Korean J. Plant Res. 19(3) : 427~431 (2006)

Somatic Embryogenesis from Various Parts of *Muscari comosum* var. *plumosum*

He Xudong, Jeong Ae Ko[1*], Jeong Ran Choi[1], Hyung Moo Kim[1], Myung Jun Kim[1], So Ra Choi[2], Young Gon Kim[3], Dong Hee Kim[4] and Hyun Soon Kim[5]

The Key Laboratory of Forest Genetics and Gene Engineering, Nanjing Forestry University, Nanjing, 210037, China
Faculty of Biological Resources Sciences, College of Agriculture, Chonbuk National University, Chonju 561-756, Korea
[2]Jinan Medicinal Herbs Experiment Station, Jinan 567-804, Korea
[3]Faculty of Forest Science, Chonbuk National University, Chonju 561-756, Korea
[4]Interdisciplinary Program of International Tea Culture, Mokpo National University, 534-729, Korea
[5]International Technical Cooperation Center Rural Development Administration, Suwon 441-707, Korea

Abstract - *In vitro* high-frequency plant regeneration of *Muscari comosum* var. *plumosum* through somatic embryogenesis was obtained via two developmental pathways: direct embryos and multiple shoots regenerated from embryogenic callus. Flower bud with pedicel, receptacle, petal and ovary wall, floral stalk and leaf as explants were cultured in MS medium supplemented with various plant growth regulators. Embryos formed directly from pedicel, receptacle and floral stalk. Depending on explant sources, the optimal medium was MS medium supplemented with 0.2 mg/L IBA and 0.3 mg/L BA, 3.0 mg/L IBA and 3.0 mg/L BA, and MS-free medium for pedicel, receptacle, and floral stalk, respectively. Multiple shoots regenerated from embryogenic calli which was initiated from petal, ovary and leaf were observed in MS medium with different concentrations and combinations of hormone. The most suitable medium for each type of explant was 3.0 mg/L IBA and 3.0 mg/L BA (petal and ovary) and 5.0 mg/L IBA and 5.0 mg/L BA (leaf). Furthermore, the combination of 0.1 mg/L 2,4-D and 1.0 mg/L BA was also good for all sources of explants not only for direct embryo formation, but also, for embryogenic callus induction.

Key words - Floral stalk, Flower buds, Leaf, Muscari, Somatic embryogenesis

Introduction

Muscari comosum var. *plumosum* belongs to the family Liliaceae, and is generally called 'muscari' or 'grape hyacinth'. It is a kind of distinctive, widespread ornamental plant and cultivated for pot and garden uses. Muscari is easily recognized by its purple or cobalt-blue 'tassel' consisted of small flowers with long stalks. Because of the different habitat and climate, it takes a long time to produce plantlets through seed germination, and the production is very low. Vegetative propagation by bulbils can also be used in *Muscari comosum* var. *plumosum*, but only three to four bulbils are formed from the mother bulbs.

Recently, *in vitro* rapid and mass propagation system of several species has already been established (Swamy *et al.*, 1992; Xiao and Branchard, 1995; Han *et al.*, 2004). But like other bulbous plants (Liu and Xu, 2001; Wang *et al.*, 2004), the underground bulbs of Muscari are still easily contaminated by fungi after being transferred to medium if using routine sterilization method in tissue culture. In order to solve this serious problem, previous studies used other plant parts as explants for mass propagation, such as pedicel of *Hippeastrum hybridum* Hort. (Ko *et al.*, 2005), receptacle of *Lilium longiflorum* (Nhut *et al.*, 2001), petal of asiatic lily (Yang *et al.*, 2004), ovary of *Lilium* (Chen *et al.*, 1997), anther of *Lilium* asiatic hybrid (Ko, 1996), flower stalk nodes of *Scilla violacea* Hutch. (Lee *et al.*, 2004) and leaf of oriental lily hybrids (Zhang *et al.*, 2004). The development of protocols aiming to establish *in vitro* propagation by using more readily available material explants and plant growth regulators could alleviate many problems associated with underground bulb with plentiful fungi. In this study, various explants and plant growth regulators were used to establish a simple and high-frequency somatic embryogenesis system of *Muscari comosum* var. *plumosum*.

Materials and Methods

Plant material

Young leaves, floral stalks and flower buds of *Muscari comosum* var. *plumosum* were used as explants for shoot or callus formation. Healthy

*Corresponding author. E-mail : kjam@chonbuk.ac.kr

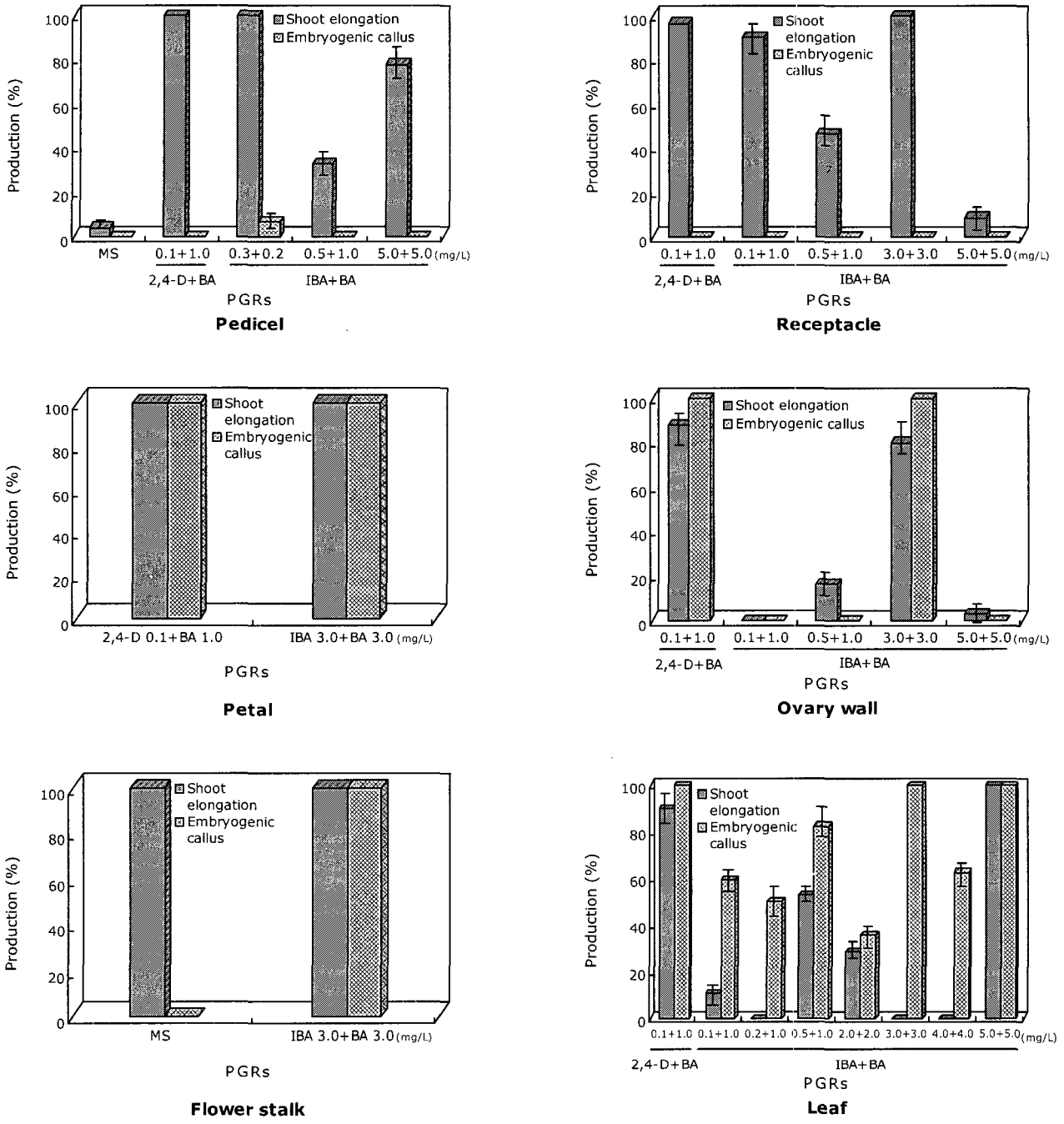

Fig. 1. Effect of explant sources and plant growth regulators on embryogenic callus and shoot regeneration in *Muscari comosum* var. *plumosum* after 30 days of culture.

flower buds before 5-10 days of anthesis containing pedicel, receptacle, petal and ovary wall were chosen. The explants were sterilized individually by dipping in 70% EtOH for 1 minute, followed by soaking in 7% calcium hypochlorite added 1 or 2 drops tween-20 for 15 minutes and rinsed five times with distilled water. Leaves and floral stalks were cut transversely into segments 5-7 mm and 1 cm long, respectively. Two longitudinal edges of leaves were also cut off for the purpose.

Culture condition

The three kinds of explants were cultured continuously on MS me-

dium containing either 0.1 mg/L to 5.0 mg/L IBA and BA, or NAA, 2,4-D and BA in 9-cm petri-dishes with six to eight explants per dish. All media used in this study were supplemented with 30 mg/L sucrose and 3.3 mg/L phytagel, and adjusted to pH 5.8 before autoclaving at 121℃ for 15 minutes. All cultures were incubated at 25±2℃, 50μ mol·m^{-2}·s^{-1}, 16/8 hour photoperiod per day in a growth chamber.

Results

Direct embryos emerged from pedicel, receptacle and floral stalk

In most of the plant growth regulator combinations tested, the different positions of flower buds showed the ability to induce direct embryos after 30 days of culture. The flower buds initially expanded entirely, then from pedicel and receptacle. Embryos grew and then elongated on MS medium with various plant growth regulators (Fig. 2A-C and D-F, Fig. 1). Especially on MS medium with 0.3 mg/L IBA and 0.2 mg/L BA or with 0.1 mg/L 2,4-D and 1.0 mg/L BA, 100% production of direct embryos could be achieved from pedicel without callus. But from receptacle, the best medium for embryo formation was 3.0 mg/L IBA and 3.0 mg/L BA wherein the production could reach 100%. The combination of 0.1 mg/L 2,4-D and 1.0 mg/L BA was also good for shoot regeneration from receptacle with production reaching 96.7%. High concentration of 5.0 mg/L IBA and 5.0 mg/L BA caused the low frequency (8.6%) of multiple shoots. There was also no callus, but a few roots formed from receptacle (Fig. 2E). Enlarged bulbs from embryos could be induced from floral stalks in MS medium without any auxin and cytokinin (Fig. 2M and O). However, embryogenic calli with regenerated shoots were observed from floral stalks in MS medium with 0.1 mg/L 2,4-D and 1.0 mg/L BA (Fig. 2N).

Multiple shoot regenerated from embryogenic callus

Yellowish and compact embryogenic calli were induced from the epidermis of petals, ovary walls and leaf after 30 days of culture (Fig. 1). Various types of embryo produced from visible embryogenic callus were obtained from MS medium containing 0.1 mg/L 2,4-D and 1.0 mg/L BA (Fig. 2G-I) wherein the embryogenic callus production was 100%, and multiple shoots could form from all these calli on each explant. Furthermore, the same case was observed on MS medium containing 3.0 mg/L IBA and 3.0 mg/L BA (Fig. 1). The difference between these two combinations was that the number of shoots per explant in 3.0 mg/L IBA and 3.0 mg/L BA was higher than those in 0.1 mg/L 2,4-D and 1.0 mg/L BA.

Figure 1 showed that in MS medium containing 0.1 mg/L 2,4-D and 1.0 mg/L BA or 3.0 mg/L IBA and 3.0 mg/l BA, the percentage of embryogenic callus formation from ovary wall (Fig. 2J-L) was the same (100%), but the production of shoot regeneration from embryogenic callus in MS media with 3.0 mg/L IBA and 3.0 mg/L BA was slightly higher (87.8%) than in 0.1 mg/L 2,4-D and 1.0 mg/L BA (80%). Similar as petal, more shoots per explant could be obtained in MS media with 3.0 mg/L IBA and 3.0 mg/L BA from ovary wall. The combination with high concentration of IBA (5.0 mg/L) and BA (5.0 mg/L) inhibited shoot formation with frequency of only 2.9%.

The earliest visible signs of embryogenic callus from leaf were noticeable within 30 days of culture. Before inoculation, the surface of leaf was green and smooth. After culture in MS medium with various auxin and cytokinin, the explants expanded gradually. Subsequently, embryogenic callus initiation was seen developing from the cut edges of leaf (Fig. 2P-R) in all treatments with a large number of concentrations and combinations of hormone (Fig. 1). The highest production of embryogenic callus could reach 100% in MS media with each 3.0 or 5.0 mg/L BA or 0.1 mg/L 2,4-D and 1.0 mg/L BA. However, only in MS medium supplemented with 5.0 mg/L IBA and 5.0 mg/L BA all these embryogenic calli could form multiple shoots. No response occurred in MS-free medium with flower buds and leaf.

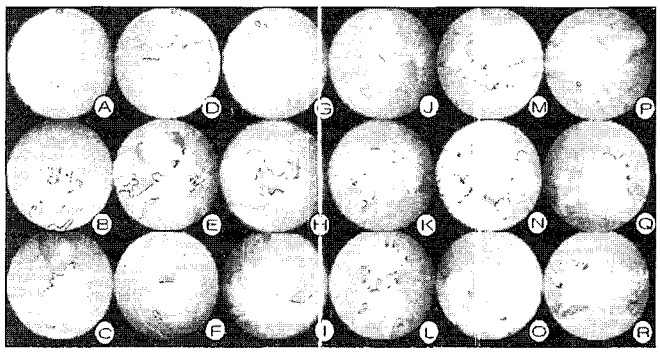

Fig. 2. Somatic embryogenesis induced from various parts of *Muscari comosum* var. *plumosum* after 30 days of culture. A-C: shoot elongated directly from pedicel in MS medium with 0.2 mg/L IBA+0.3 mg/L BA; D-F: multiple shoots and roots formed from receptacle in MS medium with 3.0 mg/L IBA+3.0 mg/L BA; G-L: embryogenic callus and various embryos produced from petal in MS medium with 0.1 mg/L 2,4-D+1.0 mg/L BA (G-I) and from ovary wall in MS medium with 3.0 mg/L IBA+3.0 mg/L BA (J-L); M-O: bulbs produced directly from floral stalks in MS-free medium (M and O) and embryogenic callus induced in MS medium with 0.1 mg/L 2,4-D+1.0 mg/L BA (N), and P-R: embryogenic callus produced from the cut edge of leaves and multiple shoots elongated in MS medium with each of 5.0 mg/L IBA and BA after 30 days of culture.

Discussion

Bulbous plants are always the object for plant regeneration and propagation from bulb scales like lilies (Li *et al.*, 2004; Wang *et al.*, 2005) and lycorises (Zhu *et al.*, 2002; Wang *et al.*, 2005). However, contamination is a serious problem in mass production of tissue culture from bulb scales, even with rare bulbous plants like muscari. Recently, explants from various parts of inflorescence have been recognized as suitable sources for micropropagation of Liliaceous plant species. Ko *et al.* (2005) reported that in *Hippeastrum hybridum* Hort., pedicel at 15 days before anthesis was the best explant for in vitro propagation compared with anther, styles and ovaries by means of organogenesis, and the optimal medium was MS medium with 0.5mg/L NAA and 1.0 mg/L BA. In the present study, 0.2 mg/L IBA and 0.3 mg/L BA was good for direct embryo formation from pedicel. In *Lilium longiflorum*, receptacle sections from flower buds cultured in one-half MS medium plus 5.4 μM NAA or 4.9 μM IBA and 2.2 mM BAP, produced an average of 41 shoots per explant without passing through a callus phase (Nhut *et al.*, 2001). Similar to this study, the combination of 3.0 mg/L IBA and 3.0 mg/L BA was the most suitable for direct embryos induction. Lee *et al.* (2004) reported that the floral stalk attached with pedicel seven days before anthesis of *Scilla violacea* Hutch. was effective to induce shoot in MS medium supplemented with 0.5 mg/L NAA and 1.0 mg/L BA. In contrast with *Scilla violacea* Hutch., the floral stalk of *Muscari comosum* var. *plumosum* could form embryo directly and elongate in MS-free medium after 20 days of culture. It is believed that near the node of the stalk there is a meristem which has the ability to produce direct embryo without any addition of auxin and cytokinin. Petal, ovary and anther of some lily species also had been reported for plant regeneration and mass propagation (Ko, 1996; Chen *et al.*, 1997; Yang *et al.*, 2004).

In another muscari species, *Muscari armeniacum* Leichtl. Ex Bak., Suzuki and Nakano (2001) obtained two kinds of callus in leaf culture: fast-growing, yellowish nodular callus and white friable callus. The yellowish nodular callus vigorously produced shoot buds after being transferred to media containing 0.44±44 μM BA, while the white friable callus produced numerous somatic embryos upon transfer to plant growth with MS-free medium. In the present study, yellowish and compact embryogenic callus were observed from all the combinations of IBA or 2,4-D and BA, and shoots emerged from these calli on the same media after 2 weeks of culture. Even in MS medium with 5.0 mg/L IBA and 5.0 mg/L BA, shoot production could reach 100%.

Thus, an effective, simple and high-frequency somatic embryogenesis system of *Muscari comosum* var. *plumosum* has been established. Depending on the sources of explants, the optimal medium containing IBA and BA may vary. However, the combination of 0.1 mg/L 2,4-D and 1.0 mg/L BA was good for all types of explants, not only for direct embryo formation, but also, for embryogenic callus induction.

Literature Cited

Chen S.N., J.L. Gao and Z.Y. Zhen. 1997. Tissue culture of *Lilium* ovary. Journal of Yunnan University 19: 374-375.

Han B.H., H.J. Yu, B.W. Yae and K.Y. Peak. 2004. *In vitro* micropropagation of *Lilium longiflorum* 'Georgia' by shoot formation as influenced by addition of liquid medium. Scientia Horticulturae 103: 39-49.

Ko J.A. 1996. Plant regeneration by anther culture of *Lilium* asiatic hybrid 'Gran Paradiso'. Korean J. Plant Tissue Culture 26: 1-6.

Ko J.A., M.J. Kim, Y.S. Kim and H.S. Kim. 2005. Effective *in vitro* propagation from pedicel culture of *Hippeastrum hybridum* Hort. 'Dazzler'. Korean J. Plant Res. 18: 382-389.

Lee E.J., M.J. Song, M.J. Kim and J.A. Ko. 2004. Mass propagation of *Scilla violacea* Hutch. through *in vitro* culture of flower stalk nodes. Korean J. Plant Res. 22: 481-485.

Li Q.X., Q.F. Zhao and H.L. Li. 2004. Study on tissue culture and propagation of *Nettuno*. J. Northwest Normal University 40: 74-76.

Liu Y.G. and Z.Q. Xu. 2001. Tissue culture and plant regeneration of *Hyacinth orientalis* L.. J. Northwest University 31: 255-258.

Nhut D.T., B.V. Le, M. Tanaka and K.T.T. Van. 2001. Shoot induction and plant regeneration from receptacle tissues of *Lilium longiflorum*. Scientia Horticulturae 87: 131-138.

Suzuki S. and M. Nakano. 2001. Organogenesis and somatic embryogenesis from callus cultures in *Muscari armeniacum* Leichtl. ex Bak.. In Vitro Cell Dev Biol-Plant 37: 382-387.

Swamy B.V.R., K. Himabindu and G.L. Sita. 1992. *In vitro* micropropagation of elite rosewood (*Dalbergia latifolia* Roxb.). Plant Cell Reports 11: 126-131.

Wang F.L., H.G. Zhou, Z.F. Hang and Y.P. Lin. 2005. Study on techniques of rapid propagation by tissue culture of the asiatic hybrids, J. Anhui Agri. Sci. 33: 820-821.

Wang G.P., Y. Chen, J. Zhou, L. Zhang and M.R. Huang. 2005. Bulb induction and plant regeneration of *Lycoris longituba*. Plant Physiology Communication 41: 457-460.

Wang Y.J., Z.C. Zhang, L.F. Zhang, Q. Qiao and X.L. Jin. 2004. Studies on the technique of tissue culture and rapid propagation of

lily. Henan Agriculture Science 5: 55-58.

Xiao X.G. and M. Branchard. 1995. *In vitro* high frequency plant regeneration from hypocotyl and root segments of spinach by organogenesis. Plant Cell, Tissue and Organ Culture 42: 239-244.

Yang W.H., Y.L. Zhang, B. Tong, H. Du and R. Yang. 2004. Tissue culture and rapid propagation of asiatic lily from flower organs. Chinese Agricultural Science Bulletin 20: 193-195.

Zhang Y.L., A. Xu, J.C. Wang and Z.J. Yang. 2004. Tissue culture of oriental lily hybrid. Journal of Northwest Sci-Tech Univ. of Agri. and For. 32: 47-50.

Zhu J., Q. Zhuge, S.S. Yu and N.J. Chen. 2002. Study on tissue culture propagation technique of *Lycoris radiate*. J. of Zhejiang For. Sci. & Tech. 22: 45-48.

(Received 15 May 2006; Accepted 12 June 2006)