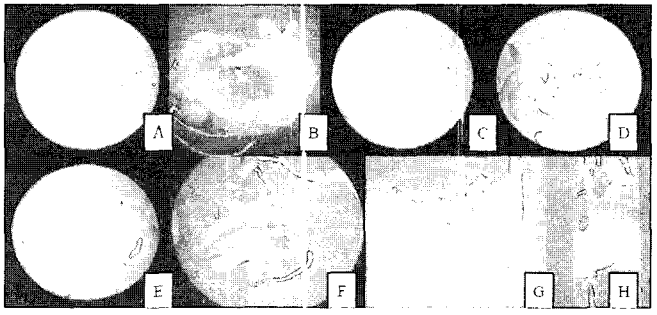

Fig. 3. Rapid and mass production of regenerated plantlets from bulb scale culture of *Muscari comosum* var. *plumosum* after 30 days of culture. A-D: Shoots emerged and grew from the surface or cut edge of the bulb scale after 2 weeks, multiple shoots formed and elongated from calli after 30 days on MS medium with 2.0 mg/L IBA and 2.0mg/L BA (A-D) or 5.0 mg/L IBA and 5.0 mg/L BA (F-H). E: A single shoot produced from compact and yellowish calli on MS medium with 0.5 mg/L NAA + 1.0 mg/L BA.

The earliest shoots emerged on the bulb scale from middle part, on which the shoots could form rapidly and become shoot clusters (Fig. 3C, 3D). We selected five kinds of media at random showed as Figure 2. We could get the highest production of shoot formation from the scale of middle part showed as Table 1.

Discussion

We found that shoot formation was stimulated by BA but not IBA. In our experiment, there were no obvious differences on MS medium containing 0.1 mg/L to 1.0 mg/L IBA and 1.0 mg/L BA. In comparison with MS medium supplemented with high concentration of BA, many shoots were formed on each bulb scales. Previously studies on *Lilium* also reported that BA was critical to promote the formation of adventitious shoot (Maesato *et al.*, 1994) and the addition of BA to the solidified medium was effective for increasing the number of shoots induced from shoot clusters (Godo *et al*,. 1998).

The effect of different auxin was shown in this experiment. MS medium with 1.0 mg/L BA and 0.1mg/L IBA, and the other with 1.0 mg/L BA and 0.1mg/ 2,4-D, same concentration but different response. We could find only 1~2 direct shoots per explants on MS medium containing 1.0 mg/L BA and 0.1 mg/L IBA, but numerous shoots on MS medium containing 0.1 mg/L 2,4-D and 1.0mg/L BA. With the same concentration of BA, 0.1 mg/L 2,4-D was more favorable for shoot formation than IBA in this experiment.

Bulb scales have a strong regeneration ability than callus and the time required for bulblet regeneration from scale is less than from callus (Takayama and Misawa, 1983). On Sego Lily, more shoots formed from the basal sections of bulb, which contain the meristematic basal plate, than from the middle or top sections (Hou *et al.*, 1997). And the shape and size of bulb scale explants from hyacinthwere critical factors in maximizing bulb production *in vitro* (Pierik and Post, 1975). In our experiment some cases showed that the bottom part ofbulbs was better than middle part on MS media containing 0.3 mg/L IBA + 0.2 mg/L BA or 1.0 mg/L IBA + 1.0 mg/L BA in this procedure. But we got the highest production of shoot formation from the scale of middle part. We thought that the initial bulb of *Muscari comosum* var. *plumosum* was small, and the scales of bottom part with smaller meristematic basal plate were not big enough to induce extensive direct shoots like the scales of middle part.

In order to obtain the rapid and mass propagation from the bulb scale of *Muscari comosum* var. *plumosum*, the middle parts were cultured on MS medium supplemented with 2.0 mg/L IBA and 2.0 mg/L BA. After thirty days, they were subcultured on the same medium for enlargement. The bulblets were transferred on MS medium for rooting. This method may be very convenient for rapid and mass propagation from bulb scale culture of *Muscari comosum* var. *plumosum.*

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