

ASSESSMENT OF GENETIC FIDELITY OF MOTHER PLANT AND *IN VITRO* RAISED MEDICINAL PLANT *EPHEDRA GERARDIANA* THROUGH MOLECULAR MARKERS

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Abstract: *Ephedra gerardiana* is an important medicinal gymnosperm shrub. It has been traditionally use for an assortment of medicinal purpose. Molecular markers analysis was conducted to screen genetic fidelity among *in vitro* raised plantlets compare with mother plant of *Ephedra gerardiana*. Genetic fidelity of regenerated plants was assessed using Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) Primers. A total of 50 RAPD primers and 30 SSR primers were utilized in the present study to analyze genetic fidelity of mother plant and among tissue culture raised plants of *Ephedra gerardiana*. Out of 50 RAPD primers, 19 primers exhibited DNA amplification in all the DNA samples and out of 30 SSR primers, 18 were show amplification. The amplified products of the regenerated plants showed similar banding patterns to that of the mother plant thus demonstrated the homogeneity of the micropropagated plants. The banding pattern ruled out presence of any kind of somaclonal variation. Thus, the results revealed that genetic fidelity between the micropropagated and mother plant in *Ephedra gerardiana* and supports the suitability of tissue culture technique for generation of genetically similar plants. Hence, the results obtained confirmed genetic stability of regenerated plants.

Keywords: *Ephedra gerardiana*, Micropropagation, Genetic fidelity, RAPD, SSR

REFERENCES

- Cordeiro, G.M., Pan, Y.B., and Henry, R.J.** (2003). Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. *Plant Sci.*,**165**:181-189.
- Devarumath, R.M.** (2007). An assessment of somaclonal variation in micropropagated plants of sugarcane by RAPD markers. *Sugar Tech.* **10**:124-127.
- Doyle, J.J. and Doyle, J.L.** (1990). Isolation of plant DNA from fresh tissue. *Focus.* **12**:13-15.
- Gupta, R., Modgil, M., and Chakrabarti, S.K.** (2009). Assessment of genetic fidelity of micropropagated apple root stock plants EMLA 11 using RAPD markers. *Indian Journal of Experimental Biology*, **47**:925-928.
- Jain, A., Chaudhary, S. and Sharma, P.C.** (2014). Mining of microsatellites using next generation sequencing of seabuckthorn (*Hippophae rhamnoides* L.) transcriptome. *Physiology and Molecular Biology of Plants.* **20**:115-123.
- Jain, A., Ghangal, R., Grover, A., Raghuvanshi, S. and Sharma, P.C.** (2010). Development of new EST based SSR markers in seabuckthorn. *Physiology and Molecular Biology of Plants.* **16**:375-378.
- Jain, R., Srivastava, S., Singh, J. and Gupta, P.S.** (2005). Assessment of genetic purity of micropropagated plants of sugarcane by isozyme and RAPD analysis. *Sugar Tech.* **7**:15-19.
- Jody Aaron, Death Over the Counter:** Dangers of Ephedrine, *Trial*, (December 1, 1997) (1997 WL 9957878).
- Katti, M.V., Ranjekar, P.K. and Gupta, V.S.** (2001). Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Mol. Biol. Evol.*, **18**(7):1161-67.
- Kumar, S., Mangal, M., Dhawan, A.K., and Narender, S.** (2011). Assessment of genetic fidelity of micropropagated plants of *Simmondsia chinensis*(Link) Schminder Using PAPD and ISSR markers. *Acta Physiol Plant.***33**:2541-2545.
- Lal, M., Singh, R.K., Srivastava, S., Singh, N., Singh, S.P., and Sharma, M.L.** (2008). RAPD based analysis of micropropagated plantlets of sugarcane for early assessment of genetic fidelity. *Sugar Tech.***10**: 99-103
- Morgante, M., Hanafey, M., and Powell, W.** (2002). Microsatellites are preferentially associated with non-repetitive DNA in plant genomes. *Nature Genet.***30**: 194-200.
- Nei, M.** (1971). Analysis of genetic diversity in subdivided population. *Proc. Natl. Acad. Sci.* **70**: 3321-3323.
- Pandey, R.N., Singh, S.P., Rastogi, J., Sharma, M.L. and Singh, R.K.** (2012). Early assessment of genetic fidelity in sugarcane (*Saccharum*

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officinarum) plantlets regenerated through direct organogenesis with RAPD and SSR markers. *Australia Journal of Crop Science*. **6**:618-624.

Parida, S.K., Kalia, S.K., Kaul, S., Dalal, V., Hemaprabha, G., Selvi, A., Pandit, A., Singh, A., Gaikwad, K., Sharma, T.R., Srivastava, P.S., Singh, N.K. and Mohapatra, T. (2009). Informative genomic microsatellite markers for efficient genotyping applications in sugarcane. *Theoretical and Applied Genetics*. **118**:327-338.

Parida, S.K., Yadava, D.K., and Mohapatra, T. (2010). Microsatellites in Brassica unigenes: Relative abundance, marker design and use in comparative physical mapping and genome analysis. *Genome.*, **53**:55-67.

Paul, D. Rheingold. (2001). The Prospects for PPA and Ephedra Litigation—and How it Differs From Prescription Drug Cases. *Mealey's Emerging Drugs & Devices*. 28 (January 18, 2001)

Pinto, L.R., Oliveira, K.M., Ulian, E.C., Garcia, A.A.F. and De-Souza, A.P. (2004). Survey in the sugarcane expressed sequence tag database (SUCEST) for simple sequence repeats. *Genome*. **47**:795-804.

Rani, V., and Raina, S.N. (2000). Genetic fidelity of organized meristem derived micropropagated plants: a critical reappraisal. *In vitro Cell dev Biol Plant*, **36**:319–330.

Roorkiwal, M., and Sharma, P. C. (2011). Mining functional microsatellites in legume unigenes. *Bioinformation*, **7**: 264-270.

Saini, N., Saini, M.L., and Jain, R.K. (2004). Large scale production, field performance and RAPD analysis of micropropagated sugarcane plants. *Ind J Gent*. **64**:102-107.

Sharma, M.D., Dobhal, U., Singh, P., Kumar, S., Gaur, A.K., Singh, S.P., Jeena, A.S., Koshy, E.P., and Kumar, S. (2013). Assessment of genetic diversity among Sugarcane cultivars using novel microsatellite markers. *Afri. J. Biotechnol.*, **13**(13):1444-1451.

Sharma, O. P., Bambawale, O. M., Gopali, J. B., Bhagat, S., Yelshetty, S., Singh, S. K., Anand, R., and Singh, O.M. (2011). Field guide Mung bean and Urd bean. Government of India, Department of agricultural and co-operation, NCIPM, ICAR, New Delhi, India

Sharma, P.C., Grover, A. and Kahl, G. (2007). Mining microsatellites in eukaryotic genomes. *Trends in Biotechnology*. **25**:490–498.

Sharma, V., Gupta, K.S. and Diman, M. (2012). Regeneration of plant from nodal and internodal segment cultures of ephedra gerardiana using Thidiazuron. *Plant tissue culture and biotechnology*, **22**(2): 153-161.

Shenoy, V.B, and Vasil I.K. (1992). Biochemical and molecular analysis of plants derived from embryogenic tissue cultures of napier grass (*Pennisetum purpureum* K. Schum). *Theor. Appl. Genet.*, **83**: 947-955.

Suprasanna, P., Desai, N.S., Choudhari, R.S. and Bapat, V.A. (2007). RAPD markers for assessing culture induced variation in somatic embryogenesis derived plants of sugarcane. *Sugar Tech*. **9**:284-289.

Suprasanna, P., Desai, N.S., Sapana, G. and Bapat, V.A. (2006). Monitoring genetic fidelity in plants derived through direct somatic embryogenesis in sugarcane by RAPD analysis. *Journal of New Seeds*. **8**:1-9.

Tawar, P.N., Sawant, R.A., Dalvi, S.G., Nikam, A.A., Kavar, P.G and Devarumath, R.M. (2008). An assessment of somaclonal variation in micropropagated plants of sugarcane by RAPD markers. *Sugar Tech.*, **10**(2): 124–127.

Taylor, P.W.J., Geijskes, J.R.K.H.L., Fraser, T.A., Henry, R. J. and Birch, R.G. (1995). Sensitivity of random amplified polymorphic DNA analysis to detect genetic change in sugarcane during tissue culture. *Theoretical and Applied Genetics*. **90**:1169-1173.

Thiel, T., Michalek, W., Varshney, R.K., and Graner, A. (2003). Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics*. **106**:411-422.

Tod, L., Stewart (1997). Getting High with a Little Help from the FEDS: Federal Regulation of Herbal Stimulants. *Journal of Pharmacy & Law*.(6 JPHARML 101).

Trivedi, S. (2004). Microsatellites (SSRs): puzzles within puzzle. *Indian journal of Biotechnology*. **3**:331–347.

Victoria, F.C., da Maia, L.C., and de Oliveira1, A.C. (2011). In silico comparative analysis of SSR markers in plants. *BMC Plant Biol.*, **11**: 11-15.