RESEARCH ARTICLE

AN IMPROVED AND EFFICIENT ORGANOGENIC REGENERATION PROTOCOL USING EPICOTYL SEGMENT OF *IN VITRO* GROWN KAGZILIME (*CITRUS AURANTIFOLIA*) SEEDLING

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Abstract: In the present study, an improved and efficient plant regeneration protocol of Kagzilime (*Citrus aurantifolia*) using epicotyl segment of *in vitro* grown seedlings was developed. Kagzilime seed sterilized with Bavistin @ 0.1% for 30 min followed by Mercuric chloride @ 0.1% for 15 min was found to be optimum to reduce the contamination and efficient seed germination. About 0.75-1.0 cm long epicotyl segments of *in vitro* grown 21 days old seedlings were found suitable explants for efficient plant regeneration. The best regeneration efficiency of 84% with 5 shoots/explant was obtained at BAP @ 2.0 mg/l. The higher efficiency of root induction of 60.60% with 4.40 roots/shoot was observed at lower concentration of NAA@ 0.5 mg/l. Over 90% of plantlets were acclimatized and grown at pot mixture of soil, sand and vermiculite @ 1:2:1 in greenhouse. The efficient regeneration protocol developed in this study will be useful for mass propagation of root stock, biological indexing of virus diseases, production of disease free elite planting material, plant transformation and *in vivo* expression of desired viral gene.

Keywords: Age of explants, Epicotyl segment, Kagzilime, Multiple shoots, Regeneration

REFERENCES

Al-Khayri, J.M. and Al-Bahrany, A.M. (2001). *In vitro* micropropagation of *Citrus aurantifolia* (lime). *Curr Sci*. 18: 1242-1246.

Bond, J.E. and Roose, M.L. (1998). *Agrobacterium*-mediated transformation of the commercially important citrus cultivar Washington navel orange. *Plant Cell Rep.* 18: 229–234.

Button, J. and Kochba, J. (1977). Tissue culture in the Citrus Industry. In: *applied and fundamental aspects of plant cell, tissue and organ culture (Reinert, J. and Bajaj, Y. P. S., eds)* Springer-Verlag, Berlin, 70-72.

Cervera, M., Lopez, M.M., Navarro, L. and Pena, L. (1998). Virulence and supervirulence of *Agrobacterium tumefaciens* in woody fruit plants. *Physiol Mol Plant Pathol.* 52: 67-78.

Chaturvedi, H.C. and Mitra, G.C. (1975). A shift in morphogenetic pattern in Citrus callus tissue during prolonged culture. *Ann Bot*. 39: 683.

Chaturvedi, H.C. and Sharma, A.K. (1985). Production of androgenic plants of *Citrus aurantifoli., J Plant Physiol.* 119: 473.

Costa, M.G.C., Alves, V.S., Lani E.R.G., Mosquim, P.R., Carvaltho, C.R. and Otoni, W.C. (2004). Morphogenic gradients of adventitious bud and shoot regeneration in epicotyl explants of *Citrus. Sci Hort.* 100: 63-74.

Gloria, F.J.M., Mourao Filho F.A. A., Camargo, L.E.A. and Mendes, M.E.J. Caipira. (2000). sweet orange + Rangpur Lime: A potential somatic hybrid to be used as rootstock in the Brazilian citrus industry. *Genet Mol Biol*. 23: 661-669.

Goswami, K., Sharma, R., Singh, P.K. and Singh, G. (2013). Micropropagation of seedless lemon (*Citrus limon* L. cv. Kaghzi Kalan) and assessment of genetic fidelity of micropropagated plants using RAPD markers. *Physiol Mol Biol Plants*. 19: 137–145.

Kaneyoshi, J., Kobayashi, S., Nakamura, Y., Shigemoto, N. and Doi, Y.A. (1994). Simple and efficient gene transfer system of trifoliate orange (*Poncirus trifoliata* Raf.). *Plant Cell Rep.* 13: 541-545.

Khan, I.A. (2007). Citrus genetics, breeding and biotechnology, CABI International, Wallingford, UK.

Khawale, R.N., Singh, S.K., Garg, G., Baranwal, V.K. and Alizadeh Ajirlo, S. (2006). *Agrobacterium*-mediated transformation of Nagpur mandarin (*Citrus reticulata* Blanco). *Current Science*, 91: 1700-1705.

Luth, D. and Moore, G. (1999). Transgenic grapefruit plants obtained by *Agrobacterium tumefaciens*-mediated transformation. *Plant Cell.* 57: 219-222.

Mitra, G.C. and Chaturvedi, H.C. (1972). Embryods and complete plants from unpollinated overies and from ovules of *in vivo* grown emasculated flower buds of *Citrus* sp. *Bull Torrey Bot Club*. 99: 184.

Moore, G.A., Jacono, C.C., Neidigh, J.L., Lawrence, S.D. and Cline, K. (1992). *Agrobacterium*-mediated transformation of Citrus stem explants and regeneration of transgenic plants. *Plant Cell Rep.* 11: 238-242.

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Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture, *Physiol Plant*. 15: 473-479.

Normah, M.N., Hamidah, S. and Ghani, F.D. (1997). Micropropagation of *Citrus halimii* stone. *Plant Cell Tiss Organ Cult*. 50: 225–227.

Pena, L., Cervera, M., Juarez, J., Navarro, A., Pina, J.A. and Navarro, L. (1997). Genetic transformation of lime (*Citrus aurantifolia* Swing.)**:** factors affecting transformation and regeneration. *Plant Cell Rep*. 16: 731-737.

Shah, S.K., Sharma, H.C., Goswami, A.M. and Saxena. (1999). *In vitro* seed germination for enhanced polyembryony in *Citrus.* spp. *Proc*. *Intl. Citrus Symposium.* 211-215.

Sim, G.E., Goh, C.J. and Loh, C.S. (1989). Micropropagation of *Citrus mitis* Blanco Multiple bud formation from shoot and root explants in the presence of 6-Benzylaminopurine. *Plant Science*, 59: 203-210.

Singh, S. and Rajam, M.V. (2009). Citrus biotechnology: Achievements, limitations and future directions. *Physiol Mol Biol Plants*. 15: 3-22.